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Preliminary Observation of Physicochemical Changes associated with the Decomposition of a Partially Submerged Buried Cadaver in a Mangrove Soil System

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ABSTRACT

The decomposition process of a partially submerged-buried body is not well understood causing difficulties in locating of a partially submerged clandestine grave. In order to address the influence of the excessive moisture on the decomposition of a cadaver, therefore, a simulated burial experiment was performed using fatty flesh of commercial pig (Sus scrofa). The fatty flesh was shallowly buried in mangrove soil system that consistently experiencing low and high tides. The fatty flesh was allowed to decompose for 120 days, and the associated soils were collected at 16 designated sampling points. The decomposition rate and ~~soil~~ post-experimental soil pH were measured. Whilst the soil cadaveric derived lipids were extracted using modified Bligh Dyer extraction method and analysed with Gas Chromatography Flame Ion Detector (GC-FID) to establish the lipid distribution profile for each of the decomposition stages. The post-experimental soil pH increased to be more alkali at the early decomposition stages and became acid towards completion of experiment. Similar trend was also observed for decomposition rate, with a rapid incline after day 3 of burial interval. The distribution of extractable lipids was different for different decomposition stages, with massive introduction of the lipids into the soil was observed between the initial and black putrefaction stages. The observed physicochemical changes of soil pH, decomposition rate and distribution of cadaveric derived lipids shall provide useful information to aid in locating the partially submerged clandestine grave, subsequently, to determine the postmortem interval of the deceased.

Keywords: Partially submerged-buried body, low and high tides, mangrove soil, simulated burial experiment

INTRODUCTION

The burial of a partially submerged body in a mangrove soil ecosystem is a complex process that can significantly impact the surrounding environment (Abdel-Razik, 1991) and lead to the release of cadaveric-derived lipids (Carter *et al*., 2007; Sofo Ismail *et al*., 2020; Caballero-Moreno *et al*., 2024). These lipids are compounds originating from the decomposition of fatty tissues in the cadaver (Carter, 2005; Goff, 2009; Bull *et al*., 2009). The presence and analysis of these cadaveric-derived lipids offer valuable insights into the decomposition process, the environmental conditions in which it occurred, and the potential forensic implications of the burial site (Vass, 2001; Megyesi *et al*., 2005; Wilson *et al*., 2007; Standen *et al*., 2010; Sofo Ismail, 2013). Microbial communities in the mangrove soil play a crucial role in the decomposition of the body, as they consume and decompose the organic matter left behind (Hopkins *et al*., 2000; Lazaroaie, 2009; Hyde *et al*., 2013). The presence and composition of these lipids can provide insights into the impact of decomposition on nutrient cycling and microbial community dynamics within mangrove ecosystems. Additionally, the release of cadaveric organic matter and nutrients into the soil can potentially alter the nutrient dynamics

and microbial composition of the mangrove soil ecosystem (Clark *et al*., 1997; Aitkenhead-Peterson *et al*., 2012; Lessin *et al*., 2018).

Extensive research has shown that the decomposition of a cadaver releases a wide range of nutrients into the soil. This is because 96% of the human body contains vital nutrients required for nutrient cycling, such as carbon, hydrogen, oxygen, and nitrogen (Gill-King, 1997; Fiedler *et al*., 2003; Dent *et al*., 2004). Another 3.45% of the body comprises calcium, phosphorus, potassium, sulphur, chlorine, sodium, and magnesium, with a small percentage of trace elements (Forbes, 2008; Perrault & Forbes, 2016). It has been found that the effect of cadaver decomposition on the soil is minimal in the early stages of the process, but adverse soil disturbances can be detected towards the active decay stage (Francher *et al*., 2017; Debruyn, 2018). The cadaveric-derived nutrients introduced into the soil include volatile fatty acids (primarily butyric and propionic acids), organic acids (e.g., acetic and oxalic acids), and organic nitrogen (such as nucleic acids, peptides, and amino acids), all of which enhance biogeochemical processes in the soil (Carter & Tibbett, 2007; Cobaugh *et al*., 2015; Chock & Sofo Ismail, 2017). Therefore, the decomposition process plays a crucial role in the nutrient cycle, facilitating the succession of microbial community structures and the mobilization of nutrients in the ecosystem (Cobaugh *et al*., 2015; Debruyn, 2018). Moreover, the by-products of decomposition serve as a major source of carbon, hydrogen, and oxygen for structural and metabolic purposes (Dekeirsschieter *et al*., 2009; Craine & Dybzinski, 2013). A recent study conducted at a decomposition research facility in Southern Ontario, Canada, demonstrated a positive nutrient response and lateral diffusion surrounding pig (*Sus scrofa domesticus*) carcasses (Perrault & Forbes, 2016).

