Decomposition patterns of buried remains in the central Highveld region of South Africa

by

Anàtulie Marais-Werner

Submitted in fulfilment of the requirements for the degree:

Masters in Science (MSc. Anatomy)

In the School of Medicine, Faculty of Health Sciences, University of Pretoria
South Africa

January 2016

DECLARATION

I, Anàtulie Marais-Werner, declare that this dissertation is my own work. It is being submitted for the degree of Masters of Science in Anatomy at the University of Pretoria, South Africa. It has not been submitted before for any other degree or examination at this or any other University.

Sign_____

This 4th day of January, 2016

ACKNOWLEDGEMENTS

I would like to take this opportunity to thank my supervisors, Professor Maryna Steyn and Ms Jolandie Myburgh for their guidance, enthusiasm, patience, advice, support and encouragement during this project. You always managed to make me even more excited about research.

I would also like to thank the following individuals/institutions for their contributions during this study:
Mr R Coertzer and the Faculty of Natural and Agricultural Sciences for assistance and permission to use the Miertjie le Roux Experimental Farm.
Mr S Mouton and Mr JD Viljoen for donating the pigs used in this study.
The South African Weather Services who supplied temperature and rainfall data for the duration of the study.
A special thank you to the Chairperson of the School of Health Care Sciences, Professor FM Mulaudzi, for allowing me additional hours to complete data collection.
Ms A Meyer and Mr WC Nienaber from the Department of Anatomy for their invaluable help in logistics, burial and excavation of carcasses.
Students in the Departments of Anatomy and Archaeology; thank you for volunteering for burial and excavation of carcasses. Without you this would have been a much more challenging task.
Professor PJ Becker for his assistance in the statistical analyses.
The National Research Foundation for funding the research project (through Professor Maryna Steyn).

Lastly I would like to extend my heart-felt gratitude towards my family whom has been a constant source of love, support and strength. Most importantly, none of this would have been possible without Sonet Marais-Werner, who always pushed, believed and supported on days when writing felt very difficult and overwhelming. I value your inputs and am grateful for your patience and encouragement with every endeavour. Thank you for showing genuine interest, support and always bragging about my achievements.

Thank you.

ABSTRACT

Understanding the process of decomposition is extremely important and aids in criminal investigations, especially when attempting to estimate the post mortem interval (PMI). Although several studies have been conducted on the decomposition patterns in surface remains, much less is known about this process in buried remains. By quantifying decomposition rates, factors influencing decomposition and research on the process of decomposition can be standardised and validated in a South African setting.

The aim of this study was to record decomposition stages and rates of buried remains and to compare it to that of remains decomposing on the surface. Twenty five pigs (*Sus scrofa*; 45-80 kg) were buried and excavated at different post mortem intervals [7 days (1 week), 14 days (2 weeks), 33 days (1 month), 92 days (3 months) and 183 days (6 months)]. Stages of decomposition were scored according to separate categories for different anatomical regions based on standardised methods, and photographed. The point values for each region were added to determine the total body score (TBS), which represents the stage of decomposition for each pig. When studying decomposition, accumulated degree days (ADD) are effective in standardising the effect of variables (i.e., temperature) that influence the decay process. It also enables researchers to replicate experiments and compare results. In this study, ADD were used to measure the rate of decomposition and to compare decomposition rates between buried and surface remains.

Results indicated that early stages of decomposition occured rapidly for both surface and buried remains within 7-33 days. Differences in the degree of decay were especially noticeable with the buried, 7 day interval pigs that displayed variations in discolouration in the lower abdomen and trunk. Between 14 and 33 days, buried pigs displayed common features associated with the early stages of decomposition, such as discolouration and bloating. The pigs then reached a stage of advanced decay where little change was observed in the next ±90-183 days after internment. Similar patterns of decomposition were observed for surface remains with rapid decay during the early stages of decay where after a plateau phase was reached during advanced decay. However, as expected, the surface remains reached higher TBS scores during similar intervals.

In this study, the decomposition rates of buried remains were mostly influenced by being buried at an average depth of 0.75 m which could have resulted in lower in-soil temperatures and limited insect activity at a depth of 0.75 m on the remains. Also, adipocere presented itself on the remains with the 33 day PMI pigs, the 92 day PMI pigs and 183 day PMI pigs. Adipocere is capable of degrading over a prolonged period which reduces the rate of decay in a conducive environment (i.e., burial in soil).

Overall, surface and buried pigs decompose with similar patterns, but buried pigs decompose at a much slower rate, reaching lower TBS values relative to similar PMIs in surface remains. This suggests that burial does have a significant effect on the rate of decomposition. Results from this study suggest that when using TBS guidelines on buried remains in the Central Highveld region of South Africa, buried remains will have, on average, a lower TBS score (7.4) than surface remains within a similar post mortem interval.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADD	Accumulated degree days
Cm	Centimeter
FABF	Forensic Anthropology Body Farm
FARC	Forensic Anthropology Research Centre
GPR	Ground penetrating radar
Kg	Kilogram
M	Meter
Mm	Millimeter
PMI	Post mortem interval
SE	Standard error
TBS	Total body score
TSD	Time since death
W	Width

CHAPTER 1
INTRODUCTION

Taphonomic analyses focus on understanding the relationship between human or other remains and its environment. Research into the effects on the remains from the surrounding environment is of high interest because of the potential it offers in understanding ante-, peri- and post mortem events surrounding death. With the rising statistics of violent crime and death in South Africa (5% average increase from 2004-2014; Crime Situation in South Africa 2014) the need for knowledge of decomposition patterns in different settings is becoming increasingly important. Decomposition studies within a forensic context are often aimed at estimating the post mortem interval (PMI) (Beary and Lyman, 2012). This interval provides important information used by forensic investigators to assist in solving crimes, and provides a measurable date to assist law enforcement in providing a reference point to evaluate a possible suspect's alibi and to identify victims (Parsons, 2009). To determine a likely PMI, all factors associated with and influencing the remains should be taken into account. In addition to simply focusing on biological profiling, the inclusion of taphonomic models in forensic anthropology highlights issues such as decomposition rates and scavenger activity which might alter the conditions of remains (Dirkmaat *et al*., 2008). Therefore, a better understanding of the post mortem fate of remains (i.e., forensic taphonomy) is of utmost importance.

Decomposition of remains is a process that is highly influenced by various interrelated variables including, but not limited to, the conditions of the remains, scavenger activity and depositional setting. Even though the pattern of decomposition is generally similar in all remains, variables such as temperature and insect activity can cause the pattern and rate of decay to differ between individuals, even within similar settings (buried, exposed, etc.). Few researchers have studied the decomposition patterns and stages of buried remains (Thew, 2000; Forbes *et al.*, 2005; Vass *et al.*, 2008; Fründ and Schoenen, 2009); hence, this was the primary focus of this study.

Even though stages of decay are very similar in some respects (Galloway *et al*., 1989; Megyesi *et al*., 2005; Comstock *et al*., 2014), it is important to note that decomposition stages will not always accurately describe the decaying process, as decomposition occurs under varying conditions and many factors play a role in the patterns and tempo of decay.

The first attempt at classifying decomposition processes into stages was published by Megnin in 1896, and a host of studies in this field has followed since (Payne, 1965; Rodriguez and Bass, 1985; Megyesi *et al*., 2005; Goff, 2009; Comstock *et al*., 2014). Eight stages of decay were reported by Megnin (1896) which were characterised by the type of insects found on the remains. Almost a century later, in 1965, Payne established six stages based on observations of appearance, odour and insect activity. In 2014, Comstock *et al*. proposed four stages of decomposition of buried remains based on observations from remains which excluded insect activity. Conditions that might alter the pattern of decomposition will also alter the rate at which decay progresses.

In this study, observations on the decomposition process were assigned point values to better assess the state of decomposition in the head and neck, trunk and limbs. Each stage has an assigned point value depending on the appearance and characteristics of the decomposing remains. The sum of these three values is referred to as the Total Body Score (TBS). In 2005, Megyesi *et al*. proposed a sequential ranking model in order for the decomposition scores to reflect the smaller sequential changes that took place, focusing specifically on the accumulative effect of temperature the remains were exposed to (ADD). Accumulated degree-days (ADD) are the product of time and temperature between the lower and upper developmental thresholds needed for an organism to develop from one point to another in its life cycle for each day. This scoring system of Megyesi *et al*. (2005) has since been used in taphonomic research within a South African setting (Myburgh *et al*, 2013; Sutherland (now Marais-Werner) *et al*., 2013).

Decomposition leads to skeletonisation of the remains, but numerous factors can influence the decomposition process of a buried body. Factors such as temperature are deemed important for decomposition as bacteria need optimal conditions (between 25°C and 35°C) for development which is hindered by lower in-soil temperatures (Campobasso *et al*., 2001). Access by insects has a very significant effect on the biomass reduction of remains.

Burial (depending on the soil texture and depth of remains) may prevent oviposition and development of larvae, thereby slowing down the decay process (Mann *et al*., 1990). A number of studies have been conducted on post mortem changes within different settings (e.g., Mann *et al*., 1990; Dix and Graham, 2000; Campobasso *et al*., 2001; Vass, 2001; Adlam and Simmons, 2007; Goff, 2009; Simmons *et al*., 2010; Myburgh *et al*., 2013) however none refer to decomposition patterns of buried remains within South Africa.

Most studies of decomposition deal with surface finds (Galloway *et al*., 1989; Archer, 2004; Megyesi *et al*., 2005; Carter *et al*., 2007; Goff, 2009; Cross and Simmons, 2010; Keough, 2013; Myburgh *et al*., 2013; Sutherland *et al*., 2013; Keough *et al*., 2015), but, due to the difficulty in studying buried remains, much less is known about the decomposition processes involved (Rodriguez and Bass, 1985; Thew, 2000; Breitmeier *et al*., 2005; Forbes *et al*., 2005; Vass *et al*., 2008; Fründ and Schoenen, 2009). As it is important to be able to provide accurate PMI estimations, a baseline for decomposition of buried remains (specifically shallow burial in a clandestine grave) in a South African setting is necessary.

As new data and data sources are needed, taphonomic studies have been conducted at the Forensic Anthropology Body Farm (FABF) belonging to the University of Pretoria. These studies included an assessment of the usability of ADD to estimate the PMI in a South African setting (Myburgh *et al*., 2013), a comparison between decomposition rates of small and large pig carcasses (Sutherland *et al*., 2013), skeletal changes after post mortem exposure to fire during decomposition (Keough *et al*., 2015) and a time-lapse GPR survey on decomposing remains (van Schoor *et al*., 2015). Although most remains of forensic interest are found on the surface, a number of cases are received annually by the Forensic Anthropology Research Centre (FARC), University of Pretoria which involves the burial of human remains by criminals that require exhumation for anthropological analyses. Between 1996 and 2013, 786 cases were received and analysed by FARC of which 36 involved the burial of remains in shallow graves (4.7% of cases within this time frame). Therefore, understanding the process of decomposition in buried remains is extremely important to aid in criminal investigations, especially when attempting to estimate the PMI which requires the evaluation of a multitude of factors that contribute towards and affect the decomposition process.

Researchers have been trying to establish standardised decomposition stages for decades. By standardising decay stages by means of, for example, TBS and ADD, as is the case with this study, data can be compared with similar studies, replicated and validated. A clear description of decomposition patterns within specific geographical areas may assist investigators to estimate a more reliable PMI. An accurate PMI is essential in criminal investigations as it establishes a time frame for the death of the victim and narrows the pool of possible victims. This study contributes to the understanding of decomposition patterns of buried remains versus surface remains and eventually more accurate PMI estimations.

The aim of this study was to record decomposition patterns and rates of buried remains using a pig model, and to compare these to data from a previous study on remains decomposing on the surface conducted at the same facility (Myburgh, 2010). The pattern and rate of decomposition was evaluated at various intervals and recorded according to existing guidelines in a controlled environment to better understand how the process is affected by environmental variables, e.g., the presence of soil. The objective was to characterise the processes of decomposition of buried remains, and to highlight the differences in the decomposition process between buried and surface remains.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

It has been reported that the decomposition process begins approximately four minutes after death (Vass, 2001) and involves different phases and processes (Mann *et al*., 1990; Goff, 2009; Finley *et al*., 2014). The processes that occur between time of death and skeletonisation are influenced by many factors, among them environmental conditions. Many studies have contributed to our understanding of decomposition and the processes/stages associated with human decomposition (e.g., Galloway *et al*., 1989; Mann *et al*., 1990; Dix and Graham, 2000; Campobasso *et al*., 2001; Vass, 2001; Archer, 2004; Megyesi *et al*., 2005; Adlam and Simmons, 2007; Carter *et al*., 2007; Goff, 2009; Simmons *et al*., 2010; Myburgh *et al*., 2013; Sutherland *et al*., 2013; Keough *et al*., 2015).

While bodies decomposing on the soil surface may reach skeletonisation within weeks, decomposition of buried remains may take months or even years under favourable conditions (Mann *et al*., 1990; Gennard, 2012). According to Fiedler and Graw (2003), the rate of decomposition in both buried and surface remains depends mainly on three factors namely, (1) scavenger activity, (2) insect colonisation, and (3) temperature. Remains are usually protected from scavenger activity when buried at a depth of about a meter and/or deeper. Buried remains also reduce oviposition of insects as it can only be colonised by a minority of insects (Fiedler and Graw, 2003). Remains on the surface are vulnerable to scavengers, which normally leads to the destruction of soft tissue, gnawing on the bones and acceleration of the decomposition process (Mann *et al*., 1990).

The interdisciplinary field of taphonomy forms an integral part of forensic anthropology and focuses on reconstruction of events during and after death. The study of taphonomy is achieved through the collection and analyses of data concerning the depositional context, distinguishing between peri- and postmortem modification of tissue and bone and estimating the PMI (Haglund and Sorg, 1997).

2.2 Taphonomy

Taphonomy is a complex subject which offers a body of knowledge to forensic anthropological analyses in order to reconstruct a broad sequence of events to aid in victim identification. A taphonomic approach views remains within the context of discovery (Sorg *et al*., 2012) as it necessitates the reconstruction of peri- and post mortem processes associated with the crime scene. The application of taphonomic principles to crime scenes has been described as "the most important development altering the field of forensic anthropology" (Dirkmaat *et al*., 2008: p34). The scientific data that are collected at a crime scene can sometimes allow for informed and factual decisions to be made regarding a person's innocence or lack thereof when involved in court cases (Dirkmaat *et al*., 2008).

With the development of forensic taphonomy, more emphasis is placed on studying remains within the context of discovery (Sorg *et al*., 2012). Taphonomic analyses have 5 primary goals: (1) to estimate the post mortem interval, (2) to differentiate between human and non-human agents of bone modification, (3) to understand the transport of remains, (4) to identify variables associated with preservation of bone, and (5) to reconstruct perimortem circumstances. In order to meet these goals, taphonomic models, concepts and analyses are of utmost importance (Komar and Buikstra, 2008).

The impact of taphonomic methods on forensic anthropology requires a brief historical review of development during the last few decades. Taphonomy derived from the disciplines of palaeontology and archaeology which focused mainly on fossil records. Literature suggests that the study of taphonomy was first introduced by a Russian geologist, Efremov, in 1940 (Dodson, 1980; Bristow *et al*., 2011). Efremov defined taphonomy as the laws of burial: "transition of animal remains from the biosphere into the lithosphere" (Bristow *et al*., 2011: p279). In more recent years the application of taphonomic principles has been modified, adapted and adopted by forensic anthropologists for a variety of studies concerned with reconstructing events that took place after death.

Haglund and Sorg (1997) define forensic taphonomy as "the use of taphonomic models, approaches, and analyses in forensic contexts to estimate the time since death, reconstruct the circumstances before and after deposition, and discriminate the products of human behavior from those created by the earth's biological, physical, chemical, and geological subsystems" (Haglund and Sorg, 1997: p3).

Parsons (2009) states that taphonomy focuses on events that took place during the post mortem interval, and assists with the reconstruction, collection and analyses of data from forensically relevant cases.

Beary and Lyman (2012) suggest that taphonomic research within a forensic setting has 5 basic goals:

- determining the forensic significance of recovered remains
- estimating the PMI
- establishing how the remains reached the location of discovery
- determining what actions may have been taken by the assailant to conceal information related to the crime
- determining taphonomic factors that might have influenced the remains and interpretation of the information.

A taphonomic approach integrates aspects such as the influence of plants, animals, microorganisms, processes of decomposition and the ecology which become associated with the decomposing remains. Due to this wide range of agents which can affect the process of decomposition, multidisciplinary approaches to taphonomic studies are recommended. For example, entomologists can contribute to the identification and stages of development of insects, and botanists can supply expert information on plants associated with the remains. Environmental factors are considered just as important as information about the condition of the remains. Environmental factors can be seen as independent variables or exogenous parameters. These are characteristic of the environment that affects the decomposition process, i.e., temperature, scavengers and soil characteristics (Sorg *et al*., 2012).

Dependent or endogenous conditions, on the other hand, refer to factors such as body mass or the use of substances. Recent studies have demonstrated that remains exposed to similar conditions as well as similar PMIs can have different stages of decomposition (Dix and Graham, 2000; Pinheiro, 2006; Myburgh *et al*., 2013; Bilheux *et al*., 2015).

2.3 Biological decomposition of remains

It is necessary to understand the decomposition process associated with human remains when undertaking a study in taphonomy. Human decomposition is a continuum and even though decomposition may follow similar sequences, it does not necessarily follow similar tempos of decomposition. The environment is a major determinant of the stages of decomposition the remains undergo as well as the rate at which it proceeds. The rate of decomposition in buried remains is extremely variable as it is not uncommon for two bodies buried for the same length of time, to appear different (Amendt *et al*., 2004).

Decomposition involves the destruction of cells by chemical processes (autolysis) and fermentation (putrefaction). In a forensic context decomposition has a much broader meaning which covers all stages from the moment of death until skeletonisation (Pinheiro, 2006). Therefore, dividing decomposition into categories is useful to establish a correlation between each phase/stage and a known PMI (Dix and Graham, 2000).

Physical changes to remains can be observed in various stages of decomposition. Table 2.1 indicates these changes, as well as modifiers that can influence decay. Within approximately 2 hours post mortem, the body temperature decreases (algor mortis sets in). This is followed by the reddening of the skin, also known as livor mortis or lividity. Stiffening of the facial muscles due to the breakdown of glycogen and onset of rigor mortis takes place 2-3 hours post mortem and reaches its maximum after 24 hours. This is followed with skin slippage, putrefaction gases and green discolouration manifesting in the body. All these stages occur within 72 to 96 hours after death. However, the rate of decomposition is affected by a range of variables associated with the body and the surrounding environment (Clark *et al.*, 1997; Amendt *et al*., 2004).

In previous studies, decomposition has been divided in as many as nine stages, depending on variables associated with the geographic region (Megnin, 1896; Rodriguez and Bass, 1985; Goff, 2009). The use of stages allows for the physical appearance of decomposition processes to be more concise when described in general terms. For consistency and purposes of this study, the 4 stages proposed by Megyesi *et al*. (2005) will be used as a broad outline to describe the patterns of decomposition observed. These stages are fresh, early decomposition, advanced decomposition and skeletonisation.

Table 2.1: Post mortem changes and modifiers in the human body (Clark *et al*., 1997; Amendt *et al.*, 2004)

Time after death	Post mortem changes	Modifiers
0 Minutes	Circulation and breathing stops	Temperature
	Pallor	Humidity
	Early lividity	Outdoor location
	Muscular relaxation	Indoor location
2 Hours	Vascular changes in the eyes	
	Rigor mortis begins	
	Algor mortis begins	
	Lividity easily seen	
4 - 5 Hours	Coagulation of blood	
	Fixation of lividity	
24 Hours	Drying of cornea	
48 Hours	Rigor disappears	
72 Hours	Loss of hair and nails	
96 Hours	Skin slippage	Insect activity
	Bacterial overgrowth	Animal activity
Days/Months	Green discolouration	
	Bloating	Mummification
	Release of gases	Adipocere formation
	Gradual loss of soft tissue	
	Partial skeletonisation	
	Complete skeletonisation	

2.3.1 Fresh stage

This stage spans from the time the heart stops, which leads to the depletion of internal oxygen (Carter *et al*., 2007) (internal, aerobic microorganisms deplete the soft tissue of oxygen), to observable bloating of the body (Goff, 2009).

This results in the termination of aerobic metabolism and destruction of the cells by means of self-digestion, also known as autolysis. This earliest biochemical process of decomposition can begin within minutes after death (Vass, 2001). Anaerobic microorganisms originate from the gastrointestinal track and respiratory system. The actions of these anaerobic microorganisms transform carbohydrates, lipids and proteins into acids and gases. This results in colour changes, odour formation and bloating of the remains, also known as putrefaction. These changes are observable within 48 hours after death (Carter, 2007).

Autolysis thus creates favourable conditions for the anaerobic microorganisms which take the remains through the putrefactive stages. Insect invasion (flies (*Diptera*) are usually the first to arrive at a death scene) of the body begins which leads to the hatching of eggs and internal feeding activity (Campobasso *et al*., 2001). Other changes associated with autolysis include *algor mortis* (cooling of the body), *livor mortis* (post mortem hypostasis/lividity) and *rigor mortis* (stiffening of the muscles) (Vass, 2001; Bristow *et al*., 2011).

a.) *Algor mortis*

Algor mortis takes place shortly after death as the body ceases heat production and its temperature reaches equilibrium with the surrounding ambient temperature. Newton's Law of Cooling states that the rate of cooling of an object is determined by the difference between passing temperature of the warmer object to the cooler environment, during which the temperature of the object will fall (Pounder, 2000; Shepherd 2003). Four mechanisms of heat transfer are involved namely radiation, conduction, convection and evaporation (Tracqui, 2000). This cooling process may occur between 18 and 20 hours after death (Goff, 2009; Bristow *et al*., 2011). Several factors may influence the rate of cooling, for instance, obesity (fat is a good insulator), exposure to sunlight and clothing (type of material). A smaller individual will cool more rapidly than a larger individual, even in the same set of environmental conditions (Shepherd, 2003; Goff, 2009).

According to Tracqui (2000), three distinct periods of algor mortis can be identified: (1) the initial phase when the temperature remains relatively stable for 30 minutes to 3 hours; (2) the intermediate phase in which the body cools rapidly and; (3) the terminal phase when the rate of cooling slows as the core temperature reaches equilibrium with the surrounding temperature.

b.) *Livor mortis:*

Livor mortis, also referred to as lividity, is the first change in external appearance of the deceased. When circulation of blood flow ceases, red blood cells and blood plasma are influenced by gravity and manifests in the lowest levels within the vascular system, causing the area to become discoloured (reddish). The first signs of lividity can be observed after more or less one hour following death, with full development within 2-4 hours (Goff, 2009). After approximately 9-12 hours a change in position of the deceased will not change the position of livor mortis as it is described as being fixed until putrefaction (Tracqui, 2000; Bristow *et al*., 2011). Any areas of pressure resulting from e.g., contact of one area of the body with another during this period, will not show discolouration (Shepherd, 2003; Goff, 2009). Change in the position of the body after the development of lividity will result in the redistribution of the red blood cells due to gravity. This may result in two overlapping patterns (Shepherd, 2003).

c.) *Rigor mortis:*

Rigor mortis is due to complex physicochemical changes in the myofibrils of the muscle tissues. As lactic acid is produced resulting in a lowered cellular pH, locking chemical bridges are formed between actin and myosin which lead to the formation of rigor (Goff, 2009). The onset is observable within 2-6 hours following death, and fully developed over the first 12 hours (Bristow *et al*., 2011). Rigor mortis lasts between 24 and 84 hours after which the muscles begin to relax again and putrefaction sets in (Goff, 2009). Main factors affecting the onset of rigor mortis include temperature and the metabolic state of the deceased.

Lower temperatures tend to delay the onset of rigor and increase the duration, whereas higher temperatures increase the onset but delay its duration (Goff, 2009). Violent activity prior to death, muscular development and cause of death would also influence the onset and duration of rigor mortis (Tracqui, 2000; Shepherd, 2003).

2.3.2 Early decomposition

Putrefaction begins during this stage (up to 1 week post mortem) with a greenish discolouration on the abdominal area as the anaerobic bacteria in the body begin to digest the tissue. This results in the production of gases and inflation of the abdomen, an enlarged scrotum and protrusion of the eyes and tongue. Internal pressure caused by gases results in the putrefactive fluids purging from the nostrils, ears and mouth, as well as a distinct odour of decomposing flesh (Tracqui, 2000; Pinheiro, 2006; Goff, 2009; Bristow *et al*., 2011). Marbling is observed as a result of colonisation of the venous system by intestinal bacteria which haemolyse the blood (Pinheiro, 2006).

Up to 1 month post mortem a change in colour from green to purple to black is observed. The outer layer of the skin separates from the dermis (skin slippage) and the gases from the abdomen escape as the body deflates, leading to the excretion of urine and faeces (Tracqui, 2000). Large masses of feeding *Diptera* (fly) larvae are associated with this stage, both internally and externally. A definite increase in numerous species of insect feeding on the remains is observable which contributes to rapid mass loss (Goff, 2009).

Mass loss is also largely attributed to the loss of fluids through seepage (Catts, 1992). By the end of this decomposition stage, *Calliphoridae* (blow flies) and *Sarcophagidae* (flesh flies) would have completed development and left the remains. *Diptera* larvae will have removed most of the flesh, leaving only skin, bone and cartilage (Goff, 2009).

2.3.3 Advanced decomposition

This stage is marked by the progressive drying of the remains (up to 2 months post mortem) (Tracqui, 2000) and bone exposure particularly on the extremities.

Stages vary in length of time and are dependent on factors such as insect abundance and activity, geographic location, temperature, humidity, rainfall, habitat, season and carcass size (Sutherland *et al*., 2013). Decomposition is largely inhibited during this stage due to the loss of cadaveric material. The putrid odour is absent and a great part of the soft tissue is removed which leads to reduced insect activity on the remains. This stage is also typically associated with discolouration and death of underlying and nearby vegetation. This might be due to nitrogen toxicity or excretion of antibiotics by blowfly larvae. The amount of nitrogen released by decomposing remains might result in a loss of nitrogen from the ecosystem through de-nitrification, volatilisation and leaching (Carter *et al*., 2007).

2.3.4 Skeletal stage

This process can develop within months and/or years depending on internal and external factors that may influence the decomposition process. This stage is reached when remaining soft tissue disappears and only bones and hair remain (Tracqui, 2000; Goff, 2009; Vass, 2010). In an extreme case, Clark *et al*. (1997) reported skeletonisation that occurred within 3 days. It is important to note that in this case, environmental conditions were very humid and intense insect activity was present. The other extreme will also be true in cases of freezing temperatures where skeletonisation may take years (Pinheiro, 2006).

The higher the temperature and humidity, the greater the rate of decomposition and skeletonisation (Clark *et al*., 1997; Pinheiro, 2006). Remains buried in a hot environment may skeletonise as rapidly as remains exposed to air in a temperate region; decomposition is therefore dependent on factors such as depth of the burial and soil type (Clark *et al*., 1997).

At a later stage during skeletonisation, bone may retain some grease or become completely dry (Parsons, 2003). Disarticulation is a common phenomenon with skeletonised remains. Even if each bone is in place, a skeleton is considered disarticulated when the soft tissues do not join the bones together (Pinheiro, 2006). Decompositional stages follow one another but no clear distinction can be made between when one stage ends and the other begins due to the variation in time of onset, overlap of changes, environmental factors, and the rate of progression of putrefaction (Campobasso *et al*., 2001).

2.4 Determination of the post mortem interval

The post mortem interval estimates provide important information on a measurable period from death to recovery. Two main factors are taken into account when estimating the PMI, namely the last time the victim was reliably known to be alive and the time at which the body was discovered (Goff, 2009).

This estimation is important for the following reasons:

- Establishing a time frame for the death of the decedent and differentiating peri- and post mortem activities
- Identifying and possibly narrowing the number of potential victims
- Verifying the accused's alibi (Komar and Buikstra, 2008).

Disciplines such as forensic pathology, anthropology and entomology have developed criteria to improve the estimation of PMI. Traditionally, the pathologist best ascertains the PMI using the soft tissue indicators of algor mortis, rigor mortis and livor mortis (Tibbett, 2010). These methods can produce PMI estimates that are accurate within days in cases where the PMI is relatively short. A method fairly successful in estimation of the PMI is entomology that uses the developmental biology of blowflies and other insects (Tibbett, 2010). However, a decompositional formula for calculating an accurate PMI remains elusive due to the vast amount of factors influencing and associated with human decomposition (Vass, 2010). Anthropologists have become increasingly astute at PMI estimation by temperature correlation (Megyesi *et al*., 2005; Myburgh *et al*., 2013). Therefore, the application of a typological qualitative approach seems more reasonable for cases involving advanced decomposition (Megyesi *et al*., 2005). According to Pounder (2000) and Amendt *et al*. (2004), the longer the PMI, the less accurate the time since death estimation based on the post mortem changes to the body. Decomposition is a process during which the body breaks down and decompose. It is a continuous process which is difficult to divide into discrete categories. Decomposition follows a known sequence, however the rate at which these phases occur is highly variable. Unfortunately processes involved with decomposition are potentially overlapping, complex, and it is almost impossible to assign a precise time interval (Haglund and Sorg, 1997). The rate of decomposition is affected by a range of variables associated with the body and the surrounding environment (Clark *et al.*, 1997; Amendt *et al*., 2004).

2.5 Accumulated Degree Days (ADD)

Insects are poikilothermic and can therefore not control their body temperatures - they use the environment as a heat source (degree days) to grow and develop. Energy needed to achieve/reach certain life stages can be calculated, and is a common future of integrated pest management predictions. Degree days can thus be added together to reflect periods of insect development. Minimum temperatures needed for growth will vary between species, and vice versa, for maximum temperatures resulting in maximum growth. Temperature readings during each 24 hour period will vary, but the area between the upper and lower thresholds of growth represents a predictable time frame, also known as ADD (Gennard, 2012).

