Particles of everyday life

Past diet and living conditions as evidenced by micro-debris entrapped in human dental calculus: a case study from Medieval Leicester and surrounding

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ABSTRACT

Dental calculus, or tartar, is commonly found on archaeological skeletons since its inorganic nature remains stable even after many thousands of years. Dental calculus has long been seen as a valuable source of information on the nutrition and dental hygiene of past populations. As calculus forms in the mouth food consumption has been the major focus of research conducted so far, looking almost exclusively at dietary remains entrapped in it. The current PhD approaches the human mouth as a 'depositional environment', in which solid microscopic debris of different origins can become entrapped in the dental calculus matrix during its formation. The overall potential of dental calculus as a reservoir of dietary and non-dietary debris, which can offer insights to the natural and anthropogenic environment, is explored. Populations from Medieval Leicester (St Michael's and St Peter's, Leicester, c. 1250-1450 AD) and its surroundings (Empingham, Rutland and Rothley, Leicestershire, c. 500-900 AD) were used as the study material. A wide range of microscopic remains of staple food crops were retrieved during analysis together with luxury foods, among others, as well as non-dietary debris, potentially from the indoor environment and craft activities, such as wool and plant fibers. Diachronic changes in their occurrence were detected between the Early and Later Medieval periods, often statistically significant, implying important shifts in life quality during Medieval times. The originality of this research and its contribution to the field lies in the fact that it demonstrates the potential of dental calculus microdebris at a population level. The results provide strong evidence regarding the archaeological value of human dental calculus in offering new insights not only into diet, but also into past environment and living conditions.

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AUTHOR'S DECLARATION

I declare this to be my own work.

Over the time that this PhD thesis has been written, several sections or chapters have contributed to a number of published works that have necessarily involved co-authors. The contribution of each chapter to published material and the contribution of other individuals to this work are indicated below:

Chapter 2 – This chapter represents an extended version of part of a paper and invited contribution to the Yearbook of The American Journal of Physical Anthropology:

> **Radini,** A., Nikita, E, Buckley, S., Copland, L. and Hardy, K., (2017). The rich and varied pathways for inclusion of microscopic remains into ancient dental calculus. *American Journal of Physical Anthropology* 162, S3, 71-83. DOI 10.1002/ajpa.23147

Karen Hardy and Efthymia Nikita co-wrote the session on the formation process. Efthymia Nikita helped with information concerning osteoarchaeology, while Steven Buckley, Les Copland and Karen Hardy provided the majority of the section on chemical analytical methods. All authors commented and helped with the editing of the final paper.

Chapter 3 – This chapter represents an extended version of two papers, the one mentioned above and one recently published on Antiquity:

> **Radini,** A., Buckley, S., Rosas, A., Estalrrich, A., de la Rasilla, M. and Hardy, K. (2016)a. Neanderthals, trees and dental calculus: new evidence from El Sidron. *Antiquity*, *90*(350), pp.290-301

Hardy provided access to the Neanderthal material mentioned in the text and contributed to the section on oral hygiene, commented and help with the editing of the final paper, Buckley conducted chemical analysis, and the other authors provided contextual information on the material.

Chapter 5 – This methodological chapter was written entirely by the author of this PhD and subsequently published in the supplementary material of a paper co-authored with Dr. Tina Warinner (Warinner et al. 2014).

Warinner, C., Rodrigues, J.F.M., Vyas, R., Trachsel, C., Shved, N., Grossmann, J., **Radini, A**., Hancock, Y., Tito, R.Y., Fiddyment, S. and Speller, C., et al. (2014). Pathogens and host immunity in the ancient human oral cavity. *Nature genetics*, *46*(4), pp.336-344

Chapter 6 and 7- Some of the criteria of identification and the descriptions adopted for some of the most recurring finds in this study, and in dental calculus samples studied by the author in other collaborative projects, were also used as criteria of identification/description in collaborative work by the author. This approach was chosen in order to be peered reviewed before analysis. In addition to the paper cited above:

Buckley, S., Usai, D., Jakob, T., **Radini, A**. and Hardy, K. (2014). Dental calculus reveals unique insights into food items, cooking and plant processing in prehistoric central Sudan. *PloS one*, *9*(7), p.e100808.

Cristiani, E., **Radini, A.**, Edinborough, M., Boric, D. (2016). Dental calculus reveals Mesolithic foragers in the Balkans consumed domesticated plant foods *PNAS*

Chapter 9 – Parts of the discussion were published in a pilot study and proof of concept for this research. Dr. Efthymia Nikita provided comments on the osteoarchaeological parameters used in reconstructing occupational health in past societies. Dr. Lisa Marie Shillito checked and commented on the phytolith section.

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CHAPTER 1:
AN INTRODUCTION TO THIS STUDY

1.1 Introduction

This PhD is the first large scale study conducted on human dental calculus from historic populations. It aims to survey the variety of debris, beyond starch granules and phytoliths, entombed in its matrix and to develop a new methodology and approach to human dental calculus studies. It focuses on the identification and quantification, by light microscopy, of all types of micro-debris entrapped in the dental calculus samples from individuals who were buried and most likely lived in an urban environment within the North East Quarter of Medieval Leicester (1250-1450 AD). The results from these Late Medieval individuals are compared to those of Anglo-Saxon date from the Leicester surroundings (500-900 AD), where the natural and built environments are considered to be rural. The data are evaluated thanks to existing information generated by recent excavations in the area, conducted by the University of Leicester Archaeological Services (ULAS). This integrated approach makes this research the first study of its kind. This PhD is therefore a methodological work aimed to widen the current approach to the study of all lines of evidence entombed in dental calculus.

1.1.1 Background to the research: tartar and the "paleodiet"

Access to food (quantity, quality and variety of foodstuffs), hygiene, and exposure to pollutants are and have been influential in the quality of life and wellbeing in both modern and past societies. It has become increasingly clear that human actions are directly behind the creation of some of the most adverse environments people are exposed to (Whitehead 1992, cited in Panter-Brick and Fuentes 2009, 2). The pivotal role of diet in human health has been highlighted in the recent past thanks to the development of the discipline of Evolutionary Medicine. Studies have shown that a complex interaction exists between health status and the physical, biological and social environment experienced by people during life (e.g. Goodman 2009; Norgan 2000). The understanding of such complexity has been, however, limited by how deep in the past

such interactions can be directly studied (Roberts 2009, 14), preventing the better understanding of health inequality observed in many modern societies.

A pathology on teeth known as *dental calculus* or *tartar* (mineralised dental plaque: see Chapter 2), is becoming an important line of evidence in Bioarchaeology for its potential to retain a wealth of biological information on diet and health, sometimes very deep into the human past; it has been found on the teeth of a Miocene *Sivapithecus* dating to between 12 - 8 million years ago (Hershkovitz et al., 1997) as well as on late Pliocene hominins (Blumenschine et al., 2003). This provides potentially new insights into the links between pathology, diet and health from a single individual to the population level.

Dental calculus is a mineralized deposit of calcium phosphate, which adheres to the tooth enamel (fig. 1). It is formed by the activity of bacteria in the mouth, and if not removed, it can become mineralised in as little as two weeks (Lieverse 1999, 220). While forming, calculus can entrap particles of different natures, including starch granules and phytoliths (plant opal), but also human cells and mineralized bacterial structures (e.g., Blondiaux & Charlier 2008; Charlier et al. 2010; Dobney & Brothwell 1987; Fox et al. 1996; Hardy et al. 2012). Moreover, Preus et al. (2011), De La Fuente et al. (2012), Adler et al. (2013) and Warinner et al. (2014a) have recently proven that human dental calculus is a novel source of bacterial genetic material and holds potential as biomolecular reservoir.

As the formation process of this type of deposit ceases at death because minerals in the saliva are needed for its formation (MacPhee & Cowley 1975), any line of evidence (from particles to genetic material) in the deposit and the deposit itself are simultaneous, providing in situ material for study with very high archaeological integrity. It must be stressed that it is currently not possible to assess what portion of the life time of an individual is represented in the calculus matrix, such limitation will be discussed in detail in Chapter 2 (paragraphs 2.2.1 and 2.2.2).

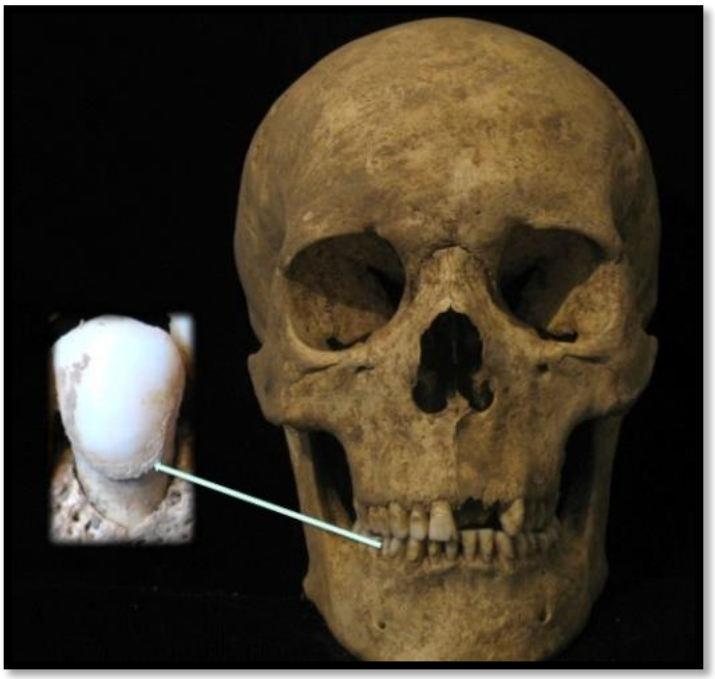

Figure 1. Exemaple of human dental calculus (male individual, Medieval Parish of St. Michael's, Leicester 1250-1400 AD)

Due to the fact that the calculus forms in the mouth, food consumption has been seen as the major source of debris recovered in the calculus matrix and most of the research conducted so far has focused almost exclusively on dietary remains entrapped in it (e.g., Hardy et al. 2009; Henry & Piperno 2008). Assessing the use of plants in diet is very difficult in prehistoric individuals as, unlike animal bones, plants are less often retrieved in the archaeological record, especially in the deep human past (Leaonard et al. 2015). Therefore, the focus of dental calculus research in recent years has been inevitably on prehistoric populations and hominins, and plant micro-debris entrapped in calculus samples from such individuals/populations (e.g., Buckley et al. 2014; Hardy et al. 2012; Henry 2012; Juan-Tresserras et al. 1997). In addition, there has been a general assumption that the presence of plant micro-remains in dental calculus is somewhat direct proof of the wide importance of plants in ancient diet (Henry et al. 2011, 2012).

As a result of the general interest of the non-academic world in the subject of 'paleodiet', papers published in the field of dental calculus research in prehistoric populations often receive wide media attention. Consequently, the general assumption that calculus inclusions represent dietary debris is transmitted directly to the wider public (for an

example see figure 2). However, where such an assumption is examined against the complexity of human behaviour, for instance the practice of oral breathing, the use of the mouth as a third hand, and technologies such as wood working, fibres working for textile and clothing that can generate a high amount of dust, some questions naturally arise:

- Is all the debris entrapped in ancient human dental calculus really the result of deliberate food consumption?
- Is all plant-derived micro-debris in calculus generated by the presence of plants in the diet?
- Is such debris direct evidence of the role that such food/plants played in the diet of an ancient individual?

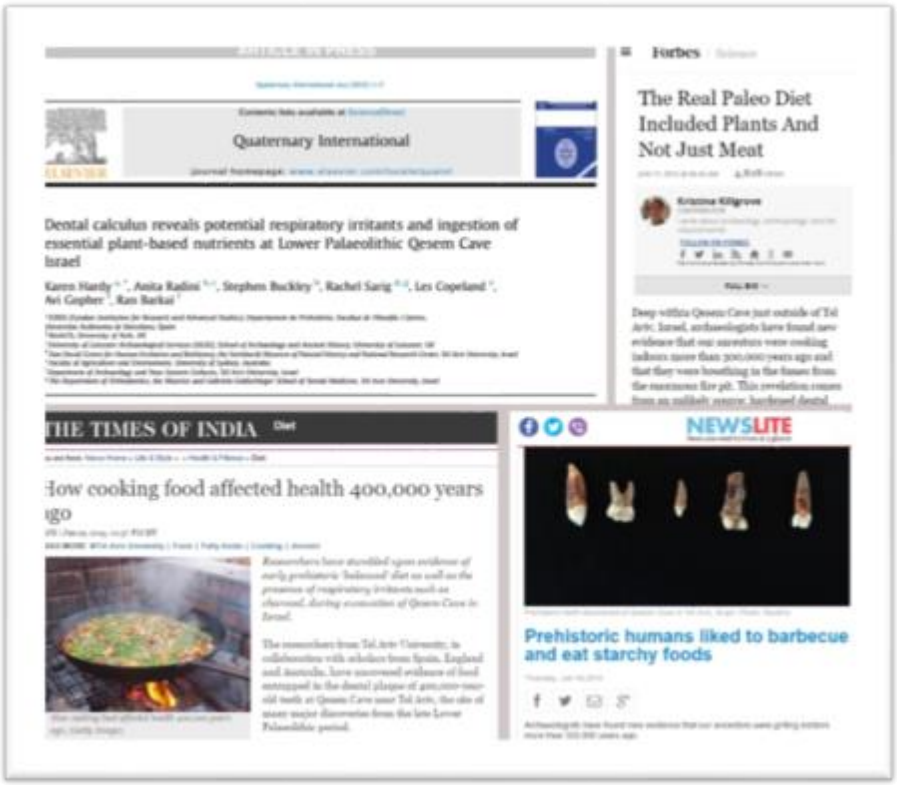

Figure 2. Media coverage of the results of the work conducted on dental calculus samples from Qesem Cave (Hardy et al. 2015), clockwise from the top: original paper, features on Forbes, Newslite blog and The Times of India

Although the majority of published work only reports evidence of plants, a few studies exist that can provide some preliminary indication on the complexity of the information

retained in the calculus matrix. Such studies will be presented briefly in the following section.

1.1.2 The archaeological complexity of human dental calculus

The tendency of focusing on dietary evidence was pointed out by Blondiaux & Charlier (2008) only a year before the current study begun. Since then, a few studies have shown that a variety of both micro-debris and chemical compounds are preserved in the calculus matrix. Blatt et al. (2011) recovered evidence of cotton fibres (*Gossypium* spp.) in situ in the dental calculus from skeletal remains from the site of Danbury, dated to 900–1100 AD, representing the first evidence of this fibre in Ohio. Their finds also proved the survival of plant debris that entered the mouth due to the plant being used for material culture. Charcoal debris and grit have been found in the dental calculus of a Brazilian Sambuque population, but in the absence of other comparative material, they were interpreted as possible contamination (Wesolowski 2010), rather than inhaled smoke. However, Blondiaux & Charlier (2008), in their study to assess the forensic potential of dental calculus, found plant fibres, potentially generated by the use of the mouth as a third hand, and several types of mineral debris either breathed in or ingested from grinding stones. Elemental analysis of some of these crystals trapped in the calculus matrix allowed them to detect environmental habits and possible work-related intoxication in one individual from the Etruscan population of Monterenzio Vecchia (Blondiaux & Charlier 2008). Moreover, the identification of specific chemical compounds has allowed Hardy et al. (2012) to detect exposure to smoke and bitumen in individuals of the Neanderthal population unearthed at El Sidrón, Spain. Therefore, it is safe to assume that a variety of micro-debris and chemical compounds can be recovered from the dental calculus matrix and it is reasonable to assert that not all the debris retrieved so far from a variety of case studies has been generated by deliberate food consumption (Figure 1-3 provides an example).

The survival of evidence generated by other human activities rather than eating, points to the complexity of the evidence preserved in the calculus matrix, as briefly mentioned above. It also highlights that several pathways are possible to the inclusion of micro-debris in human dental calculus apart from diet. In particular, the use of plants as material culture, and not just as food, together with the role of the mouth as a third hand

are very rarely explored in the interpretation of the record consisting of starch granules and phytoliths, the most common lines of evidence studied in dental calculus research. This is partially due to a lack of understanding of how representative calculus is of the diet and environment experienced by ancient people during life (see Chapter 2) and further limited by the fact that only a few studies have been conducted so far on a large number of individuals. No studies are yet available on recent historic populations, where existing archaeological and historical records would provide an important comparison with the evidence preserved in the calculus.

In the past year, however, a number of papers have begun to stress the need for better understanding what portion of the life of an individual is represented in the calculus samples (Leonard et al. 2015) and that caution needs to be exercised when considering plant remains in the calculus matrix as the sole result of plant consumption (Buck et al. 2015). Finally, problems of misidentification of micro-remains have also been pointed out by Morrow and Reinhard (2016), who stressed the need of rigour in using and presenting the criteria of identification used. Although not available in 2009, when this PhD started, the publication of such works makes the subject of this research (see Chapter 2) particularly timely.

1.1.3 Particles of 'life': the human mouth as depositional environment

The main concept behind this project is that any solid particle of anthropogenic and natural origin that enters the human mouth, whether deliberately (Chapter 2) or by accident (Chapter 3), has a chance to become entrapped in the dental calculus matrix, if such a deposit is forming. The greater the quantity of a certain type of debris or even pollutant (from food to micro-charcoal) in the environment, and the closer and the more prolonged the contact of an individual (e.g., figure 3) with the particles, the higher the possibility for such debris to enter the archaeological record by becoming entrapped in the calculus matrix of that individual.

Figure 3. Child worker in a brick factory, Nepal: note the cloud of fine dust the child is exposed to and how it settles on the skin and clothes. [1]

Dental calculus is therefore considered here not as a pathology but as a 'deposit' on teeth. This deposit will be tested for its potential and limitations to provide direct evidence about diet and, for the first time, about the natural and constructed environment experienced by ancient people during life.

[1] The imagine was taken by the following web site, and it is on public domain http://garyschapman.com/blog/2013/07/30/nepal-child-labor/

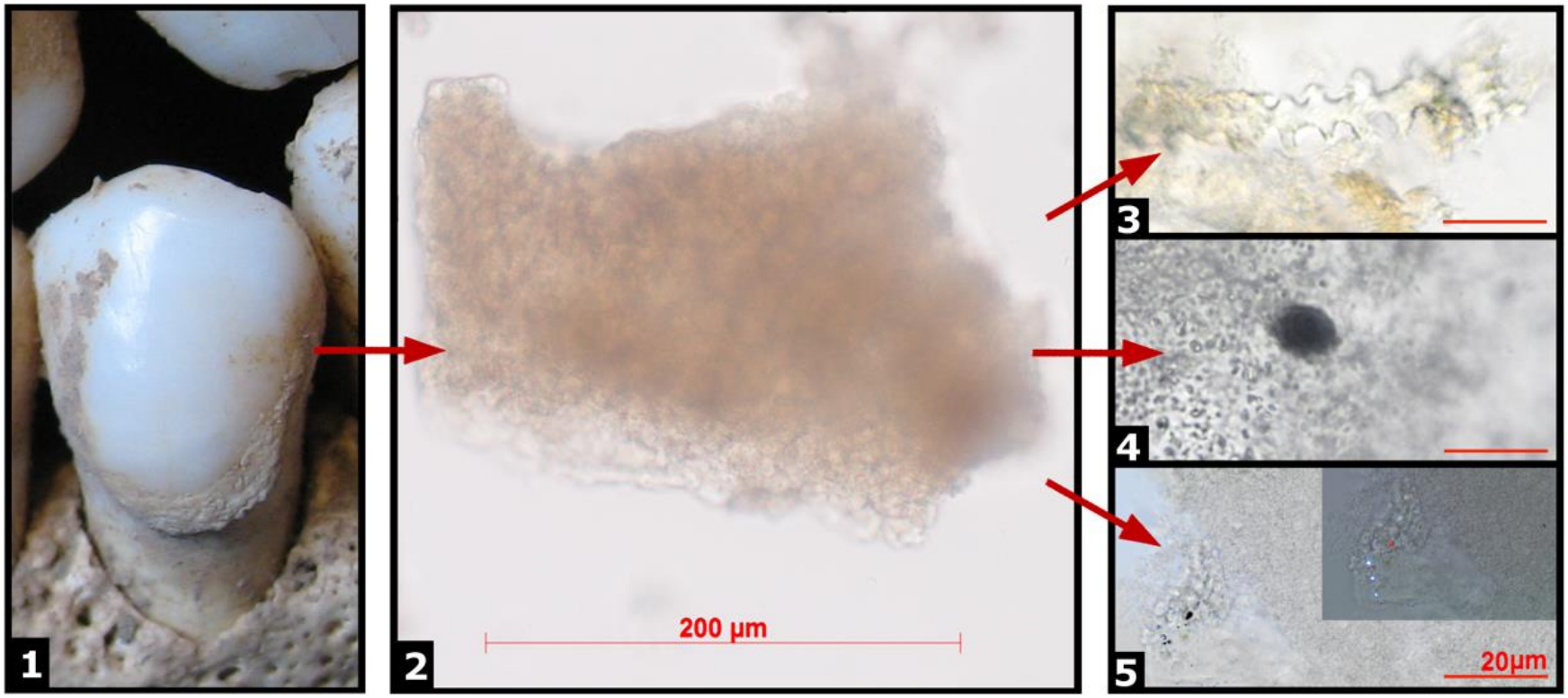

Figure 4. Dental calculus in situ (1); a fleck of calculus in the process of dissolving in HCl acid (2) - a variety of debris can be seen in situ; plant opal (3); mold spore (4); mineral grit (5) - note how soil minerals are more clearly visible under polarized light.

1.4 Aims and objectives of this the study

1.4.1 Aims

This study aims to survey and identify all typologies of micro-remains entombed in dental calculus matrix from Medieval (125-1450 AD) skeletal remains from the parishes of St Michael's (site A2.2003) and St Peter's (site A22/24.2003), Leicester and Anglo-Saxon (500-900 AD) skeletal remains from Empingham, Rutland and Rothley La Grange, Leicestershire. The main target is to gain a better understanding of the potential of all micro-debris entombed in calculus to reveal aspects of living condition beyond diet, in order to develop a new research framework in this promising field.

The methodological nature of this PhD must also be stressed at this stage. As pointed out at the beginning of this chapter, a huge emphasis of dental calculus research is on dietary remains, and conceptual approaches to other pathways of inclusions are lacking, together with any form of review of current research on human dental calculus. One of the main aims of this PhD is to overcome such issues by setting a new framework of methodologies and concepts to apply to dental calculus analysis. Finally, a considerable amount of time was spent in the survey and identification of all lines of evidence in calculus and therefore the broad contextualisation of the results is part of the future stage of this study. Finally, this PhD aims to contribute new data to the Archaeological research Agenda of East Midlands (see section 4.3.5 pg 74).

1.4.2 Objectives

The main objectives of this study are the following:

1. establish a method of extraction that maximise the recovery of all particles entombed in calculus;
2. record and quantify micro-debris in human dental calculus;
3. establish, with the use of published resources and with a purpose-built reference collection, to which extent the micro-debris in calculus can be securely identified;
4. assess if pathways of inclusion of the debris beyond diet are present;
5. assess if the findings are also present in the archaeological record of the area of origin of the skeletal populations;

6. assess if patterns are visible that can be linked to known main aspects of the living conditions known for the periods of time and locations examined;
7. establish future directions of the work, wherever needed.

1.5 Thesis outline

1.5.1 Thesis structure

Chapter 1 The first chapter of this PhD broadly frames the research project and outlines the aims, objectives and structure of the thesis.

Chapter 2 This chapter introduces dental calculus as a line of archaeological evidence, its formation process and its archaeological integrity, as well as known limitations. It then focuses on how archaeologists have recently approached the calculus as direct evidence of the importance of plants in the diet of past populations, and it outlines the problems of this assumption by using evidence of secondary eating. The chapter, therefore, has the purpose of identifying pitfalls in the current approaches in human dental calculus research. Finally, the chapter focuses on dietary remains and represent a review of current work on the subject.

Chapter 3 The third chapter focuses the non-dietary evidence that can be potentially found in ancient human dental calculus. It focuses on the potential of non-dietary pathways of inclusion of micro-debris in the calculus matrix, setting the 'theoretical' framework for the methods used in this PhD. This chapter introduces clear evidence that the human mouth can be considered a 'dust' trap, and therefore provides the justification to this study.

Chapter 4 This chapter presents the criteria adopted for the selection of the Medieval populations for this study. It provides the broad archaeological background to the selected populations, by contextualising such data in the broad trend known for the period. It finally shows how such a study can potentially contribute new lines of evidence towards the archaeology research agenda of the East Midlands, and enhance our understanding of human dental calculus in archaeology as a whole.

Chapter 5 This chapter provides the criteria of selection of individuals among the Medieval populations targeted. It details the new methodologies of extraction and

identification and recording developed during this study. It finally provides archive information on the material studied for this research.

Chapter 6 The sixth chapter presents the results regarding plant micro-remains (Kingdom Plantae). It frames the procedures and limits of the identification. It also considers potential pathways to inclusion of the debris and compares these with the archaeological record.

Chapter 7 This chapter follows a similar structure to Chapter 6, but the evidence reported is pertinent to fungal and animal debris, as well as inorganic remains.

Chapter 8 This chapter analyses the results obtained by exploring their distribution across the populations in order to identify trends in the data set. It also provides the first large statistical analysis conducted to date on dental calculus debris.

Chapter 9 This chapter discusses the results of the project and the analysis contextualising them with the other fields of the Discipline of Environmental Archaeology. It also discusses potential and limitations of addressing questions related to food access and exposure to pollutants. It also outlines the contribution of this research to our understanding of diet and living conditions in Medieval Leicester and its surroundings. Finally, it outlines current work in progress on the material.

CHAPTER 2
HUMAN DENTAL CALCULUS IN ARCHAEOLOGY: CURRENT RESEARCH FRAMEWORK

2.1 Introduction

The value of human dental calculus as archaeological evidence has been known from at least the 1970's; however, it is only since 2008 that bioarchaeologists have begun to study human dental calculus in a systematic way. This chapter will provide information on calculus composition and its formation process in order to explain why it is important for archaeologists. It will also outline the limitations to be taken into account during the interpretation of the results. Finally, the current research framework will be reviewed, highlighting why the current study makes an important contribution to the field of archaeology. This chapter contains parts (expanded) of the following published paper:

Radini, A., Nikita, E., Buckley, S., Copeland, L., Hardy, K., (2017). Beyond food: The multiple pathways for inclusion of materials into ancient dental calculus *American Journal of Physical Anthropology* 162, S3, 71-83. DOI 10.1002/ajpa.23147

2.2 Dental calculus: Formation process, structure and composition

2.2.1 Formation process

Dental calculus forms on teeth through a complex interaction between saliva and bacteria on the dental surface. Saliva is made up of water (99.5%), electrolytes, mucus, antibacterial compounds, enzymes and bacterial cells (Roberts 1979). Saliva lubricates the mouth and moistens food to create a bolus, which is then swallowed. It contains the enzyme α-amylase, which commences the breakdown of starch into simple sugars and also immunoglobulins, which control the microorganisms in the mouth and can restrict the build-up of plaque (de Almeida et al. 2008). Saliva is primarily formed by three pairs of glands, the parotid glands, the submandibular glands and the sublingual glands, and empties into the mouth through their respective ducts. The parotid gland ducts are located inside the cheeks near the upper molars (fig. 5), while the submandibular and sublingual gland ducts are located under the tongue (Edgar et al. 1996).

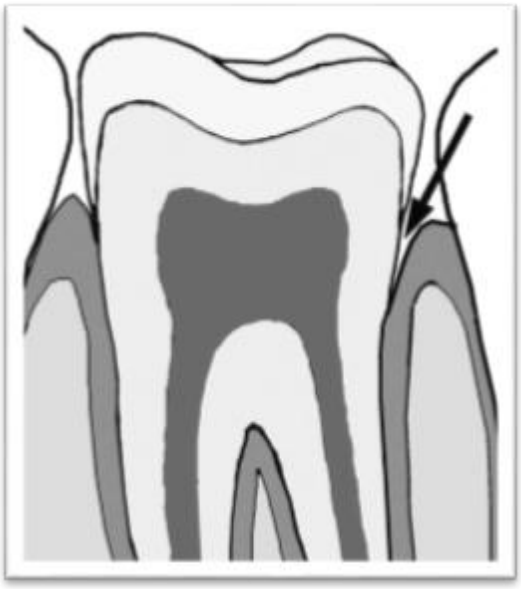

Figure 5**.** Crevice between tooth and gum where calculus forms (from Radini et al. 2017)

The mouth contains many species of bacteria (Hillson 2005), including spherical gram-positive cocci (e.g. streptococci and staphylococci), and rod-shaped gram-positive bacilli (e.g. lactobacilli and corynebacteria). When food is chewed, plaque is formed by the adsorption of proteins and bacteria, predominantly the facultative anaerobe *Streptococcus mutans* (Marcotte and Lavoie 1998). They metabolise salivary sugars form a film that adheres to the surface of the tooth. The bacteria are rapidly replaced by calcium phosphate salts and, if the plaque is not cleaned off, microorganisms nearest the tooth surface ferment sugars in the saliva and produce acids that demineralise the tooth, ultimately producing caries (Hillson 2005). The plaque hardens rapidly, beginning with the cell walls of the bacteria (Hillson 2005) and can be fully calcified within two weeks (Lieverse 1999) and preserved through time (see fig. 6). The rough surface of dental calculus serves to attract other bacteria, which adhere to those already attached, while the gaps between the cells are filled by other components of the saliva, to produce a layered structure (fig. 7) (Dobney and Brothwell 1987; Hardy et al. 2013). The build-up of dental calculus can be greatest near the salivary ducts; this results in deposits that are more prominent on the lingual surfaces of incisors and canines and the buccal surfaces of maxillary molars (Hillson 2005). However, calculus is not restricted to these locations and in extreme cases it can almost cover all teeth.

Figure 6. Dental calculus deposit on the teeth of individual sk218, St Michael's Parish, Medieval Leicester (1250-1400 AD).

When food is introduced into the mouth, the mechanical process of chewing can lead to material becoming entrapped in the gingival crevice of the teeth. The bacteria in the gingival crevice are predominantly proteolytic (utilising protein as substrate rather than sugars, contrary to those in the mouth, which are saccharolytic); this favours the preservation of starch granules once they have reached the gingival crevice (Marcotte and Lavoie, 1998). The resulting metabolic by-products of proteolytic metabolism, such as ammonia, result in localized raised pH, which in turn favours plaque mineralization by stimulating precipitation of calcium phosphate. The anti-microbial constituents of the saliva (e.g., immunoglobulins and degradative enzymes) break down the microorganisms that have survived in the mouth (Marcotte and Lavoie 1998*).* However, some material escapes breakdown and can become covered with plaque within hours. It is then protected from the effects of the α-amylase and becomes entombed in the calculus matrix (Scannapieco et al., 1993; Marcotte and Lavoie 1998). Dental calculus has a multi-causal etiology. Until recently, it was widely assumed that calculus deposits indicated diets rich in protein since such diets increase oral alkalinity, which in turn facilitates calculus formation (Hillson 1979; Meiklejohn and Zvelebil 1991; Lillie and Richards 2000; but see Lieverse 1999 for a review of

calculus formation processes). It is now clear that the dietary information provided by the extent of calculus deposits is not as straightforward as originally assumed. Specifically, high calculus deposition combined with low incidence of caries, have been considered to suggest a high protein intake (Lillie 1996; Keenleyside 2008), whereas occurrence of both high calculus and caries is understood to predominate in diets high in carbohydrates (White 1994; Humphrey et al. 2014). In any case, non-dietary factors such as the rate of salivary flow, mineral and silicon content consumed in food and water, phosphate and calcium levels in the blood and genetic factors also influence the occurrence of calculus deposits, possibly more so than diet (Lieverse et al. 2007). Finally, mechanical factors, such as chewing, have a contradictory effect; the act of chewing may promote calculus formation by increasing salivary flow rate (Dawes 1970) whereas chewing abrasive materials may mechanically remove calculus deposits (Gaare et al. 1989). Besides the multifactorial nature of calculus formation processes and the challenge this can cause to the interpretation of the embedded microdebris, additional issues arise from the fact that the morphological variation of teeth, expressed in the co-existence of smooth surfaces, fissures and pits, creates different surface morphologies that facilitate or hinder calculus formation (Hillson 2005). Furthermore, within each plaque deposit there may be marked variations in pH, nutrient availability and temperature, which can affect the formation and structure of the calculus (Hillson 2005).

2.2.2 Relevant aspects for Archaeology

For what concerns the discipline of archaeology, there are three main aspects of the dental calculus formation process that are most relevant.

- First, the processes described above cease at death. This implies that dental calculus forms only during the lifetime of an individual; therefore, it has high archaeological integrity due to the fact that post-mortem inclusions of micro-debris from soil are very unlikely (Middleton & Rovner 1994).

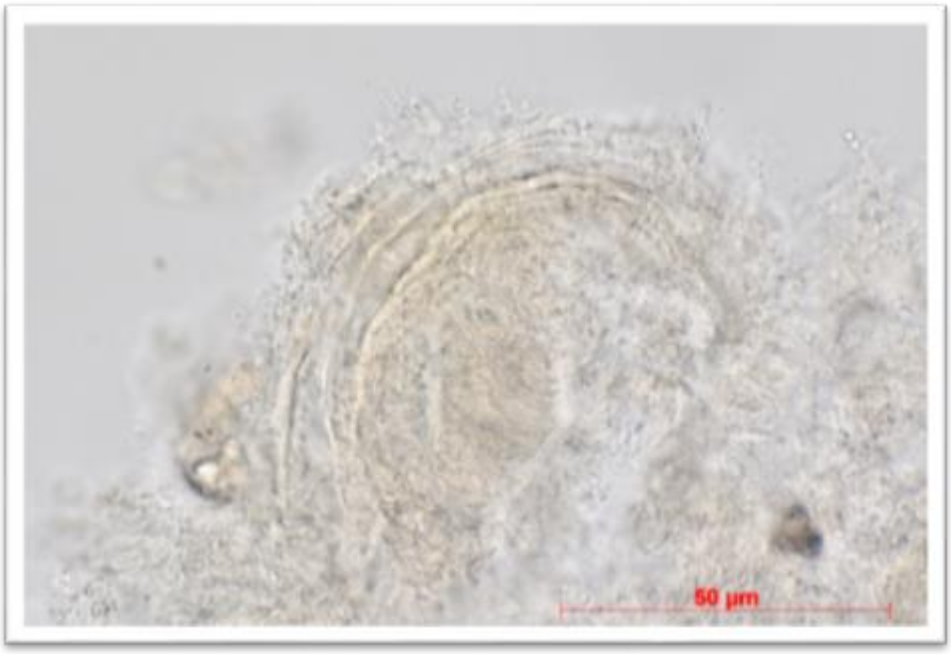

Figure 7. A fleck of calculus 'dissolving', showing the layers of calculus 'build-up' (Individual B61, Dalheim, Germany, c. 1100-1250 AD, picture by the author)

- Secondly, the stability of the calculus mineralised matrix in soil is very high, a fact that has been recently proven by Warinner et al. (2014)a. In their study, Warinner et al. used Raman microscopy to verify the integrity of the calculus matrix in Medieval individuals from Dalheim in Germany, by comparing it with calculus samples of modern origin. It was found that the stability and integrity of its matrix can be extremely useful in archaeology.

