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Effects of hydrated lime and quicklime on the decay of buried human remains using pig cadavers as human body analogues

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ABSTRACT

Recent casework in Belgium involving the search for human remains buried with lime, demonstrated the need for more detailed understanding of the effect of different types of lime on cadaver decomposition and its micro-environment. Six pigs (*Sus scrofa*) were used as body analogues in field experiments. They were buried without lime, with hydrated lime ($Ca(OH)_2$) and with quicklime (CaO) in shallow graves in sandy loam soil in Belgium and recovered after 6 months of burial. Observations from these field recoveries informed additional laboratory experiments that were undertaken at the University of Bradford, UK. The combined results of these studies demonstrate that despite conflicting evidence in the literature, hydrated lime and quicklime both delay the decay of the carcass during the first 6 months. This study has implications for the investigation of clandestine burials and for a better understanding of archaeological plaster burials. Knowledge of the effects of lime on decomposition processes also has bearing on practices involving burial of animal carcasses and potentially the management of mass graves and mass disasters by humanitarian organisations and DVI teams.

Keywords: Taphonomy; Pig cadavers; Lime; Differential decomposition; Desiccation; Histology

1. Introduction

The search, detection and recovery of buried human remains by forensic archaeologists rely on an understanding of the taphonomic processes that occur within the immediate buried body environment and specifically their effect on the rate and extent of decomposition. The depositional environment influences the rate of decomposition, and unless microbiological growth is slowed or arrested, the soft tissues eventually liquefy and disintegrate, leaving skeletonised remains [1,2]. Certain conditions may have an impact on the rate of change and bring about conditions which differ from the usual decay process [3,4], There is a paucity of scientific information on the effects of specific chemicals on decay. Recent casework in Belgium involving the search for a clandestine burial of a body covered with lime, demonstrated a need for a more detailed understanding of the effect of lime on cadaver decomposition and on the micro-environment created within the grave itself.

It is a commonly held belief that lime can be used to enhance the speed of decay, to reduce the likelihood of detecting a body, to destroy evidence and that ultimately lime will lead to the rapid and total destruction of human remains. For this reasons lime is often observed in clandestine burials [5-9].

Besides the association with criminality, in conventional archaeology there are specific traditions of burial in materials such as lime, chalk, gypsum or a mixture of it, called plaster burials. These customs are interpreted as preservation rites for physical resurrection in early Christianity, but also linked to disposal practices associated with safeguarding against disease and contagion. Lime has been evidenced in Roman and early Christian burials [10-16], in medieval burials [17,18], in post-medieval burials [19], and during modern times by the Nazis within mass graves [20], or in civil wars as in Spain, Africa and former Yugoslavia [21,22].

The effects of lime on the decomposition of human remains are poorly understood, with the available information rather limited and often conflicting. Forbes et al. [23] investigated burial factors such as soil pH on adipocere formation in laboratory experiments with pig adipose tissue. Hydrated lime was used to create a highly alkaline environment. The results showed that the lime burial environment considerably inhibited decomposition and the formation of adipocere after a 12 month period. Thew [24] studied the effects of hydrated lime on pigs interred for 6 and 30 months. As a pilot study she made a qualitative and quantitative assessment of the degree of decomposition of the pigs. Where lime was present, the burials revealed retardation in the decomposition process.

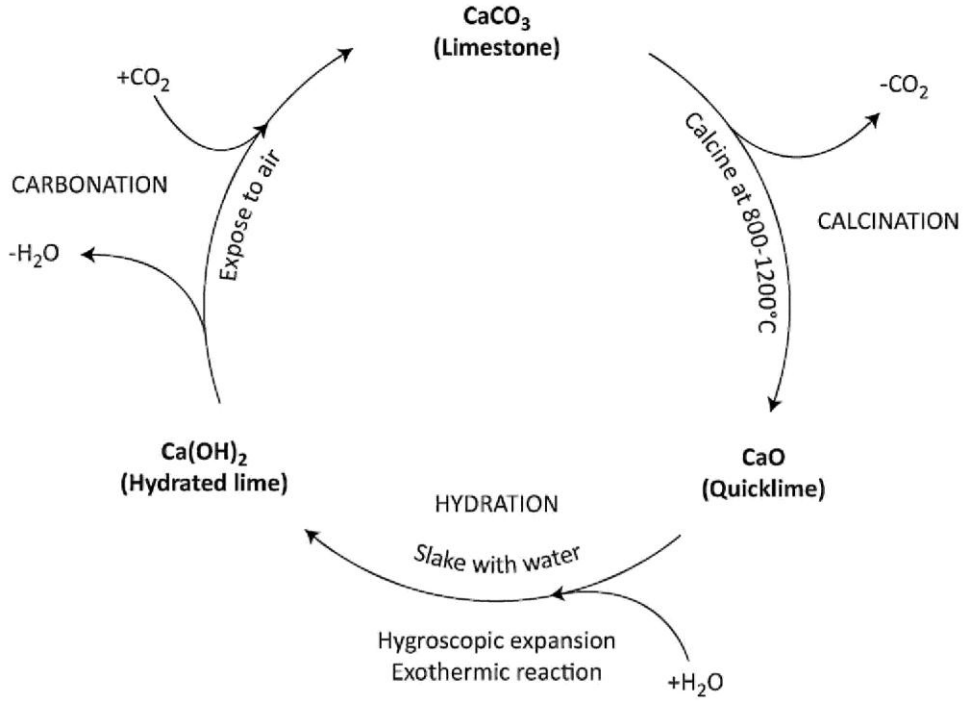
There is no research on the longer term outcome of lime within graves, other than observations from archaeological burials. A range of surviving evidence has been noted with lime burials dating from the Roman to post-medieval period [11,13,15] and last 100 years [25,26], ranging from lime casts with otherwise skeletonised remains, to textile fragments, soft tissue and surviving hair.