A simulated burial experiment using pigs (*Sus scrofa*) as human surrogates, due to ethical concerns, was conducted in response to the urgent need to monitor changes in soil pH, assess the decomposition rate of the partially submerged buried cadaver, and quantify cadaveric-derived lipids that could potentially be introduced into the surrounding soil. The decomposition of a partially submerged buried cadaver in mangrove soil is believed to lead to significant physicochemical changes, including pH fluctuations, an increased decomposition rate during the early stages, and a substantial amount of extractable cadaveric-derived lipids. Furthermore, the unique characteristics of mangrove soil, such as high salinity and organic matter content, influence the cadaver decomposition process differently compared to other ecosystems. In this study, the five stages of cadaver decomposition were identified as follows: initial decay (0-3 days), putrefaction (4-10 days), black putrefaction (11-20 days), butyric fermentation (20-50 days), and dry decay (> 50 days). These stages, each with its duration and set of characteristics, can facilitate record-keeping, observation, and documentation. Understanding the dynamics of how water and soil properties affect cadaver decomposition, along with the role of microbial communities and their interactions with cadaveric-derived lipids, is essential for forensic investigations involving submerged buried bodies in mangrove soil. The study of these cadaveric-derived lipids can also contribute to the development of forensic tools and techniques for more accurate and efficient crime scene investigations. Additionally, the analysis of cadaveric-derived lipids in mangrove soil has important implications for environmental and ecological studies.

MATERIALS AND METHODS

Simulated burial experiment

Location of the burial site

A mangrove area located at the UMT Biowalk was selected as the burial site to simulate the partially submerged burial of a cadaver in a shallow clandestine grave. The location was chosen

based on several key features, including anthropogenic activities, soil type, proximity to water, and its variable morphology and productivity (Fatemeh *et al*., 2014). This mangrove site, a brackish water ecosystem with sandy soil, is classified as a riverine mangrove forest with floodplains that grow along a tidal river. As such, the area experiences continuous tidal flushing, with flooding occurring during the highest tides and drying up during most low tides. **Figure 1** provides a satellite view of the UMT Biowalk at University Malaysia Terengganu.

Figure 1: Satellite view of study area UMT Biowalk in University Malaysia Terengganu (5.406941, 103.093663)

Experimental design

Approximately 20 g of fatty flesh from a commercial pig (*Sus scrofa*) was used as a substitute for the human cadaver. The belly fatty flesh was chosen for this study due to its high fat content. The initial weight of the flesh was accurately recorded using a weighing balance (Sartorius, AX224) before placing the flesh into a small pot (10 cm × 10 cm), which served as the burial vessel (Szelecz *et al*., 2018). A gauze bag was used to enclose the flesh to prevent access by scavengers (Debruyn, 2018). The fatty flesh was placed in a pot half-filled with the soil of interest, and then the flesh was fully covered with the soil. The pots were buried 15 cm deep from the surface and 20 cm from the water column, mimicking a burial in a shallow grave (**Figure 2**). Each pot, properly labelled, was placed approximately 3 cm apart from the others within the mangrove soil system.

