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Patterns of Mortuary Practice Associated with Ethnoreligious Genocides of the Silk Roads: The Impact of Wrapping on the Decomposition of Body Parts

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Abstract

Body part detachments caused by accelerated decay, as well as dismemberments, may alter the growth and activity of the microbial necrobiome in the absence of necessary gastrointestinal organs. The effect of depositional settings on the decomposition of individual body parts is, in addition, inadequately researched, leaving long-term implications for the recovery of mass grave remains. The present study is the second in a sequence of taphonomic reconstructions focusing on the role of microbial necrobiome in the decay of detached body parts. It assessed the early decomposition of wrapped remains using the domestic pig (*Sus scrofa*) as an animal analogue. The aim was to evaluate how the remains decompose under differing temperature levels, as tested by Total Viable Count, Gram stain, body part mass loss and gross morphological changes of soft tissue. It was hypothesised that an increase in temperature would not result in an increased decomposition rate. Statistical analyses yielded significant differences in biomass loss between the two microenvironments. The results further demonstrated differential decomposition between body parts and indicated that facultative aerobic and anaerobic bacteria were responsible for the decay. The controls suggested a specific decay pattern in reduced oxygen settings. This study advances taphonomic knowledge of the postmortem history of wrapped body parts, aiding the recovery and identification of victims of genocide.

Keywords: *Sus scrofa*, Necrobiome, Turkmen genocide, Wrapped body parts, Conflict archaeology

Introduction

Previous taphonomic studies that examined the decay in wrapped microenvironments have primarily focused on whole corpses, resulting in both contradictory findings and a limited understanding of the postmortem history of individual body parts. Early works, such as Mann and colleagues (1990), noted that if a body is wrapped in cling film or a bin liner, it will decompose slower in comparison to the unwrapped corpse. This way, the role of the wrapper may be first to limit access to insects until they have reached the body tissue. The wrapping then may provide shelter from weathering conditions and allow faster decomposition. Other contributions, such as those of Matuszewski and colleagues (2014) and Kelly and colleagues (2009), however, reported body wrapping to have an insignificant effect on decomposition rates. Their results again are in contradiction with those of Sukchit and colleagues (2015), who reported faster decomposition during summers and monsoons on hanging *Sus scrofa*. Moreover, the study of Teo and colleagues (2013) suggested that although the fabric of clothing itself has no significant effect on the decomposition rates, a combination with arthropod succession that may find shelter under the covering can impact the pattern of whole body decay.

Body parts, in addition, are also frequently found accompanied by various coverings (Galloway 1996; Gitto *et al.* 2015),

with little research available on the association between wrapping and decay, aiding the complexity of clandestine recovery and victim identification. Using *Sus scrofa* remains, this study combines taphonomic and microbiological methods to investigate the effect of ambient temperature on the early postmortem history of wrapped remains as tested by Total Viable Count, Gram stain, body part mass loss and gross morphological changes of soft tissue. It was hypothesised that an increase in temperature will not result in an increased rate of decomposition. The analysis of clothed or wrapped remains has forensic potential in examining mass graves resulting from civil conflicts. An example is the Iraqi Turkmen genocide carried out by ISL (2014 – 2017), resulting in an estimated 3000 Turkmen killed, over 600,000 displaced, and 5000 still missing (UNPO 2024).

A significant aspect of the Turkmen demographic is the near-even distribution between Sunni and Shia affiliations, a factor that has contributed to their historical persecution (Oğuzlu 2004). Since the establishment of the Iraqi State in 1921, the Arabs held governing power, the Kurds received support from the international community, while the Turkmen remained marginalised. The community faced human rights violations by successive Iraqi governments, with the study of the Turkmen language terminated in 1932. They endured displacement and deportation, were denied cultural rights, prevented from identifying as Turkmen in censuses, and coerced into changing their nationality (UNPO 2024). The Turkmen tragedy included the Gavurbağı massacre in 1946**,** the Kirkuk massacre in 1959 and the Altun Kupri massacre in 1991. The killings continued after the 2003 occupation of Iraq and especially during the ISL occupation, with neighbouring countries and the international community remaining largely uninformed or indifferent to their plight (UNPO 2024).

