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Patterns of Mortuary Practice Associated with Ethnoreligious Genocides of the Silk Roads: The Use of Bacterial Necrobiome in Investigating Mass Burials

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Abstract

Whilst previous contributions have mainly focused on the forensic aspects of investigating mass graves, this study presents a microbial technique for interpreting the postmortem history of buried remains. The case study model is the first in a series of taphonomic reconstructions that focus on the role of bacteria in the decay of detached body parts. The domestic pig (*Sus scrofa*) heads, legs, and pork belly were used to model human decay. Measurement of body mass loss was supported by a series of experiments that included soil pH, the microbiological activity of soil bacteria, Total Viable Count, and Gram stain analysis. It was hypothesised that temperature and soil moisture would not make a significant impact on the decay rates of animal body parts. The findings demonstrated that: 1) mostly Gram-negative bacteria were involved in the decay; 2) microbial decomposers were higher in numbers in higher temperature and soil moisture settings; 3) animal heads and feet decomposed at different rates. The results add to the current body of published work by providing a decomposition pattern specifically for detached and/or commingled body parts that form an essential part of mass burials. This is significant as no previous microbial experiments have been conducted on a larger scale to aid methods in recovery, analysis and identification of the victims of genocide.

Keywords: *Sus scrofa*, Necrobiome, Yazidi genocide, Buried body parts, Conflict archaeology

Introduction

Taphonomical studies have documented human decomposition in different environments (Zuha *et al.* 2016; Anderson and Bell 2014), under various climatic settings (Symes *et al.* 2013), and body conditions (e.g. Bhandari *et al.* 2015). However, the processes associated with the decay of individual body parts are scarcely studied (Scholl and Moffatt 2017). This gap in the research could be because it has been presumed that whole and dismembered human bodies exposed to identical environmental conditions undergo similar microbiological and chemical processes and will have a comparable decomposition pattern. Previous studies also focused on either morphological (qualitative) changes of the decay (e.g. Rodriguez and Bass 1983), quantitative methods of establishing time since death (Megyesi *et al.* 2005) or microbiological studies explaining the causes of differential decomposition (Cobaugh *et al.*2015). This resulted in a partial picture of the taphonomic history.

To maximise the accuracy of the results, this study combines taphonomic and microbiological methods to investigate the impact of ambient temperature and soil moisture on buried remains by means of recording soil pH, soil microbial activity, Total Viable Count, Gram stain the body mass loss and gross morphological changes of soft tissue.

The organic matter factor is expressed in terms of *Sus scrofa* remains acting as a nutrient for microorganisms as sources of nitrogen and carbon. It was hypothesised that temperature and soil moisture would not make a significant impact on the decay rates of animal body parts.

Individual body parts, whether detached or commingled, often form part of mass burials resulting from genocides (Komar 2008). An example is the Yazidi genocide perpetrated by the Islamic State in Iraq between 2014 and 2017. Since the establishment of their faith in the 12th century, the Yazidis have resided in the governorates of Nineveh and Duhok in northwestern Iraq. The locations of their holy sites along the trade routes and their syncretic religion, which blends elements of Islam, Zoroastrianism, and ancient Persian beliefs, suggest the influence of the Silk Road cultural exchange. Yazidi pray facing the Sun and believe in a Creator God and the Peacock Angel Melek Taûs, who intermediates humanity and divinity. They are considered an ethnic and religious group, which has historically made them targets of violence and repression from surrounding Muslim communities (Del Re 2022). Centuries-long campaigns of brutality go back to the birth of Sharia law in the 7th century and an acceptance of Malak Taûs as a representation of a figure of the devil (Usman 2021). During the Islamisation of the Ottoman Empire, over 3000 Yazidis were killed around Mount Sinjar (Porkka 2017). The massacres persisted in recent history, first during Saddam Hussein's regime (1979–2003) and later under ISIL rule (2014 to 2017), resulting in the displacement of half a million Yazidis (Porkka 2017). A decade after ISIL's genocidal campaign, the community still struggles with the aftermath, with over 200,000 remaining in refugee camps and some 3000 missing (ibid.). Inhumation of graves in northwestern Iraq (Nineveh Governate) so far revealed 10,000 victims (UNAMI/OHCHR 2018). Another 200 mass grave sites are suspected to be found in the governorates of Kirkuk, Anbar and Salah al-Din, which, due to the risk of explosives and the bureaucracy of the Iraqi armed forces, are still not investigated (ibid.).