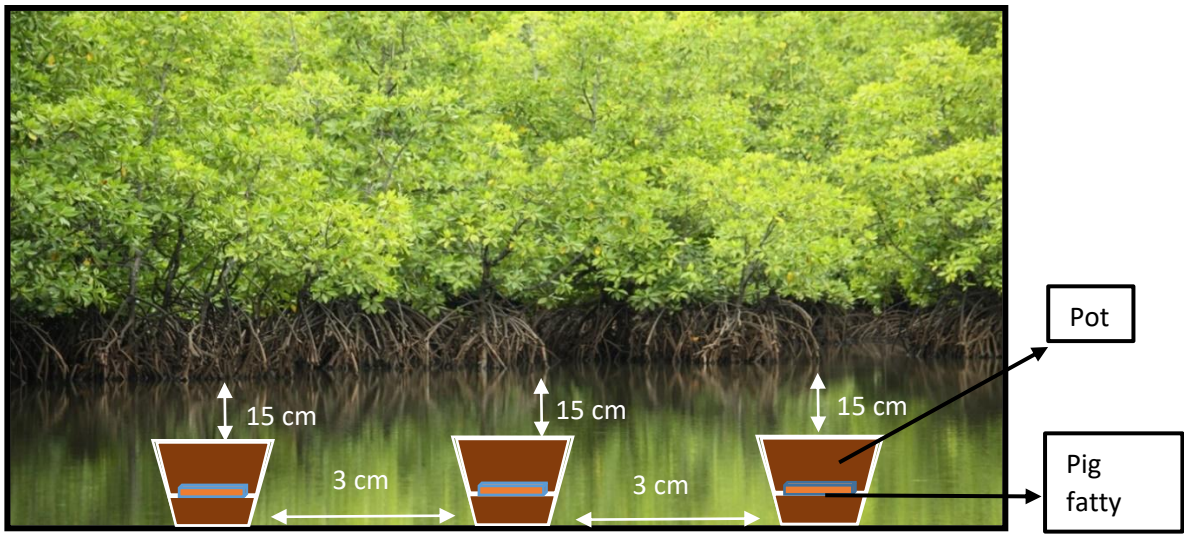

Figure 2: Burial site of the simulated burial experiment at the mangrove area during high tide

The burial site was surrounded by a fence to prevent disturbances from animals and humans. The fatty flesh was allowed to decompose for a designated burial interval, representing each stage of decomposition. Each burial interval was replicated. The control set, which did not contain fatty flesh, was prepared following the same procedures as the set with fatty flesh. The burial site was regularly visited throughout the 120-day burial period, with a total of 16 sampling days, to maintain the facility and collect samples (Daud & Sofo Ismail, 2018). The gap between adjacent sampling points was two days for the first thirty days and ten days for the remaining ninety days of the burial period. During sampling, the pots were removed from the burial site and transported to the laboratory. The remaining flesh was removed from the pots and thoroughly cleaned to remove excess soil that had adhered to it due to its damp texture. The flesh was handled carefully to avoid disturbances that could result in inaccurate mass measurements and was weighed accurately. The decomposition rate was calculated after obtaining the final weight of the remaining flesh, using Equation 1.

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

The soil surrounding the flesh was then collected and packed in the aluminium foil before storing in the freezer at a temperature of -20 ℃ before the subsequent analyses.

Pre-soil treatment

The collected soil was thoroughly homogenised to break up compacted and bulky particles. It was then freeze-dried and transferred into an airtight glass sample bottle, and stored in darkness at -80°C for seven consecutive days before further lipid extraction and analysis.

Post-experimental soil pH

The post-experimental soil pH was measured using a calibrated pH meter (Eutech Instruments, PC 700). A 15 mL aliquot of deionised water was added to 3 g of the soil sample to create a 1:5 soil-to-water solution (Perrault & Forbes, 2016). The mixture was left to settle for 5 minutes before pH readings were taken. Three measurements were recorded for each soil replicate to ensure accuracy.

