EVALUATION OF SOIL CHEMISTRY IN HUMAN DECOMPOSITION SITES

by

James P. Fancher

A thesis submitted to the Graduate Council of
Texas State University in partial fulfillment
of the requirements for the degree of
Master of Arts
with a Major in Anthropology
December 2015

Committee Members:

Michelle D. Hamilton, Chair

Jacqueline A. Aitkenhead-Peterson

Ken Mix

Daniel J. Wescott

FAIR USE AND AUTHOR'S PERMISSION STATEMENT

Fair Use

Duplication Permission

ACKNOWLEDGEMENTS

Many thanks to my committee that supported, guided, and corrected me throughout this project. As my chair, Dr. Hamilton was especially important in helping me filter ideas and offer encouragement to look beyond my dreams. Dr. Wescott allowed free access to FARF and data for the donations, plus reviews and statistical analysis. Dr. Mix gave me open access to his lab, his mind, and his experience in soil science. We had many short appointments, but always long conversations. Dr. Peterson and Texas A&M University gave me more cheerful support than I dreamed of in soil analysis and professional review. I especially want to note the statistical support of Dr. Robert Dixon, Geography, who taught me the multivariate approach to analysis that was crucial to this project. It is also important to mention that the genesis of this project occurred at an archeology field camp in the stark and challenging Pecos region of Texas staffed by Dr. Steve Black and Dr. Carolyn Boyd. The idea of soil analysis and decomposition sites came together after a presentation by Ken Lawrence on his use of soil analysis to map archaeological sites; it was a small step to applying the same concepts to human decomposition sites and forensic science.

I have a special respect and appreciation for my classmates Marilyn Isaacks, Brittany McClain, and Cassie Skipper. Throughout this educational journey we shared many classes, challenges, and a lot of laughs. These special three people are the friends that will always stand out in my memory.

There are many other colleagues in my cohort, faculty, and staff that deserve acknowledgement, but they are too many to mention individually. To all I say thank you for sharing our experiences in graduate studies.

The last group to acknowledge are the people at home who gave constant encouragement and support. First, my wife Ruth, who knows me better than anyone else, including my limits of life. Second, third, and fourth, are my three dogs. Casey the old one, Tess the strong but not so brave one, and Grace the little one so full of entertaining personality. They constantly reminded me of the need to take walks, enjoy naps, stretch and play no matter how busy the days were.

This research was supported by a Thesis Research Support Fellowship awarded by Texas State University, Fall 2014. This award covered much of the research expenses and was sincerely appreciated.

TABLE OF CONTENTS

Page

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

ABSTRACT

Soil samples were analyzed from cadaver decomposition islands (CDI) of 63 human decomposition sites at the Forensic Anthropology Research Facility (FARF), Texas State University, in order to develop a method to estimate the postmortem interval (PMI) for each donation from these data. An additional 5 control site samples were also analyzed to establish a baseline record of undisturbed soils. Fifteen soil parameters were measured or calculated that included pH, electroconductivity, nitrate-N, ammonium-N, total dissolved nitrogen, dissolved organic nitrogen, orthophosphate-P, organic carbon, inorganic carbon, total carbon, sodium, potassium, magnesium, calcium and carbon/nitrogen ratio. Accumulated Degree Days (ADD) at a base temperature of 4°C (ADD_4) were also calculated for each donation. The data were analyzed using multiple regression with PMI and ADD_4 as the dependent variables and the soil parameters as independent variables. Regression formulae for 13 models were run that had R^2 values ranging from 0.60 to 0.98 All of the models were statistically significant. The regression formulae results for ADD_4 were similar to PMI, indicating that ADD_4 may be used as a surrogate for estimating PMI. Measures of soil parameters derived from predator and microbial mediated decomposition of human remains show promise in estimating PMI accurately for a period up to nearly 5 years at FARF. This persistent change in soil chemistry extends the ability to estimate PMI in consort with the traditional methods of entomology and forensic anthropology in support of medical-legal investigations,

humanitarian recovery efforts, and criminal and civil cases. This project has also recorded a baseline soil analysis for a new human decomposition research site.

I. INTRODUCTION

This research is an investigation of the post-depositional events associated with the effects of surface human decomposition on the underlying soils of the border lands of the Texas Hill Country at the Freeman Ranch near San Marcos, Texas. The purpose of this study is to define the postmortem interval (PMI) for surface human depositions based on associated changes in soil chemistry. This study also improves the PMI estimation methodologies by adding a soil analysis technique to entomology and anthropology methods that refines and extends the ability to estimate PMI. This aids forensic anthropologists and other investigators who conduct medical-legal analysis for criminal, civil, and humanitarian cases in similar ecological or geographical regions.

Research conducted on the decomposition byproducts of human cadavers deposited on the soil surface serves to document the taphonomic processes of human tissue breakdown and soil deposition of decomposition products (Tibbett and Carter 2008). Decomposition affects the surrounding soil chemistry by leaving behind volatile and persistent compounds that are associated with different stages of decay. These compounds can be predictably identified using both routine and advanced quantitative soil analysis techniques (Aitkenhead-Peterson et al. 2012; Damann et al. 2012). These and other research results support experimental methods to predict PMI using decompositional byproducts (Benninger et al. 2008; Pringle et al. 2010; Anderson et al. 2013).

The characterization of soil associated with decomposition sites has been used to identify large carrion remains in wilderness settings for periods up to five years (Towne 2000). Similar techniques may also be used in medical-legal investigations to help

identify the location of human remains, the site of primary decomposition where human remains were once deposited, or the length of time remains were on the surface. This can be useful for field and laboratory analysis of criminal cases including clandestine surface or burial depositions and missing persons cases, as well as humanitarian efforts following disasters and cases of genocide (Tibbett and Carter 2008; Tersigni-Tarrant and Shirley 2013).

Decomposition is a complex process that begins at the time of death and continues through the skeletonization of the body and beyond to eventual destruction of skeletal elements. Forensic anthropologists have generally used stages of decomposition to give estimates of PMI that incorporate local environmental conditions (Megyesi et al. 2005). The qualitative stages of decomposition are progressive and are commonly divided into four or five stages that allow only general time interval estimates that are highly variable and influenced by environmental, climatic, and geographical factors (Rodriguez and Bass 1985; Galloway et al. 1989; Rhine and Dawson 1998). However, many factors affect the rate of soft and hard tissue decomposition that can confound the quantitative estimation of PMI. Local environmental conditions, cause of death, extent of clothing, body mass, age at death, diseases, animal scavenging, insect activity, vegetation, and many other factors affect decomposition and hamper PMI estimates when they are based solely on decomposition stages (Rodriguez and Bass 1985; Vass et al. 1992; Benninger et al. 2008; Carter and Tibbett 2008; Dadour and Harvey 2008; Parks 2011; Spicka et al. 2011; Vass 2011).

Although the fields of forensic anthropology and forensic entomology utilize different approaches to work on the problem of estimating PMI, both specializations are

intertwined in investigating the same common processes of biological decomposition. The decomposing human cadaver provides a complex food web that yields the chemicals necessary for nutrients and complex signal mechanisms that allow microbes and arthropods to cooperate in scavenging the cadaver in an orderly succession (Dadour and Harvey 2008; Hopkins 2008; Sagara et al. 2008). The processes of autolysis, putrefaction, and decay create both the gross physical signs of decomposition of interest to the anthropologist, and the medium for arthropod and microbial succession of interest to the entomologist and microbiologist. The biological agents of cadaver consumption and the processes of decomposition itself result in a flow of organic and inorganic products to the soil that are measurable over time related to species succession and physical signs of decomposition (Johnson 1975; Rodriguez and Bass 1985; Galloway 1997; Gill-King 1997; Davis and Goff 2000; Wilson et al. 2007; Janaway 2008; Sharanowski et al. 2008)

The passive loss of cadaveric fluids leaching into the soil during the decomposition process also alters the ecosystem, leaving behind a cadaver decomposition island (CDI) that is a long-lasting component of terrestrial death scenes (Tibbett and Carter 2008). This CDI is of interest as a further aid in establishing PMI through analysis of the chemical deposits and soil chemistry (Aitkenhead-Peterson et al. 2012). Additionally, continuing investigations into the microbiology and mycology of this soil component may contribute to the understanding of soil changes, especially in the later stages of decay when it may be hard to differentiate progression of decomposition based on visible changes (Dadour and Harvey 2008; Sagara et al. 2008).

Many studies of surface decomposition soil chemistry have been completed over relatively short time spans of 100 days or less (Carter et al. 2008; Sasha et al. 2008;

Howard et al. 2010; Parks 2011; Spicka et al. 2011). Currently, much longer term studies ranging from 200 days to 3 years are being reported (Pringle et al. 2010; Lovestead and Bruno 2011; Aitkenhead-Peterson et al. 2012; Damann et al. 2012; Anderson et al. 2013). However, in spite of increasing research in this area, many of the soil deposition processes associated with human cadaver decomposition are still not well understood and are dependent on local environmental factors (Benninger et al. 2008).

Research conducted at the University of Tennessee's Anthropological Research Facility has shown that continual reuse of sites at their smaller (relative to FARF) decomposition research facility has left the soil saturated with decomposition products that may affect their research outcomes (Damann et al. 2012). Reuse of surface decomposition sites at FARF has not yet occurred, leaving an undisturbed record of soil changes for nearly all decomposition sites over the time period ranging from 2008 to the present (summer, 2014). The flora, fauna, and microbes associated with surface decomposition at FARF reflect natural biomes in a relatively pristine ranchland environment, and they have left a record of soil changes that are unique to the area. These soil changes have never been studied.

Recent studies of other surface decomposition sites have looked at changes in carbon and nitrogen content, diffuse reflectance near infrared (DR-NIR) spectroscopy, soil pH, soil moisture content, soil extractable phosphorus, lipid-phosphorus, and total extracted DNA (Benninger et al. 2008; Aitkenhead-Peterson et al. 2012; Damann et al. 2012; Aitkenhead-Peterson et al. 2015). The research described in this thesis extended beyond these studies by measuring the common elements of nitrogen, carbon, and phosphorus for comparative value and adding several other elemental measures to

determine if any other factors that are commonly available for measure from a soil laboratory were significant in predicting PMI. A cross-sectional design was used to sample as many individual cadaver decomposition sites as possible over the previous three to five years of research placements conducted at FARF. Single soil samples were taken to a depth of 5 cm with a 2 cm diameter soil corer from the central cadaver decomposition island, and from 5 control sites away from the decomposition sites. Wet chemistry was completed on the parameters of pH, electro-conductivity (EC), nitrate, ammonium, total dissolved nitrogen, total organic nitrogen, phosphate, total carbon, total inorganic carbon, total organic carbon, sodium (Na^{+}), potassium (K^{+}), Magnesium (Mg^{2+}), calcium (Ca^{2+}), carbon-nitrogen ratio (C:N) after soil was extracted with ultra-pure H_2O in order to give a broad set of measurements of soil elements that were used to evaluate the following three outcomes. The first is that parameters of common soil measurements associated with surface human decomposition observed over a multi-year period at FARF were identified. The second is that these parameters were used to create a method to estimate PMI in the Central Texas region and similar environments. The third is that it established a baseline study of FARF soils that can be used to document and plan future research and ecological use of the site.

The purpose of this thesis project was to investigate the differential effects of surface human decomposition on soil chemistry over time in order to predict PMI. This is important because the changes in soil chemistry reflect the ecological conditions created by the decomposition process, and these changes may provide a quantitative method of estimating PMI. This study was undertaken by sampling and analyzing soil samples from surface decomposition sites that were stratified by date over a three to five year period

using a cross-sectional design (i.e. taking all samples at the same time period). The research questions posed for this study included:

1. What is the soil chemistry profile at undisturbed up-slope and down-slope control sites?
2. What is the soil chemistry profile at surface decomposition sites over time using date of cadaver placement as the beginning reference time?
3. Do any parameters change in a quantitatively predictable manner that can be used to predict PMI?

The next chapter will provide a brief literature review on how soil is influenced by the biological factors of surface human decomposition. The process of human decomposition will be discussed in relation to the living decomposers and the by-products of their metabolism that are left behind.

II. LITERATURE REVIEW

Introduction

Human remains that are either buried or allowed to decompose on the soil surface are affected by a number of variables including the climate and environment that surrounds them (Janaway 1996; Wilson et al. 2007). One of the main environmental factors that can influence the decomposition process is the soil that interacts with the cadaver (Carter and Tibbett 2008). This literature review will survey how soil environments are characterized and influenced by the biological factors of human decomposition. The process of human decomposition will be presented as a soil modifying factor that contributes to changes over time in soils. These alterations in the basic chemistry of soils represent taphonomic processes that may be monitored and subsequently analyzed for the estimation of the postmortem interval (PMI) (Dent et al. 2004; Benninger et al. 2008; Forbes 2008b; Pringle et al. 2010; Anderson et al. 2013).

Human Decomposition

The living human organism exists in an environment where the limits of temperature, pH, concentration of metabolic substrates, and elimination of waste products are regulated within very narrow tolerances (Gill-King 1997). Everything changes at the time of death. Decomposition technically begins when respiration and circulation cease and the internal environment of the body changes from an aerobic, oxygen rich environment, to an anaerobic, oxygen depleted environment (Gill-King 1997; Carter and Tibbett 2008; Forbes 2008a). Changes visible to the naked eye may not show for hours or days depending on the local environment, but microscopic observations can be made

almost immediately as the cellular structures of the cells change due to enzymatic actions (Di Maio and Di Maio 1993; Mello de Oliveira and Santos-Martin 1995). Cellular activity and attendant functions may continue for up to two hours after somatic death, but this is influenced by such factors as the cause of death and the condition of tissues and their oxygen requirements (Janaway 1996).

Autolysis, or cellular death, begins very quickly, resulting in the enzymatic denaturing of the structural and functional components of cells (Di Maio and Di Maio 1993; Gill-King 1997). Initially this is a sterile process that is independent of bacterial action (Janaway 1996). However, it results in the progressive destruction of internal soft tissue organs in the gut and thoracic cavity, leading to more widespread decomposition due to putrefaction that is largely associated with *in situ* florescence of anaerobic bacteria from the bowel and respiratory systems (Micozzi 1986; Di Maio and Di Maio 1993; Hyde et al. 2013).

The process of putrefaction, or active decomposition, often becomes visibly evident within 24-72 hours as the result of sequential enzymatic degradation processes initiated by cellular destruction and concomitant bacterial activity (Janaway 1996). Physiologic changes of early putrefaction such as discoloration are the signs that are often associated with the onset of established decomposition, usually leading to either artificial intervention to preserve a corpse (such as refrigeration) or prompt disposal of the remains (Di Maio and Di Maio 1993; Cantor 2010b). In historic times putrefaction was used as a positive index of death. Roman and Greek mortuary workers waited three or four days for putrefaction to begin before disposing of bodies, and 19th century

European and American cemeteries had “waiting mortuaries” where bodies were kept above ground in open coffins until unmistakable decay occurred (Cantor 2010a).

During putrefaction, bacteria migrate to local and distant tissues through tissue spaces and passive pathways such as the lymphatics, blood vessels and integuments (Cantor 2010b). These bacteria act in concert with the cellular elements that are released during autolysis to form the processes that lead to bloating, marbling, purging of fluids, and continual decomposition of organs and tissues into their elemental components (Clark et al. 1997; Gill-King 1997). Due to the lack of oxygen, the bacteria that survive and become metabolically active are facultative or obligate anaerobes (Hyde et al. 2013), meaning that they have the ability to live without oxygen. Early in the decomposition process the source of microbes is primarily enteric (from the gut or digestive system), but soil organisms may also be introduced through open tissue spaces by the end of the first week, especially in moderate climates (Micozzi 1986).

The exact bacterial species native to the cadaver that are involved are assumed to represent genera such as *Bacteroides, Lactobacillus, Proteus, Enterococci, Clostridia, Streptococcus, Staphylococcus,* and *Pseudomonas* (Gill-King 1997), but this is far from certain. It is estimated that up to 99% of bacterial species found in nature cannot be cultured by conventional means, and even with advanced methods of biochemical and genetic typing most species are still missed (Hyde et al. 2013). Also, it is difficult to get good samples from the internal spaces of either a living body or a cadaver that accurately represent the bacterial biomass for an individual (Stearns et al. 2011; Human Microbiome Project 2012b). Even with approximately 800 reference strains isolated and sequenced from the human body, there are thousands not yet classified taxonomically (Human

Microbiome Project 2012a). Rather than assuming a narrow group of known organisms function as the standard agents of decomposition, it is most likely that the conditions in the decomposing environment will select for a particular community of microbes based on factors such as temperature, concentrations of nutrients, and availability of water and oxygen (Hopkins et al. 2000; Tibbett and Carter 2003; Hopkins 2008; Carter et al. 2010).

The main effect that a decomposing human cadaver placed on the soil surface has on soil processes is the heavy inoculation and inundation of the local soil environment with a matrix of organic matter laden with a complex microbial community (Hopkins et al. 2000; Carter and Tibbett 2008; Hopkins 2008; Spicka et al. 2011; Stearns et al. 2011; Hyde et al. 2013). The process of human decomposition from the point of deposition as a fresh cadaver to the advanced skeletal stage is relatively rapid and depends on environmental factors, especially temperature (Rodriguez and Bass 1985; Galloway et al. 1989; Mann et al. 1990; Gill-King 1997; Andrews et al. 2000; Megyesi et al. 2005; Prangnell and McGowan 2009; Min et al. 2014). Although the long-term effects of surface human decomposition on soil chemistry and microbiology have not been thoroughly studied, the orderly process of decomposition for carrion with body mass equal to or greater than humans has been documented to leave a chemical signature in the soil that will last 5 years or longer (Towne 2000; Macdonald et al. 2014). Towne (2000) noted that ungulate carcass sites left disturbed patches of enriched soil and altered vegetation 5 years after death in a montane environment. Macdonald et al. (2014) argued for leaving large carrion in place as an ecosystem management tool because they provide a large and long-lasting resource for soil nitrogen cycling.

Stages of Human Decomposition

The human decomposition process has been described by many investigators as having a variable number of differentiated stages, most often four to six (Payne 1965; Johnson 1975; Rodriguez and Bass 1985; Galloway et al. 1989; Galloway 1997; Rhine and Dawson 1998; Davis and Goff 2000; Dent et al. 2004; Carter and Tibbett 2008; Sharanowski et al. 2008). The stages have value in creating subjective descriptions in the progressive and somewhat predictable stages of decomposition, although they are poorly correlated with the continuum of carrion arthropod succession (Schoenly and Reid 1987). For consistency this study will refer to the five major categories classified by Galloway and coworkers (1989, 1997): *fresh, early decomposition, advanced decomposition, skeletonization, and decomposition of skeletal material*. This scheme is chosen because it best represents the decomposition stages seen in the course of research in the naturally occurring environment at the Forensic Anthropology Research Facility (FARF).

As outlined by Galloway and coworkers (1997, 1989), the *fresh stage* refers to remains that have no visible trace of maggot activity and no discoloration of the body except for the results of lividity (pooling of blood to dependent areas of the body). *Early decomposition* includes the beginning of discoloration and extends to the bloating and post-bloat stages. *Advanced decomposition* occurs when there are moist changes such as sagging of tissues and extensive maggot activity. The processes of mummification and desiccation may also be seen at this stage depending on the local environment, although they have been documented as occurring as early as 12 days postmortem in the Central Texas region (Parks 2011). *Skeletonization* occurs when the majority of the bones are exposed. *Decomposition of the skeletal remains* is the final stage of decomposition that

includes osseous destruction indicated by bleaching, exfoliation, and cortical breakdown (Galloway et al. 1989; Galloway 1997).

The processes of decomposition proceed to produce a liquefied mass of unrecognizable putrefied tissue in the advanced decomposition stage (Forbes 2008a). The constant interplay between the substrate of the cadaver and the continual processing and degradation by microbes (Hyde et al. 2013), insects (Sharanowski et al. 2008), scavengers (DeVault et al. 2003), and the environment (Cantor 2010b) results in the complex tissues of the body being reduced to simple compounds and elements. Although the focus of much research has been on the decomposition roles of bacteria and invertebrates, a more robust multi-partite symbiotic association between plants, animals, microbes, and their environment appears necessary to explain this habitat cleaning system (Sagara et al. 2008).

Living body composition starts as approximately 64% water, 20% protein, 10% fat, 5% minerals, and 1% carbohydrate (Dent et al. 2004) and is transformed via death into organic acids, gases, aldehydes, ketones, alcohols, elemental minerals, adipocere, and skeletonized remains (Forbes et al. 2004; Carter and Tibbett 2008; Forbes 2008a). The nitrogenous compounds that are mainly derived from the amino acids of proteins enrich the soils for vegetative growth by increasing available ammonia ions. The carbohydrates are collectively converted to organic acids that acidify the local environment. The lipids present in adipose tissue largely convert to aldehydes and ketones due to the action of fungi. Fatty acids derived from lipids may be converted to adipocere in a variety of environments, especially those that are relatively anaerobic, contain sufficient moisture and bacteria, and when the temperature is in the range of 22°C

– 38°C (71.6°F – 100.4°F) (Forbes et al. 2002; Forbes et al. 2004; Forbes et al. 2005b; Forbes et al. 2005a; Forbes 2008a).

The products of decomposition that are not gaseous are deposited directly into the soil and groundwater system (Dent et al. 2004). The spatial extent of deposits result in an identifiable cadaver decomposition island (CDI) that has a variable extent depending on what element is analyzed, amount of scavenging and spread of elements, local topography, and whether the cadaver was autopsied (Aitkenhead-Peterson et al. 2012). Chemical compounds derived from decomposition have been found to persist for months to years and to travel away from the site of decomposition (Towne 2000; Wilson et al. 2007; Damann et al. 2012).

Soils

Soils have a complex biological, chemical, physical, and mineralogical set of properties that constantly change over time (Fitzpatrick 2008). They are a naturally occurring geologic medium that function as a living membrane in the Earth's ecosystem (Gardiner and Miller 2008). The USDA defines soil as "a natural body comprised of solids, liquid, and gases that occurs on the land surface, occupies space, and is characterized by one or both of the following: horizons or layers that are distinguishable from the initial material as a result of additions, losses, transfers, and transformations of energy and matter, or the ability to support rooted plants in a natural environment" (Soil Survey Staff 2010:1).

Soil is often thought of as a passive medium that can be altered by the external influences of biological, physical, and chemical actions, but it is actually a dynamic

medium that can respond to environmental changes such as pollution or disturbances due to deposition (Bongers 1990; Carter and Tibbett 2008). Soil is a complex assemblage of minerals, organic matter, salt and organic solutions, and living organisms that exert varying influences on human decomposition (Dent et al. 2004). Although the relationship between cadaver decomposition and soil is poorly understood (Carter and Tibbett 2008), soil characteristics have been investigated to estimate PMI and to locate clandestine graves with varying degrees of success (Vass et al. 1992; Carter et al. 2008; Carter et al. 2009; Van Belle et al. 2009; Vass 2011).

