Detection of Biogenic Amines from soil samples

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ABSTRACT

Complex compounds that are released during the active stage of decomposition such as Volatile Organic Compounds (VOC's) can aid in estimating the time of death, especially in cases of homicide, missing persons and mass disasters. In this paper, a new equipment, Field Asymmetrical Ion Mobility Spectrometry (FAIMS) was used to identify and study these biogenic compounds. Field Asymmetrical Ion Mobility Spectrometry, a variation of the ion mobility spectrometry, is used to detect compounds at sensitivity as low as 10 ppb and with different proton affinities. Over 96 soil samples from three pig carcasses were collected and weighed into vials. These were analysed using FAIMS and compared to blank samples. Due to competitive ionisations, it was difficult to differentiate between cadaverine and putrescine; however, the peaks of the biogenic amines were distinctly different from the blank samples. The results showed that the differences between blank samples and those with biogenic amines were highly distinguishable. The discussion covers the outcomes of the experiment and the various problems faced as this study has not been conducted before. This is a preliminary study in to the use of FAIMS in the detection of biogenic amines for the estimation of time of death and the future scope is vast, particularly in quantification of biogenic amines detected and understanding the effects of various soil types and conditions. The goal of this study was to prove that biogenic amines can be detected from soil samples using a unique equipment, the Owlstone Lonestar with the FAIMS chip. The purpose is to shed light on this field of death studies to estimate time since death and illustrate the importance of soil samples in such investigations. This can support Human Remain Detection (HRD) canines and facilitate on site detection of soil samples.

Chemical profiling of decomposition odours is an emerging area in the field of forensic science. These biogenic amines such as cadaverine and putrescine that are released into the soil during decomposition can provide valuable information and understanding on the time of death, decomposition changes after burial and the Post Mortem Interval (PMI) effects. In this study, the soil samples were collected from pig carcass which had been buried for over two months and sufficiently decomposed. Over the years, various equipment has been used to detect volatile organic compounds such as Gas chromatography–mass spectrometry (GC MS), two- dimensional gas chromatography using a time-of-flight mass spectrometric detector (GC x GC TOF MS), Ultra-performance liquid chromatography (UPLC) and Gas Chromatography – Flame Ionisation Detector (GC FID). A large number of VOC's have been detected using these techniques but cadaverine and putrescine were not detected at all. The problems faced with these compounds and the lack of detection for several years caused it to be reported as non-existent. Detection of such sensitive and specific bioamines requires complex instrumentation. They require instruments that can detect at ppb amounts and effectively show differentiation from other VOC's. Field Asymmetrical Ion Mobility Spectrometry is a variation of ion mobility spectrometry and is sensitive up to 10 parts per billion. On detection of biogenic amines, there was a change in peaks produced that aids in differentiation from other compounds. It works on the basis that the charged particle can be differentiated on its time of flight in the given electric field. The Owlstone FAIMS has increased selectivity by which it can further sperate these particles using alternative Direct Current (DC) voltages. To ensure highest accuracy, the equipment conducts simultaneous detection in positive and negative ion mode. This paper reports on the use of FAIMS to detect cadaverine and putrescine from soil samples of buried remains.

The aim of this experiment is to detect biogenic amines in soil samples collected from gravesites, using Field Asymmetrical Ion Mobility Spectrometry (FAIMS). FAIMS utilises the proton affinity of the ions from unknown compounds to create peaks in positive and negative DF matrices in the form of graphs. The DF matrices derived from the analysis

were then studied using the EasySpec software. This aims to prove that the equipment can firstly identify these biogenic amines from internal standards, then compare them to those found in soil samples and differentiate them from blank samples.

In order to achieve this aim, the following have been formulated:

2.2 OBJECTIVES

- To thoroughly study and review the literature on stages of decomposition and factors affecting integrity of soil samples.
- To be trained in instrumentation and working of Owlstone Lonestar FAIMS.
- To practice the use of the equipment and develop a method to analyse soil samples using pure compounds.
- To test the sensitivity of the equipment by serial dilution of internal standards.
- To analyse bioamines from grave soil samples using FAIMS.
- To clear the contamination in equipment and ensure equipment is uncontaminated during sample runs.
- To compare and differentiate between DF matrices of sample and blank.
- To interpret data accurately using EasySpec software.

