Comparison of the Decomposition of Buried *Mus musculus* (House Mouse) Between Two Soil Types with Contrasting pH and its Potential to Assist Forensic Taphonomy

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Abstract

In murder cases, it is essential for investigators to be able to understand forensic taphonomy in order provide an accurate post mortem interval (PMI). However, a popular method of disposing of a corpse is done through burying in soil and this can be a problem for investigators as this will affect the PMI. The decomposition process of a human corpse in soil is rarely observed, so often animal carcasses are substituted in place. This report has used house mouse carcasses (*Mus musculus*) as human surrogates and aimed to compare the decomposition rate of these carcasses when buried in two contrasting soil types. The report then aimed to aid forensic taphonomy by differing from existing literature on this subject by replicating more realistic conditions that a potential human cadaver would usually be exposed to. This being by: not altering the soil from field standard, using whole organisms and allowing temperature to naturally fluctuate. The soil types chosen for the report were a podzolic soil (podzol) and a lithomorphic soil (rendzina) due to their contrasting pH. The method was conducted through burying 20 mice carcasses in each of the two soil types; 5 mice from each soil were exhumed at weekly intervals and the experiment concluded after 4 weeks. Decomposition was calculated by weighing the carcasses before burial and then once again after they had been exhumed. The result of this experiment indicated that there was no significant difference of decomposition in mice between the two soils ($p= 0.248$). This result contrasts from existing literature on animal decomposition in soil which was likely due to the differences in the methods applied. The result has potential implications for forensic taphonomy as other factors (moisture, temperature, oxygen supply and internal microbial activity) may have a larger impact on decomposition in soil than soil pH. Furthermore, the result highlights how whole organisms decompose differently in soil than just muscle tissue. The report also reveals how mice are effective surrogates for human cadavers due to their biological similarity. This subject requires further research as the abiotic conditions produced in the method did not completely replicate a real-life scenario.

Acknowledgements

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Contents

Introduction

The decomposition of an organism in soil is a complex process and the rate of which depends on numerous biotic and abiotic factors (Guo et al 2016). This rate of decomposition is important to forensic taphonomy in murder cases because, if understood accurately, then it can allow investigators to predict a precise post mortem interval (PMI) (Tibbett and Carter 2009; Metcalf et al 2013). This is important as it can be a defining piece of evidence on determining whether a suspect is guilty of causing a death or not. Therefore, conducting investigations on human decomposition is vital however, the process is often not observed; this being mainly due to ethical issues and the lack of available human cadavers (Tibbett 2010; Hitosugi et al 2006). Only a select number of reports in recent decades have been able to use human cadavers for testing (Morovic-Budak 1965; Spennemann and Franke 1995; Rodriguez and Bass 1983; Rodriguez and Bass 1985; Cobaugh, Schaeffer and DeBruyn 2015; Wilson et al 2007; Hyde et al 2013; Megyesi, Nawrocki and Haskell 2005). This lack of human cadavers often means animal carcasses are substituted as surrogates instead. Preferably, animals most similar to humans are used; Pigs (*Sus scrofa*) are popular because they are also mammals with similar sized bodies and organs (Payne 1965; Payne, King and Beinhart 1968; Gill 2005). However, other mammals or skeletal muscle tissue (SMT) can be used for observation. There have been many reports that have used animal or tissue surrogates to observe the decomposition process over the decades, with some dating back to the 19th century (Motter 1898). However, more recent reports demonstrate a more accurate understanding of the process in soil (Haslam and Tibbett 2009; Carter and Tibbett 2008a; Carter, Yellowlees and Tibbett 2008a; Stokes et al 2009; Carter, Yellowlees and Tibbett 2010; Hopkins, Wiltshire and Turner 2000; Tibbett et al 2004; Forbes et al 2004; Turner and Wiltshire 1999; Matuszewski et al 2014; Sagara 1976). In this report, conducted to observe the rate of decomposition in contrasting soils, house mice (*Mus musculus*) were used as surrogates and the soils that were selected were a podzolic soil (podzol) and a lithomorphic soil (rendzina).

The overall aim of this report was to compare the differences in decomposition of mice that were buried in two contrasting soil types; the main difference in soil type being their pH levels. This result would then be interpreted, with assistance from other literature, to conclude whether it could contribute to forensic taphonomy. To achieve this aim, several objectives were designed. Firstly, an experiment was conducted using the mice carcasses and involved several controlled variables including: temperature, soil weight, storage, soil depth and moisture to focus the report on just the soil properties. These variables were also set up to replicate realistic conditions that a buried organism would naturally be exposed to; the validity of the experiment would be assessed through literature that have conducted similar testing. Another objective was to attempt to explain the likely processes that these buried carcasses would have gone through and how different variables may have affected them; this to was examined through further literature. The final objective was to assess whether this result could be applied to forensic taphonomy. Literature was used to compare the similarities between mice carcasses and human cadavers and evaluate whether this comparison is valid; if valid then suggestions were made on how this result influences forensic taphonomy. The null hypothesis of this report was that soil type will not have a significant effect on the decomposition rates of the mice carcases. The alternative hypothesis was that as the time left buried increases, then so will the decomposition rates of the buried mice.

The intention behind the experiment was to replicate the more natural conditions that an organism would be exposed to whilst decomposing. This being because most other literature that have conducted observations on decomposition in a laboratory setting, vastly alter these natural conditions (Carter and Tibbett 2008a; Carter, Yellowlees and Tibbett 2010; Haslam and Tibbett 2009; Stokes, Forbes and Tibbett 2013; Tibbett et al 2004; Ross et al 1985; Carter and Tibbett 2006). These conditions are major factors that affect the decomposition rate. These reports often alter the soil from its natural state, add moisture and then incubate the samples at a constant temperature; all of which influence decomposition. This is done to keep the factors affecting the samples more similar to each other and reduce the variability. However, this does not reflect the natural conditions that an

organism would be exposed to whilst decomposing; therefore, meaning this literature may not actually offer much towards forensic taphonomy. This report kept conditions more natural through actions in the method. Firstly, the soil obtained in this report was kept at field standard; this being that the soil was not: sieved, dried out and then re-moistened. Secondly, all samples were exposed to natural temperature fluctuations of night and day. These other reports also use skeletal muscle tissue as their cadaver surrogate (Carter and Tibbett 2008a; Haslam and Tibbett 2009; Tibbett et al 2004; Carter and Tibbett 2006). Although this allows all samples to be the same starting weight, it also is not effective at replicating a whole human cadaver's decomposition. It would more likely aid forensic taphonomy in cases where peripheral body parts had been separated from a cadaver (Haslam and Tibbett 2009). Using mice carcasses as human surrogates may be more effective as there are more decomposition processes that occur inside an organism than just in muscle tissue. Mice and humans share many similar types of bacteria due to their similar gastrointestinal tract, comparable gut anatomy and are omnivores (Nguyen et al 2015). Mice are a favourite in biomedical research as human surrogates, however they are often not selected for decomposition analysis despite sharing these characteristics (Heshmati, Keene and Villano 2015). Mostly skeletal muscle tissue or pig carcasses are favourites for decomposition analysis; although, some previous studies have been conducted using rodents (Lauber et al 2014; Carter, Yellowlees and Tibbett 2008a; Carter, Yellowlees and Tibbett 2008b; Burcham et al 2016; Rosenthal and Brown 2007; Metcalf, Carter and Knight 2014; Stokes et al 2009; Metcalf et al 2013; Metcalf et al 2016; Micozzi 1986). In previous reports, it was revealed that there is no animal surrogate that would respond in the way human cadaver/tissue would (Stokes, Forbes and Tibbett 2013). Moreover, vegetation was not used as it is not an effective human surrogate as vegetation receives significantly different types of microbes that conduct decomposition (Olakanye, Thompson and Ralebitso-Senior 2015).

It is important that investigators understand decomposition because it can be very beneficial to forensic investigators when dealing with serious crimes such as manslaughter and murder (Taylor 2011). An accurate understanding of the stages

of decomposition and the factors that affect these stages will lead to an accurate estimation of the post-mortem interval (PMI) (Hayman and Oxenham 2016; Tibbett and Carter 2009; Metcalf et al 2013; Guo et al 2016; Vass et al 2002). This is a vital component when investigating a death (Taylor 2011). One of the existing PMI estimation techniques involves insect analysis from forensic entomologists. They observe what species are colonising the organism and can make an estimation on the time since death from the age of the insects that have hatched there (Megyesi, Nawrocki and Haskell 2005; Guo et al 2016). However, in criminal cases, a popular method of disposing of a body is to bury the cadaver under soil (Carter and Tibbett 2008b). This is because of the obvious fact that a body is less likely to be found if not left on the surface. This is a problem for investigators because soil can significantly reduce insect activity as it acts like a barrier. Therefore, making it more difficult to get an accurate estimation in this way if the body is discovered (Goff 2009). The burial of an organism will also cause it to decompose at a slower rate and mean the remains will be better preserved (VanLaerhoven and Anderson 1999; Megyesi, Nawrocki and Haskell 2005). The preservation will be due to this reduction in insect activity and soil characteristics will too have an effect. Instead microbial activity inside the organism and the soil will be the largest contributor to decomposition. This highlights a potential use in forensic taphonomy because microbial activity may be an effective method to calculate the PMI (Burcham et al 2016; Metcalf, Carter and Knight 2014; Metcalf et al 2013). Microbial activity may also be an effective indicator of a clandestine grave (Metcalf, Carter and Knight 2014). It is currently challenging for investigators to search for a cadaver under soil but it can be done through using certain methods. These methods include cadaver dogs which search for the scent of a decomposing corpse (Armstrong et al 2016). Another method is by searching for ninhydrin reactive nitrogen which is released into the soil following the decomposition of a corpse (Carter, Yellowlees and Tibbett 2008b). Vegetation can also give away signs of a clandestine grave; firstly, most vegetation in close proximity may die due to very high nitrogen levels in the soil following decomposition (Riggs and Hobbie 2016). However, this will subside and vegetation in the area will rebound due to the introduction of nutrients from the cadaver; some species of fungi can be particularly good indicators of clandestine graves (Tibbett and Carter 2003; Tranchida, Centeno and Cabello 2014; Hitosugi et al 2006; Ishii et al 2006). Once a corpse is discovered and exhumed,

identification of the corpse can be conducted through DNA analysis but can also be done in other ways: including facial recognition techniques (Işcan 2001).

