MOLECULAR ANALYSIS OF THE IMPACT OF CADAVER BURIAL ON THE SOIL ENVIRONMENT AND THE IMPLICATIONS FOR FORENSIC INVESTIGATION

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by

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Abstracts

Following death, in the absence of any impeding environmental factors, a cadaver will begin to decay after approximately 4 minutes. Fuelled by the nutrient rich fluids released by autolysis, microorganisms (bacteria, fungi and protozoa), derived largely from the intestinal tract, proceed to consume the soft tissue of the cadaver giving rise to the process known as putrefaction. One of the major processes known to occur during this period is the decomposition of adipose tissue. At 60-85%, lipids constitute the largest proportion of adipose tissue and between 90-99% of this lipid fraction comprises triacylglycerols.

Triacylglycerol decomposition is mediated by a suite of tissue lipases and proceeds *via* hydrolytic cleavage of ester bonds to yield a mixture of free fatty acids (as their sodium and potassium salts) dominated by oleic acid and, to a lesser extent, linoleic, palmitoleic and palmitic acid. When left in a suitable environment (e.g. buried, submerged) the sodium and potassium ions may be displaced by calcium and magnesium ions thereby forming the insoluble, white, greasy substance commonly referred to as adipocere; this is the saponifcation theory of adipocere formation. As early as 1875, Ebert recognized that adipocere also contained a hydroxy-fatty acid which subsequent studies have shown to be 10-hydroxyoctadecanoic acid, a microbially mediated hydration product of the oleic acid that is the major component of human adipose fat.

A large number of factors influence the formation of adipocere following the onset of decomposition, these include: pre-mortem body conditions, the cause of death, the time elapsed between death and burial, the method of burial, environmental conditions of the burial site and also any anthropogenic effects wrought on the site. Of particular importance to this study is the depositional environment and whether it was conducive to adipocere formation tissue/adipocere. Whilst the associated corpses had been preserved through the formation of adipocere the results revealed that phosphorous, dissolved organic carbon and cadaverine had all leeched from the graves into the surrounding soil. Interestingly, microbial activity and biomass were observed to be higher in the control soil, this was ascribed to the inert character of adipocere. Indeed, under near anoxic to anoxic conditions degraded adipose tissue may exist indefinitely as adipocere requiring the presence of gram-positive bacteria and oxygen for further degradation and eventual mineralization (complete conversion of organic matter to simple substrates such as H_2O, CO_2, NO_3^- etc.) to occur.

To further understanding on the decomposition process, a comparative study of soil lipids collected from eleven crime scenes across Malaysia, with different post-depositional intervals (PDI) of cadavers, was conducted. A laboratory degradation study of porcine flesh in soil was also carried out in parallel. The overall premise for this work was that organic molecular components in soil will be useful in determining the provenance of a cadaver and/or calculation of its post-mortem interval (PMI).

The results from the Malaysian samples show that the concentrations of palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acids are higher in the cases with a low PMI. In addition, their unsaturated analogues, palmitoleic ($C_{16:1}$) and oleic ($C_{18:1}$) acids are also present at high concentration. The higher concentration of cholest-5-en-3β-ol (cholesterol) in the cases with low PMIs indicates that this component is most likely derived from the decomposing body. The cases with longer PDI demonstrate a large shift towards plant-derived organic material. Differences observed are presumably due to the transformation of the cadaver derived lipids and can be associated with PMI and/or PDI.

Subsequently, porcine flesh was degraded in aerobic and anaerobic soil mesocosms, representative of tropical and temperate climates, for a year. Mesocosms were sampled throughout the experiment and target analytes were chosen to provide information about both materials derived from the flesh and the response of the microbial community to its presence in the soil environment. Initial results reveal that flesh derived triacylglycerol components degrade within the first 120 days resulting in a corresponding increase in concentration of free fatty acids, i.e. palmitic and stearic acids. Similar patterns of degradation are observed under both aerobic and anaerobic conditions. Preliminary analysis of phospholipid fatty acid distributions, derived from the soils, reveals a concentration increase in the region of 180-400% for soils incubated with porcine flesh after 121 days, with a currently unidentified $C_{18:1}$ fatty acid moiety becoming the dominant component in the latter samples.