Malaysian Journal of Analytical Sciences (MJAS)
Published by Malaysian Analytical Sciences Society

BELOWGROUND DECOMPOSITION OF SHOULDER AND RUMP FATTY FLESH IN SANDY CLAY LOAM SOIL OF RUBBER PLANTATION OF BUKIT PAYONG, MARANG

(Pereputan Bawah Tanah Bagi Daging Bahu dan Pinggul dalam Tanah Loam Berpasir Ladang Getah Bukit Payong, Marang)

Siti Sofo Ismail*, Thivialosini Siva, Lee Xin Pei, and Loh Kit Yee

Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia

**Corresponding author: sofo@umt.edu.my*

Received: 17 August 2023; Accepted: 4 November 2023; Published: 29 December 2023

Abstract

Understanding the decomposition process of different body parts is crucial in estimating the postmortem interval (PMI) and/or locating a clandestine grave. Therefore, we conducted a laboratory controlled simulated burial experiment, mimicking a burial in a shallow grave under tropical climate, to comprehensively observe the changes of soil pH and the trend of decomposition rate. Fatty flesh of commercial pigs (*Sus scrofa*) from the shoulder and rump parts was buried in sandy loam soil. Firstly, a vial was firstly half-filled with the soil. The fatty flesh was placed and covered completely with the soil, then, allowed to decompose for 150 days of burial period. The associated soils were collected at 15 designated sampling points. Soil post-experimental pH was recorded, and the remaining fatty flesh was weighed. The lipids were extracted using modified Bligh-Dyer extraction method and analyzed with a gas chromatography-flame ionization detector (GC-FID). We noted differences in the trend of pH, decomposition rate, and total lipid extract concentration. The results demonstrated that generally the soil pH of both body parts changed from acidic at the early decomposition stage, to increasingly alkaline after several days of burial, and the alkalinity decreased back towards the completion of the experiment. Besides this, the soil pH of the rump increased, and the shoulder decreased at the early butyric fermentation stage. The decomposition rate of shoulder fatty flesh was higher than that of the rump at the early decomposition process with a maximum rate at the same burial interval. At the butyric fermentation stage, the total cadaveric derived lipid of the rump was higher than that of the shoulder. The findings of this study shall provide important information on the fates of different body parts and their impact on the surrounding soil pH, which can be used in forensic investigations to locate the clandestine graves and/or to estimate the PMI of the body.

Keywords: cadaveric derived lipids, rump fatty flesh, clandestine grave, postmortem interval, sandy clay loam soil

Abstrak

Pemahaman tentang proses penguraian bahagian badan yang berbeza adalah penting dalam menganggarkan selang postmortem (PMI) dan/atau untuk mencari kubur rahsia. Oleh itu, kami menjalankan eksperimen pengebumian simulasi terkawal makmal, meniru pengebumian di dalam kubur cetek di bawah iklim tropika untuk memerhati secara menyeluruh tentang perubahan pH

tanah dan trend kadar penguraian. Daging babi komersial (*Sus scrofa*) yang berlemak dari bahagian bahu dan pinggul ditanam di dalam tanah liat berpasir. Mula-mula, sebuah vial terlebih dahulu diisi separuh dengan tanah tersebut. Kemudian, daging berlemak itu diletakkan dan ditutup sepenuhnya dengan tanah, dibiarkan mereput selama 150 hari tempoh pengebumian. Tanah yang berkaitan telah dikumpulkan di 15 titik persampelan yang ditetapkan. pH tanah selepas eksperimen direkodkan dan baki daging berlemak ditimbang. Lipid telah diekstrak menggunakan Kaedah pengekstrakan Bligh-Dyer Terubahsuai dan dianalisis dengan gas kromatografi- pengesan nyalaan pengionan (GC-FID). Kami mencatatkan perbezaan dalam trend pH, kadar penguraian dan jumlah kepekatan ekstrak lipid. Keputusan menunjukkan bahawa secara amnya pH tanah kedua-dua bahagian badan berubah daripada berasid pada peringkat penguraian awal, meningkat kepada beralkali selepas beberapa hari pengebumian dan menurun kembali menghampiri selesai eksperimen. Selain itu, pH tanah bagi pinggul meningkat dan bahu menurun pada peringkat pembusukan hitam. Kadar penguraian daging lemak bahu adalah lebih tinggi daripada pinggul pada proses penguraian awal dengan kadar maksimum pada selang pengebumian yang sama. Pada peringkat penapaian butirik, jumlah lipid kadaver yang diperolehi adalah lebih tinggi daripada bahu. Penemuan ini memberikan maklumat penting tentang nasib bahagian badan yang berbeza dan kesannya terhadap pH tanah sekeliling yang boleh digunakan dalam penyiasatan forensik untuk mengesan kubur rahsia dan/atau untuk menganggarkan PMI mayat.

Kata kunci: Lipid terbitan kadaver, daging berlemak pinggul, kubur rahsia, selang masa postmortem, tanah liat berpasir

Introduction

Decomposition is defined as a process in which the tissue of dead organisms is degraded through the chemical and biological degradation of the tissue to simpler forms of matter and the physical removal of soft tissue by arthropods and scavengers [1-3]. It is a complicated process primarily dependent on a number of biotic and abiotic factors such as temperature, pH and moisture of the soil, the presence of microbial communities, burial environment and cadaver condition [3-6]. Several physical, chemical and climatic factors may delay and accelerate the decomposition process [2, 3]. Due to these factors, the decomposition rate of a buried body has been found to be much lower than that of a body on the soil surface [7, 8]. The decomposition process, which involves two principal processes of autolysis and putrefaction, can be described in a series of decay stages based on physicochemical changes [2, 7, 9, 10]. For this study, the decomposition stages were described as initial decay (0- 3 days), putrefaction (4-10 days), black putrefaction (10-20 days), butyric fermentation (20-50 days) and dry decay (> 50 days), each stage having its duration (burial interval) and characteristics [11]. The stages shall ease the documentation of physical and chemical changes that may be observed throughout the decomposition process. Furthermore, the progress of the decomposition can be measured, in particular, to evaluate the extent of tissue degradation, and subsequently, to estimate the postmortem interval (PMI).

