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LIPID DISTRIBUTIONS ASSOCIATED WITH CADAVER DECOMPOSITION IN MANGROVE AND OIL PALM PLANTATION SOILS UNDER TROPICAL CLIMATE

(Taburan Lipid Berkaitan dengan Pereputan Kadaver dalam Tanah Bakau dan Ladang Kelapa Sawit Di Bawah Iklim Tropika)

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Abstract

The burial environment has a significant effect on decomposition process. The most influential environmental factors that determine the fate of the process are temperature, moisture, pH, and partial pressure of oxygen. In this study, the decomposition of a cadaver buried in soils with contrasting pH under a tropical climate was investigated. Due to ethical issues, the fatty flesh of a commercial pig (*Sus scrofa*) was buried in mangrove (mild alkaline pH) and oil palm plantation (acidic pH) soils, which are amongst the most common body disposal sites encountered in forensic investigations. A controlled laboratory experiment was carried out. Soils were collected at different sampling points, corresponding to different decomposition stages. Modified Bligh-Dyer extraction method was used to extract soil lipids. Gas chromatography-flame ionisation detector (GC-FID) was used to identify the obtained lipids. A similar trend in the rate of decomposition was observed for both soils. The decomposition rate was higher at the initial and putrefaction stages. A sharp increase in the decomposition rate was observed between day 3 and day 5 of the burial interval. However, mangrove soil demonstrated a higher decomposition rate at all decomposition stages. Furthermore, the concentrations of palmitic ($C_{16:0}$), stearic ($C_{18:0}$), and oleic ($C_{18:1}$) acids were higher in mangrove soil compared to that of oil palm plantation soil. The significant differences observed between these contrasting pH soils may indicate that soil pH will eventually impose a different effect on decomposition.

Keywords: Cadaver decomposition, soil pH, clandestine grave, decomposition rate, fatty acids

Abstrak

Persekitaran pengebumian mempunyai kesan yang signifikan terhadap proses penguraian. Faktor persekitaran yang paling berpengaruh yang menentukan nasib proses adalah suhu, kelembapan, pH, dan tekanan separa oksigen. Dalam kajian ini, penguraian kadaver yang ditanam dalam tanah dengan pH yang berbeza di bawah iklim tropika telah disiasat. Disebabkan isu etika, daging lemak babi komersial (*Sus scrofa*) ditanam di ladang bakau (pH sedikit alkali) dan ladang kelapa sawit (pH berasid). Kedua-dua jenis kawasan ini adalah antara tapak pelupusan mayat yang paling biasa ditemui dalam siasatan forensik.

Eksperimen makmal terkawal telah dijalankan. Tanah dikumpulkan pada titik pensampelan yang berbeza, sepadan dengan peringkat penguraian yang berlainan. Kaedah pengekstrakan Bligh-Dyer yang telah diubah suai digunakan untuk mengekstrak lipid tanah. Kromatografi gas-pengesan nyalaan ion (GC-FID) digunakan untuk mengenal pasti lipid yang diperoleh. Corak yang sama dalam kadar penguraian telah diperhatikan untuk kedua-duanya. Kadar penguraian lebih tinggi pada peringkat awal dan pembusukan. Kadar penguraian meningkat mendadak telah diperhatikan antara hari 3 dan hari 5 selang pengebumian. Walau bagaimanapun, tanah bakau menunjukkan kadar penguraian yang lebih tinggi pada semua peringkat penguraian. Selain itu, kepekatan asid-asid palmitik ($C_{16:0}$), stearik ($C_{18:0}$), dan oleik ($C_{18:1}$) lebih tinggi di dalam tanah bakau berbanding dengan tanah ladang kelapa sawit. Perbezaan yang ketara antara pH tanah yang berlainan ini mungkin menunjukkan bahawa pH tanah akan akhirnya memberi kesan yang berbeza terhadap proses penguraian.

Kata kunci: pereputan kadaver, pH tanah, kubur rahsia, kadar pereputan, asid lemak

Introduction

Death under suspicious circumstances usually occurs without any witnesses, with no obvious clues of the cause, manner, and actual time of death. In most cases, inhumation is a common method to dispose of the body to hide the crime. It has been recognised that different burial environments will affect the decomposition process differently as each burial environment has its distinctive and complex system interrelated to the biological, chemical, and physical processes [1-5]. The four widely recognised factors influencing the rate of decomposition are temperature, moisture, pH, and partial pressure of oxygen [6]. Soil microorganisms take an important part in decomposition as they release enzymes that help in further breakdown of organic matters [7]. Besides, it also holds across a variety of spatial scales, i.e. continental scales, land-use types, small and sub-meter scales [8, 9]. It has been known that acidic and alkaline soils are dominated by fungi whereas neutral soil is dominated by bacterial communities [10].