Yet, the Turkmen are one of the oldest communities of the ancient Silk Roads. The term 'Turkmeneli', also referred to historically as 'Turcomania', encompasses the Iraqi Turkmen homelands, extending from the country's borders with Turkey and Syria and diagonally down to the border with Iran (Anderson and Stansfield 2009). The early Turkmen were essentially Oghuz Turks, the epic medieval rulers who enlightened the world with Islam's new cultural character (Baumer, 2016).

The migration of Turkic people from the Central Asian Altai Mountains to Iraq dates back to the 7th century when around 2,000–5,000 Oghuz Turks joined the Muslim armies of Ubayd-Allah ibn Ziyad (Baumer, 2016). Subsequently, Turkic forces settled in the region during the 8th century, spanning from Bukhara to Basra and Baghdad (Saatçi 2018). Throughout the Abbasid era, Turkmen warriors were further introduced to Iraq; however, the overall number of those who established roots in the region was minimal, resulting in their assimilation into the indigenous Arab population (Taylor 2004). Modern Iraqi Turkmen constitute the country's third largest ethnic group and still inhabit the Turkmeneli historical region, which Arabs geographically border to the south and Kurds to the north (Oğuzlu 2004).

The largest concentration of the Turkmen mass killings took place in these regions, too: the northwestern and northeastern parts, including Mosul, Erbil, and Kirkuk. In contrast to the arid climate of central and southern Iraq, where the average annual rainfall does not exceed 166 mm, these areas experience a yearly precipitation of around 303 mm (Al-Ansari *et al.* 2014). Consequently, the preservation of human remains is likely to be poorer, as body decay is primarily influenced by ambient temperature and soil moisture (Archer 2004; Carter *et al*. 2010). This way, taphonomic research of the wrapped depositional microenvironment may help recover, analyse, and identify genocide victims.

Materials

Sus scrofa (domestic pig) is generally used as an animal analogue in forensic research, as it is believed to decompose similarly to human remains (Matuszewski *et al.* 2014). In this study, a total of 22 pieces of pork belly and 32 pig body parts (heads and feet) were utilised. The animals were obtained from a Yorkshire abattoir, John Penny & Sons in Leeds and slaughtered on their premises by separating the head at the front and four limbs from the torso when they reached a weight between 30 to 40 kilograms. Domestic pigs were killed a maximum of seven hours before the start of the trials, kept in refrigerated conditions and delivered in plastic bags to the Taphonomy lab

at the University of Bradford. All animal analogues were antibiotic-free.

Methods

Microbiological analyses

For the Total Viable Count (TVC), the 'dilution plate method' with broad-spectrum bacterial enumeration was utilised (Hopkins *et al.* 2000; Weaver 1994). Gram staining was carried out on the swabbed skin and muscle of representative samples. Bacterial colonies were observed under a microscope with a 100X objective before being categorised into Gram-positive or Gram-negative. Samples with over 100 colonies were marked as TMTC (too many to count) and discussed in terms of approximate numbers. Three types of media plates were used: R2A (for soil-water-borne microflora), Rose Bengal agar (for fungi growth), and MacConkey agar medium (for the *E. coli* and other coliforms for growth purposes). The plates were examined at 24 hours for R2A and MacConkey agars and at 48 hours for Rose Bengal agar.

Taphonomic analyses

For the taphonomic analyses, the decay model was modified from Davis and Goff (2000) and Prieto *et al.* (2004) to accommodate a combination of terrestrial and aquatic post-mortem changes in the moist microenvironment (Table 1). To establish the biomass loss, the weight of each body part was calculated at the start and end of the experiment using an electronic scale.

Table 1: Description of morphological appearance for wrapped 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, a thin, greyish substance on bone trauma. 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 impact of ambient temperature on body part mass loss was analysed using the Kruskal-Wallis test. TVC and Gram staining were considered qualitative microbiological methods unsuitable for statistical analyses of the current study design; their characteristics were hence used to support or contrast other statistically analysed results.