Soils are composed of various combinations of solids, fluids, and living elements (Gardiner and Miller 2008). The fluids are composed of air and water that occupy pores between soil solids. The solids are divided into two groups, the mineral and organic substances. The mineral portion is dominated by the elements of sand and clay which are typically derived from the parent material or bedrock on which the soil sits. There is generally a lesser amount of organic matter called humus that is derived from the degradation of plant and animal matter. The live organisms include plants (Gardiner and Miller 2008), microorganisms mainly represented by bacteria, protists and fungi (Hopkins et al. 2000; Hopkins 2008; Sagara et al. 2008), a large variety of invertebrates (Dadour and Harvey 2008), and vertebrates (DeVault et al. 2003; DeVault et al. 2004).

Soil formation is the result of the combination of five key factors that result in the properties of the individual soil (Simonson 1959; Jenny 1994; Fitzpatrick 2008). The first is the parent material from which the mineral soil is formed from. The second is the climate that interacts with other environmental forces to break down the parent material. The third is the array of organisms that live on or in the soil such as above ground

vegetation and its associated microflora. The fourth is the topography that affects how the climate interacts with the soil. This includes how the slope might affect drainage and aspects of the site that influence the incidence of sunlight, moisture and plant growth. The fifth factor is time. This may be measured in the extensive temporal periods that are required to denature complex parent material elements or the short times that are affected by rapid addition or removal to the soil such as addition by decomposition products or removal by glaciation, landslide, or erosion events caused by rain or wind.

Microbiology

The natural mix of soil microorganisms and how they may respond to cadaver decomposition products is very complex and not well understood. A working concept of how the soil microbial community responds to the influx of organic material from human decomposition may be that an indigenous community undergoes selection based on environmental factors and an innate ability to decompose the residue (Hopkins 2008). Although the influence of soil microorganisms may not be evident until later stages of the process, shifts in the composition of the soil microbial community may provide an indication of grave sites and PMI (Tibbett and Carter 2003).

At any one time segments of the microbial community may be either active or dormant depending on environmental factors and the availability of readily exploitable nutritional resources (Hopkins 2008). Some microorganisms respond rapidly to the addition of fresh substrate (zymogenous organisms) (Winogradsky 1924). Others simply eke out an existence in the older, more stable layers of organic matter (autochthonous organisms) (Winogradsky 1924; Langer et al. 2004). Later work identified and redefined

the microorganisms as r-strategists (zymogenous) and K-strategists (autochthonous) (Andrews and Harris 1984).

The anaerobic, oxygen-starved soil environment that develops during decomposition after the cadaver has purged changes the biochemical pathways of oxidation/reduction reactions that microorganisms use to gain energy. Chemical bonds must be broken that transfer electrons away from the decomposing substrates. The technical term for losing electrons is called oxidation, and the term for chemicals that gain or accept electrons is reduction. In oxidation a chemical loses negatively charged electrons and becomes relatively more positive in charge. In reduction a chemical gains negatively charged electrons and becomes relatively more negative in charge (Brown et al. 2014; Solomons et al. 2014). In the anaerobic soil after purge, or more generally in riparian soils where the water table is high, microorganisms can no longer use oxygen as a final electron acceptor in enzyme mediated metabolic pathways (Bauman 2015). Alternate pathways of metabolism become mandatory for the surviving bacteria (Gill-King 1997; Forbes 2008a; Janaway 2008). Aerobic respiration based on oxygen reduction is replaced by less efficient microbial-based anaerobic processes that include denitrification, fermentation, iron reduction, sulphate reduction, and methanogenesis (Hopkins 2008). The dominant end products of metabolism shift from the fairly innocuous compounds of carbon dioxide and water in aerobic metabolism to a complex assortment of organic and inorganic metabolic byproducts in anaerobic metabolism. The noxious byproducts of anaerobic metabolism include volatile gases (methane, hydrogen and hydrogen sulphide), organic acids (lactic, acetic, acetoacetic, propionic, and butyric), fermentation products (ethanol, butanol, and acetone), and denatured products of protein

breakdown (peptides and amino acids) (Di Maio and Di Maio 1993; Gill-King 1997; Van Belle et al. 2009; Rath and Rousk 2015).

Mycology

There has been a growing interest in studying fungal organisms (mycology) that have an association with the soils of graves and decomposition sites (Tibbett and Carter 2003; Sagara et al. 2008). Fungi have traditionally been studied as part of botany, but they have more in common with the animal kingdom because they do not manufacture their own food. Fungi for example, must obtain nutrients directly from other organic materials, either living or dead (Hawksworth and Wiltshire 2011). According to Hawksworth and Wiltshire (2011), there are forensic applications for mycology in a number of categories including providing trace evidence; estimating PMI; ascertaining time of deposition; investigating cause of death; causes of hallucinations, or poisonings; locating buried corpses; and biological warfare. The clearest association with the science of soil analysis is the study of post-putrefaction fungi that provide visible markers of the sites of cadaver decomposition and follow repeated patterns of successional change as decomposition proceeds (Tibbett and Carter 2003). The greatest limit for this academic and forensic field appears to be a limited number of trained specialists who can identify and analyze fungal organisms in forensic settings (Sagara et al. 2008; Hawksworth and Wiltshire 2011).

Arthropods and Entomology

A large variety of arthropods, most notably insects, work alongside microbes as active and sometimes primary participants in the decomposition process (Dadour and

Harvey 2008; Sharanowski et al. 2008). The decomposing cadaver provides a nutrient-rich substrate for many different organisms that interact with the fermented and putrefied products of autolysis and bacterial digestion. While it is possible for bacteria alone to decompose tissues (Carter and Tibbett 2006), the exclusion of insects decreases the speed and efficiency of the total process (Bachmann and Simmons 2010).

Besides the decomposing cadaver, the arthropods find a beneficial substrate in the underlying soil itself as it is modified by decomposition products (Dadour and Harvey 2008). The changes in nutrient substrates and environmental factors over time creates a succession of organisms that can be used to estimate PMI based on knowledge of locality-specific succession patterns of insects found on carrion or a corpse following death (Payne 1965; Johnson 1975; Rodriguez and Bass 1983; Dadour and Harvey 2008; Voss et al. 2008; Voss et al. 2011; Magni et al. 2012). Patterns of insect succession occur in predictable sequences that vary with different habitats and seasons (Sharanowski et al. 2008). It is the detailed knowledge of this variation that proves beneficial for estimating PMI for cadavers. The soil arthropod fauna also show changes in identity and number of species over time, and may not return to baseline for long periods (Anderson and VanLaerhoven 1996). Knowledge of arthropod biology and succession, especially entomology and insect life cycles, is acknowledged as beneficial for forensic sciences in the estimation of time since death intervals (Magni et al. 2012).

Most forensic techniques of entomology rely on the character of the carrion community, arthropod succession within this community, and the biology of arthropods that are most associated with the decomposing corpse (Catts and Goff 1992; Brown et al. 2012; Richards et al. 2012). There is a temporal sequence of arthropod progression

following death that includes a period of rapid invasion by surface feeding dipterans (flies) and hymenopterans (ants), a period of maximum arthropod diversity when decomposing tissues are most attractive to consumers, and a period of decline in arthropod quantity and diversity (Schoenly and Reid 1987). The estimation of PMI requires knowledge of the temperature-dependent succession of insects following death in a particular locality (Dadour and Harvey 2008). Tracing this arthropod succession can be used to estimate PMI and seasonality, even for a cold case nine years after a death event (Magni et al. 2012).

Volatile Organic Compounds (VOCs)

The polyamines putrescine and cadaverine have classically been associated with the foul odor of death (Gill-King 1997; Vass et al. 2008; Vass 2012). These biogenic amines are also commonly recognized as odiferous products indicating food spoilage that may result in serious illness if consumed (Lange et al. 2002; Pessione et al. 2005). However, analysis of organic compounds released by pig carcasses using the advanced analytical methods of thermal desorption interfaced with gas chromatography and mass spectrometry has found that 104 cadaveric volatile organic compounds (VOCs) can be identified in various environmental settings, but not all are at every location (Dekeirsschieter et al. 2009). A common core of 35 VOCs were found in three selected biotopes, and the olfactory signature of decaying pig carcasses was similar to human decomposition. The main cadaveric VOCs found were organic acids, cyclic hydrocarbons, oxygenated compounds, sulfur, and nitrogen compounds. The decompositional odor was shown to change over time and to vary with different environments of decomposition. Variation between the biotopes based on soil,

vegetation, air, microorganisms and insects suggests that there were different decompositional processes and production of cadaveric VOCs were based on environmental factors (Dekeirsschieter et al. 2009).

A headspace analysis for VOCs was also completed by Hoffman et al. (2009) on 14 samples of human remains. Tissues examined included skeletal muscle, fat, bone, blood, and teeth. They found a total of 33 VOCs associated with these tissues. No single sample contained all of the VOCs, and it was found that there was 1 high, 15 medium, and 17 low-frequency compounds. The VOCs identified were classified as acid/acid esters, alcohols, aldehydes, halogen, aromatic hydrocarbons, ketones, and sulfide (Hoffman et al. 2009).

Vass et al. (2008) examined VOC evolution from multiple mammal species including humans and identified 478 volatile and semi-volatile compounds of which 30 were key markers of human decomposition that were detectable at the surface of the soil. For humans the highest ranked VOCs were carbon tetrachloride; toluene; ethane; 1,1,2-trichloro-1,2,2-trifluoro, 1,4 dimethyl benzene; and benzene.

Environmental Factors - Temperature

The rate of cadaver decomposition and deposition of elements onto the underlying strata and soil is controlled by many environmental factors including temperature, access by insects, depth of burial versus surface, presence and types of predators, trauma, humidity, rainfall, forest type, marine environment, body mass, enclosures, effects of chemicals, soil types, and clothing (Rodriguez and Bass 1983; Mann et al. 1990; Davis and Goff 2000; DeVault et al. 2003; DeVault et al. 2004; Voss et al. 2008; Parks 2011;

Voss et al. 2011; Tumer et al. 2013; Schotsmans et al. 2014). Out of all of the factors studied, the effects of temperature are frequently found to be the most influential (Binford 1978; Galloway et al. 1989; Micozzi 1991; Galloway 1997; Gill-King 1997; Carter and Tibbett 2006; Carter and Tibbett 2008; Forbes 2008b; Prangnell and McGowan 2009; Stokes et al. 2009; Suckling 2011; Stokes et al. 2013).

The ambient temperatures of the decomposition environment may vary by the local environment and directly affect the rate of decomposition (Gill-King 1997; Prangnell and McGowan 2009; Suckling 2011). The thermal principle of chemistry that affects floral, faunal, and microbial actions is known as Van't Hoff's rule, or the temperature coefficient "Q_{10}" (Gill-King 1997; Hopkins 2008). This physical chemistry principal states that the velocity of chemical reactions increases or decreases by two or more times with each 10°C of increase or decrease in the 10°C to 40°C (50°F to 104°F) range.

Chemistry of the living human body functions near 37°C (98.6°F), and changing the ambient body temperature will speed up or slow down the metabolic processes of all organisms associated with decomposition. The Q_{10} for many cellular enzymatic processes is in the range of 1.1 to 3 (Gill-King 1997). This means that each change of 10°C will speed up or slow down chemical processes one to three times normal. The ambient temperature also affects the total insect activity and succession that is seen in normal seasonal variations (Payne 1965; Johnson 1975; Dadour and Harvey 2008; Prangnell and McGowan 2009). Microbial diversity is affected by temperature variations with species richness being significantly lower in temperatures lower than 4°C (39.2°F) as compared to 22°C - 40°C (71.6°F-104°F) (Andrews et al. 2000). Low temperature is a constraint on

the decomposition of organic matter in soil, with virtually no decomposition at 0°C (32°F) and doubling of the decomposition rate between 5°C and 10°C (41°F and 50°F) (Tibbett et al. 2004; Carter and Tibbett 2006).

The activity of each species of bacteria is affected by a specific optimal temperature range. For instance, the bacterium *Clostridium perfringens*, found in both the gut and soil, functions best at 15°C - 25°C (59°F - 77°F), while the bacterium *Clostridium putrefaciens*, found primarily in soil, has an optimal range of 0°C - 35°C (32°F -95°F) (Prangnell and McGowan 2009). These two bacteria are frequently isolated from decomposition sites, and they are active in breaking down lipids and complex carbohydrate associated with human tissue (Hyde et al. 2013). It has also been noted that 4°C (39.2°F) provides an effective low temperature threshold below which bacterial growth is severely retarded, with optimal growth rates found in the temperature range of 15°C - 35°C (59°F - 95°F) (Micozzi 1991). It has also been observed that many bacteria associated with mammals have a high temperature threshold approaching the body temperature of homoeothermic animals (Micozzi 1997). At this upper limit of 37°C (98.6°F) bacterial the time of each cell division approaches infinity, and soft tissue decomposition rates are reduced.

Binford (1978) has a lengthy observation on the preservation of meat by Alaskan Natives (the Nunamiut) that is germane to the study of decomposition. He observed that the two factors of temperature and moisture are most closely related to spoilage due to bacterial activity. Desiccation reduces spoilage by reducing moisture to levels below optimal reproduction of decomposers. In lower temperatures less dehydration of food was required because the reproductive potential of decomposers was also reduced. He

discussed the evidence that *Bacillus mycoides*, a common soil bacteria that may contaminate food sources, has two thresholds of cell division activity significant to food preservation and decomposition. The first is the cool range below 16°C (60.8°F) when cell division is retarded. Below 5°C (41°F) cell division essentially stops, and at 0°C (32°F) the time for cell division approaches infinity. The second threshold is at normal human body temperature, 36.8°C (98.6°F), when cell division for the bacterium also stops and time for division approaches infinity above this threshold. The optimum temperature range for active food decomposition is in the range of 15°C - 37°C (59°F - 95°F) where very rapid decomposition occurs unless the food is severely dehydrated. Decomposition was found to be delayed between 6°C - 15°C (43°F - 60°F), especially with desiccation. Below 5°C (41°F) decomposition was severely retarded with minimal desiccation, and below 0°C (32°F) food storage was optimized without desiccation because water was not available for microbial use.

The influence of temperature on microbial activity in decomposition processes suggests that there is a lower threshold of microbial cell division at 4°C - 5°C (39.2°F - 41°F) with a definite cessation of microbial activity at 0°C (32°F) when water freezes at sea level (Binford 1978; Micozzi 1986; Micozzi 1991; Tibbett et al. 2004; Carter and Tibbett 2006; Prangnell and McGowan 2009). The diversity of microbes is also affected by low temperature (Andrews et al. 2000). This also suggests a reasonable base temperature for calculating accumulated degree days (ADD) is 4°C - 5°C (39.2°F - 41°F) because significant bacterial-generated decomposition may only occur above this temperature. The upper temperature limit of activity suggests that soft tissue decomposition is also dependent on microbes that optimally function in a normal

mammalian body temperature range (Binford 1978; Micozzi 1997). This may be especially significant in the warm, dry southwest region of the Freeman Ranch.

Summary

The overall goal of soil analysis in conjunction with cadaver decomposition is to provide an estimate of PMI based on the taphonomic changes related to decomposition that result in altered soil chemistry (Carter and Tibbett 2008). It is possible that soil chemistry can be used to complement estimates of PMI in conjunction with entomological findings during early decomposition. It is also possible that soil chemistry alone may be a robust predictor of PMI in extended intervals. Only a few studies to date have related soil changes with PMI (Rodriguez and Bass 1985; Vass et al. 1992; Davis and Goff 2000; Benninger et al. 2008; Vass et al. 2008; Pringle et al. 2010; Damann et al. 2012), and the opportunity exists to bridge the fields of entomology, microbiology, and soil science in estimating PMI.

This chapter has presented a brief review of the taphonomic processes associated with surface human decomposition. The focus has been on the primary events that occur in a predictable manner, as well as the major biological and environmental variables that affect the decomposition process. The soil associated with the cadaver is the medium that interacts with the process and receives and stores the elemental end products of decomposition. Analysis of this soil holds promise in defining PMI and the biological processes of decomposition. The next chapter will describe the methods that have been used to attempt to answer these questions in this research project.

III. MATERIALS AND METHODS

This chapter describing the materials and methods used in this study is divided into five sections. The first section describes the research site itself. The second section describes how the soil sample sites were selected and cataloged. The third section describes how the soil samples were taken. The fourth section describes the laboratory analysis and data collection phase. The final section describes the data organization and statistical methods used to analyze the final data set. A dictionary of all data abbreviations used is listed in Appendix A.

Research Site

The Forensic Anthropology Research Facility (FARF) at Texas State University has been conducting surface human decomposition studies for over six years dating to 2008. One of the features of this facility is that it contains a large surface area in comparison to other similar outdoor human decomposition laboratories such as those at the University of Tennessee and Sam Houston State University. A second feature is that it is located in the biotic province classically known as the Balconion province (Blair 1950), but currently identified as the Edwards Plateau or the Texas Hill Country. FARF is situated less than five miles from the Balcones Fault Line that defines the eastern edge of this region by an abrupt uplift of over 61 meters (200 feet) above the adjacent prairie (Alvarez and Plocheck 2014).

The physical location of FARF at Freeman Ranch is near the intersection of many climatic and physical regions of North America in general and Texas specifically that creates unique combinations of soil, weather, and other environmental factors. FARF lies at the boundary of the eastern and western climates of the North American continent, as

well as at the southern and lower extension of the Great Plains. It is also near the boundaries of the physical regions of Texas that include the diversity of the Blackland Belt, the Post Oak Belt, the Coastal Prairies, the Llano Basin, and the Rio Grande Plain (Alvarez and Plocheck 2014). It is characterized by rugged limestone and granite hills, karst hydrology, variable climate and high species diversity in a diverse area surrounded by prairie, savanna, and desert biomes (Texas State University 2013).

Seasonal and climatic variations of the area create both arid conditions of neighboring desert biomes as well as periods of high humidity from the coastal plains. The Freeman Ranch area is subject to a wide variety of conditions associated with a humid sub-tropical climate that is interrupted with drought and semi-arid conditions (Dixon 2000). Summers are generally long and hot, winters are short and mild, and spring and fall seasons are temperate transitions interspersed between the two. Although the ecology of this location is unique in many features, its variable climate and biomes mimic many of the features found in the desert southwest that currently is a forensic investigation focus due to the high number of deaths of undocumented border crossers (Galloway 1997; Anderson 2008; Anderson and Parks 2008; Birkby et al. 2008; Armendariz 2013).

The original FARF site examined occupies five enclosed acres that have a gentle slope from northwest to southeast, with a maximum elevation of 228.80 m (750.7 ft) near the northwest corner and a minimum elevation of 224.80 m (737.5 ft) at the southeast corner (Texas State University 2013). Annual precipitation is 857 mm (about 34 inches) and mean annual temperature is 19.4°C (about 68°F) (Dixon 2000). There is a slight tendency for increased precipitation in September associated with tropical systems. There

are occasional severe rainfall events of 300-500 mm (12 – 20 inches) that are associated with localized flooding (Dixon 2000).

The soils are characterized as relatively shallow, rocky soils that have developed over hardened limestone (Carson 2000). There are two soil series that have been identified in the FARF area of Freeman Ranch that are not precisely mapped out by Soil Survey but are included with the site map at Figure 1 (Carson 2000). The first soil series is known as the Rumple-Comfort Association, Undulating (RUD). It tends to be on broad ridgetops and side slopes with gentle sloping topography as is found at FARF. The surface soil is a cherty-clay (flint-like rocks mixed with clay) with up to 75% limestone in the subsoils. The second soil series is the Comfort-Rock Outcrop Complex, Undulating (CrD). This is dominated by an extremely stony clay soil with rock outcrops and soil less than 10 cm (about 4 inches) thick. The common feature of these soils is that they are both high in clay and rocky. They are limited in moisture storage by a high percentage of rock and rock fragments in the profiles that reduce the volume of space available for water. The soils are mildly alkaline with a relatively high carbonate content due to the limestone (Carson 2000), which is a source of inorganic carbon that has low availability for plant growth (Soil Survey Staff 2010). They also are lacking in organic matter which keeps nitrogen and phosphorus content low. Typical of the harshness of this environment, the Freeman Ranch is much like the nearby biomes of central and south Texas (Carson 2000; Alvarez and Plocheck 2014). Because of the size, dedicated location, and ecology at FARF it is an ideal arena for human decomposition studies in a diverse South Central Texas location. There are forensic applications for casework in south and central Texas

such as unidentified border crosser deaths, and for death investigations involving decomposed remains in similar environmental settings across North America and beyond.

CDI Sample Site Selection

A total of 63 human cadaver decomposition sites and 5 non-decomposition control sites were sampled in this study (Table 1). All samples came from the Forensic Anthropology Research Facility (FARF) at Texas State University. This facility operates a willed-body donation program that serves as a resource for researching forensic questions such as time since death, PMI, and decomposition processes for human remains (FACTS 2015). FARF recorded 201 donations that were placed on the site between 2008 and the start of this study in July, 2014 (Table 1).

An attempt was made to sample sites scattered across all of the years and widely dispersed on the layout of the FARF site. Three *a-priori* conditions had to exist before a site could be accepted for sampling. The first was that the exact location and donation number had to be confirmed in order to have an accurate recorded history of the cadaver placement (regardless whether the cadaver was still present or had been removed previously). This was accomplished by identifying a wooden stake marker that was in place at the head of the cadaver placement, as well as physically identifying the presence of a cadaver decomposition island (CDI). The second was that all placement sites had to be at least one meter away from any adjacent site. This eliminated double placement sites and any site that had close spatial overlap with another identified cadaver placement site. The third condition was to select sites that were widely dispersed across FARF in order to include the soil variability of the soils of the overall facility.

Evaluation of existing records and a physical survey of the FARF site found that there was no consistent and accurate mapping of donation placement sites until the fall of 2011. Routine marking of decomposition sites with stakes did not begin until November, 2011. Before this time only general sketch maps exist for cadaver placement sites. Most pre-2011 placement sites are not accurately recorded or identifiable. This made the identification of exact sites to sample before November, 2011, impossible except for a very few sites. Some cadaver decomposition islands were identified that may have come from early dates, but if they could not be definitely identified and linked to a specific donation they were rejected as sites for this study.

Five control sites were also sampled. These were taken at the four corners of the FARF area, plus one sample from near the center. The corner control sites were located by measuring a 5 m by 5 m square along the fence lines of each corner and taking the sample at the corner of the square farthest from the fence corner at a diagonal distance of approximately 7.07 m. The central control site soil was taken in the shaded area mixed tree cover near the center of the site. At the time of the control sample removals, all sites were greater than 20 meters from the nearest cadaver decomposition sites except for Control #3 at the southwest corner and Control #5 at the center. These two control sites were greater than 10 meters away from the closest surface decomposition site, but less than 20 meters (Figure 1). Note that the location of the control sites is only approximated by direction of the corner sites in Figure 1.

Soil Sample Technique

The sample technique was guided by the methods used in recently published articles (Aitkenhead-Peterson et al. 2012; Damann et al. 2012), although some

modifications had to be made due to the very rocky soil types. All samples were taken from the center of either the cadaver decomposition island if the body had been removed or from under the groin area if the body was still in place. A preliminary soil removal trial method was tested using a Marshalltown trowel to remove a sample, but this proved to be ineffective because of the rocky soil. Instead, a one piece stainless steel tubular soil sampler (15”L x ¾” diameter, Tubular Soil Sampler, Forestry Suppliers, Inc., Jackson, MS, USA) was used to take samples to the first 5 cm (2 in) of the O to A-horizon (Gardiner and Miller 2008; Soil Survey Staff 2010). Care was taken to remove overlying organic debris such as grass, leaves, twigs, or matted gravel before the sample was taken. A thin metal probe was used to locate sites that could be consistently sampled to the 5 cm depth. One soil sample was taken from each of the identified sites.