In comparison to central and southern Iraq, where the climate is arid with average annual rainfall not exceeding 166 mm, the northwestern region receives a yearly precipitation of around 303 mm (Al-Ansari *et al*. 2014). The preservation of human remains in this area is hence likely to be poorer, as body decay is mainly influenced by ambient temperature and soil moisture (Archer 2024; Carter*et al.* 2010). This way, the taphonomic research of the depositional microenvironment may benefit the recovery, analysis, and identification of human remains.

Materials

Pork belly (94 pieces) and pig heads and feet (28 pieces) that originated from a Yorkshire abattoir, John Penny & Sons in Leeds, were used as a proxy for human remains. The former allowed detailed microbial and soil analyses, and the latter served for taphonomic trials. Adult pigs from two years of age that had reached a weight of 30 to 40 kilograms were utilised. Dismemberments included five cuts to separate the head and the front and back limbs from the torso. The animals were slaughtered professionally seven hours before the experiments started and kept refrigerated before being transported in double plastic bags to the University of Bradford Taphonomy lab. No antibiotics had been administered to any of the animals during their life.

Methods

Compost analyses

The Levington Organic Blend Top compost (Suregreen Ltd.) was utilised to simulate shallow-grave soil (up to 30cm deep). The techniques for studying the organic content of compost, soil particle characteristics, soil bulk density and porosity, water content, water potential and retention were tailored from a series of soil analysis methods (Klute 1986; Weaver 1994; Sparks 1996; Dane and Topp 2002 and Ulery and Drees 2008). The techniques used to assess the soil pH were adopted from the Association of Official Analytical Chemists (Kalra 1995).

A scanning electron microscope (SEM) method was used to understand what elements constitute the largest portion of compost. Three samples of compost were prepared for analysis, having been dried and 2mm-sieved to eliminate

outgassing from water and organic contamination. Following the coating, the samples were mounted on stubs by double-sided conductive carbon tapes connected from the top of the sample to the sample holder. Coated samples were placed in the SEM sample chamber, and a picture was taken at 20 kV under 30X magnification. Inorganic compost components were identified through a scanning electron microscope, FEI Quanta 400. The elemental analysis was completed using Oxford Instruments Inca X-Sight hardware and Inca software. Samples were dried by a Thermo Savant SC250EXP SpeedVac system.

Microbiological analyses

Standard Gram staining was carried out on the skin, muscle, and soil colonies collected from representative samples before the experiment to indicate whether it was feasible to differentiate between potential body and soil microbial decomposers. Bacteria were observed under a microscope with a 100X objective. Total Viable Count (TVC) included the dilution plate method and broad-spectrum bacterial enumeration technique (Hopkins *et al*. 2000; Weaver 1994). The growth media plates used were R2A agar, MacConkey agar and Rose Bengal agar with Chloramphenicol. Plates were examined at 24 hours for R2A and MacConkey agars and 48 hours for Rose Bengal agar. The Fluorescein Diacetate Hydrolysis (FDA) method used to measure metabolic activity of soil microbes was tailored from Adam and Duncan (2001).

Taphonomic analyses

The taphonomic decomposition model was modified from Davis and Goff (2000) and Prieto *et al.* (2004) to encompass gross post-mortem changes typical for buried microenvironments. The bespoke description of the decomposition of the body parts was categorised into six stages (Table 1). The weight per body part was recorded at the start and the end of each experiment.

Table 1: Description of morphological appearance for dismembered body parts

Decay category	Description
Fresh	No discolouration or signs of lividity, intact skin
Putrefaction	The pinkish appearance of the skin; cream to light brown discolouration of the skin with slight skin slippage
Early disintegration	Ash white with green and black stripes visible under the skin, further skin slippage
Active decay	The skin colour is dark red, and the skin texture, crispy. Skin is sagging and flaking in most parts. Skin structure is leathery to stringy with evident fatty tissues
Advanced decay	Substantial greasy substance, decomposed tissue, cartilage and tendons exposed. Formation of moisture, thin, greyish substance on bone trauma ends. Bone exposure of most of the samples with greasy substances and decomposed tissue
Skeletonisation	Complete bone exposure with no tissue left

Methods of data analysis

The Kruskal-Wallis test was used to analyse compost pH and body part mass loss statistically, and Friedman's ANOVA was used to analyse bacterial metabolic activity.

