The Factors Affecting the Recovery of Bloodstain Evidence from Buried Clothing

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Abstract

In 2018, there was a record number of 726 homicides across England and Wales. A third of male victims of homicide were found in open area environments, mainly fields. Victims were found either fully buried or partially buried on the surface. The current project investigates the potential loss of bloodstain evidence on clothing that occurs following violent crimes. Throughout the current project, it is identified that there is a prominent lack of literature that focuses on determining what soil parameters impact the survival of buried bloodstain evidence. Several experiments were carried out to visually identify if bloodstain evidence is lost on clothing samples that are buried within and placed on the surface of the soil, and to determine how seasonal variations within the soil parameters affect the bloodstains survival. From an observational study conducted it is found that the bloodstains on the buried samples are visually undetectable, indicating that bloodstain evidence on a victims clothing will be lost when buried unless chemiluminescent techniques are used to identify the presence of blood. It is also determined that the soil moisture content and soil pH work together to impact the bloodstains survival, as these factors are both identified to significantly alter the fluorescence emitted by the blood when chemiluminescent techniques are used. ultimately the results gained from this research mostly agree with the hypothesis set, determining that pH, soil moisture and microbial activity all impacted the survival of the bloodstains, However, disagreeing with the statement that the bloodstains survival rate would be lower on the organic natural fibre fabrics, and that the bloodstain survival would also be more affected in the autumn and winter seasonal periods.

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1 Introduction

In 2018, it was recorded that 726 homicides occurred across England and Wales, which is a 3% increase from the previous year. The term Homicide covers murder, manslaughter and infanticide and these crimes invariably involved violence. For example, out of these homicides, 285 involved the use of sharp instruments (ons.gov.uk 2019); this is the highest involvement of sharp instruments since the Home Office index began in 1946. Males were the predominant victims in these homicides, accounting for 69% of victims, with the remaining 31% classed as female. 77% of female victims were found in and around their residential dwelling, whereas a third of male homicides took place in a street, path or alleyway, with another third taking place in open areas (ons.gov.uk 2019). With a high proportion of male homicide victims being located in open areas, it is vital that the scenes are secured and investigated as quickly as possible, to prevent environmental factors from affecting any present evidence (Galloway et al. 2010). Open area homicide scenes often contain bloodstain evidence from either the victim or the suspect and this evidence must be collected before any environmental interference (Keel et al. 2009).

Blood is a common and highly important form of evidence found at violent crime scenes. Like all bodily fluids, blood is a key component in forensic investigation, providing valuable evidence. This includes the reconstruction of a crime scene, victim and suspect's movement through a scene, and importantly DNA evidence, which will aid in identifying both the victim and possible suspects (Virkler and Lednev 2009). Blood is often found on the victim's clothing, but the survival of blood evidence is affected by environmental factors (de Castro et al. 2012) until collected by forensic investigators. These environmental factors will degrade the bloodstains and, in some cases, can remove any bloodstains from the crime scene (Bremmer et al. 2012).

Some homicide cases involve the victim being fully or partially buried (Menez 2005). Most of these victims are found buried in their clothes or found with their clothes nearby to the burial location (Haglund and Sorg 2002). Under these circumstances, it is important to find and recover all evidence as soon as possible. It is also important that all evidence from, and around, the body is collected correctly to ensure no loss of evidence (Galloway et al. 2010). When collecting evidence from a buried victim, soil samples must be collected from around the body and surrounding area to identify potential biological samples from the victim, or suspect, that have been transferred to the area (Galloway et al. 2010).

Bloodstain pattern analysis covers the collection, categorization and interpretation of bloodstains connected to crimes. These stains occur frequently in homicide cases (Peschel et al. 2010). Bloodstains are primarily used to reconstruct the pHysical events that occurred at the crime scene. By following patterns within the bloodstaining it is possible to identify areas where victims or suspects may have been during the event (Slemko 2017). Alongside this, DNA evidence may also survive in well-preserved bloodstains (Virkler and Lednev 2009). The production and distribution of bloodstains are determined by several factors. The first factor being the force with which a victim has been struck. This impacts the spread of the blood across an area, which is also altered by the type of weapon used to strike the victim (Peschel et al. 2010). The second factor affecting bloodstaining is environmental variation. Changes in environment, such as wind, can affect the movement of blood droplets through a scene; while, the temperature at the scene will alter the rate at which the bloodstains dry onto a surface or evidential object, like an article of clothing. (Peschel et al. 2010). The third factor that impacts bloodstaining at a scene is human variation and involvement. Each person at the crime scene, be it the victim or the suspect, will have varying lifestyles. Changes within a person, such as hydration levels, will alter the viscosity of their blood, causing it to spread further throughout the scene if they well hydrated (Larkin et al. 2012). Parallel with this is the formation of bloodstain

pools, which occurs when a victim is lying down for a length of time, especially if they have suffered trauma to the head (Slemko 2017). These pools of blood create bloodstain trails from the pool area that can be used to determine if the victim has been moved through the scene as well as indicate the area where most of the event occurred (Slemko 2017). These factors should be taken into consideration when investigating scenes of homicide at both indoor and outdoor crime scenes (Peschel et al. 2010).

Currently, there is no evident literature regarding the survivability of bloodstains within soils. However, forensic tapHonomy gives an insight into how chemical and biological factors within the soil affect buried human remains (Tibbett and Carter 2008), which can indicate that soil factors may affect the survival of blood-based evidence. Due to the importance of bloodstain evidence, this lack of research needs to be addressed by investigating the survivability of blood in soils.

1.1 Literature Review

1.1.1 Criminal investigations

Blood is often found at crime scenes (Peschel et al. 2010) and is considered an important forensic tool due to the information that can be gained from the blood itself and the direction of bloodstains at a crime scene (Pokupcic 2017). During a criminal investigation, blood is often the most useful source of DNA evidence that can be gained from the scene (Gill 2001). With DNA evidence, people at the scene of a crime when it occurred can be identified, aiding the forensic investigation (Gill 2001). Additionally, blood evidence could be used to estimate a sequence of events that occurred at the scene. Bloodstains and blood patterns can provide insight into the events that took place at the crime scene. For example, trails of blood through the scene can show the movement of victims or suspects; whereas, bloodstains can be measured to determine the angle of origin from which the

bloodstain originally came from, identifying where any injury caused to the victim may have taken place (Slemko 2017).

A key problem with blood-based evidence is that blood degrades or perpetrators may have tried to clean the crime scene and this may result in bloodstains becoming invisible to the naked eye. If blood is not visible in natural light, chemical analysis can be conducted to reveal if the bloodstains are still on fabrics. When investigating a violent crime scene, the presence of blood may be obvious and in such cases, presumptive tests can be conducted to identify if probable bloodstains are blood. These presumptive tests are known as non-chemiluminescent techniques (Webb et al. 2006). Presumptive tests involve the use of chemicals being added to the potential bloodstains, and if the stain changes to a positive colour, an investigator can presume that blood is present (Webb et al. 2006).

In some violent crime cases, bloodstaining may not be easily visible as the suspect may have attempted to clean the area to cover up the crime (Creamer et al. 2005). At these types of crime scenes, chemiluminescent techniques can be used to visually show the presence of blood. These chemical reagents are sprayed onto an area where blood is suspected at the scene and begin to fluoresce in the presence of blood, making it easy for an investigator to identify areas of bloodstaining (Barni et al. 2007). Once the knowledge has been gained that blood is present at the scene, the blood evidence can then be collected and analysed following the usual evidence collection protocol.

1.1.2 Detection of bloodstains: Non-chemiluminescent techniques

During an investigation and once evidence has been brought to a laboratory for examination, several presumptive tests can be conducted to detect the presence of blood on the evidence. The most commonly used presumptive test is the Kastle-Meyer test. This involves a drop of pHenolpHthalein reagent being added to the sample area, then after a few seconds, a drop of hydrogen peroxide is added. If the sample rapidly turns pink, the test is presumptive positive for blood (Webb et al. 2006). Mushtaq et al. (2015), investigated the detection of dry bloodstains on different fabrics after washing with commercially available detergents. A key part of Mushtaq et al.'s, research was to identify if Kastle-Meyer, Leucomalachite green, Tetramethylbenzidine or Hemastix tests would best determine the presence of blood on the washed clothing samples. It was established that the Hemastix test was the most sensitive in detecting the bloodstains; while, Leucomalachite green was the least sensitive (Mushtaq et al. 2015). Moreover, they found that the cotton-polyester blend fabric retained more blood after being washed compared to the other fabric types (Mushtaq et al. 2015). From Mushtaq et al. (2015) it can be determined that cotton blend fabrics will retain more blood when washed, which may assist in the survival of the bloodstains on these fabrics when left in an open environment where rainfall and the moisture of the soil will "wash" the fabric.

1.1.3 Detection of bloodstains: Chemiluminescent techniques

Chemiluminescent techniques are presumptive chemical tests used to detect latent blood by the emission of light produced from a chemical reaction (Barni et al. 2007). Currently, there are two commonly used chemiluminescent techniques, BlueStar® and Luminol, which both use chemical reagents that react with blood. When BlueStar® is sprayed onto a surface, the bloodstains begin to fluoresce blue, but when luminol is used the bloodstains will appear blue under a fluorescent light. Both chemical reagents have been extensively tested and have been confirmed to not damage any DNA evidence that can be gained

from the bloodstains (Barni et al. 2007). The main difference between the two reagents is that to use Luminol, the area used must be in near-complete darkness to get maximum effectiveness from the reagent, whereas BlueStar® can work effectively in low light conditions. One of the main differences to consider between these two reagents is the preparation method. BlueStar® simply requires a set of two BlueStar® tablets to be dissolved into a spray bottle of water and once dissolved the solution is ready to be sprayed onto the target surface. Luminol, on the other hand, requires the luminol powder, distilled water and hydrogen peroxide to create the luminol solution, which is not easily prepared at a crime scene (Jakovich 2007). Tobe et al. (2007) conducted research evaluating non-chemiluminescent and chemiluminescent presumptive tests for blood, comparing their sensitivity to detecting blood and the recovery of DNA from the bloodstains. Alongside BlueStar® and Luminol, the Kastle-Meyer, Leucomalachite green, Hemastix and Hemident tests were compared. Their research showed that the chemiluminescent tests and the Hemastix test were the most sensitive to the presence of blood. Tobe et al. (2007) also found that DNA could be recovered from all but the Leucomalachite green and the Hermident tests. From the conclusions of this research, it can be determined that BlueStar® and Luminol have the best efficacy for detecting blood at a crime scene due to their ability to detect blood without compromising DNA evidence. Furthermore, BlueStar®'s ease of preparation and use make it the better blood detection presumptive test when at a crime scene. BlueStar® Forensic is used globally by police authorities during crime scene investigations and laboratory work (Andrade et al. 2014).

1.1.4 Natural vs synthetic fibres

Articles of clothing are made up of woven fibres and these can be produced from natural or synthetic materials. It has been identified that 21% of clothes are made from cotton and 65% from synthetic fibres, primarily polyester (Ethical Fashion Group 2018). It has also been identified that natural fibre materials, from articles of clothing to household furniture,

are commonly found at crime scenes (Michielsen et al. 2015). Natural fibres come from animal sources, such as wool or vegetable sources (i.e. cotton). These fibres are used to produce articles of clothing because of their natural strength, flexibility, abrasion-resistance and elasticity. Articles of clothing produced from natural fibres are susceptible to microbial decomposition, especially vegetable-based fibres, due to their cell structure which consists mainly of cellulose. These fibres will rapidly decompose in warmer humid climates, in areas where light is not present (Müssig 2010).

Synthetic fibres are produced entirely from chemical polymers. The polymers used to produce synthetic fibres are similar to those that are used in the production of plastics and rubbers. The most common synthetic fibres used to produce clothes are polyester, rayon and nylon (Vigneswaran et al. 2014). Due to their chemical composition, these fibres are very strong and are often somewhat water-resistant in comparison to natural fibres. Unlike natural fibres, synthetic fibres do not contain any form of biopolymer, such as cellulose, which means they do not decompose at the same rate as natural fibres (Jawaid and Abdul Khalil 2011).

When natural or synthetic fibres are used to produce articles of clothing, both fibre types give the clothing similar properties (Kilic and Okur 2010). However, due to the difference in pHysical structure, the rate of decomposition for a piece of clothing will alter depending on the types of fibres it is made from. Research has been conducted comparing the rate of biodegradability between articles of clothing made from 100% cotton and 100% polyester (Li et al. 2010). This research consisted of clothing garments, made of both cotton and polyester, being buried in soil under controlled laboratory conditions and in a large-scale composting environment for three months. During the burial period, the carbon dioxide produced due to decomposition of the clothes was monitored. It was concluded

that the cotton clothing samples released larger quantities of carbon dioxide due to micro-organisms feeding on the cellulose within the natural fibre (Li et al. 2010). However, in the laboratory-controlled tests, the polyester samples biodegraded rapidly over the first 30 days due to the enzymes in the controlled soil being able to break down the chemical structure of the synthetic fibres (Li et al. 2010). Li et al.'s (2007) research indicated that cotton fabric will decompose faster in a natural burial environment due to the cellulose structure of the cotton. Consequently, the bloodstains on cotton clothing may likely be more degraded than polyester clothing because of the decomposition of the cellulose structure of the cotton samples by microorganisms.

One of the fibre properties that can potentially impact on bloodstain evidence is the water-resistance of the fibres. It is known that natural fibres have an affinity to water; whereas, the chemical composition of synthetic fibres tends to make an article of clothing water-resistant (El-Naggar et al. 2003). The fibres' resistance to water will impact how the fibres react to staining from substances like blood (Wang et al. 2010). Without treatment, clothes made from natural fibre will be more likely to absorb the blood and increase the rate at which the blood will stain the clothes (Wang et al. 2010). In comparison, while blood will stain synthetic fibres, the stains will be easily washed off by water as the blood will not interact to the synthetic fibres due to their resistance to water (Mushtaq et al. 2015). Currently, there has been little research investigating how blood interacts with different fabric types (de Castro et al. 2012). This highlights that there is a large knowledge gap that needs to be examined to ensure that potential evidence is not lost or overlooked during a criminal investigation.