Lime is a generic term used to cover quicklime (CaO), hydrated lime ($Ca(OH)_2$) and non pure derivatives such as hydraulic lime. When limestone ($CaCO_3$) or other forms of calcium carbonate is calcined at a temperature in excess of 800°C, carbon dioxide is evolved, resulting in a white residue of calcium oxide (known variously as quicklime, unslaked lime or burnt lime). By adding water to quicklime calcium hydroxide is formed (known variously as hydrated lime or slaked lime) in an exothermic reaction. On exposure to air, absorption of carbon dioxide by the hydrated lime occurs resulting in the reversion of calcium carbonate (Fig. 1).

Lime is an alkaline product. Contact with lime can cause several types of skin reactions from mild irritation to full thickness burns. When calcium oxide reacts with water to form calcium hydroxide, heat is released while the pH immediately reaches 12-13 and further increases over the next 30 min [27]. Therefore lime is used in leather tanning processes whereby the depilatory effect of alkaline solutions is caused by the instability of keratin to alkalies [28]. Furthermore, lime is applied in agriculture to raise the pH and thereby reduce the acidity of soils. Because bacteria operate best within an optimal pH range, the addition of lime to soils will increase the rate of organic matter breakdown. This supports the common belief that covering a body with lime will lead to its rapid decomposition. However, the pH range tolerated by soil bacteria is generally between pH 4 and pH 10. The greater the departure from these conditions of optimal pH, the less the bacterial activity will be [29]. If the soil is too alkaline bacteria will not flourish and for this reason lime has traditionally been used in carcass disposal and during mass disasters as a disinfectant [30]. The World Health Organisation specifically advises against the use of lime as disinfectant and instead recommends use of chlorine solutions or other medical disinfectants [31, 33]. Besides disinfection, various sources suggest the use of lime to reduce putrefactive odours and discourage scavenging by predators [6,7,21]. The only published study on odours and lime suggests that it is only effective at reducing an initial odour within the first few weeks post-mortem [34].

The current study serves as an addition to the analyses of Forbes et al. [23] and Thew [24].The experiments were designed to lookat the macroscopic and microscopic effects of both hydrated lime and quicklime on decomposition of buried human body analogues. Experimental field trials were used to mimic the effects of lime within clandestine burials. Additional laboratory simulations allowed the investigation of tissue desiccation and lime.

Fig. *1. The lime cycle.*



2. Material and methods

2.1. Field site

The field experiments were conducted in 2008 (from February to August) on land owned by the Belgian Military in Meerdaal wood, near the city of Leuven in Flanders, Belgium. The wood is dominated by European beeches (*Fagus sylvatica* L.) and pedunculate oaks (*Quercus robur* L) with a limited ground flora of ferns and mosses. The Belgian soil map indicates a dry sandy loam with strong drainage. In advance of sample burial, a test pit was dug to better understand the soil characteristics. The soil profile consisted of a humic topsoil A-horizon which overlay a subsoil B-horizon with clear eluviation and illuviation layer. Parent material appeared at a depth of 45 cm. The A-horizon indicated a pH of 2.1, both B-horizon and parent material had a pH of 3.9. These low pH values are not unusual for well drained sandy soils.

2.2. Human body analogues and pre-burial records

Pigs are the most widely used human cadaver analogues used in forensic experiments. They have been used extensively in entomological and thanato-chemistry studies [35,36] and have been utilised in taphonomic investigations in different burial environments and climates [3,4,23,37,38]. Unlike other animals, they are largely hairless and have a similar body mass, skin structure, fat-muscle ratio and physiology to humans [39-41]. The greatest dissimilarity between pigs and humans are the bones which have a different microstructure [42]. Furthermore the limbs of these animals are proportionately much shorter than human limbs. Because the architecture of their skeleton is adapted to a quadrupedal stance, it is not possible to bury pigs supine. This means that they are most commonly buried on their side which has potential implications for the localised decomposition environment. However, so long as burials were consistent this issue was seen as minor, given that human clandestine burials are not always buried supine either.

In this experiment 6 juvenile pigs (*Sus scrofa*), aged between 1.5 and 2 months old, were used as substitutes for human bodies. All pigs died of natural causes and had no notifiable diseases. Sex was difficult to identify because of their young age. Carcasses were obtained as available, so size, weight and interval between death and burial were not standardised. Time since death for the first replicate pig set was 3 days and for the second set was 10 days. Previous information together with carcass condition and core body temperature (anus) was recorded prior to burial (Table 1). A fence was placed around the site to protect the graves from scavenging.

Laboratory simulations to investigate the impact of tissue desiccation were carried out with soil from the field site and the same lime as used in the field experiments. Pig tissue for the simulations consisted of fresh belly pork and rump pork as purchased at the market.

Table 1 : Pig source data and grave details.

		Pig data				Condition of carcass at burial							Grave details			
Pig/grave		Weight	Girth	Length (no tail)	Time since death (days)	Bloating	Lividity	Green abdom. spot	Purging	Wounds (AM-PM)	Oral temp. (°C)	Anal temp. (°C)	Depth (cm)	Size (cm)	Hydrated lime	Quicklime
Set 1	A2	14	60	85	3		*V*				8.1	7.7	34	90 x 64		
	B2	15	63	85	3	*V*	*V*	*V*			8.1	7.7	37	90 x 72	*V*	
	C2	20	68	96	3	*V*	*V*	*V*	*V*		7.8	8.1	33	114 x 72		*V*
Set 2	A3	20	70	95	10	*V*	*V*	*V*	*V*		8.4	8.6	31	89 x 69		
	B3	19	70	94	10	*V*	*V*	*V*			8.6	7.9	36	78 x 70	*V*	
	C3	19	72	90	10	*V*	*V*	*V*	*V*		8.2	7.7	35	107 x 68		*V*

2.3. Burial construction

The pig graves and control pits were dug by hand. Because of site characteristics (rough terrain, tree roots) the graves could not be dug to exactly the same dimensions (Table 1). The burials were laid out on a grid system to facilitate recovery, recording and survey. They comprised two replicate pig sets and a corresponding control set. The spacing between the graves was just over a meter. The pigs were all buried on their left side. The control set consisted of pigs without lime ('A' set), a second set consisted of pigs with powdered hydrated lime ('B' set) (*Supercalco - Carmeuse Natural Chemicals - CAS n° 1305-62-0*) and a further set consisted of pigs with powdered quicklime ('C' set) (*Supervical - Carmeuse Natural Chemicals - CAS n° 1305- 78-8*). The same weight of lime (12.5 kg) was poured on top of the pigs. This meant that there was a difference in volume between the burials with quicklime and hydrated lime, given that quicklime is heavier than hydrated lime because it is not saturated with water. As a consequence the hydrated lime layer on the pigs was roughly 5-6 cm and the

quicklime layer was roughly 1-2 cm. The control pits contained the corresponding lime but no pig carcasses. Within 3.5 h the pits were backfilled with the excavated spoil in random fashion and compacted by gentle trampling.