Pork belly experiment set up

Pork belly samples were exposed to four controlled buried microenvironments (Table 2). A total of 48 meat pieces were used for the compost pH, and a further 22 for the analyses of TVC and Gram stain (16 in aerobic and 6 in anaerobic conditions). Finally, 24 pieces of meat were utilised to measure the activity of soil microbes. Meat pieces cut to measure 5cm width x 5cm length were placed in autoclaved clear glass jam jars with proportions of volume 228ml, height 85mm, diameter 63mm with lid/neck of 63mm. Approximately 3cm of Levington Organic Blend Top compost was filtered through a 5mm sieve, added at the bottom of the jar under the meat slices, and filled to the top. Jar lids were pierced to allow oxygen access. Compost water content was established at the start.

Table 2: Summary of experiment setups for buried pork belly samples

Duration	Microenvironments/Category	Tested variables	Applied methods	Control
28 days	1. 20^0C and 10% soil moisture (cat B) 2. 20^0C and 40% soil moisture (cat C) 3. 30^0C and 40% soil moisture (cat E) 4. 30^0C and 10% soil moisture (cat F)	Ambient temperature/soil moisture	1. Compost pH 2. Total Viable Counts 3. Gram stain 4. Metabolic activity of soil microbes	Surface decay

For categories E and F, the compost moisture level was kept in its original state, and in categories B and C, the compost moisture was dried. Meat in the jam jars was left to decompose in incubators at designated temperatures.

Pork belly data collection

Compost pH levels were established prior to the experiment, at weekly intervals and at the end of the experiment for each category. Control included pH measurement of compost with no contact with the meat at the start, at weekly intervals and at the end of the experiment. Samples were discarded once pH was established, with the new sample always being tested for each category and harvest. TVC was established from compost and pork belly. Microbial swabs were taken from the muscle and the skin areas of pork belly, animal heads and feet. Both TVC and Gram stain techniques were applied to pieces of meat under aerobic conditions halfway through the experiment (day 14) and again at the end. The control sample for each category was a piece of pork belly left to decompose in jars with no contact with soil. Six pork belly slices were further buried to assess the TVC and Gram stain of anaerobic bacteria; three were exposed to 20°C and three to 30°C. TVC of anaerobic bacteria was not attempted halfway through the experiment, as the samples were kept in the anaerobic chamber continuously for 28 days.

The metabolic activity of soil microbes was assessed in compost before the experiment commenced. The activity was measured halfway through the experiment and at the end. To avoid disturbing soil microbial communities, pork belly tissues sampled after 14 days were discarded, and fresh samples were tested after 28 days. Belly meat slices were decomposed in categories C and E. Control included measuring microbial activity from compost that was not in contact with the organic material.

Body part experiment set up

A total of 28 body parts were used for the decomposition of buried body parts. Animal heads weighed an average of 5.25kg and animal feet 0.75kg. Pig heads and feet were exposed to an average of 20.8°C and 10% soil moisture (category B) and 40% soil moisture (category C), an average of 26.1°C and 10% soil moisture (category F) and 40% soil moisture (category E). Body parts were weighed and photographed immediately after delivery from the butcher. All remains were deposited individually into IKEA fabric storage boxes (33 x 38 x 33 cm) in approximately five centimetres of compost, covering up to one-third of the bag. All samples were left to decompose undisturbed. Tinytag® Gemini PT100 loggers were used to monitor ambient temperatures hourly.

Skin and muscle tissues of animal heads and feet were swabbed at the start and end of all experiments to ensure body flora and microbial numbers (TVC and Gram stain) were in line with the parallel study of pork belly slices. Compost was sprinkled with sterilised water regularly to keep the moisture level steady. Control samples used in the burial trials were body parts left to decompose without contact with compost, approximating surface decomposition. At the end of the experiment, the researcher again visually observed samples for any signs of insect succession. They were photographed, weighed, and cleaned before being categorised into morphological appearances and the decay rates were calculated.

