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A STUDY OF CADAVER DECOMPOSITION IN SANDY SOIL OF EAST COAST OF PENINSULAR MALAYSIA

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ABSTRACT

A simulated burial experiment was carried out to evaluate the potential of soil lipids, as indicator for estimating the post mortem interval. The findings of this study will provide baseline data for decomposition of cadaver in sandy soil. The fatty flesh of pig carcass (Sus scrofa) was buried in sea soils, mimicking the burial in a shallow grave. The modification of Bligh-dyer extraction was used for the lipids extraction process. The soil pH was measured to identify the effect of decomposing body on the soil acidity. The soil pH was acidic in early stage and gradually increased corresponding to the next stage of decomposition. The gas chromatography with flame ion detector was employed to determine the concentration of cadaveric material that may be introduced at each stage of the decomposition process. The results showed the abundance of free fatty acids, palmitic ($C_{16:0}$) and stearic acids ($C_{18:0}$). The concentrations of these acids vary upon decomposition stages. Conclusively, the composition of lipids for each stage of decomposition can be developed as potential biomarkers in order to estimate the post-mortem interval.

Keywords: *post-mortem interval; cadaver decomposition; free fatty acid; soil lipid.*

1. INTRODUCTION

Based on Locard's exchange principle, it is stated that all the contacts leave a trace. The theory governed that when two objects come into contact with one another, each will take something or leaves something from one another. Therefore, this principle imposes a great influence in forensic science.

Decomposition is a natural process that will undergo when organism dies. During this process, the tissue of dead organisms is degraded through the chemical and biological degradation which initially may not be visible to naked eyes as it starts in cellular level. Tissue degradation involves the formation of simpler forms of matters and the physical removal of soft tissue by arthropods and scavengers (Clark *et al.*, 1997). Five stages of decomposition described by Payne (1965) then adapted by Anderson and Van Laerhoven (1996) are usually utilized in forensic taphonomic to assist in the description of stages of cadavers or carrion decomposition. These five stages are fresh (autolysis), putrefaction, black putrefaction, butyric fermentation and dry decay.

For this study, the soil associated with buried body is taken into consideration to investigate the decomposition process. Generally, soil is influenced by the surrounding environment such as pollution. Therefore, the soil biology and chemistry have been investigated as a mean to estimate post-mortem interval (PMI) and to locate clandestine graves (Vass *et al.*, 2001;

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Carter and Tibbett, 2003; Tibbett *et al*, 2004). Although some studies have proven successful in actual casework, the majority of them are in the early stages of development (Vass *et al.*, 2002).

Generally, soil microorganisms have basic functions in decomposing plant residues both above and below ground. The activities of soil microbial communities are influenced by environmental and agriculture system in soil (Melero *et al.*, 2005; Araújo *et al.*, 2008). The quality of the soil can determine the decomposition and formation of organic matter, where the high quality soil is important in order to maintain the ecological functions (Doran *et al.*, 1996). One of the soil quality indicators is microbial respiration in soil (Brendecke *et al.*, 1993).

In order to enhance our understanding of the relationship between cadaver decomposition and types of soil, a controlled laboratory simulated burial experiment was conducted, mimicking a burial in a shallow grave. This experiment shall enable a detailed organic geochemical assessment of burial activity via a collection and analysis of samples at designated sampling points. Furthermore, this experiment will provide a baseline data on the decomposition sandy soil under tropical climate as the previous study focus on season countries (Forbes *et al.*, 2002). Hence, the purpose of this study is to determine the potential of lipids found in soil during decomposition process as a tool in determining the post-mortem interval.

Pig carcasses are commonly utilized in decomposition studies as human body analogues (Gennard, 2012). Forensics analysis has exposed more of the pigs used in the various studies especially in forensic. It is reasonably important to use pig fatty flesh together with the skin for a comparison of human skin. Furthermore, pig has similarity in saturated fatty composition with human and provides a good estimate during decomposition (Stokes *et al.*, 2013).