A third aspect is that dental calculus can entrap and, therefore, entomb for thousands of years, debris, micro-organisms, molecules of various natures and trace elements (Capasso et al. 1995) that enter the mouth during the lifetime of an individual, providing a novel archaeological record to examine, in situ on the skeletons and with no contamination inside the mineralized matrix.

Finally, it must be stressed that dental calculus was often cleaned off in old excavations, it is important that dental calculus deposits are not removed during cleaning of skeletal remains.

2.2.3 Known limitations

A limitation little explored so far is that the rate of calculus formation is variable and associated with individual differences in diet, salivary flow, health, local pH and genetic factors (Marcotte and Lavoie 1998), as already pointed out by Henry and Piperno (2008). There are, therefore, two main categories of individuals: 'slow formers' and 'fast formers', with many possibilities in between. Moreover, some people may form calculus while others do not, and some people form calculus only in certain periods of their lives. Finally, the amount of calculus varies enormously from one individual to another and between populations. So far, there is no known way to tell when and how rapidly dental calculus builds up on the teeth of different individuals, and/or which part or parts of the life on an individual is represented in calculus.

Despite the above limitation, the high archaeological integrity of dental calculus as a deposit, its longevity and stability in the soil, its ubiquity in past populations, and its proven ability to entrap a number of particles in its matrix (see below), highlight beyond any doubt the extraordinary potential that dental calculus holds in archaeology.

2.2.4 Food preparation, food stickiness and oral clearance

Food preparation practices, which can significantly alter the structure and nutritious quality of plants (Butterworth et al. 2016), can have an important effect on dental calculus in terms of the formation and the survival of micro-debris. Starch granules and phytoliths can be degraded by grinding, as is evident in material extracted from stone tools (e.g., Lucarini et al. 2016). Grinding can also introduce grit and stone particles into the food, which causes tooth wear and can also lead to particles becoming embedded in dental calculus. Chewing starchy food, even when uncooked, also disrupts the granules to enhance the effects of amylase in saliva in the further breakdown of the granules (BeMiller and Whistler 2009).

The way food preparation practices may affect the formation of calculus has been given little consideration. Chewing abrasive materials can mechanically remove calculus deposits (Gaare et al. 1989) but the way food is prepared and its relative softness affects dental calculus build-up just as it affects the rate of dental wear. Several studies have demonstrated the association between food processing and dental wear levels, whereby

processed soft-textured food with low fibre content generates reduced dental wear (Molnar 1971; Eshed et al. 2006; Deter 2009). Alternatively, the ingestion of sand, grit and other hard particles that become embedded in the food during grinding, drying and other food processing practices, results in increased dental wear (e.g., Smith 1984; Lev-Tov Chattah and Smith 2006). However, the correlation between dental calculus build-up and preservation, and dental wear as a proxy for food processing practices, has yet to be explored. In addition to its potential effect on dental calculus preservation, cooking tends to alter the properties of various foodstuffs and other inclusions, rendering many of them essentially impossible to identify in dental calculus deposits. The effect of cooking on starch granules is well established and results in their disruption and gelatinisation (Radley, 1968), at which point they are no longer visibly present as microfossils in dental calculus. Furthermore, by means of cooking, proteins become denatured and various food borne pathogens are killed (Radley 1968; Gaman and Sherrington 1996; Svihus et al. 2005; Tester et al. 2006; Carmody and Wrangham 2009). The role of food stickiness, salivary clearance and oral hygiene is also relevant when evaluating the potential for dietary patterns to be reflected by the microfossils entrapped in calculus, because it may affect the quantity of dental calculus build-up as well as the typology of the entrapped debris. Food stickiness refers to the ability of certain foods to adhere to dental and other oral surfaces. Many factors determine stickiness, including water content in the food, its viscosity, as well the nature of the food ingredients (e.g., sugars of low molecular weight generate increased stickiness, whereas carbohydrates and proteins of high molecular weight tend to minimize stickiness) (see review in Adhikari et al. 2001). Food stickiness is an important aspect to be taken into consideration during dental calculus studies as particles of stickier food are more likely to become embedded in dental calculus.

Clearance from the oral cavity is one of the most important salivary functions since it removes food and dirt and maintains healthy levels of pH and oral biofilm in the oral cavity (Humphrey and Williamson 2001). Different food types (sugars and fat) clear at different rates, though no direct link between food stickiness and speed of clearance has been detected (Dawes et al. 2015). The extent to which food-related micro-debris can become incorporated into the calculus matrix is likely to depend on how long and how much the food and other debris sticks to the tooth and how fast each category of

ingested debris takes to be swallowed and removed from the mouth. Therefore, the nature of food preparation may further affect the archaeological record entrapped in the calculus matrix, though further work is needed to address this.

Food stickiness, as well as fibrous debris that can become entrapped between teeth, generates the need for oral hygiene practices.

Finally, oral hygiene may be responsible for the removal or remodelling of dental plaque during life; this further complicates the understanding of the part of a person's life that may be reflected into the deposit.

2.3 Dental calculus and ancient diet

2.3.1 Microscopy studies on dietary micro-remains

In archaeology, the study of dental calculus has been approached macroscopically (quantity and location of calculus deposits on teeth), microscopically (debris entrapped in it), and more recently, biomolecularly. Early work on dental calculus focused on macroscopic quantification and location of calculus found (Dobney and Brothwell 1987) and this approach is still sometimes used (e.g., Lillie 1996; Jankauskas and Palubeckaite 2006; Keenleyside 2008; Humphrey et al. 2014). Different recording protocols have been proposed for this purpose, ranging from simple presence/absence (e.g., Belcastro et al. 2007) to more detailed ordinal schemes (Brothwell 1981; Dobney and Brothwell 1987) or even continuous schemes that measure the maximum extent of the deposit (Hillson 2000). Such studies generally treat the amount of dental calculus per individual, combined with observations on other dental diseases such as caries, as dietary indicators as well as markers of oral health and oral hygiene. A major limitation in this approach is that dental calculus deposits, particularly the supra-gingival ones, can detach from the teeth both during life and post-depositionally (Buikstra and Ubelaker 1994), which can bias the results. Detachment is less common for sub-gingival deposits as these are protected to some extent by the gum (Figure 3), unlike supra-gingival deposits, which accumulate on the exposed surfaces of the teeth.

The potential of dental calculus to be a trap for a variety of microscopic dietary and environmental micro-debris was first recognized in 1975 when phytoliths, opaline silica deposits that form in certain types of plants, were extracted from samples of ungulate dental calculus (Armitage 1975). Following this, Dobney and Brothwell (1987 1988) were the first to explore the potential for obtaining information on archaeological samples of dental calculus, using scanning electron microscopy (SEM) to identify pollen grains, phytoliths, charcoal, microscopic fragments of cereal chaff and animal hairs from a range of different historical and archaeological material. Following this, using SEM, fossilised bacteria were detected in the calculus matrix of Neanderthal (Vandermeersch et al. 1994; Pap et al. 1995), Natufian (Arensburg 1996) and multi-period Chilean samples (Linossier et al. 1996). A more recent SEM analysis identified microfossils related to diet, including palm phytoliths and diatoms, which were used to examine water sources in an Easter Island population (Dudgeon and Tromp 2012).

Samples of dental calculus were first decalcified to extract the embedded microfossils, which were then studied using light microscopy, from the extinct ape *Gigantopithecus blacki*, in order to identify the presence of embedded phytoliths (Ciochon et al., 1990). This was followed by Middleton and Rovner (1994), who identified phytoliths and starch granules from samples of herbivores. Subsequently, starch granules (Juan-Tresserras et al. 1997; Scott Cummings and Magennis 1997) and phytoliths (Lalueza Fox et al. 1996) were extracted from human samples while a range of phytoliths was recovered from a sample of mastodon calculus (Gobetz and Bozarth 2001). Capasso et al. (1995) evaluated the utility of synchrotron radiation microprobe analysis with potentially promising results, though this method has yet to be developed further. While many of these early publications highlighted the potential for extracting useful paleodietary information from dental calculus, none attempted to identify the specific sources of the microfossils.

Since 2008, the analysis of material remains entrapped in dental calculus has increased significantly. While this has largely focused on the extraction and identification of microfossils, in particular starch granules and phytoliths which emerge from the dental calculus matrix as it is decalcificed, a range of morphological and analytical methods are now used to access the material entrapped in the calculus (Hardy et al. 2012; Power et al. 2014; Warinner et al. 2014a, 2014b). However, the largest number of publications

describes microfossil extraction and identification using optical microscopy. Most studies have focused on the identification of starch granules, and to a lesser extent phytoliths (e.g.,Henry and Piperno 2008; Piperno and Dillehay 2008; Mercader 2009; Li et al. 2010; Wesolowski et al., 2010; Mickleburgh and Pagán-Jiménez, 2012; (Horrocks et al. 2013). Henry et al. 2012, 2014; Tao et al. 2015; Wang et al. 2015). The principal aim of these studies has been to identify dietary components, though food processing through evaluation of starch granule alteration has also been explored (Henry et al., 2009 2011). However, the complexities of starch granule alteration have been highlighted, and suggest that further work is required to explore potential diagenetic processes (Collins and Copeland 2011; Barton and Torrence 2015).

Since 2015, an increasing number of papers have highlighted problems regarding the uncritical identification and interpretation of the data, most notably in terms of the integrity of the link between diet and the microfossils found, and their identification (Buck and Stringer 2014; Buck et al. 2015; Wang et al. 2015; Leonard et al. 2015; Power et al. 2015; Radini et al. 2016b). It has also become clear that accumulation of debris in calculus is more random than previously thought, which suggests that sampling as a proxy for full reconstruction is problematic. In terms of starchy food, it is clear that starch granules and phytoliths present in dental calculus do not represent dietary breadth though they may be more reliable at the population level (Leonard et al., 2015) while Power et al (2015) also detected dietary correlations at the level of populations, using phytoliths, though not with the starch granules. This suggests that broad interpretations of diet based solely on microfossil material should be viewed with caution. The morphological study of all archaeological plant-based materials is based on comparison with reference collections. In relation to this, the need for a better understanding of taphonomic processes affecting starch granule survival is as applicable to material extracted from dental calculus as it is to the analysis of other archaeological materials, such as residues extracted from stone tools (Barton and Torrence 2015). Likewise, although the preservation of starch granules and phytoliths is not in itself problematic due to the integrity of the mineralized matrix of calculus through time, the overlapping size and shape of starch granules as well as phytoliths that occur among and between species of plants, even in terms of widely used domesticated plants such as millets (Wang

et al. 2015; Lucarini et al. 2016, Shillito 2013), means use of starch granule and phytoliths morphology alone for detailed species identification can also be problematic.

2.3.2 Analytical methods

A range of analytical techniques have been used, often in combination with microscopy, to further characterize entrapped material. A study that combined scanning electron microscopy (SEM) with elemental analysis using energy-dispersive X-ray spectroscopy (EDX) has shown potential in detecting food and micro-debris linked to occupational habits (e.g. Charlier et al. 2010); however, it does not recover and identify as many particles as light microscopy (Power et al. 2014). Inductively coupled plasma mass spectrometry (ICP-MS) analysis of trace elements in dental calculus samples also seems promising as it has so far been used in the identification of carbohydrates and fish (Lazzati et al. 2015).

Stable isotope analysis has also been investigated for its potential to study the composition of materials embedded in dental calculus as a proxy for ancient dietary reconstruction (Scott and Poulson 2012). However, Salazar-García et al. (2014) have demonstrated that the isotopic values obtained from dental calculus are not equal to those from bone collagen, possibly due to the diversity of material entrapped in the calculus and the variable amounts of calculus accumulation in individuals. However, Wang et al. (2015) correlated carbon isotope data taken from samples of bone collagen, with starch granules extracted from dental calculus.

Thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) can be used to characterise a wide range of organic materials in dental calculus. The mineral component of dental calculus traps organic compounds and provides them with a protective environment, which allows biological marker compounds ('biomarkers') characteristic of the original source to survive. The identification of specific chemical compounds enabled Hardy et al. (2012) to detect exposure of Neanderthal individuals from the site of El Sidrón, Spain, to wood smoke, bitumen or oil shale and a range of plant items, providing the earliest direct evidence for the use of medicinal plants in human history. Ingestion of the plant *Cyperus rotundus* L., was detected throughout the multi period sequence at Al Khiday, Sudan

(Buckley et al., 2014). Today, this plant is considered primarily as a problematic weed in many countries (Sivapalan 2013) suggesting that dental calculus has the potential to recover evidence for foods and medicinal plants that have been forgotten. The earliest direct evidence for plant foods in the genus *Homo* has been identified at the 1.2 million year old site of Sima del Elefante, Atapuerca, Spain (Hardy et al. 2016) while chemical evidence for plant-based nutrients was identified at the 300,000-400,000 year old Lower Palaeolithic site of Qesem Cave, Israel (Hardy et al. 2015).

Dental calculus is also a source of bacterial genetic material and holds potential as a biomolecular reservoir (Preus et al. 2011). A number of papers have reviewed the recent application of biomolecular techniques to the study of ancient dental calculus and its relevance to ancient diet and oral and overall health (Metcalf et al. 2014; Pacey 2014; Huynh et al. 2015; Weyrich et al. 2015). De la Fuente et al. (2012) amplified DNA and identified bacteria from 4000-year-old samples from Chile. Similarly, Adler et al. (2013) identified a wide range of oral bacteria, demonstrating a shift in their composition as carbohydrates from domesticated plants became prominent. Warinner et al. (2014a) applied shotgun DNA sequencing to samples of historic age dental calculus. This study highlighted the link between oral pathogens, host immunity and dietary patterns by identifying in the oral environment, opportunistic pathogens, antibiotic resistant genes, bacterial and human proteins, as well as DNA sequences from dietary sources. Only a portion of the material identified using biomolecular evidence has been securely linked to diet (e.g. Warinner et al. 2014b). However, much of this evidence, such as consumption of leafy crops of the Brassicaceae family traditionally have a low archaeological visibility (Warinner et al. 2014a). Warinner et al. (2015) also detected the first direct evidence of milk consumption by identifying the protein □-lactoglobulin (BLG) in human dental calculus from the Bronze Age (ca. 3000 BCE). The use of biomolecular techniques may be limited in some cases, such as for the earlier stages of the Palaeolithic, due to the apparent degradation of biomolecular evidence in the deep past (e.g. Hardy et al. 2016), but they appear to hold great potential for more recent samples.

2.4 Dietary remains as a result of accidental and secondary eating: non-deliberate consumption associated to diet

As shown above, the main focus of the dental calculus work to date has been on dietary reconstruction and pathogen identification. The assumption that all plant micro-remains are the result of deliberate consumption of food has recently been questioned. Here, I will explore two important alternative pathways to inclusion: gastrophagy and accidental consumption of debris incorporated or settled on the eaten food.

2.4.1 The role of gastrophagy

In their recent work, Buck et al. (2015) discuss the potential for gastrophagy to confound palaeodietary reconstructions. As recently as last year, Buck and Stringer (2014) challenged the view that the chemical compounds indicative of yarrow and camomile found in the dental calculus of the El Sidrón Neanderthals (Hardy et al. 2012) were the result of direct self-medication of non-nutritious plants. Instead, they proposed that these results are the outcome of the eating of the stomach contents of an animal that had previously eaten these plants. Though this is not correct in the case of the individuals from El Sidrón (Hardy et al. in 2016), there is little doubt that Neanderthals ate chyme, as many modern human groups do (Buck et al. 2015). Gastrophagy is, therefore, a potential pathway whereby micro-debris, most notably phytoliths which are likely to survive the partial digestion process that forms chyme, may become embedded in dental calculus.

2.4.2 Secondary eating of debris embedded or settled in food or drinks

Apart from the paper by Buck et al. (2015), it is becoming important to differentiate dietary versus non-dietary micro-remains and to consider secondary eating as a possible pathway to inclusion of micro-debris in human dental calculus. A number of particles of different natures, fully assessed in Chapter 3, can become incorporated into the eaten food accidentally. A recent example of such a complex dynamic of pathways has been provided by Tromp and Dudgeon (2015) from their work on dental calculus samples from Rapa Nui. In their first study, Dudgeon and Tromp (2012) retrieved evidence of large quantities of globular echinate palm phytoliths, which were also almost ubiquitous in the calculus samples. Such debris was of problematic interpretation due to the lack of palms that produced such type of phytoliths in the island at the time the studied individuals

lived. In their more recent work, Tromp and Dudgeon (2015) investigated the consumption of sweet potato in Rapa Nui through time using starch granules entrapped in dental calculus from individuals from different periods of time and areas of the island. The authors were able to prove that palm phytoliths could have become embedded in the external part of the tubers growing in the ground where phytoliths were present as sedimentary microfossils from periods of time when the trees were common in the island (see Tromp & Dudgeon 2015, 61). As sweet potato tubers were eaten as a staple food by the population in question and grew in soil rich in such phytoliths, the predominance of globular echinate phytoliths in the calculus was not actually the result of deliberate consumption. Phytoliths adhering or being incorporated to tubers from the soil and then ingested is an important pathway of inclusion of such remains in the calculus matrix and will surely need to be carefully considered in future studies. Equally important, though investigated to a limited extent, is the role of settled dust and dirt on the food. The number of particles of grit for example found in human dental calculus in a number of studies could be the result of dirt on food (e.g., Charlier et al. 2010; Buckley et al. 2014). As pointed out by Tromp and Dudgeon (2015, 60), contamination by plant interactions with soil and environment in general during growth can generate a flux of microfossils in dental calculus as a result of secondary eating. Future work is therefore required in this direction.

2.5 Summary and overview to the proceeding chapter

Dental calculus, which results from the calcification of plaque, is common in archaeological skeletal material from all periods. Indicative of its preservation potential is the fact that it has been found on the teeth of a Miocene *Sivapithecus* dating to between 12 - 8 million years ago (Hershovitz et al. 1997) as well as on late Pliocene hominins (Blumenschine et al. 2003). This has led, unsurprisingly, to a growing interest in ancient dental calculus as a source of direct evidence for plant remains that were in the mouth during life. As dental calculus forms in the human mouth, the assumption so far has been that much of this material represents food consumed, and therefore offers direct information on items that were intentionally eaten.

Important recent work conducted on modern humans, the Twe forager-horticulturalists, by Leonard et al. (2015) has highlighted that even when the food ingested is known and identified, such remains may not directly reflect the actual role/portion of different plants in the diet, posing additional limitations in the exploration of ancient diets based on dental calculus microdebris. Furthermore, certain human behaviours observed even in modern populations, such as gastrophagy, can complicate the interpretation of the dietary evidence.

Due to the increased interest in dental calculus for assessing the role of plants as food in ancient people's lives, and the aforementioned limitations in the interpretation of relevant findings, it has become paramount to understand how representative dental calculus is of the diet and overall living conditions of ancient people.

In the following chapter potential "non-dietary" pathways to inclusion of micro-debris in human dental calculus will be addressed and supported by existing archaeological evidence. Having defined the complexity of the archaeological record retrieved from the calculus matrix, the methodological implications will also be discussed.

CHAPTER 3
NOT JUST FOOD:
THE HUMAN MOUTH AS A 'DUST TRAP'

3.1 Introduction

In the previous chapter it was shown that a number of particles retrieved in the calculus are not strictly of dietary origin. It has also been demonstrated that there is a general lack of consideration of non-dietary pathways to inclusion of micro-debris in human dental calculus. Particles can reach the human mouth in different ways and they can also be of different natures, from dust present in the environment, of both natural or human origin, to debris generated by the use of the mouth as a third hand. The main goal of this chapter is to provide clear evidence of the multiple pathways that micro-debris can take to become entombed in the dental calculus matrix. This chapter contains published work from two papers: the one mentioned in the previous chapter and the following one:

Radini, A., Buckley, S., Rosas, A., Estalrrich, A., de la Rasilla, M. and Hardy, K. (2016)a. Neanderthals, trees and dental calculus: new evidence from El Sidron. *Antiquity*, *90*(350), pp.290-301

3.2 Micro-debris in the mouth as a result of extra-masticatory uses of teeth and oral hygiene

This section provides evidence of the use of the mouth as a third hand as a potential source of micro-debris and non-edible material being introduced into the mouth. Examples of archaeological evidence of non-masticatory activities that engage the human mouth in ancient populations are discussed.

3.2.1 The use of the mouth as a third hand in modern populations

A potential source of micro-debris in human dental calculus is the use of the mouth as a third hand. The use of teeth to hold, soften or shred material is widespread in the ethnographic record, while physical evidence for the non-masticatory use of teeth, based on tooth wear and attrition, occurs across the geographical and temporal spectra

(Bonfiglioli 2004; Eshed et al. 2006; Hinton 1981; Lozano et al. 2009; Lukacs & Pastor 1988; Molnar 2008; Ryan & Johansen 1989; Volpato et al. 2012). Kreuger and Ungar's (2012) publication on the Krapina Neanderthal tooth wear, provides a summary of some ethnographic evidence for the use of anterior teeth as a third hand among some North American and Arctic populations. Activities include grasping hides, chewing leather, softening wood and root fibres and a range of abrasive minerals and sand, associated with their diets. In Papua New Guinea, plant fibres were sometimes chewed to soften them before making string (MacKenzie 1991). A recent study (Clement & Hillson 2012) on the Inuit of Igloolik found extreme dental wear among the anterior teeth, which was linked to the use of these teeth in the preparation of walrous and seal hides for clothing, as well as in making threads out of animal sinew, and other activities (Mayhall 1976; Pedersen 1947). This is particularly relevant in relation to the use of the embedded material for dietary reconstruction since all evidence for the non-masticatory use of the teeth needs to be identified and excluded before dietary characterisation can occur.

In addition, when interpreting dental wear data from palaeoanthropological and archaeological samples, it is essential to bear in mind that dietary factors, that is, diets with hard inclusions and the degree of cooking/softening of the food, also affect greatly the extent of dental wear (Deter 2009). Therefore, it is important to disentangle the dietary from the non-dietary induced wear based on the observed patterns on the occlusal and interproximal surfaces of the crown.

3.2.2 Oral hygiene

Dental hygiene is neither a product of modern society, nor an exclusively human phenomenon. Chimpanzees have been recorded cleaning each other's teeth using their fingers and sticks (McGrew and Tutin 1973). Japanese macaques use their hair or the hair of another individual to floss between their teeth (Leca et al. 2010), while long-tailed macaques in Thailand, which interact intensively with humans, have been observed to use human hair as flossing material (Watanabe et al. 2007). Among prehistoric human populations, a well-known case proposed as evidence for oral hygiene activities are the interproximal grooves on hominin teeth (Brothwell 1963) for which tooth picking has long been suggested as a cause (Ubelaker et al. 1969). Interproximal grooving has been found on hominin species since *H. habilis* (Puech and Gianfarani 1988) and, while a

number of hypotheses have been put forward that attempt to explain interproximal grooves, tooth picking to extract food stuck in between teeth is still considered to be the most likely cause (Ungar et al. 2001). A range of materials have been suggested as tooth picks, including wood and grass (Eckhardt and Piermarini 1988; Brown and Molnar 1990; Hlusko 2003). The use of chewing sticks to maintain oral hygiene is widespread in traditional societies (Almas 2002; Idu et al. 2009; Jose et al. 2011); an example witnessed by the author is the use of the wood of *Savadora persica* as a tooth brush in Libya (an example of this is provided in figure 8).

Figure 8. Wood sticks made of *Salvadora persica*.[2]

Many of the plants used for chewing have anti-bacterial qualities, which may aid oral hygiene (Idu et al. 2009; Jose et al. 2011). Among the Dakshina Kannada, for example, 25 different plants are used in oral hygiene, either being chewed, or applied to the mouth. Each plant has a specific role, including treatment of oral ulcers, gum disease, tooth

[2] The image is from The Museum of Healthcare's blog, and it is on public domain at the following link: https://museumofhealthcare.wordpress.com/2013/06/27/the-humble-toothbrushs-extravagant-past/

decay, toothache, caries and stomatitis. Other material, including charcoal, soot, clove oil, ghee, honey and salt are also used to clean teeth and relieve pain (Jose et al. 2011).

The possibility that plant parts and grass were used in oral hygiene suggests that phytoliths found in dental calculus need to be viewed with an open mind as to their origin, and that reference collections need to be sufficiently broad to incorporate the identification of grasses that may have been used in this way.

3.2.3 Existing archaeological evidence of extra-masticatory uses of teeth

The most common evidence for the non-masticatory use of teeth in archaeological samples is based on tooth wear (Bonfiglioli et al. 2004; Eshed et al. 2006; Hinton 1981; Lozano et al. 2009; Lukacs & Pastor 1988; Molnar 2008; Ryan & Johansen 1989; Volpato et al. 2012). Ethnographic evidence for the use of teeth for non-masticatory purposes is also very common. Kreuger and Ungar (2012) provide a summary of recorded uses of anterior teeth among North American and Arctic populations. These include chewing raw materials such as leather, wood and root fibres as well as grasping or holding materials as they are being processed. In Papua New Guinea, plant fibres were sometimes chewed to soften them before making string (MacKenzie 1991). Non-masticatory tooth wear has been found widely in many Neanderthal populations (Clement et al. 2012), including El Sidrón (Estalrrich et al. 2011). Most of the heavy wear thought to result from using the teeth as a third hand is found on the anterior teeth with little evidence of wear on post-canine teeth (Clement et al. 2012). Fiorenza et al. (2011) differentiate between anterior teeth, used principally as a third hand for holding, and posterior teeth, used for conducting tasks. Lozano et al. (2008) also identify evidence for holding, pulling or handling materials on the anterior teeth from Sima de los Huesos, Atapuerca, and note less evidence for non-masticatory wear on the canines and premolars. In several cases, the distribution of microwear (in this case, labial scratches) has demonstrated the handedness of the individuals, suggesting about the same proportion of right and left handers as today (e.g. Lozano et al. 2013).

3.3 Dust in the environment and the concept of particulate matter

Apart from the 'deliberate insertion' of non-dietary items in the mouth, particles and small fragments of different natures (e.g., wood dust, pollen, spores) present in high concentration in the environment can reach the oral cavity by oral breathing and accidental ingestion (e.g., as settled dust on food). Dust deposited on the face can also reach the mouth and be accidentally ingested. This section will introduce the main concepts and terminology associated with the potential presence of dust in the calculus matrix.

3.3.1 Dust and particulate matter

Dust can be defined as an "assemblage" of particles of various nature, of organic and inorganic origin, of different size and shape, that are normally present in the atmosphere around us. "Dust" has a biological (e.g., pollen, spores, fibres, insect parts, dust mites and bacteria) and a lithological component (mineral fragments of natural and anthropogenic origin), and can also contain smoke, micro-charcoal and soot. Dust is normally divided into airborne and settled dust, where airborne dust is normally below 100 microns in size. Here the main focus is on just one category of dust known as 'particulate matter' (PM) (fig. 9). PM consists of tiny fragments of debris that is airborne, at least for part of its life, ranging in size from below 1 µm up to at least 100 µm.

As noted by Yang and Omaye (2009), particles larger than 10 µm in diameter rarely reach and settle in the lungs; particles smaller than 10 µm can reach the large upper branches; particles smaller than 5 µm can reach the bronchial tubes and particles smaller than 2.5 µm can penetrate the deepest (alveolar) portions of the lung. For this reason, particles below 10 µm are often called 'respirable PM'. Fine particles (between 2.5 µm to 0.1 µm in diameter, see figure 10) and ultrafine particles (diameter smaller than 0.1 µm) can enter the bloodstream if soluble in water (Yang & Omaye 2009).

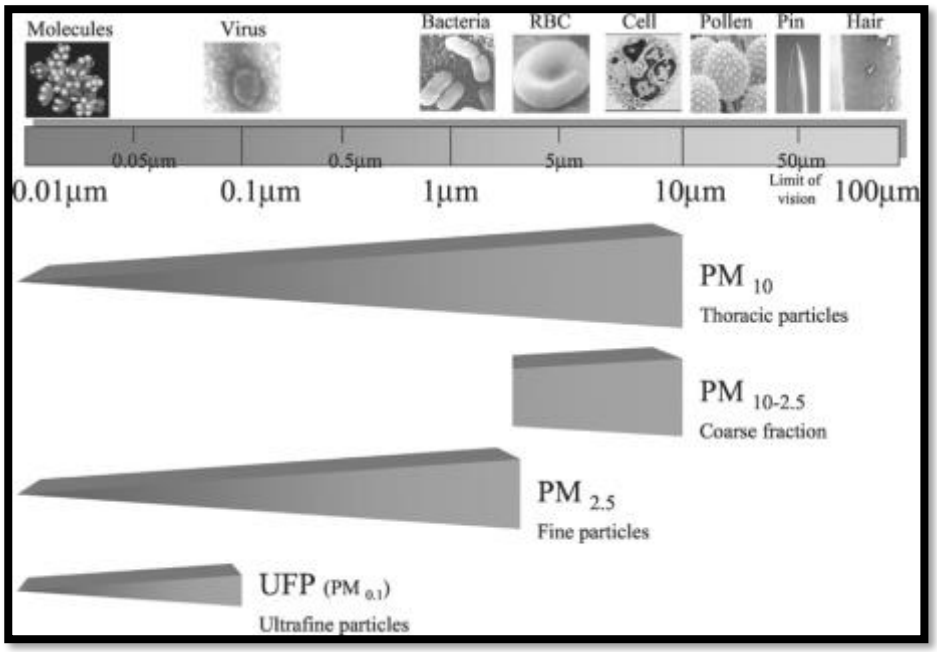

Figure 9. Examples of particulate matter in different size categories (from Brook et al. 2004, fig. 1)[3].

It should be stressed that studies have proven that the diameter of the particles, not their overall size, is the most important factor determining whether they will reach the lungs (Pope & Dockery 2006). This explains why fibres as long as 100 µm, with diameter below 5 µm have been found in the lower respiratory system. Such aspects result in occupational dust from certain industries, such as textile work, to be particularly irritating to the respiratory system.

[3] The image is on public domain and can be downloaded as power point slide from the following link: http://circ.ahajournals.org/content/109/21/2655.figures-only

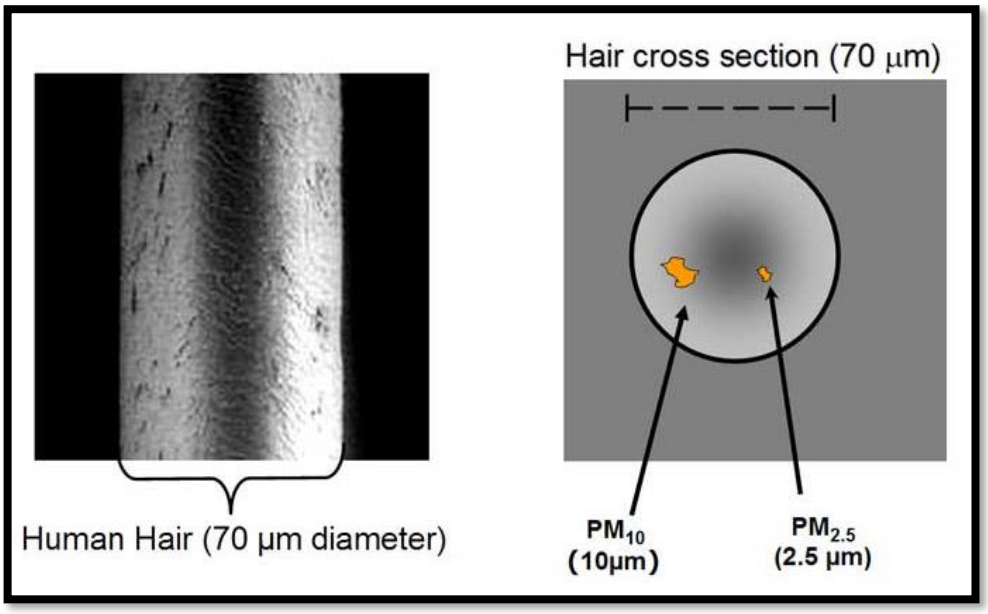

Figure 10. Particles of 'respirable' size (from Pope & Dockery 2006).

3.3.2 Types of dust

Dust composition can vary hugely from one environment to another (an example of indoor dust in an ancient dwelling is provided in figure 11) and in the same environment through the years. In the current study particular emphasis is given to 'occupational' dust that can cause health problems and is generated by the breakdown of larger objects during human activities, for example wood dust that results from sawing blocks of wood. However, natural dust is also present in the environment and can have serious health effects. For example, outbreaks of Saharan dust caused by incoming southerly winds in the city of Rome have resulted in an increased risk of cerebrovascular, circulatory, and respiratory mortality in the city (Mallone et al. 2011). Soil dust can increase the amount of particulate matter in the air and it can also influence the health of the general population (Smith & Lee 2003).

When we combine anthropogenic and natural dust, we are immersed in a 'micro environment' of debris not always visible to the human eye. Natural sources of dust are specific to the region where we live, such as geology and climate (e.g., presence of sand, volcanoes, prevailing winds) and vegetation cover (e.g., forested areas enrich the PM of pollen and have lower levels of soil-derived dust, due to lower levels of soil exposure to winds and erosion). The second typology of debris is consistent with dust generated by

human activities, in both indoor and outdoor environments, such as smoke, wood and grain dust. In this study I will refer to this debris as a whole, as the 'micro-environment' experienced by past populations. Airborne particulate matter characterizes a great variety of human industries including stone-working and manufacture of pottery (: lithological material); grain storage (mold spores); food production, such as bakeries (flour), wood working (wood dust). Finally, the term 'air pollution' will be used to indicate the deterioration of air quality as a result of human activities.

