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The interactive effect of the degradation of cotton clothing and decomposition fluid production associated with decaying remains

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ABSTRACT

Textiles are a commonly encountered source of evidence in forensic cases. In the past, most research has been focused on how textiles affect the decomposition process while little attention has been paid to how the decomposition products interact with the textiles. While some studies have shown that the presence of remains will have an effect on the degradation of clothing associated with a decaying body, very little work has been carried out on the specific mechanisms that prevent or delay textile degradation when in contact with decomposing remains. In order to investigate the effect of decomposition fluid on textile degradation, three clothed domestic pig (*Sus scrofa domesticus*) carcasses were placed on a soil surface, textile specimens were collected over a period of a year and were then analysed using ATR–FTIR spectroscopy and GC–MS. Multivariate statistical analysis was used to analyse the data. Cotton specimens not associated with remains degraded markedly, whereas the samples exposed to decomposition fluids remained relatively intact over the same time frame. An investigation of the decomposition by-products found that the protein-related bands remained stable and unchanged throughout the experiment. Lipid components, on the other hand, demonstrated a significant change; this was confirmed with the use of both ATR–FTIR spectroscopy and GC–MS. Through an advanced statistical approach, information about the decomposition by-products and their characteristics was obtained. There is potential that the lipid profile in a textile specimen could be a valuable tool used in the examination of clothing located at a crime scene.

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1. Introduction

A body located on the surface is often scavenged or scattered from the original deposition site, or the discovery of remains might occur years after death causing all soft tissue to be removed. Under these circumstances, materials associated with the remains, such as clothing, may be the only remaining evidence. The condition of textiles may therefore prove valuable in certain death investigations.

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 [1–5], while little attention has been paid to how the decomposition process affects the textiles. Of the reported studies, findings from Janaway [6] indicated that the presence of a body will influence the textile degradation. This study found that cotton only survived when placed underneath an actively decomposing pig, where, due to its location, the cotton was covered in a semi-liquid soft tissue. Similar results were reported by Lowe et al. [7], who found that natural textile specimens buried in contact with decomposing remains, remained intact longer than those buried without remains, in identical soil burial conditions. Both studies suggest that the presence of decomposing remains may inhibit or delay textile degradation in a burial environment. Nevertheless, there has been very little work studying the specific mechanisms that prevent or delay textile degradation when in contact with decomposing remains. 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 embedded within the textiles. In addition, there are no similar studies that have investigated textile degradation when associated with remains that are located above ground, on the soil surface, which is the most commonly encountered location for decomposing remains of homicide victims [8].

During the natural decomposition process, lipids, proteins and carbohydrates are broken down into smaller molecules. These

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decomposition by-products are the major components in decomposition fluid [9]. The decomposition process also causes the release of gases such as methane and hydrogen sulphide [10]. The build-up of gases (known as bloating) causes the fluids and gases to escape from the orifices of the body and eventually leads to rupturing of the skin. The adipose tissue in the body, which is comprised mostly of lipids in the form of triglycerides, will break down into separate free fatty acid chains through enzymatic action [9,11]. These fatty acids are then hydrolysed and hydrogenated [11,12]. 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.

Attenuated total reflectance–Fourier transform infrared (ATR–FTIR) spectroscopy has been previously used to analyse triglycerides that have been deposited onto cotton fabric [13] and to determine and monitor adipocere development on textile specimens [14]. ATR–FTIR spectroscopy is also an established technique for the investigation of protein structure [15] and has proven to provide both protein and lipid information simultaneously [16]; therefore making this technique particularly valuable in the study of decomposition by-products. 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 [17–19]. 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.

Previously reported studies examining the effect of decomposing remains on textile degradation have been based predominantly on visual observations and microscopy [6]. Attempts have been made more recently to analyse the lipid profile found on textile specimens associated with buried remains, but little statistical analysis was completed on the resultant data [7]. The current paper aims to provide 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. 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 and GC–MS.

2. Materials and methods

2.1. Sample collection and storage

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 and 5.

Three domestic pig (*Sus scrofa domesticus*) carcasses were used. Pig carcasses 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 [20,21]. The pig carcasses were clothed in white, 100% cotton t-shirts (alpha ♂, KMart, Australia), placed on the soil surface, and caged to avoid scavenging from large animals, while still allowing insect access. Three control sites were also created, which contained 100% cotton t-shirts placed directly on the soil surface.

The study was carried out during January 2013–January 2014. The study started in January which is during the Australian summer; with an average temperature of 23 °C (high of 47 °C and low of 14 °C) and a total of 278 mm in the three summer months. The average temperatures then dropped to 17 °C in autumn (high of 30 °C and low of 15 °C) and total rainfall decreased to 98 mm. Average temperatures in the winter months of June through to August were 11 °C (high of 24 °C and low of 2 °C) with 64 mm rainfall in total. In spring temperatures began to rise, with an average of 18 °C between September and November, with a high of 37 °C and a low of 6 °C. The total rainfall during this time was 171 mm.

Three 5 cm × 5 cm sections of the t-shirt material 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. Sampling occurred on days 0, 2, 4, 6, 8, 10, 14, 17, 21, 24, 31, 48, 59, 94, 149, 184, 212, 268 and 365, post-mortem. The textiles were photographed on each sampling day and any visible changes were noted before the textile samples were collected and packaged into a small envelope, placed into individually labelled paper bags and stored in a cooler for transportation to the laboratory. To impede bacterial and fungal growth, the textile specimens were air-dried and any adhering tissue, soil, or hair was removed after drying. The textiles were then packed in new individually labelled paper bags and stored at −18 °C until further analysis.

2.2. ATR–FTIR spectroscopy analysis

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. Textile sections were placed directly onto the crystal. 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.

2.3. GC–MS 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. [22]. Herein, three 3 cm × 3 cm t-shirt sections were prepared from the original 5 cm × 5 cm cotton specimens. 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 10 N 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 sulphuric 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

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