Biogenic amines are metabolites that are produced by decarboxylation of amino acids causing significant physiological changes in prokaryotic and eukaryotic cells. (Pugin *et al*., 2017) Cadavers are therefore rich sources for organic and essential elements and become hotspots for biotic and abiotic transformation of compounds. (Stokes *et al*., 2013) Putrescine and cadaverine are produced by the decarboxylation of amino acids lysine and arginine and both consist of short hydrocarbon chains with primary amine group at each end. (Izquierdo *et al*., 2018) They are highly toxic, have a foul odour and, along with hydrogen sulphide and methane, can be found in the soil of decomposing human remains. The distinctive odour served the purpose of triggering avoidance or

attractive response as the survival of many species depend on these necromones. Cadaverine, putrescine and methanethiol are some of the most common molecules found in rotting flesh. (Cooke *et al*, 2003)

DECOMPOSITION STAGES

Decomposition occurs in various stages. The fresh stage is between time of death till the first signs of bloating. The bloated stage begins when the putrefaction mechanism begins and that generates the breakdown of gases. Bloating first starts at the abdomen and eventually the whole body swells up. The active decay stage causes darkening and breaking up of skin as the body deflates. The proteins, fats and muscles break down and, in this stage, putrescine and cadaverine are released into the soil. Insect activity is at peak as putrefaction continues due to degradation and release of chemical components. In the advanced decay phase, the corpse dries and the only remains are bones skin, cartilage, hair and some flesh fragments. In the final skeletisation phase, bones and hair are the only remainders. (Swann *et al*., 2010) (Finley *et al*., 2015) The various stages of decomposition show different body processes such as algor mortis, rigor mortis and livor mortis. (Mathur and Agrawal, 2011) (Lee Goff, 2009)

In the decaying process, several Volatile Organic Compounds (VOC's) are released into the environment. More recently, the VOC's are being profiled by forensic departments as they are valuable in decomposition odour profiling. (Statheropoulos *et al*., 2011) Studies have shown that the state of the body, whether autopsied or not do not have much impact on the rate on decomposition. However careful examination is required when studying the insect activity and process of decomposition. (Bates and Wescott, 2016)

FACTORS AFFECTION DECOMPOSITION

Experiments have also been conducted to study the effect of freezing on decomposition rate as perpetrators often employ it to avoid detection of crime and to mislead

investigators. This study has proven that much contrast in PMI colour changes and maggot activity can be found between frozen and unfrozen body. The victim may appear to be in the early decomposition stages due to exposure to cold temperatures even though the PMI may be lengthy. (Roberts and Dabbs, 2015) In post mortem cases, contaminations may occur due to delayed refrigeration of the corpse, inadequate sample procedures and during transportation. (Palmiere *et al*., 2016) The temperature, moisture content, type of soil and weather can have effects on the VOC production and deposition. (Iqbal *et al*, 2017) Absence of insects heavily affect decomposition rates as well. (Simmons *et al*., 2010) Rate of decomposition can be lowered due to lack of any soil microbial activity. The growth of organisms in the soil quickly decompose the carcass by utilising the resources derived. (Lauber *et al*., 2014) Insect succession is better understood if a relationship is present between the growth phase of insect and the VOC found at that particular time. (Kasper *et al*., 2012)

Embalmment of the corpse can cause various alterations to the biological matrix and dilution of sample due to metabolism of drug or poison in the body. Storage instability must also be carefully evaluated and can occur do to physical, chemical or metabolic processes. (Skopp, 2004) Seasonal variations can also have an effect on the collection and detection of decomposing VOC's. Even though the warmer season provides for ease in collection, the same VOC's can be detected during cold winters. (Forbes *et al*., 2014) Widely used method for PMI estimation is appearance of the cadaver and the interpretation may be highly subjective and sometimes may show two difference phases at the same time. Insect colonisation in dead bodies give a better insight of PMI as it shows time dependent succession pattern. (Paczkowski *et al*., 2015)

