A geophysical and biochemical investigation of buried remains in contrasting soil textures in southern Ontario

by

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Abstract

Ground penetrating radar (GPR) is a non-invasive, geophysical tool used for the detection of clandestine graves. GPR operates by detecting density differences in soil by the transmission of high frequency electromagnetic (EM) waves from an antenna. A 500 Megahertz (MHz) frequency antenna is typically used for forensic investigations, as it provides a suitable compromise between depth of penetration and sub-surface resolution. Domestic pig (Sus scrofa) carcasses were clothed in 100% cotton t-shirts and 50% cotton/50% polyester briefs, and buried at a consistent depth at three field sites of contrasting soil texture (silty clay loam, fine sand and fine sandy loam) in southern Ontario. GPR was used to detect and monitor the graves for a period of 14 months post burial. Analysis of collected data revealed that GPR had applicability in the detection of clandestine graves containing remains in silty clay loam and fine sandy loam soils, but was not suitable for detection in fine sandy soil. Specifically, within a fine sandy loam soil, there is the potential to estimate the post burial interval (PBI), as hyperbolic grave response was well defined at the beginning of the 14 month burial duration, but became less distinctive near the completion of the study.

Following the detection of a clandestine grave containing a carcass, collection of gravesoil, tissue and textile samples is important for the estimation of the stage of decomposition and the post burial interval (PBI) of the remains. Throughout the decomposition process of a carcass, adipose tissue is subjected to hydrolytic enzymes that convert triglycerides to their corresponding unsaturated, saturated and salts of fatty acids. The composition of fatty acids in the decomposed tissue will vary with the post mortem period, but it is unknown what affect the soil texture has on lipid degradation. As decomposition proceeds, fatty acids can leach from the tissues into the surrounding burial environment. Fatty acid analysis of gravesoil, tissue and textile samples, exhumed at two, eleven and fourteen month post burial intervals, was conducted using diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS), attenuated total reflectance – Fourier transform infrared (ATR-FTIR) spectroscopy and
gas chromatography – mass spectrometry (GC-MS). Infrared (IR) spectroscopy analysis of the samples provided a qualitative profile of lipid degradation. Analysis of gravesoil samples did not reveal IR spectroscopy bands attributable to fatty acid degradation or adipocere formation. IR spectroscopy analysis of tissue samples is applicable for the estimation of carcass decomposition in all of the soil textures tested. Results of textile IR spectroscopy analysis revealed limited potential to estimate the stage of carcass decomposition in silty clay loam soil. GC-MS was used to quantify the peak area ratio (area/int std area) (PAR) of myristic (C_{14:0}), palmitic (C_{16:0}), palmitoleic (C_{16:1}), stearic (C_{18:0}) and oleic (C_{18:1}) acids. GC-MS results revealed that analysis of both tissue and textile samples can be useful in the estimation of the stage of decomposition and the PBI of carcasses in all three of the soil textures tested.

The results of this research may have applicability within forensic investigations involving decomposing bodies by aiding in the location of clandestine graves in silty clay loam and fine sandy loam soil through the use of GPR. Infrared spectroscopy and GC-MS analysis of the fatty acid composition of tissue and textile samples may also be incorporated into investigational protocols to aid in the estimation of the stage of decomposition and the PBI of a body.

Key Words: forensic science, ground penetrating radar, soil texture, buried remains, fatty acids, gas chromatography – mass spectrometry (GC-MS), infrared spectroscopy
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° Degree
°C Degree Celsius
% Percent
ANOVA Analysis of Variance
ATR Attenuated Total Reflectance
BSTFA + TMCS N,O-Bis(trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane
cm Centimetre
$\text{cm}^{-1}$ Inverse Centimetre (Wavenumber)
DRIFTS Diffuse Reflectance Infrared Fourier Transform Spectroscopy
EI Electron Ionization
EM Electromagnetic
ft Feet
FTIR Fourier Transform Infrared
g Gram
GC-MS Gas Chromatography – Mass Spectrometry
GPR Ground Penetrating Radar
Int Std Internal Standard
KBr Potassium Bromide
LOD Limit of Detection
LOQ Limit of Quantitation
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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</thead>
<tbody>
<tr>
<td>LOS</td>
<td>Limit of Saturation</td>
</tr>
<tr>
<td>m</td>
<td>Metre</td>
</tr>
<tr>
<td>MHz</td>
<td>Megahertz</td>
</tr>
<tr>
<td>min</td>
<td>Minute</td>
</tr>
<tr>
<td>mL</td>
<td>Millilitre</td>
</tr>
<tr>
<td>mm</td>
<td>Millimetre</td>
</tr>
<tr>
<td>m/z</td>
<td>Mass to Charge Ratio</td>
</tr>
<tr>
<td>↑N</td>
<td>North</td>
</tr>
<tr>
<td>PAR</td>
<td>Peak Area Ratio</td>
</tr>
<tr>
<td>PBI</td>
<td>Post Burial Interval</td>
</tr>
<tr>
<td>PMI</td>
<td>Post Mortem Interval</td>
</tr>
<tr>
<td>rpm</td>
<td>Revolutions Per Minute</td>
</tr>
<tr>
<td>TIC</td>
<td>Total Ion Count</td>
</tr>
<tr>
<td>µg</td>
<td>Microgram</td>
</tr>
<tr>
<td>µL</td>
<td>Microlitre</td>
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Chapter 1 – Introduction
1.1 Introduction

The ability of forensic identification officers and crime scene workers to efficiently and accurately locate clandestine graves and analyze remains to estimate the post mortem interval (PMI) and/or post burial interval (PBI) is of central importance to the field of forensic science. Establishing the PMI and/or PBI can aid in the accurate identification of a decedent based upon missing person’s reports, or accept/reject a suspects alibi. Published research findings, which can rapidly and beneficially be translated into protocols within criminal investigations, are necessary to deal with the high volume of cases faced by the criminal justice system.

Techniques currently used to locate clandestine graves include non-invasive, observational methods, e.g. reduction in plant growth due to soil disturbance of a freshly dug grave (Rodriguez and Bass, 1985) and invasive methods, e.g. ground probing (Owsley, 1995; Schultz et al., 2006). However, due to the fact that existing methods do not determine with certainty if remains will be found, and destructive methods, e.g. digging, must be employed, non-invasive techniques are required. Ground penetrating radar (GPR) is a non-invasive, geophysical tool commonly used in the search for archaeological sites and utility lines (Miller, 1996; Neubauer, 2001). Schultz et al. (2006) demonstrated the applicability of GPR in the search for clandestine graves through controlled research using pig carcasses. The authors showed that GPR could be applied, with varying degrees of success, to the detection of carcasses in sandy soils and clay soils over a time period of 21.5 months (Schultz et al., 2006). Unfortunately, little is known regarding the use of GPR in other soil textures and to what degree of decomposition a carcass can be located and the PBI estimated accurately.

Once a clandestine grave has been located and the remains exhumed, biochemical analysis of the by-products of decomposition can lead to a more accurate estimation of PMI and/or PBI (Forbes et al., 2004). Previous studies have focused on the
quantification of fatty acid composition and adipocere formation based upon the rate of decomposition of remains and the burial environment (Forbes et al., 2003, 2005a-c; Notter et al., 2008, 2009; Nushida et al., 2008). Results of these studies have shown that the saturated fatty acids, e.g. myristic, palmitic and stearic acid, unsaturated fatty acids, e.g. palmitoleic and oleic acid, and minor components, e.g. 10-hydroxy stearic acid, can be detected in samples in trace quantities. However, current methods of analysis have either failed to reproduce reliable results, or are not cost or time efficient for a high through-put of samples (Notter et al., 2008). Therefore, optimization of existing methods and continued research into the effects of burial duration and conditions, rate of decomposition and soil texture are necessary to advance this area of forensic biochemistry and incorporate methods into medicolegal investigations.

In the current study, field experiments were carried out to investigate the applicability of using ground penetrating radar (GPR) to locate clandestine graves and to detect and quantify fatty acid composition of gravesoil, tissue and textile samples taken from carcasses based upon the post burial interval (PBI). Three field sites, of contrasting soil texture, located in southern Ontario were utilized to investigate the effect of soil texture on carcass decomposition.

1.2 Locating Clandestine Graves

The ability to determine the location of a grave and a buried carcass is of importance to forensic investigators, as discovery allows investigative efforts to move forward. Traditional methods of locating a potential clandestine grave site include observations of foliage, soil changes, and determination of soil conductivity, temperature and pH (Rodriguez and Bass, 1985; Ruffell et al., 2009). Abrupt changes in the foliage over a freshly dug grave are normally evident, as the disturbance of the soil reduces plant growth (Rodriguez and Bass, 1985). However, an older grave of approximately one year or more may have an increased amount of foliage over it and in
the surrounding area due to the organic materials that are released from a decomposed carcass into the soil (Rodriguez and Bass, 1985). Soil sinking or compaction as decomposition proceeds, such as when the chest cavity collapses, can also be apparent. Traditional methods used to locate a grave are presumptive and cannot determine with certainty if a carcass is located at that site.

1.2.1 Ground Penetrating Radar (GPR)

One method of locating buried carcasses is through the use of ground penetrating radar (GPR). GPR is a non-invasive, geophysical tool that can be used for the location of unexploded ordnance, buried utility lines, landfill debris, mineral resources and artifacts at prehistoric sites (Miller, 1996; Neubauer, 2001; Ruffell et al., 2009). Recent applications of GPR include locating clandestine graves (Miller, 1996; Ruffell, 2005; Ruffell and McKinley, 2008; Ruffell et al., 2009). Law enforcement search teams and military organizations have used GPR to search for buried organic remains (Ruffell, 2005; Ruffell et al., 2009). GPR is increasingly used in the search for forensic evidence because of its non-destructive nature and because it can be used in combination with other non-invasive methods to locate areas for further testing (Schultz et al., 2006). Other methods for locating clandestine graves and carcasses include; magnetometry, electrical resistivity, probing, cadaver dogs and geochemical sampling (Owsley, 1995; Nobes, 2000; Buck, 2003; Schultz et al., 2006; Ruffell et al., 2009). The applicability of GPR to forensic investigations involving homicide victims buried in clandestine graves was demonstrated by controlled research (Schultz et al., 2006; Schultz, 2008). This controlled research consisted of burying pig carcasses as human body analogs, and subsequently detecting and monitoring the carcasses for a period of time. However, research was only conducted in sandy and clay soils, and therefore, does not represent the wide variety of soil textures that may be encountered in forensic scenarios.
Ground penetrating radar has also proven useful in the search for historic burial grounds. Ruffell et al. (2009) used GPR for the location and assessment of an unmarked, historic burial ground in north-west Ireland, believed to contain decedents of the Great Famine of 1845-1851. Modern clandestine graves (less than tens of years) have a thermal signature and have a strong influence on surrounding vegetation (Ruffell et al., 2009). Alternatively, older clandestine burial sites (tens to hundreds of years) no longer give such clues, and therefore, GPR is a more practical option in these scenarios (Ruffell et al., 2009).

1.3 Process of Decomposition

Decomposition is a process that begins with the irreversible cessation of all vital functions, indicated by permanent stoppage of the heart, respiration and brain activity (Mish, 2005). The termination of heart function leads to a deprivation of oxygen to the tissues, followed by chemical disorganization, failure of the cellular metabolism and repair mechanisms, and gross tissue effects associated with the observable stages of decomposition (Gill-King, 1997). The chemical processes associated with the decomposition of soft tissue may require a time span of days to years to reach completion, and progress through a number of stages dependent upon the surrounding environment (Forbes, 2008).

1.3.1 Autolysis

Body composition is approximately 64% water, 20% protein, 10% fat, 1% carbohydrate and 5% minerals (Van Haaren, 1951; Dent et al., 2004). Autolysis (early stage decomposition) is the post mortem disintegration of cells and tissues in the body due to the destructive action of hydrolytic enzymes that denature molecules and cell membranes, resulting in cell detachment and the digestion of proteins, carbohydrates and lipids (Mello de Oliveira and Santos-Martin, 1995; Gill-King, 1997; Dent et al., 2004;
Macroscopic changes, such as skin slippage of the extremities and marbling, associated with autolysis become apparent within the first few days following death (Love and Marks, 2002; Forbes, 2008). Marbling under the skin is caused by the reaction of degenerated blood with hydrogen sulphide produced by bacteria, resulting in a black staining of blood vessels near the skin surface (Gill-King, 1997; Clark et al., 1997; Forbes, 2008).

### 1.3.2 Putrefaction

Putrefaction (later stage decomposition) is the bacterially induced breakdown of soft tissue and the conversion of protein, carbohydrate and lipid constituents (Dent et al., 2004; Forbes, 2008). It is initiated by autolytic processes and generally not observed until 48-72 hours post mortem (Dent et al., 2004; Prieto et al., 2004; Forbes, 2008). Aerobic organisms deplete tissues of oxygen and set up favourable conditions for the anaerobic microorganisms that decompose the remains through the putrefactive stages (Dent et al., 2004). Anaerobic organisms, e.g. *Bacteriodes*, *Clostridia* and *Streptococci* species, derived from the intestinal canal and surrounding soil, convert proteins, carbohydrates and lipids into acids and gases (Janaway, 1997; Gill-King, 1997; Dent et al., 2004; Forbes, 2008). Putrefactive macroscopic changes produced by bacteria include; bloating, colour changes and odours (Love and Marks, 2002; Forbes, 2008).

### 1.3.3 Protein Degradation

Proteins are degraded through the process of proteolysis, as a result of the action of enzymes (Sabucedo and Furton, 2003; Dent et al., 2004; Forbes, 2008). Proteolysis results in the breakdown of proteins to proteoses, peptones, polypeptides and amino acids (Forbes, 2008). Proteolysis is affected by temperature, moisture and bacterial activity (Sorimachi et al., 1996; Vass et al., 2002), and does not occur at a uniform rate throughout tissues (Dent et al., 2004; Forbes, 2008). Generally, the lining
membrane of the gastrointestinal tract, pancreatic epithelium, brain, liver and kidneys will degrade during the early post mortem interval (Gill-King, 1997; Dent et al., 2004; Forbes, 2008). Alternatively, reticulin, epidermis and muscle protein are more resistant to the effects of proteolysis and will remain intact for longer periods of time (Linch and Prahlow, 2001; Forbes, 2008). Keratin, an insoluble fibrous protein found in the skin, hair and nails, is resistant to most proteolytic enzymes, which is the reason it is often found intact, specifically in burial environments (Macko et al., 1999; Dent et al., 2004; Gupta and Ramnani, 2006; Forbes, 2008).

By-products that can form during proteolysis include; histamine, tryptamine and phenylethylamine (Oliver et al., 1977; Saccani et al., 2005), which are accompanied by the gases carbon dioxide and methane (Gill-King, 1997; Forbes, 2008). Amino acids that contain sulphur atoms can be reduced by desulphhydralation to produce ammonia, thiols, pyruvic acid, sulphides and hydrogen sulphide, the latter of which will produce a black precipitate with the reaction of iron from the body (Shelef and Tan, 1998; Dent et al., 2004; Knight and Presnell, 2005; Forbes, 2008). Should hydrogen sulphide react with haemoglobin, the green pigment sulph-haemoglobin will form (Clark et al., 1997; Forbes, 2008). The reaction of phenyl pyruvic acid with ferric iron in the soil environment will result in a green precipitate being formed in the tissues (Gill-King, 1997; Forbes, 2008).

In aerobic burial environments, sulphides are oxidized to sulphate by bacteria to produce sulphurous acids in the soil (Hartikainen et al., 2001; Forbes, 2008). The nitrogen component of proteins is liberated as ammonia (NH₃), which may be converted to ammonium (NH₄⁺) in a soil environment with low pH and utilized by surrounding vegetation (Shelef and Tan, 1998; Forbes, 2008). As a result, the vegetation growing on the burial surface may be increased, or have different fruiting and flowering patterns, when compared to the surrounding vegetation; a useful tool for locating burial sites (Hunter and Martin, 1996; Forbes, 2008). Ammonium ions not utilized by surrounding
vegetation can undergo nitrification in aerobic environments and denitrification in anaerobic environments (Forbes, 2008).

1.3.4 Carbohydrate Degradation

Carbohydrates within the soft tissue will degrade as decomposition proceeds. The degradation occurs during the early stages of decomposition, and may lead to sugars being completely oxidized to carbon dioxide and water, or incompletely decomposed to form organic acids and alcohols (Ellis and Wilson, 2003; Dent et al., 2004; Forbes, 2008). In an aerobic environment, fungi decompose sugars to produce glucuronic, citric and oxalic acids (Forbes, 2008). Those decomposed in an anaerobic environment by bacteria will produce lactic, butyric and acetic acids (Ueno et al., 2001; Forbes, 2008). These organic acids are responsible for the acidic environment sometimes created around a decomposing body (Forbes, 2008). Additional fermentative products include; acetone, methane, hydrogen and hydrogen sulphide gases, and alcohols, e.g. ethanol, the latter of which creates problems regarding the determination of antemortem blood alcohol concentrations (Collison, 2005; Ziavrou et al., 2005; Forbes, 2008).

1.3.5 Lipid Degradation

Adipose tissue is comprised of 60-85% lipids, of which comprise 90-99% triglycerides, 5-30% water and 2-3% proteins (Dent et al., 2004; Forbes, 2008). Triglyceride molecules, also referred to as triacylglycerols, consist of a glycerol esterified with three fatty acids (Notter et al., 2008). Decomposition results in triglyceride molecules being hydrolyzed by bacterial enzymes that cleave the fatty acids from the glycerol molecule to form a mixture of unsaturated and saturated free fatty acids (Forbes et al., 2004). The most common fatty acids contained within the human body are; stearic \((C_{18:0})\), oleic \((C_{18:1})\), linoleic \((C_{18:2})\), palmitic \((C_{16:0})\) and palmitoleic \((C_{16:1})\) acids
Hydrogenation then occurs, thereby increasing the amount of saturated fatty acids. Fatty acids may attach to sodium or potassium present in the interstitial fluid and cell wall, or to calcium or magnesium ions present within the soil environment to form salts of fatty acids (Gill-King, 1997; Dent et al., 2004; Forbes et al., 2004). Dependent upon the environment and the presence of microorganisms, hydroxy fatty acids, such as 10-hydroxystearic acid, may form (Forbes, et al., 2004). Under suitable conditions, the original adipose tissue is reduced to a mass of fatty acids, yielding adipocere.

1.3.6 Adipocere

Adipocere, commonly referred to as ‘grave wax’ or ‘corpse wax’ due to its grayish-white, paste-like appearance (Fiedler and Graw, 2003; Forbes et al., 2004, 2005c; Notter et al., 2008), is the result of unsaturated fatty acids forming their corresponding saturated fatty acids through hydrogenation by bacterial enzymes. The major components of adipocere are the saturated fatty acids; myristic, palmitic and stearic acids, and the unsaturated fatty acids; palmitoleic, and oleic acids (Forbes et al., 2003; Notter et al., 2008). Documented minor components include triglycerides, hydroxy fatty acids, such as 10-hydroxy stearic and 10-hydroxy palmitic acids, and salts of fatty acids, such as sodium, potassium, calcium and magnesium salts (Notter et al., 2008). The degradation of adipose tissue to unsaturated and saturated free fatty acids, and eventually to adipocere, may be useful as a method to aid in the estimation of the post burial interval (PBI) (Forbes et al., 2004). As decomposition proceeds, triglycerides are hydrolyzed into unsaturated and saturated fatty acids (Forbes et al., 2004). The concentration of unsaturated fatty acids will decrease during decomposition with a concomitant increase in saturated fatty acid concentration (Forbes et al., 2004). Adipocere formation may then occur depending on factors such as the burial environment and the method of burial. Adipocere is not an end product, and will eventually decompose. Although the factors necessary for the decomposition of
adipocere are not well documented, studies suggest that the presence of adipocere in a burial environment is related to the exclusion of gram positive bacteria, e.g. *Bacillus*, *Cellulomonas* and *Nocardia*, which are able to decompose adipocere (Pfeiffer et al., 1998; Forbes, 2008).

1.3.7 Liquefaction

Following the putrefaction stage of decomposition, remains will progress through the liquefaction stage to skeletonization. Liquefaction of tissues and organs is due to the action of digestive enzymes and results in a mass of putrefied tissue and frothy discoloured natural fluids, which purge from natural orifices due to increased gas pressure (Clark et al., 1997; Gill-King, 1997; Dent et al., 2004; Forbes, 2008). The result of this stage of decomposition is skeletonized remains held together by ligaments and surrounded by a putrescent or liquefying mass, which will eventually be incorporated into the surrounding soil environment and water systems (Dent et al., 2004; Forbes, 2008).

1.3.8 Skeletonization

Skeletonization is the removal of soft tissue from bone. In an aerobic environment, decomposition and skeletonization are rapid processes (Forbes, 2008), whereas, in an anaerobic environment, the process will take longer (Forbes, 2008). The organic collagen phase of bone is degraded by bacterial collagenases, which cause hydrolysis of the protein-mineral bond to peptides and their constituent amino acids (Nawrocki, 1995; Dent et al., 2004; Forbes, 2008). Collagen may also be degraded by the activity of gas-gangrene bacteria (*Clostridium* spp.) (Janaway, 1997; Dent et al., 2004). The inorganic phase of bone is also degraded as a result of the loss of mineral hydroxyapatite by chemical weathering, leading to weakened bone structure (Hare, 1976; Dent et al., 2004; Forbes, 2008). Bone is degraded by physical breaking,
decalcification and dissolution, and may retain its general shape and form, crumble and disintegrate, or become fossilized and preserved (Forbes, 2008).

1.4 Decomposition in Soil Environments

1.4.1 Soil Surface

A carcass that has been deposited onto the soil surface to decompose is subject to both intrinsic processes, e.g. autolysis, and the activity of non-cadaveric organisms (Carter and Tibbett, 2008a). Insects may arrive at a carcass immediately following death, and maggot activity can represent the primary driving force behind soft tissue removal (Mann et al., 1990; Carter and Tibbett, 2008a). Additionally, scavengers can consume up to 75% of a carcass in terrestrial ecosystems (DeVault et al., 2003), and up to 100% during winter months when insects and microbes are less active (Carter and Tibbett, 2008a). Other factors that can influence decomposition on the soil surface include; temperature, moisture, trauma and the presence of clothing (Carter and Tibbett, 2008a). Generally, an increase in temperature results in an increase in the rate of carcass decomposition, as higher temperatures are associated with increased biological activity (Mann et al., 1990; Rodriguez and Bass, 1985; Vass et al., 1992; Carter and Tibbett, 2006, 2008a). Moisture can delay the process of decomposition; dry environments, e.g. deserts, can promote desiccation (Galloway et al., 1989; Galloway, 1997) and wet environments can promote waterlogging and the formation of adipocere (Forbes, 2008; Carter and Tibbett, 2008a). The presence of trauma on a carcass can increase the decomposition rate, as insects are attracted to open wounds (Mann et al., 1990; Carter and Tibbett, 2008a). Clothing on an exposed carcass results in an increase in the rate of decomposition, as it provides a shaded area for maggots to feed (Mann et al., 1990; Carter and Tibbett, 2008a; Janaway, 2008).
1.4.2 Buried Carcasses

Carcasses buried in soil will demonstrate a slower rate of decomposition than carcasses deposited on the soil surface. The environment in which a carcass is buried is generally defined by the chemical, biological and geological conditions that exist at a location (Forbes et al., 2005a). Factors that affect decomposition within a burial situation include; the depth of the burial, the presence or absence of a coffin, clothing type, the physical composition of the body (Forbes et al., 2005a,b) and the physical conditions of the soil, such as texture, pH, moisture, temperature (Carter and Tibbett, 2008a) and oxygen content (Forbes et al., 2005a). Environmental conditions can produce varying levels of preservation, e.g. mummification within a hot, dry environment (Forbes et al., 2005b).

