INVESTIGATION OF THE INTERACTION OF REMAINS AND TEXTILES IN SOIL GRAVES

By

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A thesis submitted for the

Degree of Doctor of Philosophy (Science)

University of Technology, Sydney

2015

Certificate of Authorship and Originality

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of the requirements for a degree except as fully acknowledged within the text.

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Date: 17.12.2015

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Acknowledgements

First and foremost I would like to acknowledge and thank my wonderful supervisor, Assoc. Prof. Barbara Stuart, for all of your assistance and support throughout this PhD project. I am also deeply grateful for my co-supervisor, Prof. Shari Forbes, for giving me numerous opportunities and taking me in as a part of her research team. This PhD would not be possible without the guidance and encouragement from both of you.

Field work is no easy task and not one that can be done by a single person only, it is a big team effort and I would not be able to do it without the help and support from a multitude of individuals. A special thank you goes out to the original Team Safari (Dr. Katelynn Perrault, Christopher Watson, Rebecca Buis, LaTara Rust and Kate Trebilcock). I would also not be able to complete my field work without the aid of Robert Chatterton who has truly been invaluable. I would also like to thank all the volunteers who have so graciously agreed to help me out at the field site, whether it would be to help with heavy lifting, clearing land or just keeping me and the goannas company.

I would also like to thank all the academic and professional staff at UTS who provided me with assistance over the course of my PhD. I particularly wish to thank Dr. Ronald Shimmon, Dr. David Bishop and Dr. Linda Xiao for all their assistance in lab and for providing technical support with the instruments.

Thank you to my fellow PhD students past and present for all the good times. A special mention goes out to Dr. Anna Molnar, Alexandria Hunt and Verena Taudte, for going

through this experience with me. My PhD time would not have been the same without your company, and I would probably go crazy without your support and our wine sessions.

Lastly I would like to thank my family, especially my mom Eva and my sister Silje for all their support and love throughout this PhD period.

Publications and Conference Proceedings

Refereed publications directly related to this project

Ueland, M., Nizio, K., Forbes, S., Stuart, B.H. The interactive effect of the degradation of cotton clothing and decomposition fluid production associated with decaying remains, Forensic Science International, (2015) Vol 255, pp 56-63, DOI 10.1016/j.forsciint.2015.05.029. (Chapter 5 and 6).

Stuart, B.H., **Ueland, M.** Degradation of Clothing in Depositional Environments. In *Taphonomy of Human Remains: Forensic Analyses of the Dead and the Depositional Environment*, eds. E. Schotsmans, N. Marquez-Grant and S. Forbes, Wiley-Blackwell, Chichester, in press (accepted 12/6/2015). (Chapter 1)

Other publications from related research conducted during candidature

Forbes, S.L.; Troobnikoff, A.N.; **Ueland, M**.; Nizio, K.D.; Perrault, K.A. Profiling the decomposition odour at the grave surface before and after probing. *Forensic Science International*. 2016, 259, 193-199.

Nizio, K.D., Perrault, K.A. Troobnikoff, A.N, **Ueland, M.,** Shoma, S., Iredell, J.R., Middleton, P.G., Forbes, S.L. Volatile Organic Compound Profiling using GC×GC-TOFMS to Differentiate Bacteria Associated with Lung Infections: A proof-of-concept study. *Journal of Breath Research,* 2016, 10, 026008.

Stuart, B.H., **Ueland, M**. Decomposition in Aquatic Environments. In *Taphonomy of Human Remains: Forensic Analyses of the Dead and the Depositional Environment*, eds. E. Schotsmans, N. Marquez-Grant and S. Forbes, Wiley-Blackwell, Chichester, in press (accepted 12/6/2015).

Refereed conference proceedings

Ueland, M., Forbes S.L., Stuart, B.H. Degradation patterns of natural and synthetic textiles associated with decomposing remains, 7th European Academy of Forensic Science Conference, September 6-11, 2015, Prague, Czech Republic.

Ueland, M., Forbes, S.L., Stuart, B.H. The influence of decomposition fluids on the degradation of clothing textiles in soil graves, 20th World Meeting of the International Association of Forensic Sciences, October 12-18, 2014, Seoul, South Korea.

Ueland, M; Forbes, S.L.; Stuart, B.H. Textile degradation patterns of clothing associated with remains, Australian and New Zealand Forensic Science Society 22nd International Symposium on the Forensic Sciences, August 31st – September 4th, 2014, Adelaide, SA.

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List of Abbreviations and Symbols

ADD	Accumulated degree day
ATP	Adenosine triphosphate
ATR	Attenuated total reflectance
CDI	Cadaver decomposition island
ECD	Electron capture detector
FID	Flame ionization detector
FTIR	Fourier transform infrared
GC	Gas chromatography or gas chromatograph
ID	Inner diameter
MS	Mass spectrometry or mass spectrometer
N	Molar concentration
NIST	National Institute of Standards and Technology
NSW	New South Wales
PC-1	First principal component
PC-2	Second principal component
PCA	Principal component analysis
PET	Polyethylene terephthalate
PMI	Post-mortem interval
RAAF	Royal Australian Air Force
RPM	Revolutions per minute
TEA	Thermal energy analyser
UV	Ultraviolet
VWC	Volumetric water content

Abstract

Textiles are a common source of evidence in forensic scenarios and can provide valuable insight into a crime event. In the past research has focused mainly on the effect of the presence of clothing on the decomposition timeline of the remains used to estimate time since death, rather than how remains affect the textiles. The hypothesis is that the presence of decomposing remains will alter the degradation patterns of textiles. It is therefore suggested that analysing textile samples collected from a crime scene might give further information about the post-mortem or post-burial interval. This is particularly valuable when only clothing is recovered from a scene.

In order to investigate textile degradation patterns associated with remains, clothed pigs were either buried or placed directly on the soil surface. Clothing in the absence of remains were also collected and analysed for comparison purposes.

The clothing samples were analysed using Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy in order to investigate any chemical damages to the textiles, in addition to looking at the presence of decomposition fluid. Samples from certain sampling days were analysed using Gas chromatography-mass spectroscopy (GC-MS) for the further elucidation of the lipid profile absorbed by the textile samples associated with decaying remains.

At the completion of the project it was confirmed both visually and through the chemical analysis that the natural textile degradation in a soil environment was inhibited in the presence of decomposing remains. Principal component analysis of the data obtained for cotton in absence of remains demonstrated a clear separation in the data sets and degraded samples could clearly be distinguished from the non-degraded ones. Seasonal variety was determined to be a factor in the timeline of textile degradation of the natural material.

The apparent inhibition of the degradation of natural textiles associated with the decomposing remains is a significant finding. It suggests that the processes leading to the release of decomposition fluid into the environment might play an important role in the textile degradation timeline. Investigating the textile degradation stage as well as analysing the lipid composition will provide invaluable information for the resolution of future cases of clandestine deaths.

Chapter 1: INTRODUCTION

Chapter 1: INTRODUCTION

Textiles have long been present as part of everyday life and, as a consequence, provide a valuable source of information in forensic and archaeological contexts [1]. In forensic scenarios, textiles are a common source of evidence and can provide valuable insight into a crime. In the field of forensic taphonomy, the nature and condition of textiles collected from a crime scene can assist investigators in determining the nature of the death and in the identification of the victim. The presence of clothing and other body coverings at the site of a death can have a significant effect on the determination of the post-mortem interval. Determining time since death is incredibly important when identifying a perpetrator or excluding suspects in a homicide investigation. A carefully constructed estimate of the post mortem interval (PMI) can reduce the potential pool of decedents and thereby contribute to the eventual identification of the culprit [2]. Knowing the PMI can also help to establish the range of natural events and environmental forces which may have affected the remains with the passing of the seasons, permitting a more thorough taphonomic analysis.

Taphonomic analysis is the application of taphonomy, the study of decaying organisms over time which focuses on the reconstruction of the events and processes that have occurred from the time of death to the time of recovery [3]. The term was coined by Ivan Efremov in 1940 from the Greek word *taphos* which means burial, and *nomos* meaning law. In the field of archaeology, taphonomic processes are used to determine if remains at an archeologic site are associated with human activity. Within forensic taphonomy the analysis considers the decomposition processes a carcass has undergone since the time of death, with the goal of determining how different factors affect the rate of decomposition.

Decomposition is the process of resolving the constituent parts of a compound body into its elementary parts through physical, chemical and biological activities. The stages of decomposition are used to estimate time since death as decomposing bodies undergo such processes in a predictable manner [4]. Environmental factors including temperature, fauna, and the medium in which the human remains are located greatly affect their taphonomy [5]. The variability in temperature will determine the overall rate of the process as well as the length of each process [4].

1.1 Post-mortem Process

The decomposition process begins immediately with the cessation of the heart. The decomposition of the soft tissue may take as little as days to completion or progress through years. During decomposition the body will undergo certain processes highly influenced by environmental factors.

1.1.1 Early Post-mortem Changes

The decomposition process occurs immediately after the heart stops beating and results in a decrease of blood pH and an overall loss of skin colour, knows as pallor [6]. These early post-mortem changes mark the onset of the stage referred to as the fresh stage of decomposition. During this process the early insect colonisers such as blow flies (*Calliphoridae)* and flesh flies (*Sacrophagidae)* are seen [6]. Egg masses will be found in moist areas of the body such as the natural orifices and trauma wounds. The eyes often exhibit some of the earliest changes, if these are left open after death a thin film will cover they eyes within minutes.

1.1.2 Late Post-mortem Changes

Late post-mortem changes such as the three mortis', algor mortis, livor mortis and rigor mortis, then becomes evident; the body will initially start equilibrating with the ambient temperature, a process known as algor mortis, this process tends to take between 18–20 hours [7]. There will be an accumulation of blood in the small vessels due to gravity now that the heart no longer pumps the blood throughout the body, referred to as livor mortis. Rigor mortis is another process that may occur; this can occur in the fresh and bloated stages and is a chemical reaction in the body causing rigidity in the muscles and joints. This process occurs due to the oxygen depletion and therefore the loss of adenosine triphosphate (ATP) in the muscles. The onset of rigor mortis is first observed in the first 2-6 hours following death [7]. It tends to start with the muscles in the face and then it spreads throughout the muscles until the entire body is experiencing rigor mortis. The initialisation and duration of rigor mortis is governed by two primary factors: temperature and the metabolic state of the body [7, 8].

1.1.3 Autolysis

As blood is no longer pumped through the body, there is no stream of oxygen, thus leading to oxygen depletion in the body. As cells of the body are deprived of oxygen, carbon dioxide in the blood increases, pH decreases, and waste accumulate, thereby poisoning the cells. Aerobic metabolism is inhibited causing the destruction of cells by enzyme digestion [6, 8]. This destruction of cells by enzymatic digestion is referred to as autolysis [9]. According to Carter, Yellowlees [6], autolysis can begin within minutes of death.

Microbial activity is initialised by cellular destruction and the release of protein, carbohydrates and lipids presented in the fresh stage. These macromolecules are broken down by anaerobic microorganisms in the gastrointestinal tract and respiratory system. Organic acids are formed in this process along with gases such as methane and hydrogen sulfide, creating a build-up of gases within the organism [6]. This marks the onset of the bloat stage. The bloat stage is derived from the increasing size of the abdomen which is a result of the internal pressure from the gas accumulation in the cadaver. At the onset of the bloat stage several carrion beetles are often seen such as *Histeridae* (clown beetles) [10]. These beetles tend to feed upon the larva present on the decomposing remains as well as the flesh itself.

Skin slippage and the presence of fluid filled blisters can be observed during the onset of autolysis due to the dissolving of cellular membranes [4]. Skin slippage of the limbs is often seen if the carcass is in water or there is evident moisture in the air [4].

1.1.4 Putrefaction

Putrefaction results from microbiological activity and is initiated by autolytic processes. During putrefaction the degradation of proteins, carbohydrates and lipids into gases, liquids and simple molecules such as amino acids, fatty acids and glucose [8] observed in the bloat stage will continue and this causes the fluids and gases to escape from the orifices (such as the mouth, anus, nose or trauma wounds) of the body due to the gas build-up [6]. A common physical change to the carcass during this stage is a green to purple discolouration of the skin due to the formation of sulfhemoglobin in the settled blood [8]. This change generally occurs between 36 and 72 hours after death [4]. Fluid blisters will be apparent during putrefaction; this is also when the distinctive odours will be evident.

1.1.5 Liquefaction/disintegration

Following the purging of gases the active decay stage begins. This stage begins when the skin ruptures [6]. A rapid mass loss occurs due to a peak in maggot activity and microorganisms; mass loss also results from a significant release of cadaveric fluids that also occurs during this stage [6]. The fluid loss will lead to the formation of a cadaver decomposition island (CDI) around the carcass [6], if the liquid is allowed to escape from the cadaver, and is not contained. The microorganisms will break down the soft tissue [4]. Bacterial action will degrade the muscle tissue into volatile fatty acids, and compounds such as putrescine and cadaverine have also been detected and identified as decomposition products [4].

When most of the soft tissue of the carcass has been consumed *Coleoptera* beetles typically become the more dominant insects compared to the *Diptera* blow flies. Their increasing presences is due to the beetles preference to feed on the leathery, dry flesh and cartilage rather than the soft tissue, as is preferred by the *Diptera* blow flies [7]. The end of his stage is marked by only cartilage, skin and bone remaining.

The vegetation surrounding the carcass will change due to the influx of a nutrient supply [8]. This large increase in nutrients is likely to cause the death of underlying and nearby vegetation at this time [6] and can impact the vegetation even after this stage.

1.1.6 Remains

At the onset of the remains process there is minimal cadaver mass loss and there will be no carrion left [4]. The vegetation will again flourish as the initial large influx of nutrients start to re-equilibrate with the surrounding vegetation, providing a large amount of nutrients, but not large enough to create the toxic effects observed in the previous process [6]. The presence of increased vegetation around the edge of the cadaver island is likely to indicate that the carcass is in the dry stage [6]. Skeletonization occurs, when all the soft tissue has disintegrated and predominately hard tissue and bone is remaining on the carcass. Skeletonization is characterised by the exposure of over 50 % of the bones in the body [4]. Chemical and physical weathering will further break down the remains according to the environmental conditions [4]. This process takes much longer than the previous decomposition processes.

1.1.7 The Stages of Decomposition

The processes mentioned above are often used to explain the chemical and biological process occurring during decomposition, however these processes might occur in clear succession or they can overlap causing several of them to occur at once. In order to facilitate the comparison of different decomposition experiments and better compare results a set of stages used to describe the physical state of the remains based on observations has been created.

The visual changes observed during decomposition of remains are categorised using the stages of decomposition proposed by Payne [11] and adapted by Anderson and Vanlaerhoven [12]. The five different stages can be seen represented in Figure 1-1a-e. The first stage is referred to as the fresh stage and occurs immediately following death. During this stage the stiffening of the joints (rigor mortis) is evident, livor mortis and pallor is also observed. The second stage is known as the bloat stage; here the abdomen becomes distended due to the build-up of gases in the body (Figure 1-1b). Following gas accumulation the skin will rupture & the body enters active decay. Purging of fluids is seen in this stage. Active decay is followed by advanced decay, here a large amount of soft tissue has been removed & the skin takes on a dark colour. Lastly the dry/remains stage will occur, here most soft tissue has been removed and any remaining tissue will be mummified.

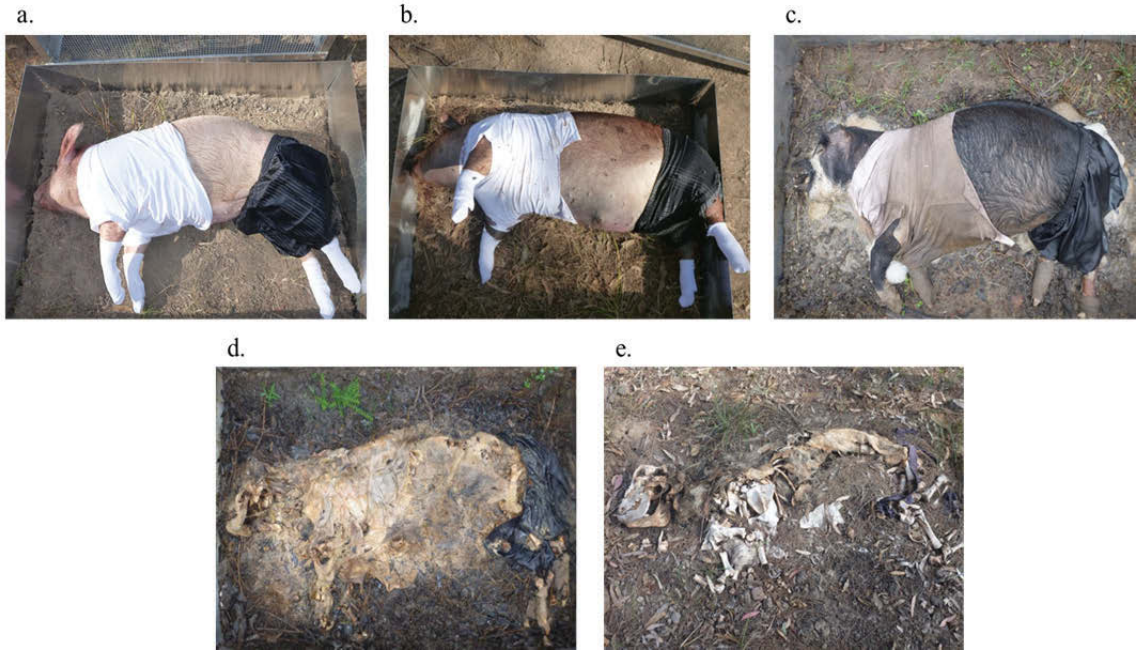

Figure 1-1: Stages of decomposition a) fresh; b) bloat; c) active decay; d) advanced decay; and e) dry/remains

1.1.8 The Environmental Factors and Rates of Decomposition

Environmental factors are crucial in the development and timing of these stages of decomposition [4, 8]. Temperature is known to be one of the main influences of decomposition, as it plays a major role in determining at which rate a body progress through the stages of decomposition. A body located in a cold environment is likely to go through the stages of decomposition in a different manner than bodies located in a hot, dry environment [7]. In areas of high acidity the body can be preserved for years [13].

Two contrasting types of soft tissue survival known as desiccation and adipocere formation may occur if the appropriate conditions are present. Desiccation results from the dehydration of soft tissues with the skin eventually becoming dark, dry and leathery [14]. Desiccation affects body parts which are generally exposed to either airflow or dry aerated burial conditions as they allow rapid drying of the soft tissues limiting putrefaction by destructive micro-organisms. Within a stable environment a desiccated body can remain preserved for years [14, 15].

The second form of preservation is referred to as adipocere. In moist environments, inhibition of decomposition can occur through the formation of adipocere [15]. Adipocere is a greyish white wax-like substance composed of fatty acids [16, 17]. During the early stages of decomposition, adipocere is typically wet whereas in the later stages it is characterised as being more solid, dry and resistant [18]. The major constituents of adipocere are saturated free fatty acids containing an even number of carbon atoms. This includes palmitic and stearic acids and their 10-hydroxy counterparts [17]. Adipocere is formed by the hydrolysis and hydrogenation of adipose fat tissue in the body [14, 16]. The adipocere formation begins in the neutral fat and is initiated by intrinsic lipases which degrade triglycerides into fatty acids [19]. The cell membrane is a lipid bilayer; its main function is to maintain equilibrium between the cell and its external environment. In the presence of water the cell will absorb water until it raptures, causing the release of the cells fatty acid content. These fatty acids are then hydrolysed and hydrogenated [14, 19], by degenerative anaerobes such as *Clostridium perfringens* [19]. The bacteria will convert the neutral fat into hydroxyl fatty acids. This process causes cell destruction and ammonia rich waste that promotes the formation of an alkaline environment. This change in the environment will inhibit bacterial growth which arrest putrefaction [19]. Over time the formation of adipocere

results in a hard, brittle substance that retards decomposition [17]. Many mammals such as human, pig, cow and sheep contain sufficient fat and moisture content to form adipocere in a moist coarser-textured soil [6].

1.2 Burial Environments

The location of remains greatly impacts the decomposition process. Whether the remains are deposited on the surface or buried changes the immediate surrounding environment and can alter access from insects and other scavengers. Temperature above the ground is found to be generally higher than below the surface [13, 20]. Burial environments tend to undergo decomposition over a longer period of time due to this temperature difference as well as due to protection from scavenging, restricting access of carrion such as blow flies (*Calliphoridae),* flesh flies (*Sacrophagidae)* and beetles (*Coleoptera)* [6] and general weathering activities [13]. This has been recorded in several studies where it was found that a shallow burial results in faster decay than that of deeper burial [10, 21].

Several factors are found to influence decomposition within a burial environment; these include the depth of the burial site, presences and type of clothing, physical condition of the body, absence or presence of a coffin and pH, texture, oxygen content and moisture of the soil, and finally the temperature.

1.2.1 Method of Burial

A study done by the Federal Bureau of Investigation (FBI) found that the most common burial depth for clandestine graves was found to be 0.46 to 0.75 m [22]. This was supported by Mitchell et al. [23] who stated that based on cases in the United Kingdom (UK) the average depth of burial was found to be 0.5 m. Hoffman et al. [22] also found that during the 10 year period from 1993 to 2003 it was found that most burial sites were located a short distance off of infrequently travelled roads, with burial sites close to bushes or heavy foliage [22].

1.2.2 Soil Conditions

Soil moisture can influence the decomposition process [24]. This is mostly due to the impact of moisture on the effect of the microorganisms involved in the

decomposition process. Moisture content of soil is closely related to the soil texture and the soils ability to retain water.

Another factor that will greatly influence the decomposition process is the soil texture. Course-texture soils, also known as sandy soil with low moisture content will often promote dessication [6, 18]. A course textured soil has a high rate of gas diffusion that allows gas and moisture to move through the soil matrix rapidly. Any moisture present in the soil will quickly run through the matrix, resulting in the nutrient cycling being slowed down due to limiting moisture content and will again promote dessication [6]. Fine-textured (clayey) soil is associated with an inhibition of the decomposition process. These soils have very low gas diffusion rates [6, 25] and this results in wet soil that will cause the decomposition process to slow down. A wet clayey soil may inhibit oxygen and CO_2 exchange rate and thereby cause anaerobic microorganisms to dominate the decomposition process. These microorganisms are less efficient than aerobes [6].

The most crucial factor pertaining to the soil conditions that will affect the decomposition process is the soil pH. There are conflicting views on how soil pH affects decomposition, specifically during adipocere formation. According to Forbes et al. [26] the ideal pH range for adipocere formation is around 5-9, whereas a highly acidic soil was found to limit adipocere formation. A low pH (2.6 – 4) appears to be the ideal soil conditions for dessication [14]. It has been speculated that a highly acidic soils may result in a retardation of the decomposition process [27] due to the fact that in such soil, plants will produce a great number of tannins. Tannins can combine with the carbohydrates and proteins in organic matter, that again results in decreased microbial activity [27]. The decomposition process which leads to leaching of decomposition fluid can have a significant effect on the soil pH. Surface decomposition has been linked with highly alkaline soil, whereas burials are believed to yield a more acidic soil [28].

1.3 Textiles

Textile damage has been the subject of many forensic and archaeological investigations. The recovery of textiles and associated death materials on buried bodies is essential in both an archaeological and forensic sense. The fibres used to produce these clothing items vary in composition. Their origin can be either natural or synthetic or a mixture of the two. Traditionally, natural plant and animal based fibres such as

wool and cotton have been used, but the development of synthetic fibres during the 20th century resulted in a modification of fibre types in use.

1.3.1 Natural Textiles

Cellulosic fibres, such as cotton and linen, are derived from plants. Natural textiles are commonly worn as clothing due to their hygienic properties [29]. Natural plant fibres used as clothing includes hemp, cotton, linen and jute. Included under this classification are also the animal fibres such as wool and silk.

Protein-based fibres from animal sources include wool and silk. Wool was the dominant fibre used for clothing until the emergence of the cotton industry in the 19th century [30-32]. Cotton is the most widely used natural textile and has been in use since antiquity. It is used in its pure form as well as in synthetic blends [30, 31]. It was for this reason that cotton was selected in this study to represent a commonly encountered natural material. Cotton fibre is obtained from the seed hair of the cotton seed and is predominantly comprised of cellulose (90 – 99 %), a polysaccharide made up of glucose residues (the structure is illustrated in Figure 1-2) [33]. The structure of the fibre consists of a thin but dense outer shell, referred to as the primary wall. The primary wall is composed of cellulose with small amounts of waxes and vegetable oil. This primary wall surrounds many concentric cellulose layers, the second wall; it encloses a hollow core, the lumen. Morphologically cotton cellulose is highly crystalline.

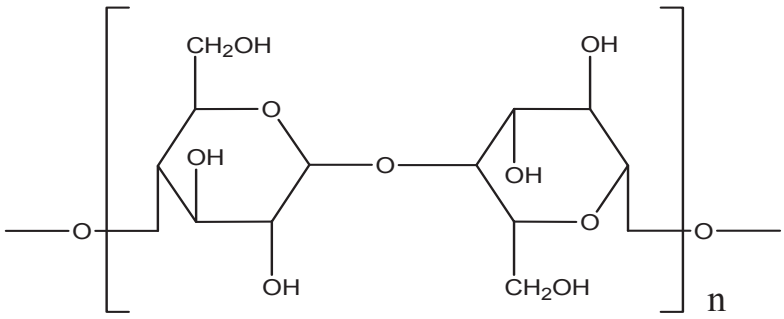

Figure 1-2: The structure of cotton

1.3.2 Synthetic Textiles

Four types of synthetic fibres dominate in textile production – polyester, nylon, acrylic and polyolefin [34, 35]. Polyester accounts for about 60 % of synthetic fibre production and Terylene and Dacron are common trade names. Polyester was therefore

chosen as the most appropriate material used to investigate synthetic textiles. Poly(ethylene terephthalate) (PET) is the major form and finds use in clothing and furnishings. Polyester is used on its own or in cotton mixtures for textile manufacture, and has advantageous mechanical properties, minimal shrinkage and good colour retention. Polyesters are compounds of high molecular weight with repeated ester bonds in the main chain. The properties of PET depend on the degree of crystallinity and the modifying agents present. PET consist of polymerised units of the monomer ethylene terephtalene, it has repeating $C_{10}H_8O_4$ units (Figure 1-3).

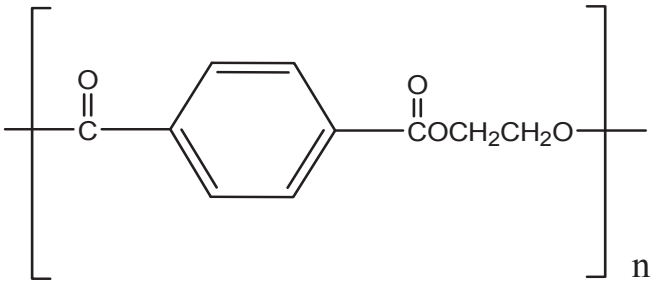

Figure 1-3: Polyester (PET) structure

1.4 Textile Degradation Methods

In addition to the activity of insects and scavenging animals, there is a number of environmental factors that are critical to the degradation or preservation of textiles. These include the presence of microorganisms, moisture content, temperature, pH, electrochemistry and surface treatments [13, 31, 36, 37]. How these factors affect textiles is dependent on the structural nature of the fibres that make up materials as the biodegradation or chemical degradation mechanism will depend on the fundamental chemistry.

Many factors define the fabric composition, including thread thickness and linear density, these have been found to affect rate of deterioration [29]. However the most crucial factors are the composition and the origination of the textile raw materials [29].

1.4.1 Mechanical Degradation

Mechanical degradation classifies damage due to wear and tear, abrasions, punctures, cuts and insect activity.

1.4.2 Light Degradation

In the presence of light, especially ultraviolet (UV) light textile degradation is initiated or enhanced. This is referred to as light degradation or photodegradation. Dyes, both natural and synthetic, are very highly susceptible to light degradation. UV light causes oxidation and the breaking of intermolecular bonds in the fibres and helps facilitate the penetration of enzymes [38] and causes reduced tensile strength [39]. Infrared (IR) spectroscopy has been used to establish that both hydroxyl- and carboxylic end-groups along with carbon monoxide and carbon dioxide as main products of photodegradation in polyester samples [40]. Light degradation causes fading of colour and will cause the material to become more brittle.

1.4.3 Chemical Degradation

Chemical degradation includes damage caused by chemicals, heat and natural aging. Cellulose based fibres are one group of textiles that are highly sensitive to attack by acids [31]. In an acidic environment the glycosidic link will be broken through hydrolysis. This results in a shortened cellulose chain which again results in a structural weakening of the fibre [31]. Cellulose may also oxidize where the hydroxyl groups are to form oxycellulose. The oxycellulose formation disrupts the hydrogen bonding leading to a reduction in fibre strength. Oxycellulose formation will also expose the fibre to further degradation.

1.4.4 Biodegradation

Another degradation process classified as chemical is biodegradation. Biodegradation is a major source of deterioration of textiles in soil environments. Microorganisms such as fungi and bacteria present in the soil, water and air produce enzymes that are capable of attacking the molecular structure of the textile fibres [41, 42]. Biodegradation can lead to changes in colour or staining of the textile, unpleasant odours and changes in the mechanical properties of the textile such as tensile strength and elasticity [29, 41]. Microorganisms can degrade textiles both mechanically and chemically [43]. Mechanically they break down the textile by penetrating, branching out and growing within the fibres. Chemically the microorganisms will secrete enzymes that break down the insoluble materials using hydrolysis.

The availability of oxygen in the burial environment determines the nature of the biological agents that are present and, therefore, determines the type of biodegradation. Certain biodegradation mechanisms require aerobic conditions, while other microorganisms thrive in anaerobic conditions. Oxygen is required for fungal metabolism so it will be anaerobic bacteria that attack textile materials in anaerobic environments. As bacteria require moisture to flourish, the amount of water in the burial environment will also play a role in determining the rate and type of degradation. Waterlogged soils have little available oxygen and will therefore be limited to a certain type of bacteria; similarly a very dry environment will reduce the bacterial populations and inhibit biodegradation. Microorganisms are also sensitive to temperature and pH, where extremes on either end will inhibit degradation.

The degree of biodegradation of the textiles also depends on the chemical and physical properties of the fibres [41]. The properties include chemical structure, molecular mass, and degree of polymerisation and orientation, crystallinity, hydrophobicity and carbon availability.

Natural textiles are more easily and rapidly affected by degradation from microorganisms than synthetic fibres due to the high presence of cellulose [29, 41]. Initially the microorganisms will gather the degradable sugars; starch, hemicellulose, pectin, wax and the other non-cellulosic materials [41]. After this the degradation of cellulose occurs. Enzymatic processes are catalysed by cellulase enzymes and result in the destruction of the linkages between the glucose components [43]. Cotton is very absorbent and swells in a wet or moist environment. The cellulose component is readily broken down by microbial agents and the presence of water is important for these processes. Cotton is highly vulnerable to hydrolysis in acidic environments. Cotton is degraded through three types of hydrolytic enzymes that releases glucose from the cellulose [29]. Exoglucanase, is the first one, it will cleave disaccharide residues in a step-wise manner. The second enzyme is called endoglucanase (formerly cellobiase); this enzyme cleaves cellulo-oligosaccharides in a random matter. The third enzyme, β-D--glucosidase will hydrolyse cellobiose and lower oligosaccharides to glucose via the breakdown of the glycosidic link [29, 31]. Before this hydrolytic breakdown of the cellulose can occur, the waxy outer cuticle has to be destroyed by other enzymes to expose the cellulose [29, 43].

Bacteria will attack the natural textiles and degrade the material from the surface towards the inside. Fungi on the other hand will penetrate the secondary wall and start

growing in the lumen. When cotton textiles are subjected to degradation by fungi, the germinating hyphae from the fungi spores inside the lumen will grow and form mycelium [29]. The mycelium will secrete cellulolytic enzymes that are responsible for the destruction of the textile.

Synthetic textiles demonstrate a considerably higher resistance to the degradation by microorganisms [31, 41, 43, 44]. This is due to the presence of chemicals that are not occurring normally in nature. However, some classes of synthetic fibres are subject to degradation due to the presence of functional groups that are vulnerable to oxidation and enzymatic attack.

Polyester fibres show a very good resistance to biological and chemical factors, but if certain finishes are used in production fungal growth may occur on the surface and result in discolouration. Should the polyester be subjected to biodegradation, the process is initiated through the hydrolysis of the ester bonds and this is achieved via an enzyme such as cutinase or lipase [41]. This degradation is very slow as the stereochemical hindrance of the ester bonds by the benzene rings prevents these enzymes from attacking the polymer chain [41].

The rate of degradation of polymer blends is initially controlled by the degradation properties and rate of the more readily biodegradable component [45].

Overall the chemical changes occurring with the growth of microorganisms result in decreased fabric strength and lead to partial or total destruction of the material [29, 45]. The study of microbiological degradation is mostly carried out by identifying the microorganisms involved and study their properties [43].

1.5 Decomposition and Textile Degradation

In addition to damage and irregularities in the raw material and from manufacturing, and damage accrued as the result of wear and tear, archaeological textiles suffer from burial-induced damage and contamination [43].

In criminal cases the clothing will often be intact, but may be disturbed by taphonomic processes [31], this is especially true if the body is recovered over a longer post mortem interval. With most forensic cases with shorter burial intervals the textiles will be subjected to differential decay depending on the type of textile [31].

A survey conducted by Manhein [46] revealed that out of 87 cases, 54 of the bodies were wrapped in some type of fabric prior to burial. Plastic was one of the most

common coverings; however sleeping bags, rugs, blankets and clothing were also observed [46]. Decomposing bodies are frequently found to be at least partially clothed [22, 47]. Covering on the body such as clothing has been found on both surface deposits and in graves, these coverings produce a variety of effects and will influence the decomposition process [48].

The presence of clothing will have a significant influence on the decomposition of a cadaver [21, 49-52]. The burial environment itself will again alter the decomposition rates of the textiles [31, 53].

1.5.1 Decomposition of Clothed Remains

Several studies have been conducted in order to investigate the effect of clothing on decomposition rate [10, 21, 54]. Clothing may slow down the rate of cooling of the body and provide an environment that is warmer and more humid and so is favourable for insects. Clothing may also protect insects from predators and environmental factors such as sunlight and rain [10, 21, 55]. However, sometimes clothing acts as a barrier for colonising insects and so inhibits decomposition [55, 56]. Adipocere formation may also be enhanced by the presence of clothing depending on the material's ability to retain moisture. The formation of adipocere has been observed to be more likely in dressed bodies [49, 54].

The nature of the material used for body coverings plays an important role in the decomposition process. As synthetic fibres are significantly more resistant to degradation in a burial environment, they provide protection of the body for a longer period of time and, therefore, change the rate of body decomposition. Adipocere formation has been shown to be dependent on the type of material present. For example, the formation of adipocere is observed to be enhanced by the presence of polyester clothing, most likely due to the ability to retain moisture as well as its resistance to biodeterioration [49].

A study by Voss et al. [52] looked at decomposition of three clothed and two unclothed pig carcasses (*Sus scrofa*) during surface decomposition, they found that the progression of decomposition differed between the two groups. Specifically they observed a change in the duration of the wet decay stage, with clothed remains spending as much as 6 days longer in this stage. The increased time spent in the wet decay stage lead to an increase in maggot masses and it also lead to a second wave of insect colonisation in the clothed cadavers.

Mant [54] conducted a large number of exhumations (over 150) in Germany after World War II. He found that many of the exhumed bodies were clothed. It was determined that in the context of burials clothing had a profound effect on the post-mortem changes to the decomposed body. The clothed parts of the body demonstrated great preservation even after periods of two years [54].

1.5.2 Soil and Textile Degradation

Degradation of natural textiles will occur over a relatively short period under the majority of soil conditions. If the body is preserved, such as during dessication, this will retard microbial action and thus slow down the degradation of the textile [31]. In waterlogged soils with low to no oxygen content, aerobic fungi will be eliminated; however anaerobic bacteria may still flourish. In acidic soils wool, animal hair and silk are favoured and will not degrade rapidly, whilst cellulosic materials will degrade rapidly in this environment [31]. Degradation will occur at sites where the textiles are in direct contact with the microorganism [29] such as they will be in a soil environment or in the presence of a decomposing body, where microorganisms generally thrive. Untreated cotton textiles will generally show signs of decay by 60 days in a biologically active soil [31].

1.5.3 Decomposition Fluid and Textile Degradation

During the natural decomposition process lipids, proteins and carbohydrates are broken down. Lipids are the major component of adipose tissue, the main types are triglycerides. Lipids will undergo hydrolysis by intrinsic tissue to produce a mixture of fatty acids. In an aerobic environment, unsaturated fatty acids undergo oxidation due to bacteria, fungi or atmospheric O_2, resulting in the formation of ketones and aldehydes. In an anaerobic environment unsaturated fatty acids are transformed into saturated fatty acids. The degradation of proteins is referred to as proteolysis and results in the formation of amino acids and biogenic amines. Overall the biodegradation pathways of these lipids, proteins and carbohydrate suggest that fatty acids, triglycerides, amino acids and biogenic amines would be present in decomposition fluid [57, 58]. In addition to these products the decomposition fluid will also produce water and carbon dioxide and volatile substances along with gas formation which causes the release of nitrogen, methane, hydrogen sulfide and ammonia to mention a few [55]. As all the organic and

inorganic components resulting from the decomposition of the soft tissue and bone will migrate into the surrounding soil environment, ending up in the water table or are liberated as gases. Trace elements in the surroundings can yield important information, especially in the event that the body has been moved after the initial deposition site.

1.5.4 Effects of the Decomposition Process on the Degradation of Textiles

Little work has been completed in order to analyse the way the decomposition affects the degradation of the textiles themselves, rather than how clothing affects decomposition.

Rodriguez and Bass [10] studied decomposition rates of buried human cadavers as well as cadavers deposited on the surface. Some of the cadavers were clothed, however, the state of the textiles was only mentioned briefly and no means of examining them other than visually with the naked eye were completed.

Spennemann and Franke [20] found that a body found after a burial time of 58 months had socks that were partially disintegrated around the hems, but were otherwise consisted of synthetic material that was found to be strong and fully intact.

Ongoing experiments from Janaway et al. [13, 31, 36] have indicated that the presence of a body will influence the textile degradation. He found that cotton only survived when placed underneath an actively decomposing pig. Due to its location the cotton was covered in semi-liquid soft tissue. During a case study by Janaway [31] conducted near Kasr-el-Yahud in Jerusalem, where an ancient mass grave excavation occurred, they found clothed skeletal remains. They observed that the textiles covering the limbs were best preserved, whereas the area around and below the body cavity were in the worst condition. They also found clothing with small circular holes (2 – 6 mm in diameter). These holes were later attributed to damage done by fly larvae when they were attacking the flesh of the body.

Lowe et al. [59] conducted an experiment where 45 clothed pig carcasses were buried, in addition control graves containing the clothing only were created. She found that the natural textiles in the control graves (which did not include a pig carcass) showed significant degradation whereas the clothing on the carcasses showed little sign of degradation.

These findings may be indicative of the fact that the decomposition process and the subsequent release of decomposition fluid products may have leached into the textile during soft tissue degradation, and may result in an inhibition of the textile

deterioration. This is also evident from case work where cotton, a textile that has found to degrade rapidly in soil [60], has been found many years after burial [31].

Differential degradation of the textiles associated with the remains may help give insight into the decomposition process and even post-mortem interval, even when the remains are in the skeletal stage. The use of textiles degradation as an indicator of post-mortem interval may also be useful due to the fact that the use of the decomposition stages remains a very subjective science.

1.6 Instrumentation

1.6.1 Fourier Transform Infrared (FTIR) Spectroscopy

Fourier transform infrared (FTIR) spectroscopy is a commonly used method for the analysis of textile degradation by monitoring the different functional groups in the material [38, 61, 62]. Attenuated total reflectance (ATR)-FTIR spectroscopy has the advantage that it can use a small sample size, it requires little preparation, it is fast and reproducible and, most importantly, it is a non-destructive technique. Since ATR spectroscopy functions by penetrating anywhere from a few to hundreds of micrometres into the material, it is thus appropriate for the identification of structural changes on the surface of objects [38].

In this thesis the use of ATR-FTIR spectroscopy is focused on the degree of deterioration of the textile. However, its role in the analysis of lipids and proteins will provide further insight into the changes in decomposition fluid profile that remains on the textile after removal from the carcass. ATR-FTIR spectroscopy has been previously used in order to analyse tissue samples [63, 64], as well as triglycerides that had been deposited onto cotton fabric [65]. This technique is highly beneficial as it relies on the absorbance of molecular vibrations in any given sample, and thus eliminating the need for dyes or labelling of the molecules for visualisation [66]. The non-destructive properties of ATR-FTIR spectroscopy are one of the reasons why it is a beneficial tool in the analysis of textile degradation and subsequent analysis of the associated lipid profiles.

1.6.2 Gas Chromatography – Mass Spectroscopy

Although FTIR has successfully been used to provide profiles of decomposition fluid, it provides qualitative data only; therefore additional analysis using a chromatographic method was also investigated. Gas chromatography (GC) is used to separate a sample into its substituent compounds. The volatile liquid or gaseous sample is introduced through a heated sampling port and the vapour is carried through a thin capillary column which is coated with a viscous liquid or polymer stationary phase using a carrier gas [67]. The analytes are separated due to their difference in interaction with the stationary phase casing them to elute and reach the detector at different times. Several different detectors can be used to analyse the compounds separated by GC such as Flame ionization detector (FID), thermal energy analyser (TEA), electron capture detector (ECD) to name a few. Mass spectroscopy (MS) is another possible detector that has proven to provide valuable information when coupled to GC [67].

GC-MS has proven to be highly successful in the investigation of decomposition fluid in tissue, soil and the fluid itself [58, 68, 69]. It is therefore a suitable method to examine the decomposition fluid and the changes in fluid composition over time. Observed changes will be correlated with the preservation of the textile samples and can indicate the preserving part of the liquid. Due to GC-MS being a more sensitive and accurate technique for the quantitative analysis of decomposition fluid on the textile samples, compared to ATR-FTIR spectroscopy, and will thus increase the validity of the decomposition fluid profiles.

1.7 Chemometrics

Several methods for the evaluation and analysis of data resulting from FTIR data have been employed. Previous studies have looked at the physical comparison of the spectra where the spectra of an earlier sampling day will be directly superimposed over the spectra of a later sampling day [70]. As spectral data is very complex in nature and encompasses a lot of information, chemometrics is particularly useful compared to a simple visual comparison of the spectra. Chemometrics involves the use of mathematical and statistical means to extract data from chemical systems. FTIR analysis often result in data with a large number of variables, thus methods have been developed in order to try to reduce the number of variables, but still retaining the original information. Principal component analysis (PCA) can be used for that purpose.

PCA is used in order to convert a set of possible correlated variables into a set of uncorrelated variables called principal components. The first principal component (PC-1 or Factor 1) will explain the largest variance in the data, followed by the second principal component (PC-2 or Factor 2), the third (PC-3 or Factor 3), and so on. PCA is often used in exploratory data analysis in order to further explore the data rather than confirmatory data analysis.

Principal component analysis has proven itself as a successful tool for the analysis of FTIR data in previous studies[71], and is also frequently used in decomposition research [72-74].

1.8 Objectives

The main aim of this research was to investigate the effect of the presence of decomposing remains on textile degradation patterns.

During this study the following objectives were addressed:

1. To examine the visual degradation patterns of both decomposing remains and their associated textiles under different environmental conditions.
2. To analyse the natural decomposition pattern of both natural and synthetic materials in an outdoor environment.
3. To investigate the composition and change over time of the decomposition fluid absorbed into textile samples using ATR-FTIR spectroscopy.
4. To investigate the lipid components of decomposition fluid from textile samples during different seasons using gas chromatography-mass spectrometry.
5. To examine the textile degradation patterns in a burial situation with and without the presence of decomposing remains.

Through these objectives it will be possible to investigate whether the presence of a body will alter the textile degradation method and timeline. The inclusion of two major outlining textile groups; natural and synthetic provides additional information about the influence of the nature of the textile samples. Looking at specific structural changes in the textile samples will aid in locating the target regions for degradation, and will further demonstrate the influence of the introduction of decomposition fluid.

The addition of the examination of the buried remains is valuable in the development of the post mortem interval, especially in cases where the body has gone through freeze-thaw cycles and the general appearance of the body may be very misleading in terms of post burial interval. Other instances where the body is completely skeletonized, the PMI of skeletal remains is often given with a large interval, and can rarely be determined within years of the when the death actually occurred. Both of these issues can possibly be excluded if one is able to develop a timeline based solely on the condition of the textiles.

Chapter 2: MATERIALS AND METHODS

Chapter 2: MATERIALS AND METHODS

In order to document and follow the textile degradation over time in a natural Australian environment several field studies were conducted. The current chapter outlines the details the sampling procedures and sampling frequency of each field trial.

2.1 Field Site Location

The field site used for this study was located in an open eucalypt woodland on the Cumberland Plain in Western Sydney. The soils in this area contain sandy clay topsoil to a depth of approximately 0.70 – 1.00 m. Shale clays represent the next horizon to a depth of approximately 1.50 – 1.80 m. Beyond this is sandstone bedrock (yellow and grey). The topsoil at the study location is acidic and typically ranges between pH 4 – 5.

A vegetation survey was also completed in order to obtain a general idea of the vegetation landscape that could influence the decomposition process. Several dominant tree species were determined; these included grey gum *(Eucalyptus punctata),* forest red gum (*Eucalyptus tereticornis),* broad-leaved ironbark *(Eucalyptus fibrosa ssp. fibrosa)* and thin-leaved stringybark *(Eucalyptus eugenoides).*The understory at the field site location is dominated by the invasive shrub lantana (*Lantana camara*). The ground layer is a mix of native and exotic graminoids and forbs, with an abundant presence of kangaroo grass (*Themeda australis*) and large-leaved rush (*Lomandra longifolia)* (Figure 2-1).

Figure 2-1: University of Technology Sydney research facility in Yarramundi, NSW.

2.1.1 Surface Depositions

Surface trials (two summer trials and one winter trial) were created. Here, three domestic pigs (*Sus scrofa domesticus*) carcasses per trial were used, these pigs were all clothed in a 100 % cotton t-shirt (alpha ♂, KMart, Australia), 100 % polyester briefs (alpha ♂, Kmart, Australia) and polyester – cotton blend socks (Tiny Little Wonders, Kmart, Australia). Prior to placing the pigs the area was cleared, thus removing the vegetation and ensuring direct contact with the soil below. The pigs were placed on the soil surface, and caged to avoid scavenging by large animals, while still allowing insect access (Figure 2-2). Cages were created using an aluminium frame covered with 1 cm diameter mesh. The length of the cages was 130 cm, and width and height of 90 and 60 cm, respectively. Separate to the cages, two long aluminium sheets (136×30 cm) and two short aluminium sheets (92×30 cm) were trenched into the soil around the cage for further protection from burrowing animals.

Three control sites were created, which consisted of the identical clothing to those on the pig carcasses placed directly on the soil surface, with the area again cleared prior to deposition of the clothing. Chicken wire was placed on top of the clothing to protect them from animal activity. In order to ensure that no contamination from one

site to another occurred, the experimental or pig sites in all surface trials had a minimum distance of two meters between them. The control sites were located a minimum of three meters from the experimental sites in each of the three trials. After trial one all subsequent trials were placed in a newly cleared area over five meters from previously used sites in order to make sure that no changes observed was due to contamination in soil from the previous trials.

Figure 2-2: Surface set-up with cage used to prevent scavenging from larger animals whilst still allowing insect access through the wire mesh.

All pigs used for the research project were euthanised with a head bolt by professionals and buried within hours after death. Pig carcasses were obtained from a licenced abattoir in the nearby area (Hawkesbury Valley Meat Processors, Wilberforce, NSW, Australia). Pig carcasses were used as they are widely accepted as analogues for human decomposition studies due to their similarity in internal anatomy, fat distribution, size of chest cavity, skin, gut fauna, and lack of heavy fur [75, 76]. All three pigs used for Trial 1 (T1) were found to weigh between 75 – 80 kg. For the second trial (T2) the pigs obtained from the abattoir were smaller in size weighing around 50 kg, this was due to the available pigs at the time. For Trial 3 (T3), pig A was approximately 60 kg, pig B 50 kg and Pig C was the largest with a weight around 65 kg.

For all surface trails three 5×5 cm sections of each clothing type were collected from each of the pig carcasses and control sites using sterilised scissors on each sampling day. The scissors were washed with acetone between each replicate and between each site. Care was taken to make sure the body was disturbed as little as possible as the pigs were sampled multiple times throughout each trial. The textiles were photographed on each sampling day and any visible changes were noted (see Appendix A-D) before the textile samples were collected and packaged into a small paper envelope, placed into individually labelled paper bags and stored in a cooler for transportation to the laboratory. Paper was selected as the packaging material over plastic in order to prevent the textile samples from degrading during transport and storage.

To impede bacterial and fungal growth, the textile samples were air-dried under ambient temperature in the laboratory and any adhering tissue, soil, or hair was removed after drying. The textile cut-out were taken out of the coin envelopes and placed flat down on clearly marked pieces of paper for drying. The textiles were then packed in new individually labelled paper bags and stored at -18 °C until further analysis.

The trial sampling schedules, duration and starting months can be found in table 2-2. The sampling days were frequent initially for all trials in order to obtain samples throughout the initial stages of decomposition. As the decomposition stages slowed down and became more prolonged, the sampling frequency was reduced.

Table 2-1: Overview of the starting time and season as well as sampling days for each surface trial.

Trial name	Date	Starting season	Sampling days post-mortem
Trial 1 (T1)	Jan 2013 – Jan 2014	Summer	0, 2, 4, 6, 8, 10, 14, 17, 21, 24, 31, 48, 59, 94, 149, 184, 212, 268, 365
Trial 2 (T2)	July 2014 – Jan 2015	Winter	0, 3, 6, 9, 13, 20, 37, 51, 65, 79, 100, 140, 210, 269, 325, 381, 443, 499, 565
Trial 3 (T3)	Jan 2014 – Jan 2015	Summer	0, 3, 6, 10, 17, 24, 31, 45, 73, 100, 129, 185, 247, 303, 369

2.1.2 Burial

Field experiments in the burial trial consist of burying and subsequently exhuming clothed domestic pig (*Sus scrofa domestica*) carcasses over a 24 month period. A total of seven pigs were buried in January 2013. The carcasses were exhumed after 1, 3, 6, 9, 12, 18 and 24 months. In addition seven control graves were used; these graves contained the textiles only without the presence of a body. Identical clothing to the surface depositions was used in the burial scenario in order to be able to compare the deposition situations to each other.

The pigs were placed in approximately 50 cm deep graves (Figure 2-3). The t-shirt, socks and shorts were collected as a whole from the exhumed pig carcasses and their respective control graves. All clothing samples were brought back to the laboratory and processed and stored identically to the surface samples. Due to the larger nature of the materials collected, these were air-dried by hanging the items vertically inside a fume cupboard.

Figure 2-3: Fresh pig remains clothed in a white 100 % cotton t-shirt, 100 % polyester briefs and two polyester – cotton blend socks in the grave prior to backfilling.

2.2 Environmental Data

A HOBO® No Remote Communication weather station base with sensors (OneTemp) was set-up in the field close to the decomposing carcasses (Figure 2-4) in order to monitor ambient temperature (°C), rainfall (mm), relative humidity (%), solar radiation (W/m^2), wind direction (Ø), wind speed (m/s) and gust speed (m/s). Soil volumetric water content (VWC) and pH were collected using a moisture sensor with a LabQuest 2 interface (Scientrific Pty Ltd), and a direct soil pH measurement kit (Hanna Instruments Pty Ltd), respectively. The moisture probe and the pH probe were both directly inserted into the soil at an approximate 10 cm distance from the abdomen of each pig carcass, and in a similar position within each control site, giving a total of three experimental and three control moisture and pH values for each sampling day.

Accumulated degree days (ADD) were used to monitor the duration of the decomposition stages during each trial. ADD is used to account for any differences in the rate of decomposition based on temperature. It was calculated by taking the sum of the average daily temperatures each day, using the average hourly measurement obtained from the weather station per day.

On the 16th of October, 2013, there was a large forest fire in the area close to the field site location; in order to protect the equipment should the fire reach the site, the weather station was removed on this day. The weather station was unavailable until the 5th of December, 2013. The weather station also had some instrument issues in the following year leading to another section of missing data. During these periods the environmental data was collected from the Royal Australian Air Force (RAAF) weather station, which was the closest weather station, located approximately 15 km from the research facility. A correction was applied to the data where possible. First, the average difference between field weather data and RAAF weather data prior to the data loss was determined, and then this correction was applied to the data recorded when the HOBO weather station was out of commission.

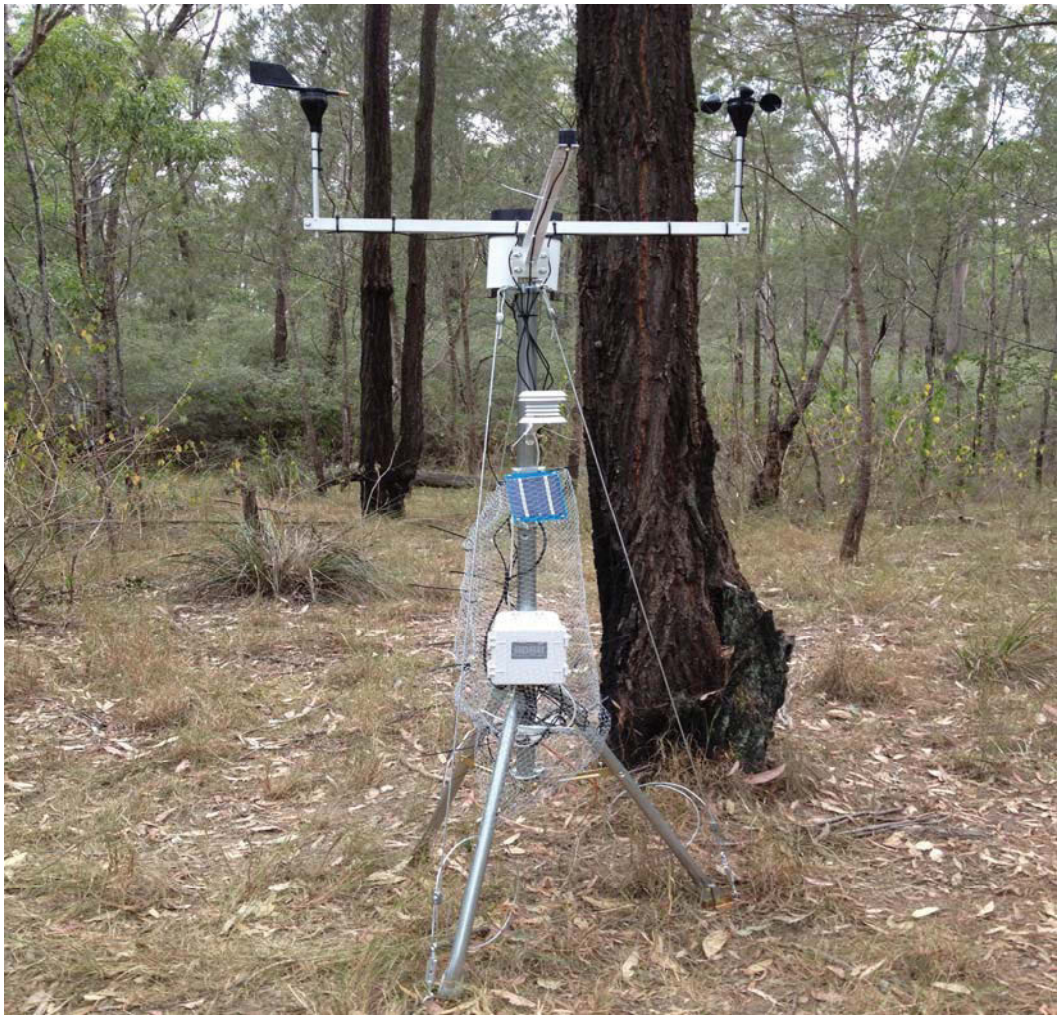

Figure 2-4: Weather station set-up, recording rainfall, temperature, relative humidity, wind speed and direction and solar radiation.