The recovery of a clandestine grave or body and estimation of PMI are crucial in forensic investigation to give a closure to the cases. The PMI indicates the time of a person's death, potentially assisting in the identification of the deceased and reducing the number of suspects in homicide investigations [12-14]. There are a number of methods that have been used to estimate PMI, including the potassium levels of vitreous humor, analysis of body temperature or algor, livor and rigor mortis, ages of insects in immature stages that feed on a corpse, and DNA degradation [15]. However, the estimation of PMI becomes more difficult and less accurate with the longer time of death [16]. This is because at the more advanced stage of the decomposition process, the uncertainty and errors in estimating PMI will be significantly increased [15]. With this, the identification of the deceased will be more difficult, especially for a buried body, where DNA identification may not be possible as the DNA may have vanished due to the chemical reactions in the soil [17].

This paper details a simulated burial experiment in sandy clay loam soil, where the aim is to investigate the decomposition rate of different body parts, the changes in soil pH, and the concentration of cadaveric derived lipids that may be introduced into the surrounding soil. The fatty flesh of the domestic pig (*Sus scrofa*) was used to replace human tissues due to ethical issues. Pigs are known to have similar anatomical structures and functions, such as skin, body hair, cardiovascular and urinary system, and fatty acid distributions as humans

[2, 18, 19]. This simulated burial experiment was conducted with the ultimate aim to establish an alternative approach using cadaveric derived lipids to locate the clandestine graves and/or to estimate the PMI.

Lipids are one of the products that are liberated from a decomposed human body [8, 20-22]. The lipids are known to have the characteristics of chemical stability, ubiquity, diagenetic, and extreme diversity in the structures [23-24]. Hence, lipids have higher persistent characteristics and preserve longer within soil. The soil lipids have also been recognized to contain several diagnostic markers of soil environmental conditions, where the simple lipids such as fatty acids and alcohol have been considered to be good markers of origin and evolutions of lipid material in soils [25-27]. Hence, together with the unique properties of soil, the decomposition rate of different body parts, the changes in the soil pH and distribution of the extractable lipids were measured in order to investigate the extent of the decomposition process, and subsequently, elucidate the potential of cadaveric derived lipids as decomposition biomarkers. The documented lipid patterns and other decomposition information for every stage of decomposition event would yield valuable insights about the duration elapsed since death. Besides, a comparative analysis of the quantity of cadaveric lipids extracted from the soil at a particular place would prove the existence of a body in the past and, consequently, the location of a clandestine grave.

Materials and Methods

Simulated burial experiment: Sandy clay loam soil

Sandy clay loam soil was the soil of interest for this study, which is known to consist mainly of sand and silt, with a low amount of clay [28]. Sandy loam soil, with a composition of 60% sand, 30% silt, and 10% clay, was collected from a rubber plantation which is located at Bukit Payong, Marang, Terengganu (5.2248°N, 103.1075° E), to mimic the burial of one of the common body disposal sites which used to be encountered in forensic investigations.

Particle size analyzer (PSA 1190) was used to identify the mixture composition of the soil. 5-10g of oven dried soil sample was sieved to remove the small branches, and tiny stones. The sieved sample have to tumble slowly to prevent the brazil-nut-effect during the analysis. Glucagon (0.1%) was used to help the soil rich with organic matters to disperse completely to obtain the result in soil triangle graph with the composition percentage.

Experimental design

A laboratory controlled simulated burial experiment was conducted to investigate and compare the decomposition process of a different part of cadaver, i.e., shoulder and rump that was buried in sandy loam soil, under tropical climate. First, the soil was collected from approximately one meter depth, i.e., the depth of graves that are commonly encountered in forensic investigations. The soil was used to bury about 20g of pig (*Sus scrofa*) fatty flesh for each, shoulder and rump body parts in vials, mimicking a burial in a shallow grave. The accurate mass of fatty flesh at each sampling point was measured and recorded. The vials were labeled and allowed to be exposed to the ambient environment. Then, the fatty flesh was allowed to decompose for a number of designated sampling points, corresponding to the decomposition stages, with specific burial intervals. The gaps between adjacent samplings were two days for the first ten days of sampling points, representing the initial decay and putrefaction stages, and three days for the black putrefaction stage. The stages have been known as active decay stages, which was postulated to be accompanied with massive introduction of cadaveric derived materials. The soil moisture was checked consistently. At each sampling point, the remaining fatty flesh was removed from the soils and weighed. The difference of mass was used for the calculation of decomposition rate. The formula of decomposition rate is as shown below. The associated soils were stored in a freezer before being freeze-dried and subjected to further subsequent analyses. The soil was then freeze dried prior to lipid extraction.

$$Decomposition\ rate = \frac{Initial\ mass\ of\ fatty\ flesh - Final\ mass\ of\ flatty\ flesh}{Sampling\ Time\ Period} \quad (1)$$

Soil post-experimental pH

The pH of control and experimental soils was measured using a calibrated pH meter. Distilled water was added to dried soil samples in disposable culture tubes in a ratio of 1:5 of soil: water. The mixture was then mixed well and the soil was allowed to settle for at least 5 minutes. After that, the pH of the soil was measured by completely immersing the pH probe into the solution. The probe was ensured to avoid contact with the soil that had settled at the bottom of the tube. The pH reading was allowed to stabilize before recording the value. All pH measurements for every sample were repeated three times and recorded.

Total lipid extraction

Modified Bligh-Dyer extraction method was used to extract the lipids from the associated soils. Approximately 3 g of dry soil was transferred into a Pyrex culture tube. Then, 3 mL of 2:1 DCM/methanol (2:1, v/v) and 100 µL of internal standard of tetratriacontane (C-34) were added into the sample. The sample was sonicated for 15 minutes at 30°C, and this was followed by centrifuging it for 5 minutes (~3000 rpm). The supernatant was then transferred into a clean vial and the process was repeated three more times using 2 mL of DCM/methanol (2:1, v/v). After being treated with 3 mL Bligh-Dyer solvent the soil sample was sonicated for 15 minutes and centrifuged for 5 minutes (~3000 rpm). The obtained supernatant was transferred into the same vial. The Bligh Dyer solution was prepared by mixing buffered water, chloroform and methanol in a ratio of 4:5:10 [29]. The extraction was repeated three times with 2 mL Bligh-Dyer solvent. To break the organic phase, 2 mL of each buffered water and chloroform were added to the supernatant, and the mixture was centrifuged for 5 minutes. The organic layer at the bottom was transferred to a new clean vial. The process was repeated three times with 2mL chloroform. The excessive solvent was then evaporated using a mild nitrogen flow. The total lipid extract (TLE) was weighed using the following formula equation 2 and stored in the freezer prior to subsequent analyses.