1.1.5 Decomposition of buried organic matter

There is a large array of soil types that vary due to natural plant life in the area, nature of the parent material, weathering of rock, the movement of weathered rock particulates and

climatic conditions (Bockheim and Gennadiyev 2000). Simply, soils can be assigned to four groups based on the proportion of sand, silt and clay: (1) clay, (2) sandy, (3) silt and (4) loam, which is a nearly even mixture of clay, sand and silt. Clay soils tend to be higher in nutrients and are often baked dry in warmer climates (Chenu et al. 2000). Sandy soils are often lower in nutrients compared to clay soils, alongside this, sandy soils tend to be more acidic (Yao et al. 2012). The silt soils are often more fertile compared to the other soil types, and are more moisture-retentive (RHS 2018). Alongside these, there are peat soils, which are mainly made up of organic matter, and chalky lime-rich soils, which are mainly very alkaline containing a high calcium content (RHS 2018). The nature of the soil, specifically its pHysiochemical properties, have a large impact on the fate of buried organic materials (Withington and Sanford 2007).

When in soil, organic material, such as human remains, will begin to decompose. The rate of decomposition is determined by three factors - the quality of organic matter in the burial area, environmental factors and the presence and activity of decomposer organisms (Tibbett and Carter 2008). It has been found that human remains tend to survive better in peat soils due to the larger amount of organic material surrounding the remains (Tibbett and Carter 2008). The organic material in the peat soils stabilises the chemical composition of the soil, ensuring that remains do not come into contact with harsh chemicals that can impact the rate of decomposition (Dent et al. 2004). The acidic nature of peat soils also negatively impacts the activity of micro-organisms, which will slow down the rate of decomposition within these soils (Dent et al. 2004).

Environmental factors affecting the rate of decomposition of buried human remains include soil water, oxygen availability, pH, temperature, and pHysical protection around the remains provided by the soil, which inhibits the access of animals (Tibbett and Carter

2008). Water is key to microbial decomposition; without water, all biological processes cease (Stott et al. 1986; Manzoni et al. 2012). However, even in dry arid areas, decomposition still occurs due to films or water persisting over soil particles, which means that decomposition will still occur but at a slower rate (Tibbett and Carter 2008). pH severely impacts the rate of organic decomposition, as a lower pH will tend to increase the activity of decomposer microorganisms (Aciego Pietri and Brookes 2008). However, if the pH is too low (pH < 5.5) the rate of decomposition will decrease as bacteria do not tolerate acidic environments (Tibbett and Carter 2008). Research has been conducted into the preservation of human remains in soils and demonstrates that human remains survive best in soils that have a more neutral to low alkaline nature with a pH around 7-9 (Dent et al. 2004). Despite the inhibition of microbial activity in acidic soils, research has found that most organic materials will still completely decompose in acidic soils below pH 5 (Dent et al. 2004).

One factor that also needs to be taken into considerations during organic decomposition is the carbon:nitrogen (C:N) ratio of the soil (Carter et al. 2006). When organic matter decomposes it releases carbon, shifting the C:N ratio of the soil. The larger the decomposing organic source the greater the C:N ratio shift, thus altering the rate of decomposition (Carter et al. 2006). It has been found that most micro-organisms require an organic nutrient source with a C:N ratio of 25:1, any variation of this will alter the rate of decomposition (Hodge et al. 2000). With this knowledge it can be determined that the presence of both an organic fibre material and blood when buried may alter the C:N ratio of the soil which in turn may impact the rate of microbial activity, ultimately affecting the rate of decomposition.

1.1.6 Decomposition of blood

During human decomposition, when cell break down and circulatory activity ceases, blood will begin to settle at the lowest points of the body, causing visible red areas to appear on the skin. This usually occurs an hour post-mortem and will take up to eight hours to complete (Baden and Hennessee 2005). 8 – 10 days post-mortem the blood begins to decompose. Like most organic material, the decomposition rate of blood is dependent on environmental factors. During burial, the main factor impacting the blood decomposition rate is the microbial activity in the burial area (Orf and Cunnington 2015). The destruction of red blood cells is known as haemolysis. It has been identified that there are two types of bacteria within the soil that conduct haemolysis, these being alpHa and beta haemolytic bacteria (Tambekar and Gadakh 2013). The difference between these haemolytic processes is that alpHa haemolysis is the reduction of haemoglobin in the red blood cells, and beta haemolysis is the complete destruction of red blood cells (Misawa and Blaser 2000). The presence of these two types of haemolytic bacteria within the soil may directly impact the outcome of the present study, as they could visually alter the appearance of the bloodstains making them more difficult to be identified on the sample materials, or by fully removing the bloodstains from the sample materials. This indicates that microbial activity will have a large impact on the survival of the bloodstains.

1.2 Aims and Objectives

The main factors within the soil that may affect the survival of bloodstain evidence are the pH of the soil, the total microbial activity within the soil, and the moisture level of the soil (Tibbett and Carter 2008). The pH of soils can vary due to many variables, the main one being the soil parent material, which will alter the soil pH. Soil is comprised of weathered rock sediment and decomposed organic remains of plants and animals, the chemical composition of the soil will vary depending on what it is primarily made up of (Anderson 1988). Parent material also affects soil texture and therefor water and nutrient retention.

Other variables affecting the pH of the soil are chemical fertilizers and falling leaves, which act as a natural fertilizer that will lower the pH. (McCauley et al. 2009). All soils contain microorganism, the activity of these microorganisms will vary depending on the soil pH, as different micro-organisms will thrive better at varying pH levels (Lauber et al. 2009) and on the soil moisture level (Barros et al. 1995). Micro-organisms under the correct conditions have been found to breakdown, blood thus removing any visible bloodstain evidence (Ogdur et al. 2018). Currently, there is a gap in our knowledge regarding the survival of bloodstains on buried clothing and how survival is affected by cloth type and soil parameters. The present study aims to begin to address this.

The overall aim of this research project was to determine how soil affects the survival of bloodstains on clothes. This was achieved by exploring several additional aims, firstly, to investigate the survival of blood under varying environmental settings. Secondly, to determine the effect varying fabric types have on the bloodstain survival. Thirdly, to ascertain how seasonal variation impacts the rate of bloodstain survival. Finally, determine the effect of key soil parameters, i.e. pH and microbial activity, on bloodstain survival.

With the knowledge gained from present literature, it has been hypothesized that pH, soil moisture and microbial activity will all be major factors impacting the bloodstains survival, with the bloodstains survival rate being lower on the organic natural fibre fabrics compared the synthetic fibre fabrics. Alongside this, the bloodstains will be most affected in the colder, wetter seasons Autumn and Winter.

In order to achieve the aims of this research a set of experiments were carried out:

1. An observational study visually comparing the bloodstains of the fabrics subject to burial in the soil, left outdoors on the soil surface and a control sample placed in the laboratory kept at room temperature.
2. Statistical data analysis to identify and compare each fabric type during each season to determine which fabric the bloodstains survived best, during each seasonal period, and to identify which seasonal period had most impacted the bloodstains survival.
3. Soil sample collection from the project area both before and after bloodstain burial and testing of each soil parameter.
4. Statistical analysis of the soil testing to determine the relationships between these environmental factors and which factors impact the bloodstain survival.

2 Materials and Methods

2.1 Producing Blood Stains

Oxalated horse blood was used as a proxy for human blood due to the difficulties of obtaining human blood and the similarities between both human and horse blood (Larkin and Banks 2016). The main difference between the species is that horse blood has a higher amount of the protein fibrinogen, which increases the rate at which horses blood clots; it was found that horses have 40% more fibrinogen in their bloodstream compared to humans (Equine Health Labs 2015). This difference should not affect the results of this research project as the horse blood is in oxalate, an anti-coagulant, which prevents the blood from clotting (Bernardo-Filho et al. 1994).

Four articles of clothing were obtained from a second-hand source to ensure they were in a similar worn condition to that of a clothed victim. All garments were made from varying materials: 100% cotton, 70% cotton blend, 100% polyester, and 70% polyester blend. Research has shown that 21% of clothing materials are made from cotton, with 65% being made with synthetic fibres, mainly polyester, which is why these material types were selected for this research. 12 9x9 cm squares were cut out of each garment, using a pipette one 100 µl drop of horse blood was dropped onto each sample and left to dry in a laboratory environment at room temperature (19-24^{o}C).

2.2 Site Description

The field site was situated in Wytch Farm, Isle of Purbeck, Dorset, UK, as shown in Figure 1. A field previously used for pasture and which had remained undisturbed since it was last ploughed in 2000 was selected for the study area. The burial area was in the corner of the field close to a tree line. The soil in the field is an acidic podzol with a topsoil pH

varying between pH 5.97-4.43 (UKSO Org 2019). Acidic, sandy conditions are commonly found in the top horizon of podzolic soils, which does not extend lower than 50cm below the surface (Rosling et al. 2003). Podzolic soils are often linked to human interference caused by woodland clearance through grazing and burning (Sanborn et al. 2011). An archaeological excavation was recently carried out at Wytch Farm and has revealed that no archaeological organic matter survived within the soil (D. Pitman, Bournemouth University, pers. comm. 15 July 2019). The archaeological features from this excavation were found over a 1m below the ground surface, the present research was 20cm below the ground surface in an area on the field with no archaeological features. The purpose for using this field to conduct this research is due to the common use of fields as a disposal site following a homicide, especially where light-textured sandy soils are present, which enable a criminal to make an easy shallow grave cut (Donnelly and Harrison 2017).

The topsoil moisture in the working area ranges from 33.72%- 27.22% (UKSO Org 2019). With the assistance of weather data gained from Hurn weather station, it was identified that over each seasonal burial period the daily average rainfall ranged from 0.8mm- 3.0mm of rain. 1-2 days of the burial period had the occasional increased rainfall, the highest being in autumn at 25.4mm of rain.

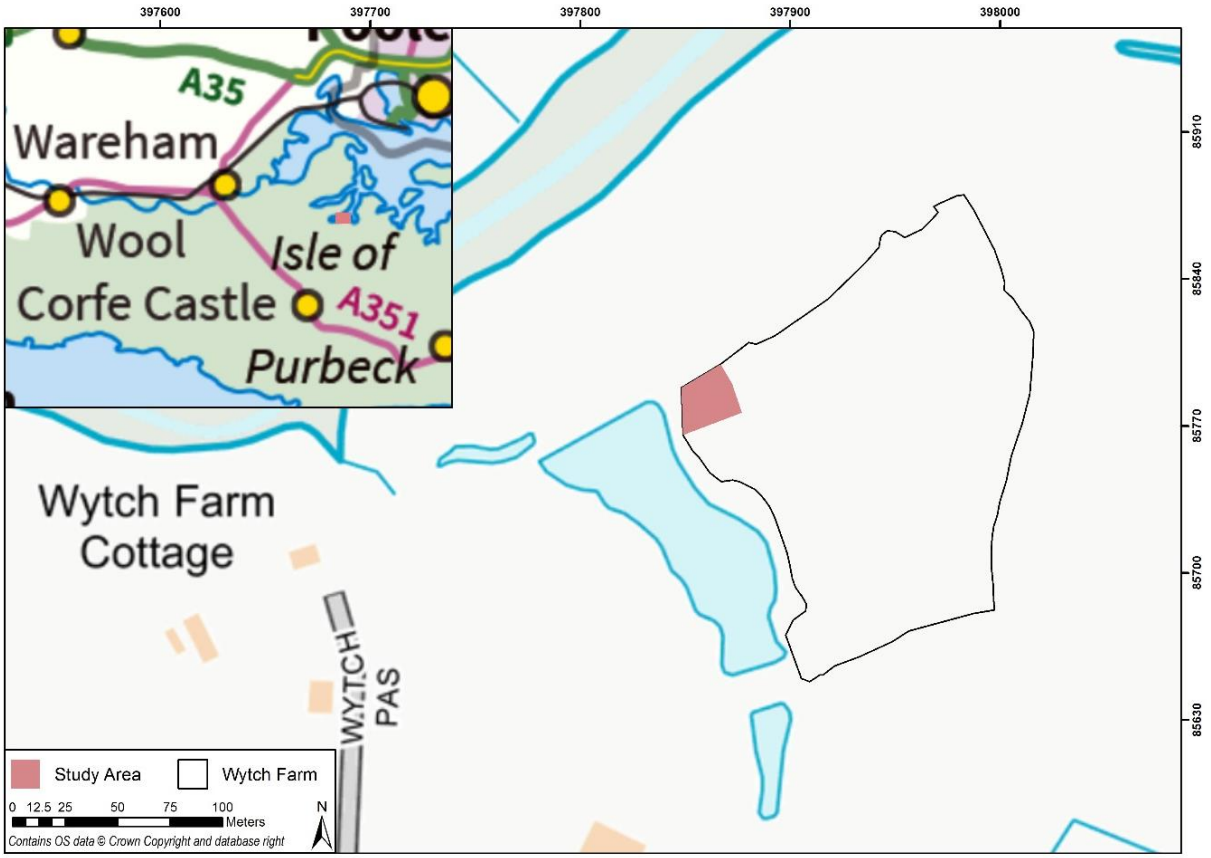

Figure 1: A map indicating the location of the study area at Wytch Farm. Highlighting specifically the area in which experimentation was carried out, in the north-west corner of the field (in pink).

2.3 Burial of Clothing Samples

A 1m X 0.50m area was measured and de-turfed to a depth of 3-5 cm to ensure all grass was removed. Once de-turfed, 5 soil samples were collected. The area was then excavated to a depth of 20cm below the surface level and 5 more soil samples were collected from this base level. After all soil samples were collected, the 24 bloodstained clothing samples, 6 replicates of each type of material, were placed into the pit, which was then backfilled. When backfilling was completed and the pit was re-turfed, the remaining 24 samples were placed on the surface of the pit, 6 being placed face up and 6 placed face down. Images showing the buried and surface samples *in situ* at the study area are provided in Figure 2. Soil samples taken on the day of experimentation were stored at 4^{o}C until analysis was conducted.