2.3 Fourier Transform Infrared (FTIR) Spectroscopy

2.3.1 Sample Preparation

Three 2×2 cm cut-outs were taken from the swatches collected in-field from the three different materials. The textile previously air-dried textile cut-outs were analysed by placing them directly onto an ATR sampling accessory. The ATR-FTIR spectra were obtained using a Nicolet Magna-IR 6700 spectrometer (Thermo Scientific, USA) using a liquid nitrogen cooled mercury-cadmium-telluride (MCT) detector and ATR accessory consisting of a germanium crystal with a 45° angle of incidence. The instrumental set-up can be seen in Figure 2-5. Spectra were recorded over a range of 4000 – 400 cm^{-1}, with a spectral resolution of 4 cm^{-1} and averaged over 128 scans. OMNIC software (Version 8.1.11, Thermo Scientific, USA) was used to record and

baseline-correct the spectra. Despite some of the textile swatches being covered in decomposition fluid, no issues were encountered during the analysis using this technique. The crystal was cleaned well between each textile cut-out.

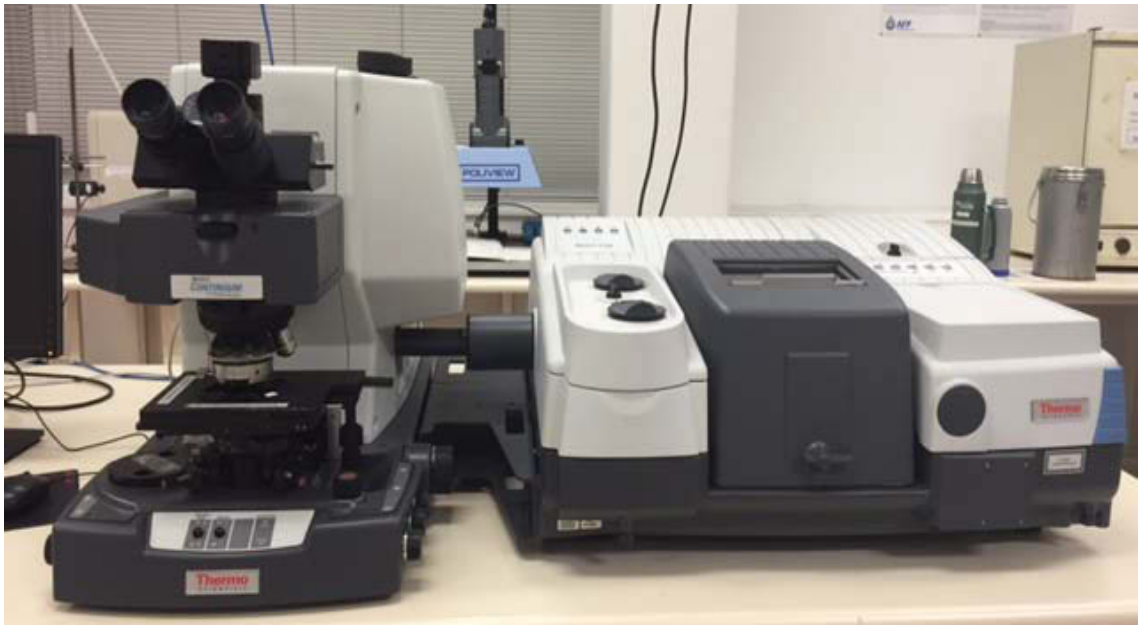

Figure 2-5: Nicolet Magna-IR 6700 spectrometer (Thermo Scientific, USA) with liquid nitrogen cooled mercury-cadmium-telluride (MCT) detector and ATR accessory.

2.3.2 Data Processing

Investigation of the spectra by visually comparing the earlier days to later sampling days was the first step in the data processing; this was done with the aim to discover certain areas or peaks of interest. The ATR-FTIR spectroscopy results were then analysed using Microsoft Excel 2010, SigmaPlot (version 11.0, Systat Software Inc., USA) and Past (version 3.14, Hammer and Harper, Norway). A one-way analysis of variance (ANOVA) statistical test was used to compare the absorbance ratios of selected FTIR bands with a significance level of 0.05. In order to conduct statistical analysis, the bands of interest were normalised to the most intense band [77], to avoid any instrument variability between the analysis days. The 985 and 1001 cm^{-1} bands were normalized using the 1033 cm^{-1} band in the cotton samples. The 1033 cm^{-1} band was selected as it is always present in the samples at a very significant absorbance level. The 1090 cm^{-1} and 1245 cm^{-1} polyester bands were both normalised using the 1715 cm^{-1} band for the initial statistical analysis.

ATR-FTIR spectral data was also directly imported into The Unscrambler® X (version 10.3, CAMO, Norway) statistical software for further analysis. The baseline

corrected spectra were initially smoothed using the Savitzky-Golay smoothing 2nd derivative [78]. The Savitzky-Golay alogorithm is useful for removing noise in the spectral data and can sharpen spectral features while still maintaining the chemical information. The Savitzky-Golay algorithm is based on performing a least squares linear regression fit of a polynomial around each point in the spectrum to smooth the data. After the second derivative was taken the selected region of the full spectra was marked. For cotton samples this region was taken from wavenumber 950 to 1200 cm^{-1}, whereas the polyester regions collected 1800-1600 cm^{-1} and 1300-950 cm^{-1}, in order to encompass the regions where degradation was predicted to occur. Initial trials using the full spectral region for the cotton and polyester samples also confirmed that changes between the spectra over time would result from these regions. A multiplicative scatter correction was then performed in order to compensate for additive effects in the spectral data. Finally PCA was conducted in order to identify and visualise any groupings or separations in the data sets.

2.4 Gas Chromatography – Mass Spectroscopy

2.4.1 Fatty acid methyl ester derivitisation

Fatty acids are reactive and polar substances, which are not suitable for direct GC-MS analysis. The poor volatility of fatty acids makes gas chromatography analysis particularly difficult and the samples were therefore derivatised. Two methods were investigated; the first method has been used successfully in previous work by the author and involves the esterification of the fatty acids into their respective ester derivatives, esters have the preferred properties of being volatile, non-polar and easy to separate. However, this previous work dealt with pure tissue samples so some modifications were needed in order to use the method on textile samples.

2.4.1.1 Sample Preparation

The method used to prepare the samples for GC-MS analysis was a modified version of the direct FAME synthesis published by O'Fallon et al. [79]. Herein, three 3×3 cm t-shirt sections (different from those used for the ATR-FTIR analysis described in section 2.3.1) were prepared from the original 5×5 cm cotton samples. The textile sections were placed in individually labelled scintillation vials (Gerresheimer

Shuangfeng Pharmaceutical Packaging (Zhenjiang) Co. Ltd., China) with 8 mL of HPLC grade chloroform (Burdick & Jackson, USA). The samples were sonicated for 30 min, vortexed for 2 min, and subsequently left for 12 h at 4 °C in order to extract the fatty acids. Following extraction, the textile squares were removed from the scintillation vials and discarded. A 1.5 mL aliquot of the extracted fatty acid solution was added to a Pyrex™ screw cap tube (ThermoFisher Scientific, USA). 6.3 mL of methanol (Burdick & Jackson, USA) was added to the tube followed by 700 µL of 10N KOH (Sigma-Aldrich, USA). The sample was shaken well before incubation at 55 °C for 1.5 h with additional vigorous shaking for approximately 5 s every 20 min, in order to dissolve and hydrolyse the sample. The sample was cooled in a cold water bath before adding 580 µL of sulfuric acid (Sigma-Aldrich, Germany) and placed back onto the heating block for an additional 1.5 h at 55 °C, again with vigorous shaking every 20 min. The sample was once again cooled in a cold water bath and 3 mL of hexane (SK Chemicals, Korea) was added to the sample. The Pyrex™ tube was vortexed twice before being centrifuged at 3000 rpm for 5 min. 200 µL of the top layer from the sample tube was removed by pipette and added to a GC vial. 100 µL of the internal standard (1000 ppm nonadecanoic fatty acid methyl ester, Sigma Aldrich, USA) was also added to the vial and the volume was made up to 2 mL using HPLC grade hexane and the GC vial inverted. The remainder of the top layer of the sample was added to a scintillation vial for storage in a freezer at -18 °C.

2.4.1.2 GC-MS Parameters

The GC-MS analysis was performed using an Agilent 7890A gas chromatograph coupled to an Agilent 5975C mass selective detector (Agilent Technologies, Australia). This instrument can be seen in Figure 2-6. An HP-5MS (5 % diphenyl, 95 % dimethyl polysiloxane) ultra-inert column (30 m × 0.25 mm ID, 0.25 µm film thickness, J & W Scientific, USA) was used during the analysis. Helium (high purity, BOC, Australia) was used as the carrier gas at a constant flow rate of 1.2 mL/min. All injections were performed in split mode with a split ratio of 25:1 and an injection volume of 1 µL using an Agilent 7683B Series autosampler. The oven temperature program was as follows: 135 °C (held for 4 min) – 180 °C at 4 °C/min, 180 °C (held for 5 min) – 200 °C at 4 °C/min, 200 °C (held for 5 min) – 250 °C at 4 °C/min and finally held at 250 °C for 10 min. The total analysis time was 52.75 min. The mass selective detector was operated in full electron ionisation (EI) scan mode was with an emission

current of 30 uA, a scan time of 0.50 s/scan, and a mass range of 50 – 450 m/z. A fatty acid methyl ester mix (C_5 – C_{23}) purchased from Supelco (USA) was used to confirm the identification of peaks.

Figure 2-6: Agilent 7890A gas chromatograph coupled to an Agilent 5975C mass selective detector.

2.4.2 Trimethyl Silylation Derivisation

The second method was adapted from Folch et al. [80] and involved the silylation of the fatty acids.

2.4.2.1 Sample Preparation

Three by three cm sections of the textile samples collected in-field were created. These cut-outs were then placed into individually labelled scintillation vials, and 8 mL of chloroform was added. The samples were then sonicated for 30 min, vortexed for 2 min, and left for 12 h at 4 °C. The textile squares were removed from the scintillation vials and discarded. 1.8 mL of the extracted fatty acid solutions were transferred to screw-top culture tubes, to which 0.2 mL of N,O-Bis(trimethylsilyl) trifluoroacetamide with 1 % trimethylchlorosilane (BSTFA + TMCS) and 0.2 mL of internal standard (1000 ppm nonadecanoic acid, Fluka Analytical, Switzerland) was added. The samples were then vortexed for 1 min and heated for 15 min at 70 °C using a heating block. Upon cooling, the samples were transferred to gas chromatography vials.

2.4.2.2 GC-MS Parameters

The GC-MS analysis was performed using the same instrumental set-up and column as explained in 2.3.1.2. The initial oven temperature was 50°C, followed by a programmed temperature ramp of the following parameters; an initial ramp of 15°C/min until a temperature of 190 °C was reached. This temperature was held for 4 min. The temperature was then increased to 200 °C at a rate of 1 °C/min held for 3 min, followed by an increase to 240°C at a rate of 15 °C/min held for 5 min. The total run time was 34.0 min. A full scan was performed with an emission current of 30 uAmp and a scan time of 0.50 s/scan. The scan used a range of 50 – 450 m/z ratio. Individual fatty acid standards of palmitic acid, myristic acid, oleic acid, palmitoleic acid, linoleic acid, and stearic acid (all from Sigma Aldrich, USA) were derivitized using the process described in section 2.4.2.1 for confirmation of the identification of peaks. GC-MS data analysis was performed using Agilent MSD ChemStation (version E 02.01.1177, Agilent Technologies, Australia) and Microsoft Excel 2010.

Chapter 3: VISUAL AND ENVIRONMENTAL DATA

Chapter 3: VISUAL AND ENVIRONMENTAL DATA

3.1 Introduction

Environmental factors are crucial in the onset and development of human decomposition and the textile degradation processes (section 1.1.8). In this chapter the environmental factors such as rainfall, temperature, UV radiation, soil moisture and pH (see section 2.2 for methodology) were all monitored and their effect on both rate of decay for the remains as well as the rate of degradation of the different textile types investigated. In addition initial visual analysis of the decomposing remains and the textiles in association with remains, as well as those without, was conducted in order to get initial decomposition and degradation data. Initial analysis of the remains and textiles is the natural first step of any decomposition study as it is non-destructive and does not inhibit any further analysis. The results obtained from the visual analysis greatly influenced the duration of any one experiment and helped define the subsequent chemical and analytical analysis conducted in the successive chapters (Chapter 4 – 6).

3.2 Environmental Factors

3.2.1 Total Rainfall and Average Daily Temperatures

A graphical representation of the temperatures and total rainfall for each of the three surface trials can be found in Figure 3-1. The first trial demonstrated that the weather conditions were found to be as expected, starting with a hot Australian summer, with temperatures then decreasing going through autumn and into winter (Table 3-1). The second summer (T3) had an average temperature of 23 °C, with a high of 29 °C and a low of 18 °C (Table 3-3). Thus, the second summer was on average milder than the previous year and also exhibited a lot less variability in the temperatures, however the total rainfall between the trials was comparable. The winter trial (T2) started in the winter of 2013 (July 2nd) and ran until summer 2015 (Table 3-2). Thus T2 initially overlapped with the some of the winter period, spring and summer for T1 and ran over the entire period of T3. T2 overall experienced two winter and spring seasons and almost two full summer seasons over the duration of the study.

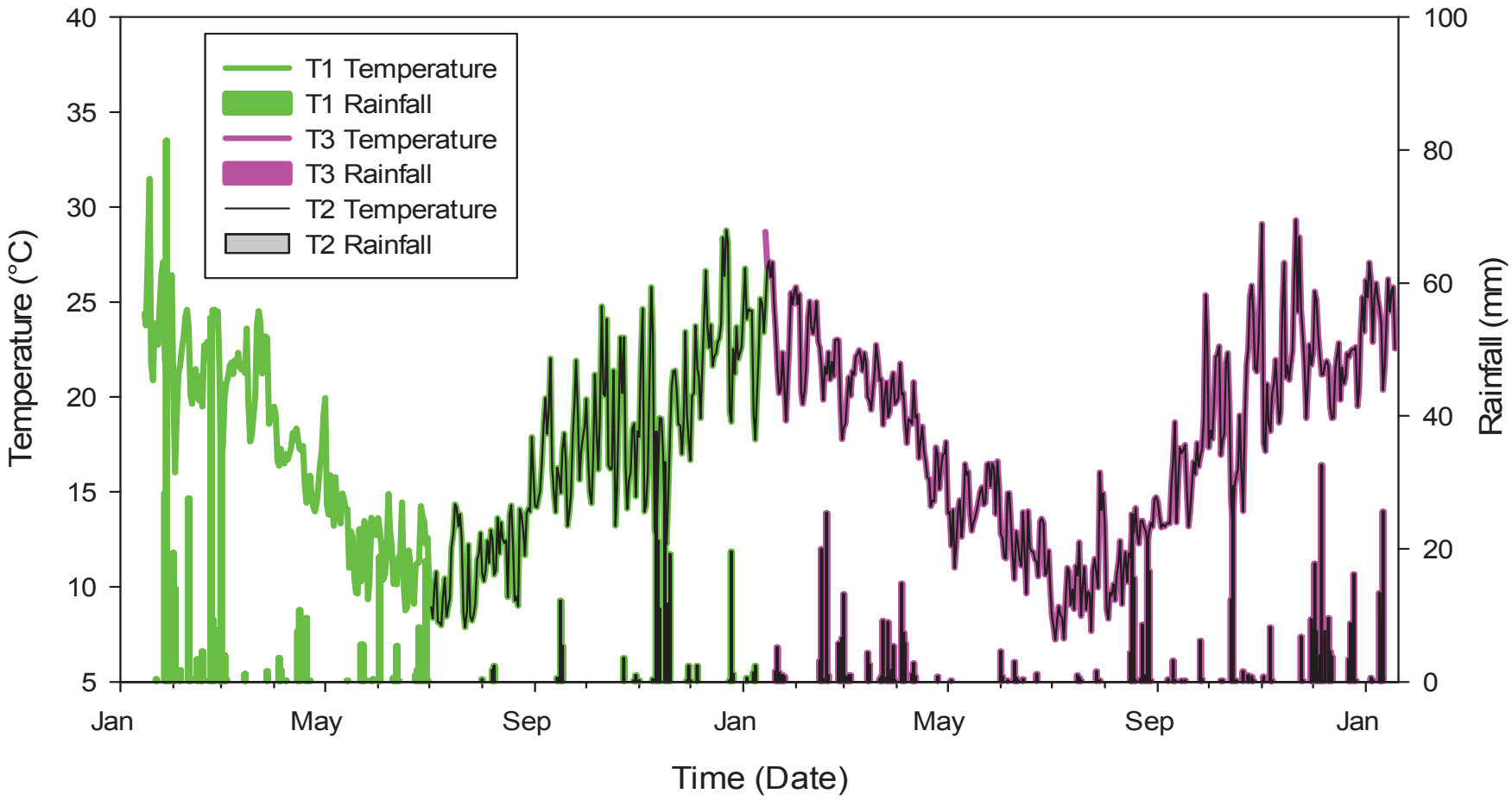

Figure 3-1: Daily average temperature (°C) and total daily rainfall (mm) for T1-T3.

Table 3-1: Average daily temperature, highest and lowest recorded temperatures and total rainfall for Trial 1.

Season	Months	Average daily temp (°C)	Highest temp recorded (°C)	Lowest temp recorded (°C)	Total rainfall (mm)
Summer	Jan – Feb 2013*	23	47	14	274
Autumn	March – May 2013	17	30	15	98
Winter	June – Aug 2013	11	24	2	64
Spring	Sept – Nov 2013	18	37	6	171
Summer	Dec – Jan 2014*	24	29	17	28

*The trial started January 15th 2013 and ended January 15th 2014.

Table 3-2: Average daily temperature, highest and lowest recorded temperatures and total rainfall for Trial 2.

Season	Months	Average daily temp (°C)	Highest temp recorded (°C)	Lowest temp recorded (°C)	Total rainfall (mm)
Winter	July – Aug 2013*	11	24	2	5
Spring	Sept – Nov 2013	18	37	6	171
Summer	Dec – Feb 2014	23	29	17	104
Autumn	March – May 2014	18	23	11	85
Winter	June – Aug 2014	11	16	7	108
Spring	Sept – Nov 2014	19	29	13	83
Summer	Dec – Jan 2015*	23	27	19	175

* The trial started July 2nd 2013 and ended January 18th 2015.

Table 3-3: Average daily temperature, highest and lowest recorded temperatures and total rainfall for Trial 3.

Season	Months	Average daily temp (°C)	Highest temp recorded (°C)	Lowest temp recorded (°C)	Total rainfall (mm)
Summer	Jan – Feb 2014*	23	29	18	76
Autumn	March – May 2014	18	23	11	85
Winter	June – Aug 2014	11	16	7	108
Spring	Sept – Nov 2014	19	29	13	83
Summer	Dec – Jan 2015*	23	27	19	175

* The trial started January 14th 2014 and ended January 18th 2015.

3.2.2 Solar Radiation

UV light has been found to degrade certain textile types by causing oxidation and the breaking of intermolecular bonds in the fibres, facilitating the penetration of the enzymes capable of hydrolysing the textile [38]. UV light also causes discolouration and visual changes to the fabrics. It was thus beneficial to this study to monitor the solar radiation over time. The results of this can be seen in Figure 3-2.

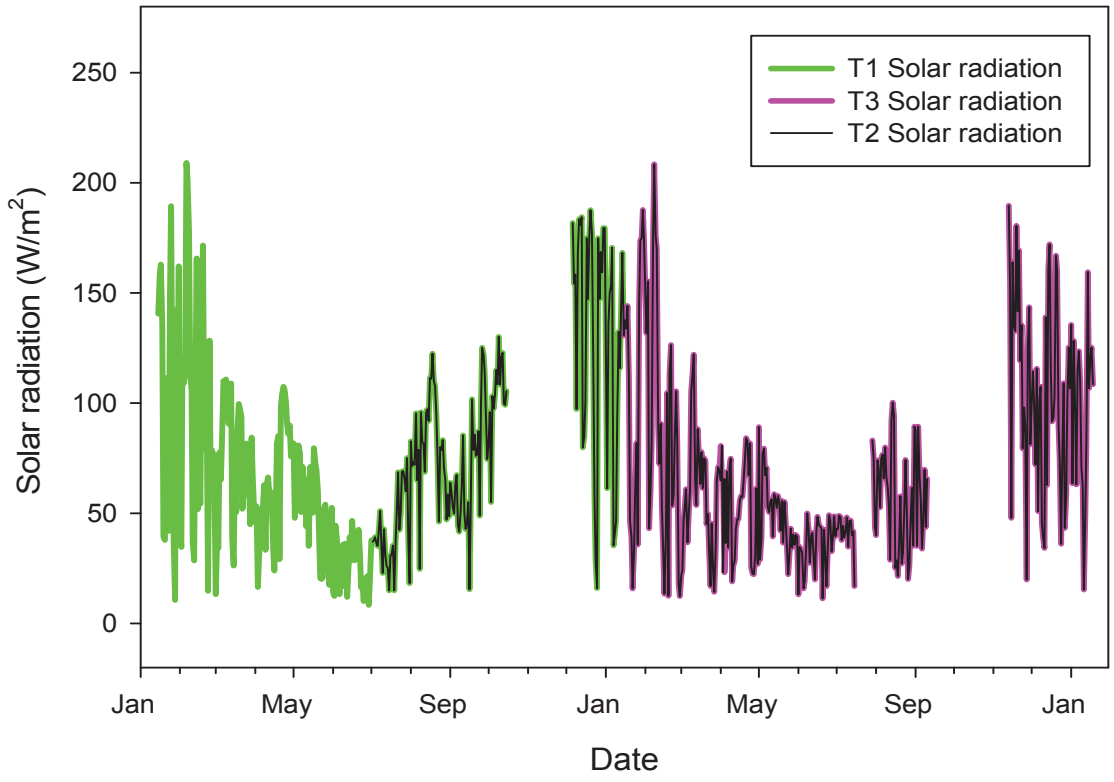

Figure 3-2: Solar radiation throughout the three surface trials

The solar radiation was found to be very variable during the warmer periods, with a large range between the highest and lowest radiation levels (Figure 3-2). For T1 the range spanned from a high daily average of 209 W/m^2 to a low daily average of 10.6 W/m^2 in January, this was the largest range during any trial. During winter season it was found to fluctuate less.

Bushfire near the location of the research facility explains the absence of information in November – December of 2013. Thus, the solar radiation was not recorded between days 275 and 325 of T1, which corresponded to days 107 till 157 of T2. There was also a gap in the collected data of T2 between days 437 and 499. This gap was due to the weather station malfunctioning. This also affected T3 as this corresponded to days 45 to 107 of the trial.

From Figure 3-3 a clear trend was observed as the overall average solar radiation decreased as the remains entered the colder seasons whereas it was high during warmer times. Thus, the solar radiation corresponded to the temperature readings taken from the same period. The summer of 2015 was found to be milder in temperatures than the year

before, this is likely why the solar radiation for spring 2015 was higher than summer 2015. The overall seasonal average reached the maximum solar radiation with an average of 113 W/m^2 in the summer of 2014, which was towards the very end of T1, a third through T2 and the very beginning stages of T3 (Figure 3-3). T3, thus, experienced a large amount of solar radiation in the beginning stages of decomposition, when the majority of the decompositional changes are occurring.

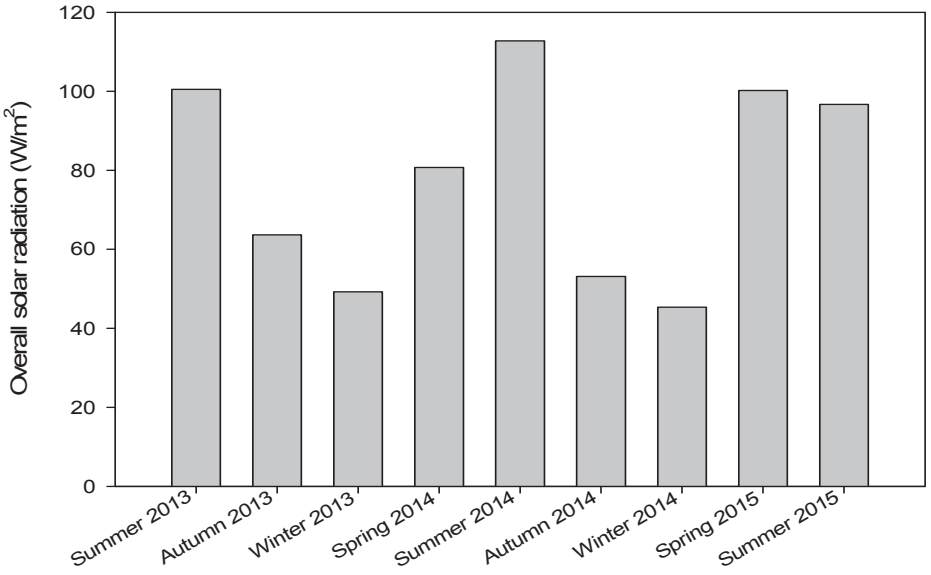

Figure 3-3: Seasonal average solar radiation through the duration of all three surface trials.

3.2.3 Soil pH

Overall there was little change in the pH of the control sites during T1, fluctuating around pH = 5 for the duration of the trial. The experimental sites initially had a pH of around 5 as well; it remained at this level until Day 8 (ADD 197) when an increase was seen, this increase continued until it reached a high of 7.5 on Day 31 (ADD 708) before it again started to decrease before reaching 4.8 on Day 365 (ADD 6281). Initially the pH readings for the control and experimental sites in T1 were comparable until Day 21 (ADD 490), between Day 24 and Day 48 (ADD 554 and 1074, respectively) the pH was found to be statistically different between the control and experimental sites, which can also be observed in Figure 3-4. From Day 149 (ADD 2717) the average pH from the experimental sites started to drop, until it became comparable with that of the control site. Both sites then had similar pH values. On the

final day of sampling the pH for both sites was around 4.7, which is about a pH unit lower than the initial average readings on Day 0 for both sites. These results are comparative to the literature, where during certain stages a difference in pH can be seen as the body releases certain chemicals causing the pH to change. After that the soil then equilibrates and the experimental pH goes back to its original pH level [10].

T2 started during the winter months, the pH fluctuated between 5 and 7, this was seen with both the experimental and control sites. Significant differences between the control and experimental sites were seen on some days (13, 79, 140, 381 and 443), but there were no apparent overall trends.

For T3 an increase in pH values was observed for both the control and experimental sites during the initial sampling days (until Day 73 or ADD 1614). After this date there was a decline in the values before they increased for both control and experimental sites. The changes in pH for T3 were seen for both the control and experimental sites, indicating that the fluctuations were due to environmental and seasonal factors rather than the decomposition process.

T1 consisted of pig carcasses that were larger than those of T2 and T3; this may explain why only the recorded pH values from T1 demonstrated a trend in the differences in pH between the control and experimental sites. With the pig carcasses weighing approximately 80 kg, the fluid release may have been more significant than that of the pig carcasses from the other trials. Prior to sampling Day 21 of T1 there was also a large amount of rainfall (162 mm), this might have caused the decomposition by-products to be carried away from the decomposing remains and to permeate into the surrounding soil where the pH readings were collected. The increase in pH values on Day 21 supports observation this as surface decomposition has been linked with alkaline soil pH values [28]. T3 also experienced some rainfall during the first 50 days of the trial, however the elevated average daily solar radiation values in January 2014, may have caused the rain to evaporate, preventing the same movement of decomposition fluid away from the remains as was observed in T1.

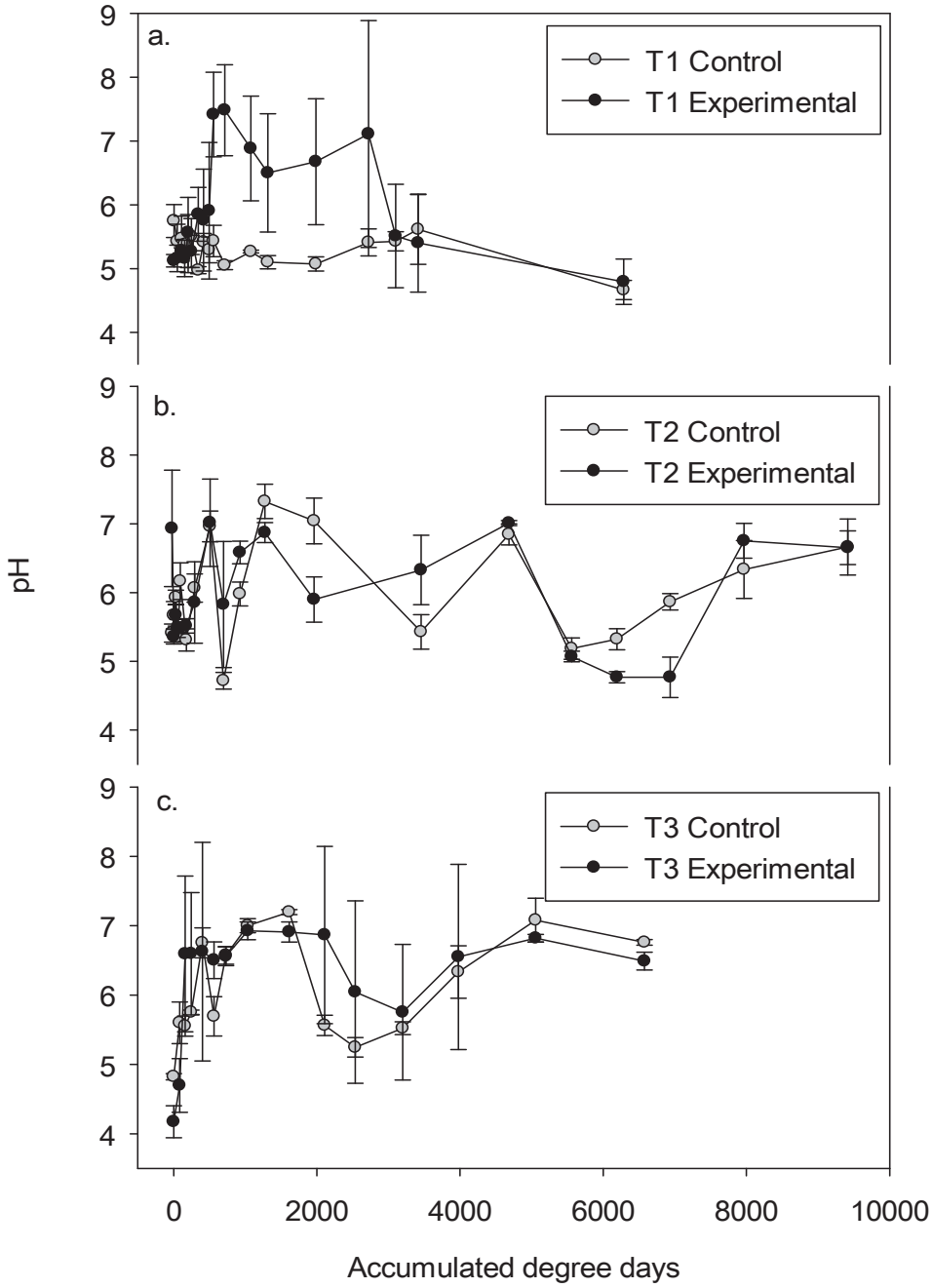

Figure 3-4: a) Soil pH from T1 with control (grey) and experimental values (black). b) Soil pH from T2 with control (grey) and experimental values (black). c) Soil pH from T3 with control (grey) and experimental values (black).

3.2.4 Moisture

The soil volumetric water content (VWC) was monitored each trial and the results can be seen summarised in Figure 3-5. Overall, a similar trend was observed for each of the trials, with an initial increase in the moisture content associated with the

remains that would later equate that of the control sites. The only exception was found on Day 73 (ADD 1614) of T3 where the soil moisture content depicted a spike after starting to go down initially.

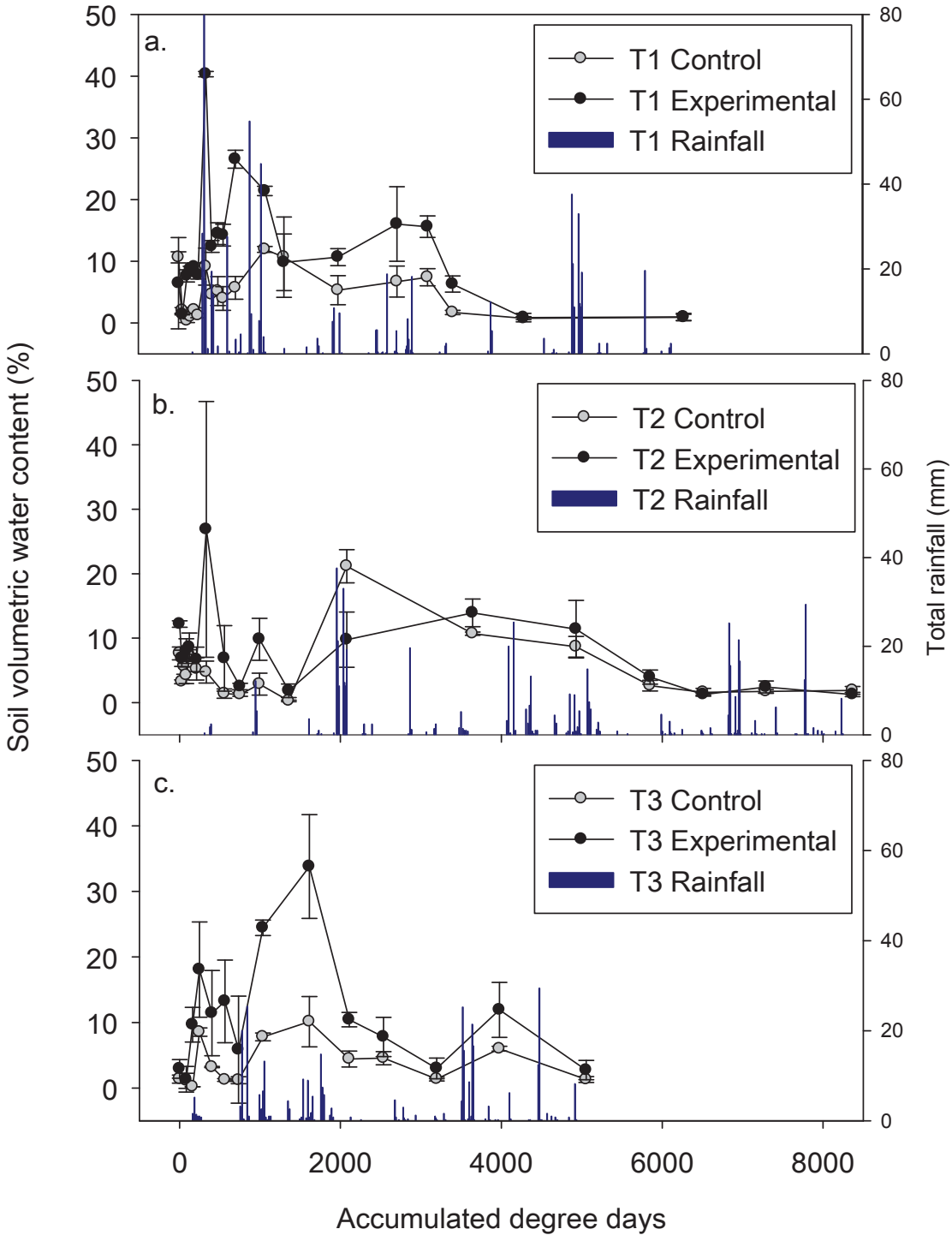

Figure 3-5: a-c) Soil volumetric water content (%) with both experimental (black, circle) and control (grey circle) values and total rainfall (mm) shown using bar graph (blue) for each of the three trials over time.

A large spike in the experimental moisture reading was seen on Day 14 (ADD 340) of T1. Prior to this sampling day there had been a large amount of rainfall, with 80 mm of rain recorded. The large amount of total rainfall may explain the increase in soil volumetric water content; however as the experimental site average was still markedly higher than that of the control sites, the added moisture is likely to be due to the release of decomposition by-products. After Day 31, the moisture in the experimental samples decreased in value, this decrease in moisture for the experimental sites was seen until Day 59, where the moisture content values for both control and experimental sites were found to be comparable. A one-way ANOVA ($p = 0.05$) was used to compare the moisture readings for the control sites over time, significant changes were found between Day 0 and days 2, 4, 6, 8 and 10. Day 0 moisture readings were also different from days 24, 212, 268 and 365. Day 14 was significantly different from days 2, 4, 6, 8 and 10. Inspection of the experimental moisture readings showed that Day 14 was found to be statistically different from all days except Day 31 and Day 48. These three days all represent the readings taken after large rainfalls occurred.

The first 20 days (ADD 211) of T2 appeared to be fairly similar to the trend observed in T1 (Figure 3-5). On Day 37 (ADD 331) there was a large increase in one of the experimental sites with a soil volumetric content of 49.3 %. The two other experimental sites were only around 12 – 19 % and, thus, the standard deviation was quite large. This corresponds to the first sign of leaching of decomposition fluid. The difference between the experimental sites is likely to be due to one of the pig carcasses rupturing before the two remaining experimental sites. On this day it was also raining, but only around 2-3 mm total rainfall was recorded, again suggesting that the large increase in soil volumetric content resulted mainly from the leaching of decomposition fluid into the surrounding environment. The experimental sites then decreased on Day 51 (ADD 557) until Day 65 (ADD 752), before increasing again on Day 79 (ADD 994). The moisture content data corresponded with the weather data collected, as no rainfall was recorded between days 37 to 74, but prior to the sampling on Day 79 (days 74 – 77), a total rainfall of around 17 mm was seen. From Day 210 (ADD 3644) and onwards the control and experimental sites were comparable and fluctuated in the same pattern. The last three sampling days the soil volumetric content was around 1 – 2 % for both site averages.

The final surface trial (T3) appeared to have comparable soil volumetric moisture content between the control and experimental sites initially. On Day 6 (ADD

161), a spike in experimental moisture content was recorded; this was also reflected statistically as this day was found to be statistically different between the controls and experimental values. Day 10 (ADD 246) saw another increase in the experimental site, but an increase was also seen with the controls. On Day 31 (ADD 731) the moisture values decreased, before increasing again, cumulating in a large soil volumetric content percentage (33 %) in the experimental sites on Day 73. Beyond this point the recorded values of the experimental sites decreased until Day 100 (ADD 2109) when the control and experimental sites were comparable. Between sampling days 45 – 73 there was approximately 50 mm rainfall which might aid in the explanation as to why the soil volumetric content spiked on Day 73. However the large increase in soil moisture content was only recorded for the experimental sites. The difference in moisture between the control and experimental sites was also reflected in the general wet appearance of the sites as noted in Appendix C.

The increase in soil VWC at the experimental sites of each trial on days 14, 37 and Day 6 for T1, T2 and T3, respectively was most likely due to the release of decomposition by-products as the remains entered the active decay stage. The large amount of total rainfall prior to some of these days might help explain the increase in soil volumetric water content; however as the soil moisture content readings were still markedly higher in the experimental sites than that of the control sites, the added moisture is likely due to the release of decomposition by-products.

All three trials demonstrated an increase in soil volumetric content for the experimental sites. Despite heavy rainfall occurring prior to some of these sampling days, a lack of a similar increase in soil moisture for the control sites suggests that the change resulted due to the presence of the decomposing remains. Fluids leaching into the surroundings are a common occurrence following the rupture of the skin of the remains, a phenomenon known as CDI around the carcass [6, 81, 82].The increase in soil moisture observed indicates that although clothing is present on the remains, the fluid goes through the fabric and enters the soil. These results are encouraging as it indicates that the textile material will be soaked in the decomposition fluid.

3.3 Decomposition Stages

The visual changes observed during decomposition of the porcine remains were categorised using the different stages of decomposition proposed by Payne [11] and

adapted by Anderson and Vanlaerhoven [12] (i.e. fresh, bloat, active decay, advanced decay and dry/remains). Where differential decomposition occurred, the dominant stage was selected (see Appendix A, B and C).

For the summer trials (T1 and T3) the remains were in the fresh stage for 2 days (ADD 24) for T1 and 3 days (ADD 55.5) for T3, prior to entering the bloat stage (Figure 3-6). The bloat stage was very short in both trials, until ADD 106 (Day 4) and ADD 136 (Day 3) for T1 and T3, respectively. Up until the start of the active decay stage the two replicates were fairly similar, however, the remains in T3 experienced a much shorter time in the active decay stage compared to the remains in T1 (Figure 3-6). The transition from active to advanced decay started on ADD 490 (Day 21) for T1 but some maggot activity was seen after this point underneath the clothing. The maggot masses remained shielded from the outside environment underneath the clothing; this is believed to be the reason why they were found after the onset of advanced decay. The hind legs were found to be skeletonised by day 21, however, the rest of the remains were in advanced decay. T3 saw the onset of the advance decay at ADD 246 (Day 10). The last stage commenced at ADD 3100 (Day 184) and ADD 2109 (Day 100) where they remained for the duration of the study. Overall the remains in the summer trial progressed through the first three stages quite rapidly, before spending a much larger interval of time in the advanced decay stage, before finally becoming skeletonized in the dry/ remains stage.

The winter trial (T2) saw the remains progress through the same stages with the fresh stage lasting until ADD 121 (Day 12) (Figure 3-6). The bloat stage then began and lasted until ADD 378 (Day 36). Both of these stages were found to be more rapid in summer as these two stages only lasted 3-4 days during summer, but were evident until day 36 during winter. A significant difference in the summer and winter trials was therefore very evident initially. The remains in the winter trial (T2) entered the active decay stage at ADD 391 (Day 37). The onset of the advanced decay stage occurred at ADD 994 (Day 79), before entering the final stage dry/remains at ADD 3644 (Day 210).

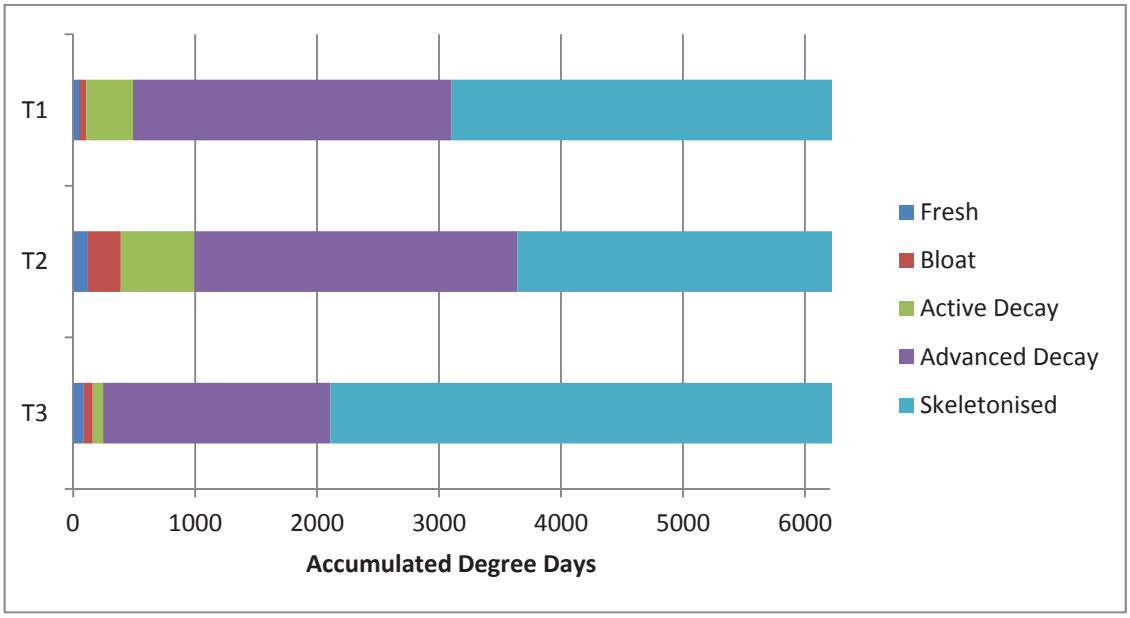

Figure 3-6: Decomposition stages of the surface trials (T1-T3) over time

The variable size and weight of the pig carcasses used between the trials might be one of the factors as to why T1 and T3 were different in the onset and length of the stages of decomposition. The pigs obtained for T1 were between 75 – 80 kg, which was noticeably heavier than the ones used in T3 (50 – 60 kg on average). Ideally T2 and T3 should be indicative of the seasonal variety seen between summer and winter decomposition stages as the pigs were of more comparable sizes, with both trials having pigs weighing around 50 kg. The remains placed at the research facility in the winter (T2) were in the beginning stages for longer than both T1 and T3, however, it was most evident that the decomposition was inhibited throughout when comparing T2 to T3. The increased duration spent in each decomposition stage during the winter trial was expected and corresponds to literature findings stating that remains in a colder environment will decompose slower than those in a warmer climate [83]. The increased rate during warmer temperatures is attributed to increased variation and amount of insect activity associated with the cadavers [55].

In addition to the variation in initial weight of the pig carcasses between T1 and T3, the solar radiation values were found to be very high in the beginning stages of T3; this might also help indicate why the remains reached skeletonization faster than the remains of T1 despite both trials starting in the same month (albeit different years) and being allowed to decompose for a similar time period.

3.4 Textile Degradation Stages

3.4.1 Cotton T-shirt

Until Day 149 (approximately 4 months, ADD 2721) there was no visual textile damage to any of the textile control samples from T1 and all replicates remained intact; although some discolouration was observed in the form of yellowing of the cotton t-shirts. On Day 149, two of the cotton control replicates were visually damaged, with the presence of small holes in the neck region of the t-shirts observed (Figure 3-7a). The textile samples were in direct contact with the soil where the damage had occurred. The second summer trial (T3) exhibited a similar trend, with the initial damage being observed on day 129 (ADD 2536). From day 149 onward for T1 and day 129 for T3, the cotton samples from the control sites continued to degrade visually, with additional holes developing and the overall t-shirt becoming thinner and more transparent. Generally, the damage occurred more rapidly and frequently where the textile samples were in direct contact with the soil. On the final day of sampling of T1 (i.e. day 365) the control t-shirts were visibly damaged in certain regions and the fabric markedly thinner, especially around the frayed edges of the holes (Figure 3-7c).

T2 had a longer sampling period than the two summer trials; this was due to findings from the summer trials, indicating that a longer post-mortem interval might be beneficial. The winter trial also commenced during winter, subjecting both textile samples and the remains to colder temperatures, hypothesised to slow down any degradation or decomposition.

At the beginning of T2 there was severe wet weather and wind, this caused the fabric to become wet, and due to the surrounding vegetation dying because of the colder temperatures, dead organic matter covered the t-shirt and caused discolouration of the cotton. The leaves and twigs caused the t-shirt to display orange/ yellow spots on the white fabric. This was seen from Day 9 (ADD 82) and lasted for the duration of the trial. On Day 140 (ADD 2078), the t-shirt displayed a clear yellow discolouration, which could have resulted from early degradation by microorganisms [29, 41]. On Day 269 (ADD 4929) the first signs of visual damage to the textile were observed, again there was a presence of small holes in certain regions of the t-shirt. On this day, parts of the t-shirt were covered in soil and several ant hills were found around the edges of the clothing. The damage continued to develop as seen with the summer trials (T1 and T3)

until Day 565 where several holes were observed and the edges of the damaged area were clearly frayed and weakened.

A distinct difference in the rate of the textile degradation of the cotton t-shirt was observed between the summer trials and the winter trial, with the summer trials experiencing visual damage from Day 149 onward for T1 and Day 129 for T3. T2 did not have any cotton degradation signs until Day 269.

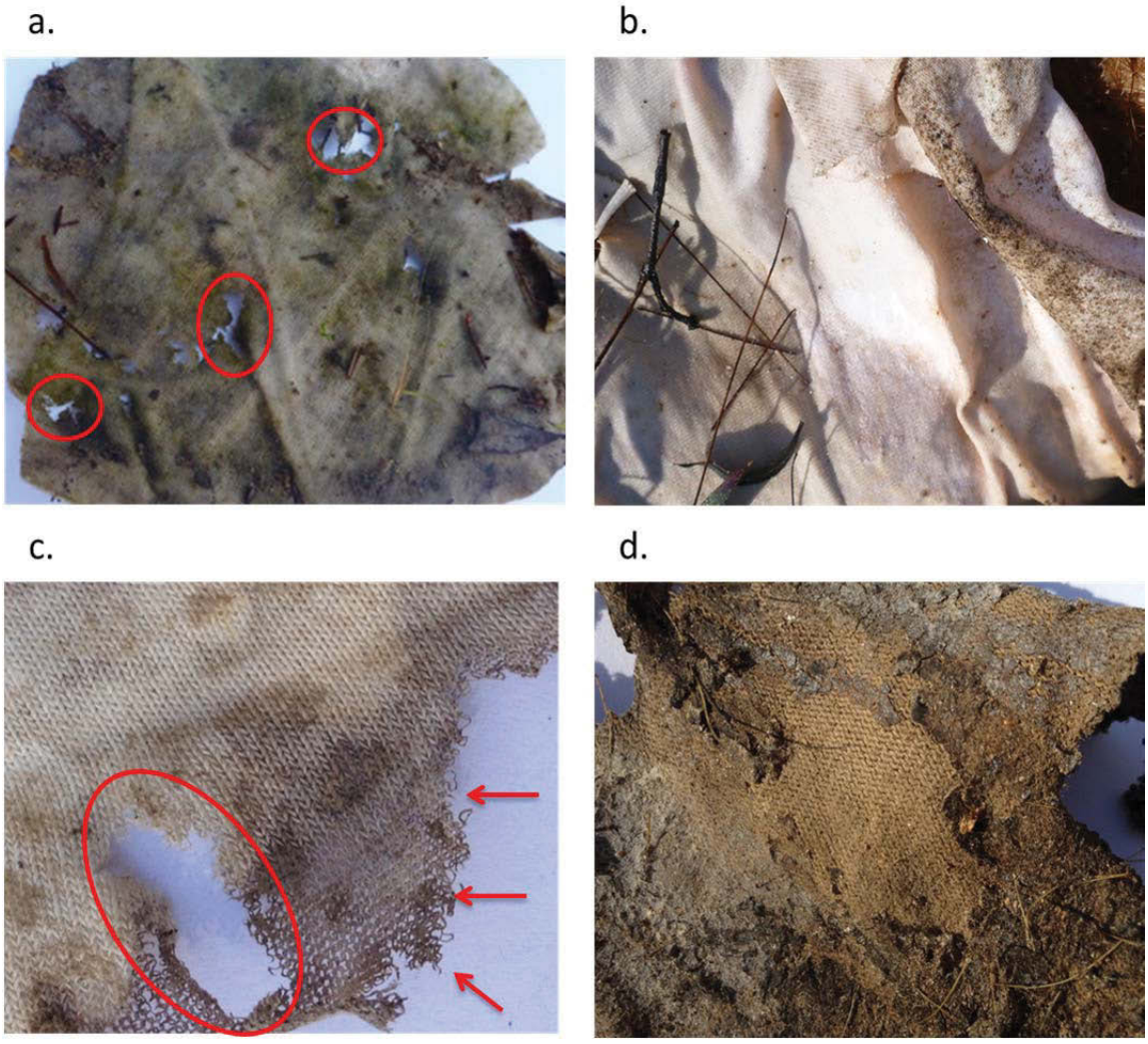

Figure 3-7: Visual damage to textile samples from a) a control site after 149 days; b); an experimental site after 149 days c) a control site after 365 days; and d) an experimental site after 365 days. Red circles indicate presence of holes, whereas he red arrows mark the frayed edges.

A small amount of blood was transferred from the carcasses to the clothing on Day 0 of T1. As the remains entered the bloat stage on Day 2 the t-shirt was stretched across the pig's abdomen, but no tearing occurred. As the remains entered active decay on Day 4 (characterised by the release of fluids), the cotton t-shirts became visibly stained yellow/orange in colour with decomposition by-products in the abdominal region and underside. Following rainfall the entire t-shirt exhibited a brownish discolouration for all replicates on Day 6. A heavy period of rainfall (127 mm) between days 12 – 14 caused large areas of the cotton samples to be "washed" and the t-shirt demonstrated several white solid deposits indicative of adipocere formation, in addition to pink staining in localised regions (Figure 3-7b).

Until Day 184 (6 months) there was no visual textile damage to the experimental cotton t-shirts in T1, but on Day 184 several small holes in the shoulder region of the t-shirt on one pig carcass were visible. The portion of the t-shirt that was damaged was not in direct contact with the soil nor did it show any visual staining by decomposition by-products; in addition, none of the other experimental samples showed similar damage. On Day 212 a second experimental site exhibited damage to the cotton t-shirt; again small holes were found in a very dry region of the t-shirt, which was not in direct contact with the soil or visibly stained by decomposition by-products. Damage to the textiles associated with the remains was quite sporadic and only occurred in two of the three replicates. Most of the textile regions were visually stained by decomposition by-products and remained visually intact even after one year (Figure 3-7d).

The second summer trial demonstrated similar trends to T1, with the t-shirt initially being stained by blood from the head bolt used to euthanise the pigs, followed by yellowing of the t-shirt. As the remains entered active, the purging of fluids staining the textiles black/brown was again observed. Prior to Day 73 there was a significant amount of rainfall, this caused the t-shirt on Day 73 (ADD 1614) to take on a pink tinted colour indicative of the presence of adipocere, similar to that observed in T1 after heavy rainfall. On Day 100 (ADD 2109) one of the replicate pigs was found to have visual damage to the cotton textile, this damage occurred on one pig site only. This one replicate pig was also found to progress through the stages of decomposition at a much faster rate than the other two sites and was already skeletonized by the day the damage to the textile occurred. The other two experimental sites did not show any damage to the t-shirt. The t-shirt on this pig was visibly degraded by Day 100 (Figure 3-8a), which was at an earlier time than the control samples (Day 129). It is hypothesised that the

reason why the experimental t-shirt demonstrated damage earlier than the control site was due to the fact that the pig was fully skeletonized and very dry. The t-shirt on the pig was also stretched across the skeleton and the front and back portion of the shirts were not in contact with each other, whereas the t-shirts on the control sites the front of the shirt was in direct contact with the back of the shirt creating a double layer. This double layer might have slightly delayed the degradation.

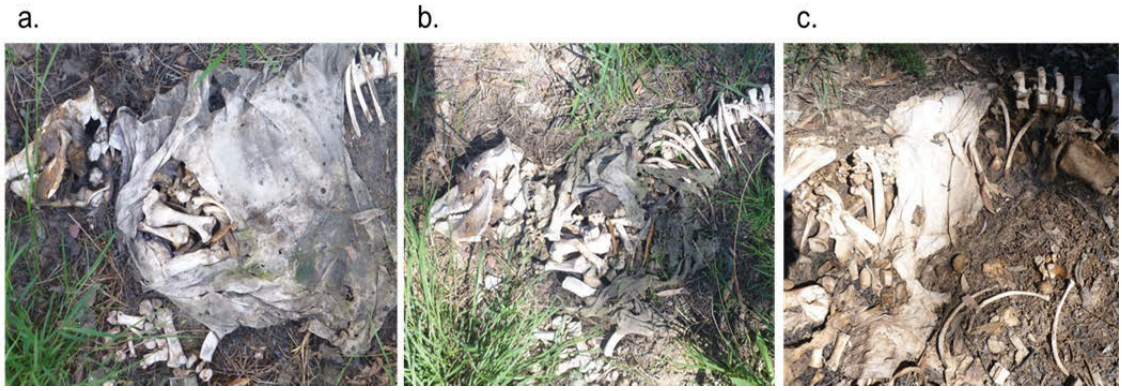

Figure 3-8: a) Pig site B t-shirt damage after 100 days; b) Pig site B t-shirt damage after 247 days; and c) a representative of the other pig sites on day 247.

Minimal damage to the cotton t-shirt was found associated with the remains in the summer trials, T2 was therefore prolonged to go beyond a year post-mortem. On Day 381 (ADD 6511) the t-shirt underneath the pig carcasses were completely disintegrated and only the seams remained. The t-shirt on top of the carcass was still relatively intact that this point (there was minimal damage by Day 269), however, on Day 565 (ADD 9889) damage was also seen in this section of the t-shirt (Figure 3-9).