3.3.3 From the air to the calculus

Examples of environmental material found in dental calculus, and most likely the result of inhalation, include insect parts, pollen, micro-charcoal, soot (which most likely represents smoke inhalation), minute grit and dust particles, plant fibres and phytoliths potentially derived from cultural practices (Buckley et al. 2014; Hardy et al. 2015; Radini et al. 2016b), or from accidental ingestion, for example, from bedding during sleep.

Figure 11. An example of possible sources of "dust" from roofing material: roof (A), with indoor lining of crop processing by-product consisting of bread-wheat (B) and phytoliths of the same species at the reference collection.

An example of accidental inhalation/ingestion is fungal debris such as spores and hyphae, which have been found in several studies (e.g., Warinner et al. 2014a; Hardy et al. 2015; Radini et al. 2016a). Fungal spores are notoriously difficult to identify to species level,

especially on the basis of a single spore as there are millions of species, and they are very common in the environment, on food, objects, soil and even in the air. The fruiting bodies of fungal spores may become useful food items such as mushrooms; however, assuming that fungal spores in calculus are the result of deliberately ingested mushrooms (e.g., Power et al. 2015) needs to be viewed with caution.

What becomes clear from the examples provided above is the potential of ancient human dental calculus to provide evidence of occupational dust and exposure to pollutants. Is there any evidence therefore of such typologies of remains in ancient human dental calculus? The following paragraph will provide a positive answer to this question.

3.3.4 The human mouth as a "depositional environment": pathways to inclusion

While food and drink are probably the most common and most regular sources of material deliberately placed in the mouth, air inhalation is constant and significant amounts of time are likely to have been spent each day on crafting activities, often breathing through the mouth, during eating, communicating and panting after exertion. Dust generated by a number of human activities can produce debris in high concentration in the environment and may naturally settle on the head, face, or, as suggested above, on food, and can, therefore, reach the mouth by transfer. Se et al. (2010) demonstrated that particles up to 70 microns are inhaled through the mouth in most circumstances while larger particles can also be inhaled under certain conditions.

3.4 Archaeological evidence for ancient polluted environments from skeletal remains

Respiratory health and air quality in past societies has received little or no attention by scholars so far, in part due to the paucity of evidence (Brimblecome 2011, 2). Despite the scarcity of the data, it is a fact that exposure to smoke and pollutants of variable nature can be considered as old as humanity itself (Naeher et al. 2007, 67).

3.4.1 Dust and health

Exposure to high levels of particulate matter can affect both the upper and lower respiratory tract. In the lower respiratory tract (trachea and lungs), pollutants in the air

can cause cancer, bronchitis, emphysema, asthma and various allergies. The effect of different sizes of particles in the respiratory system is summarized in figure 12.

Dust reaching the lungs can result either in a simple accumulation, or generate a reaction in the lung tissues that may be severe enough to impair lung function, such as byssinosis (caused by cotton and flax dust), or cause fibrotic lung diseases, such as silicosis (caused by free crystalline silica dust). On the other hand, inhalation of high levels of smoke and soot causes anthracosis (Amoli 1998; also see fig. 12).

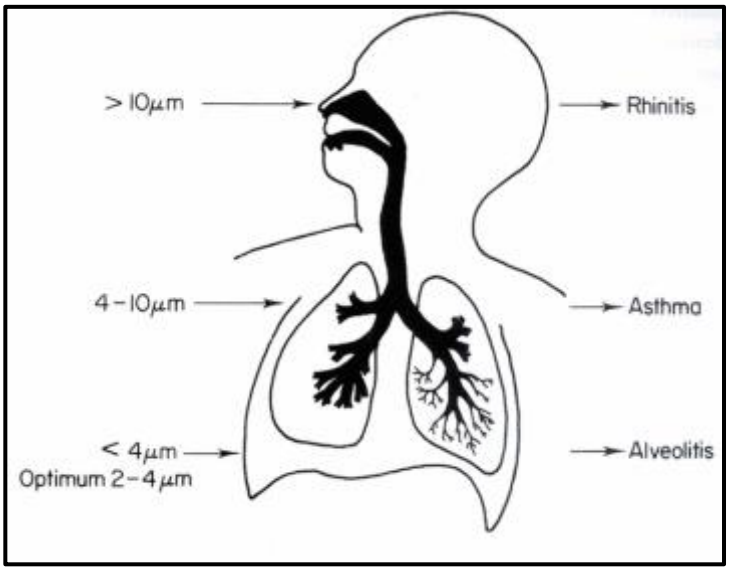

Figure 12. Particulate matter diagram, showing the potential effect of particles according to their size (modified from Lacey et al. 1972).

The upper tract of the respiratory system (nose, maxilla, sphenoid, ethmoid, and frontal sinuses) is often affected, too, and one of the commonest conditions is maxillary sinusitis, an inflammation of the paranasal sinuses that causes severe discomfort and alteration of the soft and even the bone tissues of the sinuses (Slavin et al. 2005). Maxillary sinusitis has a complex pathology; however, PM pollution has been proven to be a common contributing factor.

3.4.2 Lower respiratory system

Evidence of pollutants reaching the lower respiratory system can be found in ancient populations in lung tissues preserved by freezing or desiccation (e.g., Prats-Muñoz et al. 2013, 4705: fig. 12). Histological assessment of the lungs of the Tyrolean Ice Man dating

back to 5400BC, as well as of ancient human mummies (Egyptian, Peruvian, and Aleutian) has shown that anthracosis (fig. 13), was a regular disorder in ancient times (Zimmerman et al. 1981; Mirsadraee 2014). Additionally, a wide range of mineral and organic 'dust' from materials such as traditional pigments are also known (Murr 2009). Therefore, it is safe to state that people from a broad temporal and spatial context have been exposed to high levels of smoke, soot, organic and inorganic particulate matter. As becomes clear, lung tissues are the only remains in archaeology that allow us to assess the presence of 'pollution' and its effect on human health simultaneously. Unfortunately, the survival of this type of tissue is very limited. We should note that pitting and/or new bone formation on the visceral surfaces of the ribs has also been

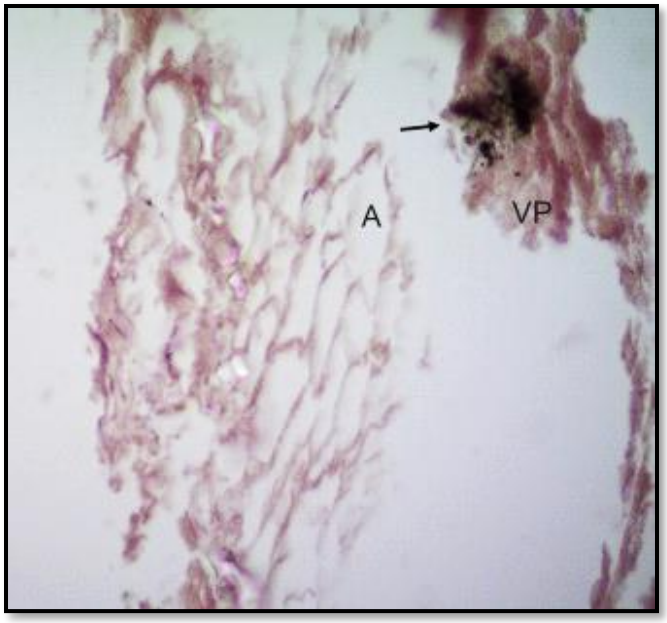

Figure 13. Histological section of intrathoracic material from Individual No. 2 from Cova des Pas site (Minorca Bronze Age), showing remains of lung parenchyma, such as alveoli (A) or visceral pleura (VP): Anthracotic pigment was also seen in the latter (arrow) (Prats-Muñoz et al. 2013, 4705).

accepted as evidence of non-specific lung disorders (Roberts 2007); however, such lesions cannot be directly linked to pollution.

3.4.3 Upper respiratory system

Maxillary sinusitis is a pathology characterized by pitting or new bone formation on the visceral internal surfaces of the maxillary sinuses (Roberts 2007), which may survive along with the skeletal remains, though drilling of the bones is required to detect it. Studies have linked this pathology to polluted environments in Medieval urban dwellers (see Roberts 2007 for a detailed review of maxillary sinusitis in archaeological contexts). We should note that by examining cases of maxillary sinusitis, we can only study the outcome of the pollution but not the pollutants that may have been responsible for it; moreover, other causes are possible for this pathology.

3.5 Summary and overview to the proceeding chapter

The research on ancient human dental calculus has gained increasing interest in the field of bioarchaeology for its undisputable potential to preserve a variety of lines of evidence not otherwise available. While the potential of dental calculus to entrap micro-remains of dietary origin was recognized very early, during the past year the direct link supposed to exist between such debris and the deliberate consumption of plants by ancient people and their role in diet has been questioned. Through a number of pathways, such as accidental eating, oral breathing and the use of the mouth as a third hand, the human mouth experiences a constant flux of organic and inorganic particles that are present in the environment or derived from human activities. If dental calculus is forming in the human mouth, such particles can become entombed in the calculus together with those of dietary origin. If this is the case, the human mouth, with the calculus forming on the teeth, may be approached as a peculiar depositional environment. In such conditions the calculus itself can then be considered as a small 'environmental deposit'. As a result of an increasing awareness of such pathways, a number of studies have highlighted the presence of debris of non-dietary origin entrapped in human dental calculus. In the past few years it has become clear that dental calculus acts as a trap for microscopic fragments of debris of a variable nature that has been ingested or inhaled during life, both deliberately and accidentally (Buckley et al. 2014; Hardy et al. 2015; Radini et al. 2016). As such, dental calculus provides *in situ* material and direct evidence for a variety of accumulated debris. There is a primary need to distinguish and separate the paleo-

environmental material from the dietary remains in the microfossil assemblages, in order for reconstructions of food items to be secure. Finally, the lack of understanding of what portion of the life of an individual is represented in the calculus matrix, due to the lack of large datasets, needs to be overcome. The current PhD introduces the evaluation of the full range of micro-debris entrapped in dental calculus, using an archaeological population where such debris can be compared with what is known about the population studied so that the contribution of dental calculus against more traditional lines of evidence can be fully understood. In the following chapter the research background of the selected populations will be provided in order to demonstrate that such a dataset represents an ideal population sample to address how representative human dental calculus is of ancient diet and living conditions in archaeological populations.

CHAPTER 4
THE DATA SET AND ITS BACKGROUND

4.1 Introduction

In Chapters 2 and 3 it was shown that the majority of studies in human dental calculus are conducted on prehistoric populations and the focus of such studies has been deliberate plant consumption. The number of samples analysed was small, and the majority of them can be considered as case studies. It has also been highlighted how a variety of debris that may become entrapped in calculus can confound the interpretation of lines of evidence related to diet. At the moment, there is a lack of research that explores the variability of entrapped remains in human dental calculus in more recent populations for which we have more archaeological and historical information. After careful consideration of the aims and objectives of this study, it was decided that a dataset with the following characteristics would be best suited for this study:

- A substantial skeletal assemblage or assemblages that are sufficiently preserved to allow sex and age at death to be estimated and with dental calculus deposits present in the majority of the individuals;
- A large number of individuals who likely shared the same natural environment and resources, in order to evaluate what portion of such environment is represented in the dental calculus matrix across a population;
- A population where archaeological excavations and historical resources would provide a solid comparative background to understand how representative the results from the analysis of the debris were and how they compare with the existing archaeological record;
- A location where plants would be readily available for collection wherever needed in order to facilitate the creation of a purpose-built reference collection to implement the identification of micro-remains found, wherever possible
- A social environment in which sexes would potentially be involved in different tasks during working age, in order to test differential exposure to debris derived from different activities.

Having carefully evaluated the aspects above, it was decided that a Medieval collection would best suit the purposes of this PhD. As the author was employed with ULAS for the majority of the time during this PhD, she was directly involved in excavation and post-excavation analysis of both skeletal and environmental samples in Leicester and its surroundings. She was also familiar with the recent excavations in Leicester from which there is a wealth of information, archaeological and historical, which would serve as an ideal dataset for this project.

This chapter focuses on the following points:

- Introducing the recent excavations in the North East Quarter of Medieval Leicester, conducted by the University of Leicester Archaeological Services, during which two remarkable skeletal assemblages were unearthed, together with a wealth of other archaeological features. Such excavations allowed the reconstruction of diet and living conditions experienced by Late Medieval individuals, therefore providing the solid archaeological background needed for the study
- Briefly reviewing broad aspects of Medieval lifeways that are most relevant to this PhD, providing evidence of the archaeological data available in the area for the selected period/area;
- Introducing the Archaeological Research Agenda for the East Midlands, in order to show how the analysis conducted also potentially address research needs in the region, and how such needs were behind the selection of the Early Medieval assemblages used as comparative dataset to that of the Later Medieval period.

As this project focuses on the potential of dental calculus as a line of evidence to investigate past lifeways, aspects of Medieval diet and lifestyle most relevant to this research will be contextualised in the discussion section of this PhD.

4.2 Leicester and its surroundings: location, past human occupation and the Highcross Project

Leicester lies in the middle of the county to which it gives its name (fig. 14), in the heart of the English Midlands, near the River Soar. The area has therefore a river, flood plains and forests, which in the past could provide wood, making Leicester a good place to live from very ancient times (Matthew Morris, personal comment). There is archaeological evidence of occupation from the Mesolithic, while the urban settlement in Leicester dates to the late 1st century BC, and it is represented by an Iron Age community believed to be the southern tribal centre of the *Corieltavi*, (Buckley 2015). There has been human occupation in Leicester ever since.

As pointed out by Morris et al. (2011), in recent year the historic centre of Leicester has undergone intensive renovation and urban development. Such development resulted in a number of industrial buildings built in the 60's and 70's being demolished in order to build new commercial and residential areas. This provided archaeologists with the opportunity to expose and study a number of archaeological sites and revealed a new and detailed picture of life in Leicester since the Roman period. All such excavations were supported by extensive environmental samples, to allow a better understanding of the human activities, diet and living conditions of the inhabitants of Ancient Leicester, providing new and valuable data, a summary of which can be found in the recent review by Monckton (2015). The environmental data from such studies are an important resource for this study. The very recent Highcross project (2003-2009), conducted by the University of Leicester Archaeological Services, is the most relevant to this project as the two Medieval populations analysed in this study were dug during this project and will be introduced below. Finally, there is a very good summary of the archaeological and historical work conducted in Leicester in recent years in the recent volume edited by Elkin (2015) on Medieval Leicestershire.

4.2.1 The Highcross Project and the Medieval populations

Between 2003 and 2009, the University of Leicester Archaeological Services was involved in large excavations in the Leicester City Centre, associated to the re-development of a large retail area (Morris et al. 2011), known as The Highcross Project. Such excavations have contributed hugely towards our understanding of the nature and extent of past

human occupation in the city and its surroundings. The majority of the excavated area lied in a part of the North-East quarter of the Medieval town, also known as 'The Lanes', and revealed extensive human activity in the North-East corner of Medieval Leicester (e.g. Coward and Speed 2009; Monckton and Radini 2009; Radini 2009).

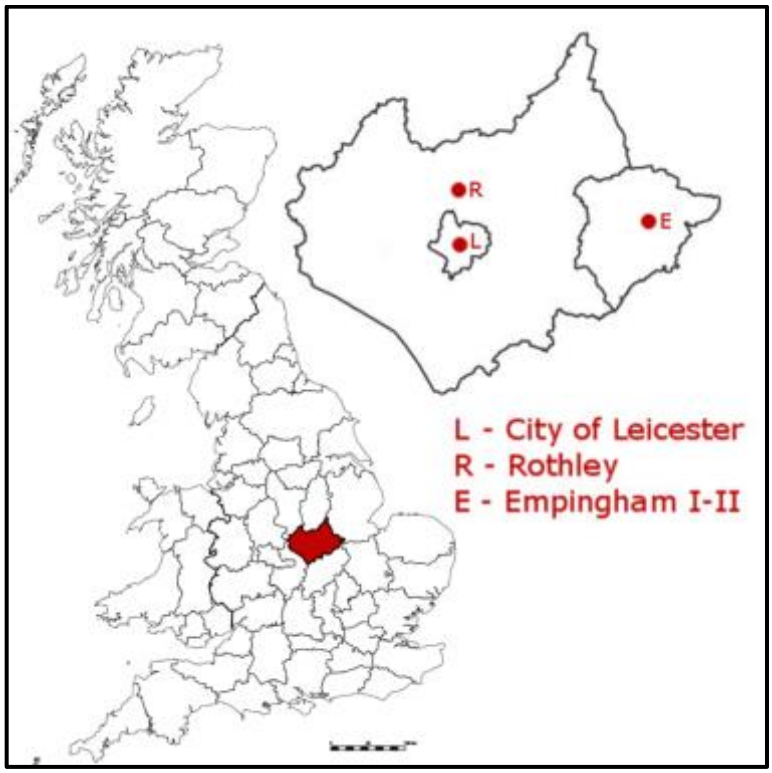

Figure 14. Location of the city of Leicester (L) in the heart of Leicestershire (in red) and locations of the studied populations.

Particularly outstanding was the recovery of two large Medieval populations excavated between 2005 and 2006. A total of 1553 skeletons (Jacklin, 2009, 2015) were unearthed from two cemeteries, those of St Michael's (figure 16-1, pg 78 Site A-A22/24.2003) and St Peter's (figure 16-2, pg 78 Site B-A2.2003), dating between the 10th and 16th century AD. Both cemeteries served the town's north-east quarter. The skeletal assemblages from both cemeteries show that the dental health of those living in Medieval Leicester was indeed poor, dental calculus was found in 76% of the adult population and 20% of the non-adult population (Jacklin 2015). This meant a large set of samples of human dental calculus could be obtained from those individuals while the archaeological background of the area was available. While skeletal remains of the Roman period were

available for this study, their number was not sufficient for this study. Furthermore, less then 50% of the skeletal remains had dental calculus, often in very low amounts. This prevented a sampling strategy sufficiently wide to allow the fulfilment of aims of this study.

Finally, the data obtained from recent excavations in the area and not only from the Highcross project were kindly made available for this study by ULAS and provide important sources of information regarding diet and living conditions for the county in the period under consideration, and also constitute comparative material for the evidence eventually retrieved from human dental calculus on the samples selected for this study.

4.2.2 The Anglo-Saxon populations of Rothley and Empingham

For the scope of this PhD, it was thought it would have been useful to contrast the urban populations with more rural setting, in order to establish if dental calculus could provide a resolution of environmental data sufficient to contrast urban versus rural setting. No rural skeletal assemblages were however available for the Later Medieval period, it was thought that population dating the Anglo-Saxon period, that lived in a less 'urbanised' environment would provide a valid comparison. Populations that lived in the Anglo-Saxon period in Leicester were not available for this project, however access was given to two populations from surrounding areas Empingham in Rutland and Rothley (Figure 14). Both Empingham and Rothley appear to have been substantial settlements in the Anglo-Saxon period (Mays 1996); by the Late Anglo-Saxon period, Rothley was a royal manor and had a priest (Upson-Smith 2016). The Anglo-Saxon cemetery of Empingham, dug in the 1970's, dates between the 6th and 8th century AD, and the grave goods suggest the participation of the population in long distance trade. There were 151 inhumations, many in poor state of preservation at the time of the sampling and only 18 individuals produced calculus samples large enough to be analysed (see Chapter 5). Mays (1996, 29) reports average estimated age at death to be between 18 and 25 years, lower than that known for the period, and low levels of both dental caries and enamel hypoplasia, potentially due to the young age at death of the individuals. The Anglo-Saxon cemetery in Rothley was dug in the past decade, by Northamptoshire Archaeology and dates between the 7th and the 9th century. Osteoarchaeological analysis was conducted but it

was limited by the poor state of preservation of the material, however there was a high number of identified males and the population appeared more robust than others of the same period for reasons that remain unknown (Upson-Smith, 133). Given the small sample sizes available due to preservation, the two populations are considered together as representative of the Anglo-Saxon (or Early Medieval) period and assumed to be rural in contrast with the urban populations of Leicester.

It must be stressed that comparisons between the Late Medieval urban samples and the Anglo-Saxon rural ones should take into account both their temporal distance and the different nature and scale of their habitation sites. As such, the Anglo-Saxon and the Late Medieval assemblages examined in the context of this PhD, represent two 'extremes', both temporally and with regard to living conditions complexity and are more likely to produce visible patterns.

4.3 General Archaeological Background

In this section I will introduce broad archaeological and historical information for the Medieval Period, providing an overall view of the data available regarding diet and living condition for the locations examined.

4.3.1 A thousand years of great changes

The period of about ten centuries considered in this study stretches from the decline of the Roman Empire to the Late Medieval period, that is, AD 400-1500, based upon the regional chronological division adopted by the Archaeological Research Agenda of the East Midlands (Cooper 2006). This period started with an Early Saxon society that was almost entirely rural and ended at the Late Medieval period with a socially complex, politically and economically organized, increasingly urbanised society, where urban centres in some cases were home to over 5000 people (Holmes 2014, Swanson 1999). Overall, the period witnessed the incoming of new peoples (i.e. Saxons, Vikings, Normans), the Christianization of Britain, the emergence and consolidation of the ecclesiastical power, the re-introduction and implementation of the monetary economy and the rise and consolidation of a feudal system. This is set against periods of great famine and outbreaks of pandemic diseases on a monumental scale (Campbell and

Grada, 2011). This period was also affected by great changes in the natural environment, such as large deforestation processes and the deterioration of climate that brought Europe into the Little Ice Age towards the 14th century (Büntgen et al. 2011; Fagan 2000).

4.3.2 The Anglo-Saxon Period

Initially the Anglo-Saxon period was characterized by rural settlements, very similar to one another, which consisted of kin-based groups that lived under one roof and produced crafts and food on a domestic scale as summarised by Holmes (2014), Hinton (1999), and Hooke (1998), although not all activities necessary happened under the same roof (Vince 1994). Gradually these settlements developed, first with the re-organisation of conglomerates of localized chiefdoms that characterized the Middle Anglo Saxon period, around the 8th century, and eventually the Medieval Kingdom of England was consolidated after the Norman conquest in the 11th century, as stressed by Lewis (2006, 185). Housing conditions are not very well known, as few left archaeological evidence, however, grubenhaus or sunken buildings are thought to be the main form of housing. These were made of wattle and daub walls and had wooden floors for food storage as well as hall houses (Hamerow 2011). Leahy (2011) provides a good overview of current work on the subject of crafts in the Anglo-Saxon period. In the context of the current PhD, what is particularly important is that all activities necessary for the functioning of the group from pottery to wood-work, would mostly happen inside the settlement, however there is also evidence of long distance trade (Huggett 1988). The scale of the activities as well as the structure of the buildings would be such that particulate matter generated by them would not be high outdoors and exposure to occupational pollutants would be very likely of indoor origin (Bernofsky 2010). For what concerns the natural environment, the Anglo-Saxon period considered here saw a rise in temperature before the decline known for the Medieval Period (Lamb 1995). The extent of forest cover is an open debate among scholars, however it is thought that woodland recovered in part from the deforestation caused during the Roman period. In addition, there is a strong cultural bond between Anglo-Saxon people and trees/forests evidenced in mythology, literature and place-names (Hooke 2010).

There is a general lack of information for the Anglo-Saxon period in the East Midlands, where all classes of environmental evidence have been found to be under-represented

(Monckton 2006, 279). Regarding the dietary patterns of these Anglo-Saxon groups, there is archaeological evidence in Leicestershire, mainly the site of Eye Kettelby (Monckton 2006) and Leicester (Radini 2009, see also Appendix I), that staple diet of the period consisted of cereals (bread wheat, barley, rye and oats) and legumes (peas and beans), as well as for the consumption of meat and game such as sheep, pigs and cattle, while poultry and fish are rarer but present (see Table 2), while the pollen record shows the cultivation of hemp in the area (Monckton 2006, 278), showing the importance of this fiber during this period. Table 1 and 2 in this Chapter provide an overview of the food available in Leicester and its surroundings for the period, while Appendix I lists in more detail the sites where plant remains have been found in Leicester and its surroundings. For what concerns the environment and use of land, pollen records from archaeological sites for the period are scarce, however, Greig (2004) have shown evidence for traditional hay meadows and floodplain cultivation.

4.3.3 The Medieval Period

An increased growth in population sizes, food production, wealth and trade networks began at the turn of the first millennium, a process which has been documented and studied across the whole of Europe (Lilley 2002, 1; Zupko and Laures 1996, 3). The need to feed a larger number of people prompted new technologies to allow better and more intensive use of the land: such as ploughing, crop rotation and manuring (Monckton 2006, 281), while studies have shown that the need for fuel resulted in forest clearance. The implementation of roads led to longer and more efficient trade networks (Zupko and Laures 1996, 3) and by the early 14th century 'almost everyone in England was within a daily reach of a market' (Swanson 1999, 10). The increased wealth, trades and markets generated new desires and occupations and resulted in an increasingly diversified society. For what concerns the Medieval period, Leicester was not different from the general trend of other Medieval urban centres, with an increased evidence of food production, consumption and trade across town from the 13th century onwards (Monkton 2015), together with an increased commercial and industrial activity (Buckley 2015). Archaeobotanical and archaeozoological analysis of a variety of deposits excavated during the work at the Highcross, thanks to an intensive sampling strategy that followed English Heritage prtotocols, is showing that a variety of food items became

increasingly available from the Norman Conquest onwards in the city (Browning et al. forthcoming; Monckton 2005). Table 1 (food of plant origin) and Table 2 (food of animal origin) provide an overall picture of the archaeobotanical and archaeozoological evidence available from the area where the selected populations supposedly lived based upon the excavation at Eye Kettelby, Leicester and Rothley. A full list of plant species and sites is provided in Appendix I at the end of this PhD.

Period	**Cereals**	**Vegetables and Garden products**	**Fruit and Nuts**
Anglo-Saxon c. 500-800 AD	Bread wheat Barley Oats Rye	Beans Peas Mustards Hemp (pollen)	Hazelnuts Bramble Sloe Elder
Medieval c. 1200-1450 AD	Bread wheat Rivet wheat Barley Rye Oats	Beans Peas Cultivated Vetch Flax (pollen) Leeks Mustards	Hazelnuts Blackberry Raspberry Figs Sloe Elder Apple Plum Cherry Grape

Table 1. Anglo-Saxon and Medieval most common plant foods available in Leicester and its surroundings for the periods of time considered in this study (based on review by Monckton 2006, Monckton 2015; Radini 2009).

Looking at Table 1, it is clearly visible that there is an increased archaeological visibility related to plant remains between the Anglo-Saxon and the Later Medieval Period. This is due to a combination of facts, from increased size of population, generating more waste, to the fact that often Medieval activity in town has truncated the existing Archaeological evidence for the Anglo Saxon period. It also makes clear the general lack of Archaeobotanical evidence for the Anglo-Saxon Period pointed out in the previous paragraph.

Period	Meat and Game	Poultry and Game	Fish from Freshwater	Sea fish and Shellfish
Anglo-Saxon c. 500-800 AD	Cattle, Sheep/Goat Pig Red deer Roe deer Hare	Fowl Goose	Eel Carp Family Stickleback Pike Perch	Herring
Later Medieval *c*.1250-140 Phase	Cattle Sheep Pigs Hare Rabbit	Fowl +eggs Goose +eggs Duck +eggs Teal Swan Pigeon Wood pigeon Starling	Eels Perch Carp Family Pike Stickleback Trout Roach	Herring Thornback- ray Cod Ling Haddock Oysters

Table 2. Anglo-Saxon and Medieval and environmental evidence: Foods of animal origin available in Leicester and its surroundings for the periods of time considered in this study (based on reviews by Browning et al. forthcoming; Monckton 2006; Monckton 2015).

A further and important aspect for this study is the following. An increasing number of people not involved in agricultural activities were attracted to or lived in urban areas, where they could make a living in occupations related to market and trades, manufacturing work and servicing industries of various kinds (Swanson 1999, 30). Such occupations may have varied between men and women. Both men and women would have been involved in agricultural work, although they would have performed different activities, such as food processing and spinning (Dyer 2000; Lilley 2002).

The occupational diversity and mobility that characterized urban centres by the 13th-14th century (see for examples from Dyer 2002) is of particular interest for this study as it would have generated exposure to different types of occupational debris across the urban dwellers, an aspect that will be explored in the relevant sections of this PhD. The result of many people living and converging in urban centres was a large amount of different types of waste and air, water and soil pollution in the urban centres and their surroundings. The problem of disposing of anthropogenic waste must have been monumental, especially considering the limited space within the city walls, as already noted by Zupko and Laures (1996, 50). The effort to regulate the manner of the disposal

of urban waste has left different forms of records, such as written regulations relating to when and how to dispose of waste, and the location in which various activities perceived as polluting could be carried out, or in the form of archaeological deposits. Evidence of pollution generated by human activities and resulting in pollutants in water and air is also documented archaeologically and in the written records. Lead and copper contamination of water and air, for example, has been recovered from soil, peat and sediments, and shows high levels of water and air pollution even in the pre-industrialized era, and in particular in the Late Medieval Period: recent studies of peat bogs in Central England and Wales have provided records of atmospheric pollution caused by lead and copper in the same period (Le Roux et al. 2004; Mighall et al. 2009). An increased sense of awareness of 'bad air quality' resulted in a proclamation in London in 1307 banning the use of coal in the city. This is thought to be the first law to control air pollution in an urban environment (Brimblecome 2011). It is therefore safe to assume that individuals that lived in the rural settlements of the Anglo-Saxon period experienced different conditions from those who lived in an urban centre.

During the above project, evidence of tanning, malting, iron smelting and corn drying kilns (see fig. 15 for an example) have been attested within Medieval Leicester and its suburbs, and in particular in the north-east quarter, in particular for the period between AD 1100 and AD 1500 (e.g., Coward and Speed, 2009; Monckton and Radini 2009; Radini 2009). All of these activities required large quantities of fuel, be it wood or coal, which is also documented in the archaeological record (e.g., Radini, 2009).

Intestinal parasites were common in the soil in Medieval Leicester (Monckton 2006, 281) and therefore the population must have been exposed to this type of pathogens too. Moreover, there is archaeological evidence of animal keeping, with finds of piglets, in the urban area in the North East quarter of the city, for example from the site of Freeschool Lane (Coward and Speed 2009) and domestic pets, such as dogs but also cats, being regular people companions (Browning et. al. forthcoming). All of these could have contributed to dirt and pathogens. Further details on the archaeological evidence relevant to this study will be given in the Results Chapters (Chapter 6 and 7) and in the Discussion and Conclusions (Chapter 9).

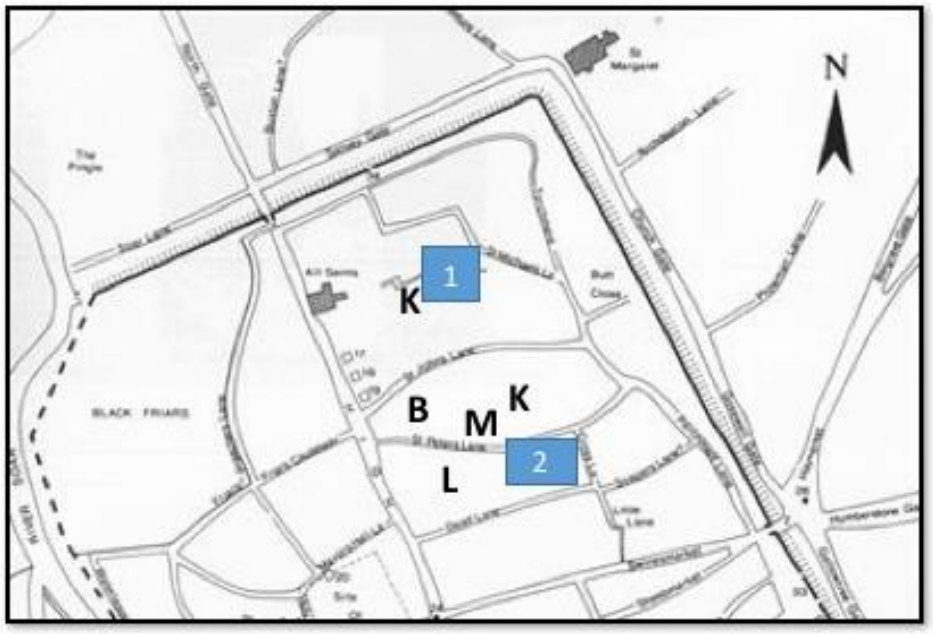

Figure 15. An example of human activities in the North-East corner of Medieval Leicester. 1= St Michael's Cemetery; 2=St Peter's Cemetery; K=kilns; B=Brewery; L=leather work; M=metal work.

This is very likely to have resulted in pollution in the form of chemical compounds, organic and inorganic dust, and smoke.

4.3.4 Implications for this study and hypotheses

When we take into consideration the points explained above, it can be said that the urbanization process generated more 'crowded' urban environments, where an increasing number of people lived closer to the sources of various different types of pollutants and occupational dust. The exposure to 'dust' and 'micro environments' experienced by individuals living in an urban setting must have been linked not only to housing conditions but to differential exposure to 'pollutants' generated by how the work was distributed across the population and likely depended on age (age in which the individual entered the work force), sex (in the sense of sexual division of labour) and social status (type of occupation). These environments were very different from those experienced by people living in the same area in earlier periods, characterized by more rural societies such as that of the Anglo-Saxon period. Due to established trade networks, Later Medieval populations could potentially gain access to a greater variety of imported

foods than those living in preceding periods. If dental calculus is to be approached as a source of information on pollutants and different types of dust and debris of low archaeological visibility, and how they change across a population and throughout time, targeting a large Late Medieval urban population for which a large set of archaeological data are also available and comparing it with a smaller dataset from a 'rural' way of life is an ideal place to start. In the following section I will highlight why the populations selected fit the purposes of this study and at the same time may help to provide some new information against specific areas of research that are needed, as identified by the Research Agenda of the East Midlands since 2006 (see dedicated paragraph in this chapter).