2.4. Environmental monitoring of burial sites

Monitoring of environmental conditions at the Meerdaal site was undertaken using two methods. Semi-local weather data including daily atmospheric temperature and precipitation values were obtained from the nearest weather station (at 7 km in Beauvechain) of the KMI, the Royal Meteorological Institute of Belgium. These data were compared to microclimate data obtained by a Tinytag dual channel temperature logger (TGP1520, Gemini Dataloggers, Chichester, UK). This datalogger is environmentally robust and has the capability to take surface and sub-surface temperatures with its two external temperature probes. It has been used successfully in other fieldwork projects at the University of Bradford. Prior to placement in the field, the datalogger was programmed to take hourly readings using GLM Version 2.6, software produced by Gemini dataloggers. One of the non-limed pig corpses was fitted with one probe to measure temperature variations in the core (anus) of the pig's body. Another probe was placed 5 cm beneath the ground surface to measure immediate subsurface temperatures of the grave fill. Additionally, thermal imagery was carried out to detect differences in thermal radiation between the graves and surrounding soil. In April, after 2.5 months of burial, airborne infrared prospection was recorded with a Wescam 12DS/TS-200 Dual Sensor. After 3 and 6 months of burial (May and August) a ground based infrared camera Agema 570 was used for thermography.

The pH of each grave was measured at the time of burial and following exhumation of the pig carcasses. On the day of interment, soil samples were collected from the humic topsoil horizon and from the B-horizon. At exhumation, soil samples were taken from the topsoil, above and beneath the pigs and at the base of the graves. For each soil sample a pH analysis was carried out in duplicate with a portable pH meter (Consort C 562) by placing the electrode in a mixture of 10 g soil and 25 ml 1 M KCl solution. The electrode was calibrated in buffer solutions of pH 4 and pH 7 and rinsed with deionised water between every measurement.

2.5. *Carcass exhumation, sample collection and analysis*

After 6 months of burial the pig and control graves were exhumed in August 2008 using standard forensic archaeological methods. Observations on insect activity were recorded during exhumation with the assistance of a forensic entomologist to discriminate forensically important insects.

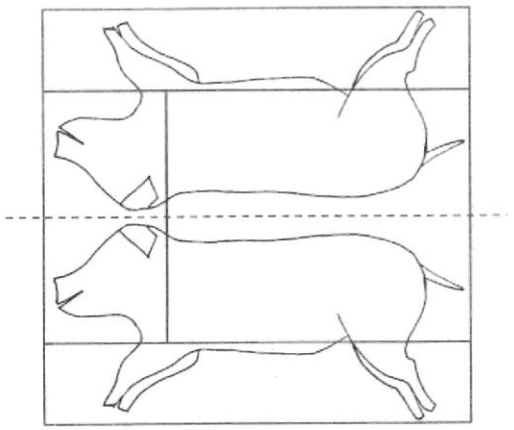
A description was made of the consistency and physical state of the lime. Voids in the lime cast were sketched in relation to the pigs' body and the dimensions of the voids were recorded. The carcasses were both qualitatively and quantitatively assessed to ensure consistency in recording differential decomposition. A qualitative assessment of the state of decomposition made reference to Wilson et al. [3]. In this method an evaluation of the state of decomposition (stages I-VI) of the buried remains was made according to modifications of criteria developed largely for surface-exposed carcasses. However, this scoring system could only be used properly for the pigs buried without lime. Based on the assumption that lime may desiccate Aufderheide's Soft Tissue Index [15, 43] was also considered. But like the scoring system of Wilson et al. [3], the Soft Tissue Index was not easy to apply in the field in this instance due to a superficial desiccation and the limited period of interment. It was clear that the limed pigs were not in a bloat stage (II and III), nor in an active or advanced decay stage (IV and V) because of the effects of the lime. A revised condition scoring method was therefore developed at the University of Bradford initially based upon Aufderheide's mummy autopsy protocol [15]. Briefly this method uses a four point scale to describe the condition of soft tissue for each of the pig carcasses. The scale ranges from 0 to 3 to indicate the amount of degradative change that is observed across the body. 0 indicates a minimal change to the remains while 3 indicates severe soft tissue changes. An outline of the scale is given in Fig. 2. The carcass is divided into three major regions: head, torso and extremities. Every region is subdivided in (I) integument (hair and skin), (II) fat layers, (III) muscles and (IV) ligaments (Fig. 2). A degradation score using the four point scale is assigned to each region. The upper and under surfaces of the pig are scored separately. To calculate the total degradative change per pig, the values of the upper and under surface of the pigs are added up and divided by the total possible score which is 2 times 36 (if the maximum score for each region is 3, the total possible score for each side would be 36, assuming that none of the body parts are missing). Finally, the value is multiplied by 100 (i.e. total degradative change = [(total score upper surface + -total score under surface)/total possible score] × 100).

Two times four soft tissue samples (1 cm × 1 cm) were collected from each pig in order to undertake histological analysis and desiccation experiments. These samples contained muscular tissue, connective tissue (skin) and adipose tissue (fat), although different layers were not easily distinguishable for liquefying tissue. A first sample was taken from the belly and a second sample was taken from the foreleg or hind leg (depending on the solidness of the tissue) to compare the extent of decomposition between the torso and the extremities. Each of the above samples was taken from both sides of the pigs in order to compare the upper surface covered with lime to the

under surface in direct contact with the soil. In order to orientate each sample the outer skin surface was stained with blue glycerine. To preserve the tissue structure for subsequent histological treatment the specimen was fixed immediately after being removed from the body. One set of samples was fixed in buffered formaldehyde. The remaining set of pig tissue samples was fixed by freezing.

