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Investigation of sterols as potential biomarkers for the detection of pig (S. *s. domesticus*) decomposition fluid in soils☆

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ABSTRACT

This study was carried out to evaluate the potential of using cholesterol and coprostanol, as indicators for the detection of decomposition fluid of buried pigs (S. *s. domesticus*) in soils. In May 2007, four pig carcasses (~35 kg) were buried in shallow graves (~40 cm depth) at the University of Ontario Institute of Technology in Canada. Two pigs were exhumed after three months (Pig 1, Pig 2) and six months (Pig 3, Pig 4) post burial. Soil samples were collected beneath the pig carcasses (~40 cm depth) and from grave walls (~15–20 cm depth) as well as from a parallel control site. Coprostanol and cholesterol were extracted from soils, purified with solid phase extraction (SPE) and analysed with gas chromatography/mass spectrometry (GC/MS). A significant increase in cholesterol concentrations ($p < 0.05$) and amounts of coprostanol were detected in soil located beneath the pig carcasses after three months of burial. It is assumed that during the putrefaction and liquefaction stages of decomposition pig fluid which contains cholesterol and coprostanol is released into the underlying soil. Therefore, cholesterol and coprostanol could be used as potential biomarkers to detect the presence of decomposition fluid three months after burial under comparable soil and environmental conditions. Further research is suggested for additional soil sampling before and after three months to investigate the abundance of these and other sterols.

1. Introduction

Sterols are natural compounds with a characteristic tetracyclic ring structure and belong to the group of lipids. They are divided due to their origin into phytosterols (e.g. β-sitosterol), mycosterols (e.g. ergosterol) and zoosterols e.g. cholesterol and coprostanol [1]. Cholesterol (cholest-5-en-3β-ol) is an ubiquitous component of all animal cells (Table 1) but is present in trace levels only in plants which have other common sterols (e.g. β-sitosterol, stigmasterol) [2–5]. It is synthesised by extraheptic tissues in the liver and performs essential functions as a component of plasma membranes and in lipid metabolism [2,4,5]. Coprostanol (5β-cholestan-3β-ol) is formed in the intestinal tract of higher mammals as a microbial hydrogenation product of cholesterol [6]. In recent studies, zoosterols and phytosterols have been investigated as pollution biomarkers introduced to marine and terrestrial environments or as faecal markers for archeological applications [7]. Notably, coprostanol is considered a useful marker associated with the faeces of carnivores and omnivores, including pigs and humans [6]. In a study by Bethell et al. [8], coprostanol was used as a marker to detect the presence of faecal material in archaeological soils, for example from ancient latrine sites [8] demonstrating its persistence against microbial degradation. In forensic investigations degradation products of human remains are of interest because they could be used to detect the presence of decomposing human remains in soils (e.g. clandestine graves). After death the decomposition of human remains is followed by the release of decomposition fluid into the environment where the body is exposed, for example soil. The degradation of cadavers above and below the soil surface has been investigated in several studies focusing on a variety of potential component markers. Fiedler et al. [9] analysed depth profiles of organic carbon and phosphorus to locate the original site of a former mass grave. Carter et al. [10] detected amino acids in soil samples with the application of ninhydrin-reactive nitrogen in order to relocate body parts of a human cadaver which had been scavenged from its original

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Table 1
Chemical name, common name and formula of the investigated sterols cholesterol, coprostanol and β-sitosterol.

Chemical name	Common name	Formula
Cholest-5-en-3β-ol	Cholesterol	C27H46O
5β-cholestan-3β-ol	Coprostanol	C27H48O
Stigmast-5β-en-3β-ol	β-sitosterol	C29H48O

deposition site. These biomarkers could be used to locate clandestine graves, to reconstruct crime scenes or to potentially estimate time since burial [11]. However, to date very few studies have focused on the longer chain carbon compounds. The origin of cholesterol and coprostanol and their stability against degradation over time due to their chemical structure makes them a valuable compound for use in detection of decomposition fluid in soils. To the best of our knowledge, there have been no studies carried out on the sterols, cholesterol and coprostanol and their application as potential markers for the identification of decomposing buried remains. The aim of this study was to evaluate the suitability of cholesterol and coprostanol as biomarkers indicative of decomposition fluid in grave soil. We ran a six month decomposition experiment using pig carcasses as human surrogates. In addition to cholesterol and coprostanol, β-sitosterol was investigated because it is a ubiquitous plant sterol in soils and it was therefore assumed to remain stable between treated soils and control soils.

2. Materials and methods

2.1. Experimental site, sample collection and preparation

Soil samples were collected from a pig burial experiment carried out at the University of Ontario Institute of Technology, Canada. Due to ethical reasons, domestic pig (S. *s. domesticus*) carcasses were used as a surrogate to study human decomposition processes in soils [12]. A summary of the site properties is shown in Table 2.