4.3.5 The Archaeology Research Agenda in the East Midlands

In recent years, members of ULAS have been proactive in contributing data and site records to several surveys not only for the county but also for the region of the East Midlands. These collaborations resulted in the publication of the Research Agenda for the East Midlands [4], of which Leicester and its county is roughly in the middle. The research agenda set a very clear research framework for the archaeological and historical environment, and the most urgently needed work in the region of the East Midlands (the counties of Derbyshire, Lincolnshire, Nottinghamshire, Northamptonshire, Leicestershire and Rutland). The main areas highlighted by the research framework by Vince (2006, 183) and Monckton (2006, 283) and most relevant to this research are the following:

- An urgent need for any archaeological evidence for the entire Anglo-Saxon period, in particular any evidence of crafts and food consumption
- A need for archaeobotanical data for wild gathered food
- A need for data regarding plant food with low archaeological visibility, such as leafy crops, roots and legumes.

[4] available online here: https://www.le.ac.uk/ulas/publications/eastmidsfw.html

- A need for data regarding plant food imports (e.g., rice, ginger and galangal), which are present in the historical record of Leicester (Browning et al. forthcoming), and present in Britain during the period under study (Livarda 2011).

Despite the fact that 10 years have gone by these targets have remained unchanged in the updated version of the Research Agenda[5] that became available during the years of this PhD. This study therefore will potentially contribute to the Research Agenda.

4.4 Summary and conclusion

Due to a shift from a rural society to a more urbanised one, populations dating to the Anglo-Saxon and the Later Medieval Period experienced very different living conditions. Recent excavations in the North East quarter of the Medieval city of Leicester unearthed two large skeletal assemblages dating to the Later Medieval Period. The overall good state of preservation and the presence of dental calculus in the majority of the individuals that were excavated made this an ideal sample for the current study. Furthermore, a sufficiently large number of individuals dating to the Anglo-Saxon period, excavated in Empingham and Rothley, was made available as a comparative dataset. Data from the dental calculus of these populations may provide new evidence for a better understanding of how representative dental calculus is of diet and environment if approached at population level and may provide new insights regarding diet and overall living conditions throughout the Medieval period and can contribute to the fulfilment of the goals set by the Archaeology Research Agenda of the East Midlands.

The majority of the archaeological work in Leicester, and its county, has been conducted in the past 20 years by the University of Leicester Archaeological Services (ULAS), for

[5] available here: http://tparchaeology.co.uk/east-midlands-research-strategy/East_Midlands_Heritage.pdf

whom the author has worked for the past 10 years. ULAS is a commercial Archaeological Unit embedded in the School of Archaeology and Ancient History at the University in Leicester. It became clear therefore, from the very initial stages of this study that the two populations unearthed in Leicester and the Anglo-Saxon populations from Rothley and Empingham where dental calculus was recorded, would provide the ideal archaeological material for the current study. Moreover, any data retrieved from the Anglo-Saxon population would contribute to the knowledge of this period, which is very poorly represented in the archaeological record in the county and in the region.

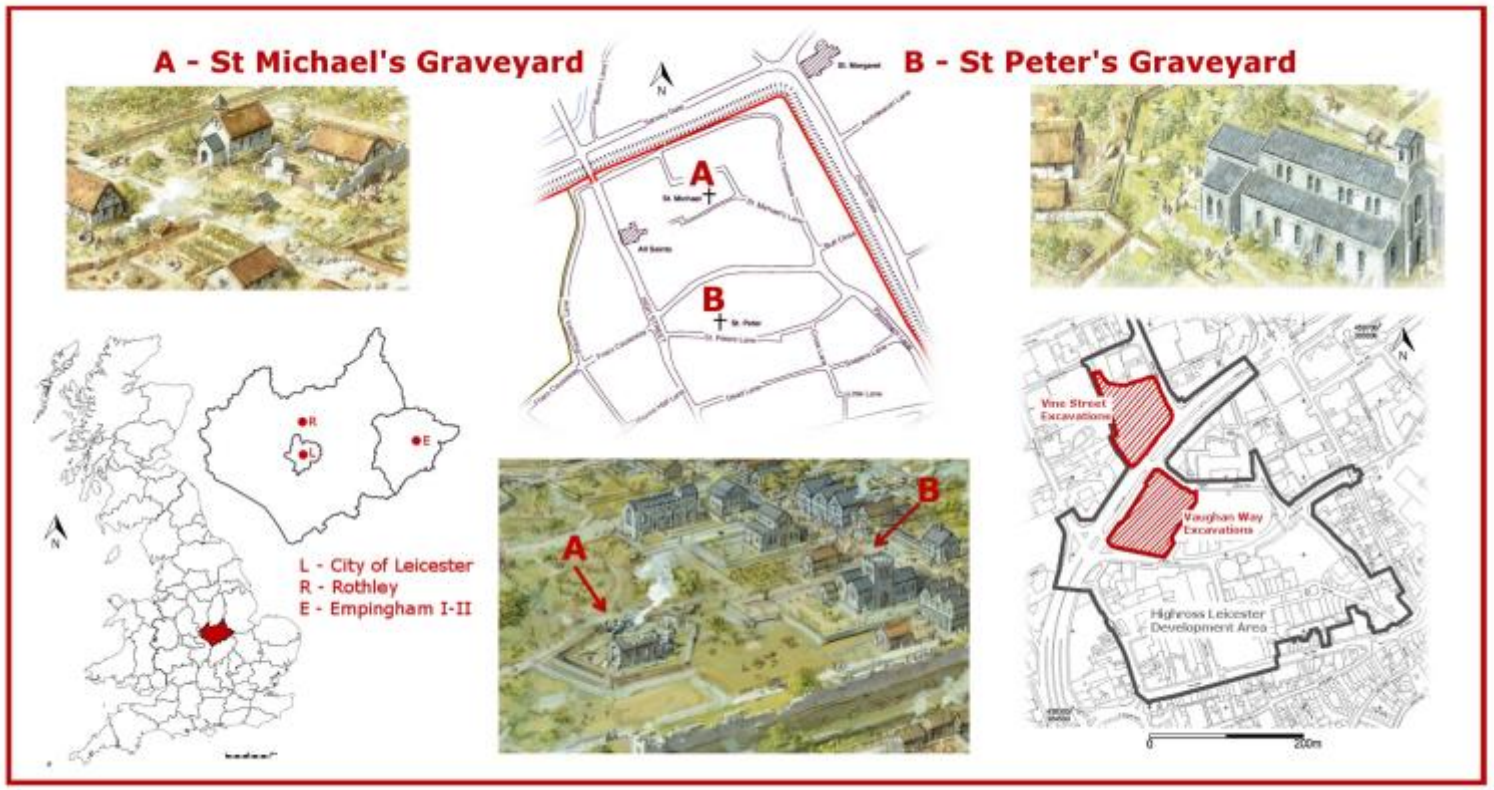

Figure 16. Clockwise, location of the city of Leicester and its county. A reconstruction of the Parish of St Michael's and its location within the Medieval city wall and its proximity to the parish of St Peter's. Locations of the graveyard within the Highcross project and, finally, an artistic impression of the North East Quarter of Medieval Leicester, with the two churches (modified artistic impressions by Mike Codd, in Morris et al. 2011)

CHAPTER 5
MATERIALS AND METHODS

5.1 Introduction

If the calculus is a reservoir of micro-debris, chemical compounds and biomolecules of both dietary and non-dietary origin, all lines of evidence should be carefully considered. It is, therefore, essential that the sampling and processing procedures are conducted in a manner that avoids any contamination from modern and ancient remains that are common in many soil types, such as fungal debris, pollen, microcharcoal and phytoliths.

Where extraction of microfossils is conducted, it is necessary to take into account that different remains have different properties: crushing the samples for instance (see Hardy et al. 2009; Henry et al. 2010) may damage long plant fibres and other large debris, with the consequence of losing potential useful features for identification. Equally, some studies have dissolved calculus and removed the natant: as the density of different types of remains is different, it may be necessary to scan the natant for micro-debris that may float in it for density reasons. An additional important step is the creation of reference collections that allow the identification of plant species and their part that may be of use for crafts and not just diet. Recording of remains that are not targeted in the study should also become standard practice.

In addition, different burial practices and soil types affect the contamination on the calculus surface very differently. Therefore, it must be acknowledged that a degree of flexibility is needed in the selection of technique adopted for the study of samples from distinct contexts, especially for what concerns the processing and extraction procedures of micro-debris to be studied under the microscope. However, considering the human mouth as a potential 'dust trap' and the calculus as a deposit forming in it, can affect all stages of dental calculus research from sampling to interpretation.

In Chapter 2 it was highlighted that it is not possible to know what part of an individual's life is represented in the calculus matrix and this has been identified as a potential limiting factor in dental calculus research. In an attempt to reduce the window of time

represented in the calculus samples as much as possible, the following aspects were considered when targeting the remains: estimated age at death of the individual and estimated age of tooth eruption. These were combined to obtain a 'calculus formation interval' as short as possible. Furthermore, as one of the objectives of this PhD was to test dental calculus for known non-dietary debris of potential occupational origin, individuals of working age were targeted. Finally, Warinner et al. (2014) have shown that the potential of biomolecular analysis is very high for the Medieval period. Samples therefore were taken from those individuals that had multiple calculus deposits, to allow for future studies.

In light of the above considerations, this chapter presents and details the criteria used for the selection of the individuals within the examined populations and the methodologies adopted for unlocking the evidence entombed in the dental calculus matrix. The selection criteria and the adopted methods were dictated by both the aims and objectives of this study but also by the literature review performed in Chapters 2 and 3. How such aspects affected the selection of the individuals analysed and the protocols adopted is explained at each relevant step of the research.

The methodologies of extraction and identification of the material entombed in calculus was published in supplementary information of the following paper:

Warinner, C., Rodrigues, J.F.M., Vyas, R., Trachsel, C., Shved, N., Grossmann, J., **Radini, A**., Hancock, Y., Tito, R.Y., Fiddyment, S. and Speller, C., et al. (2014). Pathogens and host immunity in the ancient human oral cavity. *Nature genetics*, *46*(4), pp.336-344

5.2 Materials

In Chapter 4 it has been pointed out that the populations dating to the Anglo-Saxon period were preserved in a poor condition, especially when compared to those of Later Medieval period (1250-1450 AD). Moreover, different osteoarchaeologists worked on the skeletal material with a gap of up to 30 years between studies. Therefore, sampling strategies followed for the populations mentioned above varied accordingly. A full list of the skeletal material examined, sex and estimated age at death are provided in Appendix 1, together with the results of this PhD.

5.2.1 Anglo-Saxon individuals (500-900 AD - Empingham and Rothley)

The main criterion of sample selection was the size of calculus on the teeth since decontamination procedures can result in the partial loss of calculus matrix (see decontamination procedures in section 5.5.3). Due to the fact that the preservation was very poor and the material had been handled by a large number of individuals, Anglo-Saxon material had low potential for biomolecular analysis (Prof. Matthew Collins, personal communication). However, Hardy et al. (2015) have shown that analytical chemistry can be successfully applied to very small samples, below 1 mg, even in material as old as 400,000 years of age. Therefore, calculus samples selected for this study were split in order to allow for future research. A total of **31** individuals were selected for this project, as many individuals had no skull or the calculus had dropped off. Given the poor preservation of these remains, in many cases calculus samples mentioned in the available reports were no longer on the teeth. Despite the large skeletal assemblages available for the Anglo-Saxon period, dental calculus samples were limited: only **18** individuals were available with dental calculus from the Anglo-Saxon cemetery of Empingham I and II (note that these are the same population, dug in two different years, in this study therefore they will be referred to as one), and **13** from the Anglo-Saxon cemetery of Rothley La Grange. These samples provided a dataset spanning 500 to around 900 AD, therefore pre-Conquest. Sample concordances with skeleton number are provided in Appendix I Table A1.1 .

5.2.2 Medieval populations (1250-1450 AD - Leicester)

As a consequence of the criteria mentioned in the introduction of this chapter, wherever possible, adult individuals were selected where the third molar (the last tooth to erupt - Buikstra and Ubelaker 1994) had erupted or was in the process of erupting, so that the gap between the formation of the calculus and the death of the individuals examined was as short as possible, similar to one another, and therefore comparable. A total of **180** individuals, **90** from each of the cemeteries of St. Michael's and St Peter's were selected. At least 38 males, 40 females and some unsexed adults/juveniles of working age, were sampled for each cemetery, to allow for a statistically valid data set, around 30 individuals of each sex (Dr. Efthymia Nikita's personal comment). Note that the sex of the individuals was assessed using standard pelvic and, secondarily, cranial sexually

dimorphic traits (see Buikstra and Ubelaker 1994). All selected individuals dated between 1250 and 1450 AD.

Sample concordances with skeleton number are provided in Appendix I Table A1.2 (St Peter's) and A1.3 (St Michael's) together with the amount of calculus that was sampled.

5.2.3 Known existing biases

The following biases that could not be overcome are present in the data set:

- The poor preservation of Anglo-Saxon populations reduced the dataset available for this time period.
- In order to allow for future research, only calculus from individuals with multiple deposits was analysed.
- Due to the fact that the Parish of St Peter's did not have such a restricted window of use as the Parish of St Michael's, skeletal material was chosen where stratigraphic or C14 dating provided a date between 1250 and 1450 in order for the results to be comparable with the dataset from St Michael's.

As a result of the above criteria, the skeletal remains cannot be considered a random selection of individuals. However, none of the employed criteria was based on cultural markers of social status and occupation, which may have biased the obtained results (e.g., only specific patterns of activity and diet would have emerged if only low status individuals with selected occupational markers had been intentionally examined). Therefore, the sampled individuals are expected to provide a dataset representative of the overall population of the North East Quarter of Medieval Leicester and its surroundings, especially during the Late Medieval period. However, it must be stressed than no known high status individuals were sampled for this project, due to lack of calculus on their teeth.

5.3 Methods

Laboratory procedures to unlock the evidence entombed in human dental calculus samples from the selected populations conformed to the literature review and the theoretical framework highlighted in Chapters 2 and 3, as described below.

5.3.1 Sampling and recording of the calculus

Samples of calculus were removed from the tooth and weighed on a micro-scale. The calculus deposit was then placed in a sterile tube and bagged, making sure the details of the individual sampled were preserved. All above stages were conducted at the facilities of the University of Leicester Archaeological Services.

5.3.2 Sample preparation

Microfossil analysis was performed at the Laboratory of Starch Analysis at the School of Archaeology and Ancient History, University of Leicester, and at the Laboratory for Microarchaeology at BioArCh, University of York. Dental calculus from each ancient human was investigated using optical microscopy for the presence of preserved microfossils. Samples were weighed before analysis and then transferred to sterile 15mL centrifuge tubes.

5.3.3 Contamination precautions

As with all microfossil analysis, it is essential to take precautions to prevent and monitor potential sources of contamination. The three main sources of contamination in ancient dental calculus research are:

- Microscopic food contamination from the hands of the analyst.
- Modern contamination from airborne pollutants.
- Modern contamination from settled dust transferred to the material during collection, preparation, and analysis.
- Fungal and bacterial remains included in postmortem putrefaction fluids (Blondiaux and Charlier, 2008: 4-5) and subsequently adhering to the external part of the calculus during decomposition, as well as soil contamination by other microorganisms in the ground.
- Burial and modern soil attached to the dental calculus in micro-cracks not visible to the naked eye (fig. 17).

The following procedures were followed to avoid analyst contamination:

- Sterile masks and gloves were worn at all times.

The following procedures were taken to avoid modern contamination by airborne pollutants or dust:

- Samples of settled dust were regularly collected in the area where the procedures of extraction and mounting were to take place.

The following precautions were taken to avoid soil contamination:

- The 15mL tubes containing dental calculus were filled with ultrapure water and vortexed at high speed to liberate any loosely-attached debris or soil contaminants present on the surface of the dental calculus.

Once washed in ultrapure water, the calculus was transferred to a new 15mL tube. This sequence was repeated until the water remained clear.

- As a final step, calculus deposits were bathed in 0.04M HCl for five minutes and sonicated for 30s to encourage the removal of their outermost layer along with any residual contaminants.
- Calculus samples that were too small were cleaned using an acupuncture needle and HCl under the microscope until the entire surface appeared clean.
- Finally, all calculus deposits were inspected under incident light microscopy at magnification up to 400x: deposits considered clean were examined to ensure that no soil adhered to the surface of the calculus itself.

The results of such decontamination procedures can be seen in figure 18, which depicts calculus during two of the main stages of decontamination, and the final fragment completely clean. As the process of decontamination generated loss of material, the calculus samples were weighed after cleaning so that the weight provided in mg is the effective weight of calculus analysed. The average loss of calculus in the processed samples ranged between 0.1 (in the small samples) to 0.5 mg (in the largest samples).

As a final precaution, the soil removed from the calculus surface was kept as reference in case soil contamination was suspected. The amount of time needed for the above procedures was found to be variable (anything between half a day to up to a week), and depended on the amount of soil adhering to the calculus.

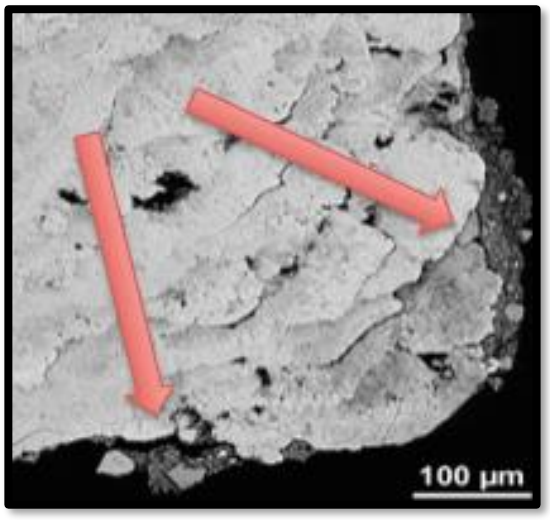

Figure 17. A fleck of dental calculus with some 'dirt' still adhering to it'. The arrows indicate areas of calculus with adhering soil, which need to be removed securely before dissolving the calculus sample (picture from Warriner et al. 2014).

5.3.4 Developing new procedures of extraction

Several recent publications were studied in order to establish a protocol of extraction of microfossils from the calculus matrix (Hardy et al. 2009; Henry & Piperno 2008; Henry et al. 2010; Piperno & Dillehay 2008). Methods of extraction varied from author to author, according to the background experience of the researcher. All of them face the same problem: in order to observe the micro-remains under the microscope, the calculus matrix needs to be dissolved. Two major approaches have been chosen in recent modern studies: chemical (e.g., Hardy et al. 2008; Li et al. 2010) and mechanical extraction of the debris (Henry et al. 2012). The non-destructive method of removing small flecks of calculus at different intervals, with the help only of a tooth pick, and placing them under the microscope (Henry et al. 2012) does not always produce the expected results of being able to see micro-fossils (Li et al. 2010). Dissolving the calculus is, therefore, necessary. Literature review highlighted that a variety of debris can survive in the calculus matrix, thus it was decided to develop a protocol that did not crush the calculus, potentially damaging the micro-debris, as done by Hardy et al. (2008), and that allowed the scanning of the entire content of the calculus including what may be present in the natant formed by HCl and dissolved calculus, which many authors actually remove (e.g.,

Li et al. 2010). The process described below also allowed the observation of particles that entered the calculus in clusters (fig. 19).

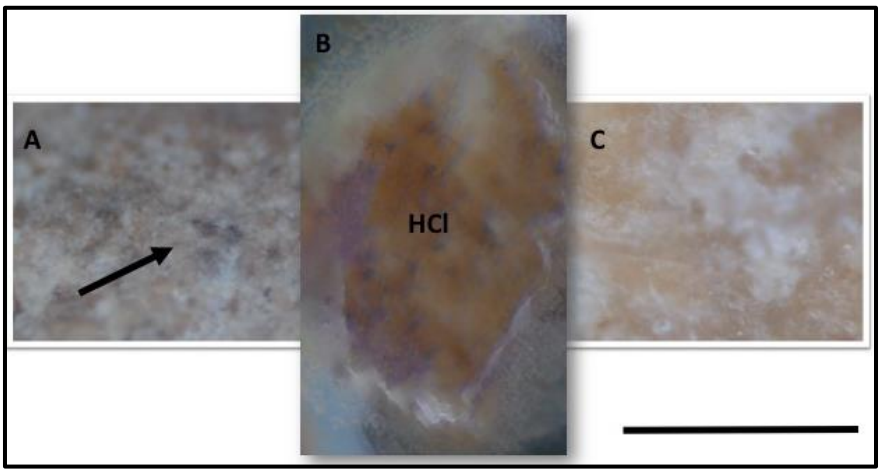

Figure 18. Fleck of calculus under incident light microscopy. A: fleck washed in ultrapure water four times, however potential dirt (see arrow) still adhering to the surface. B: calculus bathed in a weak solution of HCl (note the CO_2 generated by the calculus matrix dissolving in HCl). C: calculus fleck completely clean. Scale bar: 0.5 mm.

5.3.5 Extraction and mounting

Following decontamination procedures, the calculus samples were transferred to tubes containing 0.05M HCl and allowed to decalcify under agitation for one week at 4˚C. Microfossils and micro-debris released by the decalcifying dental calculus were then carefully collected by inserting a sterile pipette into the bottom of the tube and transferring the insoluble particles to a sterile microscope slide. To the remaining undecalcified calculus fragments, the tube was replenished with 0.05M HCl and the sample was sonicated for one minute, and then allowed to further decalcify under agitation at 4˚C for five days. The above procedure was repeated up to 11 times, until the dental calculus was completely dissolved. At each interval, the liberated microfossils and micro-debris were mounted onto a sterile microscope slide so that the decalcification process could be monitored in sequence. This method of extraction was developed because it allows for a *pseudo-in situ* visualization of the micro-debris. Using this method, it was possible to observe microfossils still partially embedded within the dental calculus matrix (an example of which is provided in figure 19), thereby validating the ancient

origin of the microfossil. Using this method, it was also observed that certain kinds of debris entered the dental calculus in discrete quantities or lumps, and it was also possible to record all the remains, as the natant was never removed.

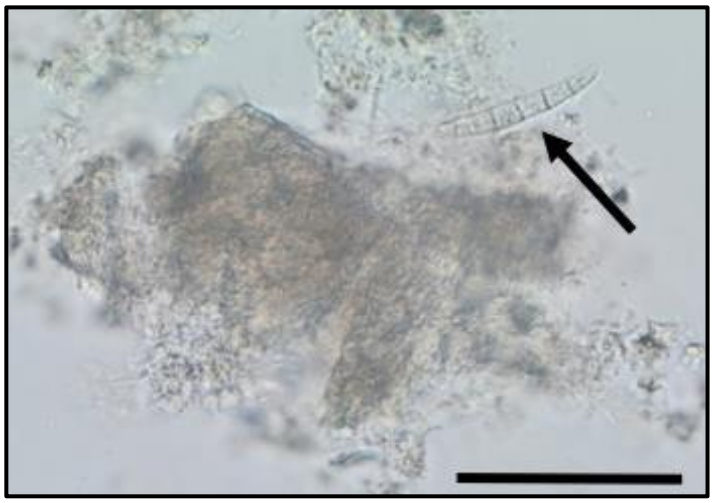

Figure 19. Fungal debris (arrow pointing at cf. *Fusarium* sp., see Chapter 7) in the process of being 'liberated' from a calculus fleck (the pale yellowish brown mass surrounding the fungal debris). Scale bar: 50 microns.

The microfossils and micro-debris removed from the decalcification tube were mounted onto glass slides to which a drop of a 50:50 mixture of glycerol and water had been added. This is necessary for microfossil description and identification, as it allows the rotation of three- dimensional objects such as phytoliths, pollen, and starch granules. A sterile cover slip was added to the slide, and a drop of clear nail polish was placed at each corner of the slide to keep it in place. This procedure leaves the side of the slide unsealed, allowing staining or rehydration if necessary. Following analysis, the slide was sealed on all sides with nail polish before archiving.

5.3.6 Identification of microfossils and micro-debris

Slides were analysed using a Zeiss and an Olympus compound microscope at magnifications of 200x, 400x, and 630x (oil immersion), as well as under polarized light. A diverse range of microfossils and micro-debris was observed, with individual finds ranging in size from sub-micron particles to structures with dimensions in excess of 200 microns. All finds were described and recorded, and any new typology was photographed. After analysis, the slides were archived at 4˚C. Material described as *in*

situ consists of micro-debris that were recovered still partially embedded within the calculus matrix or showing other clear evidence of dissolving calculus adhering to it. Micro-finds up to 50 microns in diameter could be rotated, implying that the glycerol:water layer was at least this thick. As multiple layers of debris can overlap within this space, various techniques were employed to isolate the finds for better viewing. A fine acupuncture needle was used to impart light pressure on the cover slip of the slide in order to make the micro-debris move and turn. Normally if the remains were indeed *in situ*, the dental calculus would hold the debris in place or restrict their movement. Overlapping micro-finds were also investigated by adjusting the optical zoom. When the inspected debris was located just above (rather than inside) the fleck of calculus, gentle pressure on the cover slip resulted in the micro-debris and calculus flecks separating, often moving in different directions. Criteria of identification adopted and nomenclature of the identified finds is provided in the results sections of this study. The identification of the micro-remains was made possible by a number of published and online resources, mentioned in the results section of this PhD (Chapter 6 and 7), and with the help of a very large purpose-built reference collection.

5.3.7 The reference collection

In addition to published resources, a good reference collection is essential for accurate microfossil analysis. For this analysis, a custom-built dietary reference collection was consulted that includes pollen grains, starch granules, and phytoliths for the most common known major plant crops, weeds, and wild taxa used for food, economic, and medicinal purposes in Northwest Europe (an example is provided in figure 20) with a particular emphasis on plants used and known to be present in the Medieval period and in particular in Leicester (a good summary is also available in Monckton 2015). The reference collection also expanded according to the remains found. For example the finding of potential imported items such as millet, made it necessary to collect species of the tribe Paniceae that could have been found in England during the Medieval period, in order to compare the species to one another and confirm the identification. Many species were collected from different locations (eg. York, Leicester and surroundings) to assess variability due to growing conditions. Please note that due to the very large

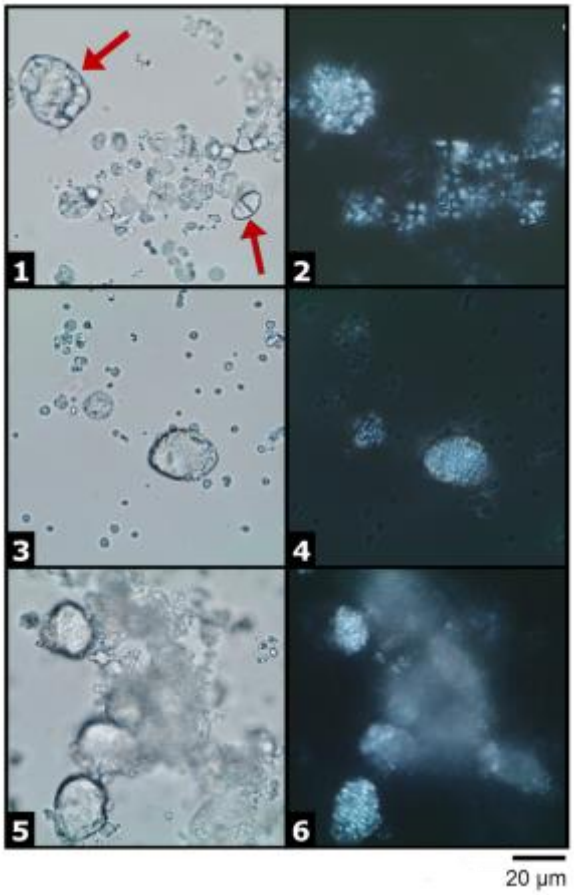

Figure 20. An example of species of the tribe Aveneae (oats) showing very similar compound granules between different genera: 1-2 *Avena sterilis*, 3-4 *Phalaris minor*, and 5-6 *Phalaris paradoxa*. Observe that it can be very difficult to distinguish the 2 genera upon the starch granules alone.

reference collection, details of species used for the identification are provided in Chapter 6 and 7 with the results. In addition to unprocessed material, the collection includes chewed, cooked, and otherwise prepared starchy material wherever needed. As for the necessity to split dietary versus non-dietary debris identified in Chapters 2 and 3, an additional reference collection at the University of Leicester Archaeological Services (ULAS) containing textiles (e.g, cotton, flax, hemp, ramie, wool and silk), pottery fragments, and a variety of environmental and occupational pollutants (e.g., wood dust, charcoal, and ash) was also consulted during identification .The reference collection was assembled by the author of this PhD and is constantly expanding, it has currently reached over 800 entries. Many plants were collected by the author herself, while other plant

reference collections present in the Department of Archaeology at the University of York were also consulted. While it was not possible for reason of times to take micrographs of all material viewed, the reference collection has been made available to other researchers and steps have been taken for this resource to be shared on line in the near future.

5.3.8 Quantificaitons and nomenclature of the micro-debris

The presence of all types of particles, such as mineral debris, pollen and spores, starch granules and phytoliths, micro-charcoal and soot was recorded. All the identified debris was counted and possible pathways to inclusion were postulated (see results and discussion), however it was not possible to securely quantify the lithological evidence, for reasons that will be highlighted in the relevant session in chapter 7. All particles apart from soil and minerals, therefore, represent absolute numbers. They were compared to one another by items/mg, to overcome the problem of different weight of calculus samples. This is detailed in the introduction of Chapter 8. The level of identification varied hugely among finds, with species identification rarely achieved and in some cases the level of Kingdom was the only taxon possible. The reliability of the identification proposed to the Family, Tribe and Genus level can be considered high, as such steps in the identification were conducted if the author felt that sufficient material had been viewed to assess variability of morphology trait in the proposed taxonomic level. Following common practice, the word *cf.* has been put before genus or species, when the comparison with other species or genus could not be carried out in full or it was not sufficiently clear. Moreover, the word *type* means in this context that the microfossil has been described only on the base of morphology, and the identification presented is an indication but cannot be considered secure. The identification described as *cf.* has a higher level of reliability than the identification described with type. Finally, plant nomenclature follows Stace (2011) for British native species, and Flora Europaea for the non-native taxa. Results are presented in Appendices at the end of this thesis.

5.3.9 Data Analysis

As the main objective of this PhD is to address the usefulness of ancient dental calculus towards archaeology, the following main aspects were tested using statistical analysis:

- Univariate analysis was used to describe the distribution of different types of debris within each sample and to explore whether statistically significant differences exist between sexes and between the Anglo-Saxon and Medieval Populations
- Mutivariate analysis was used to establish if diachronic changes existed between the Medieval and the Anglo Saxon samples, as well as between sexes, combining multiple related types of microdebris. This analysis expanded upon the univariate one, allowing the exploration of broader patterns, taking in account the fact that many different types of microdebris may relate to the same dietary and occupational activities, thus it is more meaningful to explore these together.

Also, whereas univariate tests only showed whether a statistically significant difference exists between two or more groups, multivariate tests allowed the identification of clusters of individuals with similar microdebris concentrations, thus with similar diets and activities. To be statistically analysed the counts of different categories of microdebris were transformed into concentration/mg. Given that the resulting data cannot be considered parametric, but ordinal in nature, non-parametric univariate and multivariate tests were adopted. Specifically, when only one type of microdebris was compared between males and females or between Medieval and Anglo-Saxon groups, Mann-Whitney tests were employed, while the same comparisons employing more than one categories of microdebris were performed using non-parametric MANOVA. To identify clusters of individuals exhibiting the same types of microdebris, Principal Component Analysis and Hierarchical Cluster Analysis were used. All analyses were performed using the freely available statistical software PAST v. 3.10 (Hammer et al. 2001).

5.4 Archive of the material

This research generated two archives of dental calculus mounted slides and a personal reference collection used for the identification of micro-debris.

5.4.1 Dental calculus mounted slides

It has been agreed with the Oakham Museum and City of Leicester Jewry Wall Museum that the aforementioned slides can be retained by the University of York in order to

improve the identification of the debris in the future and as teaching material (see Chapter 9: Future Directions). A full list of sampled calculus has been provided to the respective museums as a record of what has been removed. Skeletal remains have been returned to the Museums where they were originally stored.

5.4.2 The reference collection

The reference collection is currently stored at the Department of Archaeology at the University of York, which is the employer of the author of this PhD. Although only modern micro-debris relevant for this study has been so far photographed, the reference collection has already been made available to a number of external projects. A full list of species prepared for this PhD is in the process to be uploaded on the Departmental web site. I would like to stress that this PhD is submitted with an embargo of 2 years, by the end of which the web-site of the Department may have changed several times. Moreover, it is not known where the author will be working in the future and the reference collection will be moved with the person that conducted this PhD. In order to overcome the problem the list of species for the reference collection will be deposited with the PhD at the Leicester Museums Archive, where the populations are kept, at the end of March 2017. It will be therefore available by the time this PhD becomes on public domain. It was thought this was the more secure way to provide a long-term archive for such list.

5.5 Summary

A total of 211 individuals were sampled for this study following criteria to reflect the aims and objectives of this PhD. Samples were processed according to a protocol developed for the purpose of this study, which allowed the majority of debris to be viewed almost in situ. The micro-debris was quantified and described in its entirety. The remains were identified using a built for the purpose reference collection as well as published and on line resources. The reference collection and the slides containing the ancient finds are available upon request as teaching and training material and also as a comparative resource for future studies.

CHAPTER 6

RESULTS KINGDOM PLANTEAE

6.1 Introduction

This chapter presents the results of the analysis consisting of plant remains.