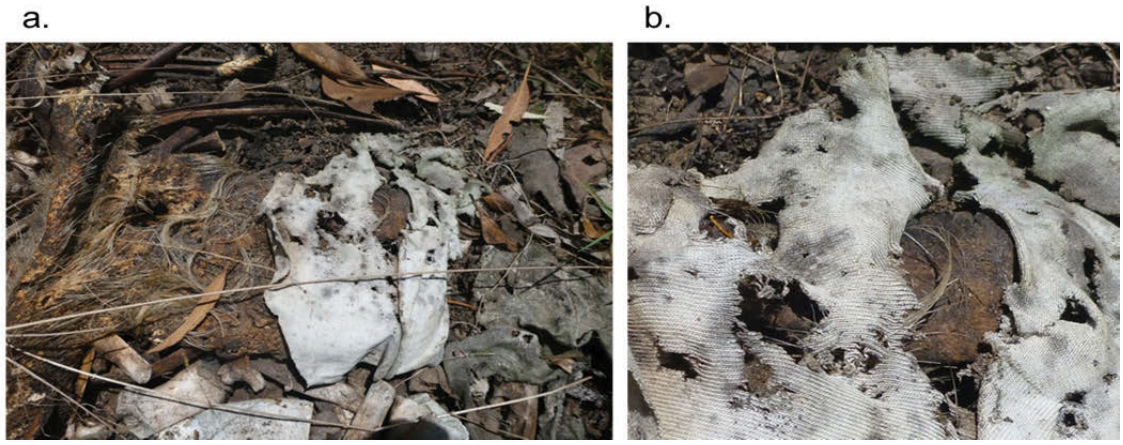

Figure 3-9: a) Visual textile damage of the experimental sites (T2) 565 days post-mortem; and b) close-up of damaged area.

Overall, there was a distinct difference between the control and experimental textile samples. Cotton in the absence of decomposing remains was observed to degrade more rapidly. After approximately four months, visual damage was observed with the control samples, which became progressively more degraded over time. The experimental samples degraded at a considerably slower rate, showing only slight visual damage after 6 months. With the control samples the damage was found to be more frequent in areas where the textile samples were in direct contact with the soil. This finding is consistent with the literature as it has been stated in other studies that degradation of cotton will occur at sites where the textiles are in direct contact with the soil microbial community [29]. In addition, earlier studies have noted that untreated cotton textiles will generally show signs of decay by 60 days in a biologically active soil [31]. The control samples did not show any degradation until approximately 4 months, suggesting that the soil at the field site used herein might be too acidic (pH 4 – 5) to have rates of degradation comparable to those reported previously by Janaway [31]. The cotton samples are in direct contact with a very complex soil matrix, but as both control and experimental samples were subjected to identical soil environments, it is not likely that soil characteristics are causing the delayed degradation of the clothing associated with the decomposing remains. Another potential explanation for the delay in the degradation of the control samples may be that the samples in the current study were not buried in the soil, as seen with the study conducted by Janaway [31], but rather placed on the soil surface, thus reducing the amount of direct contact with the soil.

3.4.2 Polyester Briefs

Throughout the entire first trial (T1) there were no visual changes to the briefs. By Day 365 (ADD 6281) the briefs were distinctly faded in colour (Figure 3-10a), but the only damage was a piece of grass growing through one of the three replicate briefs. During the trial there was evidence of insect activity on and around the briefs, including an ant hill on top of them being formed on Day 21 (ADD 490) and a spider web on Day 94 (ADD 1986), however, none of this activity resulted in any damage. Similar observations were made during T3, with some insect activity on and around the briefs (Figure 3-10b), but the only damage observed again was the fading of the colour. The only difference between the two summer trials was that in the latter the colour fading occurred much earlier, likely due to the increased solar radiation experienced by the fabric. Despite having T2 proceed for a longer time period there was again no visual

textile damage to the polyester briefs observed in the control sites. On Day 65 (ADD 752) the previously black briefs had a distinct purple shine, again likely due to the black colour fading in the sunlight. This dark colour continued to fade on the side facing the sun until Day 499 (ADD 8368) where the briefs were completely faded on the external side.

On Day 2 (ADD 48) of T1, the experimental polyester briefs were stretched as the pigs experienced the bloat stage causing the abdomen to become significantly distended. This did not result in any physical damage to the fabric, but the briefs were probably stretched to capacity. On Day 4 (ADD 107) there was a large presence of egg masses on the outside of the briefs and in one replicate pig a large maggot mass inside the briefs caused them to be pulled down towards the soil surface. Identical observations were made for T3 where again maggot masses caused the briefs to be displaced. As the pigs reached active decay the briefs became stained with decomposition fluid and some parts were fully submerged in this pool of liquid. On Day 14 (ADD 341) after the period of heavy rainfall (127 mm) the briefs were found lying in a pool of light grey liquid and several white deposits could be seen on the fabric (Figure 3-10c). Towards the end of the summer trials the decomposition fluid dried and several large chunks of tissue were found attached to the inside and outside of the briefs. In T3 this was particularly evident as an orange/brown layer covered the entire surface of the briefs (Figure 3-10d). Where there was an absence of this decomposition by-product layer, the briefs were visibly faded. No other damage was observed.

For T2 the briefs had the presence of some blood initially, as the pig carcasses had areas of blood upon arrival. On Day 37 (ADD 391) the briefs were fully submerged in froth produced by the maggot masses. A thick layer of hair and tissue was found attached to the inside of the briefs beyond this point as seen with T1 and T3. Again, there was no apparent damage other than the fading of the colour of the briefs and the addition of fungi and a greenish tint on the outside part of the briefs, even after 565 days post-mortem.

Throughout the surface trials it was evident that the polyester briefs did not show any visual signs of degradation whether they were placed directly on the soil surface or associated with the remains. Synthetic textiles have been found to demonstrate a considerably higher resistance to degradation by microorganisms as they are produced from chemical structures that are treated to oxidation of enzymatic attack [31, 41, 43]. The fact that no visual change was observed throughout any of the trials

was consistent with previous research into the degradation of polyester, and it was confirmed that this synthetic material is likely to present in nature for an extended period of time, whether placed directly on the soil surface, or found in association with remains.

Figure 3-10: Visual damage to the polyester briefs from a) a control site after 365 days; b) a control site showing the presence of insect activity on day 247; c) an experimental site after 24 days with sections of white deposits; and d) an experimental site after 31 days with visible orange/brown staining.

3.4.3 Polyester – Cotton Socks

During the first trial (T1) there were several ant hills formed around or on top of the polyester – cotton socks; these caused the socks to be buried. By the final day of sampling vegetation was growing in-between some of the sock samples, which were visibly thinner and frailer. Despite the close contact with soil both below and on top of

the socks there was no complete visual damage to any of the replicates. The polyester – cotton samples from T3 showed that on Day 185 the fibres were not held as tightly together and the material was visibly frailer as seen in T1. The polyester – cotton socks appeared to be fairly intact throughout most of T2. On Day 13, orange discolouration could be seen, most likely due to discolouring from the dead leaves that were lying on top of the textile. As the trial progressed the socks became progressively more buried under the surrounding soil. By Day 210 (ADD 3644) the textile appeared to be markedly weaker and more transparent. Vegetation growth occurring between the fibres in the socks was also observed.

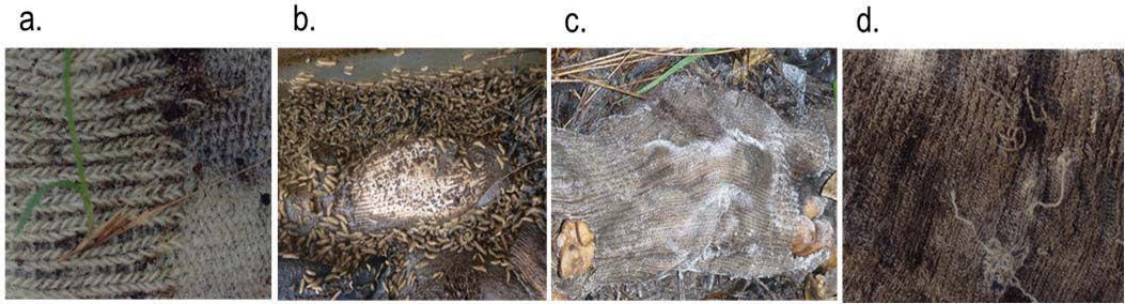

Figure 3-11: a) Control polyester – cotton sample; b) sock covered in maggots from an experimental site; c) white staining of an experimental site; and d) socks from an experimental site on Day 365.

At the onset of the active decay stage the subsequent release of decomposition fluid caused the socks to be covered in black/brown liquid and they were quickly covered in maggot masses (Figure 3-11a and Figure 3-11b). There were a few miniscule gaps between the fibres in the socks, most likely a result of the maggots travelling into the flesh underneath to feed or seeking cover inside the socks during periods of heavy rainfall. On Day 14 (ADD 341) of T1 the large influx of rain caused the socks to be washed out and become white/grey. A second large rainfall period during the same trial around Day 48 (ADD 1078) resulted in the socks taking on a pink tint in certain regions and the socks were most likely covered to an extent in adipocere (Figure 3-11c). This same pattern was observed during T3 when white deposits were seen on the outside of the socks on Day 73 (ADD 391). Beyond this point the socks were visually stained in decomposition by-products, but visually there was no apparent damage other than the disturbance of the fibres seen on the final sampling day (Day 365). Despite having a longer degradation timeframe the polyester – cotton samples from T2 did not show any visual signs of damage. Initially some regions of the socks were stained by blood from the pig remains during the dressing. These blood spots became fainter during the first

two weeks as the socks were frequently exposed to rainfall. On Day 37 (ADD 391) of T2 the socks were fully soaked in pool of brown decomposition liquid, which later dried and caused the socks to be very stiff on Day 51 (ADD 557). By the end of the trial the socks were discoloured green from the surrounding vegetation, they were less compact and frail, however, there was no visual physical damage recorded.

The polyester – cotton blend material did not show the damage to degree that was observed for the pure cotton t-shirts, however, some damage was observed and the textile was markedly changed towards the end, in clear contrast to the polyester briefs. It is hypothesised that the presence of the polyester component in the socks, aided in the preservation of this material.

3.5 Conclusions

At the completion of the trial it was found that the natural textiles from the control sites had degraded markedly with the textile being very fragile and having a presence of distinctly damaged areas. Such damage was not observed with the cotton textiles associated with the decomposing porcine remains. The clothing associated with the pig carcasses were in a very biologically active environment caused by the large presence of nutrients from the decomposing remains. This is an environment where one expects the microorganisms to increase in numbers and thrive thus breaking down the material quickly. The results from the current study contradicts this, as despite being surrounded by decomposers, the material was left relatively untouched compared to the control clothing.

The polyester briefs were in a relatively good state apart from some discolouration due to the exposure to UV light and the general outdoors environment. The briefs did not appear to be affected by the presence or absence of decomposing remains. The polyester – cotton blend showed changes intermediate between the cotton and polyester findings, with sections of the socks demonstrating some degradation through the general appearance of the fabric.

These findings were based mostly on observational data, and indicate that further analysis is required, specifically in the case of the natural and blend textile types. The visual degradation occurring in the absence of remains warranted further research into the specific chemical changes to the textile samples using more advanced techniques such as ATR-FTIR spectroscopy. The apparent inhibition of the degradation

of natural textiles associated with the decomposing remains is interesting and suggests that the decomposition processes leading to the release of decomposition fluid into the environment plays a significant role in the textile degradation timeline.

Chapter 4: INFRARED ANALYSIS OF TEXTILE DEGRADATION

Chapter 4: INFRARED ANALYSIS OF TEXTILE DEGRADATION

4.1 Introduction

The nature and timing of textile degradation in a soil environment has long been of interest for forensic and material purposes. The condition of textiles found in relation to a crime event may provide valuable information in certain death environments. Beyond the investigation of the natural textile degradation, materials associated with remains, such as clothing, may be the only remaining evidence.

The current chapter examines the degradation patterns of a natural and a synthetic textile material as well as a blend, when subjected to a natural outdoor environment. Initial analysis was conducted on textile samples placed directly on the soil surface, see section 2.1.1. This investigation was then extended to include textile samples associated with decomposing remains, also placed on the soil surface, in order to identify any changes to the degradation pattern. Three different trials were investigated, two summer trials (T1 and T3) as well as a trial that commenced during the Australian winter months (T2).

The samples were investigated using ATR-FTIR spectroscopy, a well know technique commonly employed to examine textile samples, the specific methodology can be found in section 2.3. In order to obtain baseline data on textile degradation of both natural and synthetic materials, a large dataset is required, which prompts the use of statistical tools to encompass all factors during the data analysis. Providing a statistical approach to the analysis of textile degradation patterns using ATR-FTIR spectra, multivariate statistical analysis and chemometrics was performed. Thus use of The Unscrambler® X software to further examine the degradation changes within the textile sample types was investigated for both textile samples with and without the presence of decomposing remains. The use of statistics to analyse the data will provide a better foundation for the interpretation of the data obtained using ATR-FTIR spectroscopy.

4.2 Results and Discussions

4.2.1 Band Selection

4.2.1.1 Cotton

A number of bands in the infrared region of the cotton t-shirts were initially investigated to determine their potential for monitoring degradation (Table 4-1). A preliminary visual analysis was carried out by inspection of the spectra from the earlier sampling days (i.e. days 0 – 90) compared to those from the later sampling days (beyond 100 days). A visual trend was observed with some of the IR bands in the samples in the absence of remains (control) from Trial 1 (T1): initially the 985, 1001 and 1033 cm^{-1} bands were very distinct. However, the 985 and 1001 cm^{-1} bands were observed to disappear in the later sampling days (Figure 4-1) and merging with the 1033 cm^{-1} band. It has been established that one mechanism by which cotton degrades is through hydrolysis [84]. Preliminary findings support this as the 985, 1001 and 1033 cm^{-1} bands are all in the region of the spectrum where C-O stretching occurs. The disappearance of the 985 and 1001 cm^{-1} bands might indicate that hydrolysis is occurring. A potential region where changes might occur during the cotton degradation has thus been identified. A similar disappearance of the 985 cm^{-1} band was observed in the cotton control samples from Trial 2 (T2), however this only occurred towards the very end of the trial (sampling days 499 and 565).

Table 4-1: Infrared assignments of the main vibrations in the FTIR spectra for cotton

IR region (cm^{-1})	Wavenumber (cm^{-1})	Assignments
3000-2800	2920	CH_2 asymmetrical stretching
	2850	CH_2 symmetrical stretching
1500-1200	1430	CH_2 scissoring
	1335	C-H bending
	1314	CH_2 rocking
1200-800	1110	C-O-C stretching
	1056	C-O stretching
	1033	C-O stretching
	1001	C-O stretching
	985	C-O stretching

Spectral changes to cotton samples during biodegradation has been found to occur between 1400 and 1300 cm^{-1} [38]. The ratio of the 1335 and 1314 cm^{-1} bands could be used to monitor biodegradation, with the band at 1335 cm^{-1} disappearing during biodegradation [85]. A similar trend was not observed in this study for any of the cotton related material, as there was no overall change in that band ratio over time. These bands are typical of crystalline cellulose, and only occur if there is a large presence of cellulose I. Similarly, the band at 1430 cm^{-1} is considered a crystalline marker, and should this band increase from higher to lower intensities, it is considered a sign of the degradation process of the cellulose [86]. Recent studies have focused more on the 1400 – 1800 cm^{-1} region in order to investigate cellulose degradation [87]. The initial findings from the cotton material placed directly on the soil surface in the current study might indicate that the 900 – 1033 cm^{-1} region could potentially give more information about the cotton degradation.

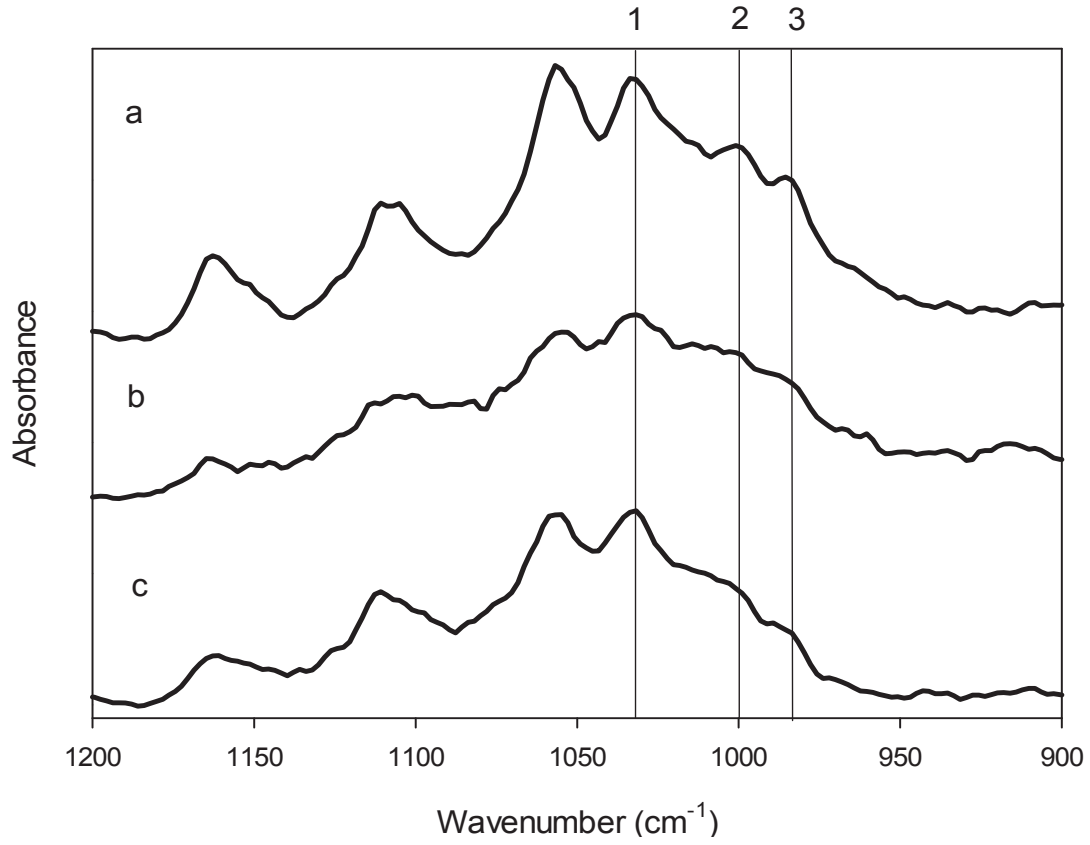

Figure 4-1: FTIR region of interest for the degradation of cotton textile samples: 1) 1033 cm^{-1}; 2) 1001 cm^{-1}; and 3) 985 cm^{-1}. Typical spectra of the cotton control collected on a) Day 0; b) Day 184; and c) Day 268 here represented by data from T1.

The experimental cotton samples from T1 did not exhibit the same trend as mentioned with the controls above as the 985 and 1001 cm^{-1} bands were found throughout (Figure 4-2). A similar trend was observed in T2, where some samples had a loss of 985 cm^{-1}, however this loss was sporadic and no trends could be identified. Trial 3 (T3) was the only trial where there appeared to be a visual trend in the experimental cotton spectra, with the 1001 cm^{-1} band disappearing towards the later sampling days, the change in presence of a distinct band at 985 cm^{-1} for the same trial was, however, more sporadic.

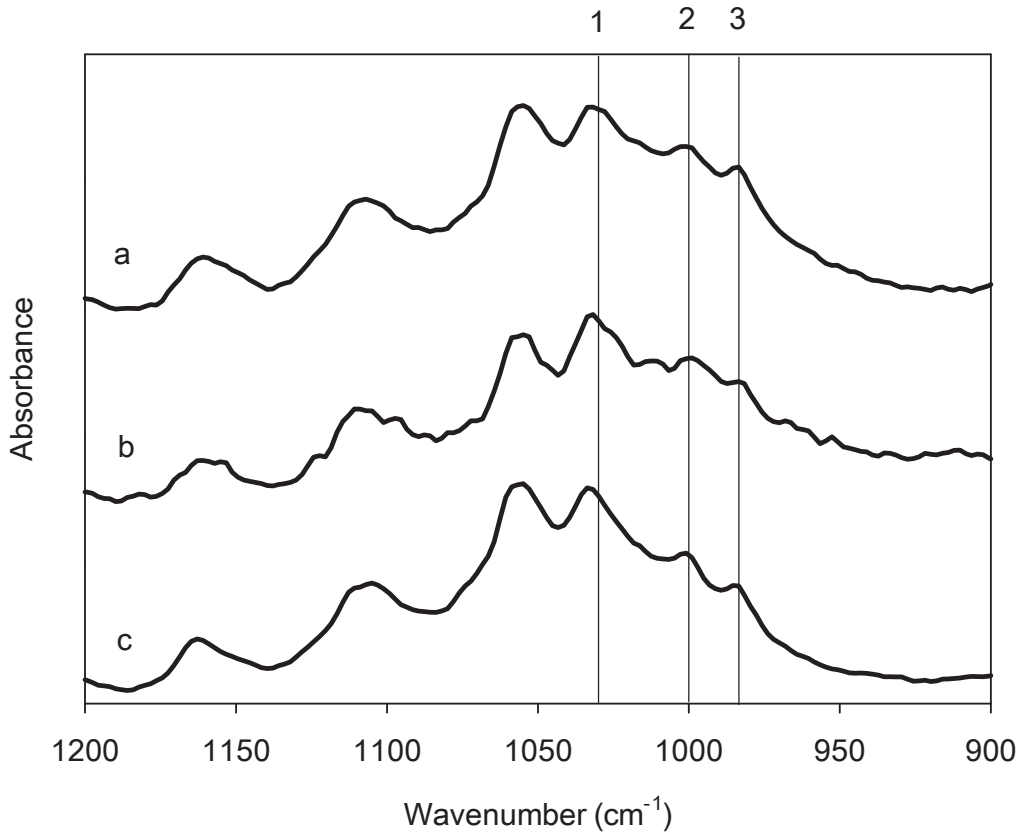

Figure 4-2: FTIR region of interest for the degradation of cotton textile samples: 1) 1033 cm-1; 2) 1001 cm^{-1}; and 3) 985 cm^{-1}. Typical spectra of the experimental cotton collected on a) Day 0; b) Day 184; and c) Day 268 here represented by data from T1.

4.2.1.2 Polyester

Polyester is suspected to degrade through hydrolysis [41], resulting with a cleavage of the ester bonds; with this in mind certain bands of interest were selected for further analysis of the polyester briefs (Table 4-2). Hydrolysis is thought to occur in the

amorphous regions of the polymer [88]. The likely result during hydrolysis of PET is an increase in carboxylic acid and alcoholic end groups, in addition to an increase in smaller chain fragments most likely through reverse esterification [89].

Table 4-2: Infrared assignments of the main vibrations in the FTIR spectrum for polyester

Vibrations (cm^{-1})	Assignments
720	C-H aromatic ring wagging
1090	C-O stretching
1245	C-O stretching
1715	C=O stretching

The regions of the spectrum where changes were predicted to occur during polyester degradation were visually compared between the sampling days (Figure 4-3). No visual differences were observed between any of the sampling days for any trial regardless of whether the polyester was placed on the soil surface or in association with the decomposing remains. This initial spectroscopic analysis is, therefore, consistent with the visual changes observed with the polyester samples in Chapter 3 and confirms that no degradation was noted throughout any trials for either the control or experimental polyester samples.

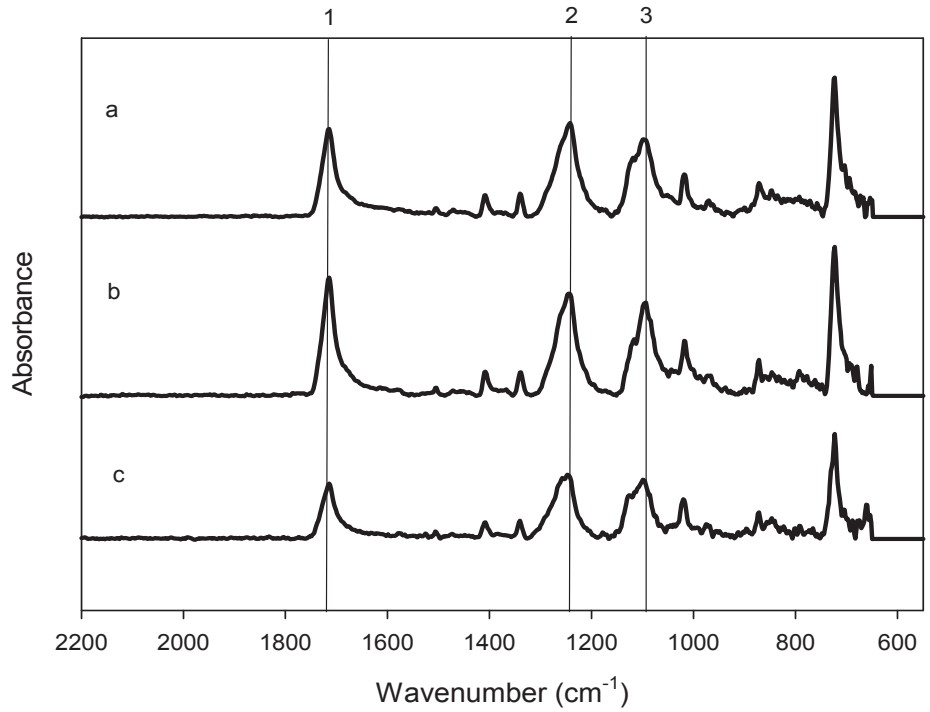

Figure 4-3: FTIR region of interest for the degradation of polyester textile samples: 1) 1715 cm^{-1}; 2) 1245 cm^{-1}; and 3) 1090 cm^{-1}. Typical spectra of the polyester samples collected from the sites on a) Day 0; b) Day 184; and c) Day 365 represented by data from T1.

4.2.1.3 Polyester – Cotton Blend

As the socks were made up of a blend of both cotton and polyester, all the aforementioned bands were investigated when analysing the sock samples. The ATR-FTIR regions containing both fibre types were able to be detected and distinguished. The polyester – cotton blend infrared spectra were separated into separate cotton and polyester spectra, and there two groups were processed separately. There were no visual changes in the spectra for the polyester component of the sock samples for neither the control textiles nor the clothing associated with the remains. This is similar to the results obtained with the 100 % polyester samples in section 4.2.1.2. In the control cotton section of the spectra, however, a similar trend as that observed for the 100 % cotton control samples was found. The control cotton section of the sock samples from both T1 and T2 section saw a loss of the 985 cm^{-1} band in the later samples (days 149 – 499). For T3 there was an evident loss of both 985 and the 1001 cm^{-1} bands towards the end of the trial.

Experimental sock samples also exhibited a spectral change in the IR region of the cotton sections, with the 985 cm^{-1} band disappearing towards the end for T1 (Day 149), decreasing in frequency after Day 51 for T2 and appearing only sporadically beyond Day 129 for T3.

4.2.2 Initial Statistical Analysis

In order to conduct statistical analysis, the bands of interest were normalised to one of the most intense bands [77]. The 985 and 1001 cm^{-1} bands were normalised using the 1033 cm^{-1} band. The 1033 cm^{-1} band was selected as it is always present in the samples at a significant absorbance level. The 1090 cm^{-1} and 1245 cm^{-1} polyester bands were both normalised using the 1715 cm^{-1} band for the initial statistical analysis.

The three control sites (clothing in the absence of remains) from T1 were treated as different sites initially, rather than replicates. However, upon visual inspection they did not appear to be different for cotton, polyester and polyester – cotton blend. A one-way ANOVA ($p = 0.05$) confirmed that there was no significant difference between the sites with $p = 0.402$, $p = 0.912$ and $p = 0.765$, respectively. The same was true for the other trials. This allowed the three sites to be treated as true replicates, thus increasing the replicate number for each sampling day and the statistical importance of the findings. All subsequent analysis was carried out using this assumption. The three

experimental sites from T1 were also treated as replicates after determining through a one-way ANOVA (p = 0.05) that the sites were comparable, with p-values of 0.987, 0.371 and 0.287 for cotton, polyester and polyester – cotton, respectively. As was seen with the control sites, the experimental sites for the remaining trials also demonstrated that there was no significant difference between the sites.

4.2.2.1 Cotton

Initial analysis of the 985/1033 ratio did not produce any significant results within the cotton control samples. However, an interesting trend was seen with the 1001/1033 cm^{-1} band in the cotton samples (Figure 4-4). From Figure 4-4 it is evident that the band ratio generally increased over time. A one-way ANOVA (p = 0.05) determined that the later sampling days (from Day 149) were statistically different from the earlier sampling days (up to Day 21). Thus, this normalised band might be a potential indicator of cotton degradation. T2 produced similar results, with the 1001 cm^{-1} band being significantly different when comparing the days from 325 until 565 with the earlier sampling days using a one-way ANOVA (p = 0.05). Again this indicates that the 1001 cm^{-1} band might be of particular interest in the mapping of cotton degradation.

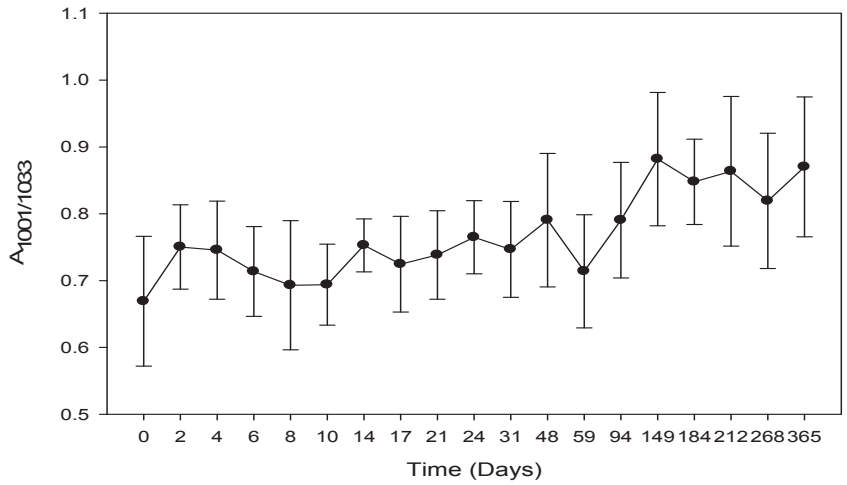

Figure 4-4: Quantity of $A_{(1001/1033)}$ from cotton control T1 as a function of time. The error bars represent one standard deviation based on 9 replicates.

The 1001 cm^{-1} band was found to be statistically different for both T1 and T2 during their respective trials. Attempting to graph and compare the occurrence of this potential degradation of the 1001 cm^{-1} band in both the summer (T1) and winter trial (T2) was carried out using accumulated degree days (which account for the difference

in temperature between the summer and winter trials). This difference was found to occur around similar ADD value, showing that the spectroscopically recorded textile damage was found to be temperature dependent (Figure 4-5).

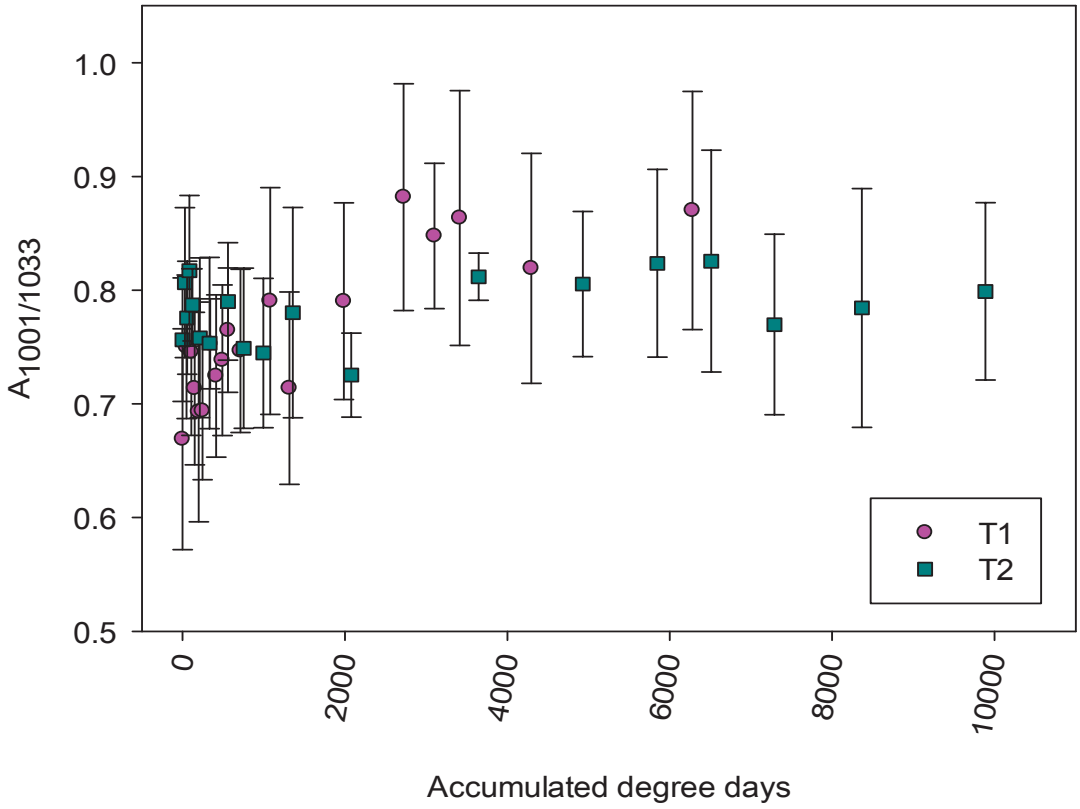

Figure 4-5: Quantity of $A_{(1001/1033)}$ from the cotton control samples from T1 (pink circles) and from cotton control samples from T2 (green square) as a function of accumulated degree days. The error bars represent one standard deviation based on 9 replicates.

T3 was slightly different from the previous trials as a one-way ANOVA ($p = 0.05$) did not find any significant differences in the normalised 1001 cm^{-1} band over time (Figure 4-6). Interestingly some significant differences in the 985 cm^{-1} band were determined instead. The band from the Day 185 samples was significantly different from the earlier days (0, 3, 6 and 31) with p values of 0.001, 0.007, 0.047 and 0.028, respectively. The same IR band from Day 247 was found to be significantly different from days 17, 73, 129 and 185, with p values of 0.014, 0.025, 0.027 and 0.001.

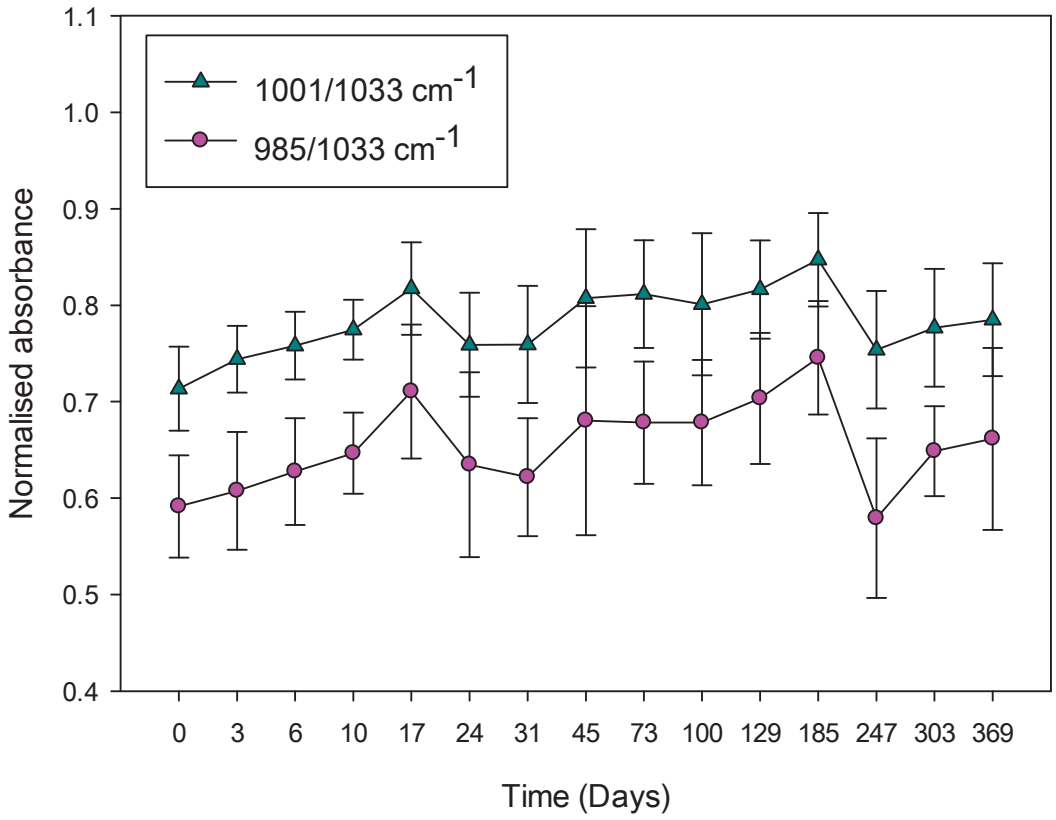

Figure 4-6: Quantity of $A_{(1001/1033)}$ from the control cotton samples as a function of time from T3 (green triangle) and the quantity of $A_{(985/1033)}$ from the control cotton samples (pink circle).

Overall there were no trends observed in the IR data from the experimental cotton samples for any of the three trials. These results suggest that the presence of decomposing remains is affecting the textile degradation process. The likely explanation for this difference in cotton degradation patterns with and without the presence of remains is the release of decomposition by-products during the decomposition process. This decomposition fluid leaches out when the carcass ruptures due to gas build-up and causes fluid to become absorbed into the textile fibers. The findings stated above are consistent with previous findings from Janaway [31] who found that cotton only survived when placed underneath an actively decomposing pig; where, due to its location the cotton was covered in semi-liquid soft tissue.

4.2.2.2 Polyester

Both normalised polyester bands were found to be unchanging in the control samples over the course of the experiment (Figure 4-7) for all three surface trials. This

helps demonstrate that the polyester did not degrade over the sampling period. The initial statistical analysis thus confirmed the lack of any visual changes in the polyester samples discussed in section 3.4.2.

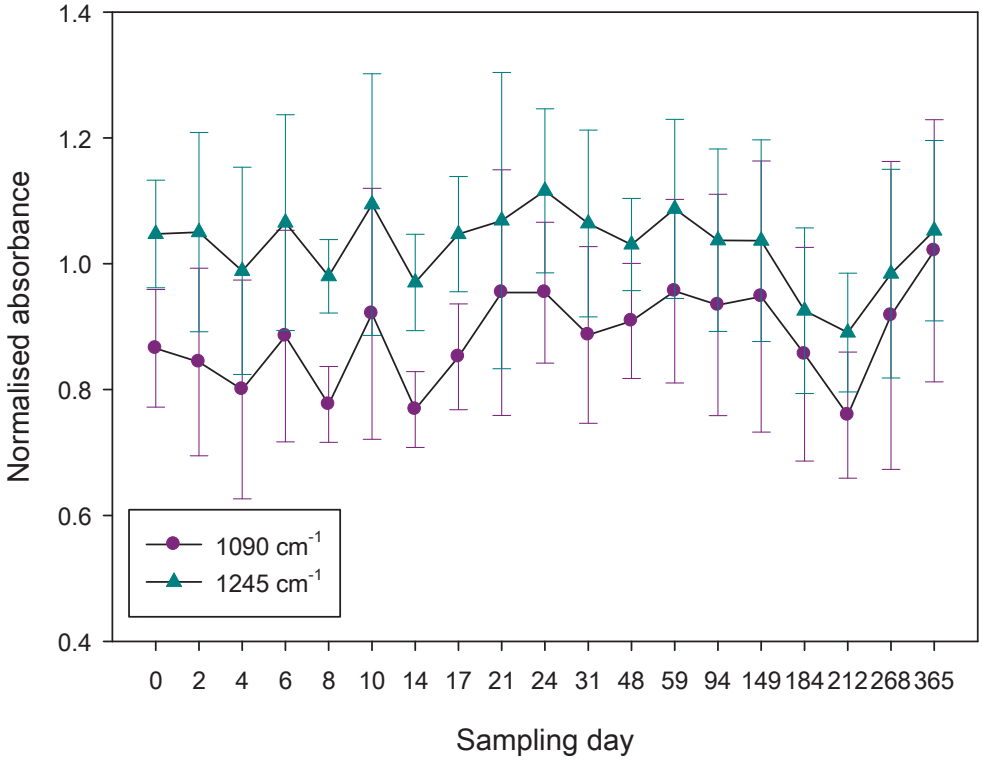

Figure 4-7: Quantity of $A_{(1090/1715)}$ as a function of time (purple circle) from T1 and the quantity of $A_{(1245/1715)}$ as a function of time (green triangle) from T1. The error bars represent one standard deviation based on 9 replicates.

There were some differences observed in the polyester related bands investigated within the experimental samples. However, no overall trends could be determined for any of the trials (T1-T3). A decrease in both FTIR bands was observed from Day 0 until Day 51 in T2, before both bands again increased the following sampling day (Figure 4-8). This difference was also determined statistically using a one-way ANOVA ($p = 0.05$), with Day 51 being different from days 0, 210 and 381 for 1090 cm^{-1}, and different from all sampling days except Day 32 for the 1245 cm^{-1} band. A difference was also found in both bands on Day 381 suggesting that these two days were outliers.

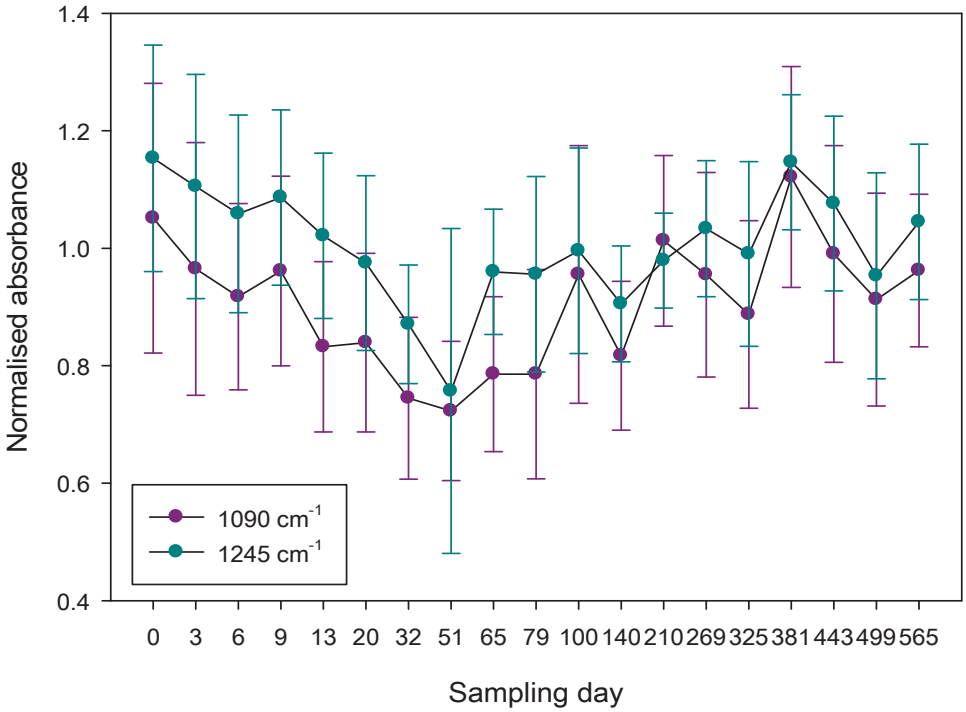

Figure 4-8: Amount of $A_{(1090/1715)}$ as a function of time (purple circle) and the quantity of $A_{(1245/1715)}$ as a function of time (green triangle) from the experimental polyester samples from T2. The error bars represent one standard deviation based on 9 replicates.

There were no trends observed with the initial statistical analysis of the polyester bands in the control or in the experimental textile samples. A study by Sammon et al. [88] found a decreased intensity of the ~1100 cm^{-1} band and an increased ~1250 cm^{-1} band in their samples as degradation occurred. As no changes were observed visually nor during the initial analysis, it is highly likely that there was no degradation to the polyester samples during the surface deposition studies. This coincides with what has been found of polyester in previous research; due to its synthetic composition polyester has been found to be very resistant to degradation in a natural environment [31, 43, 44].

4.2.2.3 Polyester – Cotton Blend

The control socks demonstrated a spike in both investigated bands in the cotton IR region on Day 94 of T1; this spike can be seen in Figure 4-9. This sampling day was also determined to be statistically different from the other sampling days through the use of a one-way ANOVA ($p = 0.05$). This day was likely an outlier as there were no other trends observed. This spike in Day 94 was not observed in the polyester related

normalised bands, in fact there were no statistical differences overall with the polyester portion of the socks. There were no trends observed with neither the cotton nor the polyester section of the polyester – cotton control samples from T2 and T3.

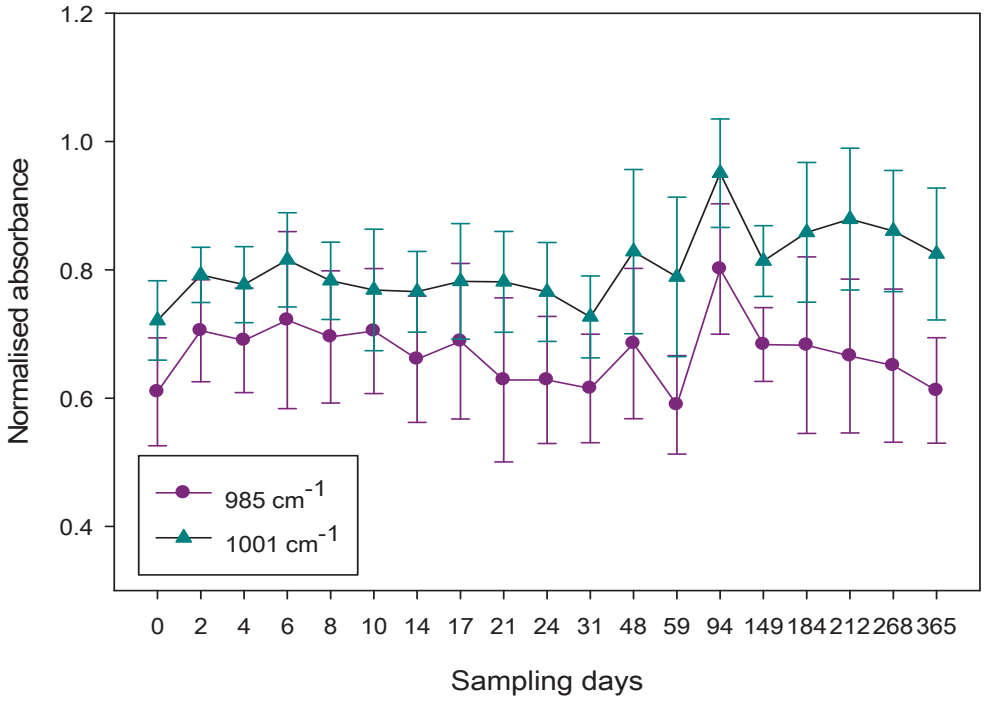

Figure 4-9: Amount of $A_{(985/1033)}$ as a function of time (purple circle) and the amount of $A_{(1001/1033)}$ as a function of time (green triangle) from the experimental polyester - cotton samples from T1. The error bars represent one standard deviation based on 9 replicates.

Similar to the 100 % cotton control samples, there appeared to be an increase in the 1001 cm^{-1} band in the experimental sock samples for all three trials (Figure 4-10a and Figure 4-10b). A one-way ANOVA ($p = 0.05$) determined that there was a statistical increase on Day 268 compared to days 0, 4, 14 and 17 in T1. Despite the visual increase in the 1001 cm^{-1} band in T2, there were no statistical differences over time. The second summer trial, T3 demonstrated a significant increase in some of the later days (days 45, 100, 185, 247 and 303) compared to days 3 and 6.

When graphing the 985 cm^{-1} band, it appeared to be decreasing over time for T2, a one-way ANOVA ($p = 0.05$) determined that the band ratio on Day 3 was significantly higher than that of days 381 and 443. No other significant changes were found.

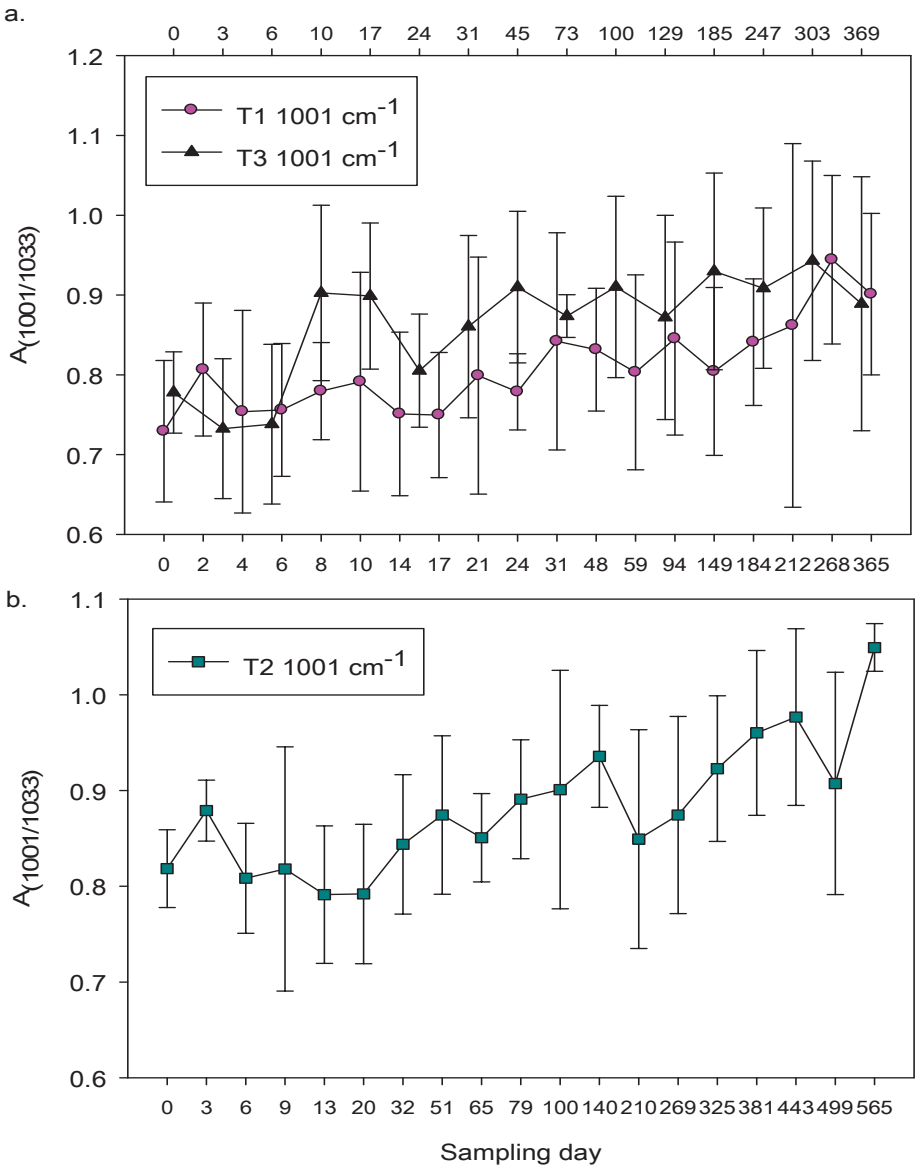

Figure 4-10: Quantity of $A_{(1001/1033)}$ as a function of time for the experimental polyester – cotton samples from: a) T1 (pink circle) and T3 (black triangle); and b) T2 (green square). The error bars represent one standard deviation based on 9 replicates.

The bands associated with the polyester material in the blend did not show any changes over time in any of the three surface trials. The initial analysis demonstrated that it is unlikely that the polyester component of the polyester – cotton blend samples degraded during the timeline of the experiment. This suggests that even when the polyester was found in blended samples, it demonstrated resistance to degradation in the soil environment.

4.2.3 Principal Component Analysis

Although the initial statistical approach helped determine certain bands of interest for the different textile types, especially for the cotton samples, additional multivariate analysis was performed. The multivariate approach was selected as spectral data has a large amount of influences that needs to be accounted for statistically. Multivariate analysis also helps visually demonstrate any differences between the samples over time and show any groupings of samples.

4.2.3.1 Cotton

In order to further validate the importance of the 1001 cm^{-1} band and determine if the 1033 cm^{-1} band is also changing and, thus, playing a part in the change observed for the 1001 cm^{-1} normalised band, principal component analysis was carried out on the cotton data from the control sites of T1. The first two factors (PC-1 and PC-2) were found to describe 45 % and 22 % of the variance, respectively. These factors were plotted against each other in order to get a two-dimensional representation of the data that captures most of the variance (Figure 4-11). The other factors were also investigated for any underlying information in the data, however no other factors were found to impact the results or provide additional information, and a scree plot was added to demonstrate that most variation was obtained with the two first factors (Figure 4-12).

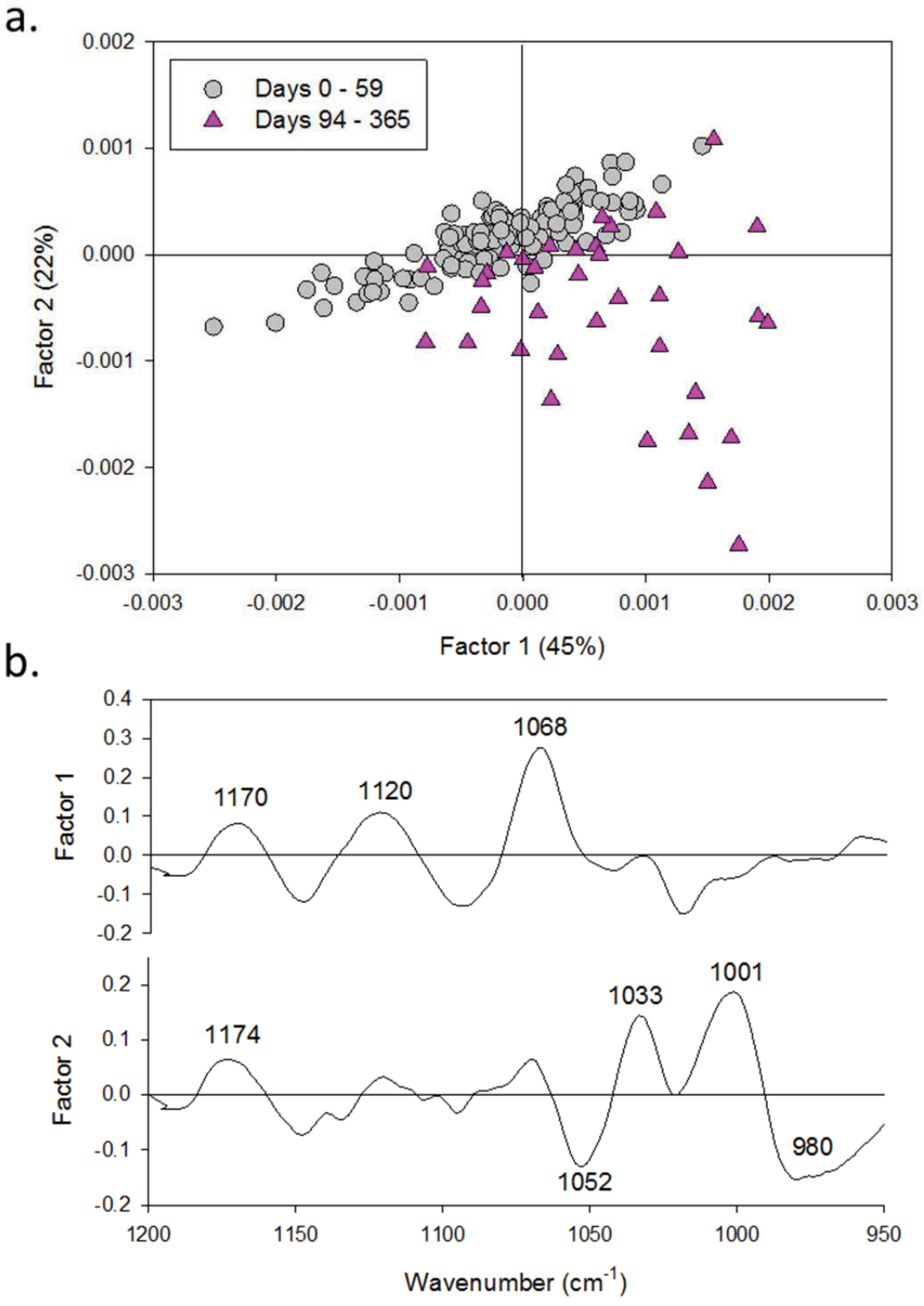

Figure 4-11: a) Principal component analysis of the cotton control spectra from T1. The PCA plot demonstrates that the later sampling days (pink triangle) could be separated from the earlier sampling days (grey circle). b) The loadings plot demonstrates the influence on the PCA groupings from bands in the spectra.