All samples were collected in the month of July in order to keep the environmental conditions as consistent and stable as possible. The weather was hot and dry, with daily high temperatures in the low to mid 30s°C (mid to high 90s°F) and overnight lows in the low 20s°C (mid 70s°F). Each sample was labeled and immediately placed in a cotton bag (Hubco Soil Sample Bags 3- ½”W x 5”D. Each sample bag was then individually placed in a brown paper sack and allowed to air dry for 14-21 days. Once the samples were dried they were placed in unsealed up-right zip-lock bags for storage until transported to the Department of Soil and Crop Sciences Nutrient and Water (NaWa) Laboratory, Texas A&M University, for soil analysis.

Laboratory Analysis and Data Collection

All samples were sieved through a 2 millimeter sieve to remove rocks and other debris. Each sample was tested for 12 chemical constituents (Table 2). An additional

three chemical constituents were calculated based on those measured variables, giving a total of 15 chemical constituents to use as variables for PMI estimation.

Soil Extraction

An aliquot of 3 g of the sieved soil was combined with 30 mL of ultra-pure water (Barnstead Ultrapure water purification system) in a 50 mL high density polyethylene (HDPE) centrifuge tube. Centrifuge tubes were shaken for approximately 20 h (generally overnight) at 50-60 rpm. The samples were then centrifuged at 19,974 g-force at 25º C for 15 min. The supernatant was removed and pH and electrical conductivity quantified using bench instruments. The supernatant was then filtered through Whatman GF/F glass fiber filters to remove any floating organic material. Recovery of extract approximated 93% and the extract was weighed and diluted with ultrapure water prior for chemical analysis.

Total carbon (TC) was measured using a high-temperature Pt-catalyzed combustion with a Shimadzu TOC-VCSH, and the sample was not acidified or sparged before analysis. Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were measured using high-temperature Pt-catalyzed combustion with a Shimadzu TOC-VCSH and Shimadzu total measuring unit TNM-1 (Shimadzu Corp. Houston, TX, USA). Dissolved non-purgeable organic carbon (NPOC) was measured as non-purgeable carbon using USEPA method 415.1 which entailed acidifying the sample and sparging for 4 minutes with C-free air. Ammonium-N (NH_4-N) was analyzed using the phenate hypochlorite method with sodium nitroprusside enhancement (USEPA method 350.1). Nitrate (NO_3-N) was analyzed using Cd-Cu reduction (USEPA method 353.3). Orthophosphate-P (PO_4-P) was quantified using the ammonium molybdate method.

Colorimetric methods were performed with a Smartchem Discrete Analyzer (Model 200 Westco Scientific Instruments Inc., Brookfield, CT, USA). Calcium (Ca^{+2}), magnesium (Mg^{+2}), potassium (K^{+}), and sodium(Na^{+}) were quantified by ion chromatography using an Ionpac CS16 analytical and Ionpac CG16 guard column for separation and 20 mM methanesulfonic acid as eluent at a flow rate of 1 mLmin−1 and injection volume of 10 μL using a Dionex ICS 1000 (Dionex Corp. Sunnyvale, CA, USA). Dissolved organic nitrogen (DON) is the difference of TDN − (NH_4-N + NO_3-N). Inorganic carbon is the difference between TC and DOC. The carbon – nitrogen ratio is the ratio between NPOC and TDN.

Assessment of PMI and ADD 4

Additional variables were calculated for each CDI that included the known postmortem interval (PMI) (days from arrival to sample), body mass index (BMI) (Mass/Stature; kg/m^2), and Accumulated Degree Days (ADD_4) using a base of 4°C (39.2°F). PMI was sometimes difficult to judge because some of the donations were not received until many days after death. Because the majority of donations were kept in coolers before surface placement at FARF, it was decided to use the day of receipt of the donation as a proxy for the date of death because decomposition had been minimized by refrigeration until placement.

Body Mass Index is a method to standardize stature and weight into one variable that can be related to body fat content and obesity (Gallagher et al. 2000). It is well known that increased BMI, especially in the obese range of over 30, increases the risk of early death due to cardiovascular diseases, diabetes, and many other chronic and acute conditions (Patel et al. 2014). Body mass has also been associated with different soil

chemistry profiles for decomposition (Spicka et al. 2011). The research conducted for this thesis presented an opportunity to use body mass as a variable to assess soil chemistry differences in the obese and non-obese. BMI was calculated for each case based on data recorded at intake.

Accumulated degree days (ADD) have also been used in decomposition studies to allow comparisons between cases and groups by standardizing a temperature based environmental factor that may explain differences in PMI calculations (Megyesi et al. 2005; Pringle et al. 2010; Michaud and Moreau 2011). A base of 4°C (39.2°F) was chosen because this represents the probable lower limit for biological activity for many microbes (Binford 1978; Micozzi 1986). Weather data was obtained from weather stations maintained at the Freeman Ranch and was used to create daily data tables with ADD and precipitation for the time period of January 1, 2009 to August 5, 2014, which included all days for this study. ADD was calculated for each donation from the time of reception to the time of sampling. Standard methods outlined in the literature (Michaud and Moreau 2011; Thevenard 2011) were used in this study.

Delineation of Soil Series at FARF Using XRF

An analysis was completed on a selection of 25 cadaver samples and 3 additional controls using an X-ray Fluorescence Spectrometer (XRF). XRF spectrometry uses X-rays for the non-destructive chemical analysis of rocks, minerals, sediments, and fluids in order to analyze major and trace elements (Suhailly et al. 2014). Portable XRF units can be used as a rapid technique to reliably survey elemental distributions in soil in order to build a profile of the soil that can be used to detect the presence or contamination of soil by heavy metals (Wu et al. 2012; Radu et al. 2013). This type of analysis was used to

detect the presence in the CDI and control soil of the following elements: Potassium (K), chromium (Cr), manganese (Mn), iron (Fe), copper (Cu), arsenic (As), zirconium (Zr), and lead (Pb) in the CDI and control soil. The purpose of this additional analysis was to attempt to delineate the accurate boundaries of the RUD and CrD soil types encountered at FARF based on differences in underlying metal concentrations.

The XRF samples were selected to represent a broad set from across the entire experimental site in order to give a spatially separated set of samples. The portable XRF instrument used in this analysis was a Delta Premium Geochem Analyzer (Olympus Corp., Houston, TX, USA). The instrument was calibrated before use and between every 10 samples using a standard calibration disk provided by the manufacturer. The instrument was calibrated before operation. All concentrations of metals examined for statistical analysis were well above the lower limit of detection (LLOD) for the instrument or were considered non-detectable.

Three readings were taken from each sample, averaged and entered into a data log for statistical analysis (see Appendix C). The assemblage of elements contained within each soil sample from this metals data was examined using a hierarchical cluster analysis with Euclidean squares using Wards method (Meyers et al. 2013). The cluster membership was set as a single solution with two clusters based on there being two soil series at the site. The individual members of the resulting two clusters were then highlighted on the site map (Figure 3) and analyzed in relation to the soil series types in order to better delineate the boundaries of the two soil series.

Data Organization and Statistical Methods

Data screening was completed using IBM SPSS (v23) following a multivariate procedure to make sure that the data are an accurate representation of what was measured and that the data meet the underlying assumptions of the analysis procedures (Meyers et al. 2013). The data were screened for missing values, outliers, and normal distribution, and adjustments to the data were considered based on these results. Descriptive statistics (mean, standard deviation and range) for the control sample chemical constituents were used to analyze potential differences across the entire sample area. A proximity matrix was created for the control samples to test similarity and dissimilarity of these soils.

The cadaver site data was first analyzed using descriptive statistics to characterize each individual variable and to look for univariate relationships between the variables and PMI. The data were evaluated for Pearson (HSD) correlation values among all variables, as well as multi-collinearity between predictor variables. The cadaver data was then subjected to multiple regression analysis in order to find predictive models for PMI and ADD_4 based on the variables that were measured and calculated.

The cadaver database was also analyzed using BMI as a discriminant variable to segment the cadaver data into samples that were analyzed independent of one another. This was done by calculating BMI for cases that had stature and mass data available (N = 55; 6 cases were excluded due to missing data) and segregating cases into groups as follows: BMI < 25 (normal body mass), BMI ≥ 25 (overweight and obese body mass), BMI < 30 (normal and overweight body mass), BMI ≥ 30 (obese body mass) (Gallagher et al. 2000). The models that resulted from multiple regression testing were tested by using the regression coefficients to create formulae that tested the known PMI and

ADD_4 values against the respective predicted values. This step of the analysis gave a statistical predictive value to each model based on the resulting coefficient of determination (r^2) and probability (p).

In summary, the data were organized and analyzed following methods used for multivariate research (Meyers et al. 2013). The computer program IBM SPSS Statistics v23.0 (Released 2015) was used for statistical analysis. This statistical analysis included descriptive statistics and multiple regression that were used to answer the three research questions that sought to define the soil chemistry profile at undisturbed control sites, the soil chemistry profile at surface decomposition sites over time, and whether soil parameters could be used in a quantitatively predictable manner to predict PMI. An additional analysis was done using the XRF data to delineate boundaries of two different soil series'. The following chapter describes the results of this analysis.

Table 1. Summary of Donations and Soil Samples at FARF by Year (2008 – July, 2014). There were 201 donations placed at FARF in this time period. 63 donation sites and 5 control sites were sampled.

Year	Number of Donations	Number of Sampled Sites
2008	3	0
2009	11	1
2010	13	0
2011	24	4
2012	49	16
2013	64	29
2014	37	13
Controls		5

Table 2. Soil Analysis Variables. Chemical Constituents: 12 are measured, and 3 are calculated.

Measured Variables	Calculated Variables
pH EC - electroconductivity Nitrate (NO_3-N) Ammonium (NH_4-N) Orthophosphate-P (PO_4-P) Total Dissolved Nitrogen(TDN) Non-purgeable Organic Carbon (NPOC) Total Carbon (TC) Sodium (Na^+); Potassium (K^+) Magnesium (Mg^{2+}); Calcium (Ca^{2+})	Dissolved Organic Nitrogen (DON = TDN - NH_4-N- NO_3-N) Inorganic Carbon (IC = TC - NPOC) Carbon:Nitrogen Ratio (C:N = NPOC/TDN)

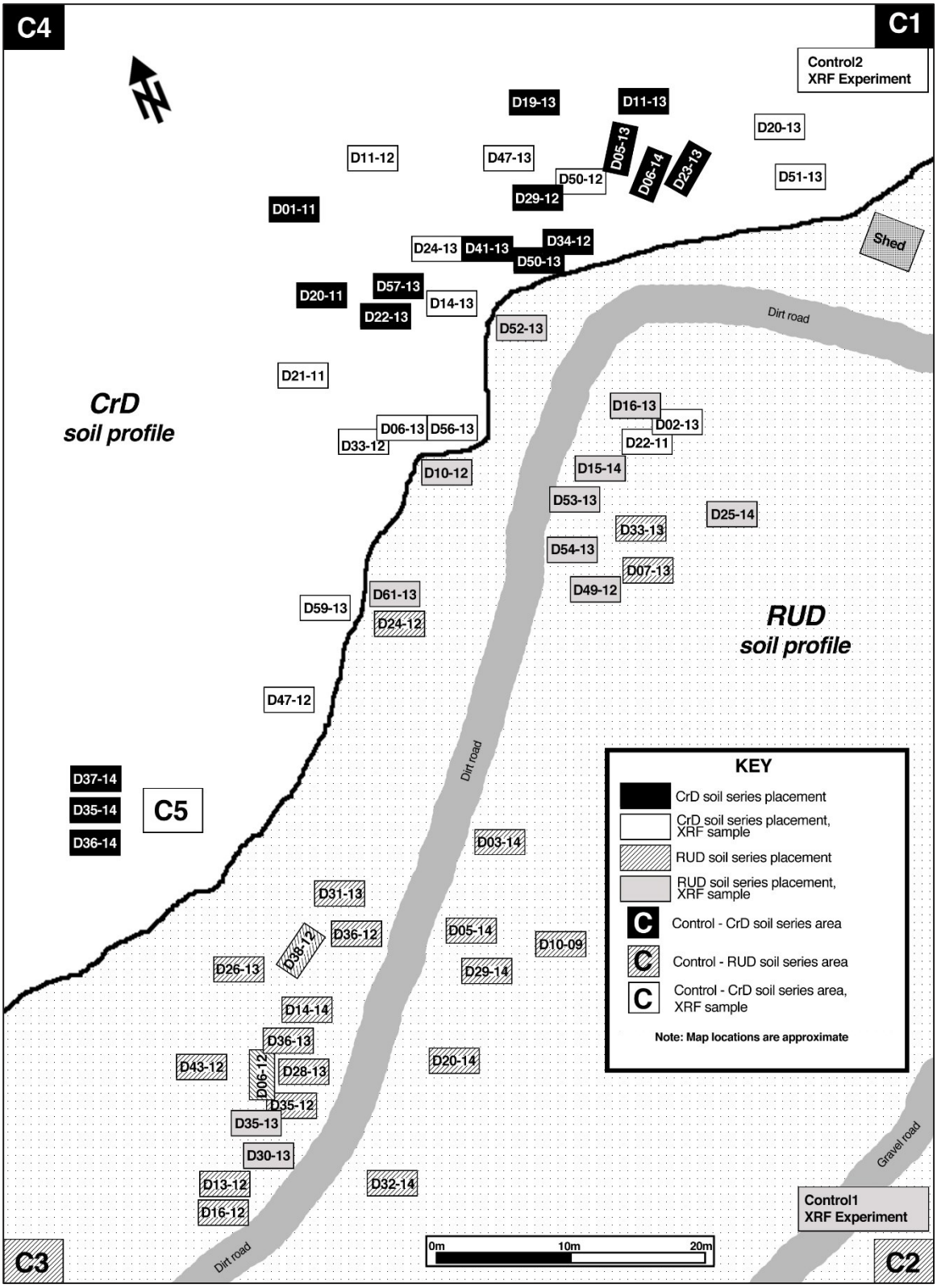

Figure 1. Site Map of FARF Samples. All sites sampled are in boxes with dark borders. The control sites (C1 – C5) are in the directions of the sampled sites and are not to scale. The irregular dark line delineates an estimation of boundary of CrD/RUD soil series.

IV. RESULTS

Appendix B contains the complete data set that was used for the analysis of the results reported in this section. Appendix C contains the complete X-ray Fluorescence (XRF) data set also reported here. This collection of data was only used to answer the research questions stated for this thesis that were posed to evaluate the soil characteristics at control sites, the soil characteristics at cadaver depostion sites over time, and whether these soil parameters could be used to create a quantitative method to estimate PMI. However, this data may be useful for developing future research questions related to decomposition processes, PMI, and soil chemistry, and therefore is included in its entirety.

Data Screening

Data screening was completed using IBM SPSS (v23) following a multivariate procedure to make sure that the data are an accurate representation of what was measured and that the data meet the underlying assumptions of the analysis procedures (Meyers et al. 2013). The first step was to inspect the data for missing values and outliers. No missing values were found in the soil sample data, although stature and/or mass data were incomplete for 6 of the donations. This prevented calculation of BMI for these cases, so it was decided to exclude these cases for any analysis that required BMI because there was no way to accurately impute or replace the missing data.

Second, outliers were assessed by calculating the Mahalanobis distance statistic D^2 that measured the multivariate distance between each case and the group multivariate mean or centroid. The D^2 statistic for each case was converted to a standardized Z Score.

These scores are listed in Appendix B under the columns of MAH representing Mahalanobis distance raw scores and ZMAH representing the standardized *Z* Scores. The criteria for exclusion as an outlier was set at $Z \geq \pm 3.0$ because this would place a score outside of the 99.7% confidence interval. No cases met this criteria, so no cases were excluded.

The third step in data screening was to assess the data distribution for normality. Descriptive statistics were calculated for the 15 soil analysis variables (Table 3). Particular attention was paid to skewness and kurtosis. Skewness describes the symmetry of the distribution, and kurtosis describes the clustering of the scores toward the center of the distribution. Scores for both values should be close to "0" for a normal distribution, and the level of skewness and kurtosis should generally not exceed ± 1.00 (Tabachnick and Fidell 2013). Note that only pH and EC have values for skewness and kurtosis that are less than ± 1.00, indicating that nearly all of the variables are not normally distributed. It is not surprising that most values over time did not follow a normal distribution because they generally would be expected to increase early in time and then return to a baseline level. It was decided to transform all of the soil values except pH and EC using a log10 transformation in order to obtain values that were closer to a normal distribution (Table 4) (Meyers et al. 2013; Tabachnick and Fidell 2013). The resulting transformation gave values that were less than ± 1.00 except for DON and Ca. This meant that except for the two variables DON and Ca, all other variables approached an accepted standard for a normal distribution by transforming the values. Even though DON and Ca were still out of the accepted range for a normal distribution, it was decided to use the log10 transformed values in the preliminary multivariate analysis to evaluate results for

improvements in the outcomes of statistical calculations. If the log10 transformation could result in improved outcomes, then further transformations might have been considered to bring DON and Ca variables closer to a normal distribution.

The last step was to carefully review the placement map developed that documented the spatial relationship of the samples (Figure 1) as well as the physical site. Close inspection found that two of the donation samples actually came from double placement sites (38-2012 and 23-2013). It was decided *a-priori* not to include double placement sites for this investigation. Therefore, these two cases were deleted from the data pool.

In summary, before proceeding with the data analysis, all variables were screened using IBM SPSS (v23) for possible descriptive and statistical assumption violations, as well as for missing values and outliers. Missing values were found for BMI, and it was decided to exclude 6 cases from analysis requiring this variable. Multivariate outliers were screened by computing Mahalanobis distance for each of the 63 cases on the 15 continuous variables, and no cases were identified as potential outliers. Due to violations of the assumption of normality, a log10 transformation was completed for variables that exceeded reasonable guidelines for skewness and kurtosis that resulted in most variables assuming a range of values that more closely fit a normal distribution. Multivariate tests were later compared between the raw data and log10 transformed data in order to assess the need to use this transformation. The final review of site maps and the physical site found that two of the cases came from double placement sites, and these two cases were excluded from final analysis. Also, it was found that six additional cases were missing data required for calculating BMI, so these cases were eliminated from BMI model

calculations. The number of sites used for model construction was 55 for those models where BMI was included and 61 for those models where BMI was not included.

XRF Samples

As previously described, the XRF data were subjected to a cluster analysis using squared Euclidian distance and Ward's analysis (Meyers et al. 2013). All 28 cases were processed, resulting in a dendogram with two clusters that contained 11 cases in one cluster and 17 in the other (Figure 2). The cases for each cluster were then mapped out on the site map using clear, white boxes to designate the first cluster and shaded, cross-hatched boxes to designate the second cluster (Figure 1). An irregular line was drawn to approximate boundaries of soil series for the spatial mapping of the sites.

The resulting distribution of the clusters appears to divide this portion of the FARF site into two zones that may closely delineate the two soil series that have been identified for this area of Freeman Ranch. Two sampled sites appear to be misplaced in the RUD area (D22-2011 and D02-20130). This may be due to the roadway that passes through these sites that could have intermingled soils, or it may be due to a natural delineation of the soil series' parameters. Additionally, it is interesting that the clusters of control samples previously mentioned also somewhat follow this soil series division. Control samples 1 and 4 are in the CrD cluster zone, and Control samples 2, and 3 are in the RUD zone. Control sample 5 is the odd sample that is located closer to 1 and 4 in the mapping distribution but clusters with 2 and 3 in the dissimilarity index described in the next section.

Control Samples

The chemical constituents of five control cases were first analyzed using descriptive statistics (Table 5). There are no complete comparison data on record, so these data represent the baseline soil analysis for the FARF site for the month of July when the CDI's were sampled. The pH was slightly acidic with a range from 5.9 to 6.3 (out of a scale of 0-14, where a pH of 7 is neutral and less than 7 is acidic), which contrasted with a previous soil survey that listed the soils for Freeman Ranch as slightly alkaline (Carson 2000). The ranges of concentrations of all other chemical compounds and elements are listed in Table 5.

The variation between the control sites is difficult to analyze and explain. A proximity matrix was created using IBM SPSS (v23) that shows the relative dissimilarity between each pair of control samples based on Euclidian distance (Table 6). The larger values indicate a greater distance between cases (Meyers et al. 2013). This type of test is often used as part of a plot of cluster membership as it shows how members of a group aggregate on multiple variables. These data show the possibility of two clusters based on dissimilarity scores. Control sites 1 and 4 appear to be close together as least dissimilar to each other, and Control sites 2, 3, and 5 appear to cluster together. This does not agree with the XRF analysis that spatially placed Control sites 1, 4, and 5 together in the CrD area, and sites 2 and 3 together in the RUD area. A sample of 5 cases is far too few to do a cluster analysis that is statistically valid, but this aggregation of control samples may suggest that control variations may be explained by more than location, including transport of decomposition compounds.

Decomposition Samples

The first step in analyzing the decomposition samples was to generate a correlation table for the complete data set (Table 7). The first row is PMI, which is the dependent variable that is the focus of this investigation. The table shows the Pearson correlations (R) of all variables with one another. Although sample size may influence the degree of significance, there is general agreement that R values of $\pm$ 0.1, 0.3, and 0.5 respectively are cutoff points for small, moderate, and large levels of significance (Cohen 1988). The values that represent moderate significance are italicized and underlined, and values with large significance are bolded and underlined. The calculated significance value for each R value is coded by a superscript letter. Note that throughout this paper Pearson product-moment correlations and coefficients of determination are represented with lower case r or r^2 for univariate values and capital R or R^2 for multivariate values due to standard notation for statistics (Meyers et al. 2013).

Inspection of the PMI row (Table 7) shows that two variables, pH and EC, have very low or no significance. Also, the carbon:nitrogen ratio (C:N) is in the low significance level. All other variables are considered to have moderate to large significance to PMI. This line of the correlation table also confirms that all variables have a statistically significant correlation with PMI except pH, EC and C:N.

The second step of analysis was to complete a multivariate regression analysis on the data set using PMI as the dependent variable and the 15 soil factors as independent variables. The initial sample size was $N = 63$. The summary result of this analysis is shown in Table 9, Model 1 (Full Data Model). This model was statistically significant,

F(15, 47) = 5.26, $p < .001$ and accounted for approximately 63% of the variance of PMI ($R^2 = 0.63$). PMI was primarily predicted by decreasing levels of total dissolved nitrogen (TDN), organic carbon (NPOC), dissolved organic nitrogen (DON), total carbon (TC), and sodium (Na^+). PMI was predicted to a lesser extent by declining levels of electroconductivity (EC), ammonium (NH_4-N), phosphate (PO_4-P), potassium (K^+), magnesium (Mg^{2+}), and calcium (Ca^{2+}). The regression coefficients of the predictors are shown in Appendix D under Model 1.