In an attempt to standardise the effects of temperature on decomposition, Megyesi *et al*. (2005) proposed that decomposition be described more precisely as a continuous variable. Instead of using qualitative categories to describe decomposition, they propose quantified stages with assigned point values to express decomposition. This would increase statistical accuracy and provide more information about the relationship between decomposition and PMI. By making use of the ADD model proposed by Megyesi *et al*. (2005), and used by Myburgh *et al*. (2013) and Sutherland *et al*. (2013) amongst others, the variables associated with temperatures can be standardised and validated. When decomposition is studied using ADD, it enables researchers to standardise the processes under different conditions which enhances their ability to replicate experiments under similar conditions. ADD represents the accumulation of heat energy units needed for the chemical and biological reactions that take place in the body during the decomposition process (Simmons *et al*., 2010). ADD is calculated by averaging the minimum and maximum temperature of a day (mean daily temperature), therefore ADD is the sum of consecutive average daily temperatures.
The equation used to calculate ADD per day is:

$$\frac{(\text{Maximum temperature} + \text{minimum temperature}) \times 1 \text{ day}}{2}$$

For decomposition to progress, temperatures must remain above a minimum value. A base temperature of 0°C is used as biological/decomposition processes cease at freezing temperatures (Megyesi *et al*., 2005). One degree day is the average daily temperature reading in 24 hours when the temperature is a degree above the base temperature. Each day the temperature is above the base temperature, more degree days are accumulated. For example, if the average temperature is 20°C above the lower threshold for 2 days the ADD is 40 (20°C x 2 days). To predict the time of death for a forensic case the TBS needs to be calculated according to the guidelines developed by Megyesi *et al*. (2005). In the study of Megyesi *et al*. (2005), it was found that the coefficient is 0.002 and the intercept 1.81 with a standard error (SE) of ±388.16 of the regression in non-logged ADD's. The predictive equation to estimate ADD is therefore:

$$ADD = 10^{(\text{coefficient x}TBS x TBS + \text{intercept})} \pm 388.16$$

For example, if a body with an unknown date of death was received and the TBS was 30, the estimated ADD would be 4073.81. The number (4073.81) is the ADD needed for the body to reach the stage of decomposition observed (TBS =30). The resulting equation is:

$$ADD = 10^{(0.002*30*30+1.81)} \pm 388.16$$

The mean daily temperatures are then subtracted backwards from the date of discovery until the actual ADD value is equal to the estimated ADD. The number of days it took for the temperatures to be the same therefore indicates the PMI.

Megyesi *et al.* (2005) incorporated mixed methods whereby both qualitative and quantitative approaches were used, however they applied a more quantitative approach to calculate a PMI estimate. This retrospective study used 68 human remains found in a variety of settings (woods, fields, ditches and indoors). The authors observed and scored the state of decomposition from photographs and case reports. The qualitative data on decomposition was converted into quantitative scores with assigned point values to assess decomposition in three specific areas of a decomposing body: (1) the head and neck, (2) the trunk, and (3) the limbs.

To account for differential decomposition in the different body segments, the regions were scored separately as not all stages of decomposition apply equally to all parts of the body. The point value allocated to each region was combined to produce a TBS (Megyesi *et al.*, 2005). Standardised variables such as TBS and ADD aid in producing comparable research results. By using the TBS and ADD models, the percentage of variability in decomposition as reflected by TBS can be accounted for by ADD.

A number of studies tested the accuracy of the ADD/TBS method of Megyesi *et al*. (2005). Parsons (2009) used two pigs (one placed during the warmer period and the other placed during the colder period) to study decomposition patterns in West Central Montana, USA.
The proposed ADD method was less accurate during the early stages of decomposition and became more accurate as decomposition progressed. This can be attributed to slower decomposition in cooler temperatures (Parsons, 2009). Also, accuracy cannot be statistically determined by such a small sample size. However, the PMI estimations were within the 80% confidence interval suggested by Megyesi *et al*. (2005).

Simmons *et al*. (2010) also suggested that the ADD model can be used during decomposition studies which would then allow for comparison across varied environments. Wild rabbits (N=114) were exposed to different environmental conditions (60 rabbits were buried and 54 rabbits were placed on the surface) where after decomposition rates were measured by TBS every 50 ADD. Although the sample size is deemed sufficient for statistical analysis, rabbits do not compare well to human decomposition. Simmons *et al*. (2010) propose that the rate of decomposition mostly depend on the insect activity on remains as the surface remains decomposed much faster. Their results indicate that when temperature is standardised as ADD, it is the presence of insects which accelerates decomposition. As burials normally exclude or reduce insect colonisation, ADD is still useful for estimating PMI for buried remains, although an insect exclusion model is proposed for the PMI calculation.

In a larger study, using 30 pigs, Myburgh *et al*. (2013) made use of ADD to estimate PMI in a South African setting and concluded that the results of this study broadly agreed with those found by Megyesi and colleagues (2005) and that the ADD model can be applied in a South African setting, although SE of the estimates were wide especially with long PMI's.

Myburgh *et al*. (2013) suggested that ADD better describes the process of decomposition than PMI. Where PMI reflects the number of days that have passed since death, the rate of decay is dependent on temperature. When examining ADD and PMI within a season, a closer statistical relationship is anticipated when temperature fluctuations are being controlled.

Following this, Sutherland *et al*. (2013) compared the findings of Myburgh *et al*. (2013) to rates of decomposition of 15 pigs (weighing between 3 and 35 kgs), with 15 pigs weighing between 60 and 90 kgs. By making use of ADD to assess the pattern of decomposition, it was concluded that smaller pigs decomposed 2.8 times faster than larger pigs within the mentioned weight ranges. The ADD method was therefore successful in replicating and comparing results across studies.

Moffat *et al*. (2015) proposed a modified regression model to predict ADD from TBS based on the findings of Megyesi *et al*. (2005). Megyesi *et al.* (2005) used a SE of ±388.16 which, according to Moffat *et al*. (2015), represents a confidence interval of 68%. To increase the confidence interval to 95%, the SE of 388.16 should be multiplied by 1.96.

For purposes of this study, ADD data of surface remains by Myburgh *et al*. (2010) were used for comparison with buried remains. The use of ADD facilitates the comparison of decomposition data regardless of local environment and seasonal fluctuations. ADD allows for the standardisation of PMI studies across different continents and climatic conditions. As concluded by Megyesi *et al*. (2005), stages of decomposition can accurately predict ADD intervals. It can therefore be assumed that ADD can also predict stages of decomposition (Adlam and Simmons, 2007). ADD would allow for the construction of taphonomic models for scoring decomposition rates which will enhance future studies on decomposition processes.

2.6 Variations in patterns of decomposition

2.6.1 Adipocere

Adipocere formation, also known as saponification, is a post mortem preservation process which results from the hydrolysis and hydrogenation of the adipose tissue, depending on environmental circumstances (Pinheiro, 2006). Adipocere has been referred to as grave wax or corpse wax (Ubelaker and Zarenko, 2011).

The ideal circumstances for the formation of adipocere require fat and a moist environment, although it is an uncommon change and does not occur on all remains. Adipocere formation is very variable and may take several weeks or in some cases months to form (Dix and Graham, 2000; Goff, 2009; Bristow *et al*., 2011). However, in some cases adipocere has been found in tombs and dry graves within a few days (Pinheiro, 2006). Its consistency gives some indication as to the rate of decomposition. Rapid decomposition results in a hard and crumbly composition when fat is bound with sodium (found in interstitial fluids). Slower decomposition is characterised by a soft, pasty consistency formed when fat binds with potassium (cell membrane breakdown) (Vass, 2001). The formation of adipocere is interconnected to the process of mummification as it has the ability to preserve evidence for a prolonged period of time. Degradation of adipocere is accelerated when exposed to air, moisture and bacteria which will eventually lead to skeletonisation of remains (Pinheiro, 2006; Ubelaker and Zarenco, 2011).

2.6.2 Mummification

During mummification the body dehydrates, usually in very dry circumstances (fluid evaporation). However, mummification may also occur in icy environments (dryness of the air and low bacterial growth), which leads to discolouration (dark), and a dry and leathery skin (Vass, 2001; Pinheiro, 2006). Mummification is characterised by dry and brittle skin and is prominent in areas like the cheeks, forehead and hips (Pinheiro, 2006).

As the skin dries and hardens, the soft tissue decomposes and the body appears preserved (Dix and Graham, 2000). The time necessary for mummification is not well documented as it relies on environmental conditions. In very hot and arid conditions (e.g., deserts), exposed remains can mummify in a matter of days or months (Pinheiro, 2006).

2.7 Scoring of decomposition

When decomposition is studied using quantitative scores, it enables researchers to standardise the processes under different conditions, which enhances their ability to replicate experiments under similar conditions. The process of decomposition can be then studied without the effects of subjectivity. Megyesi and associates (2005) scored a sample of 68 human remains from various climates and developed categories based on modified stages of decomposition. The categories, stages of decomposition and point values allocated to each region are described in Tables 2.2, 2.3 and 2.4. In these tables, decomposition is divided into four categories: fresh, early decomposition, advanced decomposition and skeletonisation. Each decomposition stage was scored with a starting value of 1, indicating the fresh stage of decomposition which increased to a maximum of 35, indicating complete skeletonisation, to calculate the TBS.

Total Body Score is a system of numerically ranked qualitative observations of decomposition which represents the overall stage of decomposition. Point values were assigned to three separate anatomical regions in order to quantify the qualitative observation. The TBS was then compared to the ADD, using a base temperature of 0˚C, as biological processes cease at zero temperatures. Megyesi *et al*. (2005) concluded that over 80% of the variation in decomposition could be accounted for by the combination of ADD and elapsed time (Megyesi *et al*., 2005).

Table 2.2: Categories and stages of decomposition for the head and neck: (Megyesi *et al*., 2005)

A.	Fresh	
1 pt	1.	Fresh, no discolouration.
B.	Early decomposition	
2 pts	1.	Pink-white appearance with skin slippage and some hair loss.
3 pts	2.	Grey to green discolouration: some flesh still relatively fresh.
4 pts	3.	Discolouration and/or brownish shades particularly at edges, drying of nose, ears and lips.
5 pts	4.	Purging of decompositional fluids out of eyes, ears, nose, mouth, some bloating of neck and face may be present.
6 pts	5.	Brown to black discolouration of flesh.
C.	Advanced decomposition	
7 pts	1.	Caving in of the flesh and tissues of eyes and throat.
8 pts	2.	Moist decomposition with bone exposure less than one half that of the area being scored.
9 pts	3.	Mummification with bone exposure less than one half that of the area being scored.
D.	Skeletonisation	
10 pts	1.	Bone exposure of more than half of the area being scored with greasy substances and decomposed tissue.
11 pts	2.	Bone exposure of more than half the area being scored with desiccated or mummified tissue.
12 pts	3.	Bones largely dry, but retaining some grease.
13 pts	4.	Dry bone.

Adlam and Simmons, (2007) scored decomposition rates using the TBS point values and ADD model as described by Megyesi *et al*. (2005). They found that scoring decomposition presented a more accurate picture of the decaying process. Their findings suggest that recording time as ADD in decomposition has been of great benefit to taphonomic research as they could compare results across geographical areas and different seasons. Myburgh *et al*. (2013) also found that they could statistically validate the accuracy of the PMI prediction when using TBS and ADD as the pattern of decay remains comparable even between periods of temperature fluctuations.

Table 2.3: Categories and stages of decomposition for the trunk: (Megyesi *et al*., 2005)

A.	Fresh	
1 pt	1.	Fresh, no discolouration.
B.	Early decomposition	
2 pts	1.	Pink-white appearance with skin slippage and some hair loss.
3 pts	2.	Grey to green discolouration: some flesh still relatively fresh.
4 pts	3.	Bloating with green discolouration and purging of decompositional fluids.
5 pts	4.	Post bloating following release of the abdominal gases, with discolouration changing from green to black.
C.	Advanced decomposition	
6 pts	1.	Decomposition of tissue producing sagging of flesh; caving in of the abdominal cavity.
7 pts	2.	Moist decomposition with bone exposure less than one half that of the area being scored.
8 pts	3.	Mummification with bone exposure less than one half that of the area being scored.
D.	Skeletonisation	
9 pts	1.	Bones with decomposed tissue, sometimes with body fluids and grease still present.
10 pts	2.	Bones with desiccated or mummified tissue covering less than one half of the area being scored.
11 pts	3.	Bones largely dry, but retaining some grease.
12 pts	4.	Dry bone.

Table 2.4: Categories and stages of decomposition for the limbs: (Megyesi *et al*., 2005)

A.	Fresh	
1 pt	1.	Fresh, no discolouration.
B.	Early decomposition	
2 pts	1.	Pink-white appearance with skin slippage on limbs.
3 pts	2.	Grey to green discolouration: marbling; some flesh still relatively fresh.
4 pts	3.	Discolouration and/or brownish shades particularly at edges, drying of limbs and other projecting extremities.
5 pts	4.	Brown to black discolouration, skin having a leathery appearance.
C.	Advanced decomposition	
6 pts	1.	Moist decomposition with bone exposure less than one half that of the area being scored.
7 pts	2.	Mummification with bone exposure of less than one half that of the area being scored.
D.	Skeletonisation	
8 pts	1.	Bone exposure over one half the area being scored, some decomposed tissue and body fluids remaining.
9 pts	3.	Bones largely dry, but retaining some grease.
10 pts	4.	Dry bone.

2.8 Factors influencing the rate of decomposition

Mann *et al*. (1990) studied and proposed a number of variables in order of the greatest to the least influential on the decomposition process. Table 2.5 shows the variables identified during their study, although it should be kept in mind that many variables influence decomposition and their study did not describe all factors in detail. From Table 2.5, it is clear that a factor such as temperature will have a greater influence on the rate of decomposition compared to variables such as body size and weight.

Table 2.5: Variables affecting the decomposition rate of human remains (Mann *et al*., 1990)

Variable	Effect on decomposition*
Temperature	5
Access by insects	5
Burial and depth	5
Trauma to the body	4
Humidity	4
Rainfall	3
Body size and weight	3
*Subjective criteria rating based on a five-point scale, five being the most influential.	

Goff (2009) clusters variables into three broad categories, namely:

(a) Physical barriers

These are variables preventing access to the remains by physical means, for example, a body in soil versus an exposed body. In similar fashion, the effect of a sealed casket can be compared to a sealed plastic bag.

(b) Chemical barriers

Chemicals used on a body would alter the decomposition process, especially embalming fluid which was designed to prevent decomposition or delay the onset for a considerable time.

(c) Climatic factors

Temperature is the most influential factor as low temperatures can delay bacterial growth as well as inhibit insect activity.

2.8.1 Temperature

According to studies undertaken by Mann *et al.* (1990) and Goff (2009), temperature appears to have the greatest effect on the rate of decomposition. Increased temperatures stimulate the rate of insect activity which leads to faster degradation of the remains. Maggots feed in dense masses, thereby elevating the temperatures which are ideal for decomposition of organic matter (Campobasso *et al.*, 2001). During cold weather, the insect activities will either retard or cease, depending on the ambient temperature. Insects will continue to lay eggs in temperatures of approximately 5˚C to 13˚C. Below 0˚C the fly eggs and maggots (larvae) exposed outside the body die. Maggots within the body will however continue to feed as they produce substantial heat which enables survival due to their large numbers (Mann *et al*., 1990). Numerous studies (e.g., Mann *et al.*, 1990; Archer, 2004; Myburgh *et al*., 2013) state that the most difficult time to estimate PMI is during change in seasons. This may be due to associated changes in the temperatures, rainfall and insect activity (Archer, 2004). It has been reported that when using ADD to standardise temperature, insects have been found to influence the rate of decomposition more than any other variable (Simmons *et al*., 2010). Temperature is the most essential variable influencing the rate of maggot development, as extreme climatic conditions can destroy eggs (Campobasso *et al.*, 2001).

2.8.2 Decomposers

Four primary categories of organisms are involved in decomposition. These organisms are also known as decomposers, and are categorised as follows (Goff, 2009):

a.) *Bacteria*

Microorganisms are found in terrestrial habitats with bacteria often being the most prevalent (Olakanye *et al*., 2014). Anaerobic bacteria are associated with the human digestive system which assists in the breakdown of nutrients. Once the individual's heart ceases to function, bacteria normally found in intestines begin to digest the body from the inside out and are particularly evident in the areas of the head and abdomen (Goff, 2009).

b.) Fungi

Bacteria and fungi are responsible for almost 90% of organic matter breakdown (Olakanye *et al*., 2014). In living bodies the outer layer of the epidermis *(stratum corneum*), consisting of dead cells (*keratinocytes*), is shed as new tissues are produced underneath. When death occurs, the outer layer of the epidermis is no longer shed, and fungi spores begin to colonise the external surface (Goff, 2009).

c.) Insects

Studies dealing with burial fauna date back to the nineteenth century. Megnin (1896) studied the biology of insects found in human cadavers in successive sequence. In doing so, he established a manner in which to estimate the time of death of an individual by means of assessing arthropod succession at different decomposition stages. A distinct relationship exists between insects and decomposing remains as the species present at any given time during the process have different functions. Essentially four basic types of insects have been identified:

(a) Necrophagous species

This group includes species actually feeding on the remains. It includes many of the *Diptera* (fly) taxa particularly the *Calliphoridae* (blow fly) and beetles such as the *Coleoptera*.

(b) Predators and parasites of necrophagous species

This category includes species (flies, beetles and wasps parasitic on fly larvae and pupae) preying on organisms encountered in the soil under the decomposing remains.

(c) Omnivorous species

This includes taxa such as wasps, ants and beetles that feed on both the remains and arthropods.

(d) Adventive species

This refers to taxa that use the remains as an extension of their own habitat, for instance spiders and centipedes (Campobasso *et al.* 2001; Goff 2009).

When insects are completely and or partially excluded from the decomposition process, the rate of decomposition decreases and visible characteristics are altered (Comstock *et al*., 2014).

d.) Vertebrate scavengers

According to Mann *et al.* (1990), carnivores accelerate the decomposition process by consumption of the soft tissue, scattering of remains and damaging the bony elements (gnawing). Scavenging and scattering are often associated with one another as carnivores have the ability to disarticulate limbs and remnants of remains can be found in defecation and regurgitation of tissue (Beary and Lyman, 2012).

2.8.3 Trauma to the body

Forensic anthropologists have suggested that human remains that display trauma will decompose faster than those remains that have not suffered either blunt force or sharp force trauma. Gross trauma offers additional access points for insects to deposit eggs (Cross and Simmons, 2010). The wounds are moist and maintain contact with air which can result in an increased rate of decomposition around the wound and modifies the decomposition sequence. For example, when one portion of decomposing remains shows more advanced decomposition than other portions, this may suggest the presence of perimortem trauma in that region (Mann *et al.,* 1990; Campobasso *et al*., 2001).

2.8.4 Humidity

Humid environments result in increased insect activity since larvae require a certain amount of moisture to survive. Dry and arid areas result in mummified remains that may show very little destruction caused by insects (Mann *et al.,* 1990). Campobasso *et al*. (2001) state that ambient temperatures have the greatest effect on decomposition, closely followed by ventilation and air humidity. Dry, windy and humid conditions dehydrate remains rapidly which impair bacterial proliferation, leading to mummification.

2.8.5 Rainfall

Mann *et al*. (1990) propose that rainfall seems to have little if any effect on insect activity as the larvae remain hidden within the cavities and continue to feed. In the study done by Myburgh (2010), however, it was evident that rainfall had a considerable influence on the decomposition pattern. In the Myburgh (2010) study, pigs that received rainfall during the later stages of decomposition showed an increased rate of decomposition compared to those with no rainfall. This is also suggested by Archer (2004) who proposed that rainfall and moist soil can potentially increase decomposition rates as it prevents the remains from drying, therefore encouraging insect activities and bacterial growth in the flesh. Archer (2004) also suggests that re-hydration of desiccated remains by rainfall in some cases allows for re-colonisation of carcasses by insects.

2.8.6 Body size and weight

Carcass size has also been found to play a significant role as smaller carcasses decompose faster compared to larger ones (Simmons *et al*., 2010; Sutherland *et al*., 2013). In a study done by Simmons *et al.* (2010) on the influence of insects and carcass size on the rate of decomposition it was found that a relationship exists between carcass mass and the rate of decomposition. Smaller carcasses decomposed quicker compared to larger carcasses, however, the slower decomposition rate in larger carcasses could be attributed to the greater body mass for insects to consume. Body size was found to be a significant factor when carcasses are accessible to insects; when insects were excluded carcass size did not seem to influence the decomposition rate.

Sutherland *et al*. (2013) observed and recorded the degree of decomposition in small pigs (<35 kg) according to guidelines developed by Megyesi (2005). Accumulated degree-days was used to standardise the effect of temperature on the rate of decomposition. The results found in the study by Sutherland *et al*. (2013) were compared to the results obtained in Myburgh *et al*. (2013) who used large pigs (>38 kg). Similar to the observations described in the study by Myburgh *et al*. (2013), rapid decomposition occurred during the early stages of decomposition for all pig samples in this study. The larger pigs in both groups (>20 kg) showed a plateau phase during the course of advanced decomposition.

During this plateau phase the soft tissue became desiccated and maggot activity was minimal. This was in contrast to the smaller pigs (<20 kg) which showed no plateau phase throughout the duration of the study. This phenomenon may be ascribed to the smaller body mass as the soft tissue was consumed by insects before the tissue became desiccated. According to the findings of Sutherland *et al*. (2013), smaller pigs (3-35 kg) decomposed 2.82 times faster than large pigs (38-91 kg).

2.9 Burial of remains

Victims are sometimes left where they are killed, but there is usually an attempt to conceal the evidence. Burial is a popular choice for assailants who are looking to dispose of human remains. Only in exceptional cases are remains buried at a depth of more than 0.9 meters (m) (Manhein, 1997). Digging of graves requires time and effort, and the longer the assailants are in contact with the remains the more likely they are to be apprehended with the remains in their possession. Therefore the digging of shallow graves for victims seems to be the obvious choice. Remains that are buried, submerged in water or even placed in the open will look much different from each other, even after the same PMI (Troutman *et al*., 2014). Buried remains generate a unique environment much different to those of surface remains. When geological and biological factors are eliminated and environmental factors (i.e., temperature) are standardised, buried remains are stated to take approximately eight times longer to decompose. This can be ascribed to the limitation of insect activity and lower ambient temperature (Troutman *et al*., 2014).

2.9.1 Factors that influence decomposition of buried remains

a.) Depth

Burial and depth are, according to Mann *et al*. (1990), the third most important variable affecting decomposition of remains, after temperature and insect colonisation. When the effect of temperature on decomposition is standardised, it is the presence of insects which accelerates decomposition (Simmons *et al*., 2010).

Pastula (2012) states that the depth of burials will make a significant difference in insect presence and abundance. In her study, insects were present at both 0.3 m and 0.6 m in depth, although the arthropods at 0.6 m were not as abundant compared to taxa at 0.3 m. Insect evidence may be of value in explaining what happened to the body before burial, for instance, how long it was exposed above ground before burial.

Gunn and Bird (2011) consider the average clandestine grave depth in the UK to be 0.4 m. In the USA the average depth is reported as 0.56 m. Unfortunately literature on average depths of clandestine graves in South Africa is not available. According to Gunn and Bird (2011), some uncertainty exists as to the ability of blowflies to exploit buried remains and how soil will affect the hatching of eggs and larvae development. As expected, soil compaction has an impact on insect colonisation of the remains. However, even in compacted soil *Diptera* managed to reach the remains. This is ascribed to the bloating of the body during decomposition which would disturb the compacted soil and facilitate colonisation.

Bodies on the soil surface follow a different sequence of decomposition as they tend to decompose more rapidly than those which are buried. As mentioned earlier, the depth at which a body is buried also affects the decomposition rate (Pastula, 2012). Bodies buried in a shallow grave of 0.3 m or 0.6 m may skeletonise within a few months, whereas bodies buried at 0.9 m or 1.2 m may take years to decompose to the same extent as those in shallower graves (Mann *et al.*, 1990).

b.) Temperature

It has been found that soil provides an efficient insulation barrier to solar radiation and restricts access of insects to the body; therefore decomposition in soil occurs at a much slower rate (Campobasso *et al.,* 2001). It has however been reported that very shallow graves (supposedly <20 cm) are likely to be affected by fluctuations of temperatures above ground as loose soil over the remains contains rocks that heat up in high temperatures. In some instances this can lead to fluctuation in temperature (Sorg *et al.*, 2012).

Gennard (2012) found that the decomposition pattern mainly depended on the season of burial and soil temperature. Insect colonisation of the remains will also depend on soil conditions. Burial of remains is therefore an important factor as it reduces, and in extreme cases, excludes oviposition by *Diptera* species, which in turn leads to modification of the decomposition process. This is in agreement with the study of Breitmeier *et al*. (2005).

Studies on human decomposition in graves have not been conducted in South Africa, therefore the data from Gennard (2012) can only be used as a very broad guideline for buried remains to reach skeletonisation. Although various non-human studies on decomposition have been reported on (Wilson *et al*., 2007; Turner *et al*., 2013; Troutman *et al*., 2014), the only accessible literature found on actual human cadavers buried in soil was that of Rodriguez and Bass (1985) and Breitmeier *et al*. (2005).

Rodriguez and Bass (1985) were first to study decomposition rates of un-embalmed buried human cadavers. Six cadavers were buried between a depth of 0.3 m and 1.2 m. Minimal soft tissue loss was observed for at least the first year after burial whereas bodies buried in shallow pits of 0.3 m skeletonised within approximately six months to a year. In general, complete skeletonisation was reached within two to three years at a depth of 1.2 m and more. This leads to the conclusion that preservation of cadavers increased with the depth of burial. This was ascribed to decreased carrion insect activities and the cooler temperatures as soil provided an insulation barrier for solar radiation.

c.) Coffined remains

Breitmeier *et al*. (2005) evaluated coffined remains of 87 exhumations. Burial varied from five days to 16.8 years. Environmental factors such as the depth of burial, condition of the soil and seasonality were found to play a role in dictating the speed of decomposition. Unfortunately the exhumations in their study were for bodies which were legally buried (coffined), whereas the criminally disposed bodies were excluded.

Dent *et al*. (2004) mention that factors such as burial in a coffin versus burial in soil would also alter the decomposition process as soil contains minerals, salt, organic matter, solutions and various organisms. During burial the excavated soil would disrupt the normal gas and water infiltration into the grave as the soil is looser in that area. When burial of remains is complete, the soil compaction proceeds over time, aided by rainfall. The decomposition process is primarily affected by factors such as the nature of the remains, site of burial, infiltration of groundwater, atmospheric gases, climate and time.

d.) Soil

The study of soil in a natural environment, also known as the science of pedology, is primarily a discipline involving agriculture and engineering but is also a component of geological, archaeological and forensic concerns. Characteristics and components of soil examined include colour, consistency, texture and structure (Pokines and Symes, 2014). Grave soil is known to undergo biochemical changes as the result of movement of fluids, nutrients and microbes. These biochemical changes provide potential in estimating the PMI and in identifying clandestine grave sites. Microbial analyses offer an additional biometric for estimating the PMI (Finley *et al*., 2014). Exposed and buried remains produce different environmental conditions. This is confirmed by Wilson *et al*. (2007) who found that buried remains modify the faunal environment of the grave during decomposition, contributing to increased microbial load and production of liquid and gaseous by-products of decomposition.

They observed that water-table movements, soil temperature and moisture strongly influenced the decomposition process. According to their findings, decomposition between carcasses in the same habitat also varied. Moisture-rich soil is conducive to adipocere formation while well-drained soil can promote mummification and soil acidity can decrease microbiological activity and decomposition. The nature of soil will therefore influence the rate and pattern of decomposition (Troutman *et al*., 2014).

Vass *et al*. (1992) collected data on volatile fatty acids from soft tissue decomposition deposited in soil from human remains. The purpose was to develop a method of determining a PMI from soil solutions. Seven nude subjects were placed in the Anthropological Research Facility at the University of Tennessee throughout a year and allowed to decompose naturally. Data analyses revealed distinct patterns in the soil for volatile fatty acids during soft tissue decomposition based on ADD. Decomposition rates were based on stages of fresh, bloating, decomposition and dry. Two variables stood out as determinants of the concentration of volatile fatty acids in soil, namely moisture present in the soil and the weight of the body prior to decomposition. These data significantly refined PMI estimations.

Due to inconsistencies identified between the PMI estimations based on entomological studies versus other evidence, Turner and Wiltshire (1999) attempted to validate PMI estimations based on insect activities in buried remains. Three pigs weighing approximately 55 kg each were buried in shallow graves. Carcasses remained in a relatively fresh state of decomposition approximately 60 days after burial. The carcasses were exposed to scavenger activity after 90 days of burial. Scavenger activity is known to provide access to the carcass and enhanced insect colonisation, in turn resulting in accelerated decomposition. At day 135 the carcasses had black skin with little soft tissue decomposition. On day 150 most of the carcasses have been consumed by scavengers with small amounts of intact soft tissue present. The study illustrated the importance of soil characteristics together with entomological activities where the victim is buried. According to their findings, soil conditions and low seasonal temperatures had preserved the carcasses for longer than anticipated. Using only the blowfly larvae to estimate PMI would have produced incorrect results. Therefore it is suggested that the burial environment and exhumation history be investigated simultaneously. A review of human decomposition processes in soil was published by Dent *et al*. (2004). They proposed that the soil environment of in-soil human decomposition studies be taken into account. According to this study, decomposition is affected by factors such as the nature of the remains and site, absence or presence of groundwater, climate, time and the mobility of oxygen within the soil type.

Schultz *et al*. (2006) monitored 12 pig burials to test the viability of using ground-penetrating radar (GPR) in identifying soil disturbances, for example such as those observed in clandestine graves. The pigs weighed an average of 63.84 kg to represent an adult human size body. Six carcasses were buried at a depth of 50-60 cm, and the remaining six at a depth of 100-110 cm. Twelve to 13 months later three carcasses from each depth were excavated to assess the decomposition state - the remaining six carcasses were excavated 21 months later. Carcasses excavated after 12-13 months displayed extensive soft tissue preservation whereas carcasses excavated at 21 months showed variable stages of decomposition, including complete skeletonisation. According to the researchers the most influential factor was the type of soil the carcass was buried in.