In May 2007 four pigs (~35 kg) from a local farm were killed by an overdose of anesthetic, wrapped in plastic, transported to the study site and buried in shallow graves (40 cm depth) within one hour after death. After back filling, the graves were secured with mesh wire to prevent scavenging. Two pig carcasses were exhumed after three (August 2007) and six months (October 2007) post burial period, respectively (3 months: Pig 1, Pig 2; 6 months: Pig 3, Pig 4). Soil samples were collected at seven different sampling positions (Fig. 1). Three samples were collected beneath the head, torso and rear of the pig carcass. Four grave wall samples

Table 2
Location, climate and vegetation type of the pig decomposition experiment study area in Southern Ontario, Canada.

Location	Southern Ontario, North of Oshawa[a]	
Coordinates	43°56′ N, 78°54′ W[a]	
Study area	Secured, 75 × 20 m[a]	
Topography	Slight gradient of 5°, depression areas[a]	
Use	Urban	
Climate	Temperate midcontinental with hot summers (~20° C average), cold winters (−4 °C average)[b]	
Vegetation	Dominant	Various grasses and herbaceous plants[a]
	Major tree species	*Thuja occidentalis* L., *Acer* spp., *Populus tremuloides* MICHX.[a]

[a] Ref. [29].
[b] Ref. [14].

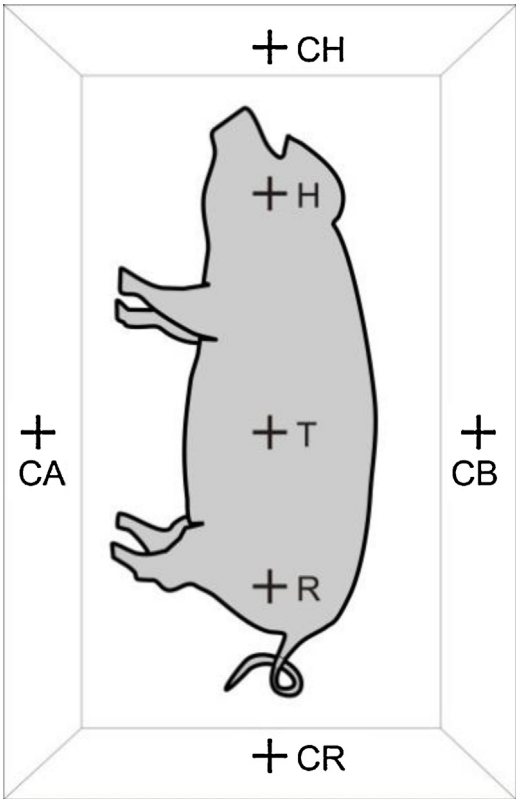

Fig. 1. Sampling positions of the pig graves after three and six months of burial of pig carcasses and control graves (same positions but without carcass). Grave wall sampling positions at ~15–20 cm depth: CH = closest to the head, CB = closest to the back, CR = closest to the rear, CA = closest to the abdomen. Sampling positions beneath the pig carcass: H = beneath the head, T = beneath the torso, R = beneath the rear.

were collected from the sides of the grave closest to the head, abdomen, rear, and back of the pig carcass. Additionally, one control grave without a pig carcass was sampled at comparable sampling positions (3 months: Control 1; 6 months: Control 2). The soil samples were transferred into capped glass vials and transported via courier to Aberdeen (UK) in dry ice packing. Samples were stored at −20 °C. Prior to analysis, samples were freeze-dried (Edwards Super Modulyo, Crawley, UK) milled (Retsch MM 301, Haan, Germany) and stored at room temperature.

2.2. Soil characterisation

Soil cores to ~50 cm depth were taken close to the former burial sites in October 2011 in order to determine the pH and the texture of the soil. Samples were separated into three soil depths (0–5 cm, 15–20 cm, 40–50 cm depth) and analysis was carried out with the finer fraction of sieved soil (<2 mm) and milled soil samples at the James Hutton Institute in Aberdeen, Scotland.

2.3. Sterol extraction

Sterol extraction follows a modification of the protocol published by Dove and Mayes, 2006 [13]. Duplicate of dried milled soil samples were weighed in glass vials and 100 μL 5-β-cholan-24-ol (Chiron, Norway; 0.08 mg g^{-1} in ethanol: *n*-heptane 50:50 v/v (Fisher, UK and VWR, UK; respectively)) as internal standard (I.S.) was added by weight. To hydrolyse any esters present, saponification was carried out by adding 1.5 mL of 1 M ethanolic KOH (both VWR, UK), and heating at 90 °C for 16 h. After cooling the samples to 50–60 °C, 1.5 mL *n*-heptane and 0.5 mL