For the scope of this PhD, all micro-remains that could be identified to a taxonomic level were separated into groups representing the anatomical part of the plants they came from (e.g., starch granules, phytoliths, pollen grains). For each category of remains the following information is provided:

- criteria of identification, their reliability and potential "look-alikes"[6] are clearly expressed
- pathways to inclusion of each typology of identified debris are postulated, although not all possibilities are included
- presence or absence from the archaeological record is assessed, using the most recent summary work in the region (Monckton 2015) and other published and unpublished resources (eg. Monkton 1995, 1996, 1998, 1999; Monckton and Radini 2009, 2010; Moffett 1991; Radini 2009)
- any future work needed to improve identification is highlighted

Wherever relevant to the identification, a micrograph is provided for each category of identified remains, often with a modern example. All debris found to be of plant origin was recorded and counted, even when it could not be securely identified to any taxonomic level, in order to provide a reliable picture of the all finds in dental calculus that belonged to the Kingdom Plantae. Due to the variable amount of calculus samples in

[6] In this context the word *look-alike* refers to all typologies of debris that could potentially be mistaken with the proposed identification

terms of weight, and the fact that the size of the deposit affects the quantity of debris entombed (Leonard et al. 2015, see also Chapter 8), results are given in items/mg. All results of this study are given in Tables in Appendix II, category by category with the following format:

1. the data follow a temporal order, giving the Anglo-Saxon data in the first rows
2. The Medieval data are given by cemetery: SM stands for St Michael's and ST stands for St Peter's.
3. Data are organised within each period and in each cemetery by sex.
4. Finally, as many categories of remains were not very common, only individuals that had debris in a specific category are listed in the tables.

Criteria of identification and observations of their pathways to inclusion were also applied by the author to a number of authored and co-authored published papers (Buckley et al. 2015; Hardy et al. 2015; Lucarini et al. 2016; Radini et al. 2016a and b; Warinner et al. 2014a) and will be quoted in support of the identification (as peer reviewed) in the relevant sections below.

6.2 Brief overview of the results

Before providing the results related to the Kingdom Planteae, here I will provide a brief overview of the finds. The analysis yielded a great variety of remains, from all Kingdoms of life and of lithological origin, ranging from starch granules to small particles of mineral grit. Level of identification varied depending on size and type of particles and their level of preservation, but in general identification to species level proved impossible for many remains. All individuals in this study had micro-debris entombed in the calculus matrix. For what concerns the plant remains, they were the most represented category of remains in the calculus matrix. The more specific identifications were reached for plant tissues consisting of the epidermis of seeds and seed capsules such as corncockle seeds (*Agrostemma githago* L.), hemp seeds (*Cannabis sativa* L.) and the capsule of flax (*Linum usitatissimum* L.), which are known to be diagnostic often to such level of identification (Hall 2003, 107). Starch granules were for the most part identified to tribe level, although in some case a more specific identification is proposed upon the modern ecology and

ancient geography of some taxa. Pollen was identified for the most part to Genus and Family level. It must be pointed out that a variety of remains could not be identified. It was possible, however, in some cases to assign the remains to a specific category (e.g., unidentified starch granules). In some cases it was possible to say they belonged to a Kingdom (unidentified plant remains) and in other cases it was not possible to tell whether the remains were of plant, animal or fungal origin. Apart from plant remains, a variety of debris of fungal and animal origin as well as mineral grit was found consistently across the samples. Fungal spores were very common, especially in the Medieval period, while animal remains mainly consisted of wool and feather barbules. Mineral grit and soil were ubiquitous across the samples, but could not be correctly quantified.

6.3 Starch Granules

Starch granules were almost ubiquitous in the samples, in fact only **7** individuals did not have remains of starch origin. Tables 11 and 12 in Appendix III report the distribution of the remains. The concentration of starch granules/mg varied across morphotypes and individuals. Starch granules were found to be of two main types:

- starch granules that did not display any type of damage, possessed distinctive visible features, such as 3D shape, lamellae, visible hilum, clear extinction cross: these were divided in typology and assigned to tribe or even species;
- starch granules damaged by some form of processing and despite the presence of some clear features, not reliable for any form of identification: these were counted but grouped into one single category of damaged starch. The majority of the starch granules belonged to staple food typical of the Middle Ages, however a total of 48 individuals showed starch granules that did not match any starch granules found in species native to or that could be grown in Medieval England. Such remains were identified as ’imports’.

6.3.1 Identification

The identification of starch granules to a taxonomic level is conducted on a number of three-dimensional features (see fig. 21 for an example from the reference collection)

such as overall size, three-dimensional shape of the granule, position, shape and features (e.g., fissures and grooves) of hilum, presence, position and number of lamellae, presentation of extinction cross, a drake feature visible under cross-polarized light (see fig. 21 for an example). The level of identification achieved varies and depends on how diagnostic such features are, due to overlaps in shape and size that sometimes exist among different species, tribes, and even Families of plants (Barton and Torrance 2015).

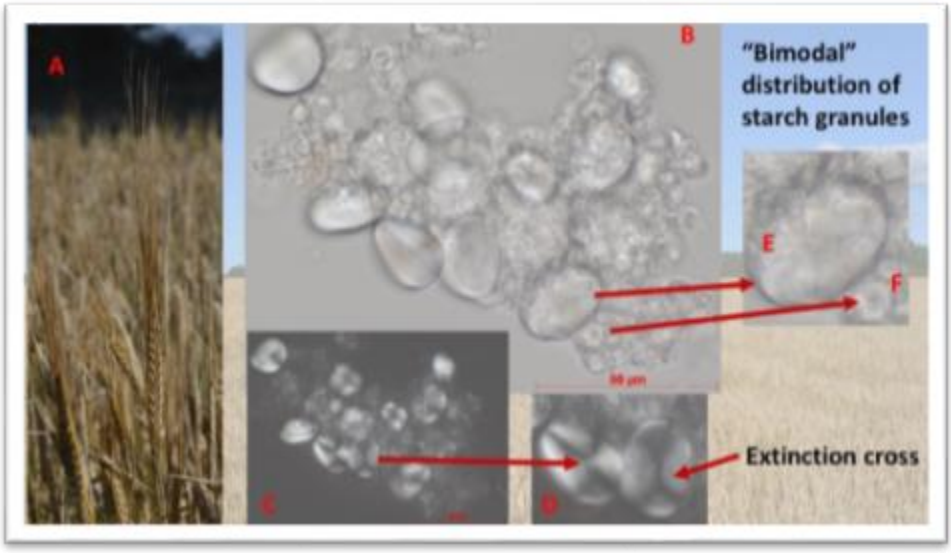

Figure 21. An example of starch granules from the reference collection. Starch granules originating from barley (*Hordeum vulgare* L.) grown in the area of Pickering, North Yorkshire (A), showing the characteristic bimodal distribution known for the Triticeae tribe and the Maltese extinction cross (D). Note the bimodal distribution consisting of large granules (E) and smaller starch granules (F).

Criteria of identification and reference used to confirm it are standardised and tabulated in Table 3 (staple food crops) and Table 4 (imported species). It must also be stressed that the identification of starch granules was greatly facilitated by the fact that all starch granules found in this study are very well known in the field of Ancient and Modern Starch analysis. This was due to the choice of the period of time and location, where food and economic plants that produce starch have been well studied.

Type	2D/3D Morphology	Hilum and extinction cross	Lamellae and fissures and other features	Proposed identification and reference
1	Round to oval Bimodal Lenticular Simple granules Their size and shape were consistent with the bimodal distribution encountered in Europe only in the members of the plant tribe Triticeae. The bimodal distribution is now known to be under genetic control (Howard et al. 2011), therefore the identification is reliable	Central Extinction cross clear and X shaped	Present across the granule, small bumps in the external surface Lamellae present, often towards the external part of the granules	**Triticeae** (eg. Cristiani et al. 2016; Henry et al. 2009;)
2	Oval to sub-oval Kidney-shape Simple granules	Central and sunken, often with cracks and fissures Extinction cross clear and X shaped	Present across the grain, often towards the middle, marginal fissures present sometimes	**Fabeae** eg. Cristiani et al. 2016; Henry et al. 2009;)
3	Very small polyhedral Compound granules	Central Extinction cross clear and X shaped	Not visible	**Aveneae** (Cristiani et al. 2016; Torrance and Barton, 2006)

Table 3. Basic morphological features and reference of starch granules belonging to staple food crops.

The identification here was therefore reached using methodologies and criteria of identification known and accepted in work conducted in the field of modern and ancient starch granules research (e.g.,Henry, Hudson and Piperno 2009; Torrance and Barton, 2006; Yang and Perry 2013) and successfully applied in archaeological settings (e.g., Blatt et al. 2011; Leonard et al., 2015; Li et al., 2012; Lucarini et al. 2016). Furthermore, criteria of identification used here in almost all cases had also been used by the author in published work and had therefore been peer-reviewed.

Morphotype 1, bimodal granules: Triticeae (fig. 22, Appendix III Table 11) Such granules were very common in the samples under study. The tribe includes the important European cereal crops wheat (*Triticum* spp.) and barley (*Hordeum vulgare* L.).

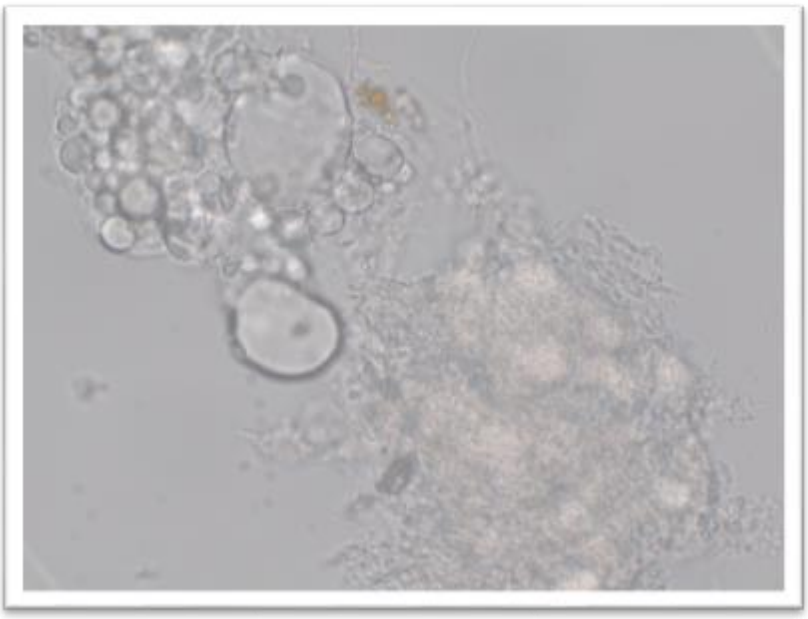

Figure 22. A clear example of starch granules showing the bimodal distribution found in the Triticeae tribe. Note on the right side of the picture and on the top left how the calculus matrix is still partially attached to the granules.

Morphotype 2, kidney shaped granules: Fabaeceae (fig. 23, Appendix III Table 11) Granules typical of the Plant Family Fabaceae and Fabeae tribe (Henry et al. 2011), which contains several edible species of legumes commonly (*Lens culinaris* Medikus), broad bean (*Vicia faba* L.), and green pea (*Pisum sativum* L.), all known to have been consumed in the Middle Ages (Dyer 2006). However, secure identification to species or genus is not

possible, due to overlaps in shape and size of starch granules at Tribe level, which was observed in the reference collection.

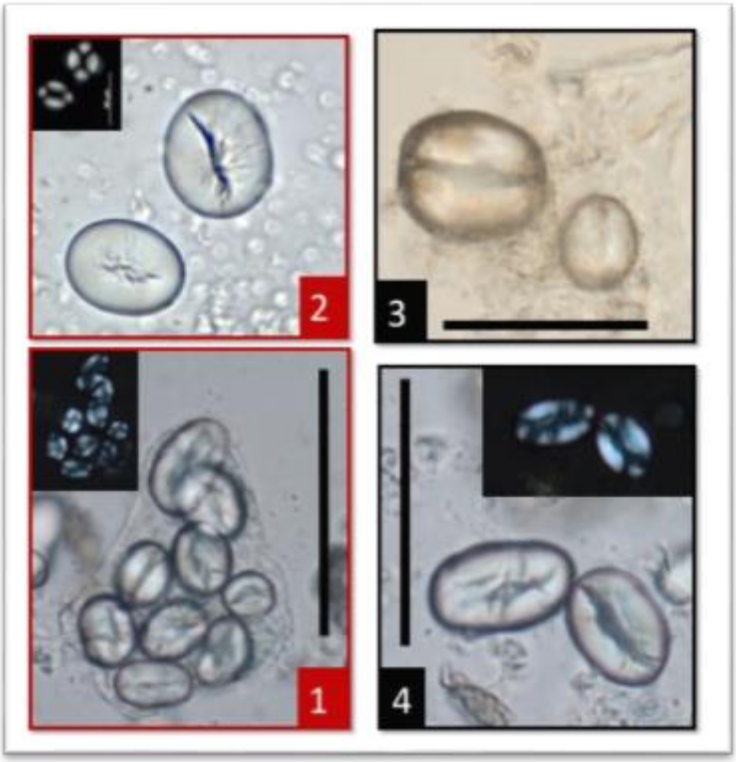

Figure 23. Modern examples of starch granules from the tribe Fabeae: *Vicia faba* L. and *Vicia cracca* L. 2 – 3-. Two examples of ancient origin that clearly show the features found in the tribe, note the deep sunken and long hilum in Figures 3 and 4, and lateral cracks visible in Figure 4, particularly clear under polarised light microscopy (inset). Also, note the presence of calculus flecks in both pictures. Scale bar: 50 microns.

Morphotype 3, compound granules: **Aveneae** (fig. 24, Appendix III Table 11) The identification of the tribe Aveneae was achieved upon comparison with published data (eg. (Henry et al. 2011), as well as evaluation of experimental starch granules in the reference collection. Compound granules are starch granules that grow in aggregates Here only intact, large aggregates of starch granules of oval to sub-spherical shape and larger than 20 microns were considered for identification and were grouped in the Tribe Aveneae, due to the fact that such large aggregates are found mostly in the genus *Avena*.

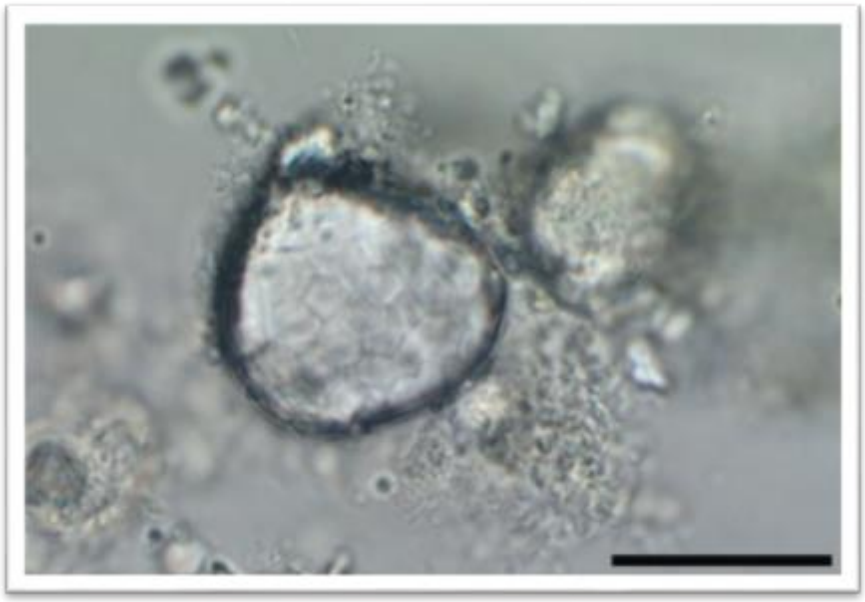

Figure 24. Archaeological compound starch granules of the tribe Aveneae, note the distinctive oval shapes and 'reticulated' surface generated by the small granules still together. The pale brown matrix encasing them is dental calculus dissolving. Scale bar 20 microns. . An example of species of the tribe Aveneae is provided in the methods section of this study (Figure 5-4), and clearly shows the similarities between the starch granules found in this study and those in the reference collection

Morphotypes 4 and 5, polyhedral granules: Paniceae and Andropogoneae (fig. 25 and 26, Appendix III Table 12) Only a low number of individuals had starch granules that very clearly belonged to the tribes Paniceae and Andropogoneae of the Grass Family Poaceae, which are very well known in ancient starch research (e.g., Madella et al. 2013 ; Yang et al. 2012). Species of these tribe are not native to Britain, and they are also very rare in the archaeological record in North West Europe (Livarda 2008). They have not been found before in the region or in the period of this study. The author of this study has assembled a very extensive reference collection and acquired familiarity with their morphology during collaborative work in the Balkans (Cristiani et al. 2016), North Africa (Lucarini et al. 2016) and Sudan (Hardy et al. 2015). The Avenaea tribe is also well published (eg. Yang et al. 2013).

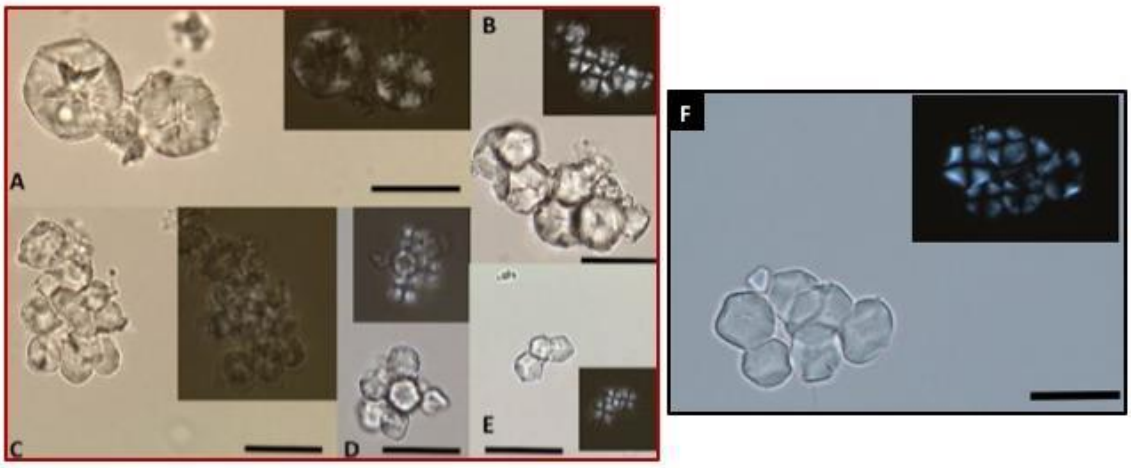

Figure 25. On the left, figures A-E: examples from the reference collection of the species of the tribe Paniceae: **A** *Cenchrus*, *Brachiaria*, *Pennisetum*, *Echinochloa*, *Setaria*, scale bar 20 microns (published by the author in Lucarini et al. 2016). On the right, some of the best preserved starch granules of the tribe millet (female EMP11) (picture by student K. Molopane), note the similarities with the genus *Setaria*, scale bar: 10

The identification to species level was not possible due to overlaps of size and shape mentioned before. However, following the same approach used by the author in other regions (see Cristiani et al. 2016) some hypotheses can be put forward. Published work leaves no doubt about the identification to tribe level; as stated before the tribes are well known in the field and extensively published. The tribe Paniceae is represented in the British Flora by a number of genera (e.g., *Stipa* spp.), however the only ones native are *Millium effusum* L., frequent in Britain in moist shady woods, and *M. vernale* M. B. Bieb. (Stace 2010, 995), which grows on sand dunes. Both are present in the reference collection. Their starch granules are 3 microns or less, and it is also very unlikely they would have been eaten as weeds of crops as they do not grow in arable fields. Therefore, these starch granules belong to the Tribe Paniceae, and are thus an imported item of one of the species normally described as millets. Madella et al. (2013,

Type	2D/3D Morphology	Hilum and extinction cross	Lamellae and fissures	Proposed identification and reference
4	Polyhedral Small single granules	Central, often with small fissures and a stellar shape	Rare and barely visible	**Paniceae** (Li et al., 2012; Lucarini et al. 2016)
5	Polyhedral Large single granules	Central, often with small fissures and a stellar shape	Rare and barely visible	**Andropogoneae** (Li et al. 2012; Lucarini et al. 2016)
6	Oval Simple granules	Central, sunken and surrounded by a large number of bumpy structures Extinction cross has multiple arms between the bumps	Present across the granule, small bumps in the external surface	Cf. *Fagopyrum esculentum* (fenugreek) The identification based upon reference collection (to be taken with caution)
7-8	Pear shape, with length of the granules more than half in length of its weidth	Hilum in extreme elliptical position, Extinction cross with arms diverging	Present across the granule	**Zingiberaceae** 7.ginger type: wide breadth 8. galangal type: small breadth (Wanton 1911)

Table 4. Morphological features and reference of starch granules belonging to imported food species.

2014) provides a very good overview on the uses and origin of such groups of important food plants of tropical origin. Such remains were found in in two individuals of the Medieval Period (one male and one female, from St Peter's), but also in one individual of the Anglo-Saxon period. The second type of starch granules (Fig. 27) was larger and the central portion of the hilum was found to be more sunken and more clearly visible, and bares an extraordinary resemblance to those found in the African crop *Sorghum bicolor*, tribe Andropogoneae, as such a tribe is not represented in British Flora. The identification is reliable.

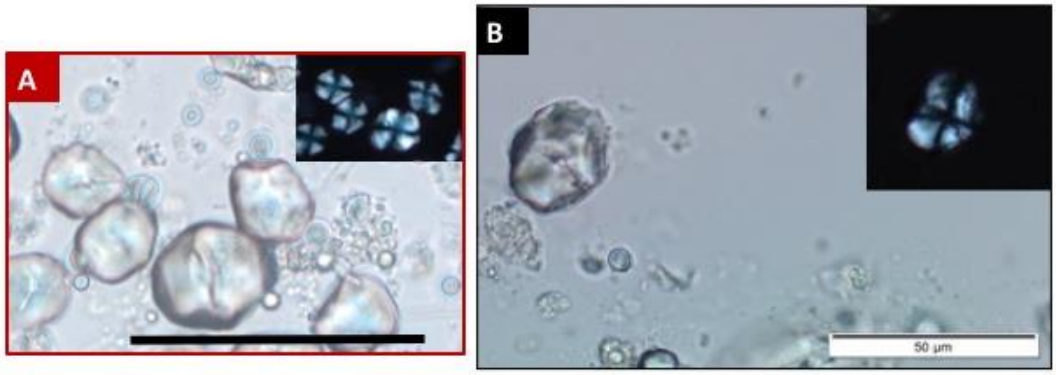

Figure 26. At the left an example of starch granules from the reference collection of *Sorghum bicolour* (A). To the right (B), archaeological starch granule of *Sorghum*.. Note the single granules have polyhedral shape, cracks radiating from the hilum, but the size is clearly larger than the other typology of polyhedral granules found.

Morphotype 7, small sub-round, pseudo-compound granules: cf. *Fagopyrum esculentum* (fig. 27, Appendix III Table 12) A few individuals across the period had starch remains that did not belong to any typologies of starch granules found or seen during this analysis and they were only present in one of the species of the reference collection of plants that would be imported or known to have been imported from outside Britain. The granules had a 'visual match' with those of the species *Fagopyrum esculentum* (buckwheat-see fig. 27.)

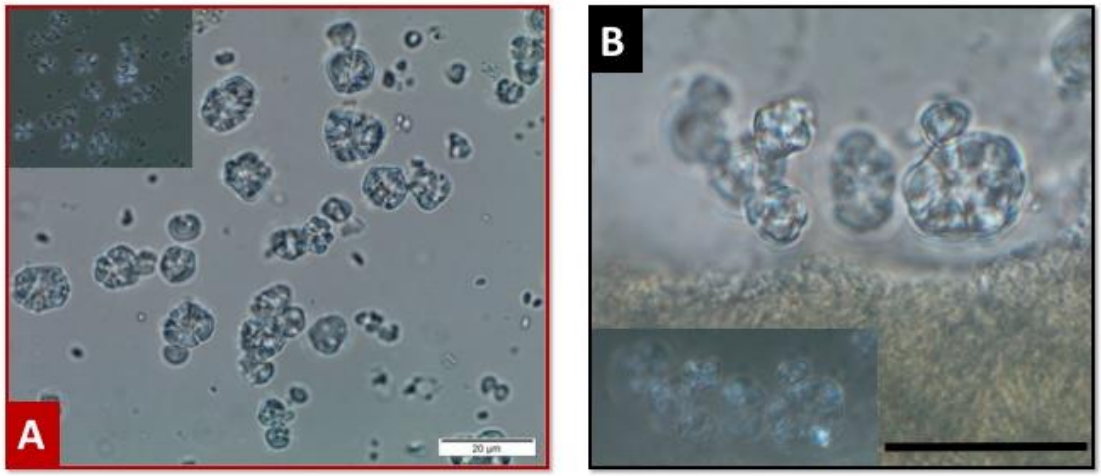

Figure 27. A starch granule from modern seeds of buckwheat. B starch granules of ancient origin detaching from the calculus fleck (pale brown matter at the bottom of the picture; note the overlap not only in the overall shape, but also in sizes). Scale bar 20 microns.

In the context of this PhD the species is treated as an import, however as not all possibilities were exhausted, the identification needs to be taken with caution. Work is currently in progress in order to confirm it. To the knowledge of the author, potentially this are the first finds of such food plant in the region and for the Anglo-Saxon period. Work is currently in progress to trace the history of this food plant in the area.

Morphotypes 8 and 9, hilum in eccentric position of sub-triangular granules: Zingiberaceae (fig. 28, Appendix III Table 12) A very distinctive final typology was retrieved in nearly a fifth of the samples. Such granules are consistent with the starch granules in the reference collection of members of the Family Zingiberaceae, which is not native to Britain, but to East Asia, and the rhizomes of such species are known to contain large quantities of starch granules. Such distinctive morphology has been known to commercial starch specialists for some time (Winton 1916, 662). Species of this family had been imported as spices and medicinal plants since Antiquity, mainly in the form of three closely related genera and their species: *Curcuma* spp., which include curcuma (*C. angustifolia* Rxb.) and turmeric (*C. longa)* and *Zingiber* spp., which includes

ginger (*Z. officinale* Roscoe). Due to the similarity of the starch granules, secure specific identification was not possible. However, during the analysis, it was noted that some of the starch granules appeared to have a larger breadth. A difference was also noticed in breadth between ginger and galangal in the reference collection. Therefore, granules were divided into two different morphologies:

- Morphotype 8 Galangal type: granules with small breadth
- Morphotype 9 Ginger type: granules with larger breadth

Examples are shown in figure 28.

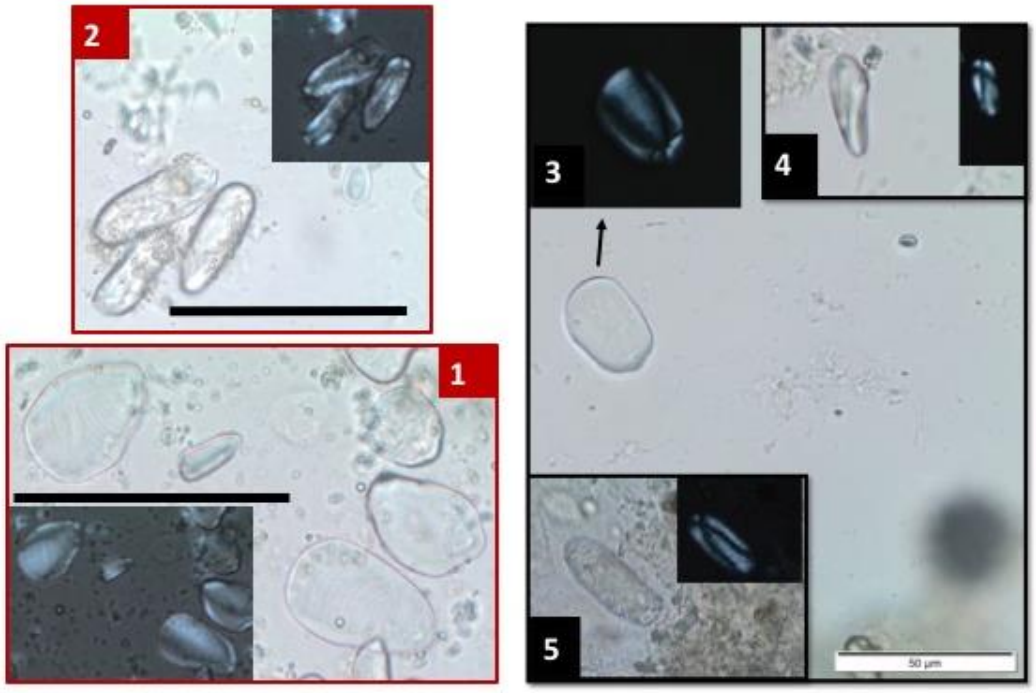

Figure 28. Example of powdered ginger (1) galangal (2) from the modern reference collection. 3-5 Examples of starch granules of the family Zingiberaceae found in dental calculus samples: note the hilum in strong elliptical position and the clearly elongated shapes. Starch granules in figure 3 are those with a wider breadth, recorded as ginger type, while figures 4 and 5 show starch granules recorded as galangal type. Scale bar: 50 microns.

Undiagnostic and damaged starch granules Although it has been suggested it is possible in many cases to identify starch granules damaged after cooking and processing (Henry et al. 2011), the author of this PhD is in disagreement, and only well preserved starch granules were used here for identification. Furthermore, many starchy food plants in Medieval times would be subject to processing and prolonged cooking resulting in many diagnostic features disappearing. Here a variable amount of starch granules were found that were either too small or too damaged (eg. loss of extinction cross, loss of hilum) to be identified, as the features visible, if any, were not sufficiently reliable. Such starch granules were counted, but no attempt was made to identify them.

6.3.2 Pathways to inclusion

Due to the nature of the finds belonging to staple plant foods and imported species that were consumed as spice or medicinal plants, the pathway to inclusion here is deliberate consumption of such plants. Both ginger and galangal are found in the Borough Record of Leicester[7] as imported. Work is currently in progress to assess if it is possible to confirm the identification to species level, suggested here to type. The identification to Family level is thought to be more solid, and considering the historic record of both plants in Leicester, it is most likely that both species were consumed either as spice or medicinal. Ginger and galangal are known to have been imported into Britain at least since Roman times and all species of this family that could be imported were viewed in the context of this PhD. In Britain spices are rare finds archaeologically; however, in this case it was fortunate that both ginger and galangal are mentioned in the Borough Records of Leicester as being used for feasts for entertaining wealthy visitors in Leicester (Browning et al. forthcoming), and their presence in the city support its identification.

[7] The Record can be view on line here:
https://archive.org/details/recordsofborough02leic

6.3.3 Comparisons with the archaeological data

Cereals grains of different species, such as rye, barley, bread wheat and rivet wheat are found in the archaeological record of Leicestershire in archaeobotanical assemblages, however legumes are rarer (eg. Monckton 2015). There is archaeobotanical evidence of domesticated oats from a malting kiln at Freeschool Lane dating to the Later Medieval period (Radini 2009), suggesting the species were also used for brewing.

There is no archaeological record in Leicester and its County of any of the potential imports found in this analysis.

6.4 Phytoliths

Plant remains consisting of plant opal, phytoliths and siliceous plant hair, were retrieved in a number of individuals, but were not as common as starch granules across the populations. In specific, phytoliths were found in 145 individuals. It is striking that they were almost ubiquitous in Anglo-Saxon populations (29 out of 30 individuals) but dropped to about 64% in the Late Medieval population.

Apart from two typologies, all phytoliths were not diagnostic to any specific Taxon. This was confirmed both in a purpose-built reference collection of modern plant specimens and published work (e.g., Lucarini et al. 2016; Piperno 2006). Some of them were found to be burnt, and therefore were potential evidence of exposure to debris that had come into contact with fire. In most of the cases fragments of dental calculus were observed still attached to the phytoliths, and in some cases the amount of dental calculus surrounding the finds was such to obliterate almost all the phytoliths (see Figure 6-9.1. for an example). In such cases further HCl was syphoned into the slide to dissolve the calculus. Such a procedure was not always successful. Finally, even if not diagnostic to a taxonomic level, they were all counted and assigned to morphotypes. For the purpose of this survey no further attempt was made to reach identification of species, as time balance in the recording of all the debris was crucial, but having counted and grouped such remains in morphotypes will allow easy access to them in the future, if new

techniques allow a better identification. However, for the scope of this study, undiagnostic phytoliths were grouped together (see Appendix III Table 13).

6.4.1 Identification

Phytoliths are characterised by multiplicity and redundancy: the same morphotype can occur in multiple species, sometime from different families, and in different parts of the plants. It must be noted that the identification of phytoliths, especially those produced by economic plants, such as wheat and barley, is often conducted based on the statistical analysis of characters present in large assemblages (Shillito 2013), and it is not possible with the low concentrations of, often damaged, phytoliths found in this study. Here, only a few phytoliths were diagnostic to a Taxon level, the majority was not. They were however grouped in "morphotypes", phytoliths with overall similar morphology. The descriptions of morphotypes is based on a combined approach of published resources (Madella et al. 2010; Piperno 2006; Rapp and Mulholland 1992).

Brief descriptions are provided below.

Undiagnostic morphotypes

- *Morphotype 1* elongate columallate/cranate with papillae (Figure 29.2);
- *Morphotype 2*: elongate cylindrical corniculate, with large numbers of horns/projections (Figure 29.3), sometimes such phytoliths are also described as dendritic in literature and are common in grasses (Piperno 2006, 189), however they are also very common in the Cyperaceae family;
- *Morphotype 3*: cylindrical, elongate tubercolated, also known as long cell phytoliths (Figure 6-9. 4);
- *Morphotype 4*: sinuate long cell phytolith (Figures 29.5 and 29-13);
- *Moprhotype 5*: long smooth cell phytolith (Figure 29.-6);
- *Morphotype 6*: tabular lacunose phytolith (Figure 29-7);
- *Morphotype 7:* tabular, perforated phytolith (Figure 29.8), sometimes found in the inflorescence of plants (Piperno 2006, 196);
- *Morphotype 8*: smooth bullyform phytolith, often found in a number of grass species (Piperno 2006), but not specific to the Family (Figure 29.9);

- *Morphotype 9*: wavy top roundel-like phytolith (Figure 29.10).