$$\text{Total Lipid Extract} = \text{Final mass of vial} - \text{Initial Mass of vial} \quad (2)$$

Where, initial mass of vial = mass of empty vial, and final mass of vial = mass of vial with the extracts

Instrumental analysis

A Gas Chromatography – Flame Ionization Detector (GC-FID) (GC-6890N, Agilent) was used to analyze the samples. The GC-FID was equipped with a column of HP-5 5% phenyl methyl siloxane column, with helium as the carrier gas. After injection at 50 °C, the oven temperature was held for 2 minutes, followed by an increase in temperature to 300 °C at 10°C min^{-1}, which was held for 20 minutes. The peaks were selected by comparing the retention times with the external standard, prepared by the mixing of several lipid components, including palmitic and stearic acids, cholesterol, tetratriacontane, and triacylglycerides.

Results and Discussion

Post experimental soil pH

The breakdown of the adipose tissue in the rump and shoulder showed a similar pattern to that of previous investigations. The pH of the soil often rises in the early stages for buried corpses, then becomes more alkaline before acidifying in the later stages [30-32]. Furthermore, a similar trend in the pH changes was observed for the associated soils of the decomposing shoulder and rump fatty flesh. The post experimental soil pH increased at the early decomposition stages and decreased towards the end of the burial interval (Figure 1). The pH was acidic at the beginning of the decomposition event, then increased to become alkaline before acidifying again towards the completion of 150 days of the burial event.

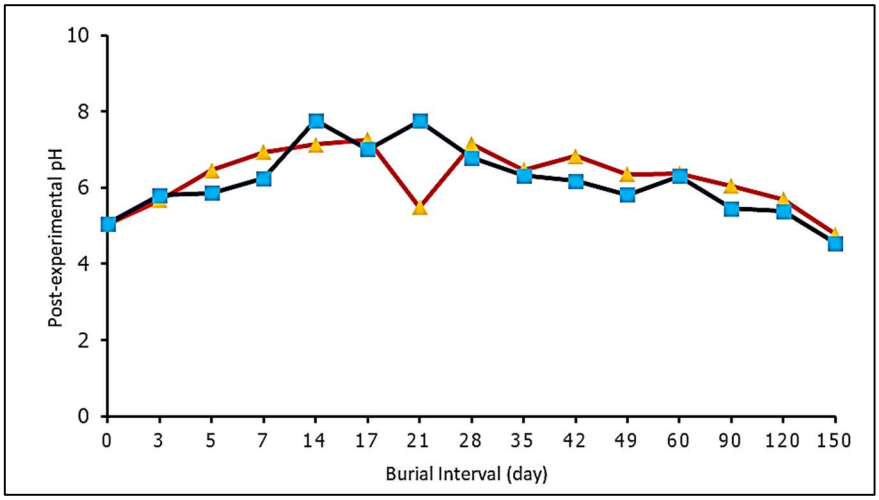

Figure 1. The changes in post experimental soil pH associated with the decomposition of pig fatty flesh of rump (blue line) and shoulder (red line) for 150 days of burial interval

The pH change was literally variable in response and slightly differed between the rump and shoulder body parts. However, the overall trend in pH changes for both body parts was similar throughout the 150 days of burial period. The pH increased between day 0 and day 14 of the burial period, corresponding to the initial and putrefaction stages of the decomposition process. The highest pH for the burial of rump and shoulder parts was observable after day 14 and day 17 of the burial period with a pH 7.77 and pH 7.26, respectively. Interestingly, on day 21 of the burial period, the soil pH of the shoulder body part increased while that of the rump fell, indicating a distinct trend in the soil pH during the early butyric fermentation stage. After 21 days of burial period, the decrease in the soil pH of decomposing rump and shoulder fatty flesh was observed, and thereafter, it continued to decline towards the completion of 150 days of burial period, with minor fluctuations. The lowest soil pH for shoulder (pH 4.78) and rump (pH 4.54) was recorded on day 150, corresponding to the dry decay stage.

The increasing in post-experimental soil pH at the first two decomposition stages, i.e., initial and putrefaction stages, may be due to the increase in the concentration of nitrogen-containing compounds, such as ammonium ions in the soil, resulting from the degradation of body macromolecules [30, 33]. After these stages, the decrease in the soil pH could be due to several reasons. Mineralization of protein and other organic nitrogen occurs via ammonification followed by nitrification of ammonia, NH_4^+, which may reduce the concentration of these nitrogen-containing compounds that are responsible for the increase in soil pH. The conversion of NH_4^+ to NO_3^- liberates protons, causing the soils to become more acidic [30, 32, 34, 35]. Besides, the continual degradation of fatty flesh may also produce non-mineralized decomposition by-products, such as acetic acid and phenolic compounds, and these may be deposited into the soil system. Organic components have been known to lower the pH and potentially inhibit subsequent decomposition events and microbial activities [34, 36]. These activities in the soil may also explain the fluctuations in the soil pH between day 17 and day 28 of the burial period for both rump and shoulder parts. These days correspond to the putrefaction and black putrefaction stages.

The decrease in the post-experimental soil pH after 21 days of burial period, continued towards the completion of the 150 days, a finding that is similar to a previous study by Haslam and Tibbett [35], which found that the soil pH became acidic when the decomposition process entered the butyric fermentation and dry decay stages. This decrease in the soil pH may also be explained by the presence of the decomposition acidic by-products as the increase in the amount of nitrate through the nitrification process has been known to result in a

decrease of soil pH [21, 35]. Moreover, the leaching of decomposition fluid which contains acidic by-products, such as volatile and long-chain fatty acids, also contributes to the decrease of soil pH (31, 38-40).

Decomposition rate

In general, the fatty flesh of both body parts demonstrated a similar trend in the decomposition rate. The decomposition rates of a cadaver for both shoulder and rump parts were high at the early stages of the process, and slowed down towards the end of the 150 days of burial interval (Figure 2). However, the trend was found to be slightly different than that commonly observed for buried cadavers [8]. Furthermore, the decomposition rate of shoulder fatty flesh was higher than that of the rump fatty flesh. This observation may indicate that the shoulder fatty flesh decomposes much faster than the rump fatty flesh.