This was confirmed by a study done by Turner *et al*. (2013) where 32 domestic pig extremities (1.9-4.2 kg wet weight) were buried in different soil types, namely loam, clay, and sand. After 3 months of burial, half the extremities of each group were excavated. In loam there was no skin or soft tissue with adipocere formation on some limbs. In clay, a small amount of soft tissue was still present with observable adipocere formation. In sand, the skin has completely decomposed with thick loose soft tissue on the bone. After 6 months the remaining extremities were excavated. In lime, the bones showed a brownish-yellow discolouration. In clay, adipocere formation was present on all extremities. Remains showed complete decomposed skin with jelly like soft tissue on the bones. Soil samples were analysed for their beta-glucosidase content (C-cycle), alkaline phosphatase activities (P cycle), urease activity (N cycle), CO_2 evolution and soil pH.

Their findings were as follows:

Nitrogen (N) – N content was highest in clay grave soil.

Soil pH – the pH of grave soil was lower than the control graves in loam soil.

Calcium carbonate (C_aC_O3) – the highest content was found in lime.

Urease – the least activity was found in sand.

CO_2 evolution – the least activity was in sand.

Beta-glucosidase – enzyme activity was highest in loam grave soil (Turner *et al*., 2013).

Soil structure and texture primarily influence decomposition of interred organic matter. This can be seen in the differences in decomposition rates in different types of soil, for instance, finer clayish soil shows slower decomposition rates and higher retention of organic matter when compared to coarse, sandy soils (van Veen and Kuikman, 1990). Techniques used for the analyses of decomposition of organic matter in soil are limited as decomposition may only be assessed through determining end-products such as CO_2 and NH_4. Bacteria and fungi are most abundant organisms in terrestrial ecosystems and therefore the primary decomposers of organic matter in soil. In literature, it is suggested that in cultivated ploughed soil, in which crop residue was incorporated into the soil, the soil food web is bacteria-dominated. With surface-placed crop residue the food web is fungi-dominated (van Veen and Kuikman, 1990).

From the literature it is clear that soil conditions such as temperature, moisture retention, pH and nutrient availability will control microbial numbers and hence their activities during the decomposition process. In-soil remains will affect the localised grave microenvironment during active decomposition which results in increased microbial loads and a shift in pH content (Wilson *et al*., 2007). Soil types should therefore be taken into account when interpreting buried decomposition patterns and estimating PMI during investigations.

2.10 Pig models as a proxy for human decomposition studies

Due to the complexity of making use of donated human remains, much research on decomposition has used pigs as a proxy for humans (Megyesi *et al*., 2005; Myburgh *et al*, 2013; Sutherland *et al*., 2013, Keough *et al*., 2015). It is necessary to conduct field based research using human cadaver proxies while continuing to use information from human cadaver decomposition studies. Although differences in the decomposition pattern of domestic pig (*Sus scrofa*) carcasses versus human cadavers can be expected, pig carcasses have been accepted as proxies for human bodies because they share many biological characteristics, such as comparable skin thickness, body size and the amount of body hair (Parsons, 2009; Taylor, 2011).

Also, putrefaction proceeds approximately at the same rate as in human remains of similar weight (Campobasso *et al*., 2001). However, care must be taken when applying data from non-human remains to estimate the PMI to human remains. The proportion of soft tissue to bone found on a domestic pig is higher than that of the average human due to the feeding habits of the pig. In addition, the morphology of the skeletons of pigs and humans differ considerably (Taylor, 2011).

In South Africa, pigs serve as acceptable proxies for decomposition studies due to ethical reasons, and also the justice system does not allow for human bodies to be used in research studies other than educational anatomical dissections performed at an approved education institution. Even if it was legal to make use of human cadavers in South Africa, difficulty would be expected in obtaining cadavers, identifying suitable areas to conduct these studies and the impact of negative public opinion. Pigs are easier to obtain and entomological studies have shown that they are the most appropriate animal model for decomposition studies (Campobasso *et al*., 2001; Schultz *et al*., 2006). It should, however, be kept in mind that decomposition is a complicated process, and differences in anatomy and intestinal bacteria can lead to variations in this process.

2.11 Taphonomy research conducted in South Africa

Most variables associated with the decomposition process need further investigation in a South African setting. According to Williams and Villet (2006), entomological assessments have been used in southern Africa as forensic evidence for decades; however, forensic research only began in southern Africa some 34 years ago. The first forensic entomological research in South Africa was conducted by André Prins at the South African Museum during 1980, who published details on the life cycle of six species of blowflies found in South Africa (Williams and Villet, 2006). Years later, in 1992, TC van der Linde from the University of the Free State in Bloemfontein established the Forensic Entomology Investigation Team.

This team, in collaboration with GS Anderson of Simon Fraser University of Canada, studied types of insect carrion, development rates of maggots and the succession of carrion in pig carcasses. During 1993, MH Villet established the Southern African Forensic Entomology Research laboratory at Rhodes University where student projects focused on ecological succession in dead cats (Williams and Villet, 2006).

Kelly *et al*. (2009) tested the influence of clothing, wrapping and a combination of both on carcass decomposition and insect activity during autumn and summer months in the Free State Province. Eight domestic pig carcasses were placed in the field within minutes of death. One carcass was clothed (t-shirt, short and male briefs) with no wrapping, one carcass had no wrapping or clothes, three carcasses were clothed and wrapped (cotton sheeting) and the remaining three were wrapped with no additional clothing. They found no delay in oviposition by insects when comparing exposed versus wrapped bodies, although advanced decomposition occurred considerably faster in the exposed carcasses. This has been ascribed to moisture retention caused by wrapping of the carcasses.

In 2013, Myburgh *et al*. published a paper on estimating the PMI by making use of ADD in a temperate region of South Africa. The purpose of the study was to evaluate the method presented by Megyesi *et al*. (2005) who proposed that TBS and ADD could be used to quantitatively estimate the PMI. Thirty pigs, weighing between 38 and 91 kgs (with an average weight of 71 kg) were placed in the open veldt. Decomposition was then visually assessed and recorded over winter and summer months, using the modified method developed by Megyesi *et al*. (2005) until complete skeletonisation.

Myburgh *et al*. (2013) proposed that decomposition rates become more variable during the advanced stages of decomposition, following a curvilinear approach when TBS values reached scores greater than 17. This may be ascribed to the decrease in the maggot mass size as the carcasses became desiccated. Also, they observed that seasonality influenced the decomposition process as decomposition occurred faster during the summer months than winter months, even with carcasses having similar ADD values. Very little rainfall occurs during the winter months in the central Highveld region of South Africa. This may result in desiccation of the remains which further delays the process of decomposition.

Following this, Sutherland *et al*. (2013) studied the effect of body size on the rate of decomposition in the central Highveld region of South Africa. Fifteen piglets (weighing between 3 and 35 kgs) were placed in the open veldt and their rates of decomposition were observed and scored, using the Megyesi *et al.* (2005) scoring method, until complete skeletonisation was reached. The rates of decomposition were then compared to that of 15 pigs weighing between 60-90 kgs. As can be expected, overall, small pigs decomposed much faster with greater TBS values relative to the same ADD in large pigs. Small pigs reached skeletonisation within a mean ADD of 875.38, compared to the larger pigs with a mean ADD of 2400.85.

Keough *et al*. (2015) investigated the skeletal changes of pig carcasses after post mortem exposure to fire as an indicator of the stage of decomposition. The sample comprised of 25 pigs (weighing between 50 and 100 kg) which were then exposed to fire during different stages of decomposition, after which the remains were processed and analysed. Each carcass was exposed to a controlled veldt fire for 30 minutes. Heat-related traits to determine the condition of the body prior to burning was identified and described. Fleshed carcasses showed distinct features such as heat lines and heat borders. As the decomposition process progressed, soft tissue degradation exposed parts of the carcasses. Delamination and heat-induced fractures were observed and associated with the advanced stages of decomposition.

A study on the pattern of decomposition of buried remains was initiated in 2014. As part of research collaboration between the University of Pretoria and the Council for Scientific and Industrial Research (CSIR), a 3 dimensional GPR survey was included in this study with the aim to detect and monitor clandestine graves using a Rock Noggin GRP system. Preliminary results indicate that burials of less than 30 days PMI are clearly discernible as hyperbolic reflections on the non-migrated radar sections.

After 60 days PMI the graves are still detectable but appear to be less sharp when compared to the 30 day data. After 90 days PMI the detectability of graves deteriorated to a great extent as data contain more clutter and contrast to a lower extent on the images. The deterioration of data on the images is mostly attributed to the heavy rainfall which occurred between early December 2015 and January 2015 (van Schoor *et al*., 2015).

From the above mentioned studies it is evident that the environment is a major determinant of decomposition of human remains as well as the rate at which it proceeds (Rodriguez and Bass, 1985; Mann *et al*., 1990; Campobasso *et al.*, 2001; Fiedler and Graw, 2003). In recent years the discipline of forensic anthropology placed stronger emphasis on quantitative methods for data collection and analyses.

Quantitative studies using decomposing remains in South Africa mostly focused on entomological evidence to determine PMI instead on stages of decomposition (Williams and Villet, 2006). As more knowledge is gained from decomposition studies in different settings, forensic anthropologists come closer to deriving a model that encompasses all parameters which may influence decomposition. In order to contribute to our knowledge on decomposition in different settings, a quantifiable approach was followed in this study to assess the pattern and rate of decomposition of buried remains in the Central Highveld region of South Africa.

CHAPTER 3

MATERIALS AND METHODS

3.1 Location of the study

The study site for this research project is situated at the Forensic Anthropology Body Farm (FABF) (Figure 3.1) on the Miertjie le Roux Experimental Farm belonging to the Faculty of Natural and Agricultural Sciences of the University of Pretoria. The farm is located at Kaalfontein 513 JR, in the Cullinan District, Gauteng Province (25˚47'20.2"S; 28˚32'34.3"E), approximately 45 km east of Pretoria, Gauteng Province. This farm consists of a total of 560 hectares, situated on the central Highveld plateau of South Africa. This is a temperate region of South Africa with warm summer days and temperatures rarely below 0˚C. Rainfall mainly occurs during the spring and summer months of September to April (with an average 650 millimetres (mm) of rainfall per year) and vegetation mostly consists of sour veldt grasslands.

Figure 3.1: Satellite image of the FABF enclosure with the map marker indicating the location of the Miertjie Le Roux Experimental Farm (Map of South Africa, from http://GoogleEarth.com).

According to a Geohydrolic Survey (Hattingh, 1987) done on the Experimental Farm, it is evident that quartzite is abundant in the area. Quartzite is known to have a low porosity therefore shallow water is present as the Elandsriver has fountains on the south-western corner of the farm forming swamps and wetlands. From the survey it was also noted that the farm has a 15º slope to the north-northeast. The FABF is situated fairly high up on the slope; thus shallow water was not a major factor when digging and excavating the burial pits.

3.2 Materials

Sus scrofa, or domestic pig carcasses, were used in this study as their pattern of decomposition is similar to that of humans (Campobasso *et al.*, 2001). Pig carcasses were donated from two local farmers for research purposes, and placed within 24 hours after death. An increase in the rate of insect colonisation has been shown at sites of trauma and or visible external wounds, which may result in an accelerated decomposition rate (Campobasso *et al.*, 2001). Therefore, only pigs that died of natural causes, had known dates of death and showed no signs of external wounds or trauma were included in the study. According to local farmers, death is usually caused by E. coli (*Escherichia coli*) and Salmonellosis (*Salmonella choleraesuis)* infection which are common causes of death of pigs on commercial farms.

Forty separate shallow burial pits were dug by means of a back-actor (additional pits were decided upon should some remains be damaged by scavenger activity necessitating reburial of carcasses). Each burial pit was measured after they were dug by a back-actor to ensure an average dimension of 2.25 m x 1.22 m with a depth of 0.75 m (Table 3.1) to simulate clandestine graves of buried victims. Manheim (1997) stated that clandestine graves seldom exceed a depth of more than 0.9 m; the average clandestine grave depth in the UK is 0.4 m, and in the USA the average depth is reported as 0.56 m (Gunn and Bird, 2011). No reference to average sizes of clandestine graves in South Africa could be found in literature. The dimensions of the burial pits were therefore chosen based on an average depth according to the studies mentioned earlier (neither shallower than 0.4 m nor deeper than 0.9 m). Also, based on body sizes of pigs in the study done by Myburgh (2010) (Table 3.2), a depth of 0.75 m was decided upon in order for the pigs to fit into the graves and to provide space to facilitate the excavation.

All pigs in this study had a weight range of between 45 and 80 kg, as it has been shown that smaller pigs decompose at a much faster rate than large pigs (Sutherland *et al.*, 2013). This weight range was also chosen as it resembles the average weight of an adult human. The sample comprised of 30 carcasses. Apart from the 25 carcasses reported on, five additional carcasses were buried as a backup to allow for unforeseen factors such as, large scavenger activity or damage during the excavation process. The additional carcasses per time category were left unexcavated and therefore undisturbed.

Table 3.1: Burial pit dimensions

Pit Number	Depth (m)	Length (m)	Width (m)
1	0.80	1.90	1.20
3	0.75	2.40	2.00
4	0.70	2.30	1.10
6	0.85	2.20	1.10
7	0.80	2.30	1.20
15	0.70	2.50	1.20
16	0.60	2.10	1.30
17	0.75	2.30	1.20
19	0.80	2.40	1.20
20	0.70	2.30	1.30
21	0.70	2.30	1.50
22	0.80	2.50	1.30
24	0.80	2.10	1.20
25	0.70	2.20	1.10
26	0.80	2.40	1.10
28	0.75	2.20	1.10
29	0.70	2.30	1.20
30	0.80	2.30	1.20
31	0.80	2.10	1.20
32	0.70	2.30	1.00
33	0.80	2.30	1.10
34	0.80	2.30	1.20
35	0.80	2.20	1.00
36	0.70	2.10	1.10
40	0.60	2.00	1.40
Average	0.75	2.25	1.22

Table 3.2: Average weight, height, length, width and belly width of the pigs used in the study done by Myburgh (2010)

Measurement	Weight (kg)	Height (cm)	Length (cm)	Width (cm)	Belly height (cm)
Minimum	38	38	102	21	21
Maximum	91	82	152	43	48
Average	71.3	58.9	130.3	28.1	34.1

Eight pigs had to be excluded and replaced during the study. Pigs 2, 5 and 18 were excluded due to scavenger activity within days after interment. Scavengers seen in the area during the study include domestic dogs, black-backed jackals and suricates. Pigs 8-12 had to be replaced as the region had intense rainfall days prior to excavation resulting in an inaccessible site due to muddy conditions (Figure 3.2), and burial pits 37-39 were waterlogged during burial of remains.

Figure 3.2: Heavy rainfall experienced during December 2014 and January 2015

The remains were placed in direct contact with soil which is similar to the type of burial characteristic of criminal activity. The pigs were not covered with additional objects (i.e., plastic) or clothed. Pigs were numbered and placed in sequence of arrival at the farm, buried approximately three meters apart to ensure that possible insect colonisation from one pig did not influence another. No pigs were moved outside the enclosure once placed.

The researcher and volunteers involved with the study were required to abide by safety regulations stipulated by the Occupational Health and Safety Act No. 85 of 1993 (Republic of South Africa) in wearing long pants, gumboots/closed shoes, safety glasses, masks and gloves when excavating and handling the remains. All pigs were observed *in situ*. Once data collection was complete, the remains were re-covered with soil, allowing future studies on the decomposition process, taking into account that these remains have been disturbed, and for conduction of workshops on bio-archaeological methods in identifying and excavating clandestine graves.

3.3 Methods

This cross-sectional study was conducted over a period of ten months from September 2014 to June 2015. All pigs were buried on the day of death (within 24 hours after death) and five pigs were excavated for each predetermined time category (one week, two weeks, one month, three months and six months) from the date of burial. Pigs were not stored in a cooler/freezer awaiting burial as cooling alters the decomposition process (Micozzi, 1997).

Each pig was allocated a number (1-25), and the date of placement (same as date of death), height, weight, length, sex and belly height were measured and recorded (Figure 3.3 and Table 3.3) prior to placement in graves. The TBS of each pig was observed and recorded during primary placement by the researcher in order to describe changes observed in decomposition patterns in all areas of the body when excavating the remains.

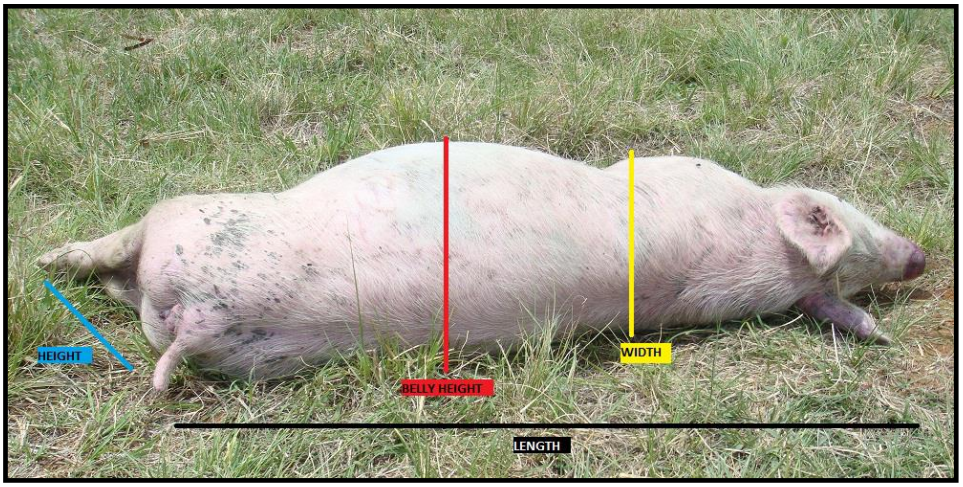


Figure 3.3: Measurements taken for each pig (Height = blue; Length = black; Width = yellow; Belly Height = red).

This will be discussed in more detail in the results. Actual measurements taken for each pig are described in Table 3.3. Although each pig was measured as described in Table 3.3, the data was not used further in this study. The measurement data is however available in Annexure 1 for possible analyses in future.

Table 3.3: Measurement of pigs (Myburgh, 2010)

Measurement	**Description**
Height (H)	Distance from the most dorsal portion of the back to the furthest point on the hind hoof
Length (L)	Maximum distance from the snout to the roof of the tail
Width (W)	Maximum distance from the ground to the exposed lateral side in the thoracic region when the pig is lying on one side
Belly Height (BH)	Maximum distance from the ground to the exposed lateral side of the abdominal region measured perpendicular to the width

Decomposing carcasses were excavated by means of standard archaeological techniques (shovels, hand shovels and trowels) (Joukowsky, 1980) within the confines of the burial pit during each predetermined time category. The application of archaeological techniques during the recovery of buried remains is valuable especially when care needs to be taken not to damage the remains during excavation. Excess soil was removed with fine paint brushes following the outline of the burial pit. None of the buried carcasses was removed from the burial pits during or after excavation.

The degree of decomposition for each pig was observed, photographed and recorded using the scoring guidelines presented in Megyesi *et al.* (2005). The three separate categories for scoring decomposition for each anatomical region (head and neck, trunk and limbs) with point values are indicated in Tables 2.2, 2.3 and 2.4 (Megyesi *et al.*, 2005). The qualitative descriptions of the stages of decomposition were converted into quantitative scores from these three (Figure 3.4) regions in the body. This was done as not all stages of decomposition apply equally to all parts of the body as stated previously.

The allotted point values of each region were then added to determine the TBS which represents the overall stage of decomposition of each pig from a minimum of three to a maximum of 35 points. Data for the PMI value, head and neck score, trunk score, limb score, total body score value, average daily temperature and accumulated degree-days value were scored on log sheets (Annexure 1).

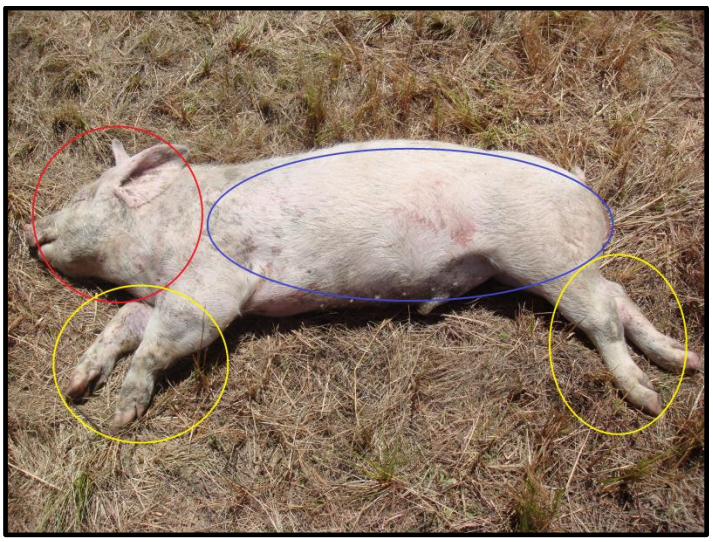


Figure 3.4: Scoring of the three anatomical regions to calculate TBS (Red = Head and neck, Blue = Trunk, Yellow = Limbs)

An additional observer recorded the stages of decomposition for the head and neck, trunk, limbs and the resulting TBS of ten pigs from randomly chosen time categories excavated without influence from the primary investigator (Annexure 2). This was performed as a means to determine the repeatability of the method used for scoring the decomposition process.

It is known that cadaver temperatures increase during the decomposition process (Rodriguez and Bass, 1985; Pastula, 2012; Niederegger *et al*., 2015), therefore a Bodycote Metech TFA temperature probe was used to establish the difference in temperature between the interred remains and the ambient temperature before excavation. An ambient temperature reading was taken above each grave before excavation. Thereafter, a shaft was made into the burial pits in order to insert the temperature probe up to the point of reaching the remains. The temperature probe was attached to a wooden stick with isolation tape markers every 10 cm to indicate the approximate depth of the carcass during the temperature reading. Temperature difference between interred remains and the ambient temperature was noted for discussion should anything out of the ordinary be observed on the remains once exposed.

Accumulated degree-days was calculated by adding together the daily average temperature for the duration of the data collection phase. This information was obtained from the closest South African National Weather Service Station (Annexure 3), approximately 23 km south-east of the burial site, based in Bronkhorstspruit. By using ADD, the effect of temperatures on the rate of decomposition is standardised and thereby allows various, similar studies to be compared.

Total Body Score methods as set out by Megyesi *et al*. (2005) were used to assess the rate and pattern of decomposition. The results in this study for buried remains were then compared to the longitudinal study done by Myburgh (2010) on surface remains with respect to TBS relative to ADD. In forensic cases, visual assessment of decomposition provides a TBS which can be used to estimate ADD. Accumulated degree-days in turn is used to estimate the day of death.

3.4 Statistical Analyses

The statistical analyses required for the results in this study are similar to the analyses used in previous studies (Myburgh *et al*., 2013; Sutherland *et al*., 2013). In order to assess the pattern of decomposition for PMI and TBS, scatter plots indicating exponential trends for each pig was compiled and interpreted by the principal investigator. A non-linear regression approach was used to model the descriptive pattern and rate of decomposition. As decomposition and the variables associated with decomposition are very complex and unpredictable, log transformation data from this study and the study of Myburgh (2010) were compared and analysed. Myburgh *et al*. (2013) found that the data followed a curvilinear pattern; therefore log transformation was used in order to allow the data and decomposition patterns to be compared.

Log transformation is useful in making highly skewed distributions less skewed. Instead of a count of events within a set time frame, the log transformation measures the time required to the event. As time is exponentially distributed, the logarithmic transformation is appropriate to convert it into a normally distributed measurement. Log transformed values have a curvilinear relationship with the original values and therefore stretch low values and compress high values. This aids in better interpretability of patterns within the data.

As PMI values were expected to result in skewed distributions similar to the findings of Myburgh (2010), PMI were log transformed so as to be linearly related with TBS to determine the difference in the rate of decomposition between surface and buried remains.

3.5 Ethical consideration

As the study involved biomass reduction of remains in a natural environment, approval for this study was granted from the MSc Committee of the School of Medicine, Faculty of Health Sciences, and ethical clearance was received from the Environmental Biohazard Committee of the Faculty of Natural and Agricultural Sciences, University of Pretoria and the Animal Ethics Committee of the Faculty of Veterinary Sciences, University of Pretoria (Annexure 4).

CHAPTER 4

RESULTS

4.1 Pig sample used in this study

The sample comprised of 25 pigs (20 males, 5 females) in total. The dates of placement on the FABF, weights and dimensions for each pig are indicated in Table 4.1. Carcasses were placed within 24 hours after death. In contrast to excavation days, timing of burials were not standardised as no pigs were culled for this study. The piggeries that supplied the carcasses are very large commercial farms, therefore most of the carcasses buried for a specific time frame were obtained on the same day, with the exception of the 92 day PMI pigs. Pigs 28, 29 and 30 died on 19 March 2015, whereas pigs 31 and 32 died 4 days later on 23 March 2015 for the 92 PMI timeframe. As a result, pigs 28–30 were excavated on 18 June 2015 and pigs 31 and 32 were excavated on 22 June 2015.

The 183 day PMI pigs were buried first, followed by 92 day PMI pigs, 33 day PMI pigs, 15 day PMI pigs and 7 day PMI pigs. Pigs were not placed during December 2014 and January 2015 as piggeries did not have deaths within the chosen weight range, and the research site was not accessible during heavy rainfall as experienced during the Summer (December to February). Body weights of the pigs ranged from a minimum of 45 kg to a maximum of 80 kg with an average weight of 64 kg. Pigs were on average 118.12 cm in length (excluding the tail length), 46.08 cm in height and 22.4 cm in width (Table 4.2).

Pigs 2, 5 and 18 were excluded due to possible scavenger activity prior to excavation. Scavenger activities mostly took place within the first half of the study period. Pigs 8, 9 10, 11 and 12 were also excluded as the graves were waterlogged due to exceptionally heavy rainfall the week prior to excavation and the moist conditions may have influenced comparative decomposition data.

Pigs 13, 14, 23 and 27 were not excavated as they served as backup pigs for possible scavenger activities. Pigs 37, 38 and 39 were buried in moist conditions which differed marginally from all other burials and were, therefore, also excluded from this study.

Figure 4.1 gives an overall layout of the carcasses placed on the FABF for the purposes of this study. Each burial pit is indicated on the layout with the individual pig number, PMI for that specific pig and grave dimensions before internment. The research area for this study encompasses 1001 m^2. The first pits were dug 45.5 m from the entrance gate in 4 x 10 rows, with the last row placed 5 m from the entrance to the site. Orientation and GPS co-ordinates are also indicated on the layout for reference.