Results

1. Qualitative and quantitative assessments before burial

Compost condition

An average bulk density yielded 1.4g/cm3 and an average porosity of 45.6%, both important for exchanging gases and liquids (Dane and Topp 2002). Soil texture indicated a larger percentage of sand than expected for organic compost (58.9%) and an average rate of silt (23.07%). The compost was categorised as a medium loam soil, rich in organic matter (12%). The loam soil type of compost had a high level of available moisture, water retention, and water potential to support bacterial activity. The high percentage of silicon allowed good soil mineral formatting that, together with organic matter, compensated for a lower organic matter percentage.

Soil bacteria analyses

A fluorescein diacetate measured the metabolic activity of bacteria on three samples of sieved compost, each weighing 5 grams, and revealed a similar amount of fluorescein released, ranging from 0.06mg to 0.08mg. TVC of bacteria isolated from R2A agar showed a similar trend between the three samples, and Gram staining indicated predominantly Gram-positive bacteria.

Pork belly and body part analyses

Mostly Gram-negative bacteria were associated with pork belly samples. No animal elements exhibited insect or scavenging succession or visible signs of skin condition before burial. Animal heads and legs were assessed against decomposition stage criteria and classified into the 'Fresh' stage. The average mass for fourteen analogue heads was 5.25 kilograms, and for fourteen animal feet, 0.75 kilograms.

2. Micro-environmental monitoring during the decay

Compost pH

Friedman's ANOVA yielded a statistically significant difference between compost pH levels ($X^2 = 17.62$, $p < 0.05$). Compost was shown as moderately alkaline in all categories throughout the experiment. This was especially evident at the end of the trial in the categories B, E and F (see Table 2 for experiment setups). In comparison, compost acidity was shown in Category C at the end of the experiment.

Metabolic activity of soil microbes

Friedman's test yielded a significant difference in microbial activity between harvests ($X2 = 24.00$, $p < 0.05$). Microbial metabolism measured by the FDA method revealed the highest activity during harvests in category E, with an average of 0.65 mg fluorescein released in compost, and the lowest in category B, with 0.13mg. Fluorescein level was the lowest in all categories at the end of the experiment, with not much difference between the tested groups, averaging at about 0.04mg.

Total Viable Count and Gram stain

The bacterial count of the aerobes in category B showed a similar trend of TVC from the start to the harvest count, which decreased at the end of the experiment. In category C, several colonies were higher in number, indicating that conditions favoured bacterial growth. TVC from the bacteria in Categories E and F also showed a similar trend of bacterial growth, rising to a peak at the harvest and decreasing at the end of the experiment. R2A and MacConkey bacteria could not be compared with the samples incubated at 20°C during harvests due to the high numbers of colonies for TVC. The anaerobic bacteria exposed at 20°C were reduced slightly compared to those exposed to 30°C.

Colonies that grew on the Rose Bengal spread plate varied in size and colour, from large white and raised to small white and pink (Figure 1). Numerous bacteria were also noted on MacConkey spread plates, where colony morphology varied from brown to white and flat to slightly raised (Figure 2). Colonies on R2A were present in the most significant numbers, mostly white, with fewer colonies small and yellow (Figure 3). The Gram stain results of the three growth media plates showed that mostly Gram-negative organisms were involved in the decomposition, compared to predominantly Gram-positive strains found in the soil samples before the experiments.

Figure 1: Typical Rose Bengal plate associated with buried tissue: pork belly muscle

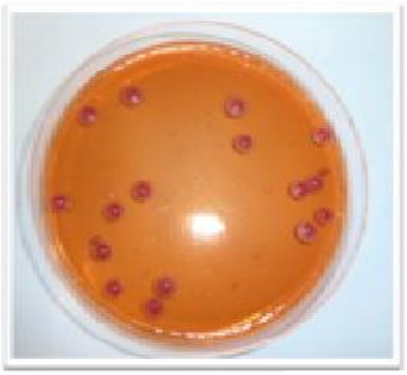

Figure 2: Typical MacConkey plate associated with buried tissue: pork belly skin

Figure 3: Typical R2A plate associated with buried tissue: pork belly muscle

The Gram stain results of the three growth media plates showed that mostly Gram-negative organisms were involved in the decomposition, compared to predominantly Gram-positive strains found in the soil samples before the experiments.