Research design for pork belly experiments

A total of 22 samples were used for TVC and Gram stain, with 16 in aerobic conditions and 6 in anaerobic conditions (Table 2; Table 3). The belly slices measuring approximately 5cm x 5cm were wrapped and incubated at two ambient temperatures: 20°C (category A) and 30°C (category D). Meat pieces designed for aerobic conditions were placed in autoclaved clear glass jam jars with a volume of 228ml, height of 85mm, and a diameter of 63mm, with a lid/neck diameter of 63mm. The jar lids were pierced to allow oxygen access. Double wrappers were used as a control. The samples designated for decay in anaerobic conditions were kept in an anaerobic chamber. Due to the nature of the treatment, control samples were not used.

Microbial swabbing designed for TVC and Gram staining was taken from the muscle and skin areas of the pork belly prior to the experiment. Swabbing (harvesting) on three

meat pieces and one control per category was again completed on day 14 (halfway through the experiment) and day 28 (end of the experiment). Swabbed samples were not re-wrapped to avoid disturbing the body microflora; instead, new sets of belly slices were used. In the anaerobic chamber, samples were not harvested but left to decompose undisturbed.

Table 2: Breakdown of sample size for wrapped pork belly

Microenvironment	Category	Harvest	End of trial
Aerobic	A	3+1 control sample	3+1 control sample
Aerobic	D	3+1 control sample	3+1 control sample
Anaerobic	A	--------	3
Anaerobic	D	--------	3

Table 3: Summary of experiment setups for wrapped pork belly

Duration	Sample size	Microenvironments/Category	Applied method	Tested variables	Controls
28 days	Aerobic: 16 Anaerobic: 6	A (20^0C) aerobic conditions D (30^0C) aerobic conditions A (20^0C °C) anaerobic conditions D (30^0C °C) anaerobic conditions	TVC Gram stain	Ambient temperature	Double wrapping

Research design for body part experiments

Six animal heads and six feet, as well as two heads and two feet controls, were used per category, making it a total of 32 body parts, including one repeated trial (Table 4). Animal elements were weighed and photographed at the start, with attention being paid to insects not entering the wrapper. Pig heads weighed approximately 5.25kg, and pig feet 0.75kg. Body parts were wrapped individually in heavy-duty plastic waste bags measuring 57 x 27 x 4 cm and sealed with gaffer tape.

Table 4: Breakdown of sample size for wrapped body parts

Category	Sample	Sample size
A	Head	6
A	Foot	6
A control	Head	2
A control	Foot	2
D	Head	6
D	Foot	6
D control	Head	2
D control	Foot	2

Samples were then deposited individually into IKEA fabric storage boxes, dimensions 33 x 38 x 33 cm and left to decompose undisturbed for 28 days. Those in category A were exposed to the room temperature averaging 20.8°C, and samples in category D decayed in the heated room at an average of 26.1°C (Table 5). The controls were body parts left to decay double wrapped in two plastic bags, the inner one packed top-up into the bottom of the outer and double sealed. Skin and muscle tissues from the remains were swabbed at the beginning and end of all trials to ensure body flora and microbial numbers were comparable with the parallel pork belly study. Ambient temperatures were monitored hourly with Tinytag® Gemini PT100 loggers. Transparent bin liners were used for two samples, with the thermometer left inside to record the temperature daily. At the end of the trial, animal elements were again visually observed for any signs of insect succession. They were then cleaned, weighed and photographed before being classified into decay stages.

Table 5: Summary of experiment setups for wrapped animal body parts

Duration	Sample size	Microenvironments/ Category	Applied method	Tested variables	Controls
28 days	32	A (20.8^0C) D (26.1^0C)	Mass loss Morphological changes of soft tissue	Ambient temperature	Double wrapping

Results

1. Qualitative and quantitative assessments prior to wrapping

Microbiological analyses

The bacterial population densities of the pork belly swabbed before the experiment showed that muscle areas had a higher number of bacterial colonies (average 4.3 x 10^5) in comparison to the tested belly slice skin (average 3.1 x 10^5). Predominantly Gram-negative bacteria were identified from the tested samples. Positive bacterial colonies were found mainly on swabbed skin areas from both belly slices and animal heads and feet, albeit in small numbers.