As a result of mice and humans sharing characteristics they will experience similar decomposition (Nguyen et al 2015). While a mammalian carcass is buried under soil, many different biological and physical processes are involved with the decomposition of the organism; the rate of these processes are influenced by many abiotic conditions. Decomposition can generally be defined as the decay and break down of the body's tissues into smaller constituents (Hau et al 2014). This process occurs shortly after the organism has deceased and the rate of this is affected by several factors**.** The major factors that affect the rate of decomposition in soil include: environment temperature, oxygen access, moisture abundance, soil texture, soil pH and burial depth (Hayman and Oxenham 2016; Wang et al 2016). All of which were controlled in this report.

There are different stages of decomposition a mammalian organism will go through. These stages can be divided into six as described by Payne (1965): fresh stage, bloat stage, active decay stage, advanced decay stage, dry stage and remains stage. For this report, the early stages (fresh stage, bloat stage and active decay stage) were expected to be observed with the advanced decay stage possibly being observed; dry stage was unlikely to be observed. The remains stage would not have been observed due to the mice carcasses only being measured for a month and therefore not left for a long enough length of time (Payne 1965). All of these stages will be slowed by the burial in soil; the main cause of this effect being the reduction in oxygen supply (Carter et al 2006; Carter and Tibbett 2008b; VanLaerhoven and Anderson 1999). To ensure the tests only variable was soil type meant sealing the tubs to not allow any potential insect activity (Carter, Yellowlees and Tibbett 2007). Despite the chance of insect activity being low due to the samples being buried, it need to be conducted; this being because if some samples were accessed from insects and some were not then it could influence the results. This lack of any potential insect activity would further slow decomposition as it can be a major source of mass loss (Reed 1958; VanLaerhoven and Anderson 1999).

A process involved with the fresh stage of decomposition includes autolysis which is where the pH levels within cells begin to decrease as carbon dioxide levels increase (Fiedler and Graw 2003; Parkinson et al 2009). This increases acidity causing lysosomes to rupture and release their enzymes instigating the breakdown of the body's cells. Moreover, during the fresh stage the organism's natural microbial collection also begins the breakdown of the body's soft tissues and internal organs (Vass et al 2002). There are numerous sites where bacteria will be highest within an organism and these include: gastrointestinal tract, respiratory tract, oral cavity and genitalia (Wilson 2005; Burcham et al 2016; Hyde et al 2013). Of these bacteria will be in highest concentration in the gastrointestinal tract and especially in the jejunum, ileum and colon (Wilson 2005; Burcham et al 2016; Melvin et al 1984). These bacteria are useful to the organism when living so are kept under control but after death there are no longer any controls in place (Hyde et al 2013). These bacteria are able to spread from these locations to anywhere in the body through the lymph nodes and blood vessels (Janaway 1996; Burcham et al 2016). Initially after death, the bacteria that respire aerobically will dominate due to there still being oxygen available; once oxygen levels decrease within the carcass, bacteria that respire anaerobically will dominate (Hyde et al 2013).

Some fresh stage processes like rigor mortis, lividity and algor mortis would not be expected in the mice carcasses however. This is because the mice that were used in this report were transported frozen and then had to be thawed out; this freezethaw process would have certain effects on the fresh stage of decomposition when compared to mice that are fresh deceased (Micozzi 1986). This is because mice that have been freeze-thawed are already experiencing some decompositional processes. The report by Micozzi (1986) examined freeze-thawed rats internally; this highlighted organ tissue damage and major blood loss from nasal and oral mucosa when compared to freshly killed rats. The Stokes, Forbes and Tibbett (2009) report also revealed that freezing caused some damage to enzymes within some cells. However, as all mice were freeze-thawed in the same conditions it should have ensured that all samples were in the same

decompositional state. Moreover, the Stokes, Forbes and Tibbett (2009) report also revealed this method of freezing before testing does not have a significant effect on the overall decomposition process when compared to freshly deceased organisms.

After the initial fresh stage, the mice would begin to experience the bloat stage. This is where the internal bacteria have built up and released gases like hydrogen sulphide and methane which cause the body to swell (Goff 2009; Hau et al 2014). Eventually the gas would have begun to escape through the carcasses mouth, genitals and other body orifices. Moreover, the colour of the cadaver would have turned dark as putrefaction begins (Armstrong et al 2016). If the carcasses were left on the surface of the soil this bloat stage would have begun as early as 2 days into being left but as they were buried in soil it would have taken longer (Goff 2009). After the bloat stage the mice carcasses will have experienced both active decay and possibly started advanced decay (Vass 2001). The putrefaction causes tissues to turn to liquid; this liquid would leak from the body into the surrounding soil causing the mice carcasses to lose mass (Hau et al 2014). Advanced decay would be where the carcass has lost most its mass; this would usually be recognised by the reduction of insect activity due to loss of available nutrients (Vass 2001).

The soil used in the report was collected from two locations within Dorset, UK. In the UK, there are many different soil types with some similar and some contrasting properties. The report conducted by Avery (1973) suggested that there are up to 10 different soil groups all containing their own subgroups. The two soils selected for this report needed to have contrasting pH levels; therefore, a podzolic soil was chosen for its acidity (3.5 – 4.5 pH) and a lithomorphic soil was chosen for its basicity (6.5 – 8.5 pH). These soils would have obtained their pH level from their parent material (Burnham 2005) but they also have other properties that would influence decomposition. Lithomorphic soils contain many sub-categories and usually have an organo-mineral surface horizon (Avery et al 1980). The soil chosen in this report was a type of rendzina from the subgroup 3.4; this type soil is calcareous and therefore normally well drained of moisture (Avery, Findlay and Mackney 1975). The podzolic soils are black or dark brown in colour and usually

have a combination of iron, aluminium and organic matter (Avery, Findlay and Mackney 1975). The sub-category soil chosen from this group was 6.3 podzol; this soil is also well drained with a compact subsurface horizon (Avery, Findlay and Mackney 1975). Each soil would usually contain different vegetation growing within each with the lithomorphic soil having more basophilic vegetation and the podzolic soil having more acidophilic vegetation (Burnham 2005). The two soils do have some similar properties when compared with other soils in the UK. Both soils freely drain so are therefore drier than others: for example, peat (Burnham 2005). Moreover, their parent materials are more permeable than other soil categories (Burnham 2005). Soil pH would also change once the carcasses have been introduced and start to decompose. Although this will not be tested in this experiment, other reports suggest that cadaver decomposition will cause the pH levels to rise initially (Hopkins, Wiltshire and Turner 2000).

Another factor which would be different in each of the two soils, due to their pH, is their microbial content (Lin and Brookes 1999). This is important because microbial activity makes up a large factor of an organisms' decomposition (Neher et al 2003; Stokes et al 2009). Moreover, since insects and other scavengers would not have access to the cadavers will mean that microbial activity will have been the main contributor to decomposition (Carter, Yellowlees and Tibbett 2010; Vass 2001). Microbes are attracted to breakdown dead animals as they contain a high level of nutrients and moisture (Haslam and Tibbett 2009; Vass 2001). Both bacteria and fungi make up the microbial decomposers and both have their productivity influenced by pH (Lin and Brookes 1999). In the reports by Rousk, Brookes and Bååth (2009) and Rousk et al (2010) they observe the microbial activity in soils which have an opposing pH that were similar to the soil pH's used in this experiment. In the report, they revealed that soils closest to pH 7 will have a higher percentage of bacteria than fungi. This should mean that bacterial activity would have been higher in the lithomorphic soil as it has a pH range of 6.5 - 8.5. The Rousk, Brookes and Bååth (2009) and Rousk et al (2010) reports then go on to reveal that fungal activity is greater in soils that are further from neutral (pH 7). Therefore, as the podzolic soil typically has a pH range from between 3.5 – 4.5, its main microbial decomposers may have been fungal. However, since the podzolic

soil's pH may have been as low as 3.5, it would make it too acidic for most bacteria and would also have had detrimental effects for fungi (Hansel et al 2008). Consequently, meaning the podzolic soil may have had a lower overall microbial community than the lithomorphic soil. The depth of burial in soil will also influence the type of microbial activity the carcasses will be exposed to (Zhou et al 2004; Child 1995). It is also unlikely that both soils will share any exact species of microbes, especially bacteria, due to the soils differing properties (Zhou et al 2004; Child 1995). However, Fungi species may be more similar (Chimutsa et al 2015). If the mice cadavers had been left to decompose for long enough then there would be a shift in the type of microbial activity. With soft tissue available, both bacteria and fungi will be present whereas once all the soft tissue has decomposed then it would be majority fungi; this is because there are many more species of fungi that specialise in the decomposition of hard tissue than bacteria (Chimutsa et al 2015; Sagara, Yamanaka and Tibbett 2008; Sagara 1976).

Soil microbial activity will also depend on other factors including: moisture, texture, oxygen, depth and temperature (Carter and Tibbett 2008b; Carter, Yellowlees and Tibbett 2010; Dilly and Munch 1998) but since they were controlled in the experiment, no samples would have an advantage. The effect of sealing the tubs would have an impact on microbial activity however. This sealing would cause the microbes to be exposed to aerobically closed conditions. This would have caused the microbial activity within the soil to reduce as the week's pass. This would have occurred because the microbial activity needing to respire aerobically to decompose tissue, would have a decreased oxygen supply (Riggs and Hobbie 2016). The microbes within the carcasses would too have a reducing oxygen supply however this would be natural. Once this oxygen supply has been exhausted decomposition will continue through some microbes being able to respire anaerobically (Hyde et al 2013). Whilst report aims to be able to link with forensics, there are other factors that affect microbial activity that are possible in criminal cases; however, these were not considered. Firstly, an aspect that would increase microbial activity would be if the organism had any trauma. Trauma allows outside microbial activity an easier access into the carcass to therefore increase the rate of decomposition (Carter and Tibbett 2008b). Other factors

include the presence of any clothing; clothing can have both a positive and a negative effect on the rate of decomposition. A positive effect would be that heat would be retained within the carcass more effectively (Carter and Tibbett 2008b). The negative effect would be that clothing may restrict outside microbes from accessing the carcass (Matuszewski et al 2014). Another factor which would affect microbial activity that may be present in a criminal case is the presence of drugs. Depending on the properties, the drug may cause an elevation in microbial activity or may inhibit the process (Fiedler and Graw 2003).