Table 4.1: Summary of the date of death and excavation, sex, weight and metric dimensions of the sample

Pig	Date of placement	Date of excavation	Sex	Weight (kg)	Length (cm)	Height (cm)	Width (cm)	Belly height (cm)
1	30/09/2014	31/03/2015	Male	80	114	57	22	38
3	30/09/2014	31/03/2015	Male	45	119	38	17	29
4	30/09/2014	31/03/2015	Male	45	108	39	20	28
6	30/09/2014	31/03/2015	Male	70	127	45	22	24
7	30/09/2014	31/03/2015	Male	73	130	52	19	26
15	09/10/2014	10/11/2014	Male	70	130	58	23	35
16	09/10/2014	10/11/2014	Male	80	132	53	21	24
17	09/10/2014	10/11/2014	Male	80	131	47	24	27
19	09/10/2014	10/11/2014	Male	65	125	46	19	24
20	09/10/2014	10/11/2014	Male	50	110	46	20	28
21	24/11/2014	08/12/2014	Male	80	135	57	25	37
22	24/11/2014	08/12/2014	Male	66	98	38	24	31
24	24/11/2014	08/12/2014	Male	60	108	35	18	29
25	24/11/2014	08/12/2014	Male	60	123	45	26	35
26	24/11/2014	08/12/2014	Male	58	114	41	23	35
28	19/03/2015	18/06/2015	Male	45	108	47	25	37
29	19/03/2015	18/06/2015	Male	60	137	49	26	32
30	19/03/2015	18/06/2015	Male	55	115	47	21	23
31	23/03/2015	22/06/2015	Female	70	118	47	24	27
32	23/03/2015	22/06/2015	Female	48	102	41	19	18
33	13/04/2015	20/04/2015	Male	80	125	47	24	28
34	13/04/2015	20/04/2015	Female	75	111	50	28	33
35	13/04/2015	20/04/2015	Female	76	127	42	25	29
36	13/04/2015	20/04/2015	Male	45	98	41	22	34
40	13/04/2015	20/04/2015	Female	64	108	44	23	32

Table 4.2: Minimum, maximum and averages of the pig weights and measurements

Measurement	Weight (kg)	Length (cm)	Height (cm)	Width (cm)	Belly height (cm)
Minimum	45	98	35	17	18
Maximum	80	137	58	28	38
Average	64	118.12	46.08	22.4	29.72

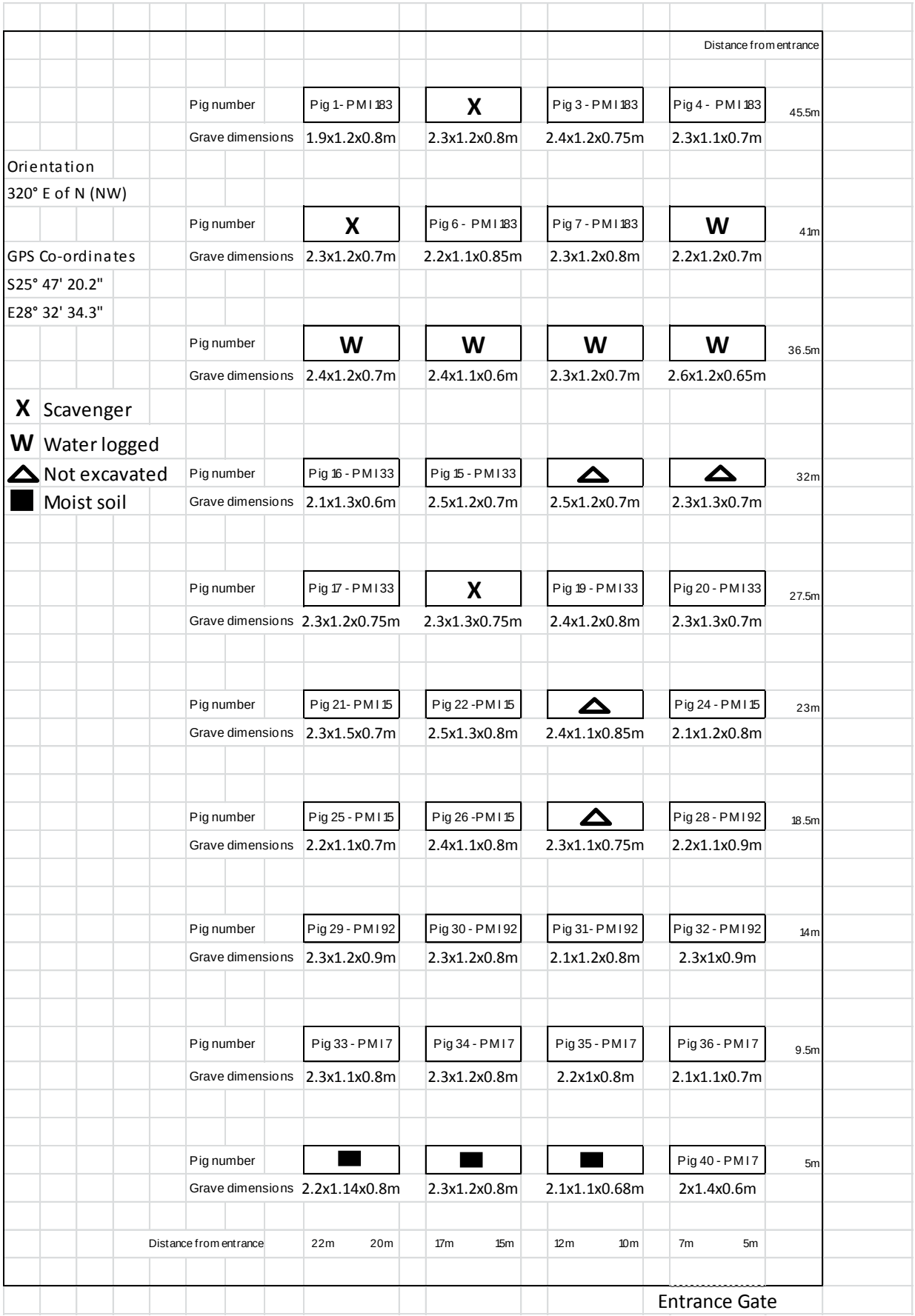


Figure 4.1: Carcass layout plan: Forensic Anthropology Body Farm

4.2 Total body scores across periods of decomposition

The observed TBS scores ranged from 8 (early decomposition) after 7 days of burial to a maximum of 25 (advanced decomposition) excavated after 183 days of burial. Early decomposition (TBS scores above 3) is marked by skin slippage, bloating and discolouration especially on the abdomen. The final stages of early decay (TBS scores averaging 16) are usually characterised by a dark (black) colour (Galloway *et al*., 1989). Advanced decomposition (TBS scores of 19+) begins with the sagging of the thoracic and abdominal cavities as bodily fluids purge from the natural openings. The purging of fluids leads to dehydration of soft tissue causing the skin to turn leathery with some bone exposure (Megyesi *et al*., 2005). No cases reached advanced decay up to the point of skeletonisation (TBS scores of 27+) within the 6 month (183 days) interval of this study. The most advanced decomposition observed within this time frame (TBS of 25) for buried remains was limited to moist decomposition with bone exposure less than one half of the area being scored.

As was expected, surface remains reached much higher TBS values compared to buried remains within similar periods (Table 4.3). Initially, all pigs followed a sigmoidal curve during decomposition. During the early decomposition stages (a PMI of 6 to 7 days), decomposition followed a linear pattern for both groups. Buried remains reached an average TBS of 9.6 after 7 days of burial (Table 4.3) compared to surface remains that decayed much faster reaching an average TBS of 13.8. Total Body Scores and decomposition rates began to vary drastically between buried and surface remains as surface remains decomposed rapidly between 8 and 15 days. After a PMI of ±14 days, buried remains had an average TBS of 13.4 (±3.8) compared to surface remains with a score of 22.6 (±8.8). This indicates a notable difference during early phases of decomposition as the process for buried remains slowed down gradually, mostly resulting from the fact that buried remains displayed moist decay compared to surface remains already showing signs of desiccated tissue and mummification. The rapid decay of surface remains within 14 days are most probably due to exposure to factors such as temperature (Prangnell and McGowan, 2009) and insect infestation (Simmons *et al*., 2010).

After rapid decay during the early decomposition phase, decay rates slowed dramatically as remains reached what seemed to be a plateau phase where little change was noted after 32 to 33 days PMI. Buried remains reached an average TBS of 17.4 and surface remains averaged 25.2. During advanced decomposition, the TBS did not increase much with TBS values of 20.4 and 22.2 observed after 92 days and 183 days, respectively. A similar plateau phase was observed with surface remains which reached an average TBS of 28.2 after 92 to 93 days and 29.8 after 162 to 175 days. A similar plateau phase indicating little change (slow decomposition) during advanced decomposition was also observed and described in the study by Sutherland *et al*. (2013). This plateau phase therefore seems to present itself regardless of size and whether remains were decomposing on the surface or buried in soil.

Table 4.3: Summary of PMI and TBS scores of buried and surface remains (*estimates)

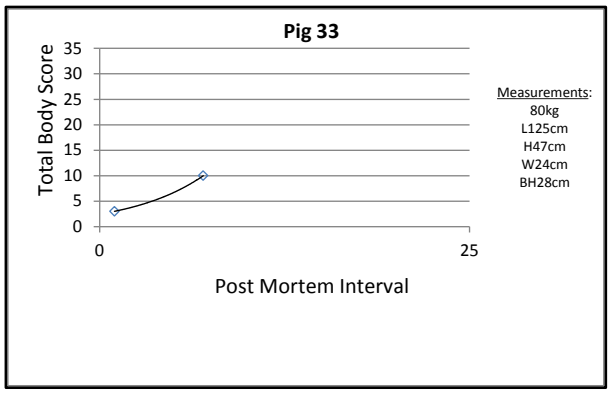
Buried Remains					Surface Remains				
Pig number	Sex	Kgs	PMI / TBS	Avg TBS	Pig number	Sex	Kgs	PMI / TBS	Avg TBS
33	M	80	7/10		7	M	38	7/9	
34	F	75	7/11		8	M	68	7/8	
35	F	76	7/8	9.6	9	M	80	7/21	13.8
36	M	45	7/9		12	M	85	7/17	
40	F	64	7/10		28	M	91	6/14	
21	M	80	14/13		20	M	65*	15/23	
22	M	66	14/14		23	F	75*	15/21	
24	M	60	14/13	13.4	24	F	75*	15/23	22.6
25	M	60	14/13		25	M	85*	15/21	
26	M	58	14/14		26	M	68	15/25	
15	M	70	33/16		10	M	80*	32/25	
16	M	80	33/20		11	F	59	33/23	
17	M	80	33/16	17.4	14	M	74	32/21	25.2
19	M	65	33/16		21	M	65*	32/26	
20	M	50	33/20		22	M	70*	32/31	
28	M	45	92/20		13	F	80*	92/24	
29	M	60	92/22		15	M	80*	93/29	
30	M	55	92/21	20.4	16	M	65*	93/31	28.2
31	F	70	92/20		17	M	75*	92/29	
32	F	48	92/19		18	F	65*	92/28	
1	M	80	183/21		1	M	82	175/31	
3	M	45	183/21		2	F	75	169/32	
4	M	45	183/23	22.2	4	F	55	168/30	29.8
6	M	70	183/25		5	F	63	163/25	
7	M	73	183/21		6	F	83	162/31	

4.2.1 Results after 7 days of internment

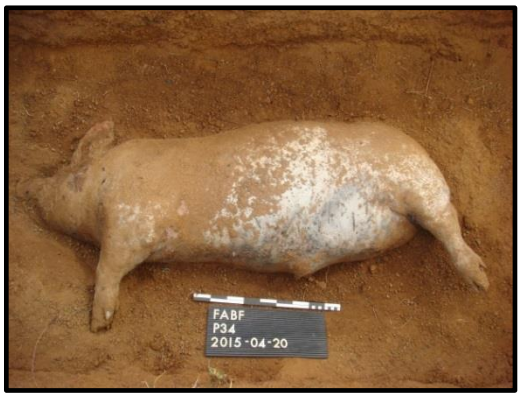
After a PMI of 7 days (133.1 ADD) pigs exhibited variations in the decomposition process and the TBS scores ranged between 8 and 11, averaging at 9.6. The ADD for surface remains within this timeframe was very similar (133.95 ADD).

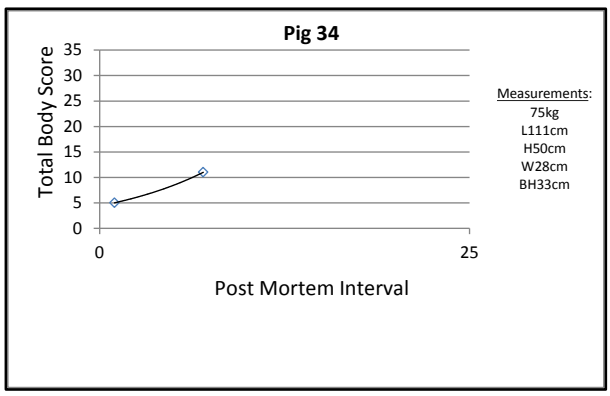
Pigs 33, 34, 35, 36 and 40 (Figure 4.2) had a mild odour that is associated with the early stages of decomposition (Pinheiro, 2006; Schoenly *et al*., 2006; Parsons, 2009).

Pig 33

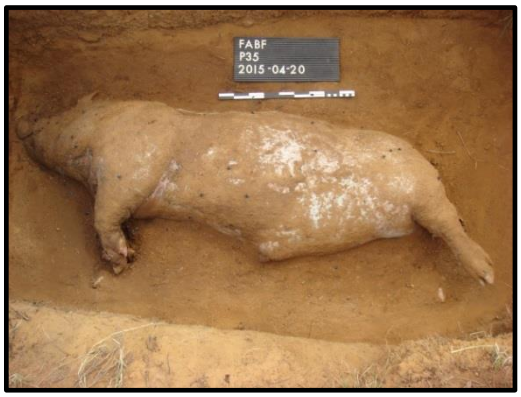


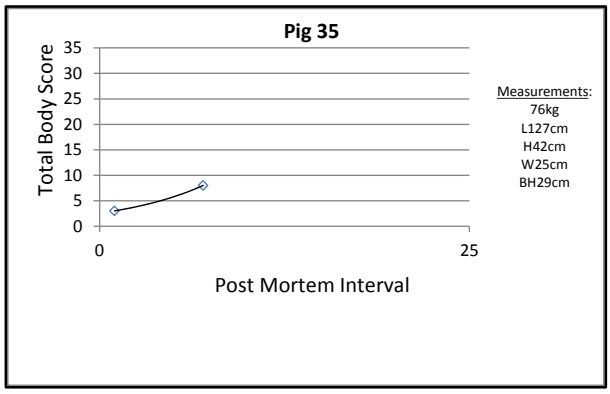
Pig 34



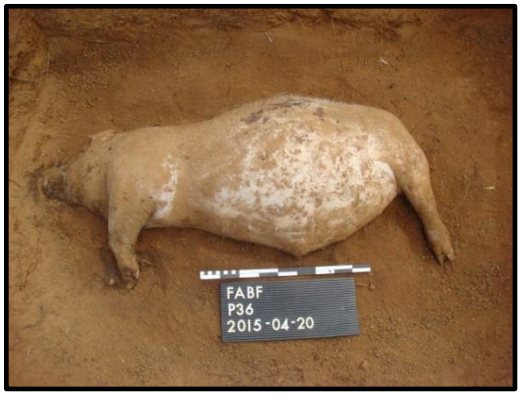


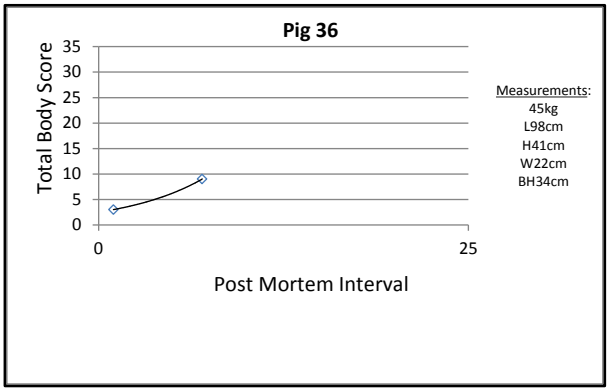
Pig 35



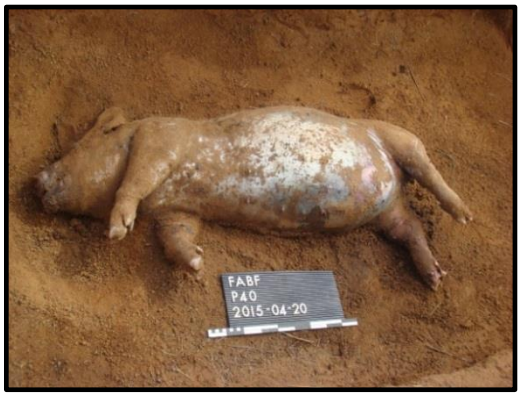


Pig 36





Pig 40



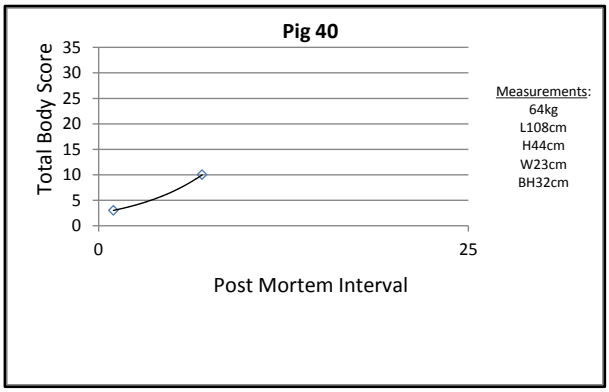


Figure 4.2: Results after 7 days of internment – the x-axis indicating the PMI in days

Furthermore, pigs 33, 34 and 40 showed a very dark purple and grey discolouration on the lower abdomen and trunk and oily skin (Figure 4.3), whereas pigs 35 and 36 had a fairly fresh pink skin on the abdomen with little to no discolouration (Figure 4.4).

Figure 4.3: Pig 33 exhibiting bloating of the abdomen with dark discolouration of the abdomen after 7 days of internment

The fresh appearance is likely due to the oxygen available to the carcass once buried. Compactness of soil would most probably inhibit gaseous diffusion and moisture availability, resulting in limited microbial activity which prolongs decomposition (Vass *et al*., 1992; Carter *et al*., 2007; Carter *et al*., 2010). This could explain the longer preservation of the remains for pigs 35 and 36.

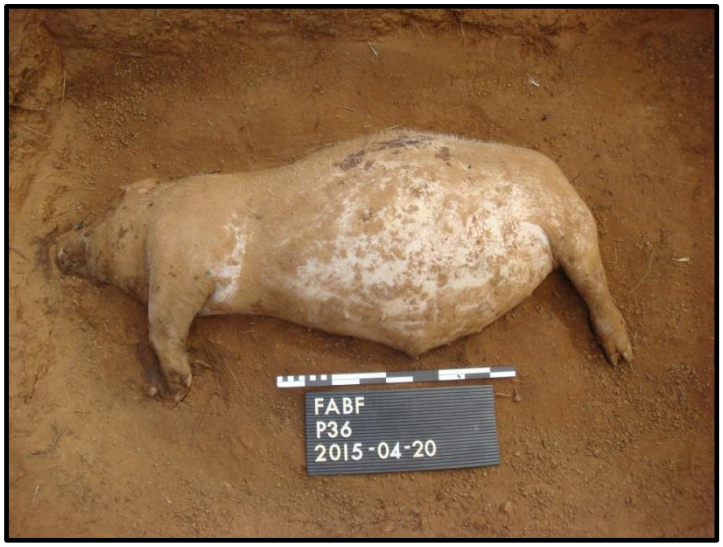


Figure 4.4: Pig 36 exhibiting bloating of the abdomen with relatively fresh skin after 7 days of internment

All carcasses in this time frame showed bloating of the trunk, intact hooves, purging of decomposition fluids from the mouth and anus, protrusion of the eyes, skin slippage and blisters on the abdomen and thorax (Figure 4.5).

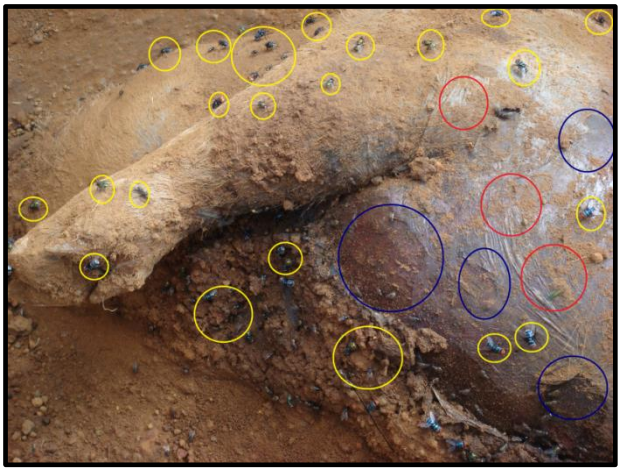


Figure 4.5: Pig 33 showing dark discolouration, bloating, skin slippage (blue), blisters (red) and blowflies (yellow) on the thorax after 7 days of internment

The epidermis became fragile and tore easily resulting in large skinless areas when exhumed. According to Galloway *et al*. (1989), remains which are in direct contact with soil show very moist decomposition patterns, therefore skin slippage is a common feature in buried remains as was also observed here. No insect activity was observed in any of the carcasses, although masses of blowflies (Diptera: *Calliphoridae*) colonised the carcasses within minutes after exposure during excavation (Figure 4.5).

In-soil temperatures measured with the temperature probe ranged between 18.9ºC and 20ºC (19.5ºC average) with the ambient temperature being 20.4 ºC. The in-soil and ambient temperatures therefore showed no distinct fluctuation as no insect activity was noticed on any of the remains within this timeframe.

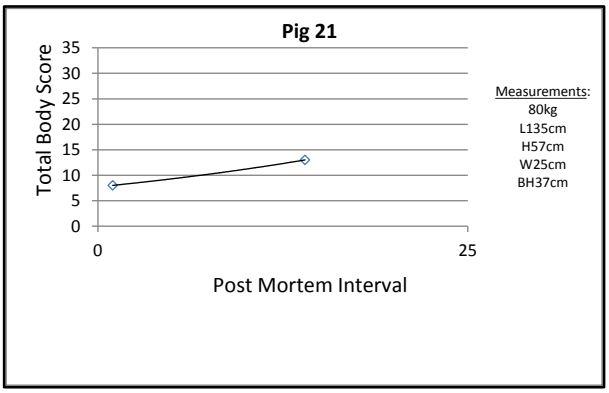
4.2.2 Results after 14 days of internment

After 14 days post mortem (313.1 ADD), pigs displayed some common features associated with the early stages of decomposition with TBS scores ranging between 13 and 14, averaging at 13.4. The ADD for surface remains within this timeframe was very similar (344.31 ADD).

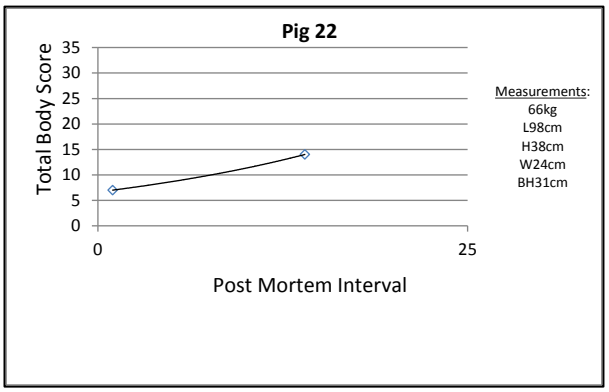
Pigs 21, 22, 24, 25 and 26 (Figure 4.6) showed obvious sagging/deflation of the abdomen, a dark pink discolouration especially on the head, decomposition fluids purging out of the natural openings of the carcass and skin slippage on the extremities (Figure 4.7).

Only pig 25 exhibited protruded intestines from the abdomen (Figure 4.7). A considerable putrid smell was noticeable from all carcasses in this time frame. In addition, all five graves caved in above the trunk area as soon as excavation started on the burial pits. The unstable surfaces for these graves were not evident before excavation started. This secondary depression of the burial pits is caused by the collapse of the abdomen during the decomposition process.

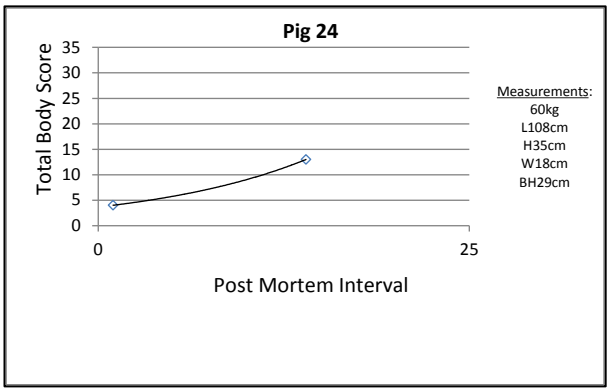
Pig 21



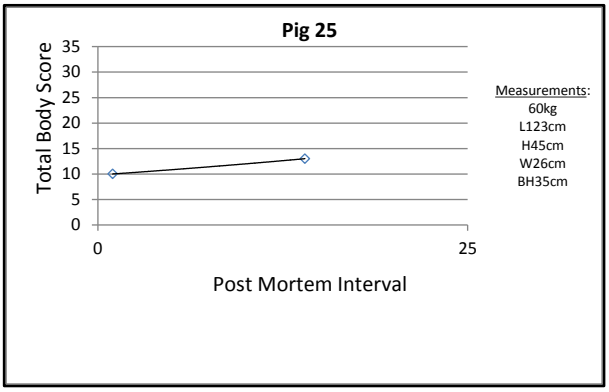
Pig 22



Pig 24



Pig 25



Pig 26

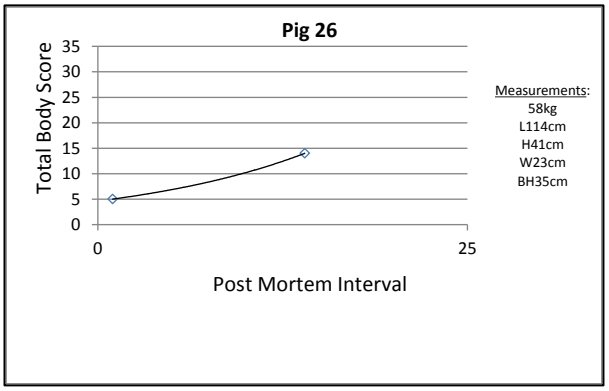
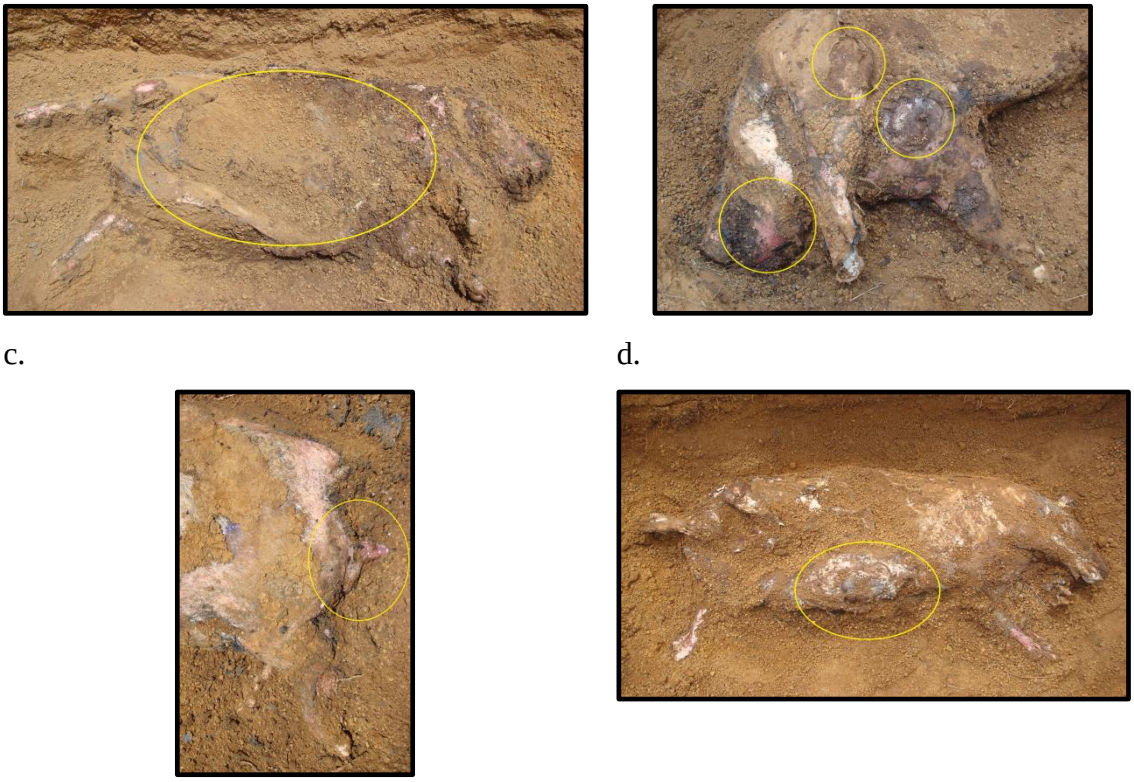


Figure 4.6: Results after 14 days of internment – the x-axis indicating the PMI in days

Temperatures in soil ranged from 23ºC to 24.3ºC (23.6 ºC average) with the ambient temperature being 23.7°C. Pig 21 had a higher in-soil temperature reading of 27.7ºC. While excavating pig 21, maggots were noticed in the abdominal area. The increased temperature reading can therefore probably be ascribed to the insect activity within the remains, which is known to generate heat, also known as maggot mass heating (Rodriguez and Bass, 1985; Haglund and Sorg, 1997; Pastula, 2012).

a. b.



c. d.

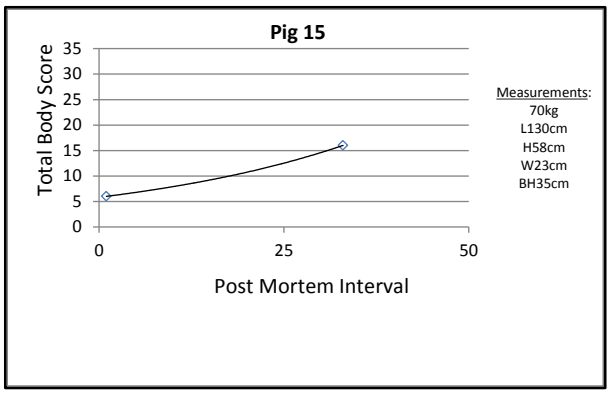
Figure 4.7: Sagging of the abdomen (a), discolouration on the head and skin slipping (b), purging of decompositional fluid (c) and protruded intestines (d) after 14 days of internment (yellow encircled areas)

4.2.3 Results after 33 days of internment

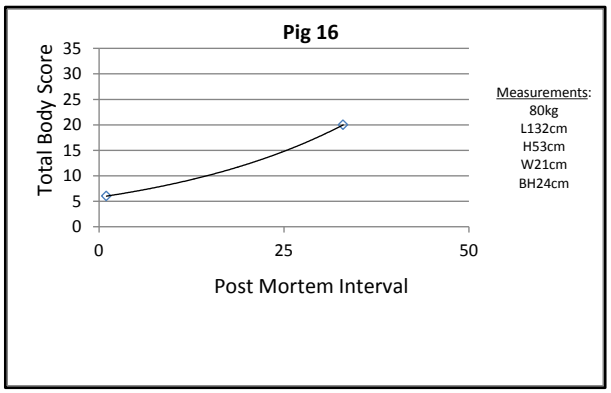
After a PMI of 33 days (623.4 ADD), pigs 15, 16, 17 and 20 (Figure 4.8) still displayed features described during early decomposition. The TBS scores ranged between 16 and 20, averaging at 17.4. The ADD for surface remains within this timeframe was very similar (702.82 ADD).Observations included bloating, skin slippage, detachable hooves, a strong putrid odour and purging of decomposition fluid from natural openings (snout, anus) and a greasy whitish texture on the abdomen with a black soil-skin interface.

In this case, the greasy texture is probably an indication of the beginning of adipocere formation. Buried remains are often associated with adipocere, also known as grave wax. Adipocere develops from body fat tissue in moist conditions. Its presence imply deposition of the remains within an environment where soft tissue could not be consumed quick enough with restricted access to oxygen, for example, burial in soil (Pokines and Symes, 2014).