1.4.3 Restricted Insect and Animal Activity

The decomposition process of carcasses buried directly in soil is slowed, as burial restricts or eliminates access of most carrion insects and a large majority of carcass soft-tissue destruction is due to feeding by insect larvae (Rodriguez and Bass, 1985; Mann et al., 1990; Spennemann and Franke, 1995; Wells and Lamotte, 2001; Forbes, 2008). Burial also restricts the carcass to a reduced set of temperatures and soil provides an efficient insulation barrier to solar radiation, both of which can slow down the decomposition process (Rodriguez and Bass, 1985; Mann et al., 1990; Spennemann and Franke, 1995; Galloway et al., 2000; Forbes, 2008). Carcasses buried during the summer season show an increased rate of decomposition compared to carcasses buried during the winter season, thought to be due to higher temperatures and increased insect activity (Morovic-Budak, 1965; Spennemann and Franke, 1995). Burial can also protect a carcass from the effects of animal scavenging by restricting vertebrate access (Haglund, 1997).
1.4.4 Method of Burial

The method of burial will have an effect on the rate of decomposition and the formation of adipocere. Decomposition will proceed at a faster rate, and adipocere formation is more conducive, when a body is buried directly in soil, as opposed to in a coffin (Forbes et al., 2005a). The use of plastic coverings to wrap bodies before being placed into the soil results in liquefaction of fatty tissue and the inhibition of adipocere formation (Forbes et al., 2005a).

1.4.5 Clothing

The presence of clothing on a buried body will have an effect on the rate of decomposition, and the burial environment itself will alter the decomposition rates of the textiles (Janaway, 1987, 2002, 2008; Wilson et al., 2007). Cotton, a natural cellulose fibre, is currently the most widely used form of textile worldwide (Shepherd, 1969; Janaway, 2008). Cotton contains 90-99% cellulose, and therefore, is highly vulnerable to decomposition (Janaway, 2008). For example, in a shallow burial in damp, aerated and biologically active soil, undyed cotton may completely decay within a period of months (Janaway, 2008). However, commercial finishes applied to inhibit cotton mildew attack can increase its resiliency and delay the process of decomposition. Under most soil burial conditions, cotton will degrade rapidly, as cellulose is the most abundant biomolecule on the planet, with a range of decomposer organisms capable of utilizing it as a carbon source (Janaway, 2008). However, extended preservation of cotton is associated with desiccation (desert soils) and freezing (permafrost) (Betts et al., 1994; Janaway, 2002, 2008).

In addition to pure cotton textiles, blends consisting of cotton and polyester are common. Polyester, a synthetic textile fibre, was developed during the 1940s, and consists of long-chain synthetic polymers composed of at least 85% by weight of an
ester of dihydric alcohol and terephthalic acid (P-HOOC-C₆H₄-COOH) (Janaway, 2008). Cotton – polyester blend textile has a low level of water absorbency, is not attacked by moth larvae, is resistant to microbial activity, and therefore, shows considerable resistance to degradation based upon the soil environment (Janaway, 2008).

The presence of clothing will retard decomposition in areas where the body is covered (Galloway et al., 1989; Komar, 1998; Weitzel, 2005; Forbes, 2008). Clothing serves to inhibit the effects that the surrounding soil environment has on decomposition, as well as to encourage adipocere formation (Galloway et al., 1989; Forbes, 2008). Overall, a clothed body will be preserved more so than an unclothed body (Forbes, 2008).

**1.4.6 Body Composition**

Body composition, physically thin or emaciated, or well-nourished, will alter the rate of decomposition. A body composition which is physically thin or emaciated at the time of death will decompose and skeletonize rapidly (Mant, 1987; Forbes, 2008). A well-nourished composition at the time of death will more readily result in a delayed rate of decomposition due to the formation of adipocere (Nawrocki, 1995; Forbes, 2008).

**1.4.7 Soil Characteristics and Depth of Burial**

Soil texture and depth of burial are also determining factors in the rate of decomposition of carcasses buried in soil. Soil texture affects water permeability and air exchange within the soil (Forbes, 2008). Finer textured soils, such as clay, will retain moisture and slow the rate of decomposition of a carcass, as opposed to more permeable soils, such as sand (Bethell and Carver, 1987; Hopkins et al., 2000; Santarsiero et al., 2000; Forbes, 2008; Carter et al., 2010). The depth of burial impacts
the moisture content within the burial environment, as deeper burials generally exhibit more moisture due to lower evaporation and their closer proximity to the water table (Forbes, 2008). Excess moisture within a burial environment, leading to retardation of decomposition, can be linked to the formation of adipocere (Fiedler et al., 2004; Forbes et al., 2005b; Forbes, 2008).

1.4.8 Soil pH

Overall, soil pH is less influential on the rate of decomposition of a carcass than soil texture or depth of burial, with the exception of highly acidic soils (Forbes, 2008). Soft tissue and bone can be reduced to a dark silhouette of the former body shape, known as pseudomorphs or burial silhouettes, in highly acidic sandy and gravelly soils (Bethell and Carver, 1987; Bethell and Smith, 1989; Hunter and Dockrill, 1997; Forbes, 2008).

1.5 Current Knowledge

1.5.1 Ground Penetrating Radar (GPR)

Soil and environmental conditions can enhance, limit, or impair ground penetrating radar (GPR) performance. Research has shown that GPR yields reliable results in sandy soils (Schultz et al., 2006), permafrost (Davis et al., 2000), glaciers and concrete/pavement (Ruffell et al., 2009). In contrast, GPR is associated with difficulties in clay soils (Schultz et al., 2006; Schultz, 2008) and after periods of heavy rain (Ruffell et al., 2009). Schultz et al. (2006) found that pig carcasses buried in sandy soils could be detected using GPR for 21.5 months, while exhibiting variable decomposition stages, including complete skeletonization. However, anomalies in the sand were produced, as there was a weak contrast between the skeleton and the surrounding soil (Schultz et al., 2006). Clay resulted in difficulties imaging the carcasses during the later stages
decomposition. During the first six months of burial, the graves and carcasses were generally detectable (Schultz et al., 2006). However, as the disturbed ground became more compact over the duration of the study, the response became increasingly difficult to interpret, even though the carcasses had undergone little decomposition (Schultz et al., 2006). Despite the fact that carcasses buried in clay were difficult to detect, Schultz et al. (2006) found that it was possible to image disruptions or breaks in the clay horizon that were the result of soil disturbance from the presence of the grave and carcass. However, the authors note that detecting clandestine graves based solely on soil features may not be possible, as the response from the disturbed soil of the grave will be reduced over time (Schultz et al., 2006).

Ruffell et al. (2009) used GPR for the location and assessment of an unmarked, historic burial ground in north-west Ireland believed to contain decedents of the Great Famine of 1845-1851. Area soils comprised post-glacial sands, glacial till and Carboniferous sandstones (Ruffell et al., 2009). Prior to GPR use, 84 possible burials were located based upon historical records, aerial photographs and landscape interpretation (Ruffell et al., 2009). The target area (area of suspected burials) was analyzed using GPR and three different antenna frequencies; 100, 200 and 400 MHz (Ruffell et al., 2009). After data interpretation, it was determined that the 400 MHz antenna centre frequency was the most appropriate antenna to use, as the location of over 300 possible burials were obtained using this antenna. Alternatively, the 100 MHz antenna gave only an indication of some possible burials, whereas the 200 MHz antenna only detected 210 possible burials (Ruffell et al., 2009).

**1.5.2 Fatty Acid Analysis**

Analysis of fatty acid composition by infrared (IR) spectroscopy and gas chromatography – mass spectrometry (GC-MS) can aid in the determination of the post mortem interval (PMI) and/or the post burial interval (PBI). Research has focused on the
qualitative and quantitative analysis of fatty acid composition and adipocere formation based upon the rate of decomposition of remains, burial environment and conditions, e.g. presence of clothing, and sample type (Forbes et al., 2003, 2005a-c; Notter et al., 2008, 2009; Nushida et al., 2008). Although it was once believed that adipocere formation required the presence of excess moisture, such as aquatic environments, research has determined that there is sufficient moisture within adipose tissue to result in the formation of adipocere in most environments (Forbes et al., 2003). Nushida et al. (2008) found that adipocere formed on a body that had been kept in a water-tight container for four years.

1.5.3 Gravesoil Sample Analysis

A study conducted by Stuart et al. (2000) used diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) to qualitatively determine the fatty acid composition of gravesoil samples that contained adipocere taken from three cemetery burials and one forensic burial. Examination of the sample spectra indicated that fatty acids, hydroxy fatty acids, triglycerides and calcium salts of fatty acids were present, and that the forensic sample (27 month old sample) contained a similar fatty acid composition to a cemetery sample (26 year old sample) (Stuart et al., 2000). However, this may be attributed to the fact that the forensic sample was taken from a site that consisted of calcrete; a soil rich in calcium carbonate, thereby leading to the formation of calcium salts of fatty acids in the adipocere (Stuart et al., 2000).

More recently, Forbes et al. (2003) developed a GC-MS method for the detection of adipocere in gravesoils. This method has a detection limit of 0.8 ppm. Precision for the fatty acids for five spiked samples was within 4% (relative standard deviation), and recovery of fatty acids from spiked soil samples ranged from 68-96%. The method developed was successfully applied to the investigation of gravesoil samples containing
trace levels of adipocere, including the identification of myristic, palmitic, stearic, oleic and 10-hydroxystearic acids (Forbes et al., 2003).

1.5.4 Tissue Sample Analysis

In order to qualitatively analyze tissue samples, Stuart et al. (2005) investigated the applicability of using attenuated total reflectance – Fourier transform infrared (ATR-FTIR) spectroscopy, as this method requires only minimal sample preparation. Pig adipose tissue was placed directly in soil and also in a mock coffin environment; adipocere that formed was then extracted and cast as a film for sample analysis (Stuart et al., 2005). The adipocere that was formed from the tissue buried directly in soil showed bands which were distinctive of later stage decomposition, such as strong bands attributed to fatty acid calcium salt carboxylate C-O stretching (Stuart et al., 2005).

Despite the successful application of the GC-MS method developed by Forbes et al. (2003) for the identification of trace levels of adipocere in gravesoils, results could not be replicated by other researchers when attempting to analyze different sample types, e.g. tissue. Notter et al. (2008) attempted to reproduce the method using wet, macroscopic adipocere formed in aquatic environments from tissue samples, but failed to produce reliable results. The lack of reproducibility may have been attributed to the complexity of the soil matrix (Notter et al., 2008). To increase the efficiency of the method, Notter et al. (2008) incorporated additional steps, such as an initial lipid extraction. Spiked adipocere samples were analyzed using this method, and were found to give recoveries of greater than 90% to the standard lipid mixture used (Notter et al., 2008).
1.5.5 Textile Sample Analysis

Although various forms of textile, e.g. cotton and cotton – polyester blends, have been studied in terms of textile rate of degradation and their influence on the rate of decomposition of a body (Galloway et al., 1989; Komar, 1998; Weitzel, 2005; Forbes, 2008; Janaway, 2008), little published literature is available regarding the analysis of fatty acids and adipocere formation on clothing. Murthy et al. (1985) investigated the use of ATR-FTIR spectroscopy to analyze triglycerides that had been deposited onto cotton fabric. The triglyceride deposited fabric, cut to 1 x 5 cm pieces, was analyzed directly on the ATR crystal at 80 ozin torque (Murthy et al., 1985). However, it was found that the addition of the triglycerides resulted in fluid that flowed towards the ATR crystal under pressure and complicated the analysis. Furthermore, often widely differing absorbances were obtained when surfaces of the same piece of cotton fabric were pressed against the ATR crystal, indicating non-uniform triglyceride distribution on the fabric surface (Murthy et al., 1985). As such, this area of research requires more attention in order to determine if fatty acid analysis and adipocere formation on textiles can beneficially aid in the estimation of PMI and/or PBI.

1.6 Thesis Objectives

The current study involved the burial of domestic pig carcasses (Sus scrofa) (Linnaeus, 1758) in contrasting soil textures (silty clay loam, fine sand and fine sandy loam) at three field sites in southern Ontario. Ground penetrating radar (GPR) was used to detect the graves based upon the post burial interval (PBI). Subsequent to exhumation, samples of gravesoil, tissue and textile were analyzed qualitatively and quantitatively for fatty acid composition using infrared (IR) spectroscopy and gas chromatography – mass spectrometry (GC-MS), respectively.
Chapter 2 – Materials and Methods
2.1 Introduction

Field experiments, which consisted of burying, and subsequently exhuming, domestic pig (*Sus scrofa*) carcasses in contrasting soil textures, were conducted over a 14 month period. Ground penetrating radar (GPR) was employed in order to determine carcass and grave response. The fatty acid composition of gravesoil, tissue and textile samples was analyzed qualitatively and quantitatively. Both GPR and fatty acid analysis were used as a means of estimating the stage of decomposition and the post burial interval (PBI) of the carcasses.

2.2 Site Locations

Three field site locations within southern Ontario, Canada were selected for the study; ‘Nashville’, a grazing field located in Nobleton, ON, ‘Springwater’, a commercial gravel pit located in Springwater, ON, and ‘Dummer’, a grazing field located in Douro-Dummer Township, ON (Figure 2.1), for GPR data collection and burial of domestic pig (*Sus scrofa*) carcasses based upon soil texture. The domestic pig (*Sus scrofa*) is commonly used as a model for humans in research involving forensic practices (Schoenly et al., 2006; Notter et al., 2009). This is due to the ethical restrictions of using human bodies for research (Notter et al., 2009), their similar internal anatomy, fat distribution, size of chest cavity, lack of heavy fur, and omnivorous diet, suggesting a similar gut fauna (Schoenly et al., 2006).
Figure 2.1 – Map of Ontario showing the three field sites. Circles represent: ● Nashville (Nobleton, ON), ▲ Springwater (Springwater, ON) and ★ Dummer (Douro-Dummer Township, ON).

2.2.1 Nashville

The Nashville field site (Figure 2.2) was located in Nobleton, Ontario, Canada (43° 54’ 08” N, 79° 41’ 10” W); an unincorporated village in south-western King Township, within the Regional Municipality of York. York lies in south-central Ontario and is bounded by Lake Simcoe and Simcoe County to the north, Ontario County to the east, Lake Ontario to the south and Peel County to the west (Hoffman and Richards, 1955). Total land area within the municipality is 564,480 acres (Hoffman and Richards, 1955).

Underlying bedrock formations within York include; the Trenton (limestone), the Billings (shale), the Dundas (shale and limestone) and the Meaford (shale, limestone,
calcareous sandstone and arenaceous shale) (Hoffman and Richards, 1955). Clay loam and clay textured tills occur within King Township, and glacial deposits exist that consist of a poorly sorted mixture of sand (dominant component), gravel and till (Hoffman and Richards, 1955). Drainage within King Township is by the Rouge, Don and Humber Rivers and Highland and Etobicoke Creeks, all of which empty into Lake Ontario (Hoffman and Richards, 1955). Both internal and external soil drainage is good, and erosion is moderate to severe (Hoffman and Richards, 1955). The vegetation predominately includes red maple (Acer rubrum), silver maple (Acer saccharinum), sugar maple (Acer saccharum), white spruce (Picea glauca), black ash (Fraxinus nigra) and white ash (Fraxinus americana) (Hoffman and Richards, 1995).

The Regional Municipality of York annual temperature ranges from -32.8°C to 40.6°C, with a daily mean temperature of 9.2°C (Environment Canada). Average annual rainfall is 709.8 mm, with 834 mm of precipitation and 133.1 cm of snowfall (Environment Canada).
2.2.2 Springwater

The Springwater field site (Figure 2.3) was located in Springwater, Ontario, Canada (44° 22’ 48” N, 79° 45’ 80” W); a Township in Simcoe County. Located in the north-western part of south-central Ontario, with a total land area of 1,064,320 acres, Simcoe County is one of the largest counties in southern Ontario (Hoffman et al., 1962).

The soils of Simcoe County are underlain by rocks of the Ordovician, Silurian and Precambrian ages (Hoffman et al., 1962). Also present are limestones of the Black River, Trenton, Medina, Cataract and Lockport formations, and shales of the Utica, Queenston and Richmond formations (Hoffman et al., 1962). The till is generally coarse textured and consists of a mixture of boulders, cobbles, gravel, sand, silt and clay (Hoffman et al., 1962). The Nottawasaga River drains most of the County and empties into Georgian Bay at Wasaga Beach (Hoffman et al., 1962). The most commonly occurring species within Simcoe are the sugar maple (*Acer saccharum*), red maple (*Acer rubrum*), American basswood (*Tilia Americana*), yellow birch (*Betula alleghaniensis*), red oak (*Quercus rubra*), white oak (*Quercus alba*), white ash (*Fraxinus americana*), black ash (*Fraxinus nigra*) and white birch (*Betula pendula*) (Hoffman et al., 1962).

Simcoe County annual temperature ranges from -35°C to 36°C, with a daily mean temperature of 6.7°C (Environment Canada). Average annual rainfall is 700.2 mm, with 938.5 mm of precipitation and 238.4 cm of snowfall (Environment Canada).
2.2.3 Dummer

The Dummer field site (Figure 2.4) was located in Douro-Dummer Township, Ontario, Canada (44° 18’ 00” N, 78° 19’ 00” W) within Peterborough County. Peterborough County is bordered by Haliburton County to the north, Hastings County to the east, Rice Lake and the Trent River to the south and Victoria County to the west (Gillespie and Acton, 1981). Total land area is 895,114 acres (Gillespie and Acton, 1981).

Precambrian bedrock formations in the area comprise granites, marble and amphibolites (Gillespie and Acton, 1981). There are two limestone formations of the Ordovician age; Black River and Trenton (Gillespie and Acton, 1981). Soils of the area are developed on shallow deposits of very stony, calcareous, recessional moraine till overlying limestone bedrock, with the predominant soil texture being sandy loam (Gillespie and Acton, 1981). Topography is irregular gently sloping and the soil drainage within Peterborough County is good to excessive. The entire drainage system is part of the Trent River system; a chain of lakes and rivers that transverse through the county.
and are connected by the Otonabee River (Gillespie and Acton, 1981). The vegetation includes tamarack (*Larix laricina*), white spruce (*Picea glauca*), yellow poplar (*Liriodendron tulipifera*), northern white cedar (*Thuja occidentalis*) and white pine (*Pinus strobes*) (Gillespie and Acton, 1981).

Peterborough County annual temperature ranges from -35.5°C to 36.5°C, with a daily mean temperature of 6.6°C (Environment Canada). Average annual rainfall is 715.3 mm, with 869.6 mm of precipitation and 165 cm of snowfall (Environment Canada).

Figure 2.4 – Dummer field site

### 2.3 Burial Parameters

A total of 45 pig carcasses were buried across the three field sites. Burial formations were in the shape of a cross (Figures 2.5 and 2.6), with the exception of the Springwater site. This grave arrangement was used for ease of data collection, as a
fellow researcher was working with magnetometry. At the Springwater site, burials were arranged in two parallel lines (Figure 2.7), due to a space constraint and potential safety hazards to researchers. Burial occurred on August 11, 2008. Pig carcasses were purchased from a dead stock company, and were sacrificed according to industry standards (head bolt) (Olfert et al., 1993). Carcasses were buried within a matter of hours after death. Each site consisted of 15 graves, each containing a carcass and 5 control graves, to establish a baseline for comparison to the decomposition process of pig carcasses (a total of 20 graves per site).

![Figure 2.5 – Nashville field site burial arrangement schematic](image-url)
Figure 2.6 – Dummer field site burial arrangement schematic

Figure 2.7 – Springwater field site burial arrangement schematic
To more closely represent forensic situations, the carcasses were buried at a depth of approximately 0.76 m (2.5 ft) in 100% cotton t-shirts and 50% cotton/50% polyester briefs (Figure 2.8), which are representative of common textiles. The control graves also contained the t-shirts and briefs (Figure 2.9), in order to determine the rate of decomposition of the fibres based upon the soil texture and microbial environment.

Figure 2.8 – Springwater experimental grave 7; pig carcass burial in 100% cotton t-shirt and 50% cotton/50% polyester briefs
2.4 Exhumation Timeline

The study was conducted over a 14 month period; from August 11, 2008 until October 23, 2009. Exhumations were performed at designated intervals; two months, 12 months and 14 months post burial, which were dependent upon the climate of south-central Ontario. This climate experiences temperatures ranging from -35°C in the winter to 40.6°C in the summer (Environment Canada). The Nashville site was exhumed on October 15, 2008 (two months/66 days post burial), July 22, 2009 (12 months/346 days post burial) and October 16, 2009 (14 months/432 days post burial). The Springwater site was exhumed on the same days as Nashville. The Dummer site was exhumed on October 16, 2008 (two months/67 days post burial), July 23, 2009 (12 months/347 days post burial) and October 23, 2009 (14 months/439 days post burial). During each exhumation, four randomly selected graves were exhumed; one control grave and three graves containing a pig carcass.
2.5 Sample Collection and Storage

Gravesoil, tissue and textile samples were collected during each exhumation for qualitative and quantitative analysis of fatty acid composition using infrared (IR) spectroscopy and gas chromatography – mass spectrometry (GC-MS), respectively. A total of three soil samples were collected from each grave following exhumation; directly beneath the head, torso and rear regions of the carcass. A total of three tissue samples were collected from the head, torso and rear regions of each pig carcass. The t-shirt and briefs from each grave were collected in whole. The gravesoil samples were collected using a trowel, manually cleaned and then sterilized with methanol between samples, and placed into individually labelled plastic bags. The tissue samples were collected using meat scissors, manually cleaned and then sterilized with methanol between samples, and placed into individually labelled glass vials. The t-shirts and briefs were collected and placed into individually labelled plastic bags.

Upon return to the laboratory, samples were stored in the freezer until extraction and analysis by IR spectroscopy and GC-MS. However, to impede bacterial and fungal growth, the textile samples were air-dried in a fume hood, and any adhering tissue, soil or hair was removed. The cleaned textile samples were then stored in paper bags at -20°C.
Chapter 3 – Soil Characterization and Stages of Carcass Decomposition
3.1 Soil Characterization

Analysis of control soil samples collected from each site to determine soil texture and electrical conductivity was performed by the University of Guelph Laboratory Services – Agriculture and Food Laboratory on February 5, 2010.

3.1.1 Nashville

The soil at the Nashville site was determined to be silty clay loam, with the following components; gravel 0.0%, sand 19.1%, very fine sand 8.6%, fine sand 6.9%, medium sand 2.3%, coarse sand 0.7%, very coarse sand 0.1%, silt 53.4% and clay 27.5%.

3.1.2 Springwater

The soil at the Springwater site was determined to be fine sand, with the following components; gravel 0.0%, sand 97.6%, very fine sand 4.4%, fine sand 61.5%, medium sand 19.4%, coarse sand 9.0%, very coarse sand 2.7%, silt 1.2% and clay 1.2%.