Total lipids extracts (TLEs)

Cadaveric-derived lipids were extracted from the associated soil using a modified Bligh-Dyer extraction method (Bligh & Dyer, 1959; Sofo Ismail, 2013). Approximately 2 g of soil was transferred into a glass centrifuge tube, and 3 mL of dichloromethane (DCM)/methanol (2:1, v/v) was added. The solution was spiked with 100 µL of an internal standard, tetratriacontane (C_{34}). The sample was sonicated for 15 minutes at 40°C and centrifuged for 5 minutes at approximately 3000 rpm. The resulting supernatant was transferred to a clean vial. This extraction step was repeated three times using 2 mL of DCM/methanol (2:1, v/v). The soil was subsequently treated with 3 mL of Bligh-Dyer solution, sonicated for 15 minutes at 40°C, and centrifuged for 5 minutes at approximately 3000 rpm. The supernatant was added to the same vial. This step was also repeated three times using 2 mL of Bligh-Dyer solution. To separate the organic phase, 2 mL each of buffered water and chloroform were added to the pooled supernatant, followed by centrifugation for 1 minute. The organic layer was carefully transferred to another clean vial using a Pasteur pipette. This process was repeated three times with 2 mL of chloroform. The solvent was evaporated under a gentle flow of nitrogen, and the total mass of the extractable lipids was measured with precision. The evaporated lipids were stored at 4°C in a refrigerator before subsequent analyses (Chock & Sofo Ismail, 2017).

Analysis of the extractable lipids

The resulting extractable lipids were analysed using a Gas Chromatography-Flame Ionisation Detector (GC-FID). The GC-FID was equipped with an HP-5 column (5% Phenyl Methyl Siloxane; 30.0 m × 320 µm × 0.25 µm), with helium as the carrier gas. The oven temperature was initially set at 40°C and held for 1 minute. Subsequently, the temperature was increased at a rate of 10°C min^{-1} until reaching 200°C, where it was held for 16 minutes. The temperature was then further increased at a rate of 3°C min^{-1} until 300°C and held for 20 minutes. The stored TLE was diluted with hexane to prepare the sample before injection into the device. An external standard was also injected for qualitative and quantitative analysis. Chromatographic peaks were identified by comparing the retention times of the sample with those of the external standard.

RESULTS AND DISCUSSION

Post-experimental Soil pH

The post-experimental soil pH of each sample was measured to assess the changes in soil acidity associated with the decomposition of partially submerged, buried fatty flesh. **Figure 3** illustrates the variations in soil pH over the 120-day burial period. Overall, the soil pH exhibited an initial acidic phase during the early stages of decomposition, followed by an increase to alkaline conditions, before acidifying again towards the end of the 120-day burial period. This trend aligns with the typical post-experimental soil pH patterns observed in other studies (Haslam & Tibbett, 2009; Larizza, 2010; Sofo Ismail *et al*., 2023). At the start of the burial period (Day 0), the initial soil pH was slightly acidic at 6.468. The pH rose rapidly during the putrefaction stage, peaking at 7.909 on Day 10. This was followed by a decrease to 6.993 on Day 12 during the black putrefaction stage. A slight increase to 7.617 was observed on Day 17, after which the pH declined again to 7.363 on Day 20. The soil pH reached its highest value of 8.25 on Day 40, coinciding with the butyric fermentation stage. Subsequently, the pH began to decline steadily as the burial period progressed, reflecting the acidic conditions characteristic of later decomposition stages as the 120-day period concluded.