Taphonomic analyses

Body part examination showed no evidence of fly and insect infestation. All remains were categorised into the 'Fresh' decomposition stage. The average weight of animal heads was 4.45kg, and for animal feet, 0.95kg.

2. Micro-environmental monitoring during the decay

Total Viable Count and Gram stain

The increase of TVC in pork belly pieces incubated at 20°C (category A) was noticeable from the start of the experiment, with numbers over 100 per swab during the harvest time. Visual examination of Petri dishes confirmed various colony types potentially associated with the decay. A single raised, oversized, mushroom cup-shaped colony grew on a Rose Bengal plate (Figure 1). Bacteria grown on R2A (Figure 2) and MacConkey (Figure 3) agar plates from muscle tissue were, in contrast, smaller in size and mostly white/creamy in colour.

Figure 1: Typical Rose Bengal plate from wrapped pork belly muscle, category A

Figure 2: Typical R2A plate from wrapped pork belly muscle, category A

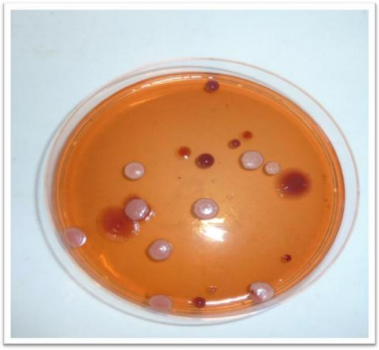

Figure 3: Typical MacConkey plate from wrapped pork belly muscle, category D

The growth pattern of colonies in (double-wrapped) control samples from both categories was similar. The main difference was the larger growth of fungi and bacteria at the end of the experiments compared with the main samples (Table 6). TVC of six samples left to decompose in anaerobic conditions also rose in numbers from the start to the harvest point and declined at the end of the experiment (Table 7). Samples exposed to higher temperatures were higher in numbers during harvest time and lower at the end of the trial. However, the number of microorganisms in both anaerobic settings was higher than in the main samples or double-wrapped controls at the end of the trials.

Table 6: Total Viable Count from pork belly samples, Category D

Samples	R2A Harvest	MacConkey Harvest	RB Harvest	R2A End	MacConkey End	RB End
1: Muscle	> 5 X 10^6	> 5 X 10^6	> 1 X 10^3	3.4 x 10^5	1.5 X 10^5	No growth
1: Skin	> 5 X 10^6	> 5 X 10^6	No growth	2.2 X 10^5	5 X 10^5	No growth
2: Muscle	> 5 X 10^6	> 5 X 10^6	No growth	1.3 X 10^5	1.2 X 10^5	No growth
2: Skin	> 5 X 10^6	> 5 X 10^6	No growth	1.0 X 10^5	1.1 X 10^5	No growth
3: Muscle	> 5 X 10^6	> 5 X 10^6	No growth	4.3 X 10^5	1.7 X 10^5	No growth
3: Skin	> 5 X 10^6	> 5 X 10^6	No growth	2.3 X 10^5	9 X 10^5	No growth
4: Control muscle	> 5 X 10^6	> 5 X 10^6	3.6 X 10^3	5.2 X 10^5	5.5 X 10^5	2.0 X 10^3
4: Control skin	> 5 X 10^6	> 5 X 10^6	1.1 X 10^3	2.4 X 10^5	3.7 X 10^5	9.0 X 10^3

Gram stain of pork belly incubated at 30°C (category D) on R2A plate revealed pink rods, suggesting Gram-negative bacteria were engaged in the decay process. This was also the case with MacConkey agar incubated at 20°C (category A). Gram stain of the control sample

in category A exhibited large purple fungi (Figure 4), and the control sample of category D showed a mixture of bacteria on R2A and MacConkey plates. Pink sphere colonies, purple rods, and yeasts, together with dark sphere-shaped colonies, were all assumed to be engaged in the decay of pork belly tissue. Gram stain analysis was further performed under anaerobic conditions to establish whether mainly Gram-positive or Gram-negative microbes were active in the absence of oxygen. Samples incubated in Category A mainly contained Gram-negative bacteria, exemplified by spherical and rod colonies. However, category D staining showed Gram-positive thick bacilli (usually characteristic of *Clostridium* spp.).