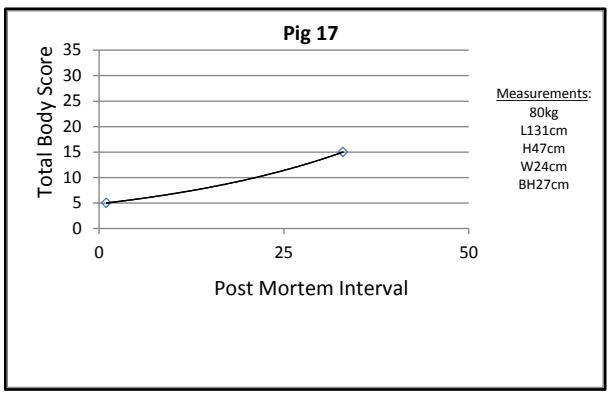
Pig 15



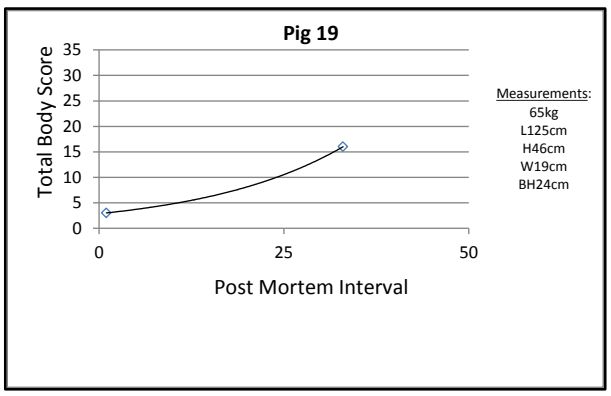
Pig 16



Pig 17



Pig 19



Pig 20

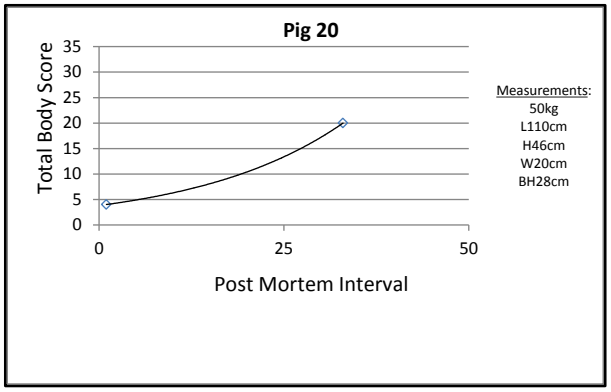


Figure 4.8: Results after 33 days of internment – the x-axis indicating the PMI in days

Pig 19, also buried for 33 days (623.4 ADD), had a collapsed abdomen and rib cage. This might be ascribed to the growing instability of the carcass, as well as pressure caused by the soil on top of the remains. However, the putrid odour associated with the other pigs in the same time frame was not similarly distinct for pig 19. It can also be argued that putrefaction of intestine took place within hours after death, compromising the stability of remains and altering stages of decomposition and preservation expected within this time frame.

In contrast to our findings, Niederegger *et al.* (2015) reported no bloating, but a flabby consistency of the remains after 20 days of burial followed by a collapsed carcass after 27 days. After 34 days, skin ablation on the tendons and ligaments of the ankle joint were visible which was not observed within this study. Also, no mention is made of insect activity on remains in this time frame, whereas maggots protruded from the hind quarter of pig 20 during this study as shown in Figures 4.9.

Figure 4.9a: Pig 20 showing maggot activity after 33 days (red encircled area)

Figure 4.9b: Close up photo of the maggot activity indicated in Figure 4.9a

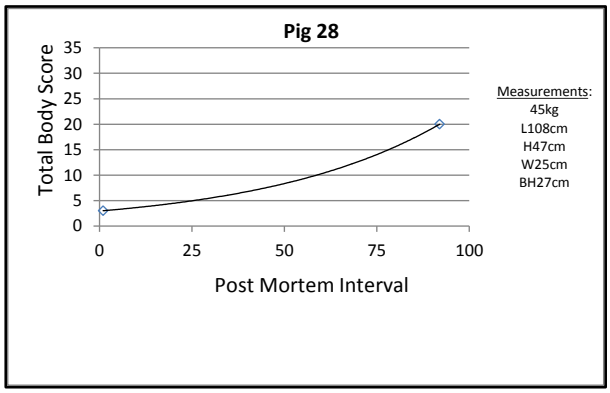
The ambient temperature reading was 24.7°C, and temperatures in soil for pigs 15 to 19 ranged from 23.1ºC to 23.9ºC. Pig 20, the carcass with insect activity, had an in-soil temperature of 25.9ºC. While excavating pig 20, maggots were first noticed at a depth of 40 cm. Figure 4.9a shows the area of maggot activity encircled in black, with a close up of the area in Figure 4.9b.

4.2.4 Results after 92 days of internment

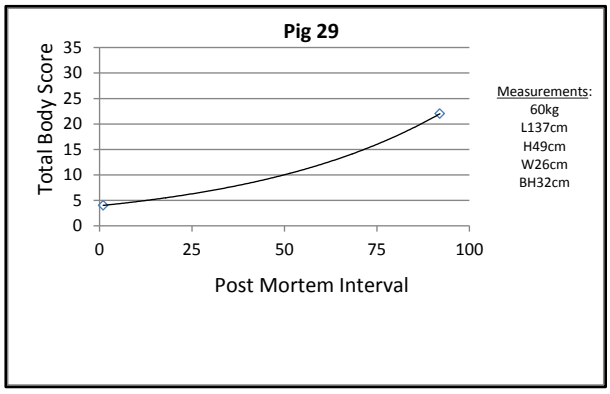
Initially, pigs 8 to 12 were buried for 92 days but had to be replaced due to exceptionally heavy rainfall in the research site area during December 2014 (150.2 mm) and January 2015 (40.8 mm). When excavation started the water table seeped through constantly to such an extent that collection of data would not have been possible. For this reason, pigs 28 to 32 were buried as replacements on 19 January 2015 for a 92 day interval.

After 92 days post mortem (1388.92-1437.1 ADD), pigs 28, 29, 30, 31 and 32 (Figure 4.10) showed black skin-soil interface on the majority of the remains. TBS ranged between 19 to 21, averaging 20.4. The ADD for surface remains within this timeframe was much higher (2096.82 ADD).Only pigs 28 and 29 displayed pink and black patches on the extremities and head (Figure 4.11). All remains within this time frame exhibited a mild ammonium smell, partial skeletonisation of the heads, adipocere formation, collapse/flattening of the trunks, disarticulated hooves and very leathery skin (especially on the vertebrae).

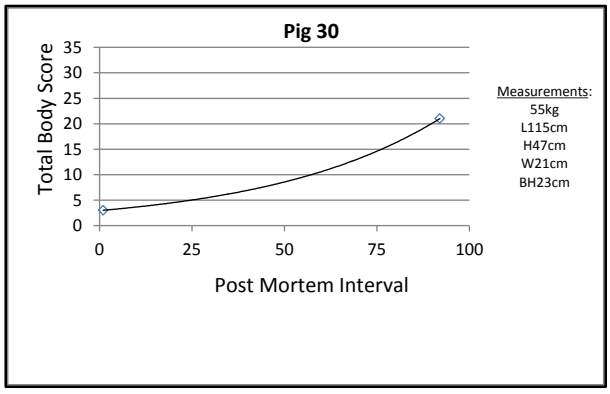
Pig 28



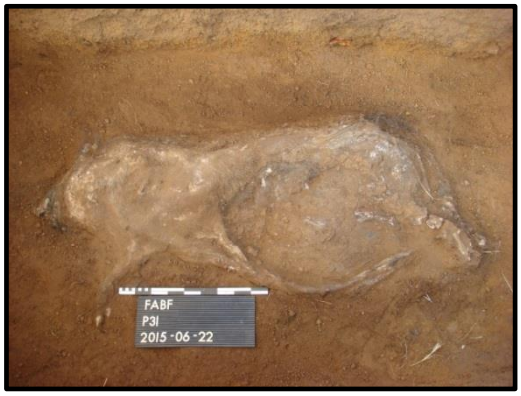
Pig 29

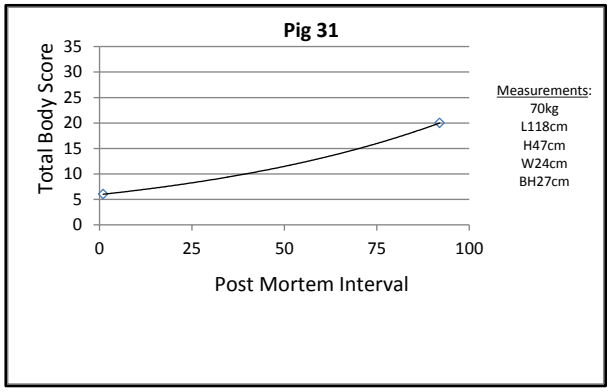


Pig 30



Pig 31





Pig 32

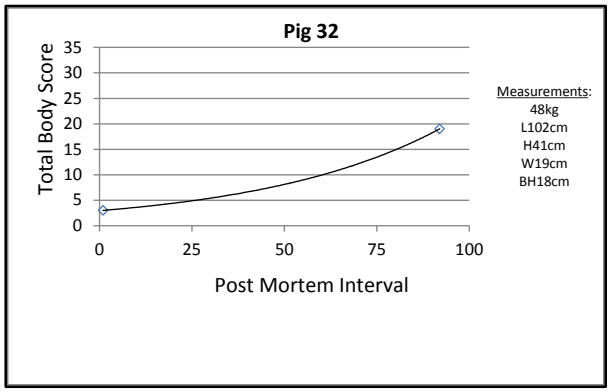


Figure 4.10: Results after 92 days of internment – the x-axis indicating the PMI in days

a.) b.)

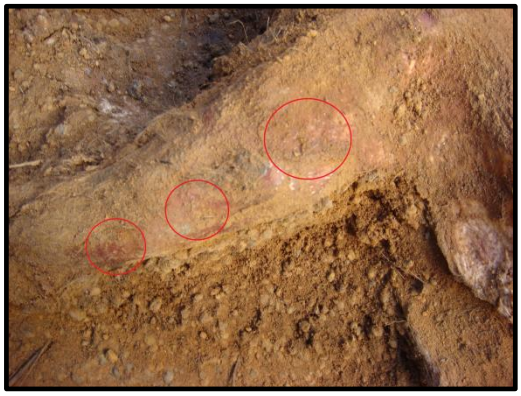


Figure 4.11: Pink and black discolouration on the extremities (a) and head (b) (encircled in red)

The lungs and large intestines of pigs 28 and 32 appeared to be preserved instead of liquefied. This phenomenon was noticed underneath the ribcage of pig 28 and the hind quarter of pig 32 (Figure 4.12). Also, the ribcage of pig 32 had a much darker (purple/black) discolouration than the rest of the pigs within this time frame. Temperatures in soil ranged between 9.5°C and 12.7°C with the ambient temperature being 12°C. Only pig 30 displayed an increase of temperature (+0.7°C) above the ambient temperature where limited insect activities were observed (a few maggots and ants protruding from the head and hind quarter).

(a) (b)

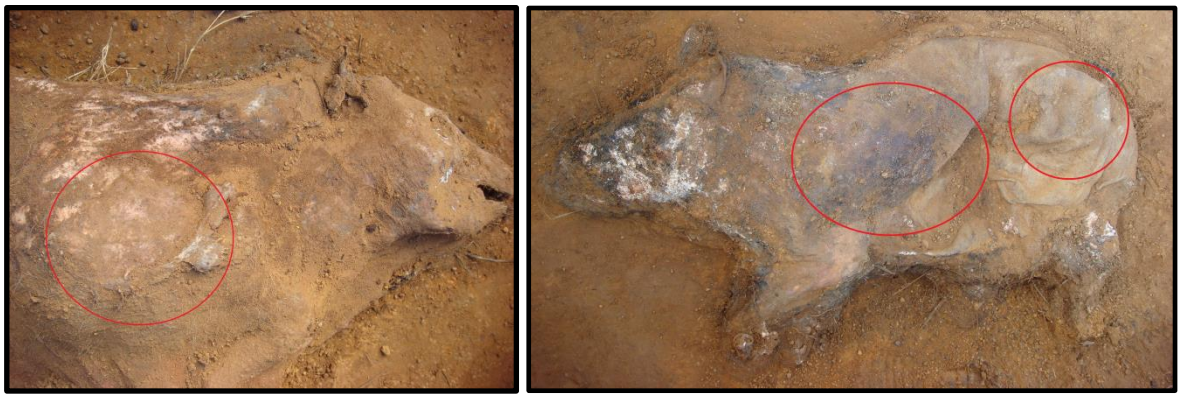
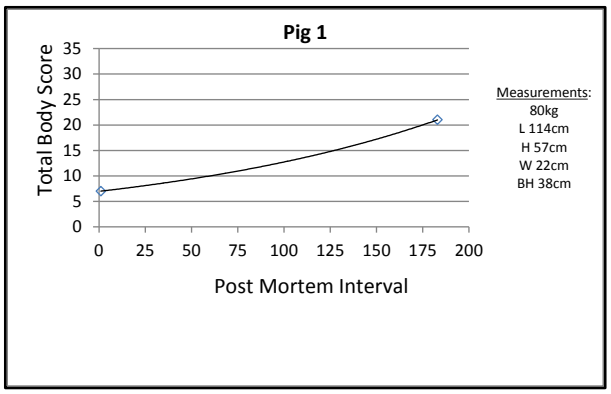


Figure 4.12: Preserved lungs in pig 28 (a) and large intestine in pig 32 (b) with dark discolouration on the rib and pelvic region (encircled in red)

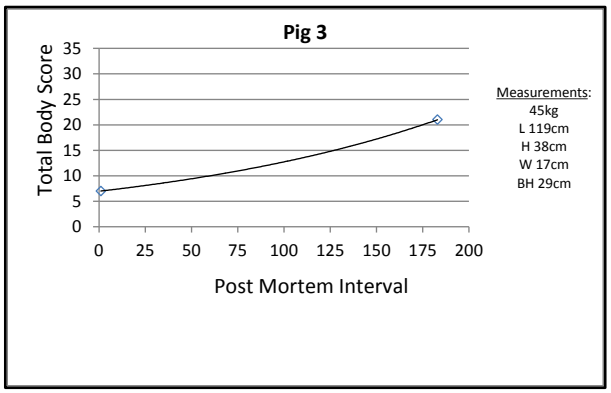
4.2.5 Results after 183 days of internment

After 183 days post mortem (3860.6 ADD), pigs 1, 3, 4, 6 and 7 (Figure 4.13) with TBS scores ranging between 21 and 25, averaging at 22.2, displayed features described in advanced decomposition. The ADD for surface remains within this timeframe was lower as not all surface remains were exposed to a maximum of 183 days (3530.8 ADD) (Table 4.3). Features include black skin-soil interface, collapsed abdomen, skeletonised heads with some areas of leathery skin and hair still present with disarticulated limbs and hooves. Leathery skin patches resulted in the protection of the underlying tissue retaining some moisture.

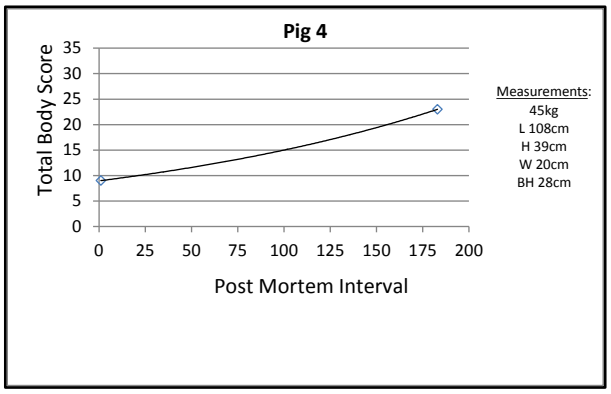
Pig 1



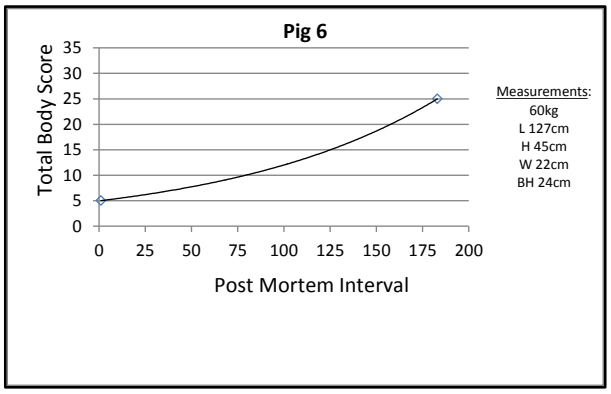
Pig 3



Pig 4



Pig 6



Pig 7

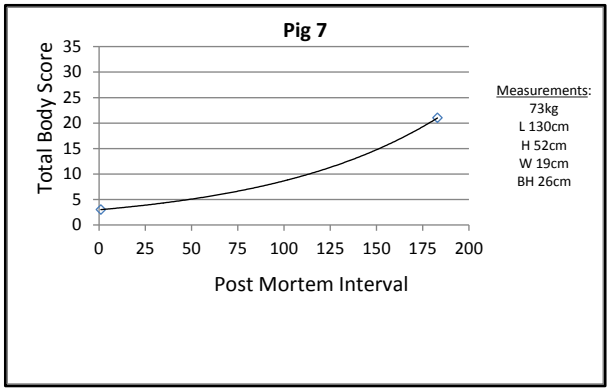


Figure 4.13: Results after 183 days of internment – the x-axis indicating the PMI in days

Greasy skin and adipocere formation were visible on all remaining flesh of all remains in this time frame (Figures 4.14 and 4.15). This can be attributed to the moist soil and high humidity due to heavy rainfall in this region throughout December 2014 to February 2015. A very strong putrid smell (ammonia) was distinct for all carcasses.

Figure 4.14: Pig 7 with a partially skeletonised head after 183 days of burial with some hair and skin still visible on the remains

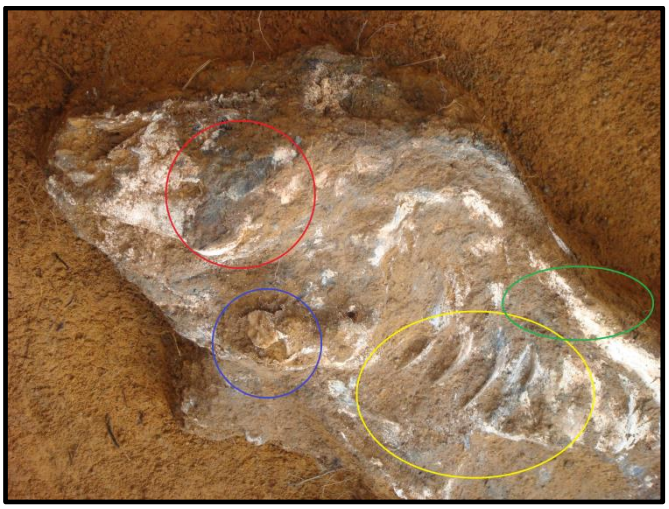


Figure 4.15: Pig 4 showing the black skin-soil interface and desiccated tissue on the skull (red), a disarticulated front limb (blue), visible rib cage (yellow) and adipocere/grease formation on the spine (green)

Only pig 7 showed obvious insect activity after burial as blowfly remains (Figure 4.16) and empty pupa cases were found in grave soil on top of the carcass, especially in the head and trunk areas. Although these pupa cases were empty, it remains important to note any insect activity as insects hasten the decomposition process. Similar to Pig 20, oviposition of larvae probably took place before internment. An interesting observation was that the TBS for pig 7 remained comparable to the other carcasses within this PMI. Therefore, in this case, pre-burial insect activities did not alter the rate of decomposition.

Figure 4.16: Pig 7 had blowfly remains in the soil on top of the abdomen and head area (encircled in red)

The ambient temperature was 23.4ºC and in-soil readings showed no exceptional fluctuations in temperatures ranging from between 23.2ºC to 24.1ºC. Therefore, for carcasses within this time frame, there did not appear to be a large difference in temperatures between surface and buried remains.

4.3 Random-effects maximum likelihood regression analyses for buried remains

A comparison of TBS vs. PMI or ADD for both buried and surface remains shows a non-linear relationship (Figures 4.17 and 4.18). In both groups, decomposition rates followed a curvilinear pattern indicating rapid decay during early decomposition where after the rate of decomposition slowed down during the later stages. As can be seen in Figures 4.17 and 4.18, in the first week of decay (133.1 ADD), surface remains had a higher TBS (an average of 4.2 score difference).

Although the averaged TBS for surface remains is slightly higher than buried remains within this timeframe, the values are much more spread, emphasising the variation on the decay process for surface remains, After two weeks (313.1 ADD) there was a noticeable difference between the groups (an averaged 9.8 score difference) after which decay slowed down to reach the plateau phase (between 665 ADD and 3860.6 ADD). TBS differences remain, on average, 7.7 between buried and surface remains during the plateau phase. Throughout the study period TBS remained higher for surface remains. The greater rate of decomposition for surface remains may be due to exposure to insects and higher temperatures where in contrast, buried remains were protected from these elements by their burial depth and the moist soil surrounding the remains.

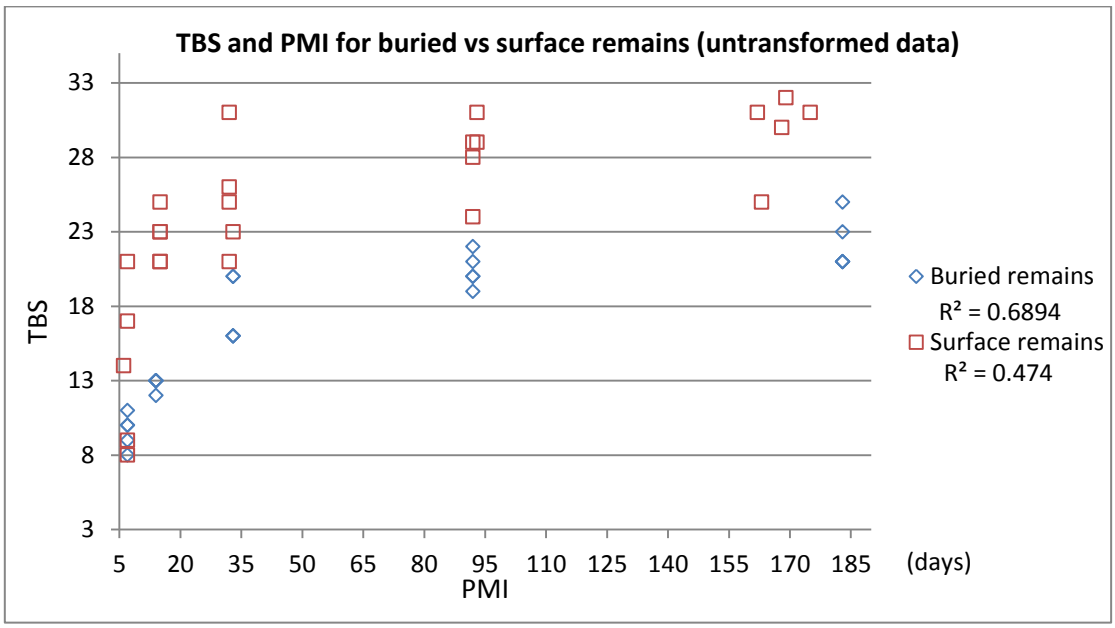


Figure 4.17: TBS vs. PMI for buried (N=25) and surface (N=25) remains with similar PMI's

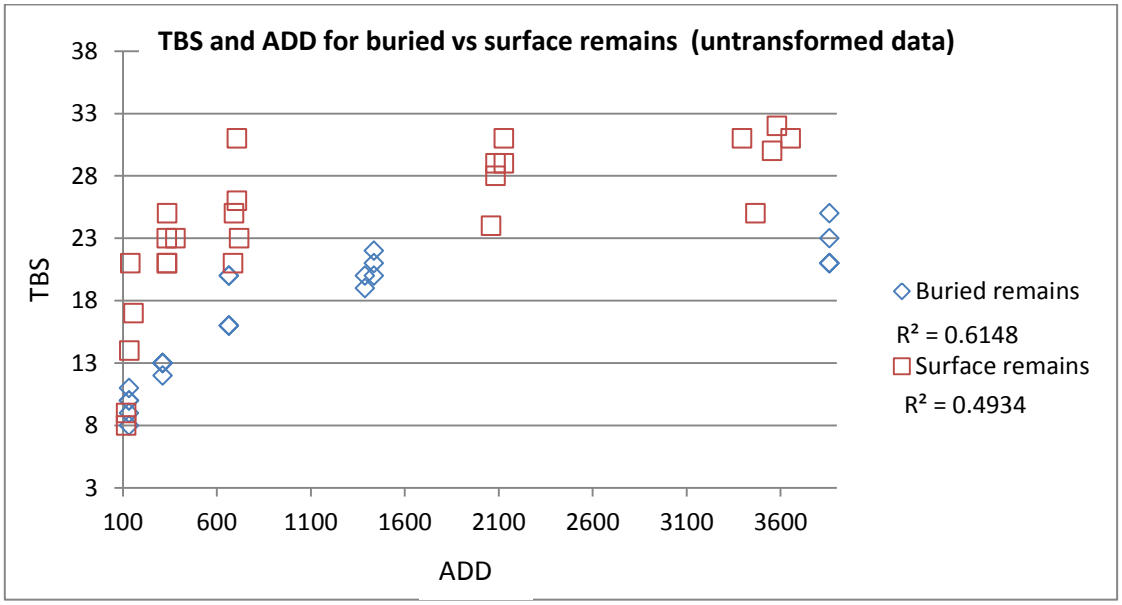


Figure 4.18: TBS vs. ADD for buried (N=25) and surface (N=25) remains with similar PMI's

In the untransformed data for TBS of buried remains, PMI accounted for 68% of the variability in decomposition, while PMI accounted for 47% of the variability for surface remains (Figures 4.17 and 4.18). When using ADD the r-value increases for surface remains to 49% and declines for buried remains to 61%. Although PMI is of course always the constant factor, and the value that we attempt to determine either by means of TBS or by means of ADD. TBS was plotted against ADD and PMI respectively. In this instance, ADD reflects the amount of temperature (degree days), whereas PMI reflects the passage of time.

Therefore, in this case, it was attempted to assess what contributes most to the observed decomposition changes; TBS, temperature or simply elapsed time. As the correlation between TBS vs. ADD and TBS vs. PMI was not too distinct for buried remains, data was log-transformed to determine the relationship between TBS vs. ADD and PMI respectively.

4.3.1 Combined data sets for buried and surface remains

By log-transformation, a more linear curve was produced which provided a better indication of the relationship between TBS and PMI and allowed the use of Random-Effects Maximum Likelihood Regression. By transforming TBS the equation took the form of:

$$Log_{10}TBS = B(x) + Constant$$

B represents the slope and the constant represents the y-intercept. For purposes of this study, the predicted variable (y) was the PMI and the independent variable (x) was the TBS. This resulted in the following equation:

$$Log_{10}PMI\ (y) = B*TBS\ (x) + Constant$$

Log transformations produced improvements in the r-squared value for TBS (Figures 4.19 and 4.20). The r-squared value increased from 0.6894 (Figure 4.17) to 0.8805 (Figure 4.19) for buried remains and from 0.474 (Figure 4.17) to 0.5705 (Figure 4.19) for surface remains. Therefore, the log-transformed data for TBS against PMI for buried remains has a strong correlation of 88% indicating that the variability in decomposition is more distinct for surface remains (57%).

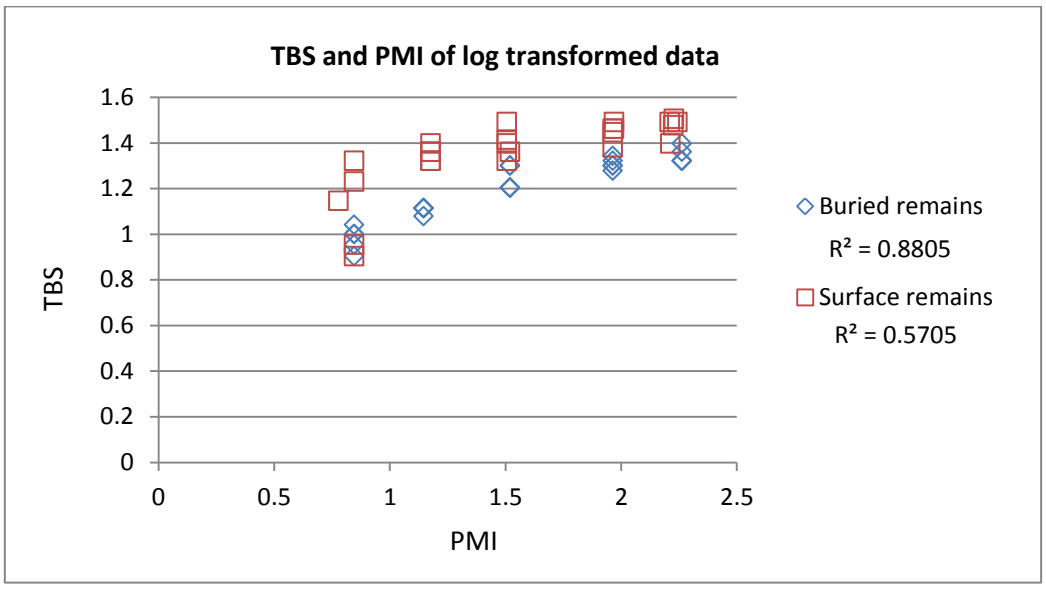


Figure 4.19: LogTBS vs. PMI for all pigs indicating the regression relationship

TBS was also plotted against ADD (Figure 4.20) and log-transformed. The predicted variable (y) was the ADD and the independent variable (x) was the TBS. This resulted in the following equation:

$$Log_{10}ADD = B*TBS + Constant$$

When ADD (y) and TBS (x) data were logged (Figures 4.18 and 4.20), the r-squared value increased from 0.6148 (Figure 4.18) to 0.8717 (Figure 4.20) for buried remains and from 0.4934 (Figure 4.18) to 0.6218 (Figure 4.20) for surface remains. In this study, the log transformed r-squared values remained very similar for buried remains (88% for PMI and 87% for ADD), irrespective of the use of PMI and/or ADD (1% difference). The log transformed r-squared value increase from 57% (PMI) to 62% (ADD) for surface remains, indicating a 5% stronger correlation. Accumulated degree-days is therefore a better descriptor of decay for surface remains since the temperature fluctuations are controlled for when using ADD. A possible reason for the improved result of using ADD in surface remains compared to buried remains may be due to greater temperature fluctuations on the ground surface. Although temperature fluctuations occur in the soil, the temperature changes are not as large as those on the surface. This emphasises the importance of the effects of temperature (for surface remains) and time (for buried remains) on the decay process.