The activity of recovering the body and estimating post-mortem interval (PMI) is crucial in forensic investigations to identify the criminals and/or the victim. Several conventional methods can be used to locate a body in a clandestine grave. Ground-penetrating radar and cadaver dog are commonly used during the search for clandestine graves [11, 12]. However, these methods have limitations, especially for the cases of longer burial interval [12]. The post-mortem investigations will be rather complicated for the body that is discovered long after it has been in contact with the surrounding soil. Hence, there is a crucial need for an alternative approach that can provide useful information for forensic investigations to find bodies in a terrestrial soil system. Recognising these deficiencies and the potential of belowground processes associated with cadaver decomposition will be useful, particularly for gathering important information to conduct criminal investigations. Locating clandestine graves and/or estimating PMI using information obtained from cadaveric materials, such as lipids extracted from the associated soil, may not be impossible and can be developed as one of the new approaches in forensic investigations [13].

The ability of forensic investigators to estimate PMI through analysis is important [14]. Hence, a simulated burial experiment was conducted to investigate the impact of different soil pH on cadaver decomposition as a burial study under tropical climate is not yet widely understood. The fatty flesh of a commercial pig (*Sus scrofa*) was used to replace human cadaver due to ethical issues. Furthermore, the pig was identified as the most reliable model due to similarities with humans in terms of organ morphology and lipid distribution [15]. The pig fatty flesh was buried in two types of soil with different acidity, i.e., mangrove (slightly alkaline, initial pH = 7.45) and oil palm plantation (acidic, initial pH = 3.74) soils. The soils were chosen for this experiment as they are known to be common disposal sites in Malaysia. Furthermore, these types of soil have different physical and chemical characteristics, including particle size and mineral content. Mangrove soil contains more sandy particles whereas oil palm plantation soil contains more clay particles.

Mangrove sandy loam soil was collected from Setiu Wetlands, Terengganu (5.6700° N, 102.7199° E),

which is a representation of most mangrove forests in the east coast region of Peninsular Malaysia. Setiu Wetland is the largest natural wetland in this region, combining various ecosystems including freshwater and seawater. This mangrove forest is much more under the influence of freshwater than seawater. This unique wetland is an area of high biodiversity with various types of flora and fauna, including *gelam* trees, coastal swamp, and freshwater swamp forest. Meanwhile, loam soil was sampled from an oil palm plantation owned by Sime Darby Industrial located in Kuala Berang, Terengganu (4.274520° N, 103.465652° E). Both mangrove and oil palm plantation sites are in isolated areas and away from villagers.

The changes in the decomposition rate and the quantity of the soil cadaveric derived lipids at different sampling points representing each decomposition stage were qualitatively and quantitatively analysed. The extent of particular soil lipid components was also analysed and evaluated. The unique characteristics that lipids possess, for example, the diagenetic pattern, are ubiquitously distributed in soil and chemically stable, making lipids an ideal marker [16]. Besides, the use of lipids as biomarkers provides higher accuracy in identifying multiple sources of organic materials [17, 18]. Furthermore, cadaveric fatty acids have demonstrated the potential to be used as burial biomarkers where the pattern and concentration of fatty acids in the soil at different decomposition stages can be used to locate a clandestine grave and estimate the PMI [17, 19]. Hence, the trend of decomposition rate and cadaveric derived lipids observed for each soil may provide preliminary data to further understand the decomposition in acidic and alkaline soils, especially for burial under tropical climate. Subsequently, the data may potentially be developed as a useful forensic tool to link the suspects with the crime.

Materials and Methods

Soil pH

The pre- and post-experimental pH of detritosphere soil were measured using a calibrated pH meter. Approximately 80 mL of distilled water was added into 20 g of the associated soil, creating a mixture of water and soil with a ratio of 4:1. The mixture was mixed well with a glass rod and allowed to settle for at least 5 minutes. The soil pH was measured three times for each soil under room temperature.