Table 7: Total Viable Count from pork belly samples in anaerobic conditions

Samples	Temperature	Harvest	End of the experiment
One	20	6.2 X 10^5	5.3 X 10^5
Two	20	6.0 X 10^5	5.8 X 10^5
Three	20	6.8 X 10^5	4.7 X 10^5
One	30	8.5 X 10^5	3.9 X 10^5
Two	30	7.9 X 10^5	4.1 X 10^5
Three	30	8.2 X 10^5	3.8 X 10^5

Body part mass loss

The Kruskal-Wallis test measured the difference in biomass loss between two microenvironments and yielded significant results for both wrapped heads (H= 8.23, p= < 0.05) and wrapped feet (H= 11.44, p= < 0.05). There was not much difference in the percentage of biomass loss between heads decomposing at an average of 20.8°C (category A) and the control samples. However, the control of animal feet lost over twice the amount of biomass (average of 38.20%) than samples decomposing in regular bin liners (average of 16.27%).

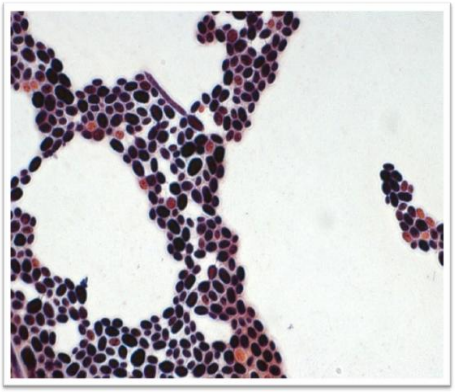

Figure 4: Fungi from the surface of animal tissue: category A control. Scale 100μm

Heads left to decompose at an average of 26.1°C (category D) lost over double the amount of biomass (average of 53.85%) compared to the control samples (average of 25.80%). A small difference in the mass loss was shown between the main feet samples and the control, with the latter losing 2.62% more weight (Figure 5). Thermometers inside the bin liners recorded the difference in temperature of up to two degrees Celsius higher in comparison with the ambient temperature. Temperature rising from the start of the experiment was demonstrated, with the peak being week 3 for all samples. Category A head decomposed at an

average of 22.3°C inside the bin liner, approximately 1.5°C above ambient temperature. Category D head decayed at approximately 28.1°C, two degrees above ambient temperature, and a foot sample just above the temperature outside the bin liner (26.8°C).

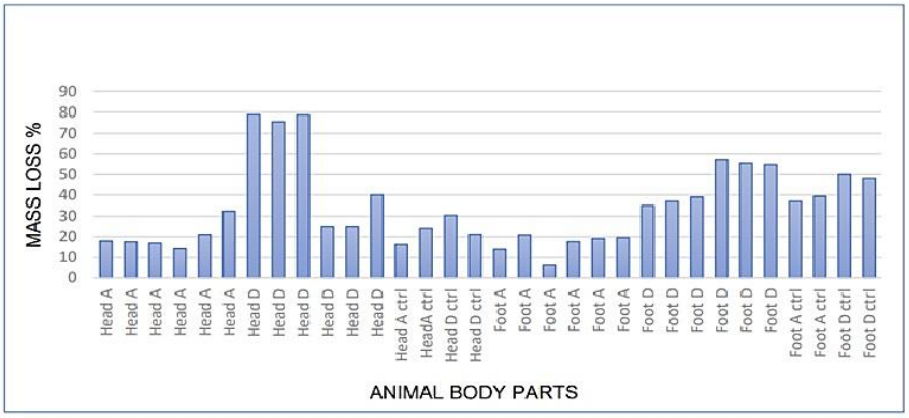

Figure 5: Percentage of biomass loss for wrapped body parts. Treatments: category A= average of 20.8°C, category D= average of 26.1°C

Morphological appearance of body parts

All animal heads in category D were classified into the Advanced decay stage. There was visible discolouration of the skin, with a detachment of body elements, including ears and mandibles. Skin and liquid were coming off, and bones started showing (Figure 6). Animal heads in category A decomposed slower and were classified into the Active decay stage. Animal feet showed more decelerated decay compared to animal heads. In category A, they mostly exemplified characteristics of the Early disintegration stage. In category D, the morphological changes were characteristic of Active decay.