3.1.3 Dummer

The soil at the Dummer site was determined to be fine sandy loam, with the following components; gravel 0.0%, sand 59.8%, very fine sand 17.4%, fine sand 21.3%, medium sand 11.7%, coarse sand 6.5%, very coarse sand 2.2%, silt 35.2% and clay 4.9%.
3.2 Stages of Carcass Decomposition

During each of the three exhumation periods, the pig carcasses were visually assigned to a decomposition stage for comparison to fatty acid analysis results obtained by infrared (IR) spectroscopy and gas chromatography – mass spectrometry (GC-MS). Figure 3.1a-c illustrates representative carcasses exhumed from the Dummer site. The carcasses exhumed from the Dummer site were representative of the stage of decomposition of the carcasses exhumed from both the Nashville and Springwater sites.

Figure 3.1a illustrates a representative carcass exhumed from the Dummer site during the October 16, 2008 (67 days PBI) exhumation period. The carcasses were considered to be in early stage decomposition (autolysis). The carcasses were still generally intact with some discolouration of the skin surface.

Carcasses exhumed from the Dummer site during the July 23, 2009 (347 days PBI) exhumation are represented in Figure 3.1b. The carcasses had progressed to later stage decomposition (putrefaction). Soft tissue had begun to degrade, colour changes were apparent and a strong odour emanated from the carcasses.

During the final exhumation period at the Dummer site, October 23, 2009 (439 days PBI), the carcasses were still in later stage decomposition (putrefaction). Figure 3.1c is representative of the carcasses exhumed during this exhumation period. Evidence of liquefaction or skeletonization of the carcasses was not present. Note that during the exhumation, there was torrential downpour in the area. The carcass pictured appears to be quite intact as a result of the wet soil.
Figure 3.1a-c – Representative domestic pig (*Sus scrofa*) carcasses exhumed from the Dummer site indicating stage of decomposition (a) October 16, 2008 (67 days PBI) exhumation; early stage decomposition (autolysis), (b) July 23, 2009 (347 days PBI) exhumation; later stage decomposition (putrefaction) and (c) October 23, 2009 (439 days PBI) exhumation; later stage decomposition (putrefaction).
Chapter 4 – Ground Penetrating Radar (GPR)
4.1 Introduction

Ground penetrating radar (GPR) is a non-invasive, geophysical tool used in the search for archaeological sites and buried utility lines (Miller, 1996; Neubauer, 2001). More recently, the use of GPR to search for clandestine graves has become apparent through controlled research in which pig carcasses were used as human body analogs (Schultz et al., 2006; Schultz, 2008). Published literature has investigated the use of GPR in sandy and clayey soils in open fields with no trees or bushes in the area, flat topography and excellent drainage (Schultz et al., 2006; Schultz, 2008). As forensic scenarios can exhibit many different soil textures and landscape parameters, further controlled research in a variety of settings is necessary to determine to what degree of decomposition a carcass can be located and the post burial interval (PBI) estimated accurately.

4.1.1 Operational Parameters

The underlying physics of GPR consist of the transmission and reflection of high-frequency electromagnetic (EM) waves into the ground from an antenna, and reflection back to the surface and detection by the receiving antenna (Ruffell, 2005; Ruffell and McKinley, 2008; Ruffell et al., 2009). The antenna transmits the EM waves, which are reflected when changes in the electrical properties of the ground are detected, such as the difference between soil texture and the presence of organic matter (Davis et al., 2000). Common GPR models use antennas of 300, 500 or 900 Megahertz (MHz) centre frequency (Miller, 1996; Davis, 2000; Schultz et al., 2006). A short pulse antenna (900 MHz) is effective with near-surface targets, such as buried ordnance (Miller, 1996). A 500 MHz antenna is useful for depth investigations of 0.5 to 3.5 m, which includes most of the items of interest in a forensic investigation (Miller, 1996). A long pulse antenna (300 MHz) is effective for sub-surface imaging of depths greater than 3.5 and up to 9.0 m, such as water-tables (Miller, 1996; Davis et al., 2000). Overall, a decrease in antenna
frequency will increase the depth of investigation, while decreasing the vertical resolution of the subsurface (Schultz et al., 2006). Alternatively, an increase in antenna frequency will decrease the depth of investigation, while increasing the resolution of subsurface objects (Schultz et al., 2006). An antenna in the frequency range of 500 MHz is ideally suited to forensic investigations, as it provides a suitable compromise between depth of penetration and resolution of subsurface features.

GPR equipment is used by passing the antenna over the ground surface, generally using a cart (Figure 4.1), while it emits continuous EM pulses of short duration (nanoseconds) downward and at a 45° conical angle into the ground (Miller, 1996; Schultz et al., 2006). The EM wave is reflected and refracted when it encounters areas of contrasting properties, and a cross-sectional picture of the subsurface is generated from the composite of the reflected waves (Schultz et al., 2006; Ruffell et al., 2009). The resulting data collected is stored and displayed digitally on a two-dimensional monitor connected to the antenna. Due to the fact that the EM waves pulse at a 45° conical angle into the ground, a detected object will appear as a stack of hyperbolas, or arc-like reflections, with the target object located at the top point of the shallowest curve (Ruffell et al., 2009). Environmental and equipment anomalies are commonly associated with GPR use due to the fact that the radar can reflect off surrounding objects, such as large rocks, and reverberate off the antenna. These anomalies are referred to as artificial reflections from ringing (Schultz, 2008), and result in a series of horizontal lines on the display screen of the GPR. However, as long as the antenna is calibrated before each use, the artificial reflections will not hinder the detection of a grave or carcass.
4.1.2 Operator Experience

Controlled forensic studies using GPR provide training for operators, determination of soil and environmental conditions that are applicable to the use of the radar and detection of burial duration. Operator experience can be a limiting factor of GPR use in a forensic setting, and therefore, research conducted in a known setting is necessary to interpret the data collected during a criminal investigation (Schultz et al., 2006). However, the difference in geology, soil texture, vegetation, ground water and human interference between controlled research settings and suspected crime scenes can, despite operator experience, result in difficulties in the efficiency of GPR use (Ruffell et al., 2009). The way in which the GPR is used and the data collected can also have an impact on the ability to locate a buried body. Experienced GPR operators may overlook a body when conducting a survey if transects are not collected over a grid or line pattern that utilizes appropriate spacing (Schultz et al., 2006). Davis et al. (2000) and Neubauer (2001) suggest applying archaeological GPR parameters to forensic cases.
by using transects separated by 0.5 m or less. The use of control graves, which consist of only disturbed backfill, are also important to demonstrate that hyperbolic anomalies are primarily the result of a decomposing carcass or skeleton.

4.2 Materials and Methods

In the current study, domestic pig (*Sus scrofa*) carcasses were clothed in 100% cotton t-shirts and 50% cotton/50% polyester briefs prior to burial in three contrasting soil textures. Ground penetrating radar (GPR) data was collected over a 14 month period. The applicability of using GPR to locate clandestine graves and estimate the post burial interval (PBI) was investigated.

4.2.1 Ground Penetrating Radar (GPR) Unit

A Sensors & Software Inc. Noggin Plus 500 (Mississauga, Canada) ground penetrating radar antenna was used for surveying the graves. A SmartCart configuration was used to allow for quick and efficient coverage of the sites. A Digital Video Logger (DVL) was used in the field as a guide for line tracking, to provide real-time display and record data. All data was stored onto a SanDisk Extreme III 1.0 GB CompactFlash, and downloaded to a computer.

4.2.2 Data Collection

GPR data was collected during the following months; August, September and October of 2008, and July, August, September and October of 2009. However, due to heavy rain in the area, GPR data was not collected from the Nashville site for the month of July, 2009.
A line survey pattern was used at the Nashville site (Figure 4.2), due to the rough terrain. The use of the line pattern adhered to procedures used by forensic identification officers (Ruffell and McKinley, 2008). A grid pattern was used at the Springwater and Dummer sites (Figure 4.3), for greater detail. The use of the grid pattern adhered to procedures used by academic researchers and archaeologists (Davis et al., 2000; Neubauer, 2001). A total of 8 lines were collected at Nashville. Springwater consisted of four grids; three 10 x 10 m and one 2 x 10 m. Dummer consisted of three grids; one 40 x 5 m and two 5 x 15 m. A transect spacing of 0.5 m was used (Davis et al., 2000; Neubauer, 2001). The grid, line and spacing parameters used ensured coverage of all sites, and overlap of undisturbed soil.

Figure 4.2 – Line survey pattern schematic

Figure 4.3 – Grid pattern schematic
4.2.3 Data Analysis

The software used to view, analyze and qualitatively interpret the GPR data was provided by Sensors & Software Inc.; GFP Edit, EKKO View, EKKO View Deluxe and EKKO Mapper 3 (Mississauga, Canada). The GFP Edit, EKKO View and EKKO View Deluxe programs are designed to create, view and edit GFP (GPR Files and Parameters) files. The EKKO Mapper 3 program is designed to create depth slices of GPR data collected in a grid.

4.3 Results

Initial data collection at the three field sites took place during the month of August, 2008. However, due to operator inexperience leading to improper parameter settings and collection of data, the August, 2008 time period was not considered within the results of this study.
4.3.1 Nashville

The Nashville field site consisted of silty clay loam soil. Figure 4.4 is a representative line collected on September 11, 2008 (32 days PBI). Line data were collected in a section containing ten graves; seven experimental and three controls. It is clear from the labelled figure that there is a response (hyperbolic shape) from ten graves. However, a difference between the size and shape of the response from the control graves was not discernable from the experimental graves.

Figure 4.4 – Representative GPR line data from Nashville – September 11, 2008 (32 days PBI). Lines bisecting graves represent: control and experimental.
Throughout the length of the study, the GPR data collected from the Nashville site remained similar in that a hyperbolic shaped response from all of the 20 graves was detected for all of the data collection dates following the initial collection day. Similar to the September, 2008 collection date, a discernable response between the control and experimental graves could not be made. Subsequent GPR data were collected on the following dates; October 9, 2008 (60 days PBI), August 25, 2009 (380 days PBI), September 28, 2009 (414 days PBI) and October 16, 2009 (432 days PBI). Figure 4.5 is a representative line collected from the October, 2009 session.

Figure 4.5 – Representative GPR line data from Nashville – October 16, 2009 (432 days PBI). Lines bisecting graves represent: control and experimental.
4.3.2 Springwater

The Springwater site consisted of a fine sand soil. In contrast to the Nashville site, hyperbolic shapes, indicating the detection of a grave, were not evident. Figure 4.6 is a representative line from a grid pattern collected from Springwater on September 11, 2008 (32 days PBI). Within the figure, it is possible that three graves were slightly visible. However, within the parameters of the collected line, there should have been four graves detected; three experimental and one control. It is possible that within this soil texture, the control grave was simply not detected, as at the Nashville site. The September 11, 2008 data set represented the only collection date where graves were evident.

Figure 4.6 – Representative GPR line collected from a grid pattern from Springwater – September 11, 2008 (32 days PBI). Lines bisecting graves represent: ----- potential experimental graves.
By the conclusion of the study, hyperbolic grave response was not detected at the Springwater site. Figure 4.7 is a representative GPR line collected from a grid pattern from the Springwater site on October 16, 2009 (432 days PBI), in which four graves (three experimental and one control) should be present, but were not detected.

Figure 4.7 – Representative GPR line collected from a grid pattern from Springwater – October 16, 2009 (432 days PBI).
4.3.3 Dummer

The Dummer site consisted of a fine sandy loam soil. Figure 4.8 is a representative line from a grid collected from the Dummer site on September 12, 2008 (33 days PBI). Within the figure, 11 hyperbolic shapes, representing graves, are present. This response is accurate, as 11 graves were dug within the section, despite the fact that only 10 were required. Similar to the Nashville site, a distinct response between the control and experimental graves cannot be made.

Figure 4.8 – Representative GPR line collected from a grid pattern from Dummer – September 12, 2008 (33 days PBI). Lines bisecting graves represent: control, experimental and extra grave.
On the September 29, 2009 (415 days PBI) GPR data collection day, the Dummer site received heavy rain, which collected and remained in the two most westerly graves. As a result, the water created a disturbance which masked the detection of the graves (Figure 4.9). However, the remainder of the graves were detectable by GPR.

Figure 4.9 – Representative GPR line collected from a grid pattern from Dummer – September 29, 2009 (415 days PBI); indicating grave interference by water. Lines bisecting graves represent: –––– control, –––– experimental and –––– experimental grave interference by water.
By the completion of the GPR data collection on October 15, 2009 (431 days PBI), the grave locations at the Dummer site were still discernable (Figure 4.10). However, the hyperbolic shapes were not as defined as at the beginning of the study (refer to Figure 4.8).

Figure 4.10 – Representative GPR line collected from a grid pattern from Dummer – October 15, 2009 (431 days PBI). Lines bisecting graves represent: --- control, ---- experimental and --- extra grave.
4.4 Discussion

Within this study, soil texture had the greatest effect on the ability to detect clandestine graves by ground penetrating radar (GPR). Results indicate that GPR provided the most valuable data when used in a soil environment of silty clay loam (Nashville site). All 20 graves at the Nashville site were detectable by GPR for the entire 14 month duration of the study. This is in contrast to results presented by Buck (2003), who found that GPR use was not successful in the location of an excavated and backfilled trench that was a matter of days old in silty clay loam soil. Although not a forensic scenario involving buried carcasses, GPR testing in areas of known soil conditions with clearly defined features of known dimensions are important to determine radar applicability based upon soil texture. Within the present study, a 500 MHz antenna was used to detect the graves, whereas in the study conducted by Buck (2003), a 400 MHz antenna was employed. Long pulse antennas (300 MHz) are effective for imaging depths of greater than 3.5 m (Miller, 1996; Davis et al., 2000). Alternatively, 500 MHz antennas are useful for depth investigation greater than 0.5 metres (Miller, 1996). The trench that was dug and backfilled in the Buck (2003) study was 2.5 m deep. It is possible that the antenna frequency selected (400 MHz) created difficulties when trying to detect the trench, and that had another antenna frequency been tested, detection may have been possible.

Schultz et al. (2006) found that carcasses buried in clayey soils were only generally detectable by GPR for the first six months post burial, and more difficult to discern after that time period. Soils which contain a high clay content can attenuate electromagnetic (EM) wave propagation resulting in a reduction of depth of penetration into the ground and prevention of the detection of burial sites and features contained within them (Schultz et al., 2006). Overall, the clay masks the carcass by limiting the dielectric permittivity of the carcass with that of the soil horizon (Schultz et al., 2006). The clay content in the silty clay loam soil at the Nashville site was determined to be
27.5%. The fact that the Nashville soil was a loam mixture and the soil in the Schultz et al. (2006) study was heavy clay, may explain why graves and carcasses were detectable using GPR in the present study, but not by Schultz et al. (2006).

In contrast to previous research (Schultz et al., 2006; Schultz, 2008), the present study found that clandestine graves could not be detected with accuracy by GPR in a fine sandy soil (Springwater site), for the entire duration of the study. Within a sandy soil environment, the carcass and its immediate surroundings are detected by GPR more so than the contrasting properties of the undisturbed soil surrounding the grave (Schultz et al., 2006; Schultz; 2008). Schultz (2008) found that the degree of skeletonization of buried carcasses appeared to have the greatest effect on whether or not a distinctive hyperbolic shaped response was discernable over the duration of a 21.5 month period. Over time, as a carcass progresses through the stages of decomposition, the dielectric permittivity surrounding the body will equalize to the surrounding soil due to the movement of the soil solution or ground water (Schultz, 2008), making detection by GPR more difficult. It is unclear why, in the present study, graves were not detectable even during the early stage of decomposition (autolysis). It is possible that the contrasting textures of sandy soil in the current study and the studies conducted by Schultz et al. (2006) and Schultz (2008) played a role. The Springwater site was located at a commercial gravel pit, and as such, the soil environment consisted of a variety of textures of sand, including; medium and very course sand, as well as gravel. The specific properties of the sand within the studies conducted by Schultz et al. (2006) and Schultz (2008) were not stated, and therefore, it is possible that those soil environments consisted of more uniform sand particles. However, Miller (1996) stated that soils that contain gravel permit the successful use of GPR in forensic contexts due to good depth of signal and high resolution. Additionally, within sand, depth of GPR penetration is dependent upon the pore water conductivity, more so than the sand material, and bedding within wet sand deposits can mask grave detection (Sensors & Software). Alternatively, clandestine grave detection in fine sandy loam soil (Dummer site) was
successful for the 14 month duration of the present study. However, the hyperbolic response from the graves became less defined as the study progressed. Based upon the combination of results obtained from all three of the field sites in the present study, it is possible that loam soils, despite varying percentages of components (i.e. fine sand vs. silty clay) represent a suitable soil environment for clandestine grave detection using GPR.

With respect to the use of GPR for the estimation of post burial interval (PBI) of clandestine graves, the present study found limited applicability in one of the soil textures investigated; fine sandy loam soil (Dummer site). The September 12, 2008 (33 days PBI) GPR data collection from Dummer (Figure 4.8) resulted in distinctive hyperbolic shaped responses from the graves. By the completion of the study, October 15, 2009 (431 days PBI) (Figure 4.10), the hyperbolic responses were still discernable, but were less distinctive. This result could potentially aid crime scene investigators who detect a body in fine sandy loam soil using GPR to estimate whether the grave was freshly dug, or was closer to one year old. The silty clay loam soil (Nashville site) and the fine sandy soil (Springwater site) did not provide results which could aid law enforcement officials to estimate PBI as there was no alteration in hyperbolic response in silty clay loam soil and graves were not detected in fine sandy soil. More accurate estimations of PBI may be possible with a GPR study of longer duration, as Schultz et al. (2006) and Schultz (2008) found that pig carcasses exhibiting varying degrees of decomposition could be detected for the full duration of a 21.5 month study.

Although the use of GPR to the application of forensic scenarios has seen increased interest in recent years, the use of other more traditional non-invasive techniques, such as observations of changes in foliage and soil depression above a grave, should not be overlooked. The most effective means of searching for a clandestine grave is to not rely solely on one technique, but to use multiple techniques in combination. Further controlled research into the applicability of GPR for the
detection of clandestine graves based upon soil texture, rate of carcass decomposition and length of burial is necessary for GPR to remain an effective tool within law enforcement.

4.5 Conclusion

Ground penetrating radar is a useful tool in the location of clandestine graves in areas of known soil conditions, specifically due to its non-invasive nature. This study has shown that GPR was successful in locating graves in silty clay loam and fine sandy loam soils, but was unsuccessful in fine sandy soil. Specifically, within a fine sandy loam soil, there is the potential to estimate the post burial interval (PBI), as hyperbolic grave response was well defined at the beginning of the 14 month burial duration, but became less distinctive near the completion of the study. However, distinction between experimental and control graves could not be made.
Chapter 5 – Gravesoil Analysis
5.1 Introduction

The detection of fatty acids and/or adipocere formation in gravesoils may be useful in forensic investigations as a means of estimating the stage of decomposition and the post mortem interval (PMI) and/or post burial interval (PBI) of remains. Adipocere formation is the result of the post mortem conversion of body fat into a lipid mixture (Forbes et al., 2003). Analysis of gravesoils may provide evidence as to where a body that has decomposed was once located, both on the soil surface and within a burial environment, as fatty acids can leach from the body. Changes in soil moisture content and pH as a result of decomposition may also be useful as presumptive tests for the location of remains. Estimation of the PMI and/or PBI is important in forensic situations, as it can help investigators identify a body based upon missing person’s reports. Gravesoil sample analysis is of particular interest once a body has reached skeletonization and soft tissue is no longer present.

The degradation of fatty acids leading to adipocere formation follows a known sequence of events (Forbes et al., 2004). This sequence can be used to estimate the stage of decomposition (e.g. autolysis vs. putrefaction) of a body. The unsaturated fatty acids palmitoleic (C\textsubscript{16:1}) and oleic (C\textsubscript{18:1}), and the saturated fatty acids myristic (C\textsubscript{14:0}), palmitic (C\textsubscript{16:0}) and stearic (C\textsubscript{18:0}) have been determined to be significant in the analysis of post mortem samples due to their stability over long time periods (Forbes et al., 2004; Forbes, 2008).

Previous research into fatty acid analysis of gravesoil samples has investigated the effect of burial duration on the leaching of fatty acids into soil from domestic pig (\textit{Sus scrofa}) carcasses buried in sandy soil (Forbes et al., 2004). Analysis of gravesoil samples containing adipocere collected from human cadavers within cemeteries has also been conducted, where the burial duration was known (Forbes et al., 2002). The use of Fourier transform infrared (FTIR) spectroscopy was employed as a confirmatory
test and gas chromatography – mass spectroscopy (GC-MS) used to quantify the fatty acid composition of samples (Forbes et al., 2002, 2004).

5.2 Materials and Methods

In the current study, domestic pig (Sus scrofa) carcasses were clothed in 100% cotton t-shirts and 50% cotton/50% polyester briefs prior to burial in three contrasting soil textures. During set exhumation intervals, gravesoil samples were collected for analysis of soil moisture content, pH and fatty acid composition, leading to adipocere formation. The applicability of using soil moisture content and pH as presumptive tests for the location of decomposing remains was investigated. Fatty acids that had leached into the soil environment from the decomposing soft tissue were analyzed by diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) as a means of estimating the stage of decomposition of the carcasses and the post burial interval (PBI).

5.2.1 Moisture Content

Moisture content of collected gravesoil samples was determined by weighing moist soil, drying the soil in an 80°C oven for 12 hours, and then re-weighing. The percentage of moisture within the soil sample was calculated using the following formula: \[ \text{Moisture content} = \left( \frac{\text{moist soil} - \text{dry soil}}{\text{dry soil}} \right) \times 100 \]. Three soil replicates from each grave were analyzed to determine moisture content.

5.2.2 pH

Gravesoil pH was determined in the laboratory, using a Mettler Toledo Seven Easy pH meter. Two grams of dry soil was mixed with 10 mL of deionized water by vortexing for one minute using a Fisher Scientific Vortex Mixture. Samples were then
centrifuged for two minutes at 6500 rpm. The pH was measured in the aqueous level. Three soil replicates from each grave were analyzed.

5.2.4 Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS)

5.2.4.1 Sample Preparation

Gravesoil samples collected during the three exhumation periods were dried in an oven at 80°C for 12 hours to remove moisture. Samples were then ground with Potassium bromide (KBr) (99+% metals basis, FTIR grade; Sigma-Aldrich, Germany) in a 1:9 ratio (sample : KBr) (Forbes et al., 2005a,b) using a glass mortar and pestle. Post-grinding, samples were immediately analyzed for qualitative fatty acid content using diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS). Three gravesoil sample replicates from each grave were analyzed.

5.2.4.2 Instrumental Sample Analysis

Prepared gravesoil samples were qualitatively analyzed for fatty acid composition using a Nicolet Fourier transform infrared dual-beam spectrometer equipped with a diffuse reflectance accessory. A sampling tray containing a sample cup and a reference cup (KBr) was used to individually analyze samples, while comparing them to the KBr background spectrum. Each sample was analyzed using the following instrumental parameters; 64 scans over a frequency range of 4000-500 cm\(^{-1}\) to provide a resolution of 4 cm\(^{-1}\) (Stuart et al., 2000; Forbes et al., 2005a,b). Following analysis of each sample, the sampling cup was cleaned with acetone to remove fatty acid residue.
5.2.5 Statistical Analysis

Statistical analysis was conducted using SPSS Statistics 17.0 software software. Two-way analysis of variance (ANOVA) was conducted to determine significance between soil moisture content (%) and soil pH levels of gravesoil and control soil samples based upon the burial duration and soil texture.
5.3 Results

5.3.1 Moisture Content

5.3.1.1 Nashville

The determination of moisture content (%) of soil samples was conducted to determine if carcass decomposition had an effect on the surrounding soil environment. At the Nashville site, there was not an overall significant difference (p = 0.329) in moisture content (%) between control and gravesoil samples (Figure 5.1). However, gravesoil moisture content during the third exhumation period (October 16, 2009 – 432 days PBI; 30.4%) was significantly higher than the control (23.5%).