Simulated burial experiment

A laboratory-controlled simulated burial experiment was conducted to investigate and compare the decomposition of a cadaver buried in oil palm plantation and mangrove soils. Using a hoe and a shovel, the soils used in this experiment were taken from the depth of 50 cm from the surface to represent the soil of a shallow grave. Vessels were used to bury the abdominal fatty flesh of a commercial pig (*Sus scrofa*) that contained some muscles and skin. The pig fatty flesh was weighed accurately (± 20 g) and buried in a vessel, mimicking a burial in a shallow grave. Then, the fatty flesh was allowed to decompose for a burial period. The burial vessels of both mangrove and oil palm plantation soils were placed outside the laboratory to experience tropical climate ambient. The changes in the weather, such as the amount of precipitation, were obtained from the meteorological station located at the Sultan Mahmud International Airport, Kuala Terengganu, Terengganu. The associated soils were collected at eight different designated sampling points (i.e., days 0, 3, 5, 7, 15, 17, 21, and 28). These sampling points correspond to each decomposition stage. The remaining fatty flesh (if any) was removed from the vessel and weighed. Before the extraction and subsequent analyses, the associated soils were homogenised and stored in a freezer before being freeze-dried. The burial intervals were duplicated and the control soils without flesh were prepared with similar procedures, and then placed under a similar ambient environment similar to the vessels with fatty flesh.

Rate of decomposition

The rate of cadaver decomposition was calculated using the following equation 1:

$$Rate\ of\ decomposition = \frac{Difference\ in\ mass}{Days\ of\ decomposition} \quad (1)$$

Total lipid extracts

Lipid extraction was performed using a modified protocol of Bligh-Dyer extraction method [20]. Approximately 4 g of freeze-dried soil was transferred into vials. The soil was then mixed with 3 mL of DCM/methanol (2:1, v/v), and 100 µL of an internal standard (tetratriacontane) was added to quantify the lipid components extracted from the soils. The mixture was then sonicated (40 °C, 15 minutes) and centrifuged (~3,000 rpm, 5 minutes). The supernatant solution formed was transferred into a clean vial. This process was repeated three times with 2 mL of DCM/methanol (2:1, v/v), followed by 3 mL of Bligh-Dyer solution. The mixture was sonicated (40 °C, 15 minutes) and centrifuged (~3,000 rpm, 5 minutes). The supernatant solution formed was transferred to the same clean vial. The extraction was then repeated three times with 2 mL of Bligh-Dyer solution. About 2 mL of buffered water and chloroform were then added to the supernatant solution to break the organic phase and centrifuged (~ 3,000 rpm, 1 minute). The organic layer formed was transferred to a new clean vial. This process was repeated three times with 2 mL of chloroform. The excess solvent was removed with a gentle nitrogen flow. The total lipid extracted from the soil was weighed and kept in a freezer prior to further analysis.

Soil lipid analysis

Soil lipid extracts were analysed using gas chromatography-flame ionisation detector (GC-FID) (GC-6890N, Agilent). The samples were re-dissolved in hexane and injected (1 µL) *via* an on-column injector. The GC- FID was equipped with HP-5 5% phenyl methyl siloxane (30.0 m × 320 µm × 0.25 µm). The initial temperature for injection was 50 °C and the temperature was held constant for 2 minutes. The oven temperature then rose to 300 °C at a rate of 10 °C/min and kept constant for 20 minutes. Helium was used as the carrier gas. The generated peaks were identified by comparing them with an external standard. The external standard consists of several lipid components, including palmitic acid, stearic acid, methyl nonadecanoate, cholesterol, tetratriacontane, and tripalmitin.

Results and Discussion

Rate of decomposition

Figure 1 shows the decomposition rate for both types of soils throughout the experiment at different sampling points. Generally, a similar trend in the decomposition rate of pig fatty flesh was observed for both mangrove and oil palm plantation soils. The decomposition rate for both soils increased slightly between day 0 and day 3, corresponding to the initial stage of decomposition. Then, the decomposition rate increased rapidly after day 3 and reached the maximum decomposition rate on day 5, corresponding to the putrefaction stage. Between day 5 and day 7, a rapid decrease in the decomposition rate was observed. After day 7, the decomposition rate decelerated for both soils towards the completion of the experiment, with a slight increase on day 21 of the burial period for oil palm plantation soil. A similar trend was observed in a previous study where the decomposition rate was slow at the early stage, rapid at the intermediate stage, and slow again towards the end of the experiment [21].