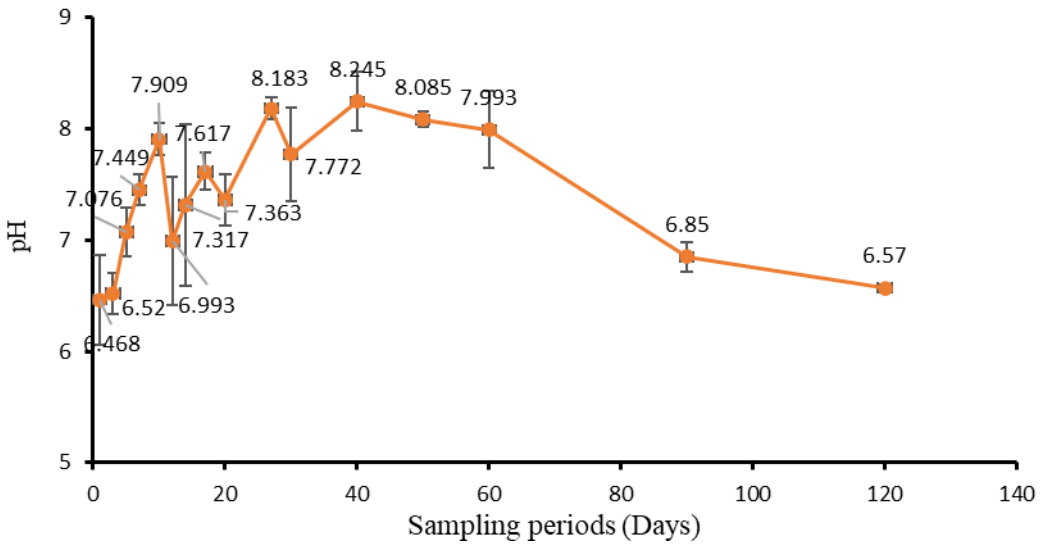

Figure 3: Graph of the post-experimental soil pH

During the decomposition process, nutrients released from the cadaver seep into the surrounding soil, forming a cadaveric decomposition island (CDI) (Carter *et al*., 2007; 2010). The mineralisation of proteins and other organic nitrogen compounds begins with ammonification, followed by the enzymatic oxidation of ammonia through nitrification. The conversion of NH_4^+ to NO_3^- releases protons, acidifying the soil (Hopkins et al., 2000; Haslam & Tibbett, 2009). Additionally, acid- and base-forming ions significantly influence soil pH. Alterations in metallic ions within the soil, such as aluminium (Al^{3+}) and iron (Fe^{2+} or Fe^{3+}), further contribute to pH changes (Brady & Weil, 2002; Ioan *et al*., 2017).

The degradation of fatty tissues generates non-mineralised decomposition by-products, including phenolic compounds, palmitic acid ($C_{16:0}$), and stearic ($C_{18:0}$) acids, which are deposited into the soil solution (Dekeirsschieter *et al*., 2009; Sofo Ismail *et al*., 2020). These organic compounds lower soil pH and may inhibit subsequent decomposition and microbial activity (Fuentes *et al*., 2006; Kumar *et al*., 2009). Similarly, oxalic acid, a by-product of muscle tissue decomposition, is known to acidify the soil (Vass *et al*., 2002; Larizza, 2010). For example, the decomposition of a 68 kg human cadaver increased ammonium concentration in the soil by approximately 525 µg g^{-1} (Carter & Tibbett, 2008). Furthermore, significant shifts in soil chemical properties beneath decomposing cadavers have been observed, including a notable increase in extractable organic carbon, nitrogen, and ammonia during the bloat and active decay stages (Cobaugh *et al*., 2015; Vonder Lühe *et al*., 2018).

The rise in soil pH to slightly alkaline levels between Day 30 (butyric fermentation stage) and Day 60 (dry decomposition stage) of the burial period may be attributed to the formation of ammonia (NH_3), often indicated by blackening of the cadaver (Tranchida *et al*., 2014). However, the gradual decline in pH after Day 40, stabilising at 6.57 by the end of the 120-day burial period, is likely due to the continuous release of cadaver-derived acidic by-products from the decomposition of fatty tissues (Davenel *et al*., 1999; Notter *et al*., 2009; Irish *et al*., 2019). This decline could also result from the nitrification of nitrogen, a natural process in soil (Ni *et al*., 2023). Moreover, the reduced release of cadaveric fluids during the later stages of decomposition may further contribute to the acidic shift in soil pH (Haslam & Tibbett, 2009; Olakanye *et al*., 2017).