The decomposition of organic matter is a series of complex chemical reactions carried out by microbial enzymes. These reactions cause the production of VOC's as

by-products, that are then released into the environment. Their disposal is ensured by physiochemical properties like low boiling point, low molecular weight, high vapour pressure etc. The major VOC's classes released by fungi are alcohols, alkenes, alkanes and ketones. While a high atmospheric pressure pushes the chemicals down the soil column, a lower atmospheric pressure draws these chemicals up to the surface making it more detectable. Soil moisture content seems to have the opposite reaction. (Vass, 2012) Dead animal remains and cadavers have huge impact on the soil and its micronutrients. Carbon, nitrogen and phosphorous are found in abundance beneath cadavers over a 100-day period. Hence knowledge in cadaver decomposition will help in a better understanding of nutrient cycle from carcasses and decomposition process in soils. (Benninger *et al*., 2008) Studies show that the air above the decomposing carcass and the soil below it provide information on the number and type of VOC's found in the decomposing remains. (Forbes and Perrault, 2014) The study by Stadler *et al*. (2015), is the first study to demonstrate reproducibility of decomposition VOC's and provides better understanding of soft tissue decomposition.

Soil can vary from one gravesite to another based on its environmental conditions such as vegetation, climate, geology, etc. Hence there cannot be just one ideal model for decomposition date. Soils used in study by Vass *et al*. were fine, mixed, thermic Typic Paleudalf sampled taken from a corpse of over 2 years. The study suggested post mortem interval could be estimated from soil solution of volatile fatty acids. (Aitkenhead-Peterson *et al*., 2012) In terms of soil, he only identified 4 variables that had a measurable impact and they were temperature, moisture, pH and partial pressure of oxygen. (Vass *et al*, 2011) Availability of moisture in the soil can be determined by soil texture and structure (its ability to hold water). Large amounts of moisture can control microbial activity (as soil microorganisms are primary decomposers), nutrient and water uptake and extracellular enzyme activity (Carter *et al*., 2010)

According to the experiments conducted by Cockle and Bell, to confirm reliability of the Universal model from PMI estimations, it was concluded that it was not practical to apply one ideal rate of decomposition to cadavers all around the globe and rejected Vass' Universal model/ hypothesis. (Cockle and Bell, 2015) Studies conducted in Central Texas also argue that no model is Universal and decomposition rate and conditions must be studied in each location. A study by Parks provides a new and original data set that is environment specific and compared against previous quantitative studies. (Parks, 2011) (Suckling *et al*., 2016) On loamy soil, the bacteria, fungi and other microorganisms are unique and their activity can be affected by various factors such as temperature and resource quality (Carter and Tibbett, 2006)

A practical application of the area of study is in the case of mass disasters as it is a method of detection of decreased bodies. Decomposition soil is highly essential in location of clandestine graves of missing persons or in a genocide event as soil acts as a sink for VOC's and they are retained for a long time. As Lonestar is a portable device (with necessary transport documents as it is a radioactive equipment), it can aid in on-site immediate detection of soil sample and avoid the hassle of transporting to the laboratory and additional contamination.

PREVIOUS STUDIES AND THE TECHNIQUES USED

Studies carried out by Vass report the odour components of human decomposition found in soil associated with dead human remains. The paper focuses on odour mortis- the smell of death and how it can be a valuable aid in determining the time and stage of death. The instrument of choice was GC MS and GC FID. VOC's are complex compounds that require complex instrumentation to detect them. GC MS are unable to quantitatively detect long and short chain hydrocarbons due to the polarity difference in molecules and show peak capacity limitations. (Rosier *et al*, 2014)

An advanced technique GC x GC was then used. In this instrument, the sample goes through two different separation systems coupled and every compound is characterised by a detention time in each dimension. Not only does it mean the needs of complex compounds but also addresses the limitations such as peak capacity limitations, dynamic range and restricted specificity of one-dimensional GC MS. (Dallüge *et al*., 2003) One particular study by Cooke *et al*. used HPLC and fluorescence detector to quantify these amines in human saliva (Cooke *et al*., 2003) while other studies were known to use UPLC. (Pugin *et al*., 2017)