This first model run also included diagnostics for multicollinearity that are shown in the Tolerance and VIF columns in Appendix D, Model 1. Multicollinearity is a condition that may exist when two predictors correlate very strongly with each other that may distort the interpretation of multiple regression results (Meyers et al. 2013). This often occurs when two varibles are measuring the same characteristic in different manners. It may be caused by variables that measure the same subscales differently, or variables that are mathematical transformations of each other. Tolerance values for a variable that are less than 0.1 and VIF values greater than 10.0 should be evaluated for multicollinearity.

This first model indicates that there are multicollineraity problems in the values for measuring nitrogen compounds (NO_3-N, NH_4-N, TDN, and DON) as well as organic carbon compounds (TC and NPOC) (Appendix D, Model 1). The variable for total nitrogen (TDN) represents the sum of the other nitrogen variables (NO_3-N, NH_4-N, and DON), and the variable for total carbon (TC) represent the sum of the other two carbon variables (NPOC and IC). It was decided to eliminate these two summative variables from further analysis as they are mathematical transformations of other variables. It was

also decided to eliminate the carbon:nitrogen ratio (C:N) variable because this also represents a mathmatical transformation and has a borderline multicollinearity problem.

The summary results of 13 different model runs calculate using IBM SPSS (v23) are shown in Table 8. The first set of models #1 – 7 used PMI as the dependent variable, and models #8 – 13 used ADD_4 as the dependent variable. The unstandardized coefficients resulting from this analysis and all subsequent model runs are in Appendix D. Model 1 was the complete set of data (N=63) run as a baseline without any changes as previously described. The dependent variable was PMI, and the independent variables were the 15 measures of each soil sample or case. Model 2 was the adjusted model (N=61) where 2 cases (due to double placement) and 3 variables (TDN, TC and C:N) were removed as previously discussed due to violation of *a-priori* assumptions and multi-collinearity concerns. This same adjustment of the data was used for Models 3 to 13 with the additional removal of 6 cases from BMI models due to cases with missing BMI data (N=55 total for non-segmented BMI models). Model 3 was run with the log10 transformed variables (N=61). Models 4 to 7 were run by subdividing the data by BMI categories; Model 4 was BMI $\geq$ 30 (obese), Model 5 was BMI < 30 (overweight and normal), Model 6 was BMI $\geq$ 25 (overweight and obese), and Model 7 was BMI < 25 (normal or underweight). Models 8 – 13 were run almost identically with Models 2 – 7 except the dependent variable was selected as ADD base 4°C (ADD_4). Model 8 was run using the adjusted independent variables, Model 9 used the log10 transformed variables, and Models 10 – 13 used the BMI subdivided data; Model 10 was BMI $\geq$ 30, Model 11 was BMI < 30, Model 12 was BMI $\geq$ 25, and Model 13 was BMI < 25. Models were

attempted but not reported for the BMI segment 25 < BMI $\leq$ 30 because there were not adequate cases to run this regression (N=8).

There was inconsistent improvement in the models using the log10 transformed data, and it was decided not to use this data except for the two preliminary runs. Model 3 using PMI as the dependent variable and the log10 transformed data had a slight increase in *R* value, and Model 9 using ADD_4 as the dependent variable and the log10 transformed data also had a slight increase in *R* value. However, the resulting 2-4% improvement of explaining the variation (based on R^2) did not improve the significance of the moels themselves. The lack of significant benefit of using the transformed data was noted and discontinued.

The test of significance of each model was done using an analysis of variance (ANOVA; Table 8). The regression effect was statistically significant for all models ($p <$ 0.001), indicating that prediction of the dependent variable is accomplished better than can be done by chance (Meyers et al. 2013).

In order to evaluate the predictive value of the models, regression equations using the coefficients listed in Appendix D were used to create graphs of the actual PMI and ADD_4 data versus the predicted or calculated PMI and ADD_4 values. This was done for Models 2 and 8 for the full adjusted sets of data (Figure 3), Models 4 and 10 for data from the BMI greater than or equal to 30 models (Figure 4), Models 5 and 11 for data from the BMI less than 30 models (Figure 5), Models 6 and 12 for data from BMI greater than or equal to 25 (Figure 6), and Models 7 and 13 for data from BMI less than 25 (Figure 7). The equation for the line of best fit for each graph and the r^2 and *p* values are

listed on each graph. The intercept of each graph was set at (0,0) in order to best visualize the over and under estimation of predicted values.

An analysis of the over and under estimations of each model is in Table 9. The means and standard deviations of each over and under estimation are listed by model. The sample sizes are equal or nearly equal except for Models 4 and 10 (BMI $\geq$ 30) as represented by "N" in each table, and the results of a two tailed *t*-test between the sets of values for each model are shown. In all cases there was no statistical significance, which means that there was no difference between the over and under values for all models and any differences are due to chance (Spatz 1997).

Summary of Results

Samples from 63 decompostion sites and 5 control sites were analyzed for 15 soil parameters. The period of the utilization of decomposition sites spanned the time period 2009 – 2014, although most samples were taken from 2012 – 2014 sites. The results of the control analysis form a baseline set of data for the FARF site representing unmodified soil products with no human decomposition by products directly introduced. The decomposition samples were subjected to multiple regression analysis that had statistically significant results for all 13 models tested. An additional XRF study provided evidence to map out different soil series' of the FARF site that might help explain differences in control and decomposition samples. Implications of these results are explored in the next section.

Table 3. Descriptive Statistics for 15 Soil Variables. N = 63 Cases

	N	Range	Minimum	Maximum	Mean	Std. Deviation	Skewness		Kurtosis	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Std. Error
pH	63	2.40	5.10	7.50	6.07	0.45	0.047	0.30	0.49	0.60
EC	63	866.00	36.00	902.00	319.41	207.67	0.605	0.30	-0.25	0.60
NO_3-N	63	553.67	0.05	553.71	79.76	125.27	2.371	0.30	5.54	0.60
NH_4-N	63	524.23	1.56	525.78	106.48	125.09	1.838	0.30	3.48	0.60
PO_4-P	63	85.80	0.61	86.42	16.55	15.79	2.194	0.30	6.25	0.60
TDN	63	1012.87	27.33	1040.20	294.25	227.72	1.005	0.30	0.84	0.60
NPOC	63	10620.50	284.84	10905.34	2311.51	2652.41	1.974	0.30	3.17	0.60
DON	63	636.27	-17.17	619.10	111.93	99.00	2.567	0.30	10.17	0.60
TC	63	10685.69	290.96	10976.65	2402.98	2667.20	1.956	0.30	3.12	0.60
IC	63	390.10	6.12	396.22	107.73	84.13	1.177	0.30	1.32	0.60
Na	63	862.67	49.71	912.39	246.60	182.77	1.928	0.30	4.37	0.60
K	63	620.88	23.23	644.11	137.10	87.12	3.197	0.30	17.80	0.60
Mg	63	162.14	8.57	170.70	37.32	30.30	2.773	0.30	9.35	0.60
Ca	63	1643.55	74.65	1718.20	307.90	343.08	2.918	0.30	8.75	0.60
C:N	63	83.45	1.06	84.51	11.05	14.64	3.475	0.30	13.26	0.60

Table 4. Descriptive Statistics for log10 Transformed Variables. N = 63 Cases

	N	Range	Minimum	Maximum	Mean	Std. Deviation	Skewness		Kurtosis	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Std. Error
pH	63	2.40	5.10	7.50	6.07	.45062	0.05	0.30	0.49	0.60
EC	63	866.00	36.00	902.00	319.41	207.67116	0.61	0.30	-0.25	0.60
log10NO_3N	63	4.09	-1.34	2.74	1.33	.82964	-0.43	0.30	0.23	0.60
log10NH_4N	63	2.53	0.19	2.72	1.68	.61889	-0.26	0.30	-0.93	0.60
log10PO_4P	63	2.15	-0.21	1.94	1.03	.45047	-0.61	0.30	0.30	0.60
log10TDN	63	1.58	1.44	3.02	2.31	.40800	-0.43	0.30	-0.87	0.60
log10NPOC	63	1.58	2.45	4.04	3.16	.40406	0.67	0.30	-0.50	0.60
log10DON	63	2.79	0.00	2.79	1.90	.40594	-1.50	0.30	6.66	0.60
log10TC	63	1.58	2.46	4.04	3.19	.39444	0.62	0.30	-0.50	0.60
log10IC	63	1.81	0.79	2.60	1.88	.41343	-0.67	0.30	-0.01	0.60
log10Na	63	1.26	1.70	2.96	2.29	.29427	0.06	0.30	-0.24	0.60
log10K	63	1.44	1.37	2.81	2.06	.26475	-0.62	0.30	1.20	0.60
log10Mg	63	1.30	0.93	2.23	1.48	.28162	0.31	0.30	0.40	0.60
log10Ca	63	1.36	1.87	3.24	2.34	.31676	1.10	0.30	1.01	0.60
log10CN	63	1.90	0.02	1.93	0.85	.39338	0.35	0.30	0.79	0.60

Table 5. Descriptive Statistics for 15 Soil Variables of Control Samples. N = 5 Cases

	N	Range	Minimum	Maximum	Mean	Std. Deviation	Skewness		Kurtosis	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Std. Error
pH	5	0.46	5.88	6.34	6.11	0.20	-0.30	0.91	-2.55	2.00
EC	5	89.00	70.00	159.00	117.40	37.65	-0.20	0.91	-2.10	2.00
NO_3-N	5	11.47	1.31	12.77	4.42	4.80	1.96	0.91	3.95	2.00
NH_4-N	5	4.14	2.22	6.36	4.48	1.70	-0.21	0.91	-1.49	2.00
PO_4-P	5	1.89	0.12	2.01	0.76	0.75	1.64	0.91	2.82	2.00
TDN	5	22.52	12.94	35.47	25.91	9.42	-0.48	0.91	-1.39	2.00
NPOC	5	171.81	192.84	364.65	294.84	65.32	-0.98	0.91	1.25	2.00
DON	5	15.54	8.07	23.61	17.02	5.80	-0.88	0.91	1.24	2.00
TC	5	134.06	284.60	418.66	351.55	52.48	0.06	0.91	-1.01	2.00
IC	5	120.98	9.75	130.73	71.58	46.94	-0.01	0.91	-0.78	2.00
Na	5	37.39	67.40	104.79	84.42	16.31	0.06	0.91	-2.17	2.00
K	5	37.93	36.63	74.55	58.14	17.12	-0.30	0.91	-2.40	2.00
Mg	5	30.08	20.90	50.98	39.00	11.52	-1.05	0.91	1.26	2.00
Ca	5	260.69	111.94	372.64	239.41	110.19	0.25	0.91	-2.23	2.00
C:N	5	6.46	8.44	14.90	12.13	2.81	-0.61	0.91	-2.28	2.00

Table 6. Proximity Matrix Showing Dissimilarity Between Control Samples Based on Euclidean Distance. Highlighted values are the most dissimilar pairs.

	1:Control-1	2:Control-2	3:Control-3	4:Control-4	5:Control-5
1:Control-1	0.00	**264.56**	168.74	123.11	**214.36**
2:Control-2		0.00	140.91	**333.05**	113.03
3:Control-3			0.00	**274.88**	163.68
4:Control-4				0.00	**259.12**
5:Control-5					0.00

This is a dissimilarity matrix

Table 7. Pearson Correlation Matrix. Values with moderate strength of correlation are italicized and underlined; large strength values are bolded and underlined. All other values have low strength of correlation. The following superscript notations indicate significance level (1-tailed): [a]$p < 0.05$; [b]$p < 0.01$; [c]$p < 0.001$. The values for the PMI row are the most important for this investigation and are unsahded for emphasis. Only pH, NO_3N and C:N are not significantly correlated with PMI.

	PMI	pH	EC	NO_3N	NH_4N	PO_4P	TDN	NPOC	DON	TC	IC	Na^+	K^+	Mg^{+2}	Ca^{+2}	C:N
PMI	1.00	0.05	*-0.30*[b]	-0.05	*-0.33*[b]	*-0.33*[b]	*-0.43*[c]	**-0.55**[c]	*-0.49*[c]	**-0.55**[c]	-0.22[a]	**-0.51**[c]	*-0.44*[c]	*-0.37*[c]	*-0.32*	-0.19
pH		1.00	0.29[a]	*0.32*[b]	*0.36*[b]	-0.07	0.29[a]	*-0.46*[c]	-0.16	*-0.45*[c]	0.19	0.08	0.03	*-0.39*[c]	-0.29[a]	**-0.58**[c]
EC			1.00	**0.55**[c]	**0.57**[c]	0.25[a]	**0.75**[c]	*0.43*[c]	*0.38*[c]	*0.44*[c]	*0.35*[b]	**0.54**[c]	**0.64**[c]	*0.48*[c]	**0.53**[c]	0.08
NO_3N				1.00	*0.33*[b]	-0.21	**0.57**[c]	-0.27[a]	-0.23	-0.26[a]	0.24[a]	0.08	0.13	0.07	0.25[a]	*-0.34*[b]
NH_4N					1.00	0.18	**0.88**[c]	0.17	*0.41*[c]	0.18	*0.34*[b]	*0.42*[c]	*0.46*[c]	-0.06	-0.07	-0.28[a]
PO_4P						1.00	*0.33*[b]	**0.54**[c]	**0.75**[c]	**0.54**[c]	0.07	**0.55**[c]	**0.73**[c]	0.20	0.05	0.06
TDN							1.00	0.28	**0.54**[c]	0.29[b]	*0.38*[c]	**0.58**[c]	**0.69**[c]	0.09	0.12	-0.29
NPOC								1.00	**0.75**[c]	**1.00**[c]	0.16	**0.54**[c]	**0.61**[c]	**0.69**[c]	**0.58**[c]	**0.65**[c]
DON									1.00	**0.75**[c]	0.17	**0.72**[c]	**0.85**[c]	0.21	0.07	0.10
TC										1.00	0.19	**0.54**[c]	**0.62**[c]	**0.70**[c]	**0.59**[c]	**0.64**[c]
IC											1.00	0.12	0.17	0.22[a]	0.29[b]	-0.02
Na^+												1.00	**0.75**[c]	0.22[a]	0.08	0.08
K^+													1.00	**0.31**[b]	0.19	0.03
Mg^{++}														1.00	**0.88**[c]	**0.77**[c]
Ca^{++}															1.00	**0.63**[c]
C:N																1.00

Table 8. Summary of Models Run Using Multiple Regression. Two independent variables were used; PMI and ADD base 4°C (ADD_4).

Model	Model Number	Dependent Variable	N	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
								R Square Change	F Change	df1	df2	Sig. F Change
Full data model	1	PMI	63	0.79	0.63	0.51	224.85	0.63	5.26	15	47	0.001
Adjusted model	2	PMI	61	0.77	0.60	0.50	230.08	0.60	5.96	12	48	0.001
log10 model	3	PMI	61	0.78	0.62	0.52	225.30	0.62	6.39	12	48	0.001
BMI $\geq$ 30	4	PMI	20	0.99	0.97	0.93	79.36	0.97	21.55	12	7	0.001
BMI < 30	5	PMI	35	0.89	0.79	0.67	185.40	0.94	6.83	12	22	0.001
BMI $\geq$ 25	6	PMI	28	0.96	0.92	0.85	108.36	0.92	14.07	12	15	0.001
BMI < 25	7	PMI	27	0.91	0.80	0.69	190.59	0.83	5.78	12	14	0.001
Adjusted model	8	ADD_4	61	0.78	0.60	0.50	3637.72	0.60	6.01	12	48	0.001
log10 Model	9	ADD_4	61	0.80	0.64	0.55	3403.61	0.64	7.31	12	48	0.001
BMI $\geq$ 30	10	ADD_4	20	0.99	0.98	0.95	1087.01	0.98	29.27	12	7	0.001
BMI < 30	11	ADD_4	35	0.90	0.81	0.70	2768.56	0.81	7.70	12	22	0.001
BMI $\geq$ 25	12	ADD_4	28	0.96	0.92	0.86	1648.83	0.92	15.32	12	15	0.001
BMI < 25	13	ADD_4	27	0.92	0.85	0.71	2855.86	0.85	6.366	12	14	0.001

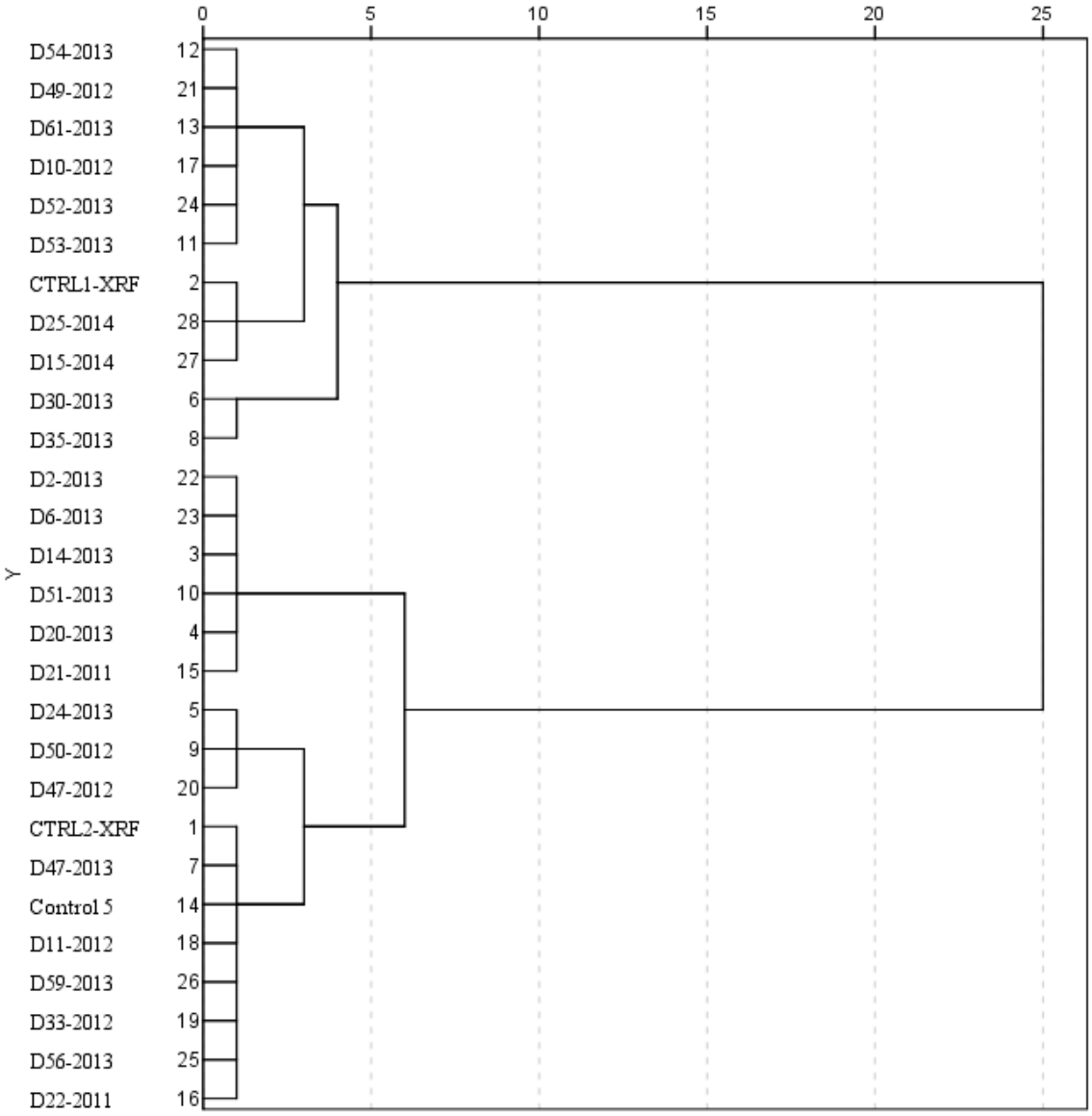

Figure 2. Cluster Analysis Dendogram for the XRF Data. Each case was analyzed on 8 elements using squared Euclidian distance and Ward's Method. The results show two clusters with a division between D35-2013 and D2-2013.

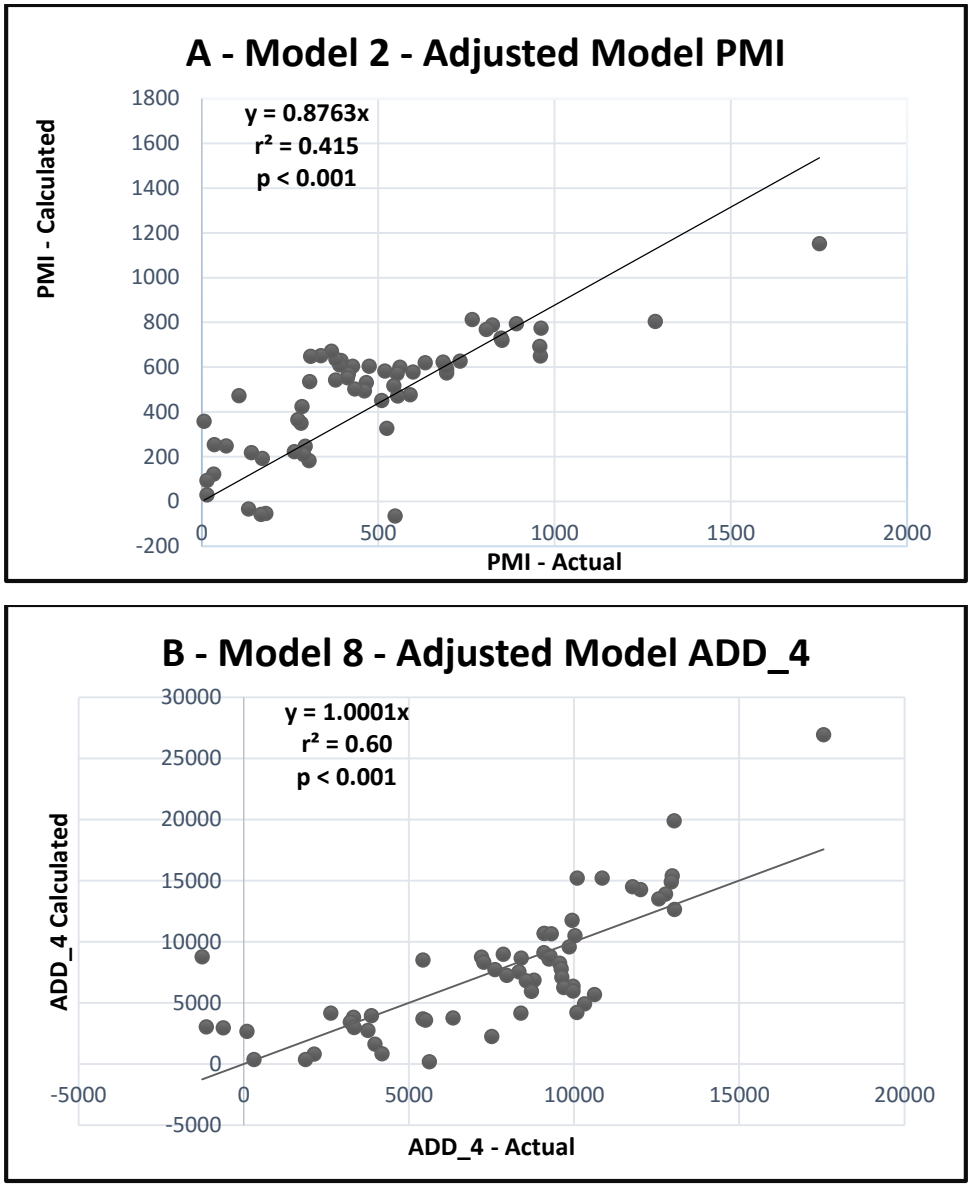

Figure 3. The Graphs and Equation of the Line of Best Fit for Models 2 and 8. These were used to compare the actual and predicted values of PMI and ADD_4 for the adjusted model using all 61 cases. Predictions were made using regression formulae using the coefficients for each model as listed in Appendix D with intercept set at 0/0.

