Lipid Distribution Associated With a Clandestine Grave: A Burial in Mangrove Soil

Xueer Chock, Siti Sofo Ismail#

School of Marine and Environmental Science, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, MALAYSIA.
Corresponding author. E-Mail: sofo@umtedu.my; Tel: +609-6683843

ABSTRACT Locating clandestine grave is difficult using the traditional methods as it is time consuming, costly and involves a lot of human power. By determining the soil lipid distribution for each decomposition stages, the prior presence of a body in a grave can be confirmed and the estimated postmortem interval (PMI) can be established. In this study, fatty flesh of commercial pig (*Sus Scrofa*) was allowed to decompose under controlled laboratory experiment, mimicking a burial in a shallow grave. The fatty flesh was buried in mangrove soil under tropical climate. Soils were collected at eight designated sampling points, representing different decomposition stages. Lipids were extracted using Modified Bligh-Dyer Extraction Method and analyzed with Gas Chromatography-Flame Ionization Detector (GC-FID). The common lipids found were palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$), oleic acid ($C_{18:1}$) and cholesterol. Obvious difference in lipid content was observed in soil with and without burial. Lipid is a suitable burial biomarker for forensic scientists and law enforcements in solving crime cases.

KEYWORDS: Lipid; Fatty acids; Decomposition; Clandestine grave; Post mortem interval

Full Article - *Biological sciences*
Received 7 May 2017 **Revised** 3 November 2017 **Accepted** 10 December 2017 **Online** 28 December 2017

INTRODUCTION

Decomposition process involves the degradation of larger compounds into simpler components by microorganisms, arthropods and scavengers (Teo *et al*., 2014). According to Vass (2001), human decomposition may start approximately four minutes after death. It begins with the autolysis process, i.e. self-destruction of cell. That is initiated by the release of intrinsic enzymes. The release of the hydrolytic enzymes such as lipases, amylase and proteases denature molecules and cell membrane. Eventually, nutrient rich substances in the cell will be released and aid the putrefaction process (Janaway *et al*., 2009; Teo *et al*., 2014). Putrefaction is the process of breaking down soft tissues by microorganisms such as bacteria, fungi and protozoa followed by catabolism of tissues into gases, liquids and simple molecules. The decomposition process then proceeds with liquefaction and skeletonization (Vass, 2001).

Generally, decomposition can be described into different stages which are a convenient means of summarizing the changes that may occur during the event. It has known that the most important events may take place during the first 345 days of the decomposition process (Hopkins *et al*., 2000) In this study, the process was divided into five stages namely as initial decay (0-3 days), putrefaction (3-10 days), black putrefaction (10-20 days), butyric fermentation (20-50 days) and dry decay (50-365 days) (Statheropoulos *et al*., 2011). These stages will facilitate the data organization and discussion. Decomposition rate can be influenced by many factors. The most widely recognized factors are temperature, moisture, pH and partial pressure of oxygen (Vass, 2001). Basically, rate of decomposition increases with the increasing in temperature. This is because reaction rate of chemical processes is faster and microorganism responsible for decomposition are more active at warmer temperature (Larizza, 2010; Vass, 2001). Water is vital for the hydrolysis process in tissue degradation. Besides, moist environment promotes the growth of microorganisms (Larizza, 2010). However, adipocere tends to form in a moist environment. The formation of adipocere, known a grave wax, will delay the decomposition process and may preserve the body (Notter *et al*., 2009).

Soils with different pH will have different effect on decomposition. Decomposition is faster in acidic soil than alkaline soil as because adipocere forms in mildly alkaline soil (Tibbett & Carter, 2009). Oxygen plays an important role in decomposition. Body buried in surrounding with lack oxygen decompose slower due to the retardation of oxidative processes (Vass, 2001).