In order to identify the effects of lime on tissue, histological analysis was carried out on one set of pigs. The analysed pigs were unlimed pig A3, hydrated lime pig B3 and quicklime pig C3. The analysis concentrated on muscular tissue with an evaluation of the overall preservation, the presence of fungi and the presence of Gram-positive and Gram-negative bacteria. Preparation and processing was difficult because of the degraded nature of the tissue. Briefly the samples were fixed in formalin, underwent paraffin embedding and were sectioned to a thickness of 5 μm with a Leitz microtome using conventional methodology. Four different stains were used. Haematoxylin and eosin (H and E) was used to demonstrate the overall structure. Toluidine blue showed up the bacteria as dark blue. Periodic Acid and Schiff s Reagent (PAS) was used to demonstrate fungi and finally Gram staining was applied to show the difference between Gram-positive and Gram-negative bacteria [44,45].

Fig. 2. Carcass condition assessment (Bradford score).



Score	Soft tissue condition
0	**Minimal Change**: some discolouration and evidence of decomposition on the body
1	**Some Change**: decomposition is readily visible; the area is only partly intact, still identifiable yet no longer structurally sound
2	**Severe Change**: the body is no longer intact due to advanced decomposition; adipocere can be present
3	**Heavy Change**: decomposition advanced to the extent that soft tissue has been lost from the remains

Upper surface	Head	Torso	Extremities	Total
Integument				
Fat layers				
Muscles				
Ligaments				
Total				(a)

Under surface	Head	Torso	Extremities	Total
Integument				
Fat layers				
Muscles				
Ligaments				
Total				(b)

Total degradative change = [(upper + under surface) / total possible score] x 100
[(a + b) / (36+36)] x 100

2.6. Laboratory simulations

Laboratory experiments were designed to investigate the moisture content and desiccation rate of fresh pork and degraded field-recovered tissue. First, a comparison of moisture content and desiccation rate of belly pork and rump pork was made. Replicates of fresh belly and rump pork composite tissue (4 cm × 4 cm) each comprising skin, adipose layers and muscle were dried in an oven at 56 °C. Moisture loss and desiccation rate were measured by weighing the tissue. They were first weighed in small open Petri dishes and then placed in the oven to desiccate. They were subsequently weighed every few hours during the first day and every 24 h from the day after until weight readings were consistent (after 18 days). The percentage dry residue of each sample was calculated by dividing their final weight (= dry residue) by their initial weight, multiplied by 100.

To investigate the desiccation effects of atmospheric air, soil, hydrated lime and quicklime on pig tissue, the experiment was repeated, with duplicates of composite tissue of belly and rump pork left for 35 days in 4 different containers. The first contained only tissue, exposed to the surrounding air. The second container was filled with soil from Meerdaal. The third and fourth containers included hydrated lime and quicklime, respectively. The tissue was weighed before and after the container experiment and then placed in the oven following the same procedure as previously described for the fresh samples.

The aim of these laboratory experiments was to provide an additional context to observations from the Meerdaal field samples. Four tissue samples (1 cm × 1 cm) of each of the excavated Meerdaal pigs were desiccated in the oven according to the same procedure as the laboratory samples to investigate desiccation rate and moisture content.

3. Results and discussion

3.1. Temperature and precipitation

Monthly averages of minimum air temperatures, maximum air temperatures and precipitation during the period of study are shown in Fig. 3. The meteorological conditions demonstrate a consistent trend of seasonal change over the 6 months of burial. Daily precipitation varied over the months. Major rainfall was recorded in March, in the second half of May and in the first half of June 2008.

The effect of body decomposition on temperature within the grave micro-environment of the unlimed pig is shown in Fig. 4 and plotted against the air temperatures. Temperature differences are observed between the core body and the subsurface, with the greatest difference up to 3.4 °C during the third month of burial (April). These temperature changes are due to microbially driven putrefactive changes which produce heat depending on the state of decay and the extent of microbial involvement. Thermal imagery with both airborne and ground based cameras demonstrated localised temperature divergence between the graves and the surrounding soil. After 2.5, 3 and 6 months of burial the temperatures of the pig graves and control pits were lower than the surrounding soil (Fig. 5). These temperature differences are due to ground disturbance from digging and not to the absence or presence of a carcass given that the control pits also showed a temperature anomaly. There were no noticeable thermal distinctions between the graves without lime and with different types of lime.

3.2. Soil pH

The soil from the Meerdaal site was acidic, related to the sandy loam texture and low water table. At exhumation there was a considerable variation in pH. Clearly the presence of lime altered the pH values of the limed control pits (without pigs). The pH of unlimed control pit A remained the same, while the pH values of control pit B (hydrated lime) and C (quicklime) both increased in alkalinity (Table 2). This confirms that lime can be used in agriculture to lessen the acidity of the soil.

In contrast to the control pits, pH values increased in the soil adjacent to the unlimed as well as the limed decomposing carcasses (Table 2). Given that the pH shifts were similar below both limed and unlimed cadavers and that the lime was only present above the bodies, the change in pH values was mainly caused by the decomposing cadavers rather than the lime. During the decay process, ammonium concentrations and carbon dioxide liberated by decarboxylation reactions cause an increase of the pH of soils surrounding decomposing remains [46-48]. However, the correlation between pH and ammonium is only noticed in acidic soils such as in Meerdaal. Research revealed that no significant increase in pH is observed during decomposition in alkaline soil types [49].

3.3. Carcass condition

The condition of the pig carcasses varied considerably between the limed and unlimed graves as well as within the carcasses themselves. A summary of the assessment of the condition of the carcass is represented in Table 3. Unlimed carcasses A2 and A3 were both in an advanced stage of decompostition, although pig A3 showed a slightly better preservation (Table 3 and Fig. 6a). In each case the upper surface of the carcasses was partially skeletonised with wet disintegrating soft tissue also present. Hair and eyelashes were noted within the liquefying tissue. The under surface of the pigs exhibited a slightly better preservation than the upper surface, although soft tissue was also wet and liquefying. The limbs were partially skeletonised, with disarticulated hoofs and ligaments present. The internal organs were in an advanced state of decomposition.