![Figure 5.1 - Mean difference of soil moisture content (%) of control and gravesoil at Nashville by exhumation period. (Unbalanced ANOVA, F_{Exhumation Period} (2, 35) = 0.897, p = 0.419, F_{Sample Type} (1, 35) = 1.621, p = 0.213, F_{Interaction} (2, 35) = 1.155, p = 0.329).]
5.3.1.2 Springwater

The moisture content (%) of soil samples collected from the Springwater site is illustrated in Figure 5.2. There was not a significant overall difference ($p = 0.155$) in moisture content (%) between control and gravesoil samples. Note that control soil moisture content (%) was not determined for the second exhumation period, as the control grave was not recovered.

![Figure 5.2 – Mean difference of soil moisture content (%) of control and gravesoil at Springwater by exhumation period. (Unbalanced ANOVA, $F_{\text{Exhumation Period}} (2, 29) = 7.294$, $p < 0.003$, $F_{\text{Sample Type}} (1, 29) = 0.039$, $p = 0.844$, $F_{\text{Interaction}} (1, 29) = 2.153$, $p = 0.155$)](image-url)
5.3.1.3 Dummer

Figure 5.3 represents the moisture content (%) of soil samples collected from the Dummer site. There was not an overall significant difference ($p = 0.694$) between control and gravesoil samples. However, gravesoil moisture content (15.8%) was significantly lower than the control (17.5%) during the first exhumation period (October 16, 2008 – 67 days PBI).

Figure 5.3 – Mean difference of soil moisture content (%) of control and gravesoil at Dummer by exhumation period. (Unbalanced ANOVA, $F_{Exhumation\ Period\ (2,\ 35)} = 0.540$, $p = 0.588$, $F_{Sample\ Type\ (1,\ 35)} = 0.253$, $p = 0.619$, $F_{Interaction\ (2,\ 35)} = 0.370$, $p = 0.694$)
5.3.2 pH

5.3.2.1 Nashville

Soil pH measurements were taken to determine the effect of a decomposing carcass on surrounding soil environment. Gravesoil pH levels were significantly lower than control pH levels ($p < 0.001$) at the Nashville field site during the first (October 15, 2008 – 66 days PBI), second (July 22, 2009 – 346 days PBI) and third (October 16, 2009 – 432 days PBI) exhumation periods (Figure 5.4).

![Field plot diagram showing pH measurement over exhumation periods.](Image)

Figure 5.4 – Mean difference of soil pH of control and gravesoil at Nashville by exhumation period. (Unbalanced ANOVA, $F_{Exhumation\ Period\ (2,\ 34)} = 37.090, p < 0.001$, $F_{Sample\ Type\ (1,\ 34)} = 75.783, p < 0.001$, $F_{Interaction\ (2,\ 34)} = 13.380, p < 0.001$)
5.3.2.2 Springwater

Similar to the Nashville site, there was a significant difference \((p < 0.001)\) between gravesoil and control pH levels at the Springwater site (Figure 5.5). Gravesoil pH was significantly lower than control pH during the first (October 15, 2008 – 66 days PBI) and third (October 16, 2009 – 432 days PBI) exhumation periods. Note that control soil pH was not determined for the second exhumation period, as the control grave was not recovered.

Figure 5.5 – Mean difference of soil pH of control and gravesoil at Springwater by exhumation period. (Unbalanced ANOVA, \(F_{\text{Exhumation Period \ (2, 29)}} = 29.144, p < 0.001\), \(F_{\text{Sample Type \ (1, 29)}} = 48.984, p < 0.001\), \(F_{\text{Interaction \ (1, 29)}} = 14.543, p < 0.001\))
5.3.2.3 Dummer

Figure 5.6 illustrates the pH levels of gravesoil and control soil at the Dummer site. Overall, gravesoil pH was significantly lower \((p < 0.001)\) than control pH during the first (October 16, 2008 – 67 days PBI), second (July 23, 2009 – 347 days PBI) and third (October 23, 2009 – 439 days PBI) exhumation periods.

![Figure 5.6: Mean difference of soil pH of control and gravesoil at Dummer by exhumation period.](image)

- **Control**
- **Experimental**

Figure 5.6 – Mean difference of soil pH of control and gravesoil at Dummer by exhumation period. (Unbalanced ANOVA, \(F_{\text{Exhumation Period}} (2, 35) = 0.430, p = 0.654\), \(F_{\text{Sample Type}} (1, 35) = 28.918, p < 0.001\), \(F_{\text{Interaction}} (2, 35) = 12.858, p < 0.001\))
5.3.3 Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS)

Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) analysis of the gravesoil samples collected from directly beneath the carcasses during each exhumation period was conducted to detect fatty acids that had leached from the tissue into the soil as a result of soft tissue degradation. Previously identified infrared spectroscopy bands associated with fatty acid degradation and adipocere formation include; triglycerides (~1740 cm\(^{-1}\)), fatty acids (~1720-1700 cm\(^{-1}\)) and salts of fatty acids (~1576-1540 cm\(^{-1}\)) (Bereuter et al., 1997; Stuart et al., 2000, 2005; Forbes et al., 2004, 2005a, 2005b, 2005c).

5.3.3.1 Nashville

Figure 5.7, plots (a-d) illustrates the infrared spectra of representative control and gravesoil samples collected from the Nashville field site during the three exhumation periods. Figure 5.7 (a) illustrates a characteristic infrared spectrum of control soil samples. The spectrum lacks bands associated with fatty acid degradation and adipocere formation.

Gravesoil samples collected from the Nashville site during the October 15, 2008 (66 days PBI) exhumation are represented by the characteristic infrared spectrum in Figure 5.7 (b). Similar to the control soil, the spectrum lacks bands associated with fatty acid degradation and adipocere formation.

The characteristic infrared spectrum of the gravesoil samples collected from the Nashville site during the July 22, 2009 (346 days PBI) exhumation is illustrated in Figure 5.7 (c). The spectrum contains C-H stretching bands in the 2950-2800 cm\(^{-1}\) region. Evidence of lipid degradation is not present.
Figure 5.7 (d) illustrates the infrared spectrum for the gravesoil samples collected from the Nashville site during the October 16, 2009 (432 days PBI) exhumation. Again, the spectrum contains C-H stretching bands in the region of 2950-2800 cm⁻¹, but evidence of lipid degradation is not apparent.
Figure 5.7, plots (a-d) – Characteristic infrared spectra of Nashville soil samples (a) control, (b) October 15, 2008 (66 days PBI) exhumation, (c) July 22, 2009 (346 days PBI) exhumation and (d) October 16, 2009 (432 days PBI) exhumation. Lines represent: (1,2) C-H Stretching (2950-2800 cm\(^{-1}\) region).
5.3.3.2 Springwater

The infrared spectra of representative control and gravesoil samples collected from the Springwater field site during the three exhumation periods is illustrated in Figure 5.8, plots (a-d). The characteristic infrared spectrum of control soil samples is illustrated in Figure 5.8 (a). The spectrum contains weak C-H stretching bands in the 2950-2800 cm\(^{-1}\) region. However, other bands associated with fatty acid degradation and adipocere formation are not present.

Figure 5.8 (b) illustrates the infrared spectrum for the gravesoil samples collected from the Springwater site during the October 15, 2008 (66 days PBI) exhumation. The spectrum lacks bands associated with fatty acid degradation and adipocere formation.

The characteristic infrared spectrum of the gravesoil samples collected from the Springwater site during the July 22, 2009 (346 days PBI) exhumation is illustrated in Figure 5.8 (c). The spectrum only contains C-H stretching bands in the 2950-2800 cm\(^{-1}\) region.

Gravesoil samples collected from the Dummer site during the October 23, 2009 (439 days PBI) exhumation are represented by the characteristic infrared spectrum in Figure 5.8 (d). One weak C-H stretching band is visible in the region of 2950-2800 cm\(^{-1}\). Evidence of fatty acid degradation and adipocere formation is not present.
Figure 5.8, plots (a-d) – Characteristic infrared spectra of Springwater soil samples (a) control, (b) October 15, 2008 (66 days PBI) exhumation, (c) July 22, 2009 (346 days PBI) exhumation and (d) October 16, 2009 (432 days PBI) exhumation. Lines represent: (1,2) C-H Stretching (2950-2800 cm\(^{-1}\) region).
5.3.3.3 Dummer

Figure 5.9, plots (a-d) illustrates the infrared spectra of representative control and gravesoil samples collected from the Dummer field site during the three exhumation periods. Figure 5.7 (a) illustrates a characteristic infrared spectrum of control soil samples. The spectrum lacks bands associated with fatty acid degradation and adipocere formation.

A characteristic infrared spectrum of the gravesoil samples collected from the Dummer site during the October 16, 2008 (67 days PBI) exhumation is illustrated in Figure 5.9 (b). Similar to the control soil, the spectrum lacks bands associated with fatty acid degradation and adipocere formation.

Figure 5.9 (c) illustrates the infrared spectrum of the gravesoil samples collected from the Dummer site during the July 23, 2009 (347 days PBI) exhumation. Again, the spectrum lacks bands associated with fatty acid degradation and adipocere formation.

Gravesoil samples collected from the Dummer site during the October 23, 2009 (439 days PBI) exhumation are represented by the characteristic infrared spectrum in Figure 5.9 (d). The spectrum contains weak C-H stretching bands in the 2950-2800 cm\(^{-1}\) region, but lacks bands attributable to fatty acid degradation and adipocere formation.
Figure 5.9, plots (a-d) – Characteristic infrared spectra of Dummer soil samples (a) control, (b) October 16, 2008 (67 days PBI) exhumation, (c) July 23, 2009 (347 days PBI) exhumation and (d) October 23, 2009 (439 days PBI) exhumation. Lines represent: (1,2) C-H Stretching (2950-2800 cm\(^{-1}\) region).
5.4 Discussion

In this study, gravesoil samples collected from directly beneath buried domestic pig (*Sus scrofa*) carcasses were analyzed for soil moisture content and pH to determine if the presence of a decomposing carcass could be confirmed. Samples were also analyzed by infrared (IR) spectroscopy to determine rate of decomposition based upon known exhumation intervals. Carcasses were buried at three field sites; Nashville, Springwater and Dummer, in three contrasting soil types; silty clay loam, fine sand and fine sandy loam, respectively. Additionally, the carcasses were buried in 100% cotton t-shirts and 50% cotton/50% polyester briefs in order to more closely represent a forensic scenario.

Overall, the moisture content percentage of gravesoil samples was not significantly different than control soil at any of the three field site locations. These results indicate that analysis of soil moisture content is not a valuable method for determining the presence of a decomposing carcass in silty clay loam, fine sand or fine sandy loam soils. Benninger et al. (2008) found similar results from the analysis of gravesoil collected from beneath domestic pig (*Sus scrofa*) carcasses that had been deposited on the surface of a loam textured soil.

Significant differences in gravesoil pH as compared to control soil pH were observed at all three field sites (p <0.001 for all three). The mean control soil pH values from the Nashville site for the first, second and third exhumation periods were 7.16, 6.21 and 7.53, respectively (neutral pH). Gravesoil sample pH values were significantly more acidic; 5.35, 5.78 and 6.77. At the Springwater site, control soil pH values were alkaline for the first and third exhumation periods; 8.41 and 8.04, respectively (control not collected for second exhumation period). A significant decrease in gravesoil pH was observed for the three exhumations periods; 7.32, 6.75 and 7.72, which resulted in a neutral pH at the Springwater site. The Dummer site was of neutral pH, with control soil.
pH levels of 7.88, 7.35 and 7.21 for the three exhumation periods. The gravesoil pH values were significantly more acidic; 6.31, 7.10 and 7.00. In all three of the soil textures tested, gravesoil pH became less acidic by the final exhumation period, and began to represent basal levels. This trend may be explained by ammonium (NH$_4^+$) entering the soil during the later stages of decomposition (Stokes et al., 2009). These results indicate that in the soil textures from the three field sites used, the presence of a decomposing carcass produces acidic pH levels, which may be beneficial as a presumptive test in forensic investigations.

Previous research has investigated the effect that a decomposing carcass has on soil pH levels (Rodriguez and Bass, 1985; Vass et al., 1992; Wilson et al., 2007; Benninger et al., 2008; Carter and Tibbett, 2008b,c; Stokes et al., 2009). Traditionally, it has been believed that carcass decomposition results in more alkaline pH levels as a result of the accumulation of ammonium (NH$_4^+$) in soil (Carter et al., 2007; Carter and Tibbett, 2008c; Benninger et al., 2008; Stokes et al., 2009). After the initial increase in pH commonly observed in association with decomposition, Carter and Tibbett (2008c) observed a decrease in pH in some gravesoils incubated at 22° C and 29° C; thought to be the result of the soil returning to its basal pH. A more recent study (Stokes et al., 2009) suggests that pH levels become more alkaline as decomposition proceeds only if basal control soil pH levels are acidic. Therefore, it is important for forensic investigators to be aware of site specific pH levels (e.g. acidic vs. alkaline) before using pH values as a presumptive test for the presence of a decomposing carcass.

Infrared (IR) spectroscopy analysis of the gravesoil samples did not reveal bands pertaining to fatty acid degradation or adipocere formation. At the Nashville field site, C-H stretching bands in the 2950-2800 cm$^{-1}$ region were present in the samples collected from the second (July 22, 2009 – 346 days PBI) and third (October 16, 2009 – 432 days PBI) exhumation periods. Similar results were obtained from the Springwater site. C-H stretching bands (2950-2800 cm$^{-1}$ region) were only present in the gravesoil
samples collected from the Dummer site during the third exhumation period (October 23, 2009 – 439 days PBI). C-H stretching bands are present within all organic material, and are therefore not an indication of fatty acid degradation and adipocere formation on their own. These results suggest that infrared spectroscopy analysis of gravesoil samples is not a viable method for determining rate of carcass degradation or making estimations regarding the post burial interval (PBI) in the soil textures tested.

In contrast to the results of the current study, Forbes et al. (2004) found that in a preliminary study consisting of a small number of gravesoil samples, adipocere formation was detectable by IR spectroscopy. Gravesoil samples were collected from decomposing domestic pig (*Sus scrofa*) carcasses that were buried in shallow graves in sandy soil (Forbes et al., 2004). Gravesoil samples had PBI’s of 13 and 14 months (Forbes et al., 2004). Results from the 13 month PBI samples revealed that adipocere was present within the soil matrix, but that the IR spectroscopy absorbance of the adipocere peaks was weak due to the soil matrix (Forbes et al., 2004). Results were not presented for the gravesoil samples of 14 months PBI (Forbes et al., 2004).

The pig carcasses within the current study were clothed in 100% cotton t-shirts and 50% cotton/50% polyester briefs prior to burial to more closely represent a forensic scenario involving a body. The carcasses used by Forbes et al. (2004) were not clothed. It is possible that the leaching effect of fatty acids into the soil environment was not observed in this study as a result of the clothing. The clothing may have absorbed and retained the fatty acids as they leached from the decomposing tissue. Further research investigating the effects of a longer PBI are necessary to determine if fatty acids would eventually leach into surrounding soil as the carcasses continued to decompose and the clothing degraded.

In addition to analyzing gravesoil samples using IR spectroscopy, Forbes et al. (2004) quantitatively analyzed samples using gas chromatography – mass spectrometry
GC-MS results indicated that adipocere was able to leach into the soil during decomposition, most likely following liquefaction of the remains (Forbes et al., 2004). Due to time constraints and the fact that IR spectroscopy analysis of the gravesoil did not reveal bands attributable to fatty acid degradation and adipocere formation, samples were not analyzed using GC-MS. It is possible that fatty acids may have been detected within the gravesoil of the current study had the samples been analyzed by GC-MS, as this is a more sensitive technique. Further method optimization is necessary to determine the effect that the soil matrix has on the ability to extract and derivatize free fatty acids from gravesoil samples. Following successful method optimization, further research must be conducted to determine if fatty acid analysis of gravesoil samples has the potential to be successfully incorporated into forensic investigations involving decomposing bodies.

5.5 Conclusion

This study was conducted to determine the effect that a decomposing carcass has on soil moisture content and pH in a burial environment. In the soil textures tested (silty clay loam, fine sand and fine sandy loam), it was determined that there was not a significant difference in moisture content percentage between control soils and gravesoils. Alternatively, gravesoil was significantly more acidic than control soil in all three soil textures. The pH levels may have applicability within forensic investigations as a presumptive test for the presence of a decomposing body, as significant differences between control and gravesoil were observed up to an including 439 days post burial interval (PBI).

The study also investigated the applicability of using infrared (IR) spectroscopy to detect fatty acids, leading to adipocere formation, that have leached from the decomposing tissue of domestic pig (Sus scrofa) carcasses buried in 100% cotton t-shirts and 50% cotton/50% polyester briefs. Fatty acids and adipocere were not detectable
within the gravesoil samples. Further research investigating longer post burial intervals (PBI) and the effect of clothing on the leaching of fatty acids into the surrounding soil environment is necessary. Continued research into the applicability of using gas chromatography – mass spectrometry (GC-MS) to detect fatty acids in gravesoils is required, as GC-MS is a more sensitive technique than IR spectroscopy.
Chapter 6 – Tissue Analysis
6.1 Introduction

Following the detection of a clandestine grave containing a body, law enforcement officials must be able to estimate the post mortem interval (PMI) and/or post burial interval (PBI) in order to further investigative efforts and potentially identify the body. More traditional means of estimating time since death (i.e. forensic entomology) are generally not effective for burial situations, as burial restricts insect access to the decomposing remains.

In the event that soft tissue is present, biochemical analysis of tissue samples to determine fatty acid composition can provide a useful estimation of time since death (Forbes et al., 2004). Fatty acid degradation can lead to adipocere formation, and follows a known sequence of events (Forbes et al., 2004). This sequence of events can be useful to forensic investigators when making estimations regarding the PMI and/or PBI. The unsaturated fatty acids palmitoleic (C₁₆:₁) and oleic (C₁₈:₁), and the saturated fatty acids myristic (C₁₄:₀), palmitic (C₁₆:₀) and stearic (C₁₈:₀) have been determined to be useful in the analysis of post mortem samples due to their stability over long time periods (Forbes et al., 2004; Forbes, 2008).

Previous research into fatty acid analysis of tissue samples has focused on the effects of burial environment (i.e. soil pH and warm vs. cold burial) (Forbes et al., 2005b; Stuart et al., 2005), the method of burial (i.e. burial of tissue directly in soil vs. coffin or plastic bag) (Forbes et al., 2005a) and the formation of adipocere in aquatic environments (Notter et al., 2008) by infrared (IR) spectroscopy and gas chromatography – mass spectrometry (GC-MS).
6.2 Materials and Methods

In the current study, domestic pig (Sus scrofa) carcasses were clothed in 100% cotton t-shirts and 50% cotton/50% polyester briefs prior to burial in three contrasting soil textures. During set exhumation intervals, tissue samples were collected for analysis of fatty acid content and adipocere formation by diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) and gas chromatography – mass spectrometry (GC-MS). The applicability of using tissue samples as a means of estimating the stage of decomposition and the post burial interval (PBI) of the carcasses was investigated.

6.2.1 Reagents

Potassium bromide (KBr) (99+% metals basis, FTIR grade) was obtained from Sigma-Aldrich (Germany). Chloroform (HPLC grade) was obtained from Fisher Scientific (United States of America). N,O-Bis(trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane (BSTFA + TMCS) was obtained from Fluka Analytical (Switzerland).

6.2.2 Standards

Myristic fatty acid trimethylsilyl ester standard (99+%%) was obtained from Sigma (Malaysia). Palmitic fatty acid trimethylsilyl ester standard (minimum 99%) was obtained from Sigma (United States of America). Palmitoleic fatty acid trimethylsilyl ester standard (≥ 98.5%, GC grade) was obtained from Fluka Analytical (United States of America). Stearic fatty acid trimethylsilyl ester standard (~ 99%, capillary GC) was obtained from Sigma-Aldrich (United States of America). Oleic fatty acid trimethylsilyl ester standard (~ 99%, reagent grade) was obtained from Sigma-Aldrich (India). Nonadecanoic fatty acid trimethylsilyl ester standard was used as an internal standard and obtained from Fluka Analytical (Switzerland). Fatty acid stock solutions were
prepared by dissolving 25 mg of each fatty acid in 25 mL of chloroform, producing a concentration of 1 mg/mL⁻¹.

6.2.3 Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS)

6.2.3.1 Sample Preparation

Tissue samples collected during the three exhumation periods were lyophilized using a Labconco Freeze Dryer for 36 hours to remove moisture. Samples were then ground with Potassium bromide (KBr) in a 1:9 ratio (sample : KBr) (Forbes et al., 2005 a,b) using a glass mortar and pestle. Post-grinding, samples were immediately analyzed for qualitative fatty acid content using diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS), as outlined in Section 5.2.4.2 of Chapter 5 – Gravesoil Analysis. Three tissue replicates from each of the exhumed carcasses were analyzed.

6.2.4 Gas Chromatography – Mass Spectrometry (GC-MS)

6.2.4.1 Sample Preparation

Tissue samples collected during the three exhumation periods were lyophilized using a Labconco Freeze Dryer for 36 hours to remove moisture. 6 mg of freeze dried tissue was weighed into a sterilized screw-top culture tube, to which 2 mL of chloroform was added. The samples were sonicated for 15 minutes using a Fisher Scientific Ultrasonic Cleaner FS110D sonicator. The samples were then centrifuged for 2 minutes using a Fisher Scientific centrifuge. The chloroform layer was drawn off using disposable glass Pasteur pipettes and transferred to sterilized screw-top culture tubes, to which 0.25 mL of N,O-Bis(trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane (BSTFA + TMCS) was added. The samples were then vortexed for 1 minute using a Fisher Scientific Vortex Mixture. The samples were heated for 15 minutes at 70°C using a
Fisher Scientific Isotemp heating block. Upon cooling, the samples were transferred to gas chromatography vials for analysis using disposable glass Pasteur pipettes. The method used was adapted from Forbes et al. (2003). Three tissue replicates from each of the exhumed carcasses were analyzed.

6.2.4.2 Instrumental Sample Analysis

A Varian 450 – GC Gas Chromatograph coupled with a Varian 240 – MS IT Mass Spectrometer was used to analyze all samples. A Varian CombiPAL Autosampler was used for all sample injections onto the column. 1 μL of each sample was analysed on a Varian WCOT Fused Silica 30 m column with a VF-5ms stationary phase, with an internal diameter of 0.25 mm, an external diameter of 0.39 mm and a film thickness of 0.25 μm. The initial temperature of the column was 50°C, followed by a programmed temperature ramp of the following parameters; 50°C held for 2 minutes, which was then increased to 150°C at a rate of 15°C/min held for 1 minute, followed by an increase to 240°C at a rate of 10°C/min held for 3.33 minutes. All samples were injected in splitless mode and analysis was carried out using total ion count (TIC) mode. An ion trap mass spectrometer (MS) was used with electron ionization as the acquisition mode. The trap was held at 250°C with a TIC target of 20,000 counts and a scan time of 0.50 seconds/scan. The lowest mass to charge ratio (m/z) analyzed was 50. The highest m/z analyzed was 450. Peaks corresponding to trimethylsilyl (TMS) fatty acid esters were identified based on comparison of their retention time to fatty acid standards and mass spectra against the NIST MS Search 2.0 Library. Instrumental parameters were adapted from Forbes et al. (2003) (Table 6.1). The peak area ratio (PAR) of the fatty acids was calculated by dividing the peak area of the fatty acid by the peak area of the internal standard (area/int std area). Nonadecanoic acid (C₁₉:₀) was used as the internal standard. PAR was used to measure the relative abundance of the fatty acids extracted from the samples.
Table 6.1 – Gas chromatography – mass spectrometry (GC-MS) instrumental parameters

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6.2.4.3 Instrumental Detection Parameters

In order to determine the detection limits of the instrument, mixed fatty acid standards were prepared, with concentration ranges from 100 µg/mL to 0.01 µg/mL, and analyzed. The limit of detection (LOD) was determined to be 0.1 µg/mL. The limit of quantitation (LOQ) was determined to be 1.0 µg/mL. The limit of saturation (LOS) was determined to be 50 µg/mL.