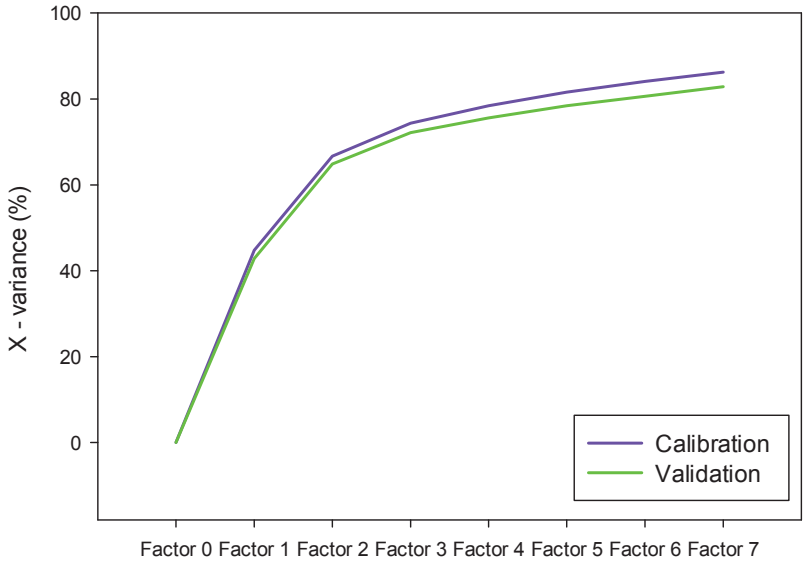

Figure 4-12: Scree plot showing the variation explained by each factor in the principal component analysis of cotton control spectra from T1.

From the PCA plot it became clear that the samples could be separated into two groups, denoted early sampling days (days 0 – 59) and late sampling days (days 94 – 365). The early sampling days were grouped well together, whereas the later sampling days were spread out from this original group. The later sampling days could not be grouped together, but were clearly identified due to their separation from the main group (early sampling days). All the later samples were spreading out from the early sampling days in a similar fashion. From the loadings plot it was determined that this was due to a decrease in both the 1001 and the 1033 cm^{-1} bands based on the factor 2 (y-axis) loadings plot (Figure 4-11b). This means that a change in the spectral region where hydrolysis occurs was observed, demonstrating that textile degradation is happening in the later sampling days. The later sampling days also exhibited an increase in the ~1052 cm^{-1} band corresponding to C-O stretching. Following the analysis of the winter trial (T2), the same pattern was observed (Figure 4-13a). The early sampling days were again grouped along the y-axis, with the later sampling days spreading out downwards towards negative y-values (Figure 4-13a). The loadings plot demonstrated that the 1001 cm^{-1} and 1033 cm^{-1} bands were again a factor in the separation along the y-axis (Figure 4-13b). The other factors were also investigated, no other factors were found to impact the results or provide additional information, although the first two factors (Factor 1 and Factor 2) only explained 51 % of the variance (Figure 4-14).

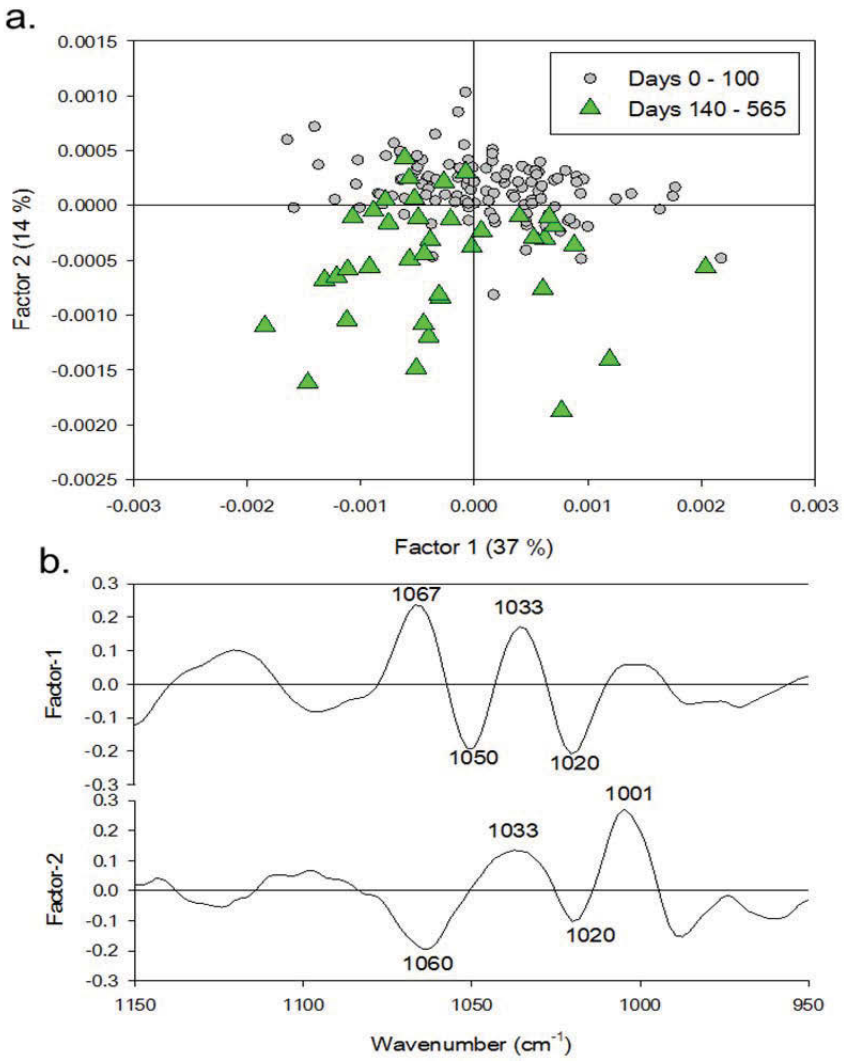

Figure 4-13: a) Principal component analysis of the cotton control spectra from T2 with the later sampling days (green triangle); and the earlier sampling days (grey circle). b) The loadings plot shows the influence on the PCA groupings from bands in the spectra.

A difference in the timing of the degradation was obtained between the summer and winter trials, this can be demonstrated by comparing the first summer trial (T1) with the winter trial (T2) as the separation in the data set from T2 did not occur until Day 140, compared to Day 94 for T1. In order to determine if this delay in degradation observed was due to the temperature difference the ADD values at the time of the data separation were compared. The summer trial (T1) was found to end the early sampling days sampling group on ADD 1315, and samples beyond ADD 1968 were classified in the later sampling days. Similar ADD values were obtained in the winter study (T2), with the end of the early sampling days extending to and including ADD 1357. The later sampling days grouping started on ADD 2078. Thus, comparing the ADD values

that account for temperature, demonstrated that the difference in ambient temperature was likely to be responsible for the delay in degradation observed in T2. This difference in temperature between the two seasons could have resulted in differences in microbiological activity between the summer and winter seasons as microbial communities are largely affected by temperature [90-92]. The difference in pH between the two trials might also be a factor in the delayed onset of textile degradation seen in T2. The control site for T1 was found to have a fairly stable pH around 5 throughout the study, whereas the soil at the control sites had pH values that were found to be a lot more variable and would fluctuate between pH 5 and 7. This fluctuation in pH might prove non – conducive for certain bacteria and fungi. The lower pH experienced in T1 might also be due to the fact that cotton is highly vulnerable to hydrolysis in acidic environments and hydrolysis may have initiated earlier in T1 than during T2.

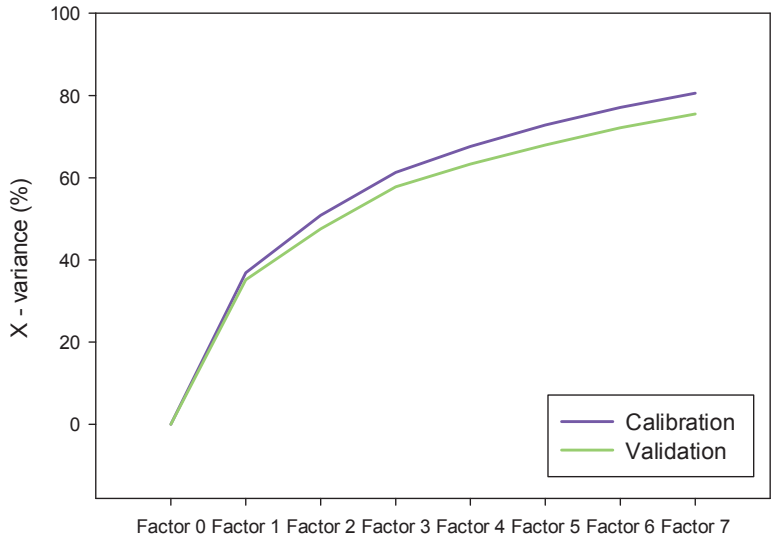

Figure 4-14: Scree plot showing the variation explained by each factor in the principal component analysis of cotton control spectra from T2.

Despite the lack of any significant trends observed during the initial statistical analysis, the experimental cotton samples were investigated using PCA in order to determine if a more powerful statistical tool could find any differences in the textile over time. No groupings were obtained with the data from any of the trials, an example PCA plot of what was commonly observed can be found in Figure 4-15a which shows the data from T1.

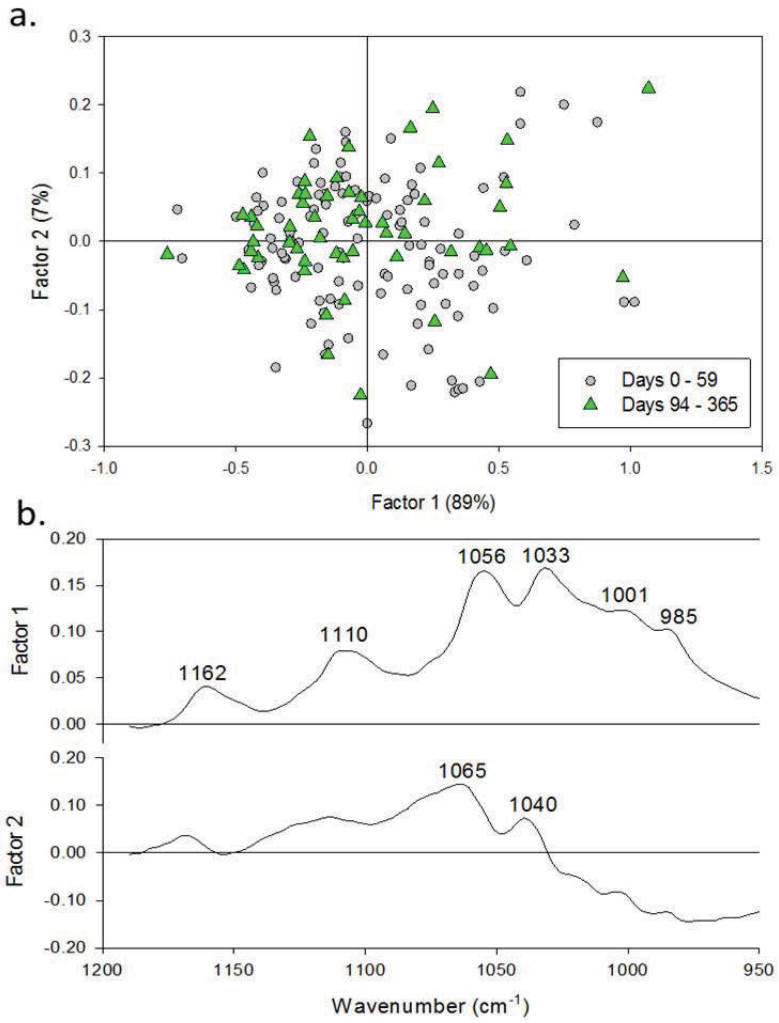

Figure 4-15: a) Principal component analysis of the cotton experimental spectra from T1. The PCA plot demonstrates that the later sampling days (green triangle) could not be distinguished from the earlier sampling days (grey circle). b) The loadings plot demonstrates the influence on the PCA groupings from bands in the spectra.

A model pig burial study carried out in Bradford in the United Kingdom has provided insight into the effect of decomposition on textile preservation [36, 93]. It was observed that while natural fibres including cotton, denim and wool were susceptible to decay in the well-drained and biologically active soil when positioned above the pigs, some preservation was seen with the same textiles when placed below the pigs in the same environment. Their findings were confirmed during the present study as the cotton samples in presence of remains did not demonstrate any signs of degradation.

4.2.3.2 Polyester

Similarly to the experimental cotton samples, PCA was conducted on the FTIR data from all the polyester samples, despite the lack of any trends during the visual and

initial data analysis. Figure 4-16a shows the principal component analysis plot obtained using the control samples from T1; no trends were observed, the graph shows the plot of Factor 1 versus Factor 2, however all factors were investigated and plotted against each other, no smaller underlying trends were observed. Identical results were found for the other surface trials (T2 and T3). The loadings plot (Figure 4-16b) shows that the bands influencing the data sets were comparable to the ones selected during the initial spectral analysis.

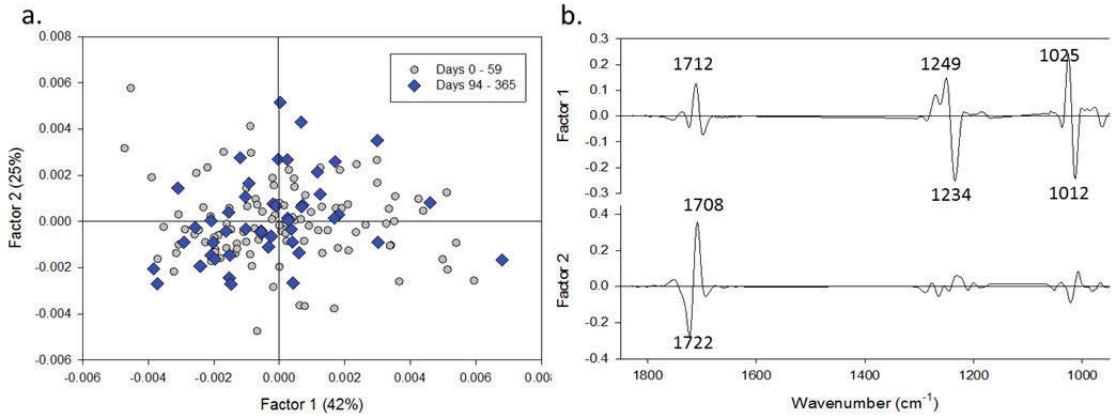

Figure 4-16: a) Principal component analysis of the control polyester spectra from T1. The PCA plot demonstrates that the later sampling days (black triangle), selected based on the cotton results from the same trial, could not be separated from the earlier sampling days (grey circle), again based on the change determined in the cotton data. b) The loadings plot demonstrates the influence on the PCA groupings from ester bands in the spectra.

The polyester PCA plots from all trials further validated that there was no difference in the spectral data for the polyester samples, and no degradation was observed. This corresponds to what is known from literature, as polyester and similar synthetic textiles have been found to degrade very slowly under natural conditions [31, 43, 44]. PCA was also conducted on the polyester samples from the experimental sites, no trends were obtained with any of the trials.

4.2.3.3 Polyester – Cotton Blend

The cotton infrared region from the control socks analysed from T1 demonstrated a similar trend to that found for the 100 % cotton control samples, however, it was seen to a lesser extent (Figure 4-17a and Figure 4-17b). When examining the data from the winter trial (T2) the same trend was observed (Figure 4-17c and Figure 4-17d). The fact that the separation between the undamaged and degraded textile samples was less distinct in the polyester – cotton blend samples compared to the

100 % cotton samples may indicate that the presence of the synthetic material, along with the cotton, slightly inhibited the cotton degradation. Again all the factors were investigated, however no trends were found, despite the first two factors only accounting for 56 and 50 % of the variation in the spectra from T1 and T2, respectively (Figure 4-18a and Figure 4-18b). There were no separations observed in the cotton components of the sock samples from T3. The polyester IR region of the control polyester – cotton blend samples did not exhibit any trends as predicted and further validated that polyester did not degrade during the time frame of any of the trials. The experimental polyester – cotton samples also did not demonstrate any trends in the data during the principal component analysis for either cotton or polyester infrared section.

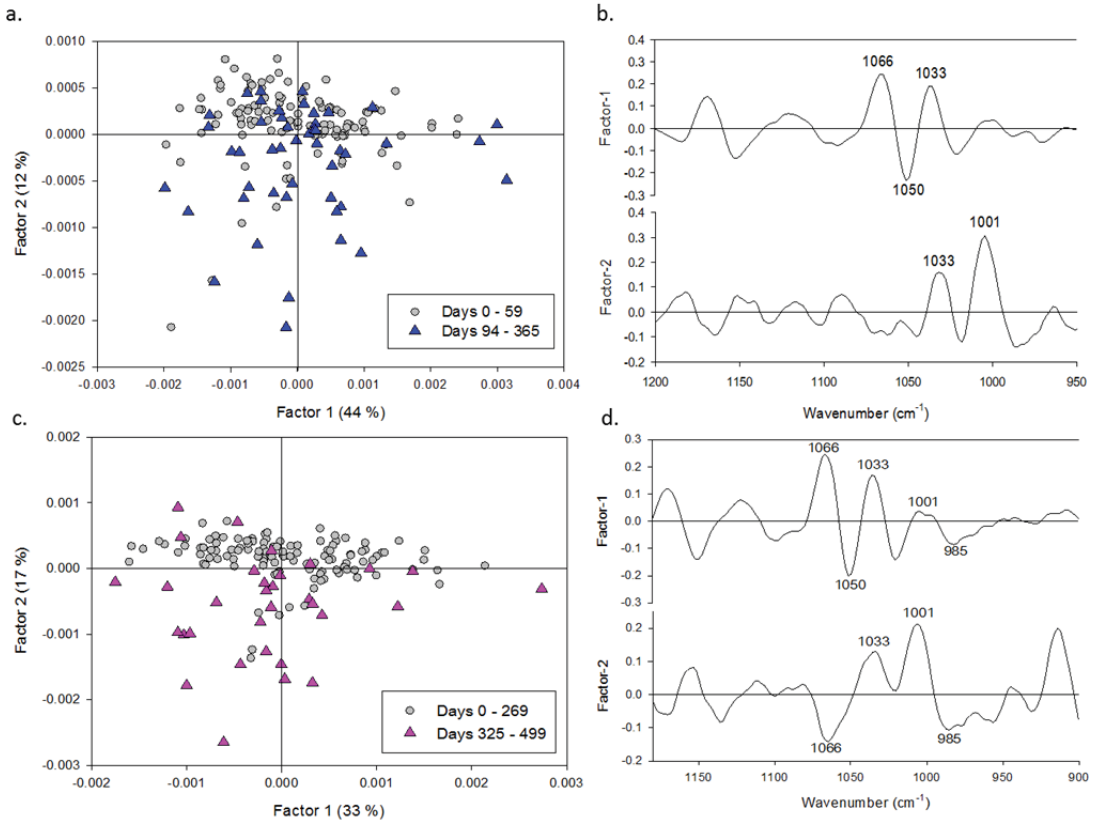

Figure 4-17: a) Principal component analysis of the cotton section of the polyester – cotton blend samples from the control sites from the summer trial (T1) showing later sampling days (blue triangle); and the earlier sampling days (grey circle). b) The loadings plot shows the influence on the PCA groupings from cotton bands in the spectra. c) Principal component analysis of the cotton section of the polyester – cotton blend samples from the control sites from the winter trial (T2). d) The loadings plot showing the influence on the PCA groupings from cotton bands in the spectra.

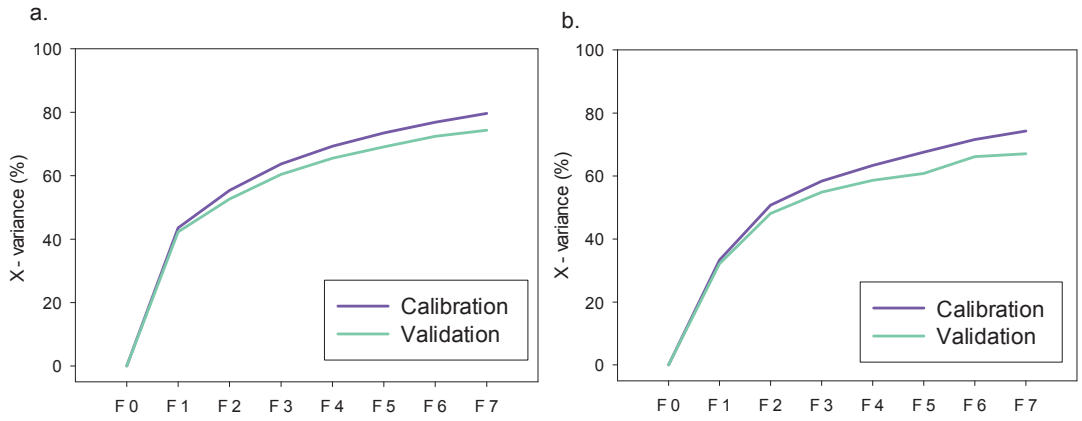

Figure 4-18: a) Scree plot showing the variation explained by each factor (F0 – F7) during principal component analysis of the control cotton section from the polyester – cotton blend samples from the summer trial (T1). b) Scree plot showing the variation explained by each factor (F0 – F7) during principal component analysis of the control cotton section from the polyester – cotton blend samples from the winter trial (T2).

4.3 Conclusions

Visual examination of the cotton IR bands saw a disappearance of some of the C-O stretching bands. The development of more advanced statistical software enabled the spectral data to be analysed using multivariate analysis and chemometrics. Principal component analysis of the cotton control data demonstrated a clear separation between the earlier sampling days and the later sampling days where degradation had occurred. Again the separation in the data was determined to be related to the C-O stretching regions of the cotton spectrum. This pattern of degradation was determined to occur at a later stage in the winter trial, as the separation in the data set from T2 did not occur until Day 140, compared to Day 94 for T1. This indicated that seasonal variation is a factor in textile degradation.

Contrary to the natural textile samples, the synthetic material did not show any signs of degradation either visually or statistically throughout the surface trials. The cotton section from the polyester – cotton blend samples were found to be very similar to the 100 % cotton samples, however the PCA analysis did show that the degradation patterns were less distinct in both the summer and winter trial for the blend samples. These findings indicated that the presence of the synthetic material might have inhibited the degradation of the natural material.

The presence of the remains was also found to greatly impact textile degradation. It was determined that the presence of the decomposing remains were preventing textile degradation, this has been hypothesised to be due to the release of decomposition fluid during the decomposition process as the fluid becomes absorbed into the textile samples and will be investigated further.

Overall the use of statistics to analyse the data provided a better foundation for the interpretation of the data obtained using ATR-FTIR spectroscopy, and also gave further insight into textile degradation in both a natural environment and in the presence of decomposing remains.

Chapter 5: INFRARED ANALYSIS OF DECOMPOSITION FLUID IN TEXTILES

Chapter 5: INFRARED ANALYSIS OF DECOMPOSITION FLUID IN TEXTILES

5.1 Introduction

Textiles associated with remains have been a subject of interest for a long time, most research has been focused specifically on how textiles affect the decomposition process [10, 21, 54-56], while little attention has been paid to how the decomposition process affects the textiles. There have been reports of textiles that would commonly degrade rapidly, being found intact in association with remains. There has been very little work studying the specific mechanisms that prevent or delay this textile degradation. It has been speculated that this difference in rate of degradation, or lack thereof, is due to the leaching of the decomposition fluid, which then becomes absorbed into the textiles.

During the natural decomposition process, lipids, proteins, and carbohydrates are broken down into smaller molecules. These decomposition by-products are the major components in decomposition fluid [48]. Monitoring the changes in protein content and the lipid profile (with the subsequent conversion of triglycerides into fatty acids) over time on textiles associated with decomposing remains may demonstrate how, and during which decomposition stages, the decomposition fluid affects the textiles. It may also aid in understanding which decomposition by-product components are responsible for the possible inhibition of textile degradation.

This chapter will focus on providing a statistical approach to the analysis of ATR-FTIR spectra of decomposition by-products by using multivariate analysis and chemometrics. The use of statistics to analyse the data will provide a better foundation for the interpretation of the data obtained using ATR-FTIR spectroscopy (see section 2.3). The aim was to better demonstrate the effect of decomposition by-products (from lipids and proteins) on textile degradation with the use of ATR-FTIR spectroscopy. Data was taken from the experimental sites (with decomposing remains) from the three different surface trials (section 2.1.1), and all textile types (cotton, polyester and cotton – polyester blend) in order to compare textile type.

5.2 Results and Discussion

The textile samples collected from the control and experimental sites were analysed using ATR-FTIR spectroscopy. Particular experimental textile samples showed large infrared bands corresponding to the presence of proteins and/or lipids, which can be attributed to decomposition fluid becoming absorbed into the textile. These bands were not observed in any of the control samples throughout the duration of the different trials.

5.2.1 Cotton

Figure 5-1 displays representative ATR-FTIR spectra of cotton samples with high lipid (Figure 5-1a) and high protein (Figure 5-1b) contents. Some of the experimental replicates were found to contain bands corresponding to only proteins or lipids; however, the presence of both was more commonly detected in the majority of experimental samples.

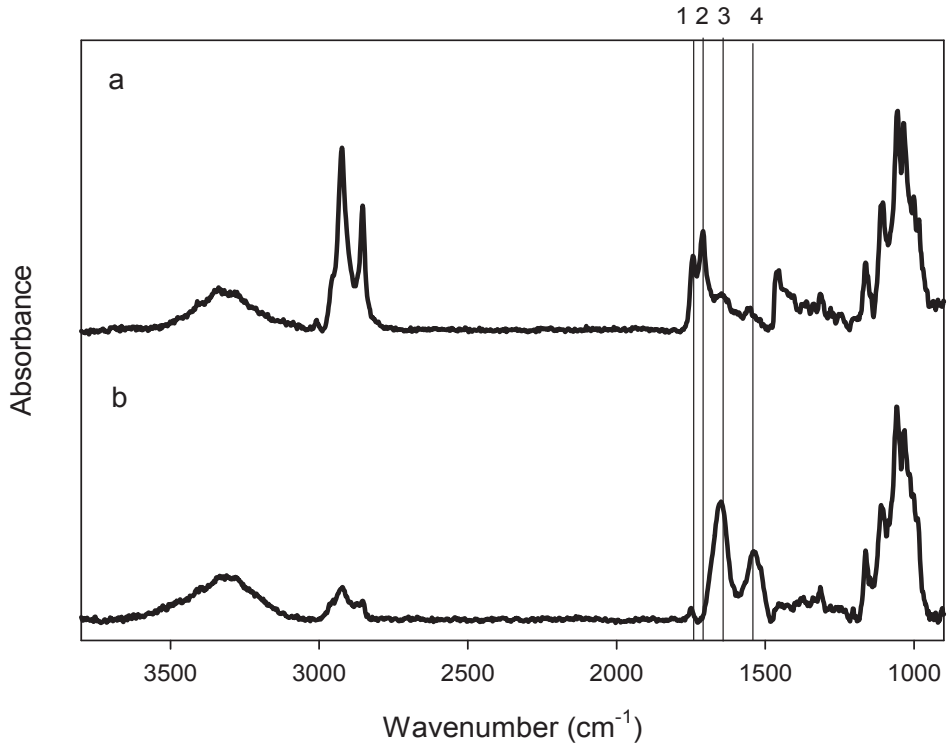

Figure 5-1: ATR-FTIR spectra of samples with a) high lipid content with large bands at 1) ~1735 cm^{-1}; and 2) ~1715 cm^{-1}; and b) high protein content demonstrated by a large occurrence of 3) ~1648 cm^{-1}; and 4) ~1543 cm^{-1}.

5.2.1.1 Protein

The bands observed at ~1545 cm^{-1} and ~1648 cm^{-1} (corresponding to amide II and amide I, respectively) were used to follow the change in protein of the decomposition fluid absorbed in the experimental textile samples. The amide I band is known to be very sensitive to the conformation of proteins and its frequency and intensity is determined by the backbone conformation and hydrogen bonding of the protein, whereas amide II is more stable [94], thus their ratio is often used to monitor protein structural change [95]. In order to account for any instrumental variation over time, the protein bands were normalised. This was done by taking the ratio of 1545 / 1648 cm^{-1}; this data was then used for the initial statistical analysis.

It was important to determine whether the protein detected resulted from blood transferred to the clothing initially on Day 0 or whether the protein bands were a result of the decomposition fluid purging from the remains as the skin ruptured. Overall, the appearance of the protein-related bands was observed more frequently during the later sampling days. This suggests that the presence of protein-related bands was most likely due to decomposition fluid, rather than from the initial blood transferred onto the t-shirt.

Figure 5-2 illustrates the absorbance ratios of the protein amide II and amide I bands as a function of time since death. A one-way ANOVA ($p = 0.05$) statistical test with a significance level of 0.05 found that the three different experimental sites were comparable and could be treated as replicates, giving a total of nine replicates for each day. During the year-long decomposition in the process there does not appear to be any significant changes to the protein by-products absorbed into the textile samples, apart from Day 59 and Day 184 where there was an apparent increase in the absorbance of the 1545 cm^{-1} band (Figure 5-2). A one-way ANOVA ($p = 0.05$) was conducted to compare the protein-related band ratio over time and significant differences were found between days 59 and 184, and all other sampling days.

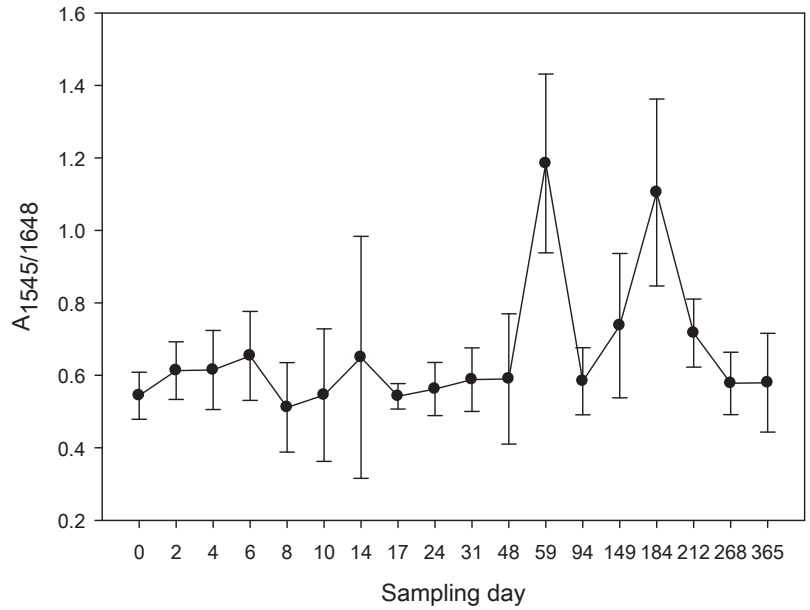

Figure 5-2: Protein infrared band ratio as a function of time since death. The error bars represent one standard deviation based on nine replicates.

Similar data was obtained for the other surface trials (T2 and T3), with the protein normalised band showing little to no visual trend over the course of the trials (for example with T3 as seen in Figure 5-3), and no statistical differences.

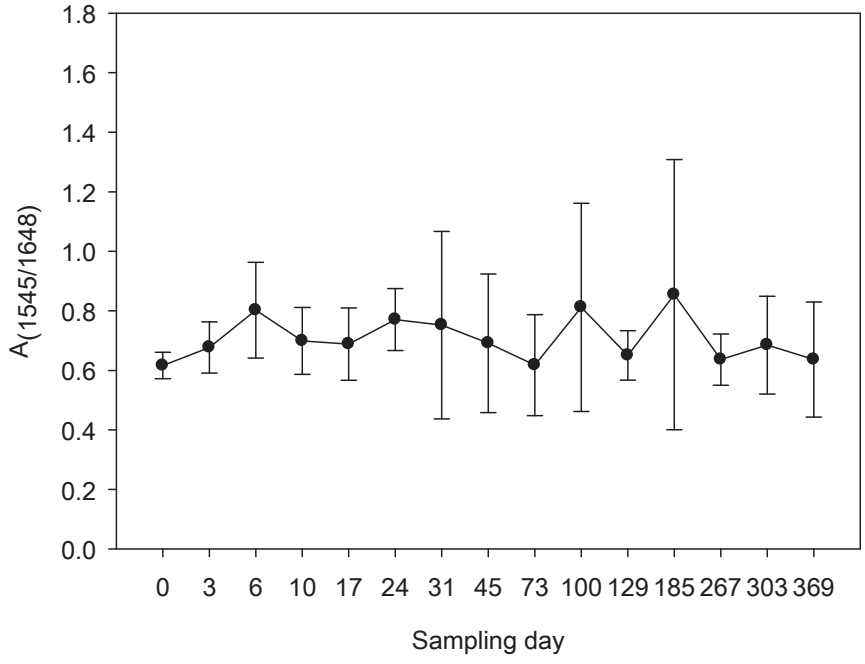

Figure 5-3: Protein infrared band ratio as a function of time since death from T3. The error bars represent one standard deviation based on nine replicates.

Based on these findings, the protein by-products do not appear to exhibit any significant structural changes during the year-long timeline. Thus, there is no evidence of any structural change in the secondary structure of the proteins [95]. Therefore, the evolution of protein-related bands may not be an accurate indication of the changes undergone by the decomposition by-products in the textile samples.

An interesting finding was observed in T2 for the protein bands, as the remains took a long time to rupture and release decomposition fluid due to the colder winter temperatures. The protein bands could be separated into pre-rupture (originating from the blood due to the head wound during euthanasia) and post-rupture (with protein bands more likely resulting from the breakdown of amino acids due to internal bacterial action). PCA was conducted on this data in order to visually and statistically demonstrate any groupings (Figure 5-4).

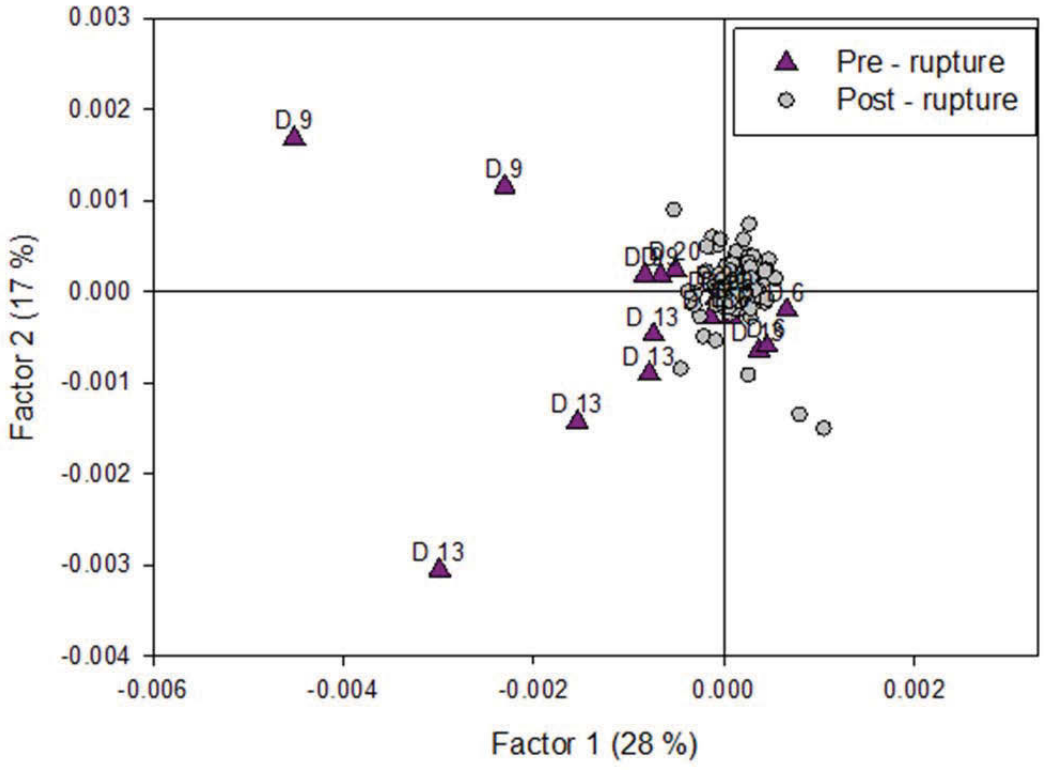

Figure 5-4: PCA plot of the protein related bands from T2 with pre-rupture samples (purple triangles) and post-rupture samples (grey circles).

Some of the replicates of days 6, 13, and 20 were found to be grouped with the post-rupture samples; however some were distinctly separated from the majority of the other samples.

5.2.1.2 Lipids

Infrared bands associated with lipid degradation as a result of soft tissue decomposition were observed in a number of the experimental textile samples; the bands corresponded to triglycerides (~1735 cm^{-1}), fatty acids (~1715 cm^{-1}), and salts of fatty acids (1570 – 1538 cm^{-1}) as identified in previous studies [26, 49, 96, 97]. In addition, a weak C-H stretching band near 2920 cm^{-1} was observed. Initially a large band at 1735 cm^{-1} corresponding to triglyceride C=O stretching was observed in the experimental samples from T1 as illustrated in Figure 5-5a. By Day 8 this triglyceride band diminished as the free fatty acid C=O stretching band at 1715 cm^{-1} began increasing as seen in Figure 5-5b. After 31 days the triglyceride band had almost disappeared and the presence of salts of fatty acids in the 1570 – 1538 cm^{-1} region became visible due to the appearance of fatty acid carboxylate C-O stretching (Figure 5-5c); this trend was observed more clearly on Day 94 (Figure 5-5d). Similar results were obtained by Stuart et al. [96] during the analysis of extracted adipocere samples, which is a type of lipid decomposition product observed when sufficient moisture is present. Wherein the adipose tissue had a large band at 1744 cm^{-1} due to triglyceride C=O stretching, this band subsequently disappeared in the samples and a C=O stretching band appeared at 1710 cm^{-1} indicating the presence of free fatty acids. Adipocere is a possible result from the breakdown of adipose tissue and thus is comparable to a later lipid degradation stage. Fatty acid salt carboxylate C-O stretching bands at 1575 cm^{-1} and 1539 cm^{-1} were also observed in the adipocere spectrum [96], corresponding to the findings in Figure 5-5 of the present study and again is indicative of a later stage of lipid degradation.

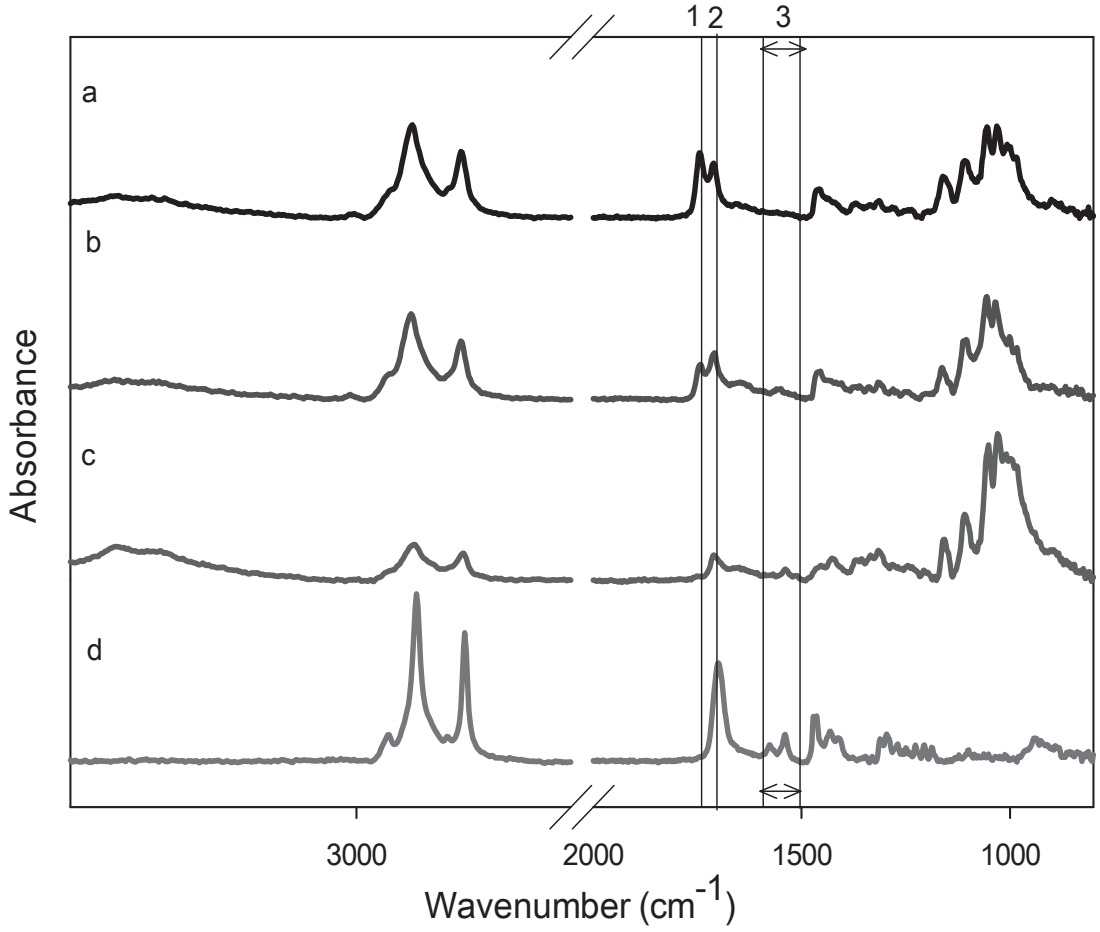

Figure 5-5: Typical infrared spectra for the experimental cotton samples on a) day 4; b) Day 8; c) Day 31; and d) Day 94. The vertical lines highlight the regions of interest: 1) triglyceride C=O stretching (~1735 cm^{-1}); 2) C=O stretching corresponding to free fatty acid (~1715 cm^{-1}); and 3) fatty acid carboxylate C-O stretching (1570 – 1538 cm^{-1}).

In order to further investigate the lipid characteristics of the decomposition by-products statistical analysis was conducted. Two different band ratios were used to examine the lipid profile from the experimental textile samples: 1715 cm^{-1} / 2920 cm^{-1} and ~1735 cm^{-1} / 2920 cm^{-1}. The 2920 cm^{-1} C-H stretching band present in all spectra provides a suitable reference band due to its intensity and relative stability [96]. The 1570 – 1538 cm^{-1} band appeared too infrequently and was not used in the statistical analysis. Figure 5-6 shows the change in triglyceride and fatty acid ratios over time. On certain sampling days the error bars were quite large and this is attributed to the fact that when performing ATR-FTIR spectroscopy only a relatively small section of the textile samples were analysed. Attempts were made to analyse areas visually affected by

decomposition fluids in order to determine if and how these by-products may be inhibiting textile degradation.

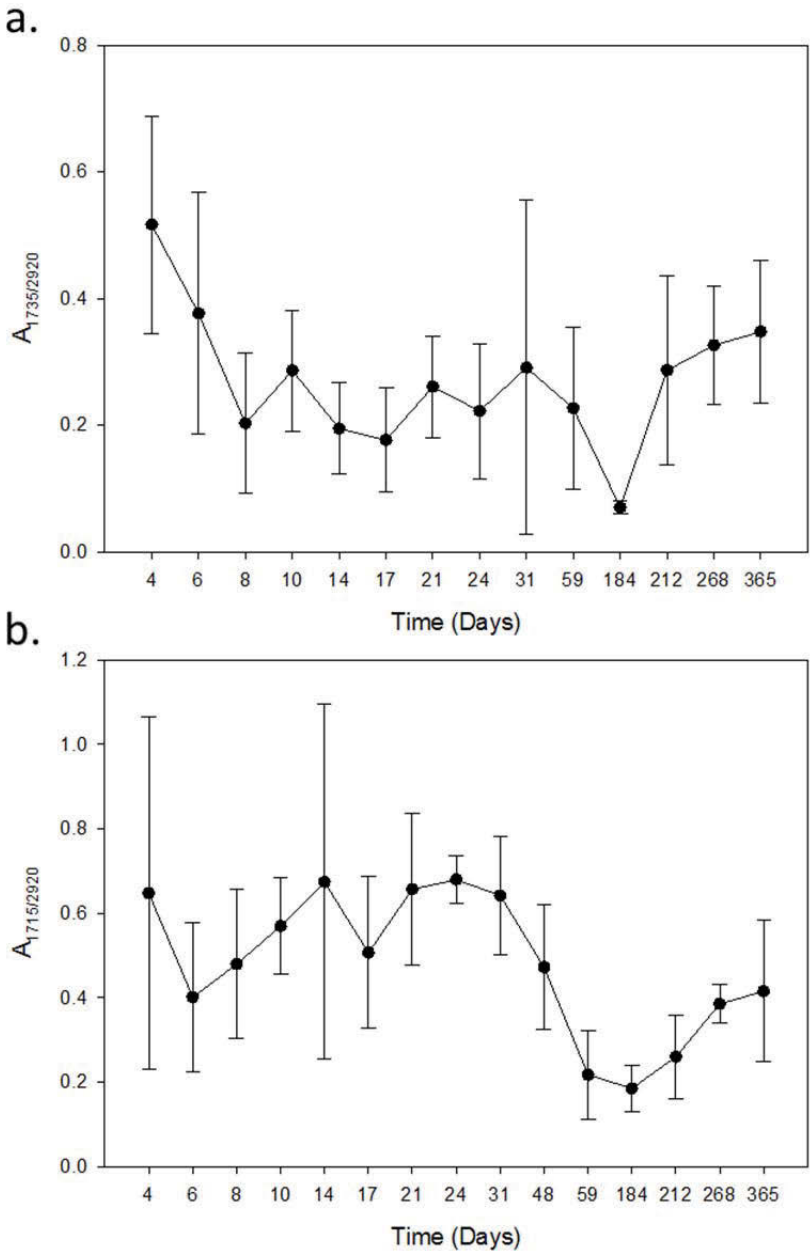

Figure 5-6: a) Quantity of triglyceride ($A_{(1735/2920)}$) as a function of time and b) free fatty acid ratio ($A_{(1715/2920)}$) as a function of time. The error bars represent one standard deviation based on 9 replicates.

Initially, the triglyceride C=O stretching band was observed to decrease during the first week before remaining relatively stable throughout the remainder of the study (Figure 5-6a), with the exception of Day 184, where the band was only present in two out of nine replicates. Day 48 was also found to have an absence of triglycerides and

was therefore not included in Figure 5-6a. Statistically Day 4 was found to be significantly different from days 8 to 212, using a one-way ANOVA ($p = 0.05$), supporting the observation that triglycerides initially decreased. The free fatty acid band appeared to be quite stable initially before decreasing after Day 31 (Figure 5-6b), and subsequently increasing between days 184 and 365. No statistical trends were obtained.

PCA was conducted on the spectra collected for samples up to, and including, Day 48 from T1 in order to better visualise the changes in lipid profile during the early stages of decomposition. Based on initial trends seen with the PCA results (Figure 5-7a) the samples appeared to be grouped into three general regions of the PCA plot based on the lipid characteristics of the samples: little to no lipid degradation (days 2 – 8), some lipid degradation (days 10 – 21), and advanced lipid degradation (days 24 – 48).

The PCA loadings plot (Figure 5-7b) indicates the bands in the spectra that drive the variability between the samples observed in the PCA scores plot. In this case, Factor-1 accounted for 33 % of the variation between the samples (Figure 5-8), and the loadings plot (Figure 5-7b) demonstrated that the separation along the x-axis in Figure 5-7a was mainly attributed to a difference in the 1700 – 1715 cm^{-1} and 1735 – 1745 cm^{-1} bands. A high absorbance of the triglyceride band (1735 – 1745 cm^{-1}) caused the samples to have a positive value along the x-axis as seen with sampling days 2 – 8, while a high free fatty acid band (1700 – 1715 cm^{-1}) absorbance caused samples to have a negative position along the x-axis (found to be occurring in the later sampling days). As the data was transformed using the second derivative, a positive peak in the loadings plot indicated a decrease in absorbance for points that had positive scores in the PCA plot. A separation in the 1450 – 1475 cm^{-1} region corresponding to C-H bending was also observed. The Factor-2 loadings (Figure 5-7b) accounted for 24 % of the variation between the sampling days and here the separation was driven mainly by a difference in triglyceride bands at 1745 cm^{-1} (negative y-value) and 1725 cm^{-1} (positive y-value). A larger absorbance in ~1710 cm^{-1} resulted in the plot being into the negative region of the y-axis.

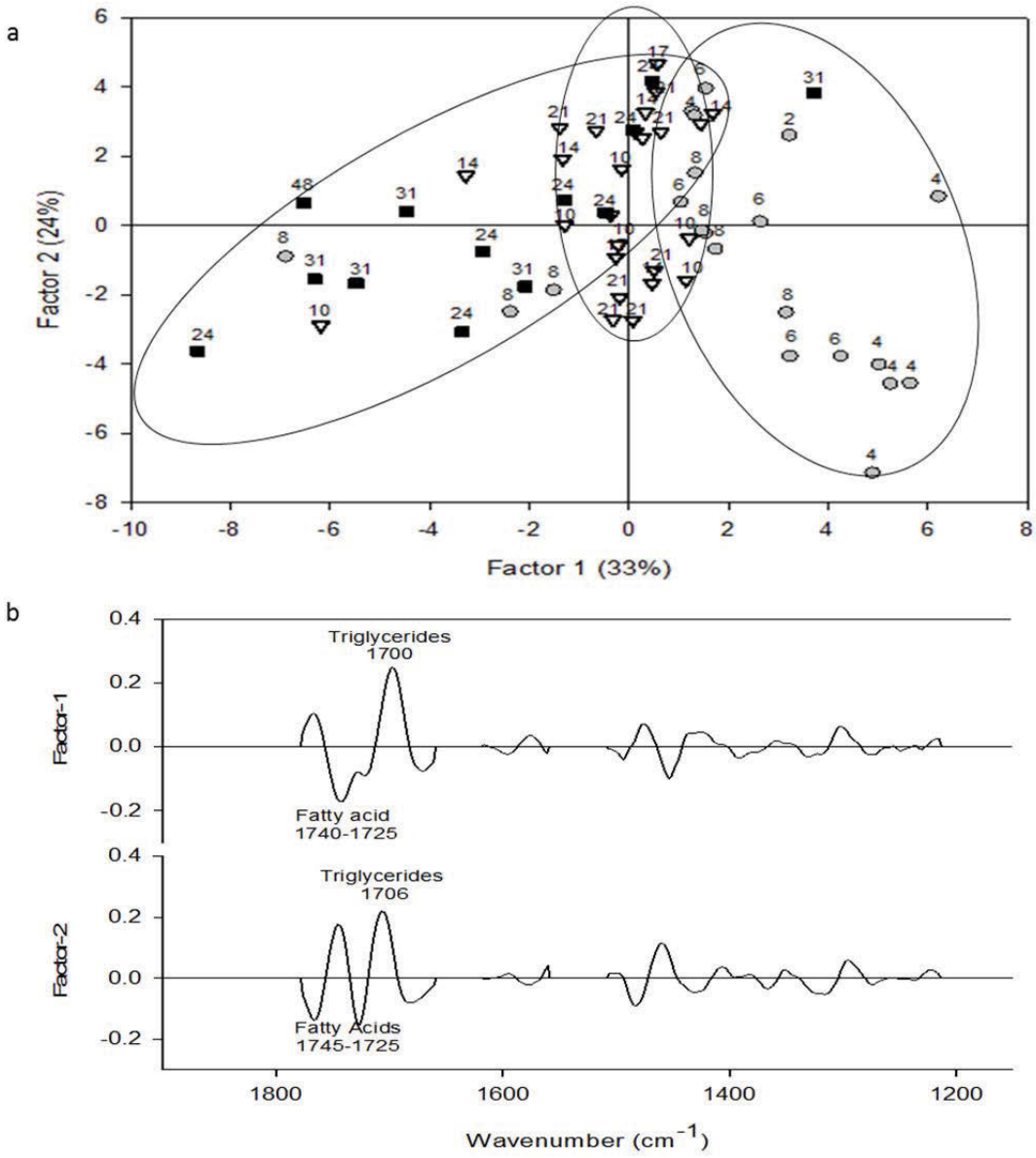

Figure 5-7: a) Principal component analysis on days 4 - 48. The PCA plot grouped into little to no lipid degradation (days 2 – 8, grey circle), some lipid degradation (days 10 – 21, triangle) and advanced lipid degradation (days 24 – 48, black square). b) The loadings plot show the influence on the PCA groupings from the lipid bands in the spectra.

It is apparent that day 2 – 8 were grouped together, with the exception of three replicates from Day 8 (Figure 5-7a). Comparing the spectra obtained on Day 8 visually, it was observed that most had the presence of a fatty acid band that was slightly larger than the triglyceride band. However, in the spectra it was observed that the outliers from the 'little to no lipid degradation' category for lipid degradation area in the PCA plot had a fatty acid band that was shifted towards 1698 cm^{-1} and was significantly larger than the fatty acid band, which was also observed to be shifting towards 1745 cm^{-1}.

From Day 24 until Day 48, there was another grouping. All but one sample (a single Day 31 replicate) has fatty acid band absorbance values that are larger than the triglyceride band absorbance in the spectrum, indicating that the textile samples contain lipids in the later stages of degradation. A single replicate on Day 31 shows the opposite relationship, indicating a much earlier lipid degradation stage for the textile. However, all other replicates were consistent with each other and were well within the grouping with the 'advanced lipid degradation' stage. The intermediate days (days 10 – 21) made the least distinct grouping, overlapping largely with the 'little to no lipid degradation' and the 'advanced lipid degradation' groupings. This overlapping was expected as the intermediate grouping was a transition stage of lipid degradation.

Both the visual and statistical analysis demonstrates that during the early days of the study the presence of triglycerides was high, showing that samples were associated with the early stages of decomposition. The appearance of this band then decreased, as seen in Figure 5-6a and determined statistically, before the band remained stable at very low to almost absent levels. An opposite trend was seen with the free fatty acid band as well as the salts of fatty acids. These were not initially detected. The fatty acid ratio remained stable initially, with a possible small increase (Figure 5-6b), during this time the salt of fatty acid bands became apparent, more specifically on Day 14. Thus, the textile samples exhibited lipid patterns consistent with tissue degradation as triglycerides were degrading into free fatty acids, and the presence of salts of fatty acids demonstrate that the remains had entered putrefaction.

However, the free fatty acid band did show a decrease around Day 31 until Day 184. The presence of both triglycerides and fatty acids was frequently observed during the first 59 and 31 days, respectively, in the spectra. After this day the bands were only encountered sporadically in the spectra (with the exception of Day 212). Towards the advanced decay stage of the remains there is no new influx of decomposition fluids into the surroundings, including the clothing. On Day 184 the remains entered the dry/remains stage, meaning that no soft tissue remained. This may represent the cause of the decrease of the normalised fatty acid band as the textiles dried out markedly. The intra-day sample replicates became highly variable after Day 94, reducing the effectiveness of the PCA.

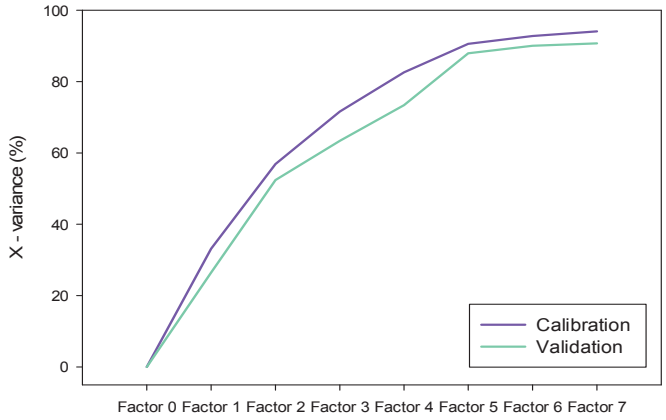

Figure 5-8: Scree plot showing the variation explained by each factor during principal component analysis of the lipid bands from the cotton samples from T1.

In order to monitor the lipid degradation in the later periods of decomposition the data from T2 was used. This trial commenced in the winter months and thus caused each decomposition stage to commence at a later stage, but also caused each stage to last longer. Due to the late onset of the active decay stage where the skin ruptures and the decomposition fluid is released, there were no lipid band until Day 32.