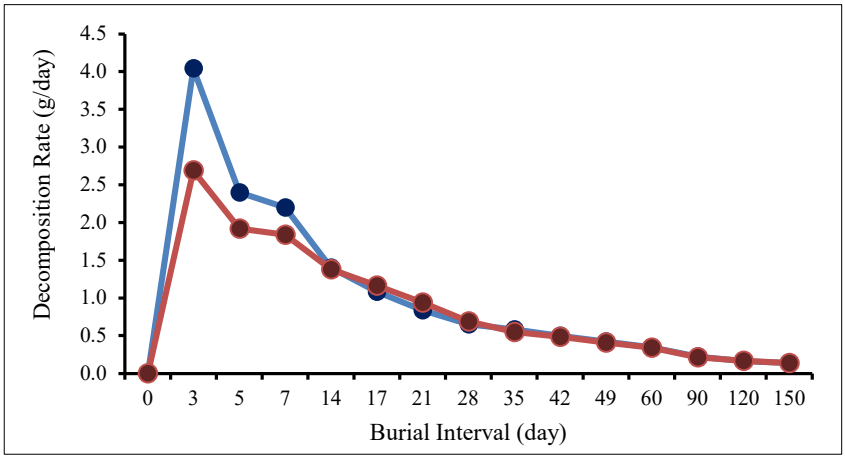

Figure 2. Graph of rate of decomposition of the burial fatty flesh of shoulder (blue line) and rump (orange line) for 150 days of burial period

The decomposition rate rapidly increased between day 0 and day 3 of the burial interval, reaching a maximum after 3 days of burial period, with magnitudes of 4.044 g/day and 2.690 g/day for the fatty flesh of shoulder and rump body parts, respectively. These days correspond to the initial decay stage. After this point, the decomposition rate decreased sharply and continued to decrease towards the end of the burial interval, reaching the lowest in the decomposition rate of shoulder and rump fatty flesh on day 150 of burial period. The lowest decomposition rates were recorded as 0.137 g/ day and 0.135 g/day for the fatty flesh of the shoulder and rump, respectively. This observation may indicate the completion of the decomposition process where most of the fatty flesh has disintegrated.

The biotic and abiotic factors of below ground are different compared to those above ground, which may reduce the activity of the decomposers [8, 34, 41]. Fiedler and Graw [42] stated that the decomposition of buried bodies depended mainly on scavenger activity, insect colonization, and temperature. Burial depth has also been identified to decrease the decomposition rate of a buried cadaver to lower the in-soil temperature, less decomposer activity, and grave moisture content [41, 43]. Considering the burial environmental factors for both rump and shoulder body parts, it has been hypothesized that the main body components of fat and muscular protein would contribute to the differences in their decomposition rates. The observation may be due to the autolysis process that took place during these days. This process has been known to involve the highest and most active degradation activity by microorganisms [4, 20, 34, 36]. The maximum decomposition rate may also be due to the higher rate of redox potential of tissues which contributed to the growth of bacteria at the end stage of autolysis during the decomposition of the fatty flesh [16, 17, 45]. Furthermore, the maximum soil microbial activity had also been observed within the first ten days of burial [44

– 46].

Total lipid extracts (TLEs)

The TLE mass recovered from the associated soils of both decomposing body parts exhibited a similar trend, i.e., increased at the beginning of the experiment, then decreased towards the completion of 150 days of the burial period. The TLE mass from both associated soils was found to slightly increase between day 0 and day 7 of the burial period, corresponding to the initial stage, and decreased towards the completion of the 150 days of the burial process. However, the mass of TLEs extracted from the associated soil of decomposing shoulder fatty flesh was lower than that of the rump fatty flesh. The mass of TLEs extracted from the soils of shoulder fatty flesh ranged from 0.0025 g to 0.0301 g/g soil dry weight, whilst, the mass of TLEs recovered from the soils of rump fatty flesh ranged between 0.0005 g/g and 0.5720 g/g soil dry weight. The lowest and the highest masses of soil TLEs of shoulder fatty flesh were recorded on day 7 (0.0025 g/g soil dry weight) and day 3 (0.0301 g/g soil dry weight), respectively. These days correspond to the initial decay (day 3) and putrefaction (day 7) stages.

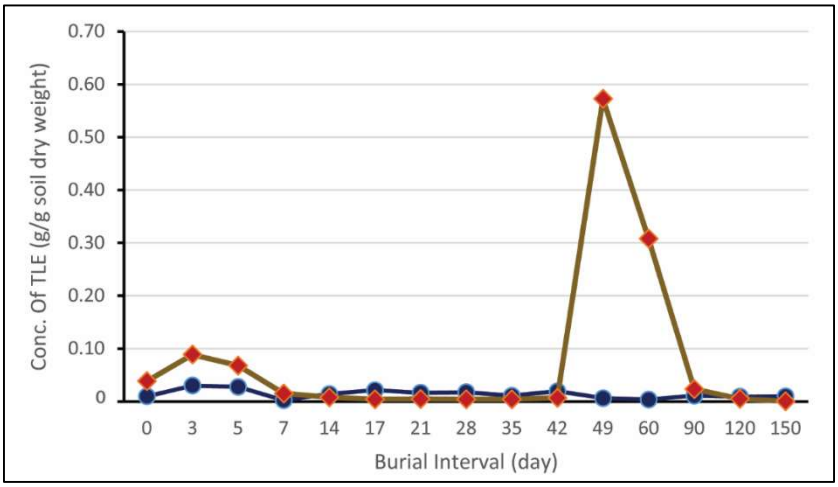

Figure 3. Total Lipid Extracts (TLEs) recovered from the soils associated with the decomposition of fatty flesh of rump (brown line) and shoulder (black line) parts for 150 days of burial interval

The trend of TLE mass of the rump fatty flesh was slightly different compared to that of the shoulder fatty flesh. Similar to that of the shoulder body part, the TLE mass increased between day 0 and day 3, corresponding to the initial decay stage. Then, the TLE mass decreased from day 5 until day 42 of the burial period, corresponding to the stages of putrefaction, black putrefaction, and butyric fermentation. After these points, the mass of TLE increased rapidly and reached the highest mass of TLE on day 49, i.e., 0.572 g/g soil dry weight. The TLE mass then decreased sharply after day 49 and this continued to the end of the burial period, reaching the lowest TLE mass on day 150, i.e. 0.0005 g/g soil dry weight.