6.2.4.4 Instrumental Reproducibility

In order to establish instrumental reproducibility, one experimental sample was injected into the instrument ten times on the same day and again ten times one week later. The coefficient of variation was determined to be 11.0%, which is within the acceptable range.

6.2.5 Statistical Analysis

Statistical analysis was conducted using VSN International GenStat Version 12 software. One-way analysis of variance (ANOVA) was conducted to determine significance between peak area ratio (area/int std area) (PAR) of fatty acids in tissue samples based upon the following parameters; fatty acid type, burial duration and soil texture.
6.3 Results

6.3.1 Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS)

Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) analysis of the tissue samples collected from the carcasses during each exhumation period was conducted to produce a qualitative lipid profile and determine if adipocere had formed. Previously identified infrared spectroscopy bands associated with fatty acid degradation and adipocere formation include; triglycerides (~1740 cm\(^{-1}\)), fatty acids (~1720-1700 cm\(^{-1}\)) and salts of fatty acids (~1576-1540 cm\(^{-1}\)) (Bereuter et al., 1997; Stuart et al., 2000, 2005; Forbes et al., 2004, 2005a-c).

6.3.1.2 Nashville

Figure 6.1, plots (a-d) illustrates the infrared spectra of representative tissue samples collected from the Nashville field site during the three exhumation periods and a representative control sample taken from a domestic pig (Sus scrofa) from a different experiment being conducted within the laboratory.

Figure 6.1 (a) illustrates the characteristic infrared spectrum of control tissue samples collected from a pig carcass that was still considered fresh (collected within a matter of hours). The spectrum contains a strong band near 1740 cm\(^{-1}\) indicating an abundance of triglycerides and a weak C=C stretching band indicative of unsaturated fatty acids at 1656 cm\(^{-1}\). C-H stretching bands are visible in the 2950-2800 cm\(^{-1}\) region, and a =C-H stretching shoulder indicative of free fatty acids is evidenced in the higher wavenumber end of this region. Notably, the control tissue lacks bands attributable to later stage decomposition or adipocere formation, such as a free fatty acid band at ~1700 cm\(^{-1}\) and salts of fatty acids in the region of 1576-1540 cm\(^{-1}\).
The characteristic infrared spectrum of the tissue samples collected from the Nashville site during the October 15, 2008 (66 days PBI) exhumation is illustrated in Figure 6.1 (b). A strong C=C stretching band at 1656 cm\(^{-1}\) indicating unsaturated fatty acids, and C-H stretching bands in the region of 2950-2800 cm\(^{-1}\) and a \(=\text{C}-\text{H}\) shoulder in the higher wavenumber (3065 cm\(^{-1}\)) of the region indicate the presence of free fatty acids. In addition, a weak fatty acid carboxylate C-O stretching band at 1556 cm\(^{-1}\) indicates small quantities of salts of fatty acids, suggesting that early stage decomposition (autolysis) has commenced.

Figure 6.1 (c) illustrates the infrared spectrum for the tissue samples collected from the Nashville site during the July 22, 2009 (346 days PBI) exhumation. Again, the spectrum contains C-H stretching bands in the 2950-2800 cm\(^{-1}\) region and a \(=\text{C}-\text{H}\) stretching shoulder in the higher wavenumber region. A moderate fatty acid carboxylate C-O stretching band at 1552 cm\(^{-1}\) indicates small quantities of salts of fatty acids. In contrast to the October, 2008 samples, a fatty acid C-O stretching band is observed at 1708 cm\(^{-1}\), which represents free fatty acids, and the continued degradation of the lipid products.

Tissue samples collected from the Nashville site during the October 16, 2009 (432 days PBI) exhumation are represented by the characteristic infrared spectrum in Figure 6.1 (d). Later stage decomposition (putrefaction) of the tissue is evidenced by a fatty acid C-O stretching band at 1713 cm\(^{-1}\) indicating conversion of triglycerides to free fatty acids, a moderate fatty acid carboxylate C-O stretching band at 1565 cm\(^{-1}\) indicating salts of fatty acids, C-H stretching bands in the 2950-2800 cm\(^{-1}\) region and a \(=\text{C}-\text{H}\) stretching shoulder band observed in the higher wavenumber region indicating free fatty acids. The lipid profile indicates advanced stage decomposition (putrefaction), but is not consistent with adipocere formation.
Figure 6.1, plots (a-d) – Characteristic infrared spectra of Nashville tissue samples (a) control, (b) October 15, 2008 (66 days PBI) exhumation, (c) July 22, 2009 (346 days PBI) exhumation and (d) October 16, 2009 (432 days PBI) exhumation. Lines represent: (1,2) C-H stretching (2950-2800 cm\(^{-1}\) region), (3) triglyceride C=O stretching (~1740 cm\(^{-1}\)), (4) fatty acid C-O stretching (1720-1700 cm\(^{-1}\) region) and (5,6) fatty acid carboxylate C-O stretching (1576-1540 cm\(^{-1}\) region).
6.3.1.3 Springwater

Figure 6.2, plots (a-d) illustrates the infrared spectra of representative tissue samples collected from the Springwater field site during the three exhumation periods and a representative control sample. Figure 6.2 (a) illustrates the characteristic infrared spectra of control tissue samples collected from a pig carcass that was still considered fresh. Refer to Section 6.3.1.2 for spectral interpretation, as the same control was used as a comparison for all three field sites.

The characteristic infrared spectrum of the samples collected from the Springwater site during the October 15, 2008 (66 days PBI) exhumation is illustrated in Figure 6.2 (b). C-H stretching bands in the region of 2950-2800 cm\(^{-1}\) and a =C-H stretching shoulder in the higher wavenumber region indicate free fatty acids. Unsaturated fatty acids are indicated by a moderate C=C stretching band at 1656 cm\(^{-1}\). A small quantity of salts of fatty acids is indicated by a broad fatty acid carboxylate C-O stretching band at 1543 cm\(^{-1}\). However, the spectrum lacks a band attributable to triglycerides (~1740 cm\(^{-1}\)). The samples are in early stage decomposition (autolysis).

Tissue samples collected from the Springwater site during the July 22, 2009 (346 days PBI) exhumation are represented by the characteristic infrared spectrum in Figure 6.2 (c). Similar to the October, 2008 samples, free fatty acids are indicated by strong C-H stretching bands in the region of 2950-2800 cm\(^{-1}\) and a =C-H stretching shoulder in the higher wavenumber region. A weak C=C stretching band at 1652 cm\(^{-1}\) indicates unsaturated fatty acids. A broad fatty acid carboxylate C-O stretching band at 1539 cm\(^{-1}\) indicates small quantities of salts of fatty acids. Additionally, a fatty acid C-O stretching band indicating free fatty acids is observed at 1708 cm\(^{-1}\), suggesting further lipid degradation.
Figure 6.2 (d) illustrates the infrared spectrum for the tissue samples collected from the Springwater site during the October 16, 2009 (432 days PBI) exhumation. Strong C-H stretching bands in the 2950-2800 cm\(^{-1}\) region and a \(=\text{C-H}\) stretching shoulder in the higher wavenumber region indicates free fatty acids. In contrast to the July, 2009 sample, the spectrum lacks the C=C stretching band relating to unsaturated fatty acids. Free fatty acids are indicated by a weak fatty acid C-O stretching band at 1717 cm\(^{-1}\). A broad fatty acid carboxylate C-O stretching band at 1569 cm\(^{-1}\) indicates small quantities of salts of fatty acids. The preliminary lipid profile indicates that the samples are in later stage decomposition (putrefaction), with little change from the July, 2009 exhumation period to the final exhumation period. Adipocere had not formed by the completion of this experiment.
Figure 6.2, plots (a-d) – Characteristic infrared spectra of Springwater tissue samples (a) control, (b) October 15, 2008 (66 days PBI) exhumation, (c) July 22, 2009 (346 days PBI) exhumation and (d) October 16, 2009 (432 days PBI) exhumation. Lines represent: (1,2) C-H stretching (2950-2800 cm$^{-1}$ region), (3) triglyceride C=O stretching (~1740 cm$^{-1}$), (4) fatty acid C-O stretching (1720-1700 cm$^{-1}$ region) and (5,6) fatty acid carboxylate C-O stretching (1576-1540 cm$^{-1}$ region).
6.3.1.4 Dummer

Figure 6.3, plots (a-d) illustrates the infrared spectra of representative tissue samples collected from the Dummer field site during the three exhumation periods and a representative control sample. Figure 6.3 (a) illustrates the characteristic infrared spectrum of control tissue samples collected from a pig carcass that was still considered fresh. Refer to Section 6.3.1.2 for spectral interpretation.

Tissue samples collected from the Dummer site during the October 16, 2008 (67 days PBI) exhumation are represented by the characteristic infrared spectrum in Figure 6.3 (b). C-H stretching bands in the region of 2950-2800 cm\(^{-1}\), and a \(=\)C-H stretching shoulder in the higher wavenumber region indicate free fatty acids. A weak C=C stretching band at 1647 cm\(^{-1}\) indicates unsaturated fatty acids. A weak fatty acid C-O stretching band at 1704 cm\(^{-1}\) indicates free fatty acids. Two moderate fatty acid carboxylate C-O stretching bands in the 1576-1540 cm\(^{-1}\) region indicate salts of fatty acids. The samples are in early stage decomposition (autolysis).

Figure 6.3 (c) illustrates the infrared spectrum of the tissue samples collected from the Dummer site during the July 23, 2009 (347 days PBI) exhumation. Similar to the October, 2008 spectrum, C-H stretching bands in the 2950-2800 cm\(^{-1}\) region and a \(=\)C-H shoulder in the higher wavenumber region indicate free fatty acids. However, the C=C stretching band, indicating unsaturated fatty acids, at 1626 cm\(^{-1}\) has decreased. Two moderate fatty acid carboxylate C-O stretching bands at 1573 and 1534 cm\(^{-1}\) indicate salts of fatty acids. The fatty acid C-O stretching band at 1708 cm\(^{-1}\) indicating free fatty acids has remained weak, suggesting that little degradation of the lipid products has taken place since the October, 2008 exhumation period.

The characteristic infrared spectrum of the tissue samples collected from the Dummer site during the October 23, 2009 (439 days PBI) exhumation is illustrated in
Figure 6.3 (d). Strong C-H stretching bands in the region of 2950-2800 cm$^{-1}$ and a $\equiv$C-H shoulder in the higher wavenumber region indicate free fatty acids. A weak fatty acid C-O stretching band at 1708 cm$^{-1}$ indicates free fatty acids. Two strong fatty acid carboxylate C-O stretching bands at 1573 and 1534 cm$^{-1}$ indicate salts of fatty acids, suggesting that the samples are in later stage decomposition (putrefaction) with no adipocere formation.
Figure 6.3, plots (a-d) – Characteristic infrared spectra of Dummer tissue samples (a) control, (b) October 16, 2008 (67 days PBI) exhumation, (c) July 23, 2009 (347 days PBI) exhumation and (d) October 23, 2009 (439 days PBI) exhumation. Lines represent: (1,2) C-H stretching (2950-2800 cm\(^{-1}\) region), (3) triglyceride C=O stretching (~1740 cm\(^{-1}\)), (4) fatty acid C-O stretching (1720-1700 cm\(^{-1}\) region) and (5,6) fatty acid carboxylate C-O stretching (1576-1540 cm\(^{-1}\) region).
6.3.2 Gas Chromatography – Mass Spectrometry (GC-MS)

Gas chromatography – mass spectrometry (GC-MS) analysis of the tissue samples collected from the pig carcasses during each exhumation period was conducted to quantify the fatty acid composition of myristic, palmitic, palmitoleic, stearic and oleic acids. These fatty acids were used to estimate the stage of decomposition and the post burial interval (PBI) of the carcasses based upon soil texture. Figure 6.4 illustrates a representative gas chromatogram of a tissue sample collected from a carcass from the Nashville site during the October 15, 2008 exhumation. The fatty acids eluted from the column in the following order; myristic acid (retention time: 10.525 min.), palmitic acid (retention time: 11.941 min.), palmitoleic acid (retention time: 12.239 min.), stearic acid (retention time: 13.281 min.), oleic acid (retention time: 13.505 min.) and nonadecanoic acid was used as an internal standard (retention time: 13.912 min.). All acids were analyzed as trimethylsilyl esters in order to increase volatility.
Figure 6.4 – Representative total ion count (TIC) gas chromatogram of a tissue sample collected from a carcass at the Nashville site during the October 15, 2008 (66 days PBI) exhumation period showing the retention times of myristic (10.525 min), palmitic (11.941 min), palmitoleic (12.239 min), stearic (13.281 min), oleic (13.505 min) and nonadecanoic (13.912 min) fatty acids in trimethylsilyl ester form.
Overall, there was a difference in the peak area ratio (area/int std area) (PAR) of each identified fatty acid, excluding palmitoleic acid, during the three exhumation periods and between fatty acids at the Nashville site (Figure 6.5). The saturated fatty acids palmitic (C_{16:0}) and stearic (C_{18:0}) were the most abundant fatty acids within the tissue samples.

Figure 6.5 – Mean peak area ratio (area/int std area) of myristic, palmitic, palmitoleic, stearic and oleic fatty acids at Nashville by exhumation period.
• **Myristic Acid**

There was a significant increase in the PAR of myristic acid in the tissue samples collected from the Nashville site (p = 0.001) (Figure 6.6). The PAR of the tissue samples collected during the second exhumation period (July 22, 2009 – 346 days PBI; 6.889 PAR) was significantly higher than during the first exhumation period (October 15, 2008 – 66 days PBI; 1.495 PAR). Furthermore, the PAR of the tissue samples during the third exhumation period (October 16, 2009 – 432 days PBI; 12.211 PAR) was significantly higher than both the first and third exhumation periods.

Figure 6.6 – Mean difference of peak area ratio (area/int std area) of myristic acid at Nashville by exhumation period. (Unbalanced ANOVA, $F_{(2, 25)} = 9.32$, p 0.001).
• **Palmitic Acid**

The change in PAR of palmitic acid in the tissue samples collected from the Nashville site was significant (p = 0.006) (Figure 6.7). The PAR of the tissue samples collected during the second exhumation period (July 22, 2009 – 346 days PBI; 41.589 PAR) was significantly higher than both the first (October 15, 2008 – 66 days PBI; 18.775 PAR) and third exhumation periods (October 16, 2009 – 432 days PBI; 21.921 PAR).

![Figure 6.7 – Mean difference of peak area ratio (area/int std area) of palmitic acid at Nashville by exhumation period. (Unbalanced ANOVA, F(2, 25) = 6.35, p 0.006).](image-url)
• **Palmitoleic Acid**

There was not a significant difference ($p = 0.967$) in the PAR of palmitoleic acid in the tissue samples collected from the Nashville site (Figure 6.8).

![Chart](image_url)

Figure 6.8 – Mean difference of peak area ratio (area/int std area) of palmitoleic acid at Nashville by exhumation period. (Unbalanced ANOVA, $F_{(2, 25)} = 0.03$, $p 0.967$).
• Stearic Acid

Overall, there was a significant difference (p < 0.001) in the PAR of stearic acid in the tissue samples collected from the Nashville site (Figure 6.9). The PAR’s of the tissue samples collected from both the second (July 22, 2009 – 346 days PBI; 31.775 PAR) and third (October 16, 2009 – 432 days PBI; 26.086 PAR) exhumation periods were significantly higher than the first exhumation period (October 15, 2008 – 66 days PBI; 11.000 PAR). However, there was not a significant difference between the second and third exhumation periods.

Figure 6.9 – Mean difference of peak area ratio (area/int std area) of stearic acid at Nashville by exhumation period. (Unbalanced ANOVA, F(2, 25) = 16.45, p <0.001).
• *Oleic Acid*

The change in PAR of oleic acid in the tissue samples collected from the Nashville site was significant ($p = 0.045$) (Figure 6.10). The PAR of the tissue samples collected during the second exhumation period (July 22, 2009 – 346 days PBI; 15.393 PAR) was significantly higher than both the first (October 15, 2008 – 66 days PBI; 6.549 PAR) and third (October 16, 2009 – 432 days PBI; 8.512 PAR) exhumation periods. However, the first and third exhumation periods were not significantly different from one another.

![Figure 6.10 – Mean difference of peak area ratio (area/int std area) of oleic acid at Nashville by exhumation period. (Unbalanced ANOVA, $F_{(2, 25)} = 3.55$, $p = 0.045$).](image-url)
Overall, the tissue samples collected from the Nashville site exhibited GC-MS lipid degradation profiles consistent with later stage decomposition (putrefaction) during the third, and final, exhumation period (October 16, 2009 – 432 Days PBI), as evidenced by the significantly higher PAR’s of the saturated fatty acids; myristic, palmitic and stearic. These results are consistent with infrared (IR) spectroscopy results (see Section 6.3.1.2).

**6.3.2.2 Springwater**

Overall, there was a difference in the PAR of each identified fatty acid in the tissue samples, excluding palmitoleic acid, during the three exhumation periods and between fatty acids at the Springwater site (Figure 6.11). Similar to the Nashville site, the fatty acids palmitic (C\textsubscript{16:0}) and stearic (C\textsubscript{18:0}) were the most abundant fatty acids in the tissue samples.

![Figure 6.11](image_url)  
*Figure 6.11 – Mean peak area ratio (area/int std area) of myristic, palmitic, palmitoleic, stearic and oleic fatty acids at Springwater by exhumation period.*
• **Myristic Acid**

The change in PAR of myristic acid in the tissue samples collected from the Springwater site was, overall, significant \((p = 0.006)\) (Figure 6.12). The PAR of the tissue samples was significantly higher during the third exhumation period (October 16, 2009 – 432 days PBI; 8.143 PAR) than both the first (October 15, 2008 – 66 days PBI; 1.620 PAR) and second (July 22, 2009 – 346 days PBI; 2.163 PAR) exhumation periods. However, exhumation periods one and two were not significantly different from one another.

![Figure 6.12](image-url)

**Figure 6.12** – Mean difference of peak area ratio (area/int std area) of myristic acid at Springwater by exhumation period. (Unbalanced ANOVA, \(F_{(2, 23)} = 6.48, p 0.006\).
• **Palmitic Acid**

There was not a significant difference ($p = 0.542$) in PAR of palmitic acid in the tissue samples collected from the Springwater site (Figure 6.13).

![Graph showing peak area ratio (area/int std area) of palmitic acid at Springwater by exhumation period.](image)

**Figure 6.13** – Mean difference of peak area ratio (area/int std area) of palmitic acid at Springwater by exhumation period. (Unbalanced ANOVA, $F_{(2, 3)} = 0.63$, $p = 0.542$).
• **Palmitoleic Acid**

Overall, there was a significant difference ($p = 0.010$) in the PAR of palmitoleic acid in the tissue samples collected from Springwater site (Figure 6.14). The PAR of the tissue samples collected during exhumation period three (October 16, 2009 – 432 days PBI; 0.6168 PAR) was significantly lower than both the first (October 15, 2008 – 66 days PBI; 1.4404 PAR) and second (July 22, 2009 – 346 days PBI; 1.3911 PAR) exhumation periods, which were not significantly different from one another.

Figure 6.14 – Mean difference of peak area ratio (area/int std area) of palmitoleic acid at Springwater by exhumation period. (Unbalanced ANOVA, $F_{(2, 23)} = 5.82, p 0.010$).
• *Stearic Acid*

Overall, there was not a significant difference (p = 0.107) in the PAR of stearic acid in the tissue samples collected from Springwater site (Figure 6.15). However, the PAR of the tissue samples was significantly higher during the third exhumation period (October 16, 2009 – 432 days PBI; 24.279 PAR) as compared to the first (October 15, 2008 – 66 days PBI; 12.668 PAR) exhumation period.

![Graph](image)

**Figure 6.15 –** Mean difference of peak area ratio (area/int std area) of stearic acid at Springwater by exhumation period. (Unbalanced ANOVA, $F_{[2, 23]} = 2.49$, p 0.107).
• **Oleic Acid**

There was no significant difference (p = 0.397) in the PAR of oleic acid in the tissue samples collected from the Springwater site (Figure 6.16).

![Graph showing mean difference of peak area ratio (area/int std area) of oleic acid at Springwater by exhumation period. (Unbalanced ANOVA, F_{2,23} = 0.97, p 0.397).](image)

During the final exhumation period, the tissue samples collected from the Springwater site exhibited GC-MS lipid degradation profiles consistent with later stage decomposition (putrefaction), as evidenced by the significantly lower PAR’s in the unsaturated fatty acid palmitoleic and significantly higher PAR’s in the saturated fatty acids myristic and stearic. These results are similar to the Nashville site, and consistent with infrared (IR) spectroscopy results (Section 6.3.1.3).
6.3.2.3 Dummer

Overall, there was a difference in the PAR of each identified fatty acid in the tissue samples, excluding palmitoleic and oleic acids, during the three exhumation periods and between fatty acids at the Dummer site (Figure 6.11).

![Bar chart showing the mean peak area ratio (area/int std area) of myristic, palmitic, palmitoleic, stearic and oleic fatty acids at Dummer by exhumation period.](image)

Figure 6.17 – Mean peak area ratio (area/int std area) of myristic, palmitic, palmitoleic, stearic and oleic fatty acids at Dummer by exhumation period.
• *Myristic Acid*

There was a significant overall difference (p < 0.001) in the PAR of myristic acid in the tissue samples collected from the Dummer site (Figure 6.18). The PAR’s of the tissue samples collected during the second (July 23, 2009 – 347 days PBI; 24.3 PAR) and third (October 23, 2009 – 439 days PBI; 23.7 PAR) exhumation periods were significantly higher than the first exhumation period (October 16, 2008 – 67 days PBI; 1.6 PAR), but were not significantly different from one another.

![Graph of peak area ratio (area/int std area) of myristic acid at Dummer by exhumation period.](image)

Figure 6.18 – Mean difference of peak area ratio (area/int std area) of myristic acid at Dummer by exhumation period. (Unbalanced ANOVA, F(2, 26) = 12.11, p <0.001).
• *Palmitic Acid*

There was not a significant difference ($p = 0.414$) in the PAR of palmitic acid in the tissue samples collected from the Dummer site (Figure 6.19).

Figure 6.19 – Mean difference of peak area ratio (area/int std area) of palmitic acid at Dummer by exhumation period. (Unbalanced ANOVA, $F_{(2, 26)} = 0.92$, $p = 0.414$).
- **Palmitoleic Acid**

Overall, there was not a significant difference \((p = 0.086)\) in the PAR of palmitoleic acid in the tissue samples collected from the Dummer site (Figure 6.20). However, the PAR of the tissue samples was significantly higher during the third (October 23, 2009 – 439 days PBI; 2.86 PAR) exhumation period as compared to the first (October 16, 2008 – 67 days PBI; 1.09 PAR) exhumation period.