Body part mass loss

A Kruskal-Wallis test that measured differences in mass loss between categories before and after the experiment yielded significant results for both the animal heads ($H= 4.92$, $p < 0.05$) and animal feet ($H= 5.04$, $p <0.05$). The biomass loss average in category B heads was 28.38%, and in category C, 31.02% (Figure 4). The control sample C, however, lost significantly less biomass (5.77%). The animal feet in category B lost approximately 18.85% and those in category C an average of 25.55% (Figure 5), however, the mass loss of the control sample was not significantly higher (30.97 %).

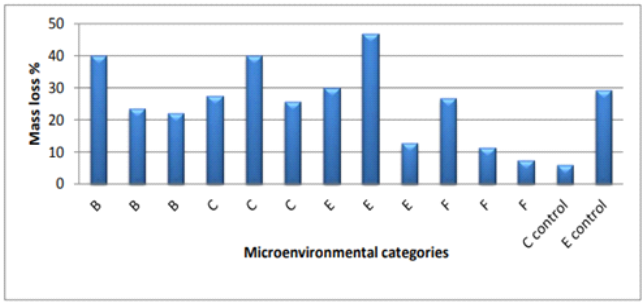

Figure 4: Summary of tissue mass loss for animal heads

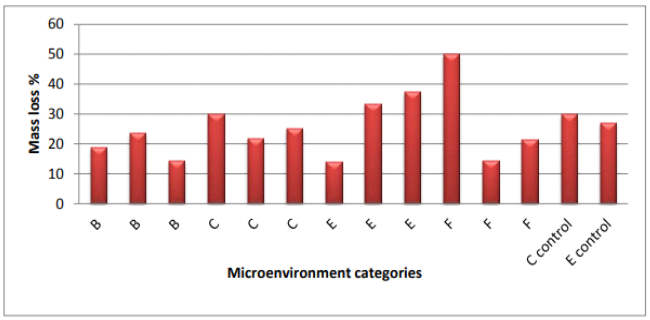

Figure 5: Summary of tissue mass loss for animal feet

Morphological appearance of body parts

Overall, animal heads showed more accelerated decomposition than animal feet. All head samples in categories B and C were classified into the Early disintegration stage. This is compared to category E and F head samples that showed morphological changes characteristic of Active decay (Figure 6). Control sample C was in line with the rest of the specimens. However, a control sample of category F was desiccated (Figure 7). Category B feet were still in the Putrefaction stage at the end of the experiment. Animal feet in all other categories were classified into the Early disintegration stage. The decomposition of the control samples followed the same pattern as that of the heads.

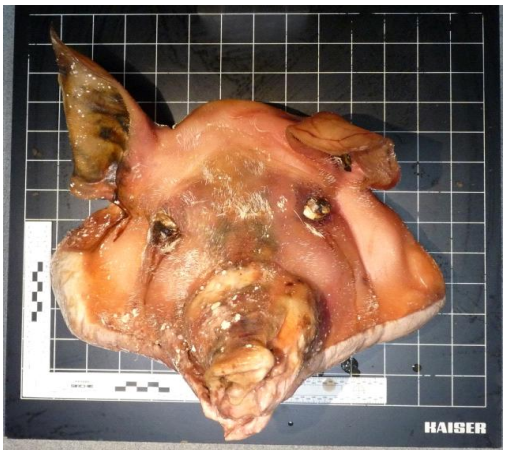

Figure 6: *Sus scrofa* head decomposition- day 28, Category E

Figure 7: *Sus scrofa* head decomposition- day 28, Category F

Discussion

This study hypothesised that temperature and soil moisture would not significantly impact the decay rates of animal body parts. However, the statistical results of soil pH, microbial activity, and body part mass loss were all significant. The null hypothesis was hence rejected, and the alternative hypothesis accepted.

The physiochemical composition of compost helped understand the burial microenvironment into which organic meat was introduced in terms of nutrient availability to microbes and suitability for their growth and activity. Due to the organic content above the average of most field soil types (Partanen *et al.* 2010), the tests revealed favourable decay conditions with the expected large spectrum of bacteria.