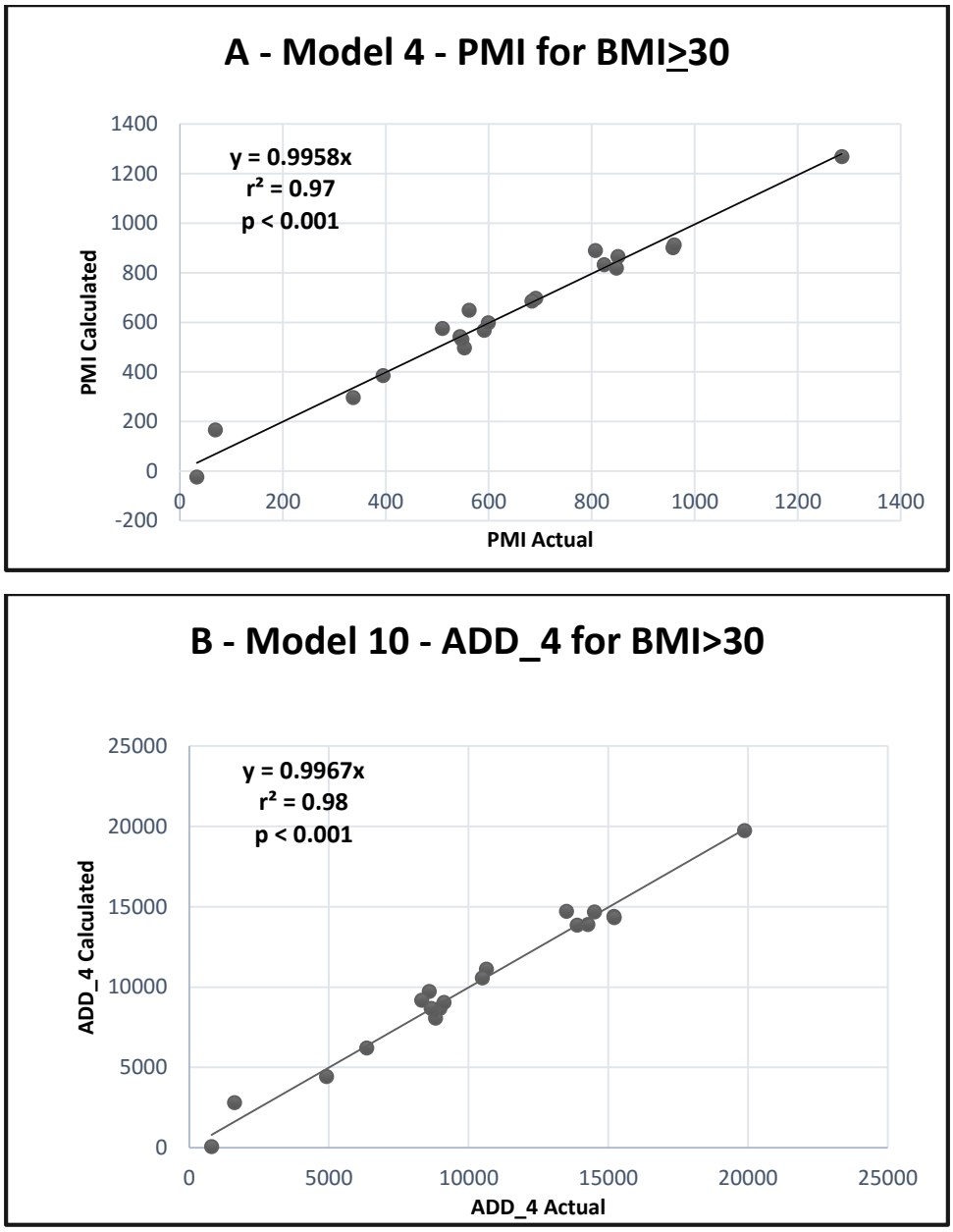

Figure 4. The Graphs and Equation of the Line of Best Fit for Models 4 and 10. These were used to compare the actual and predicted values of PMI and ADD_4 for BMI > 30. Predictions were made using regression formulae using the coefficients for each model as listed in Appendix D with intercept set at 0/0.

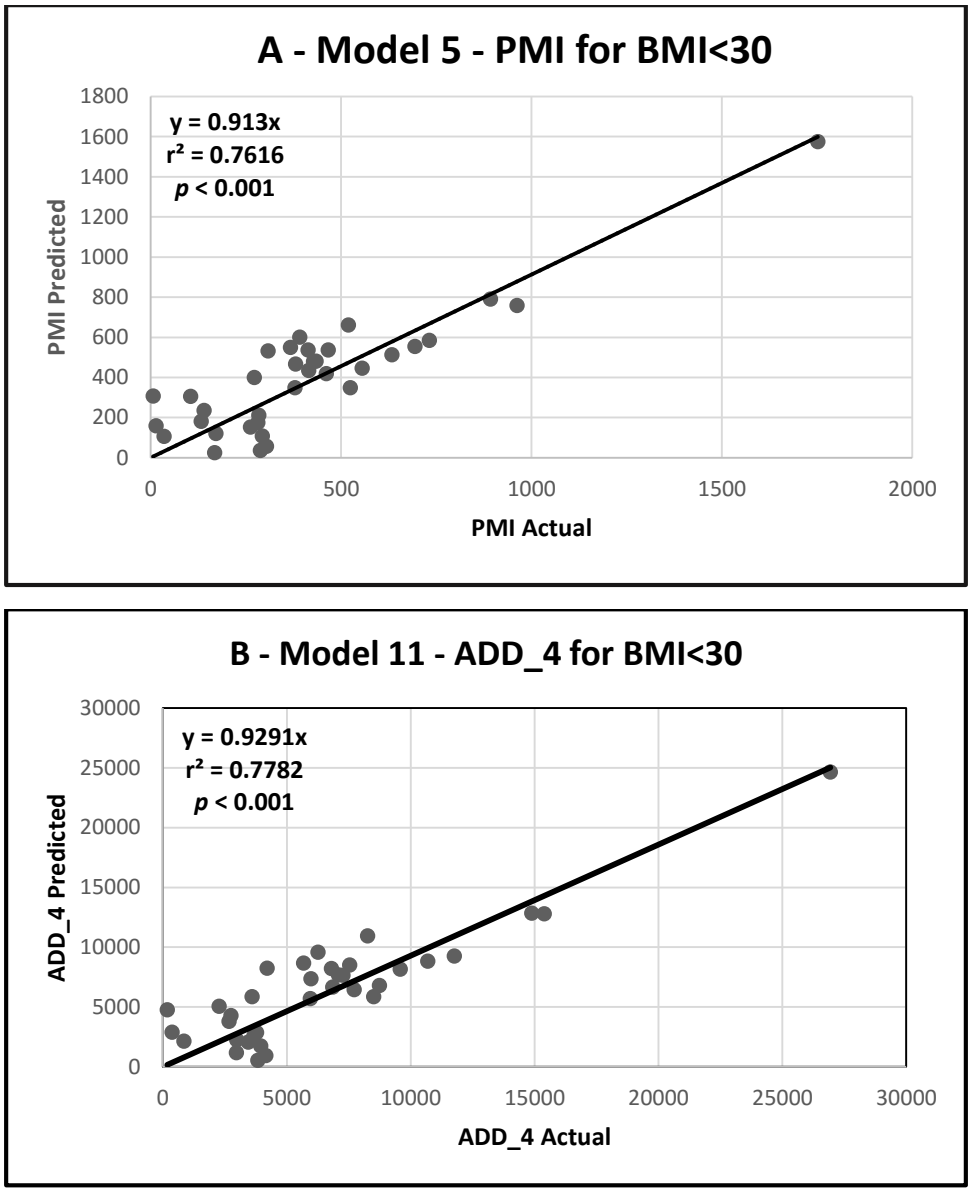

Figure 5. The Graphs and Equation of the Line of Best Fit for Models 5 and 11. These were used to compare the actual and predicted values of PMI and ADD_4 for BMI $\leq$ 30. Predictions were made using regression formulae using the coefficients for each model as listed in Appendix D with intercept set at 0/0.

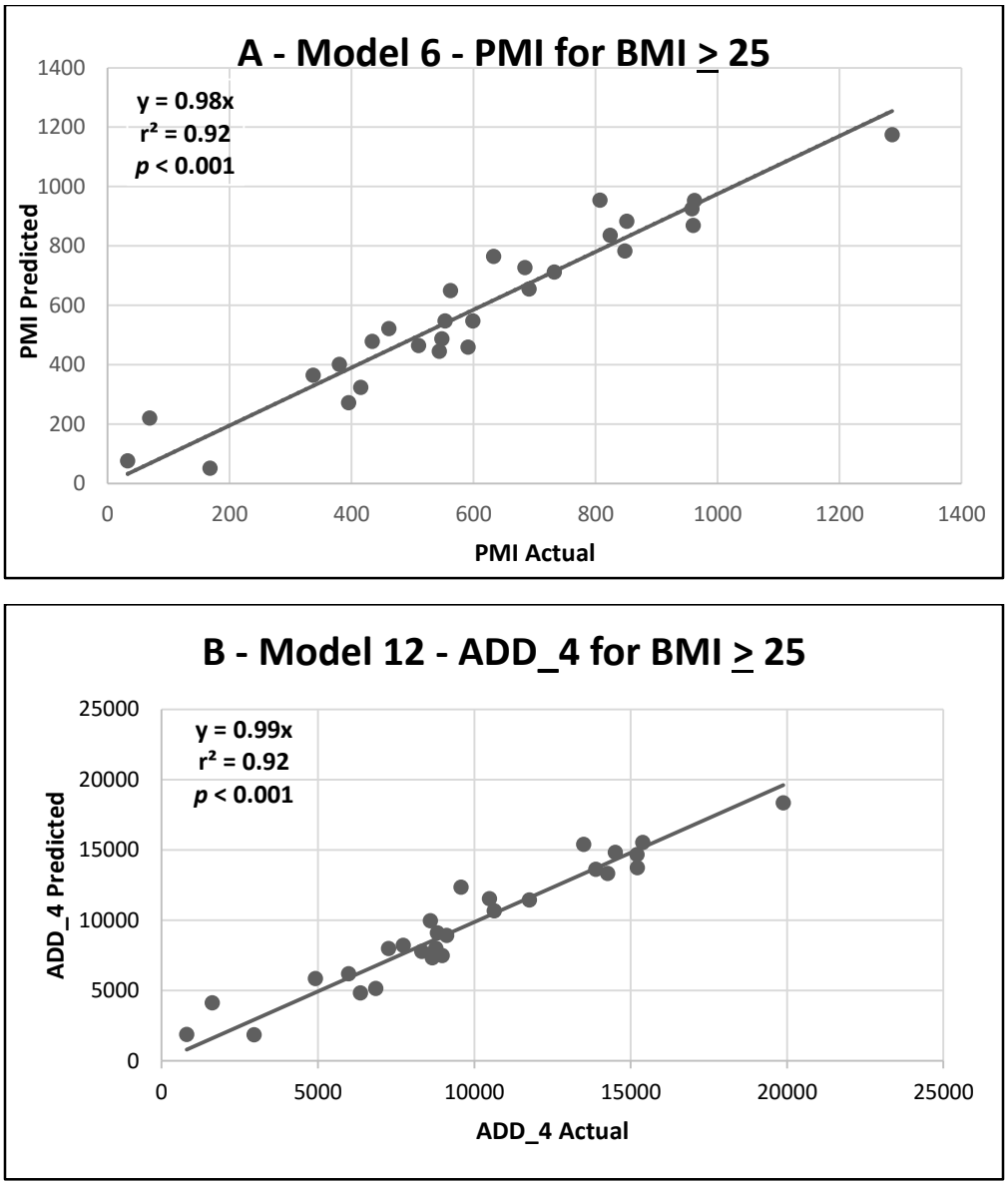

Figure 6. The Graphs and Equation of the Line of Best Fit for Models 6 and 12. These were used to compare the actual and predicted values of PMI and ADD_4 for BMI ≥ 25. Predictions were made using regression formulae using the coefficients for each model as listed in Appendix D with intercept set at 0/0.

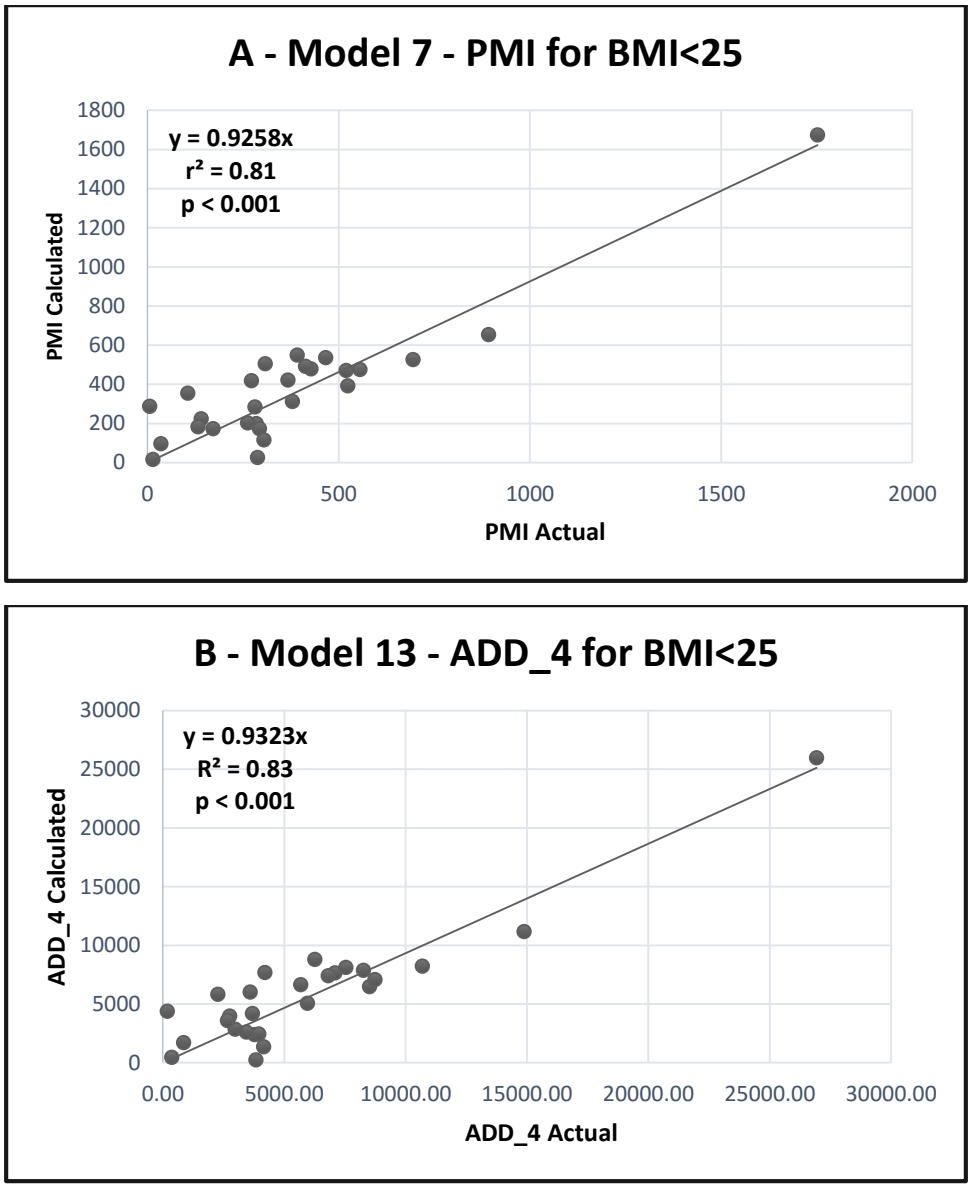

Figure 7. The Graphs and Equation of the Line of Best Fit for Models 7 and 13. These were used to compare the actual and predicted values of PMI and ADD_4 for BMI < 25. Predictions were made using regression formulae using the coefficients for each model as listed in Appendix D with intercept set at 0/0.

Table 9. Summary Data for Over and Under Predictions of Models. Each table lists the number of cases (N), mean, standard deviation**,** and significance of *t*-tests (two tailed) between over and under predictions for each model. No cases have significant differences.

A - Summary Data Adjusted Model 2				
	N	Mean	Std. Deviation	Sig. (2-tailed)
PMI over	31	152.37	107.31	0.86
PMI under	30	158.68	157.61	

B - Summary Data Adjusted Model 8				
	N	Mean	Std. Deviation	Sig. (2-tailed)
ADD_4 Over	30	2456.11	1720.17	0.89
ADD_4 Under	31	2378.82	2536.24	

C - Summary Data BMI ≥ 30 Model 4				
	N	Mean	Std. Deviation	Sig. (2-tailed)
PMI Over	8	45.41	41.47	0.35
PMI Under	12	29.70	21.31	

D - Summary Data BMI ≥ 30 Model 10				
	N	Mean	Std. Deviation	Sig. (2-tailed)
ADD_4 Over	7	723.93	489.24	0.14
ADD_4 Under	13	389.18	315.90	

E - Summary Data BMI < 30 Model 5				
	N	Mean	Std. Deviation	Sig. (2-tailed)
PMI Over	17	126.51	76.51	0.76
PMI Under	18	133.89	64.72	

F - Summary Data BMI < 30 Model 11				
	N	Mean	Std. Deviation	Sig. (2-tailed)
ADD_4 Over	16	2122.76	1227.14	0.40
ADD_4 Under	19	1768.15	899.06	

G - Summary Data BMI ≥ 25 Model 6				
	N	Mean	Std. Deviation	Sig. (2-tailed)
PMI Over	12	66.90	50.13	0.94
PMI Under	16	68.21	41.81	

H - Summary Data BMI ≥ 25 Model 12				
	N	Mean	Std. Deviation	Sig. (2-tailed)
ADD_4 Over	14	981.90	876.42	1.00
ADD_4 Under	14	982.15	540.07	

I - Summary Data BMI < 25 Model 7				
	N	Mean	Std. Deviation	Sig. (2-tailed)
PMI Over	15	99.74	88.19	0.40
PMI Under	12	127.02	71.94	

J - Summary Data BMI < 25 Model 13				
	N	Mean	Std. Deviation	Sig. (2-tailed)
ADD_4 Over	14	1602.95	1351.67	0.80
ADD_4 Under	13	1724.09	1148.11	

V. DISCUSSION

The basis for this project lies at the intersection of two fields; pedogenesis (the study of soil formation and evolution), and forensic anthropology, especially as it relates to taphonomy and decomposition processes. The physical zone of interplay of these two fields is located at the junction of human decomposition and the underlying soil where the final stages of decompostion products are deposited. These postmortem byproducts are the essential elemental traces left behind from the activities of living organisms that create an opportunity for forensic invesitgators to measure these elements in an attempt to estimate the postmortem interval for the deceased human remains.

The soils data collected in this study have been used to provide models for estimating postmortem intervals specific for the FARF site. The models established in this research and the protocols developed for this study can be used to initiate similar research projects at other localities examining for similar variables. This project goes beyond the traditional methods of estimating PMI that have primarily and traditionally relied upon entomology and invertebrate succession (Dadour and Harvey 2008) and physical decomposition stages (Galloway et al. 1989; Galloway 1997; Dent et al. 2004; Carter and Tibbett 2008). Multiple regression models based on soil chemistry analysis are introduced that predict PMI with high correlation values. This substantiates the concept that living decomposers ultimately leave measurable quantities of organic and inorganic materials behind that can be used for forensic purposes. The decomposition community comprising bacteria, fungi, invertebrate and larger scavengers is complex and varies by much more than just the locality-specific insects and scavengers. The environmental variables, especially temperature and moisture, play a major role in controlling microbial

activity that initiates and sustains decomposition processes (Gill-King 1997; Prangnell and McGowan 2009; Suckling et al. 2015).

Ultimately it is the microbial community in the cadaver itself and the underlying soil that convert complex chemical compounds into soil nutrients and other chemical constituents that can be measured postmortem. Insects and other invertebrates are easier to see and identify by their presence than microbes, but the aerobic and anaerobic metabolic processes of microbes continue throughout the cadaver decomposition cycle, and stay active in the cadaver decomposition island (CDI) until nutrients are reduced to simple elements. These end-decomposition products deposited in the soils include the essential elements required by plants and animals to sustain life (Gardiner and Miller 2008).

Those same essential elements required by plants and animals for life largely constitute the elements measured in this study. Of particular interest are the factors with moderate to high correlations with PMI, especially carbon, nitrogen (NH_4-N), phosphorus (PO_4-P), sodium (Na^+), and potassium (K^+). Comparison of the raw data of the means and standard deviations for the cadaver and control samples (Tables 3 and 5) is difficult to explain. It is obvious that the cadaver samples are increased by several magnitudes for many of the extracted soil chemical constituents, but the variation as expressed by the standard deviations are also extremely large. The large variance is due largely to gaseous losses such as CO_2 and N_2 and plant uptake of essential nutrients which will vary depending upon the age of the CDI. As might be expected, the samples are extremely rich in essential elements for plant growth, including nitrogen, phosphorus, potassium and carbon products (Gardiner and Miller 2008). In general the purge will render the CDI

anaerobic. Under these conditions NH_4-N and DON concentrations will be high and NO_3-N concentrations will decrease as soil microbes utilize the oxygen (O) in NO_3-N, denitrifying it and releasing N_2 as a gaseous release. Under anaerobic conditions NH_4-N cannot be nitrified because the function must be carried out in aerobic conditions (Aitkenhead-Peterson et al. 2015). Once the CDI is aerated by scavengers, invertebrates and plant roots, then the amino group (NH_2) of DON will be cleaved forming NH_4, and also due to the likely lack of labile C, will be nitrified forming NO_3-N. Hence the peak of NO_3-N observed during temporal studies of a single CDI is an indication that the CDI is now aerobic.

Carbon enters the decomposition cycle in various forms, largely as complex organic compounds that may be either fully metabolized to carbon dioxide and water in an aerobic environment or converted to a wide variety of mostly transient organic compounds in an anaerobic environment. This carbon turnover is also environmentally sensitive , continually responding to replenishment by new inputs and modification by other decomposing organisms. This may help explain variations in the data set as the single session of collecting samples may have overlain different phases of carbon turnover in each cadaver site.

Both carbon and nitrogen products had strong negative correlations with PMI, and organic carbon (NPOC), and nitrogen (DON) had strong positive correlations with each other (Table 7). DON is a subset of DOC in terms of it having a C-H structure with amino (NH_2 or NH_3) groups attached. Stable isotope studies would be an interesting way to evaluate the cycles of carbon and nitrogen through the plant succession at decomposition sites over several years after cadaver removal. This may relate the source

of these two essential elements as being from routine detritus or a cadaver source in the CDI.