Both mangrove and oil palm plantation soils demonstrated the highest decomposition rate on day 5 of the burial interval, i.e., 1.76 and 1.622 g/day, respectively. These findings observed during the putrefaction stage may be due to the occurrence of the autolysis process, accompanied by the release of nutrient-rich fluid. The substances in the released fluid, including sugars, amino acids, and fatty acids, serve as food and energy sources for the microbial community, thus promoting putrefaction [22, 23]. Meanwhile, another study found that the maximum microbial activity was observed within the first 10 days of the burial period [24]. The decomposition rate then decreased sharply after day 5 and continued to decrease to the end of the experiment. The decrease in the decomposition rate indicated the completion of decomposition as most of the fatty flesh decomposed, which was accompanied by the reduction in the mass of pig fatty flesh.

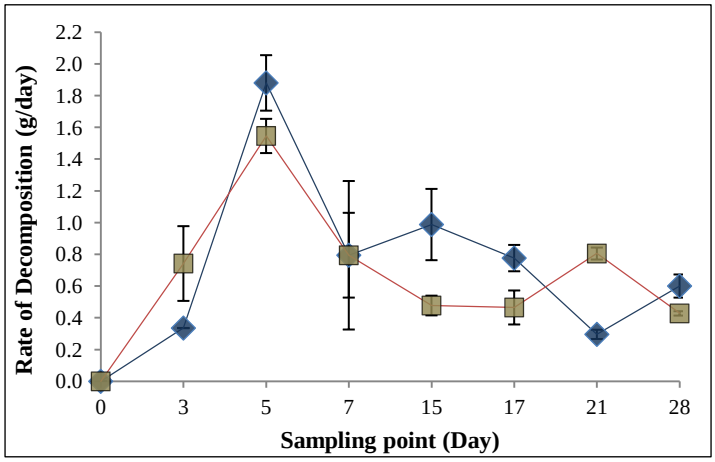

Figure 1. Plot of decomposition rate at different sampling points for 28 days of the burial period. The blue line represents the burial in mangrove soil whereas the red line represents the burial in oil palm plantation soil (n = 2)

Although a similar trend was observed for both soils, the decomposition rate of pig fatty flesh buried in mangrove soil occurred at a higher rate than that of oil palm plantation soil at most of the decomposition stages. This trend did not agree with previous studies as it is known that the rate of decomposition in the acidic soil was found to be two times faster than of the alkaline soil [25]. This observation may indicate the influence of other environmental factors on tends to occur in a mildly alkaline environment and can slow down the rate of decomposition [23, 28]. Adipocere is a decomposition product observable at the later stages of decay, usually following putrefaction [3, 29, 30]. The formation of adipocere results in unusual preservation, which also complicates the estimation of PMI [6, 31]. However, adipocere was not physically detected during sampling. Furthermore, the difference may also be due to the shift in microbial communities that is responsible for decomposition. It is well recognised that the decomposition of a cadaver will cause significant changes to the soil chemical properties and microbial communities [32]. It is also known that the changes in microbial communities across space are strongly associated with the differences in soil chemistry, particularly soil pH [33]. Besides, the introduction of cadaver decomposition materials will cause changes in many taxa contributing to the succession microbial community [34].

Total lipid extracts

Figure 2 shows the mass of total lipid extracts (TLEs) recorded at different sampling points for both soils throughout 28 days of the burial interval. The TLEs recovered from both soils showed an increasing trend in their masses throughout the experiment with minor fluctuation. The mass of TLEs extracted from the associated mangrove soil ranged between 0.076 and 0.5502 g g^{-1} soil dry weight. On the other hand, the mass of TLEs recovered from the oil palm plantation soil ranged between 0.2117 and 0.5423 g g^{-1} soil dry weight.

Even though both soils exhibited a similar trend in the decomposition rate, the mass of TLEs recovered from these soils was remarkably different. Between day 0 and day 7 of the burial interval, the mass of TLEs recovered from oil palm plantation soil was higher than that of mangrove soil. After day 7, the mass of TLEs extracted from mangrove soil was higher than that of oil palm plantation soil. The findings may be due to the shift in the soil microbial communities responsible for the decomposition of fatty flesh, as different microbial communities have a different need in nutrients and

energy [32, 35]. Thus, the response and adaptation of these microbial communities to the sudden increase in nutrient-rich fluid associated with decomposition may differ, resulting in varying mass of TLEs. These microbial communities may also involve in the mineralisation of the chemical components in the decomposition fluid into simpler components [10, 34].