Traditionally, postmortem interval (PMI) or time since death has been determined by observing physical changes, measuring body temperature, and using forensic entomology methods. Estimating PMI using the information that is obtained from the distribution of soil lipid is relatively new approach in forensic investigations. During decomposition, cadaveric substances will seep into underneath soil and will form a highly-concentrated island of fertility, which is known as cadaver decomposition island (CDI) (Carter *et al*., 2006). The burial associated lipids have a high potential to be established as burial biomarkers due to their abundancy in human tissue and longer persistency in soil compared to other compounds. Lipids have the characteristics of ubiquity, diagenetic, chemical stability and extreme diversity in the structures (Derrien *et al*., 2015).

In this study, a simulated burial experiment was carried out to study the decomposition process in mangrove soil under tropical climate. Pig fatty flesh was used to substitute human cadaver. This is due to ethical problem and pig has similarity to human in terms of anatomy and lipid distribution (Statheropoulos *et al*., 2011).

METHODOLOGY

Experimental Design

A controlled laboratory experiment was carried out. The experiment was designed to mimic a burial in shallow grave. Soils were sampled in Setiu Wetland mangrove area, Terengganu. Approximately 20g of pig fatty flesh were buried in vials and allowed to decompose according to specific burial intervals. Samplings were carried out on designated days, corresponding different decomposition stages. The sampling points are: Day 0, Day 3, Day 5, Day 7, Day 15, Day 17, Day 21 and Day 28. Pig fatty flesh was removed and weighed. Rate of decomposition is calculated using the formula shown below. The samples were then freeze dried prior to lipid extraction.

$$Rate\ of\ decomposition = \frac{Difference\ in\ mass}{Days\ of\ decomposition}$$

Preparation of Buffered Water and Bligh-Dyer Solution

To prepare buffered water, 300ml of the dichloromethane (DCM) extracted double distilled water (DDW) was transferred into a separating funnel. 2.02g of monopotassium phosphate (KH_2PO_4) were then added to create a 0.05M solution. pH of the solution was adjusted to pH 7.2 by adding 2 pellets of sodium hydroxide (NaOH). The Bligh-Dyer Solution was prepared by mixing buffered water, chloroform and methanol with a 4:5:10 ratio. The actual volumes used were 100ml buffered water, 125ml chloroform and 250ml methanol.

Modified Bligh-Dyer Extraction

Approximately 4g of soil was transferred into vials. 3ml of 2:1 DCM/Methanol were added and samples were spiked with 100µl internal standard tetratriacontane, followed by a 15 minute sonication (40°C) and 5 minute centrifugation (~3000rpm). The supernatant solution formed was transferred into a new vial. This process was repeated thrice with 2ml of 2:1 DCM/Methanol. The soil was then treated with 3ml of Bligh-Dyer solution, followed by a 15 minute sonication (40°C) and

5 minute centrifugation (~3000rpm). The supernatant was transferred to the same vial. This step was also repeated thrice with 2ml of Bligh-Dyer solution. To break the organic phase, 2ml of buffered water and chloroform were added to the supernatant solution, followed by a 1 minute centrifugation. The organic layer form was transferred into a clean vial. This process was repeated thrice with 2ml of chloroform. The solvent was then evaporated under a gentle nitrogen flow. The total lipid extracted (TLE) was weighed and stored in freezer prior to analysis.

Instrumental Analysis

Samples were analysed using Gas Chromatography-Flame Ion Detector (GC-FID) (GC-6890N, Agilent). The column used was HP-5 5% Phenyl Methyl Siloxane (30.0m x 320µm x 0.25µm). Helium gas was used as carrier gas. After injection at 50°C, the oven temperature held for 2 minute, then temperature increased 10°C min^{-1} to 300°C and was held constant for 20 minute. Peaks were identified by comparing the retention times with the external standard.

RESULT AND DISCUSSION

Rate of decomposition

Figure 1 shows the decomposition rate throughout the experiment at different sampling points.