Damann *et al.* (2012) recommend incorporating early and repeated ecological surveys throughout the lifecycle of human decomposition facilities in order to monitor the possibility of saturation or changes in the native soil environment that may result from the constant decay of human remains and constant use for research and education activities. The investigation presented in this thesis represents an effort to gather baseline soils data from a limited number of control sites at FARF and an extensive cross-sectional set of data for soils from decomposition sites representing an initial almost 5 year period of site use. The research design described in this thesis set a goal of eliminating multi-use or cross-contaminated sites, preventing the problem of site saturation for this study. However, the large amounts of nutrient materials deposited in the soils underneath human decomposition sites have been documented to persist in measurable amounts for nearly 5 years after initial placement of cadavers at FARF. The negative correlation factors listed in Table 2 (PMI row) indicate that all variables except pH decline over time, although at diverse rates. While the final end point of return to baseline measures of soil paramenters is not yet known or has not been determined, it is likely to be a timeframe measured in multiple years given such a marginally harsh environment with low soil turnover rates. This indicates that if placement sites are reused or overlapped with existing CDI's in time periods of less than five years there is a high liklihood that soil parameters will be influenced for any subsequent studies. Future studies should focus on defining a finite period for soil regeneration and a starting point for reuse and soil preservation. A section of FARF would benefit from long-term studies

of soil chemical constituents to determine the stage at which baseline conditions return and also the depth at which that decomposition products leach.

A limitation of this study from the outset was that it was cross-sectional and retrospective. It relied on gathering all data in one short time period that represented a snapshot of decompostion processes or the end result of the entire process. This approach is of vital importance for relaying to law enforcement. Most crime scenes have undisturbed soil (no monthly soil sampling) which is important when attempting to model PMI. Continual soil sampling of a CDI may initiate early aerobic conditions which would affect estimation of PMI from a physically undisturbed CDI as is often found at a crime scene. It was not possible to control for seasonal and environmental placement factors between each cadaver in an equal manner, nor was it possible to control for life history factors such as disease and physical attributes for each individual. Despite the design limitations imposed by cross-sectional studies, however, what this design approach did allow for was the collation and analysis of a large number of samples in a short period of time in order to begin baseline data collection with a reasonable number of samples. An alternative design would be to use systematic observations of the same set of cadavers over a long time period, as well as controlling for life history factors. This longitudinal approach would afford a more accurate, chronological sequencing of decomposition products in the soil record, but also may introduce sampling error due to repeatedly disturbing the soils during testing activities (Aitkenhead-Peterson et al. 2012). It also would likely limit the number of subjects entered into the observation pool, and would extend the research project time-frame by a number of years.

The control samples that were taken from a widely spaced area of the FARF site provided a baseline set of data for undisturbed sites. The variations in data have been potentially related to different soil series' that course through the site, a factor which may also influence the cadaver samples. Furthermore differences in control soils may be a factor of translocation of decomposition products downslope, or may simply be due to chance since only 5 samples were taken. The innovative use of the XRF data points to show variations in the soils can best be explored through further systematic research. Soil sampling that is spaced at regular spatial intervals (preferably seasonally) for a period of 1 year across new research sites should be completed before placement of cadavers in order to document the beginning status of soil parameters. This would also provide a rich source of baseline data for future comparisons that the present set of 5 samples could not accomplish. However, the fact that the control soils were taken during the same period as the CDI soils renders them a good comparison.

One of the most significant findings of this research is that analysis of the final 12 variables predicted PMI with a good rate of success for a maximum of almost 5 years (4 years and 8 months) after cadaver placement. How much longer than this time period is unknown because this is the longest period of time tested. Starting with the adjusted model, 60% of the variation (R^2 X 100; Table 8) was explained by the multiple regression formula. When the data were partitioned by BMI for analysis, the explanation of variation increased to 97% for BMI $\geq$ 30, and 79% for BMI less than 30; 92% for BMI $\geq$ 25 and 80% < 25. This lends validation to previous research that carcass mass of pigs can influence the decomposition rate and nitrogen release into gravesoil (Spicka et al. 2011). It also confirms other large mammal studies that have shown that carcass remains can be

detected for very long periods of 5 years or longer in xeric environments that are characterized by dry, hot or cold, environments (Towne 2000; Macdonald et al. 2014). Most significantly, these results show that by measuring elements and compounds left behind in soils associated with human decomposition they can be used to model an estimate of PMI. This is especially important because the elements and compounds chosen for this study can be measured in most standard soils laboratories.

It seems intuitive that a larger carcass will deposit greater amonts of nutrients into the soil, but this differential effect now has been quantified using the BMI scale as a body mass segregation tool. A greater sample size that includes more cases in the middle range of BMI between 25 and 30 is needed to complete the sequence of BMI ranges with statistical significance. Further analysis of the impact of changing amounts of triglyceride (fat) levels on carbon/nitrogen ratios may also give indications of underlying bacterial activity in decomposition (Gardiner and Miller 2008). This may have a practical use in predicting or refining PMI estimation through soil analysis if the stature and mass of a missing person is known. Perhaps a general regression equation can be used to estimate PMI, and then a set of equations based on BMI can be used to further refine the estimate.

Cadavers will decompose at different rates depending on the time of year they were placed due to varying environmental factors, especially temperature (Micozzi 1997; Tibbett et al. 2004; Carter and Tibbett 2008; Prangnell and McGowan 2009). An interesting finding is that the ADD_4 calculations show the same general predictability as PMI when analysed using multiple regression (Table 8). Using 4°C (39.2°F) as the base number to calculate accumulated degree days gave a set of formulas with nearly identical predictive value as the previous formulas for PMI. This supports the concept that there is

a lower limit for an optimal temperature range for human decomposition as discussed by many authors (Binford 1978; Micozzi 1986; Micozzi 1997; Tibbett et al. 2004; Carter and Tibbett 2006; Prangnell and McGowan 2009). Most likely this is based on the lower limit for nearly all microbial and arthropod activity, and essentially nearly all known life forms in nature that are not homeothermic. A base temperature of 0°C (32°F) might also work well, but the literature points to 4°C (39.2°F) as the lowest reasonable limit for most decomposition processes. It was beyond the scope of this research to find an upper limit of decomposition, but most likely this exists at or just beyond the upper limits of human and other mammalian life at approximately 37°C (98.6°F) (Di Maio and Di Maio 1993; Micozzi 1997). Investigating an upper limit temperature in human decomposition, especially in reference to mummification and delayed decomposition, needs to be accomplished.

The ADD data calculated for this study was based on the actual daily temperature measurements taken at the FARF site. Weather stations have been in place on-site continually since the site was opened in 2008. This site-specific data collection is part of the common best-practices for studying human decomposition (Dabbs 2015). Converting accumulated degree days to an estimate of PMI requires working through the ADD data tables, but the results of the regression formulae show a close relationship to PMI (Figures 4 – 7). Future studies of the relationship between PMI and ADD may create a generalizable predictive model. Furthermore, it would be interesting to see how ADD may be related to the very early stages of decomposition, especially purge and bloat. A longitudinal design in various temperature ranges, seasons, and sites should show some promising results.

There are multiple ways to create a model to estimate PMI. In this study multiple regression analysis was used that included all the soil variables, but step methods of building models such as a backward multiple regression analysis may adequately describe PMI with fewer variables (Meyers et al. 2013). The backward multiple regression method enters all the variables and then removes those variables that are co-correlated or contribute least to the model, resulting in a less complex model. A model with fewer variables and an R^2 value that approaches the values found using the standard (enter) method may result in a more parsimonious model. It could be more cost-effective or practical to measure a smaller number of soil parameters that can be justified by such a model for predicting PMI. The results of a preliminary run of the backward method using Model 2 (the Adjusted Model with 61 cases) is shown in Table 10. Five variables were removed step-wise based on the weight of their contributions with a progressive loss of R^2 values of 0.01. This means that only approximately 1% of the ability to account for variation in estimated PMI has been lost by removing these five variables in this set of models. This is a promising method to reduce elements that are measured and focus on factors that have shown higher correlations with PMI as in Table 2.

In summary, there are five major points to be taken from this research. Some are limited to FARF as a single site, but many are generalizable to the field of forensic soil science.

The first is that it is possible to acquire a baseline of soils information for a site such as FARF, but a larger number of samples that can delineate changes in soil types and contours is needed. The topographical characteristics of the study site and previous surveys should help determine the spatial definition of sample sites. The Freeman Ranch

fortunately has previous rangeland studies to guide this process (Carson 2000). An intensive baseline study of the FARF site would create a more detailed ability to understand soil characteristics and variations for this specific area.

The second is that characterization of human decompostion sites is possible with a cross-sectional approach, but there is much variation that is not fully explained. A longitudinal study design would allow better control of studying continuous variables such as environmental and seasonal factors, although the extended period of many years to complete a study would require a strong logistical commitment. Multiple regression formulas that are site specific have been created with good predictability, but need to be verified with a longitudinal study. Additionally, stepwise methods of multiple regression need to be explored in order to find the most parsimonius formulas that may drive the selection of measurable soil parameters.

The third is that accurate calculation of ADD appears to be a very predictable surrogate for estimating PMI. ADD using the most appropriate base temperature may provide insights into temperture-related variables for components and stages of decompostion. Well-designed research may also provide evidence of how temperature relates to the living organisms that participate in the decomposition process. Research projects investigating differences between human decomposition sites in contrasting environments should also analyze for common and differing qualities of decomposition processes and events.

The fourth point is that body mass matters in how a human decomposes and leaves identifiable traces in the soil. Formulas that predict PMI need to be assessed using BMI as a variable to modify calculations or use different formulas for different BMI

ranges. Investigations into the variation and quantity of nutrients from human decomposers in different BMI categories may show how the essential nutrients deposited into soil vary by body mass size. The ADD and BMI co-factors need to be further researched in order to find possible relationships with stages of decomposition, especially how body composition can be related to decomposition rate. This could be part of a longitudinal study as mentioned above.

The final point is that completing a site survey with soil parameters is needed and should be periodically repeated, especially in an environmentally sensitive area such as FARF and the Edwards Plateau. Changes in soil parameters have been detected for almost 5 years, and probably can be for a longer period. This indicates that there are persistent changes in soil chemistry from human decomposition sites in the Edwards Plateau area that may influence future investigations for many years. It also means that as the local area cycles through normal or abnormal environmental conditions, it would be good to know how these cyclical changes affect the native soils.

Table 10. Backwards Multiple Regression Results. Model summary of the preliminary trial using Model 2 data (Adjusted Model). The Change Statistics indicate that Model 1 has a statistical significance of $p < 0.001$, and each subsequent model is not significantly different from the previous run, meaning that all models are statistically significant.

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	0.77[a]	0.60	0.50	230.08	0.60	5.96	12	48	0.001
2	0.77[b]	0.60	0.50	227.75	0.00	0.01	1	48	0.91
3	0.77[c]	0.60	0.52	225.92	-0.00	0.20	1	49	0.66
4	0.77[d]	0.60	0.52	224.29	-0.00	0.27	1	50	0.61
5	0.77[e]	0.59	0.53	222.34	-0.00	0.10	1	51	0.75
6	0.77[f]	0.59	0.54	221.00	-0.00	0.36	1	52	0.55

a. Predictors: (Constant), Ca, PO4P, NH4N, pH, IC, NO3N, Na, K, Mg, EC, NPOC, DON

b. Predictors: (Constant), Ca, PO4P, NH4N, pH, IC, NO3N, Na, Mg, EC, NPOC, DON

c. Predictors: (Constant), Ca, PO4P, NH4N, pH, NO3N, Na, Mg, EC, NPOC, DON

d. Predictors: (Constant), Ca, PO4P, NH4N, pH, NO3N, Na, Mg, EC, NPOC

e. Predictors: (Constant), Ca, NH4N, pH, NO3N, Na, Mg, EC, NPOC

f. Predictors: (Constant), Ca, NH4N, pH, NO3N, Na, EC, NPOC

VI. CONCLUSIONS

This study defined the postmortem interval characteristics of surface human depositions based on the characterization of soil associated with decomposition sites. A wide net of 15 variables was cast to find if common measurements in a soil laboratory has significance in predicting PMI. The results of this study show that multivariate equations with high correlation values can be derived from these data. The primary benefit of this technique, once refined with further research, will be that medical-legal investigations will be aided to help identify and date the locations of human remains or the sites of primary decomposition where such remains were once deposited.

This research compliments and supplements other methods commonly used to predict PMI, especially entomology succession patterns and anthropological observation of decomposition sequences. The timeframe of entomology techniques tend to focus on the seasonal and temporal changes associated with early to advanced stages of decomposition when the insect decomposers are most active, a timeframe commonly extending from days to possibly weeks and a few months. The anthropology techniques of observing stages of decomposition extend from early, moist stages to advanced skeletonization and osseous deterioration, but PMI estimation becomes more difficult with advanced times. Soil analysis offers the advantage of site-specific estimations of PMI that begin in the early stage of decomposition and extend for many years, in this study almost 5 years. The final stages of microbial decomposition and deposition of identifiable elements into soils underlying human cadavers leaves an identifying signature of elements. This research showed that measurement of these elements can

predict PMI, which is a benefit to all workers who conduct medical-legal investigations for criminal, civil, and humanitarian cases in similar ecological or geographical regions.

Specifically, the results of this investigation support the following conclusions based on the questions posed in the introduction and the additional investigation of XRF data.

1. The cluster analysis of XRF data of 28 soil samples (25 cadaver sites and 3 control sites) for 8 elements showed two distinct clusters. When these individual soil samples were highlighted on the site map they appeared to distinguish the two different soil series known to traverse the FARF site. This variation in soil types may help explain some of the variation between soil samples. Future research should expand this analysis to accurately map soil profiles of this research site that may influence soil analysis variation.

2. The soil chemistry profile at undisturbed up-slope and down-slope control sites was tested at 5 sites of the original FARF enclosed site. The results of 15 variables are listed in Appendix B and summarized in Table 5. The pH was slightly acidic with a range from 5.9 to 6.3. The variation in values for the variables is likely explained by different soil types or series' that flow through the site, but also may be explained by transposition of decomposition products at downslope sites. The number of control samples analyzed proved to be too few for an accurate picture of the 5 acre site. Recommendations for future research include expanding the collection of control samples and repeating analysis of soils periodically.

3. The soil chemistry profile at surface decomposition sites over time was analyzed at 63 decomposition sites. The results of 15 variables are listed in Appendix B

and summarized in Table 3. The variables are characterized by wide variability and a general decline over time. Due to the cross-sectional design of this study, many environmental, seasonal, and life history parameters could not be controlled which probably contributed to the variability. It is recommended that future research be longitudinal in design to better control for and study environmental variations.

4. Multiple regression equations were derived from these data that are highly correlated with PMI. Similar equations were derived with high correlations using Accumulated Degree Days base 4°C (39.2°F), indicating that ADD_4 may be used as a surrogate for PMI estimations. It was also found that segregating the samples based on Body Mass Index (BMI) resulted in improvements in the PMI and ADD_4 prediction equations. Although the estimations of PMI and ADD_4 are based on site specific data, the influence of BMI is a generalizable concept that can be incorporated for all decomposition sites. It is recommended that future research consider investigating the relationship of ADD and PMI, especially in relation to stages of decomposition and the life forms that are active decomposers. A reasonable assumption is that decomposition essentially slows to infinity or stops at 0 – 4°C (32 – 39.2°F), but the upper limit of decomposition processes needs further investigation.

APPENDIX SECTION

APPENDIX A: DATA DICTIONARY

Year	Year of donation
ID #	Case ID # (XX-YYYY)
Date Received	Date received (MM/DD/YY)
Date Sampled	Date soil sampled (MM/DD/YY)
PMI	Days from date received to soil sample
ADD_4	ADD Base 4 deg C: $\sum (M^0C - 4^0C)_n$
BMI	Body Mass Index; Mass(kg)/Stature^2(m)
pH	pH of sample
EC	Electro-conductivity uS/cm
NO_3-N	Nitrate *ug*/g soil
NH_4-N	Ammonium *ug*/g soil
PO_4-P	Phosphate *ug*/g soil
TDN	Total dissolved nitrogen *ug*/g soil
NPOC	Non-purgeable organic carbon *ug*/g soil
DON	Dissolved organic nitrogen *ug*/g soil
TC	Total carbon *ug*/g soil
IC	Inorganic carbon *ug*/g soil
Na	Na^+; Sodium *ug*/g soil
K	K^+; Potassium *ug*/g soil
Mg	Mg^{++}: Magnesium *ug*/g soil
Ca	Ca^{++}: Calcium *ug*/g soil
C:N	Carbon/Nitrogen ratio = NPOC/TDN
MAH	Mahalanobis Distance
ZMAH	Standardized (Z) Score of Mahalanobis Distance
Cr	Chromium *ppm*
Mn	Manganese *ppm*
Fe	Iron *ppm*
Cu	Copper *ppm*
As	Arsenic *ppm*
Zr	Zirconium *ppm*
Pb	Lead *ppm*

APPENDIX B: COMPLETE DATA SET

Year	ID #	Date Received	Date Sampled	PMI	ADD_4	BMI	pH	EC	NO_3-N	NH_4-N	PO_4-P	TDN
9	10-2009	11/19/09	9/5/14	1752	26933.84	24.91	7.50	700.00	10.58	35.85	19.63	81.03
11	01-2011	1/6/11	7/15/14	1286	19883.44	49.79	5.33	64.00	5.58	4.23	0.61	40.92
11	20-2011	11/28/11	7/15/14	960	15208.31	58.08	5.94	36.00	0.54	4.31	1.91	34.39
11	21-2011	11/30/11	7/15/14	958	15207.31	30.57	5.80	50.00	0.05	4.87	4.29	46.14
11	22-2011	12/5/11	7/24/14	962	15383.63	27.05	5.34	178.00	6.79	1.56	2.71	30.97
12	06-2012	2/13/12	7/24/14	892	14880.60	23.11	5.57	104.00	8.09	12.77	33.36	86.74
12	10-2012	3/30/12	7/25/14	848	14265.90	43.98	5.68	68.00	11.40	10.67	25.10	68.54
12	11-2012	3/30/12	7/28/14	851	14505.06	30.44	5.62	72.00	3.44	7.82	16.17	70.49
12	13-2012	4/26/12	7/29/14	824	13884.29	30.18	5.62	67.00	14.63	11.25	13.91	78.70
12	16-2012	5/9/12	7/25/14	807	13499.50	37.01	5.55	119.00	32.37	26.81	12.92	138.49
12	24-2012	6/21/12	7/28/14	767	12629.21	0.00	5.42	51.00	2.10	6.85	5.12	49.45
12	29-2012	7/13/12	7/15/14	732	11763.91	27.10	6.24	156.00	20.04	6.46	7.69	59.03
12	33-2012	8/30/12	7/24/14	694	10687.18	0.00	6.10	77.00	1.66	7.52	3.50	54.89
12	34-2012	8/31/12	7/25/14	694	10687.15	22.55	6.05	281.00	68.80	108.87	14.31	301.61
12	35-2012	9/6/12	7/29/14	691	10641.12	40.85	6.09	67.00	16.12	16.84	6.69	69.37
12	36-2012	9/13/12	7/29/14	684	10487.96	48.06	5.82	143.00	8.16	11.97	3.34	123.27
12	38-2012	10/3/12	7/24/14	659	9959.70	26.91	6.16	96.00	3.39	7.66	0.72	27.33
12	43-2012	11/3/12	7/29/14	633	9571.87	29.21	5.97	125.00	9.71	18.33	6.40	103.40
12	47-2012	12/6/12	7/28/14	599	9119.94	32.72	5.88	268.00	106.21	92.12	16.21	274.32
12	49-2012	12/11/12	7/25/14	591	8975.68	30.75	6.31	359.00	84.55	188.62	13.26	409.57
12	50-2012	12/31/12	7/15/14	562	8594.21	38.07	6.39	638.00	375.14	160.32	8.31	518.28
13	02-2013	1/15/13	7/24/14	555	8734.46	23.57	6.62	371.00	150.25	158.13	20.68	373.85
13	05-2013	1/21/13	7/28/14	553	8820.84	35.73	6.50	760.00	397.71	156.29	9.06	576.82
13	06-2013	1/26/13	7/28/14	548	8763.37	43.40	6.45	649.00	538.85	502.76	3.10	1040.20
13	07-2013	1/28/13	7/25/14	544	8657.17	44.80	6.63	425.00	31.06	279.19	28.37	471.23

APPENDIX B, CONTINUED

Year	ID #	Date Received	Date Sampled	PMI	ADD_4	BMI	pH	EC	NO_3-N	NH_4-N	PO_4-P	TDN
13	14-2013	2/19/13	7/28/14	524	8505.88	18.82	6.20	280.00	3.90	96.19	29.51	279.40
13	16-2013	2/27/13	7/24/14	510	8317.97	45.53	6.15	535.00	1.36	367.96	14.93	582.23
13	19-2013	3/29/13	7/15/14	474	7767.59	0.00	6.21	902.00	553.71	205.46	4.01	730.59
13	20-2013	4/5/13	7/15/14	466	7531.85	24.89	6.52	497.00	259.00	51.98	5.40	378.05
13	22-2013	4/23/13	7/28/14	461	7721.82	25.46	6.49	305.00	114.97	108.11	1.55	283.13
13	23-2013	5/6/13	7/25/14	445	7441.86	21.35	6.35	435.00	109.85	151.34	18.28	371.50
13	24-2013	5/20/13	7/28/14	434	7259.09	28.47	6.46	219.00	63.17	28.25	18.53	174.78
13	26-2013	5/23/13	7/24/14	427	7083.58	22.32	6.06	370.00	198.41	90.74	13.29	354.15
13	28-2013	6/9/13	7/29/14	415	6852.98	26.91	6.35	293.00	101.99	47.55	2.38	210.32
13	30-2013	6/11/13	7/29/14	413	6805.98	24.89	6.22	153.00	35.49	30.76	7.27	147.07
13	31-2013	6/24/13	7/24/14	395	6358.02	31.56	6.25	385.00	74.83	183.15	13.13	401.90
13	33-2013	7/3/13	7/29/14	391	6258.94	21.87	5.98	355.00	93.32	177.52	29.38	414.43
13	35-2013	7/9/13	7/24/14	380	5979.77	26.18	6.13	531.00	321.11	96.48	2.02	444.01
13	36-2013	7/15/13	7/29/14	379	5951.04	19.64	6.09	254.00	91.27	12.88	7.53	186.90
13	41-2013	7/26/13	7/28/14	367	5679.67	15.22	5.97	69.00	9.86	9.37	15.47	86.57
13	47-2013	8/12/13	7/15/14	337	4913.96	34.98	5.57	395.00	9.33	144.23	19.96	274.90
13	50-2013	9/20/13	7/25/14	308	4206.16	21.53	6.00	173.00	69.19	34.35	5.92	163.57
13	51-2013	9/22/13	7/25/14	306	4169.10	0.00	5.83	383.00	51.11	192.65	12.17	455.12
13	52-2013	9/27/13	7/28/14	304	4152.78	24.22	5.10	300.00	5.98	84.97	42.88	377.14
13	53-2013	10/9/13	7/29/14	293	3946.94	21.07	5.90	280.00	146.62	98.26	8.81	384.90
13	54-2013	10/10/13	7/25/14	288	3825.37	21.89	6.50	300.00	71.00	118.75	14.78	310.65
13	56-2013	10/16/13	7/24/14	281	3681.27	24.04	6.00	390.00	292.35	162.66	20.87	491.13
13	57-2013	10/17/13	7/28/14	284	3776.56	18.11	6.30	70.00	30.15	16.70	10.37	95.31
13	59-2013	10/30/13	7/28/14	272	3598.61	18.89	6.50	180.00	92.22	51.30	56.06	242.92
13	61-2013	11/5/13	7/25/14	262	3437.59	20.94	6.70	380.00	96.25	307.07	12.92	510.41
14	03-2014	1/30/14	7/29/14	181	3033.18	0.00	5.90	310.00	6.38	26.47	22.91	285.08