(a)  (b) 

Figure 2: Images of the fabric samples *in situ* at the study area. Figure 2a shows the buried samples and Figure 2b shows the surface samples.

The surface samples were retrieved 14 days after deposition. The rationale for the experimental period taking place over 14 days was a pilot study found that the bloodstains were not identifiable on any samples past 14 days. Upon retrieval, all clothing samples were individually placed into sealable sample bags and labelled. The turf was then removed from the area, and 5 soil samples were taken in a 'W' formation from the area. The area was then carefully excavated, to exhume the buried samples of clothing, which were also individually bagged and labelled. 5 more soil samples were taken again in a 'W' formation from the base level of the burial area. Both clothing and soil samples were stored at 4^{o}C until analysis was conducted. This process was repeated once during each season, to gain comparable seasonal data. When repeated, a new pit was dug; this is to ensure that the soil has not been altered by any previous experiment.

Alongside the samples in the field, samples of each material were kept under indoor conditions in the laboratory. These samples consisted of three squares of each of material, with a 100 µl drop of horse blood placed onto them as per the buried samples. These samples were then kept in the laboratory, at room temperature (19-24^{o}C).

2.4 Analysis of Bloodstains

2.4.1 Bloodstain detection, chemical agents

BlueStar® latent bloodstain reagent was sprayed onto all samples to reveal bloodstains not visible to the naked eye. This was conducted in a dark room to allow the BlueStar® to give optimum fluorescence to identify if the blood was still present on the sample materials. PHotos were taken of the fluorescence on samples using an iPHone XR 12 megapixel camera with a pixel density of 326 ppi. Figure 3 demonstrates the fluorescing bloodstains once sprayed with BlueStar®. The fluorescence bloodstains were subsequently measured using the ImageJ open-source image processing software. An area where no fluorescence occurred on the sample image was selected and background image fluorescence was measured and recorded. Then the area of fluorescence on the sample was measured and recorded. The Corrected Total Cell Fluorescence (CTCF) was then calculated using the background and fluorescence measurements using Equation 1. ImageJ is commonly used to measure cell fluorescence (Bankhead, 2014). It has also been used to investigate drip patterns in bloodstain pattern analysis (Boos et al. 2019). The method used to measure cell fluorescence can be used to measure the fluorescence of the bloodstains used in this project. The CTCF of each bloodstain shows the survival of the bloodstains as the CTCF variation depended on the fluorescence emitted from the bloodstain when sprayed with BlueStar® latent bloodstain reagent. Likewise, the fluorescence is impacted by the amount of blood present, where less blood is present there is less fluorescence, thus affecting the samples' CTCF. If the CTCF if higher, this

indicates that the bloodstain survived better than that of a bloodstain sample with a lower CTCF.

Equation 1: Measure of Corrected Total Cell Fluorescence

$$\text{CTCF} = \text{Integrated Density - (Area of selected cell X Mean fluorescence of background)}$$

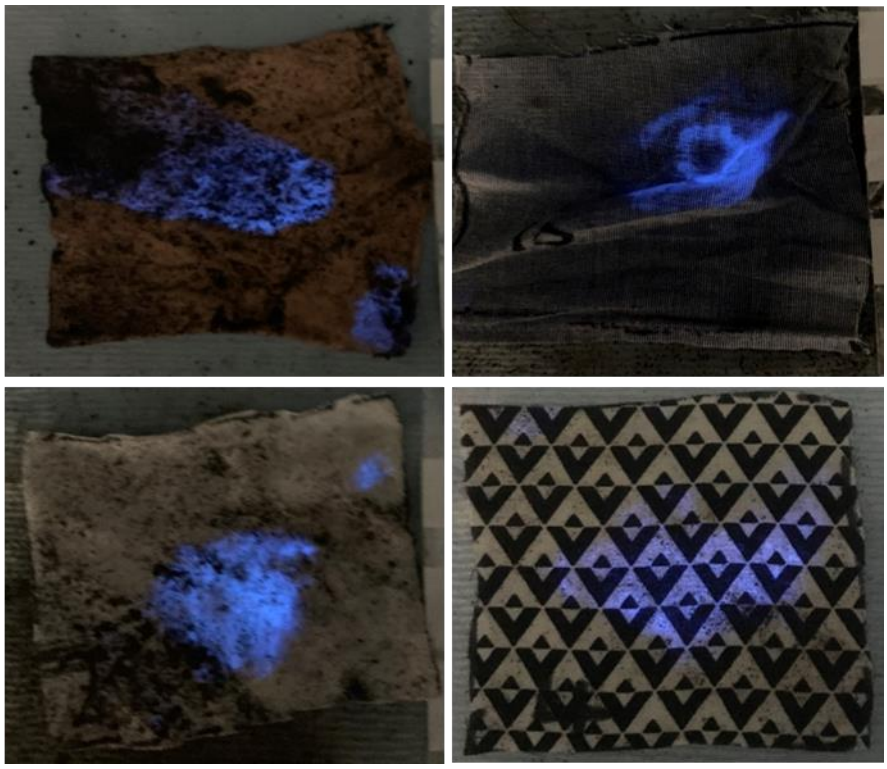

Figure 3: Images of fluorescing bloodstains once sprayed with BlueStar®, showing the movement of the bloodstains from the centre of each material, where the blood was originally dropped. (Materials from left to right, cotton, cotton blend, polyester blend, and)

2.5 Soil Analysis

2.5.1 Residual moisture content

10-20g of fresh soil taken from each soil sample were left to air dry until constant weight.

Once dried, the residual moisture content was calculated using Equation 2.

Equation 2: Measure of residual moisture content

$$P = 100 \times \frac{(W - D)}{D}$$

Where:

P= moisture content of sample (%)

W= original weight of sample (g)

D= dry weight of sample (G)

2.5.2 Determination of pH

10g of air-dried soil was placed into a 50 ml beaker, 25 ml of distilled water was then added to the sample. The beaker was stirred and left to stand for 15 minutes. The pH of the sample was then recorded using a Hanna instrument HI99121 pH meter by suspending the electrode in the liquid fraction.

2.5.3 Relative bulk density

A 70g bulk sample of air-dried soil was randomly generated from each original soil sample collected from the experimental site using the cone and quarter method. A further 10ml sample of the soil was taken from the bulk sample and then weighed. This was repeated 5 times for each sample and a mean density in g/cm^3 calculated from these repeats (Lestariningsih et al. 2013).

2.5.4 Total microbial activity

The method of determining total microbial activity described by Adam and Duncan (2001) was used to identify the activity of micro-organisms within a soil sample. This method measures the activity of the soil microbiota by measuring the amount of fluorescein released from fluorescein diacetate (FDA) by microbial hydrolase enzymes. A sub-sample of 2g of fresh soil from each sample was placed into a 50ml conical flask. 15ml of 60mM

potassium pHospHate buffer was then added to each conical flask. To start the reaction, 0.2ml of FDA stock solution was added to the conical flasks. Stoppers were then placed onto the flasks, which were then shaken in an orbital shaker/incubator at 30^{o}C for 20 minutes, at 100 rev min^{-1}. After incubating, the flasks were removed and placed into a fume cupboard, where a 2:1 mixture of chloroform:methanol was added in order to terminate the reaction. Whilst under the fume cupboard, the contents of the flask were transferred into 50ml centrifuge tubes, which were centrifuged at 2000 rev min^{-1}. Once centrifuged, the supernatant was filtered through a Whatman No. 2 filter paper and collected in a 30mL polypropylene tube. This was repeated to give two replicates. A third flask was prepared as previously described, but without FDA to provide a blank. Finally, ~2 mL of each filtered sample was transferred to a cuvette and the absorbance read at 490 nm by a spectropHotometer (Varian Cary 50, Varian Inc.). The absorbance of the flask without added FDA was subtracted from the two flasks with FDA to remove the effect of light absorbed by fluvic substances in the samples.

2.6 Data Analysis

Raw data obtained from all experimentation was collated onto a Microsoft Excel spreadsheet, where any mathematic equations were conducted. Once the data was processed on the spreadsheet, it was transferred over to a statistical package for social sciences (SPSS vs 26) database. SPSS is commonly used by researchers to perform statistical analysis (Field 2017). The reason SPSS was used for this research is that SPSS can manage and analyse large quantities of data, allowing the user to specify what areas of the data to focus the analysis on. This is especially useful when trying to identify correlations within the data set as a starting point to narrow down what further analysis needs to be conducted. Whilst conducting all statistical analyses, assumptions underlying parametric tests were conducted to identify the most robust statistical methods. The first stage of statistical analysis was to conduct a two-way ANOVA to determine the

significance of the main effects (season and clothing type) and interactions between them on the dependent variable (CTCF). This was performed twice, firstly selecting all cases where the samples were buried, then repeated selecting all cases where the samples were not buried.

The second use for SPSS was to identify seasonal variations between recorded soil parameters at the start and end of the experimentation period. The statistical analysis of this was collected by conducting Welch robust tests of equality of means as preliminary testing of data for the assumption of homogeneity of error variance showed the assumption was not always met.

The third use for SPSS for this research was to run a multiple regression to identify which of the soil parameters explored impacted the dependent variable (CTCF). Each sample was selected, and the result for each soil parameter was used as an independent variable to perform the multiple regression. This type of analysis a data file containing, correlations between each variable, including the dependent variable, and a table which can be used to identify how significantly each independent variable impacted the dependent.

3 Results

3.1 Observations

During an investigation it is clear to see that using observation alone, it is difficult to determine if bloodstains are present on clothing after burial.

Figure 4: Dried bloodstains created in a Laboratory environment after 14 days. (Materials from top left cotton, top right cotton blend, bottom left polyester blend, and bottom right polyester)

The bloodstains in Figure 4 were created and kept in a laboratory to avoid any external environmental factors that may affect the bloodstains. All bloodstains on all of the sample materials are visible, indicating that they will be identified and collected as evidence during an investigation.

Figure 5: Bloodstained samples taken from the ground surface after 14 days. (Materials from top left cotton, top right cotton blend, bottom left polyester blend, and bottom right polyester)

The bloodstains on the ground surface samples were still somewhat visible on the cotton and cotton blend samples after 14 days (as demonstrated in Figure 5). However, the bloodstains were harder to visualise on the polyester and polyester blend samples. In comparison to the samples left under indoor laboratory conditions, the bloodstains had faded, making them slightly more difficult to identify as originating from blood.

Figure 6: Bloodstained samples after being buried for 14 days. (Materials from top left cotton, top right cotton blend, bottom left polyester blend, and bottom right polyester)

After being buried for 14 days, it was clear that the bloodstains on all types of clothing samples were not visible (as shown in Figure 6), making it difficult to identify if there is any blood on the samples. When compared to the samples from indoors and left on the soil surface, it was clear that the bloodstains had completely faded, which could impact an investigators judgment and therefore collection of evidence during an investigation.

3.2 Buried vs Un-buried.

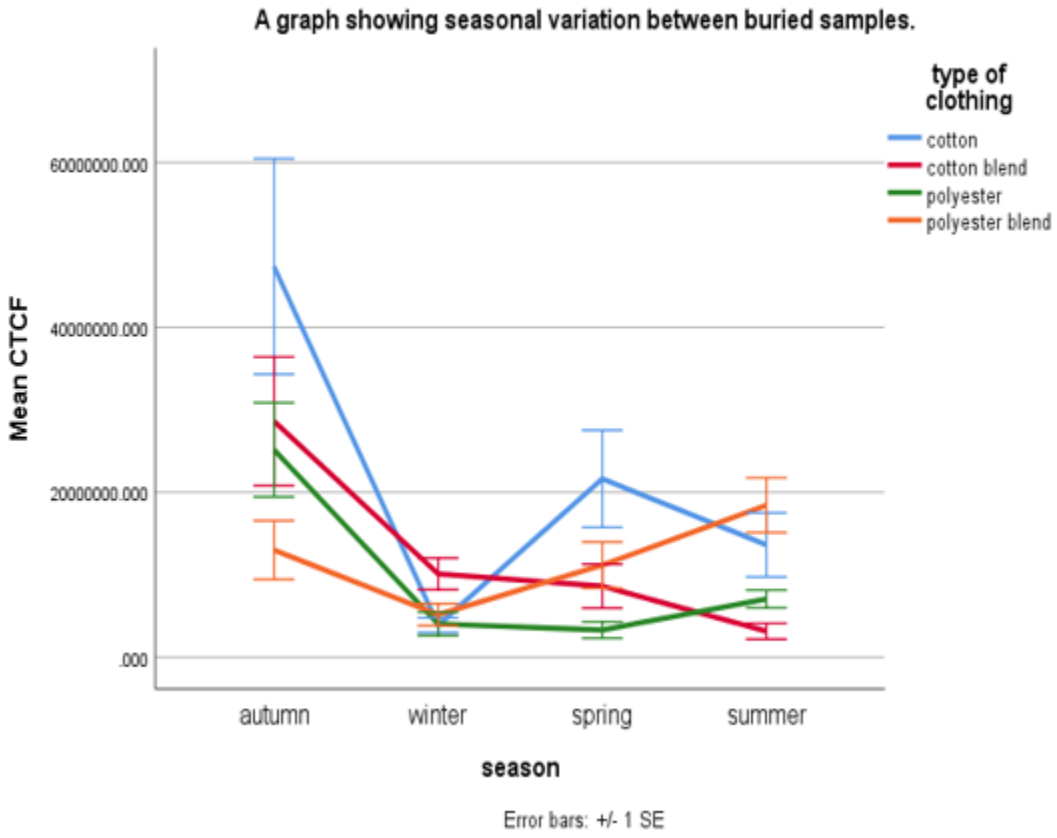

Figure 7: CTCF values following BlueStar treatment of bloodstains on buried clothing samples buried for 14 days in each seasonal period.