Rate of Decomposition

The decomposition rate exhibited a sharp increase from Day 0 to Day 3 (**Figure 4**). This period marks the onset of the initial decay stage, during which no significant changes in the mass of the fatty flesh were observed, and no noticeable odour of decomposition was detected. A rapid rise in the decomposition rate occurred between Day 3 and Day 5, resulting in dual maxima on Days 5 and 10 of the burial period, with similar rates of 1.624 g/day and 1.636 g/day, respectively. These days correspond to the early putrefaction stage. A slight decline in the decomposition rate was observed on Day 7, with a recorded rate of 1.417 g/day. The peak decomposition rates on Days 5 and 10 may be attributed to the maximum degrading activity of microorganisms acting on the fatty flesh. At this stage, the breakdown of biomolecular structures and the rupture of cell membranes released intracellular contents into the surrounding soil, providing an energy source and nutrients for microbial activity (Goff, 2009; Zhou *et al*., 2011). This phase was characterised by a strong, distinctive odour, likely due to the purging of cadaveric fluids. Optimum microbial activity is known to occur within the first ten days following the burial of a cadaver, with peak microbial activity typically detected during the early stages of decomposition (Lazaroaie, 2009; Wescott, 2018, Hyde *et al*., 2013). This heightened activity accelerates mass loss and enhances decomposition rates during this critical period.

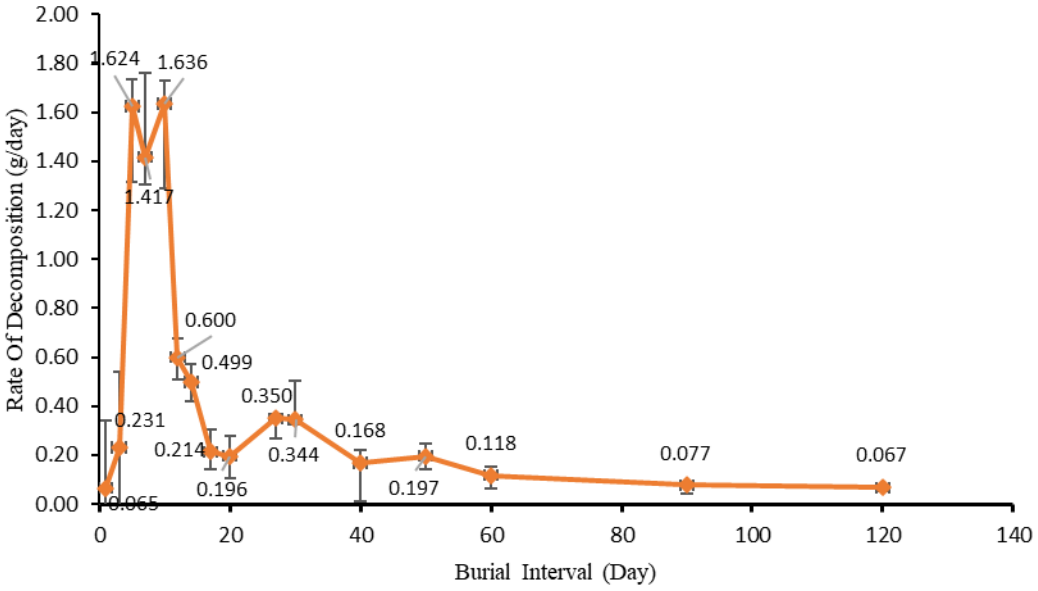

Figure 4: Plot of decomposition rate at each sampling point for 120 days of burial interval (n=3)

The slightly lower decomposition rate observed on Day 7 of the burial period may be attributed to precipitation between Days 6 and 7. The rainfall caused water levels in the mangrove site to remain elevated, even during low tide. Prolonged moisture likely resulted in soil particles becoming more tightly packed, with water filling the interstitial spaces. This condition reduces the oxygen content in the soil (Fiedler & Graw, 2003; Forbes *et al*., 2005; 2008). In low-oxygen environments, microbial activity slows, which could explain the slight decrease in the decomposition rate to 1.4172 g/day on Day 7. Variations in humidity may further influence microbial activity, as microbial populations tend to migrate or adjust in response to environmental conditions (Carter *et al*., 2010; Tranchida *et al*., 2014).