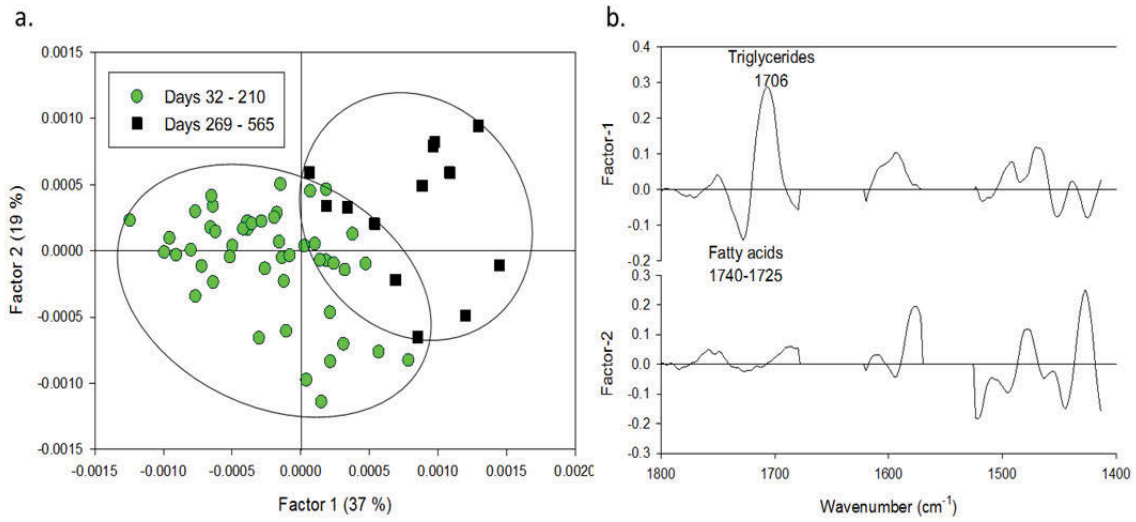

Figure 5-9: a) Principal component analysis plot of the lipid related bands from T2. The PCA plot with samples grouped as little to no lipid degradation (days 32 – 210, green circle); and advanced lipid degradation (days 269 – 565, black square). b) The loadings plot show the influence on the PCA groupings from the lipid bands in the spectra.

The biggest factors causing the separation were the presence of either fatty acids in the samples or the presence of triglycerides. The presence of triglycerides caused the earlier sampling days to lie on the negative side of the x-axis and a high presence of

fatty acid bands caused the later days to lie on the positive side of the same axis (Figure 5-9a). The separation along the y-axis was caused due to influences from biological bands related to salts of fatty acids (1570 – 1538 cm^{-1}) amongst others (Figure 5-9b). The presence of salts of fatty acids indicates a later stage of lipid degradation and was found to account for the later days to lie mostly on the positive side of the y-axis (Figure 5-9a).

5.2.2 Polyester

Figure 5-10 displays representative ATR-FTIR spectra with a presence of lipid related bands (Figure 5-10b) and protein bands (Figure 5-10c). Due to the non-absorbent nature of the polyester material, the presence of biological fluid inside the textile samples was not as prominent as seen with the cotton textile samples. Thus, most of the spectra were comparable to those obtained from the control samples (Figure 5-10a). The polyester spectra has a large band at ~1715 cm^{-1} (Figure 5-10(2)), this ester band is located in the region where lipid related bands are prominent; this caused the analysis of the lipid evolution to be slightly problematic.

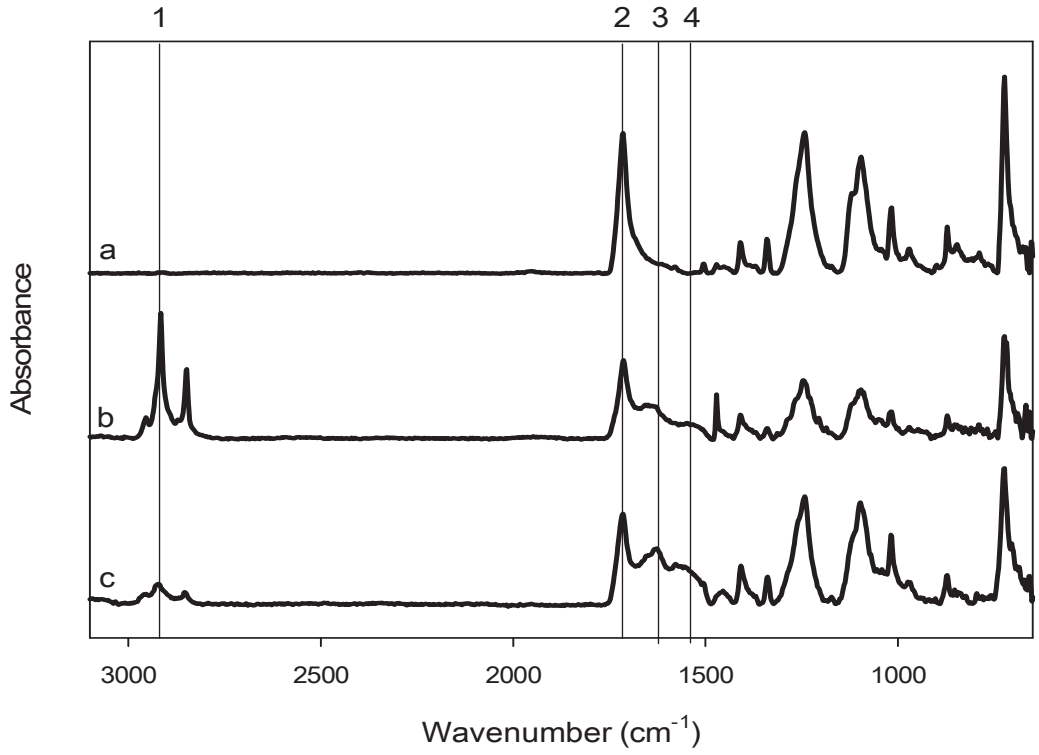

Figure 5-10: ATR-FTIR spectra of a) a control sample with the polyester bands and samples with b) high lipid content, with large bands at 1) ~2920 cm^{-1} and 2) ~1715 cm^{-1} and b) high protein content shown by a large occurrence of 3) ~1648 cm^{-1} and 4) ~1543 cm^{-1}.

5.2.2.1 Protein

The selected protein related bands were observed quite infrequently from the textile samples in T1 and T2. The only trial depicting a significant amount of protein related bands in the samples was T3, one way ANOVA ($p = 0.05$) demonstrated that the ratio of the protein bands did not alter over time. The protein bands were mostly observed during the initial sampling days (Figure 5-11).

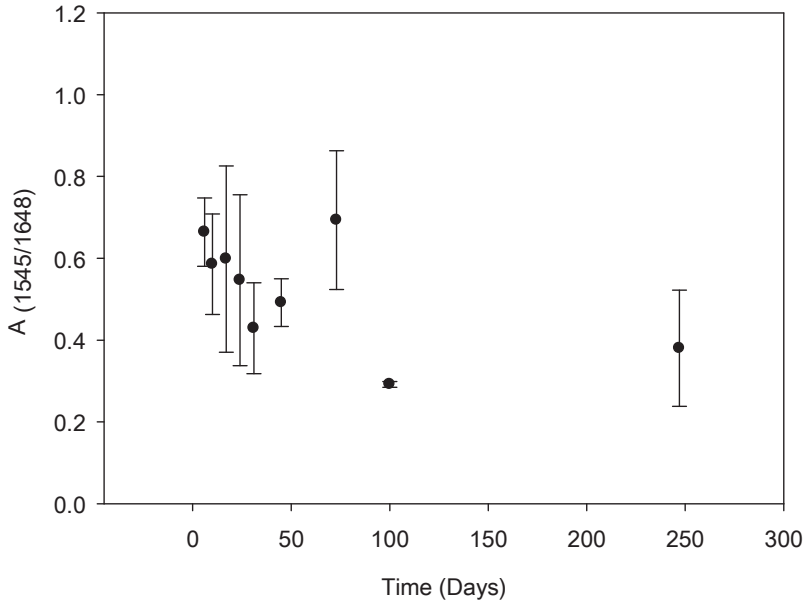

Figure 5-11: Protein infrared band ratio $A_{(1545/1648)}$ from the polyester textile samples acquired during T3 as a function of time since death.

Despite the lack of changes in the amide I and amide II bands with the initial analysis, PCA was conducted in order to determine if any changes could be found when encompassing the full protein related region and without having to use the ratio of the two for analysis. The results of this analysis using Factor 1 and Factor 2, as they explained 60 % of the variation, from T1 are depicted in Figure 5-12a. The other factors were also investigated; however this did not yield any new information regarding the data set. From the PCA plot it was determined that the samples taken at the end of T1 were found to be grouped together due to the presence of both amide I and amide II (Figure 5-12b), whereas the samples from the earlier sampling days (days 2 – 31) did not exhibit the presence of these bands. This suggest that there might be an evolution of protein related bands over time in the polyester samples, with the occurrence of amide I and amide II occurring later in this material than initially predicted. However, this pattern was only observed in the data from T1 where the occurrence of protein related

bands was quite scarce and might therefore not be the best representation of the change in protein composition.

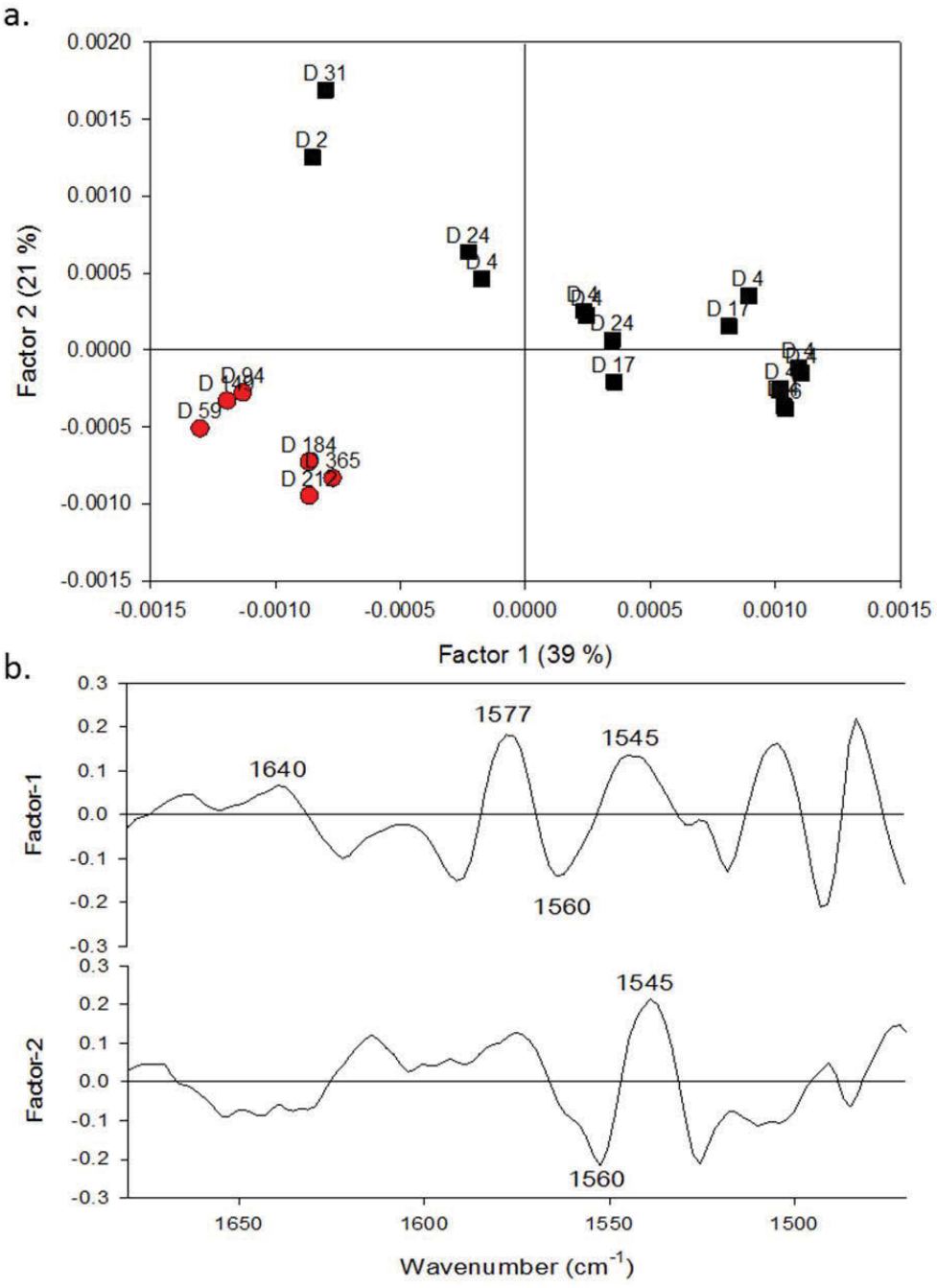

Figure 5-12: a) Principal component analysis plot of the protein related bands from T1. The PCA plot showing early sampling days (days 2 – 24, squares), and later days (days 59 – 365, circle). b) The loadings plot show the influence on the PCA groupings from the protein bands in the spectra.

The separation in protein bands due to the occurrence of amide I and amide II during the later samples days was not observed in the samples from the second summer trial (T3) where the presence of protein related bands was more frequent. This again

supported that this trend observed in T1 does not give an accurate representation of the change to the protein absorbed into the synthetic textile samples.

5.2.2.2 Lipid

No lipid bands were found in enough replicates to carry out data analysis. The 1715 cm^{-1} band from the esters in the polyester structure was most likely overlapping with the 1700-1715 cm^{-1} band representatives of free fatty acids, and thus masking this lipid band.

5.2.3 Polyester – Cotton Blend

5.2.3.1 Protein

Although protein related bands were frequently observed in the cotton spectra for all three trials, there were no changes observed, neither visually nor through the statistical analysis. A representative graph of the protein normalised band (Figure 5-13) demonstrated what was observed for each separate trial. The ratio of 1545 cm^{-1} / 1648 cm^{-1} did not change at any stage of the trial, not even in the prolonged winter trial (T2). The further PCA verified this finding and again demonstrated that there were no trends in the protein related bands over time.

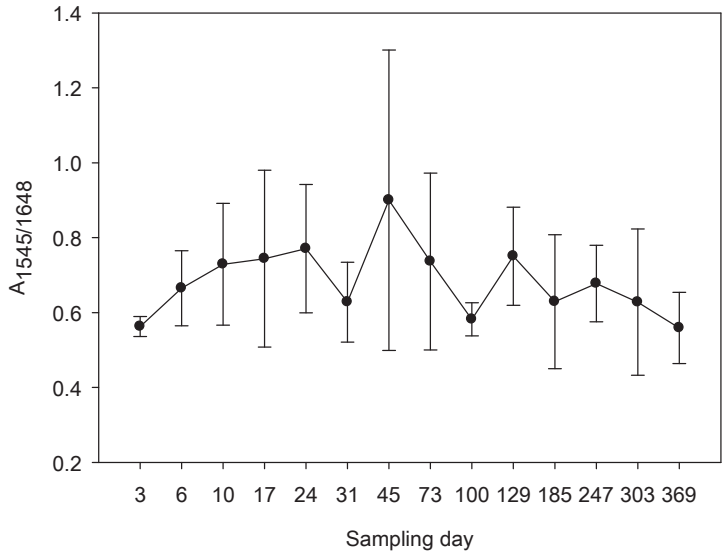

Figure 5-13: Protein infrared band ratio from the cotton section of the polyester – cotton blend textile samples during T3 as a function of time since death.

As with the protein related bands in the cotton section, there were no changes through the different surface trials during the initial statistical analysis for the polyester section, PCA was conducted and, as was observed during the initial statistical analysis, there were no groupings or patterns in the datasets from any of the trials.

5.2.3.2 Lipid

The triglyceride band from the cotton section of the polyester – cotton textile samples collected during T1 was found to decrease initially, this can be seen in the Figure 5-14, and it was also confirmed statistically using a one-way ANOVA ($p = 0.05$). The amount of triglycerides were decreasing from Day 4, it then remained comparable until sampling Day 48. Beyond this time the band was no longer found to be statistically different from Day 4. This finding corresponds with the pattern observed in the 100 % cotton samples, as there was only one sampling day after Day 48 that displayed any lipid related bands, however, on this day (Day 184) lipid bands were only observed in relatively few sample replicates causing the standard deviation to be quite significant.

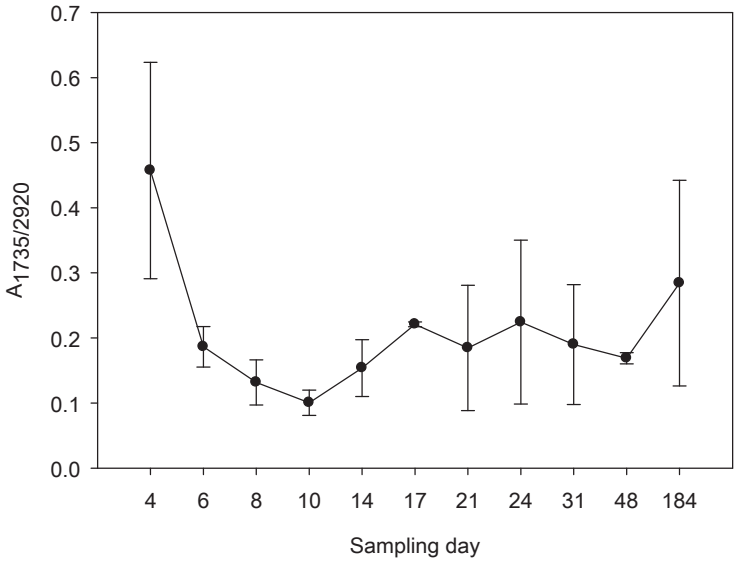

Figure 5-14: Quantity of triglyceride ($A_{(1735/2920)}$) as a function of time collected from the cotton component of the polyester – cotton blend textile samples from T1.

The subsequent trials did not show any trends over time when investigating the lipid related bands in the cotton section of the polyester – cotton blend. The statistical analysis confirmed this as a one-way ANOVA ($p = 0.05$) found no statistical differences over time. Similar results were obtained through PCA. The textile samples from T2 were again found to be lacking in lipid related bands in the first month of sampling, as

was observed in the other textile types from the same trial, beyond this time no specific trends could be determined.

As one of the lipid-related bands was found in close proximity of the one ester band of interest, the lipid band was likely masked in most polyester component of the blend. However, this band (~1715 cm^{-1}) was found to be distinct in some samples, but these were only obtained during the early sampling days of T1 (until Day 48). During this sampling period no significant differences were determined either visually or statistically. Again PCA was conducted and no trends were established.

5.3 Conclusions

Investigation of the decomposition by-products found that the protein-related bands remained stable and unchanged throughout the experiment. An interesting trend was observed during the winter decomposition trial as the protein bands could be separated into pre-rupture, originating from the blood due to the head wound during euthanasia and post-rupture, with protein bands more likely resulting from internal bacterial action during autolysis. After rupture in T2 and during the summer trials there were no trends observed with the protein samples. Similar results were obtained with the cotton and polyester sections of the polyester – cotton blend samples, again demonstrating the lack of change in protein composition.

Lipids on the other hand demonstrated a large change in the cotton samples; this was confirmed with the use of ATR-FTIR spectroscopy. Through an advanced statistical approach information about the decomposition by-products and their characteristics was obtained. The analysis demonstrated that the lipids from T1 could be grouped into three general categories; little to no lipid degradation (days 2 – 8), some lipid degradation (days 10 – 21), and advanced lipid degradation (days 24 – 48). Overall, the textile specimens exhibited lipid patterns consistent with tissue degradation, as triglycerides were degrading into free fatty acids, and the presence of salts of fatty acids demonstrate that the remains had entered putrefaction.

The presence of a large ester band at ~1715 cm^{-1} in the polyester spectra posed some problems as lipids could not be easily determined in the polyester spectra as this location also corresponds to the C=O stretching region corresponding to free fatty acid. However, due to the non-absorbent nature of the polyester material, the presence of

biological fluid in the textile samples was not as prominent as that observed for the cotton textile samples and the presence of any other lipid markers were scarce.

The findings indicate that in an absorbent material such as cotton, the decomposition fluid are becoming absorbed into the textile samples and is likely having an effect on their preservation. The fluid was likely working as a protective layer against the soil microbes responsible for the textile degradation in the control samples. The inhibition of the bacteria could have resulted due to the increased pH caused by the release of the decomposition fluid. The soil at the field site was found to be alkaline in nature, and the increase in pH observed at the experimental sites (section 3.2.3) could have resulted in a decrease in the abundance of the soil bacteria. The lipids also demonstrate potential as a marker for determining time since death of clothed individuals due to their ability to be separated into different stages of lipid degradation.

Chapter 6: GC-MS ANALYSIS OF DECOMPOSITION FLUID IN TEXTILES

Chapter 6: GC-MS ANALYSIS OF DECOMPOSITION FLUID

6.1 Introduction

The presence of decomposing remains may inhibit or delay textile degradation in a burial environment, but there has been very little work studying the specific mechanisms that prevent or delay textile degradation when in contact with decomposing remains. The difference in degradation rate or lack thereof, is likely due to the leaching of the decomposition fluid, which then becomes absorbed within the textiles.

The previous chapter used ATR-FTIR spectroscopy to characterise the protein and lipids in the decomposition by-products absorbed inside the textile samples. This data demonstrated that the lipid components were found to change over time in the textile samples, this lead to an increased interest in the change in lipids over time.

In addition to ATR-FTIR spectroscopy, another commonly used method for the analysis of lipids is gas chromatography - mass spectrometry (GC-MS). GC-MS has proven to be highly successful in the investigation of decomposition by-products in tissue, soil and the fluid itself [58, 68, 69]. ATR-FTIR spectroscopy and GC-MS are often used as complimentary techniques in order to obtain a more comprehensive view of the components of decomposition fluid. With this in mind selected experimental days were further analysed using GC-MS (see section 2.4) in order to obtain a more complete view of the lipid components. The added benefit of the GC-MS data is that in addition to confirming the presence of lipids, and in which form (triglycerides, fatty acids etc.) the lipids are in, as was achieved in Chapter 5, the GC-MS data can also identify the type of fatty acid present. Identifying the specific type of fatty acid present might give further indications of the stages of lipid degradation observed on the clothing samples, as well as provide more clues about the reason for the inhibition of textile degradation in the presence of decomposing remains.

6.2 Results and Discussion

6.2.1 Fatty Acid Derivatization Method

Fatty acids are reactive and polar substances, which are not suitable for direct GC-MS analysis. The poor volatility of fatty acids makes gas chromatography analysis

particularly difficult and the samples were therefore derivatized. Two methods were investigated; the first method has been used successfully in previous work by the author and involves the esterification of the fatty acids into their respective ester derivatives (section 2.4.1). Esters have the preferred properties of being volatile, non-polar and easy to chromatograph. However, this previous work dealt with pure tissue samples so some modifications were needed in order to use the method on textile samples. The second method was adapted from Folch et al. [80] and involved the silylation of the fatty acids (section 2.4.2).

As the cotton material is known to be very absorbent it was hypothesised that the presence of lipids would be quite prominent in these samples, and this was supported by the ATR – FTIR findings in the previous chapter. Thus, the cotton textile samples from T1 were used in order to evaluate the two different extraction methods.

The initial steps for the extraction the lipids from the textile samples were identical for both methods with a 30 minute sonication, 2 minute vortex time and overnight storage at 4 °C (section 2.4.2.1). Once this step was conducted, the fatty acids had to be derivatized, and this is where two separate methods were investigated.

The methylation method was found to be quite labour intensive and involved two 1.5 hour heating periods, where the samples had to be shaken every 20 minutes, making the total derivatization method quite long (around 5 hours per sample). In contrast the silylation method was much faster, with a total derivatization time of approximately 20 minutes per sample. The silylation method was found to yield much higher concentrations of the fatty acids, which can be seen in Figure 6-1.

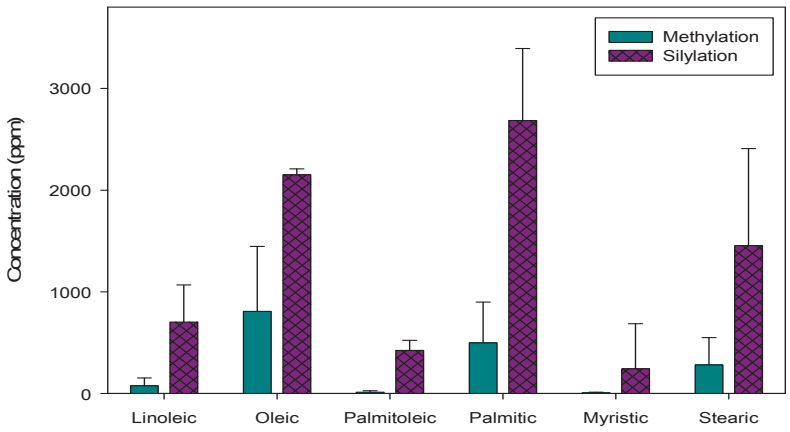

Figure 6-1: Concentration of the selected fatty acids using both methylation and silylation from the samples analysed 21 days post-mortem. Error bars represent the standard deviation based on 9 replicate samples.

The ratio of each fatty acid in the samples from Day 21 appeared to be similar between the two derivatization methods, which demonstrated that the lipid content in the samples were similar. However, the silylation method demonstrated a better sensitivity. In order to determine if this was also true for the other sampling days the component percentages of the fatty acids per sampling day were plotted (Figure 6-2).

Overall the silylation method was deemed superior as it demonstrated a higher sensitivity and greatly reduced preparation time. As the component percentage results were comparable, both methods could feasibly identify the lipid progression during the decomposition process.

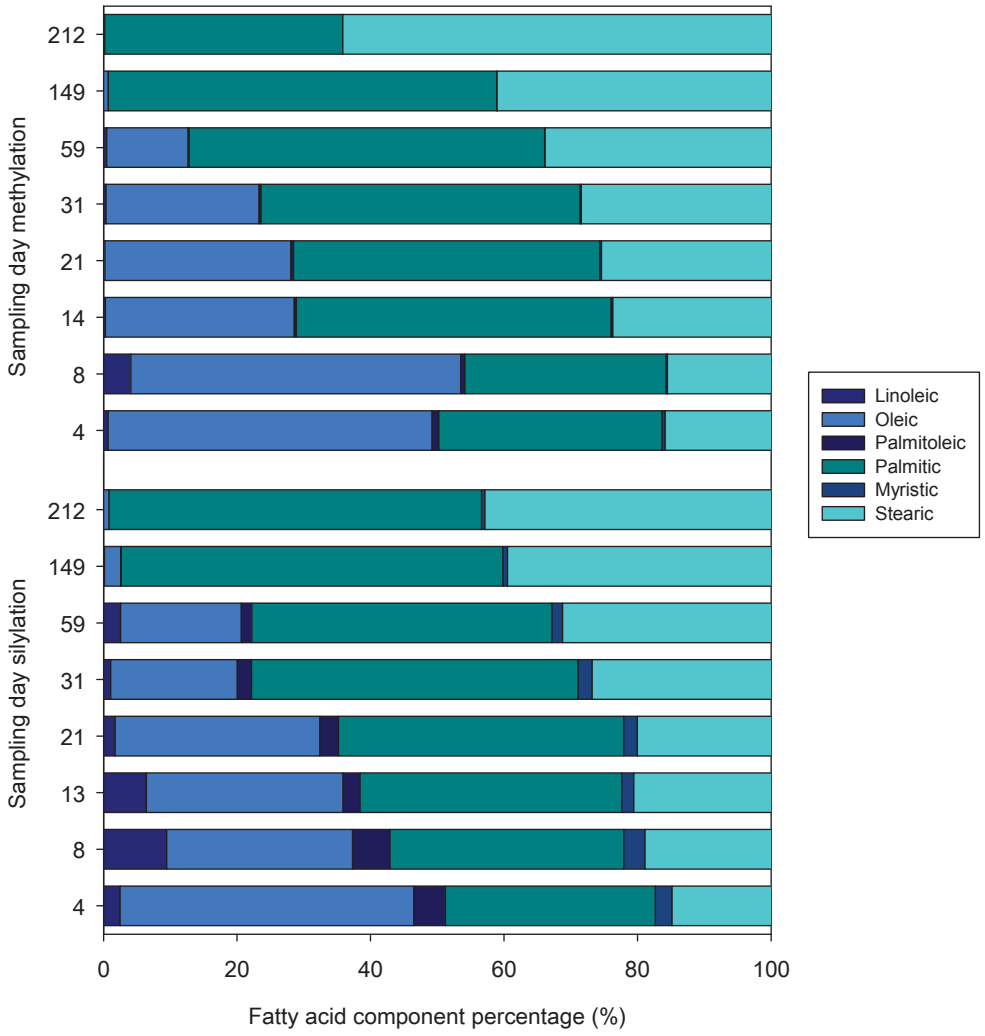

Figure 6-2: Mean fatty acid component percentage (presence of each fatty acid in relation to total fatty acid composition) of selected fatty acids per sampling day from the cotton samples using fatty acid methylation and silylation extraction methods.

6.2.2 GC-MS Analysis of Lipids Extracted From Cotton

6.2.2.1 Surface Trial 1

During the analysis the control textile samples from each trial were also examined and no fatty acids were detected from any of the sampling days. The mean component percentage of six selected common fatty acids (oleic, palmitoleic, linoleic, stearic, palmitic and myristic) is shown in Figure 6-2. The most widespread fatty acids in adipose tissue are oleic acid, linoleic acid and palmitic acid [48] in the present study both oleic acid and palmitic acid were present in significant amounts prior to decomposition.

All three unsaturated fatty acids (oleic, palmitoleic and linoleic) were initially present in the experimental samples; however, by the last sampling days linoleic and palmitoleic acids were no longer appearing in any of the textile samples (Figure 6-2). Oleic acid was found to show a significant decrease in values over time; palmitoleic acid also decreased both visually and statistically using a one-way ANOVA ($p = 0.05$). With the exception of Day 8, the amount of linoleic acid was found to show a small decrease over time. Overall, the three unsaturated fatty acids present all decreased in their relative percentage component in the textile samples associated with the remains: oleic acid decreased from 44.0 – 0.8 %; palmitoleic acid from 4.8 – 0.0 %; and linoleic acid from 2.5 – 0.0 %.

A one-way ANOVA ($p = 0.05$) found that stearic acid increased significantly throughout the study, with the earlier sampling days being significantly different from the last two sampling days (149 and 212). Palmitic acid also increased significantly with days 4 - 21 being statistically lower than Day 149 and Day 212. On Day 212 the component percentage of palmitic acid decreased compared to Day 149. However, on this day (Day 212) only myristic, palmitic, oleic, and stearic acid were present out of the six fatty acids selected. Stearic acid was found to be an influential contributor in all samples accounting for almost 45 % of the total fatty acid composition. Stearic acid results from the hydrogenation of oleic acid [48]. As the samples initially had a large presence of oleic acid, this suggests why this specific fatty acid was found in such a large abundance and thus decreasing the component percentage of palmitic acid. Overall, the percentage component of palmitic and stearic acids in the experimental textile samples increased from 31.5 – 55.8 % and 14.8 – 42.9 %, respectively.

As the study progressed myristic acid was found to be decreasing, a one-way ANOVA ($p = 0.05$) demonstrated significant differences between Day 4 and Day 8 and the later sampling days (149 and 212). The percentage component of myristic acid was very low in all experimental samples (between 3.2 and 0.5 %). This may have been due to complications extracting myristic acid from the textile samples in full, which may have altered the resulting abundance. Myristic acid was the only medium-chain fatty acid selected and might have been lost in the handling and processing of the textile samples. According to Dent et al. [48], oleic, linoleic, and palmitic acids are hydrogenated to yield stearic, oleic, and palmitic acid, respectively. Oleic acid is then further broken down by bacteria into stearic acid, palmitic acid, and myristic acid. The trend observed herein suggests that the lipids extracted from the textile samples were quite advanced in the decomposition fluid by the completion of the trial. This was supported by the fact that a decrease in unsaturated fatty acids, particularly oleic acid, and an increase in the saturated fatty acids, specifically palmitic acid could be indicative of early-stage adipocere formation, which would occur through the hydrolysis and hydrogenation of adipose fat tissue in the body [98].

6.2.2.2 Surface Trial 2

The first three sampling days were dominated by the presence of palmitic acid in the samples when plotting the respective presence of each fatty acid in relation to the total fatty acid amount (Figure 6-3).

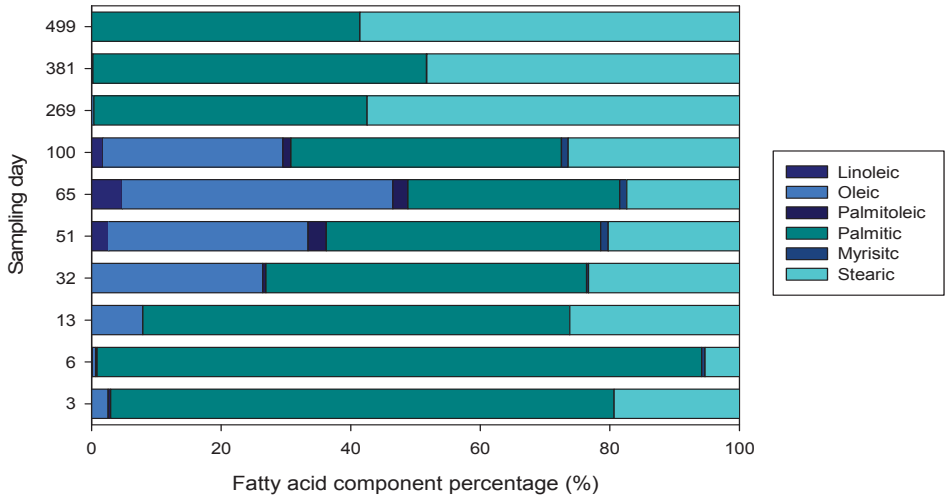

Figure 6-3: Mean percentage component (presence of each fatty acid in relation to total fatty acid composition) of selected fatty acids per sampling day from the T2 cotton samples.

It was suspected that using the component percentage as a means to map the fatty acid component during these first sampling days did not give an accurate representation as there was limited visual staining of the textiles, and sampling occurred prior to the rupture of the skin. The lipid composition was therefore likely a result of fats on the skin of the pig remains rather than those resulting from the decomposition process. As an internal standard was run with each sample, it was thus possible to estimate the concentration of each of the selected fatty acids. The results can be seen in Figure 6-4. From Figure 6-4 it is apparent that there was little to no presence of fatty acids initially, fatty acids were first observed in the samples on sampling Day 32, this sampling day corresponded to the onset of active decay, identified by skin rupture and the subsequent release of decomposition fluid.

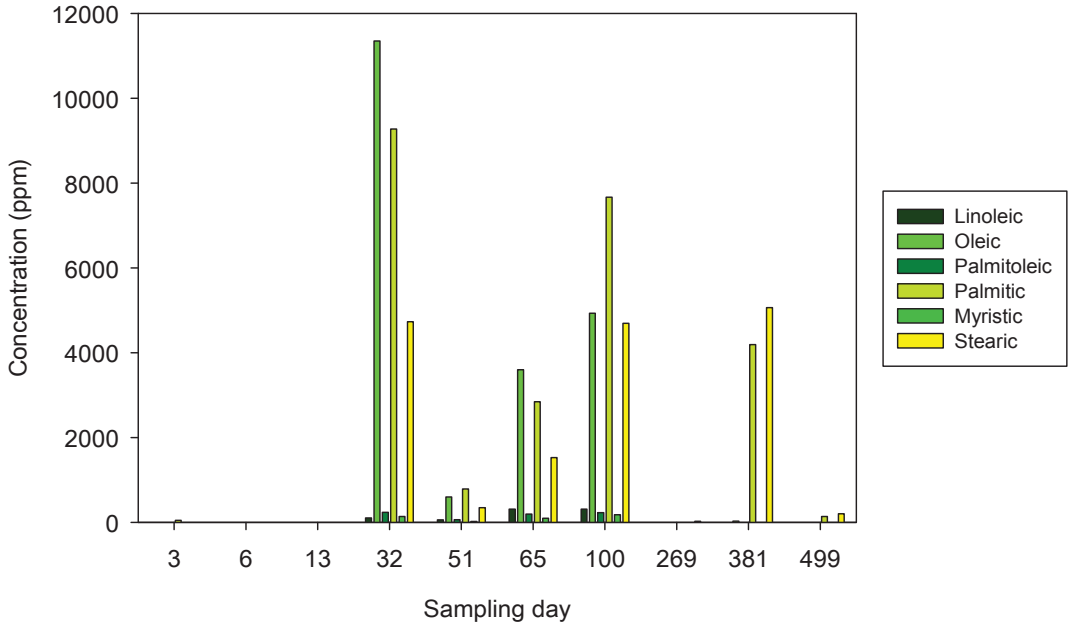

Figure 6-4: Mean concentration of selected fatty acids extracted from cotton samples from the winter trial (T2).

Due to the low levels of fatty acids in the initial and final sampling days, analysis was only conducted between days 32 and 100. The component percentage of linoleic was found to increase until Day 65, before decreasing, with Day 65 being statistically different from the other days. Oleic and palmitoleic did not demonstrate any significant differences.

Myristic acid increased significantly from Day 32 till Day 51, then remained steady throughout. Stearic and palmitic acid were increasing visually, however there were no statistical differences.

6.2.2.3 Surface Trial 3

All three unsaturated fatty acids were found to be decreasing statistically throughout the study. In fact, by the final sampling day, no triglycerides were found in any of the samples. Oleic acid decreased from 42.0 to 0 %, whilst linoleic acid decreased from 13.0 to 0 %, and finally palmitoleic acid from 3.0 to 0 %.

Palmitic acid and stearic acid both increased statistically during the sampling period with an increase from 27.6 to 53.7 %, and from 13.2 to 46.2 %, respectively. Similarly to what was found in T1, myristic acid fluctuated around 1 %, neither decreasing nor increasing overall.

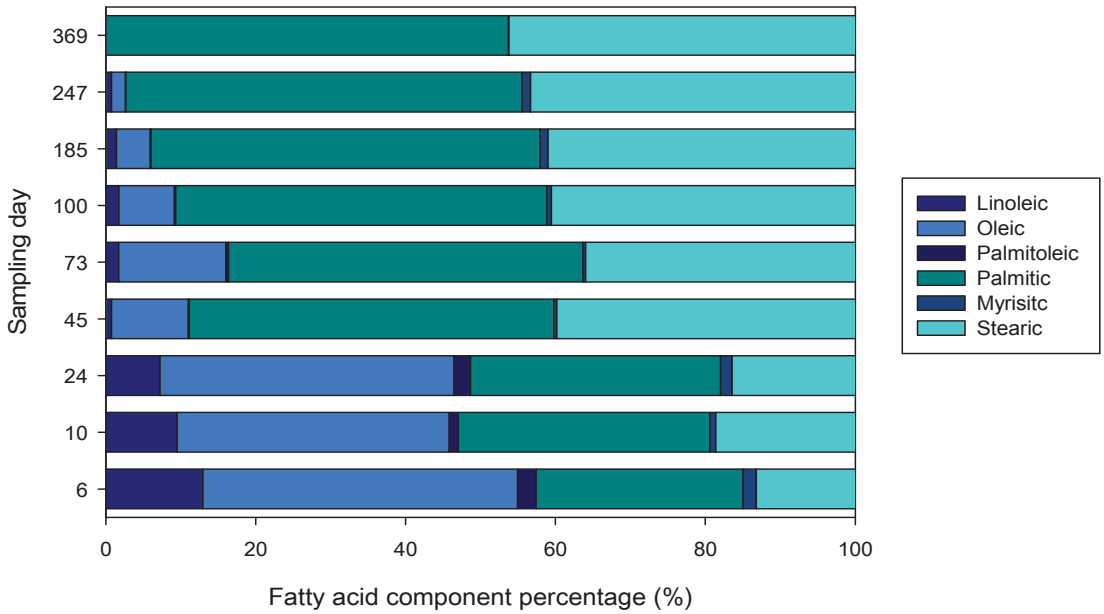

Figure 6-5: Mean percentage component (presence of each fatty acid in relation to total fatty acid composition) of selected fatty acids per sampling day from the T3 cotton samples.

Overall the lipid profiles from the cotton samples were fairly consistent to each other with the two summer trials (T1 and T3) showing identical patterns that both demonstrated that the lipids were transforming from unsaturated fatty acids into saturated fatty acids and were thus in a later stage of lipid degradation. Due to the slow onset of decomposition in the winter trial, there were no lipids until after approximately

one month post-mortem. There were also little changes in the lipid profile, likely due to the cold temperatures experienced in winter along with the slow progression of the stages of decomposition.

6.2.3 GC-MS Analysis of Lipids Extracted From Polyester

6.2.3.1 Surface Trial 1

Oleic and palmitoleic both decreased significantly, oleic acid was initially found at a component percentage of 42.3, however by the final sampling day it only accounted for 0.1 % (Figure 6-6a). Palmitoleic acid saw a much smaller drop as the initial component percentage was only 4.0 %. Linoleic acid saw a large increase in percentage on Day 8 before decreasing until it was no longer present in the study, statistically only Day 8 was found to be significantly different from the other sampling days.

Stearic acid increased from 15.1 to 42.7 %, and palmitic acid increased from 33.3 – 57.1 %, both visually when plotting the component percentage and statistically. Myristic acid did not appear to change over the course of T1.

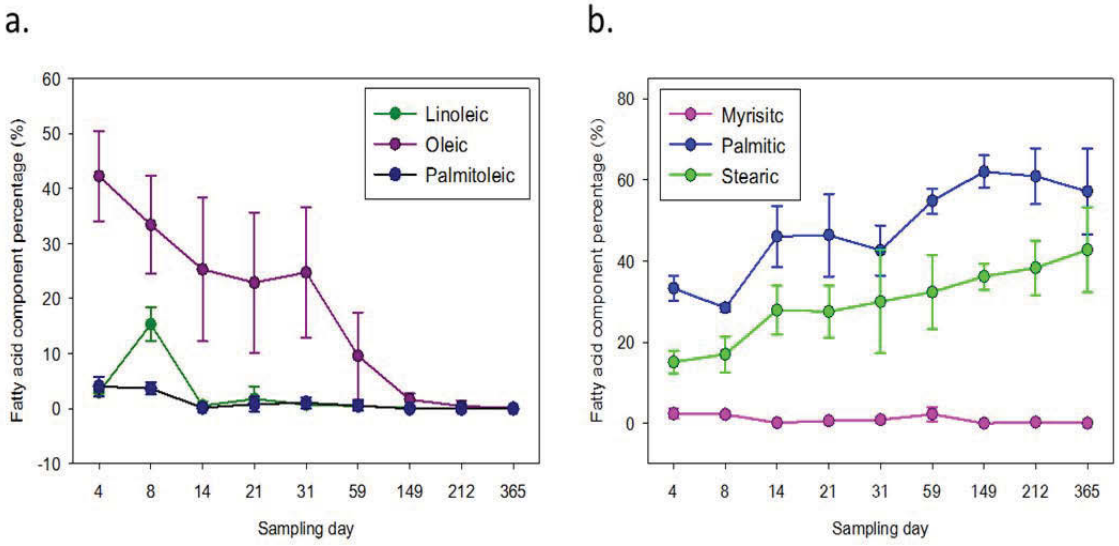

Figure 6-6: Mean fatty acid component percentage of a) unsaturated fatty acids and b) saturated fatty acids from polyester T1.

6.2.3.2 Surface Trial 2

As was seen with the lipid profiles from the cotton samples from the same trial, there was little evidence of any lipids present prior to Day 32. In addition Day 269 and Day 499 were also void of lipids (Figure 6-7).

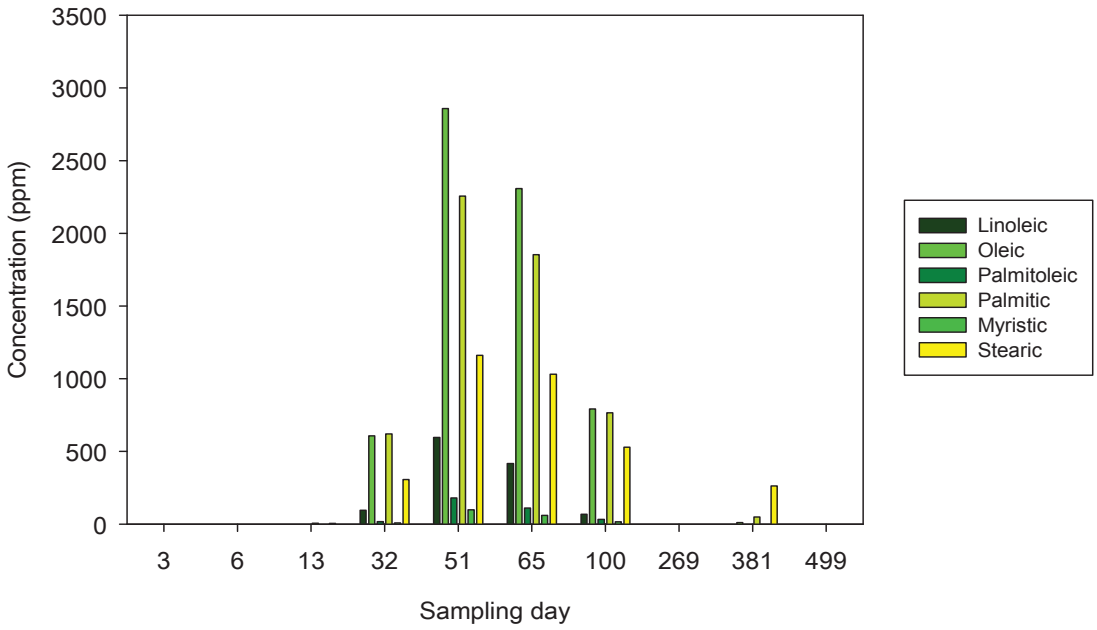

Figure 6-7: Mean concentration of selected fatty acids extracted from polyester samples from the winter trial (T2).

As a result of the lack of lipids on certain sampling days, these were removed for the subsequent statistical comparison. Overall oleic acid was found to decrease from 28.6 to 0.0 %, however it was increasing between days 32 and 65. Linoleic acid initially increased until Day 51, and then subsequently decreased over time ending with a fatty acid component percentage of 0.3 (down from the original 3.7 %). Palmitoleic acid decreased from 0.5 % to 0.0 % overall, but the component percentage initially increased on Day 51, before decreasing for the remaining time.

Stearic acid increased from 21.8 to 69.8 % (Figure 6-8). Statistically a one-way ANOVA ($p = 0.05$) determined that Day 381 was significantly higher than the other sampling days. Palmitic acid appeared to be decreasing throughout, however, a one-way ANOVA ($p = 0.05$) did not demonstrate any significant differences. Myristic acid saw a significant difference between Day 23 and Day 100 compared to Day 51.

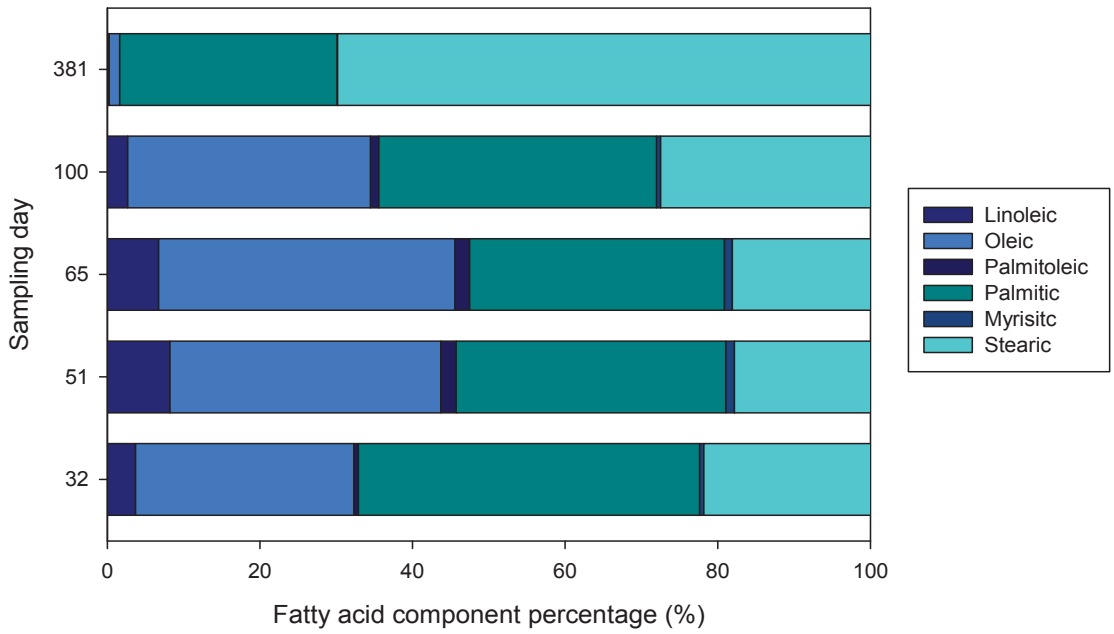

Figure 6-8: Mean percentage component (presence of each fatty acid in relation to total fatty acid composition) of selected fatty acids per sampling day from the T2 polyester samples.

6.2.3.3 Surface Trial 3

As with the previous summer trial (T1), the lipids were mostly present during the initial stages of decomposition, it was therefore determined that only the data up until Day 45 was used for the investigation of the lipid composition. Oleic acid was found decrease significantly as the earlier sampling days were determined to be statistically different from Day 45 using a one-way ANOVA ($p = 0.05$) (Figure 6-9a). Oleic acid decreased from 40.2 % to fewer than 10 % in the final sampling days. Linoleic acid was also determined to be decreasing significantly over time using a one-way ANOVA ($p = 0.05$). The final unsaturated fatty acid, palmitoleic acid was initially only present in 1.9 %; despite the low starting percentage this fatty acid decreased significantly until it reached 0 % on Day 45.

The first two saturated fatty acids, palmitic acid and stearic acid were both determined to be increasing overall as predicted, with changes from 26.8 to 45.9 % and 12.8 to 45.0 %, respectively (Figure 6-9b). Myristic acid was again found to be fluctuating around 1.0 % as was observed in T1.

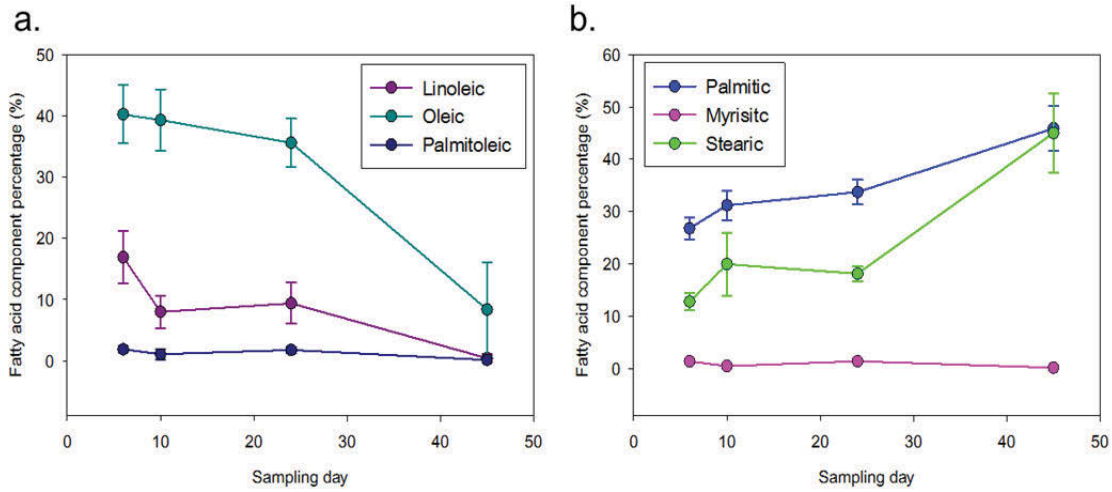

Figure 6-9: Mean fatty acid component percentage of a) unsaturated fatty acids; and b) saturated fatty acids from polyester T3.

6.2.4 GC-MS Analysis of Lipids Extracted From Polyester – Cotton

6.2.4.1 Surface Trial 1

Oleic acid decreased both visually and statistically, the first four sampling days (4, 8, 14 and 21) were statistically different from the later sampling days using a one-way ANOVA ($p = 0.05$). The second unsaturated fatty acid, palmitoleic acid, also demonstrated a decrease in the component percentage (Figure 6-10), with Day 4 and Day 8 being statistically different from the sampling days between days 31 and 212. Days 14 and 21 were also statistically different from days 59-212. Linoleic acid was decreasing significantly throughout the trial with the exception of a large spike in its component percentage on Day 8.

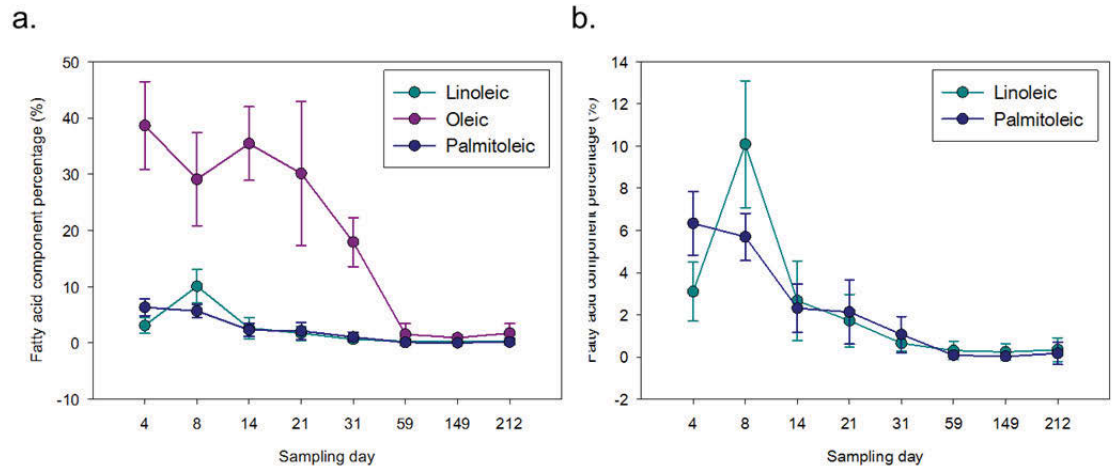

Figure 6-10: Mean fatty acid component percentage of a) unsaturated fatty acids and b) close up of linoleic acid and palmitoleic acid from polyester – cotton from T1.

The saturated palmitic and stearic acids both increased significantly, however from Day 149 until Day 212 stearic acid demonstrated a decrease in the component percentage . The last saturated fatty acid, myristic acid was found to be decreasing, a one-way ANOVA ($p = 0.05$) demonstrated significant differences between Day 4 and Day 8 and the later sampling days (149 and 212). The percentage component of myristic acid was again very low in all experimental samples (between 3.3 and 0.9 %).

6.2.4.2 Surface Trial 2

The polyester – cotton samples did not exhibit any lipids in the first sampling day, as was seen with the other textile types in the winter trial. Out of the six fatty acids of interest, none of them exhibited any statistical differences over time.

6.2.4.3 Surface Trial 3

Linoleic acid was found to decrease over time, with the exception of an increase from Day 45 to Day 73. Oleic acid decreased the first 100 days, before a spike in the component percentage of oleic acid was observed on Day 185. Palmitoleic acid initially decreased, before fluctuating around 0.00 – 0.46 %.

Palmitic acid generally increased throughout the sampling period (Figure 6-11), on Day 100 it accounted for over 60 % of the fatty acid component percentage, this value dropped slightly the next sampling day (Day 185) before again being over 60 % the final two sampling days. Stearic acid was found to increase between days 6 – 73, before it decreased. This decrease in stearic acid seen in the later sampling days was not as predicted. However, when inspecting the abundance of fatty acids in the later sampling days, they were found to be a lot less frequent and only minor amounts were found beyond Day 100. This is likely attributed to the fact that the remains entered the later stages of decomposition and were thus dry with no new leaching of decomposition fluid into the textiles. Myristic acid fluctuated between 0.0 – 1.8 % with no distinct pattern.