Figure 6.20 – Mean difference of peak area ratio (area/int std area) of palmitoleic acid at Dummer by exhumation period. (Unbalanced ANOVA, \(F_{(2, 26)} = 2.72, p 0.086\).
• Stearic Acid

There was a significant difference \( (p = 0.022) \) in the PAR of stearic acid in the tissue samples collected from the Dummer site (Figure 6.21). The PAR's of the tissue samples collected during both the second (July 23, 2009 – 347 days PBI; 27.5 PAR) and the third (October 23, 2009 – 439 days PBI; 30.2 PAR) exhumation periods were significantly higher than the first exhumation period (October 16, 2008 – 67 days PBI; 10.8 PAR), but were not significantly different from each other.

![Figure 6.21 – Mean difference of peak area ratio (area/int std area) of stearic acid at Dummer by exhumation period. (Unbalanced ANOVA, \( F_{2, 26} = 4.47, p 0.022 \).)
• **Oleic Acid**

There was not a significant difference ($p = 0.929$) in the PAR of oleic acid in the tissue samples collected from the Dummer site (Figure 6.22).

![Graph showing mean difference of peak area ratio (area/int std area) of oleic acid at Dummer by exhumation period. (Unbalanced ANOVA, $F(2, 26) = 0.07$, $p = 0.929$).](image)

The tissue samples collected from the Dummer site exhibited GC-MS lipid degradation profiles consistent with later stage decomposition (putrefaction) during the third exhumation period (October 23, 2009 – 439 Days PBI), as evidenced by significantly higher PAR’s in the saturated fatty acids myristic and stearic. These results are similar to both the Nashville and Springwater sites, and are consistent with infrared (IR) spectroscopy results (Section 6.3.1.4).
6.4 Discussion

In this study, tissue samples collected from buried domestic pig (*Sus scrofa*) carcasses were analyzed by diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) and gas chromatography – mass spectrometry (GC-MS) to determine the stage of decomposition and the post burial interval (PBI) of the carcasses based upon soil texture. Carcasses were buried at three field sites; Nashville, Springwater and Dummer, in three contrasting soil textures; silty clay loam, fine sand and fine sandy loam, respectively. Additionally, the carcasses were buried in 100% cotton t-shirts and 50% cotton/50% polyester briefs, in order to more closely represent a forensic scenario involving a body.

Infrared (IR) spectroscopy analysis of the tissue samples revealed that during the first exhumation period (October 15, 2008 – 66 days PBI for Nashville and Springwater; October 16, 2008 – 67 days PBI for Dummer) the samples, regardless of soil texture, were in early stage decomposition (autolysis). During the second exhumation period (July 22, 2009 – 346 days PBI for Nashville and Springwater; July 23, 2009 – 347 days PBI for Dummer) the tissue samples collected and analyzed from the Nashville and Springwater sites exhibited lipid profiles consistent with further degradation leading to putrefaction. However, the tissue samples from the Dummer site exhibited little change from the first exhumation period, indicating that the carcasses buried at this site were still in early stage decomposition (autolysis). By the final exhumation period (October 16, 2009 – 432 days PBI for Nashville and Springwater; October 23, 2009 – 439 days PBI for Dummer) the tissue samples collected from all three sites had progressed through the decomposition process and exhibited lipid degradation profiles consistent with later stage decomposition (putrefaction), but not consistent with adipocere formation.

The IR spectroscopy results obtained in this study are similar to those published by Forbes et al. (2005b). Forbes et al. (2005b) buried domestic pig (*Sus scrofa*) adipose
tissue in loamy sand soil microcosms to act as a control, and altered burial conditions, such as cold versus warm temperatures, for the experimental parameters. Samples were collected and analyzed after a period of 12 months (Forbes et al., 2005b), a PBI interval which is comparable to the current study. The control samples exhibited a lipid profile consistent with adipocere formation; fatty acid C-O stretching (~1700 cm\(^{-1}\)), fatty acid carboxylate C-O stretching (1576-1540 cm\(^{-1}\) region) indicating salts of fatty acids and C-H stretching bands (2950-2800 cm\(^{-1}\) region). The fact that the control samples exhibited lipid degradation profiles consistent with adipocere formation after only 12 months (Forbes et al., 2005b), whereas the current study did not exhibit profiles consistent with adipocere formation after 14 months of burial may be explained by the fact that the burials in the current study were located in the field and therefore underwent a freeze-thaw cycle. Southern Ontario can experience temperatures as low as -35.0°C during the winter season (Environment Canada). Temperatures below freezing halt decomposition, and therefore, slow progression through decomposition stages. This idea is supported by the results obtained from the cold burial environment (Forbes et al., 2005b). An abundance of triglycerides (~1740 cm\(^{-1}\)), C=C stretching (1680-1600 cm\(^{-1}\) region) indicating unsaturated fatty acids and weak fatty acid carboxylate C-O stretching (1576-1540 cm\(^{-1}\) region) indicating small quantities of salts of fatty acids all suggest that minimal decomposition of the adipose tissue took place in the cold burial environment (Forbes et al., 2005b). Further evidence to support the effects of temperature on the rate of carcass decomposition in soil was presented by both Carter and Tibbett (2006, 2008c), in that a reduction in temperature slowed the rate of decomposition and prevented the observation of later stage decomposition, respectively.

The presence of clothing on the carcasses in the current study did not result in the formation of adipocere, as indicated by the IR spectroscopy lipid degradation profiles. This is in contrast to the suggestion that the presence of clothing is conducive to adipocere formation of tissue samples (Mant, 1987; Mellen, 1993). However, the
results of the current study are supported by Forbes et al. (2005a), in that adipocere formation did not occur from tissue samples wrapped in cotton clothing and buried directly in soil. This may be explained by the limited life expectancy of cotton materials in an organic environment (Forbes et al., 2005a). Alternatively, tissue samples wrapped in polyester clothing and buried directly in soil did exhibit adipocere formation, possibly due to the ability of the polyester to retain moisture (Forbes et al., 2005a). Although adipocere formation due to the presence of polyester did not take place in the current study, it is possible that with a longer PBI (i.e. had the duration of the study been increased), formation would have occurred. Also, the briefs used to clothe the carcasses consisted of a cotton/polyester blend (50/50%), and therefore, results are not directly comparable with the Forbes et al. (2005a) study.

Tissue samples were also analyzed by GC-MS to quantify fatty acid composition. Well formed adipocere generally contains the following fatty acids in order of highest to lowest concentration; palmitic, stearic, myristic, 10-hydroxystearic and oleic acid (Forbes et al., 2005a). In the tissue samples analyzed from all three field sites, palmitic and stearic acids were present in the highest ratios, followed by myristic acid. Forbes et al. (2005a) found that the only unsaturated fatty acid regularly detected in small quantities in adipocere samples was oleic acid. In the current study, both palmitoleic and oleic acids were detected in the tissue samples collected from all three field sites. The fact that palmitic acid alone did not account for approximately half of the concentration of fatty acids in the tissue samples (Forbes et al., 2005a) by the final exhumation period, and that the unsaturated fatty acids palmitoleic and oleic had not undergone complete hydrogenation to yield their saturated fatty acid counterparts, palmitic and stearic acid, respectively, indicates that adipocere formation did not take place and that the tissue samples are in later stage decomposition (putrefaction). The GC-MS results support the results obtained from the IR spectroscopy lipid profiles.
With respect to quantifying fatty acid composition in tissue samples as a means of estimating the post burial interval (PBI), myristic acid was the most useful acid in the three soil textures tested. The peak area ratio (area/int std area) (PAR) of myristic acid was significantly higher during the third exhumation period as compared to the first exhumation period. Stearic acid also showed potential in estimating PBI, as the PAR of this acid was significantly higher during the third exhumation period than the first exhumation period. These two acids, used in combination, may allow investigators to estimate between a shorter PBI, approximately two months, or a longer PBI, approximately 10 months to over one year.

6.5 Conclusion

This study was conducted to determine the effect of soil texture on the degradation of tissue samples collected from buried domestic pig (Sus scrofa) carcasses clothed in 100% cotton t-shirts and 50% cotton/50% polyester briefs. Regardless of soil texture, all of the carcasses reached later stage decomposition (putrefaction) by the final exhumation period, 14 months post burial interval (PBI), as determined by diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) and gas chromatography – mass spectrometry (GC-MS). Myristic and stearic acids may be useful fatty acids for the estimation of PBI, as the peak area ratios (area/int std area) (PAR) of these two fatty acids were significantly higher during the third exhumation period as compared to the first exhumation period.
Chapter 7 – Textile Analysis
7.1 Introduction

The presence of clothing on a decomposing body may alter the rate of decomposition. Clothing serves to retard decomposition in covered areas, inhibit the effects of the surrounding soil environment and encourage the formation of adipocere (Galloway et al., 1989; Komar, 1998; Weitzel, 2005; Forbes, 2008). Textiles commonly used in current society include pure cotton and cotton/polyester blends (Shepherd, 1969; Janaway, 2008).

The ability of forensic investigators to make estimations regarding the post mortem interval (PMI) and/or post burial interval (PBI) by analysis of a wide variety of samples is important. Bodies can be discovered years after death, long after soft tissue has decomposed. The analysis of clothing associated with a decomposed body may allow investigators to estimate the PMI and/or PBI as a result of the composition of fatty acids that leached into the textile during soft tissue degradation. Fatty acid degradation, leading to adipocere formation, follows a known sequence of events (Forbes et al., 2004), which can be used to estimate decomposition stages. In addition to fatty acid analysis of textile materials, the degradation of the textile itself may provide clues regarding the PMI and/or PBI. The accurate establishment of PMI and/or PBI can aid investigators in identifying a body based upon missing person’s reports.

Little published literature is available regarding the analysis of fatty acids and adipocere formation on clothing. Murthy et al. (1985) investigated the use of attenuated total reflectance – Fourier transform infrared (ATR-FTIR) spectroscopy to analyze triglycerides that had been deposited onto cotton fabric. Repeated analysis of the same sample often resulted in widely differing absorbances, indicating non-uniform triglyceride distribution on the fabric surface (Murthy et al., 1985). Alternatively, studies investigating the degradation of textile materials as a result of the burial environment have been conducted (Janaway, 2002; Wilson et al., 2007). Untreated, undyed cotton
can show decay in as little as two weeks in soil and will not survive in acidic burial conditions (Morse and Dailey, 1983; Janaway, 2002). In contrast, cotton/polyester blend textile has a low level of water absorbency, is not attacked by moth larvae, is resistant to microbial activity, and therefore, shows considerable resistance to degradation based upon the soil environment (Janaway, 2008).

7.2 Materials and Methods

In the current study, domestic pig (Sus scrofa) carcasses were clothed in 100% cotton t-shirts and 50% cotton/50% polyester briefs prior to burial in three contrasting soil textures. During set exhumation intervals, textile samples were collected for analysis of fatty acid content and adipocere formation by attenuated total reflectance – Fourier transform infrared (ATR-FTIR) spectroscopy and gas chromatography – mass spectrometry (GC-MS). The applicability of using textile samples as a means of estimating the stage of decomposition and the post burial interval (PBI) of the carcasses based upon fatty acids that had leached from the soft tissue into the clothing during decomposition was investigated.

7.2.1 Attenuated Total Reflectance – Fourier Transform Infrared (ATR-FTIR) Spectroscopy

7.2.1.1 Sample Preparation – Method Development

In order to qualitatively analyze fatty acids that had leached from the decomposing tissue of the pig carcasses into the textile samples, the use of attenuated total reflectance – Fourier transform infrared (ATR-FTIR) spectroscopy was employed. Textile samples collected during the three exhumation periods were prepared for analysis by air-drying them in a fume hood, and removing any adhering tissue, soil or hair. Original method development consisted of cutting 4 X 4 cm sections of the prepared textile samples, which were visibly stained with decomposition fluid, and
analyzing them directly on the ATR-FTIR spectroscopy crystal with force applied. It was determined that direct analysis of the samples was not sufficient, as infrared bands attributable to fatty acid degradation and adipocere formation were not identified in the spectra.

Next, a fatty acid extraction method was adapted from Folch et al. (1957). Four x four cm sections of the prepared textile samples were cut-out of the t-shirt and briefs. The removed textile squares were placed into individually labelled scintillation vials, to which 8 mL of chloroform was added as an extraction solvent. The samples were then sonicated using a Fisher Scientific Ultrasonic Cleaner FS110D for 30 minutes, vortexed using a Fisher Scientific Vortex Mixer for 2 minutes, and left for 12 hours at 4°C in order to extract the fatty acids. Following extraction, the textile squares were removed from the scintillation vials and discarded. The extracted solutions were then transferred onto glass slides using disposable glass Pasteur pipettes and allowed to dry to a film (Craft, 2004). Samples were then immediately analyzed for qualitative fatty acid content using ATR-FTIR spectroscopy. Due to the fact that the glass slides on which the extracted fatty acids had been deposited were masking bands of interest in the spectra, the use of glass slides was deemed to be inappropriate. The extraction method adapted from Folch et al. (1957) was followed for all subsequent method development steps.

In order to remove the glass slides from the analysis procedure, the possibility of depositing the extracted fatty acids directly onto the ATR-FTIR spectroscopy crystal was investigated. However, this option was quickly discarded, as the chloroform used within the extraction procedure could not come into contact with the glue that held the crystal in place within the instrument. The solvent would dissolve the glue, thus creating the potential for the crystal to become dislodged from the instrument and lost.

To remove the chloroform, the extracted fatty acid solutions were transferred to disposable glass tubes using disposable glass Pasteur pipettes and evaporated to
dryness using a Thermo Electron Corporation Savant SC210A SpeedVac Concentrator. Samples were dried for 36 hours until only a fatty acid film remained in the bottom of the tubes. The films were then scraped from the tubes, deposited directly onto the ATR-FTIR spectroscopy crystal and immediately analyzed with force applied. Following sample analysis, it was determined that this method was successful, as bands attributable to fatty acid degradation and adipocere formation were present in the spectra. This method was used for all subsequent infrared (IR) spectroscopy analysis of textile samples. All reagents used for ATR-FTIR spectroscopy textile sample preparation were the same as those listed in Section 6.2.1 of Chapter 6 – Tissue Analysis.

7.2.1.2 Instrumental Sample Analysis

Prepared textile samples were qualitatively analyzed for fatty acid composition using a Nicolet 4700 Fourier transform infrared dual-beam spectrometer equipped with a Nicolet Smart Performer accessory. Samples were placed onto the ATR-FTIR spectroscopy crystal and force was applied. Each sample was analyzed using the following instrumental parameters; 64 scans over a frequency range of 4000 – 500 cm\(^{-1}\) to provide a resolution of 4 cm\(^{-1}\) (Stuart et al., 2000; Forbes et al., 2005 a,b). Following analysis of each sample, the crystal was cleaned with acetone to remove fatty acid residue.

7.2.2 Gas Chromatography – Mass Spectrometry (GC-MS)

7.2.2.1 Sample Preparation

Textile samples collected during the three exhumation periods were cleaned and prepared following a method adapted from Folch et al. (1957) in order to extract fatty acids (Section 7.2.1.1). 1.8 mL of the extracted fatty acid solutions were transferred to sterilized screw-top culture tubes, to which 0.2 mL of N,O-Bis(trimethylsilyl)
trifluoroacetamide with 1% trimethylchlorosilane (BSTFA + TMCS) was added. The samples were then vortexed for 1 minute using a Fisher Scientific Vortex Mixture. The samples were heated for 15 minutes at 70°C using a Fisher Scientific Isotemp heating block. Upon cooling, the samples were transferred to gas chromatography vials for analysis using disposable glass Pasteur pipettes. The method used was adapted from Forbes et al. (2003). All reagents and standards used for gas chromatography – mass spectrometry (GC-MS) textile sample preparation and analysis were the same as those outlined in Section 6.2.1 and 6.2.2 of Chapter 6 – Tissue Analysis, respectively. Textile samples were analyzed by GC-MS following the instrumental parameters described in Section 6.2.4.2 of Chapter 6 – Tissue Analysis.

7.2.3 Statistical Analysis

Statistical analysis was conducted using SPSS Statistics 17.0 software. Two-way analysis of variance (ANOVA) was conducted to determine significance between peak area ratio (area/int std area) (PAR) of fatty acids in textile samples based upon the following parameters; fatty acid type, textile sample type, burial duration and soil texture.
7.3 Results

7.3.1 Degradation of Control Textile

In order to investigate the rate of degradation of the 100% cotton t-shirts and 50% cotton/50% polyester briefs in the soil textures tested, control textile samples were buried and exhumed following the same schedule as the pig carcasses. Figure 7.1a-c illustrates representative control textile exhumed from the Springwater and Dummer field sites, which were also representative of the control textile exhumed from the Nashville site. In contrast to the other two field sites, the control t-shirt was recovered from the Springwater site during the third exhumation period.

Figure 7.1a illustrates representative control textile exhumed at Springwater during the October 15, 2008 (66 days PBI) exhumation period. The 100% cotton t-shirt had experienced partial degradation. Alternatively, the 50% cotton/50% polyester briefs had remained intact and were in good condition.

Control textile exhumed from the Dummer site during the July 23, 2009 (347 days PBI) exhumation are represented in Figure 7.1b. The 100% cotton t-shirt had degraded considerably, with only the lower torso portion of the t-shirt remaining. Similar to the October, 2008 exhumation period, the 50% cotton/50% polyester briefs had remained intact and were in good condition.

During the final exhumation period at the Dummer site, October 23, 2009 (439 days PBI), only the control 50% cotton/50% polyester briefs were recovered, which were intact and in good condition (Figure 7.1c). The 100% cotton t-shirt had fully degraded. In contrast to both the Nashville and Dummer field sites, the t-shirt was recovered from the Springwater site during the third exhumation period (photograph not available), and exhibited considerable degradation.
Figure 7.1a-c – Representative degradation of exhumed control 100% cotton t-shirts and 50% cotton/50% polyester briefs (a) Springwater site during October 15, 2008 (66 days PBI) exhumation; partially degraded t-shirt/intact briefs (b) Dummer site during July 23, 2009 (347 days PBI) exhumation; considerably degraded t-shirt/intact briefs and (c) Dummer site during October 23, 2009 (439 days PBI) exhumation; complete degradation of t-shirt/intact briefs.
### 7.3.2 Degradation of Experimental Textile

The experimental 100% cotton t-shirts and 50% cotton/50% polyester briefs covering the carcasses did not degrade and were intact during each of the three exhumation periods in all three of the soil textures tested. Figure 7.2a-b illustrates representative experimental textile exhumed from the Dummer field site, which were also representative of the experimental textile exhumed from the Nashville and Springwater sites.

Experimental textile exhumed from the Dummer site during the October 16, 2008 (67 days PBI) exhumation are represented in Figure 7.2a. The 100% cotton t-shirt had not degraded, nor had the 50% cotton/50% polyester briefs. Both of the textile sample types were intact.

Figure 7.2b illustrates representative experimental textile exhumed from the Dummer site during the July 23, 2009 (347 days PBI) exhumation period. Similar to the October, 2008 exhumation period, neither of the experimental textile sample types had degraded and both were intact.

A photograph illustrating experimental textile degradation for the October 23, 2009 (439 days PBI) exhumation period is not available. However, both of the experimental textile sample types did not exhibit any degradation and were intact at the time of exhumation and removal from the carcasses.
Figure 7.2a-b – Representative degradation of exhumed experimental 100% cotton t-shirts and 50% cotton/50% polyester briefs from Dummer site (a) October 16, 2008 (67 days PBI) exhumation; no degradation/intact for both t-shirt and briefs and (b) July 23, 2009 (347 days PBI) exhumation; no degradation/intact for both t-shirt and briefs.
7.3.3 Attenuated Total Reflectance – Fourier Transform Infrared (ATR-FTIR) Spectroscopy

Attenuated total reflectance – Fourier transform infrared (ATR-FTIR) spectroscopy analysis of the textile samples collected from the pig carcasses during each exhumation period was conducted to produce a qualitative lipid profile and determine if adipocere had formed.

7.3.3.1 Nashville

- 100% Cotton T-Shirt

Figure 7.3, plots (a-d) illustrates the infrared spectra of representative 100% cotton t-shirt samples collected from the Nashville field site over the three exhumation periods and a representative control.

Figure 7.3 (a) illustrates the characteristic infrared spectrum of control 100% cotton t-shirt samples. The spectrum contains a strong band near 1740 cm\(^{-1}\) indicating an abundance of triglycerides. Weak C-H stretching bands are visible in the 2950-2800 cm\(^{-1}\) region.

The characteristic infrared spectrum of the 100% cotton t-shirt samples collected from the Nashville site during the October 15, 2008 (66 days PBI) exhumation is illustrated in Figure 7.3 (b). The spectrum contains a strong triglyceride C=O stretching band near 1740 cm\(^{-1}\). Moderate C-H stretching bands in the region of 2950-2800 cm\(^{-1}\) and a =C-H shoulder in the higher wavenumber (3065 cm\(^{-1}\)) of the region indicate the presence of free fatty acids. In contrast to the control sample shown in Figure 7.3 (a), a weak fatty acid carboxylate C-O stretching band near 1540 cm\(^{-1}\) indicates small
quantities of salts of fatty acids, suggesting that early stage decomposition (autolysis) of the carcasses has commenced.

100% cotton t-shirt samples collected from the Nashville site during the July 22, 2009 (346 days PBI) exhumation are represented by the characteristic infrared spectrum in Figure 7.3 (c). Again, the spectrum contains C-H stretching bands in the 2950-2800 cm\(^{-1}\) region. A weak fatty acid carboxylate C-O stretching band at 1543 cm\(^{-1}\) indicates small quantities of salts of fatty acids. In contrast to the October, 2008 samples, a triglyceride C=O stretching band near 1740 cm\(^{-1}\) is no longer present. This finding, coupled with the presence of a fatty acid carboxylate C-O stretching band at 1708 cm\(^{-1}\) (indicating free fatty acids), suggests continued degradation of the carcasses.

Figure 7.3 (d) illustrates the infrared spectrum for the 100% cotton t-shirt samples collected from the Nashville site during the October 16, 2009 (432 days PBI) exhumation. Similar to the July, 2009 samples, the spectrum contains a fatty acid C-O stretching band at 1708 cm\(^{-1}\) and C-H stretching bands in the region of 2950-2800 cm\(^{-1}\). The presence of strong bands in the 1576-1540 cm\(^{-1}\) region indicates an abundance of salts of fatty acids. The lipid profile suggests that the carcasses have progressed to the later stage of decomposition (putrefaction). However, the profile is not consistent with adipocere formation.
Figure 7.3, plots (a-d) – Characteristic infrared spectra of Nashville 100% cotton t-shirt samples (a) control, (b) October 15, 2008 (66 days PBI) exhumation, (c) July 22, 2009 (346 days PBI) exhumation and (d) October 16, 2009 (432 days PBI) exhumation. Lines represent: (1,2) C-H stretching (2950-2800 cm⁻¹ region), (3) triglyceride C=O stretching (~1740 cm⁻¹), (4) fatty acid C-O stretching (1720-1700 cm⁻¹ region) and (5,6) fatty acid carboxylate C-O stretching (1576-1540 cm⁻¹ region).
• **50% Cotton/50% Polyester Briefs**

Figure 7.4, plots (a-d) illustrates the infrared spectra of representative 50% cotton/50% polyester briefs samples collected from the Nashville field site during the three exhumation periods and a representative control.