Diagnostic morphotypes

- *Morphotype 10*: multicellular aggregation, typically found in the epidermis of the rushes and sedges (Juncaceae and Cyperaceae species) (Piperno 2006, 197) (Figure 29.11);
- *Morphotype 11*: hat shaped phytoliths, often retrieved in numbers and attached to one another; such phytoliths are found in the stems and leaves of members of the **Cyperaceae** Family (Piperno 2006, 197) (Figure 29.12);
- *Morphotype 12*: **The most specific phytolith** was found in low numbers in one male individual from St Peter's (**SP24**, 5 phytoliths) and one male from St Michael's (**SM80**, 3 phytoliths) – **a spherical echinate/spianate**, which is **known to belong only** to the Palm family **Araceae** (Piperno 2006) (fig. 29.14-15). The fruit of the date palm, a very rare import in North West Europe in Medieval times (Livarda 2008), is rich in phytoliths of this type. Considering that Palms are not native to the British Flora, the period of time, and that the two individuals have remains of other imports, these phytoliths are here considered as evidence of the consumption of date palm. Finally, a very large reference collection of ashed plants has been built with over 300 species of Native British species (see pg. 91 for location of catalogue of species seen) as well as species that were known to be introduced in the Middle Ages. At the time during which this study was written, none of the species processed had echinate phytoliths and none of the Family of native British plants is known to have echinate phytoliths.

Siliceous hair A few individuals had siliceous hair, often broken. Siliceous hair can sometimes be diagnostic, but several families of plants common in the area and the period of time under study produced this type of siliceous remains (Poaceae, Primulaceae, Urticaceae), and therefore these were recorded as undiagnostic.

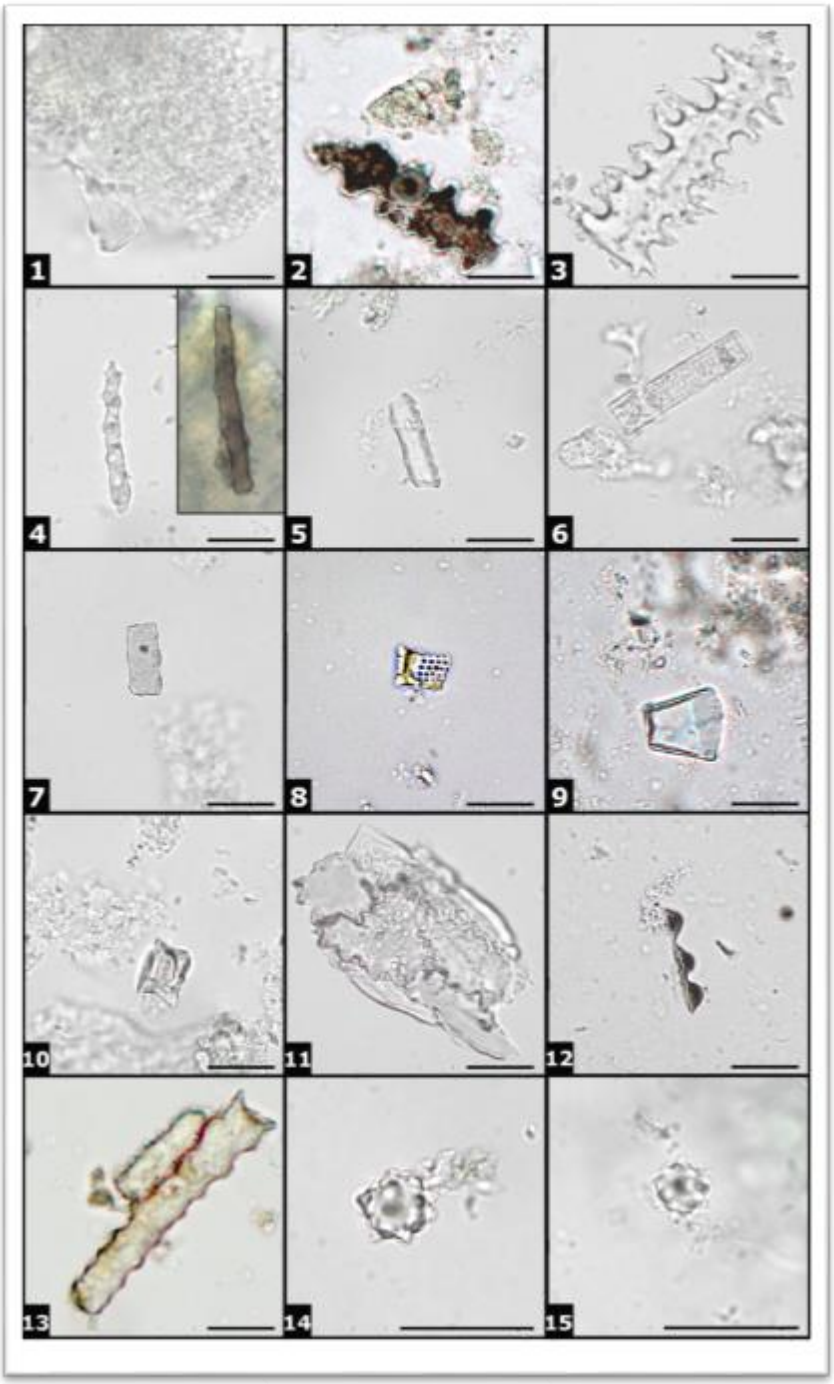

Figure 29. Examples of phytolith ‘morphotypes’ 1-10 are undiagnostic; 11-13 are morphotypes found in leaves, stems and inflorescence of Cyperaceae; 14-15 show echinate phytoliths found in the Palm Family Araceae, and in this context very likely to be from date palm.

6.4.2 Pathways to inclusion

Pathways to inclusion of phytoliths where no secure identification is achieved are difficult to address. However, many species of plants that are common in buildings, roofing material and flooring such as grasses and sedges have phytoliths similar to those found. Combined with the evidence of the diagnostic phytoliths of plants used in craft, roofing and bedding material (Cyperaceae), they are all very likely the result of the

widespread presence and use of such plants in the built and natural environment. The echinate phytoliths are most likely of dietary origin as they do represent imported items. It must be stressed that this is a very important find. To the knowledge of the author, there is no archaeological evidence of palm in England.

6.4.3 Comparison with the Archaeological Record

First of all, there is no evidence of palm date stones in the archaeological record in Leicester, and date palm finds are very rare in Medieval Europe (Livarda 2011). The relevance of these remains in this survey will be discussed in Chapter 9. In contrast, there is archaeological evidence of Cyperaceae, Poaceae and Juncaceae in the archaeological record for the city in the form of charred remains from pits (Monkton 2006, 2015; Radini 2009, 2010); however in all reports species of Cyperaceae, Juncaceae and Poaceae (with the exclusion of domesticated species) are never identified to species level, preventing a clear understanding of the specific source .

6.5 Pollen grains

Pollen grains were common but not ubiquitous in the material studied in this PhD: 156 individuals had pollen remains. Due to the diagnostic nature to at least Family level of the majority of pollen and the wide resources available both published and on line, a taxonomic level to at least family was reached for most of the remains. The majority of the pollen grains retrieved were in good to fair state of preservation, which is not surprising due to the fact that pollen is very resistant and it has been so far found in samples as old as 400,000 years in the dental calculus of *Homo erectus* at the Qesem Cave in Israel (Hardy et., 2015).

6.5.1 Identification

The identification of pollen was conducted using a variety of published resources (e.g., Moore, Webb and Collinson, 1991; Sawyer, 2006), web resources[8] and a modern reference collection of pollen specimens available at the University of York to which the author contributed. Although it is not common practice in pollen analysis to provide illustrations and descriptions of all pollen found due to the established and reliable criteria of identification, here micrographs of the species found are provided in order to show that they were found in situ in the calculus matrix. Such an approach was chosen due to the ubiquity of pollen in the environment and to counter any doubt regarding the antiquity of the retrieved material. Finally, the identification to species level is sometimes achieved in pollen analysis using very high magnification (x1000), which was not available for this study, therefore some of the identifications here are kept at level of Family, even if potential species are suggested.

To facilitate the discussion, pollen grains are here briefly described in alphabetical order, but grouped into two main categories:

- Arboreal wind pollinated (anemophilous) pollen: produced in high quantities by trees and shrubs, such pollen is naturally airborne, and very often known to constitute an inhalant allergen
- Non-arboreal pollen: produced in smaller quantities and less common in the environment than the wind pollinated arboreal one; such pollen types are normally regarded as entomphilus (insect pollinated). It must be stressed that such species are also sometimes airborne (Hyde and Adams 1958), many are bee pollinated and found in the pollen load of honey (Sawyer 2006). The reason for the above separation, which will be kept during analysis, is the assumption that plants that

[8] an example of consulted web resources can be viewed here http://pollen.usda.gov/Light_Micrographs/Oleaceae/Oleaceae.html

produce smaller amounts of pollen are less likely to come into contact with the mouth unless a very close exposure to the flowering part of the plant occurs. Finally, it must be stressed that in many cases pollen needs to be viewed from different angles, and that many features necessary for the identification need to be observed at a very high magnification. The presence of calculus flecks, even when very small, can prevent such procedures.

Arboreal pollen (Appendix III Table 14) Arboreal pollen was ubiquitous in the Anglo-Saxon population, but found in about a third of the Later Medieval Population. In general, it was better preserved than non arboreal pollen. More ecological information on pollen species will be given in Chapters 8 and 9. Arboreal pollen belonged to the following species (here only very briefly described):

- ***Alnus*** sp. (ash): spheroidal, pentaporate grains, 4 to 5 aspidate pores linked by strongly curved bands/arcs (Figure 30.A)
- ***Betula*** sp. (birch): triporae, triangular in side view, with 3 ringed and shouldered pores (Figure 30.B)
- **Coryloid type** (cf. *Corylus avellana* L.) (hazel), also referred to as *Corylus* type in the text: smooth, 3 pored grains, evenly spaced around the equator, pores 2-6 microns in diameter (Figure 30.C)
- ***Fraxinus* sp.** (Ash): tricolpate and subobalte with 3 furrows visible when zooming in and out (Figure 30.D)
- ***Populus*** sp. (poplar): grains prolate to subplorate with granulate surface still clearly evident
- ***Quercus*** sp. (oak): prolate, oval, tricolpate with short straight furrows and verrucate to scabrate surface (Figure 30.E)
- ***Salix*** sp. (willow): prolate, tricolpate, with long tapering furrows
- ***Sambucus nigra*** L. (elder): spheroidal, 3-colporate, with 3 furrows abruptly narrowing outside the pore, with circular pore and a reticulate surface (Figure 30.F) (this identification was made by Tony Goudwell, formerly laboratory technician in Environmental Archaeology at the University of Leicester)

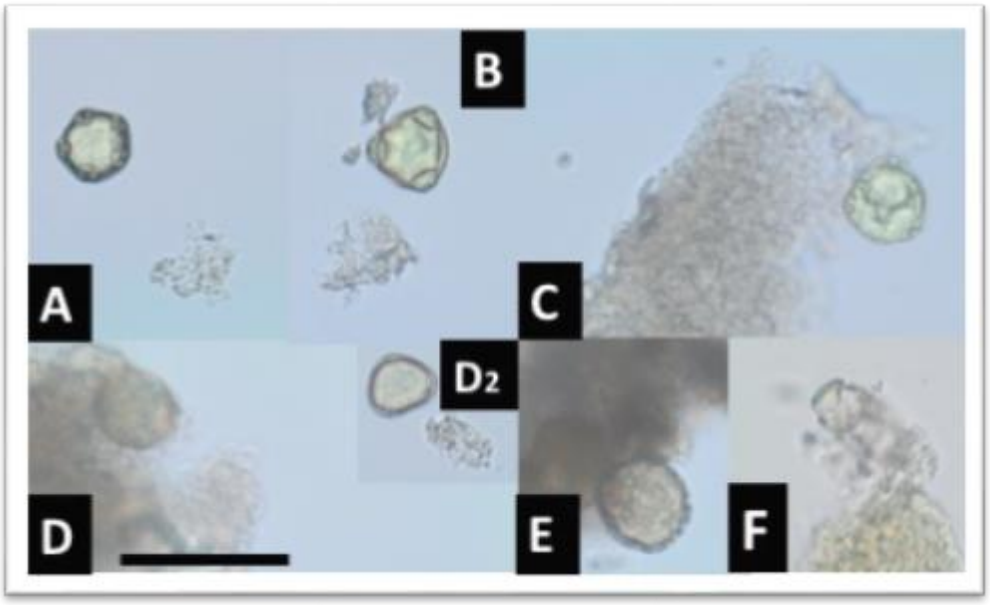

Figure 30. Arboreal pollen from ancient dental calculus: A *Alnus* sp.; B *Betula* sp.; C *Corylus* type; D-D2 *Fraxinus* sp.; E *Querqus* sp.; F *Sambucus* sp.. Scale bar: 50 microns.

Non-Arboreal pollen (Appendix III Table 15-16) Non arboreal pollen was more difficult to identify in many cases due to the lack of visibility of features necessary for the identification. The pollen grains were overall less well preserved than the arboreal ones, potentially due to consumption or cooking. Pollen grains where the identification is suggested to Family level need further analysis. Such analysis may only be possible if all the remains of calculus around them are dissolved, as they prevent observation of their fine structure. The following non arboreal species were retrieved during analysis:

- ***Artemsia* sp**. (mugwort): tricolporate, echinate, with a reticulate pattern generated by spines and rodes (Figure 30.A)
- **Asteraceae, spiny pollen granules** (daisy Family): small, spheroidal and echinate (Figure 31.B). Problems in the identification were due to calculus matter adhering to many parts of the grains.
- **Boraginaceae type** (borage or forget me not Family): 2-6 colporate, prolate toprolate spheroidal (Figure 31.C). Problems in the identification were due to calculus matter adhering to many parts of the grains. In the reference collection

pollen species of *Borago* and *Anchusa* (also referred to as *Borago/Anchusa* type in the text) were found to have similarities, but identification needs to be confirmed

- ***Centaurea*** type (cornflowers): tricolporate, radially symmetric, oblate spheroidal, to subprolate, tectum perforate and scabrate (Figure 31.D)
- **Chenopodiaceae** (goosefoots Family): spherical pantaporate, with pores up to more than half of the entire surface, with granular surface (Figure 31.E), such pollen grains are very distinctive to at least Family level.
- ***Linum type*** (flax) (Figure 31.F): large, prolate spheroidal, trizoncolpate, semitectate; here the identification is tentative, as many of the grains had large lumps of calculus that could not be removed.

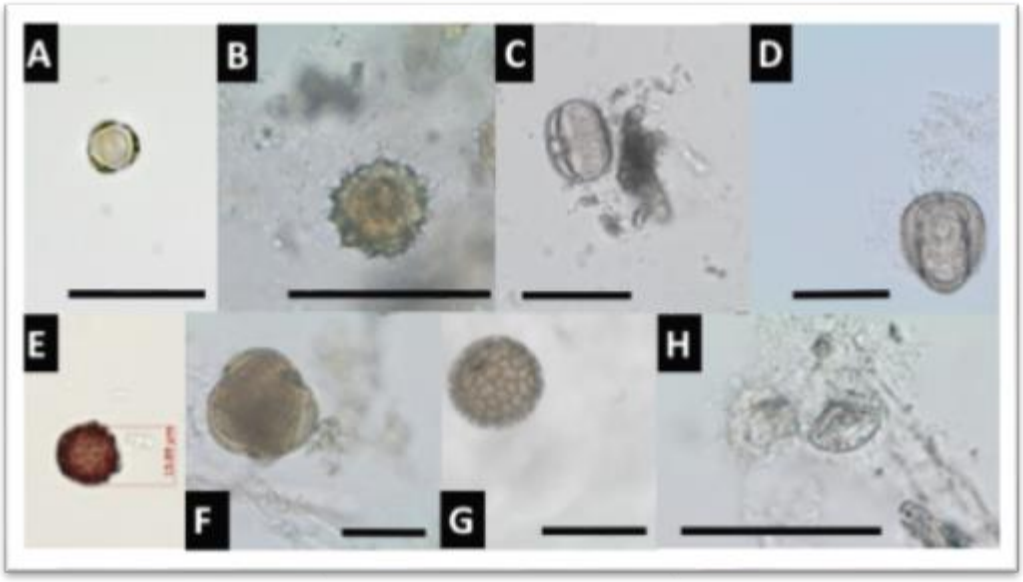

Figure 31. Non-arboreal pollen from ancient dental calculus. A *Artemisia* sp.; B Asteraceae; C Boraginaceae; D *Centaurea* type; E Chenopodiaceae; F *Linum* type; G Polygonaceae; H Rosaceae/Papaveraceae type, here a 'collapsed' pollen grain is seen still embedded in the calculus matrix.

- **Polygonaceae***:* small spherical grains with a very reticulate surface (Figure 31.G), very similar in the reference collection to *Persicaria* sp., however this is not a secure identification.

- **Rosaceae/Papaveracea** type: very badly preserved grains in all cases, small, tricolpate, oblate-spheroical, with blunt or rounded furrows (figure 31.H). The identification will need to be confirmed, however as such typology was recurrent across the assemblage a morphological description was assigned. It must be stressed that pollen grains belonging to non-arboreal species of plants will need further examination subject to removal of the calculus fragments still adhering to them. Finally, they will be discussed in more detail in the relevant session of this study.

6.5.2 Pathways to inclusion

The majority of arboreal pollen found belongs to pollen that is wind pollinated and therefore the origin of this pollen is very likely airborne. However, all the species found have uses ranging from fuel and building material (oak and hazel), material culture (e.g., birch and hazel) to some medicinal uses (such as birch and elder). Pathways to the inclusion are different, however in this study they are approached as airborne as they would be ubiquitous in the pollen load of air in antiquity. Arboreal pollen will be explored in detail in Chapters 8 and 9. Non arboreal pollen belonged to species that are normally considered bee/insect pollinated, although some times they are also caught in dust traps (Hyde and Adams 1958). Pollen of flax can be considered a crop, and the pollen of Boraginaceae, very likely either *Borago* or the closely related *Anchusa*, are often used as herbs and medicinal plants (Hatfield 2007, 49). All other species can be present in meadows, as weeds of crops but also are known to have vary medicinal uses and have edible leaves (Hatfield 2007). Such remains are explored in more detail in Chapters 8 and 9.

6.5.3 Comparison with the archaeological data

There is a range of archaeological record in the form of charcoal (see wood and charcoal section of this Chapter), pollen, charred and waterlogged remains of species of most of typologies of arboreal pollen found in this PhD in the archaeological record for Leicester (eg. Mockton 2006, 2015; Radini 2009) which also includes Flax (Greig 1999). Pollen analysis from the sites however, is better documented for the Medieval period, but is lacking for the Anglo-Saxon one, as noted in Chapter 4, that shows cultivation of hey-

meadows in the area as well as hemp (Monckton 2006). It has also been noted, again by Monckton (2015) that long term sequences of pollen diagrams are lacking for the area, with a gap between the Later Roman period and the Medieval one; such lack of evidence makes the data from this PhD particular useful. Where evidence exists, Shires and Causeway lane sites, it shows short lived species of wind pollinated trees (such as birch), cultivated and hay meadows (Greig 1994, 1999), a fact confirmed also by charred plant remains at the site of Causeway Lane (Monckton 1999); Finally, a mire found at Stamford Road Oakham with pollen evidence from Roman to medieval date showed less signs of cultivation in the middle of the profile (Monckton 2004, 61). It must also be stressed that the noon-arboreal pollen would be unlikely found in pollen diagrams, as it belongs to species insect pollinated which are under-represented in traditional pollen record. Data from calculus are therefore novel in providing evidence for the potential consumption of honey or medicinal plants as mentioned before. Finally, Appendix I provides an overview of plant remains from sites in the area, and more information on the species found in the analysis in broader context will be provided in Chapter 9.

6.6. Diagnostic plant tissues

Plant tissues sufficiently large to be diagnostic to some taxonomic level were found scattered across all populations. Due to the nature of such tissues it was possible in most cases to reach secure identification to species level.

6.6.1 Identification

The identification of the epidermis and other tissues was conducted in three distinctive stages: anatomical characteristics were checked using published work on the subject of 'powdered' microscopy of food and other plants. Although little known to archaeologists, vast resources on the subject of identifying economic plants and their weeds have existed for a while, a good example is the work by Winton (1916). Books on plant anatomy and seed atlases were also consulted, as they help with the identification of patterns on the seed surface for instance (e.g., Neef et al. 2012). Once a possible identification was confirmed, a modern reference comparison was prepared to confirm

the identification. Finally, wherever possible, the reference collection of archaeological plant tissues composed by Allan Hall was also consulted. The following plant tissues were found:

- Cereal bran
- Tissues from the seed epidermis of ***Agrostemma githago* L., *Cannabis sativa* L. and *Papaver* sp. And cf. *Silene* sp.**
- Leaf epidermis **of *Allium* cf. *porrum* L.**
- Fruit pericarp of ***Linum usitatissimum* L.**
- Palisade tissue from the seeds of ***Trigonella Foenum-Graecum* L.**

Results of plant tissues are provided in Table 17, 18 and 19. In the following part of this section I will provide details of the identification as well as a micrograph of the finds. Due to the diversity of the tissues, it was not possible to create table as previously done with starch granules.

Cereal bran (fig. 32) A few individuals had remains of tissue of cereal bran (fig. 32) of the Triticeae tribe, and potentially wheat. The diagnostic feature of such tissue is represented by the reticulate pattern created by the sub-rectangular cells intercrossing. These remains are commonly found in waterlogged cess-pits, and are very distinctive.

The author of this study has found such remains in very large quantities in many waterlogged remains (e.g., Radini 2010), and they have been recovered from the archaeological record in waterlogged conditions in many sites (Hall 2003, 187). Cereal bran mainly consists of fragments of the cereal grain pericarp and it is a very thin and flexible tissue and potentially this is why they were among some of the largest remains found in this study, although not so common.

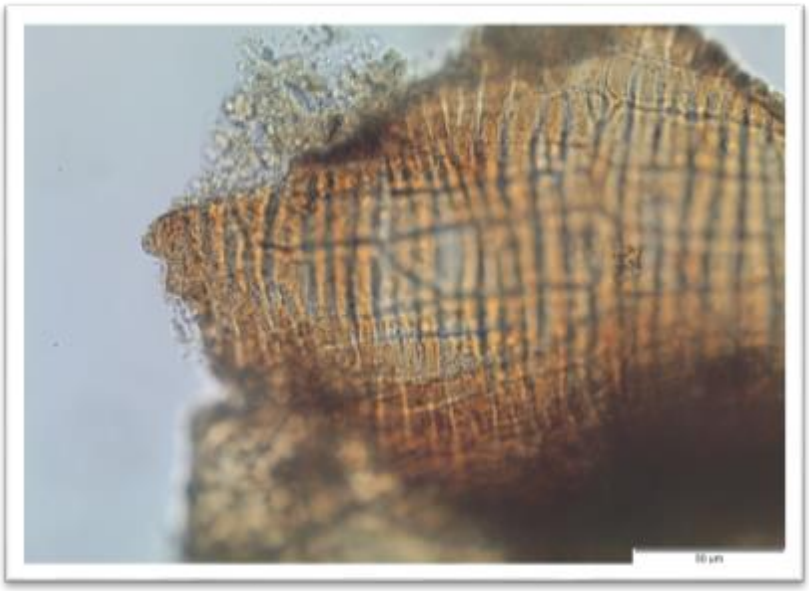

Figure 32. A large fleck of cereal bran, very likely from the Triticeae tribe, note the characteristic 'regular' reticulate surface. Also, note that the top and bottom right side of the fleck are still embedded in the calculus matrix.

***Agrostemma githago* L.** (fig. 33) Corncockle, a weed once very common in arable fields, has seeds characterized by a very strong outer epidermis, such a structure has a pigment that does not disappear even after boiling for a long period of time (figure 33 below shows corncockle outer epidermis after boiling for 3 hours - from modern reference collection). The diagnostic portion of the seeds is formed by a thickened epidermal wall with bumps that interlock with one another, the surface of which is characterised by fine warts. This diagnostic feature is retained even after boiling, so it is not a surprise that fragments are found intact even after they are ground with crops. Fragments of the outer epidermis of corncockle were recovered in one individual out of four. More information on this species is provided in the Discussion.

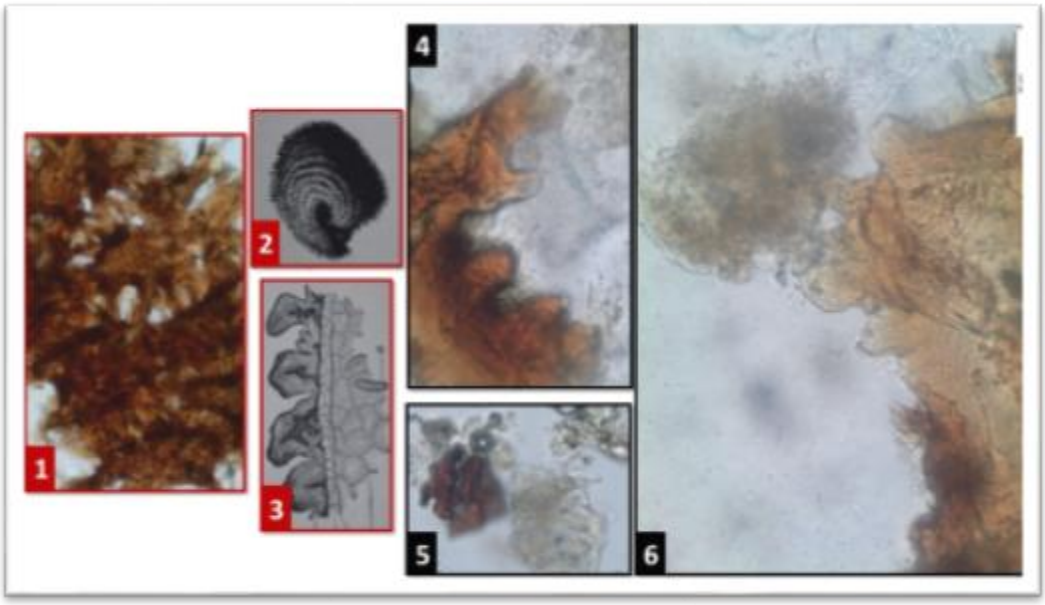

Figure 33. Modern examples of corncockle (1) outer epidermis; (2) seed; (3) forming part of the very diagnostic 'bumpy' surface; (4-6) archaeological examples from dental calculus samples.

***Allium* cf. *porrum* L.** (fig. 34) Another very diagnostic tissue was that of the leek leaf epidermis. The identification was conducted by comparison with a rich reference collection of archaeological finds from the Coppergate site from Viking York, provided and confirmed by Dr. Allan Hall. Criteria of identifications are: zig-zag patterns of the epidermal cell, the diagnostic sunken stoma and tooth shaped protrusions, all clearly visible in the remains found (fig. 34). Such finds are important evidence of the consumption of leafy greens and they are addressed in Chapter 9. The identification is solid.

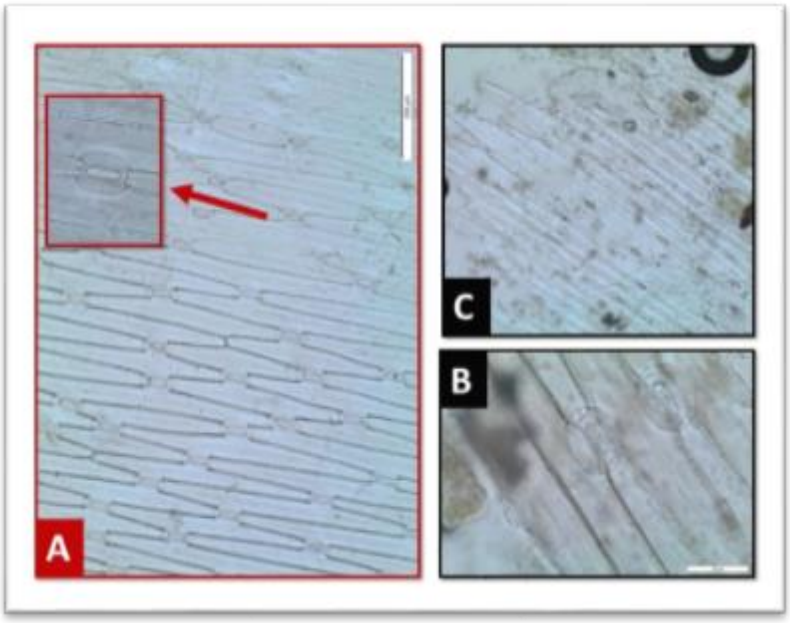

Figure 34. A Modern example of leek leaf epidermis, showing the zigzag pattern and the sunken stoma, typical of the genus *Allium* sp. B and C show an example from the archaeological record, note the calculus fragments adhering to the epidermis. Figure B shows both the sunken stoma and left side of the picture, near the calculus, the little tooth diagnostic of the leeks. Note that due to the presence of calculus such features are difficult to show clearly in pictures.

***Cannabis sativa* L.** (fig. 35.) Very interesting finds were those concerning another diagnostic seed coat, that of hemp. Here the remains included the diagnostic "waved" epicarp and palisade tissue still articulated with one another, a fact only visible under the microscope by zooming in and out (see figure 35) and the very complex and wavy outlines, still very clear in the archaeological material. Hemp has been cultivated for millennia for its very reliable bast fiber (also potentially found here). However, the seeds are edible both as food and for their mild medicinal properties, which must not be confused with those of *Cannabis sativa* v*. indica*, grown mainly as a drug. The finds in teeth are clearly of dietary origin here, as the seed is very tough and would not normally break down into such small fragments in the environment.

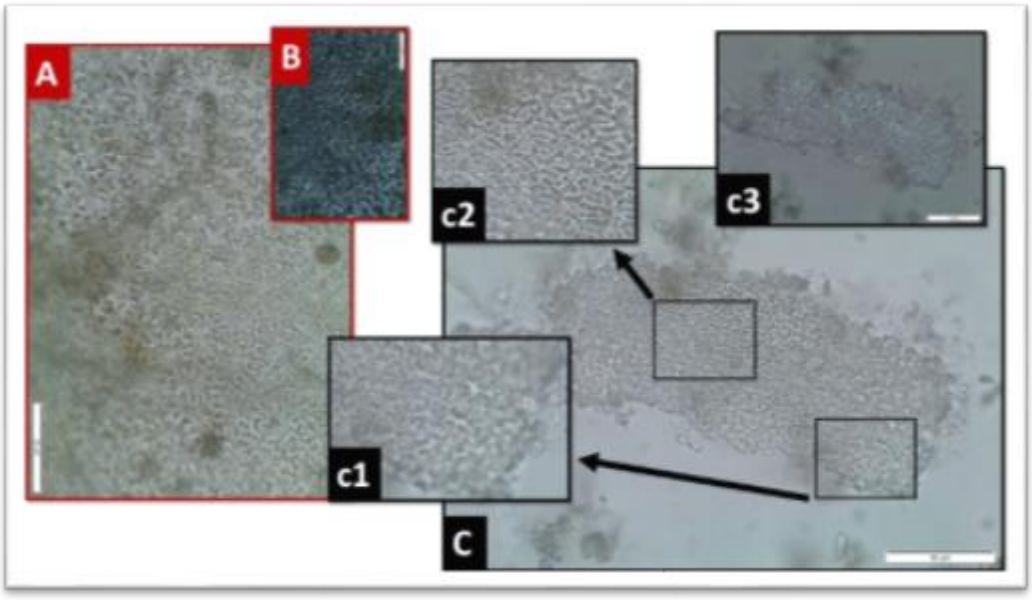

Figure 35. Modern reference material from hemp seeds under cross-polarized light (A-B); a fragment of the seed, clearly still embedded in the calculus matrix with some details (C: c2 = under polarized light (c3), and magnified to show the diagnostic pattern of the seed coat tissue (c1 and c2)).

***Linum usitatissimum* L.** Remains of the Flax fruit pericarp (fig. 36). The flax fruit pericarp is very papery and becomes very brittle when dried. It needs to be removed to reach the seeds or before fiber processing. Therefore, both dietary and non-dietary uses of the plant will produce a still edible by product, often used as fodder (Winton 1916, 202), known as 'flax bran'. Here diagnostic fragments of flax pericarp were recovered in very few individuals. This consisted of fragments of tissues with characteristics thin and thick layers of tissue, with thick cordons of pericarp running along the surface in pseudo-rectangular patterns. Such finds in dental calculus are very likely to be the result of exposure to 'occupational dust' generated by the removal of the fruits to free either the seeds or the fiber or both.

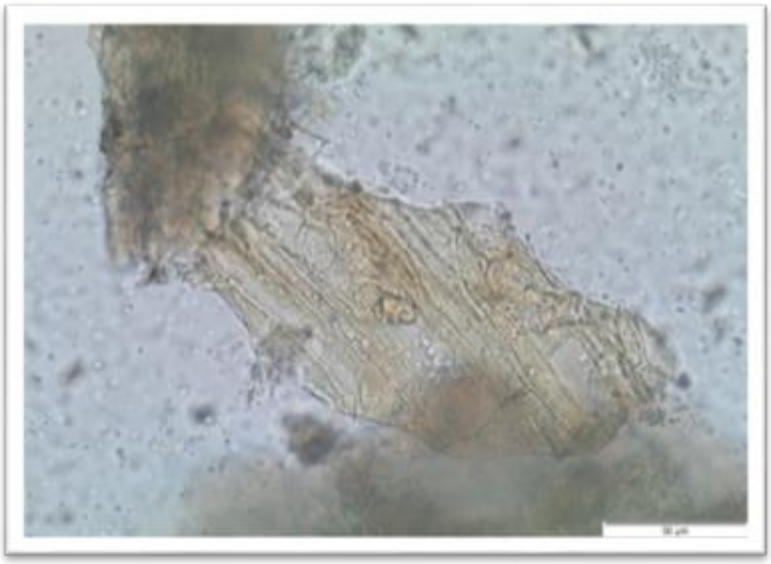

Figure 36. Archaeological example of flax fruit epicarp.

Papaver spp. (fig. 37) Another rare find in the dental calculus assemblage was that of poppy remains , although it is not possible to assess if these were from one single species or more than one, as at least two species are part of the Archaeophyte and native to Britain, while a few were imported (Stace 2010, 88).