The hydrated lime pigs B2 and B3 showed a better state of preservation than the unlimed pigs (Table 3 and Fig. 6b). After 6 months of burial, the lime had formed an endocast around the pig torso and within both graves a noticeable void was present between the deflated pig torso and cast. The regions where skin was not in direct contact with the lime cast showed a reddish brown discolouration suggestive of the initial stages of desiccation.

The upper surface of each carcass was remarkably well preserved without liquefying tissue or evidence of skeletonisation.

Most of the skin was intact and external features such as the ears were clearly defined. While taking samples, no fluids purged from the abdominal cavity and an intact structure of the skin-fat-muscle layers was noticed. The under surfaces of both carcasses were less preserved than the upper surfaces due to the absence of lime and direct contact with the soil. The under surface of B2 showed better preservation than the under surface of B3, which exhibited liquefied soft tissue and skeletonisation. The internal organs of both carcasses also showed an extremely good preservation state although they were not desiccated. Even the heart and lungs were still preserved while normally these organs dissolve rapidly [15]. The quicklime pigs C2 and C3 had a less solid lime cast compared to the casts of the hydrated lime pigs, in part probably due to the smaller volume of quicklime used. The upper surfaces of the carcasses were well preserved with a limited amount of degradative change (Table 3 and Fig. 6c). Reddish-brown discolouration could be noticed where the lime cast did not touch the skin and the outer surface had started to desiccate (as with the hydrated lime pigs). The limbs were disarticulated and some limb bones were skeletonised. Putrefactive fluids purged from the abdominal area when taking belly samples. The under surfaces of these carcasses were less preserved than the upper surfaces with significant degradative changes as liquefying, disintegrating soft tissue. The internal organs were in a more degraded state than the organs of the hydrated lime B-pigs.

Fig. 3. *Monthly averages of minimum air temperatures, maximum air temperatures and precipitation during the period of burial. Recordings obtained from the nearest weather observation station in Beauvechain at 7 km from the burial site. 1 mm of rainfall corresponds with one litre per square meter.*

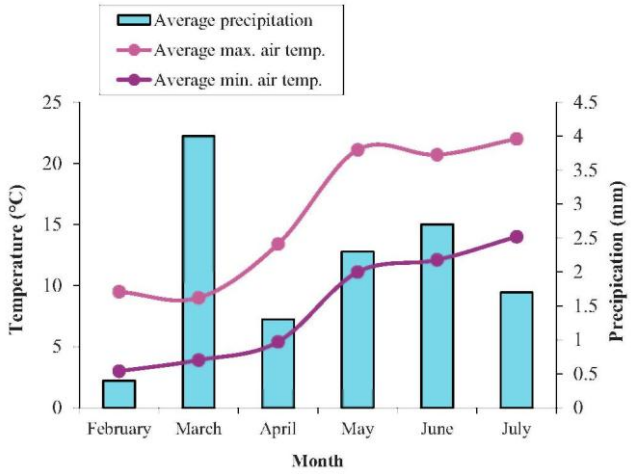


Fig. 4. *Comparison between air temperature, subsurface temperature of the grave fill recorded at 5 cm depth and core body temperature of unlimed pig carcass A2, all recorded at 8 am local time.*

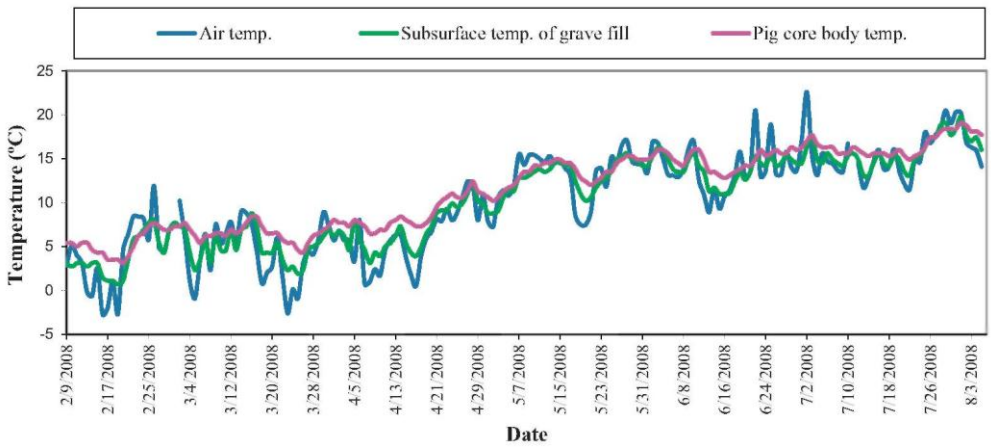


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Table 2 : *pH results measured at the time of burial and following exhumation of the pig carcasses.*

pH results		No lime			Hydrated lime				Quicklime							
		Grave A2	Grave A3	Control A	Grave B2	Grave B3	Control B	Grave C2	Grave C3	Control C						
Before burial	After 6 months of burial															
Topsoil	Topsoil	2.7	3.8	2.8	3.3	2.8	4	2.8	3.5	2.7	3.6	2.8	3.7	2.7	3.6	2.7	3.8	2.6	3.5
Eluviation	Above pig	3.1	6.4	3.1	6.9	3.2	3.9	3	3.8	2.9	5.6	3.2	7.3	3	7.1	3	6.9	3.1	4.6
Illuviation	Below pig	4.1	8	4	8.8	4	4.1	4	8.4	3.9	9	4.1	7.5	4	8.6	3.9	8.8	4	13
	Base grave		4.2		7.8		4		6.3		8.1		5.2		8.6		8.3		4

neutral pH = 7 (6.5 - 7.5)
acidic pH < 7
akaline pH > 7

Table 3 : *Summary of the assessment of the condition of the carcass, lime and entomology after 6 months of burial.*