The increase in the TLE mass that is observed at a number of burial days may potentially be the result from the leaching of cadaveric materials from the decomposing fatty flesh into the surrounding soil environment. The slight increase in the TLE mass during the initial decay and putrefaction stages agreed with the previous findings which described these first two stages as active decomposition stages [16, 47]. The decrease in the TLE mass after day 7 of burial period may explain a rapid mineralization of the introduced cadaveric materials to the simpler lipid components, such as carbon dioxide and water, as lipids are known to be vulnerable to the decomposition process when exposed to suitable conditions [48, 49]. Remarkably, the decrease in the TLE mass after this point can directly correlate with the decline in the decomposition rate of fatty flesh of both body parts.

The noticeable rapid increase in the TLE mass for rump that took place after 42 days of burial period potentially indicated a massive deposition of the cadaveric materials from the remaining fatty flesh into the surrounding soil system even though the decomposition rate started to slow down. This observation may also be a sign of slow mineralization of the cadaveric derived lipids, which may be due to the shifting in the microbial communities that were responsible for the decomposition process [45, 46, 50, 51]. The decomposition products have been suggested to impose a significant impact on the microbial communities that are responsible for the process, where they are known to either speed up or inhibit the disintegration of the fatty flesh [4, 45, 46]. It has been known that the fast-high rate of decomposition at the beginning of the experiment will be accompanied by a massive indication of the introduction of cadaveric substances into the surrounding burial soil [11, 18, 21, 31]. The mass of TLE was high at the beginning of the experiment. The potential explanation for this observation may be due to the rapid mineralization of the bigger compounds to simpler compounds. For example, the hydrolysis of triglycerides to free fatty acids and carbon dioxide. This explanation can be supported by the concentration of fatty acids detected by the GC-FID. The concentration of fatty acids was low at the beginning of the experiment, although the rate of decomposition was high.

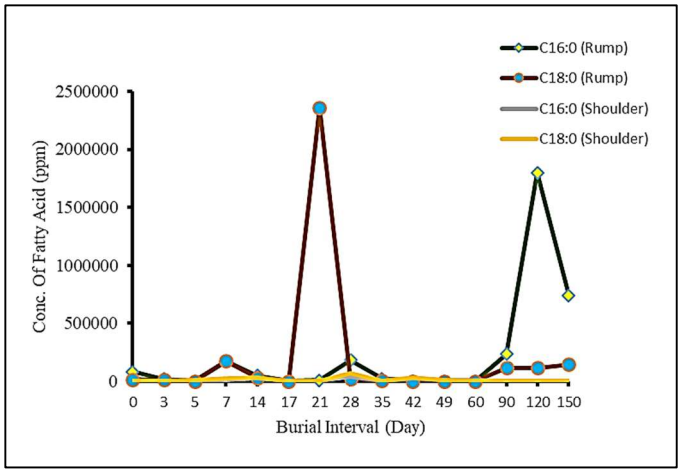

Figure 4. The concentrations of palmitic and stearic acids extracted from the soils associated with the decomposition of fatty flesh of rump and shoulder body parts

A gas chromatography–flame ionization detection (GC–FID) method was used for direct quantitative analysis of fatty acids. The analyses of cadaveric derived lipids, such as fatty acids, recovered from the associated soil was carried out to determine their potential usage for estimating the PMI. The lipid components detected were palmitic (C16:0) and stearic (C18:0) acids, together with cholesterol. Figure 4 shows the concentrations of fatty acids and cholesterol detected at different sampling points for 150 days of the burial interval. During the decomposition process, fatty acids are released from adipose tissues. The adipose tissues are made up from 60-85% lipids by weight, and 90-99% triglycerides, which would be further hydrolyzed into glycerin and free fatty acids [8]. Bull et al. [21] suggested an approximate 500-fold difference in the concentrations of palmitic (C16:0) and stearic (C18:0) acids extracted from the soils with decomposing cadaver or fatty flesh compared to that of control soils. Therefore, the presence of fatty acids in soil extracts at concentrations greater than that of the control soil can be interpreted as them having derived from anthropologically introduced organic matter such as pig adipose tissue. Furthermore, the remarkable changes in soil lipid compositions can help to confirm the prior presence of a cadaver in the suspected area. Janaway et al. [17] identified that the postmortem lipid components, such as palmitic, stearic, and oleic acids, were found as soon as 8 hours after

death, and that the most abundant lipid components in human body are oleic (C18:1), linoleic (C18:2), and palmitoleic (C16:1) acids. According to Dent et al. [8], these unsaturated fatty acids will be turned into simpler fatty acids shortly after death through hydrolysis and oxidation processes.

The majority of soil extracts showed a significant level of cholesterol along with the dominance of stearic (C18:0) and palmitic (C16:0) acids. Interestingly, the soils of both body parts had more palmitic (C16:0) acid than stearic (C18:0) acid. Furthermore, the soil of rump had greater fatty acid contents of C16:0 and C18:0 components than that of the shoulder. The total C16:0 and C18:0 concentrations of rump were determined to be 3315843.7 ppm and 2998265 ppm, respectively. The total values of C16:0 and C18:0 for the shoulder were 2108658.1 ppm and 1132487.0 ppm, respectively. There was 3840.1 ppm and 3466.9 ppm of cholesterol in the rump and shoulder, respectively.

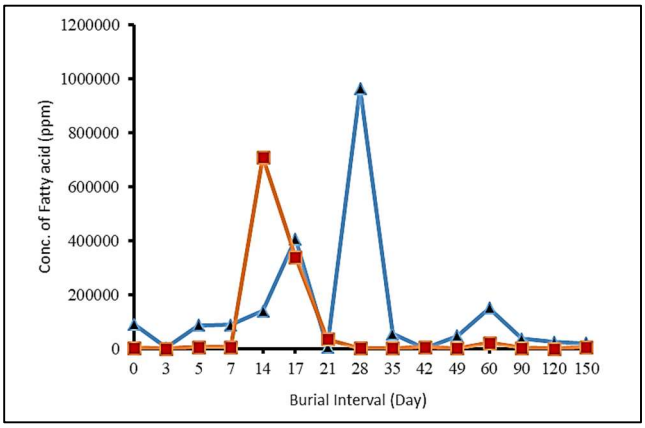

Figure 5. The concentrations of fatty acids of C16:0 and C18:0 components extracted from the soils associated with the decomposition of shoulder fatty flesh. The blue and orange lines represent the concentrations of C16:0 and C18:0 fatty acids, respectively