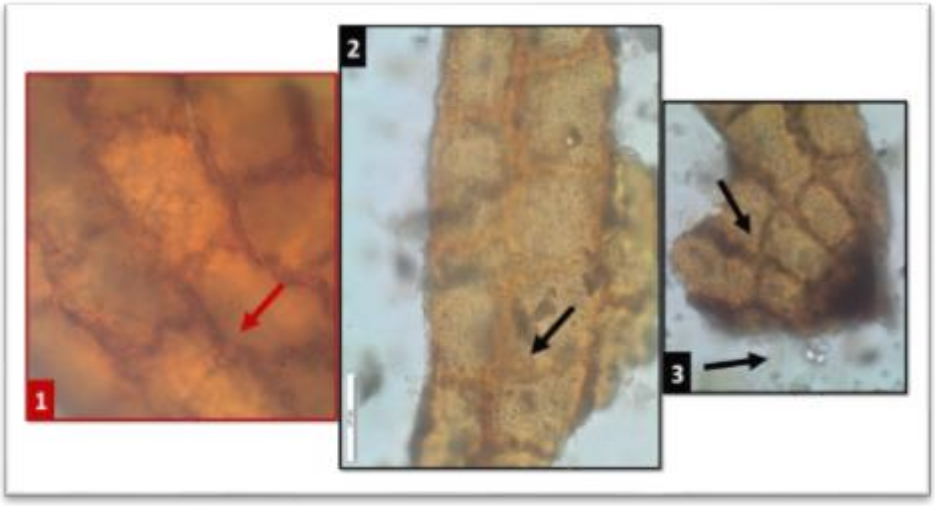

Figure 37. 1. Diagonstic polygonal cell pattern on the surface of modern common poppy (*P. rhoeas* L.); 2-3. Archaeological examples from dental calculus deposits (2-3).

Here the fragments found were consistent with the diagnostic epidermis of the seed, consisting of the very large polygonal cell, with a characteristic wavy 'wall', which are those that generate the distinctive patterns of the seed surface visible under stereomicroscope, and which normally allow archaeobotanists to reach at least the genus level of identification (fig. 37). The author could not separate the species due to the very small size of the fragments. Poppy seeds are edible, but they are also commonly found as weeds in arable fields. In this context they are very likely the result of accidental ingestion as weeds of a crop.

***Cf. Silene* spp**. (fig. 38) Very few individuals had remains of seeds of another weed. The identification was possible due to comparative work conducted to confirm the identification of corncockle. Several species of the Sub-Family Caryophyllodeae were viewed in order to eliminate the possibility that the characteristics seen in corncockle overlapped with others of the same Family. The remains had a thickened epidermal wall, with prominent warts and the cell interlocked with a distinctive star shape (fig. 38).

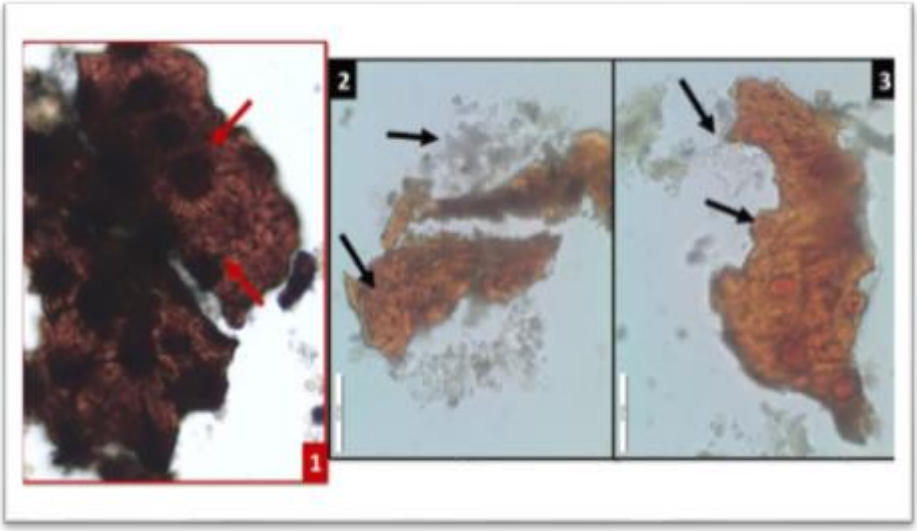

Figure 38. Modern epicarp red campion seed (*S. dioica* (L.) Clairv.) (1); archaeological example of the epicarp with characteristic worts (2-3). Arrows point to both the worths and calculus fragment. The scale bar visible in pictures 2 and 3 is 50 microns.

In the reference collection such patterns were seen in the seeds of campions and the comparison was found to be satisfactory to genus level (Figure 6-18). However, not all possibilities were exhausted (hence cf. before the genus). Campions are common weeds of crops as well as growing in natural meadows.

***Trigonella Foenum-Graecum* L.** (fig. 39) One of the most important finds in this study was that of fenugreek, a plant that is to be considered a luxury food and an import. Implications of this find are explored elsewhere. The identification was possible due to the presence of the very diagnostic element of the palisade tissue of the spermoderm (fig 6-19) of the seed, consisting with rows of sub-rectangular cell with a small apix on one side. This feature has been used for a long time to make identification to species level on this plant (Winton 1916, 259) and is well known in the field of Pharmacology (Yadav et al. 2011, 444). The identification is therefore solid.

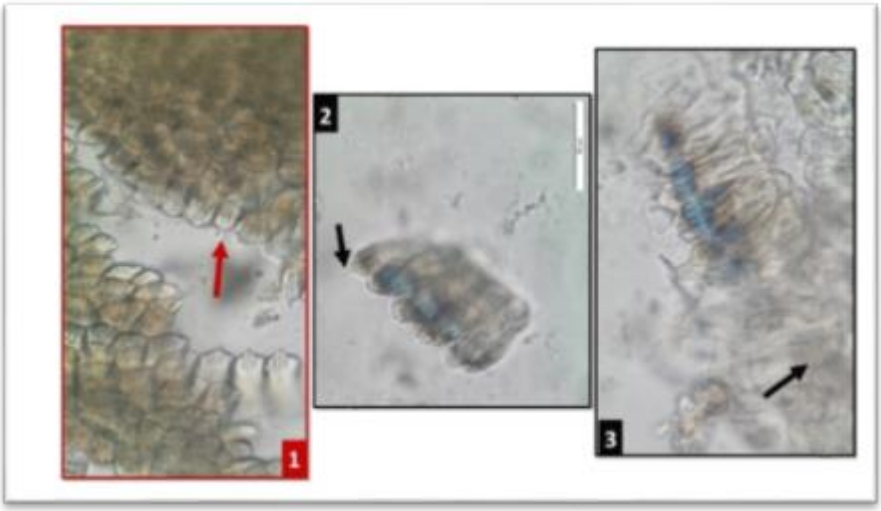

Figure 39. 1. Modern example of palisade tissue of the seed spermoderm. 2 and 3 show fragments of the distinctive cells of the palisade tissue, arrow in figure 1 shows the pointed extremity of the cell, while arrow in figure 3 is showing fragments of dental calculus still entombing the remains.

6.6.2 Pathways to inclusion

All species found here, apart from flax bran, are very likely of dietary origin. In the case of leek, cereal bran, fenugreek and hemp seeds, the parts found are the result of

deliberate consumption. In contrast, seeds of corncockle, poppy and campions are very likely ingested together with the crops that were harvested, and therefore are the result of accidental consumption associated to diet. The remains of flax fruit, although edible, are most likely the result of accidental ingestion of 'occupational dust', as the removal of the papery epicarp of the fruits during the processing of the plant for fiber and for the seeds would generate such debris in large quantities. This may have been collected as fodder for cattle.

6.6.3 Comparison with the archaeological evidence

All finds but fenugreek retrieved here had previous archaeological evidence either in the form of charred and waterlogged remains or in the form of pollen (Monkton 2006, 2015). Seeds of common poppy, corncockle as well as campions have been routinely found in Medieval Leicester (e.g. Monckton 2009; Monckton and Radini 2009; Radini 2009) as they are common weeds of crops. Neither flax or hemp seeds have been found in Medieval Leicester, however pollen of both species have been retrieved from the area (Greig 1999; Monckton 2006).

6.7 Plant Fibers

Around half of the individuals found in this study had remains of plant fibers: 20 Anglo-Saxon and 98 Medieval individuals. The identification level achieved depended on the level of preservation and the visibility of features useful for the identification.

All plant fiber finds are listed in Appendix III Table 20.

6.7.1 Identification

All fibers were counted and separated into three categories.

1) fibers where identification was not possible due to the lack of diagnostic features were described as un-diagnostic plant fibers
2) fibers where diagnostic feature were visible but overlapped between hemp and flax, this were called *Linum/Cannabis* type.

3) Fibers with a narrow lumen, which is most common in flax, this were described as likely belonging to flax, cf. *Linum usitatissimum* L.

The remains were identified by comparison with modern and archaeological reference collection fibers (fig. 40.A) and using morphological criteria: dislocation bands (sometimes referred to as 'x features'), the lumen (the fiber's small, central channel), and the aspect of the fibers under polarized light (fig. 40.B) (Petrarco and Kubic 2004, 100). The following on line resource was also consulted:

http://www.microlabgallery.com/gallery-fiber.aspx).

All bast fibers exhibited a degree of mineralization, as may be expected given their isolation from dental calculus, and many had a bluish color, possibly due to dye or to the effect of the phosphate rich environment. The author of this study has seen plant remains from phosphate rich environment encrusted of the mineral Vivianite, blue in color. It is personal opinion of the author that the bluish color maybe due to a similar process of Vivianite formation in the calculus. Although it was not possible to determine the species of origin in many cases on the basis of white light and polarized light compound microscopy alone, 10 individuals had remains clearly belonging to flax (fig.40.D). 12 Anglo-Saxon and 30 Medieval individuals had remains of bast fibers which could be either flax, hemp or nettle. Results are provided in Appendix III Table 20.

The above criteria of identification were presented and peer reviewed during the publication of work conducted by the author on Medieval samples from Germany (Warinner et al. 2014a).

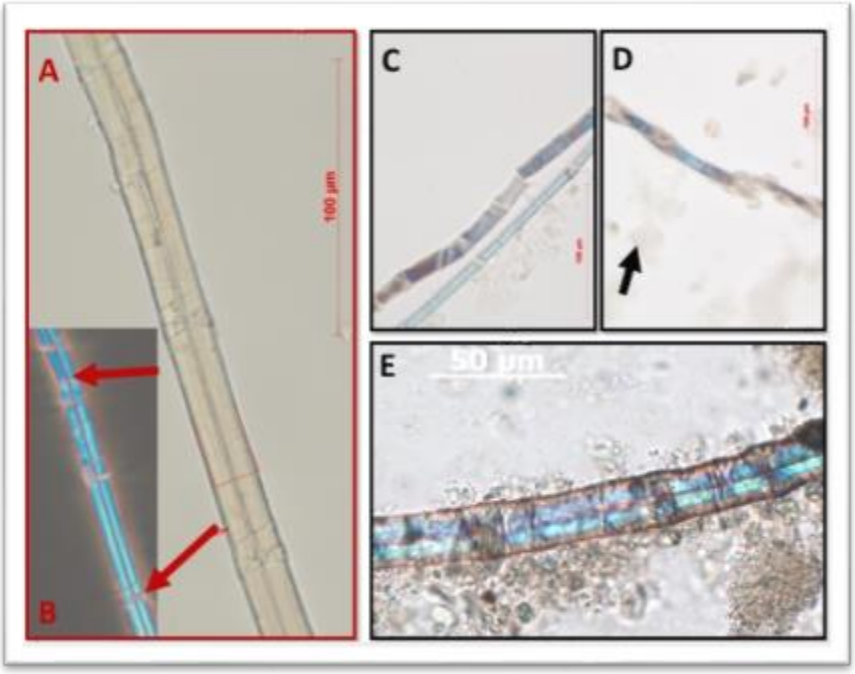

Figure 40. A-B: Flax from the reference collection. C-E: Examples of bast fibers from calculus matrix. C is an example of fiber that showed dislocation bands. In D the fiber is 'twisted' and, finally, E shows a fiber identified as flax based on the clear dislocation band, the narrow lumen and the optical properties - note the similarities with the reference material. (the arrow in D points to the fleck of calculus separating from the fiber).

6.7.2 Pathways to inclusion

Flax, nettle and hemp were used in the production of linen, a textile in widespread use during the Medieval period to produce household cloth and clothing, as well as religious textiles. Dust from the production of such items and their natural breakdown would be very common in the environment, as it is today. However, the concentration in the environment would be much higher in the environment surrounding an individual processing the fiber. Such an individual would be persistently exposed to a very high concentration of debris, in a similar manner to cotton processing. High concentration of fibers in an individual can be potentially considered evidence of exposure to occupational pollutants and will be discussed in more details in Chapter 9.

6.7.3 Comparison with the archaeological data

There is archaeological evidence in the area examined in this PhD for the cultivation and processing of flax (Leicester) and hemp (Eye Kettelby) (Greig 1999; Monckton 2006) in the Medieval and Anglo-Saxon period (see also Appendix I) , but it is general lacking in the region, and it has been identified as a research priority for the region (Monckton 2006, 289). The finds are therefore very useful in complementing our understanding of the presence and potential use of such fibers. However, it is not possible to state if the crop was grown locally.

6.8 Wood

Wood remains and wood elements were very rare across the populations but in a few cases they were found in high concentrations (see fig. 41). Remains of such types of plant material were often very small, and in many cases insufficiently preserved or organized in their structure to allow identification. It was however possible to separate them between soft wood (from conifers) and undiagnostic wood, in a few cases (fig. 41.1-2). In most cases the wood remains were represented by isolated pits and wood elements and damaged fibers (fig. 41.3-5). The very few finds of wood debris are listed in the next page in Table 5 and in Appendix III Table 21.

6.8.1 Identification

The identification of wood was not possible due to the size of the remains not being large enough. However, in a limited number of cases it was possible to identify the remains as soft wood. The characteristic used for the identification were:

- anatomy and distribution along the wood fiber of lines of bordered pits if they were visible. These were in line of small circular pits with a border (hence the name), a common way to separate soft wood from hard wood in forensic contexts (Petrarco and Kubic 2004, 96)
- the presence of the tracheid elements of the xylem, lacking visible pores which are found in hard wood vessels.

The above criteria of identification were found to be reliable in recent work by the author (Radini et al. 2016), conducted on dental calculus samples from Neanderthal material from El Sidron (Spain) and confirmed by gas-chromatography conducted on the sample.

Sample Number	Sex	Wood debris particles/mg
EMP10	**M**	0.0526
SM55	**M**	4.0333
SP1	**USC**	0.0313
SP18	**M**	3.5172
SP23	**M**	0.0323
SP24	**M**	0.3333
SP25	**M**	0.0357
SP27	**M**	0.0435
SP28	**M**	0.0370
SP29	**M**	0.0294
SP4	**M**	0.0714
SP63	**F**	0.0278

Table 5. Occurrence of wood remains across the studied populations, note the very high concentrations (in yellow) of debris in male SM55 (St Michael's) and male SP18 (St Peter's). Sk sample Id= skeleton sample identification number; F=female; M=males.

6.8.2 Pathways to inclusion

Wood debris would be common in the environment due to its many uses, including fuel, building materials, and tools. It is therefore surprising how in the current dataset, wood remains were not more common. In recent work conducted by the author on additional individuals from St Michael's, however, a small amount was found in 14 samples (Radini et al. 2016), suggesting that sample size could affect its visibility (larger sample sizes have more chances to entrap the remains).

6.8.3 Comparison with the archaeological data

Please see section on charcoal.

6.9 Charcoal and burnt debris

Small particles of micro-charcoal and burnt debris were found to be ubiquitous across the populations (fig. 42), although secure identification of particles as burnt wood was not possible in most cases. Results on charcoal and burnt debris are presented in Appendix III Table 22.

Although some minerals may be distinguished from microcharcoal due to their optical properties using polarized light microscopy (Petrarco and Kubic 2003), the identification of micro-debris below 3 microns remains challenging (Blackford 2000). In addition, it is still not known how phosphate-rich environments of dental calculus may affect the optical properties of such debris, in particular when microscopic flecks of calculus are still attached to it, as was the case in most of the finds in this study.

6.9.1 Identification

Particles of microcharcoal are here defined as microscopic fragments of charcoal below 180 microns in size (Blackford 2000), which can be securely identified as such using light microscopy when the structural features necessary for conclusive identification are present (Rhodes 1998). Several brown to black, opaque, fragments, often with sharp angular edges, in some cases only visible as black dots were recovered. It has beenpointed out by Blackford (2000) that particles below 3 microns, often interpreted as soot (elemental Carbon) when in the form of black dots, cannot be safely identified as microcharcoal using light microscopy. A range of inorganic particles in fact exists, for example minerals common in soil, such as horneblende and mica (Petrarco and Kubic 2003), pyrite, marcasite and biotite (Rhodes 1998) that have characteristics under light microscopy (appearing brown to black, opaque, with sharp angular edges) similar to the ones of microcharcoal (Blackford 2000).

Therefore, burnt debris was separated into ash and soot (below 3 microns), fine particles (between 3 and 20 microns) and large particles (above 20 microns).

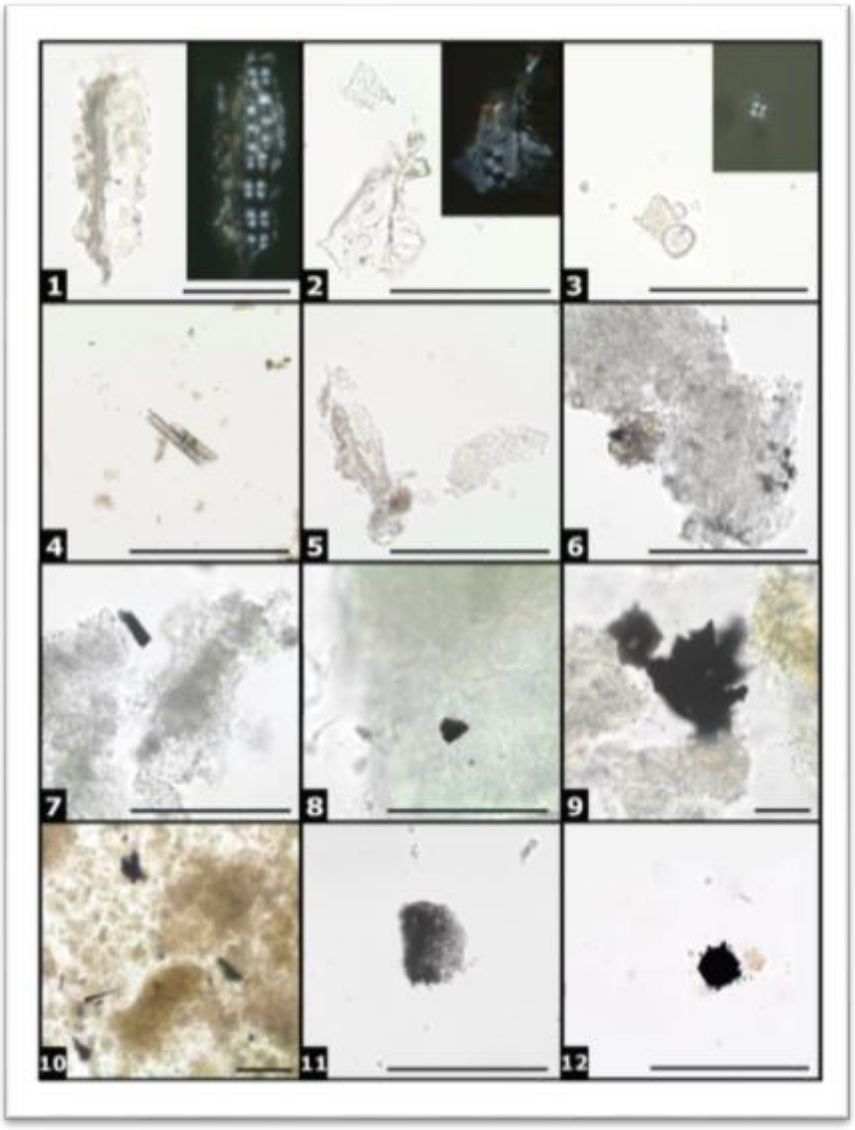

Figure 41. Wood and charcoal recurring typologies: conifer wood (1-2); wood elements (3-5); examples of micro-charcoal (6-10); soot (11-12). Scale bar 20 microns.

The criteria and considerations were used and published in a paper co-authored by the

author of this PhD, and are the sole work of the author[9], as micro-charcoal is among the most common remains found in human dental calculus.

6.9.2 Pathways to inclusion

The most likely origin of such debris is close exposure to fire and/or ingestion of food with particles of charcoal adhering to it, possibly due to cooking by roasting. Micro-charcoal is widely used to interpret fire history (Scott & Damblon, 2010) while microcharcoal found in dental calculus can be the result of exposure to natural fires.

6.9.3 Comparisons with the archaeological data

Charcoal is ubiquitous in archaeological samples in Leicester and the surrounding area. The majority of finds comes from kilns, earth and also sometime ditches, and is mainly consistent with hard wood species, such as hazel and oak in kilns (e.g. Monckton and Radini 2009; Radini 2009; Radini 2010), probably due to the fact that the wood burns at high temperature. Other remains are those of birch, willow/poplar and elder (Monckton and Radini 2009). There is however very little record of soft wood, and it is possible that this was used for artefacts rather the fuel, reducing the chances of its survival. There is a lack of summary work on charcoal and this could be a goal for future studies, mainly due to the fact that charcoal analysis has been conducted in the area with dating purposes. Monckton (2006, 279) has already pointed out the need of more information on the history and use of wood and woodland for the Anglo-Saxon period. Finally fruits and nuts found in several archaeological sites points to the presence of fruits and shrubs such as hazel and elder and its exploitation also as food resource (see Appendix I for a detailed picture of their presence in Leicester and surroundings).

[9] Hardy et al., 2015 see supplementary session: http://www.sciencedirect.com/science/article/pii/S1040618215004553

6.10 Calcium oxalate and sclereids

A number of sclereids and calcium oxalate crystals of various shapes were found still embedded in the calculus matrix. Remains of sclereids are commonly found in hard parts of plants but also fruits. Sclereids (plant 'stone cells') and crystals of calcium oxalate are cellular inclusions of plants (fig. 42):

- Sclereids: these are elements of a plant tissue called sclerenchyma, which encompasses a variety of plant tissues where cell walls are thickened and the content of lignin in them is very high. Sclereids are characterised by their isodiametric shape, the tendency to be elongated and their thick walls with often large pores. Although they can occur in tissues, they can also be found singly or in small groups.
- Crystals of calcium oxalate: these are produced in plant cells as the result of metabolic activity (Figure 43). They can be rounded (druse) or acicular (raphids).

Results are presented in Appendix III Table 23.

6.10.1 Identification

It is not possible to identify to species level sclereid and calcium oxalate and they are found in many parts of plants, in all plants.

6.10.2 Pathways to inclusion

British flora is common in fruits such as apple and pear, which were common in the Medieval period. Calcium oxalate was found in the leaves of almost all species of trees examined.

6.10.3 Comparison with the archaeological data

Although there is no record in the archaeological data from Leicester of such finds, due to the degree of preservation and the processing of environmental material, there is an existing record in calculus of crystals of calcium oxalate. Furthermore, druses of calcium oxalate are very often found in ash deposits as a consequence of the burning of wood and other plant debris that had them. Such facts potentially reinforce the possibility that the origin of calcium oxalate crystals, especially of the echinate and round type,

could be the result of accidental ingestion or inhaling of debris coming from ash, and the fact that such debris is again found in women more than in men could reinforce this assumption. This hypothesis will be examined in Chapter 9.

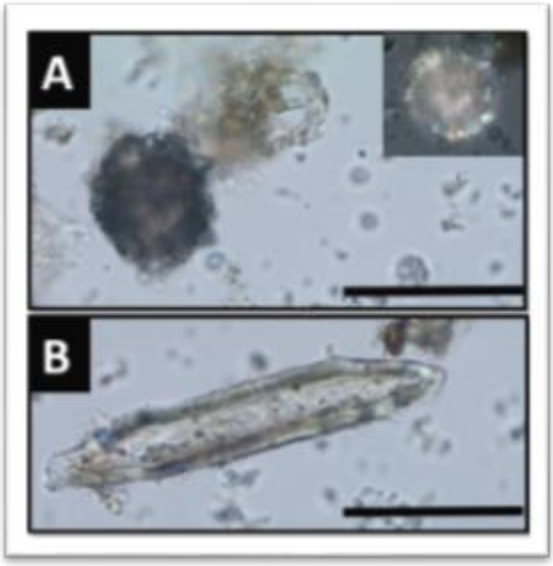

Figure 42. Example of plant inclusion consisting of druse of calcium oxalate (A). These remains dissolve in HCl, and are distinctive due to their optical properties under polarized light (top right). Example of plant sclereid (B). Scale barr: 20 microns

6.11 Unidentified plant remains

A number of plant remains were found that could not be identified because they were too damaged or too small, or no match was found in the reference collection. Such remains were still noted down and counted, but they were not included in the analysis and were not tabulated for the purposes of this PhD.

6.12 Summary and overview to the proceeding chapter

Micro-remains of plant origin were the most abundant types of remains found in the material under study and they consisted of a variety of parts of plants, including starch

granules, phytoliths, pollen grains but also a number of diagnostic and non-diagnostic plant tissues, such as seed epidermis and plant hair, or simply fragments of plant tissues/fibers too small to be identified.

Starch granules were found belonging to staple food plants (such as wheat, oats and legumes tribes) and also rare imported species, the most common of which were starch granules of the Zingiberaceae Familiy, which includes ginger and galangal.

Phytoliths and siliceous plant hairs were also found. Many appeared broken, and some had been clearly burnt. Among the phytoliths the most interesting finds were those of echinate phytoliths diagnostic of the palm Family Arcaecae (Piperno 2006, 192), and that are very likely the result of the consumption of date palms. However, those were the only remains of phytoliths that could be directly linked to diet.

Both arboreal and non arboreal plant species were found, which had a number of possible pathways of inclusion, including potential medicinal uses of some of the species.

Plant tissues included leek epidermis, hemp seed coat and the seeds of weeds, while fibers of flax and wood were found in some individuals and were considered the result of exposure to craft activities. Micro charcoal and soot were ubiquitous. Finally a number of low diagnostic or undiagnostic debris were also recorded.

The survey of plant micro-debris in human dental calculus shows that a great variety of debris can be retrieved from dental calculus, apart from starch granules and phytoliths. This study also shows the potential of plant tissues to be identified to species or genus level with the appropriate resources.

In relations to the aims and objectives of this PhD, the results of this chapter show clearly:

1. Starch granules are indeed the most common remains found in the populations that ate cereals and legumes as staple foods

2. There is a wealth of other remains but the difficulty of identification sometimes prevents a better understanding of their origin. Therefore, pathways of inclusion are often difficult if not impossible to trace.
3. Apart from starch granules, the echinate phytoliths, the plant tissues and a few species of pollen, the rest of the remains are very likely not of dietary origin.

Having surveyed the microremains of plant origin, in the next chapter I will present the results of all typologies of remains that did not belong to the Kingdom Plantae.

CHAPTER 7

RESULTS II: MICRO-REMAINS OF NON-PLANT ORIGIN

7.1 Introduction

The majority of the work conducted on micro-debris entrapped in ancient human dental calculus has focused on plant remains. One of the objectives of this PhD was to record any type of debris visible by light microscopy, not just starch and phytoliths, as well as evaluate any problems with the identification and quantification of such non-plant remains. This chapter describes all the remains that did not belong to the Kingdom Plantae, and discusses problems encountered in their identification.

Micro-debris of non-plant nature has been divided here into the following 3 groups:

- Kingdom Fungi: fungal spores and hyphae
- Kingdom Animalia: a variety of remains from different Phyla
- Debris of lithological origin: minerals and soil flecks

Following the same approaches adopted in the previous chapter, criteria of identification, including potential look-alike particles, pathways of inclusions, and archaeological finds are outlined. Finally, a short section at the end of this chapter briefly presents the typology of remains that could not be identified.

7.2 Kingdom Fungi

A great variety of fungal debris consisting of spores, conidia, and hyphae was consistently retrieved across the studied populations. Despite the ubiquity of such remains, the lack of large reference collections limited the level and accuracy of identification. Indeed, of all the micro-remains retrieved during this analysis, fungal debris is the most problematic. Nevertheless, it holds great potential and an entire

section is dedicated to it in Chapter 9. Results from the Kingdom Fungi are tabulated in Appendix IV Table 24.

7.2.1 Identification

The identification of fungal remains requires a well trained analyst with a solid background in Mycology and with a large built-for-the-purpose reference collection (De-Wei Li et al. 2007 98). Furthermore, in many cases culture based analysis of fungal colonies is necessary to achieve identification (Wu 2007 105). Naturally, this is not possible in archaeological material, and often biomolecular methodologies are the only ones that can achieve secure species identification. Finally, most identifications of fungal remains based on spores rely on prior knowledge of the origin of the samples, such as indoor rooms (e.g., bathroom floor/ceiling; kitchen floor), outdoor settings (e.g., soil, wood, crops), with a number of parameters affecting their growth well documented (such as temperature, humidity, light, nature of the surface).

Figure 43. Puffball collected for the reference collection and its spores under light microscopy (scale bar: *20 microns*)

Such parameters allow narrowing down the identification of the origin of the spores/hyphae/mycelia, but are not available for fungal debris from calculus, which

could originate from a number of locations. However, a number of mushroom spores (see fig. 43), indoor molds and plant pathological fungi were viewed for this PhD, a process kindly supported by the Royal Mycological Society and The University of Leicester Botanic Garden. Although it was not possible to achieve secure specific identification at this stage, it was possible to group fungal debris in categories based on recurring “morphotypes” and in some cases a potential identification to Genus level was suggested. Groups consisted of yeast-like cell*, Pennicillum/Aspergyllum* types, dark walled and hyaline multicelled spores, and hyaline and dark walled single celled, as well as mycelia and hyphae fragments. Such morphotypes are described below.

Morphotype 1: Yeast like fungi (cf. *Saccharomices* spp.) Fungal remains characterized by a hyaline, thin walled cell, round to sub-round in 3d shape and small, measuring 2-7µ in diameter, were considered Yeasts where the budding scar was visible (fig 44.1). A large number of yeast-like cells were observed in the dental calculus of several individuals. Although similar in size to plant starch granules, the yeast cells did not exhibit an extinction cross under polarized light and they lacked other starch features, such as a hilum and lamellae. In addition, it is possible to see through the spores by zooming in and out, like pollen, an aspect not possible with starch granules due to their 'crystalline' nature. Moreover, some of the cells exhibited a darkened circular to oval area on their surface, similar in appearance to a small hole, and resembling a budding scar[10]. Some of the larger cells seemed to have smaller cells attached to them, as found in budding yeast; however, budding could not be confirmed. Many species of non-budding yeast, especially of the genus *Candida*, are normal inhabitants of the human oral cavity, and wild yeasts (including budding yeasts) have long been used for brewing and as a raising agent for bread in Europe. Such remains were also found and described

[10] Mature yeast cells have a form of reproduction known as "budding" were a single mature mother cell forms a smaller cell which slowly detaches leaving a circular crater delimitated by a darker ring; this is the budding scar.

in work conducted by the author on Medieval German remains (Warinner et al. 2014), their identification as yeasts was therefore accepted by the dental calculus research community and has not been challenged so far.

Morphotype 2, *Pennicillium/Aspergillus* type Small spherical spores, 2.5-7 microns in diameter, hyaline in their structure, without a scar and sometimes found in chains (Fig.44.2), were assigned to fungal debris of *Aspergillus/Penicililum* type. These were small rounded or sub-spherical and colourless or bluish. It must be stressed that such a group of fungi is very difficult to identify using only their morphology and light microscopy (Lacey and West 2006, 27), and almost impossible to identify based on a single spore. Many species of *Aspergillus* are human mycosis and can cause pulmonary diseases. *Penicillum* species are often food moulds.

Morphotype 3, *Alternaria* sp. Spores that appeared dark-walled, multi-celled with several longitudinal and transversal septa, brown to pale brown in colour, with a smooth appearance, were described as cf. *Alternaria* sp. (fig. 44.3), due to the characteristic appearance of the conidia of species of this genus (Lacey and West 2006, 112). However, a number of genera, for example *Ulocladium*, develop similar conidia (De-We Li 2007, 245). *Alternaria* species are plant pathogens and cause in many cases the rot of plant stems.

Morphotype 4, *Curvularia* spp. Spores that appeared dark-walled three- to five-celled, fusiform and curved, with a characteristic enlarged central cell, were described as cf. *Curvularia* type (fig. 44.4) and are very likely to belong to a species of that genus as the curved fusiform morphology is characteristic of the genus (De-We Li 2007, 250). Such species are saprophytic and pathogens to a variety of plants.

Morphotype 5, *Chaetomium* type Spores that appeared single-celled, smooth, dark-walled (fig. 44.5), were found to be very similar to those of *Chaetomium,* found in indoor damp conditions, and very common in wooden buildings with long term water damage. Species of this genus are some of the most allergenic fungal spores in indoor environments (De-We Li 2007, 248).

Morphotype 6, cf. *Fusarium* sp. Fungal remains consisting of fusiform, hyaline-celled, colourless to pale blue spores, also known as macroconidia, were assigned to the group *Fusarium* type (fig. 44.6), due to the very characteristic morphology of the genus (Lacey and West 2006, 112). While spores of species of *Fusarium* are commonly of outdoor origin, they can be found indoors in very damp environments (De-We Li 2007, 251).

Morphotype 7, Hyline single celled type (cf. *Fusarium microconidia*) Another type of spore, potentially a micro-conidia of *Fusarium*, was also found in a number of individuals. These spores were hyaline, single-celled and smaller than the large macroconidia described above (fig.44.7), and they are harder to identify, while they can be confused with other species, such as those belonging to the genus *Acremonium* (De-We Li 2007, 252).

Morphotype 8, *Drechslera* type Another type of multicelled fungal spores, but with thick wall, thin septa and rounded extremities (fig. 44.8), were also found. These were very similar to the conidia produced by species of the genus *Drechslera* (Lacey and West 2006, 112). They were also very similar to the conidia of the genus *Bipolaris.* Species of the genus *Drechslera* are plant pathogens and their origin is normally outdoor (De-We Li 2007, 250).

Morphotype 9, *Calvatia* type (Puffballs) type Finally, small round spores, with mid-brown, thin-walled cell were recovered in a number of individuals. Such spores were remarkably similar in size, shape and colour to those belonging to puffball species in our reference collection (see fig. 44.1 and 44.9). Some species of puffball are edible, but many can be dangerous to humans when they mature, as they can release clouds of spores that cause allergies.