2. METHODOLOGY

2.1 Experimental Design

Approximately, 30 g of pig fatty flesh was buried in a 28 ml vial that was filled-up with sandy soil and was allowed to decompose to mimic a burial in a shallow grave. The decomposition period was three months with a total of seven sampling points. The vial without flesh was also prepared as a control soil for comparison. Each of burial intervals was replicated. The ambient condition of the soils was closely monitored and checked for every week to maintain the actual ambient condition of the soil.

2.2 pH measurement

A ratio 1:2 of soil and distilled water was prepared. The pH of detritosphere soil from each of decomposition event/sampling point was measured using a pH meter.

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2.3 Total Lipid Extraction (TLE) Method

Approximately 2 g of the freeze dried soil was placed into a culture tube. Then 3 ml of DCM/methanol was added, spiked with 100 µl internal standard tetratriacontane, sonicated (15 min) and centrifuged (5 min, ~3000 rpm). The supernatant was transferred into a clean vial. The process was repeated three times with 2 ml of DCM/ methanol (2:1, v/v). Then, the soil was treated with 3 ml of Bligh Dyer solvent, sonicated for 15 min and centrifuged for 5 min (~ 3000 rpm). The supernatant was then transferred into the same vial. The extraction was repeated with 2 ml of Bligh Dyer solvent. The 2 ml of each buffered water and chloroform were added to the supernatant to break the organic phase. The mixture was then centrifuged for 1 min (~3000 rpm). The obtained organic layer was then transferred into another clean vial. The solvent was evaporated under a gentle flow of nitrogen. The obtained TLE was stored in a freezer (< 20°C) prior to gas chromatography analysis.

2.4 Instrumental Analysis

Samples were analysed using a gas chromatography- flame ion detector (GC-FID) with BP1 nonpolar capillary column (30 m x 0.25 mm internal diameter, 0.25 µm film thickness). Helium was used as a carrier gas. The temperature program after injection was set at 50 °C, the oven temperature was raised to 100 °C at a rate 10 °C min^{-1} and held for 5 min., then increased to 300 °C at 4°C min^{-1}, and finally held constant for 20 min. The flame ionization was held at 350°C. Peaks were identified by comparing their retention times with those of authentic standards (Acros organics).

3. RESULTS AND DISCUSSION

3.1 pH Measurement

Figure 1 shows the soil pH during the decomposition day. The pH was slightly acidic (~6.24) between Day 0 and Day 3 burial interval, corresponding to the initial stage of decomposition. There was no significant different between sample pH and control with the decomposition day as the p-value is 0.317 (P>0.05). The fluctuating in pH during decomposition may indicate the level of introduction of cadaveric products and the microorganism activities during the stages.

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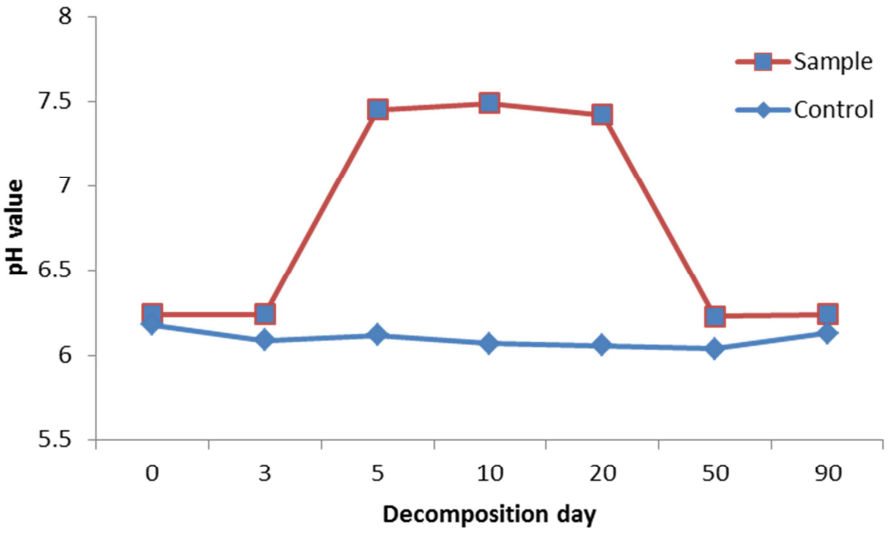

Figure 1**:** The pH during decomposition day

This finding agreed with the previous study where the soil pH was not drastically changed (Haslam and Tibbett, 2009). The slight changes in the pH may be due to mineralisation of protein and other nitrogen, which occurs *via* ammonification, followed by nitrification of ammonia by enzymatic oxidation. This process involves conversion of NH_4^+ to NO_3^- liberated H^+ which makes the soil become more acidic (Haslam and Tibbett, 2009; Hopkins *et al.*, 2000).