For the soils of the shoulder, the concentration of fatty acids generally increased between day 7 and day 21 of the burial interval, with minor fluctuations (Figure 5). These days correspond to the first three active decomposition stages of initial decay, putrefaction and black putrefaction. With a sharp increment between day 7 and day 14, the stearic acid reached maximum concentration (709127.6 ppm) on day 14, and then, the concentration of stearic (C18:0) acid decreased rapidly after this point and continued to decrease towards the completion of 150 days of the burial period. On the other hand, the palmitic acid demonstrated a first sharp increase in concentration after 17 days of the burial period, with a concentration of 138899.6 ppm, followed by a sharp decrease in the concentration of this fatty acid. The second dramatic increment in the concentration of this C16:0 component was observed after 21 days, where it reached a maximum concentration (964554.7 ppm) on day 28 of burial period. The possible explanation for this fluctuation is the introduction of different cadaveric substances during decomposition, which caused the shifting in microbial communities. Besides, there was also a slight increase in pH from day 21 to 28, which may impose an impact of the microbial community that is involved in the decomposition process. It is well known that soil microbial community has a strong correlation with soil pH [42]. The soil pH might facilitate succession of certain microbial species and induce the breakdown of lipids. The concentrations of palmitic (C16:0) and stearic (C18:0) acids decreased towards the end of the experiment. This observation was in line with the decomposition rate of the shoulder fatty flesh.

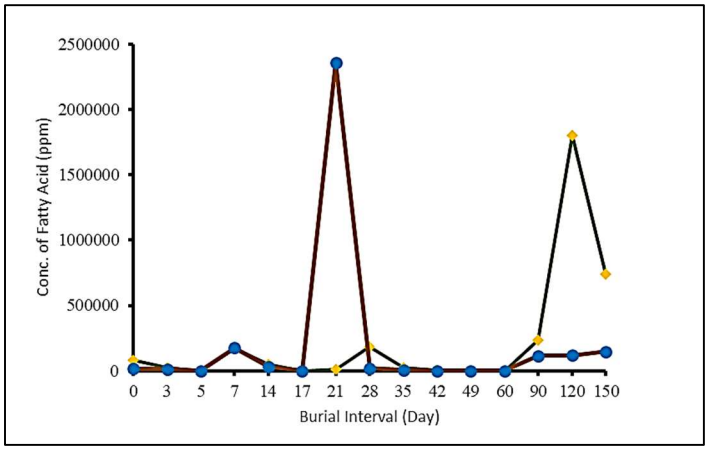

Figure 6. The concentrations of C16:0 and C18:0 extracted from the soils associated with the decomposition of rump fatty flesh. The green and brown lines represent the concentrations of C16:0 and C18:0 fatty acids, respectively.

The concentrations of palmitic and stearic acids extracted from the soils associated with the decomposition of rump fatty flesh demonstrated significantly different trends than that of the shoulder fatty flesh. Remarkably, the total concentration of C16:0 acid was higher than that of C18:0 acid. Furthermore, the concentration of palmitic acid was low at the early decomposition process before a rapid incline after 90 days of the burial event, with minor fluctuations between day 0 and day 35. The concentration of this fatty acid rapidly increased and reached a maximum (1798832.3 ppm) on day 120, corresponding to the dry decay stage. Then, the concentration of palmitic acid sharply declined after this point. The concentration of the stearic acid increased sharply after 17 days of the burial period and reached a maximum concentration (2359151 ppm) on day 21, corresponding to the black putrefaction stage. After this point, the concentration of this acid sharply declined to a much lower concentration which continued to decrease towards the completion of 150 days of the burial interval.

A higher abundance of palmitic and stearic acids extracted from the associated soils may be an indication of hydrogenation process of unsaturated acids and triacylglycerols during the decomposition of the fatty flesh of both rump and shoulder body parts. It is known that the hydrolysis of triacylglycerols is followed further by hydrogenation to form saturated components, yielding palmitic and stearic acids [8]. Moreover, the increase and decrease of the concentrations of palmitic and stearic acid may be due to the shifting of microbial communities due to the introduction of cadaveric components into the soil. The increment of fatty acid levels might be due to the initial growth and feeding stage of maggots, whereas the decrement of fatty acid might be due to the pupation period [40]. This also suggested that the activity of insects played a role in the amounts of fatty acids detected in the soil. The high concentrations of these short chain fatty acids may be explained by the observation that short-chain fatty acids had degraded faster than long-chain fatty acids [33]. This statement may also explain the low concentrations of cholesterol, and long-chain lipids extracted from the soils associated with the decomposition of both rump and shoulder body parts (Figure 7).

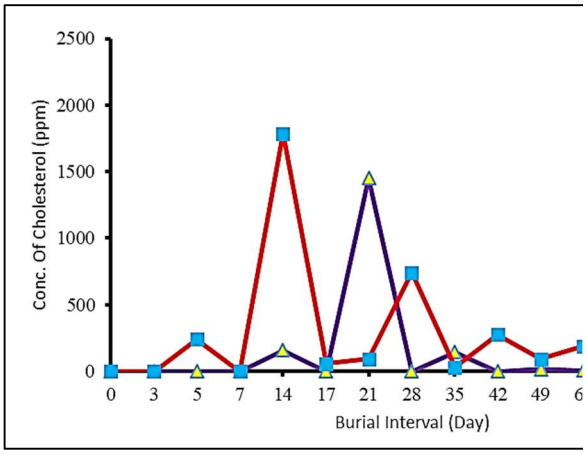

Figure 7. The concentrations of cholesterol extracted from soils associated with the decomposition of rump (purple line) and shoulder (red line).