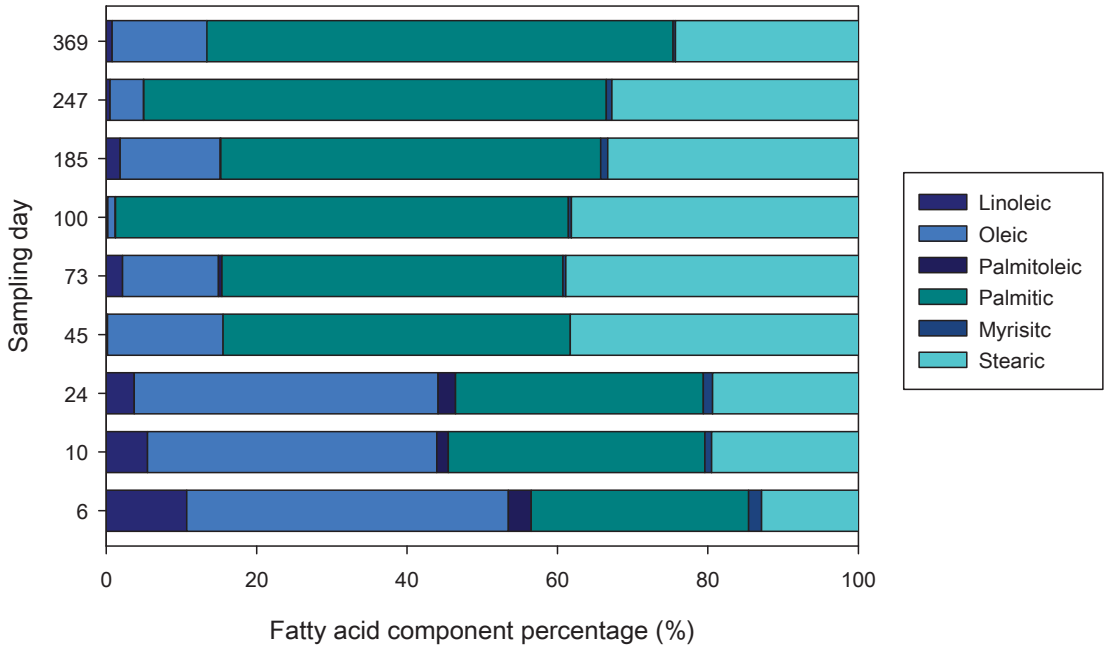

Figure 6-11: Mean percentage component (presence of each fatty acid in relation to total fatty acid composition) of selected fatty acids per sampling day from the T3 polyester - cotton samples.

6.3 Conclusions

The GC-MS data demonstrated that the lipids absorbed into the various textile types were transforming from unsaturated fatty acids into saturated fatty acids and were thus in a later stage of lipid degradation. The lipid profile in the textile specimens changed over time and could be grouped into certain timeframes, there is therefore potential that the current lipid profile in a textile specimen could be a valuable tool used in the examination of clothing located at a crime scene.

Chapter 7: **Burial Analysis**

Chapter 7: BURIAL ANALYSIS

7.1 Introduction

The previous chapters looked at both textile degradation and the stages of decomposition of surface deposited remains. In this chapter textile samples that were buried with and without the presence of decomposing remains are investigated (see section 2.1.2 for methodology). Although remains are often found disposed of on a surface after a homicide event, the occurrence of buried remains is significant enough that it warrants further investigations. As the decomposition process is greatly affected by the surrounding environment, remains found in a burial situation will experience different environmental factors to those of surface depositions, it is thus likely that the textiles will also be influenced by these changed surroundings. As with remains located on the surface a great deal of information can be obtained from textiles associated with remains in a burial scenario.

7.2 Environmental Factors

7.2.1 Rainfall and Average Daily Temperature

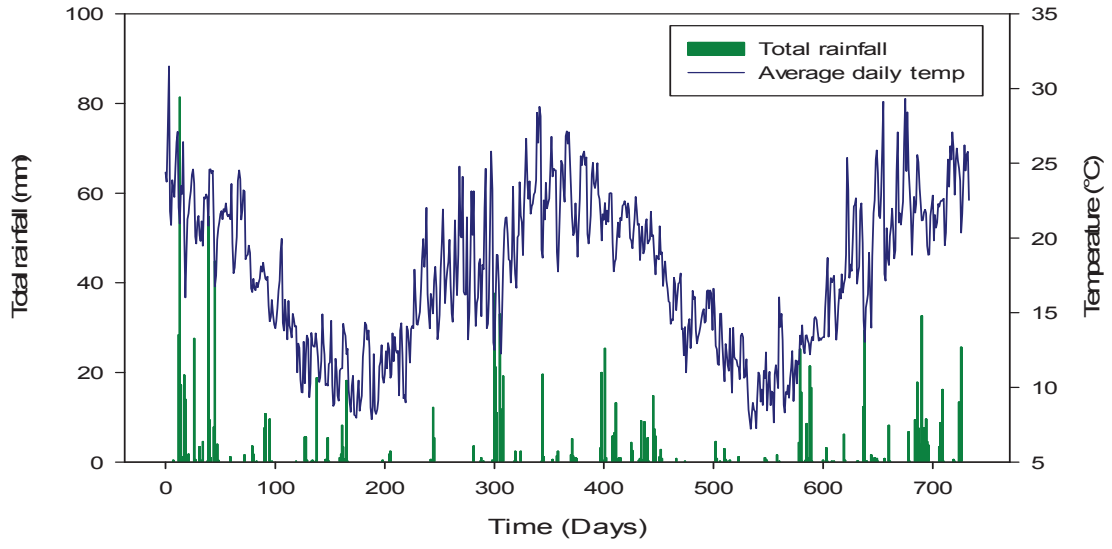

Figure 7-1: Average daily temperature and total daily rainfall for the duration of the burial.

The burial study lasted for two years, and thus the latest remains experienced two rounds of each season (Figure 7-1). The temperatures and total rainfall followed the predictive pattern for the Australian seasons, the data can be found in Table 7-1. Both the summer season and the winter in the second year of the experiment were found to be comparable in the average daily temperature to those of year one, however the first year of sampling had a lot more variability in the temperatures. During the first year the difference between the highest and lowest recoded temperatures for both summer and winter were markedly different that those observed the following year.

Table 7-1: Average daily temperature, highest and lowest recorded temperatures and total rainfall for each season during the burial study.

Season	Months	Average daily temp (°C)	Highest temp recorded (°C)	Lowest temp recorded (°C)	Total rainfall (mm)
Summer	Jan – Feb 2013	23	47	14	274
Autumn	March – May 2013	17	30	15	98
Winter	June – Aug 2013	11	24	2	64
Spring	Sept – Nov 2013	18	37	6	171
Summer	Dec – Feb 2014	23	29	17	104
Autumn	March – May 2014	18	23	11	85
Winter	June – Aug 2014	11	16	7	108
Spring	Sept – Nov 2014	19	29	13	83

7.2.2 Soil pH and Moisture Content

The pH readings for the reference control and reference experimental graves were calculated by taking the average of the control graves prior to the placement of the textile samples into the grave; similarly the average pH was calculated from the designated experimental graves prior to the addition of the remains (Figure 7-2).

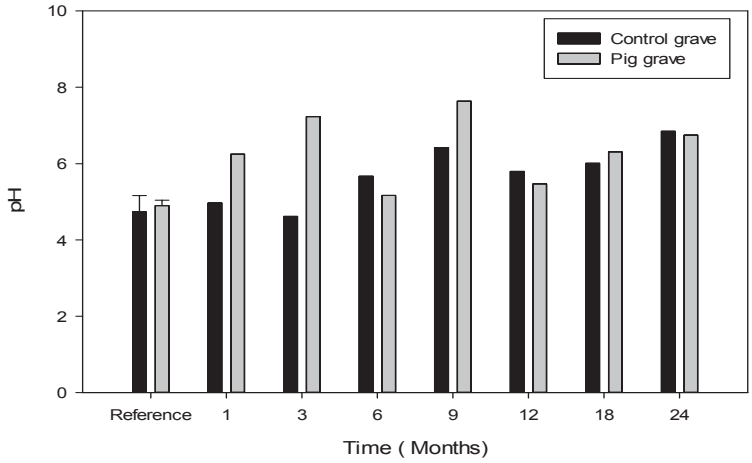

Figure 7-2: pH measurements from the control graves (black) and experimental graves (grey) for each excavation

The pH in the control graves demonstrated less variability than the pH readings from the experimental graves. The soil volumetric water content was also monitored throughout the burial study; the results can be seen in Figure 7-3. A large increase in the soils moisture content was found associated with the experimental controls. Initially the reference values for the control and experimental sites were comparable, however this quickly changed as the excavation after 1 month demonstrated a large increase in soil moisture content for the experimental site. This trend continued the following excavation with the experimental site having a soil moisture content of over 30 %, compared to ~ 10 % in the control grave. The soil volumetric water content in the experimental graves then decreased until the moisture content between the experimental and control graves were comparable, this occurred after 18 months.

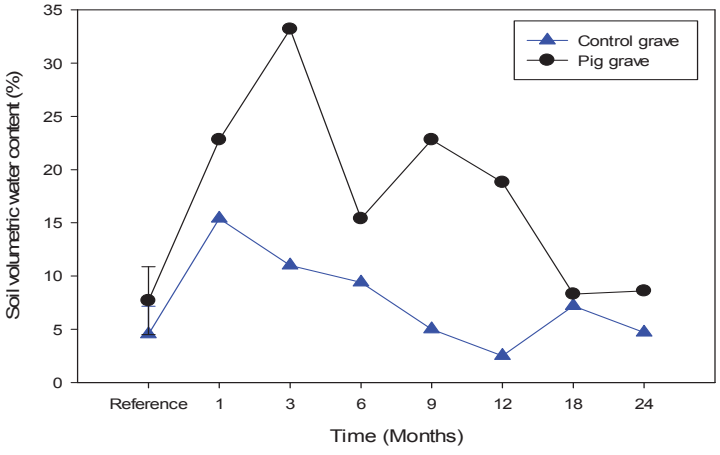

Figure 7-3: Soil volumetric water content in the graves. Readings from the experimental graves shown in black and the control grave readings in blue.

7.3 Visual Observations

7.3.1 Buried Remains

After 1 month burial the first pig grave was exhumed. At this time there was still a large amount of soft tissue present and the pig could easily be removed as a whole section (see Appendix D). The surrounding soil was found to be very moist, even up to 40 cm above the pig remains. Three months post-burial the surrounding soil was still quite wet, and the remains were intact with a large amount of soft tissue remaining. When lifting the remains, the soil beneath was found to be very dark in colour, appearing almost black.

The graves exhumed both after 9 months and one year were visually affected by adipocere, with large tissue sections being well preserved. The remains after 18 months post-burial were very dry and had a large amount of tissue loss, only skin and bone remained for the most part. After 24 months, the remains were fully skeletonised and the grave environment was again found to be very dry.

7.3.2 Textile Damage

7.3.2.1 Cotton

The control t-shirt showed the presence of yellow and red circles and was generally very discoloured from the adhering soil after 1 month; there was no apparent visual damage (Figure 7-4a). The t-shirt that was exhumed after 3 months had a medium sized rock on the top if the t-shirt in the neck region. Once removed there was a visible hole in the textile underneath. Vegetation was also growing on the shirt and it was overall highly discoloured. After 6 months the t-shirt was highly degraded, there were multiple holes in the remaining textile and all edges were frayed (Figure 7-4b). Only fragments remained. After 9 months the t-shirt was much degraded (Figure 7-4c). Only the seams and a few larger pieces that were located under the briefs remained. After a year post burial the t-shirt only had the seams left, and a small (30 x 4 cm) section left at the bottom of the t-shirt, an area that had been covered by the briefs. The control shirt from the control grave after 18 months was surprisingly a lot more intact than the samples at 6, 9 and 12 months. Large sections across the left shoulder down to the right abdomen were absent; however the t-shirt could still be lifted as a whole. And the

bottom section was intact. The t-shirt was highly discoloured with red stains and several roots were piercing the material. The final exhumation at 24 months demonstrated a t-shirt sample that was more degraded than the one at 18 months, however still more intact than the 6, 9 and 12 month samples. The remaining textile was very fragile and would disintegrate upon handling.

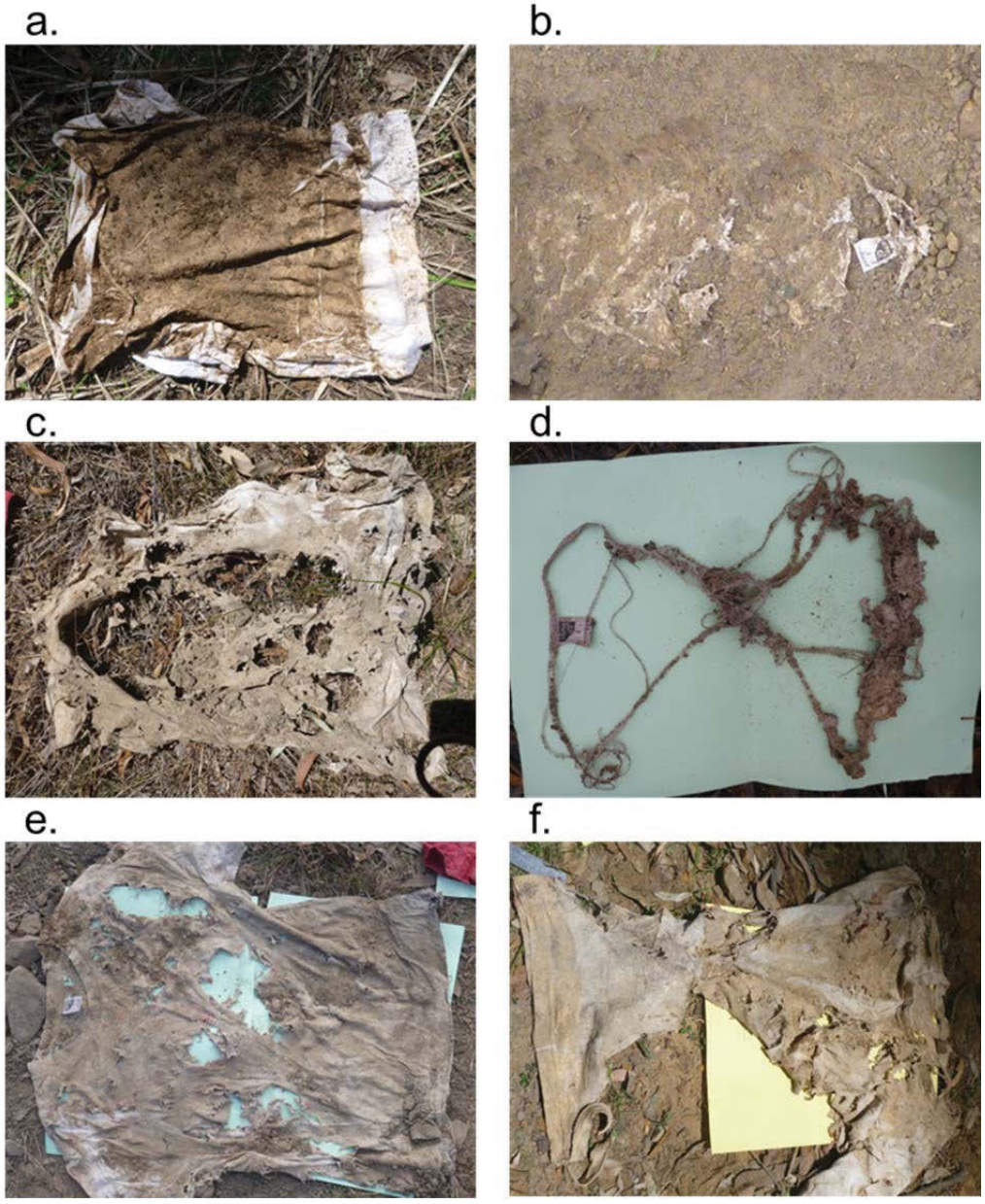

Figure 7-4: Visual damage to the cotton samples from the control graves after a) 1 month, b) 6 months, c) 9 months, d) 12 months, e) 18 months and f) 24 months post burial.

The experimental t-shirt was very discoloured with orange and brown staining after one month. There were large sections of tissue attached to the surface of the textile and a foul smell was associated with the t-shirt. The t-shirt from the experimental grave

did not have any signs of visible damage after 3 months; the t-shirt was very discoloured and stained with black and brown particles. Upon removal of the shirt from the grave, there was a large presence of flies. After 6, 9 and 12 months the t-shirt located underneath the pig was very well preserved, the section on top, less so (Figure 7-5a). The t-shirt from the pig exhumed after 18 months was similar to the previous samples, with the section on top of the pig being completely degraded, however the section underneath the pig carcass after 18 months also showed signs of degradation as only a smaller section was found. This remaining section was found to be well preserved and covered in several layers of tissue, as was observed in during the previous exhumations. On the final sampling day, 24 months, the t-shirt was almost completely disintegrated; with the seams and small sections attached to it still remaining (Figure 7-5c).

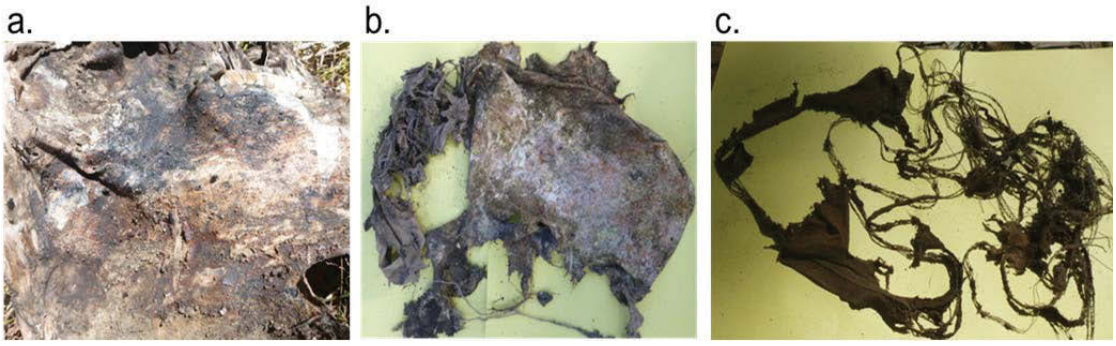

Figure 7-5: Textile damage observed with cotton samples from the pig graves after a) 9 months, b) 18 months and c) 24 months.

The drastic loss of material in the last two sampling graves (particularly after 24 months) was most likely due to the distinct difference in moisture observed on these two sampling times compared to the other graves. The increased moisture content caused the remains to be preserved, especially in the 9 and 12 month graves, this was also evident by the production of adipocere. The presence of remains with soft tissue is hypothesised to be the reason why the cotton samples in these graves were preserved.

7.3.2.2 Polyester

The briefs from the control grave were discoloured after 1 month; however no visual physical damage was seen. The briefs from 3 months were also in a good condition (Figure 7-6a). There was vegetation growing on the outside, and some had penetrated the fabric. After 6 months the briefs were again well preserved with no visible damage. The same was seen after 9 and 12 months. After 18 months there was

still no visual damage other than roots growing through the briefs. Even after 24 months, the briefs were intact and had no visual textile damage, other than some fading of the colour and a few piercing roots.

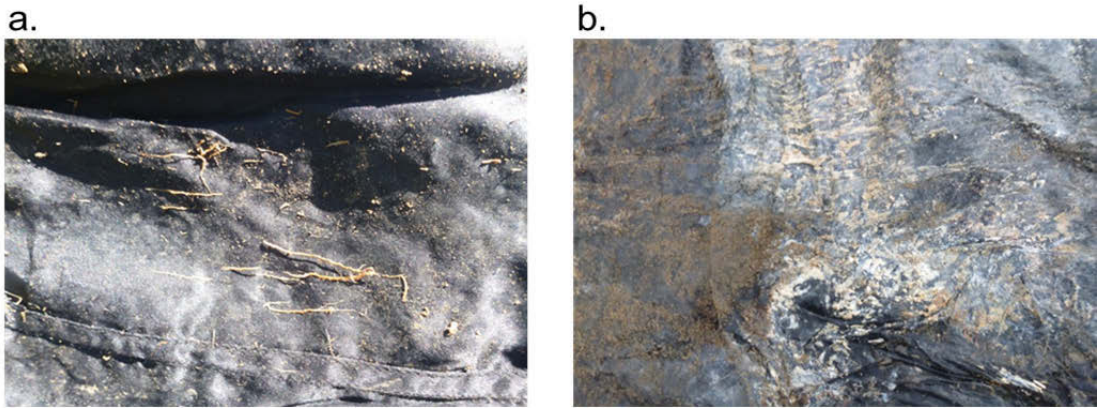

Figure 7-6: Typical polyester samples recovered from a) control grave and b) pig grave, both these samples had been buried for a duration of 3 months.

The briefs from the pig grave were also seemingly intact, again with staining from the surrounding soil. In addition tissue was adhering to the textile and there was a foul smell associated with the briefs. The briefs after both 3 and 6 months were again well preserved with large tissue pieces on the inside surface. The briefs buried for 9, 12, 18 and 24 months were again well preserved.

7.3.2.3 Polyester – Cotton Blend

At one month post-burial no visual physical damage to the polyester – cotton socks at the control site was observed, and there was a grass root intertwined with the socks and the surrounding soil caused the socks to be discoloured. After 3 months a similar observation was made, the socks were well preserved apart from some vegetation growth between some of the fibres (Figure 7-7a). The control socks after 6 months were less preserved than the earlier graves, the material was fully intact, but it appeared fragile. The same phenomenon occurred after 9, 12, 18 and 24 months.

The experimental socks had adhering tissue on the inside of the socks and were also quite discoloured. After 3 months the socks were intact, but did exhibit some vegetation growth between the fibres. The 6 month samples were also fully intact and again covered in tissue. After 9 months the socks were again fully intact as seen previously, however the material was visibly weaker and more fragile. At the yearlong burial exhumation two minor holes were seen the socks in the experimental grave. The

samples at 18 months were transparent and fragile. After 24 months the socks were fragile, and there was a large root growing through the entire sock (Figure 7-7b).

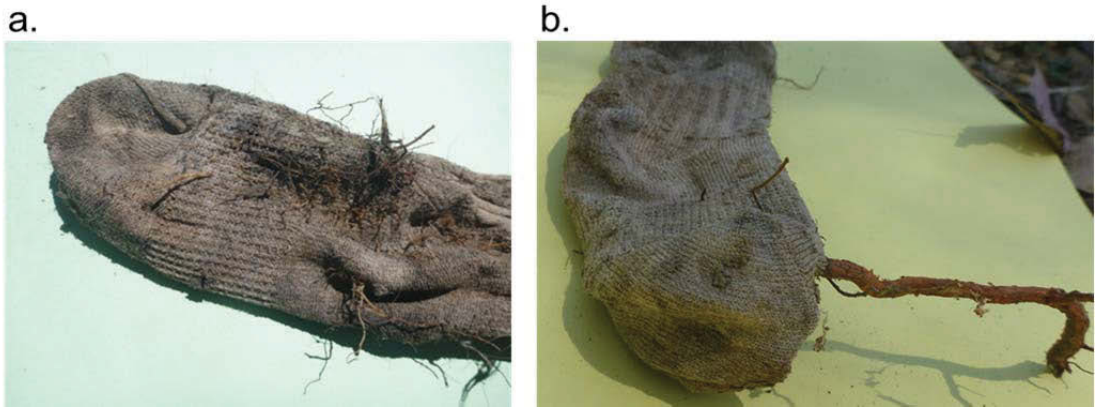

Figure 7-7: Examples of damage due to vegetation growth in between the polyester - cotton textile samples.

The visual observation of the textile types over time showed a clear distinction between the natural and synthetic materials. The cotton control samples in the graves demonstrated some damage to the material after 3 months post-burial, whereas the polyester material was found to be intact in the control graves throughout the entire burial period (2 years). These results are consistent with finding by Tomsic et al. [41] who found that when placing swatches of fabric in soil, the cellulose textile decomposed visually and chemically after 12 days of burial, whereas the polyester textile remained smooth and unchanged on the surface. The samples in the current burial study did take between 1 – 3 months to show signs of physical degradation, however the samples consisted of a much larger piece of fabric than the ones used by Tomsic et al. [41]. The polyester – cotton blend samples demonstrated a higher resistance to biodegradation than did the 100 % cotton samples. There was no visual textile damage until a year post – burial, however the material was recorded to be physically weaker after 9 months.

There were distinct visual differences between the cotton material buried without remains and those with. As seen with the surface samples (section 3.4.1), the presence of remains was found to inhibit the degradation of cotton. A study by Lowe et al. [59] reported similar findings, with cotton material in the absence of decomposing remains being partially degraded after 2 months post-burial and completely disintegrated after 14 months post-burial. Lowe et al. [59] also found that buried

clothing associated with porcine remains showed no signs of degradation after 18 months, demonstrating comparable results to the present study.

7.4 ATR-FTIR Analysis of Textiles

The textiles used for the burial studies were identical to those used in the surface trials (see Chapter 2 and 4), so the same infrared bands were selected for the analysis (Table 4-1 and Table 4-2). As for the surface cotton control samples (Chapter 4) the 985 cm^{-1} and 1001 cm^{-1} bands are observed as initially quite distinct shoulders, as can be observed in Figure 7-8a showing the control cotton samples from the burial sites. These bands broaden as their relative intensity changes relative to the 1033 cm^{-1} band for the 6 and 9 months samples (Figure 7-8b). A different trend was observed for the samples from the control graves after a year until the end of the trial (24 months). For these spectra, where the 985 cm^{-1} band remained weak, the 1001 cm^{-1} band was found to be distinct and increased in the absorbance relative to the 1033 cm^{-1} band (Figure 7-8c). The same trends were not seen with the cotton samples from the pig graves, as in these samples there were no apparent disappearance or appearance of any bands of interest.

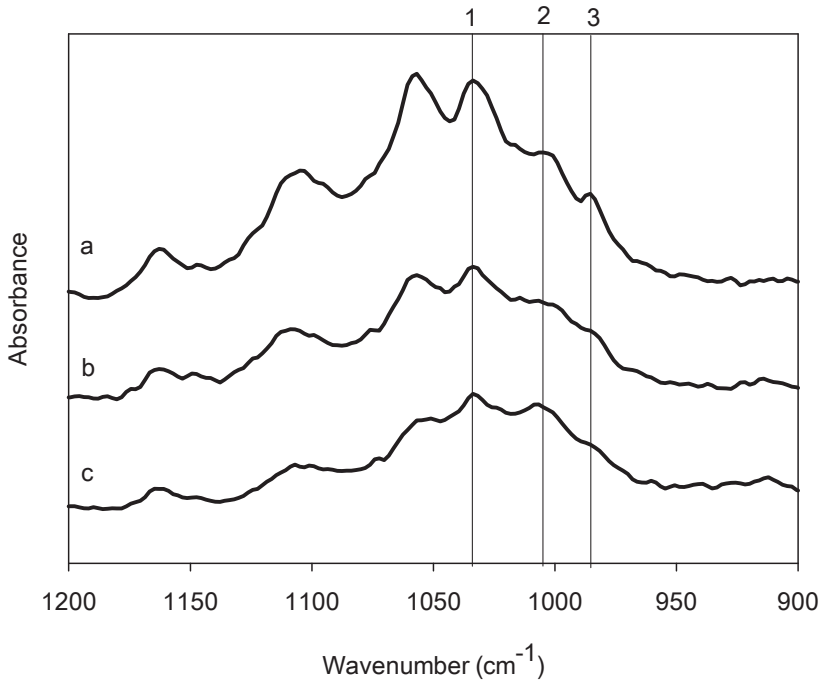

Figure 7-8: FTIR region of interest for the degradation of cotton control samples. 1) 1033 cm^{-1}; 2) 1001 cm^{-1}; and 3) 985 cm^{-1}. Typical spectra of the cotton collected from the burials after a) 3 months; b) 9 months; and c) 24 months.

Polyester samples appeared to be unchanged throughout the sampling period when examining the spectra visually. The three bands of interest were found to be comparable throughout, illustrated in Figure 7-9 where typical spectra from the 1 month and 24 month exhumations are shown.

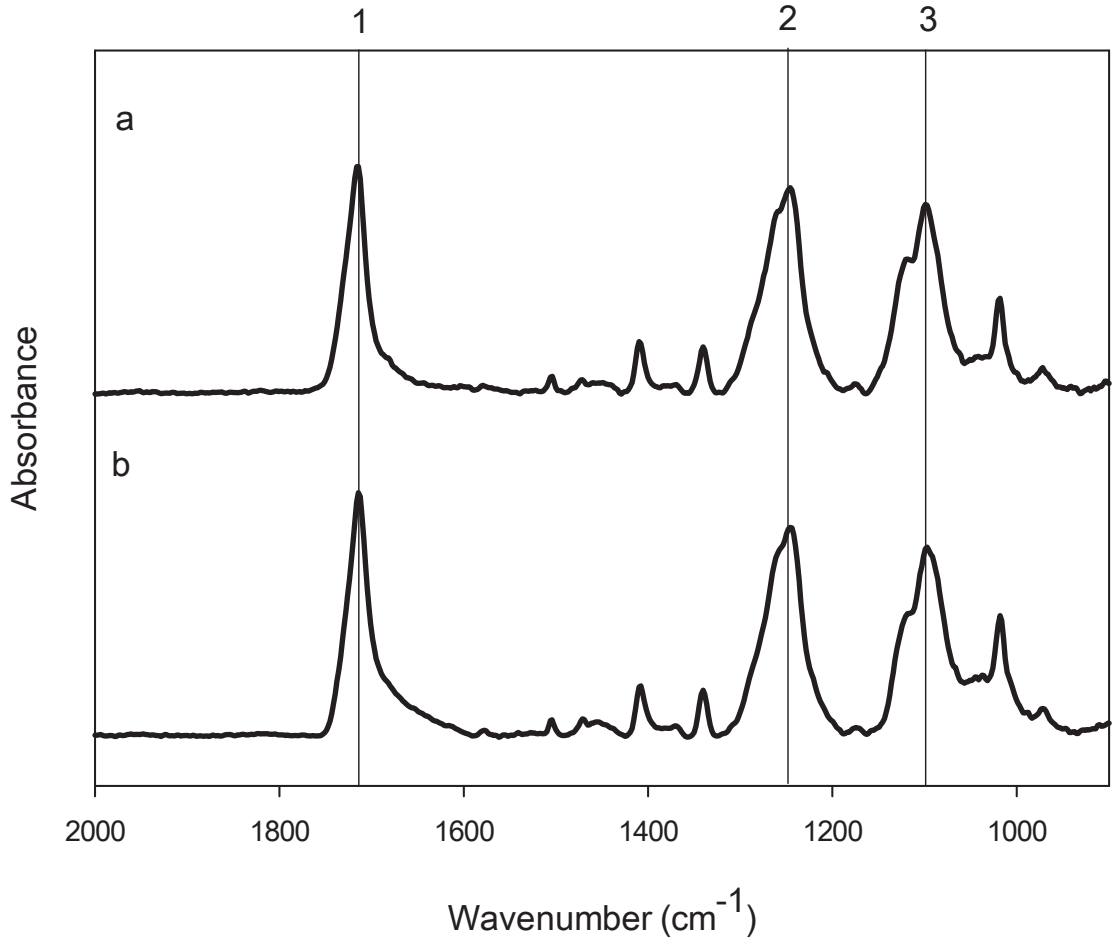

Figure 7-9: FTIR region of interest for the degradation of polyester control samples: 1) 1715 cm^{-1}; 2) 1245 cm^{-1}; and 3) 1090 cm^{-1}. Typical spectra collected from the burials after a) 1 month and b) 24 months.

For the polyester – cotton blend samples, separate spectra were recorded for the cotton section and the polyester section. A distinct trend was only observed when visually inspecting the spectra with the cotton sections of the experimental textile samples. In the region of interest it was found that the three bands were visually distinct initially, the 985 cm^{-1} band disappears after 3 months post-burial (Figure 7-10). The 1001 cm^{-1} band increased, which is also what was observed with the 100 % cotton burial samples. The 1001 cm^{-1} band increased relative to the 1033 cm^{-1} band, and a

slight shift in values from 1001 cm^{-1} until approximately 1005 cm^{-1} was also observed. After 24 months the 1001 – 1005 cm^{-1} band was higher than the 1033 cm^{-1} band.

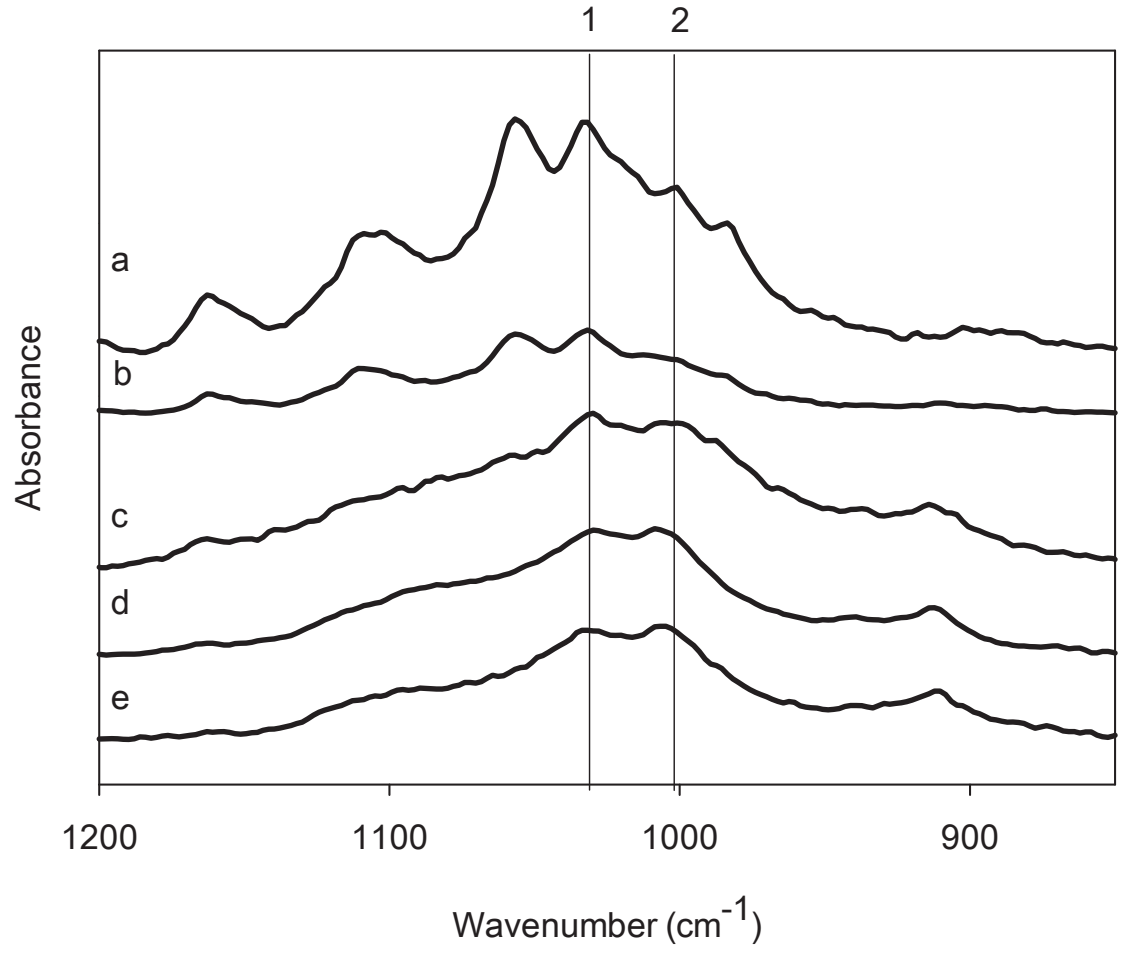

Figure 7-10: FTIR region of interest for the degradation of cotton section of the polyester – cotton blend samples 1) 1033 cm^{-1}; and 2) 1001 cm^{-1}. Typical spectra of the polyester - cotton blend collected from the pig burials after a) 0 months; b) 1 month; c) 3 months; d) 6 months; and e) 24 months.

7.4.1 Initial Statistical Analysis

7.4.1.1 Cotton

As there were significant changes observed to the spectra of the cotton textile samples, it was proposed that a normalised band absorbance would also reflect this change. When plotting the 1001 cm^{-1} normalised band it appeared that an increase occurred in both the control and experimental samples the band ratio was increasing (Figure 7-11). A one-way ANOVA ($p = 0.05$) was completed on both data sets (control and experimental) and no significant differences were calculated. Thus, the apparent

visual trend could not be confirmed statistically using the normalised band. There were also no significant changes to the initial analysis of the 985 cm^{-1} band. T-tests were conducted for the control and experimental IR bands for each sampling month, and for the cotton samples no statistical differences were calculated.

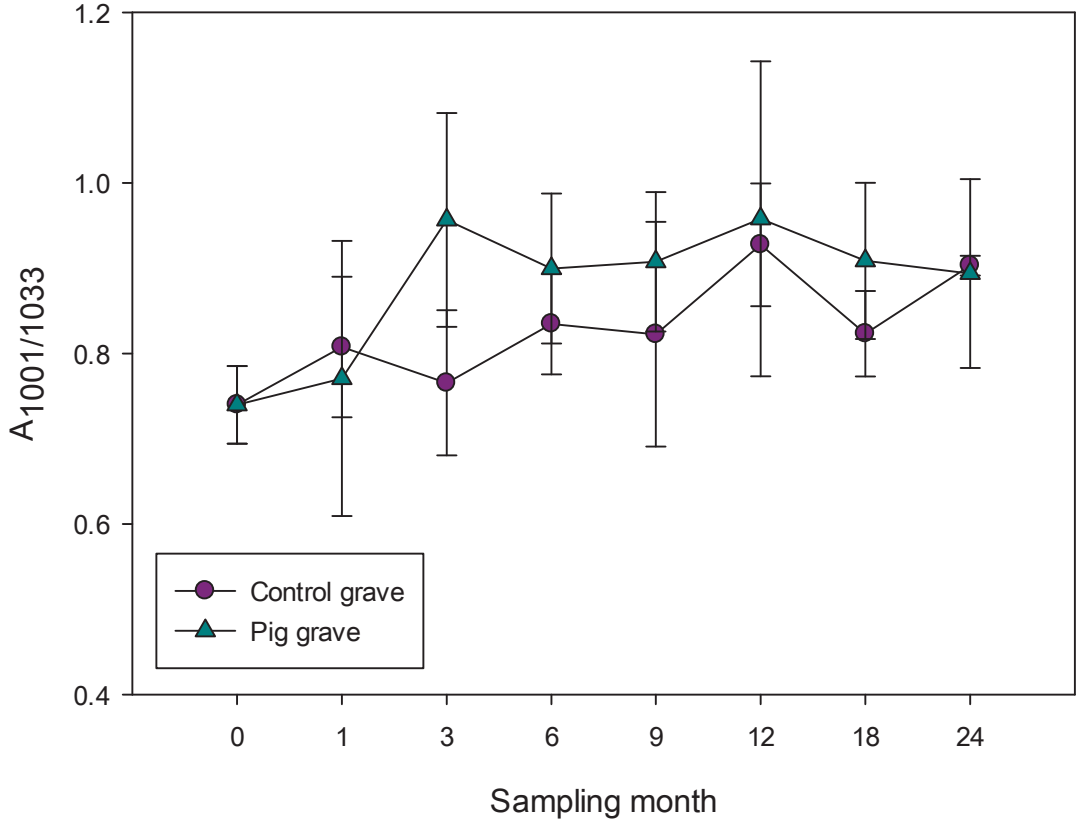

Figure 7-11: Quantity of $A_{(1001/1033)}$ from the 100 % cotton samples as a function of time for the control grave (purple circle) and the experimental graves (green triangle).

7.4.1.2 Polyester

When plotting the normalised bands investigated for the polyester samples the 1090 cm^{-1} band in the control samples appeared to increase until 9 month post-burial before decreasing again (Figure 7-12). Using a one-way ANOVA ($p = 0.05$) a statistical difference was obtained between the unburied or month 0 samples and 12 months post-burial samples ($p = 0.041$). There were no other trends or statistical differences in the bands over time for both control and pig grave samples.

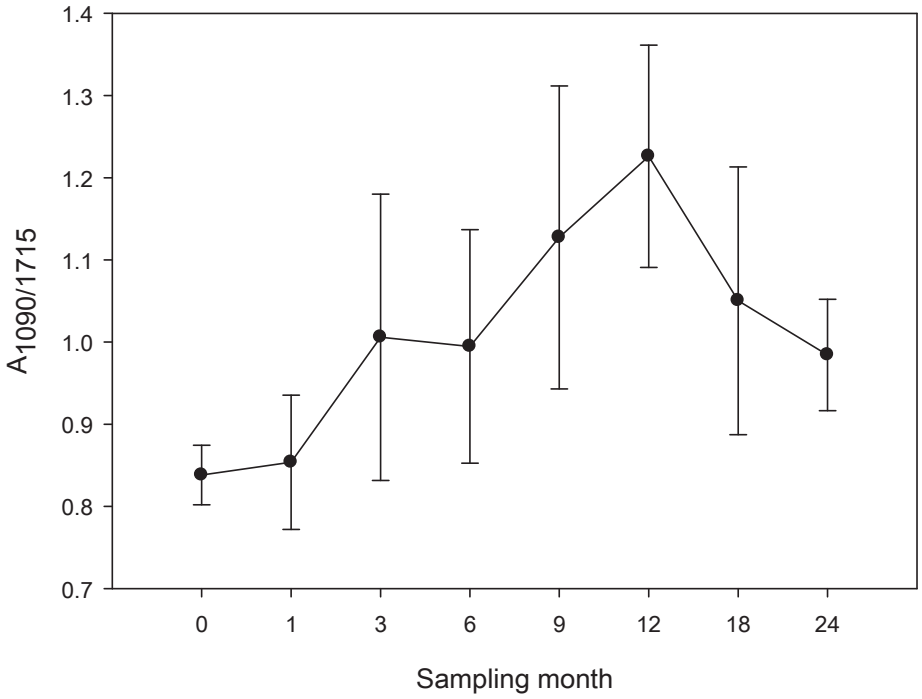

Figure 7-12: Polyester control samples showing the change in the $A_{(1090/1715)}$ band over time.

A student's t-test comparing the FTIR bands for the control and pig graves at each sampling point found a significant difference between the samples after 9 months for the 1245 cm^{-1} band ($p = 0.047$), however by month 12 they were again comparable.

7.4.1.3 Polyester – Cotton Blend

The 985 cm^{-1} band in the polyester – cotton spectra did not depict any trends over the course of the two years for either control or samples from the pig graves. The 1001 cm^{-1} band was normalised to the 1033 cm^{-1} and the resulting graph for both the control grave and pig grave can be found in Figure 7-13. For the 1001 cm^{-1} normalised band, it was found that the initial unburied Day 0 samples were statistically different from the control sample after 12 months ($p = 0.034$). The Day 0 samples were also statistically different to the samples collected on exhumation months 3, 9, 12, 18 and 24 for the cotton samples collected from the pig graves, using a one-way ANOVA ($p = 0.05$). A significant difference was also observed for the pig samples when comparing the spectra collected after 1 month to those after 24 months ($p = 0.031$). These represent the first and last exhumation times.

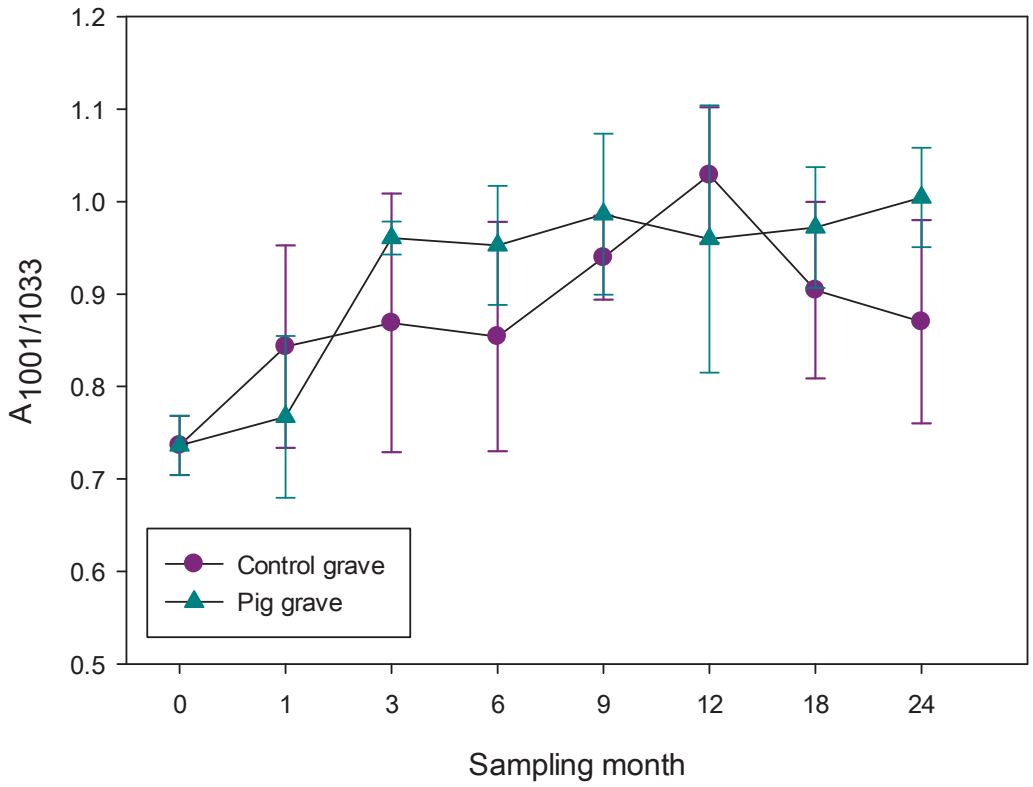

Figure 7-13: Quantity of $A_{(1001/1033)}$ from the cotton sections of the polyester – cotton blend samples as a function of time for the control grave (purple circle) and the experimental graves (green triangle).

The polyester related bands in the polyester – cotton blend samples were also investigated, and when comparing the control samples over time there were no statistical differences for either the 1090 cm^{-1} band or the 1245 cm^{-1} normalised band (Figure 7-14). A statistical difference was obtained between the 1090 cm^{-1} band recorded after 6 months and the same band at 0, 3, 9, 18 and 24 months post-burial for the pig samples, with p values of 0.002, 0.004, 0.007, 0.001, and 0.003 respectively. The 6 month samples were also statistically different from the samples obtained after 24 months when comparing the 1245 cm^{-1} band ratio ($p = 0.009$). A t-test comparing the normalised bands for the control and experimental grave found that the 1245 cm^{-1} band was significantly different between the samples after 6 months ($p = 0.032$). The 6 month samples are likely outliers.

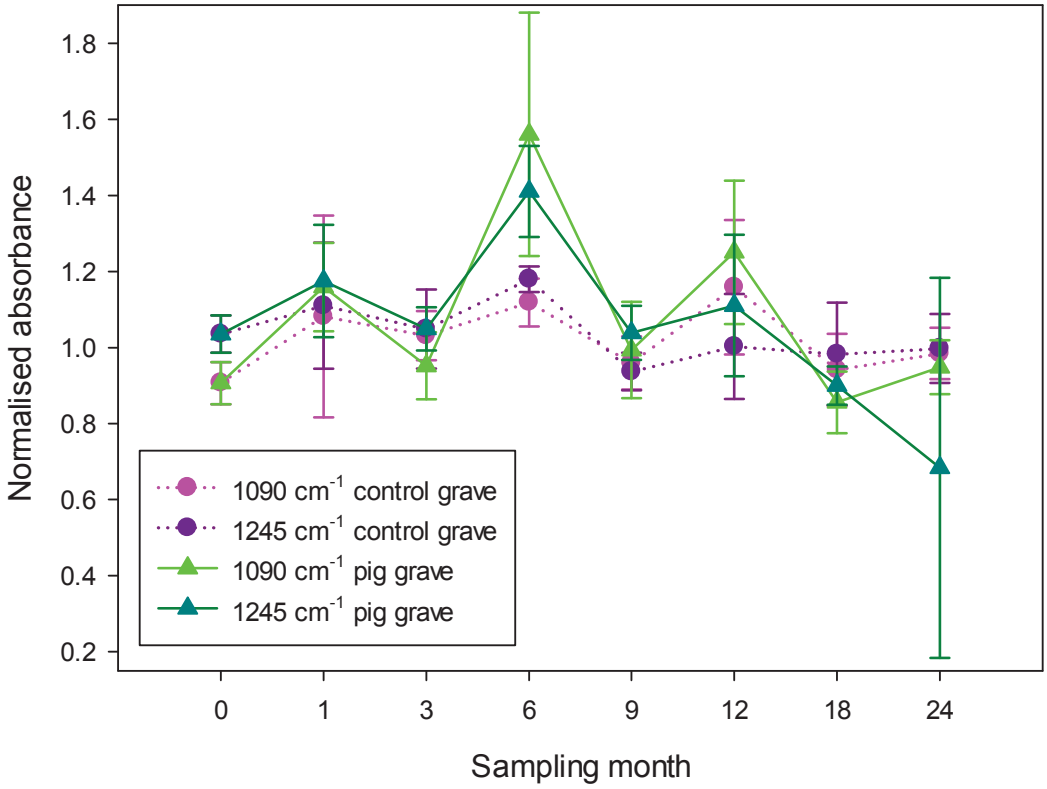

Figure 7-14: Polyester related bands over the course of the burial shown for both control (pink and purple circle) and pig sites (light green and dark green triangle).

Overall some changes could be determined, however, normalising the bands provided rather large error bars, and with only three replicates per sampling day, further statistical analysis using more sophisticated software was warranted.

7.4.2 Principal Component Analysis

7.4.2.1 Cotton

The burial cotton data were separated into three separate groups based on their exhumation date in order to get a better idea of the degradation timeline of the cotton samples. These groups also helped visualise any separation or clustering of the data (Figure 7-15a). A PCA plot of Factor 1 versus Factor 2 was created as these factors represented 86 % of the variation in the PCA. The PCA of the cotton control data demonstrated that two distinct groups could be separated, the first group included the earlier and intermediate exhumation samples (months 0 to 9), and the second grouping was found with the samples from the last two exhumations (18 and 24 months).

Inspection of the loadings plot (Figure 7-15b) reveals that the separations were mainly due to Factor-2, which included both the 985 cm^{-1} and the 1001 cm^{-1} infrared band. Experimentally there were no separations or groupings in the data set. Thus the experimental samples were not found to exhibit any statistical trends.

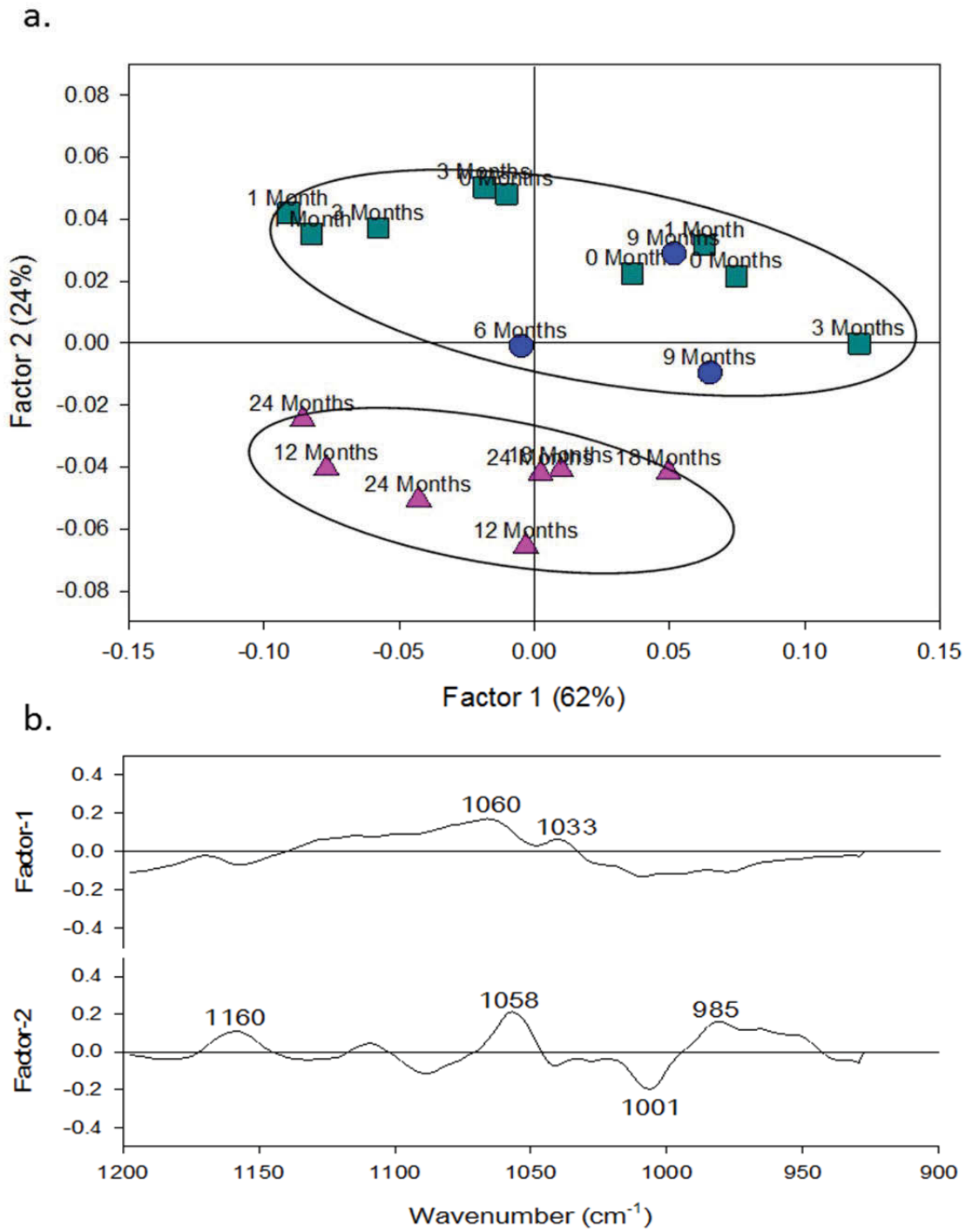

Figure 7-15: a) Principal component analysis of the cotton spectra from the control graves with early excavation times (green square); mid-excavation times (blue circles); and late excavation times (pink triangle). b) The loadings plot shows the influence on the PCA groupings from bands in the spectra.

7.4.2.2 Polyester

No separation in the control samples or in the experimental samples based on the polyester bands was observed from the PCA results (Figure 7-16a). The samples associated with the remains were found to be somewhat separated compared to the corresponding control grave data; however no distinct trends could be seen from the polyester bands (Figure 7-16b).

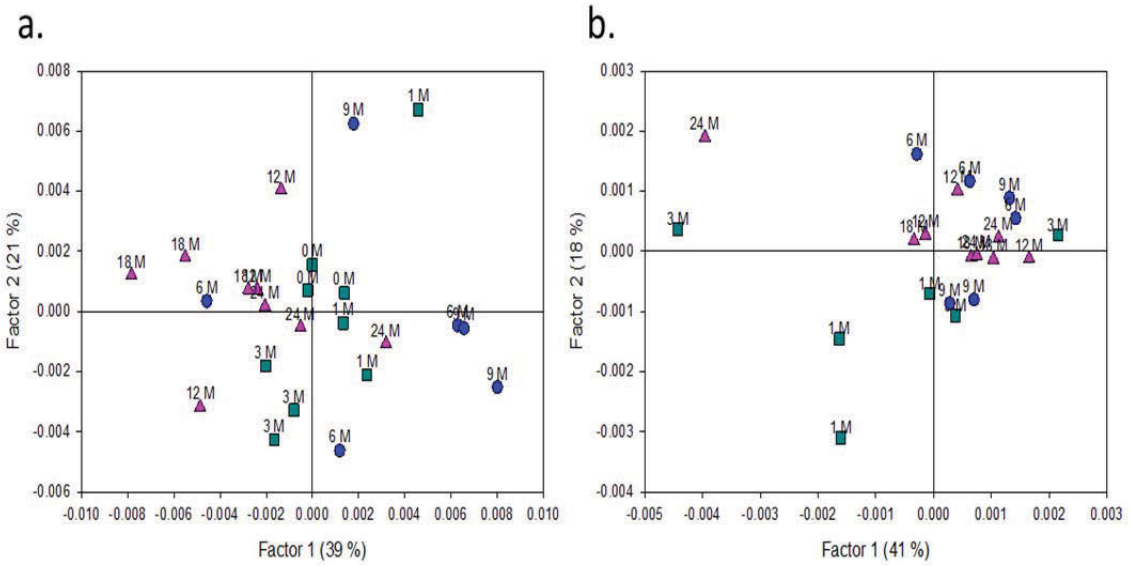

Figure 7-16: a) Principal component analysis of the polyester spectra from the control graves, and b) Principal component analysis of the polyester spectra from the experimental graves.