The pH value changed slightly into alkaline from day 5 to day 20, corresponding to the putrefaction, black putrefaction and butyric fermentation stages respectively. A study done by Haslam and Tibbett (2009) showed the rate of decomposition is three times higher in acidic soil than alkaline. Since sandy soil was slightly acidic; hence, a rapid decomposition may be observable at the stage of putrefaction (Baldrian *et al.*, 2012). The changing in pH into alkaline was due to the formation of ammonia. During the putrefaction stage, gases were purged from the cadaver and caused colour changes into black colour. The black colour indicated the excess of ammonia due to presence of ammonia fungi (Tranchida *et al.*, 2014). The presence of ammonia group caused the pH value to increase. Besides, inorganic matters such as calcium, magnesium, and others released from cadaveric material during decomposition (Payne, 1965).

After day 50 and day 90, the pH of soil became acidic (pH ~6.23 and ~6.24) corresponding to the butyric fermentation and dry decay stages, respectively. From those points, the decomposition proceeded to completion. The remains were left as skeleton and cartilage. The slowing down of decomposition during completion stage was attributed to the formation of adipocerous around cadaver or internal organ (Pfeiffer *et al.*, 1998 and Turner *et al.*, 2013).

3.2 Fatty Acid

The analysis of soil fatty acids was carried out to determine its composition at each stage of decomposition. Subsequently, the soil fatty acids were used to be developed as forensic tools to estimate the PMI. Figure 2 shows the concentrations of palmitic ($C_{16:0}$) and stearic acid

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($C_{18:0}$) during decomposition day. The palmitic and stearic acid were abundance in soil decomposition (Forbes *et al.*, 2005c). There were significant difference in concentration of palmitic and stearic acids among the day of decomposition with the 95% confidence interval (P=0.0004 and P=0.001), respectively. The concentration of both acids showed the data were significantly different as the p values (p<0.5) during each day of decomposition.

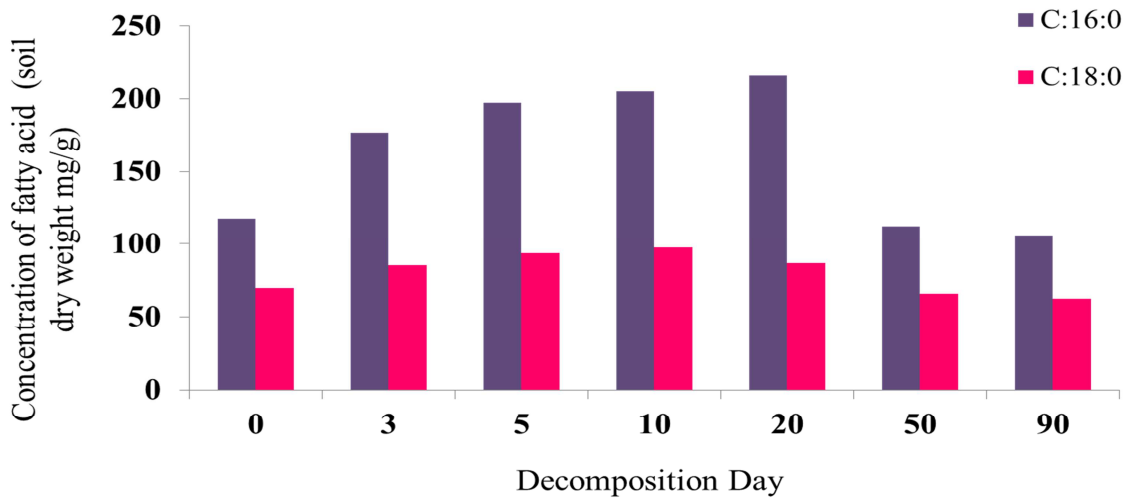

Figure 2**:** The concentrations of palmitic acid ($C_{16:0}$) and stearic acid ($C_{18:0}$) during decomposition day