Over the years, a vast number of techniques and instrumentation were used for decomposition odour analysis and these have helped better understanding and advancement within the subject. Headspace seemed to be the most used equipment for study of VOC's. Solid- phase Microextraction (SPME) was utilised for collection and characterisation of the different VOC's seen in various types of human tissues like blood clots, muscle, skin, body fat, etc. In another study by Cablk *et al*, SPME GC MS was used to differentiate the VOC's found in human from those in animals. (Iqbal *et al*, 2017)

The GC TOFMS used in the study by Dekeirsschieter *et al*., (2012) proved that it could detect 832 VOC's while conventional GC MS could detect only 85 VOC's. They also stated "cadaverine and putrescine were not detected as cadaveric VOCs in many cases as biogenic amines have a low volatility and therefore are not frequently identified with gas chromatography. Liquid chromatography (LC) is more suitable to detect biogenic amines "

In a review by Dent *et al* (2004), they state "There is an important opposing effect to also consider. With time, and for variable amounts of time, decomposition gas products like carbon dioxide, methane, hydrogen sulfide, ammonia, cadaverine and putrescine,

diffuse upwards from the remains; preferentially in the looser soils of the grave volume. These gases will compete for space with oxygen diffusing downward with unknown effects; suffice to expect the blocking of downward diffusion and displacement of entrapped gases (e.g. air) upwards. These aspects have scarcely been studied."

Further experiments conducted by Statheropoulos to counteract these arguments had air samples taken from air tight sealed body bags in order to prevent the escape of decomposing gases. In the study conducted in 2005 where air samples were taken from two bodies found in sea water in Greece with PMI of three weeks, over 60 chemicals were collected and yet putrescine and cadaverine were not detected. The second study carried out in 2007 involved a decedent who had been dead for four days, where decomposition had not yet been progressed, and was placed in a sealed body bag. 30 VOC's of major family groups such as alcohols, ketones were detected. (Statheropoulos *et al*., 2007) (Statheropoulos *et al*., 2011)

USE OF FAIMS FOR DETECTION OF BIOGENIC AMINES

The problems in detection of cadaverine and putrescine can be attributed to wrong techniques and equipment used rather than the disappearance of the compounds. An emerging technique called FAIMS has been known to detect small quantities of cadaverine and putrescine but its yet to be studied and expanded upon. Field Asymmetrical Ion Mobility Spectrometry is a variation of Ion Mobility Spectrometry. It works on the principle that gaseous molecule moves at different speeds through a buffer gas under the influence of an oscillating electric field. After ionization, the VOC ions move through channels in which a Radio Frequency (RF) field is applied. They move in a particular direction at a velocity based on the individual mobility of the ions in that electric field. As the field polarity is changed, they change speed and direction based on the new conditions. A net drift is caused towards one of the electrodes as the mobility of ions in two different waveforms are rarely equal. This net drift can be

corrected by applying an alternate DC voltage, known as the compensation field, that focuses the ions towards the detector.

In complex mixtures where more than one compound is present, there is a competition for available charge resulting in preferential ionization of compounds in the sample. Hence compounds which have higher proton or electron affinity will bind more readily than compounds with lower proton or electron affinity. Therefore, analytes whose proton affinities are lower, the concentration of water within the ionization region will have a direct effect. (Allsworth, n.d.)

This device is most suitable for detection of cadaverine and putrescine as it is highly sensitive and can detect ions up to 10 parts per billion. It can ignore chemical noise and detect VOC biomarkers with reduced possibility of false positives. This instrument is highly versatile and can also be used for early detection of diseases using breath samples. It's easy to use, portable and better value than other techniques such as GC MS.

Some of the limitations of this experiment with FAIMS are: - that the bioamines may not be detected. There may be low availability of samples and collection from grave sites may be difficult to access/ less accessible. The samples may not be sensitive or volatile enough and limited amount of time can be an issue as well. These issues can be addressed by careful planning throughout the experiment, conducting many test samples to ensure efficient working and detection of FAIMS and proper collection of samples.