Methodology

From the reports that have been conducted to observe decomposition in soil there are two clear differences in the methods used. They are either conducted in a laboratory environment with controls over the soil particle size, soil moisture and are incubated; or reports are carried out in a field laboratory conditions with samples being left and only protected by a cage against scavengers. This report was conducted with a mix of both types of laboratories with some similarities in the method to the report by Haslam and Tibbett (2009).

The two soils used in this experiment were collected from two locations within Dorset. Their proximity from each other was approximately 7.27km (Google Maps 2016). The lithomorphic soil (rendzina – horizon A) was collected from a site in Wareham in Dorset on Knitson Farm (SZ 00513 80937) (Figure 7). The podzolic soil (podzol – horizon E) was collected from another site within Wareham in Dorset (SY 94098 84408) (Figure 8). The soils were extracted and placed into separate plastic bags; the bags were then sealed to ensure that the moisture already within the soil would stay. This ensured the soils contained their natural moisture at field level so that no extra moisture was needed to be added before the experiment started. Since both soils were geographically close to each other and were collected on the same day, meant they should already contain similar moisture levels to each other. Furthermore, their similar moisture retention properties also

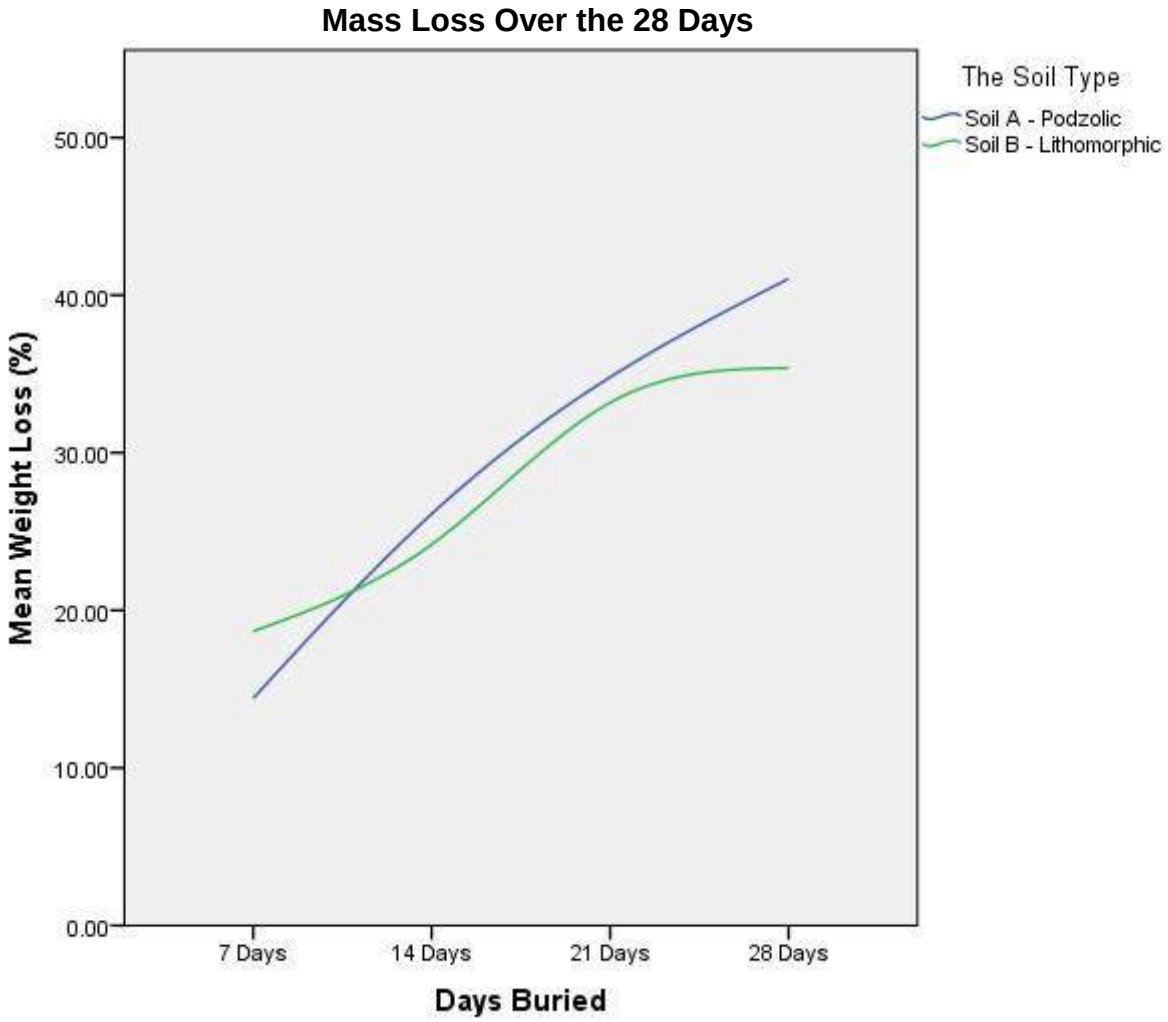
meant that both soils freely drain so one should not have retained moisture any more than the other. It is important that each soil has similar moisture levels because this is one of the main influencers to decomposition (Wang et al 2016). Ideally, pig (*Sus scrofa*) carcasses would have been selected for investigation due to their similarity to humans (Gill 2005), however this was impractical due to size and cost. Instead, laboratory mice (*Mus musculus*) cadavers were used due to their relative similarity to humans, and their availability, size and low cost (Rosenthal and Brown 2007; Nguyen et al 2015; Burcham et al 2016; Carter, Yellowlees and Tibbett 2008a; Heshmati, Keene and Villano 2015). In the report by Haslam and Tibbett (2009) they used skeletal muscle tissue cubes mainly to ensure the starting weight is the same for all samples. However, whole mice were chosen because they may portray a more realistic comparison to a human cadaver being buried.

The comparison of each of the soils decomposition rates was measured through burying the mice within each of the two soils and then calculating the carcasses change in mass (grams). This was done by weighing the mice before burial and then weighing them again once they were exhumed from the soil; this method of weighing before and after is similar to the method used in other reports (Tibbett et al 2004; Haslam and Tibbett 2009). This is because it portrays the amount of mass the mice had lost from decomposition over the time it was buried. The mice were buried for a total time of 28 days (4 weeks). Throughout the period, randomly selected mice were exhumed every 7, 14 and 21 day periods before all being exhumed after 28 days. This was done to view how the decomposition rate was progressing. This experiment required a total of 40 mice with 20 mice going to each soil. At each 7-day period, 5 randomly nominated mice were selected from each of the two soil types to be exhumed. The mice were buried separately in individual 1 Litre sized tubs and then sealed. Setting the experiment up in this way meant that the only influencing variable depended on the properties of each soil only. However, sealing off each sample also meant that the mice would decompose in an aerobically closed environment; therefore, meaning that it is expected the rate of decomposition will slow more than expected over the weeks as the oxygen levels decreased. However, the soil with the buried carcasses only

took up approximately 20% of the overall space within the tubs allowing a sufficient air supply for decomposition to progress effectively. By placing the samples into separate tubs, it allowed the same amount of soil in each. 500g of each soil were used in total to bury each mouse individually. The mice were also buried at the same depth to each other; this was 2cm below the soil surface line. None of the soil placed into the tubs were sieved to allow all sizes of soil and rock into the container allowing more natural conditions. Only large rocks were discarded from filling the tubs as they would take up much of the weight allowance. Moreover, no additional water was added to the soils to keep their moisture levels the same as from when they were collected at field level. Once this was done the lid would then be placed into the tub and not re-opened until it was being re-examined.

The tubs were given numbers from 1 up to 20 for each soil. If the mice were randomly selected to be exhumed before day 28, they were removed from the experiment; thereby meaning the sampling strategy was also destructive. A random sampling strategy was selected for choosing which mice would be exhumed at what time interval. To ensure that the mice were selected completely randomly, a random number generator (Dean 2016) was used to select each tub for analysis. Once a number was produced from the generator, the same tub number for both soils would be examined. The exhumed mice then needed to be re-weighed to calculate the percentage of mass they had loss. However due to putrefaction, it meant that the exhumed mice were moist and therefore had soil attached to them. The soil was removed from the cadavers as much as possible through scraping the soil from the cadavers. Another important control in this experiment was making sure each sample had the same experienced the same temperature conditions. All tubs were stored within a heated greenhouse which experienced average temperatures of between 15 – 21^{O}C despite being conducted in November (Figure 11 and 12). Despite this temperature range not being prime for microbial growth, it exposed the cadavers to more natural temperature fluctuations of daily highs and nightly lows rather than incubation at a continuous temperature. The temperature range that the samples would have experience was more similar to the months of May and June (MetOffice 2014). However, as the samples were buried, the soil would have insulated the samples meaning the carcasses may not have been exposed to such temperature fluctuations.

Results



(Figure 1. Graph revealing the trend of the mean mass loss from the buried carcasses over the 7-day intervals.)

The graph (Figure 1) conveys how the rates of mean mass loss progressed over the 7-day intervals. It reveals that the mice buried in the lithomorphic soil had a greater mass loss after the first 7 days. After this the mice buried in the podzolic soil over takes the lithomorphic and continues to have the greater mass loss rate. Both soils follow a positive correlation: being that as the length of time the mice spent buried increases, then more mass is lost which was expected.