Statistical analyses of the compost pH indicated that the deposition of organic meat significantly impacted compost acidity and alkalinity levels during sampling intervals and at the end of the experiment. This is significant since it indicates that soil pH may be used to detect smaller clandestine graves. The study of Vass *et al*. (1992) proposed that high pH (> 7.0) can be linked with the early and intermediate stages of decay. Their findings are supported in the present contribution, with the soil pH levels showing alkaline in all categories throughout the experiment, especially towards the end of the trial.

The ideal moisture level for compost varies between 40-60%; anything above and waterlogged conditions would cause air to leave pore spaces, negatively impacting aerobic bacteria and allowing anaerobic microbes to flourish (Jagnow *et al*. 1977). Measurement of organic Levington yielded 37%, indicating some dryness. Experimental modification of soil moisture either boosted the moisture level to 40% or dried it to the wilting point (10%). An extreme drop in moisture would, in principle, result in bacterial action significantly slowing down, but there was no evidence of it because it did not pass the wilting point.

In addition to the compost's physical and chemical characteristics, the activity of soil microbes prior to and throughout the decomposition was also tested. In principle, the more surface space is available, the easier it gets for microbes to work; digestion and heat generation speed up, as well as bacterial growth and development and the rate of organic matter breakdown. Previous studies (e.g. Paul and Clark 1996 and Carter 2005) suggested that a soil matric potential of -0.01 MPa will allow the highest metabolic activity of microbes. Therefore, lower scales of matric potential would decrease the metabolic activity of microbes. The results of the present contribution showed otherwise. With the compost MPa of -0.03, microbial activity was still high and was affected by the higher temperature and higher soil moisture. These findings are in line with Couteaux *et al*. (2002), and Carter and Tibbett (2006) who associated the metabolism of soil microorganisms with specific temperature levels. Further, the results confirm those of Carter *et al*. (2010) that linked higher moisture levels with the higher activity of soil microbes.

Gram staining identified mostly Gram-negative bacteria involved in the decay that could have originated from the intestine and other body areas. In comparison, Gram staining of soil samples before the start of the

experiment indicated predominantly Gram-positive bacteria. This is significant since it points to the chemical breakdown of organic material potentially taking place in compost and the enteric bacteria likely decomposing the tissue.

Bacterial enumeration, in addition, demonstrated that microbial decomposers were higher in numbers in higher temperature and soil moisture settings, indicating accelerated decomposition. Body part decomposition supported this, with the biggest mass loss in depositories exposed to the highest temperature and soil moisture levels.

Biomass loss measurements further demonstrated that animal heads lost more weight in both temperature settings than feet. Animal elements also decayed at different rates according to the stages of decomposition. The size of organic material is a significant factor in the decay pattern. This is because smaller pieces of organic material are more suited for microbial degradation; they are easier to digest, and microbial decomposition is faster. This way, body parts should decompose faster than whole bodies, in theory, as the latter have a larger surface area to volume ratio. Indeed, Pope (2010) and Notter and colleagues (2008) showed differential decomposition even in whole bodies. Megyesi *et al.* (2005) also provided quantitative post-mortem interval equations for different body regions. Different microbial communities have also been associated with different body regions, for instance, skin (Metcalf *et al.* 2016), mouth and rectum (Hyde *et al.* 2013), internal organs (Tuomisto *et al.* 2013) or ears and nasal canals (Johnson *et al.*2016). The body parts used in the current project were detached from the rest of the body, and this might have limited or prevented other bacteria from a gastrointestinal reservoir from invading the tissue.

Conclusions

In summary, the results showed soil pH as a potential indicator of smaller clandestine burials. Being responsive to the temperature and soil moisture variables, metabolic microbial activity levels and Total Viable Count were also found suitable for further decomposition experiments. Gram-negative bacteria were higher in numbers, pointing to potential decay within the body. Morphological changes and biomass loss showed the differential decay between body parts, serving as a basis for field research using human remains. The case study demonstrated that differential preservation in mass burials can reveal localised depositional circumstances that may aid in establishing postmortem history.

Competing Interests

The authors have no competing interests to declare.

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