A slower rate of decomposition for the burial in mangrove soil than that of oil palm plantation soil was evident by the decreasing mass of TLE between day 0 and day 3 of the burial period. After these days, the increase in the mass of TLEs recovered from this soil was observed, and a sharp increase in the mass of TLE was recorded between day 3 and day 5 of the burial interval. These observations may potentially be explained by the adaptation of microbial communities to the introduction of decomposing fatty flesh into the burial environment. The needs of nutrient sources may differ for different microbial communities; therefore, the shift in their communities can be observed towards the introduction of different cadaveric lipid components into the soil underneath [32, 36]. This shift due to the adaptation of the microbial communities to nutrient sources may lead to the decline in the degradation of fatty flesh between day 0 and day 3 of the burial period. The sharp increase in the TLE mass recorded after day 3 may be attributed to an enhanced adaptation of soil microbial communities towards the influx of cadaveric materials, which subsequently leads to the increase of decomposition. The rapid decrease and increase in the TLE mass may also be attributed to the slow and rapid mineralisation of cadaveric lipids into simpler components [32, 34, 36].

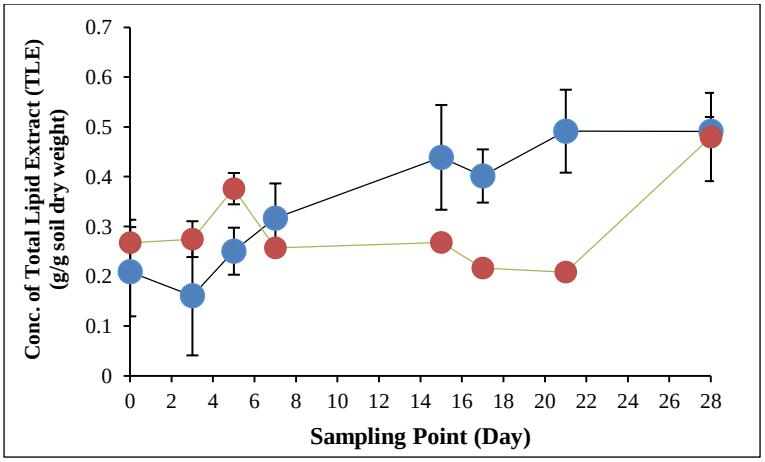

Figure 2. Mass of TLEs at different sampling points recovered from the associated mangrove and oil palm plantation soils for 28 days of the burial period. The black line represents the burial in mangrove soil whereas the green line represents the burial in oil palm plantation soil (n = 2)

For oil palm plantation soil, the mass of TLEs increased gradually from day 0 to day 3 and reached the maximum in the mass after 5 days of the burial period. The difference in the observation between that of mangrove and oil palm plantation soils at the initial decay and putrefaction stages can be regarded as an indication of different microbial responses towards the introduction of cadaveric materials. Furthermore, the findings agreed with the results from previous studies, which showed different burial soil environments have different influences on the cadaver decomposition rates [3, 4, 10]. After day 5 of the burial interval, the mass of TLEs decreased and the magnitudes in the mass were almost constant towards day 21. The observation was consistent with the decomposition rate shown in Figure 1, which may indicate less impact of cadaveric materials on the soil microbial communities after day 5 of the burial event. The results may also be an

indication of the rapid mineralisation of cadaveric materials into simpler compounds, resulting in a constant mass of TLEs. However, a rapid incline in the decomposition rate was observed after day 21, which contradicted the result of the decomposition rate; subsequently, it may explain a low mineralisation rate of cadaveric materials. Therefore, the mass of TLEs extracted from the burial associated soil will be higher compared to that of other burial days.