APPENDIX B, CONTINUED

Year	ID #	Date Received	Date Sampled	PMI	ADD_4	BMI	pH	EC	NO_3-N	NH_4-N	PO_4-P	TDN
14	05-2014	2/8/14	7/29/14	171	2976.58	21.79	6.60	360.00	122.79	188.97	57.61	524.63
14	06-2014	2/10/14	7/28/14	168	2958.72	29.29	5.40	550.00	3.06	28.43	27.18	284.91
14	14-2014	3/11/14	7/29/14	140	2750.06	24.21	6.20	450.00	3.41	525.78	23.05	775.08
14	15-2014	3/19/14	7/29/14	132	2663.37	24.28	5.40	650.00	2.59	127.63	86.42	749.32
14	20-2014	4/15/14	7/29/14	105	2263.58	22.17	5.50	280.00	3.07	19.82	17.24	171.22
14	25-2014	5/21/14	7/29/14	69	1620.79	35.14	6.20	260.00	4.51	210.72	14.05	379.62
14	29-2014	6/24/14	7/29/14	35	851.02	21.79	6.50	620.00	18.90	238.53	56.27	553.09
14	32-2014	6/26/14	7/29/14	33	807.30	58.61	6.40	610.00	2.44	506.97	26.28	814.27
14	35-2014	7/15/14	7/29/14	14	365.34	20.64	5.20	570.00	3.23	30.04	18.06	123.18
14	36-2014	7/15/14	7/29/14	14	365.34	0.00	5.30	610.00	3.08	14.54	21.25	139.28
14	37-2014	7/23/14	7/29/14	6	179.65	21.46	6.50	150.00	7.25	11.95	2.91	50.26
C	Control1		7/8/14	0	0.00		5.88	148.00	1.39	3.59	0.38	20.41
C	Control2		7/8/14	0	0.00		6.22	90.00	3.98	6.36	2.01	33.95
C	Control3		7/8/14	0	0.00		6.20	120.00	1.31	5.94	0.46	26.80
C	Control4		7/8/14	0	0.00		6.34	159.00	2.65	2.22	0.83	12.94
C	Control5		7/29/14	0	0.00		5.92	70.00	12.77	4.29	0.12	35.47

APPENDIX B, CONTINUED												
Year	ID #	NPOC	DON	TC	IC	Na	K	Mg	Ca	C:N	MAH	ZMAH
9	10-2009	637.44	34.59	667.88	30.44	137.64	138.25	19.68	104.35	7.87	42.81	2.43
11	01-2011	500.57	31.12	531.00	30.43	216.54	24.98	10.27	112.48	12.23	13.96	-0.07
11	20-2011	433.54	29.54	452.66	19.12	56.49	23.23	8.57	74.65	12.61	4.53	-0.89
11	21-2011	609.72	41.22	622.40	12.68	57.72	24.65	10.60	108.12	13.21	4.97	-0.85
11	22-2011	1701.22	29.41	1855.27	154.05	177.55	111.55	65.27	326.99	54.93	38.72	2.07
12	06-2012	744.38	65.88	779.16	34.78	82.79	104.43	38.53	185.04	8.58	11.31	-0.30
12	10-2012	491.01	46.47	474.53	125.74	82.50	97.50	41.62	148.83	7.16	18.13	0.29
12	11-2012	644.59	59.22	692.98	48.39	57.19	91.34	43.92	182.67	9.14	7.35	-0.64
12	13-2012	580.62	52.82	545.88	125.74	89.03	71.67	12.32	87.76	7.38	12.99	-0.15
12	16-2012	768.25	79.31	885.43	117.18	132.02	66.26	22.70	137.06	5.55	6.87	-0.68
12	24-2012	658.70	40.50	683.01	24.32	103.54	35.13	11.55	85.41	13.32	7.37	-0.64
12	29-2012	386.69	32.54	505.30	118.60	107.56	74.44	23.55	294.64	6.55	5.39	-0.81
12	33-2012	588.97	45.72	597.22	8.25	107.67	36.93	12.07	93.85	10.73	4.23	-0.91
12	34-2012	1611.77	123.94	1659.56	47.79	128.35	116.68	29.56	286.93	5.34	5.85	-0.77
12	35-2012	491.19	36.41	521.83	30.64	63.25	62.29	11.37	101.97	7.08	3.98	-0.93
12	36-2012	1574.13	103.13	1567.43	125.74	183.00	61.36	21.16	128.39	12.77	11.19	-0.31
12	38-2012	284.84	16.28	290.96	6.12	140.95	81.52	48.15	174.94	10.42	11.15	-0.31
12	43-2012	954.37	75.36	1022.89	68.52	106.93	64.87	24.39	181.04	9.23	2.57	-1.05
12	47-2012	1496.98	75.99	1522.56	25.58	254.38	93.19	26.10	180.73	5.46	3.98	-0.93
12	49-2012	1832.54	136.40	2095.23	262.68	305.65	140.34	28.63	146.81	4.47	6.41	-0.72
12	50-2012	548.45	-17.17	707.14	158.69	221.14	121.84	34.39	767.25	1.06	19.30	0.39
13	02-2013	919.14	65.46	1030.38	111.24	250.03	161.43	22.72	167.79	2.46	5.39	-0.81
13	05-2013	911.04	22.82	1155.62	244.58	176.80	212.50	81.64	1005.77	1.58	21.50	0.58
13	06-2013	1107.43	104.95	1268.54	161.12	482.02	204.12	33.60	298.46	1.06	34.72	1.73
13	07-2013	1703.29	160.98	1928.25	224.96	299.00	141.48	19.21	117.72	3.61	8.61	-0.53
13	11-2013	883.98	100.00	1185.01	301.03	213.86	121.86	31.99	350.00	4.25	13.12	-0.14

APPENDIX B, CONTINUED												
Year	ID #	NPOC	DON	TC	IC	Na	K	Mg	Ca	C:N	MAH	ZMAH
13	14-2013	3040.76	179.31	3172.01	131.25	304.02	170.85	56.83	282.40	10.88	6.49	-0.72
13	16-2013	4158.04	212.91	4194.64	36.60	305.79	182.63	50.33	197.19	7.14	19.50	0.41
13	19-2013	1266.57	104.95	1459.99	193.42	241.13	195.29	60.05	928.94	1.73	44.52	2.57
13	20-2013	715.76	67.07	883.43	167.67	212.72	128.83	42.42	562.47	1.89	10.99	-0.33
13	22-2013	994.31	60.05	1134.39	140.08	157.45	93.90	23.74	223.72	3.51	3.38	-0.98
13	23-2013	1417.39	110.30	1657.02	239.63	104.48	171.22	42.60	314.32	3.82	6.78	-0.69
13	24-2013	815.21	83.35	983.73	168.51	139.23	84.62	34.68	256.22	4.66	6.00	-0.76
13	26-2013	915.18	65.00	1028.61	113.43	289.47	141.36	29.99	217.61	2.58	4.45	-0.89
13	28-2013	784.15	60.78	827.97	43.82	275.15	115.33	16.80	132.48	3.73	4.08	-0.92
13	30-2013	886.16	80.82	895.13	8.97	104.09	136.87	20.27	138.22	6.03	6.55	-0.71
13	31-2013	1696.29	143.92	1777.62	81.34	235.73	183.07	13.12	110.54	4.22	4.58	-0.88
13	33-2013	2201.81	143.58	2262.69	60.88	247.26	200.89	19.86	122.96	5.31	4.98	-0.85
13	35-2013	554.21	26.41	620.84	66.63	372.60	137.08	41.27	256.93	1.25	16.00	0.11
13	36-2013	1148.57	82.75	1125.08	125.74	340.43	103.81	27.85	167.37	6.15	11.17	-0.31
13	41-2013	800.41	67.34	778.66	125.74	49.71	64.15	8.96	84.69	9.25	11.75	-0.26
13	47-2013	4649.48	121.34	4644.54	125.74	258.92	115.99	30.57	356.36	16.91	17.68	0.25
13	50-2013	867.28	60.03	906.50	39.22	74.47	70.47	18.35	168.65	5.30	2.57	-1.05
13	51-2013	3813.79	211.36	3827.75	13.96	137.92	179.45	33.17	232.00	8.38	15.26	0.04
13	52-2013	8699.13	286.19	8767.19	68.06	190.85	199.26	58.35	560.59	23.07	22.75	0.69
13	53-2013	3785.59	140.02	3999.56	213.97	208.39	165.56	46.35	331.37	9.84	13.57	-0.10
13	54-2013	1764.16	120.90	1843.21	79.05	533.44	190.03	31.82	154.80	5.68	11.21	-0.31
13	56-2013	1017.87	36.12	1049.46	31.58	260.68	116.45	54.79	495.86	2.07	15.35	0.05
13	57-2013	687.54	48.45	706.32	18.77	157.73	61.29	11.11	105.84	7.21	5.68	-0.79
13	59-2013	1181.31	99.40	1233.91	52.59	144.56	169.37	30.60	252.08	4.86	22.13	0.64
13	61-2013	1835.01	107.09	1889.86	54.85	342.96	185.50	22.02	134.62	3.60	12.02	-0.24
14	03-2014	6811.76	252.23	6775.53	125.74	705.12	162.54	33.45	244.08	23.89	27.75	1.12

APPENDIX B, CONTINUED												
Year	ID #	NPOC	DON	TC	IC	Na	K	Mg	Ca	C:N	MAH	ZMAH
14	05-2014	2271.52	212.87	2558.59	287.07	443.53	194.69	34.92	188.66	4.33	24.97	0.88
14	06-2014	9743.67	253.42	10009.29	265.61	302.89	189.66	115.39	1718.20	34.20	36.18	1.85
14	14-2014	5423.95	245.88	5820.17	396.22	198.63	187.38	30.07	132.36	7.00	31.29	1.43
14	15-2014	10905.34	619.10	10976.65	71.31	912.39	644.11	69.76	330.34	14.55	47.73	2.85
14	20-2014	4812.04	148.33	4861.83	49.80	340.59	142.71	52.82	247.15	28.11	8.36	-0.55
14	25-2014	3040.86	164.40	3169.57	128.71	361.45	168.26	23.88	162.32	8.01	5.64	-0.79
14	29-2014	4566.17	295.66	4629.61	63.44	902.23	284.64	23.94	119.12	8.26	25.49	0.93
14	32-2014	4984.04	304.86	5074.49	90.45	613.30	255.13	25.85	214.30	6.12	23.15	0.73
14	35-2014	10409.14	89.91	10502.84	93.70	179.80	137.57	160.01	1707.17	84.51	33.96	1.66
14	36-2014	9174.56	121.66	9283.46	108.90	488.58	168.53	170.70	1310.31	65.87	30.04	1.32
14	37-2014	721.38	31.06	807.52	86.14	164.31	54.67	51.20	385.62	14.35	9.60	-0.45
C	Control1	282.39	15.44	386.19	103.80	67.40	74.24	50.98	333.98	13.84		
C	Control2	334.99	23.61	344.74	9.75	104.79	36.63	20.90	111.94	9.87		
C	Control3	364.65	19.55	418.66	54.01	68.56	44.87	36.40	206.50	13.61		
C	Control4	192.84	8.07	323.57	130.73	94.52	74.55	46.11	372.64	14.90		
C	Control5	299.32	18.40	284.60	59.60	86.81	60.39	40.63	172.00	8.44		

APPENDIX C: XRF DATA SET

Yr	ID#	K	Cr	Mn	Fe	Cu	As	Zr	Pb
11	D21-2011	4484.0	78.0	1358.0	18979.0	11.0	9.6	778.0	22.7
11	D22-2011	5525.0	76.0	661.0	20312.0	12.0	8.4	824.0	22.6
12	D10-2012	5329.0	78.0	683.0	17153.0	10.5	7.1	740.0	24.0
12	D11-2012	6333.0	90.0	642.0	20041.0	12.5	8.3	787.0	23.5
12	D33-2012	5832.0	79.0	857.0	20193.0	13.5	8.2	760.0	27.4
12	D47-2012	6159.0	79.0	720.0	21707.0	11.5	10.2	751.0	24.3
12	D49-2012	6183.0	69.0	840.0	17654.0	10.0	6.8	847.0	22.1
12	D50-2012	5231.0	88.0	832.0	21782.0	17.0	9.0	900.0	22.0
13	D14-2013	5393.0	72.0	942.0	18994.0	14.0	9.0	872.0	22.0
13	D20-2013	4995.0	80.0	616.0	18294.0	18.0	8.0	772.0	25.0
13	D2-2013	6078.0	75.0	888.0	18889.0	17.0	9.2	920.0	22.5
13	D24-2013	5364.0	84.0	1008.0	21383.0	15.0	10.0	816.0	25.0
13	D30-2013	7645.0	79.0	998.0	17643.0	15.0	8.0	689.0	24.0
13	D35-2013	7692.0	79.0	864.0	18848.0	15.0	9.0	740.0	24.0
13	D47-2013	5056.0	83.0	785.0	20052.0	13.0	8.0	774.0	23.0
13	D51-2013	5319.0	70.0	545.0	19035.0	15.0	9.0	724.0	20.0
13	D52-2013	5079.0	72.0	758.0	16633.0	12.0	9.5	771.0	17.2
13	D53-2013	5859.0	75.0	643.0	16918.0	12.0	7.0	1103.0	21.0
13	D54-2013	6126.0	79.0	766.0	17660.0	16.0	9.0	964.0	23.0
13	D56-2013	5866.0	83.0	994.0	19964.0	13.0	10.0	749.0	25.4
13	D59-2013	6591.0	85.0	936.0	20079.0	14.0	11.1	739.0	22.3
13	D61-2013	6371.0	77.0	669.0	17876.0	13.0	8.0	896.0	25.0
13	D6-2013	5602.0	84.0	1125.0	18381.0	15.0	8.2	773.0	22.7
14	D15-2014	6157.0	69.0	611.0	14958.0	13.5	4.5	840.0	16.6
14	D25-2014	6512.0	66.0	1186.0	16260.0	13.0	9.0	714.0	22.3
	Control 5	5233.0	84.0	1265.0	19911.0	14.0	10.2	847.0	27.3

APPENDIX C, CONTINUED									
Yr	ID#	K	Cr	Mn	Fe	Cu	As	Zr	Pb
	CTRL1-XRF	6938.0	69.0	837.0	16023.0	12.0	8.0	784.0	22.0
	CTRL2-XRF	4829.0	84.0	655.0	20197.0	11.0	8.0	886.0	25.0

APPENDIX D: MULTIPLE REGRESSION RESULTS

Model 1 PMI	Full Data Model Unstandardized Coefficients		Collinearity Statistics	
	B	Std. Error	Tolerance	VIF
Constant	2991.58	737.12		
pH	-376.08	121.24	0.28	3.57
EC	1.79	0.40	0.12	8.12
NO_3N	2.05	1.99	0.01	74.66
NH_4N	2.96	2.35	0.01	102.7
PO_4P	2.70	3.19	0.33	3.00
TDN	-3.94	2.37	0.00	341.71
NPOC	-0.67	0.74	0.00	4529.24
DON	2.45	2.02	0.02	47.61
TC	.588	0.75	0.00	4733.58
IC	-0.16	0.72	0.24	4.18
Na^+	-.562	0.30	0.28	3.62
K^+	.571	1.17	0.08	12.47
Mg^{2+}	-3.54	3.04	0.10	10.23
Ca^{2+}	-.328	0.28	0.09	10.82
CN	0.90	6.35	0.10	10.36

Model 2 PMI	Adjusted Model Unstandardized Coefficients	
	B	Std. Error
Constant	2998.51	685.91
pH	-383.852	116.25
EC	1.811	0.40
NO_3N	-1.391	0.50
NH_4N	-.857	0.39
PO_4P	1.617	3.19
NPOC	-.094	0.04
DON	-.642	1.24
IC	0.20	0.44
Na^+	-0.63	0.28
K^+	0.12	1.12
Mg^{2+}	-2.10	2.82
Ca^{2+}	-0.32	.274

APPENDIX D, CONTINUED

Model 3 PMI	log10 Model Unstandardized Coefficients	
	B	Std. Error
Constant	4723.01	945.41
pH	-127.30	101.06
EC	1.14	0.32
log10NO_3N	-37.99	64.70
log10NH_4N	-152.69	98.93
log10PO_4P	10.29	104.29
log10NPOC	-442.27	217.92
log10DON	-12.09	135.08
log10IC	50.74	89.55
log10Na^+	-315.73	170.06
log10K^+	-160.84	328.85
log10Mg^{2+}	348.36	284.43
log10Ca^{2+}	-708.67	261.54

Model 4 PMI	BMI $\geq$ 30 Model Unstandardized Coefficients	
	B	Std. Error
Constant	4142.32	672.64
pH	-516.04	114.62
EC	1.60	0.47
NO_3N	-2.49	0.63
NH_4N	1.75	0.63
PO_4P	-10.29	3.78
NPOC	-0.24	0.04
DON	-2.55	1.70
IC	0.46	.383
Na^+	0.16	0.45
K^+	-2.59	1.10
Mg^{2+}	6.42	2.37
Ca^{2+}	-0.55	.37

Model 5 PMI	BMI <30 Model Unstandardized Coefficients	
	B	Std. Error
Constant	2544.17	872.06
pH	-345.82	142.75
EC	2.49	0.44
NO_3N	-2.34	0.60
NH_4N	-1.01	0.47
PO_4P	2.52	3.12
NPOC	-0.14	0.06
DON	-0.56	1.59
IC	0.29	0.53
Na^+	-1.32	0.32
K^+	1.48	1.25
Mg^{2+}	0.08	4.04
Ca^{2+}	-0.30	0.29

APPENDIX D, CONTINUED

Model 6 PMI	BMI ≥ 25 Model Unstandardized Coefficients	
	B	Std. Error
Constant	4100.45	619.26
pH	-530.41	106.87
EC	0.31	0.39
NO_3N	-1.25	0.48
NH_4N	1.90	0.48
PO_4P	-6.41	3.81
NPOC	-0.22	0.05
DON	1.04	1.25
IC	0.36	0.41
Na^+	-0.25	0.41
K^+	-3.56	1.15
Mg^{2+}	3.11	2.32
Ca^{2+}	0.55	0.23

Model 7 PMI	BMI < 25 Model Unstandardized Coefficients	
	B	Std. Error
Constant	1233.39	1116.38
pH	-137.10	183.74
EC	2.42	0.48
NO_3N	-1.08	0.80
NH_4N	-1.09	0.50
PO_4P	2.47	3.35
NPOC	-0.09	0.06
DON	0.21	1.87
IC	-0.26	0.62
Na^+	-1.50	0.34
K^+	0.27	1.47
Mg^{2+}	5.23	5.53
Ca^{2+}	-0.94	0.51

Model 8 ADD_4	Adjusted Model Unstandardized Coefficients	
Adjusted	B	Std. Error
Constant	50927.07	10844.62
pH	-6714.63	1837.94
EC	29.18	6.38
NO_3N	-23.89	7.96
NH_4N	-13.15	6.11
PO_4P	25.73	50.39
NPOC	-1.718	0.70
DON	-8.38	19.58
IC	4.95	6.98
Na^+	-9.51	4.49
K^+	2.71	17.73
Mg^{2+}	-26.78	44.53
Ca^{2+}	-5.00	4.33

APPENDIX D, CONTINUED

Model 9 ADD_4	log10 Model Unstandardized Coefficients	
	B	Std. Error
Constant	79902.96	14247.86
pH	-2673.64	1523.48
EC	19.29	4.647
log10NO_3N	-796.84	936.34
log10NH_4N	-2489.15	1479.77
log10PO_4P	641.66	1457.12
log10NPOC	-8056.75	3235.11
log10DON	275.79	2027.47
log10IC	1596.28	1291.05
log10Na^+	-4831.44	2472.67
log10K^+	-2648.51	4748.89
log10Mg^{2+}	5096.18	4158.19
log10Ca^{2+}	-11347.38	3937.78

Model 10 ADD_4	BMI $\geq$ 30 Model Unstandardized Coefficients	
	B	Std. Error
Constant	70434.16	9213.158
pH	-9028.94	1569.882
EC	20.08	6.476
NO_3N	-36.70	8.557
NH_4N	33.27	8.588
PO_4P	-141.87	51.767
NPOC	-4.04	0.547
DON	-30.61	23.226
IC	7.73	5.252
Na^+	-2.02	6.187
K^+	-35.75	15.121
Mg^{2+}	93.61	32.487
Ca^{2+}	-5.44	5.038

Model 11	ADD_4 Model Unstandardized Coefficients	
BMI<30	B	Std. Error
Constant	44134.93	13022.69
pH	-6190.42	2131.74
EC	40.95	6.51
NO_3N	-39.55	8.89
NH_4N	-16.00	7.09
PO_4P	39.83	46.63
NPOC	-2.42	0.83
DON	-6.79	23.80
IC	6.64	7.98
Na^+	-20.41	4.72
K^+	23.25	18.67
Mg^{2+}	7.35	60.30
Ca^{2+}	-4.71	4.38

APPENDIX D, CONTINUED

Model 12 – ADD_4	BMI ≥ 25 Model Unstandardized Coefficients	
	B	Std. Error
Constant	64440.069	9422.569
pH	-8346.256	1626.078
EC	2.378	5.990
NO_3N	-18.644	7.378
NH_4N	30.266	7.243
PO_4P	-85.738	57.977
NPOC	-3.484	.761
DON	17.840	19.059
IC	4.976	6.236
Na^+	-4.803	6.312
K^+	-49.259	17.424
Mg^{2+}	55.671	35.319
Ca^{2+}	8.831	3.473

Model 13 – ADD_4	BMI <25 Model Unstandardized Coefficients	
	B	Std. Error
Constant	26835.54	16727.88
pH	-3477.93	2753.22
EC	40.38	7.14
NO_3N	-21.92	11.97
NH_4N	-16.77	7.50
PO_4P	42.17	50.16
NPOC	-1.70	0.93
DON	3.15	27.96
IC	-1.27	9.33
Na^+	-23.09	5.13
K^+	6.26	21.98
Mg^{2+}	93.92	82.87
Ca^{2+}	-15.05	7.67

LITERATURE CITED

Aitkenhead-Peterson JA, Alexander MG, Bytheway JA, Carter DO, and Wescott DJ. 2015. Applications of Soil Chemistry in Forensic Entomology. In: Tomberlin JK, and Benbo ME, editors. Forensic Entomology: International Dimensions and Frontiers.

Aitkenhead-Peterson JA, Owings CG, Alexander MB, Larison N, and Bytheway JA. 2012. Mapping the lateral extent of human cadaver decomposition with soil chemistry. Forensic Science International 216(1–3):127-134.