The characteristic infrared spectrum of control 50% cotton/50% polyester briefs samples is illustrated in Figure 7.4 (a). Similar to the 100% cotton t-shirt control, the spectrum of the control briefs contains a strong band near 1740 cm\(^{-1}\) indicating an abundance of triglycerides and weak C-H stretching bands in the region of 2950-2800 cm\(^{-1}\) indicating free fatty acids.

Figure 7.4 (b) illustrates the characteristic infrared spectrum of the 50% cotton/50% polyester briefs samples collected from the Nashville site during the October 15, 2008 (66 days PBI) exhumation. The lipid profile suggests that little decomposition of the carcasses has occurred, as the spectrum is comparable to that of the control.

50% cotton/50% polyester briefs samples collected from the Nashville site during the July 22, 2009 (346 days PBI) exhumation are represented by the characteristic infrared spectrum in Figure 7.4 (c). Strong C-H stretching bands in the region of 2950-2800 cm\(^{-1}\) and a =C-H stretching shoulder in the higher wavenumber region indicate free fatty acids. A moderate fatty acid C-O stretching band indicating free fatty acids is observed at 1700 cm\(^{-1}\) and weak fatty acid carboxylate C-O stretching bands in the 1576-1540 cm\(^{-1}\) region suggest small quantities of salts of fatty acids are present. The lipid profile suggests that early stage decomposition (autolysis) of the carcasses has commenced.
Figure 7.4 (d) illustrates the infrared spectrum for the 50% cotton/50% polyester briefs samples collected from the Nashville site during the October 16, 2009 (432 days PBI) exhumation. The spectrum is similar to that of the samples collected during the July, 2009 exhumation. However, a strong fatty acid C-O stretching band is now present at 1708 cm⁻¹, as opposed to a moderate band. The lipid profile indicates that the carcasses are in later stage decomposition (putrefaction). Adipocere has not formed.
Figure 7.4, plots (a-d) – Characteristic infrared spectra of Nashville 50% cotton/50% polyester briefs samples (a) control, (b) October 15, 2008 (66 days PBI) exhumation, (c) July 22, 2009 (346 days PBI) exhumation and (d) October 16, 2009 (432 days PBI) exhumation. Lines represent: (1,2) C-H stretching (2950-2800 cm⁻¹ region), (3) triglyceride C=O stretching (~1740 cm⁻¹), (4) fatty acid C-O stretching (1720-1700 cm⁻¹ region) and (5,6) fatty acid carboxylate C-O stretching (1576-1540 cm⁻¹ region).
7.3.3.2 Springwater

- **100% Cotton T-Shirts**

Figure 7.5, plots (a-d) illustrates the infrared spectra of representative 100% cotton t-shirt samples collected from the Springwater field site during the three exhumation periods and a representative control. Figure 7.3 (a) illustrates the characteristic infrared spectrum of control t-shirt samples. Refer to Section 7.3.3.1 for spectral interpretation, as the same control t-shirt was used as a comparison for all three field sites.

100% cotton t-shirt samples collected from the Springwater site during the October 15, 2008 (66 days PBI) exhumation are represented by the characteristic infrared spectrum in Figure 7.5 (b). Moderate C-H stretching bands in the region of 2950-2800 cm⁻¹ are present. The spectrum also contains a weak fatty acid C-O stretching band at 1708 cm⁻¹ indicating free fatty acids. The lipid degradation profile suggests that the carcasses are in early stage decomposition (autolysis).

The characteristic infrared spectrum of the 100% cotton t-shirt samples collected from the Springwater site during the July 22, 2009 (346 days PBI) exhumation is illustrated in Figure 7.5 (c). Similar to the t-shirt samples collected during the October, 2008 exhumation period, the spectrum contains moderate C-H stretching bands in the 2950-2800 cm⁻¹ region and a fatty acid C-O stretching band at 1708 cm⁻¹. However, the band at 1708 cm⁻¹ is now strong, indicating an increased presence of free fatty acids and continued degradation of the lipid products of the carcasses.

Figure 7.5 (d) illustrates the infrared spectrum of the 100% cotton t-shirt samples collected from the Springwater site during the October 16, 2009 (432 days PBI) exhumation. Strong C-H stretching bands in the region of 2950-2800 cm⁻¹ and a strong
fatty acid C-O stretching band at 1708 cm\(^{-1}\) indicate free fatty acids. However, a triglyceride C=O stretching shoulder band near 1740 cm\(^{-1}\) and weak fatty acid carboxylate C-O stretching bands in the 1576-1540 cm\(^{-1}\) region (indicating small quantities of salts of fatty acids) suggest that the carcasses have not progressed to the later stage of decomposition (putrefaction). Furthermore, adipocere has not formed.
Figure 7.5, plots (a-d) – Characteristic infrared spectra of Springwater 100% cotton t-shirt samples (a) control, (b) October 15, 2008 (66 days PBI) exhumation, (c) July 22, 2009 (346 days PBI) exhumation and (d) October 16, 2009 (432 days PBI) exhumation. Lines represent: (1,2) C-H stretching (2950-2800 cm$^{-1}$ region), (3) triglyceride C=O stretching (~1740 cm$^{-1}$), (4) fatty acid C-O stretching (1720-1700 cm$^{-1}$ region) and (5,6) fatty acid carboxylate C-O stretching (1576-1540 cm$^{-1}$ region).
• **50% Cotton/50% Polyester Briefs**

Figure 7.6, plots (a-d) illustrates the infrared spectra of representative 50% cotton/50% polyester briefs samples collected from the Springwater field site during the three exhumation periods and a representative control. Figure 7.6 (a) illustrates the characteristic infrared spectrum of control briefs samples. Refer to Section 7.3.3.1 for spectral interpretation, as the same control briefs sample was used as a comparison for all three field sites.

Figure 7.6 (b) illustrates the infrared spectrum for the 50% cotton/50% polyester briefs samples collected from the Springwater site during the October 15, 2008 (66 days PBI) exhumation. Moderate C-H stretching bands in the region of 2950-2800 cm\(^{-1}\) and a weak fatty acid C-O stretching band at 1708 cm\(^{-1}\) indicate free fatty acids. The lipid profile suggests that the carcasses are in early stage decomposition (autolysis).

50% cotton/50% polyester briefs samples collected from the Springwater site during the July 22, 2009 (346 days PBI) exhumation are represented by the characteristic infrared spectrum in Figure 7.6 (c). Similar to the samples collected during the October, 2008 exhumation period, the lipid profile indicates that the carcasses have remained in early stage decomposition (autolysis). Weak C-H stretching bands in the region of 2950-2800 cm\(^{-1}\) are present. The spectrum also contains a moderate fatty acid C-O stretching band at 1704 cm\(^{-1}\) indicating free fatty acids.

The characteristic infrared spectrum of the 50% cotton/50% polyester briefs samples collected from the Springwater site during the October 16, 2009 (432 days PBI) exhumation is illustrated in Figure 7.6 (d). Strong C-H stretching bands are present in the 2950-2800 cm\(^{-1}\) region. Strong fatty acid carboxylate C-O stretching bands in the region of 1576-1540 cm\(^{-1}\) indicate an abundance of salts of fatty acids. However, the presence of a strong triglyceride C=O stretching band at 1739 cm\(^{-1}\) indicates conflicting lipid
degradation results. Based upon the lipid profile, the carcasses are still in early stage decomposition (autolysis), with no indication of adipocere formation.
Figure 7.6, plots (a-d) – Characteristic infrared spectra of Springwater 50% cotton/50% polyester briefs samples (a) control, (b) October 15, 2008 (66 days PBI) exhumation, (c) July 22, 2009 (346 days PBI) exhumation and (d) October 16, 2009 (432 days PBI) exhumation. Lines represent: (1,2) C-H stretching (2950-2800 cm⁻¹ region), (3) triglyceride C=O stretching (~1740 cm⁻¹), (4) fatty acid C-O stretching (1720-1700 cm⁻¹ region) and (5,6) fatty acid carboxylate C-O stretching (1576-1540 cm⁻¹ region).
### 7.3.3.3 Dummer

- **100% Cotton T-Shirts**

Figure 7.7, plots (a-d) illustrates the infrared spectra of representative 100% cotton t-shirt samples collected from the Dummer field site during the three exhumation periods and a representative control. Figure 7.7 (a) illustrates the characteristic infrared spectrum of control t-shirt samples. Refer to Section 7.3.3.1 for spectral interpretation, as the same control t-shirt was used as a comparison for all three field sites.

The characteristic infrared spectrum of the 100% cotton t-shirt samples collected from the Dummer site during the October 16, 2008 (67 days PBI) exhumation is illustrated in Figure 7.7 (b). Weak C-H stretching bands in the 2950-2800 cm\(^{-1}\) region are present. A strong triglyceride C=O stretching band near 1740 cm\(^{-1}\), coupled with a weak fatty acid carboxylate C-O stretching band indicating small quantities of salts of fatty acids in the 1576-1540 cm\(^{-1}\) region, indicate a lipid profile consistent with carcasses in early stage decomposition (autolysis).

100% cotton t-shirt samples collected from the Dummer site during the July 23, 2009 (347 days PBI) exhumation are represented by the characteristic infrared spectrum in Figure 7.7 (c). Free fatty acids are indicated by strong C-H stretching bands in the region of 2950-2800 cm\(^{-1}\) and a =C-H stretching shoulder in the higher wavenumber of this region. Strong fatty acid carboxylate C-O stretching bands in the region of 1576-1540 cm\(^{-1}\) indicate an abundance of salts of fatty acids. However, the remaining presence of a strong triglyceride C=O stretching band near 1740 cm\(^{-1}\) and the lack of a fatty acid C-O stretching band in the 1720-1700 cm\(^{-1}\) region suggests that the carcasses are still in early stage decomposition (autolysis).
Figure 7.7 (d) illustrates the infrared spectrum for the 100% cotton t-shirt samples collected from the Dummer site during the October 23, 2009 (439 days PBI) exhumation. Moderate C-H stretching bands in the region of 2950-2800 cm$^{-1}$ indicate free fatty acids. Moderate fatty acid carboxylate C-O stretching bands in the 1576-1540 cm$^{-1}$ region indicate the presence of salts of fatty acids. Similar to the July, 2009 samples, the lipid profile indicates that the carcasses are in early stage decomposition (autolysis). Adipocere has not formed.
Figure 7.7, plots (a-d) – Characteristic infrared spectra of Dummer 100% cotton t-shirt samples (a) control, (b) October 16, 2008 (67 days PBI) exhumation, (c) July 23, 2009 (347 days PBI) exhumation and (d) October 23, 2009 (439 days PBI) exhumation. Lines represent: (1,2) C-H stretching (2950-2800 cm\(^{-1}\) region), (3) triglyceride C=O stretching (~1740 cm\(^{-1}\)) and (4,5) fatty acid carboxylate C-O stretching (1576-1540 cm\(^{-1}\) region).
50% Cotton/50% Polyester Briefs

Figure 7.8, plots (a-d) illustrates the infrared spectra of representative 50% cotton/50% polyester briefs samples collected from the Dummer site during the three exhumation periods and a representative control. Figure 7.8 (a) illustrates the characteristic infrared spectrum of control briefs samples. Refer to Section 7.3.3.1 for spectral interpretation, as the same control briefs sample was used as a comparison for all three field sites.

50% cotton/50% polyester briefs samples collected from the Dummer site during the October 16, 2008 (67 days PBI) exhumation are represented by the characteristic infrared spectrum in Figure 7.8 (b). Strong C-H stretching bands in the 2950-2800 cm\(^{-1}\) region and a weak fatty acid C-O stretching band near 1720 cm\(^{-1}\) indicate free fatty acids. Weak fatty acid carboxylate C-O stretching bands in the region of 1576-1540 cm\(^{-1}\) indicate small quantities of salts of fatty acids. The lipid profile suggests that the carcasses are in early stage decomposition (autolysis).

Figure 7.8 (c) illustrates the infrared spectrum of the 50% cotton/50% polyester briefs samples collected from the Dummer site during the July 23, 2009 (347 days PBI) exhumation. Strong C-H stretching bands in the region of 2950-2800 cm\(^{-1}\) indicate free fatty acids. A moderate triglyceride C=O stretching band is present at 1734 cm\(^{-1}\). Strong fatty acid carboxylate C-O stretching bands in the 1576-1540 cm\(^{-1}\) region indicate an abundance of salts of fatty acids. The remaining presence of a triglyceride C=O stretching band and the lack of a fatty acid C-O stretching band in the 1720-1700 cm\(^{-1}\) region suggests that the carcasses are still in early stage decomposition (autolysis).

The characteristic infrared spectrum of the 50% cotton/50% polyester briefs samples collected from the Dummer site during the October 23, 2009 (439 days PBI) exhumation is illustrated in Figure 7.8 (d). The spectrum is comparable to that of the
control briefs samples, suggesting a lack of lipid degradation. However, torrential downpour at the time of the exhumation may have diluted any fatty acids that had leached from the tissue into the textile.
Figure 7.8, plots (a-d) – Characteristic infrared spectra of Dummer 50% cotton/50% polyester briefs samples (a) control, (b) October 16, 2008 (67 days PBI) exhumation, (c) July 23, 2009 (347 days PBI) exhumation and (d) October 23, 2009 (439 days PBI) exhumation. Lines represent: (1,2) C-H stretching (2950-2800 cm⁻¹ region), (3) triglyceride C=O stretching (~1740 cm⁻¹), (4) fatty acid C-O stretching (1720-1700 cm⁻¹ region) and (5,6) fatty acid carboxylate C-O stretching (1576-1540 cm⁻¹ region).
7.3.4 Gas Chromatography – Mass Spectrometry (GC-MS)

Gas chromatography – mass spectrometry (GC-MS) analysis of the textile samples collected from the pig carcasses during each exhumation period was conducted to quantify the fatty acid composition of myristic, palmitic, palmitoleic, stearic and oleic acids. These fatty acids were used to estimate the stage of decomposition and the post burial interval (PBI) of the carcasses based upon soil texture. Figure 7.9 illustrates a representative gas chromatogram of a tissue sample collected from a carcass from the Nashville site during the October 15, 2008 exhumation. The fatty acids eluted from the column in the following order; myristic acid (retention time: 10.525 min.), palmitic acid (retention time: 11.941 min.), palmitoleic acid (retention time: 12.239 min.), stearic acid (retention time: 13.281 min.), oleic acid (retention time: 13.505 min.) and nonadecanoic acid was used as an internal standard (retention time: 13.912 min.). All acids were analyzed as trimethylsilyl esters in order to increase volatility.
Figure 7.9 – Representative total ion count (TIC) gas chromatogram of a 100% cotton t-shirt sample collected from the Nashville site during the October 15, 2008 (66 days PBI) exhumation period showing the retention times of myristic (10.525 min), palmitic (11.941 min), palmitoleic (12.239 min), stearic (13.281 min), oleic (13.505 min) and nonadecanoic (13.912 min) fatty acids in trimethylsilyl ester form.
7.3.4.1 Nashville

Figure 7.10a-c illustrates the peak area ratio (area/int std area) (PAR) of the five identified fatty acids at the Nashville site during the three exhumation periods. The saturated fatty acids palmitic (C₁₆:0) and stearic (C₁₈:0) were the most abundant within the textile samples, followed by myristic acid.
Figure 7.10a-c – Mean peak area ratio (area/int std area) of myristic, palmitic, palmitoleic, stearic and oleic fatty acids at Nashville during (a) October 15, 2008 (66 days PBI) exhumation, (b) July 22, 2009 (346 days PBI) exhumation and (c) October 16, 2009 (432 days PBI) exhumation.
• **Myristic Acid**

Overall, there was not a significant difference (p = 0.730) in the PAR of myristic acid between the four textile sample types collected from the Nashville site (Figure 7.11). However, the PAR of both of the experimental sample types was significantly higher than the PAR of both of the control sample types. During the third exhumation period (October 16, 2009 – 432 days PBI), the PAR of the experimental t-shirt samples (28.99 PAR) was significantly higher than the PAR of the experimental briefs samples (10.71 PAR). The PAR of the experimental t-shirt samples during the second (July 22, 2009 – 346 days PBI; 35.02 PAR) and third (October 16, 2009 – 432 days PBI; 29.40 PAR) exhumation periods were both significantly higher than the first (5.20 PAR) exhumation period.

![Figure 7.11 - Mean difference of peak area ratio (area/int std area) of myristic acid at Nashville by exhumation period. (Unbalanced ANOVA, F_{Exhumation Period} (2, 58) = 0.648, p = 0.527, F_{Sample Type} (3, 58) = 1.551, p = 0.214, F_{Interaction} (5, 58) = 0.561, p = 0.730)](image-url)
• **Palmitic Acid**

There was not an overall significant difference ($p = 0.879$) in the PAR of palmitic acid between the four textile sample types collected from the Nashville site (Figure 7.12). The PAR of the experimental briefs samples was significantly higher than the PAR of the control briefs samples. The PAR of the experimental briefs samples (16.14 PAR) was also significantly higher than the PAR of the experimental t-shirt samples (4.66 PAR) during the first exhumation period (October 15, 2008 – 66 days PBI). During the third exhumation period (October 16, 2009 – 432 days PBI), the PAR of the experimental briefs samples (35.83 PAR) was significantly higher than the first (16.14 PAR) exhumation period. The PAR during both the second (July 22, 2009 – 346 days PBI; 37.25 PAR) and third (25.54 PAR) exhumation periods for the experimental t-shirt samples were significantly higher than the first (4.76 PAR) exhumation period.

![Figure 7.12](image-url)

Figure 7.12 – Mean difference of peak area ratio (area/int std area) of palmitic acid at Nashville by exhumation period. (Unbalanced ANOVA, $F_{\text{Exhumation Period}} (2, 58) = 0.452$, $p = 0.639$, $F_{\text{Sample Type}} (3, 58) = 0.847$, $p = 0.475$, $F_{\text{Interaction}} (5, 58) = 0.351$, $p = 0.879$)
• **Palmitoleic Acid**

Similar to both myristic and palmitic acids at the Nashville site, the PAR of palmitoleic acid was not significantly different ($p = 0.659$) between the four textile sample types collected (Figure 7.13). Both the PAR’s of the experimental t-shirt and briefs samples were, however, significantly higher than the PAR’s of their control counterpart samples. During the third exhumation period (October 16, 2009 – 432 days PBI), the PAR’s of both the experimental t-shirt (0.89 PAR) and briefs (0.87 PAR) samples were significantly lower than the first (October 15, 2008 – 66 days PBI; t-shirt: 2.65 PAR, briefs: 2.43 PAR) and second (July 22, 2009 – 346 days PBI; t-shirt: 3.90 PAR, briefs: 2.30 PAR) exhumation periods.

![Graph showing the mean difference of peak area ratio (area/int std area) of palmitoleic acid at Nashville by exhumation period.](image)

Figure 7.13 – Mean difference of peak area ratio (area/int std area) of palmitoleic acid at Nashville by exhumation period. (Unbalanced ANOVA, $F_{\text{Exhumation Period}} (2, 58) = 1.389$, $p = 0.259$, $F_{\text{Sample Type}} (3, 58) = 4.081$, $p = 0.012$, $F_{\text{Interaction}} (5, 58) = 0.656$, $p = 0.659$)
• **Stearic Acid**

The PAR of stearic acid at the Nashville site was not significantly different ($p = 0.866$) between the four textile sample types collected (Figure 7.14). Although, the PAR’s of both experimental sample types were significantly higher than the PAR’s of their control sample counterparts. The PAR of the experimental t-shirt samples (33.99 PAR) was significantly higher than the PAR of the experimental briefs samples (15.57 PAR) during the third exhumation period (October 16, 2009 – 432 days PBI).

![Figure 7.14 – Mean difference of peak area ratio (area/int std area) of stearic acid at Nashville by exhumation period. (Unbalanced ANOVA, $F_{Exhumation Period}(2,58) = 0.287, p = 0.752, F_{Sample Type}(3,58) = 0.971, p = 0.414, F_{Interaction}(5,58) = 0.371, p = 0.866)
• **Oleic Acid**

There was not a significant difference \( (p = 0.916) \) in the PAR of oleic acid at the Nashville site between the four textile sample types collected (Figure 7.15). The PAR’s of both of the experimental sample types were significantly higher than the PAR’s of the control sample types. During the first exhumation period (October 15, 2008 – 66 days PBI), the PAR of the experimental briefs samples (7.44 PAR) was significantly higher than the PAR of the experimental t-shirt samples (3.11 PAR). The PAR of the experimental t-shirt samples during both the second (July 22, 2009 – 346 days PBI; 17.61 PAR) and third (October 16, 2009 – 432 days PBI; 21.03 PAR) exhumation periods were significantly higher than the first (3.11 PAR) exhumation period.

![Figure 7.15 – Mean difference of peak area ratio (area/int std area) of oleic acid at Nashville by exhumation period. (Unbalanced ANOVA, \( F_{\text{Exhumation Period}} (2, 58) = 0.742, p = 0.482, F_{\text{Sample Type}} (3, 58) = 2.133, p = 0.108, F_{\text{Interaction}} (5, 58) = 0.290, p = 0.916) \)]
Overall, the textile samples collected from the Nashville site exhibited GC-MS lipid degradation profiles consistent with association with carcasses in later stage decomposition (putrefaction) during the third, and final, exhumation period (October 16, 2009 – 432 Days PBI), as evidenced by the significantly higher PAR’s in the saturated fatty acids myristic and stearic.

7.3.4.2 Springwater

Figure 7.16a-c illustrates the PAR of the five identified fatty acids at the Springwater site during the three exhumation periods. Similar to the Nashville site, the saturated fatty acids palmitic ($C_{16:0}$), stearic ($C_{18:0}$) and myristic ($C_{14:0}$) were abundant within the textile samples.
Figure 7.16a-c – Mean peak area ratio (area/int std area) of myristic, palmitic, palmitoleic, stearic and oleic fatty acids at Springwater during (a) October 15, 2008 (66 days PBI) exhumation, (b) July 22, 2009 (346 days PBI) exhumation and (c) October 16, 2009 (432 days PBI) exhumation.
• Myristic Acid

Overall, there was not a significant difference ($p = 0.766$) in PAR of myristic acid at the Springwater site between the four textile sample types collected (Figure 7.17). However, the PAR’s of both of the experimental samples were significantly higher than the PAR’s of both of the control sample types. During the first exhumation period (October 15, 2008 – 66 days PBI), the PAR of the experimental t-shirt samples (8.61 PAR) was significantly higher than the PAR of the experimental briefs samples (5.11 PAR).

![Diagram showing the mean difference of peak area ratio (area/int std area) of myristic acid at Springwater by exhumation period.](image)

**Figure 7.17** – Mean difference of peak area ratio (area/int std area) of myristic acid at Springwater by exhumation period. (Unbalanced ANOVA, $F_{\text{Exhumation Period}} (2, 58) = 1.489$, $p = 0.236$, $F_{\text{Sample Type}} (3, 58) = 9.769$, $p < 0.001$, $F_{\text{Interaction}} (4, 58) = 0.459$, $p = 0.766$)
• **Palmitic Acid**

The PAR of palmitic acid at the Springwater site was not significantly different ($p = 0.876$) between the four textile sample types collected (Figure 7.18). Although, both the PAR’s of the experimental sample types were significantly higher than the PAR’s of their control sample counterparts.