Free fatty acids

The GC results of TLEs for both mangrove and oil palm plantation soils revealed similar distributions of fatty acid components. Major free fatty acids (FFAs) recovered from both soils were saturated fatty acids of palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) components, together with the unsaturated fatty acid of oleic ($C_{18:1}$) component. Relatively, the concentrations of these FFAs were higher in mangrove soil compared to that of oil palm plantation soil. This finding agrees with the finding of the higher decomposition rate of fatty flesh buried in mangrove soil compared to that of the oil palm plantation soil at most of the sampling points. It has been known that neutral fats of a decomposing cadaver will eventually be hydrolysed into a mixture of saturated and unsaturated fatty acids as the decomposition process involves the breaking down of larger compounds into smaller chemical components [23, 37, 38]. Furthermore, the fatty acids of oleic ($C_{18:1}$), linoleic ($C_{18:2}$), and palmitoleic ($C_{16:1}$) components have been identified to be the most abundant lipid components in the human body [38, 39].

Figure 3a shows that the concentration of FFAs recovered from the associated oil palm plantation soil varied with the burial interval. Remarkably, their concentrations were higher at the early stages of decomposition. The observations may suggest a massive introduction of cadaveric materials occurred at the early stages of decomposition for this type of soil. These early stages have been regarded as the active stages of decomposition [23, 38, 40]. The concentrations of $C_{16:0}$ ranged from 0.1808 to 61.0164 ppm g^{-1} soil dry weight. Meanwhile, the concentrations of $C_{18:0}$ were lower than that of $C_{16:0}$, ranging between 0.2548 and 21.6411 ppm g^{-1} soil dry weight. The fatty acid of the $C_{18:1}$ component exhibited the lowest concentration compared to that of $C_{16:0}$ and $C_{18:0}$, in the range between 0.0071 and 0.3839 ppm g^{-1} soil dry weight. It has been known that unsaturated fatty acids will be further hydrolysed and oxidised into simpler fatty acids shortly after death [39]. Furthermore, the mineralisation of $C_{18:1}$ is quicker in all the different soils [41]. The concentrations of these FFAs decreased towards the end of the experiment as the mass of decomposing fatty flesh will decrease with burial period.

Similarly, three major FFAs were extracted from the associated mangrove soil (Figure 3b). The $C_{18:0}$ component exhibited higher concentrations, ranging from 6.6374 to 3405.403 ppm g^{-1} soil dry weight, followed by $C_{18:1}$ with the concentration between 0.5949 and 2666.279 ppm g^{-1} soil dry weight. On the other hand, the $C_{16:0}$ component was found to have the lowest concentration, ranging from 0.00 to 36.0937 ppm g^{-1} soil dry weight. Remarkably, these FFAs demonstrated low concentration for the first 17 days of the burial period, contradicting to that of oil palm plantation soil. The concentrations of $C_{18:0}$ and $C_{18:1}$ increased rapidly after day 21 of the burial interval; however, the concentrations of $C_{16:0}$ were almost constant from the beginning of the burial event. The decomposition rate of pig fatty flesh in mangrove soil was almost similar to that of oil palm plantation soil (Figure 1); therefore, the finding may be an indication of rapid conversion of these FFAs into simpler compounds, such as carbon dioxide and water that occurred in mangrove soil. This rapid decomposition has been known to result in extensive hydrolysis and hydrogenation of fats, which eventually increases the mixture of saturated fatty acids, i.e., $C_{16:0}$ and $C_{18:0}$, and decreases the relative amount of unsaturated fatty acids, i.e., $C_{18:1}$ [39]. Subsequently, $C_{16:0}$ and $C_{18:0}$ dominated the oil palm plantation and mangrove soils for most of the sampling points. Previous studies identified that $C_{18:1}$ is the most abundant fatty acid found in adipose tissues, followed by $C_{18:2}$ and $C_{16:1}$, where the hydrogenation of unsaturated $C_{18:1}$, $C_{18:2}$, and $C_{16:1}$ will yield homologous saturated fatty acids, i.e., $C_{16:0}$ and $C_{18:0}$ [39, 42].