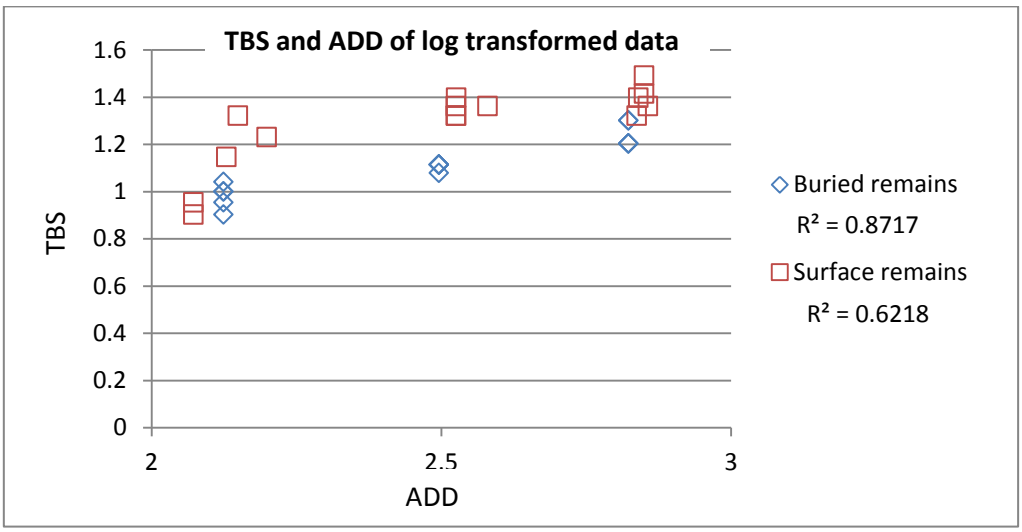


Figure 4.20: LogTBS vs. ADD for all pigs indicating the regression relationship

The TBS difference between buried and surface remains differed significantly ($p<0.0001$) (Table 4.4). Buried remains had a mean TBS of 16.52 compared to surface remains with a value of 23.92 (-7.4 difference). The SE for buried remains was narrower (0.10) where surface remains displayed a wider range (1.32). Similar results can be seen in the 95% confidence interval with surface remains having a wider 95% confidence interval than buried remains with a difference of 10.72 between buried remains and surface remains for the lower limit and 4.08 between buried and surface remains for the upper limit. The variation in the decay process is therefore much less for buried remains than compared to surface remains as can be seen in the narrower margins for buried remains (Table 4.4). As a result, the 95% confidence interval lower and upper limits for buried remains relative to surface remains are 55.2% and 82.9%. Overall, buried pigs decomposed at a much slower rate, reaching lower TBS values relative to similar PMI's than in surface remains.

Table 4.4: Welch two sample t-test with unequal variances (TBS)

Group	Observations	Mean	Standard Error	95% Confidence Interval	
				Lower limit	Upper limit
Buried remains	25	16.52	0.9951888	14.46603	18.57397
Surface remains	25	23.92	1.315193	21.20557	26.63443
Combined	50	20.22	0.9723903	18.26591	22.17409
Difference		-7.4		-10.71902	-4.080982
T	=	-4.4868			
Welch's degree of freedom	=	46.4227			
P	=	<0.0001			
-10.72/23.92+1	=	0.55183946			
-4.08/23.92+1	=	0.82943144			

4.3.2 Inter-observer analyses

If experience plays a detrimental role in accurate TBS observations, then this must be examined further. Decomposition stages are in general considered subjective, however inter-observer analyses are suggested when recording decomposition stages with the TBS system (Suckling, 2011).

Inter-observer analyses were conducted to determine the accuracy and repeatability of the TBS system. Table 4.5 reports on the intra-class correlation coefficients between the primary investigator and the additional observer. The additional observer information (Annexure 3) was kept separate until statistical analyses in order to assess the inter-observer repeatability of scoring. This was tested by means of an intra-class coefficient. If the intra-class correlation coefficient, which measures inter-observer repeatability, is one, perfect agreement exists; values between 0.75 and 0.99 indicate a high degree of agreement; 0.5 to 0.74 indicates moderate agreement and values less than 0.49 indicate a poor agreement. If the intra-class correlation is above 0.75 it indicates that the degree of decomposition, as reflected by TBS, can be consistently repeated (Allan, 1982).

Table 4.5: Intra-class correlation coefficients depicting the inter-observer agreement in the anatomical regions of pigs

Description score	Correlation coefficient
Head and Neck	0.979
Trunk	0.838
Limbs	0.913
Total Body Score	0.953

Although the trunk scores had lower r-values compared to the head and neck, limbs and TBS, the r-values remained high (0.979 for the head and neck, 0.838 for the trunk, 0.913 for the limbs and 0.953 for TBS) (Table 4.5). The head and neck had the highest r-value of all the description scores and can be correctly repeated 97.9% of the time. The overall high degree of agreement suggests that the method is successfully repeatable between observers even though, in this study, the additional observer scored the regions from photographs taken by the primary investigator.

CHAPTER 5
DISCUSSION

5.1 Introduction

Decomposition is a biological process driven by a vast number of variables (Dent *et al*., 2004; Goff, 2009; Mann *et al*., 2009; Carter *et al*., 2010; Vass, 2011). These variables, some of which are still poorly researched and understood, often hinder criminal investigators from establishing a reliable PMI based on the visible state of decomposition. These factors have mostly been studied for remains decomposing on the surface, whereas much less is known for buried remains. For this reason, the aim of this study was to characterise the process of decomposition of buried remains, and to compare this with decomposing surface remains.

For the purpose of this study a pig model was used as pigs are considered acceptable proxies for humans, and to allow standardisation and initiation of the process of burial and decomposition is a South African setting. In order to allow for greater control over the excavation process which involved the removal of soil in equal layers, and also because damaging the remains during excavation were of concern, remains were excavated by means of archaeological techniques. In an attempt to maximise recovery and documentation of decomposing remains, no mechanical equipment was thus used during excavation and burial. As a result, approximately 120 tons of soil had to be shovelled by hand to bury the remains initially, then removed to do the observations, and eventually backfilled for future projects.

The decomposition patterns and rates for buried remains were observed at different time intervals over a period of 10 months (September 2014 – June 2015) using TBS guidelines and ADD developed by Megyesi *et al*. (2005). Total Body Score and ADD were used to standardise the scoring of decomposition which enables studies to be replicated and compared under similar conditions. To demonstrate the progression of decomposition, TBS's were plotted against PMI and ADD for both buried and surface remains.

5.1.1 Interpretation of decomposition patterns

Table 5.1 summarises the average TBS for all anatomical regions scored on buried remains. As mentioned earlier, the most prominent change in decay was observed during the early stages of decomposition (7 to 14 days), which slows down considerably after 33 days of burial. When regions are viewed individually, a gradual increase in the rate of decomposition is evident. From Table 5.1 it can be argued that decomposition progressed more rapidly in the head and neck regions than the rest of the body. As cells becomes deprived of oxygen soon after death and the acidity increases due to the toxic by-products of chemical reactions, enzymes start to digest cell membranes and leak out as the cells break down. This usually begins in the brain, which has high water content (Costandi, 2015). Following rigor mortis and the predictable pattern known as Nystens' Law, rigor first appears in the small muscles of the face, and then spreads to the neck, trunk, upper limbs and lower limbs (Tracqui, 2000). Also, due to the small amount of fat and biomass on the head and ample bacteria in the mouth, it is possible that decay could progress faster in the head and neck regions (Costandi, 2015). As is expected, decay is slowest in the limbs.

Table 5.1: Summary of average TBS scores of all anatomical regions of buried remains

	PMI 7	PMI 14	PMI 33	PMI 92	PMI 183
Head and Neck	4	5	6.8	8.4	9
Trunk	3.2	5.8	6	6.4	7.2
Limbs	2.4	2.6	4.6	4.6	6
TBS	9.6	13.4	17.4	20.4	22.2

With a multitude of factors affecting decomposition, it can be assumed that differences exist. Megyesi *et al*. (2005) state that limbs do not bloat. However, bloating of the limbs was observed during early decomposition, more specifically on the 7 day PMI pigs. Pigs 35 and 36 showed distinct bloating of the limbs especially around the joint areas of the extremities (Figure 5.1). Where the head is scored 5 points for bloating and the trunk 4 points, no allocation is made for bloating of limbs. When the limbs are compared to similar scores, Megyesi *et al*. (2005) refers to drying of limbs (4 points) and leathery skin (5 points).

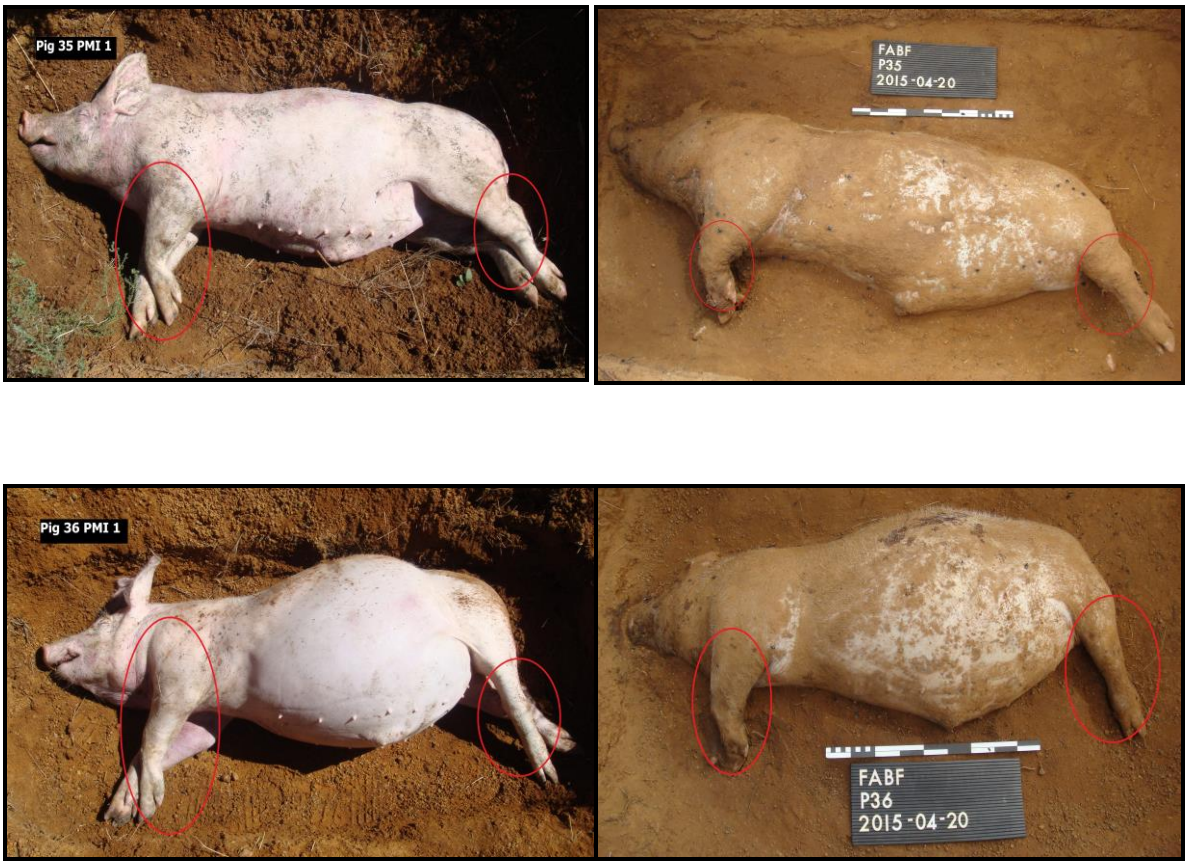


Figure 5.1: Pigs 35 and 36 showing bloating of limbs after 7 days of internment (encircled in red)

Very similar patterns of decomposition were observed between buried and surface remains even with the exclusion of insect activity for the majority of buried remains. Both groups followed patterns where minimal putrefaction is observed during the fresh phase (no discolouration), followed by bloating, discolouration of some regions, blisters and purging of fluids. Literature reports on a distinct foul smell, observable maggot masses, skeletonised head, expulsion of liquids from the mouth, collapse of the abdomen and spilling of intestines after 13 days of burial (Pinheiro, 2006; Schoenly *et al.*, 2006; Parsons, 2009; Niederegger *et al.*, 2015). From our observations the flesh on the head was still relatively fresh after 14 days of burial. During active decay the remains displayed a great loss of mass as a result of purging of decompositional fluids, and the odour remained very distinct. When advanced decay manifested on the remains, the skin became leathery, the flesh caved in and bone exposure was visible especially on the head, ribs and vertebrae.

During advanced decay, surface remains entered the plateau phase and almost reached skeletonisation within 60 days where the tissues became desiccated and maggot activity ceased. In contrast to surface remains, buried remains displayed moist decomposition throughout the study and during advanced decay the skin discoloured and became leathery. However, soft tissue loss was minimal at 33 days of internment. Only after 92 days were partial skeletonisation observed on the heads of buried remains. Even after 183 days of internment, buried remains still displayed moist tissue.

Microorganisms are the primary decomposers in soil. The influence of moisture on decomposition in soil is therefore, in general, due to its effect on soil microbial activity. The decline of microbial activity is primarily ascribed to slower rates of gas diffusion (Carter *et al*., 2010). At greater burial depths there is typically more moisture due to the lesser degree of evaporation attributed to the soil barrier (Haglund and Sorg, 1997). Soil structure and texture should be taken into account when studying in–soil decomposition. Literature clearly states that decomposition rates will differ depending on the type of soil (Turner *et al*., 2013). The soil in the research area for this study was identified as a clay soil. As all burials took place in clay soil no further analysis on soil type and characteristics was done for this study. Clay soil shows slower decomposition rates and higher retention of organic matter in comparison to sandy soil (van Veen and Kuikman, 1990). Clay soil properties have the ability to entrap decomposition fluids around the remains which aids in adipocere formation (Hau *et al*., 2014). Thus, the increased amount of soft tissue visible on the remains in the current study was due to the high moisture content of the clay soil.

The presence of ground water, especially in clayish soil (which retains moisture), produces an environment conducive to adipocere formation (Haglund and Sorg, 1997; Carter *et al*., 2007). Adipocere is capable of degrading over a prolonged period; however, it does reduce the rate of decay in an unchanging environment such as with burial in soil (Pinheiro, 2006; Bristow *et al*., 2011). Adipocere could have manifested on remains between 15 and 32 days, but was first noticed when buried remains were excavated after 33 days PMI. Adipocere also presented itself on both 92 and 183 day PMI carcasses. In total, 15 out of the 25 buried pigs showed signs of adipocere formation.

Adipocere was not only associated with weeks in which heavy rainfall was experienced. For instance, the 33 day pigs received 113.4 mm of rain during internment (October – November 2014), the 92 day pigs 43.2 mm of rain (March – June 2015) and the 183 day pigs received the most rainfall during burial (September 2014 – March 2015) 444 mm. However, it can be argued that the 92 day pigs were buried in soil that already reached saturation with moisture content due to heavy rainfall prior to the 92 day burials. Hence the smallest amount of rain could keep the soil moist for a prolonged period or until the underground water table dropped. Other researchers (e.g. Pounder, 2000; Pinheiro, 2006) report that adipocere formation may be visible as early as three weeks after death in a warm, moist and anaerobic environment and may last for decades. This is also in agreement with Bristow *et al*. (2011) stating that adipocere forms during late post mortem changes. Adipocere commonly develops in the subcutaneous tissues, and its importance is in the preservation of remains, aiding in identification and recognition of injuries (Pounder, 2000). The primary importance of adipocere is the preservation of remains pertaining to the conditions during burial. For example, in the current study the remains were buried in an environment where soft tissue could not degrade quickly enough due to restricted oxygen in the clay soil. Thus, the presence of adipocere during this project is attributed to the fact that heavy rainfall was experienced at the research site during Spring and Summer (November to February). Also, the natural water table was very shallow at certain areas within the site, retaining moisture, which aids in the formation of adipocere (Pinheiro, 2006). The absence of insects and/or minimal insect activity may also have played a role in increased adipocere formation. Due to minimal insect activity, carcasses retained a large amount of moist tissue, therefore the environment was more favourable for the formation of adipocere (Brown and Peckmann, 2013).

Moist conditions also hindered the accessibility of insects to the remains as the clay soil restricts the availability of oxygen needed for development. Gunn and Bird (2011) reported on adult flies laying eggs in cracks caused by, for example, secondary depression or rodent burrows. These eggs then hatch and the larvae migrate through the soil to the food supply. According to their research, even muscids *M. prolapse* and *M. stabulans* can colonise remains at a depth of at least 40 cm. With shallow graves it was found that some remains were partially accessible by insects (Gunn and Bird, 2011). Rodriguez and Bass (1985) also published on their observations of blowflies accessing buried remains by burrowing through cracks in the soil trying to reach the cadavers.

Blowflies were also found to deposit eggs in the soil cracks following significant rain. Although a secondary depression was observed on some burial pits during this study, it is highly unlikely that the insects were able to burrow down to the remains for oviposition, due to the average depth of the pits (0.75 m). Only 4 out of the 25 buried pigs showed signs of insect activity throughout the study. Pig 21 (PMI 14) had live maggots protruding from the abdomen once excavated, pig 20 (PMI 33) displayed masses of live maggots in the hind quarter, pig 30 (PMI 92) had a few maggots protruding from the head and hind quarter and pig 7 (PMI 183) had blowfly remains and empty pupa cases on the head and trunk. It is proposed that these maggots were ovipositioned on the remains before internment took place.

Insects assist in the decay of remains through their feeding activities, and they also produce salivary substances containing enzymes which break down tough tissue (Turner, 1987). As a result of burial, the loss of biomass of surface remains would be much more than that of buried remains which is sheltered from insect access. Insects have the ability to locate decomposing remains within minutes/hours after death and cause the greatest amount of soft tissue destruction during the process of decomposition (Campobasso *et al*., 2001). As insects have the ability to oviposition on remains within minutes of death, it may be possible that remains could have been exposed to infestation before internment. The decomposition process is slowed down when insects are limited to the carcass. Insects hasten liquefaction and disintegration by dissemination of bacteria and secretion of digestive juices (Payne, 1965). Even with pre-burial exposure to insect activity for pigs 7, 20, 21 and 30, their influence on the rate of decay seemed insignificant due the lack in subsequent insect succession on the remains, as TBS scores remained average for all carcasses in these time frames (Table 4.3). Decomposition rates for these pigs were therefore not enhanced due to the minimal insect activities observed. As no literature could be found on the ability of insects to borrow down to a depth of 0.75 m in clayish soil, the maggot activity observed in this study must have been due to colonisation before internment. In agreement with Megyesi *et al*. (2005) and Simmons *et al*. (2010), when insects are excluded from remains, it was found that temperature is the greatest accelerator for decomposition. In addition, soil provides an efficient insulation from solar radiation, resulting in a reduced rate of decomposition (Mann *et al*., 1990) especially when decay rates of buried remains are compared to decay rates of surface remains.

Temperature is one of the most important taphonomic factors contributing to degradation or preservation of buried remains (Mann *et al*., 1990; Prangnell and McGowan, 2009). Putrefaction is limited at temperatures below 10°C as the optimum temperature for soft tissue degradation is between 20°C and 40°C (Pounder, 2000; Prangnell and McGowan, 2009). Temperatures in soil decreased in general with soil depth. Rodriguez and Bass (1985) states that thermal stabilisation normally occurs at 0.60 m of depth, while at depths of 0.30 m and or less, the in-soil temperature is very similar to the ambient temperature.

As the burial pits were dug to an average of 0.75 m (Table 3.1), temperature fluctuations were suggested not to play a major role on these buried remains as with surface remains. Temperatures higher than the ambient temperature were only observed in buried remains where insects were active. This is ascribed to the heat generated by the insects during decomposition (Simmons *et al*., 2010). Also, with increased burial depth, soil displayed higher moisture content due to reduced evaporation and the closer proximity to natural underground water tables.

The 7 day interval pigs were buried and excavated during Autumn, 14 day interval pigs buried in Autumn and excavated in Summer, 33 day interval pigs buried and excavated during Spring, 92 day interval pigs were buried in Autumn and excavated in Winter and the 183 interval pigs were buried in Spring and excavated during Autumn. Numerous researchers have observed increased insect activity and decomposition rates during summer (Archer, 2004; Prieto *et al*., 2004; Myburgh *et al*., 2013). Similar to Archer (2004), it can be suggested that decomposition varies between seasons and is known to take longer during Winter and Autumn than compared to Spring and Summer (Myburgh *et al*., 2013). However, the delay in decomposition within this study may not be due to seasonality. Rather the depth the remains were buried in may have insulated the remains from seasonal temperature fluctuations and the rates observed were much less variable than those on the surface throughout.

5.1.2 Interpretation of decomposition stages

Recent studies have demonstrated that remains exposed to similar conditions and PMIs can have different stages of decomposition (Dix and Graham, 2000; Pinheiro, 2006; Wilson *et al*., 2007, Bilheux *et al*., 2015). Different stages of decomposition within similar PMIs were also observed in this study. The 7 day PMI pigs displayed variation in appearance as pigs ranged between pink to very dark purple/grey discolouration. Even though similar discolouration patterns were observed in both groups, the variation observed with buried remains was still slower than variations described on surface remains.

Table 5.2 indicates that none of the buried remains were classified in the fresh stage of decomposition at day 7. Remains all progressed to the early stage of decomposition displaying inflated abdomens due to the build-up of gases. Between 7 and 14 days post-burial, remains entered early decay which is similar to the observations described by Gennard (2012) (Table 5.3). Carcasses deflated, odour was prominent and the skin began to peel.

Table 5.2: Speed of pig decomposition in burial pits

Decomposition stage	Description	Average time	Range
Fresh decomposition	No maggot activity No discolouration	0 - 6 days	Days
Early decomposition	Discolouration Bloating Purging of fluids	7 - 14 days	Days
Advanced decomposition	Moist changes Sagging of trunk Tissue desiccation	14 - 183 days	Weeks to Months
Skeletonisation	Majority of bones are exposed	Not observed within a timeframe of 6 months	Months to Years

However, in contrast to what was reported by Gennard (2012), buried remains entered what is described as advanced decomposition by Megyesi *et al*. (2005) anywhere between 14 and 183 days (caving in of the abdominal cavity, some bone exposure and adipocere development) whereas Gennard (2012) refers to an average time of 2.8 years for advanced decay to manifest on buried remains (Table 5.3). This shows that the rate of decay is variable and that advanced decay can be reached after unexpectedly short time periods.

Even within a small catchment area, underlying geology and soil structure can alter decomposition patterns and rates of buried remains, which can lead to significant differences within the same carcass (Wilson *et al*., 2007). However, intrinsic factors such as constitution of the body (obese corpses decompose faster due to the greater amount of liquid in soft tissue) can also alter the rate of decomposition (Campobasso, 2001). Carcasses in this trial remained in the advanced stages of decomposition for the remainder of the study. No clearly defined beginning or end of decomposition stages was observed. Therefore the stage changeover time is fairly subjective.

Table 5.3: Speed of human decomposition in graves (Gennard, 2012)

Decomposition stage	Average time	Range
Early decomposition	22 days	5-48 days
Moderate decomposition	93 days	8 days-8.7 months
Advanced decomposition	2.8 years	5.7 months-10years
Skeletonisation	12.7 years	8.4-16.8 years

Attempts to standardise decomposition stages assist in the description of the decomposition process, and aid in the estimation of the PMI of remains. Comstock and colleagues (2014) proposed four new stages of decomposition to characterise decomposition on carcasses that are completely excluded from insect activity.

These stages are: fresh (no visible external changes); bloating (discolouration and bloating); deflation (purging of fluid and limbs fall limp); and dry decomposition (flattening of the carcass). The four stages proposed by Comstock *et al*. (2014) are more in agreement to the rate of decay described within this study. However, despite defined stages of decay, the process of decomposition is not uniform and variations occur in terms of rates and time elapsed between stages. For example, Comstock *et al.* (2014) did not list active decay descriptors (strong odours of ammonia, discolouration and moist decomposition) for carcasses completely excluded from insect activity. Active decay was however observed in the current study. In addition, the delay in the onset of the dry stage is due to the amount of soft tissue still present on the remains. The majority of skin and hair remained on the buried carcasses and moisture loss was particularly slow.

5.1.3 Estimating the PMI in buried remains

The estimation of a reliable PMI is of utmost importance in forensic investigations. It remains a very complicated calculation due to the vast number of variables influencing the patterns and rate of decay (Pinheiro, 2006). To further complicate an accurate PMI estimation, decomposition rates of buried bodies decrease with burial depth due to lower in-soil temperatures, less insect activity and grave soil moisture content (Bachmann and Simmons, 2010). For this reason, developmental and succession data taking into consideration a great number of geographical regions and death scene scenarios are essential (Amendt *et al*., 2004). However, fewer variables are associated with in-soil decay than surface decay, as there are no access by scavengers, limited insect activity and reduced temperature fluctuations. Therefore one should potentially be able to get a narrower estimate than what is the case for surface remains. TBS is valuable to assess the progress of decomposition in relation to ADD. A quantitative method to accurately calculate the PMI from gross morphological changes of decomposed remains could prove useful (Marhoff *et al*., 2015) should other evidence be unavailable (e.g. insects, soil analyses). TBS could currently be the most valued tool in estimating the PMI (Bachmann and Simmons, 2010).

When ADD and PMI were logged (Figures 4.17 and 4.19) against TBS, the log transformed r-squared values remained very similar for buried remains (88% for PMI and 87% for ADD). For surface remains however, ADD seemed a better descriptor with a stronger log transformed r-squared value increase of 5%. Accumulated degree-days is therefore useful for controlling the effect of temperature on the decay of surface remains while TBS is a better descriptor of observations for buried remains due to constant temperatures in soil.

According to Troutman *et al*. (2014), the delay in decay is mostly ascribed to the lower in-soil temperature and accessibility by insects on the remains. They state that buried remains can take up to eight times longer to decompose than surface remains (Troutman *et al.*, 2014). The results from this research suggest that buried remains, at an average depth of 0.75 m, can have a decay rate of between 55.2% to 82.9% less than surface remains with similar PMI's. The average TBS score difference for buried remains will therefore be 7.4 times less than that of surface remains. It should be taken into account that some remains might have been exposed to insects prior to burial, which was also the case in this study.

Nevertheless, the presence of insects may necessitate the collection of grave soil for the identification of insects and assessment of developmental stages when applicable. Although insects most probably did not accelerate the rate of decay in this study, the mere presence of insects suggests a variation in the decomposition pattern and rate of buried remains and should be accounted for when estimating a PMI.

With the observations made within this study, it is attempted to make an estimation for calculating a PMI from the decomposition stages of clandestine burials (Table 5.4). The estimation would however pertain to remains with an average weight of 64 kgs based on the average weights of the pigs used in this study (Table 4.2). Also, the estimations will only be applicable for remains found at the same depth in the Central Highveld region of South Africa with similar environmental factors as it is not yet known if the process of decay would be different in other regions within South Africa.

Cooler temperatures in soil and moisture slowed down discolouration of the remains and tend to vary between shades of grey, green, purple and black. Green discolouration is still categorised as early decay and was only noticed at 14 days PMI, however it could have manifested on the carcasses sooner. The same applies to the darker discolouration such as a black skin which indicates advanced stages of decomposition.

Insects were only noticed on the remains after 14 days of burial, which probably allowed sufficient time for development due to the availability of oxygen in the soil when backfilled. When insects or insect activity are visible on buried remains, it is suggested that the estimated PMI be narrowed as insects might accelerate the rate of decomposition due to the consumption of soft tissue. On the other hand, adipocere formation on remains should result in a broader PMI estimation as adipocere can delay the rate of decomposition on remains as discussed throughout the study.

As suggested by Bachmann and Simmons (2010), retrospective studies on buried remains using similar TBS and ADD models could assist in more reliable PMI estimations in the future. When applying PMI estimates based on pig data to humans, it should be kept in mind that differences in intestinal bacteria, anatomy and fat ratios between pigs and humans can lead to variations in the decay process.

Table 5.4: PMI estimation for buried remains in the Central Highveld region of South Africa

Estimated PMI	Estimated ADD	Estimated TBS	Description of remains
4 - 9 days	90 - 200	8 – 11	Bloating Purging of fluids Skin slippage
10 – 18 days	250 – 450	9 – 15	Sagging of trunk Discolouration
24 – 40 days	550 – 900	14 – 19	Bloating Adipocere Insect infestation
56 – 100 days	1300 – 2300	17 – 22	Dark discolouration Strong putrid smell Adipocere Partial skeletonisation Collapsed trunk
152 days +	3500 +	20 – 35	Black skin interface Adipocere Skeletonisation of the head

5.2 Limitations of the study and difficulties experienced

The study observed decomposition patterns of buried remains which are an important aspect of decomposition as many factors can influence the rate of decay. The study encountered a number of limitations, which need to be considered as outlined below:

5.2.1 Pigs as proxies for human decomposition studies: The substitution of pigs for human cadavers is not ideal, but continues to be a viable alternative for decomposition studies due to the complications of conducting actualistic research on donated bodies. Pig carcasses are more readily available and therefore sample sizes can be increased with future studies.

5.2.2 TBS guidelines: Distinction of different colours during early decomposition on the whole carcass was at times difficult, especially with moist pits as soil dried quickly on the remains when excavated, covering the carcass. The carcasses were not rinsed before scoring as this would add additional moisture to the already wet research site.

Cleaning the remains of excess soil for scoring purposes might be a viable option should the research site consist of sandy soil which does not retain moisture. This may aid in retaining skin on the carcasses for observation purposes. Colour was also challenging at times as shades of grey, green and brown did not always appear on the remains during early decomposition, and skin slippage was visible only after the remains discoloured, with no visible fresh flesh present. Various colours also appeared on the same anatomical region of the remains, more especially on the trunk with variations of grey and black. When using the TBS guidelines of Megyesi *et al*. (2005), the scores differ for grey (3 points) and black (5 points) discolouration in the trunk. Adding scores for bloated limbs might proof useful for observational purposes. However, keeping in mind that the TBS guidelines of Megyesi *et al*. (2005) are based on human cadavers, the differences in observations do not seem to be to such an extent that the guidelines of Megyesi *et al*. (2005) cannot be successfully applied.