	Carcass assessment										Lime				Entomology		
	Condition of the carcass								Other		Cast intact	Cast collapsed	Void at abodmen	Void at mouth	*Conicera tibialis* (adults)	*Conicera tibialis* (pupae)	*Anoplotrupes stercorosus* (adults)
Pig/grave	Head upper surface	Head under surface	Torso upper surface	Torso under surface	Extremities upper surface	Extremities under surface	Internal organs	Overall decomposition	Discoloured area (upper surface)	Putrefactive liquid in abdomen							
A2 (no lime)	3	2	3	2	3	3	3	3	0	*V*					*V*		*V*
A3 (no lime)	2	2	2	2	2	2	3	2	0	*V*					*V*	*V*	
B2 ($Ca(OH)_2$)	1	2	1	2	1	2	1	1	1		*V*		*V*				
B3 ($Ca(OH)_2$)	1	2	1	3	1	3	1	1	3	*V*	*V*		*V*				
C2(CaO)	1	2	1	3	1	3	2	2	3	*V*	*V*		*V*				
C3(CaO)	1	2	1	2	1	2	2	2	2	*V*		*V*	*V*	*V*	*V*		

V, present; 0, no changes (as in fresh pig); 1, limited change (0-50/100 Bradford score); 2, significant change (51-75/100 Bradford score) and 3, extensive change (76-100/100 Bradford score).

Fig. 5. *Thermal image of graves A2, B2, C2 and A3 taken with the ground based infrared camera at three months after burial. The graves in blue and purple are colder than the surrounding soil. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)*

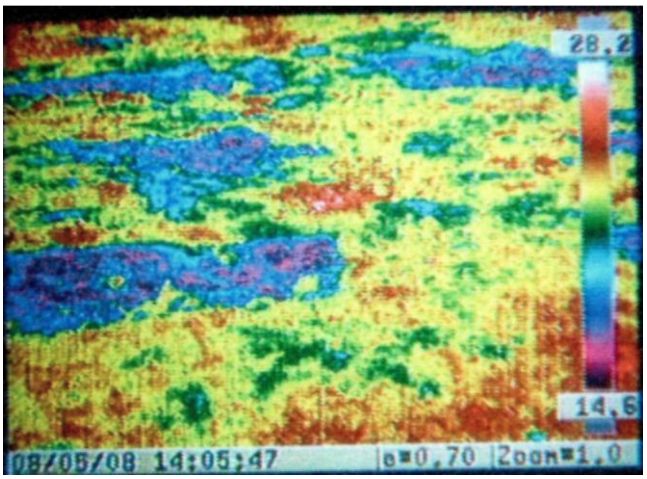
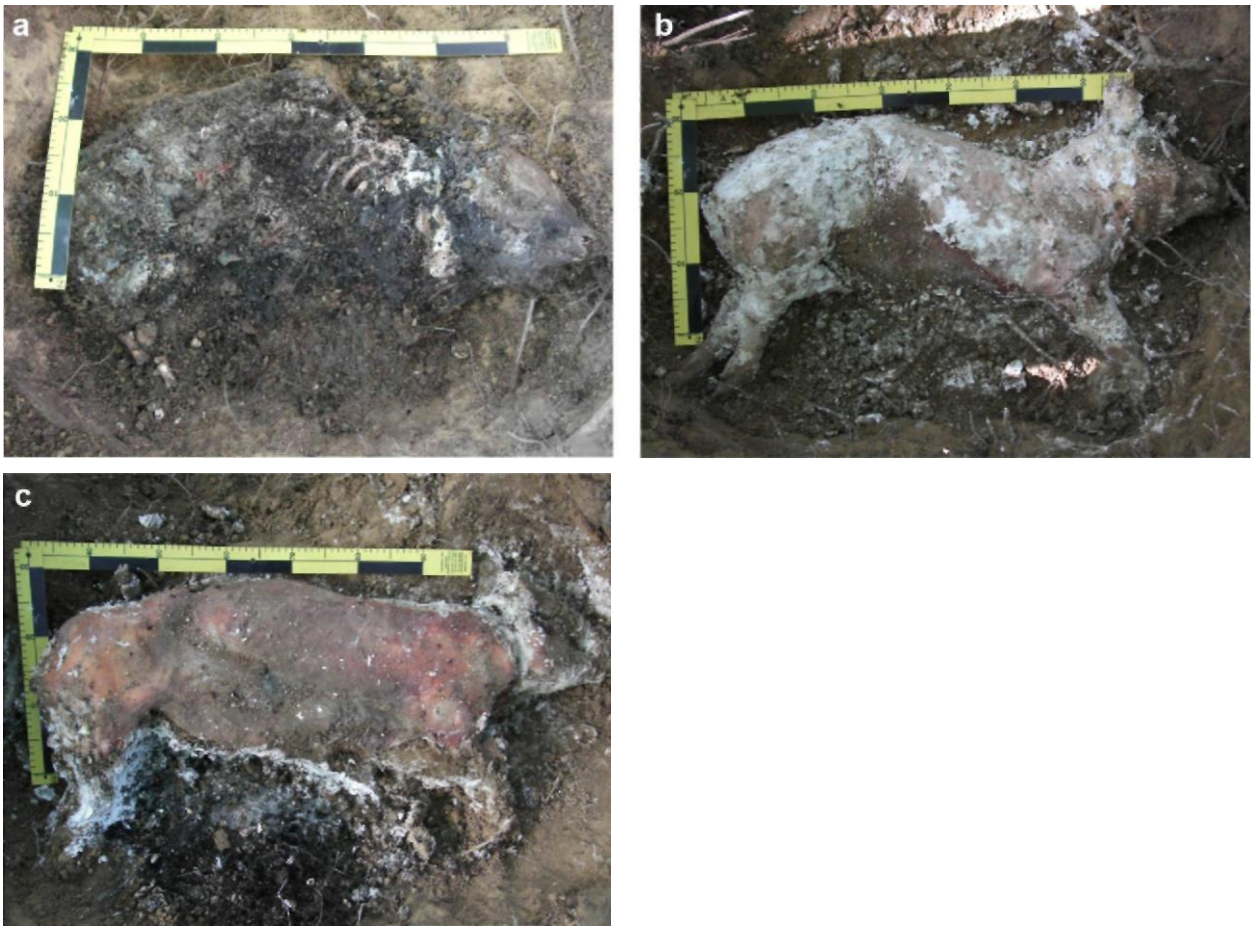