Figure 7 shows that the buried cotton samples have a very large variation in CTCF values during autumn, with the cotton blend samples also having a large CTCF value over this period. However, over the later seasons, all sample materials begin to coincide with each other, as they maintain an average CTCF of 2 X 10^7 and below, which indicated that none of the sample bloodstains fared well during summer. To identify differences between the survival of bloodstains on buried and surface samples, a two-way ANOVA was conducted. It was identified that there was a significant between the buried and surface samples CTCF values, during each seasonal period, and between season and the sample material type which impacted the variations between the samples CTCF values. The results of the ANOVA demonstrated the main effects of clothing type ($F = 4.761$, $P = <.004$) and season

(F = 17.487, P = <.0001) had a significant effect on CTCF. Likewise, the interaction between material type and seasonality was also significant (F = 3.052, P = ≤ .003), indicating that the interaction between the material type and the season was significant.

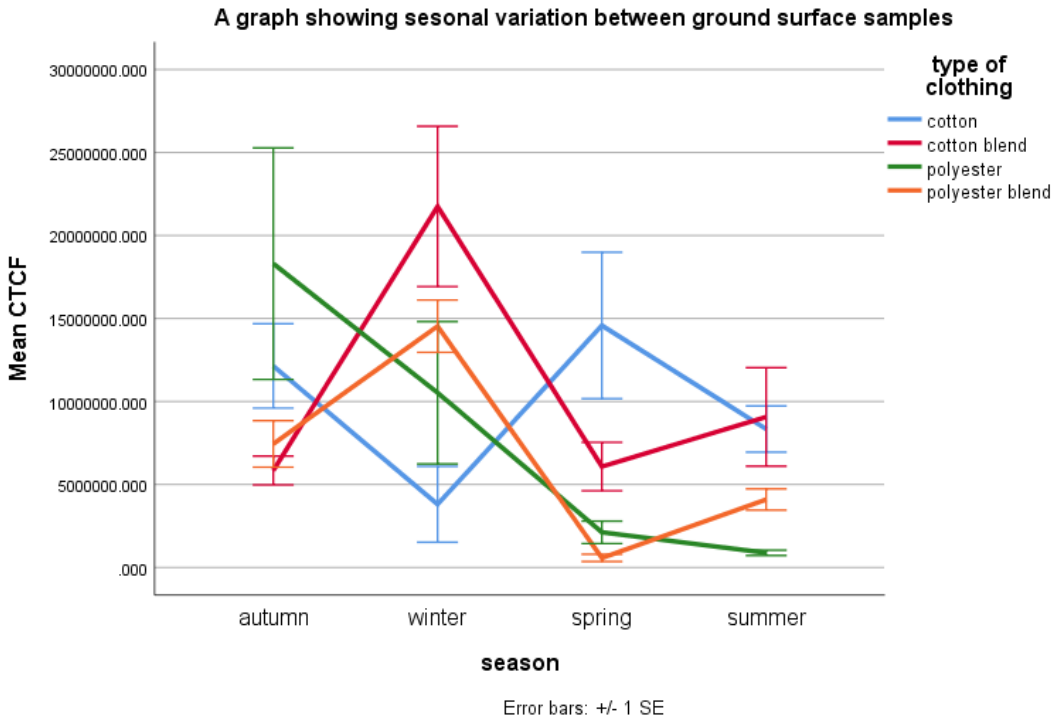

Figure 8: CTCF values following BlueStar treatment of bloodstains on surface clothing samples buried for 14 days in each seasonal period.

Figure 8 indicates that all sample material types have maintained an average CTCF value below 25 X 10^6, which is similar to the buried samples during winter, spring and summer. However, when comparing the two grapHs during the autumn period, especially looking at the cotton samples, it shows that the bloodstains on the surface did not survive as well as the buried bloodstains, indicating that the surface environment may not be the best placement for the preservation of bloodstains on cotton fabric materials. A two-way ANOVA demonstrated the main effects of clothing type (F = 41.468, P = <0.230) and season (F = 5.838, P = <.001) interaction between material type and season was significant (F = 4.889, P = ≤ .0001).

3.3 Clothing Type

From the results gained in Figure 7 and Figure 8, it has been identified that the bloodstain survival rate changed throughout all seasonal periods across each clothing material type when buried and when not buried. As shown in Figure 7, the bloodstains on all of the materials except for the polyester blend samples have a mean CTCF over 2 X 10^7 during the autumn period, and then begin to decrease and stay below a mean CTCF of 2 X 10^7 over the other seasonal periods, with cotton going slightly above during spring. In comparison from the data shown on Figure 8, it is clear that none of the sample materials had a mean CTCF over 2 X 10^7 with the only exception being the cotton blend samples, having a slightly higher CTCF during winter.

3.4 Soil Parameters

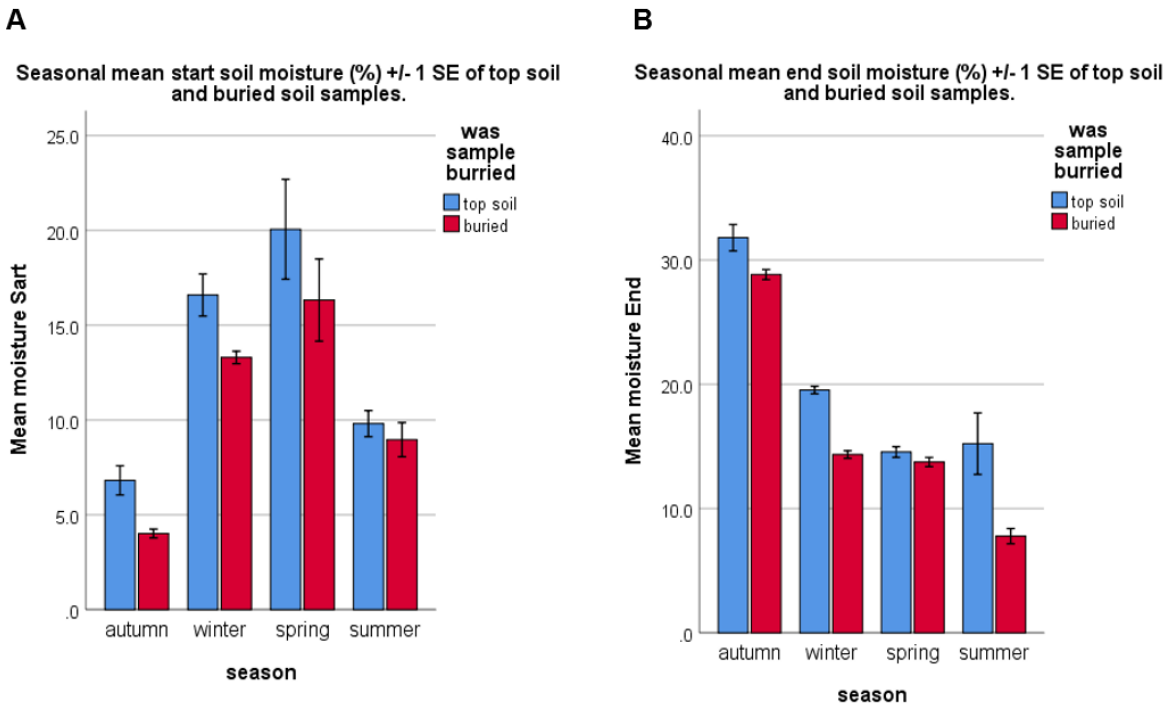

Figure 9: GrapHs showing the mean soil moisture (%) +/-1 SE during each seasonal period of both topsoil and burial soil samples. Figure 9a (left) showing the start of the experimentation period, and Figure 9b (right) showing the end of the experimentation period.

From Figure 9a it can be seen that soil moisture was at its highest for both the topsoil and soil from the burial level during spring, with autumn having the lowest average soil moisture. Whereas, Figure 9b indicates that during the autumn experimental period the soil moisture was at its highest increasing by 25% over the soil moisture at the start of the experimentation, with the soil moisture in spring decreasing by 5% on the topsoil samples. Welch robust tests of equality means demonstrated the variation between the soil moisture start ($F_{(3,19.324)}$ = 37.717, P = <0.0001) and the end soil moisture ($F_{(3,17.286)}$ = 133.024, P = <.0001) were significantly different.

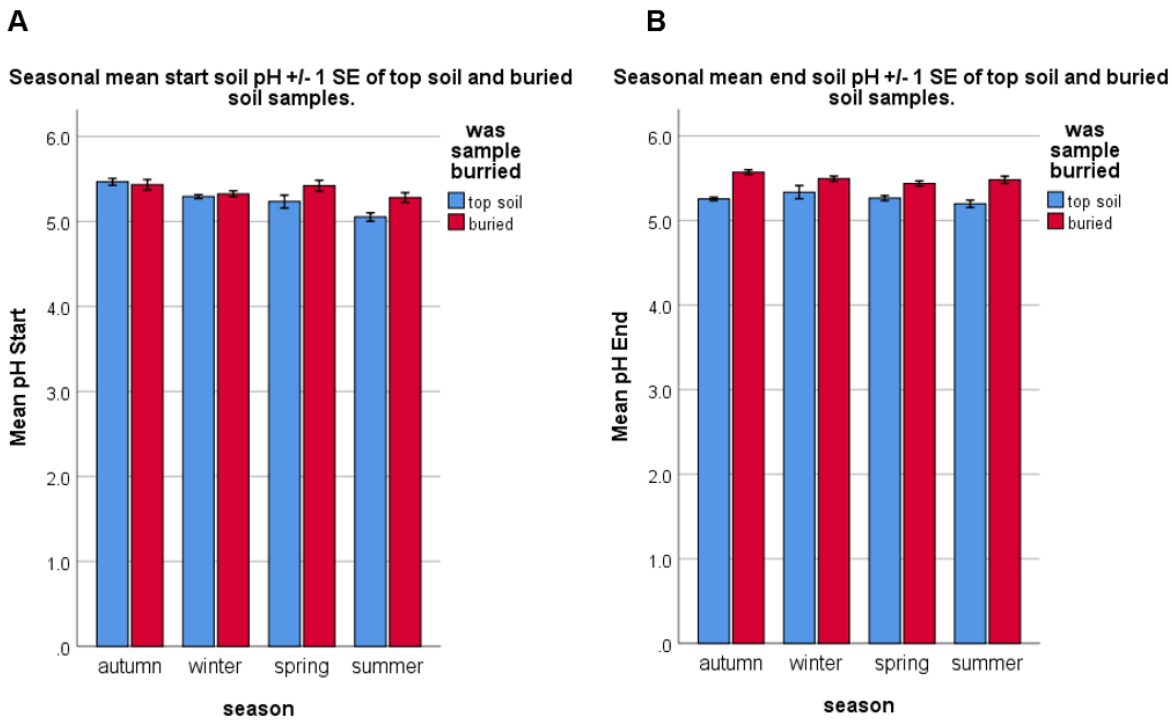

Figure 10: GrapHs showing the mean soil pH +/-1 SE during each seasonal period of both topsoil and burial soil samples. Figure 10a (left) showing the start of the experimentation period, and Figure 10b (right) showing the end of the experimentation period.

In Figure 10a it can be identified that the topsoil pH decreased over each seasonal period with the burial soil samples maintaining a similar pH throughout each season. Both soil samples, topsoil and buried, maintain a near-constant pH during each season, with the topsoil summer samples having a slightly higher average pH than the topsoil summer

samples seen at the start of the extermination. Welch robust tests of equality means demonstrated the variation between the soil pH start ($F_{(3,18.548)}$ = 6.949, P = <.003) and end soil pH ($F_{(3,19.627)}$ = .634, P = <.602).

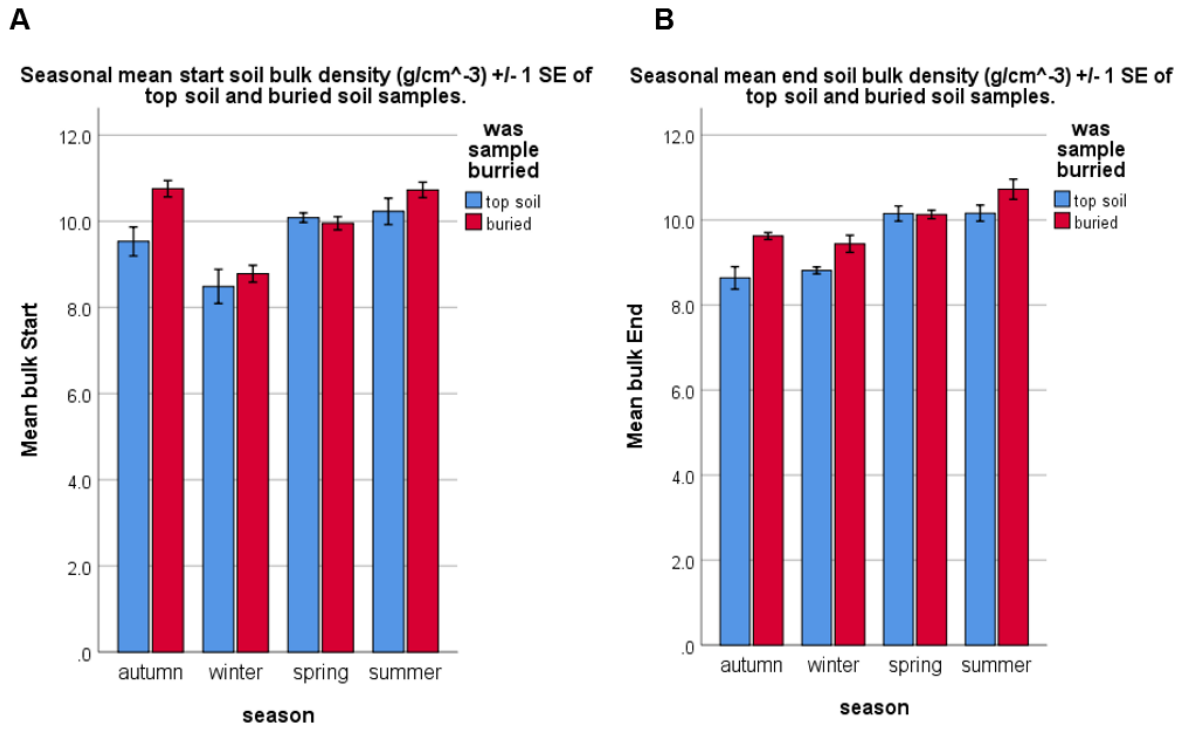

Figure 11: GrapHs showing the mean soil bulk density (g/cm^{-3}) +/-1 SE during each seasonal period of both topsoil and burial soil samples. Figure 11a (left) showing the start of the experimentation period, and Figure 11b (right) showing the end of the experimentation period.