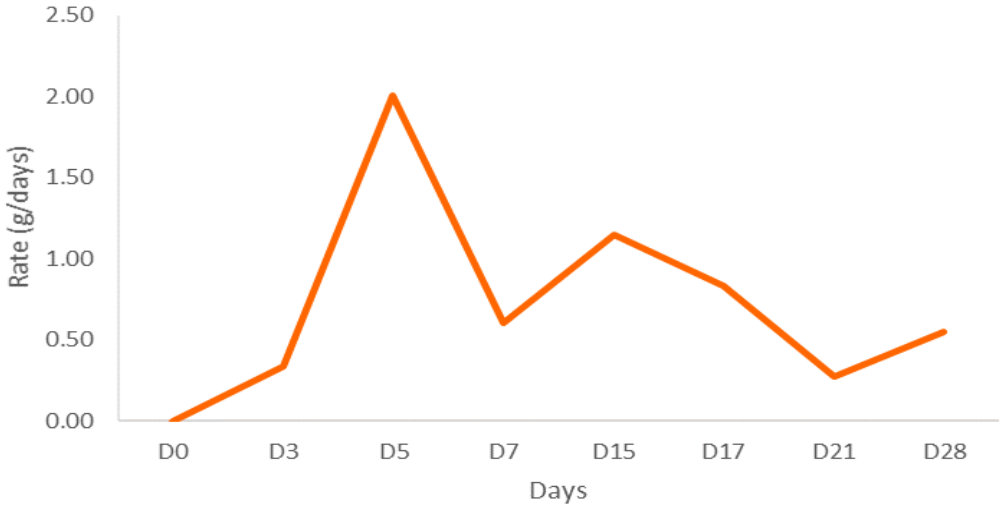

Figure 1. Plot of decomposition rate at different sampling points for 28 days of burial period.

The rate of decomposition increased from day 0 to day 3, corresponding to the initial stage of the process. Then, a rapid increase in the decomposition rate was observed between day 3 and day 5. The rate reached maximum at day 5, corresponding to the putrefaction stage of the decomposition process. This stage exhibit a rapid mass loss due to high maggot activity. Maximum microbial activity was observed within the first 10 days of burial. Microbial activity shows a higher peak level at the initial stages of decomposition and later return to a stable level of activity (Stokes *et al*., 2009). At these points, a massive introduction of cadaveric substances was also observed. The flux of cadaveric substances into soil increases the microbial activity (Carter *et al*., 2006). Decomposition rate sharply decreased after day 5 and continued towards the end of the burial period. The observation indicates the completion of the decomposition process. These days are corresponding to the stages of black putrefaction and butyric fermentation where most of the fatty flesh has disintegrated.

Total Lipid Extraction (TLE)

Mass of lipid was weighed after solvent was reduced under a gentle nitrogen flow. Figure 2 shows the mass of TLE from the associated soil at different sampling points.

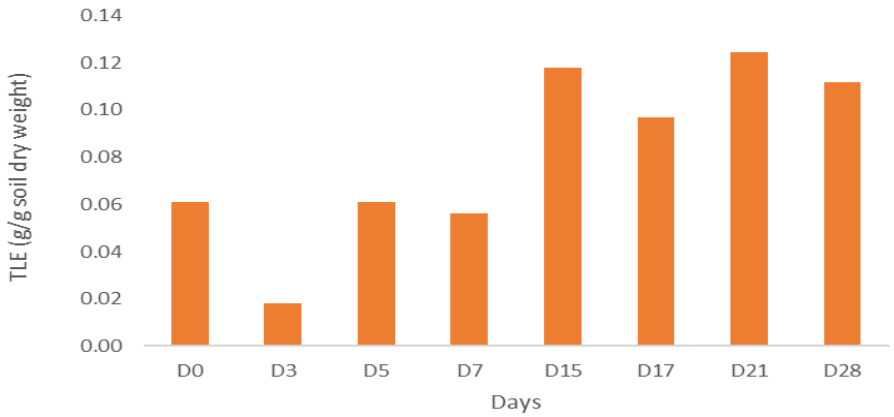

Figure 2. Mass of Total Lipid Extracted

The mass of TLE were found to range between 0.02g and 0.12g/g soil dry weight. Generally, the mass of TLE increased througout the experiment with minor fluctations. The soil of day 3, corresponding to the initial stage of decomposition, contained lowest mass of TLE, i.e. 0.02g/g soil dry weight. Whilst, the soil of day 21 (Black putrefaction) contained the highest mass of TLE, i.e. 0.12g/g. The potential explaination for the obeserved trend many due the adaptation of microbial community to the introduction of cadaveric substances. The introduction of nutrient-rich cadaveric substances will cause changes in many taxa contributing to the succession of microbial community structure (Cobaugh *et. al*., 2015).