Fig. 6. *(a) Upper surface of unlimed pig A2 after six months of burial. The carcass exhibited an advanced stage of decomposition and skeletonisation. (b) Upper surface of pig B2 after six months of burial with hydrated lime. The carcass is well preserved. Reddish brown discolouration of the skin can be noticed on the abdomen, (c) Upper surface of pig C2 after six months of burial with quicklime. The trunk is well preserved and exhibits a reddish brown skin discolouration. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)*



3.4. Lime observations and analysis

Within the literature on historic lime burials it is often assumed that a complete lime cast can only be formed by pouring lime in a liquid state over and around the body rather than in a powdered state [12]. This clearly contradicts the findings of this study. In the pig graves both powdered hydrated lime and quicklime readily formed a cast around the pigs.

Given the discovery of air voids above the deflated carcasses it is apparent that the formation of these lime casts had occurred while the pigs were in the active bloat stage as moisture was released during the wet phase of putrefaction. Subsequent deflation of the carcass left a void between the cast and the torso of the pig (Fig. 7). Those parts which did not remain in direct contact with the lime began to undergo desiccation, discolouration (turning reddish-brown) and became hard and leathery. The body parts which remained in contact with the lime cast were able to retain their moisture.

The consistency of the lime was different depending on the nature and location of the lime as applied to the body. Where the lime still remained in contact with the body, it was of a soft and creamy texture. Towards the outside of the cast and above the voids the lime had become hard, as the lime underwent carbonation. On exposure to air, hydrated lime is known to absorb carbon dioxide, resulting in the formation of a hard crust of calcium carbonate. With these burials, carbon dioxide could be derived from decomposition gases, the soil and from microbial respiration (Fig. 1). The different composition of the lime types also had an effect, with the casts of hydrated lime being more solid than the casts of quicklime. A part of quicklime cast C3 was collapsed. As explained before, the same weight of lime was poured on both graves, resulting in a different volume and thus a less-thick layer of quicklime relative to the hydrated lime. Even after the hygroscopic expansion the quicklime casts remained less solid.

Fig. 7. *The formation of the lime casts occurred while the pigs were in the active bloat stage. Subsequent deflation of the carcass left a void between the cast and the torso of the pig. The body parts which remained in contact with the lime cast were able to retain moisture (left). Those parts which did not remain in direct contact with the lime desiccated (right).*

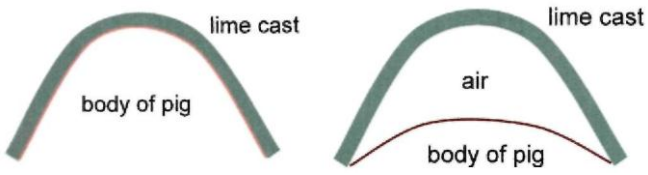


Table 4 : *Dry weight, moisture content and moisture loss from experimental composite tissue of belly pork and rump pork left for 35 days in 4 different environments.*

Tissue	Environment	Specimen number	% Dry weight	% Total moisture content	% Moisture loss after 35 days
Belly pork	Isolated (air)	1	31.5	68.5	29.3
	Isolated (air)	2	36.8	63.2	23.8
	Soil	3	59.3	40.7	1.8
	Soil	4	52.6	47.4	8.5
	Hydrated lime	5	35.9	64.1	38.5
	Hydrated lime	6	36.3	63.7	36
	Quicklime	7	33.8	66.2	62.9
	Quicklime	8	32.6	67.4	64.3
Rump pork	Isolated (air)	1	33.9	66.1	31.9
	Isolated (air)	2	25.5	74.5	40
	Soil	3	54.3	45.7	8.7
	Soil	4	41.8	58.2	27.8
	Hydrated lime	5	28.2	71.8	41.5
	Hydrated lime	6	30.6	69.4	43.7
	Quicklime	7	31.9	68.1	65.4
	Quicklime	8	34	66	63.4

3.5. Entomology

Entomological observations during exhumation were sparse. Differences were noticed between the graves with respect to the presence and the absence of species (Table 3).

In this study, the only necrophagous species present in the graves was the coffin fly or *Conicera tibialis* (Diptera, Phoridae). There are 300 species of Phoridae in France and Belgium and 600 in Europe [50]. In Belgium, only four species of Phorid flies are discovered on human corpses since 1947 [50]. *C. tibialis* is one of them. Adult coffin flies were found in the two graves without lime (A2 and A3) and in quicklime grave C3. Pupae of coffin flies were only noticed in unlimed grave A3. There was a clear absence of coffin flies in both hydrated lime graves and in quicklime grave C2. Mann et al. [51] showed that insects prefer not to infest remains exposed to certain chemicals. Following this reasoning it is assumed that flies would avoid a body treated with lime and that maggots have difficulties to survive on this chemical. Nevertheless coffin flies were observed in quicklime grave C3. It can be argued that this was due to the collapse of the lime cast. As such the scent plume was not shielded anymore. Cadaveric volatile organic compounds entered the grave soil and became detectable by coffin flies.

A single adult dung beetle or *Anoplotrupes stercorosus* (Coleop-tera, Geotrupidae) was observed in unlimed grave A2. Most Geotrupidae are coprophagous and eat dung excreted by mammals [52,53]. But according to observations they might also feed on human and animal cadavers [53,54].

Immediately upon exposure of the carcasses *Anoplotrupes* and blowflies (Diptera, Calliphoridae) were attracted to the cadavers. Extensive fresh oviposition was observed by the blowflies. The cadaveric volatile organic compounds released by carcasses during decomposition attract necrophagous insects [36]. It was clear that the lime acted as a physical barrier and restricted the release of decomposition chemicals. From the moment the lime covering was removed Geotrupidae and Calliphoridae amassed, reinforced by the sunny weather during excavation which is a convenient factor for insect activity.