Seen in Figure 11a, the topsoil and the buried samples have the lowest bulk density at the start of the winter period, this slightly increases at the end of the winter period, seen in Figure 11b. The bulk density of the autumn samples can be seen to decrease as the experimentation period occurs, by 0.5 g/cm^{-3} in the topsoil samples, and 2 g/cm^{-3} in the buried samples. Welch robust tests of equality means demonstrated the variation between the start soil bulk density ($F_{(3,\ 18.220)}$ = 14.507, P = <.0001) and the end soil bulk density ($F_{(3,19.184)}$ = 17.913, P = <.0001)

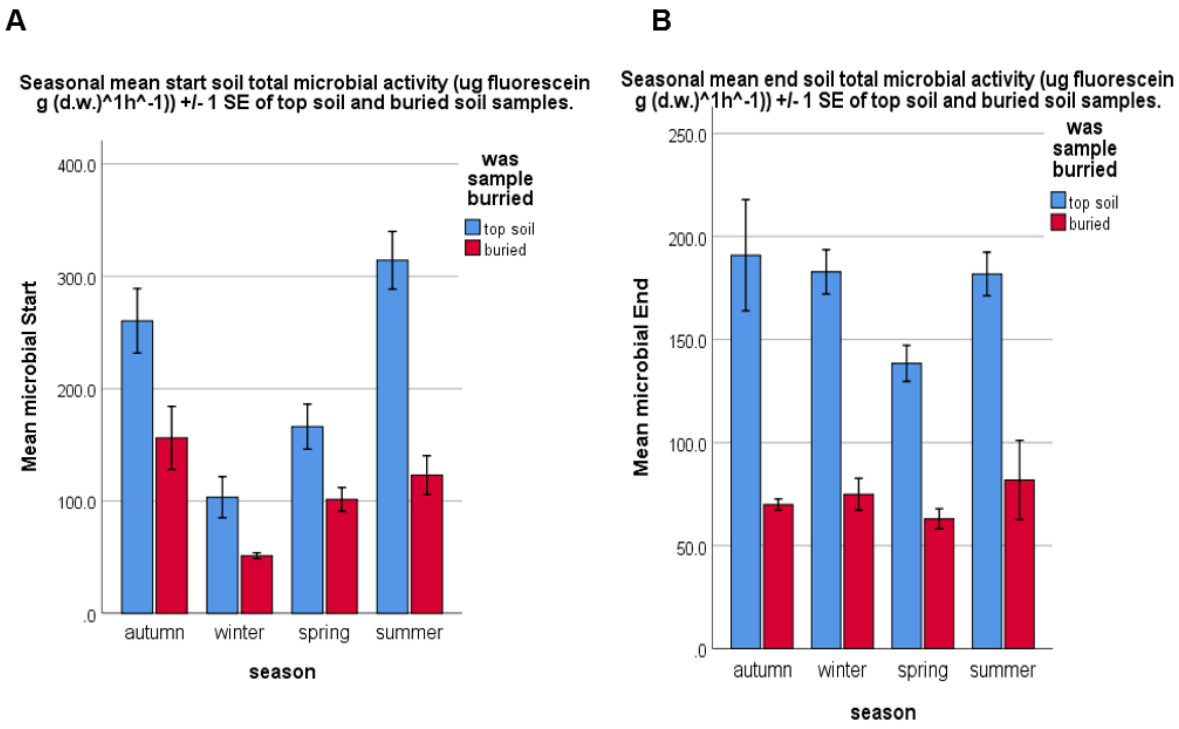

Figure 12: GrapHs showing the mean soil total microbial activity (µg fluorescein g (d.w.)1h-1) +/-1 SE during each seasonal period of both topsoil and burial soil samples. Figure 12a (left) showing the start of the experimentation period, and Figure 12b (right) showing the end of the experimentation period.

The microbial activity is larger in the topsoil samples in both Figure 12; however, it can also be seen that the microbial activity of the topsoil samples during autumn and summer are considerably larger at the start of the experimentation compared to the end of experimentation. However, microbial activity largely increased during the winter experimentation period. Welch robust tests of equality means demonstrated the variation between the start soil total microbial activity ($F_{(3,18.978)}$ = 10.164, P = <.0001) and end soil total microbial activity ($F_{(3,19.559)}$ = .856, P = <.480).

Table 1: CTCF of bloodstained clothes when buried (N=96)

	Zero-Order *r*							
variable	Bulk End	Microbial End	pH End	Moisture End	CTCF	β	Sr^2	p
Moisture End					.412			**
pH End				.820	.375	6.377	.257	**
Microbial End			.161	-.432	-.124	-.027	-.103	**
Bulk End		.373	-.476	-.657	-.029	.402	.097	*
						Intercept =	-30.200	
Mean	9.97685	72.40475	5.49500	16.17650	6.9142	R2 =	.522	
SD	.501120	6.922122	.048257	7.785291	.48539	Adjusted R2 =	.249	

*=	P=≤.001
**=	P=≤.0001

A multiple regression was run on data pooled from the buried bloodstains from each season to model Bloodstain CTCF from the parameter of soil moisture, soil bulk density, soil pH and soil microbial activity determined after the burial period, i.e. when a forensic investigator would be able to sample the soil. Table 1 shows basic descriptive statistics and regression coefficients. Moisture, pH and microbial predictor variable had a significant (P = <.0001) impact on the CTCF, with only bulk density (Bulk end) having a lower significance (P = <.001). These variables statistically significantly predicted CTCF ($F_{(3,92)}$ = 11.512, P = <.0001, R^2 = .522). All four variables added could account for 52.2% of the variance in CTCF, which was statistically significantly (P = < .0001). Preliminary tests of the data to check that the necessary assumptions were met showed that untransformed data failed the heteroscedasticity assumption. A $\log_{10}$ transformation of the CTCF data was conducted to correct this and multiple regression conducted on the transformed data. Some values for the soil moisture are missing from Table 1, due to its high correlation with pH (.820) resulting in a tolerance of 0. This meant that SPSS excluded the variable on the grounds of multicollinearity.

4 Discussion

4.1 Observational Study

From the evidence gained by conducting an observational study, bloodstains differ in survival depending on the environment in which the bloodstained clothing has been left. For the laboratory samples, the bloodstains are visible after 14 days, which means during an investigation a crime scene investigator would be able to identify the possibility of blood on the clothing of a victim; whereas, the samples left on the soil surface have faded. It is still possible to see the bloodstains on the cotton and cotton blend samples, which means that a crime scene investigator may be able to identify the presence of blood on these samples. However, on the polyester and polyester blend samples, bloodstains are difficult to visually identify without the aid of chemiluminescent reagents. In comparison, the buried clothing samples, it is not clear that any blood was placed onto any of the sample materials. On initial inspection, these samples simply look like dirty pieces on clothing with no bloodstain evident, which turn lead the potential loss of important evidence. It is important to note that any changes in the size and shape of the bloodstains, would impact the recreation of the crime scene as it will be difficult for an investigator to determine the angle of the origin of the blood once altered by any environmental and soil factors.

The introduction of the present study touched upon the variations in chemical techniques that are used and can be used during a forensic investigation, both non-chemiluminescent and chemiluminescent. Any of these techniques could have been used to identify the presence of blood on the clothing material samples, as all techniques used have been created for this purpose., However, the only technique used for this research was BlueStar® forensic reagent. The reason for using BlueStar® can be seen in the research by Tobe et al. (2007), which consisted of multiple presumptive bloodstain techniques, being compared. Tobe et al. (2007) found that the chemiluminescent techniques were able

to identify the presence of blood better than most of the non-chemiluminescent techniques, as the two tested did not destroy any trace DNA evidence which could be gained from the blood staining. The reason for using BlueStar® is also supported by Jakovich (2007), who stated the preparation of BlueStar® is a quicker process than that of luminol, and that BlueStar® is more user-friendly, as the target surface does not need to be in complete darkness for the bloodstains to fluoresce. A further rationale for using any chemiluminescent techniques over non-chemiluminescent techniques was that the fluorescence emitted from the blood was easier to measure quantitatively, as this makes the entire bloodstain fluoresce. Conversely, non-chemiluminescent techniques only make the target area of a bloodstain change colour; this would provide a sufficient measurement for the amount of blood present.

It is clear to see that all of the bloodstains no matter what material they were placed on, were affected differently depending on the environment they were placed. The laboratory samples may have been preserved due to the lack of environmental changes, whereas the surface bloodstain samples may have faded, due to any rainfall that occurred during the experimentation period, as supported by work of Mushtaq et al. (2015), which found that the bloodstains will not bind to the synthetic fibre samples, and will be easily washed off as a result. As seen in Figure 5, the blood has potentially been washed off by the rain on the synthetic fibre samples, whereas the blood has bound itself to the natural fibre samples, hence why the bloodstains are still somewhat visible on the cotton and cotton blend materials. As shown in Figure 6, the samples that were buried are near impossible to identify any visible presence on blood without the use of non-chemiluminescent and chemiluminescent techniques. This supports the research conducted by Li et al. (2010) which identified that cotton and cotton blend materials decompose quicker dependent on the microbial activity of the soil and the micro-organisms attraction to the cellulose structure of the cotton and cotton blend sample materials. The problem with this is that it

does not explain why the buried polyester and polyester blend samples appear to have no blood present. This indicates further research is needed to determine the factors are impacting the clothing materials, as most research only focuses on natural fibre-based materials, and not synthetic fibres.

The lack of visible blood may have been caused by bacterial haemolysis (Orf and Cunnington 2015). Haemolysis is the premature destruction of red blood before the end of their normal life span (Orf and Cunnington 2015). The process of haemolysis occurs when the membrane of a red blood cell (RBC) is broken down by a bacterial protein known as Hemolysin. This causes the release of haemoglobin. Many types of bacteria possess haemolytic proteins (Orf and Cunnington 2015). Two types of haemolysis can occur, alpHa haemolysis, being the reduction of haemoglobin in red blood cells, and beta haemolysis, involving the destruction of red blood cells (Misawa and Blaser 2000). The presence of either alpHa or beta haemolytic bacteria within the soil may have caused the process of haemolysis to occur within the bloodstains. AlpHa haemolysis would explain the visual loss of the red colour produced from the haemoglobin of the blood (Stuart 1982). Although, the process of beta haemolysis would explain the total lack of blood evidence on a sample. Research has found the presence of fourteen alpHa and beta haemolytic bacteria species within soils (Tambekar and Gadakh 2013). Tambekar and Gadakh (2013) identified that out of these fourteen bacterial species, seven conduct beta haemolysis. It was found that *Pseudomonas* were commonly found in the soil, with *P. aeruginosa* being the most prominent bacterium to conduct beta haemolysis within the soil. From this, it can be inferred that haemolytic bacteria are present within the soil, which explains why the bloodstains on the buried samples are not detectable by the naked eye. This further identifies the importance of buried bloodstain evidence being collected as quickly as possible, as these haemolytic bacteria within the soil will remove any visible evidence, thus causing the loss of important evidence. With the presence of haemolytic bacteria in the

soil, it is important to ensure that any buried bloodstained clothing is stored in a cool storage unit until further tests can be conducted as the colder environment will decrease the rate of bacteria reproduction, thus decreasing the loss of evidence between collection and testing (Ogdur et al. 2018).

4.2 Environment Placement

From the results in Figure 7 and Figure 8, the mean CTCF for all of the samples were all near to 2 X 10^7 and below during each seasonal period with the only exception being the buried cotton, cotton blend and polyester samples during autumn, which were all above an average CTCF of 2 X 10^7. During spring, the cotton samples mean CTCF values were also over 2 X 10^7 indicating that these bloodstains survived better on the cotton sample. From this analysis, it was identified that the material type and season significantly ($P \leq .003$) affected the average CTCF of the samples over the seasonal periods. Whereas the surface samples CTCF values were very significantly ($P \leq .0001$) effected by the material type and the season. When focusing on the buried samples, the majority of the bloodstains were visible when BlueStar® was applied. However, the emitted fluorescence measured was considerably lower during winter, spring and summer, when compared to the recorded average fluorescence emitted from the bloodstains during autumn, especially those on the cotton samples which varied between a CTCF of below 4 X 10^7, to above 6 X 10^7. This indicated that the buried bloodstains survived better during the autumn period. Unlike the buried bloodstain samples, the surface samples did not show an average CTCF above 2.5 X 10^7, which suggests that the surface sample bloodstains did not survive as well as the buried bloodstains. This is also the case during spring and summer. However, the error bars for the surface samples fluctuated largely for each sample clothing type at each season, which suggests a large variation of CTCF values recorded for each of these sample materials. From this, it is indicated that although the mean CTCF values are in most cases below that of the buried samples, the individual surface samples' CTCF values

fluctuated a lot, showing that some of the surface sample bloodstains survived better than some of the buried samples.

By comparing the results of Figure 7 and Figure 8, it can be determined that the buried bloodstains survived the best during autumn; whereas, the surface bloodstains fared better in the winter than the buried bloodstains. During spring and summer, both buried and surface bloodstains are seen to be relatively equal. Despite these results suggesting similarly average CTCF values between either environment samples, this does not explain the visual observations described in Section 3.1 that indicate the buried samples are nearly undetectable by the naked eye. However, they fluoresce similarly, and in some cases more so than the surface bloodstain samples. During an investigation it is likely that because of the lack of visually identifiable bloodstain evidence on buried clothing materials the evidence will not be assessed to determine the presence of blood, despite the bloodstain evidence on average, surviving better than the surface samples.