Figure 6: Category A *Sus scrofa* head decay at the end of the experiment

Control samples from both categories and for both types of body parts visually appeared to decay much more slowly, with all eight specimens being categorised into the Putrefaction stage. On touch, however, it became apparent that they either crumbled or disintegrated into a paste. No adipocere or desiccation was recorded on any of the specimens.

Discussion

This study hypothesised that an increase in temperature would not result in an increased rate of decomposition of wrapped remains. However, the statistics yielded significant

results; the null hypothesis was rejected, and the alternative hypothesis accepted. TVC in both categories of pork belly samples peaked at the sampling interval (day 14) and then lowered in numbers towards the end of the experiment, indicating the highest microbial activity halfway through. It was not possible to differentiate between the numbers of R2A and MacConkey plates at sampling intervals due to the sheer amount of microbes present. At the end of the trials, however, the microbial count was higher in Category A, confirming that decomposition was slower at a lower temperature and was still taking place. In category D, colonies were also more varied and prominent, further pointing to the higher temperature affecting the development of bacteria. The slow decay between the double-wrapped control and especially anaerobic conditions further showed that reduced oxygen influenced the decay. So, it can cautiously be concluded that neither strict aerobic nor strict anaerobic bacteria were mostly involved in decomposing the meat, but facultative bacteria that survive and thrive with or without oxygen. This contrasts with other studies, such as Schotsmans (2013), Hyde *et al.* (2013) or Metcalf *et al.* (2013), which reported a switch from obligate aerobes to facultative anaerobes until oxygen is reintroduced into the body during rupture.

Gram staining completed throughout the trials showed mostly Gram-negative bacteria, pointing to potential decay within the body. By exemplifying a mix of yeast, Gram-positive and Gram-negative colonies, the control conditions, however, indicated that the reduced oxygen levels combined with temperature can influence the type of bacterial species. The experiments involving the biomass loss of animal body parts also proved that ambient temperature has an effect on the decomposition. Differential decay was shown between the main samples, with animal heads losing more weight overall (36.96%) than feet (31.32%). The temperature measured inside the wrapper of animal heads revealed an increase of up to two degrees Celsius, explaining to an extent the more advanced decay and indicating higher microbial growth and activity.

Decay patterns exemplified by a variation of biomass loss in double-wrapped controls were unlikely caused by temperature itself. It is probable that different microbial activities were taking place due to reduced oxygen levels. Indeed, prior studies, such as those by Zhou and Byard (2011), demonstrated that a limited oxygen microenvironment increases humidity levels, leading to variable microbial activity under favourable temperature conditions. Others, such as Pokiness (2013), came to similar conclusions, showing that fungi and facultative anaerobic bacteria will dominate in an environment with low oxygen and no fly infestation. Morphological changes supported these findings, showing that animal elements decayed faster in higher temperature settings. The control also revealed the decay specific to tightly wrapped remains, where they could not be fitted into any decomposition stage once out of the bin liner. A study by Forman (2015) reported a ‘greenhouse effect’ in the wrapping, suggesting a third variable being dominant other than time and temperature. As demonstrated in the present contribution, this is likely to be oxygen.

Conclusion

In summary, the temperature, as the main variable known to affect the decay of whole bodies, was tested for its impact on the decay of wrapped body parts. Microbiological analyses indicated a probability of facultative anaerobic and aerobic bacteria being involved in the decomposition. *Sus scrofa* body parts confirmed the importance of temperature in biomass loss, and the controls indicated a specific decomposition pattern in reduced oxygen settings. Because the wrapping was not an obstacle to microbial decomposers under controlled conditions, it is unlikely to be in the field either. Established decomposition patterns, hence, may serve as the basis for further research on single limb decay. The results are relevant for understanding the postmortem history of clothed and wrapped human remains, often found in mass graves resulting from genocides.

Competing Interests
The authors have no competing interests to declare.

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