Following Day 10, the decomposition rate decreased sharply to 0.600 g/day on Day 12, and subsequently, to 0.196 g/day by Day 20 of the burial interval. This trend reflects the progression into the black putrefaction stage, during which the decomposition rate continued to decline until the end of the 120-day burial period. On Days 27 and 30, a slight increase in the decomposition rate was recorded, with values of 0.350 g/day and 0.344 g/day, respectively, corresponding to the butyric fermentation stage. These minor fluctuations in decomposition rate could be influenced by the variable weather conditions in the Kuala Terengganu region. The sharp decline in decomposition rate after Day 10 may also result from the depletion of cadaveric materials and fluids, as most of the fatty tissues had decomposed by this stage. Additionally, heavy precipitation and flooding from nearby seawater could have caused significant cadaveric fluids to drain away from the surrounding soil. During this phase, a grey-white layer formed on the flesh after fat liquefaction, which later solidified into a crumbly consistency. The white, waxy film observed on the flesh and adjacent soil was identified to be potentially a substance known as adipocere (**Figure 5**).

Figure 5: The white layer (yellow circle) that was suspected to be adipocere, which was observed on both of fatty flesh (left) and surrounding soil that was in closed-contact with the flesh (right).

Adipocere formation is known to occur around internal organs and tissues with lower fat content, such as the heart, liver, and kidneys, as a result of fat liquefaction from other decaying tissues during the putrefaction process (Forbes *et al*., 2005; Kumar *et al*., 2009; Ubelaker & Zarenko, 2011). The odour associated with adipocere has been described as "earthy, cheesy, and ammoniacal," and it is more commonly observed in submerged bodies (Adachi *et al*., 1997; Kumar *et al*., 2009; Mähler *et al*., 2022). Water or moisture plays a crucial role in the bacterial and enzymatic processes that lead to adipocere formation. This layer subsequently hinders further decomposition (Kumar *et al*., 2009; Teo *et al*., 2014). Adipocere formation can preserve soft tissue to varying degrees by inhibiting the activity of microorganisms (Fiedler & Graw, 2003; Kumar *et al*., 2009). This preservation effect helps explain why the decomposition rate of submerged bodies differs from those buried in other environments (Wescott, 2018b; Ubelaker & Zarenko, 2011). From Day 50 to Day 120 of the burial period, the decomposition rate gradually decreased, reaching a minimal rate of 0.067 g/day on Day 120, corresponding to the dry decay stage. This slowdown in decomposition can be attributed to reduced microbial

activity, which results from the depletion of cadaveric nutrients in the surrounding soil and the production of adipocere (Carter *et al*., 2007).

Total Lipid Extracts (TLEs)

The mass of total lipid extracted at each sampling point is presented in **Figure 6**. Within the associated soils of partially submerged, buried fatty flesh undergoing decomposition, the mass of TLE recovered ranged from 0.0010 g/g soil dry weight to 0.0610 g/g soil dry weight. Between day 1 and day 3, corresponding to the initial decay period, there was an increase in the mass of extracted TLE, with concentrations of 0.0039 g/g soil dry weight and 0.0240 g/g soil dry weight, respectively.

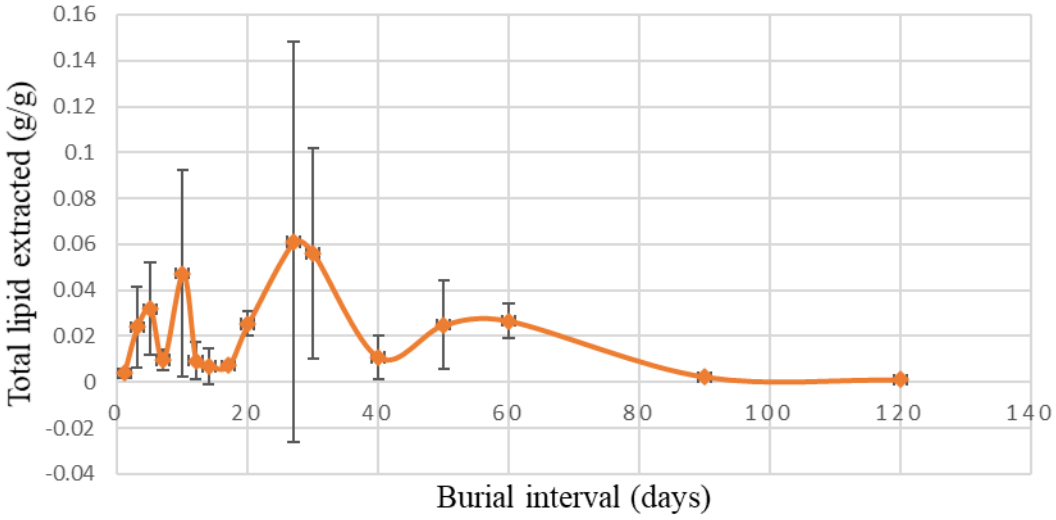

Figure 6: Graph of concentration of extractable lipids recovered from the associated soils of the partially submerged clandestine grave, as determined by GC analysis (n=3).