4.3 Best Clothing Type

When evaluating the findings gained by identifying the interaction between material type and season on the buried samples, it can be determined that the clothing type that retained the bloodstains best when buried was the 100% cotton samples. The cotton samples maintained the highest mean CTCF over the two seasonal periods, of autumn and spring. However, the results from both the surface and the buried samples do not clearly show an overall best material for retaining bloodstains. The cotton, cotton blend, and polyester samples all held the highest mean CTCF for one of the seasons, with the cotton and polyester both having very similar CTCF values during summer. The only sample material type that was consistent in either environment was the polyester blend as these samples maintained a mean CTCF value below 2×10^7. The buried polyester blend samples during

summer held the highest mean CTCF of all of the sample materials during summer, however, this was still below a CTCF of 2 X 10^7. When comparing CTCF values for buried and surface clothing types, it can be determined that on average the cotton samples retained the bloodstains better than the other materials, as it held the highest mean CTCF over the most seasonal periods. These results support the conclusions of Wang et al. (2010) which determined that natural fibre-based materials would absorb and retain bloodstains better than treated synthetic fibre-based materials. However, Li et al.'s (2010) research found despite the cotton sample having a faster decomposition rate than the synthetic fibre materials, the micro-organisms attracted to the cellulose structure of the cotton have not visibly effected the bloodstaining on the cotton samples which indicates that microbial activity may not have affected the bloodstaining on the cotton samples, or that micro-organisms that fed on blood may not have been present in the soil.

As mentioned above there has been a lack of research investigating the interaction between bloodstains on clothing and soils, with most research conducted either focusing on blood pattern analysis, soil sciences and the variations between fabrics and their composition. The lack of research investigating the interaction between blood and fabrics has was acknowledged by de Castro et al. (2012) which has further supported the need for research to investigate the factors that will impact bloodstains survival on buried clothing. Despite this, some research has been conducted which can be evaluated and compared to the findings of the present study. As discussed previously, the results of the present study do support the finding of both Tibbett and Carter (2008) and Lauber et al. (2009), in that they state that soil parameters and environmental seasonal changes will impact the decomposition of organic materials. Though, the results of the present study do not support the findings of Li et al. (2010), as it has been found that bloodstains on cotton samples appear to survive better compared to other materials, which does not support the theory that decomposer micro-organisms are attracted to the cellulose

structure of the cotton samples causing the cotton bloodstain samples to decompose faster. This, however, did not appear to dramatically alter the survival of the bloodstains. It was not identified in the research conducted by Li et al. (2010) what soil type was used, and what micro-organisms were present in the soil. On this topic, Figure 3 demonstrates that when BlueStar® was sprayed onto some of the samples the corners of the sample began to fluoresce, potentially indicating the presentence of blood-feeding micro-organism being present in the soil.

El-Naggar et al. (2003) identified that synthetic fibres are often produced to have basic natural water resistance. Wang et al. (2010) further support this, as they determined that natural fibres were found to absorb blood whereas the blood did not bind to the synthetic fibres, which is supported by the findings that the bloodstains appear to have survived best on the natural fibre materials. From a basic observation, it is perceptible that the bloodstains under laboratory conditions (shown in Figure 4) are clearer on the cotton and cotton blend samples, compared to the polyester blend samples, where the blood is still visible but is not as obviously identifiable. This is more evident in surface samples, where the bloodstains on the polyester and polyester blend samples resembled a dirty smudge on the sample. Once BlueStar® was sprayed onto some of the samples the corners of the sample began to fluoresce; however, alongside this, some of the samples also fluoresced in a larger area around the bloodstains. This fluorescence potentially indicates that the moisture of the soil or the rainfall in the area may have washed the samples, dragging the blood across the sample materials. Using the results gained from Figure 9a and 9b, it can be determined that during the autumn period the soil moisture largely increased, affecting both the surface and buried samples. This increase was potentially the main cause for the movement of the bloodstains during the autumn. The movement of the blood has been altered thus changing the direction in which the blood originally travelled, which indicates that the crime scene will not be reconstructed using blood pattern analysis methods

correctly and suggests that blood pattern analysis will not be successfully conducted. The soil moisture during the other seasons did not change as drastically as the autumn period, which indicates that soil moisture still impacted the bloodstains but not to the same effect as the autumn samples.

4.4 Soil Factors

The soil factors investigated as a part of this research all had a unique, significant effect on the survival the sample bloodstains (as demonstrated in Table 1). The multiple regression results demonstrated that microbial activity had the smallest (sr^2 = -.103) correlation with the CTCF values, however, the microbial activity significantly (P = <.0001) affected the CTCF values. This supports the findings of Tibbett and Carter (2008) who stated that microbial activity is a major factor in the decomposition of organic matter. The present study shows that microbial activity did largely affect the survival of the bloodstains, similarly to soil moisture and pH.

One of the main findings of the multiple regression was the positive correlation between pH and microbial activity (0.161). This correlation does not support the research of Lauber et al. (2009) which indicated that microbial activity is determined by the pH of the soil, with microbial activity rapidly decreasing when the pH drops below pH 5.5. The mean pH of the soils used at the experimentation site overall seasonal periods was pH 5.38, and by using the findings of Lauber et al. (2009) this pH would have been low enough to directly decrease the rate of the microbial activity in the soil, which does not support the significance (P = <.0001) of the microbial activity on the CTCF of the bloodstains. Although it was previously mentioned, that micro-organisms present in the soil may not have been attracted to the bloodstains, the results in Table 1, clearly show that the microbial activity of the soil compromised the survival of the bloodstaining, on all of the material types.

However, per the research of Spohn (2015), it is possible that the presence of organic material, such as blood and natural fibre materials, decomposing and releasing carbon, will have increased the rate of microbial activity.

During decomposition, organic remains release carbon (Carter et al. 2006). This release of carbon in more prevalent with the decomposition of larger organic remains. The released carbon shifts the balance of the C:N ratio of the soil, which can alter the stability of important ecosystem processes (Carter et al. 2006). Most micro-organisms require a nutrient source with a C:N ratio of 25:1, ratios outside of this will result in a decrease of decomposition rate (Hodge et al. 2000). It has been found that the average human has a base C:N ratio of 7:2, with average adult human cadavers having a carbon:nitrogen ratio of 5:8 (Tortora and Derrickson 2014). Carter et al. (2006) noted that most large cadavers used for experimentation, are approximately 20% C, and act as a specialised habitat for decomposer microorganisms. Extensive research into this has been conducted, investigating the presence of these microorganisms, within decomposing cadavers, however, little research has been conducted investigating these microorganisms when cadavers are buried (Carter et al. 2006).

Research has been conducted to investigate the relationship between terrestrial carbon and soil microbial respiration (Spohn 2015). It was found that the C released from decomposing soil litter layer positively increased the microbial activity of the soil; whereas, N decreased the rate of microbial activity. It was determined that as long as the C released from decomposing organic materials can be sustained at a larger rate than the released N, then the microbial activity will increase with the amount of present C (Spohn 2015). Using the findings of Carter et al. (2006) and Spohn (2015), it can be determined that the presence of decomposing blood within the soil will increase the C:N ratio of the soil, which

will increase the rate of microbial activity. This will mean that the survival of the bloodstains is likely to decreases, especially on natural fibres, as the decomposition of the natural cellulose structure will also increase the C:N ratio of the soil (Spohn 2015).

As mentioned previously, research has been conducted, investigating the effect, of which the decomposition of organic materials affects the C:N ratio of soil (Carter et al. 2006). Carter et al. (2006) conducted their research into this by using pig cadavers as a proxy for human remains. The pig cadavers were left on the surface of the soil until the cadaver reached a dry stage of decomposition. The carbon released from the cadaver was monitored, and micro-organisms attracted to the decomposition process were observed. It was noted that microbial activity, increased in and around the area of the cadaver. When comparing the finding of Carter et al. (2006) to the results of the present study, it can be determined that the microbial activity on the soil's surface may have increased due to the decomposition of the bloodstains. However, Carter et al. (2006) did not explain the outcome of the buried samples, as their research did not investigate the soils C:N ratio when organic remains are buried. Likewise, the research conducted by Spohn (2015) also only focused on the presence of decomposing organic remains on the soil surface, which determined that the carbon released by decomposing organic remains increased the rate of microbial activity. Similarly, Carter et al.'s (2006) research also only gives an insight into conditions that may have affected the bloodstains survival on the surface, and how the bloodstains themselves may have altered the experimentation area. However, no research has investigated what changes may occur to the soil when decomposing organic materials are buried. Experimentation conducted on the soil at Wytch Farm has found that the topsoil had a C:N ratio of 9.97 and for the buried soil at depth of 20cm it was 11.20 (I. Green, Bournemouth University, pers. comm. 16 September 2019). From the results of this, it can be determined that the C:N ratio of the soil surrounding both the buried and surface samples, would have resulted in the decrease of microbial decomposition, as this is below

the optimum 25:1 ratio. Indeed, the soils total microbial activity was lower at the end of the experimental period over each season. This does not, however, explain the findings that soil microbial activity had a significantly large effect CTCF recorded from samples.

The results of the multiple regression analysis also determined that the soil moisture had a strong positive correlation (.820) with the measured CTCF values. This suggests that the soil moisture has majorly affected the CTCF, thus the survival of the bloodstains, following the research conducted by Tibbett and Carter (2008). All together the multiple regression analysis demonstrated that the survival of the bloodstaining was affected mainly by the soil moisture and the soil's pH. Using the results shown in Figure 10a and 10b it is evident that the soil pH did not vary much throughout from each seasonal period, whereas the soil moisture in Figure 9a and 9b fluctuated during each seasonal period due to rainfall and temperature. Despite the changes in the soil moisture and stability of the soil's pH, these two factors would have worked in conjunction with each other as a natural organic stain remover (Pager 2000). This explains why the bloodstains are not visible on the buried samples, as these samples were fully covered by the soil, so they had the most direct interaction with the moist, acidic soil, which would have acted as a natural stain remover. These findings support the low CTFC values of the samples during winter, spring, and summer, but not during autumn. During autumn the buried samples yielded their greatest CTCF values, however, the soil moisture was also at its highest, at the end of the autumn period which suggests that the CTCF should have been lower for the buried samples. Further analysis must be conducted to determine how much of an impact the soil moisture is having on the survival of the bloodstains. Alongside this, no evident research has been conducted investigating the interaction between soil moisture and pH. The present study demonstrates that this relationship needs to be researched further to determine how these soil parameters impact the survival of bloodstains on clothing materials as they have had a significant impact on the buried bloodstains' survival.

The possible issue arising from the way the multiple regression was conducted, is that it only takes into consideration the buried bloodstain samples, so a generalised model has been created using data collected from all types of clothing material and the burial environment they were placed, on one specific soil type. If another separate model was to be created for the surface samples, it would allow for each model to be used when investigating buried victims bloodstaining or bloodstains from victims found on the surface. Currently, the created model can be used to generalise how the soil parameters impact the survival of buried bloodstains, the model can also be used to predict the CTCF values for bloodstained clothing within a similar soil type.

The multiple regression analysis used the data gained from each soil parameter at the end of each seasonal period when the bloodstain samples were collected. The purpose for this was that it was determined that these results would mostly represent the soil conditions in which the bloodstains would have been found when collected by a crime scene investigator. Whereas, if this analysis was conducted with the soil parameters collected at the start of the burial, this would not have given a good representation of the disturbance of the soil when a suspect has dug a shallow grave.

4.5 Research Relevance and Impact

Research has been conducted identifying the effects of clothing on human decomposition and the implications of estimating the time of death (Miller 2002). This research consisted of cadavers, nude and clothed, being placed lying down facing upwards over different seasonal periods. It was found that the clothing slowed down the rate of decomposition during the spring and summer; whereas, in the winter the clothing was not a significant variable and no comparison between cadavers in the autumn. The study concluded that

the presence or absence of clothing must be taken into consideration when estimating time since death. This research is useful as it identifies that clothing needs to be considered when investigating human decomposition, however, it does not take into account the burial of clothed humans remains and how this may impact the rate of decomposition. Further supporting the point that more research needs to be conducted on the decomposition of organic remains. As identified by the present study, bloodstaining did not survive as well during the spring and summer compared to the autumn and winter. This finding is contrary to the results from research conducted by Miller (2002), which further indicates that research needs to be conducted on the decomposition clothed humans and human tissue on clothing when buried and left on the surface, and to identify why there is a change in results when comparing the decomposition of clothed human remains and human tissues found on clothing at crime scenes.

Blood pattern analysis is an extremely important form of evidence analysis used during the forensic investigation of violent crime. In the introduction of this topic, it is stated that 726 homicides took place across England and Wales in 2018 (ons.gov.uk 2019). This was a 3% increase from the previous year. Any loss of crucial evidence at these types of crime scenes could change the outcome of an investigation, which is why the research was needed to be conducted. From a visual point of view, the research has identified that it may not always be possible for a crime scene investigator to see any bloodstaining through the naked eye on a buried victim, and this is somewhat difficult when observing clothing on a victim left in an open environment. This research has also found that the material of the victims' clothes will also affect the retention of bloodstains on a victim, which need to be taken into account when assessing the victim and the scene. Also identified in the research is the need to recover buried victims quickly, as the bloodstains were detectably after 14 days when buried. However, bloodstains became difficult to visibly see without the use of chemiluminescent techniques, which indicates that after 14 days if the victim is not

recovered all potential bloodstain evidence may be lost, ultimately impacting the investigation as a whole. This research needed to be completed to ensure to identify that important bloodstain evidence is potentially being lost during a forensic investigation due to environmental factors within the soil and the soil's surface.

5 Conclusion

The purpose of this research was to investigate factors within the soil that affect the recovery of bloodstain evidence from buried clothes. From the results gained, it is apparent that there is a visible change in bloodstains on clothes depending on their environmental placement the most notable change being on the buried samples, where the bloodstain is near undetectable by the naked eye. It was also noted that the survival of the bloodstains differed depending on the fabric type on which they have been placed. The results showed that on average the cotton samples yielded the highest CTCF values over each seasonal period, whereas, the bloodstains on the polyester blend samples constantly maintained the lowest CTCF values, which indicated that the bloodstains survived better on cotton samples. It is also shown that the bloodstains survived best during autumn as the mean CTCF values for the samples were highest during autumn when the samples were buried and placed on the surface. However, the surface samples maintained similar CTCF values over all seasonal periods. Finally, the main focus of this research was to determine what factors within the soil, would most impact the survival of the bloodstains. By conducting a multiple regression analysis, it was found that the bloodstains were majorly affected by the soil moisture, which is supported by the findings of Tibbett and Carter (2008). Alongside this, it was also noted that pH and microbial activity both significantly impacted the survival of the bloodstains. It was also determined the relationship between the soil moisture and soil pH were the main impactors on the bloodstain's survival.