5.2.3 Logistics: To unload the carcasses, the burial pit rows were dug 4.5 m apart and did not allow enough space to drop off the carcasses at the designated burial pit with a vehicle, which resulted in some of the carcasses having being dragged some meters for burial, keeping in mind that care was taken not to damage the remains. It could be helpful when attempting similar studies, to dig the pits wider apart in order for vehicles to drive up to the designated grave and unload the carcass. Also, when removing the soil for observations, it is suggested to place the soil in the length of the grave to ease the process of backfilling pits. When soil is placed in the width of a pit it results in soil being thrown to the middle and opposite side to fill up the grave, which is intense manual labour.

Although the collection of so many carcasses was useful pertaining to logistical aspects, it was a difficult task to co-ordinate enough excavators to assist with the removal of soil as we needed 2 people per grave (due to limited space inside the pit). Each grave took an average of 3 hours to excavate. Back filling the remains was also done with shovels, taking at least 1 hour. To have a designated team of excavators would be more viable as they could be available on short notice and also know what is expected and familiar with techniques to expose buried remains.

5.2.4 Scavengers: Even though scavenger activity was not expected due to the depth of the burials, three pigs had to be replaced due to disturbance by, most probably, black-backed jackals. These were seen on the farm at numerous occasions while burying carcasses. Some pits were disturbed within days and another after months of internment with remains scattered around the graves. Shortly after noticing scavenger activity, galvanised mesh was placed over each pit and covered with large rocks to prevent the mesh from lifting. An interesting observation was that the scavengers (presumably black-backed jackals) never dug up remains from the side; they always dug up the remains from the middle. Hence, when placing the mesh it was ensured that the middle part was covered completely, which seemed to solve the problem experienced with scavengers.

5.2.5 Seasonality: It would be valuable to observe the rate of decomposition when remains are buried and excavated on different days with similar PMIs, more especially in different seasons to compare seasonal fluctuations if applicable. With this study no difference in the rate of decay which could be ascribed to (in soil) season fluctuations was observed. In the study done by Myburgh (2010) however, it was suggested that seasonality have a great influence on the rate of decomposition for surface remains.

5.2.6 In-soil temperature: Equipment to measure in-soil temperatures would have enabled a comparative reading between surface and in-soil ADD to further analyse the effects of temperature on the rate of decomposition.

5.3 Future research projects

Despite limitations and challenges experienced with this study, the results are of considerable importance for research projects on buried remains within South Africa. This research provides baseline data for future research studies on buried remains which has not been tested before within a South African setting. Although taphonomy studies can be very complex and labour intensive, the knowledge gained from these research initiatives are of imperative value. The following areas of research for the future are recommended:

5.3.1 From observations within this study, it might be useful to replicate the methods used with buried remains using carcasses that have been treated with chemicals (i.e., antibiotics) before death as this seemed to preserve remains to a better extend which might alter the rate of decay and PMI estimations significantly. In commercial piggeries, death is commonly caused by E. coli (*Escherichia coli*), which is an intestinal bacterial infection, and Salmonellosis (*Salmonella choleraesuis)* which leads to respiratory distress. As preservation of intestines were observed on pigs 28 and 32, it seems possible that these pigs died of a lung or intestinal infection and it is possible that medication (antibiotics) was administered shortly before the death of these pigs, which might have led to preservation of these organs. This suggests that substance intake may also play a significant role in the pattern and rate of decomposition, although this cannot be confirmed at this stage.

5.3.2 Similar studies, following the same methods, should be conducted throughout different geographical areas in Southern Africa as burial of remains influenced the decay rate considerably when compared to decay rates of surface remains. It would be interesting to compare the rate of decay found with this study to the rate of decay on remains buried in, for instance, sandy soil.

5.3.3 As burial depth and clay soil was also found to protect remains from extrinsic factors (i.e., insects) it is suggested that a multidisciplinary approach be followed with future research on buried remains to try and determine if insects are capable of burrowing down to the carcasses at a certain depth, keeping the soil structure in mind. If this is possible, the science of entomology might be able to narrow down an estimated PMI if one can determine how long insects can survive in such dense and moist soil as clay.

5.3.4 Grave soil is reported to be rich in certain minerals and elements, grave soil can be collected from the natural openings underneath a carcass at different timeframes to try and establish if minerals and elements enrich (i.e., nitrogen) or deprive (i.e., iron) soil. If there are remarkable differences in pre- and post- burial soil, this could possible lead to another method in estimating the PMI, or detect if a decomposing body may have been present in soil.

CHAPTER 6
CONCLUSIONS

This study took a quantitative approach to observe decomposition of buried remains, an area in the realm of forensic anthropology with few experimentally supported findings within a South African setting. Data collection for taphonomic studies are extremely important to understand the complex process of decomposition in different settings. It must be kept in mind that decomposition is a continuous process which makes it difficult to characterise all regions of a body into the same decompositional state due to interindividual variation. As the possibility of subjectivity exists when characterising remains into stages of decomposition, the need for standard stage definitions that would reduce the potential for personal interpretation is accentuated. From this study, the following conclusions were made:

The stages of decomposition for both surface and buried remains were very similar, however there was a vast difference as far as the rate of decay was concerned. It was evident in this study that decomposition of buried remains occurred at a much slower rate than that of surface remains, which is in agreement with previous studies (Mann *et al*., 1990; Dent *et al*., 2004; Goff, 2009; Comstock *et al*., 2014).

1. Variables such as insect activity and temperature are related to aspects of decomposition. Where insects are considered the most significant environmental decomposer (Simmons *et al*., 2010), temperature is considered the most important variable (Mann *et al*., 1990). Throughout this study, TBS remained higher for surface remains emphasising a decreased rate of decay for buried remains; this decay rate was ascribed to exposure to insects and higher temperatures where in contrast, buried remains were protected from these elements by limited insect access, lower in-soil temperatures due to their burial depth, soil characteristics (clay soil) and moisture content surrounding the remains.
2. Overall, similar patterns of decomposition were observed between buried and surface remains with rapid decay during early decomposition stages where after reaching a plateau phase during advanced decay where minimal change was noted. However, no clearly defined beginning or end of decomposition stages was observed.

3. In porous soils (e.g. sand), desiccation is promoted as moisture is drawn away from the interned remains due to better drainage. This, in contrast to the soil (clay) found at the FABF, as clay exhibits poor drainage (retains moisture) and would be more likely to assist in the formation of adipocere (Schotsmans *et al*., 2011), which has been reported to preserve remains (Pinheiro, 2006). With the formation of adipocere, buried remains displayed moist decay compared to surface remains already showing signs of desiccated tissue and mummification.
4. It is generally agreed that variables may alter decomposition differently between remains exposed to similar conditions. This was also observed within this study, more specifically on the 7 day PMI pigs. Even within a small catchment area, underlying geology and soil structure can alter decomposition patterns and rates of buried remains (i.e. differences in discolouration rates of remains), This emphasises the importance of taking both extrinsic (i.e., insect activity) and intrinsic (i.e., constitution of the body) factors into account when interpreting the environment at the death scene to aid in PMI estimations.
5. The standard uses of quantitative variables such as ADD and TBS facilitated the comparison of data regardless of environmental factors as this approach incorporates variability associated with temperatures and seasons. This allows for the development of prediction models for a specific geographical region. With this study, ADD was found to be a better descriptor of decay for surface remains as ADD is useful for standardising the effect of temperature. On the other hand, TBS was found to be a better descriptor of observations for buried remains.
6. The impact of various factors, both external and internal, that influences decomposition i.e., different soil types, depth, presence of groundwater, etc. can be researched in future studies, and the results compared to those obtained from this study.
7. Although extensive and detailed, the materials and methods used in this research are repeatable and future studies are encouraged as it is impossible to assign predictability to the pattern and rate of decomposition. However, with a regional database on similar studies, a specific model to identify variables associated with the altering of decay particular to that area, can be created.

8. It is imperative that a support tool for the estimation of a reliable PMI be created to aid forensic investigators (i.e., identifying the victim). A model to estimate a reliable PMI should be kept in focus as the conduction of forensic taphonomy studies continue to develop in the future.

REFERENCES

Adlam RE, Simmons T. 2007. The effect of repeated physical disturbance on soft tissue decomposition – are taphonomic studies an accurate reflection of decomposition? Journal of Forensic Science, 52(5): 1007-1014.

Allan JC. 1982. Learning about statistics: a primer in simple statistical methods for students of the medical, biological, paramedical, social and behavioural sciences. MacMillan South Africa (publishers) (PTY) Ltd. Johannesburg, South Africa: 122-140.

Amendt J, Krettek R, Zehner R. 2004. Forensic entomology. Naturwissenschaften, 91: 51-65.

Archer MS. 2004. Rainfall and temperature effects on the decomposition rate of exposed neonatal remains. Science and Justice, 44(1): 35-41.

Bachmann J, Simmons T. 2010. The influence of preburial insect access on the decomposition rate. Journal of Forensic Sciences, 55(4): 893-900.

Beary MO, Lyman, RL. 2012. The use of taphonomy in forensic anthropology: Past trends and future prospects. In: A companion to forensic anthropology. Dirkmaat DC. 1st Edition. Blackwell Publishing Ltd: 477-525.

Bilheux HZ, Cekanova M, Vass AA, Nichils TL, Bilheux JC, Donnell RL. Finochiarro V. 2015. A novel approach to determine post mortem interval using neutron radiography. Forensic Science International. http://dx.doi.org/10.1016/j.forsciint.2015.02.017.

Breitmeier D, Grafe-Kirci U, Albrecht K, Weber M, Troger HD, Kleemann WJ. 2005. Evaluation of the correlation between time corpses spent in in-ground graves and findings at exhumation. Forensic Science International, 154: 218-223.

Bristow J, Simms Z, Randolph-Quinney P. 2011. Taphonomy. In: Forensic Anthropology 2000 to 2010. Black S, Ferguson E editors. Taylor and Francis Group: CRC Press: 279-317.

Brown C, Peckmann T. 2013. Decomposition rates and taphonic changes associated with the estimation of time since death in a summer climate: A case study from Urban Nova Scotia. Canadian Society of Forensic Sciences Journal, 46(4): 209-230.

Campobasso CP, Di Vella G, Introna F. 2001. Factors affecting decomposition and Diptera colonization. Forensic Science International, 120: 18-27.

Carter DO, Yellowlees, D, Tibbett, M. 2007. Cadaver decomposition in terrestrial ecosystems. Naturwissenschaften 94: 12-24.

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

Catts EP. 1992. Problems in estimating the postmortem interval in death investigations. Journal of Agricultural Entomology, 9(4): 245-255.

Clark MA, Worrell MB, Pless JE. 1997. Postmortem changes in soft tissue. In: Haglund WD, Sorg MH editors. Forensic taphonomy: The post mortem fate of human remains. Boca Rato: CRC Press: 151-164.

Cockle LC, Bell SB. 2015. Human decomposition and the reliability of a 'Universal' model for post mortem interval estimations. Forensic Science International 253: 136e1-136e9.

Comstock JL, Desaulniers JP, LeBlanc HN, Forbes SL. 2014. New decomposition stages to describe scenarios involving the partial and complete exclusion of insects. Canadian Society of Forensic Science Journal: 1-18.

Costandi, M. 2015. Life after death: the science of human decomposition. Forensic Science: Neurophilosophy. http://www.theguardian.com/science/neurophilosophy/2015/may/05/life-after-death.

Crime Situation in South Africa, 2014. South African Police Services. RSA: April to March 2004-2014: Provincial and national figures and ratios. http://www.saps.gov.za/resource_centre/publications/statistics/crimestats/2014/crime_stats.php.

Cross P, Simmons T. 2010. The influence of penetrative trauma on the rate of decomposition. Journal of Forensic Science, 55(2): 295-301.

Dent BB, Forbes SL, Stuart HH. 2004. Review of human decomposition processes in soil. Environmental Geology, 45: 576-585.

Dirkmaat DC, Cabo LL, Ousley SD, Symes SA. 2008. New perspectives in forensic anthropology. Yearbook of physical anthropology, 51: 33-52.

Dix J, Graham M. 2000. Time of death, decomposition and identification: An atlas. Boca Raton, CRC Press: 10-12.

Dodson P. 1980. Vertebrate burials. Paleobiology, 6(1): 6-8.

Fiedler S, Graw M. 2003. Decomposition of buried corpses, with special reference to the formation of adipocere. Naturwissenschaften, 90: 291-300.

Finley SJ, Benbow ME, Javan GT. 2014. Microbial communities associated with human decomposition and their potential use as postmortem clocks. International Journal of Legal Medicine: 1-10.

Forbes SL, Stuart BH, Dent BB. 2005. The effect of soil type on adipocere formation. Forensic Science International, 154: 35-43.

Fründ HC, Schoenen D. 2009. Quantification of adipocere degradation with and without access to oxygen and to the living soil. Forensic Science International, 188: 18-22.

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

Gennard D. 2012. Forensic entomology: An introduction. Hoboken, NJ, USA: Wiley-Blackwell: 35-36.

Goff ML. 2009. Early post-mortem changes and stages of decomposition in exposed cadavers. Experimental and Applied Acarology, 49: 21-36.

Gunn A, Bird J. 2011. The ability of the blowflies *Calliphora vomitoria* (Linnaeus), *Calliphora vicina* (Rob-Desvoidy) and *Lucilia sericata* (Meigen)(Diptera: Calliphoridae) and the muscid flies *Muscina stabulans* (Fallen) and *Muscina prolapse* (Harris)(Diptera: Muscidae) to colonise buried remains. Forensic Science International, 207: 198-204.

Haglund WD, Sorg MH.1997. Introduction to forensic taphonomy. In: Forensic taphonomy: The post mortem fate of human remains. Boca Rato: CRC Press: 3-12.

Hattingh PJ. 1987. Geohidrologiese ondersoek van gedeeltes van Kaalfontein 513 JR: 1-30. (Unpublished report).

Hau TC, Hamzah NH, Lian HH, Hamzah SPAA. 2014. Decomposition process and post mortem changes: Review. Sains Malaysiana 43(12): 1873-1882.

Joukowsky M. 1980. A complete manual of field archaeology. Tools and techniques of field work for archaeologists. Prentice-Hall, Englewood Cliffs, USA: 1-630.

Kelly JA, van der Linde TC, Anderson GS. 2009. The influence of clothing and wrapping on carcass decomposition and anthropod succession during the warmer seasons in Central South Africa. Journal of Forensic Science, 54(5): 1105-1112.

Keough N. 2013. Skeletal changes after post-mortem exposure to fire as an indicator of decomposition stage. PhD dissertation. University of Pretoria, South Africa: 1-298.

Keough N, L'Abbé EN, Steyn M, Pretorius S. 2015. Assessment of skeletal changes after post-mortem exposure to fire as an indicator of decomposition stage. Forensic Science International, 246: 17-24.

Komar DA, Buikstra JE. 2008. Forensic anthropology: Contemporary theory and practice. Oxford University Press: 18-207.

Manhein MH. 1997. Decomposition rates of deliberate burials: A case study of preservation. In: Forensic taphonomy: The post mortem fate of human remains. Haglund WD and Sorg MH, Eds. Boca Raton, FL: CRC Press: 469-481.

Mann RW, Bass WM, Meadows L. 1990. Time since death and decomposition of the human body: Variables and observations in case and experimental field studies. Journal of Forensic Science, 35(1): 103-111.

Marhoff SJ, Fahey P, Forbes SL, Green H. 2015. Estimating post-mortem interval using accumulated degree-days and a degree of decomposition index in Australia: A validation study. Australian Journal of Forensic Sciences. http://dx.doi.org/10.1080/00450618.2015.1021378

Megnin P. 1896. La fauna des cadavres. The British Medical Journal, 1(1838): 736-737.

Megyesi MS, Nawrocki SP, Haskell NH. 2005. Using accumulated degree-days to estimate the post-mortem interval from decomposing human remains. Journal of Forensic Science, 50(3): 618-626.

Micozzi M. 1997. Frozen environments and soft tissue preservation. In: Haglund WD, Sorg MH, Editors. Forensic Taphonomy: The post-mortem fate of human remains. Boca Raton: CRC Press:171-180.

Moffat C, Simmons T, Lynch-Aird J. 2015. An improved equation for TBS and ADD: Establishing a reliable post-mortem interval framework for casework and experimental studies. Journal of Forensic Science. Doi: 10.1111/1556-4029.12931.

Myburgh J. 2010. Estimating the post-mortem interval using accumulated degree-days in a South African setting. MSc dissertation, University of Pretoria, South Africa: 1-151.

Myburgh J, L'Abbé EN, Steyn M, Becker PJ. 2013. Estimating the postmortem interval (PMI) using accumulated degree-days (ADD) in a temperate region of South Africa. Forensic Science International, 229: 165e1-165e6.

Niederegger S, Schermer J, Höfig J, Mall G. 2015. Case Report: Time of death estimation of a buried body by modeling a decomposition matrix for a pig carcass. Legal Medicine, 17: 34-38.

Olakanye AO, Thompson T, Ralebitso TK. 2014. Changes to soil bacterial profiles as a result of *Sus scrofa* domesticus decomposition. Forensic science International, 245: 101-106.

Parsons HR. 2009. The postmortem interval: A systematic study of pig decomposition in West Central Montana. MSc Thesis, University of Montana, Montana: 1-89.

Pastula EC. 2012. Insect timing and succession on buried carrion in East Lansing, Michigan. MSc Thesis, Michigan State University: 1-33.

Payne JA. 1965. A summer carrion study of the baby pig *Sus Scrofa Linnaeus*. Ecology 46(5): 592-602.

Pinheiro J, 2006. Decomposition process of a cadaver. In: A. Schmitt, E. Cunha, J. Pinheiro (Eds.) Forensic Anthropology and Medicine: Complementary sciences from recovery to cause of death**.** Humana Press, New Jersey: 85–116.

Pokines, JT, Symes, SA. 2014. Manual of forensic taphonomy. CRC Press. Taylor & Francis Group: 73-88.

Pounder DJ. 2000. Postmortem interval. Encyclopaedia of Forensic Sciences. London Academic Press: 1167-1172.

Prangnell J, McGowan G. 2009. Soil temperature calculation for burial site analysis. Forensic Science International, 191: 104-109.

Prieto JL, Concepción Magaña DDS, Ubelaker DH. 2004. Interpretation of post-mortem change in cadavers in Spain. Journal of Forensic Science, 49(5): 1-6.

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

Schultz JJ, Collins ME, Falsetti AB. 2006. Sequential monitoring of burials containing large pig cadavers using ground-penetrating radar. Journal of Forensic Science, 51(3): 607-616.

Schoenly KG, Haskell NH, Mills DK, Bieme-Ndi C, Larsen K, Yer L. 2006. Recreating death's acre in the school yard: Using pig carcasses as model corpses to teach concepts of forensic entomology & ecological succession. The American Biology Teacher, 68(7): 402-410.

Schotsmans EMJ, van de Voorde W, de Winne J, Wilson AS. 2011. The impact of shallow burial on differential decomposition to the body: A temperate case study. Forensic Science International, 206: e43-e48.

Shepherd R. 2003. Simpson's forensic medicine. 12th Edition. Oxford University Press Inc: 37-48.

Simmons T, Cross PA, Adlam RE, Moffat C 2010. The influence of insects on decomposition rate in buried and surface remains. Journal of Forensic Science, 55(4): 889-892.

Sorg MH, Haglund WD, Wren JA. 2012. Current research in forensic taphonomy. In: A companion to forensic anthropology. Dirkmaat DC. 1st Edition. Blackwell Publishing Ltd: 477-525.

Suckling JK. 2011. A longitudinal study on the outdoor human decomposition sequence in Central Texas. MSc Thesis, Texas State University, San Marcos: 1-106.

Sutherland A, Myburgh J, Steyn M, Becker PJ. 2013. The effect of body size on the rate of decomposition in a temperate region of South Africa. Forensic Science International, 231: 257-262.

Taylor M. 2011. A taphonomic study and post-mortem interval formula comparison. MSc dissertation, Cranfield University, p1-98.

Thew HA. 2000. Effects of lime on the decomposition rate of buried remains. MSc dissertation, University of Indianapolis Archaeology and Forensics Laboratory.

Tibbett M. 2010. Blood, guts, gore and soil: decomposition processes in graves and forensic taphonomic applications. 19th World congress of soil science, soil solutions for a changing world.

Tracqui A. 2000. Time since death. Encyclopaedia of Forensic Sciences. London Academic Press: 1357-1363.

Troutman L, Moffat C, Simmons T. 2014. A preliminary examination of differential decomposition patterns in mass graves. Journal of Forensic Science, 59(3): 621-626.

Turner AR, Karacaoglu E, Namli A, Keten A, Farasat S, Akcan R, Sert O, Odabasi AB. 2013. Effects of different types of soil on decomposition: An experimental study. Legal Medicine. http://dx.doi.org/10.1016/j.legalmed2012.11.003

Turner B. 1987. Forensic entomology: insects against crime. Science Progress, 71(1): 133-144.

Turner B, Wiltshire P. 1999. Experimental validation of forensic evidence: a study of the decomposition of buried pigs in a heavy clay soil. Forensic Science International 101: 113-122.

Ubelaker DH, Zarenco KM. 2011. Adipocere: What is known after two centuries of research. Forensic Science International 208: 167-172.

Van Schoor M, Nienaber WC, Marais-Werner A. 2015. A controlled monitoring study of simulated clandestine graves using 3D ground penetrating radar. EAGE Near Surface Geoscience 2015 Conference and Exhibition, Turin, Italy. (Unpublished study).

Van Veen, JA, Kuikman, PJ. 1990. Soil structural aspects of decomposition of organic matter by micro-organisms. Biogeochemistry 11: 213-233.

Vass AA, Bass WM, Wolt J, Foss J, Ammons J. 1992. Time since death determinations of human cadavers using soil solutions. Forensic Science, 37: 1236-1253.

Vass AA. 2001. Beyond the grave – understanding human decomposition. Microbiology Today, 28: 190-192.

Vass AA, Smith RR, Thompson CV, Burnett MN, Dulgerian N, Eckenrode BA. 2008. Odor analysis of decomposing buried human remains. Journal of Forensic Science, 53(2): 384-391.

Vass AA. 2010. Dust to dust. The brief, eventful afterlife of a human corpse. Scientific America. www.ScientificAmerica.com.

Williams KA, Villet MH. 2006. A history of southern African research relevant to forensic entomology. South African Journal of Science, 102: 59-65.

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

ANNEXURES

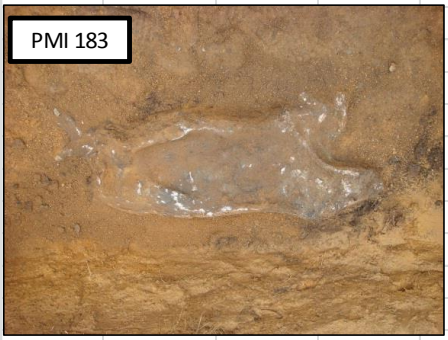
Annexure 1: Data for the post mortem interval values, head and neck scores, trunk scores, limb scores, total body score values, average temperature and accumulated degree-days values of TBS vs PMI (in calendar days) for each pig

Pig	Season	Date	Observation	han_sco	tru_sco	lim_sco	PMI	tbs_val	tem_avg	add_val
Pig 1	Spring	30/09/2014	1	2	4	1	1	7	17.7	17.7
	Autumn	31/03/2015	2	8	7	6	183	21	21	3860.6
80 kg										
L 114 cm										
H 57 cm										
W 22 cm										
BH 38 cm										
Male										

Pig	Season	Date	Observation	han_sco	tru_sco	lim_sco	PMI	tbs_val	tem_avg	add_val
Pig 3	Spring	30/09/2014	1	2	3	1	1	6	17.7	17.7
	Autumn	31/03/2015	2	9	6	6	183	21	21	3860.6
45 kg										
L 119 cm										
H 38 cm										
W 17 cm										
BH 29 cm										
Male										

Pig	Season	Date	Observation	han_sco	tru_sco	lim_sco	PMI	tbs_val	tem_avg	add_val
Pig 4	Spring	30/09/2014	1	2	4	3	1	9	17.7	17.7
	Autumn	31/03/2015	2	10	7	6	183	23	21	3860.6
45 kg										
L 108 cm										
H 39 cm										
W 20 cm										
BH 28 cm										
Male										

Pig	Season	Date	Observation	han_sco	tru_sco	lim_sco	PMI	tbs_val	tem_avg	add_val
Pig 6	Spring	30/09/2014	1	2	2	1	1	5	17.7	17.7
	Autumn	31/03/2015	2	10	9	6	183	25	21	3860.6
70 kg										
L 127 cm										
H 45 cm										
W 22 cm										
BH 24 cm										
Male										



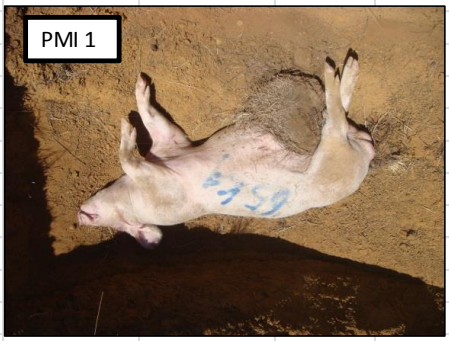
Pig	Season	Date	Observation	han_sco	tru_sco	lim_sco	PMI	tbs_val	tem_avg	add_val
Pig 7	Spring	30/09/2014	1	1	1	1	1	3	17.7	17.7
	Summer	31/03/2015	2	8	7	6	183	21	21	3860.6
73 kg										
L 130 cm										
H 52 cm										
W 19 cm										
BH 26 cm										
Male										

Pig	Season	Date	Observation	han_sco	tru_sco	lim_sco	PMI	tbs_val	tem_avg	add_val
Pig 15	Spring	09/10/2014	1	3	2	1	1	6	21.2	21.2
	Spring	10/11/2014	2	5	6	5	33	16	20.1	665
70kg										
L 130cm										
H 58cm										
W 23cm										
BH 35cm										
Male										

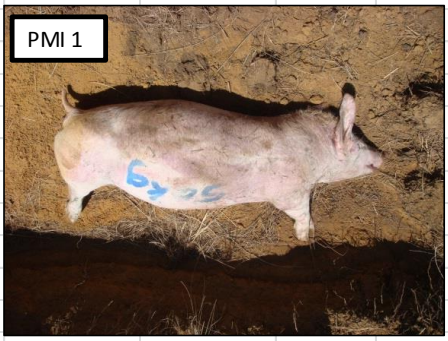
Pig	Season	Date	Observation	han_sco	tru_sco	lim_sco	PMI	tbs_val	tem_avg	add_val
Pig 16	Spring	09/10/2014	1	2	3	1	1	6	21.2	21.2
	Spring	10/11/2014	2	8	6	6	33	20	20.1	665
80kg										
L 132cm										
H 53cm										
W 21cm										
BH 24cm										
Male										

Pig	Season	Date	Observation	han_sco	tru_sco	lim_sco	PMI	tbs_val	tem_avg	add_val
Pig 17	Spring	09/10/2014	1	2	2	1	1	5	21.2	21.2
	Spring	10/11/2014	2	6	6	3	33	15	20.1	665
80kg										
L 131cm										
H 47cm										
W 24cm										
BH 27cm										
Male										

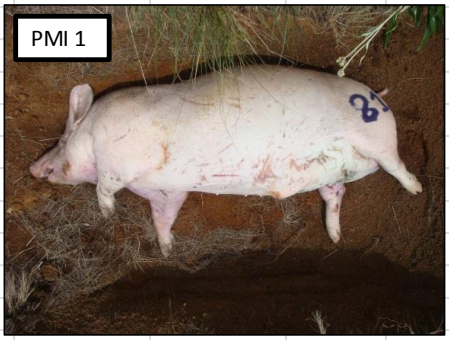
Pig	Season	Date	Observation	han_sco	tru_sco	lim_sco	PMI	tbs_val	tem_avg	add_val
Pig 19	Spring	09/10/2014	1	1	1	1	1	3	21.2	21.2
	Spring	10/11/2014	2	7	6	3	33	16	20.1	665
65kg										
L 125cm										
H 46cm										
W 19cm										
BH 24cm										
Male										



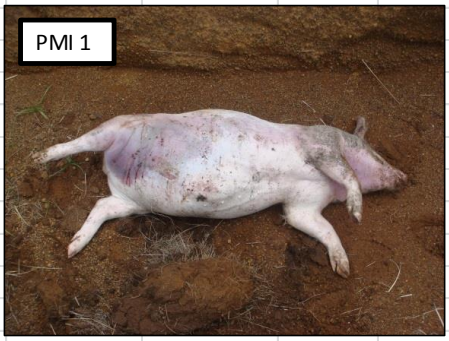
Pig	Season	Date	Observation	han_sco	tru_sco	lim_sco	PMI	tbs_val	tem_avg	add_val
Pig 20	Spring	09/10/2014	1	2	1	1	1	4	21.2	21.2
	Spring	10/11/2014	2	8	6	6	33	20	20.1	665
50kg										
L 110cm										
H 46cm										
W 20cm										
BH 28cm										
Male										



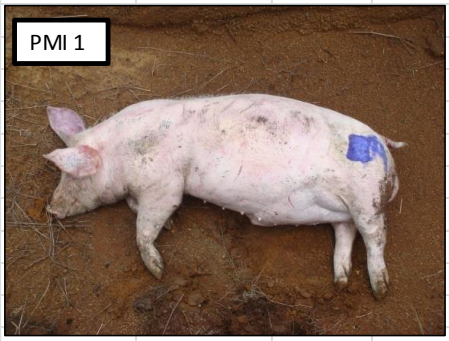
Pig	Season	Date	Observation	han_sco	tru_sco	lim_sco	PMI	tbs_val	tem_avg	add_val
Pig 21	Spring	24/11/2014	1	2	4	2	1	8	19.6	19.6
	Summer	08/12/2014	2	5	6	2	14	13	22.3	313.1
80kg										
L 135 cm										
H 57 cm										
W 25 cm										
BH 37 cm										
Male										