Figure 7.18 – Mean difference of peak area ratio (area/int std area) of palmitic acid at Springwater by exhumation period. (Unbalanced ANOVA, $F_{\text{Exhumation Period}} (2, 58) = 2.984, \ p = 0.060$, $F_{\text{Sample Type}} (3, 58) = 1.033, \ p = 0.386$, $F_{\text{Interaction}} (4, 58) = 0.301, \ p = 0.876$)
• **Palmitoleic Acid**

There was not a significant difference ($p = 0.875$) in the PAR of palmitoleic acid at the Springwater site between the four textile sample types collected (Figure 7.19). The PAR’s of both of the experimental samples were significantly higher than the PAR’s of the control samples.

![Graph showing the mean difference of peak area ratio (area/int std area) of palmitoleic acid at Springwater by exhumation period.](image)

*Figure 7.19 – Mean difference of peak area ratio (area/int std area) of palmitoleic acid at Springwater by exhumation period. (Unbalanced ANOVA, $F_{\text{Exhumation Period}} (2, 58) = 1.122, p = 0.334$, $F_{\text{Sample Type}} (3, 58) = 5.526, p = 0.002$, $F_{\text{Interaction}} (4, 58) = 0.329, p = 0.875$)*
• **Stearic Acid**

There was not an overall significant difference ($p = 0.328$) in the PAR of stearic acid at the Springwater site between the four textile sample types collected (Figure 7.20). The PAR’s of both of the experimental sample types were significantly higher than the PAR’s of the control sample types. The PAR of the experimental t-shirt samples (25.78 PAR) was significantly higher than the PAR of the experimental briefs samples (13.86 PAR) during the third exhumation period (October 16, 2009 – 432 days PBI). The PAR of the experimental t-shirt samples collected during the third (25.78 PAR) exhumation period was significantly higher than those collected during the second (July 22, 2009 – 346 days PBI; 6.94 PAR) exhumation period.

![Graph showing peak area ratio over exhumation periods](image)

Figure 7.20 – Mean difference of peak area ratio (area/int std area) of stearic acid at Springwater by exhumation period. (Unbalanced ANOVA, $F_{\text{Exhumation Period}} (2, 58) = 2.073, p = 0.137$, $F_{\text{Sample Type}} (3, 58) = 3.760, p = 0.017$, $F_{\text{Interaction}} (4, 58) = 1.189, p = 0.328$)
• **Oleic Acid**

Similar to the other fatty acids at the Springwater site, there was not a significant difference ($p = 0.857$) in the PAR of oleic acid between the four textile sample types collected (Figure 7.21). However, the PAR of the experimental briefs samples was significantly higher than the PAR of the control briefs samples. The PAR of the experimental t-shirt samples (3.77 PAR) was significantly higher than the PAR of the control t-shirt samples (0 PAR) during the first exhumation period (October 15, 2008 – 66 days PBI). The PAR of the experimental briefs samples was significantly higher during the third exhumation period (October 16, 2009 – 432 days PBI; 6.00 PAR) as compared to the second exhumation period (July 22, 2009 – 346 days PBI; 3.47 PAR).

![Graph showing mean difference of peak area ratio (area/int std area) of oleic acid at Springwater by exhumation period.](image)

Figure 7.21 – Mean difference of peak area ratio (area/int std area) of oleic acid at Springwater by exhumation period. (Unbalanced ANOVA, $F_{\text{Exhumation Period}} (2, 58) = 6.074$, $p = 0.004$, $F_{\text{Sample Type}} (3, 58) = 9.192$, $p < 0.001$, $F_{\text{Interaction}} (4, 58) = 0.330$, $p = 0.857$)
Overall, the textile samples collected from the Springwater site exhibited GC-MS lipid degradation profiles consistent with association with carcasses in later stage decomposition (putrefaction) during the third, and final, exhumation period (October 16, 2009 – 432 Days PBI), as evidenced by the significantly higher PAR’s in the saturated fatty acids myristic, palmitic and stearic.

7.3.4.3 Dummer

Figure 7.22a-c illustrates the PAR of the five identified fatty acids at the Dummer site during the three exhumation periods. The saturated fatty acids palmitic (C_{16:0}), stearic (C_{18:0}) and myristic (C_{14:0}) were abundant within the textile samples.
Figure 7.22a-c – Mean peak area ratio (area/int std area) of myristic, palmitic, palmitoleic, stearic and oleic fatty acids at Dummer during (a) October 16, 2008 (67 days PBI) exhumation, (b) July 23, 2009 (347 days PBI) exhumation and (c) October 23, 2009 (439 days PBI) exhumation.
• **Myristic Acid**

The PAR of myristic acid at the Dummer site was significantly different \((p = 0.007)\) between the four textile sample types collected (Figure 7.23). The PAR’s of both of the experimental sample types were significantly higher than the PAR’s of their control sample counterparts. Likewise, the PAR’s of both of the experimental sample types were significantly higher during the second (July 23, 2009 – 347 days PBI) and third (October 23, 2009 – 439 days PBI) exhumation periods than the first exhumation period (October 16, 2008 – 67 days PBI). The PAR’s of both the experimental t-shirt and briefs samples were significantly higher during the second (t-shirt: 15.43 PAR, briefs: 16.31 PAR) and third (t-shirt: 11.19 PAR, briefs: 18.39 PAR) exhumation periods as compared to the first (t-shirt: 1.85 PAR, briefs: 1.85 PAR) exhumation period.

Figure 7.23 – Mean difference of peak area ratio (area/Int std area) of myristic acid at Dummer by exhumation period. (Unbalanced ANOVA, \(F_{\text{Exhumation Period}} (2, 61) = 9.709, p < 0.001\), \(F_{\text{Sample Type}} (3, 61) = 12.771, p < 0.001\), \(F_{\text{Interaction}} (5, 61) = 3.623, p 0.007)\)
• **Palmitic Acid**

Overall, there was a significant difference (p = 0.028) in PAR of palmitic acid at the Dummer site between the four textile sample types collected (Figure 7.24). The PAR of the experimental briefs samples was significantly higher than the PAR of the control briefs samples. During the second exhumation period (July 23, 2009 – 347 days PBI; 27.33 PAR), the PAR of the experimental briefs samples was significantly higher than the first exhumation period (October 16, 2008 – 67 days PBI; 14.65 PAR). Likewise, the PAR of the experimental briefs samples during the third (October 23, 2009 – 439 days PBI; 40.27 PAR) exhumation period was significantly higher than both the first and second exhumation periods. The PAR of the experimental t-shirt samples was significantly higher during the third (35.71 PAR) exhumation period than both the first (12.95 PAR) and second (14.01 PAR) exhumation periods.

![Graph showing the peak area ratio (area/int std area) of palmitic acid at Dummer by exhumation period](image)

Figure 7.24 – Mean difference of peak area ratio (area/int std area) of palmitic acid at Dummer by exhumation period. (Unbalanced ANOVA, $F_{\text{Exhumation Period}}(2, 61) = 17.649, p < 0.001$, $F_{\text{Sample Type}}(3, 61) = 15.424, p < 0.001$, $F_{\text{Interaction}}(5, 61) = 2.759, p 0.028$)
- *Palmitoleic Acid*

There was not a significant difference ($p = 0.597$) in the PAR of palmitoleic acid at the Dummer site between the four textile sample types collected (Figure 7.25). The PAR of the experimental briefs samples was significantly higher than the PAR of the control briefs samples. The PAR of the experimental briefs samples was significantly higher during the second exhumation period (July 23, 2009 – 347 days PBI; 2.26 PAR) than both the first (October 16, 2008 – 67 days PBI; 0.89 PAR) and third (October 23, 2009 – 439 days PBI; 0.55 PAR) exhumation periods. During the third (0.26 PAR) exhumation period, the PAR of the experimental t-shirt samples was significantly lower than both the first (1.60 PAR) and second (1.96 PAR) exhumation periods.

![Figure 7.25](image-url) - Mean difference of peak area ratio (area/int std area) of palmitoleic acid at Dummer by exhumation period. (Unbalanced ANOVA, $F_{\text{Exhumation Period}} (2, 61) = 4.819$, $p = 0.012$, $F_{\text{Sample Type}} (3, 61) = 5.318$, $p = 0.003$, $F_{\text{Interaction}} (5, 61) = 0.740$, $p = 0.597$)
- **Stearic Acid**

There was not an overall significant difference ($p = 0.941$) in the PAR of stearic acid between the four textile sample types collected from the Dummer site (Figure 7.26). Similar to palmitoleic acid at this site, the PAR of the experimental briefs samples was significantly higher than the PAR of the control briefs samples. The PAR of the experimental briefs samples was significantly higher during the second exhumation period (July 23, 2009 – 347 days PBI; 18.50 PAR) than the first exhumation period (October 16, 2008 – 67 days PBI; 5.63 PAR).

Figure 7.26 – Mean difference of peak area ratio (area/INT std area) of stearic acid at Dummer by exhumation period. (Unbalanced ANOVA, $F_{\text{Exhumation Period}} (2, 61) = 2.225$, $p = 0.118$, $F_{\text{Sample Type}} (3, 61) = 4.510$, $p = 0.007$, $F_{\text{Interaction}} (5, 61) = 0.243$, $p = 0.941$)
• **Oleic Acid**

The PAR of oleic acid at the Dummer site was not significantly different \( (p = 0.541) \) between the four textile sample types collected (Figure 7.27).

![Graph showing mean difference of peak area ratio (area/int std area) of oleic acid at Dummer by exhumation period.](image)

**Figure 7.27** – Mean difference of peak area ratio (area/int std area) of oleic acid at Dummer by exhumation period. (Unbalanced ANOVA, \( F_{\text{Exhumation Period}} (2, 61) = 2.299, p < 0.111 \), \( F_{\text{Sample Type}} (3, 61) = 7.175, p < 0.001 \), \( F_{\text{Interaction}} (5, 61) = 0.820, p = 0.541 \))

Overall, the textile samples collected from the Dummer site exhibited GC-MS lipid degradation profiles consistent with association with carcasses in later stage decomposition (putrefaction) during the third, and final, exhumation period (October 23, 2009 – 439 Days PBI), as evidenced by the significantly higher PAR’s in the saturated fatty acids myristic, palmitic and stearic.
7.4 Discussion

This study investigated the applicability of analyzing textile samples associated with decomposing carcasses for fatty acid composition as a means of estimating the stage of decomposition and the post burial interval (PBI) of the carcasses. Domestic pig (*Sus scrofa*) carcasses were buried at three field sites of contrasting soil texture (silty clay loam, fine sand and fine sandy loam). Prior to burial, the carcasses were clothed in 100% cotton t-shirts and 50% cotton/50% polyester briefs, which are representative of common textiles in current society (Shepherd, 1969; Janaway, 2008). The presence of clothing is indicative of forensic scenarios involving bodies.

An understanding of the degradation rates of textile samples in control burial environments is important for forensic investigators to make accurate estimations regarding the post mortem interval (PMI) and/or post burial interval (PBI) during a forensic investigation. This is of particular interest in cases where the clothing of a homicide victim has been buried in a separate location from the body. In the current study, the 100% cotton control t-shirt samples had completely degraded in the silty clay loam and fine sandy loam soils by the completion of the 14 month study. However, the control t-shirt samples were recovered from the fine sandy soil. It is possible that the ability of water to move freely through the fine sand due to its particle size, 2.0 to 0.005 mm (Voroney, 2007), prevented the fibres from swelling, lengthening and degrading as a result of absorbing excess amounts of moisture in this soil texture (Janaway, 2002). Alternatively, the 50% cotton/50% polyester control briefs samples did not exhibit signs of degradation and were intact during the final exhumation period from all three of the soil textures tested.

Morse and Dailey (1983) conducted field experiments which consisted of burying cotton to determine its rate of degradation. Deterioration became readily apparent between 2 weeks and 10 months (a time period comparable to the current study), with
total destruction typical between 5 and 10 months (Morse and Dailey, 1983). However, the rate of degradation of cotton textiles is largely dependent on the populations of cellulose decomposing organisms present within a given soil environment. As such, further research into the microbial communities present within the three soil textures tested is necessary to elucidate the differences in textile degradation between the sites.

The results of the current study demonstrate the reduced rate of degradation of textile materials when associated with a decomposing carcass. By the completion of the experiment, both the t-shirt and briefs samples had not degraded and were intact in all three of the soil textures tested. Evidence from case studies has shown that cotton can be recovered many years after burial (Janaway, 2002). An ancient mass grave, estimated to be from 614 A.D., was found to contain preserved pieces of cotton from the sleeve, shoulder, neck region and the lower skirt of long-sleeved, ankle-length tunics (Janaway, 2002). Little information is available regarding synthetic textiles, such as cotton/polyester blends. However, it has been reported that the synthetic portion (polyester) experiences reduced rates of degradation due to its low level of water absorbency, the fact that it is not attacked by moth larvae and its resistance to microbial activity (Janaway, 2002).

Infrared (IR) spectroscopy analysis of the textile revealed that the soil texture of the burial environment may impact lipid degradation and thus the accurate estimation of the stage of decomposition. By the completion of the experiment (October 16, 2009 – 432 days PBI for Nashville and Springwater; October 23, 2009 – 439 days PBI for Dummer), the textile samples were considered to be in different stages of decomposition based on site. Overall, the type of textile analyzed (i.e. 100% cotton t-shirt vs. 50% cotton/50% polyester briefs) did not appear to impact the decomposition stage estimation.
At the Nashville site, both the t-shirt and the briefs samples were identified as being associated with carcasses in later stage decomposition (putrefaction), based on the lipid profile. In contrast, both of the textile sample types collected from the Springwater site indicated association with carcasses that were still in early stage decomposition (autolysis). The t-shirt samples collected from the Dummer site were indicative of association with carcasses in early stage decomposition (autolysis). The briefs samples collected from the Dummer site suggested association with carcasses that had yet to reach early stage decomposition (autolysis). The lipid profile obtained for the experimental briefs samples was consistent with that of the control briefs profile. However, the briefs samples collected from the same site during the second exhumation period (July 23, 2009 – 347 days PBI) suggested association with carcasses in early stage decomposition (autolysis). The lack of IR spectroscopy bands attributable to fatty acid composition and adipocere formation at the Dummer site during the third exhumation period may be explained by the weather. During the exhumation there was torrential downpour. To maintain collection consistency over the three field sites, sample collection followed a specific order. The t-shirt samples were always collected prior to the briefs samples. It is possible that fatty acids that had been absorbed in the briefs as a result of soft tissue degradation were diluted by the rain during the additional time that they were exposed to the elements.

Although the carcasses had consistent post burial intervals (PBI) and were exhumed following a similar timeline across the three field sites, IR spectroscopy analysis of the textile suggested varying stages of decomposition of the carcasses. Only the samples collected from the Nashville site suggested association with carcasses that had progressed to the later stage of decomposition (putrefaction), which was consistent with both visual classification (Section 3.2) and tissue analysis (Section 6.3.1.2). This site consisted of a silty clay loam soil texture. Silt-sized soil particles range from 0.005 to 0.002 mm (Voroney, 2007). Clay-sized soil particles (< 0.002 mm) are the smallest of the mineral particles and form a sticky mass when wet (Voroney, 2007). The small particle
size associated with this soil can restrict water movement through the soil. Restricted water movement may have prevented the dilution of fatty acids that had been absorbed by the textile as a result of soft tissue degradation. Conversely, sand-sized particles are considerably larger (2.0 to 0.05 mm) (Voroney, 2007). The soil textures at the Springwater and Dummer sites were fine sand and fine sandy loam, respectively. Water movement throughout the soil at these two sites may have resulted in the loss of some of the leached fatty acids, thus resulting in lipid profiles consistent with early stage decomposition (autolysis), despite the fact that the carcasses had actually progressed to later stage decomposition (putrefaction).

In contrast to Murthy et al. (1985), the current study found that repeated analysis of the same textile sample by attenuated total reflectance – Fourier transform infrared (ATR-FTIR) spectroscopy produced comparable lipid profiles. Murthy et al. (1985) used ATR-FTIR spectroscopy to analyze triglycerides that had been deposited onto cotton fabric. Repeated analysis of the same sample, by direct contact of the cotton with the ATR-FTIR spectroscopy crystal, often resulted in widely differing absorbances, which the authors attributed to non-uniform triglyceride distribution on the fabric surface (Murthy et al, 1985). Difficulties associated with direct textile analysis were experienced within the current study. However, fatty acid solvent extraction of the textile allowed for consistency throughout sampling.

Textile samples were also analyzed by gas chromatography – mass spectrometry (GC-MS) to quantitate fatty acid composition. In the textile samples analyzed from all three field sites, the saturated fatty acids palmitic, stearic and myristic were shown to have the greatest peak area ratio’s (area/int std area) (PAR) of the five fatty acids analyzed. Alternatively, the unsaturated fatty acids palmitoleic and oleic had consistently lower PAR’s by comparison. Forbes et al. (2005a) found that palmitic, stearic and myristic acids were most abundant in samples of well formed adipocere, with oleic present in only small quantities. Palmitoleic acid was not detected in the well
formed adipocere samples (Forbes et al., 2005a). In contrast to the IR spectroscopy results, the GC-MS results suggest textile samples that were in association with carcasses that had reached later stage decomposition (putrefaction), but had not formed adipocere. The GC-MS results of the textile are consistent with those of the tissue samples (Section 6.3.2).

The fatty acids myristic and stearic both showed some applicability with respect to estimating the post burial interval (PBI) based on fatty acid composition. The PAR’s of both of these acids were significantly higher during either the second or third exhumation periods as compared to the first. These two acids, used in combination, may allow investigators to estimate between a shorter PBI, approximately 2 months, or a longer PBI, approximately 10 months to over one year. In contrast, the rate of textile degradation does not currently offer investigators strong evidence regarding estimations of the PBI. Textile degradation is dependent upon many factors (e.g. association with a decomposing carcass, soil environment, microbial community of the soil environment and type of textile) and further controlled research is necessary before accurate estimations regarding PBI can be drawn from recovered articles of clothing in a forensic investigation.

7.5 Conclusion

This study was conducted to determine the applicability of analyzing the fatty acid composition of clothing materials associated with decomposing carcasses as a means of estimating both the stage of decomposition and the post burial interval (PBI) of the carcasses. Attenuated total reflectance – Fourier transform infrared (ATR-FTIR) spectroscopy indicated that carcasses were in different stages of decomposition by the completion of the study. Gas chromatography – mass spectrometry (GC-MS) analysis resulted in consistent estimations of the stage of decomposition of the carcasses over the three field sites, and was comparable with previous analysis of tissue samples. GC-
MS is a more sensitive and accurate technique for the quantitative analysis of textile samples as compared to ATR-FTIR spectroscopy. Myristic and stearic acids may be useful fatty acids for the estimation of PBI, as the peak area ratio (area/int std area) (PAR) of these acids was significantly higher during the second or third exhumations periods as compared to the first. Estimations of PBI based upon textile degradation is currently not a viable method within forensic investigations, as many factors alter the rate of degradation.
Chapter 8 – Conclusion and Future Considerations
8.1 Conclusion

The current study investigated the applicability of using ground penetrating radar (GPR) to locate clandestine graves. Domestic pig (Sus scrofa) carcasses were buried at three field sites of contrasting soil texture (silty clay loam, fine sand and fine sandy loam) and monitored for a period of 14 months. Additionally, carcasses were exhumed at 2, 11 and 14 months post burial interval (PBI) and samples of gravesoil, tissue and textile were analyzed for the detection and quantification of fatty acid composition as a means of estimating the stage of decomposition and the PBI of the carcasses.

The use of GPR may aid in the detection of clandestine graves within forensic investigations in known soil textures due to its non-Invasive nature. Graves, which contained decomposing pig carcasses, were successfully detected in silty clay loam and fine sandy loam soil textures, but not in fine sandy soil. The use of GPR to estimate the PBI of buried remains in fine sandy loam soil may be possible, as hyperbolic grave response became less distinctive over time.

Infrared (IR) spectroscopy analysis of the fatty acid composition of gravesoil samples did not provide information regarding the stage of decomposition or the PBI of the carcasses in the soil textures tested. Therefore, this is not a viable method for use within forensic investigations in these soils. Furthermore, soil moisture content as a presumptive test for the location of decomposing remains was also not useful in the soil textures tested, as significant differences between control soil and gravesoil were not observed. Soil pH levels, however, may have applicability within forensic investigations as a presumptive test for the location of decomposing remains in the three soil textures tested, as gravesoil pH was significantly more acidic than control soil.
Of the three sample types analyzed, tissue samples showed the highest level of applicability within a forensic investigation for the estimation of the stage of decomposition of a body and the PBI. IR spectroscopy and gas chromatography – mass spectrometry (GC-MS) analysis of the fatty acid composition of the tissue samples gave results that corresponded to the visual classification of the stage of decomposition of the carcasses during the exhumation periods in all three of the soil textures tested (silty clay loam, fine sand and fine sandy loam). Additionally, myristic and stearic fatty acids may be useful in estimating the PBI, as the peak area ratio (area/int std area) (PAR) of these fatty acids in the tissue samples was significantly higher during the second or third exhumation periods as compared to the first exhumation period. This was observed in all three of the soil textures tested.

GC-MS analysis of 100% cotton and/or 50% cotton/50% polyester blend textiles may also aid in determining the stage of decomposition of a body and estimations of the PBI. GC-MS analysis of the composition of fatty acids that had leached from the decomposing tissue of the carcasses into the clothing gave results consistent with the visual classification of the stage of decomposition of the carcasses. Similar to the tissue samples, myristic and stearic fatty acid composition within textile may aid in the estimation of the PBI as the PAR ratio of these two acids was significantly higher during the second or third exhumation periods as compared to the first exhumation period. Conversely to the tissue samples, IR spectroscopy analysis of the textile did not produce lipid profiles consistent with carcass stage of decomposition. Using the rate of degradation of the actual textile fibres as a means of estimating the PBI is not currently a reliable method within forensic investigations, as many factors (e.g. microbial community within soil environment) may alter degradation.
8.2 Future Considerations

Further research into the use of ground penetrating radar (GPR) in additional soil textures is necessary, as forensic investigations can take place in a wide variety of locations. Studies investigating GPR grave response of carcasses with longer post burial intervals (PBI) is required to determine detection time-frame limits. Additionally, controlled research investigating potential detection differences between pig carcasses and human cadavers is important for the successful use of GPR within forensic investigational procedures. Finally, studies to determine the ability of graves and carcasses to be detected by GPR during the spring and winter seasons must be conducted, as data collection only took place during the summer and fall seasons in the current study.

Similarly, continued research into the analysis of gravesoil, tissue and textile samples is necessary in additional soil textures in order to effectively incorporate fatty acid analysis of these samples into investigational protocols. Controlled burial studies investigating longer PBI’s and the contrast between pig carcasses and human cadavers are required to determine how fatty acid composition changes over time.

Currently, minimal literature exists regarding the degradation of textile materials in soil, both in control scenarios and associated with decomposing carcasses. Research investigating both degradation scenarios in a wide variety of soil textures and over extended PBI’s is necessary to determine the applicability of estimating the PBI of a body based on recovered textile. The applicability of using infrared (IR) spectroscopy to detect fatty acid composition leading to adipocere formation requires further investigation. Longer PBI’s may allow for more fatty acids to leach from the tissues of the carcasses into the textile material, thus allowing for more accurate IR spectroscopy lipid profiles to be obtained.
Finally, more extensive sampling parameters (increased number of exhumation periods and more sample replicates) may provide forensic investigators with more information regarding the use of fatty acids as a means of estimating the stage of decomposition and PBI of a body based on changes in the peak area ratios (area/int std area) (PAR) of fatty acids.
References