5.1 Further Research

This research has opened up several areas that need to also be investigated. Most importantly, different soil types need to be investigated to create a more appropriate model to be used in forensics investigations. This should be investigated by collecting a larger variety of soil types and by conducting further statistical analysis, using more complex

methods of analysis. It also needs to be determined how much the seasonal change in soil moisture is impacting the bloodstains survival, by repeating the experimentation, collecting a larger reference database of soil moistures throughout the experimentation period, and by having a portable weather station near the experimentation site to ensure accurate analysis of rainfall and temperature in the area whilst experimentation is occurring. It would also be best to conduct an in-depth study into how the bloodstains are affecting the C:N ratio of the soil, and in conjunction to this, identify what micro-organisms are present in the soil that will impact the bloodstains survival, despite any variation within the soils carbon: nitrogen. Finally, an investigation into what specific proteins are surviving on the bloodstains, mainly to identifying the presence of any DNA evidence, to identify if the surviving bloodstains are still useful in a criminal investigation.

It has been discussed the need for this research to be conducted, due to the lack of research being conducted into soil factors impacting the survival of bloodstains. By conducting this research, it has been made evident those subject areas that can be related to this research are currently lacking more up to date literature, as most of the literature linked this topic was published in 2012 and earlier. The most in need of updating is the data gained from the UK Soil Observatory, as the majority of the data from this database was collected in 2007, with only some area of the UK containing data from 2012. This has not impacted the finding of this research, however, as the data used from this database was only used for a small section of this research to gain a basic understanding of the recorded soil parameters around the Wytch Farm experimental site. Despite the desperate need of an update, the literature used for this research has been extremely helpful to gain the advanced knowledge needed to conduct this research.

5.2 To Conclude

In conclusion, the research carried out aimed to primarily investigate factors within the soil that affect the recovery of bloodstain evidence from buried clothes, and has been successful in doing this by identifying that the burial of bloodstained clothes affects the visual presence of blood, which can impact a crime scene investigators interpretation. It has determined that the bloodstains survived best on the cotton samples during the autumn, and it has determined that the main factors that impact the bloodstain survival, is the soil moisture, and its relationship with the soil pH. Ultimately this research has shown that the burial of a victim, and their clothes, may impact the outcome of an investigation as there is a high potential that important bloodstain evidence may be lost during the investigation. This research has also shown the large gaps in the literature that need to be covered going further and the need for existing literature to be updated.

6 References

Aciego Pietri, J. and Brookes, P., 2008. Relationships between soil pH and microbial properties in a UK arable soil. *Soil Biology and Biochemistry*, 40 (7), 1856-1861.

Adam, G., Duncan, H., 2001. Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. *Soil Biology & Biochemistry*, 33 (7-8), 943-951.

Andrade, A., Siqueira, M., Arantes, L., Queiroz, L., Silva, R. and Ramalho, E., 2014. Forensic identification of human blood: Comparison of two one-step presumptive tests for blood screening of crime scene samples. *Revista Brasileira de Criminalística*, 3 (1), 12.

Anderson, D., 1988. The effect of parent material and soil development on nutrient cycling in temperate ecosystems. *Biogeochemistry*, 5 (1), 71-97.

Baden, M. and Hennessee, J., 2005. *Unnatural death*. New York: Ivy Books.

Bankhead, P., 2014. Analyzing fluorescence microscopy images with ImageJ. *Queens University Belfast*. May 2014.

Barros, N., Gomezorellana, I., Feijoo, S. and Balsa, R., 1995. The effect of soil moisture on soil microbial activity studied by microcalorimetry. *Thermochimica Acta*, 249 (1-2), 161-168.

Barni, F., Lewis, S., Berti, A., Miskelly, G. and Lago, G. (2007). Forensic application of the luminol reaction as a presumptive test for latent blood detection. *Talanta*, 72(3), 896-913.

Bernardo-Filho, M., Gutfilen, B. and Souza Maciel, O., 1994. Effect of different anticoagulants on the labelling of red blood cells and plasma proteins with 99Tcm. *Nuclear Medicine Communications*, 15(9), 730-734.

Bockheim, J. and Gennadiyev, A., 2000. The role of soil-forming processes in the definition of taxa in Soil Taxonomy and the World Soil Reference Base. *Geoderma*, 95(1-2), 53-72.

Boos, K., Orr, A., Illes, M. and Stotesbury, T., 2019. Characterizing drip patterns in bloodstain pattern analysis: An investigation of the influence of droplet impact velocity and number of droplets on static pattern features. *Forensic Science International*, 301, 55-66.

Bremmer, R., de Bruin, K., van Gemert, M., van Leeuwen, T. and Aalders, M., 2012. Forensic quest for age determination of bloodstains. *Forensic Science International*, 216(1-3), 1-11.

Carter, D., Yellowlees, D. and Tibbett, M., 2006. Cadaver decomposition in terrestrial ecosystems. *Naturwissenschaften*, 94(1), 12-24.

Chenu, C., Le Bissonnais, Y. and Arrouays, D., 2000. Organic matter influence on clay wettability and soil aggregate stability. *Soil Science Society of America Journal*, 64(4), 1479.

Creamer, J., Quickenden, T., Crichton, L., Robertson, P. and Ruhayel, R., 2005. Attempted cleaning of bloodstains and its effect on the forensic luminol test. *Luminescence*, 20(6), 411-413.

de Castro, T., Nickson, T., Carr, D. and Knock, C., 2012. Interpreting the formation of bloodstains on selected apparel fabrics. *International Journal of Legal Medicine*, 127 (1), 251-258.

Dent, B., Forbes, S. and Stuart, B., 2004. Review of human decomposition processes in soil. *Environmental Geology*, 45 (4), 576-585.

Donnelly, L. and Harrison, M., 2017. Ground searches for graves and buried targets related to homicide, terrorism and organised crime. *Episodes*, 40 (2), 106-117.

El-Naggar, A., Zohdy, M., Mohammed, S. and Alam, E., 2003. Water resistance and surface morpHology of synthetic fabrics covered by polysiloxane/acrylate followed by electron beam irradiation. *Nuclear Instruments and Methods in PHysics Research Section B: Beam Interactions with Materials and Atoms*, 201(4), 595-603.

Equine Health Labs, 2015. *Equine Health Labs - Sticky Blood in Horses* [online]. Equinehealthlabs.com. Available from: http://www.equinehealthlabs.com/sticky-blood-in-horses [Accessed 09 Nov 2018].

Ethical Fashion Group, 2018. *What Are Our Clothes Made From?* [online]. Common Objects. Available from: https://www.commonobjective.co/article/what-are-our-clothes-made-from [Accessed 7 Aug 2020].

Field, A., 2017. *Discovering statistics using IBM SPSS statistics +SPSS 24*. Sage Publications.

Galloway, A., Walsh-Haney, H., Byrd, J., 2010. *Recovering buried bodies and surface scatter: The associated anthropological, botanical and entomological evidence.* Forensic Entomology: The Utility of Arthropods in Legal Investigations. Boca Raton, Fla.: CRC. 2010. 223-257.

Gill, P., 2001. *Application of low copy numbers DNA profiling.* Croatian Medical Journal. Forensic Science Service, Birmingham, UK. 229-232.

Haglund, W. and Sorg, M., 2002. *Advances in forensic tapHonomy*. Boca Raton: CRC Press. 380-399.

Hodge, A., Robinson, D. and Fitter, A., 2000. Are microorganisms more effective than plants at competing for nitrogen?. *Trends in Plant Science*, 5 (7), 304-308.

Jakovich, C., 2007. *STR Analysis following latent blood detection by luminol, fluorescein, and BlueStar*., Journal of Forensic Identification. (Technical Note). 57 (2),193- 198.

Jawaid, M. and Abdul Khalil, H., 2011. Cellulosic/synthetic fibre reinforced polymer hybrid composites: A review. *Carbohydrate Polymers*, 86 (1), 1-18.

Keel, T., Jarvis, J. and Muirhead, Y., 2009. An exploratory analysis of factors affecting homicide investigations. *Homicide Studies*, 13(1), 50-68.

Kilic, M. and Okur, A., 2010. The properties of cotton-Tencel and cotton-Promodal blended yarns spun in different spinning systems. *Textile Research Journal*, 81(2), 156-172.

Klintschar, M. and Neuhuber, F., 2000. Evaluation of an alkaline lysis method for the extraction of DNA from whole blood and forensic stains for STR analysis. *Journal of Forensic Sciences*, 45 (3).

Larkin, B. Banks, CE., 2016. *Exploring the applicability of equine blood to bloodstain pattern analysis.* US National Library of Medicine National Institutes of Health. 2016

Larkin, B., El-Sayed, M., Brownson, D. and Banks, C., 2012. Crime scene investigation III: Exploring the effects of drugs of abuse and neurotransmitters on Bloodstain Pattern Analysis. *Analytical Methods*, 4(3), 721.

Lauber, C., Hamady, M., Knight, R. and Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied and Environmental Microbiology*, 75 (15), 5111-5120.

Lestariningsih, I., Widianto and Hairiah, K., 2013. Assessing Soil Compaction with Two Different Methods of Soil Bulk Density Measurement in Oil Palm Plantation Soil. *Procedia Environmental Sciences*, 17, 172-178.

Li, L., Frey, M. and Browning, K., 2010. Biodegradability study on cotton and polyester fabrics. *Journal of Engineered Fibers and Fabrics*, 5 (4).

McCauley, A., Jones, C., Jacobsen, J., 2009. *Soil pH and organic matter.* Nutrient Management, Montana State University, 2009.

Manzoni, S., Schimel, J. and Porporato, A., 2012. Responses of soil microbial communities to water stress: results from a meta-analysis. *Ecology*, 93 (4), 930-938.

Menez, L., 2005. The place of a forensic archaeologist at a crime scene involving a buried body. *Forensic Science International*, 152 (2-3), 311-315.

Michielsen,, S., Taylor, M., Parekh, N. and Ji, F., 2015. *Bloodstain Patterns on Textile Surfaces: A Fundamental Analysis*.

Miller, R A. 2002. The Affects of Clothing on Human Decomposition: Implications for Estimating Time Since Death. University of Tennessee- Knoxville.

Misawa, N. and Blaser, M., 2000. Detection and characterization of autoagglutination activity by *Campylobacter jejuni*. *Infection and Immunity*, 68 (11), 6168-6175.

Mushtaq, S., Rasool, N. and Firiyal, S., 2015. Detection of dry bloodstains on different fabrics after washing with commercially available detergents. *Australian Journal of Forensic Sciences*, 48 (1), 87-94.

Müssig, J., 2010. *Industrial applications of natural fibres*. Chichester: John Wiley.

Ogdur, M., Cakan, H. and Cevik, F., 2018. Investigation of the microorganisms decaying bloody evidences. *Medicine Science | International Medical Journal*, 1.

ons.gov.uk., 2019. *Homicide in England and Wales - Office for National Statistics*. [online] Available from: https://www.ons.gov.uk/peoplepopulationandcommunity/crimeandjustice/articles/homicideinenglandandwales/yearendingmarch2018 [Accessed 15 April. 2019].

Orf, K. and Cunnington, A., 2015. Infection-related hemolysis and susceptibility to Gram-negative bacterial co-infection. *Frontiers in Microbiology*, 6.

Pager, C., 2000. Streptokinase versus alteplase and other treatments for acute and delayed thrombolysis of blood stains in clothing. *BMJ*, 321 (7276), 1554-1556.

Peschel, O., Kunz, S., Rothschild, M. and Mützel, E., 2010. Blood stain pattern analysis. *Forensic Science, Medicine, and Pathology*, 7(3), 257-270.

Pokupcic, K., 2017. Blood as an important tool in criminal investigation. *Journal of Forensic Sciences & Criminal Investigation*, 3 (2).

Rosling, A., Landeweert, R., Lindahl, B., Larsson, K., Kuyper, T., Taylor, A. and Finlay, R., 2003. Vertical distribution of ectomycorrhizal fungal taxa in a podzol soil profile. *New PHytologist*, 159 (3), 775-783.

Royal Horticultural Society, 2018. *Soil types*. [online] Available at https://www.rhs.org.uk/advice/profile?pid=179 [Accessed 13 January 2019].

Sanborn, P., Lamontagne, L. and Hendershot, W., 2011. Podzolic soils of Canada: Genesis, distribution, and classification. *Canadian Journal of Soil Science*, 91 (5), 843-880.

Slemko, J., 2017. Bloodstain tutorial, *properties of blood*, [online] forensic consulting INC Canada, available from: http://www.bloodspatter.com/bloodstain-tutorial-page-2.

Spohn, M., 2015. Microbial respiration per unit microbial biomass depends on litter layer carbon-to-nitrogen ratio. *Biogeosciences*, 12(3), 817-823.

Stott, D., Elliott, L., Papendick, R. and Campbell, G., 1986. Low temperature or low water potential effects on the microbial decomposition of wheat residue. *Soil Biology and Biochemistry*, 18 (6), 577-582.

Stuart, J., 1982. The function of red blood cells: Erythrocyte pathobiology. *Journal of Clinical Pathology*, 35 (1), 122-122.

Tambekar, D. and Gadakh, P., 2013. Biochemical and molecular detection of biosurfactant producing bacterial from soil. *International Journal of Life Sciences Biotechnology and PHarma Research*. 2 (1), 204-211.

Tibbett, M. and Carter, D., 2008. Cadaver Decompostion and Soil: Processes., *Chemical and biological effects of buried human remains*. Soil analysis in forensic tapHonomy. Boca Raton: CRC Press.

Tobe, S., Watson, N. and Daéid, N., 2007. Evaluation of six presumptive tests for blood, their specificity, sensitivity, and effect on high molecular-weight DNA. *Journal of Forensic Sciences*, 52(1), 102-109.

Tortora, G. and Derrickson, B., 2014. *Principles of anatomy and pHysiology*. Hoboken: Wiley.

ukso.org, 2019. *UK Soil Observatory*. [online] Available at: http://www.ukso.org/ [Accessed 23 March. 2019].