Descriptive Statistics

Dependent Variable: Weight Loss (%)

The Soil Type	Days Buried	Mean	Std. Deviation	N
Soil A - Podzolic	7 Days	14.4140	1.71078	5
	14 Days	26.1156	5.21456	5
	21 Days	34.7951	4.97418	5
	28 Days	41.0444	5.68385	5
	Total			20
Soil B - Lithomorphic	7 Days	18.6633	4.52757	5
	14 Days	24.1825	7.00019	5
	21 Days	33.1782	5.67583	5
	28 Days	35.3768	6.59341	5
	Total			20
Total	7 Days	16.5386	3.92774	10
	14 Days	25.1491	5.90781	10
	21 Days	33.9866	5.10300	10
	28 Days	38.2106	6.52706	10
	Total			40

(Figure 2. Table revealing the mean mass loss for podzol soil, lithomorphic soil and both soils together.)

All mice samples experienced mass loss that decreased in the manor that was expected. This being that the mice experienced their highest mass loss over the first 7 days then the percentage gradually decreased as the other weeks followed (Figure 2). For the mice in the podzol (soil A), they experienced a -14.41% mean

mass loss after the first 7 days. After 14 days buried they lost a further -11.70% mean mass loss. After 21 days buried they lost a further -8.68% and after 28 days they lost a further -6.25% mean mass loss (total mean mass loss being -41.04%). For mice buried in the lithomorphic soil (soil B), they too experienced their major mean mass loss during the first 7 days: this being -18.66%. Following this after 14 days, the mean mass loss has reduced a further -5.52%. After 21 days, the mean mass loss had reduced another -8.99% and after 28 days, it had reduced a further -2.19% (total mean mass loss being -35.38%).

Levene's Test of Equality of Error Variances[a]

Dependent Variable: Weight Loss (%)

F	df1	df2	Sig.
.767	7	32	.619

a. Design: Intercept + Soil_Type + Days + Soil_Type * Days

(Figure 3.Tests the null hypothesis that the error variance of the dependent variable is equal across groups.)

This test reveals that all samples have equal variances so meaning a two-way ANOVA can be conducted. This being because the significance (0.619) is greater than 0.05.

Tests of Between-Subjects Effects

Dependent Variable: Weight Loss (%)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2928.314[a]	7	418.331	14.403	.000
Intercept	32424.462	1	32424.462	1116.369	.000
Soil_Type	15.428	1	15.428	.531	.471
Days	2786.988	3	928.996	31.985	.000
Soil_Type * Days	125.898	3	41.966	1.445	.248
Error	929.427	32	29.045		
Total	36282.203	40			
Corrected Total	3857.741	39			

a. R Squared = .759 (Adjusted R Squared = .706)

(Figure 4. Table revealing the effects days buried, soil type and both these together had on the mass loss of the mice.)

The significance of the differences in mass loss between the two soil types over the time period was determined by a two-way ANOVA (Figure 4). The test indicated that the time period had a statistically significant effect on the percentage mass loss ($F = 31.985$, $p < 0.001$). However, the observed difference in mass loss between the two soil types was not statistically significant ($F = 0.471$, $p = 0.471$). The interaction between soil type and the time period was also not significant ($F= 1.445$, $p= 0.248$). This indicates that soil type had no significant effect on the percentage mass loss over the time period.

Discussion

The analysis reveal that there was no significant difference between the decomposition rates of the mice carcasses between the two soils types. Despite the graph (Figure 1) suggesting that the podzolic soil had a slightly higher decomposition rate, when the results were analysed statistically through SPSS (2017), it revealed there was no significant difference between the two (Figure 4). This result contrasts from other reports that have observed mammalian decomposition between the two soil types (Carter and Tibbett 2008a; Carter and Tibbett 2008b; Haslam and Tibbett 2009). The Haslam and Tibbett (2009) report revealed that the decomposition rate was three times greater in the podzol compared to the rendzina. A cause of this result may be due to several differences in the way the method was conducted. This being: the way the soils were readied, the human surrogates used, incubation temperature and moisture content. The result confirms the null hypothesis, being that the soil type would have no effect on decomposition, will be accepted. Despite this contrasting from other literature it may still have some implications for forensic taphonomy.

In many other reports, the soils that were used had been altered from the state they were collected in (Carter and Tibbett 2008a; Carter, Yellowlees and Tibbett 2010; Haslam and Tibbett 2009; Stokes, Forbes and Tibbett 2013; Tibbett et al 2004; Carter and Tibbett 2006). In those reports the soil was often sieved to make sure all the soil particles were the same size; the soils would then be dried out and then re-moistened. For example, Haslam and Tibbett (2009) weighed out 100g of soil then added moisture to 60% of the soils water holding capacity. Although this method allows the soil samples to be more similar to each other, it does not replicate a likely scenario. In forensic cases, it is not likely that a cadaver will be buried in soil that has been dried out, sieved to have all the same soil particle sizes and then re-moistened; therefore, these reports with similar methods may not actually offer as much towards forensic taphonomy. Whereas in this report, soil moisture and particle size were all kept in the same condition that they were collected in to match more realistic circumstances. Another characteristic that may

explain as to why a result of no significant difference was recorded was because the soil's properties were too similar. The soils already share properties including coming from a highly permeable parent material and both freely draining from moisture (Burnham 2005). The main assumed difference was their pH but if this was actually more similar than expected then they may have had similar microbial activity levels (Cobaugh, Schaeffer and DeBruyn 2015; Rousk, Brookes and Bååth 2009; Rousk et al 2010). This assumption being the lithomorphic soil (rendzina) will be between 6.5 – 8.5 pH and the podzolic soil (podzol) being between 3.5 – 4.5 pH (Avery, Findlay and Mackney 1975). The Metcalf et al (2016) report conducted similar testing on mice carcass decomposition rates under contrasting soil types; it discovered that soil type was not the dominant factor in driving microbial communities. Therefore, revealing that soil pH alone may not be enough to significantly alter the rates mice decomposition (Metcalf et al 2016). Microbial activity within soil would not have been totally irrelevant in the decomposition of the mice carcasses however. The report by Lauber et al (2014) revealed that soil with microbial activity showed a substantially greater decomposition rate on mice carcasses than soil which was sterile.

The soil's pH would have been altered from decomposition itself. As the carcasses decompose they would have released by-products into the soil which would have modified the pH; therefore, affecting the microbial activity already within the soil. It has been observed in other literature that decomposition byproducts cause the soils pH to initially increase then after time decrease (Haslam and Tibbett 2009). The increase in soil pH would likely have been caused by ammonification with the breakdown of protein products (Haslam and Tibbett 2009; Hopkins, Wiltshire and Turner 2000). This process causes nitrogen levels within the soil to increase; this increased level has been observed to reduce microbial activity (Riggs and Hobbie 2016; Hopkins, Wiltshire and Turner 2000). The decrease in soil pH would likely have been caused by the release of acetic acids, oxalic acids and fatty acids from the carcasses (Vass et al 2002; Carter and Tibbett 2008a). Along with additional by-products this change in pH may have had an effect on the microbial activity conducting decomposition within the soil (Cobaugh, Schaeffer and DeBruyn 2015). The interchanging pH would also have

altered the microbial activities effectiveness within the carcasses too (Burcham et al 2016). However, soil pH is just one component that effects the microbial activity within soil and therefore the decomposition process; other influencing components include temperature, oxygen and moisture (Wilson et al 2007; Parkinson et al 2009). Despite all these being controlled to keep decomposition down to difference in soil type, they might overall be a more important contributor to microbial activity and hence decomposition (Hansel et al 2008); therefore, causing the results of decomposition from the soil types to be not significant.

All samples in the experiment would have been exposed to temperatures that would have fluctuated between 15 - 21^{O}C as they were all stored the same. This was done to replicate the natural temperature changes of night and day. However, this temperature range is under the prime conditions for microbial activity so therefore would have reduced the effectiveness of both bacteria and fungi (Goff 2009; Tibbett et al 2004; Turner and Wiltshire 1999). Other literature reveals decomposition is highly dependent on temperature being adequate (Rodriguez and Bass 1985; Carter and Tibbett 2008b; Tibbett et al 2004). The Prime temperature for microbial activity in both a high pH soil and low pH soil would have been between 25 – 30^{O}C (Carter, Yellowlees and Tibbett 2008a; Pietikåinen, Pettersson and Bååth 2005). As microbial activity was not able to be measured throughout the experiment, conclusions about what soil type had a higher percentage of either bacteria or fungi cannot be made. However, as the decomposition rates of both soils showed no significant difference then it could be assumed that the overall microbial activity for both soils were similar. Other literature can portray a more accurate assumption of what soil types contained more bacteria or fungi at 15 – 20^{O}C. In the report by Pietikåinen, Pettersson and Bååth (2005) a soil with a pH similar to the rendzina recorded that bacterial activity was slightly higher than fungal activity at 15 – 20^{O}C. This result was also similar to a soil with a pH similar to the podzol as this revealed that bacterial activity too was slightly higher than fungal activity at 15 – 20^{O}C (Pietikåinen, Pettersson and Bååth 2005). Overall the report revealed that 15 – 20^{O}C was approximately half the potential microbial activity output (Pietikåinen, Pettersson and Bååth 2005). This reduction in microbial activity from the soil may be a cause of why a result of no

significant difference was recorded (Carter, Yellowlees and Tibbett 2008a). Moreover, the soil may have also insulated the carcasses from the temperature highs meaning that they would have been kept more at a more continuous low temperature (Turner and Wiltshire 1999).