Conclusion

This study was conducted to investigate the potential of cadaveric lipids to be developed as burial biomarkers that may aid in locating clandestine graves and/or to determine the PMI. The distribution of the cadaveric derived lipids was documented to further confirm their potential to provide useful information to aid forensic investigators as their concentrations may differ significantly, which can act as fingerprints for each of the decomposition stages. The fatty flesh of shoulder and rump demonstrated similar trends in the post experimental soil pH and decomposition rate. The post experimental soil pH was acidic at the early decomposition stages, then became alkaline, and then acidic again towards the completion of 150 days of the burial interval. The decomposition rate was found to be high at the initial stage and had reached the maximum after 3 days of the burial interval. Then, the decomposition rate then eventually decreased after this point and continuously decreased towards the completion of the process. Even though, both the body parts exhibited similar trends, the decomposition rate of fatty flesh of the shoulder was higher than that of the rump. A similar trend was also observed for their extractable derived lipids throughout the burial period. The concentrations of lipids slightly increased at beginning of experiment and decreased towards the end of experiment with minor fluctuations, which may due to the activity of the microbial community that is responsible for the decomposition process. However, the concentration of the extractable cadaveric lipids of the rump was higher than that of the shoulder. Moreover, the concentration of cadaveric lipids of the rump rapidly increased after 42 days of the burial period and decreased thereafter. The concentrations of cadaveric derived fatty acids and cholesterol extracted from the soils of both rump and shoulder were also different in their trends throughout the 150 days of the burial period. Hence, these findings may indicate that the analysis of soil cadaveric lipids can be developed as an alternative forensic tool to locate a clandestine grave and to estimate the PMI and/or to identify the victim and the criminal. It is recommended to investigate the breakdown of other body components in order to provide more evidence that different body parts break down at various rates. More information about the destiny of various body parts can therefore be obtained.

Acknowledgement

The authors would like to thank the Ministry of Higher Education Malaysia FRGS/1/2021/WAB02/UMT/03/3 for the financial aid, Faculty of Science and Marine Environment, and the Centre of Research and Field Service of Universiti Malaysia Terengganu (UMT) for providing the necessary equipment and facilities for the completion of this study.

References

1. Clark, M.A, Worrell, M.B and Pless, J.E (1997). Postmortem changes in soft tissue, in: W.D. Haglund, M.H. Sorg (Eds), Forensic taphonomy: The postmortem fate of human remains, CRC Press, Boca Raton, Florida, pp 151-160.
2. Goff, M.L. (2009). Early post-mortem changes and stages of decomposition in exposed cadavers. *Experimental and Applied Acarology*, 49: 2-36.
3. Zhou, C. and Byard, R.W. (2011). Factors and processes causing accelerated decomposition in human cadavers-An overview. *Journal of Forensic and Legal Medicine* 18(1): 6-9.
4. Vass, A.A. (2001). Beyond the grave: understanding human decomposition. *Microbial Today*, 28: 190-192.
5. Forbes, S.L, Dent, B.B. and Stuart, B.H. (2005).

The effect of soil type on adipocere formation. *Forensic Science International*, 154:35-43

6. Carter, D.O., Yellowless, D. and Tibbett, M. (2008). Temperature affects microbial decomposition of cadavers (*Rattus rattus*) in contrasting soils. *Applied Soil Ecology*, 40: 129-137.
7. VanLaerhoven, S.L. and Anderson, G.S. (1999). Insect succession on buried carrion in two biogeoclimatic zones of British Columbia. *Journal of Forensic Science*, 44(1):32-43.
8. Dent, B.B., Forbes, S.L., and Stuart, B.H. (2004). Review of human decomposition in soils. *Environmental Geology*, 45:576-585.
9. Payne, J. A. (1965). A summer carrion study of the baby pig *Sus scrofa Linnaeus. Ecology,* 46(5):592-602.
10. Eline M.J. Schotsmans, Van de Voorde, W., De Winne, J. and Andrew S.W. (2011). The impact of shallow burial on differential decomposition to the body: A temperate case study. *Forensic Science International*, 206(1-3): 43-48.
11. Ismail, S.S. and Chong, Z.Y. (2019). Decomposition of abdomen fatty flesh of cadaver buried in Nami series soil of Bukit Kor Terengganu. *Materials Today: Proceedings*, 19: 1426-1433.
12. Mann, R.W., Bass, W.M. and Meadows, L. (1990). Time since death and decomposition of the human body: variables and observation in case and experimental field studies. *Journal of Forensic Science,* 35(1): 103-111.
13. Catts, E.P. (1992). Problems in estimating the postmortem interval in death investigations. *Journal of Agricultural Entomology*, 9(4): 245-255.
14. Megyesi, M.S., Nawrocki, S.P. and Haskell, N.H. (2005). Using accumulated degree-days to estimate the postmortem interval from decomposed human remains. *Journal Forensic Science*, 50(3): 618-626.
15. Swann, L., Forbes, S. and Lewis, S. (2010). Analytical separations of mammalian decomposition products for forensic science: A review. *Analytica Chimica Acta*, 682(1-2): 9-22.
16. Teo, C.H., Hamzah, N.H., Hing, H.L. and Hamzah, S.P. (2014). Decomposition process and post mortem changes: Review. *Sains Malaysiana*, 43(12):1873-1882.
17. Janaway, R., Percival, S. and Wilson, A. (2009). Decomposition of human remains. *Microbiology and Aging*. Totowa, NJ: Humana Press, pp. 313-334.
18. Notter, S.J., Stuart, B.H., Rowe, R. and Langlois, N. (2009). The initial changes of fat deposits during the decomposition of human and pig remains. *Journal of Forensic Sciences*, 54(1): 195-201.
19. Štembírek, J., Kyllar, M., Putnová, I., Stehlík, L., and Buchtová, M. (2012). The pig as an experimental model for clinical craniofacial research. *Laboratory Animals,* 46(4): 269-279.
20. Janaway, R.C. (1987). The decay of buried remains and their associated material; in Studies in Crime: An introduction to forensic Archaeology (J. Hunter, C. Roberts, and A. Martin, Eds.). London: Routledge, 58-85.
21. Bull, I.D., Berstan, R., Vass, A. and Evershed, R.P. (2009). Identification of a disinterred grave by molecular and stable isotope analysis. *Science & Justice*, 49(2): 142-149.
22. Ioan, B.G., Manea, C., Hanganu, B., Statescu, L., Solovastru, L.G. and Manoilescu, I.R.I.N.A. (2017). The chemistry decomposition in human corpses. *Revista de Chimie*, 68(6): 1450-1454.
23. Derrien, M., Cabrera, F.A., Tavera, N.L., Manzano, C.A. and Vizcaino, S.C. (2015). Sources and distribution of organic matter along the Ring of Cenotes, Yucatan, Mexico: Sterol markers and statistical approaches. *Science of the Total Environment*, 511: 223-229.
24. Collins, S., Stuart, B. and Ueland, M. (2020). Monitoring human decomposition products collected in clothing: an infrared spectroscopy study. *Australian Journal of Forensic Sciences,* 52(4): 428-438.
25. Ambles, A., Magnoux, P., Jacquesy, R. and Fustec, E., (1989). Effects of addition of bentonite on hydrocarbon fraction of a podzol soil (A1 Horizon). *Journal of Soil Science*, 40: 485-694.
26. Jambu, P, Ambles A., Dinel H, and Seqouet B. (1991). Incorporation of natural hydrocarbons from plant residues into a hydromorphic humic podzol following afforestation and fertilization. *Journal of Soil Science*, 42: 629-636.
27. Jambu, P., Ambles, A., Jacquesy, J.C., Secouet, B., and Parlanti, E. (1993). Incorporation of natural