The mass of TLEs continued to rise from 0.0318 g/g soil dry weight on day 5 to 0.0473 g/g soil dry weight on day 10, corresponding to the putrefaction stage. However, the amount of lipids dropped slightly on day 7. Since this trend closely mirrored that of the decomposition rate, the finding may also be attributed to the rainfall, which resulted in excessive moisture that reduced the release of cadaveric lipids into the surrounding soil, as the decomposition process was temporarily depressed. After day 10, a sharp decline in TLE concentration was observed, with values recorded at 0.0069 g/g soil dry weight on day 12, 0.0610 g/g soil dry weight on day 14, and 0.0071 g/g soil dry weight on day 17 of the burial period. Following these days, a rapid increase in TLE concentration was recorded, with 0.0610 g/g soil dry weight extracted on day 27, corresponding to the black putrefaction stage. Remarkably, the TLE concentration increased during the first four stages of the decomposition process, with fluctuations at certain points. This trend may be explained by the adaptation of the microbial community to the introduction of cadaveric substances into the soil environment. The introduction of nutrient-rich cadaveric substances causes changes in many taxa, contributing to the succession of microbial community structure (Cobaugh et al., 2015). With the increased breakdown of the buried fatty flesh, more lipids from the cadavers would therefore leak into the nearby soil.

From day 27 onward, there was a notable decline in the TLE concentration, which persisted with very slight variations until the end of the burial experiment. The lowest quantity of TLEs detected on day 120 of the burial interval was 0.0010 g/g soil dry weight. Decomposition drastically slows down as the cadaver reaches the skeletal state, as seen by a

drop in TLE mass. By the end of the experiment, other findings included a decrease in the amount of fatty tissue, a reduced quantity of cadaveric material, and the development of adipocere. Additionally, the remaining flesh may have broken down within the developed adipocere (Forbes *et al*., 2005). Fluctuations in TLE concentration are common during decomposition, as the breakdown of tissue into its building blocks takes time and largely depends on both intrinsic and extrinsic factors (Teo *et al*., 2014). The increase and decrease in TLE mass indicated the adaptation of microbial communities to the introduction of cadaveric nutrients (Chock & Ismail, 2017). The decomposition of cadaver-derived organic matter facilitates successional shifts in the microbial communities of the burial soil (Cobaugh *et al*., 2015). Thus, this contributed to the fluctuation of TLE mass from day 0 to day 120.

CONCLUSION

The post-experimental soil pH, decomposition rate, and cadaveric-derived lipids changed significantly as a result of the decomposition of partially submerged buried fatty flesh in the mangrove soil system. These changes provide valuable insights for forensic investigations. Early in the decomposition process, the pH of the post-experimental soil was found to be acidic; however, as the process progressed to subsequent stages, it became alkaline before turning acidic again as the process neared completion. It was observed that the early stages of decomposition of the partially submerged buried fatty flesh exhibited a much higher rate of decomposition, indicating rapid degradation of the fatty flesh. The amount of lipids derived from the cadavers extracted from the associated soils, however, did not show any clear pattern. The related changes in post-experimental pH, decomposition rate, and cadaveric-derived lipids hold great potential for enhancing forensic investigations. Future studies could take into account the effects of environmental variables with a longer study timeframe. Additionally, comparisons with varying submersion levels, similar mangrove systems, and other ecosystems could provide valuable insights, highlighting distinctive forensic indicators. These could include soil microbial profiling to identify important decomposer species and their roles in lipid breakdown and pH shifts. In summary, these aspects of the decomposition of a partially submerged buried cadaver provide crucial information with significant implications for forensic investigations.

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