Lipid Analysis

In this study, the fatty acid concentrations exhibit an extremely high input of anthropogenic organic material, potential from decomposing fatty flesh, into the underneath soil during decomposition. Fatty acids were detected even just after a short period of burial. The compounds detected were palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$) acids and cholesterol.

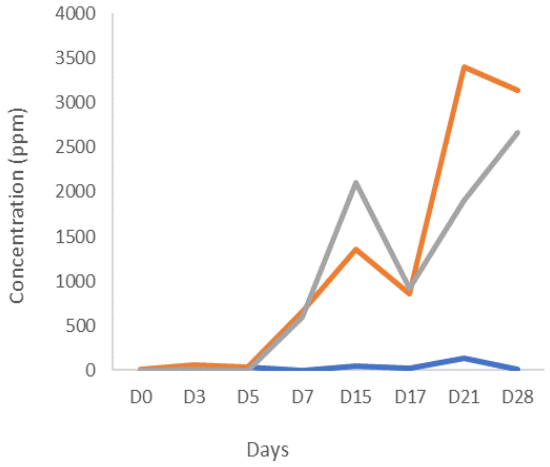

Figure 3. Concentration of free fatty acids at different sampling points. Orange line represents $C_{18:0}$; grey line represents $C_{18:1}$ and blue line represents $C_{16:0}$

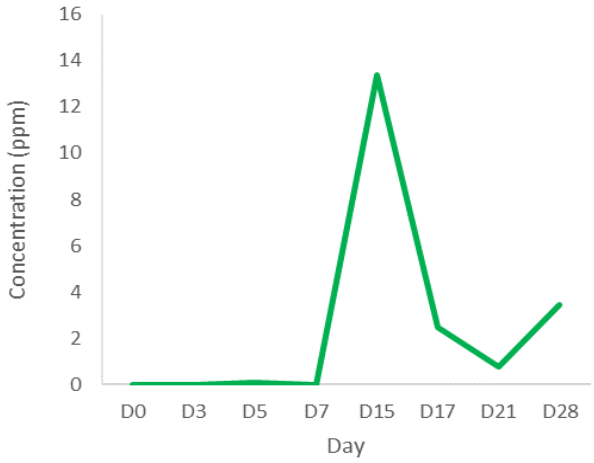

Figure 4. Concentration of cholesterol different sampling points.

The result of this study agrees with the previous studies. Oleic acid dominated the associated soils throughout the experiment, exhibit the highest concentration, followed by stearic and palmitic acids. Body's adipose tissue contained 60-85% of lipids. The monounsaturated oleic acid ($C_{18:1}$) is the most abundant, followed by the unsaturated linoleic acid ($C_{18:2}$), palmitoleic acid ($C_{16:2}$) and saturated palmitic acid ($C_{16:0}$). Shortly after death, the neutral fats will eventually be hydrolysed or oxidized into simpler fatty acids such as stearic, oleic and palmitic acids (Dent *et. al*., 2004). Between day 0 to day 5, the concentrations of these fatty acids were relatively low. However, their concentrations started to increase from Day 7 to Day 28 with minor fluctuations. A high abundance of the saturated fatty acids (stearic and palmitic acids) may indicate a rapid hydrogenation of adipose tissues (Lu̇he, 2016). Overall, the concentrations of fatty acids increased from day 0 to day 28 of burial intervals. It clearly shows that different concentration of fatty acids was detected at different stages of decomposition.