Another control measure that may have effected decomposition significantly was oxygen availability. This is because some of the microbial activity within both soil and carcass will only respire aerobically (Bucheli and Lynne, 2016). Other reports, which have targeted the observation of microbial activity in soil during decomposition, revealed that aerobic decomposers are the main contributor to active decay (Cobaugh, Schaeffer and DeBruyn 2015). To keep this the same for each sample and controlled throughout, all mice were buried with the same amount of soil. This being 2cm below the surface and in the same shaped tub meaning they all experienced the same air supply. However, since the tubs were sealed, it would have eventually reduced the oxygen supply and therefore reduced the output from some microbes as the days passed (Riggs and Hobbie 2016). Despite this, decomposition would still have been able to continue through certain microbes that respire anaerobic (Hyde et al 2013). This would have especially occurred within the mice carcasses as oxygen supply would have been exhausted more rapidly (Bucheli and Lynne, 2016; Hyde et al 2013). Anaerobic microbial activity however favours the early stages of decomposition and will significantly reduce during the latter stages (Cobaugh, Schaeffer and DeBruyn 2015). This reducing oxygen supply may have been another contributor as to why a result of no significance was recorded; the microbial activity in the soil may not have enough oxygen to respire effectively enough to portray a significant difference. This method of having the carcasses closed off from the air was a contrast to other reports (Carter and Tibbett 2008a; Carter, Yellowlees and Tibbett 2010; Haslam and Tibbett 2009; Stokes, Forbes and Tibbett 2013). In the report by Haslam and Tibbett (2009) they used ventilation to constantly replenish oxygen. If there had been a significant absence of oxygen to the samples then adipocere may have formed which is a further inhibitor of decomposition (Fiedler and Graw 2003; Motter 1898; Forbes et al 2004). Adipocere is formed when the organism's fat is alternated from triglycerides into glycerine and free fatty acids which liquefy and

penetrate surrounding muscle tissue (Fiedler and Graw 2003; Forbes, Dent and Stuart 2005); the process also requires very moist conditions. However, it is unlikely that adipocere would have formed around the samples as it usually takes 30 days to begin (Fiedler and Graw 2003). In the Forbes, Dent and Stuart (2005) report which examined soil type effect on adipocere formation, revealed that sandy soils are more likely to produce the substance. Therefore, meaning in this report the podzol would have been more likely to produce adipocere if the samples had been left for long enough.

Moisture within the soil would have also played a key role in the productivity of the microbial biomass. Moisture has been observed to be one of the most important contributors when it comes to decomposition in soil (Wang et al 2016; Carter, Yellowlees and Tibbett 2010). This is because the bacteria and fungi within the soil require it to control diffusion of nutrients to respire; they also need it for microbial motility and the excretion of waste (Carter, Yellowlees and Tibbett 2010). Moisture will affect the soils structure and texture; the availability of moisture between soil particles is known as its matrix potential (Carter, Yellowlees and Tibbett 2010). However, the relationship between moisture and microbial activity does not follow positive correlation. A lower matrix potential (wetter soil) is not ideal for microbial activity because gas diffuses more slowly through this large volume of moisture; a higher matrix potential (drier soil) is also not ideal for microbial activity as this lack of moisture reduces microbial motility (Carter, Yellowlees and Tibbett 2010). Typically, a matrix potential of -0.01 megapascals (MPa) is accepted as being the ideal moisture level for microbial activity to thrive (Carter, Yellowlees and Tibbett 2010). The moisture within each soil was kept as it was found at field level. Although this had the potential to mean that the two soils would not have the same moisture levels, this was controlled by collecting the soils from similar locations and on the same day; meaning they should have experienced similar weather. This was also done to allow the soil to be in their naturally moist state so therefore would represent a more realistic scenario for if an organism was buried in it. The method used in other reports (Haslam and Tibbett 2009; Carter and Tibbett 2008a; Stokes, Forbes and Tibbett 2013; Tibbett et al 2004; Carter and Tibbett 2006) of drying out the soils and then re-moistening them was not conducted in case this

drying method affected the soil in any way; therefore, altering their natural state. However, in a report carried out by Duboc et al (2016) tests were conducted on whether drying out soils affects their organic matter; the results revealed that there was only a small-scale effect on organic matter.

The report by Carter, Yellowlees and Tibbett (2010) carried out tests on the decomposition of rodents in soil with varying levels of moisture. Their report included obtaining 3 different soils which were then calibrated to hold moisture at matrix potentials of -0.3MPa (driest), -0.05MPa and -0.01MPa (wettest). Their results concluded that all soils had a more effective decomposition rate at the soil with 0.01MPa. If this result is compared to the Haslam and Tibbett (2009) report which used the same soils to the ones in this report (rendzina and podzol), then an assumption could be made about the state of moisture in each of the soils used in this report. In the report by Haslam and Tibbett (2009) they recorded that the podzol is a more significant decomposer than the rendzina. However, as this reports results showed no significant difference between the two, then the assumption of the rendzina having a matrix potential closer to 0.01MPa could be made. This is because moisture is known to be one of the most important decomposition factors and therefore may have equalled out the rate microbial activity between the soils (Wang et al 2016). Moisture for microbial activity would also have not have just been obtained from the soils. Moisture would have also been attained from the putrefaction of the mice carcasses during the active decay stage (Vass 2001; Carter and Tibbett 2008b). This moisture leaking would benefit microbial activity in the soil if it was in a relatively dry state, because it would reduce the matrix potential to a more favourable condition. However, if a soil had a significantly high matrix potential this would greatly affect the overall decomposition rate. This is because this dryness would cause desiccation of the carcasses before the decay stages of decomposition can occur (Carter and Tibbett 2008b). Thereby, stopping decomposition as the moisture is diffused into the surrounding soil and which effectively mummifies the carcasses as this moisture level is still too low for microbial activity (Carter and Tibbett 2008b; Goff 2009). This highlights an issue with this reports method of measuring decomposition by weighing mass before and after; this being because when desiccation causes a carcass to diffuse its moisture, the carcass will lose a large portion of weight without actually going through decomposition (Carter and Tibbett 2008b). Thus,

producing a false positive result. However, despite the report not conducting tests on moisture content, the soils were not visibly in a very dry state; furthermore, on exhumation the mice carcasses were visibly in a state of decay (Figure 9 and 10).

Another way in which this report's method is different from others is the human cadaver surrogate; some reports use skeletal muscle tissue (Carter and Tibbett 2008a; Haslam and Tibbett 2009; Carter and Tibbett 2006). Skeletal muscle tissue will differ from whole organisms as there will be limited microbial activity from within just muscle tissue. Using muscle tissue would allow the soil to have a more distinctive impression on decomposition as the majority of microbial activity would need to come from the soil. However, skeletal muscle tissue will undergo desiccation at a greater rate meaning that it will be recorded as losing a greater percent of mass (Haslam and Tibbett 2009). The method of using whole carcasses will represent a whole human more accurately due to the internal microbial activity; especially within the Gastrointestinal Tract (Goff 2009; Burcham et al 2016; Metcalf et al 2013); mice and humans have a similar gastrointestinal tract along with nervous, cardiovascular and musculoskeletal systems (Rosenthal and Brown 2007; Nguyen et al 2015). The Burcham et al (2016) report used mice carcasses to analyse microbial activity after death. It revealed that previously sterile organs such as kidney, liver, spleen, and heart had been colonised with bacteria just a couple of hours - days after death. These internal bacteria will experience changing conditions as oxygen, moisture, temperature and nutrients levels all change (Burcham et al 2016; Metcalf et al 2016). The comparison of microbial activity within humans and mice has been studied and revealed that despite there being some differences, both respond to decomposition in similar ways (Nguyen et al 2015). Therefore, meaning the comparison between mice and human cadavers is valid. The report conducted by Melvin et al (1984) looked at microbial activity within deceased mice; the results revealed that aerobic bacteria were the first to migrate from the intestines to other parts of the body. Anaerobic bacteria would then follow once the oxygen within the mice carcasses had reduced (Melvin et al 1984; Metcalf et al 2013). This Melvin et al (1984) report provides an understanding into what likely happened within the mice in this report.

This result of no significance could reveal that decomposition of an organism is caused more from the microbial activity within an organism than the microbial activity within the soil (Burcheli and Lynne 2016; Hyde et al 2013). This could be assumed since all mice were kept as whole carcasses and the soil type was the only differing variable. In the Burcheli and Lynne (2016) report, decomposition of whole organisms were observed for microbial activity and the initial stage of decomposition is predominantly conducted by the internal microbes; only later does the microbial activity in the soil take over. The result suggests that maybe the carcasses were not exposed to the microbes in the soil for a significant result, because they were not left for a long enough time; further enhanced by the lack of other prime growth conditions. If this assumption is correct and since the comparison between mice and humans is valid then it would have implications for forensic taphonomy.

To assess the overall validity of the results a similar report can be used for comparison. In the report conducted by Carter, Yellowlees and Tibbett (2008a) in which rodents were also buried in contrasting soils to observe the decomposition rate also shared some similarities with this reports result. Firstly, their results revealed that mass loss between soils with contrasting pH's were very similar when exposed to 15 – 22^{O}C; despite them not conducting statistical analysis of the relationship between mass loss, soil and days passed it is clear their figures would also give a result of no significant difference; mainly because their figures do not stay consistently different from each other. This being because a soil with a high pH (rudosol) started with a higher mass loss than the soil with a low pH (brown sodosol) after 14 days. However, after 28 days the low pH soil (brown sodosol) recorded a higher mass loss than the soil with the high pH (rudosol). This inconstancy in mass loss is similar to this report; being as it too saw the mass loss start greater in the high pH soil (rendzina), but concluded with the low pH soil (podzol) ending better. Despite this similarity, the comparison is not completely valid as the soils may only share the characteristic of similar pH.

Overall, this report may offer some assistance for forensic taphonomy especially since there are similarities between a mouse carcass and a human cadaver. Firstly, the result of no significance may suggest that soil pH is not one of the dominant factors when it comes to organism decomposition in soil (Metcalf et al 2016). If this result is applied to forensic taphonomy then it may allow investigators to reduce consideration into how soil pH will affect the PMI. Other factors like temperature, moisture and oxygen supply may all play a larger role in the decomposition process (Wilson et al 2007); this being because they were all consistently applied across all samples making the result of no difference expected. Another way in which this result may assist forensic taphonomy is through its use of whole organisms. With the comparison between a whole organism and muscle tissue then it is clear that the two will decompose differently. Moreover, this result may also suggest that if a human cadaver is known to be buried as a complete corpse, then the soil type it has been buried in may not affect the PMI significantly in the short term. This being because the microbial activity within the organism is conducting the majority of the process (Bucheli and Lynne 2016). Whereas, if the corpse has been dismembered and then these sections buried, then soil type will have a significant effect; this is emphasised by reports that conducted decomposition analysis with muscle tissue (Haslam and Tibbett 2009). Furthermore, forensic taphonomy may also be aided through the use of microbial activity as a PMI indicator. Other reports have discovered that microbial activity in the soil surrounding the buried organism has good potential for estimating PMI (Vass 2001; Metcalf, Carter and Knight 2014; Burcham et al 2016; Guo et al 2016; Cobaugh, Schaeffer and DeBruyn 2015; Melvin et al 1984; Metcalf et al 2013). Soil microbial analysis would too be a good indicator for clandestine graves as grave soil and regular soil have vastly differing microbial communities (Vass 2001; Metcalf, Carter and Knight 2014); this difference in microbial communities in soil have been found to last up to 30 days after a carcass was removed meaning microbial analysis has also great potential for locating where a body had been buried (Metcalf, Carter and Knight 2014).