7.4.2.3 Polyester – cotton Blend

The cotton section of the polyester – cotton blend samples could be clearly separated into three groups, as was also observed for the data from the 100 % cotton samples (Figure 7-17). The groups were, however, slightly different as the Day 0 samples were distinctly different from all other excavated samples. The two other groups consisted of the early excavation times (1 – 9 months) and late excavation times (18 – 24 months). The 12 month samples were removed as they were considered outliers.

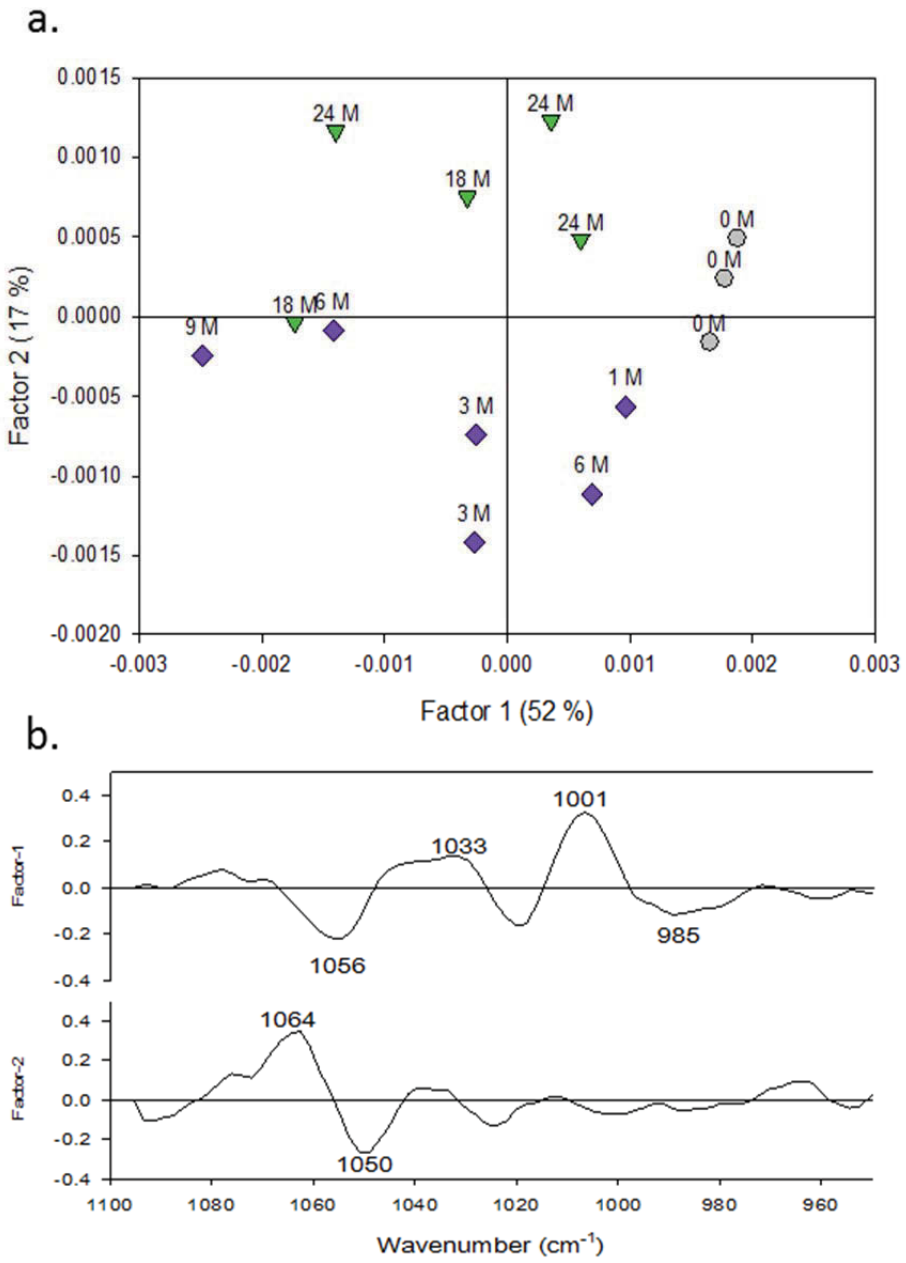

Figure 7-17: a) Principal component analysis of the cotton section from the polyester – cotton blend samples from the control graves with Day 0 (grey circle); early excavation times (purple diamond); and late excavation times (green triangle). b) The loadings plot showing the influence on the PCA groupings from bands in the spectra.

Only the first two factors were found to represent the trends in the data set, despite Factor 1 and Factor 2 only explaining 52 and 17 % variation, respectively. The other factors were also investigated; however no other information about the data set could be retained as the other factors only explained minimal variation in the data.

7.5 ATR-FTIR Analysis of the Decomposition Fluid in the Textile Samples

7.5.1 Protein

The presence of protein related bands were only observed in the 100 % cotton samples and in the cotton region of the polyester – cotton blend material. The polyester materials did not show these bands in enough regularity in either the pure sample or the blend. The ratio between 1545 cm^{-1} and 1640 cm^{-1} bands appeared to be visually changing with burial time. Initially the 1545 cm^{-1} band was higher compared to the 1640 cm^{-1} band (Figure 7-18a), but as time progressed this ratio would change, with the 1640 cm^{-1} band becoming higher in absorbance than 1545 cm^{-1} after 9 months (Figure 7-18c). A similar trend was seen for the cotton sections of the polyester – cotton samples, at 3 months and for one 6 month replicate, the ratio of the two bands was fairly even, then at 9 and 18 months there was a clear change with the 1648 cm^{-1} band increasing relative to the 1545 cm^{-1} band.

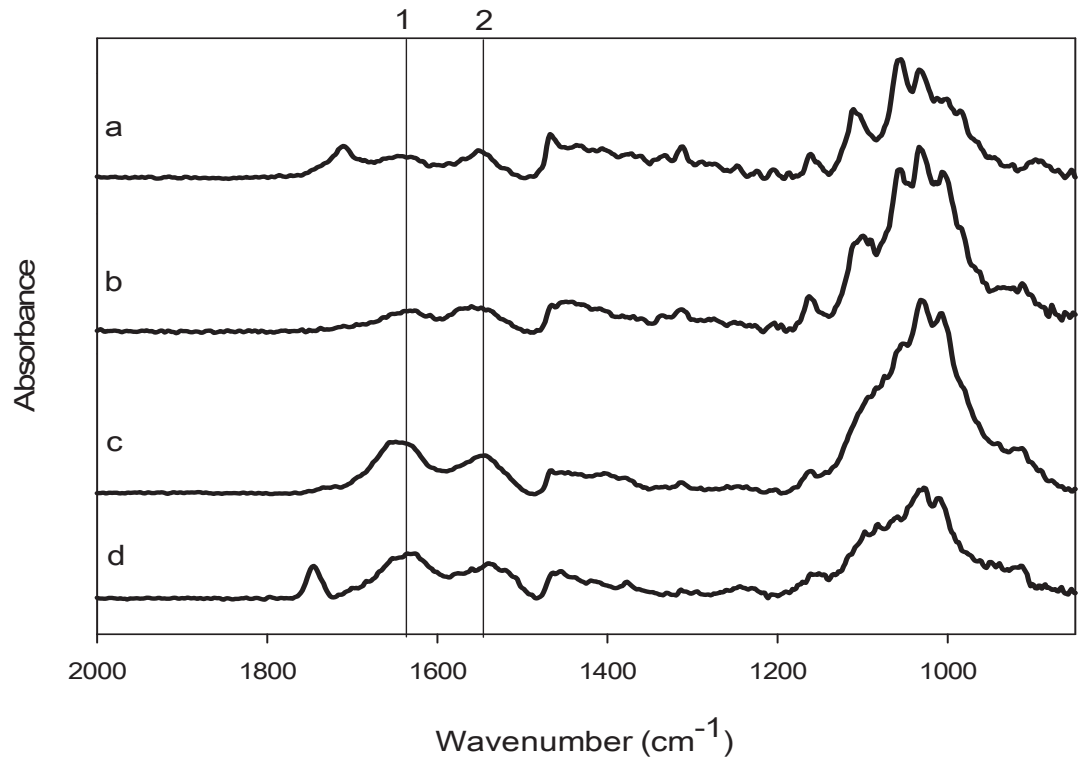

Figure 7-18: Typical infrared spectra for the experimental cotton samples after a) 1 month; b) 3 months; c) 9 months; and d) 18 months. The vertical lines show the regions of interest: 1) amide II (~1545 cm^{-1}); 2) amide I (~1648 cm^{-1}).

Statistical analysis was conducted on the normalised protein related bands in the cotton samples. With the cotton t-shirt there was no statistical difference when conducting a one-way ANOVA ($p = 0.05$). When plotting the normalised protein bands found in the spectra from the cotton section of the polyester – cotton blend, the ratio was found to be initially decreasing (Figure 7-19). A statistical difference was obtained between the samples from the exhumation after 3 and 9 months ($p = 0.043$). Thus, analysing the ratio of the two amide bands demonstrated that there was a significant difference between the samples collected at 3 months and those at 9 months. This could be due to the presence of blood on the cotton material initially due to bleeding from the head wound acquired when the pigs were euthanized. The amide ratio could therefore be indicative of blood, whereas the ratio after 9 months could indicate a presence of protein from the decomposition process. Care should be taken when comparing the protein evolution over time as the samples were taken from different graves, and not from one grave over time which would be more comparable to the method used for the surface trials (section 2.1.1.).

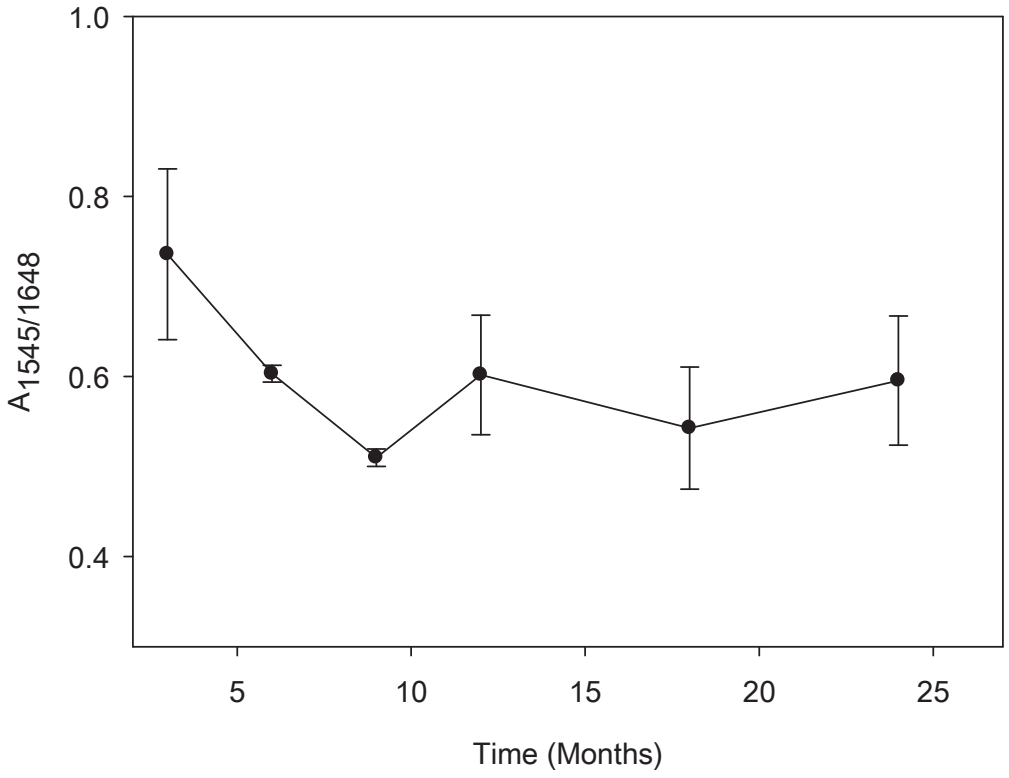

Figure 7-19: Protein infrared band ratio as a function of time since death taken from the cotton section of the polyester – cotton spectra. The error bars represent one standard deviation based on three replicates.

7.5.2 Lipid

The lipid profile absorbed into the textile samples was also examined. During the sample collection sections of the fabrics with visible staining from decomposition fluids were selected for the analysis. However, as there were only three replicate samples from each textile types, a much larger variation in the lipid infrared profile was observed compared to what was seen in the surface trials (Section 5.2.1.2). As sampling occurred on textile samples from different pig carcasses each time this made a comparison over time more complicated as the lipid profile content in one grave could be distinctly different between the experimental graves. The cotton t-shirts from the 1 month samples displayed a band at 1715 cm^{-1} demonstrating the presence of free fatty acids, no distinct band at 1735 cm^{-1} and a single band at (1570 – 1538 cm^{-1}) (Figure 7-20a). The presence of free fatty acids in the absence of a band indicative of triglycerides, suggests that the lipids are undergoing decomposition. However the band single band at 1570 – 1538 cm^{-1} should be investigated further as this band could potentially be indicative of a short chain carboxylic acid. The spectra collected following the 6 month exhumation showed a presence of a minor band at ~1735 and a larger one at 1715 cm^{-1}. The same trend was observed after 12 months (Figure 7-20c) when focusing in on the specific region. In addition a single bond was again found around 1560 cm^{-1} for the 6 month samples (Figure 7-20b). The spectra obtained from the 18 month samples had a fatty acid band around 1705 cm^{-1} indicative of free fatty acids. In addition a large band at 1745 cm^{-1} was observed; this band likely corresponded to the COOH ester band (Figure 7-20d). There was no distinct doublet in the 1570 – 1538 cm^{-1} throughout the sampling period which would indicate salts of fatty acids occurring as seen with the 100 % cotton surface samples. Upon excavation of the last two graves (18 and 24 months) the graves themselves were found to be quite dry (Appendix D), this was also reflected in the soil volumetric water content measured on these exhumation times (Figure 7-3). There was also little evidence of any presence of adipocere in the final two graves; this may have contributed to the lack of any evidence of lipids in the final stages of decomposition.

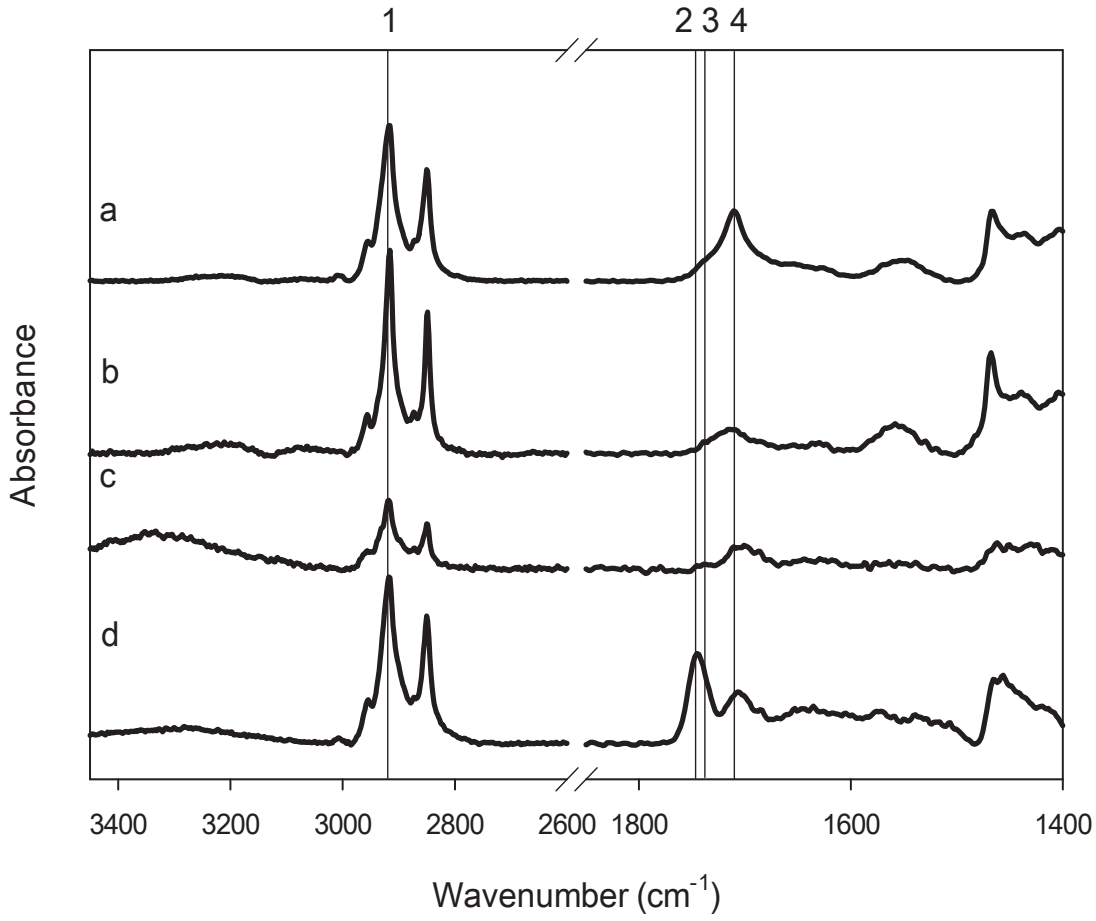

Figure 7-20: Infrared spectra for the experimental cotton samples on a) 1 month; b) 6 months; c) 12 months; and d) 18 months. The vertical lines demonstrate the regions of interest: 1) C-H stretching (2920 cm^{-1}); 2) COOH ester band (~1745 cm^{-1}); 3) triglyceride C=O stretching (~1735 cm^{-1}); and 4) C=O stretching corresponding to free fatty acid (~1715 cm^{-1}).

Only one other textile type had the presence of lipid related bands, the cotton section of the polyester – cotton blend socks (Figure 7-21). The one month samples had a large doubled in the 1578 – 1540 cm^{-1} region corresponding to fatty acid carboxylate C-O stretching (Figure 7-21a). A band at 1700 cm^{-1} was also observed in a higher abundance than the band at 1735 cm^{-1}. These bands were all suggesting that the lipids were in a later stage of lipid decomposition. The three month samples were found to have the presence of a large free fatty acid band (1715 cm^{-1}), as well as two large sharp bands at 1540 cm^{-1} and 1570 cm^{-1} corresponding to salts of fatty acids. There were no triglyceride bands (~ 1735cm^{-1}) in the three month samples again supporting that the lipids were in a later stage. One replicate at 9 months post-burial was found to have a more abundant band at 1735 cm^{-1} than the band at 1700 cm^{-1}. The doublet in the 1570 –

1538 cm^{-1} region corresponding to fatty acid salt carboxylate was found to be a lot less distinct and not as sharp compared to the 1 and 3 month samples. The evidence suggests that the lipids in the 9 month sample were in a very early lipid degradation stage as there is a larger presence of triglycerides than free fatty acids.

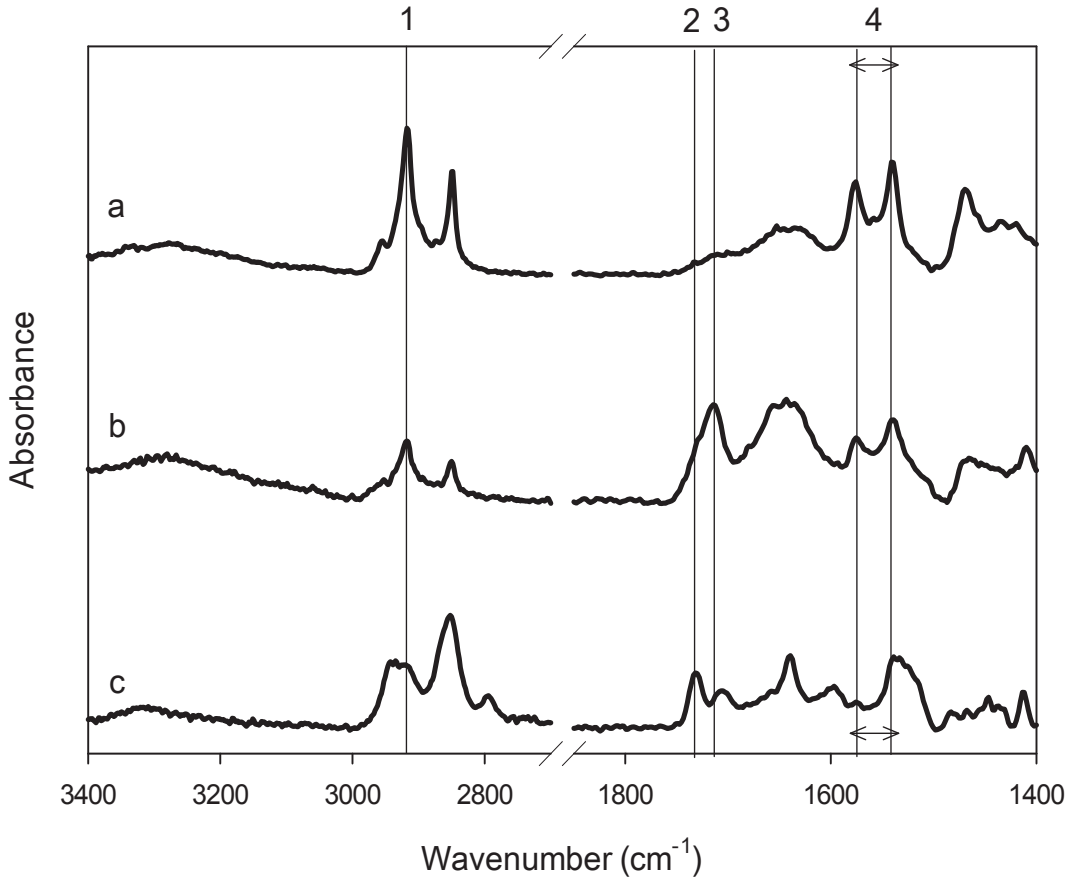

Figure 7-21: Infrared spectra for the cotton sections of the experimental polyester – cotton samples on a) 1 month; b) 3 months; and c) 9 months. The vertical lines demonstrate the regions of interest: 1) C-H stretching (2920 cm^{-1}); 2) triglyceride C=O stretching (~1735 cm^{-1}); 3) C=O stretching corresponding to free fatty acid (~1715 cm^{-1}); and 4) fatty acid carboxylate C-O stretching (1570 – 1538 cm^{-1}).

The triglyceride band found in the infrared spectra of the experimental cotton samples was plotted and there appeared to be an increase in the samples between the 12 month and 18 month excavations (Figure 7-22a). However, the band was not present in enough of the replicate samples in order for statistical analysis to be conducted. A trend was observed when the normalised band free fatty acid band was plotted over time (Figure 7-22b). Statistically it was confirmed that the 6 month samples were

significantly different from the 1 and 12 month samples. The 9 month samples were statistically different from 1, 12 and 18 month samples.

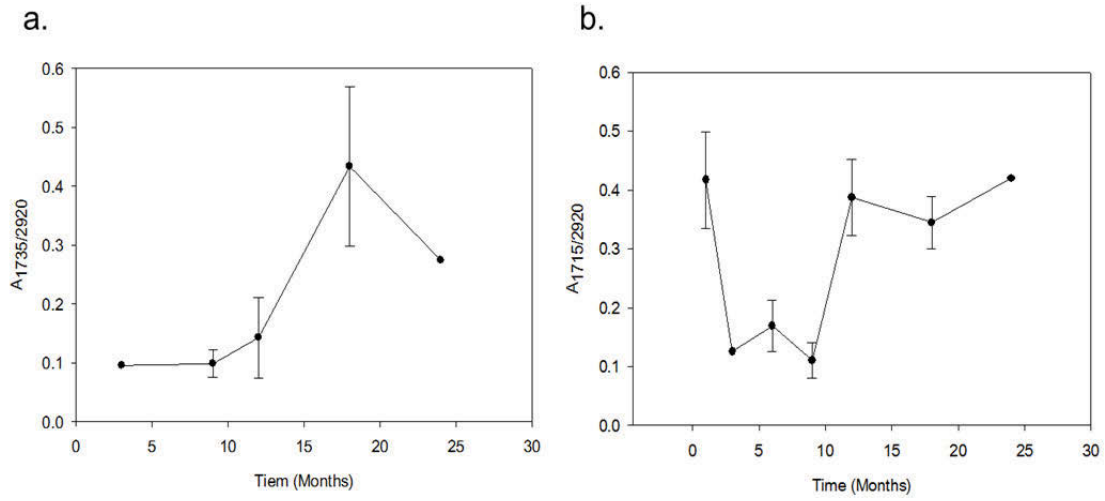

Figure 7-22: a) Quantity of triglyceride ($A_{(1735/2920)}$) as a function of time and b) free fatty acid ratio ($A_{(1715/2920)}$) as a function of time from the 100 % cotton samples.

Free fatty acid bands were observed in the cotton section of the polyester – cotton blend samples (Figure 7-23). It appeared to be a large increase in free fatty acids after 3 months post-burial, this normalised band then decreased after 9 months before remaining comparable for the remainder of the trial. As there were limited replicate spectra displaying these bands, no statistical analysis could be conducted using the normalised band.

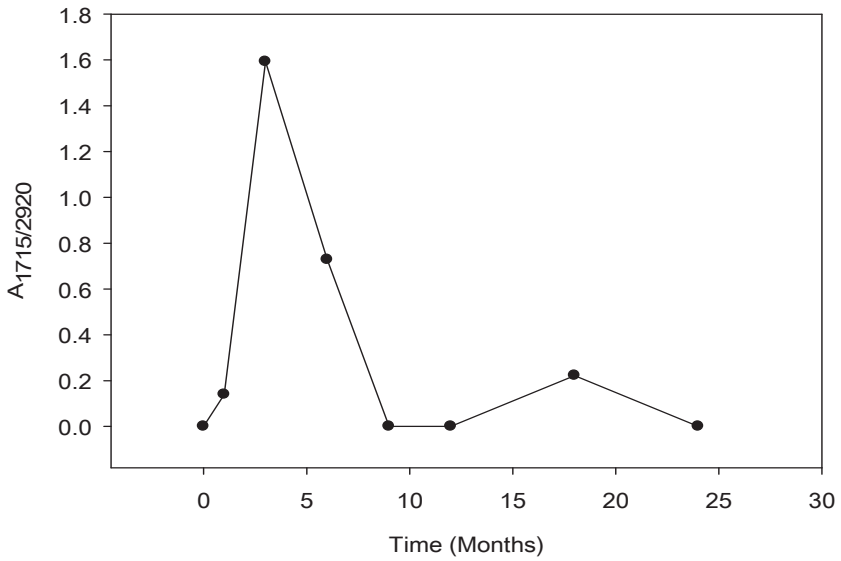

Figure 7-23: Quantity of free fatty acid ($A_{(1715/2920)}$) as a function of time from the polyester – cotton blend samples.

7.5.3 Control Grave Versus Pig Grave

In addition to looking at the variation in the types of lipids within the experimental samples, the presence of lipids and the proteins was deemed to be useful for separating samples that had been in contract with decomposing remains with those that had not (Figure 7-24). The cotton control samples could be clearly distinguished from the experimental samples when the decomposition fluid bands were selected as the influential wavenumber factors.

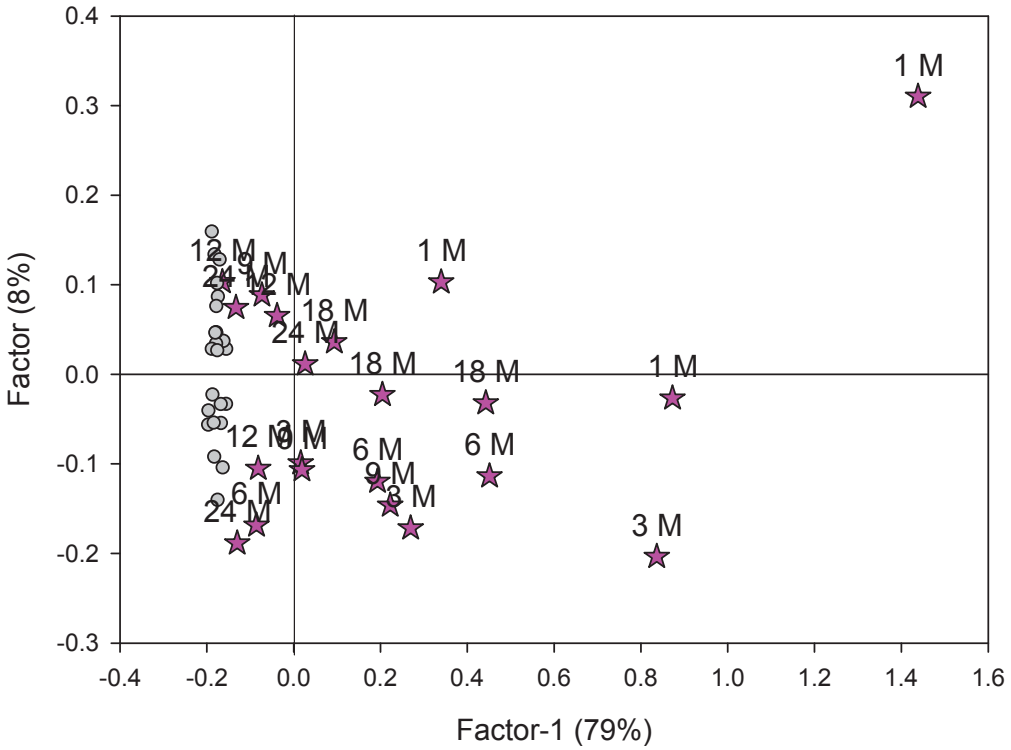

Figure 7-24: Principal component analysis of decomposition fluid bands in control cotton samples (grey circle) and from cotton samples associated with pig carcasses (pink star).

When the infrared bands associated with cotton were compared for the polyester – cotton control and experimental samples, the two groups could not be separated (Figure 7-25a). However, when the selected bands used for the loadings plot were taken from the regions encompassing the protein and lipid related bands, a clear distinction could be made between the control and pig samples (Figure 7-25c). This separation based on the presence of decomposition fluid related bands could be useful if clothing is found at the scene of a crime, but away from remains, as it might demonstrate if the clothing item was originally on decomposing remains and then later removed.

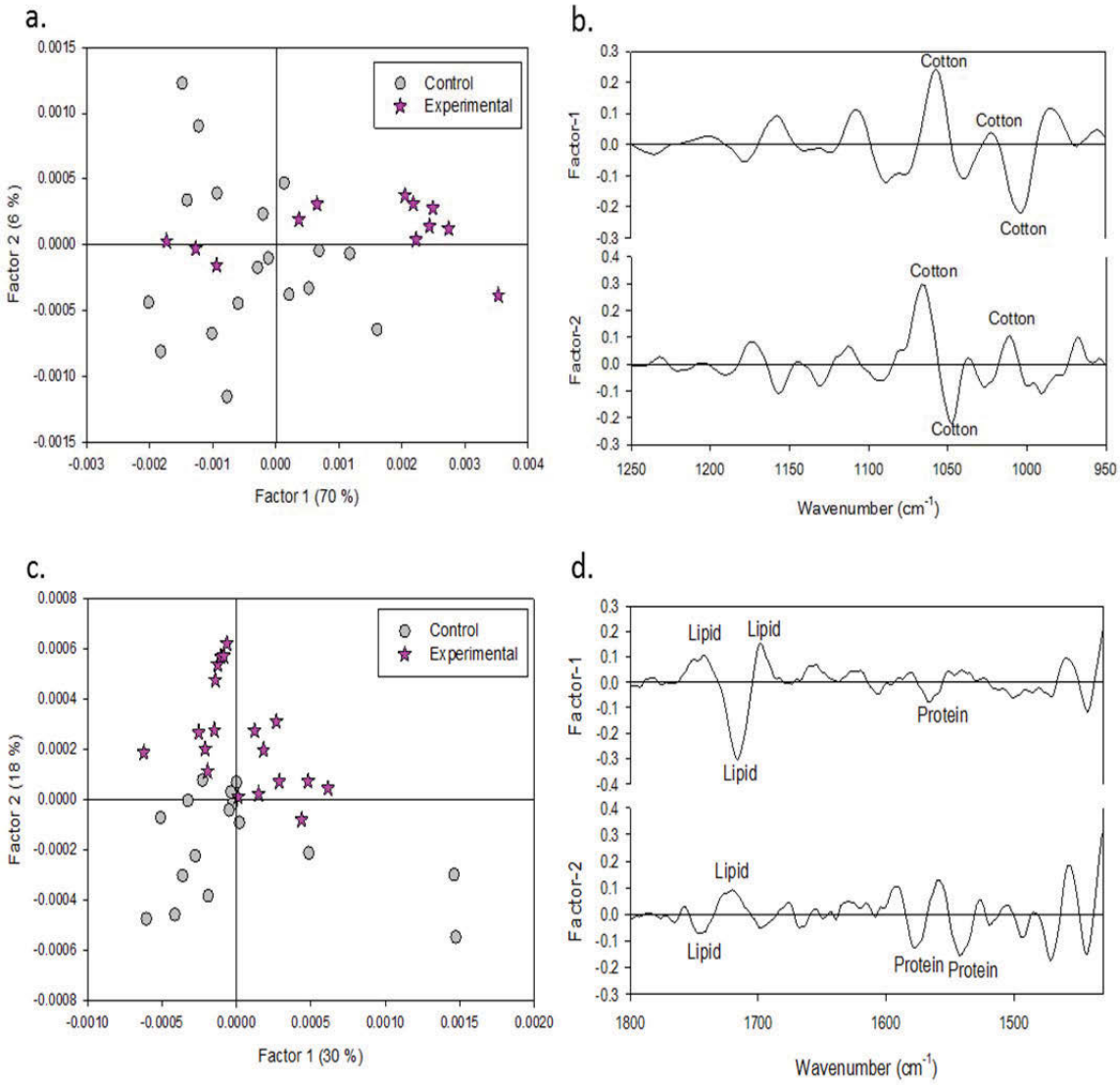

Figure 7-25: a) Principal component analysis of polyester - cotton control (grey circle) and experimental (pink star) samples based on the cotton related infrared bands. b) Loadings plot using the cotton related infrared bands. c) PCA plot of the polyester - cotton control (grey circle) and experimental (pink star) samples separated based on the decomposition fluid infrared bands. d) Loadings plot with the decomposition fluid infrared bands.

The decomposition fluid bands also enabled the detection of the exhumed clothing samples from the 'wetter' graves in the trial as these were well separated from the control samples as well as the clothing from the later graves which were found to be quite dry in nature. This was demonstrated using the data from the polyester section of the polyester – cotton blend samples (Figure 7-26).

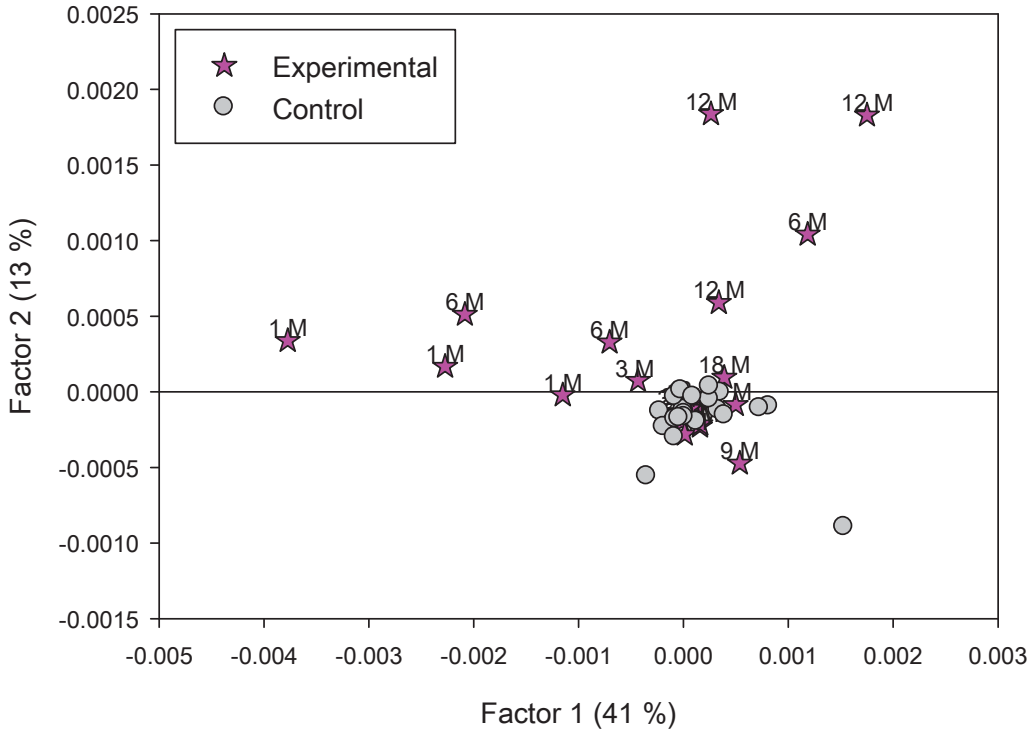

Figure 7-26: Polyester - cotton polyester section showing the PCA plot based on the decomposition fluid infrared bands.

7.6 GC-MS Analysis

As some changes were observed with the lipid components observed in the ATR-FTIR spectra from the burial samples, further lipid analysis was conducted. The mean component percentage of six selected common fatty acids (oleic, palmitoleic, linoleic, stearic, palmitic and myristic) was monitored over time.

7.6.1 Cotton

Two of the three unsaturated fatty acids were found to be decreasing both during the visual inspection of the data and statistically (Figure 7-27). The amount of oleic acid decreased notably between the first two exhumations (from 43.4 to 14.7 %), this was confirmed with a one-way ANOVA ($p = 0.05$) showing that the one month samples were significantly higher than the remaining samples ($p = 0.002$, 0.009, 0.001, 0.001, 0.001 and 0.001 for the 3, 6, 9, 12, 18 and 24 month samples, respectively). The amount of palmitoleic acid also decreased, with the one month samples again being significantly higher than the other excavation days (with p-values of 0.018, 0.024, 0.001, 0.001,

0.001 and 0.001 for the 3, 6, 9, 12, 18 and 24 month samples, respectively). The third unsaturated fatty acid, linoleic acid was found to fluctuate around 1 % in component percentage abundance, with the exception of the three month samples where the abundance was around 4 %.

All three saturated fatty acids (myristic acid, palmitic acid, stearic acid) were found to be increasing (Figure 7-27). Myristic acid increased until the 12 month samples, and the samples on this excavation were significantly different to all the other sampling days using a one-way ANOVA ($p = 0.05$). A decrease in the component percentage was then observed when comparing the 12 month to the 18 month samples, before a second increase was detected. The percentage of palmitic acid increased significantly overall from 32.6 to 52.9 %. The amount of stearic acid also increased as predicted with the samples from the one month excavation being significantly lower than the remaining excavation times.

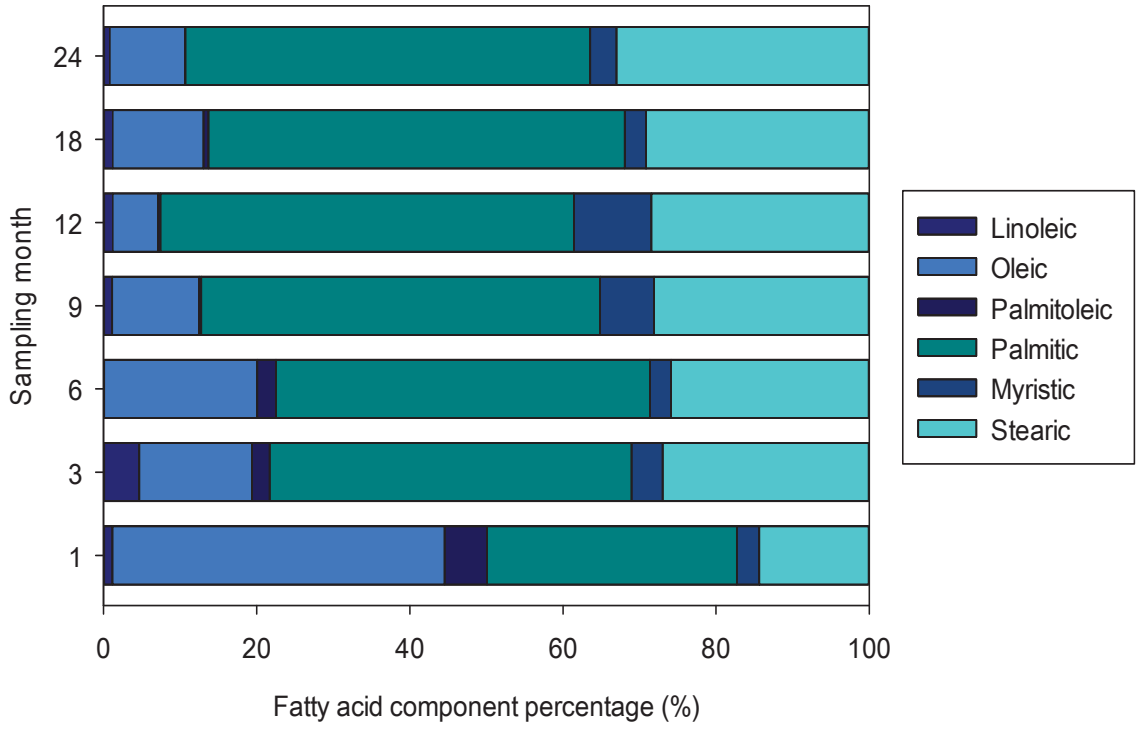

Figure 7-27: Mean percentage component (presence of each fatty acid in relation to total fatty acid composition) of selected fatty acids per sampling month from the cotton textile samples.

7.6.2 Polyester

The polyester material was also analysed using GC-MS despite the lack of a significant presence of decomposition fluid bands in the FTIR data. The amount of oleic acid was found to be decreasing the first year of sampling (Figure 7-28), but an increase was then observed in the last two sampling days. This increase was most likely due to the very low levels of most of the other fatty acids investigated from the samples taken from the last two exhumations. The other two unsaturated fatty acids, palmitoleic and linoleic acid were found in very low levels and were found to fluctuate between the sampling days.

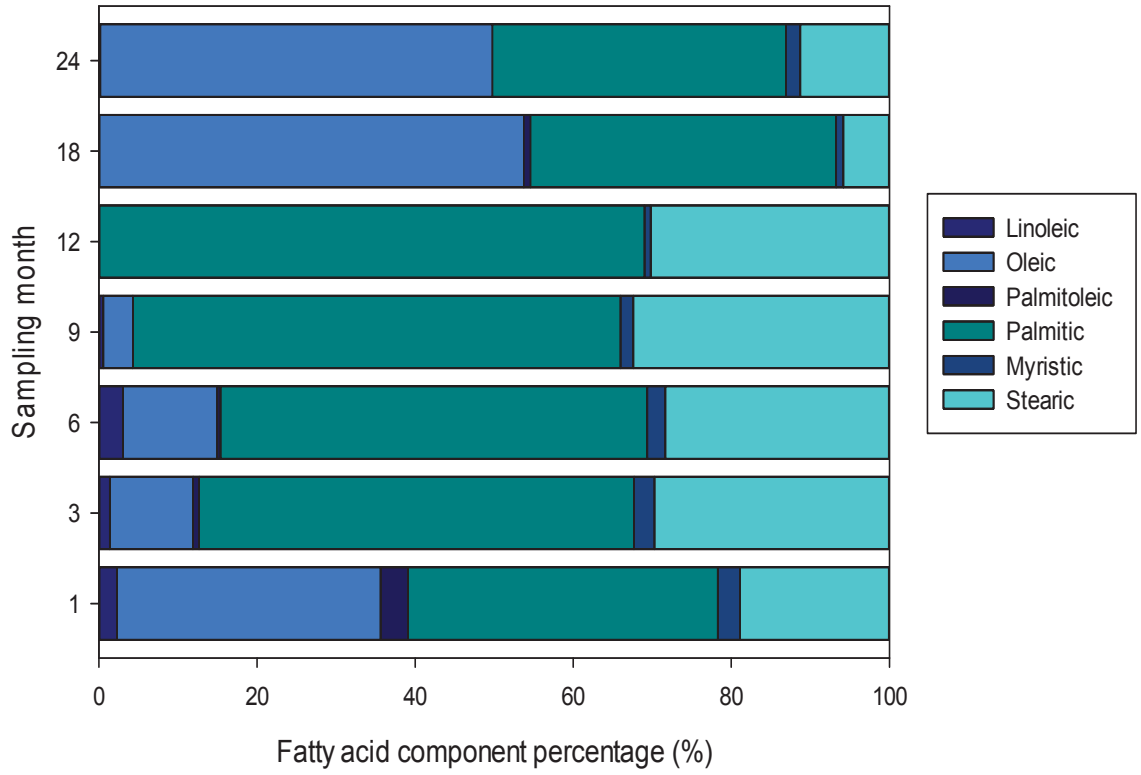

Figure 7-28: Mean percentage component (presence of each fatty acid in relation to total fatty acid composition) of selected fatty acids for the first year post-burial from the polyester textile samples.

The percentage of myristic acid was found to decrease visually – however there were no significant differences detected. Palmitic and stearic acid values were both found to increase statistically, using a one-way ANOVA ($p = 0.05$) for the first year of sampling, with increases observed from 39.2 to 69.0 % and 18.9 to 30.2 %, respectively (Figure 7-28). However, the quantity of both of these saturated fatty acids then

decreased during the final two exhumations. Overall it was likely that not all compounds were retained after 12 months, causing the component percentage to shift drastically during these final two sampling periods (Figure 7-28). This would also explain why oleic acid, which was found to be decreasing the first year suddenly increased during the last two exhumations.

7.6.3 Polyester – Cotton Blend

The polyester – cotton material was represented by socks that were applied to one front limb and one posterior leg. When the graves were backfilled this caused the legs to be somewhat separated from the torso. When excavating the samples beyond one month, the socks were difficult to locate in the grave as the limbs became separated from the rest of the body and were fully surrounded by soil. The location of the socks in the later graves caused there to be little to no presence of lipids in the material. The one month samples demonstrated high lipid levels, at this time the legs were still attached and the body demonstrated a high presence of soft tissue (Figure 7-29). However, beyond this point little data was obtained from the GC-MS analysis.

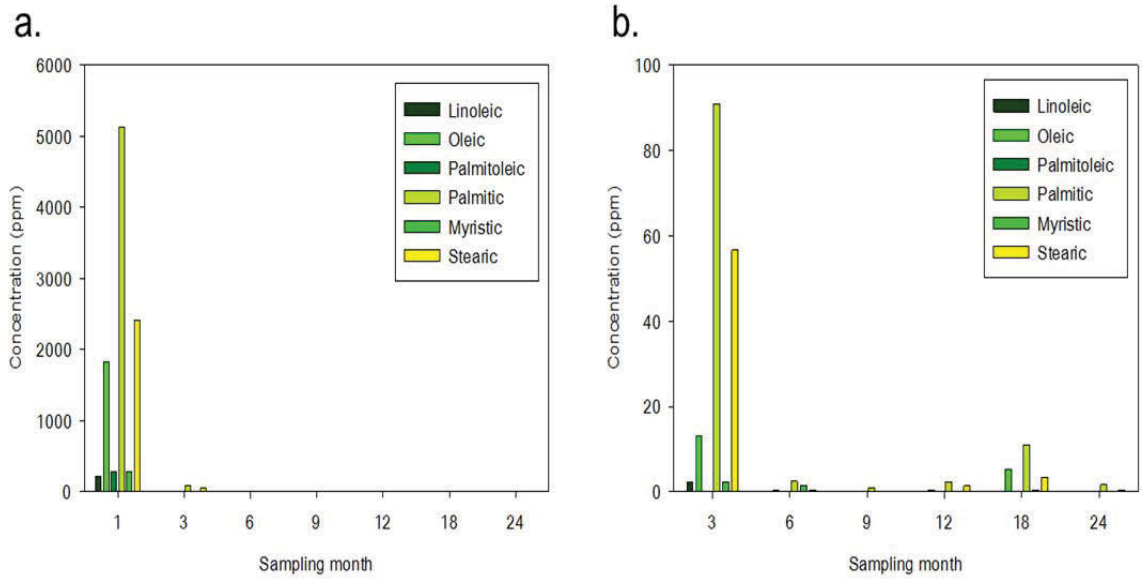

Figure 7-29: Lipid concentration of the selected fatty acids from the polyester - cotton samples from the grave sites.

7.7 Conclusions

The burial period lasted for a two year period with 7 exhumation points. Soil pH and moisture readings determined that the immediate soil environment inside the pig graves was changing more than in the control graves. Upon exhumation, the cotton t-shirts in the control graves degraded visibly throughout the burial period, and the size of remaining textile was reduced between each exhumation until the t-shirts had almost completely disintegrated after 12 months. The samples collected at 18 and 24 months did not fully follow this trend as they were present in larger quantities than the 6 – 12 month samples. The cotton clothing associated with the pig remains were notably more preserved, particularly the section underneath the pig carcass.

The synthetic polyester material was found to be virtually unchanging throughout the burial period for both control and pig graves, with the exception of some discolouring and staining of the fabric.

The control polyester – cotton material after 6 months were found to be less preserved than the prior graves. Overall the blend material was mostly intact however it was visibly weakened. The same phenomenon occurred after 9, 12, 18 and 24 months.

During the initial statistical analysis of the FTIR bands some changes could be determined, mostly in the natural materials, however normalising the bands provided significant variation, and with only three replicates per exhumation, further statistical analysis using more sophisticated software was warranted. The principal component analysis of the cotton control data demonstrated that two distinct groups could be separated, the first group included the earlier and intermediate exhumation samples (months 0 to 9), and the second grouping was found with the samples from the last two exhumations (18 and 24 months). As predicted, no degradation patterns were observed with the polyester samples. The cotton section of the polyester – cotton samples were found to be comparable to the 100 % cotton samples, with the exception of a third grouping in the PCA plot due to the Day 0 samples.

Due to the difference in the rate of textile degradation between the control and pig graves, the decomposition fluid resulting from the active decay stage of decomposition was further analysed. Investigations into the protein and lipids absorbed into the textile samples using FTIR revealed an initial change in the abundances of amide I and amide II in the natural materials. The 100 % cotton samples had various stages of lipid degradation, and it was evident that the last two exhumation times had a

lesser extent of lipids than the preceding exhumed graves; this was also reflected in the moisture recordings. The three month samples from the cotton sections of the polyester – cotton blend were found to have the presence of a large 1715 cm^{-1} band, as well as two large bands at 1540 cm^{-1} and 1570 cm^{-1} corresponding to salts of fatty acids, indicating a presence of adipocere in the grave, whereas the 9 month samples were determined to be in an early stage of lipid degradation. The presence of decomposition fluid bands (lipid and proteins) in the infrared spectra were determined to be useful for separating samples that had been in contract with decomposing remains with those that had not. The decomposition fluid bands also enabled the detection of the exhumed clothing samples from the "wetter" graves in the trial as there were well separated not only from the control samples but also from the clothing from the later graves which were found to be quite dry in nature.

Using GC-MS the lipid components in the textile samples were further investigated in order to determine if any trends could be established using this technique. In the absorbent cotton material the unsaturated fatty acids were determined to be decreasing, and the saturated fatty acids were increasing. This demonstrated that the lipids were changing over time and could be of use during the crime investigation, however due to the limited availability of pig carcasses the replicate number for each exhumation time-point was less than that collected for the surface trials. There is potential for the lipid profile to give valuable information to an investigator, however more research with more replicate data is required.

Chapter 8: CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

Chapter 8: CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

8.1 Overview

Clothing is a commonly recovered item at the scene of a crime and knowing more about these evidence items can help assist the investigators. Currently the condition of the clothing material is used to help with the determination of the nature of the death and in the identification of the victim. Clothing has also been found to have a profound effect on the timing of the post-mortem interval. Being able to determine the time since death is incredibly important when identifying a perpetrator or excluding suspects in a homicide investigation. However, it has also been suggested that the presence of decomposing remains will have a large influence on the textiles themselves. Analysing how the presence of decomposing remains affect textile degradation patterns can help increase the evidentiary value of clothing samples found at the scene of a crime.

Three trials, two commencing in the Australian summer and one starting during the Australian winter, consisting of clothed pig remains along with the identical clothing placed directly on the soil surface, were completed. The field site was located in an open eucalypt woodland in western Sydney. The soil in the area was acidic with a pH range of 4 – 5. Three different clothing types were investigated, a synthetic material, a natural material and a blend of the two. A longer burial study with a total of seven exhumation times with graves consisting of either clothed remains or the clothing samples without the presence of decomposing remains was also conducted. Textile samples were collected each sampling day and analysed using ATR-FTIR spectroscopy and GC-MS. ATR-FTIR spectroscopy was successfully used to investigate textile degradation and the presence of decomposition by-products absorbed into the textile samples. GC-MS was then employed to further explore the lipid composition in the textiles. Multivariate statistical analysis was used to process the data.

At the completion of the project it was confirmed both visually and through the chemical analysis that the natural textile degradation was inhibited in the presence of decomposing remains. The 100 % cotton samples showed visual signs of degradation after approximately four months and continued to degrade beyond this point. The

development of more advanced statistical software enabled the spectral data to be analysed using multivariate analysis and chemometrics. PCA of the cotton control data demonstrated a clear separation in the data sets, and degraded samples could clearly be distinguished from the non-degraded ones. This separation was determined to be related to the C-O stretching regions of the cotton spectrum, and indicated that the cotton samples degraded through the process of hydrolysis. Seasonal variety was also determined to be a factor in the timeline of textile degradation of the natural material, as the degradation occurred at later stage during the winter trial.

The synthetic polyester material from the surface trials was in a relatively good state apart from some discolouration. The polyester – cotton blend was found to be somewhere in between as predicted, with sections of the socks demonstrating some degradation through the apparent decreased strength and general appearance of the fabric. The cotton sections from the polyester – cotton blend samples were found to be very similar to the 100 % cotton samples, however the statistical analysis did demonstrate that the distinction between degraded and non-degraded samples was less distinct compared to the 100 % cotton samples. Thus, the presence of synthetic material in close proximity with the cotton section was found to slightly inhibit the degradation of the natural material.

The textile samples from the burial experiment produced similar trends to those observed for the surface studies for all three textile types. However, some notable differences in the degradation behaviour were observed as a result of burial. The 100 % cotton control samples demonstrated greater visual degradation after 3 months. These samples showed a much greater degree of visual damage over time compared to the identical material on the surface. In addition, there was also some visual damage to the 100 % cotton material associated with the remains, however, this damage was found to be located where no decomposition by-products were apparent. Overall, the use of multivariate statistics provided a better foundation for the interpretation of the data obtained using ATR-FTIR spectroscopy, and also gave further insight into textile degradation in both burial and surface environments.

It was determined that the presence of the decomposing remains prevented textile degradation and this was hypothesised to be due to the release of fluid during the decomposition process. The more absorbent cotton materials were found to have a greater presence of decomposition by-products, whereas for the non-absorbent polyester, the presence of biological fluid was not as prominent. The fluid was absorbed

in the textile samples and was likely working as a protective layer against the soil microbes responsible for the textile degradation in the control samples. The inhibition of the bacteria could have resulted due to the increased pH caused by the release of the decomposition fluid. The soil at the field site was found to be alkaline in nature, and the increase in pH observed at the experimental sites could have resulted in a decrease in the abundance of the soil bacteria.

Further investigation of the decomposition by-products showed that the amide II/amide I ratio used to monitor the protein component remained stable and unchanged throughout the surface experiment. Lipids, on the other hand, demonstrated a significant change; this was confirmed with the use of ATR-FTIR spectroscopy, advanced multivariate analysis and GC-MS. The lipids in the 100 % cotton samples could be grouped into three general categories; little to no lipid degradation (days 2 – 8), some lipid degradation (days 10 – 21), and advanced lipid degradation (days 24 – 48). The winter trial could be separated into two lipid groups. The lack of a third group is likely due to the slow onset of active decay during the winter season due to the colder temperatures being less advantageous for bacteria and inhibited insect activity. Overall, the textile specimens exhibited lipid patterns consistent with tissue degradation, as triglycerides were degrading into free fatty acids, and the presence of salts of fatty acids demonstrate that the remains had entered putrefaction. Thus, the lipids demonstrate potential as a marker for determining time since death of clothed individuals due to their ability to be separated into different stages of lipid degradation.