alcohols from plant residues into a hydromorphic forest-podzol. *Journal of Soil Science*, 44: 135-146.
28. Ritchey, E.L., McGrath, J.M. and Gehring, D. (2015). Determining Soil Texture by Feel. *Agriculture and Natural Resources Publications*. pp.139.
29. Ismail, S. S. and Daud, N. A. (2016). Lipid analysis on potential grave soil products. *Transactions on Science and Technology*, 3(3): 489-494.
30. Hopkins, D.W., Wilthire, P.E.J. and Turner B.D. (2000). Microbial characteristic of soils from graves: an investigation at the interface of soil microbiology and forensic science. *Applied Soil Ecology*, 14(3): 283-288.
31. Laura, A.B., David, O.C. and Shari, L.F. (2008). The biochemical alteration of soil beneath a decomposing carcass. *Forensic Science International*, 180(2-3): 70-75.
32. Szelecz, I., Koenig, I., Seppey, C.V., Le Bayon, R.C. and Mitchell, E. A. (2018). Soil chemistry changes beneath decomposing cadavers over a one-year period. *Forensic Science International*, 286: 155-165.
33. Comstock, J.L., Leblanc, H.N. and Forbes, S.L. (2016). Analysis of decomposition fluid collected from carcasses decomposing in the presence and absence of insects. *Soil in Criminal and Environmental Forensics Soil Forensics*, pp. 275-296.
34. David, O.C., David, Y. and Mark, T. (2007). Cadaver decomposition in terrestrial ecosystems. *Naturwissenschaften*, 94: 12-24.
35. Haslam, T.C. and Tibbett, M. (2009). Soils of contrasting pH affect the decomposition of buried mammalian (*Ovis aries*) skeletal muscle tissue. *Journal of Forensic Sciences*, 54(4): 900-904.
36. Vass, A.A., Barshick, S.A., Sega, G., Caton, J., Skeen, J.T., Love, J.C. and Synstelien, J.A. (2002). Decomposition chemistry of human remains: a new methodology for determining the postmortem interval. *Journal of Forensic Science,* 47(3): 542-553.
37. Szelecz, I., Sorge, F., Seppey, C.V., Mulot, M., Steel, H., Neilson, R., ... and Mitchell, E.A. (2016). Effects of decomposing cadavers on soil nematode communities over a one-year period. *Soil Biology and Biochemistry,* 103: 405-416.
38. Larizza, M. (2010). Physical and chemical analysis of pig carcass decomposition in fine sand (Master's thesis). Retrieved from https://ir.library.dc-uoit.ca/bitstream/10155/115/1/Larizza_Melina.pdf
39. Swann, L, Chidlow, G.E., Forbes, S.L. and Lewis, S.W. (2010). Preliminary studies into the characterization of chemical markers of decomposition for geoforensics. *Journal of Forensic Sciences*, 55(2): 308-314.
40. Swann, L., Forbes, S.L. and Lewis, S. W. (2010b). Observations of the temporal variation in chemical content of decomposition fluid: A preliminary study using pigs as a model system. *Australian Journal of Forensic Sciences*, 42(3):199-210.
41. Mann, R.W., Bass, W.M. and Meadows, L. (1990). Time since death and decomposition of the human body: variables and observations in case and experimental field studies. *Journal of Forensic Science*, 35(1): 103-111.
42. Fiedler, S. and Graw, M. (2003). Decomposition of buried corpses, with special reference to the formation of adipocere. *Naturwissenschaften*, 90: 291-300.
43. Bachmann, J. and Simmons, T. (2010). The influence of preburial insect access on the decomposition rate. *Journal of Forensic Science*, 55(4): 893-900.
44. Stokes, K.L., Forbes, S.L., Benninger, L.A., Carter, D.O. and Tibbett, M. (2009). Decomposition studies using animal models in contrasting environments: evidence from temporal changes in soil chemistry and microbial activity. Ritz, K., A. *Criminal and Environmental Soil Forensic*, pp.1-519. Porirua, New Zealand: Springer.
45. Rachel, A.P., Kerith-Rae, D., Jacqui, H., Paul, G., Natasha, B., Mark, T. and Arpad, A. V. (2009). Microbial community analysis of human decomposition on soil. *In book of Criminal and Environmental Soil Forensic*, pp 379-394.
46. DeBruyn, J.M., Hoeland, K.M., Taylor, L.S., Stevens, J.D., Moats, M.A., Bandopadhyay, S., ... and Steadman, D.W. (2021). Comparative decomposition of humans and pigs: soil biogeochemistry, microbial activity and metabolomic profiles. *Frontiers in Microbiology,*

11: 608856.

47. Sukchit, M., Deowanish, S. and Butcher, B. A. (2015). Decomposition stages and carrion insect succession on dressed hanging pig carcasses in Nan Province, Northern Thailand. *Tropical Natural History,* 15(2): 137-153.
48. Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S. and Trumbore, S.E. (2011). Persistence of soil organic matter as an ecosystem property. *Nature*, 478: 49-56.
49. Jansen, B. and Wiesenberg, G.L.B. (2017). Opportunities and limitations related to the application of plant-derived lipid molecular proxies in soil science. *Soil*, 3: 211-234.
50. Janaway, R.C. (1996). The decay of buried human remains and their associated materials. In: Studies in crime: An introduction to forensic archaeology (Eds. J Hunter, C Roberts and A Martin), pp. 58-85. Batesford, London.
51. Carter, D.O. and Tibbett, M. (2006). Microbial decomposition of skeletal muscle tissue (*Ovis aries*) in a sandy loam soil at different temperatures. *Soil Biology and Biochemistry*, 38(5): 1139-1145.