However, other factors in the report also suggest that the result is also not completely reliable for forensic taphonomy. One of the objectives of this report was to replicate more realistic conditions for decomposition than existing literature; despite all the techniques used to keep conditions as realistic as possible, the main factor that was not realistic was oxygen availability. The fact that the samples were sealed in tubs would have reduced the capacity for microbes to respire aerobically as the days passed reducing decomposition (Riggs and Hobbie 2016).

There is a chance that this is the main cause of the result of no significant difference. Another factor in this report which meant the experiment did not replicate realistic conditions was through its objective to just observe the soils effects on a buried organism's decomposition. Decomposition in natural conditions would include other contributors to the process: this being the roll of insects and scavengers. Depending on circumstances these two other contributors can massively influence decomposition in a natural environment (Payne, King and Beinhart 1968; Gill 2005). It has been observed that the amount of insect activity and the rate of decay follow a positive correlation to a point (Rodriguez and Bass 1983). An organism that is buried would have a reduced impact from insects and scavengers but would still likely be effected if not buried too deep (Turner and Wiltshire 1999; VanLaerhoven and Anderson 1999). The type of insects/ scavengers may also be different in locations where the soil type contrasts and this is another factor that needs to be considered (Reed 1958). For this report to replicate more realistic conditions and therefore contribute more towards forensic taphonomy, it would have to compromise on the method of observing decomposition in a laboratory setting. The human cadaver surrogate also indicates that this report is not completely reliable for forensic taphonomy. Despite the similarities between a human cadaver and a mouse carcass, the two do not respond to decomposition in identical ways. As other literature identifies, there is no perfect human surrogate (Stokes, Forbes and Tibbett 2013). The only way to truly observe human decomposition would be to use actual human cadavers.

Conclusion and Summary

To conclude, this report produced a result of no significant difference which contrasts from existing literature on decomposition in contrasting soils. However, this may have been due to differences in the method used to conduct the experiment. In all this report has further emphasised that there will be never be a universal PMI that could be applied to all forensic cases (Taylor 2011). This being due to the vastly varying number of contributors that either: conduct, promote or inhibit decomposition. The aim of the report was to observe carcass decomposition in soil with more realistic conditions than other literature, and if possible, be able to apply this to forensic taphonomy. This has been achieved to a degree as more realistic soil conditions were able to be produced; this being through not editing the soil from its natural state by changing particle size and moisture holding capacity. Moreover, all samples were exposed to more realistic temperature fluctuations of night and day than other literature. The only way in which realistic soil conditions were not produced was through the closed oxygen supply. The other half of the aim, which was to see whether this report could produce a result that would assist forensic taphonomy, has also been achieved to a degree. Firstly, other literature has revealed that there are some similarities between a mouse carcass and a human cadaver. With this information and this reports result, it may suggest that if a human cadaver is buried in soil, then soil pH will not be a major contributor towards decomposition in the short term and therefore not effecting the PMI. Especially if other conditions like: temperature, moisture availability and oxygen supply are not prime for microbial activity. This being because the effectiveness of soil pH on microbial activity, was not able cause a significantly different result. Furthermore, the result may also reveal that a whole organism's decomposition is more effected by microbial activity within itself than the microbial activity within the soil. This is emphasised by the fact that all microbial activity within the carcasses would have been similar. Moreover, other reports, which just used muscle tissue, saw that decomposition was significantly affected by soil type. However, the result cannot be completely applied to forensic taphonomy due to completely realistic conditions not being created. This being due to the experiment being conducted in a laboratory environment and that no other animal can completely replicate human decomposition.

The report has highlighted that more research into the decomposition process is needed especially if it has the intention to assist forensic taphonomy. This is due to the great number of variables that influence decomposition and attempting to control too many of these makes the observation unrealistic. If further research is going to be conducted on decomposition with the aim of assisting forensic taphonomy then these reports need to be conducted with more natural contributors and conditions. Further research could also be conducted into what insects, which live in environments with contrasting soil properties, would be attracted to a buried organism. Especially since they can be a major source of decomposition. The depth of burial could also be further studied to see what depths significantly influence the rate of decomposition. Moreover, trauma on an organism needs to be investigated to observe how much more it increases decomposition; this is especially needed since trauma on a cadaver is likely in criminal cases. The report highlighted a benefit for using mice as human surrogates for future research. This is because of the biological similarities to a human and they are also much more cost and space effective than other surrogates.

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Appendices

Evaluation Supplement

There were several strengths highlighted to my attention whilst conducting this report. Firstly, this report has allowed me was to expand my knowledge in a subject that interests me. Through conducting a hands-on experiment, meant that I could gain a deeper understanding into the decomposition process and appreciate other factors that affect it in greater detail. Another strength this report produced, was through some of the findings. Decomposition was observed at a more natural standard than other existing literature. This being through the way in which the method was conducted. For example: the soils were not altered in particle size or moisture from their natural state, temperature could naturally fluctuate and whole carcasses were used. Other reports, which changed these, would have had the intention to reduce variability between all samples; however, this would not reflect decomposition at a natural standard and therefore may not actually offer much towards forensic taphonomy. The choice to use whole mice carcasses is another strength to this report. This being because they can be linked to a whole human cadaver; backed up through literature which have compared mice and humans.

Here then reveals a potential for further work; since there is a valid comparison, further work can be conducted using the burial of mice for observation of decomposition. Existing observations either involve actual human cadavers or use large animals like the pig; nevertheless, there are numerous issues with using these including: availability, morality, cost and space. However, mice carcasses are easily accessible, cheap and do not require large amounts of space for burial.

Although the comparison between mice and humans is not completely similar, it would allow further investigations into what the most important influencers on decomposition are in greater detail.

However, producing the report revealed several limitations. The report attempts to address what the result of no significance was caused from but can only make assumptions on other influencers, without actually identifying the cause. For example, the result of no significant difference may have been caused from a lack of prime microbial conditions in the soil; these conditions being: moisture, oxygen or temperature. The outcome may also have been caused due to the internal microbial activity doing most of the decomposition or could have been caused from a combination of these factors. To actually reveal that soil pH is not a driving contributor to decomposition then the effect of these other factors would need to be known. The cause of these assumptions was due to limitations within the reports method. Firstly, if soil pH was measured beforehand then it would have guaranteed that the two soils were contrasting in pH and not just assumed from their classification. Moreover, if moisture was measured it would reveal exactly which soil had the closer level for prime microbial activity; Instead of this information being gained through literature and assuming they had similar levels due to their natural retention properties. Oxygen levels could also have been measured throughout the experiment because it was known that levels would reduce as the days passed; this may have been a determining factor in the cause of no significance. The impact of internal microbial activity could have been observed through burying samples of muscle tissue along with the mice carcasses and comparing their rates. If this investigation was to be carried out again then it would be essential for these factors to be measured.

The technique of weighing the carcasses to analyse decomposition was another limitation with the method. By weighing the samples before burial and then after

may not actually be an accurate judge of decomposition. This being because the carcasses could have lost weight through desiccation, meaning they have not actually decomposed; despite this being unlikely due to examination upon exhumation (Figure 9 and 10), it is still possible. The method of measuring decomposition could have been boosted through the testing of soil for decompositional by-products, which would have been released by the carcasses. The technique of scraping the soil off the carcasses also presented a further issue with this measurement of decomposition. Upon exhumation, the carcasses would often be moist so would have soil attached to them. With this, and the latter stages of decomposition when the carcasses would be disintegrating, meant that removing soil from flesh was challenging. To combat this the carcasses should have been dried out to allow for easier soil removal.