The breakdown of adipose tissues during the decomposition will produce free fatty acid (Dent *et al.*, 2004). This study was performed to obtain the baseline data of extracted fatty acids from sandy soil associated with decomposing carcass. The most abundance saturated fatty acids that are commonly found in human and pig adipose tissues are palmitic acid ($C_{16:0}$) and stearic acid ($C_{18:0}$) (Pfeiffer *et al.*, 1998 and Dent *et al.*, 2004). The concentrations of palmitic and stearic acids were increased from day 0 and reached maximum at day 20. The concentrations of stearic and palmitic acids at day 0 were 117.2 mg/g and 69.6 mg/g soil dry weight, respectively, corresponding to the initial stage. For day 3, the concentrations of stearic and palmitic acids were 177 mg/g and 85.2 mg/g soil dry weight, respectively. These concentrations started to increase corresponding to the autolysis process. During this stage proliferation of anaerobic bacteria from the gut will lead the body to become distended due to gas production and accumulation. Therefore, the anaerobic environment will provide the proper condition for bacteria to breakdown proteins, carbohydrates and lipids producing gases, acids, volatile organic compounds, carbohydrates and fat components into diverse acids, gases, liquids and simple molecules (Gill-king, 1997; Vass *et al.*, 2002; Dent *et al.*, 2004; Stadler 2013).

The concentrations of palmitic and stearic acid increased drastically between day 5 and 10, corresponding to the putrefaction and black putrefaction. The increasing concentrations of palmitic and stearic acids were due to the introduction of decomposition fluid into the surrounding environment. The pressure gasses allowed the fluid such as fatty acid flow out from natural cadaveric openings during this stage and contribute into the soil (Tibbett and Carter, 2008).

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The concentrations of stearic and palmitic acids were highest at day 20 corresponding to the black putrefaction stage (active stage). Under oxygen deficient environment, the mixture of unsaturated and saturated fatty acids released during hydrolysis process will undergo further hydrolysis and hydrogenation (Dent *et al.,* 2004). Thus, the increasing concentration of these fatty acids may be associated with the presence of bacterial enzyme that involved in the decomposition process.

The decreasing concentrations of stearic and palmitic acids at day 50 were corresponded to the butyric fermentation stage. At this stage the bloating begins to subside and the body begins to flatten due to the breakdown of the tissues. This causes the release of the fluid of decomposition and gases as the thoracic and abdominal walls disintegrate as well as consumption by insect and predator activity. Prior to the basal level in human body during bloating stage, fatty acids oxidised into anaerobic degradative product (Hopkins *et al.,* 2000 and Dent *et al.,* 2004).

The drastic decreased in the concentrations of stearic (62.3 mg/g soil dry weight) and palmitic (105.7 mg/g soil dry weight) acids were observed at day 90, which corresponding to dry decay. The decrease in these concentrations was indicative of the completion of decomposition process. Furthermore, the deterioration of the remains was very slow and involved the loss of bone-stem strength.

4. CONCLUSION

Lipids analysis of decomposition using modified Bligh dyer methods was successfully carried out in this study. The chemical changes in soil associated with pig decomposition in particular and variations in pH have the potential to be used in forensic science as means of identifying a clandestine grave. The pH was slightly changed throughout this study. The soil pH showed acidic at the early stage and slightly increased during the putrefaction stage. Soil microbial activity played an important role in the change in pH and the concentrations of fatty acids. The objective of the study was to establish baseline data of decomposition of pig in sandy soil using GC-FID was achieved. Palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acids were the most abundant fatty acids detected in decomposition soil with varying concentrations. Thus, it may be considered as a potential tool for the estimation of PMI. This study may also be used to indicate that lipid extract from soil has the potential to be developed as a tool or biomarker to estimate the PMI.

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