8.2 Future Recommendations

The burial study did show a difference in protein ratio over time, as well as some changes in the lipids throughout the sampling time, however, as there was a limited number of replicates, further work should be conducted with an increased number of buried remains. Increasing the number of replicates would greatly increase the influence of the findings and their statistical validity. In addition the results of the ATR-FTIR spectroscopic analysis of both proteins and lipids in the textile samples may be more comparable to the findings of the surface trials if the samples could be taken from the same body over time rather than using bodies excavated from different graves over different time points.

Another aspect that would be beneficial for future studies would be to investigate the difference between the natural degradation of the textile samples and any intentional damage to the fabric pre-post or during a criminal activity. If clothing evidence if found any natural textile degradation needs to be able to be separated from intentional damage such as a stab wound, as this can potentially prevent the discovery of cause of death. It will therefore be of value to replicate the experiments conducted during this project and include intentional damage that can be monitored over time.

As the methodology employed in this study was found to be successful in separating the degraded from non-degraded clothing samples using materials commonly encountered in criminal cases, it demonstrates potential for its use in criminal cases. However, using more standardised materials, and a larger variety of materials, in order to create a more comprehensive database of textile degradation would be advised.

It would also be highly beneficial to look at the microbial populations responsible for the degradation of the textile samples. A difference due to season of commencement of the trials was observed, and likely attributed to the differing microbial communities that can be found in the soil during the seasons. The lack of degradation of textiles associated with decomposing remains is likely due to inhibition of soil microbes caused by the fluid released from the remains. Following the microbial population over time in the absence and presence of remains could show the specific mechanism behind this inhibition.

These experiments were all conducted using pigs as human analogues. Despite the frequent use of pigs as human substitutes, and the similarity between pigs and humans in terms of internal anatomy, gut fauna and the lack of fur, there is no conclusive work demonstrating that the decomposition is identical. Replicating the surface and burial studies using human remains should be completed in order to determine if similar trends as observed here also occur in the case of human remains.

8.3 Concluding Remarks

Through both the surface and the burial experiments it was demonstrated that the presence of textiles as the scene of an event can provide more information than initially evident. Not only does the presence of textiles alter the decomposition timeline, the decomposition process itself affects the degradation of the textiles. The apparent inhibition of the degradation of natural textiles associated with the decomposing

remains is a significant finding. It suggests that the decomposition processes leading to the release of decomposition fluid into the environment might play a significant role in the textile degradation timeline. The ability to monitor the degradation of the textiles, particularly the natural textiles using a combination of visual observation and the statistical analysis of microstructural changes indicates that there is potential to use the textile degradation patterns in order to create a timeline of events. The analysis of the decomposition by-products also revealed that mapping the lipid composition and lipid degradation stage may also be useful for the estimation of post-mortem interval.

Overall the findings demonstrate that investigating the textile degradation stage, as well as analysing the decomposition fluid of textile samples encountered at the scene of a crime may help in the estimation of the post-mortem or post-burial interval. An additional means of estimating time of death can provide invaluable information that has great potential to help solve future cases.

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APPENDICES

APPENDIX A: SUPPORTING INFO FOR DECOMPOSITION T1

Day	Site	Stage	Notes	Photo
0	Pig A	Fresh	• Quite large pigs about 75 – 80 kg • Pigs fresh, starting to enter rigor mortis	
0	Pig B	Fresh	• Quite large pigs about 75 – 80 kg • Some blood on the t-shirt from a head wound	
0	Pig C	Fresh	• Quite large pigs about 75 – 80 kg • A very small amount of blood on the t-shirt	
0	Control A	N/A	• No visual physical textile damage	
0	Control B	N/A	• No visual physical textile damage	
0	Control C	N/A	• No visual physical textile damage	

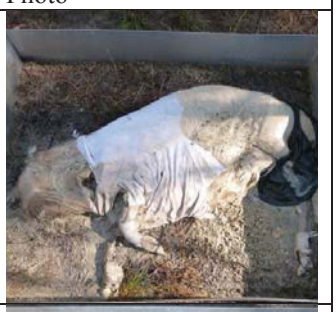
Day	Site	Stage	Notes	Photo
2	Pig A	Bloat	• High fly activity • Obvious smell from the site, sour • Hydrogen sulfide smell • Pooling of blood underneath • Sack of internal organs protrude from the abdomen • Marbling on lower abdomen	
2	Pig B	Bloat	• Maggots under the head • Blistering on abdomen and some on upper limbs • T-shirt very bloody • Protrusions on lower side of the back • Very greenish discolouration in head region	
2	Pig C	Bloat	• Foul smell • Marbling, very dark in head region • Maggots in mouth • Fly activity, mostly around briefs • Reddish discolouration on lower abdomen • Some blisters on left side	
2	Control A	N/A	• No visual physical textile damage	
2	Control B	N/A	• No visual physical textile damage	
2	Control C	N/A	• No visual physical textile damage	

Day	Site	Stage	Notes	Photo
4	Pig A	Post bloat early active	• Hair loss • Maggots on clothing and near lower abdomen • 3rd instar larvae surrounding pig • Some mummification on right front limb • Ammonia smell	
4	Pig B	Post bloat early active	• Large maggot mass under briefs • Larger CDI than Pig A • Skin exposed, no hair • Left ear dry and leathery skin • Red mark above briefs (coloured off?)	
4	Pig C	Post bloat early active	• Large areas of hair loss on abdomen, behind left ear and on right front limb • High maggot activity around lower legs • Briefs covered in 1st instar	
4	Control A	N/A	• No visual physical textile damage • Some discoloration	
4	Control B	N/A	• No visual physical textile damage • Some discoloration	
4	Control C	N/A	• No visual physical textile damage • Some discoloration	

Day	Site	Stage	Notes	Photo
6	Pig A	active	• Some localised maggot masses • Less hair • T-shirt wet and discoloured	
6	Pig B	active	• Shorts pulled down by the weight of the maggot masses • Socks very muddy, have been in the maggot mass • Yellowish areas on t-shirt between front limbs • Large amount of hairless maggots in the surrounding soil	
6	Pig C	active	• Frothing from hole in abdomen • Shorts has dry white and yellowish material on them • T-shirt yellowish on front • Teeth and parts of mandible exposed	
6	Control A	N/A	• No visual physical textile damage • Some discoloration	
6	Control B	N/A	• No visual physical textile damage • Some discoloration	
6	Control C	N/A	• No visual physical textile damage • Some discoloration	

Day	Site	Stage	Notes	Photo
8	Pig A	Dry decomp Haltered active	• Lots of hair on briefs • Large amount of maggot under clothing • Some of the molars visible in mouth • Can see where the briefs used to be, no hair and lighter colour	
8	Pig B	Dry decomp Haltered active	• Layer of decomp fluid on t-shirt • Black layer on the socks • Bone protruding under briefs • Maggots under clothing are protected from the rain • Can see most of the mandible • Sections of skin in head region still dry and leathery	
8	Pig C	Dry decomp Haltered active	• Large holes in abdomen from maggots • T-shirt dark in front and light in the back • Can see the hairs through the back of t-shirt • Neck dry and hairless • Maggot crater below left ear	
8	Control A	N/A	• No visual physical textile damage • Some discoloration	
8	Control B	N/A	• No visual physical textile damage • Some discoloration	
8	Control C	N/A	• No visual physical textile damage • Some discoloration	

Day	Site	Stage	Notes	Photo
10	Pig A	Localised active Mummification	• Not much change • Large maggot masses under briefs and front of t-shirt	
10	Pig B	Localised active Mummification	• Dry and leathery where t-shirt was removed • Yellowish dry discolouration on left front limb • Head dry and leathery	
10	Pig C	Localised active Mummification	• Large beetle on the pig • Higher maggot activity, all under clothing • Bone exposed under t-shirt • Pelvis evident	
10	Control A	N/A	• No visual physical textile damage • Some discoloration	
10	Control B	N/A	• No visual physical textile damage • Some discoloration	
10	Control C	N/A	• No visual physical textile damage • Some discoloration	

Day	Site	Stage	Notes	Photo
14	Pig A	Wet mummification Localised activity under clothing	• Lots of drowned maggots under briefs • Adipocere in the CDI next to hind legs • Very wet and white in appearance • Upper limbs almost skeletonized	
14	Pig B	Wet mummification Localised activity under clothing	• Jaw protruding, white in colour • Likely adipocere formation • Maggot activity under clothing only • Limbs falling apart when touched • Very rubbery look overall	
14	Pig C	Wet mummification Localised activity under clothing	• Possible adipocere on t-shirt • Pink and white discolouration • Adipocere formation in CDI, head region and abdomen as well as on lower left limb • Orange discolouration on the inside of the socks	
14	Control A	N/A	• No visual physical textile damage • Some discoloration	
14	Control B	N/A	• No visual physical textile damage • Some discoloration • Presence of leaves and organic matter	
14	Control C	N/A	• No visual physical textile damage • Some discoloration	

Day	Site	Stage	Notes	Photo
17	Pig A	active	• Maggots and lots of fluid under briefs • High beetle activity (associated with active stage) • Bone exposed under t-shirt • Socks immersed in decomp fluid • Maggots mostly drowned	
17	Pig B	active	• Very leathery region in abdomen • Lots of beetles when removing clothing • Maggot mass between t-shirt and right side of underbelly • T-shirt soaked and discoloured	
17	Pig C	active	• Dark brown pupae • Very dark in colour, especially in abdominal region • White discolouration in abdomen • Lower limbs skeletonized	
17	Control A	N/A	• No visual physical textile damage • Clothing damp	
17	Control B	N/A	• No visual physical textile damage • Clothing damp	
17	Control C	N/A	• No visual physical textile damage • Clothing damp	

Day	Site	Stage	Notes	Photo
21	Pig A	Advanced	• Partially skeletonized • Eyes missing • Fatty fluid along the back • Very dark and dry around head region excluding the head itself • Minimal fly activity	
21	Pig B	Advanced	• Some maggot activity in rear inside pool of fluid • Very dry and darkly discoloured • White on skull and front limbs • Legs skeletonized and mandible exposed	
21	Pig C	Advanced	• Hind legs fully skeletonized • Front legs very white and preserved • Skin very orange • Fluid pool in rear with maggots • Some fly activity	
21	Control A	N/A	• No visual physical textile damage • Discoloration from organic matter	
21	Control B	N/A	• No visual physical textile damage • Discoloration from organic matter	
21	Control C	N/A	• No visual physical textile damage • Discoloration from organic matter	

Day	Site	Stage	Notes	Photo
24	Pig A	Advanced	• Still fat deposits on the back • Fluid in rear, still maggot masses in the "pool" • White deposits inside CDI • Limbs, ribs and part of vertebral column skeletonized • Possible fungal growth in head region and shoulder	
24	Pig B	Advanced	• Lots of white deposits on head, back and abdomen • Very, dry and leathery abdominal region • Soft layer of white deposits on the front of the t-shirt • Limbs skeletonized	
24	Pig C	Advanced	• Large pool of fat under t-shirt • Lower limbs skeletonized • Front limbs preserved • Brittle white substance on abdomen • Maggots along the trenches in head region	
24	Control A	N/A	• No visual physical textile damage • Discoloration from organic matter	
24	Control B	N/A	• No visual physical textile damage • Discoloration from organic matter	
24	Control C	N/A	• No visual physical textile damage • Discoloration from organic matter	

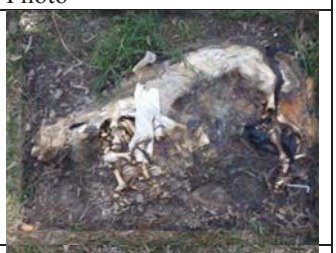
Day	Site	Stage	Notes	Photo
31	Pig A	Mummified Local skeletonization	• Mummified limbs, left shoulder • Frothing in rear • Maggot activity under ribs • Growth of grass and plants in CDI on upper side of carcass and lower left corner	
31	Pig B	Mummified Local skeletonization	• A lot of pupae in rear • White/yellow discolouration on torso and shoulders • Very white left front limb • White deposits on t-shirt • Some maggots in dark fluid in rear	
31	Pig C	Mummified Local skeletonization	• Ribs exposed • Lower limbs skeletonized • White/grey deposits on abdomen/front limb/rear/head region • Maggots in decomp fluid • Some grass growth	
31	Control A	N/A	• No visual physical textile damage • Discoloration from organic matter	
31	Control B	N/A	• No visual physical textile damage • Discoloration from organic matter	
31	Control C	N/A	• No visual physical textile damage • Discoloration from organic matter	

Day	Site	Stage	Notes	Photo
48	Pig A	Mummified Local skeletonization	• Massive amount of pupae along lower trench wall and above head • Large amounts of fluid • Still living maggots in rear • Red discol. t-shirt left side • Adipocere in some regions • Dry and leathery skin on the back and side of pig	
48	Pig B	Mummified Local skeletonization	• White deposits on exposed shoulder blade • Reddish discol. on t-shirt • Some fly activity • Wet fluid on lower part • Some maggot activity • White region on the left side of the chin	
48	Pig C	Mummified Local skeletonization	• Maggot activity under rib cage • Adipocere chunks on and below abdomen • Front limbs still fairly intact • Some fly activity	
48	Control A	N/A	• No visual physical textile damage • Has been a flooding, soil on top of the clothing • Surrounding ants and ant hills	
48	Control B	N/A	• No visual physical textile damage • Very wet • Lots of dirt on top of clothing • Increased surrounding vegetation	
48	Control C	N/A	• No visual physical textile damage • Ant activity around • Increased vegetation, some white flowers • Very wet and textile very dirty	

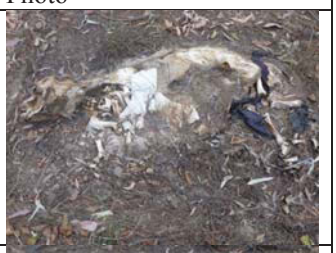
Day	Site	Stage	Notes	Photo
59	Pig A	Advanced	• Some fly activity • Further skeletonized in neck region • Surrounding soil is dark • Some increase in vegetation growth	
59	Pig B	Advanced	• Plastic looking white/clear layer in rear on top of the lower limbs • Little apparent activity, but a lot under the clothing • Very pale in colour	
59	Pig C	Advanced	• A lot of dead vegetation • Very dark soil	
59	Control A	N/A	• No visual textile damage • Very wet and dirty from soil	
59	Control B	N/A	• No visual textile damage • Very wet and dirty from soil	
59	Control C	N/A	• No visual textile damage • Very wet and dirty from soil	

Day	Site	Stage	Notes	Photo
94	Pig A	Advanced	• Orange coloured liquid • Some parts of skin coloured pink • T-shirt pink discolouration • Front limbs and shoulder and parts of ribs skeletonized • Maggots under some parts of clothing	
94	Pig B	Advanced	• Limbs, mandible and most of skull skeletonized • Shoulder bones and ribs exposed • Very flat with some fluid under the left front shoulder • Jaw very discoloured, pink gold in colour	
94	Pig C	Advanced	• T-shirt very pink • Hind limbs skeletonized and all ribs exposed • Front limbs still very intact and skin is white and wrinkly • Still covered in hair in most areas	
94	Control A	N/A	• Clothing wet • T-shirt discoloured orange/yellow and brown but no other damage	
94	Control B	N/A	• T-shirt is less discoloured • No apparent damage to textile structure • Some fungi on the briefs	
94	Control C	N/A	• T-shirt has a yellow tint to it • One sock almost completely buried in the soil	

Day	Site	Stage	Notes	Photo
149	Pig A	Advanced	• Orange coloured liquid by the rear and abdomen • Parts of skin coloured pink • T-shirt pink discolouration over the back area • Front limbs and shoulder and parts of ribs skeletonized	
149	Pig B	Advanced	• Limbs, mandible and most of skull skeletonized • Shoulder and ribs exposed • Very flat with some fluid under the left front shoulder • Jaw very pink gold in colour • Skin very white	
149	Pig C	Advanced	• T-shirt very pink • Hind limbs skeletonized and all ribs exposed • Front limbs still very intact and skin is white and wrinkly • Still covered in hair	
149	Control A	N/A	• Clothing wet • T-shirt discoloured orange/yellow and brown • Visual damage to the t-shirt in the form of small holes	
149	Control B	N/A	• T-shirt is less discoloured • Visual damage to the t-shirt in the form of small holes • Some fungi on the briefs	
149	Control C	N/A	• T-shirt has a yellow tint to it • One sock almost completely buried in the soil	

Day	Site	Stage	Notes	Photo
184	Pig A	Dry/Remains	• Still some maggots under the t-shirt • Some small holes in the socks	
184	Pig B	Dry/Remains	• Limbs, mandible and most of skull skeletonized • Shoulder bones and ribs exposed	
184	Pig C	Dry/Remains	• Holes in the region of the t-shirt that was laying on the top of upper limbs	
184	Control A	N/A	• Visible damage to the t-shirt • Leafs grown within the t-short • Roots piercing the briefs • Some small areas of damage at the end of the socks	
184	Control B	N/A	• Visible damage to the t-shirt • Roots piercing the briefs • Socks damaged in some areas	
184	Control C	N/A	• The t-shirt is very frail and damaged in the neck region • Grass is piercing the socks	

Day	Site	Stage	Notes	Photo
212	Pig A	Dry/Remains	• Dry section of the t-shirt on the underside that is not covered in liquid is starting to show small holes	
212	Pig B	Dry/Remains	• Large maggot mass underneath the t-shirt	
212	Pig C	Dry/Remains	• Holes in the region of the t-shirt that was laying on the top of upper limbs	
212	Control A	N/A	• Roots piercing the t-shirt, holes in the neck region still • Some orange rust on the briefs, but still well preserved • Socks discoloured, but intact	
212	Control B	N/A	• T-shirt visibly thinner • Briefs colour is very faded • Socks have no visible damage	
212	Control C	N/A	• T-shirt have some small holes in certain areas • Briefs colour faded • Socks have no visible damage	

Day	Site	Stage	Notes	Photo
268	Pig A	Dry/Remains	• Dry section of the t-shirt on the underside that is not covered in liquid is starting to show small holes	
268	Pig B	Dry/Remains	• Large maggot mass underneath the t-shirt	
268	Pig C	Dry/Remains	• Holes in the region of the t-shirt that was laying on the top of upper limbs	
268	Control A	N/A	• Roots piercing the t-shirt, holes in the neck region still • Some orange rust on the briefs, but still well preserved • Socks discoloured, but intact	
268	Control B	N/A	• T-shirt visibly thinner • Briefs colour is very faded • Socks have no visible damage	
268	Control C	N/A	• T-shirt have some small holes in certain areas • Briefs colour faded • Socks have no visible damage	

Day	Site	Stage	Notes	Photo
365	Pig A	Skeletonized Mummified	• Cotton mostly intact, some holes under where there is contact with soil • Briefs well preserved • Socks have frayed ends and small holes	
365	Pig B	Skeletonized Mummified	• T-shirt damaged on the top • T-shirt covered in liquid is less damaged • Briefs well preserved • Green discolouration of the socks, one area has visible damage	
365	Pig C	Skeletonized Mummified	• Pig is very dry under • The cotton is a lot more damaged than pig A and B • Briefs colour fading	
365	Control A	N/A	• Holes in t-shirt, very degraded • Very discoloured briefs on the side facing the sun • Socks seem weaker and more degraded	
365	Control B	N/A	• Holes in t-shirt, very degraded • Very discoloured briefs on the side facing the sun • Socks seem weaker and more degraded	
365	Control C	N/A	• Only degraded on the t-shirt that is in direct contact with the soil and slightly packed under it • Piercing grass and roots on the briefs • Briefs faded	

APPENDIX B: SUPPORTING INFO FOR DECOMPOSITION T2

Day	Site	Stage	Notes	Photo
0	Pig A	Fresh	• Black fur • Some bloody spots of t-shirt and socks • Body still warm • Smaller than the other two pigs	
0	Pig B	Fresh	• Very bloody t-shirt • Black briefs	
0	Pig C	Fresh	• Black fur • Some blood on t-shirt • Red briefs	
0	Control A	N/A	• No visual physical textile damage	
0	Control B	N/A	• No visual physical textile damage	
0	Control C	N/A	• No visual physical textile damage	

Day	Site	Stage	Notes	Photo
3	Pig A	Fresh	• Blood from snout • Some flies • No egg masses • Some bloating in head region	
3	Pig B	Fresh	• Blood from snout and in ear • Two large coppery flies • Increased fly activity as the sun came out	
3	Pig C	Fresh	• In direct sunlight • Some larvae in upper lip • Hair very stiff and sandy • Very large copper/brown flies	
3	Control A	N/A	• Frost droplets on clothing • No visual physical textile damage	
3	Control B	N/A	• Frost droplets on clothing • No visual physical textile damage	
3	Control C	N/A	• Frost droplets on clothing • No visual physical textile damage	

Day	Site	Stage	Notes	Photo
6	Pig A	Fresh	• Neck region bloated • Some fly activity – all centred around the mouth • Bloody snout • Larvae in the mouth • Some egg masses in mouth	
6	Pig B	Fresh	• Higher fly activity • Pool of blood under snout • Larvae in the mouth (only in the front) • Joints very stiff – rigor mortis	
6	Pig C	Fresh	• Some fly activity • Dark blood from snout • High larval activity in the mouth • Joints very stiff – rigor mortis	
6	Control A	N/A	• No visual physical textile damage	
6	Control B	N/A	• No visual physical textile damage	
6	Control C	N/A	• No visual physical textile damage	

Day	Site	Stage	Notes	Photo
9	Pig A	Autolysis	• Marbling, some greenish discolouration on abdomen • Large maggot mass in mouth • Pooling of liquid underneath • Liquid purging from mouth • Limbs still a bit rigid • Large fly activity	
9	Pig B	Autolysis	• Some fly activity • Maggots in mouth, under the lip • Joints very rigid • Some discolouration in abdominal region	
9	Pig C	Autolysis	• Appear a bit bloated • High fly activity • Large maggot masses inside entire mouth • Pooling of liquid • Some eggs in ear • Joints still a bit stiff	
9	Control A	N/A	• No visual physical textile damage • Some discoloration	
9	Control B	N/A	• No visual physical textile damage • Some discoloration	
9	Control C	N/A	• No visual physical textile damage • Some discoloration	

Day	Site	Stage	Notes	Photo
13	Pig A	Autolysis Partial bloat	• Large maggot mass in the mouth • Egg masses in head region • Fly activity high – 2 types; blue bottle and unidentified • Greenish discol of abdomen • Egg masses in ear	
13	Pig B	Autolysis Partial bloat	• Egg masses in rear and eyes • Larvae in mouth breaking down the flesh • Fly activity high – collected orange fly • Evidence of rain	
13	Pig C	Autolysis Partial bloat	• A lot of activity in head region • Large and small larvae • Part of lower lip removed • Sticky around eye, egg masses throughout entire head region • High fly activity • Larvae in rear	
13	Control A	N/A	• No visual physical textile damage • Some discoloration	
13	Control B	N/A	• No visual physical textile damage • Some discoloration	
13	Control C	N/A	• No visual physical textile damage • Some discoloration	

Day	Site	Stage	Notes	Photo
20	Pig A	Autolysis Partial bloat	• Eyes and jaw gone • Frothing under head • Lots of maggots around head • White layer in rear – mould? • Egg masses in rear • High fly activity • Marbling on front limbs	
20	Pig B	Autolysis Partial bloat	• Half of head (mouth) eaten • Lots of larval activity • Egg masses under chin • Egg masses on abdomen and rear • Mould in rear	
20	Pig C	Autolysis Partial bloat	• Clothing in neck region and under front limbs is dark and wet • Maggot masses clumping inside the t-shirt • Egg masses under chin • Eyes removed	
20	Control A	N/A	• No visual physical textile damage • Some discoloration	
20	Control B	N/A	• No visual textile damage • Some discoloration	
20	Control C	N/A	• No visual physical textile damage • Some discoloration	

Day	Site	Stage	Notes	Photo
37	Pig A	Active decay	• Frothing lots around head and rear, some around abdomen • High maggot activity internally and under clothing • Limbs still fresh • Rupture in abdomen, head and rear	
37	Pig B	Active decay	• Still a little bloated in abdomen • Hind limbs mummified (skin leathery, hair fallen off) • High maggot activity • A lot of drowned maggots around the head and front limbs • T-shirt brownish/grey discol	
37	Pig C	Active decay	• Large beetle spotted • Large amount of drowned maggots (large white) & soil has been turned up • Front limbs – skeleton exposed • Very distended abdomen • Maggot masses on the inside	
37	Control A	N/A	• No visual textile damage • Some discoloration	
37	Control B	N/A	• No visual physical textile damage • Some discoloration	
37	Control C	N/A	• No visual physical textile damage • Some discoloration	

Day	Site	Stage	Notes	Photo
51	Pig A	Active decay	• A lot less frothing • High maggot activity internally and under clothing • Limbs still fresh	
51	Pig B	Active decay	• Large areas of foam • Large amount of drowned maggots (large white) & soil has been turned up • Front limbs – skeleton exposed • Very distended abdomen, very discoloured	
51	Pig C	Active decay	• Very dark areas under pig • Large amount of drowned maggots (large white) & soil has been turned up • Front limbs – skeleton exposed • Very distended abdomen, very discoloured	
51	Control A	N/A	• No visual physical textile damage • Some discoloration	
51	Control B	N/A	• No visual physical textile damage • Some discoloration	
51	Control C	N/A	• No visual physical textile damage • Some discoloration	

Day	Site	Stage	Notes	Photo
65	Pig A	Active decay	• Skull partly exposed • High maggot activity under clothing • Fluid and frothing in rear • Limbs still not exposed • Soil filled with maggots • Vegetation growth above pig	
65	Pig B	Active decay	• Soil has been very wet and overturned • Vegetation growth above pig • Mandible exposed • Right front limb skeletonized • Fly activity • Maggot mass under clothing	
65	Pig C	Active decay	• Soil very damaged and churned • Mandible and front limbs skeletonized • Large dry mass in rear • High maggot activity under clothing • Shoulder exposed and head starting to become mummified	
65	Control A	N/A	• No visual physical textile damage • Discoloration	
65	Control B	N/A	• No visual physical textile damage • Some discoloration	
65	Control C	N/A	• No visual physical textile damage • Some discoloration	

Day	Site	Stage	Notes	Photo
79	Pig A	Dry/Remains	• Large mass loss • Minimal fly activity • Looks very dried up	
79	Pig B	Dry/Remains	• Large mass loss • Minimal fly activity • Looks very dried up	
79	Pig C	Dry/Remains	• Large mass loss • Minimal fly activity • Looks very dried up	
79	Control A	N/A	• No visual physical textile damage • Some discoloration	
79	Control B	N/A	• No visual physical textile damage • Some discoloration	
79	Control C	N/A	• No visual physical textile damage • Some discoloration	

Day	Site	Stage	Notes	Photo
100	Pig A	Dry/Remains	• Large mass loss • Minimal fly activity • Looks very dried up	
100	Pig B	Dry/Remains	• Large mass loss • Minimal fly activity • Looks very dried up	
100	Pig C	Dry/Remains	• Large mass loss • Minimal fly activity • Looks very dried up	
100	Control A	N/A	• No visual physical textile damage • Discoloration from organic matter	
100	Control B	N/A	• No visual physical textile damage • Discoloration from organic matter	
100	Control C	N/A	• No visual physical textile damage • Discoloration from organic matter	

Day	Site	Stage	Notes	Photo
140	Pig A	Dry/Remains	• Pink sections on the t-shirt along the back	
140	Pig B	Dry/Remains	• Large mass loss • Minimal fly activity • Looks very dried up	
140	Pig C	Dry/Remains	• Large mass loss • Minimal fly activity • Looks very dried up	
140	Control A	N/A	• No visual physical damage • Yellow discolouration of the t-shirt	
140	Control B	N/A	• No visual physical damage • Briefs has a purple shine • Colour change from black • Yellow discolouration of the t-shirt	
140	Control C	N/A	• No visual physical damage • Yellow discolouration of the t-shirt	

Day	Site	Stage	Notes	Photo
210	Pig A	Dry/Remains	• Still very hairy, tail fully intact • T-shirt is grey/black towards abdomen and limbs • Teeth missing and mandible is exposed • A lot of white fungi on the underside • A lot more vegetation present	
210	Pig B	Dry/Remains	• Skull has been moved • A lot of vegetation growth above pig (liquid has been moving downwards) • T-shirt had maggot inside a crease • T-shirt ripped easily where it had been folded	
210	Pig C	Dry/Remains	• A lot of fungi underneath and inside • Hind limbs well preserved • Skull has been moved (goanna) • Grey wet spot on the t-shirt • Dry skin with hair remaining • Some vegetation growth	
210	Control A	N/A	• T-shirt has no visible damage, other than some yellow and green discolouration • Briefs have faded slightly • Socks have no visible damage, but the textile seems weaker	
210	Control B	N/A	• T-shirt has no visible damage, only colour change and the fabric seems weaker • Briefs have faded slightly • Socks have no visible damage, but the textile seems weaker	
210	Control C	N/A	• T-shirt has no visible damage, other than some yellow and white spots • Briefs have faded slightly • Socks have grass piercing through the fabric in one area	

Day	Site	Stage	Notes	Photo
269	Pig A	Dry/Remains	• White/grey deposits on t-shirt • T-shirt has black dots (circles) all over • Mostly skeletonized • Very compressed in abdomen • A lot of vegetation growth	
269	Pig B	Dry/Remains	• Adipocere on parts of the skeleton • T-shirt and socks have a lot of green discoloration • A lot of vegetation growth	
269	Pig C	Dry/Remains	• Black section of the t-shirt • White deposits (web/fungi) all over the dumping site • Adipocere present (orange/pink colour) • A lot of vegetation growth	
269	Control A	N/A	• T-shirt has is very frail and has some small holes • White dots on the t-shirt • Briefs have faded slightly • Socks have no visible damage, but the textile seems weaker	
269	Control B	N/A	• T-shirt has no visible damage, only colour change and the fabric seems weaker • White and yellow dots on the t-shirt • Briefs have faded slightly • Socks have no visible damage, but the textile seems weaker	
269	Control C	N/A	• T-shirt has no visible damage, other than some yellow and white spots • Briefs have faded slightly on the part facing the sunlight • The socks have grass piercing through the fabric in one area	

Day	Site	Stage	Notes	Photo
325	Pig A	Dry/Remains	• White/grey deposits on t-shirt • T-shirt has black dots (circles) all over • Mostly skeletonized • Very compressed in abdominal region • A lot of vegetation growth	
325	Pig B	Dry/Remains	• Adipocere on parts of the skeleton • T-shirt and socks have a lot of green discoloration • A lot of vegetation growth	
325	Pig C	Dry/Remains	• Black section of the t-shirt • White deposits (web/fungi) all over the dumping site • Adipocere present (orange/pink colour) • A lot of vegetation growth	
325	Control A	N/A	• T-shirt has is very frail and has some small holes • White dots on the t-shirt • Briefs have faded slightly • Socks have no visible damage, but the textile seems weaker	
325	Control B	N/A	• T-shirt has no visible damage, only colour change and the fabric seems weaker • White and yellow dots on the t-shirt • Briefs have faded slightly • Socks have no visible damage, but the textile seems weaker	
325	Control C	N/A	• T-shirt has no visible damage, other than some yellow and white spots • Briefs have faded slightly on the part facing the sunlight • The socks have grass piercing through the fabric in one area	

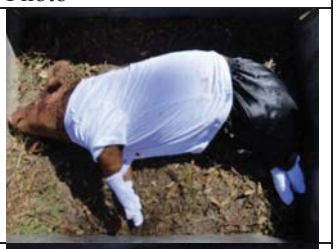
Day	Site	Stage	Notes	Photo
381	Pig A	Dry/Remains	• Most of the hair still present • Skull skeletonised • T-shirt not present on the underside, only seams left • Socks are a little see through • Briefs have no visual physical damage	
381	Pig B	Dry/Remains	• A lot of hair and skin • Legs are almost skeletonised • No cotton underneath the pig • Black spots on the t-shirt • Cotton very fragile • Briefs very discolored • Socks less compact	
381	Pig C	Dry/Remains	• Large amount of pig still intact • All skin and hair present • Skull and front limbs skeletonised • No cotton underneath pig • Cotton intact in front of abdomen in decomposition fluid • Briefs fully intact	
381	Control A	N/A	• Cotton visibly weaker and breaks easily • Some holes in the t-shirt • Green discoloration of the socks	
381	Control B	N/A	• T-shirt has holes	
381	Control C	N/A	• Small holes on t-shirt, not as many as control A & B • Socks are a little see through • Fungi on briefs • Briefs are very bleached by the sun	

Day	Site	Stage	Notes	Photo
443	Pig A	Dry/Remains	• Most of the hair still present • T-shirt not present on the underside, only seams left • Socks are a little see through • Briefs have no visual physical damage • Large vegetation growth	
443	Pig B	Dry/Remains	• A lot of hair and skin • Cotton t-shirt very stiff • No cotton underneath the pig • Black spots on the t-shirt • Cotton very fragile • Briefs very discolored but no other damage	
443	Pig C	Dry/Remains	• All skin and hair present • Even hair and skin on hind limbs • Skull and front limbs completely skeletonised • No cotton underneath pig • Cotton intact in front of abdomen in decomposition fluid • Briefs fully intact	
443	Control A	N/A	• Cotton visibly weaker and breaks easily • Some holes in the t-shirt • Green discoloration of the socks	
443	Control B	N/A	• T-shirt has holes	
443	Control C	N/A	• Small holes on t-shirt, not as many as control A & B • Socks are a little see through • Small bush growing in between sock fibres • Fungi on briefs • Briefs are very bleached	

Day	Site	Stage	Notes	Photo
499	Pig A	Dry/Remains	• Most of the hair still present • T-shirt not present on the underside, only seams left • Socks are a little see through • Briefs have no visual physical damage • Large vegetation growth	
499	Pig B	Dry/Remains	• Cotton t-shirt very stiff and brittle, ant colony underneath • No cotton underneath the pig • Black spots on the t-shirt • Cotton very fragile, rips upon handling • Briefs very discolored	
499	Pig C	Dry/Remains	• All skin and hair present • Skull and front limbs skeletonised • No cotton underneath pig • Cotton intact in front of abdomen in decomposition fluid • Briefs fully intact	
499	Control A	N/A	• Briefs faded by the sun • T-shirt has small holes, could be from ant bites • Socks are intact	
499	Control B	N/A	• Briefs faded by the sun, but intact • T-shirt material very fragile • Socks are intact	
499	Control C	N/A	• Briefs faded by the sun • Socks are intact	

Day	Site	Stage	Notes	Photo
565	Pig A	Dry/Remains	• Most of the hair still present • T-shirt not present on the underside, only seams left • Socks are a little see through • Briefs have no visual physical damage • Large vegetation growth	
565	Pig B	Dry/Remains	• Cotton t-shirt very stiff and brittle, ant colony underneath • No cotton underneath the pig • Black spots on the t-shirt • Cotton very fragile, rips upon handling • Briefs very discolored	
565	Pig C	Dry/Remains	• All skin and hair present • Skull and front limbs skeletonised • No cotton underneath pig • Cotton intact in front of abdomen in decomposition fluid • Briefs fully intact	
565	Control A	N/A	• Briefs faded by the sun • T-shirt has small holes, could be from ant bites • Socks are intact	
565	Control B	N/A	• Briefs faded by the sun, but intact • T-shirt material very fragile • Socks are intact	
565	Control C	N/A	• Briefs faded by the sun • Socks are intact	

APPENDIX C: SUPPORTING INFO FOR DECOMPOSITION T3

Day	Site	Stage	Notes	Photo
0	Pig A	Fresh	• Fairly big • Approx. 60 kg • Full rigor mortis • Bloody snout • A large number of surrounding flies	
0	Pig B	Fresh	• A lot smaller in size • Approx. 50 kg • Not yet in full rigor, limbs only • In the shade • Less fly activity	
0	Pig C	Fresh	• The largest one • Approx. 65 kg • Blood cloths in the face • Full rigor, starting to bloat • Foaming from an area near the edge of the briefs	
0	Control A	N/A	• No visual physical textile damage	
0	Control B	N/A	• No visual physical textile damage	
0	Control C	N/A	• No visual physical textile damage	

Day	Site	Stage	Notes	Photo
3	Pig A	Bloat	• Maggots on the underside, and under t-shirt & front limbs • Socks on front limb are bloody • Large maggot masses in the mouth and ear • Can see the formation of CDI	
3	Pig B	Bloat	• Beetles under the rear • Large maggot mass underneath • Eggs on the jaw • Very liquid in CDI	
3	Pig C	Bloat	• Front limb badly eaten by maggots • Massive maggot mass going from snout, into the mouth, and all along the neck, into the front right limb • A lot of fly activity in the front of the briefs (near the tag)	
3	Control A	N/A	• T-shirt yellowish/brown where sun exposure has occurred • Same discolouration seen with socks • Some flies around	
3	Control B	N/A	• T-shirt yellowish/brown where sun exposure has occurred • Same discolouration seen with socks • Some flies around	
3	Control C	N/A	• T-shirt yellowish/brown where sun exposure has occurred • Same discolouration seen with socks • Some flies around	

Day	Site	Stage	Notes	Photo
6	Pig A	Active	• Head very decomposed already • Large maggot masses under the clothing and entire underside • Front limbs decomposing quickly • Front of t-shirt has a brown discolouration with dried spots • Red discolouration on the underside with dry and leathery skin	
6	Pig B	Active	• A lot of maggots under the clothing • More decomposed than the others • Heading towards advanced • Hair loss and large maggots in CDI • T-shirt and socks full of dark brown spots (fly poop) • Socks of the front limbs pink	
6	Pig C	Active	• Small maggots in the mouth • Clothing is very wet and the t-shirt is brown everywhere but the left shoulder and back side • Hair on the backside thickened and clumpy with sawdust looking deposits	
6	Control A	N/A	• No visual physical textile damage	
6	Control B	N/A	• No visual physical textile damage	
6	Control C	N/A	• No visual physical textile damage	

Day	Site	Stage	Notes	Photo
10	Pig A	Active	• Frothing all along lower side • Large maggot mass in abdomen • Egg masses • Purging of fluids and maggots • T-shirt dark everywhere but shoulder and upward facing section	
10	Pig B	Skeletonized	• Skeleton visible throughout • One sock with bones inside has moved • Frothing and maggots under t-shirt and briefs • All clothing black and wet	
10	Pig C	Active / Advanced	• A lot of flies • Clothing less discoloured • A lot of egg masses on head, front limbs and rear • Massive maggot mass inside the skull and inside abdomen • Socks not as wet as Pig A and B	
10	Control A	N/A	• Yellow/orange on t-shirt • Blue discolouring of certain areas of the socks • Socks also has area with yellow/orange discolouring • Briefs have no evident damage	
10	Control B	N/A	• Yellow/orange on t-shirt • Blue discolouring of certain areas of the socks • Socks also has area with yellow/orange discolouring • Briefs have no evident damage	
10	Control C	N/A	• Yellow/orange discol of t-shirt • Blue discolouring of certain areas of the socks • Socks also has area with yellow/orange discolouring • Briefs have no evident damage	

Day	Site	Stage	Notes	Photo
17	Pig A	Advanced	• A lot of green metallic beetles under clothing • Maggot mass inside abdomen, protected by t-shirt • A lot of decomp fluid under head	
17	Pig B	Skeletonized	• Socks and briefs have dried tissue on them • High beetle activity, at least 3 different types • Skeletonized even under shirt and briefs	
17	Pig C	Advanced	• A lot of flies around, but they pay little attention to the pig • Maggot masses under the clothing • CDI filled with frothing and decomposition fluid	
17	Control A	N/A	• No visual physical textile damage • Yellowish staining of t-shirt	
17	Control B	N/A	• No visual physical textile damage • T-shirt stained, a lot more orange tint • Some orange/yellow stains on the socks (from leaves)	
17	Control C	N/A	• No visual physical textile damage • Staining from the soil on the t-shirt • Brown/orange stains on the t-shirt	

Day	Site	Stage	Notes	Photo
24	Pig A	Advanced	• Pig immersed in warm black liquid • Clothing very wet • T-shirt dry over the shoulder • Skin remaining on the pig, but most of tissue is removed • Single plant growing above pig	
24	Pig B	Skeletonized	• All clothing is very dry and covered in dirt and decomposition fluid • A lot of flies around • Only some skin left on the head, nowhere else	
24	Pig C	Advanced	• Pig immersed in warm black liquid • Clothing very wet • Some vegetation growth above pig • Still some tissue present, a lot of skin still remaining • More fly activity	
24	Control A	N/A	• Yellow/brown discolouration on t-shirt • Very purple shine on the briefs on side facing the sun • Yellow/brown discolouration of the socks	
24	Control B	N/A	• Yellow/brown discolouration on t-shirt • Very purple shine on the briefs on side facing the sun • Yellow/brown discolouration of the socks	
24	Control C	N/A	• Yellow/brown discolouration on t-shirt • Very purple shine on the briefs on side facing the sun • Yellow/brown discolouration of the socks	

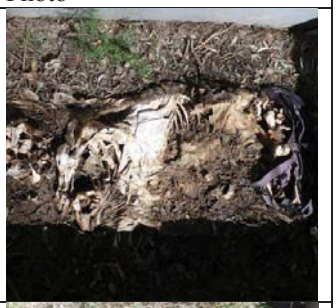
Day	Site	Stage	Notes	Photo
31	Pig A	Advanced	• Orange/yellow fat deposits on the t-shirt and briefs • Green metallic beetles present • Some fly activity	
31	Pig B	Skeletonized	• Large amount of pupae inside socks • Beetles under the socks and briefs • Beetle larvae and pupae around	
31	Pig C	Advanced	• Pink discolouration on the front of t-shirt, plus orange/brown (coppery) colour • Insect activity under clothing • A lot of pupae around • Still in wet decomposition	
31	Control A	N/A	• Yellow/brown discolouration on t-shirt • Very purple shine on the briefs on side facing the sun • Yellow/brown discolouration of the socks	
31	Control B	N/A	• Yellow/brown discolouration on t-shirt • Very purple shine on the briefs on side facing the sun • Yellow/brown discolouration of the socks	
31	Control C	N/A	• Yellow/brown discolouration on t-shirt • Very purple shine on the briefs on side facing the sun • Yellow/brown discolouration of the socks	

Day	Site	Stage	Notes	Photo
45	Pig A	Advanced	• Very wet • White deposits on the clothing • Pink discoloration of the t-shirt • Lots of empty pupae shells	
45	Pig B	Skeletonized	• Very wet • T-shirt dark brown and dirty • Briefs also coloured brown from dirt layer • Large amount of pupae inside socks • Otherwise no change	
45	Pig C	Advanced	• Pink discolouration on the front of t-shirt, in addition to orange/brown (coppery) colour • Parts of t-shirt still white • Large accumulation of water in front of the abdomen • Vegetation growth above pig	
45	Control A	N/A	• Yellow/brown discol. on t-shirt • T-shirt very grey and wet • Very purple shine on the briefs on side facing the sun • Yellow/brown discol. of the socks	
45	Control B	N/A	• Yellow/brown discolouration on t-shirt • T-shirt very grey and wet • Very purple shine on the briefs on side facing the sun • Yellow/brown discolouration of the socks	
45	Control C	N/A	• Yellow/brown discolouration on t-shirt • T-shirt very grey and wet • Very purple shine on the briefs on side facing the sun • Yellow/brown discolouration of the socks	

Day	Site	Stage	Notes	Photo
73	Pig A	Advanced	• Very wet • White deposits on the clothing, a lot of adipocere formation • Pink discoloration of the t-shirt • Overall very orange/pink • Increased vegetation	
73	Pig B	Skeletonized	• Very wet • T-shirt dark brown and dirty • Briefs also coloured brown from dirt layer • White fungi/web on the clothing • Increased vegetation around pig	
73	Pig C	Advanced	• Very wet, adipocere present • Pink discolouration on the front of t-shirt, in addition to orange/brown (coppery) colour • Large accumulation of water in front of the abdomen • Vegetation growth above pig	
73	Control A	N/A	• T-shirt very grey and wet • Very purple shine on the briefs on side facing the sun • Yellow/brown discolouration of the socks • Grass piercing the socks • Increase in vegetation growth	
73	Control B	N/A	• Yellow/brown discol on t-shirt • T-shirt very grey and wet • Very purple shine on the briefs • Yellow/brown on the socks • Increase in vegetation growth	
73	Control C	N/A	• T-shirt very grey and wet • Very purple shine on the briefs • Yellow/brown discolouration of the socks • Increase in vegetation growth • Lizard under one of the socks	

Day	Site	Stage	Notes	Photo
100	Pig A	Skeletonized	• White deposits on the clothing, a lot of adipocere • Pink discol of the t-shirt • T-shirt very fragile and breaks upon touch (on top of pig) • Overall very orange/pink • Increased vegetation	
100	Pig B	Skeletonized	• T-shirt dark brown and dirty • Briefs also coloured brown from dirt layer • White fungi/web on the clothing • Increased vegetation around pig	
100	Pig C	Skeletonized	• Pink discolouration on the front of t-shirt, in addition to orange/brown (coppery) colour • Large accumulation of water in front of the abdomen • Vegetation growth above pig	
100	Control A	N/A	• T-shirt very grey and wet • Very purple shine on the briefs on side facing the sun • Yellow/brown discolouration of the socks • Large increase in vegetation growth	
100	Control B	N/A	• Yellow/brown discol on t-shirt • T-shirt very grey and wet • Very purple shine on the briefs • Yellow/brown discol. of the socks • Increase in vegetation growth	
100	Control C	N/A	• T-shirt very grey and wet • Very purple shine on the briefs on side facing the sun • Yellow/brown discolouration of the socks • Increase in vegetation growth	

Day	Site	Stage	Notes	Photo
129	Pig A	Skeletonized	• White deposits on the clothing, a lot of adipocere formation • Pink discoloration of the t-shirt • T-shirt very fragile and breaks upon touch (on top of pig) • Overall very orange/pink • Increased vegetation	
129	Pig B	Skeletonized	• T-shirt dark brown and dirty • Briefs also coloured brown from dirt layer • White fungi/web on the clothing • Increased vegetation around pig	
129	Pig C	Skeletonized	• Pink discolouration on the front of t-shirt, in addition to orange/brown (coppery) colour • Large accumulation of water in front of the abdomen • Vegetation growth above pig	
129	Control A	N/A	• T-shirt very grey and wet • Very purple shine on the briefs on side facing the sun • Yellow/brown discolouration of the socks • Increase in vegetation growth	
129	Control B	N/A	• Yellow/brown discolo on t-shirt • T-shirt very grey and wet • Very purple shine on the briefs • Yellow/brown discolouration of the socks • Increase in vegetation growth	
129	Control C	N/A	• T-shirt very fragile, the underside is gone • Very purple shine on the briefs • Yellow/brown discolouration of the socks • Increase in vegetation growth	

Day	Site	Stage	Notes	Photo
185	Pig A	Skeletonized	• Still hair and skin • Only one plant in upper right section • Some small holes in the t-shirt • On underside of pig in contact with soil t-shirt seems fragile • Socks very fragile and see through	
185	Pig B	Skeletonized	• Large amount of surrounding vegetation • Ground very dry • T-shirt on underside gone • Very dry and stiff cotton • Cotton breaks easily	
185	Pig C	Skeletonized	• Large amount of very fresh green grass above pig • Very wet underneath pig still • Large amount of skin and hair • Still skin on skull, very pink • Socks very see through material • Underside of pig, cotton well preserved	
185	Control A	N/A	• Cotton stained very green • Very purple shine on the briefs on side facing the sun • Yellow/brown discolouration of the socks	
185	Control B	N/A	• Yellow/brown discol on t-shirt • T-shirt very grey and wet • Very purple shine on the briefs on side facing the sun • Yellow/brown discolouration of the socks	
185	Control C	N/A	• Very purple shine on the briefs on side facing the sun • Yellow/brown discolouration of the socks	

Day	Site	Stage	Notes	Photo
247	Pig A	Skeletonized	• Still very wet underneath pig • Briefs have a lot of white fungi on the underside • Green discolouration of the socks	
247	Pig B	Skeletonized	• T-shirt very dry and brittle • Socks discoloured green	
247	Pig C	Skeletonized	• Large amount of very fresh green grass above pig • Socks very see through material	
247	Control A	N/A	• There is a nest of sorts (spider) under the **BRIEFS**	
247	Control B	N/A	• Very purple shine on the briefs on side facing the sun • Yellow/brown discolouration of the socks	
247	Control C	N/A	• Very purple shine on the briefs on side facing the sun • Yellow/brown discolouration of the socks	

Day	Site	Stage	Notes	Photo
303	Pig A	Skeletonized	• Briefs have a lot of white fungi on the underside • Green discolouration of the socks, thinner and more see through	
303	Pig B	Skeletonized	• T-shirt very dry and brittle • Socks discoloured green, thinner and more see through	
303	Pig C	Skeletonized	• T-shirt very dry and brittle • Socks discoloured green, thinner and more see through	
303	Control A	N/A	• Socks are very see though and thin • Briefs very faded by the sun • Increased vegetation growth	
303	Control B	N/A	• T-shirt sections very brittle and break when handled • Socks are very see though and thin • Briefs very faded by the sun • Increased vegetation growth	
303	Control C	N/A	• Socks are very see though and thin • Briefs very faded by the sun	

Day	Site	Stage	Notes	Photo
369	Pig A	Skeletonized	• Briefs have a lot of white fungi on the underside • Green discolouration of the socks, thinner and more see through	
369	Pig B	Skeletonized	• T-shirt very dry and brittle • Socks discoloured green, thinner and more see through	
369	Pig C	Skeletonized	• T-shirt very dry and brittle • Socks discoloured green, thinner and more see through	
369	Control A	N/A	• Socks are very see though and thin • Briefs very faded by the sun • Increased vegetation growth	
369	Control B	N/A	• T-shirt sections very brittle and break when handled • Socks are very see though and thin • Briefs very faded by the sun • Increased vegetation growth	
369	Control C	N/A	• Socks are very see though and thin • Briefs very faded by the sun	

APPENDIX D: SUPPORTING INFO FOR THE BURIAL TRIAL

Grave 1	Grave 2	Grave 3	Grave 4	Grave 5
				
Grave 6	Grave 7	Grave 8	Grave 9	Grave 10
				
Grave 11	Grave 12	Grave 13	Grave 14	
				

Month	Site	Notes	Photo
1	Grave 2 	• T-shirt very discoloured • Yellow and red circles	
		• The briefs are very wet • No visible damage	
		• No visible damage • One grass root embedded into the sock • Brownish discolouration	

1	Grave 11  • Maggots and wet soil about 10 cm down	• Very discoloured • Tissue stuck on the textile • Very foul smell	
		• Very discoloured • Tissue stuck on the textile • Very dark in colour • Very foul smell	
		• Very discoloured • Tissue stuck on the textile	

Month	Site	Notes	Photo
3	Grave 5 	• A rock was on top of the t-shirt, when removed a visible hole had was found • Vegetation growing on the shirt • Very discoloured overall • Red in shoulder/neck area	
		• Overall well preserved • Vegetation attaching to the outside • Some vegetation has also penetrated the fabric	
		• Overall well preserved • Vegetation growing between sock fibres	

<table>
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<td rowspan="3">3</td>
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• No maggots
• Soil very moist 30 cm down, foul smell and flies are attracted to the carcass
• Soil underneath is black</td>
<td>• Black discolouration
• Part facing upwards has a reddish/brown discolouration
• Very dark black over the shoulder
• A lot of flies once removed</td>
<td>  </td>
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<tr>
<td>• Dirty but otherwise in good condition
• Large tissue pieces inside
• A lot of flies once removed</td>
<td>  </td>
</tr>
<tr>
<td>• A few pieces of vegetation growing in-between the fibres</td>
<td>  </td>
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Month	Site	Notes	Photo
6	Grave 6 	• T-shirt broken down a lot • Only small pieces remaining • Holes and frayed edges	
		• Briefs well preserved • Soil on the outside, but no visible damage	
		• Socks well preserved • Material is visibly weakened, but still intact	

6	Grave 9 	• T-shirt underneath the pig very well preserved • Very discoloured • Covered in tissue	
		• Very well preserved • Very discoloured • Covered in tissue	
		• Very well preserved • Very discoloured • Covered in tissue • A lot drier than the t-shirt and briefs	

Month	Site	Notes	Photo
9	Grave 3 	• T-shirt almost gone • Only seams, a few pieces and some that was left under the briefs	
		• Briefs well preserved • No visible damage	
		• Socks well preserved • Material is visibly weakened, but still intact	

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• Large amount of adipocere in the grave</td>
<td>• T-shirt underneath the pig very well preserved
• T-short above the pig is decomposing
• Very discoloured
• Covered in tissue</td>
<td>  </td>
</tr>
<tr>
<td>• Briefs well preserved
• No visible damage</td>
<td>  </td>
</tr>
<tr>
<td>• Very well preserved
• Very discoloured
• Covered in tissue
• A few piercing roots
• The textile is more fragile</td>
<td>  </td>
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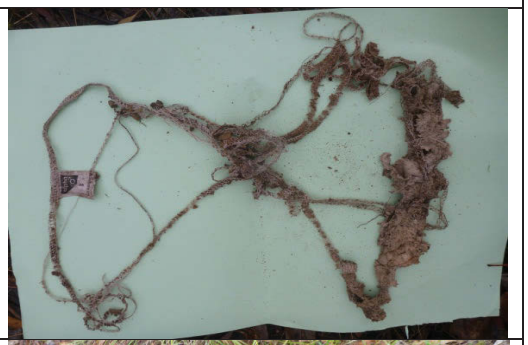
Month	Site	Notes	Photo
12	Grave 7 	• T-shirt pretty much gone • Only the outer seams and some that was under the briefs left	
		• Briefs well preserved • No visible damage	
		• Socks well preserved • Material is visibly weakened, but still intact • A lot of piercing roots	

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<td rowspan="3">12</td>
<td rowspan="3">Grave 10
 
• Large amount of adipocere in the grave</td>
<td>• T-shirt on top of pig gone
• Underneath it is covered in adipocere and well preserved
• Slightly discoloured</td>
<td>  </td>
</tr>
<tr>
<td>• Briefs well preserved
• No visible damage
• Liquid pig residues inside the shorts</td>
<td>  </td>
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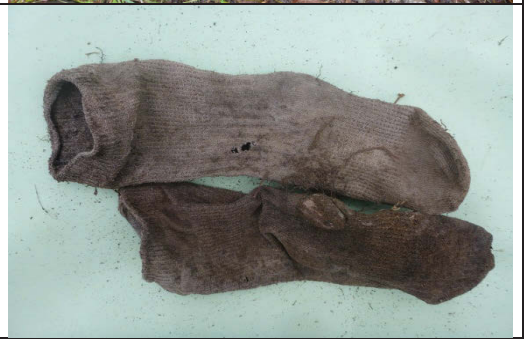
<tr>
<td>• Socks have two minor holes in one of them
• Pig tissue on the socks
• Some piercing roots</td>
<td>  </td>
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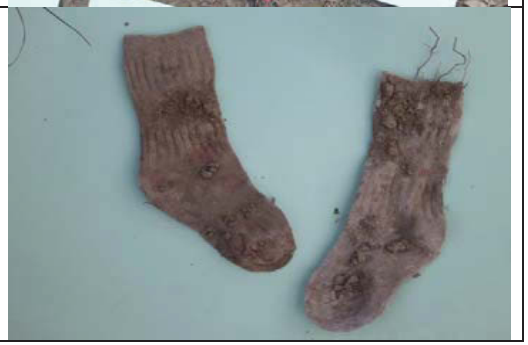
Month	Site	Notes	Photo
18	Grave 4 	• T-shirt could be lifted up as a whole and was markedly less degraded than at 12 months • T-shirt was stained • Roots growing through • T-shirt had large red stains (possible mould)	
		• Good condition overall • Piercing root • Some roots were growing through the briefs and connected the briefs and socks together	
		• The socks are in pretty good condition • They had roots growing through them	

18	Grave 13 	• T-shirt on top of pig was completely gone other than hemline and tag • Underside very intact and very strong, layers of tissue on top making it stronger	
		• Briefs were in good condition	
		• Socks very visibly more see through • More fragile than when new	

Month	Site	Notes	Photo
24	Grave 1 	• T-shirt still has a large section present • Textile is very fragile • Discoloured	
		• Good condition overall • Piercing root • There was a section of the t-shirt stuck to the button of the briefs	
		• The socks are in pretty good condition • They had roots growing through them	

24	Grave 14 	• T-shirt pretty much gone • Only the outer seams and some small sections in the shoulder region remained	
		• Briefs were in good condition	
		• Socks very visibly more see through • More fragile than when new • A large root was stuck through the entire sock