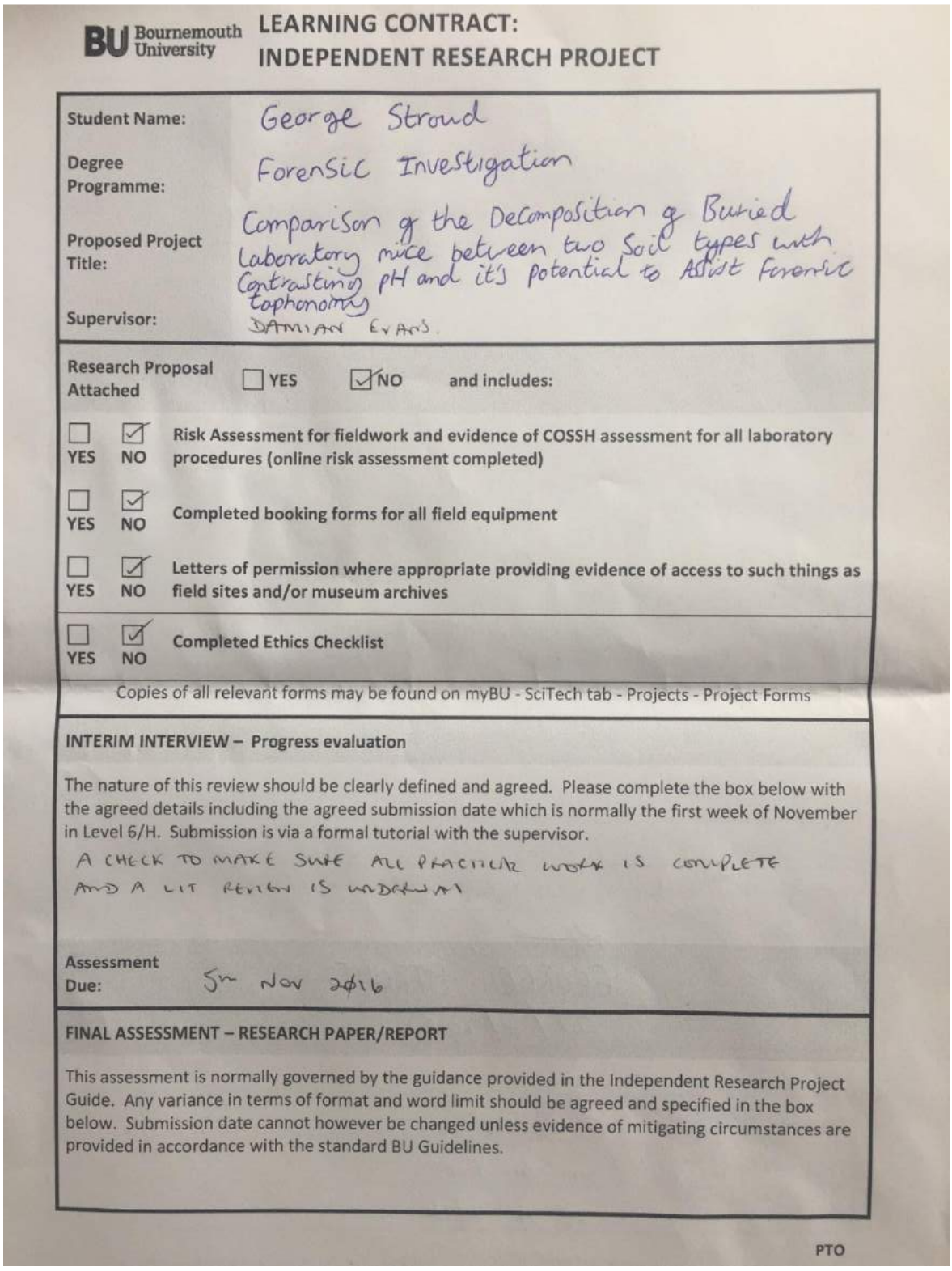
One part of the aim of the report was to observe decomposition at a more natural standard. Despite this being achieved to a level, there were drawbacks within the method that meant this was not achieved. Firstly, a limitation was that the samples were kept in a closed atmosphere and therefore meant oxygen would reduce. This would have negatively affected the amount of aerobic microbial activity and consequently the decomposition rate. This was done to stop insect activity but hereby highlights another limitation against replicating a natural scenario. If the report aimed to convey a truly natural scenario then insects would need to have access to the carcasses. Another limitation was availability to certain literature. Despite most literature being available for access, there were still numerous journals that required payment. This especially presents an issue when attempting to research literature that have reviewed human decomposition. This being because there have only been a limited number of these reports conducted which therefore impacted on background research.

Further research should be conducted on decomposition in more natural conditions; especially if the aim is to link to forensic taphonomy. Laboratory research can reduce the effects of decomposition to just one variable however, this would not happen in a criminal case. Further research needs to also be conducted on the potential for microbial activity to be a PMI indicator. Most PMIs are worked out on insect activity but this is highly dependent on insects being able to access the cadaver. Whereas, microbial activity will always be present within a

cadaver as long as it is not exposed to extreme conditions. Finally, further research needs to be carried out on carcasses that have been exposed to: external trauma or drug. This being because these are likely in criminal cases and can massively effect the PMI.

Comments from Interim Interview

A check to make sure all practical work is complete and a literature review is done (Figure 5 and 6). – Assessment due: 5th November 2016



BU Bournemouth University

LEARNING CONTRACT:
INDEPENDENT RESEARCH PROJECT

Student Name:	George Stroud
Degree Programme:	Forensic Investigation
Proposed Project Title:	Comparison of the Decomposition of Buried Laboratory mice between two Soil types with Contrasting pH and it's potential to Assist Forensic Taphonomy
Supervisor:	DAMIAN EVANS.

Research Proposal Attached ☐ YES ☑ NO and includes:

☐ YES	☑ NO	**Risk Assessment for fieldwork and evidence of COSSH assessment for all laboratory procedures (online risk assessment completed)**
☐ YES	☑ NO	**Completed booking forms for all field equipment**
☐ YES	☑ NO	**Letters of permission where appropriate providing evidence of access to such things as field sites and/or museum archives**
☐ YES	☑ NO	**Completed Ethics Checklist**

Copies of all relevant forms may be found on myBU - SciTech tab - Projects - Project Forms

INTERIM INTERVIEW – Progress evaluation

The nature of this review should be clearly defined and agreed. Please complete the box below with the agreed details including the agreed submission date which is normally the first week of November in Level 6/H. Submission is via a formal tutorial with the supervisor.

A CHECK TO MAKE SURE ALL PRACTICAL WORK IS COMPLETE AND A LIT REVIEW IS UNDERWAY

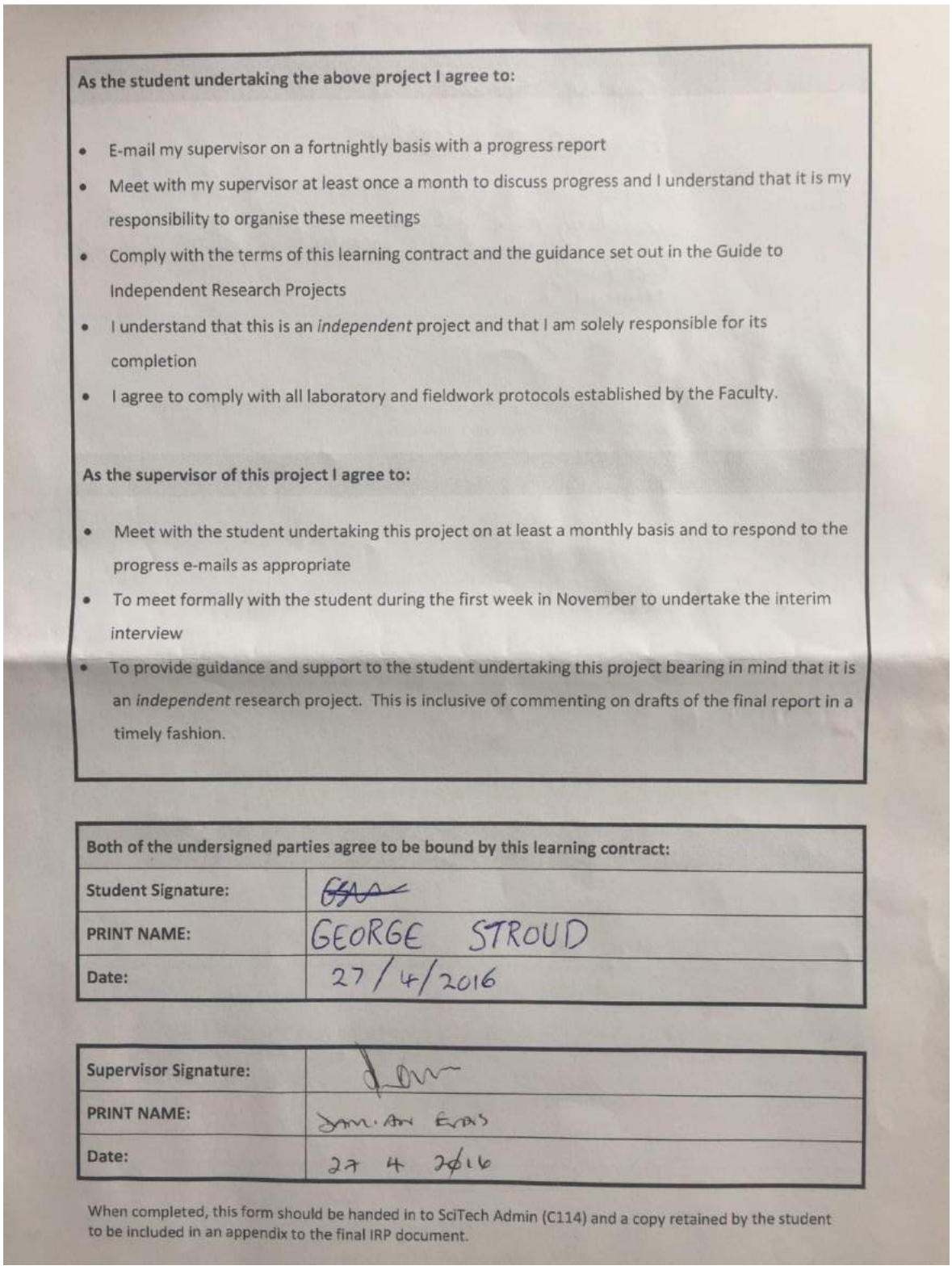
Assessment Due: 5th Nov 2016

FINAL ASSESSMENT – RESEARCH PAPER/REPORT

This assessment is normally governed by the guidance provided in the Independent Research Project Guide. Any variance in terms of format and word limit should be agreed and specified in the box below. Submission date cannot however be changed unless evidence of mitigating circumstances are provided in accordance with the standard BU Guidelines.

PTO

Figure 5. Learning contract side 1



As the student undertaking the above project I agree to:

- E-mail my supervisor on a fortnightly basis with a progress report
- Meet with my supervisor at least once a month to discuss progress and I understand that it is my responsibility to organise these meetings
- Comply with the terms of this learning contract and the guidance set out in the Guide to Independent Research Projects
- I understand that this is an *independent* project and that I am solely responsible for its completion
- I agree to comply with all laboratory and fieldwork protocols established by the Faculty.

As the supervisor of this project I agree to:

- Meet with the student undertaking this project on at least a monthly basis and to respond to the progress e-mails as appropriate
- To meet formally with the student during the first week in November to undertake the interim interview
- To provide guidance and support to the student undertaking this project bearing in mind that it is an *independent* research project. This is inclusive of commenting on drafts of the final report in a timely fashion.

Both of the undersigned parties agree to be bound by this learning contract:	
Student Signature:	GSA
PRINT NAME:	GEORGE STROUD
Date:	27/4/2016

Supervisor Signature:	
PRINT NAME:	JAMIE ANN EVANS
Date:	27 4 2016

When completed, this form should be handed in to SciTech Admin (C114) and a copy retained by the student to be included in an appendix to the final IRP document.