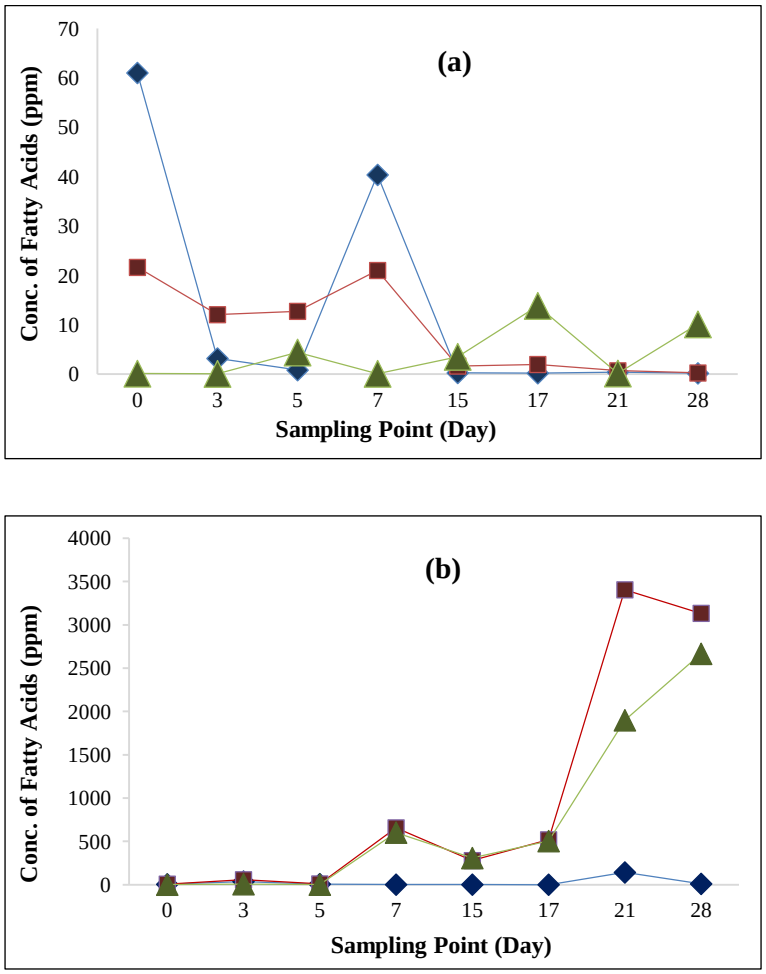

Figure 3. The concentration of fatty acids detected in oil palm plantation soil for 28 days of the burial interval. The blue line represents $C_{16:0}$, the red line represents $C_{18:0}$, and the green line represents $C_{18:1}$

Conclusion

In this study, three main parameters, namely decomposition rate, TLEs, and FFAs were the focus of discussion to describe the burial of a cadaver substituted by pig fatty flesh in mangrove and oil palm plantation soils. For a burial period of 28 days, a similar trend in the decomposition rate of pig fatty flesh was observed for both mangrove and oil palm plantation soil environments. However, the decomposition rate of the pig fatty flesh buried in mangrove (alkaline) soil was slightly higher compared to that of oil palm plantation (acidic) soil at sampling points of day 5, day 15, and day 17 of the burial period. Furthermore, both soils demonstrated a rapid increase in the decomposition rate after three days of the burial interval and reached maximum on day 5. Then, a rapid decline in the decomposition rate of both soils was observed between day 5 and day 7 of the burial period. The decomposition rate of both mangrove and oil palm soils continued to decrease towards the completion of the burial experiment as the mass of decomposing fatty flesh decreased with time. The mass of TLEs recovered from oil palm plantation soil was high at the first seven days of the burial period and low after this point.

Meanwhile, an opposite trend was observed for the mass of TLEs recovered from mangrove soil, i.e., the mass of TLEs was low at the first seven days of the burial period and high after this point. Both soils demonstrated a similar trend of decomposition rate; therefore, the different trends in the mass of TLEs may be an indication of different mineralisation rates occurring in both soils. Different responses of the microbial communities towards the introduction of different cadaveric materials at different decomposition stages may also lead to a different trend in the mass of TLEs recovered from the soils of different acidity. Even though both soils showed a similar trend of decomposition rate, the concentrations of FFAs recovered from mangrove soil were found to be higher than that of oil palm plantation soil. The findings may indicate different mineralisation rates and the response of microbial communities on the introduction of FFAs into the underneath of both soils. For oil palm plantation soil, the concentrations of FFAs were higher for the first 17 days of decomposing and lower after that point. In contrast, the concentrations of FFAs were low for the first 17 days and high after that point.

Remarkably, the introduction of decomposing fatty flesh in soils with different soil pH led to a significant difference in the decomposition rate for both soils, and subsequently resulted in a different mass of TLEs and other lipid components. Hence, the findings of this study may propose that the mass of TLEs and concentrations of FFAs can be developed as an alternative forensic tool to locate a clandestine grave and to estimate the PMI for different types of soils with different acidity.

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