A significant relationship was observed between the fatty acids and cholesterol. When the concentration of cholesterol decreased, the concentration of fatty acids increased. The highest concentration of fatty acids was observed on day 21, which corresponds to butyric fermentation stage. On a contrary, decrease in concentration of cholesterol was recorded on the same day. This is because cholesterol is not stable in soil and will be further reduced into simpler compounds (Bull *et. al*., 2002).

CONCLUSION

The introduction of a cadaver into soil will elevate the concentration of lipids. Hence, this study evident that soil lipids have high potential to be established as burial biomarkers that may aid in locating clandestine graves Furthermore, these lipids may also provide information to estimate PMI as different concentrations of fatty acids at different decomposition stages were obtained. Subsequently, the identification victim and/or criminal may also possible to be established.

ACKNOWLEDGEMENTS

The authors wish to thank all the staffs in Oceanography Laboratory (MOSEA) and School of Marine Science and Environmental (PPSSMS) of Universiti Malaysia Terengganu (UMT) for their technical assistance and wonderful collaboration throughout the study. The authors also wish to express their gratitude to the Royal Malaysia Police for their fully support and encouragement.

REFERENCES

[1] Bull, I. D., Lockheart, M. J., Elhmmali, M. M., Roberts, D. J. & Evershed, R. P. (2002). The origin of faeces by means of biomarker detection. *Environment International*, **27**(8), 647-654.

[2] Carter, D. O., Yellowlees, D. & Tibbett, M. (2006). Cadaver decomposition in terrestrial ecosystems. *Naturwissenschaften*, **94**(1), 12-24.

[3] Cobaugh, K. L., Schaeffer, S. M. & Debruyn, J. M. (2015). Functional and Structural Succession of Soil Microbial Communities below Decomposing Human Cadavers. *Plos One*, 10(6), e0130201.

[4] Dent, B. B., Forbes, S. L. & Stuart, B. H. (2004). Review of human decomposition processes in soil. *Environmental Geology*, **45**(4), 576-585.

[5] Derrien, M., Cabrera, F. A., Tavera, N. L., Manzano, C. A. & 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.

[6] Hopkins, D. W., Wilthire, P. E. J. & 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

[7] Janaway, R. C., Percival, S. L. & Wilson, A. S. (2009). Decomposition of Human Remains. *In:* Percival, S. L. (eds). *Microbiology and Aging*. Humana Press.

[8] Lühe, B. V. (2016). *The fate of human decomposition products in soils.* Ph.D. dissertation. Johannes Gutenberg University of Mainz, Germany.

[9] Notter, S. J., Stuart, B. H., Rowe, R. & 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.

[10] Statheropoulos, M., Agapiou, A., Zorba, E., Mikedi, K., Karma, S., Pallis, G., Eliopoulos, C. & Spiliopoulou, C. (2011). Combined chemical and optical methods for monitoring the early decay stages of surrogate human models. *Forensic Science International,* **210**(1-3), 154-163.

[11] Stokes, K. L., Forbes, S. L., Benninger, L.A., Carter, D. O. & Tibbett, M. (2009). Decomposition Studies Using Animal Models in Contrasting Environments: Evidence from Temporal Changes in Soil Chemistry and Microbial Activity. *In:* Ritz, K., Dawson, L. & Miller, D. (Eds.). *Criminal and Environmental Soil Forensics*. Bradford: Springer Science & Business Media.

[12] Teo, C. H., Hamzah, N. H., Hing, H. L. & Hamzah, S. P. (2014). Decomposition Process and Post Mortem Changes: Review. *Sains Malaysiana,* **43**(12), 1873-1882.

[13] Tibbett, M. & Carter, D. O. (2009). Research in Forensic Taphonomy: A Soil-Based Perspective. In: Ritz, K., Dawson, L. & Miller, D. (Eds.), *Criminal and Environmental Soil Forensics*. Bradford: Springer Science & Business Media.

[14] Vass AA (2001) Beyond the grave: understanding human decomposition. *Microbiol Today*, **28**,190–192.