Vigneswaran, C., Kandhavadivu, P. and Ananthasubramanian, M., 2014. *Bioprocessing of textiles*. Bioprocessing of synthetic fibres, 189-250

Virkler, K., Lednev, I., 2009. Analysis of body fluids for forensic purposes: From laboratory testing to non-destructive rapid confirmatory identification at a crime scene. *Forensic Science International*, 188 (1-3), 1-17.

Wang, H., Ding, J., Lin, T. and Wang, X., 2010. Super water repellent fabrics produced by silica nanoparticle-containing coating. *Research Journal of Textile and Apparel*, 14 (2), 30-37.

Webb, J., Creamer, J. and Quickenden, T., 2006. A comparison of the presumptive luminol test for blood with four non-chemiluminescent forensic techniques. *Luminescence*, 21(4), 214-220.

Withington, C. and Sanford, R., 2007. Decomposition rates of buried substrates increase with altitude in the forest-alpine tundra ecotone. *Soil Biology and Biochemistry*, 39 (1), 68-75.

7 Appendices

Appendix A - SPSS Raw Data, Autumn

11/10/2018				25/10/2018		
Moisture content		Average		Moisture content		Average
T1	4.040			T1	27.602	
T2	8.153			T2	32.936	
T3	7.214			T3	32.382	
T4	6.415			T4	33.317	
T5	8.251	6.815		T5	32.745	31.796
B1	3.486			B1	28.368	
B2	4.305			B2	27.458	
B3	3.625			B3	29.249	
B4	4.753			B4	29.569	
B5	3.897	4.013		B5	29.520	28.833
pH				pH		
T1	5.455			T1	5.260	
T2	5.425			T2	5.225	
T3	5.354			T3	5.195	
T4	5.495			T4	5.305	
T5	5.595	5.465		T5	5.285	5.254
B1	5.485			B1	5.620	
B2	5.300			B2	5.645	
B3	5.625			B3	5.570	
B4	5.300			B4	5.490	
B5	5.440	5.430		B5	5.525	5.570
Bulk density				Bulk density		
T1	9.415			T1	9.169	
T2	9.277			T2	8.368	
T3	8.485			T3	8.821	
T4	10.021			T4	7.74	
T5	10.456	9.5308		T5	9.085	8.6366
B1	10.133			B1	9.751	
B2	10.671			B2	9.672	
B3	11.1			B3	9.381	
B4	10.666			B4	9.802	
B5	11.2	10.754		B5	9.496	9.6204

Microbial				Microbial		
T1	232.30			T1	142.12	
T2	291.05			T2	138.96	
T3	162.34			T3	267.34	
T4	292.37			T4	243.97	
T5	324.14	260.44		T5	162.06	190.89
B1	111.15			B1	64.71	
B2	229.95			B2	64.63	
B3	119.43			B3	76.45	
B4	101.89			B4	76.33	
B5	218.80	156.24		B5	67.43	69.91

Cotton CTCF			Cotton blend CTCF	
1	22228036.6		1	15570838.9
2	30652271.7		2	6700608.89
3	102069713		3	34859815.8
4	69654655.8		4	42689792.4
5	37155167.1		5	15506276.8
6	22498727.8		6	56361776.3
7	18368506.1		7	5741345.83
8	16542533.5		8	5801341.18
9	14245802.7		9	5647531.53
10	14870003.6		10	5573561.97
11	2931647.85		11	9478485.61
12	5879512.36		12	2808994.52
Polyester CTCF			Polyester blend CTCF	
1	2942820.79		1	8050024.48
2	30342067.2		2	27659838.2
3	43665626.1		3	17808454
4	21058825.7		4	12760262.6
5	19525306.1		5	2944529.79
6	33383067.7		6	8706742.69
7	6270635.33		7	6147229.87
8	24396280		8	4029333.2
9	8803478.45		9	9272395.78
10	50592803.6		10	12966267.6
11	10937237.4		11	4086969.46
12	8806209.27		12	8155457.2

Appendix B - SPSS Raw Data, Winter

07/01/2019				21/01/2019		
Moisture content		Average		Moisture content		Average
T1	13.218			T1	19.616	
T2	16.636			T2	20.372	
T3	15.960			T3	18.622	
T4	17.000			T4	19.900	
T5	20.156	16.594		T5	19.178	19.538
B1	12.529			B1	13.621	
B2	13.112			B2	14.516	
B3	13.478			B3	13.992	
B4	14.468			B4	15.413	
B5	12.898	13.297		B5	14.189	14.346
pH				pH		
T1	5.310			T1	5.270	
T2	5.265			T2	5.450	
T3	5.360			T3	5.435	
T4	5.220			T4	5.465	
T5	5.305	5.292		T5	5.055	5.335
B1	5.180			B1	5.475	
B2	5.350			B2	5.535	
B3	5.360			B3	5.575	
B4	5.350			B4	5.385	
B5	5.375	5.323		B5	5.495	5.493
Bulk density				Bulk density		
T1	9.586			T1	8.859	
T2	8.986			T2	8.573	
T3	8.430			T3	8.782	
T4	8.214			T4	8.780	
T5	7.217	8.487		T5	9.071	8.813
B1	8.403			B1	9.569	
B2	9.419			B2	8.807	
B3	8.363			B3	10.028	
B4	8.752			B4	9.248	
B5	8.973	8.782		B5	9.538	9.438

Microbial				Microbial		
T1	101.593			T1	166.101	
T2	94.335			T2	186.387	
T3	137.010			T3	170.697	
T4	143.012			T4	223.355	
T5	40.637	103.317		T5	167.454	182.798
B1	58.595			B1	74.108	
B2	47.436			B2	66.509	
B3	46.646			B3	56.046	
B4	56.648			B4	102.651	
B5	47.097	51.284		B5	75.232	74.909

Cotton CTCF			Cotton blend CTCF	
1	2166692		1	4785231
2	5252260		2	6761063
3	4908642		3	15069706
4	264461.6		4	6382081
5	4685087		5	15430578
6	5925463		6	12121207
7	4846669		7	10221462
8	229334.9		8	27727860
9	14694331		9	16852051
10	443828.6		10	8957124
11	848329.3		11	27345427
12	1777536		12	39404148
Polyester CTCF			Polyester blend CTCF	
1	9642500		1	7237490
2	812178.8		2	4008325
3	5996488		3	2038760
4	1644411		4	2544108
5	1049580		5	4445304
6	5017184		6	10649317
7	4621530		7	8655129
8	17195515		8	12439304
9	4716398		9	15777144
10	4091579		10	13505185
11	29132911		11	17283470
12	3410756		12	19553163

Appendix C - SPSS Raw Data, Spring

11/04/2019				25/04/2019		
Moisture content		Average		Moisture content		Average
T1	25.027			T1	14.193	
T2	13.850			T2	14.156	
T3	21.719			T3	15.062	
T4	25.889			T4	15.941	
T5	13.810	20.059		T5	13.422	14.555
B1	14.052			B1	14.119	
B2	13.066			B2	13.296	
B3	15.188			B3	12.541	
B4	24.893			B4	14.144	
B5	14.431	16.326		B5	14.628	13.745
pH				pH		
T1	5.360			T1	5.335	
T2	5.350			T2	5.330	
T3	5.240			T3	5.245	
T4	5.270			T4	5.180	
T5	4.945	5.233		T5	5.230	5.264
B1	5.300			B1	5.370	
B2	5.615			B2	5.515	
B3	5.280			B3	5.505	
B4	5.390			B4	5.415	
B5	5.510	5.419		B5	5.380	5.437
Bulk density				Bulk density		
T1	10.030			T1	10.702	
T2	10.502			T2	9.835	
T3	9.863			T3	10.365	
T4	10.063			T4	10.123	
T5	9.955	10.083		T5	9.721	10.149
B1	9.954			B1	10.019	
B2	10.134			B2	10.257	
B3	10.345			B3	10.293	
B4	9.893			B4	10.286	
B5	9.431	9.951		B5	9.785	10.128

Microbial				Microbial		
T1	212.809			T1	131.368	
T2	106.781			T2	122.157	
T3	204.586			T3	122.452	
T4	137.598			T4	167.776	
T5	169.447	166.244		T5	148.346	138.420
B1	118.899			B1	56.195	
B2	61.476			B2	56.413	
B3	104.466			B3	52.728	
B4	119.049			B4	76.178	
B5	103.379	101.454		B5	73.426	62.988

Cotton CTCF			Cotton blend CTCF	
1	17356244		1	15658595
2	13618010		2	8806184
3	11893343		3	2615595
4	41158974		4	17417108
5	7421174		5	3700359
6	38302826		6	3433508
7	8629673		7	10433128
8	29245749		8	6373651
9	1872318		9	1604576
10	6871969		10	10023157
11	15754906		11	3454547
12	25100052		12	4585949
Polyester CTCF			Polyester blend CTCF	
1	2118465		1	13829575
2	1943848		2	1215950
3	1950242		3	21860274
4	7642452		4	11664962
5	4718958		5	7317749
6	1348719		6	11040902
7	4389869		7	504258.6
8	1994790		8	788260.9
9	116953.1		9	-415294
10	793651.6		10	1190552
11	3725179		11	754311.7
12	1680236		12	622357.1

Appendix D - SPSS Raw Data, Summer

11/06/2019				25/06/2019		
Moisture content		Average		Moisture content		Average
T1	11.637			T1	25.109	
T2	10.033			T2	13.227	
T3	7.625			T3	12.616	
T4	10.672			T4	12.588	
T5	9.074	9.808		T5	12.549	15.218
B1	9.915			B1	9.716	
B2	10.092			B2	7.200	
B3	5.564			B3	5.945	
B4	10.525			B4	7.933	
B5	8.718	8.963		B5	8.114	7.782
pH				pH		
T1	5.035			T1	5.280	
T2	5.045			T2	5.190	
T3	5.045			T3	5.135	
T4	5.220			T4	5.305	
T5	4.910	5.051		T5	5.075	5.197
B1	5.270			B1	5.370	
B2	5.145			B2	5.400	
B3	5.185			B3	5.610	
B4	5.465			B4	5.520	
B5	5.335	5.280		B5	5.500	5.480
Bulk density				Bulk density		
T1	9.050			T1	10.485	
T2	10.279			T2	9.954	
T3	10.646			T3	10.637	
T4	10.415			T4	9.577	
T5	10.751	10.228		T5	10.136	10.158
B1	10.764			B1	10.920	
B2	10.337			B2	10.121	
B3	11.385			B3	11.059	
B4	10.514			B4	10.207	
B5	10.629	10.726		B5	11.300	10.721

Microbial				Microbial		
T1	273.136			T1	190.984	
T2	235.844			T2	145.820	
T3	339.431			T3	183.698	
T4	348.439			T4	210.587	
T5	374.390	314.248		T5	177.927	181.803
B1	89.343			B1	68.717	
B2	176.555			B2	75.146	
B3	150.448			B3	35.636	
B4	106.132			B4	152.624	
B5	92.590	123.013		B5	76.940	81.812

Cotton CTCF			Cotton blend CTCF	
1	6738161.225		1	6697488
2	11161479.28		2	3223084
3	31606952.68		3	5016532
4	6847296.698		4	444650.4
5	16465586.96		5	1174024
6	8897365.507		6	2319022
7	12025873.69		7	3213653
8	3567309.32		8	12284206
9	12474128.89		9	6647515
10	7106889.908		10	22065206
11	8368807.667		11	2511309
12	6490198.61		12	7683098
Polyester CTCF			Polyester blend CTCF	
1	7277482.719		1	22269218
2	10879680.88		2	33326813
3	2648899.523		3	14645901
4	7627488.996		4	12165888
5	7033378.242		5	15298532
6	6832992.264		6	12838551
7	1105151.608		7	3956757
8	464178.585		8	1630824
9	695502.777		9	3539746
10	1147463.585		10	4001514
11	460152		11	5180909
12	1411653.591		12	6257735

Appendix E - Rainfall Data

Autumn	Daily Total Rainfall (0900-0900)(mm)	Average
11/10/2018	3.8	
12/10/2018	0.8	
13/10/2018	25.4	
14/10/2018	2.4	
15/10/2018	0.2	
16/10/2018	1.2	
17/10/2018	1.8	
18/10/2018	0.0	
19/10/2018	0.0	
20/10/2018	0.2	
21/10/2018	0.0	
22/10/2018	0.0	
23/10/2018	0.0	
24/10/2018	0.2	
25/10/2018	0.0	2.4

Winter	Daily Total Rainfall (0900-0900)(mm)	Average
07/01/2019	Trace	
08/01/2019	0.0	
09/01/2019	0.2	
10/01/2019	Trace	
11/01/2019	Trace	
12/01/2019	Trace	
13/01/2019	Trace	
14/01/2019	0.0	
15/01/2019	3.6	
16/01/2019	3.8	
17/01/2019	0.0	
18/01/2019	5.8	
19/01/2019	7.0	
20/01/2019	0.0	
21/01/2019	6.4	3.0

Spring	Daily Total Rainfall (0900-0900)(mm)	Average
11/04/2019	0.0	
12/04/2019	0.0	
13/04/2019	0.0	
14/04/2019	0.0	
15/04/2019	0.8	

16/04/2019	0.4	
17/04/2019	0.0	
18/04/2019	0.0	
19/04/2019	0.0	
20/04/2019	0.0	
21/04/2019	0.0	
22/04/2019	0.0	
23/04/2019	Trace	
24/04/2019	10.4	
25/04/2019	0.0	0.8

Summer	Daily Total Rainfall (0900-0900)(mm)	Average
11/06/2019	1.4	
12/06/2019	13.4	
13/06/2019	2.2	
14/06/2019	Trace	
15/06/2019	1.8	
16/06/2019	0.0	
17/06/2019	0.4	
18/06/2019	2.2	
19/06/2019	1.6	
20/06/2019	Trace	
21/06/2019	0.0	
22/06/2019	0.0	
23/06/2019	n/a	
24/06/2019	0.0	
25/06/2019	0.4	2.0

Total seasonal average
1.9