3.6. Histology

Histology provided a localised picture of changes to the pig carcasses. Overall it could be observed that more solid structures such as connective tissue (collagen, fascia, blood vessels) and adipose tissue were better preserved than muscle. The nuclei had not survived in any of the tissue. The tissue of the limed upper surfaces of the hydrated lime pig B3 and quicklime pig C3 was best preserved. Furthermore, in all three pigs the tissue which overlay the limbs showed a better preserved structure compared to the belly region. Such core-periphery differences can be explained by the sites of greatest putrefactive change and moisture increase, i.e. the intestines which contain a considerable microbial load that will migrate into the local tissues after death [1].

The tissue samples contained both fungi and bacteria, although the presence of fungi was minimal. The greatest amount of bacteria was found on unlimed pig A3 and on the under surface of hydrated lime pig B3. This is consistent with the normal putrefactive process, given that the under surface of pig B3 was not limed.

Another interesting observation was the switch from Gram-negative bacteria to Gram-positive bacteria. The Gram-negative bacteria were likely responsible for the initial tissue breakdown followed by a rear guard of Gram-positives who came afterwards and populated more necrotic areas. Gram-negative bacteria do survive a long time, but the oxygen tension and pH of their postmortem environment progressively depart from values optimal for these organisms and approach the optimum for the anaerobic, Gram-positive bacteria. Eventually the latter will become the predominant organisms [15].

3.7. Desiccation results

Tissue desiccation as observed within the limed graves was investigated further by a series of laboratory experiments using moisture measurement of fresh pig tissue. In the first experiment replicates of fresh belly pork and rump pork, each comprising skin, adipose layers and muscle were dried in an oven at 56 °C for 18 days. The initial weight loss of both belly and rump pork samples was rapid, resulting in loss of over 50% of their moisture content by the end of day 1. After this point, the tissue continued to lose moisture at a slower rate. This can be explained by the way water is held within these tissues. Water removed by rapid desiccation such as in an oven is principally free or unbound water [55]. Persistence of the desiccation process may be accompanied by a change in tissue composition producing a change in its water activity (i.e. the intensity of the tissue's affinity for bound water) and resulting in tissue loss of additional water from the tissue. Any water that has been lost after 65% of the original weight, is most likely to be bound water from proteins and other compounds [55].

The second laboratory experiment investigated the effects of different environments upon desiccation. Eight pieces of composite tissue of belly pork and rump pork were left for 35 days in different environments (air, soil, hydrated lime and quicklime). The results indicated that quicklime had the most desiccating effects with the tissue loosing over 60% of its moisture (Table 4). This is supported by the chemistry of quicklime, given that it will immediately absorb water from its environment. Hydrated lime and air had a medium dehydration effect and

soil was the least desiccating (Table 4). Only the tissues in the soil were wet, liquefying and disintegrating.

These laboratory experiments provided an additional context to the observations from the Meerdaal field observations. Samples from four locations on each excavated pig were assessed on desiccation. Despite the field observation of dehydration and reddish brown discolouration to the superficial layers of the skin, the oven experiment indicated that samples obtained from these locations in the field were not wholly desiccated. However, it should be borne in mind that experiments with cubes of composite tissue can not be easily compared to whole carcasses. Quicklime can easily desiccate a small cube of tissue, with its large surface area to volume ratio, but dehydration of an entire pig carcass, complete with internal organs, does not happen so readily. Research revealed that larger tissue pieces desiccate at a slower rate than smaller ones because of the relation between surface area and volume [56]. In the laboratory experiments a whole cube of tissue was surrounded by lime while in the field, only the upper surfaces of the pigs were covered with lime. Tissue depth also has a significant effect, with a longer time needed to transfer moisture from the internal organs (such as the heart tissue) of the body core to the skin surface than it will to remove moisture from the muscle of more peripheral structures overlying the limbs. These observations explain why the best preserved soft tissues in spontaneously desiccated bodies are commonly the fingers, toes, ear lobes and other skin-covered extremities with little underlying soft tissue [15, 57].

4. Conclusion

Forensic investigation cannot base all its information on case studies entirely. This research provides a valuable insight into the effects of lime on the decomposition of buried pig carcasses as human analogues. The studies generated by Forbes et al. [23] and Thew [24] were extended to the analysis of complete carcasses amended with two types of lime, entomology, histology and the investigation of moisture content and desiccation. The results showed that lime retards the rate of decomposition if present in a burial environment. It was evident that the limed pigs were better preserved than the unlimed pigs. It can be argued that the encasement of a body in lime, although as here only present on the upper surfaces of the pigs, served as a barrier for the whole carcass. It partially negated the effects of the general soil environment, delayed the decay process, restricted the release of cadaveric volatile organic compounds and therefore attracted fewer insects.

Histological analysis also revealed better preserved tissue on the limed surfaces of the pigs, as compared with the under surfaces. Furthermore it provided a localised picture of the changes to the pig carcasses with core-periphery differences and the presence of Gram-negative and Gram-positive bacteria. Additional laboratory experiments showed that quicklime had the most desiccating effects. Although parts of the skin of the limed pigs in the field looked desiccated, tissue samples from the field pigs were not as desiccated as anticipated. It is clear that one has to be careful in translating information derived from microcosm experiments using cubes of tissue in the laboratory to whole carcasses in the field. Lime can rapidly desiccate a cube of tissue, but dehydration of a whole carcass is more complex.

These observations have a potential impact on the search, detection and recovery of human remains, and also for considerations regarding post-mortem interval. Research is ongoing at the University of Bradford into the longer term effects of hydrated lime and quicklime on the decay of human remains and the associated micro-environment. More histological and chemical analysis is been carried out. In the absence of other studies on lime in graves, this research produces useful and novel information of interest to forensic scientists, archaeologists, historians, humanitarian organisations and those concerned with disposal of animal carcasses or human remains in mass disasters.

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