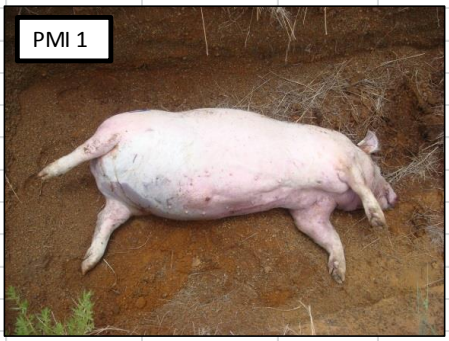
Pig	Season	Date	Observation	han_sco	tru_sco	lim_sco	PMI	tbs_val	tem_avg	add_val
Pig 22	Spring	24/11/2014	1	1	4	2	1	7	19.6	19.6
	Summer	08/12/2014	2	5	6	3	14	14	22.3	313.1
66kg										
L 98 cm										
H 38 cm										
W 24 cm										
BH 31 cm										
Male										



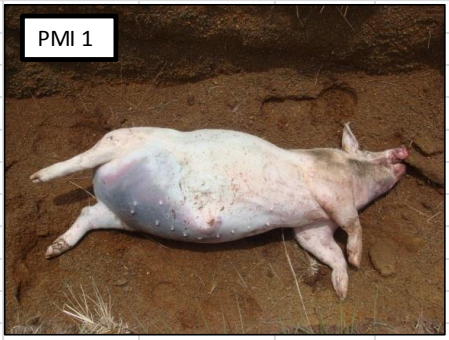
Pig	Season	Date	Observation	han_sco	tru_sco	lim_sco	PMI	tbs_val	tem_avg	add_val
Pig 24	Spring	24/11/2014	1	2	1	1	1	4	19.6	19.6
	Summer	08/12/2014	2	5	5	3	14	13	22.3	313.1
60 kg										
L 108 cm										
H 35 cm										
W 18 cm										
BH 29 cm										
Male										



Pig	Season	Date	Observation	han_sco	tru_sco	lim_sco	PMI	tbs_val	tem_avg	add_val
Pig 25	Spring	24/11/2014	1	4	4	2	1	10	19.6	19.6
	Summer	08/12/2014	2	5	6	2	14	13	22.3	313.1
60 kg										
L 123 cm										
H 45 cm										
W 26 cm										
BH 35 cm										
Male										

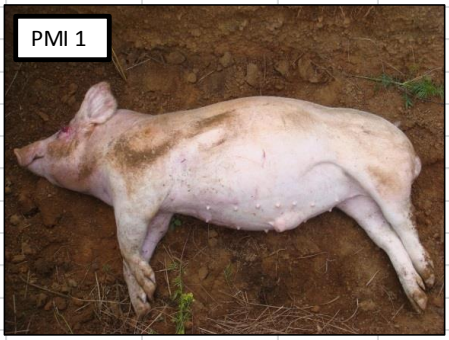


Pig	Season	Date	Observation	han_sco	tru_sco	lim_sco	PMI	tbs_val	tem_avg	add_val
Pig 26	Spring	24/11/2014	1	1	3	1	1	5	19.6	19.6
	Summer	08/12/2014	2	5	6	3	14	14	22.3	313.1
58 kg										
L 114 cm										
H 41 cm										
W 23 cm										
BH 35 cm										
Male										



Pig	Season	Date	Observation	han_sco	tru_sco	lim_sco	PMI	tbs_val	tem_avg	add_val
Pig 28	Autumn	19/03/2015	1	1	1	1	1	3	21.2	21.2
	Winter	18/06/2015	2	9	6	5	92	20	7.3	1437.1
45kg										
L 108cm										
H 47cm										
W 25cm										
BH 37cm										
Male										

Pig	Season	Date	Observation	han_sco	tru_sco	lim_sco	PMI	tbs_val	tem_avg	add_val
Pig 29	Autumn	19/03/2015	1	2	1	1	1	4	21.2	21.2
	Winter	18/06/2015	2	8	8	6	92	22	7.3	1437.1
60kg										
L 137cm										
H 49cm										
W 26cm										
BH 32cm										
Male										



Pig	Season	Date	Observation	han_sco	tru_sco	lim_sco	PMI	tbs_val	tem_avg	add_val
Pig 30	Autumn	19/03/2015	1	1	1	1	1	3	21.2	21.2
	Winter	18/06/2015	2	9	6	6	92	21	7.3	1437.1

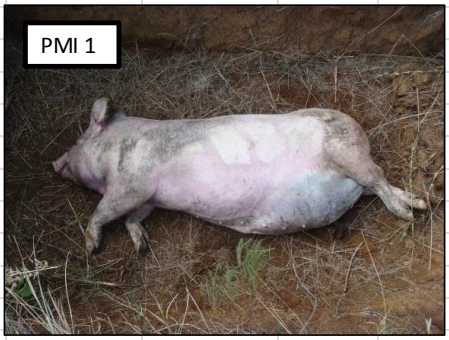
55kg
L 115cm
H 47cm
W 21cm
BH 23cm

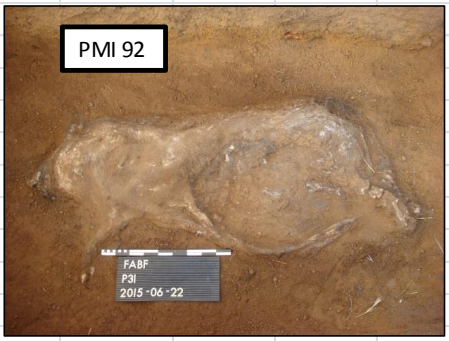
Male

Pig	Season	Date	Observation	han_sco	tru_sco	lim_sco	PMI	tbs_val	tem_avg	add_val
Pig 31	Autumn	23/03/2015	1	2	3	1	1	6	20.8	20.8
	Winter	22/06/2015	2	8	6	6	92	20	9.5	1388.95

70kg
L 118cm
H 47cm
W 24cm
BH 27cm

Female





Pig	Season	Date	Observation	han_sco	tru_sco	lim_sco	PMI	tbs_val	tem_avg	add_val
Pig 32	Autumn	23/03/2015	1	1	1	1	1	3	20.8	20.8
	Winter	22/06/2015	2	8	6	5	92	19	9.5	1388.95

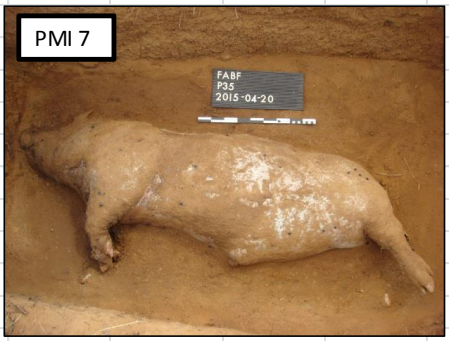
48kg
L 102cm
H 41cm
W 19cm
BH 18cm

Female

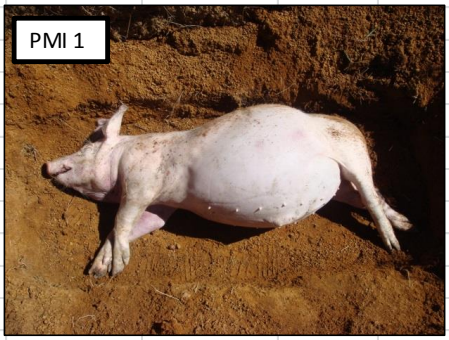
Pig	Season	Date	Observation	han_sco	tru_sco	lim_sco	PMI	tbs_val	tem_avg	add_val
Pig 33	Autumn	13/04/2015	1	1	1	1	1	3	17	17
	Autumn	20/04/2015	2	3	4	3	7	10	16.3	133.1
80kg										
L125cm										
H47cm										
W24cm										
BH28cm										
Male										

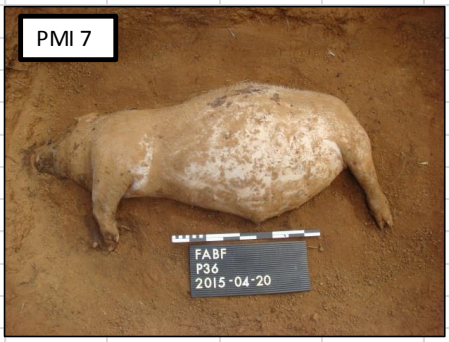
Pig	Season	Date	Observation	han_sco	tru_sco	lim_sco	PMI	tbs_val	tem_avg	add_val
Pig 34	Autumn	13/04/2015	1	1	3	1	1	5	17	17
	Autumn	20/04/2015	2	5	4	2	7	11	16.3	133.1
75kg										
L111cm										
H50cm										
W28cm										
BH33cm										
Female										

Pig	Season	Date	Observation	han_sco	tru_sco	lim_sco	PMI	tbs_val	tem_avg	add_val
Pig 35	Autumn	13/04/2015	1	1	1	1	1	3	17	17
	Autumn	20/04/2015	2	2	3	3	7	8	16.3	133.1
76kg										
L127cm										
H42cm										
W25cm										
BH29cm										
Female										

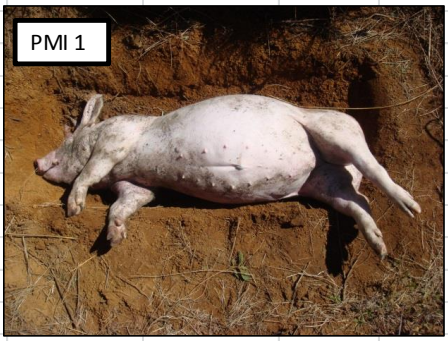


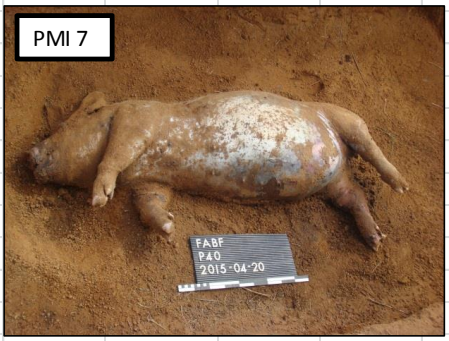
Pig	Season	Date	Observation	han_sco	tru_sco	lim_sco	PMI	tbs_val	tem_avg	add_val
Pig 36	Autumn	13/04/2015	1	1	1	1	1	3	17	17
	Autumn	20/04/2015	2	5	2	2	7	9	16.3	133.1
45kg										
L98cm										
H41cm										
W22cm										
BH34cm										
Male										





Pig	Season	Date	Observation	han_sco	tru_sco	lim_sco	PMI	tbs_val	tem_avg	add_val
Pig 40	Autumn	13/04/2015	1	1	1	1	1	3	17	17
	Autumn	20/04/2015	2	5	3	2	7	10	16.3	133.1
64kg										
L108cm										
H44 cm										
W23 cm										
BH32 cm										
Female										





Annexure 2: Interobserver data analyses

Annexure 2a: Results of the repeatability of head and neck scores (Orig = Original score and Inter = Interobserver score)

Pig	Observation	han_sco (Orig)	han_sco (Inter)
1	1	2	2
3	1	2	2
3	2	9	9
4	1	2	2
4	2	10	9
6	1	2	1
6	2	10	10
7	1	1	1
7	2	8	7
15	2	5	5
16	2	8	8
17	1	2	2
17	2	6	6
19	2	7	7
20	2	8	10

Head and Neck

Inter-Class Correlation Score:

0.979

Annexure 2b: Results of the repeatability of trunk scores (Orig = Original score and Inter = Interobserver score)

Pig	Observation	tru_sco (Orig)	tru_sco (Inter)
1	1	4	1
3	1	3	3
3	2	6	6
4	1	4	3
4	2	7	8
6	1	2	1
6	2	9	6
7	1	1	1
7	2	7	6
15	2	6	6
16	2	6	5
17	1	2	2
17	2	6	6
19	2	6	8
20	2	6	6

Trunk

Inter-Class Correlation Score:

0.838

Annexure 2c: Results of the repeatability of limb scores (Orig = Original score and Inter = Interobserver score)

Pig	Observation	lim_sco (Orig)	lim_sco (Inter)
1	1	1	1
3	1	1	1
3	2	6	6
4	1	3	1
4	2	6	7
6	1	1	1
6	2	6	8
7	1	1	1
7	2	6	6
7	2	6	6
15	2	5	3
16	2	6	7
17	1	1	1
17	2	3	3
19	2	3	3
20	2	6	8

Limb

Inter-Class Correlation Score:

0.913

Annexure 2d: Results of the repeatability of Total Body Scores (TBS) (Orig = Original score and Inter = Interobserver score)

Pig	Observation	tbs_sco (Orig)	tbs_sco (Inter)
1	1	7	4
3	1	6	6
3	2	21	21
4	1	9	6
4	2	23	24
6	1	5	3
6	2	25	24
7	1	3	3
7	2	21	19
15	2	16	12
16	2	20	20
17	1	5	5
17	2	15	10
19	2	16	18
20	2	20	24

Total Body Score

Inter-Class Correlation Score:

0.953

Annexure 3: Temperature and rainfall data received from the South African Weather Service (Bronkhorstspruit Station)

Date	Min Temperature (°C)	Maximum Temperature (°C)	Average Temperature (°C)	Rainfall (mm)
30-Sep-14	8.1	27.3	17.7	0
01-Oct-14	6.7	27.1	19.9	0
02-Oct-14	8.2	24.5	16.3	0
03-Oct-14	8.4	24.6	16.5	1.2
04-Oct-14	7	22.9	14.9	0.2
05-Oct-14	6.7	25.9	16.3	0
06-Oct-14	7.5	28	17.7	0
07-Oct-14	8.3	28.7	18.5	0
08-Oct-14	6.7	30.7	18.7	0
09-Oct-14	9.4	33	21.2	0
10-Oct-14	14	28.5	21.2	1.8
11-Oct-14	12.3	21.9	17.1	0
12-Oct-14	13.8	30.4	22.1	0.2
13-Oct-14	13.9	33.1	23.5	0
14-Oct-14	11.8	29.1	20.4	0
15-Oct-14	15.2	27.2	21.2	0.4
16-Oct-14	12.8	28.1	20.4	0.2
17-Oct-14	8.9	23.4	16.1	0
18-Oct-14	6.3	25.2	15.7	0
19-Oct-14	5.4	29.7	17.5	0
20-Oct-14	8.1	30.6	19.3	0
21-Oct-14	12.5	29.5	21	0.6
22-Oct-14	11.3	28.8	20	2.6
23-Oct-14	10.9	28.5	19.7	4.2
24-Oct-14	13.2	31	22.1	0.2
25-Oct-14	11.8	27.5	19.6	19.4
26-Oct-14	12.5	20.6	16.5	0.2
27-Oct-14	10.1	23.3	16.7	0

28-Oct-14	11.1	27.6	19.3	0
29-Oct-14	12	29.9	20.9	0
30-Oct-14	14.4	32.7	23.5	0
31-Oct-14	15.7	32.7	24.2	0
01-Nov-14	16.1	26.7	21.4	0
02-Nov-14	13.7	24.3	19	6.2
03-Nov-14	15.4	26.4	20.9	2
04-Nov-14	14.1	25.8	19.9	0.2
05-Nov-14	10.5	28.8	19.6	0
06-Nov-14	11.2	26.8	19	0
07-Nov-14	14.8	30.3	22.5	0
08-Nov-14	14.2	27.8	21	7
09-Nov-14	15.6	30.4	23	1.4
10-Nov-14	15.6	23.5	19.5	33.4
11-Nov-14	14.7	20.2	17.4	2.2
12-Nov-14	15.4	20.4	19.9	0.2
13-Nov-14	15	27.1	21	0.2
14-Nov-14	13.9	31.1	22.5	0
15-Nov-14	12.8	22	17.4	8.6
16-Nov-14	9	27.2	18.1	5.4
17-Nov-14	9.3	19.5	14.4	7.8
18-Nov-14	9.3	20.9	15.1	0
19-Nov-14	7.5	24	15.7	0.2
20-Nov-14	11.5	27.2	19.3	0
21-Nov-14	10.5	29	19.7	0
22-Nov-14	15.2	29.1	22.1	8.8
23-Nov-14	13	21.7	17.3	5.6
24-Nov-14	11.1	28.2	19.6	0.8
25-Nov-14	14.8	30.4	22.6	0
26-Nov-14	14	30.4	22.2	44
27-Nov-14	14	20.6	17.3	0
28-Nov-14	12.9	28.3	20.6	0
29-Nov-14	14.3	27.1	20.7	14

30-Nov-14	12.1	25.5	18.8	6
01-Dec-14	11.4	28	19.7	0.2
02-Dec-14	14.2	28.2	21.2	0
03-Dec-14	13.4	29.2	21.3	0
04-Dec-14	14.6	30.9	22.7	0
05-Dec-14	14.4	32.2	23.3	1
06-Dec-14	14.5	28.6	21.5	4
07-Dec-14	14.5	27.9	21.2	1.4
08-Dec-14	13.9	26.9	20.4	15.6
09-Dec-14	14.5	21.5	18	3.2
10-Dec-14	15.3	27.8	21.5	4.6
11-Dec-14	15.4	28.9	22.1	6.4
12-Dec-14	15.4	25.7	20.5	0.4
13-Dec-14	15.4	28.2	21.8	0
14-Dec-14	16.4	26.3	21.3	0
15-Dec-14	15.7	28.7	22.2	1.6
16-Dec-14	15	21.3	18.1	1.6
17-Dec-14	14.9	20.1	17.5	10.2
18-Dec-14	16.6	26.9	21.7	5
19-Dec-14	15.8	28.6	22.2	22.4
20-Dec-14	16.2	29.8	23	0
21-Dec-14	13.3	31.1	22.2	1.4
22-Dec-14	17.3	29.8	23.5	0.2
23-Dec-14	18.2	28.2	23.2	2.6
24-Dec-14	16.4	31.3	23.8	0.2
25-Dec-14	17.3	32	24.6	0.4
26-Dec-14	18.8	31.5	25.1	33.6
27-Dec-14	17.4	23.1	20.2	29.4
28-Dec-14	18.3	25.7	22	4.6
29-Dec-14	16.4	25.8	21.1	0
30-Dec-14	13.3	30.9	22.1	0.2
31-Dec-14	17.4	26	21.7	0
01-Jan-15	14.2	25.8	20	7.6

02-Jan-15	16.6	29.6	23.1	0
03-Jan-15	16.1	30.5	23.3	0.4
04-Jan-15	13.2	32.5	22.8	0
05-Jan-15	16.9	31.6	24.2	0
06-Jan-15	16.1	33.1	24.6	0
07-Jan-15	16.9	28.5	22.7	0
08-Jan-15	17.4	30.6	24	0
09-Jan-15	17	31.6	24.3	0
10-Jan-15	17.3	31.3	24.3	0
11-Jan-15	17.6	31.2	24.2	0
12-Jan-15	14.4	30	22.2	0
13-Jan-15	15.8	32.2	24	6.2
14-Jan-15	17.5	24	20.7	0.4
15-Jan-15	16.5	28.5	22.5	0
16-Jan-15	18.3	28.2	23.2	0
17-Jan-15	15.8	31.7	23.7	0
18-Jan-15	16.3	26.4	21.3	0
19-Jan-15	15.4	27.7	21.5	1
20-Jan-15	14.6	27.3	20.9	0
21-Jan-15	12.9	29.3	21.1	0
22-Jan-15	15.6	31.4	23.5	3.4
23-Jan-15	13.1	31.8	22.4	0
24-Jan-15	15.5	32	23.7	0
25-Jan-15	16.1	30.3	23.2	0
26-Jan-15	15.2	30.7	22.9	1.4
27-Jan-15	18.6	31.4	25	9.8
28-Jan-15	16.3	22.9	19.6	1.2
29-Jan-15	15.5	25.2	20.3	9.2
30-Jan-15	14.9	28.5	21.7	0
31-Jan-15	17	28.8	22.9	0.2
01-Feb-15	17	28.8	22.9	2.2
02-Feb-15	17.2	29.2	23.2	0.2
03-Feb-15	12.6	30.3	21.4	0

04-Feb-15	16.4	31.2	23.8	0
05-Feb-15	14.3	31.1	22.7	0
06-Feb-15	13.1	31.2	22.1	0
07-Feb-15	13.6	32	22.8	0
08-Feb-15	12.9	32	22.4	0
09-Feb-15	16.7	33	24.8	0.4
10-Feb-15	16.6	33.1	24.8	6.4
11-Feb-15	15.1	34.1	24.6	0.2
12-Feb-15	16.5	31.4	23.9	0
13-Feb-15	17.3	31.4	24.3	5.6
14-Feb-15	17.7	30	23.8	0.2
15-Feb-15	12.2	30.5	21.3	0
16-Feb-15	15.3	27.7	21.5	0
17-Feb-15	15.5	27.2	21.3	2.6
18-Feb-15	15.2	29.9	22.5	0
19-Feb-15	12.7	32.8	22.7	0
20-Feb-15	13.8	32.2	23	0
21-Feb-15	14.4	30.8	22.6	0
22-Feb-15	15	31.8	23.4	0
23-Feb-15	15.1	28.6	21.8	0.2
24-Feb-15	13.6	30.3	21.9	5.6
25-Feb-15	15.4	28	21.7	0
26-Feb-15	14.6	30.5	22.5	0
27-Feb-15	16.1	25.5	20.8	0.2
28-Feb-15	14.8	27.8	21.3	0
01-Mar-15	12.9	28.3	20.6	1.4
02-Mar-15	14.4	23.5	18.9	0
03-Mar-15	12.6	26.2	19.4	0
04-Mar-15	8.5	29	18.7	0
05-Mar-15	10	30.7	20.3	0
06-Mar-15	15.8	29.3	22.5	2.6
07-Mar-15	15.1	25.7	20.4	6.4
08-Mar-15	13.3	29.8	21.5	1.6

09-Mar-15	13.8	28.8	21.3	0
10-Mar-15	10.6	29.7	20.1	0
11-Mar-15	12.5	30.9	21.7	0
12-Mar-15	14.5	26.9	20.7	0.6
13-Mar-15	13.5	29.9	21.7	0
14-Mar-15	13.8	29.4	21.6	1.4
15-Mar-15	15.2	31.3	23.2	1.8
16-Mar-15	15.3	30.1	22.7	0.2
17-Mar-15	12.8	31.5	22.1	0
18-Mar-15	13.9	28.1	21	1
19-Mar-15	14.6	27.9	21.2	16.8
20-Mar-15	13.1	30.1	21.6	0
21-Mar-15	14.3	28.1	21.2	5.8
22-Mar-15	15.5	24.9	20.2	1.6
23-Mar-15	14.9	26.8	20.8	0
24-Mar-15	12.4	28.4	20.4	0
25-Mar-15	17.1	28.3	22.7	1
26-Mar-15	14.6	26.8	20.7	1.2
27-Mar-15	12.6	29.8	21.2	0
28-Mar-15	13	27.5	20.2	0
29-Mar-15	13	26	19.5	0.6
30-Mar-15	11.4	26.8	19.1	0
31-Mar-15	14.2	25.6	19.9	0
01-Apr-15	12.5	29.3	20.9	0
02-Apr-15	11	29.6	20.3	0
03-Apr-15	12	26.2	19.1	0
04-Apr-15	15.4	24.5	19.95	0
05-Apr-15	10.8	26.5	18.65	0
06-Apr-15	9.7	27.9	18.8	0
07-Apr-15	15.1	25	20.05	4
08-Apr-15	11.2	23.4	17.3	0
09-Apr-15	10.7	25.6	18.15	2.8
10-Apr-15	9.3	23.1	16.2	0

11-Apr-15	8.5	24.6	16.55	0
12-Apr-15	11.7	27.3	19.5	0
13-Apr-15	7.7	26.3	17	0
14-Apr-15	6.8	27.2	17	0
15-Apr-15	9.2	27.1	18.15	0
16-Apr-15	5.4	26.6	16	0
17-Apr-15	11.6	24.6	18.1	0.2
18-Apr-15	9.9	18.3	14.1	0
19-Apr-15	10	22.8	16.4	0.2
20-Apr-15	8.2	24.5	16.35	0.4
21-Apr-15	6.3	27	16.65	0.2
22-Apr-15	10.6	23.6	17.1	0.2
23-Apr-15	10	26.7	18.35	0
24-Apr-15	11.5	22.7	17.1	2.6
25-Apr-15	9.4	27.4	18.4	0.4
26-Apr-15	8.2	28.7	18.45	0
27-Apr-15	7.4	28.4	17.9	3.2
28-Apr-15	6.4	27.5	16.95	0
29-Apr-15	6.3	28.1	17.2	0
30-Apr-15	5.7	27.2	16.45	0
01-May-15	-0.1	26.9	13.4	0
02-May-15	1.3	25.4	13.35	0
03-May-15	1.6	27.8	14.7	0
04-May-15	2.2	28	15.1	0
05-May-15	3.5	27.1	15.3	0
06-May-15	4.3	28.8	16.55	0
07-May-15	4.3	24.3	14.3	0
08-May-15	8.4	24.1	16.25	0
09-May-15	6.1	26	16.05	0
10-May-15	6	25.9	15.95	0
11-May-15	6.8	23.9	15.35	0.4
12-May-15	6.9	23.8	15.35	0
13-May-15	5.1	24.6	14.85	0

14-May-15	0.7	27.5	14.1	0
15-May-15	1.4	28.3	14.85	0
16-May-15	4.5	28.1	16.3	0
17-May-15	3.9	27	15.45	0
18-May-15	6.9	26.1	16.5	0
19-May-15	3.4	26.7	15.05	0
20-May-15	2.4	27.3	14.85	0
21-May-15	3.3	26.5	14.9	0
22-May-15	3.1	23.1	13.1	0
23-May-15	3.7	22.4	13.05	0
24-May-15	2	25.1	13.55	0
25-May-15	1.7	25.3	13.5	0
26-May-15	2	27	14.5	0
27-May-15	2.3	27.2	14.75	0
28-May-15	2	24.9	13.45	0
29-May-15	1.1	27.1	14.1	0
30-May-15	1.6	27.6	14.6	0
31-May-15	1.6	25.9	13.75	0
01-Jun-15	2.3	24.4	13.35	0
02-Jun-15	3.9	21.1	12.5	0
03-Jun-15	9.4	20.8	15.1	0
04-Jun-15	2	17.2	9.6	0
05-Jun-15	-1.6	17.8	8.1	0
06-Jun-15	-1.3	16.2	7.45	0
07-Jun-15	-2	18.1	8.05	0
08-Jun-15	-2.5	20.9	9.2	0
09-Jun-15	-2.6	21.5	9.45	0
10-Jun-15	-1.5	18.2	8.35	0
11-Jun-15	-0.3	19.5	9.6	0.4
12-Jun-15	3.6	19.8	11.7	0.2
13-Jun-15	-0.5	21.3	10.4	0
14-Jun-15	-1.9	22.8	10.45	0
15-Jun-15	-2.7	23.9	10.6	0

16-Jun-15	-2.4	21.9	9.75	0
17-Jun-15	-0.2	15.2	7.5	0
18-Jun-15	-4.3	18.9	7.3	0
19-Jun-15	-3.7	20.8	8.55	0
20-Jun-15	-1.6	18.8	8.6	0
21-Jun-15	-1.9	20.6	9.35	0
22-Jun-15	-1.2	20.3	9.55	0

Annexure 4: Ethics clearance certificates from the Faculty of Natural and Agricultural Sciences, Animal Ethics Committee, Faculty of Veterinary Sciences, and the MSc Committee of the School of Medicine, Faculty of Health Sciences, University of Pretoria

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

ETHICS COMMITTEE

Faculty of Natural and Agricultural Sciences

21 August 2014

Ms A Marais

School of Health Care Sciences

Faculty of Health Sciences

University of Pretoria

0002

Dear Ms Marais

EC140721-068 Decomposition patterns of buried remains in the northern region of South Africa

Your application conforms to the requirements of the NAS Ethics Committee

Kind regards

Prof NH Casey

Chairman: Ethics Committee

Agriculture Building 10-20
University of Pretoria
Private bag X20, Hatfield 0028
Republic of South Africa

Tel: 012 420 4107
Fax: 012 420 3290

ethics.nas@up.ac.za

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**Decomposition patterns of buried remains in the central Highveld region of South Africa**
PROJECT NUMBER	**H009-14**
RESEARCHER/PRINCIPAL INVESTIGATOR	**A Marais**

STUDENT NUMBER (where applicable)	**025 704 29**
DISSERTATION/THESIS SUBMITTED FOR	**MSc**

ANIMAL SPECIES	**Pigs**	
NUMBER OF ANIMALS	**30**	
Approval period to use animals for research/testing purposes		**September 2014 – September 2015**
SUPERVISOR	**Prof. M Steyn**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date	**29 September 2014**
CHAIRMAN: UP Animal Ethics Committee	Signature	

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA
Faculty of Health Sciences
Fakulteit van Gesondheidswetenskappe

Prof Riana Cockeran
Chair: MSc Committee
School of Medicine
Dr Savage Rd, Private Bag x323, Pretoria, 0001
Tel.: (012) 319-2624 Fax: (012) 323-0732
E-mail: riana.cockeran@up.ac.za

7 October 2014

Prof M Steyn
Department of Anatomy
Faculty of Health Sciences

Dear Prof Steyn,

Ms A Marais, Student no 02570459, MSc Committee submission

Thank you for submitting the above mentioned student's revised protocol to the MSc Committee. Hereby please accept the following recommendations as discussed during the meeting.

Student name	Ms A Marais	**Student number**	02570459
Name of study leader	Prof M Steyn		
Department	Anatomy		
Title of MSc	Decomposition patterns of buried remains in the central Highveld region of South Africa		
Date of first submission	August 2014		
September 2014	• Thank you for nominating the examiners, and submitting a copy of ethics approval.		
Decision	This **protocol** has been **approved**. **Ethics approval** obtained. The **internal** and **external examiners** have been **approved**.		

Yours sincerely
Prof Riana Cockeran
Chair: MSc Committee

Department of Immunology
University of Pretoria
PRETORIA 0002
Republic of South Africa

Tel: Number +27 (0)12 319 2624
Fax: Number +27 (0)12 323 0732

Email address riana.cockeran@up.ac.za
www.up.ac.za