Alvarez EC, and Plocheck R, editors. 2014. Texas Almanac; Physical Regions of Texas. Texas State Historicl Association: University of North Texas.

Anderson B, Meyer J, and Carter DO. 2013. Dynamics of Ninhydrin-Reactive Nitrogen and pH in Gravesoil During the Extended Postmortem Interval. Journal of Forensic Sciences 58(5):1348-1352.

Anderson BE. 2008. Identifying the Dead: Methods Utilized by the Pima County (Arizona) Office of the Medical Examiner for Undocumented Border Crossers: 2001–2006. Journal of Forensic Sciences 53(1):8-15.

Anderson BE, and Parks BO. 2008. Symposium on Border Crossing Deaths: Introduction. Journal of Forensic Sciences 53(1):6-7.

Anderson GS, and VanLaerhoven SL. 1996. Initial Studies on Insect Succession on Carrion in Southwestern British Columbia. Journal of Forensic Sciences 41(4):617-625.

Andrews J, and Harris H. 1984. Relevance of r- and K-theory to the ecology of plant pathogens. . In: Klug M, and Reddy C, editors. Current Perspectives in Microbial Ecology. Washington, DC: American Society for Microbiology. p 1-7.

Andrews JA, Matamala R, Westover KM, and Schlesinger WH. 2000. Temperature effects on the diversity of soil heterotrophs and the δ13C of soil-respired CO2. Soil Biology and Biochemistry 32(5):699-706.

Armendariz J. 2013. Unidentified immigrant body count remains grave issue in Brooks County. Monitor, The (McAllen, TX).

Bachmann J, and Simmons T. 2010. The Influence of Preburial Insect Access on the Decomposition Rate. Journal of Forensic Sciences 55(4):893-900.

Bauman RW. 2015. Microbiology. Boston: Pearson.

Benninger LA, Carter DO, and Forbes SL. 2008. The biochemical alteration of soil beneath a decomposing carcass. Forensic Science International 180(2–3):70-75.

Binford LR. 1978. Nunamiut Ethnoarchaeology. New York: Academic Press.

Birkby WH, Fenton TW, and Anderson BE. 2008. Identifying Southwest Hispanics Using Nonmetric Traits and the Cultural Profile. Journal of Forensic Sciences 53(1):29-33.

Blair W. 1950. The biotic provinces of Texas. The Texas Journal of Science 2:93-116.

Bongers T. 1990. The Maturity Index: An Ecological Measure of Environmental Disturbance Based on Nematode Species Composition. Oecologia 83(1):14-19.

Brown K, Thorne A, and Harvey M. 2012. Preservation of Calliphora vicina (Diptera: Calliphoridae) pupae for use in post-mortem interval estimation. Forensic Science International 223(1–3):176-183.

Brown TE, LeMay HE, Bursten BE, Murphy C, Woodward P, and Stoltzfus ME. 2014. Chemistry: The Central Science, 13th Edition. New York: Pearson.

Cantor NL. 2010a. Chapter 3 The Legal Sttus of the Postliving: Do Corpses Have Rights? The Life and Times of the Human Cadaver. Washington DC: Georgetown University Press.

Cantor NL. 2010b. Chapter 4 Decomposition of the Body and Efforts to Slow its Disintegration. After We Die. Washington DC: Georgetown University Press.

Carson D. 2000. Soils of the Freeman Ranch, Hays County, Texas. Freeman Ranch Publication Series No 4-2000. San Marcos, TX.

Carter DO, Filippi J, Higley LG, Huntington TE, Okoye MI, Scriven M, and Bliemeister J. 2009. Using Ninhydrin to Reconstruct a Disturbed Outdoor Death Scene. Journal of Forensic Identification 59(2):190-195.

Carter DO, and Tibbett M. 2006. Microbial decomposition of skeletal muscle tissue (Ovis aries) in a sandy loam soil at different temperatures. Soil Biology and Biochemistry 38(5):1139-1145.

Carter DO, and Tibbett M. 2008. Cadaver Decomposition and Soil: Processes. In: Tibbett M, and Carter DO, editors. Soil Aalysis in Forensic Taphonomy. Boca Raton, FL: CRC Press. p 29-51.

Carter DO, Yellowlees D, and Tibbett M. 2008. Using Ninhydrin to Detect Gravesoil. Journal of Forensic Sciences 53(2):397-400.

Carter DO, Yellowlees D, and Tibbett M. 2010. Moisture can be the dominant environmental parameter governing cadaver decomposition in soil. Forensic Science International 200(1–3):60-66.

Catts EP, and Goff ML. 1992. Forensic Entomology in Criminal Investigations. Annual Review of Entomology 37(1):253-272.

Clark MA, Worrell MB, and Pless JE. 1997. Postmortem Changes in Soft Tissues. In: Haglund WD, and Sorg MH, editors. Forensic Taphonomy: The Postmortem Fate of Human Remains. Boca Raton, FL: CRC Press. p 151-164.

Cohen J. 1988. Statistical Power Analysis for the Behavioral Sciences. Hillsdale, N.J.: Erlbaum Associates.

Dabbs GR. 2015. How Should Forensic Anthropologists Correct National Weather Service Temperature Data for Use in Estimating the Postmortem Interval? Journal of Forensic Sciences 60(3):581-587.

Dadour IR, and Harvey ML. 2008. The Role of Invertebrates in Terrestrial Decomposition: Forensic Applications. In: Tibbett M, and Carter DO, editors. Soil Analysis in Forensic Taphonomy. Boca Raton, FL: CRC Press. p 109-122.

Damann FE, Tanittaisong A, and Carter DO. 2012. Potential carcass enrichment of the University of Tennessee Anthropology Research Facility: A baseline survey of edaphic features. Forensic Science International 222(1–3):4-10.

Davis JB, and Goff ML. 2000. Decomposition patterns in terrestrial and intertidal habitats on Oahu Island and Coconut Island, Hawaii. Journal of Forensic Sciences 45(4):836-842.

Dekeirsschieter J, Verheggen FJ, Gohy M, Hubrecht F, Bourguignon L, Lognay G, and Haubruge E. 2009. Cadaveric volatile organic compounds released by decaying pig carcasses (Sus domesticus L.) in different biotopes. Forensic Science International 189(1–3):46-53.

Dent B, Forbes S, and Stuart B. 2004. Review of Human Decomposition Processes in Soil. Environmental Geology 45(4):576-585.

DeVault TL, Brisbin IL, Jr, and Rhodes OE. 2004. Factors influencing the acquisition of rodent carrion by vertebrate scavengers and decomposers. Canadian Journal of Zoology 82(3):502-509.

DeVault TL, Rhodes OE, Jr., and Shivik JA. 2003. Scavenging by Vertebrates: Behavioral, Ecological, and Evolutionary Perspectives on an Important Energy Transfer Pathway in Terrestrial Ecosystems. Oikos 102(2):225-234.

Di Maio DJ, and Di Maio VJ. 1993. Forensic Pathology. BocaRaton, FL: CRC Press.

Dixon R. 2000. Climatology of the Freeman Ranch, Hays County, Texas. Freeman Ranch Publication Series(3):1-9.

FACTS. (2015). Forensic Anthropology Research Facility. Retrieved from http://www.txstate.edu/anthropology/facts/labs/farf.html. May 10, 2015.

Fitzpatrick RW. 2008. Nature, Distribution, and Origin of Soil Materials in the Forensic Comparison of Soils. In: Tibbett M, and Carter DO, editors. Soil Analysis in Forensic Taphonomy. Boca Raton, TL: CRC Press. p 1-28.

Forbes SL. 2008a. Decomposition Chemistry in a Burial Environment. In: Tibbett M, and Carter DO, editors. Soil Analysis in Forensic Taphonomy: CRC Press. p 203-223.

Forbes SL. 2008b. Potential Determinants of Postmortem and Postburial Interval of Buried Remains. In: Tibbett M, and Carter DO, editors. Soil Analysis in Forensic Taphonomy: CRC Press. p 225-246.

Forbes SL, Stuart BH, Dadour IR, and Dent BB. 2004. A preliminary investigation of the stages of adipocere formation. Journal Of Forensic Sciences 49(3):566-574.

Forbes SL, Stuart BH, and Dent BB. 2002. The identification of adipocere in grave soils. Forensic Science International 127(3):225-230.

Forbes SL, Stuart BH, and Dent BB. 2005a. The effect of the burial environment on adipocere formation. Forensic Science International 154(1):24-34.

Forbes SL, Stuart BH, and Dent BB. 2005b. The effect of the method of burial on adipocere formation. Forensic Science International 154(1):44-52.

Gallagher D, Heymsfield SB, Heo M, Jebb SA, Murgatroyd PR, and Sakamoto Y. 2000. Healthy percentage body fat ranges: an approach for developing guidelines based on body mass index. The American Journal Of Clinical Nutrition 72(3):694-701.

Galloway A. 1997. The Process of Decomposition: A Model from the Arizona-Sonoran Desert. In: Haglund WD, and Sorg MH, editors. Forensic Taphonomy: The Postmortem Fate of Human Remains. Boca Raton, FL: CRC Press. p 139-150.

Galloway A, Birkby WH, Jones AM, Henry TE, and Parks BO. 1989. Decay rates of human remains in an arid environment. Journal Of Forensic Sciences 34(3):607-616.

Gardiner DT, and Miller RW. 2008. Soils in Our Environment, 11th Edition. New Jersey: Pearson Education.

Gill-King H. 1997. Chemical and Ultrastructural Aspects of Decomposition. In: Haglund WD, and Sorg MH, editors. Forensic Taphonomy: The Postmortem Fate of Human Remains. Boca Raton, FL: CRC Press. p 93-108.

Hawksworth DL, and Wiltshire PEJ. 2011. Forensic mycology: the use of fungi in criminal investigations. Forensic Science International 206(1–3):1-11.

Hoffman EM, Curran AM, Dulgerian N, Stockham RA, and Eckenrode BA. 2009. Characterization of the volatile organic compounds present in the headspace of decomposing human remains. Forensic Science International 186(1–3):6-13.

Hopkins DW. 2008. The Role of Soil Organisms in Terrestrial Decomposition. In: Tibbett M, and Carter DO, editors. Soil Analysis in Forensic Taphonomy. Boca Raton, FL: CRC Press. p 53-66.

Hopkins DW, Wiltshire PEJ, and Turner BD. 2000. Microbial characteristics of soils from graves: an investigation at the interface of soil microbiology and forensic science. Applied Soil Ecology 14(3):283-288.

Howard GT, Duos B, and Watson-Horzelski EJ. 2010. Characterization of the soil microbial community associated with the decomposition of a swine carcass. International Biodeterioration & Biodegradation 64(4):300-304.

Human Microbiome Project C. 2012a. A framework for human microbiome research. Nature 486(7402):215-221.

Human Microbiome Project C. 2012b. Structure, function and diversity of the healthy human microbiome. Nature 486(7402):207-214.

Hyde E, Haarmann D, Lynne A, Bucheli S, and Petrosino J. 2013. The Living Dead: Bacterial Community Structure of a Cadaver at the Onset and End of the Bloat Stage of Decomposition. PLoS ONE 8(10):e77733.

Janaway RC. 1996. The decay of buried human remains and their associated materials. In: Hunter J, Robersts C, and Martin A, editors. Studies in Crime: An Introduction to Forensic Archaeology. London: B.T. Batsford Ltd. p 58-85.

Janaway RC. 2008. The Decomposition of Materials Associated with Buried Cadavers. In: Tibbett M, and Carter DO, editors. Soil Analysis in Forensic Taphonomy. Boca Raton, FL: CRC Press. p 153-201.

Jenny H. 1994. Factors of Soil Formation: A System of Quantitative Pedology. New York: Dover Publications.

Johnson MD. 1975. Seasonal and Microseral Variations in the Insect Populations on Carrion. American Midland Naturalist 93(1):79-90.

Lange J, Thomas K, and Wittmann C. 2002. Comparison of a capillary electrophoresis method with high-performance liquid chromatography for the determination of biogenic amines in various food samples. Journal of Chromatography B 779(2):229-239.

Langer U, Bohme L, and Bohme F. 2004. Short communication: Classification of soil microorganisms based on growth properties: A critical view of some commonly used terms. Journal of Plant Nutrition and Soil Science 167:267-269.

Lovestead TM, and Bruno TJ. 2011. Detecting gravesoil with headspace analysis with adsorption on short porous layer open tubular (PLOT) columns. Forensic Science International 204(1–3):156-161.

Macdonald BCT, Farrell M, Tuomi S, Barton PS, Cunningham SA, and Manning AD. 2014. Carrion decomposition causes large and lasting effects on soil amino acid and peptide flux. Soil Biology and Biochemistry 69(0):132-140.

Magni PA, Harvey ML, Saravo L, and Dadour IR. 2012. Entomological evidence: Lessons to be learnt from a cold case review. Forensic Science International 223(1–3):e31-e34.

Mann RW, Bass WM, and Meadows L. 1990. Time Since Death and Decomposition of the Human Body: Variables and Observations in Case and Experimental Field Studies. Journal of Forensic Sciences 35(1):103-111.

Megyesi MS, Nawrocki SP, and Haskell NH. 2005. Using accumulated degree-days to estimate the postmortem interval from decomposed human remains. Journal Of Forensic Sciences 50(3):618-626.

Mello de Oliveira JA, and Santos-Martin CC. 1995. Enzyme histochemistry of the liver in autopsy material at different post-mortem times. Medicine, Science, And The Law 35(3):201-206.

Meyers LS, Gamst G, and Guarino AJ. 2013. Applied Multivariate Research. Los Angeles: Sage.

Michaud J-P, and Moreau G. 2011. A Statistical Approach Based on Accumulated Degree-days to Predict Decomposition-related Processes in Forensic Studies. Journal of Forensic Sciences 56(1):229-232.

Micozzi MS. 1986. Experimental study of postmortem change under field conditions: Effects of freezing, thawing, and mechanical injury. Journal of Forensic Sciences 31(3):953-961.

Micozzi MS. 1991. Postmortem Change in Human and Animal Remains. Springfield, IL: Charles C. Thomas.

Micozzi MS. 1997. Frozen Environments and Soft Tissue Preservation. In: Haglund WD, and Sorg MH, editors. Forensic Taphonomy. Boca Raton, FL: CRC. p 171-180.

Min K, Lehmeier CA, Ballantyne F, Tatarko A, and Billings SA. 2014. Differential effects of pH on temperature sensitivity of organic carbon and nitrogen decay. Soil Biology and Biochemistry 76(0):193-200.

Parks CL. 2011. A Study of the Human Decomposition Sequence in Central Texas. Journal of Forensic Sciences 56(1):19-22.

Patel AV, Hildebrand JS, and Gapstur SM. 2014. Body Mass Index and All-Cause Mortality in a Large Prospective Cohort of White and Black U.S. Adults. PLoS ONE 9(10):1-14.

Payne JA. 1965. A Summer Carrion Study of the Baby Pig *Sus Scrofa Linnaeus*. Ecology 46(5):592-602.

Pessione E, Mazzoli R, Giuffrida MG, Lamberti C, Garcia-Moruno E, Barello C, Conti A, and Giunta C. 2005. A proteomic approach to studying biogenic amine producing lactic acid bacteria. Proteomics 5(3):687-698.

Prangnell J, and McGowan G. 2009. Soil temperature calculation for burial site analysis. Forensic Science International 191(1–3):104-109.

Pringle JK, Cassella JP, and Jervis JR. 2010. Preliminary soilwater conductivity analysis to date clandestine burials of homicide victims. Forensic Science International 198(1–3):126-133.

Radu T, Gallagher S, Byrne B, Harris P, Coveney S, McCarron S, McCarthy T, and Diamond D. 2013. Portable X-Ray Fluorescence as a Rapid Technique for Surveying Elemental Distributions in Soil. Spectroscopy Letters 46(7):516-526.

Rath KM, and Rousk J. 2015. Salt effects on the soil microbial decomposer community and their role in organic carbon cycling: A review. Soil Biology and Biochemistry 81(0):108-123.

Rhine S, and Dawson J. 1998. Estimation of time since death in the southwestern United States. In: Reichs K, editor. Forensic osteology: Advances in the identification of hyman remains, 2nd edition. Springfield, IL: Charles C. Thomas. p 145-159.

Richards CS, Simonsen TJ, Abel RL, Hall MJR, Schwyn DA, and Wicklein M. 2012. Virtual forensic entomology: Improving estimates of minimum post-mortem interval with 3D micro-computed tomography. Forensic Science International 220(1–3):251-264.

Rodriguez WC, 3rd, and Bass WM. 1985. Decomposition of buried bodies and methods that may aid in their location. Journal Of Forensic Sciences 30(3):836-852.

Rodriguez WC, and Bass WM. 1983. Insect Activity and its Relationship to Decay Rates of Human Cadavers in East Tennessee. Journal of Forensic Sciences 28(2):423-432.

Sagara N, Yamanaka T, and Mark T. 2008. Soil Fungi Associated with Graves and Latrines. In: Tibbett M, and Carter DO, editors. Soil Analysis in Forensic Taphonomy. Boca Raton, FL: CRC Press. p 67-107.

Sasha V, Forbes SL, and Dadour IR. 2008. Decomposition and insect succession on cadavers inside a vehicle environment. Forensic Science, Medicine and Pathology 4(1):22-32.

Schoenly K, and Reid W. 1987. Dynamics of Heterotrophic Succession in Carrion Arthropod Assemblages: Discrete Seres or a Continuum of Change? Oecologia 73(2):192-202.

Schotsmans EMJ, Denton J, Fletcher JN, Janaway RC, and Wilson AS. 2014. Short-term effects of hydrated lime and quicklime on the decay of human remains using pig cadavers as human body analogues: Laboratory experiments. Forensic Science International 238(0):142.e141-142.e110.

Sharanowski BJ, Walker EG, and Anderson GS. 2008. Insect succession and decomposition patterns on shaded and sunlit carrion in Saskatchewan in three different seasons. Forensic Science International 179(2–3):219-240.

Simonson RW. 1959. Outline of a Generalized Theory of Soil Genesis. Soil Sci Soc Am J 23:152-156.

Soil Survey Staff S. 2010. Keys to Soil Taxonomy, 10th Edition. In: Service U-NRC, editor. Washington, DC.

Solomons G, Fryhle CB, and Snyder SA. 2014. Organic Chemistry, 11th Edition. New Jersey: John Wiley and Sons.

Spatz C. 1997. Basic Statistics: Tales of Distributions. New York: Brooks/Cole.

Spicka A, Johnson R, Bushing J, Higley LG, and Carter DO. 2011. Carcass mass can influence rate of decomposition and release of ninhydrin-reactive nitrogen into gravesoil. Forensic Science International 209(1–3):80-85.

Stearns JC, Lynch MDJ, Senadheera DB, Tenenbaum HC, Goldberg MB, Cvitkovitch DG, Croitoru K, Moreno-Hagelsieb G, and Neufeld JD. 2011. Bacterial biogeography of the human digestive tract. Sci Rep 1.

Stokes KL, Forbes SL, and Tibbett M. 2009. Freezing skeletal muscle tissue does not affect its decomposition in soil: Evidence from temporal changes in tissue mass, microbial activity and soil chemistry based on excised samples. Forensic Science International 183(1–3):6-13.

Stokes KL, Forbes SL, and Tibbett M. 2013. Human Versus Animal: Contrasting Decomposition Dynamics of Mammalian Analogues in Experimental Taphonomy. Journal of Forensic Sciences 58(3):583-591.

Suckling J. 2011. A Longitudinal Study on the Outdoor Human Decomposition Sequence in Central Texas (Thesis). San Marcos, TX: Texas State University.

Suckling JK, Spradley MK, and Godde K. 2015. A Longitudinal Study on Human Outdoor Decomposition in Central Texas. Journal of Forensic Sciences:n/a-n/a.

Suhailly J, Zaidi E, and Ismail B. 2014. Elemental Composition Study of Heavy Metal (Ni, Cu, Zn) in Riverbank Soil by Electrokinetic-Assisted Phytoremediation using XRF and SEM/EDX. AIP Conference Proceedings 1584:221-227.

Tabachnick BG, and Fidell LS. 2013. Using multivariate statistics. Boston: Pearson Education.

Tersigni-Tarrant MTA, and Shirley NR, editors. 2013. Forensic Anthropology. New York: CRC.

Texas State University. (2013). Freeman Center: Present day operation. Retrieved from http://www.txstate.edu/freemanranch/about/History/Present-Day-Operation.html. December 8, 2013.

Thevenard D. 2011. Methods for Estimating Heating and Cooling Degree-Days to Any Base Temperature. ASHRAE Transactions 117(1):884-891.

Tibbett M, and Carter DO. 2003. Mushrooms and taphonomy: the fungi that mark woodland graves. Mycologist 17(1):20-24.

Tibbett M, and Carter DO, editors. 2008. Soil Analysis in Forensic Taphonomy. Boca Raton: CRC Press.

Tibbett M, Carter DO, Haslam T, Major R, and Haslam R. 2004. A laboratory incubation method for determining the rate of microbiological degradation of skeletal muscle tissue in soil. Journal Of Forensic Sciences 49(3):560-565.

Towne EG. 2000. Prairie Vegetation and Soil Nutrient Responses to Ungulate Carcasses. Oecologia 122(2):232-239.

Tumer AR, Karacaoglu E, Namli A, Keten A, Farasat S, Akcan R, Sert O, and Odabaşi AB. 2013. Effects of different types of soil on decomposition: An experimental study. Legal Medicine 15(3):149-156.

Van Belle LE, Carter DO, and Forbes SL. 2009. Measurement of ninhydrin reactive nitrogen influx into gravesoil during aboveground and belowground carcass (Sus domesticus) decomposition. Forensic Science International 193(1–3):37-41.

Vass AA. 2011. The elusive universal post-mortem interval formula. Forensic Science International 204(1–3):34-40.

Vass AA. 2012. Odor mortis. Forensic Science International 222(1–3):234-241.

Vass AA, Bass WM, Wolt JD, Foss JE, and Ammons JT. 1992. Time since death determinations of human cadavers using soil solution. Journal Of Forensic Sciences 37(5):1236-1253.

Vass AA, Smith RR, Thompson CV, Burnett MN, Dulgerian N, and Eckenrode BA. 2008. Odor Analysis of Decomposing Buried Human Remains. Journal of Forensic Sciences 53(2):384-391.

Voss SC, Cook DF, and Dadour IR. 2011. Decomposition and insect succession of clothed and unclothed carcasses in Western Australia. Forensic Science International 211(1–3):67-75.

Voss SC, Forbes SL, and Dadour IR. 2008. Decomposition and insect succession on cadavers inside a vehicle environment. Forensic Science, Medicine, And Pathology 4(1):22-32.

Wilson AS, Janaway RC, Holland AD, Dodson HI, Baran E, Pollard AM, and Tobin DJ. 2007. Modelling the buried human body environment in upland climes using three contrasting field sites. Forensic Science International 169(1):6-18.

Winogradsky S. 1924. Sur la microflore autochthone de la terre arable. Compte Rendu Acad Sci (Paris) 178:1236-1239.

Wu C-M, Tsai H-T, Yang K-H, and Wen J-C. 2012. How Reliable is X-Ray Fluorescence (XRF) Measurement for Different Metals in Soil Contamination? Environmental Forensics 13(2):110-121.