Figure 6. Learning contract side 2

Data

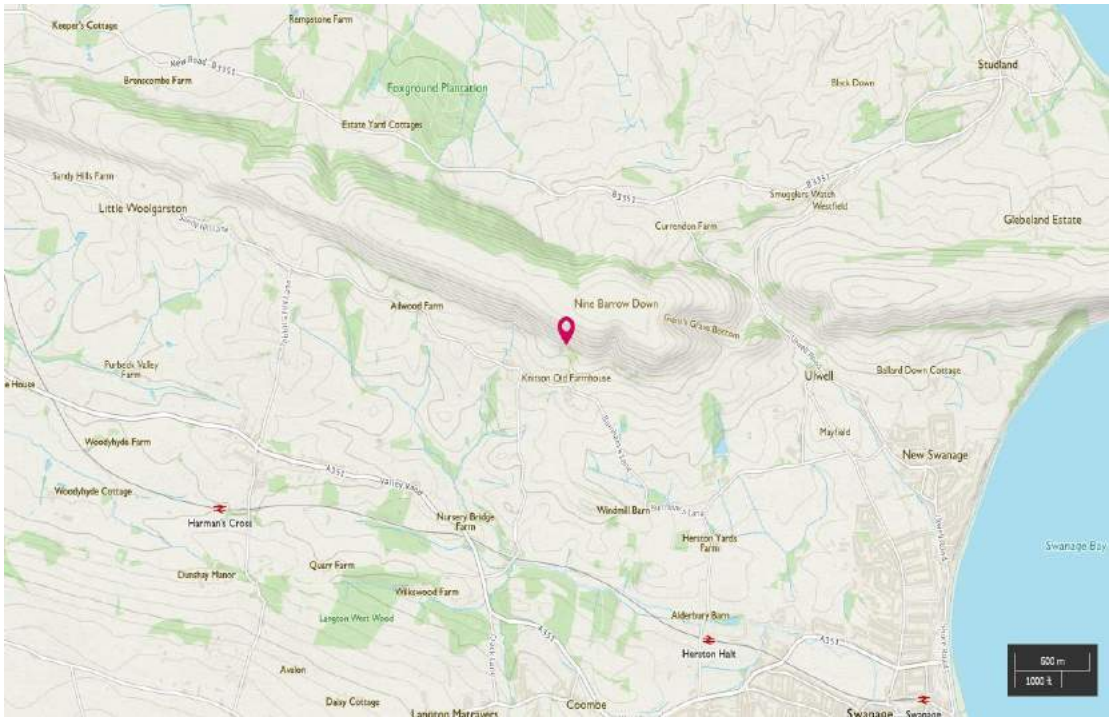


Figure 7. Location the Lithomorphic soil (rendzina) was collected from. Located in Wareham, Dorset, UK. Nearest postcode BH20 5JB. (50.628134, -1.994107) (SZ 00513 80937). (Ordinance Survey 2017).

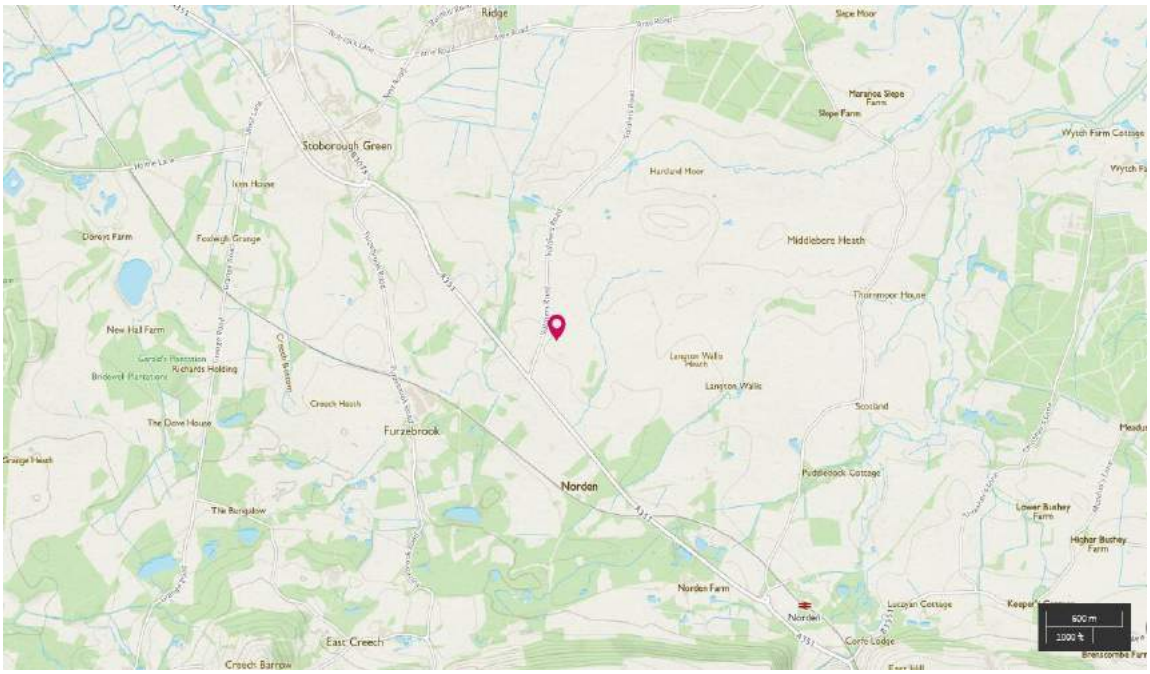


Figure 8. Location the Podzol soil was collected from. Located near Corfe Castle, Wareham, Dorset, UK. Nearest postcode BH20 5DU. (50.659316, -2.0848604) (SY 94098 84408). (Ordinance Survey 2017).

Figure 9. Example of mouse carcass after exhumation from podzol (soil A). Pre-soil removal and clearly in a state of decomposition.

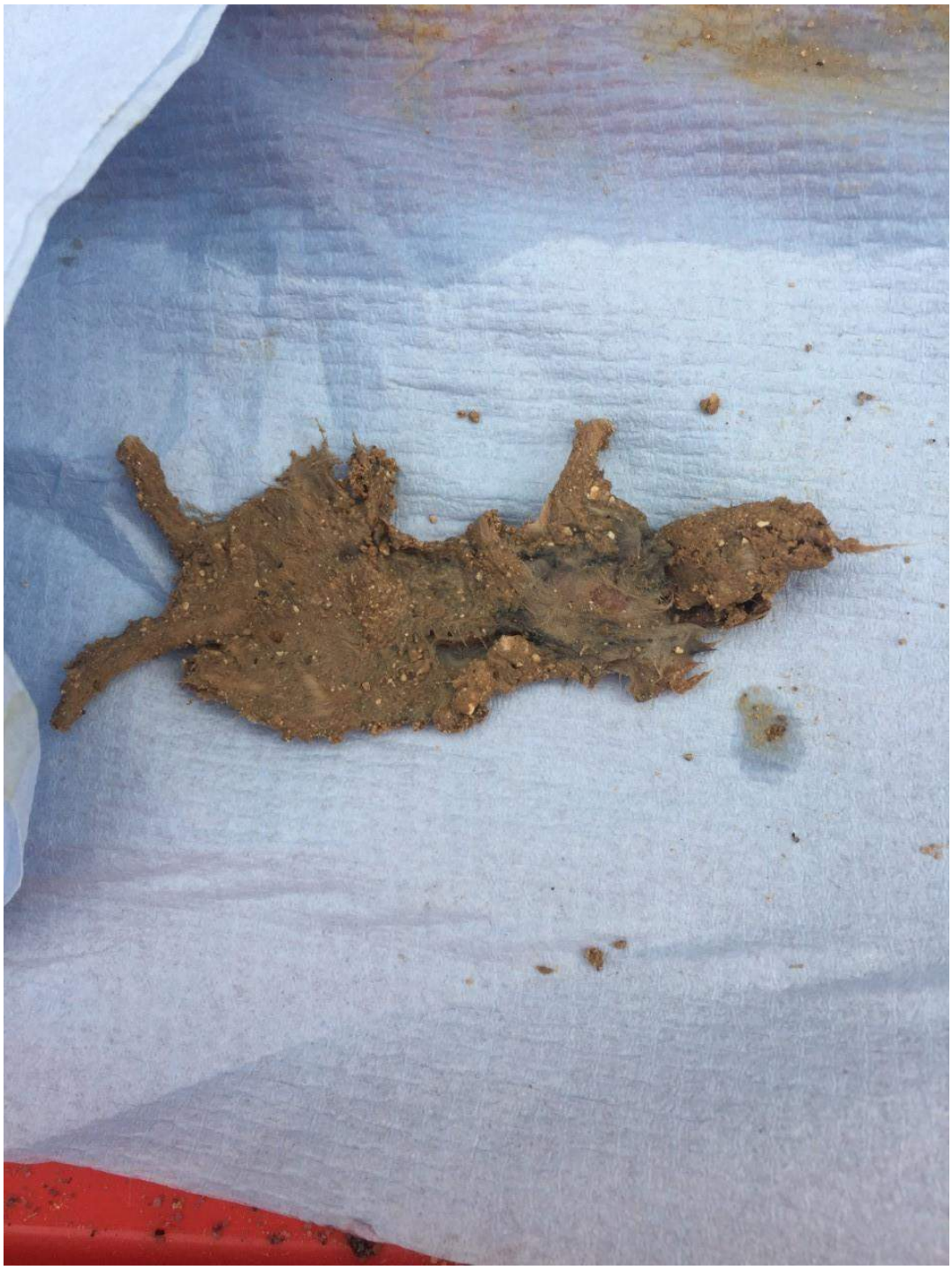


Figure 10. Example of mouse carcass after exhumation from rendzina (soil B). Pre-soil removal and clearly in a state of decomposition.

Figure 11. Example of tub storage with podzol. Also, portraying soil level inside tubs.

Figure 12. Example of tub storage with rendzina. Also, portraying soil level inside tubs.