Other fungal debris A variety of other fungal remains, with low diagnostic features, were found scattered across the individuals under study. These consisted mainly of fragments of hyphae, sometimes in masses, also known a ”mycelium” (fig. 44.10), or fragments of single hypha, sometimes very small (fig. 40.11). Finally, a number of fungal spores/fruiting bodies were also found, in particular bi-celled large fungal spores were recurring (fig. 44.12), but could not be assigned to any viewed morphology.

7.2.2 Pathways to inclusion

Fungal remains, spores and hyphae are ubiquitous in the world around us. Pathways of inclusions could be securely narrowed down only upon identification to species level.

Yeast like fungi, *Pennicillum/Apsergillus* and the Puffball type could be of dietary origin. The yeast *Saccharomyces* is commonly used together with other yeast naturally found on fruits, as a rising agent and in the production of beer/alcoholic beverages. *Pennicillium* species are found on bread mould but some species like *P. casei* are responsible for the white mould on cheese. Some species of puffball are edible, although medicinal properties are also known for them (Coetze and Wyk 2009). The majority of fungal spores found belong to plant pathogens and they may well have been ingested or inhaled as a consequence of ingestion or contact with contaminated plant matter. Finally, species of *Aspergillus* spp*., Penicillium* spp., and *Chaetomium* spp. are often found indoor in contaminated humid environments, and they could be the result of exposure to unhealthy living conditions, they can also be found in soil, and therefore their interpretation is difficult unless species level is reached.

7.2.3 Comparison with the archaeological data

There is no analysis on fungal debris in the archaeological record of Leicester, although sometimes fungal remains, such as puffballs, have been found in waterlogged conditions, for example in a number of British archaeological sites, including Vindolanda (Watling and Seaward 1976) and despite fungal remains are normally found in pollen samples (eg. Haas 2010; Mighall et al. 2006); however, no remains of mushrooms or fungi have been found in Medieval Leicester and its surroundings. Fungal remains have been found in the dental calculus record from samples of *Homo erectus* at Qesem Cave in Israel, of age older than 400,000 years (Hardy et al. 2015), showing that this type of remains has the potential to survive from very deep in the human past.

Figure 44. Fungal micro-debris 1-Yeast; 2- *Pennicillium* type; 3. Cf. *Alternaria* sp.; 4- Cf. *Curvularia* sp.; 5- *Chaetomium* type – 6- Cf. *Fusarium* sp.; 7- single celled *Fusarium type*; 8- *Drechslera* type; 9- *Calvatia* type; 10- Fungal growth *Aspergillum/Penncillium* type; 11- hyphal fragment; 12- unknown fungal fruiting body. Scale bar 20 microns.

7.3 Kingdom Animalia-Phylum Nematoda: Trichuridae

A single evidence of the Phylum Nematoda was found in one male individual from Medieval Leicester (see fig. 45), who also had remains of soil embedded in the calculus matrix. Such remains belong to the egg of species of intestinal parasites *Trichuris* spp. (whipworms).

7.3.1 Identification

The identification was conducted using publications and atlases on the subject of animal and human parasitology (e.g., Ash and Orihel 1990; Spencer and Monroe 1961; Zaman 1979) and by comparison with archaeological material found in Leicester, from the site of Freeschool Lane (identified by Dr. Bone Jones). The identification of species of whipworms is based upon average measurements of a large data set, which is not available in this context, therefore, identification to species level is not possible in this case. Most recent work on the identification of whipworms eggs from soil in Leicester has failed to achieve secure identification to species level, so it is not possible to see if this egg belongs to *T. suis* (parasite of pigs) or *T. trichiura*, which is the one that affects humans. However, the range is within *T. suis*. Finally, one child from St Michael (which was not included in this PhD due to young age) also had remains of parasite ova in his/her calculus. Sub samples of such remains were sent to Dr. Tina Warinner in order to attempt DNA identification and any other information that can help to understand the origin of such ova. Results are not yet available, but the work is in progress.

7.3.2 Pathways to inclusion

Intestinal parasite ova in the human mouth can only be the result of accidental ingestion of such remains by eating contaminated meat or potentially ingestion of contaminated soil if dung or faecal material was used as fertilizer. This issue is discussed in detail in sections "filth and food hygiene" in Chapter 9.

7.3.3. Comparison with the archaeological data

Intestinal parasites of ova have been found very often in waterlogged deposits and cess pits in Medieval Leicester and are almost ubiquitous in latrine deposits of ancient times (Connor and Buckley 1999; Monckton 1995; Radini 2010); recently parasite ova have been even retrieved from the grave of King Richard III (Mitchell et al. 2013), suggesting high status people were equally exposed to them. This is, however, the first record of an intestinal parasite in human dental calculus.

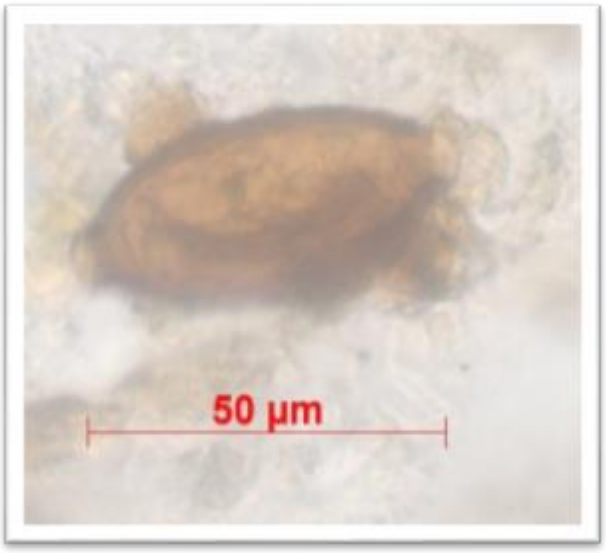

Figure 45. *Trichuris* sp. egg. Note the egg is still embedded in the calculus matrix (the cloudy nature of the image is due to the dissolving calculus matrix in HCl). The egg is also surrounded by amorphous organic matter, very likely humic substance from soil.

7.4 Kingdom Animalia-Phylum Arthropoda

Micro-debris positively identified as belonging to the Phylum Arthropoda was found scattered across all groups. The remains were mainly in very small fragments and could not be identified to species level. Results from the Phylum Arthropoda are presented in Appendix V table 25.

7.4.1 Identification

Insect remains in the majority of cases did not retain anatomical features to allow precise identification aside from that fact that they belonged to the Phylum Arthropoda. It was however possible to separate debris in 2 main categories: 1) unidentified insect remains (see example in Fig. 7.4) and 2) scales or scale fragments belonging to the wings of moths and butterflies (Lepidoptera). Lepidoptera scales have in fact a very distinctive morphology (See fig. 46.1 and 47.3-8) characterized by a sort of flattened sac, with a reticular pattern formed by the intersection of longitudinal ridges and transversal ribs

and a pedicel (Ghiradella 1994, 430). Morphology of scales can vary within the same species according to their position on the wings and the sex of the individual. Although it was not possible to reach a specific identification during this study, anatomical differences in the micro- and nano-scale anatomy are present in different species of Lepidoptera, as shown by a number of studies (e.g., Girlado et al. 2008; Prum et al. 2006), therefore, it may be possible in the future to identify such remains to species level if an appropriate reference collection is built.

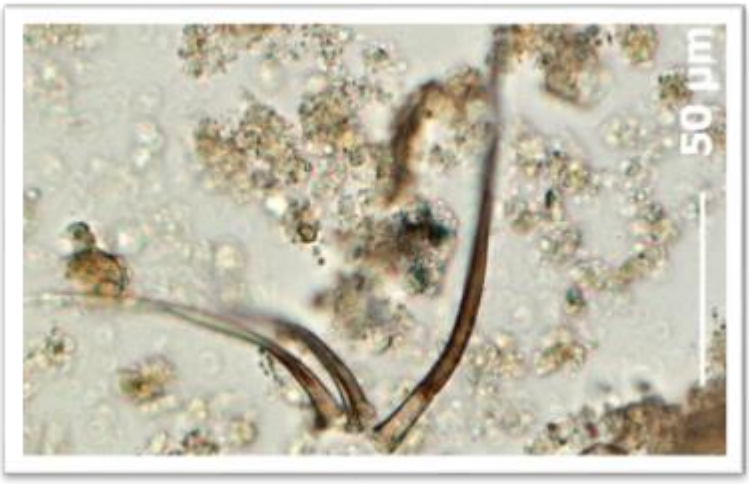

Figure 46. An example of insect remains, potential larva hair (Dr. David Smith's personal comment). Note also the dissolving fleck of calculus with some mineral grit and micro charcoal surrounding the debris.

Furthermore, calcium oxalate deposits found during phytolith extraction for the building of the reference collection of leaves of *Vitis vinifera* (grapes) highlighted the fact that such deposits could be confused for badly preserved remains of Lepidoptera scales (fig. 47.2). However, such deposits have different optical properties and they dissolve fast in weak solutions of HCl acid, leaving no doubts regarding the integrity of the proposed identification. The reticulate surface differentiates such remains form those of any palisade tissue of plants. Finally, it must be stressed that under fluorescent light insect remains can be distinguished for the optical properties of chitin present in their exoskeleton and other parts of their body, making the identification to the Phylum

Arthropoda secure wherever needed, although this was not required in this case due to the overall good preservation of the debris.

7.4.2 Pathways to inclusion

Settled dust in buildings and outdoor air very often contain remains of insects, sometimes in large quantities. The presence of butterfly/moth scales could have been of indoor or outdoor origin. Moths would be attracted to indoor light naturally, while butterflies also spend the winter indoor. This was observed in the modern churches where remains of these species were found abundantly during a survey (see ch. 9 for further information). Finally, a number of moths feed on wool; while remains of wool are rare in the archaeological record, it is commonly accepted this would have been the most common fibre in the production of clothing so it is also possible that wool moth scales would be present in the environment potentially in high concentrations in the indoor environment. Insect fragments are also normally found in the micro-debris load of honey, and many insects are pests of stored crops. Pathways of inclusion into the dental calculus matrix of such debris are multiple, and such pathways cannot be narrowed down unless a more specific identification is reached in the future.

7.4.3 Comparison with the archaeological data

Ancient insect remains analysis exists for the city of Leicester but mainly consists of fly pupa retrieved from cess pits (Smith 2013, 528). It is very interesting to be able to see that small fragments of insects are found in the human mouth. Finally, remains of Lepidoptera have been retrieved by the author in work conducted in dental calculus

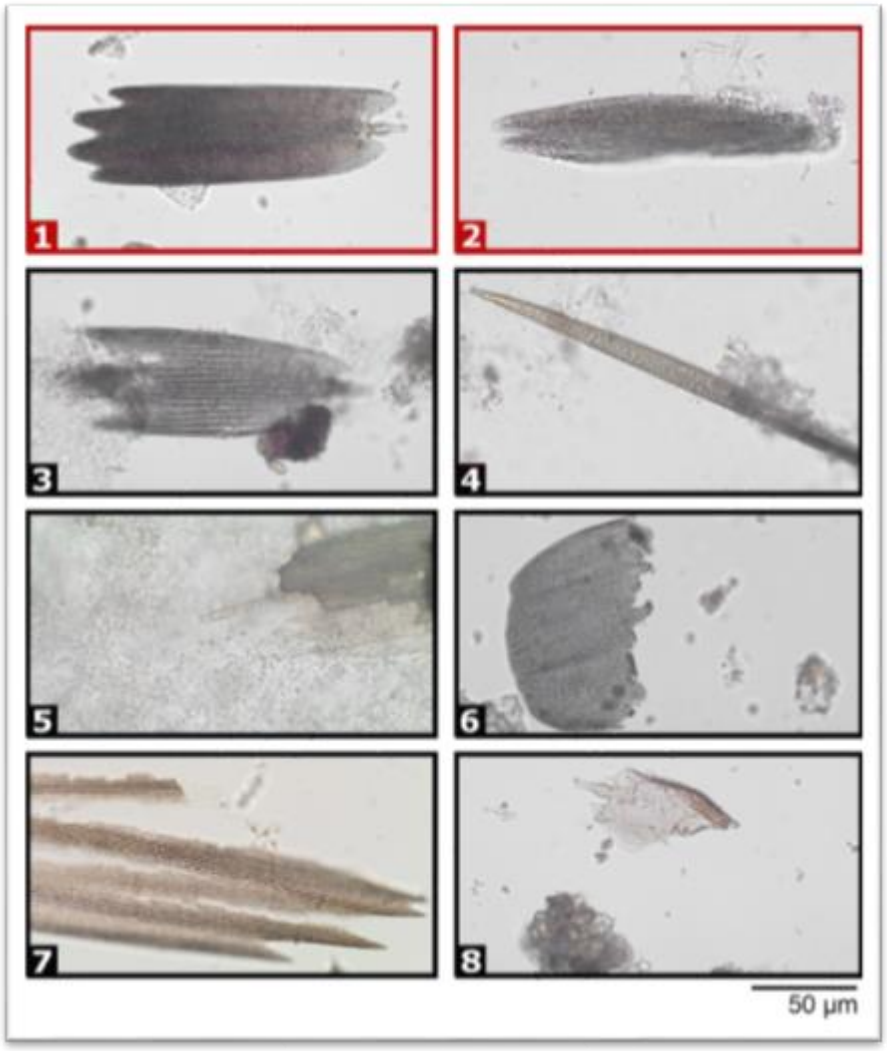

Figure 47. Modern scale. **2.** Calcium oxalate deposit from the leaf of *Vitis vinifera* L. **3-8**. Examples of Lepidoptera scales from dental calculus, showing different level of preservation and potentially different morphologies.

samples of *Homo erectus* from Qesem Cave in Israel (Hardy et al. 2015)[11] and it shows the survival of this line of evidence very deep into the human past, like the fungal remains, which are incidentally composed of chitin too.

[11] The identification of the debris as Lepidoptera scale fragments has, therefore, been peered reviewed in a publication co-authored by the author of this PhD.

7.5 Kingdom Animalia-Phylum Chordata: Class Mammalia

Although a variety of potential fibres were found during analysis, only a portion appeared to be of animal origin. The most common remains of such fibres were identified as animal hair, belonging to sheep and are therefore interpreted as evidence of wool. Results are presented in Appendix V Table 26.

7.5.1 Identification

The identification of the debris was conducted using the atlas *Hair of West European Mammal* (Teerink 1991) and supplemented by other work on the subject of textile fibres (e.g., The Textile Institute 1953) Such textbooks allowed a first step in the identification to species or group of animals, which was then confirmed by the use of reference material. The criteria of identification used were the scale pattern of the cheratine cuticle of the hair, which is the outermost part of the animal hair, as well as overall size, and where visible in the medulla, the innermost layer (see fig. 48.6). Ancient remains and relevant modern reference material are shown in the figure below (fig. 48.6). The cuticle often contains overlapping keratin scales. The morphology, size and the way such scales "overlap" one another is a criterion of identification, sometimes even to species level (Hausman, 1920; Petraco and Kubic, 2003; Titus, 1980; Tobin, 2005). Petraco and Kubic (2003) have pointed out that in many cases, a combination of cuticles, medulla and other features is more reliable. For most of the remains, cuticle patterns were visible, in their characteristic regular, mosaic scale patterns, which combined with birefringence of about 0.01 and a positive sign of elongation, pointed at sheep wool. However, in a number of them they were not sufficiently preserved to allow identification. Debris was therefore divided into two main categories: animal hair identified as sheep, and unidentified animal hair. An

excellent source of online microscopy pictures as comparative material can be viewed from the open resources hosted by the website Micro-lab [12].

7.5.2 Pathways to inclusion

Wool was one of the most important fibres in the Medieval world. Dust containing fragments of such an item would be very common in the Medieval environment. Furthermore, a large portion of the meat consumed originated from sheep. Wool debris in high quantity would also be the result of occupational dust where large amount of such remains would be inevitably inhaled. The use of the mouth as a third hand in the process of wool work would also be a very likely pathway of inclusion for such remains in the human mouth. Differences in the distribution of wool debris that appeared to be statistically significant were found, see Chapter 8.

Furthermore, in Chapter 9 such remains and their distribution are discussed in light of exciting osteoarchaeological evidence of dental wear.

7.5.3 Comparison with the archaeological data

To the knowledge of the author, no wool based textile remains have been found in recent excavations in the North East quarter of Medieval Leicester. However, a number of finds associated to wool spinning and animal remains were found across the town. Wool is also known to have been an important industry in Medieval Leicester, as was leather production. Both were documented in the historic and archaeological record in the North East Quarter. There is no previous record of animal hair in ancient human

[12] http://www.microlabgallery.com/hair.aspx

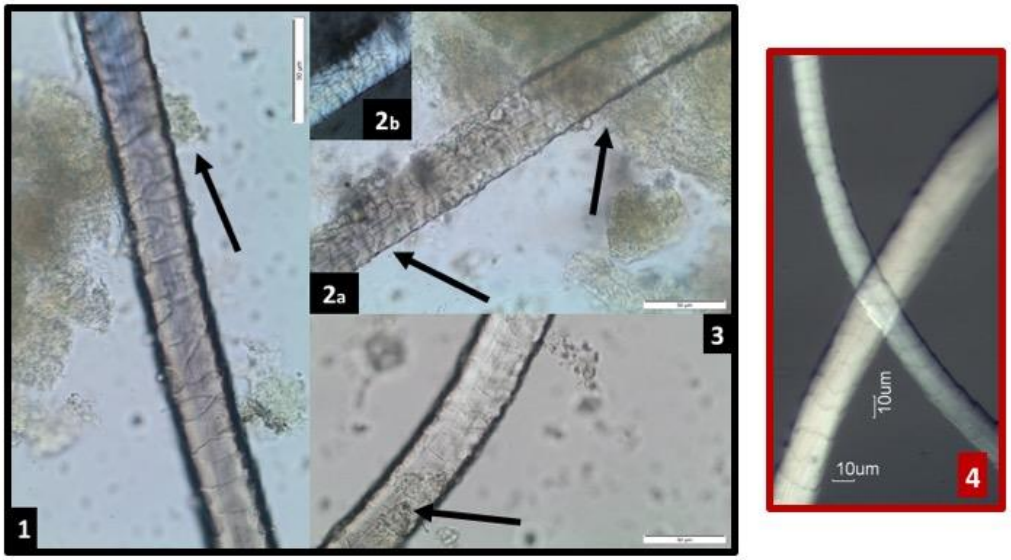

Figure 48. Ancient remains of wool, note the regular scale pattern. Arrows show the calculus embedding the scale. **4.** Modern example of wool (source: http://www.microlabgallery.com/hair.aspx).

dental calculus. However, there is record of potential fibres of corium, possibly occupational dust during leather work, from dental calculus samples of a Medieval male individual at Dalheim, Germany, published by the author in Warinner et al. (2014). Such finds therefore suggest that dust originated by clothing and other textile items can be retrieved from human dental calculus, complementing the evidence of bast fibers of plant origin already found in other studies (Blatt et al. 2010; Radini et al. 2016; Warinner et al.2014).

7.6 Kingdom Animalia: Phylum Chordata Class Avia

One of the most striking finds of animal origin were the very distinctive and often diagnostic fragments of downy feather barbules. The downy barbule is a long subunit of the feather structure with tiny branches. Downy barbules are found in the plumulaceous region at the base of wing, tail, contour, and semi-plume feather types, and throughout the length of down feathers. The characteristics of downy feather

barbules which can identify bird order, family, and sometimes even species are: shape of nodes, distance between nodes, presence and shape of prongs, and pigmentation.

7.6.1 Identification

The identification of down feather remains was divided into two stages. First, a preliminary division was made based on published work (eg. Dove and Koch 2011) and the remains of barbules fragments were grouped accordingly, a process often used by charcoal analysts to divided items of similar morphology. Then a reference collection of modern material was built to confirm such identifications. It must be stressed that the reference collection is not very large, and included birds that were found in the archaeological record of Leicester, especially in the recent excavations at the Shires (eg. chickens, ducks, owls, swans). As a cautionary note, modified setae or bristle hairs on the larvae of some species of the Carpet Beetle (Family Dermestidae) and rodent hair could appear similar to feather barbule nodes and prongs when viewed with light microscopy (see Croft et al. 2016 for more details. Figure 49 shows the anatomy of a downy feathers and the element diagnostic for the identification, while examples of feathers from dental calculus are also provided, with their modern reference material for comparison. Results are presented in Appendix V Table 27.

Remains were grouped in the following categories, described below:

- **Galliformes** (eg. chicken, quails, Pheasants)
- **Anseriformes** (ducks, geese, swans)
- **Strigiformes** (Owls, Barn-owl)
- **Un-diagnostic remains**

Galliformes (eg. chicken, quails, Pheasants, fig. 49 and fig. 50.1). The diagnostic feather for the order has ring shaped structures in the distal part of the internodes. Chickens (like turkey) typically have two different types, which were visible in the material, suggesting these to be very likely chicken, with slightly expanded proximal nodes with some “spine”.

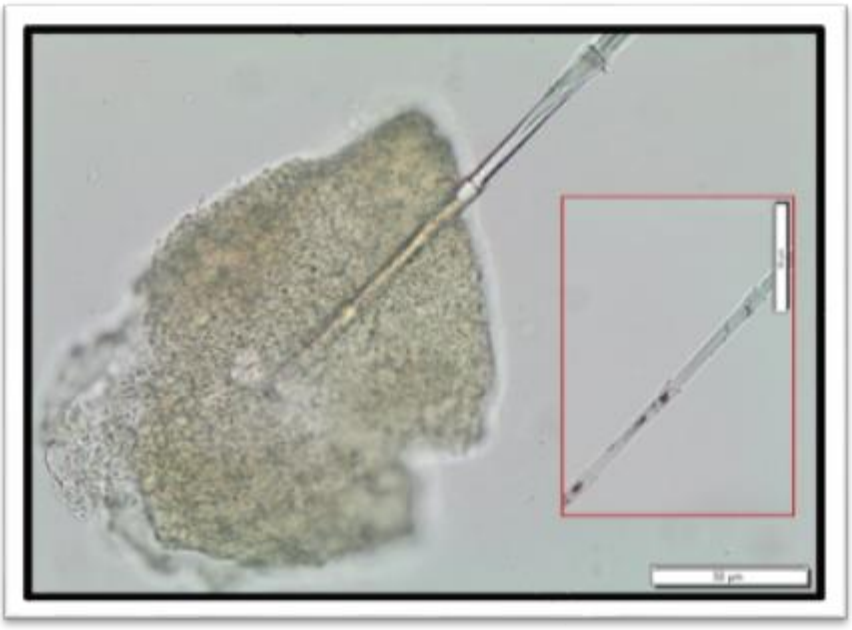

Figure 49. Feather barbules fragment of Galliformes (chicken) still in situ in the calculus fleck, note the similarity of a barbules from modern reference material of downy feathers from chicken Oxford game, male individual, where the presence of pigmentation is higher than in the female.

An interesting feature in some of the remains were ring structures that shows the tendency to slip free from the node and moved close together along the barbule. In the reference collection there is an extraordinary resemblance to feather barbule found in male and female Oxford game.

Anseriformes (ducks, geese, swans). A second typology of feather barbule was consistent with Anseriforms due to the diagnostic triangular nodes located in the distal portion of the feather barbules, of which preserved remains were found in a number of individuals (See fig. 50.2,3) These were then separated in duck type feathers and swan type feathers based on the presence of prongs in the distal part of the barbule, but such division needs to be taken with caution (see picture for more details).

Strigiformes (Owls, Barn-owl). The most diagnostic feature in the material retrieved was the presence of elongated internode (see fig. 50.4), which were constantly pigmented along the visible length of the barbule. This also shows the remarkable potential of

preservation of the human dental calculus matrix. It is thought that the thin and elongated barbules present in the feathers of this order of birds is responsible of their characteristic silent flight (Dove and Koch 2011, 50). The preservation of pigments also allowed a better identification and suggests that the calculus matrix has the extraordinary potential to preserve this line of evidence in very good condition in the period under study. Microscopically, it was possible to identify the feather barbules of owls as they were sufficiently long to present diagnostic features: two to three triangular nodes at the end of the barbule and regular and uniform pigmentation at nodes visible even when the remains are still partially embedded in the calculus matrix (see fig. 50.4).

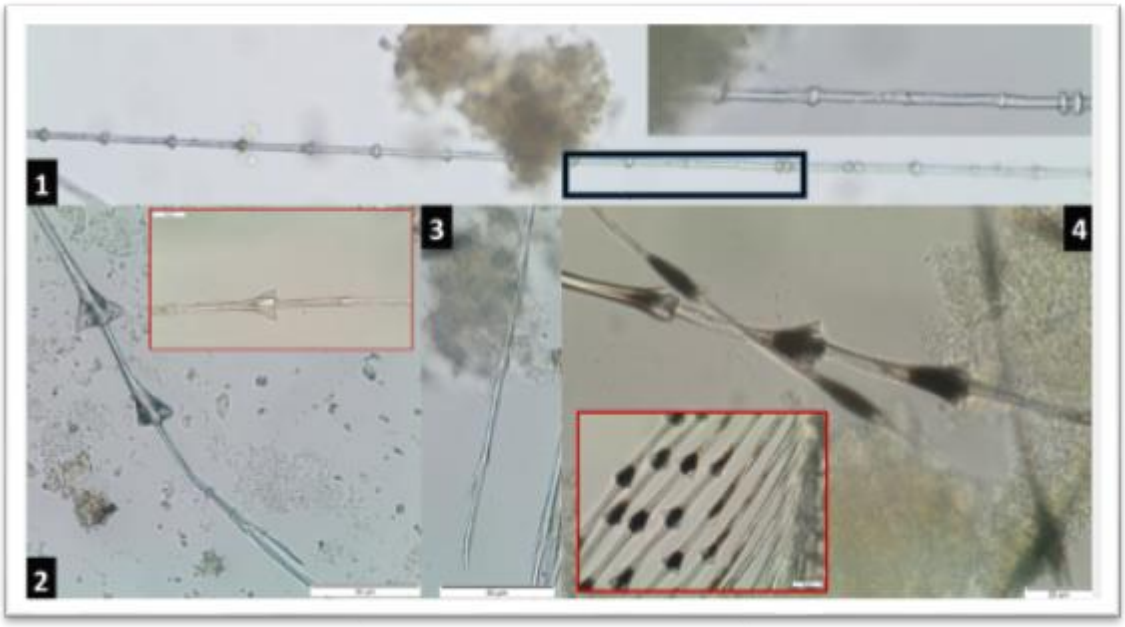

Figure 50. 1. Ancient remains of feather barbules Galliformes, very likely to be chicken, note the characteristics ‘nodes’, 2. Anseriformes barbule fragment (modern reference material from duck is shown in the box) 3. Anseriformes swan type. 4. The pictures show the fragment of feather barbules of Strigiformes (owls) still embedded in calculus matter, note the pigmented internode, in the square a modern example of owl, .

Un-diagnostic remains. A number of feather fragments were found that preserved no traits that would allow their identification. These were found in low numbers in the

same individuals that had higher amounts of other feathers, therefore, they very likely belong to the same type of birds from where the identifiable parts of the feathers were retrieved.

7.6.2 Pathways to inclusion

The small amount of down feathers of chicken remains often attached to egg shells suggests that a possible, though unlikely, source of feathers in women could be the consumption of crushed egg shells for oral hygiene or medical purposes (Richard Thomas' personal comment). However, the most likely pathway of inclusion is the presence of feathers in the environment due to plucking of birds for food consumption. Incidentally down feathers are the most airborne part of bird feathers and their remains can be present in rooms where plucking took place for long periods of time, a fact noticed by the author during the preparation of modern reference material. Particularly dusty is the so called dry-plucking. The presence of such remains can be explained as a result of dust present in the environment due to the plucking of birds for human consumption.

7.6.3 Comparison with the archaeological data

Almost all the feathers retrieved come from birds that are found in the archaeological record in the Anglo-Saxon and Later Medieval deposits in Leicester (Monckton 2015). However, the groups of owls, crow and tits are very rarely found. The historic record also mentions poultry and game as a form of payment for transactions in the Borough of Leicester (Browning et al. forthcoming).

7.7 Inorganic debris

The analysis yielded a variety of 'inorganic' debris, ranging from small mineral particles, to mineral aggregates up to 100 microns in length, small particles of quartz, and possibly flecks of pottery/clay minerals and maybe soil. Moreover, remains of possible pigments were recovered in all human samples.

In general, the minerals present in the human calculus were similar to each other, apart from possible pigments, which were of different colours (see pigments section). The inorganic component of the debris found in the calculus was carefully examined under polarized light. It was noticed that the calculus flecks, in the condition prepared for the analysis (see ch5), became 'dark', invisible, at an angle between 52 to 82 degrees under polarized light. The calculus was first recognized based on its characteristic filamentous but compacted nature, due to the bacterial structure often still visible, the polarizer was then rotated so that the calculus would disappear while the mineral debris behaved differently from the calculus as each mineral changed colour and brightness according to different optical properties associated with different mineral structures and chemical make up.

In this report brief comments are made on the various appearances of the inorganic debris found in the analysis, according to the following categories:

1) Single minerals and mineral 'aggregates' that entered the calculus as a discrete 'entity' (fig.51)
2) Soil

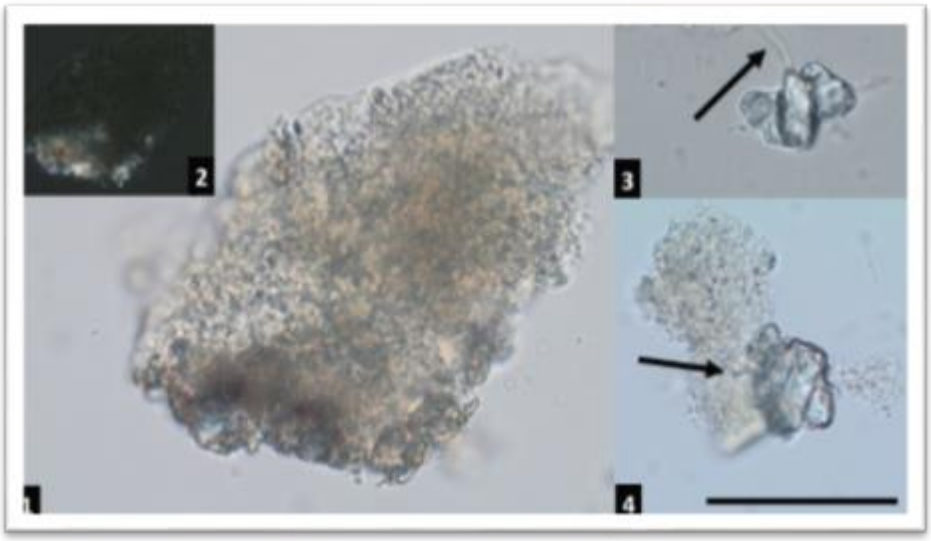

Figure 51. Examples of mineral grit in lumps (1), visible also under cross-polarised light (2) and single minerals (3 and 4). Note flecks of calculus adhering to them (arrows). Scale bar: 50 microns.

7.7.1 Single minerals and mineral aggregates

In all samples single minerals were recovered (fig 51). These were sometimes similar to the ones visible in the flecks of aggregates, suggesting part of them may have come from the flecks and were freed during the preparation of the calculus. One of the most common minerals was quartz. It was recovered in all samples and in high quantity. The particles size ranged from very small (around 2 to 5 microns), to larger flecks of just above 100 microns. Some of the quartz particles showed many cracks and they were of a flattened shape, often embedded in mineral flecks, while more rounded and larger particles were often recovered on their own. All dental calculus samples appeared to have the same type of 'lumps' of fine, unsorted minerals held together by finer, possibly binding, media. These 'lumps' have entered the calculus as small flecks of debris or in larger lumps and they were clearly visible in situ. The fact that they have entered the calculus as 'flecks' was put to the test by poking the coverslip with a fine acupuncture needle, in the same way as it was used to turn the organic debris around. It was noted that the flecks, when not in situ, would move without changing shape and without 'dispersing' their mineral contents. Finally, one individual had a large quantity of very fine particles of what was interpreted as clay (fig. 52).

7.7.2 Soil

Lumps of soil (fig. 53), often still embedded in dental calculus were found in every individual studied. It was possible to establish the nature of such remains as soil flecks when they were composed of a matrix with minerals, organic matter, and sometimes small flecks of charcoal, all below 300 microns in size. The presence of soil was also visible using polarised light microscopy where the small mineral particles had different optical properties from the rest of the remains.

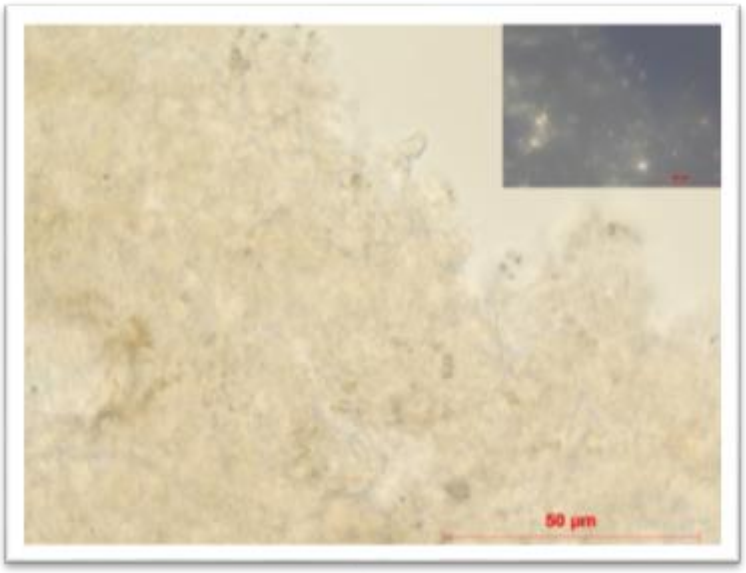

Figure 52. Microscopic particles of “clay” scattered across the entire dental calculus of male individual from St Peter’s, suggesting prolonged exposure to dust consisting with the same type of mineral debris in the form of fine dust. This is potentially interpreted as “occupational dust” generated by working bricks or pottery. The high quantity of fine debris in the form of dust is visible in the cross polarised light micrograph on the top right of the picture.

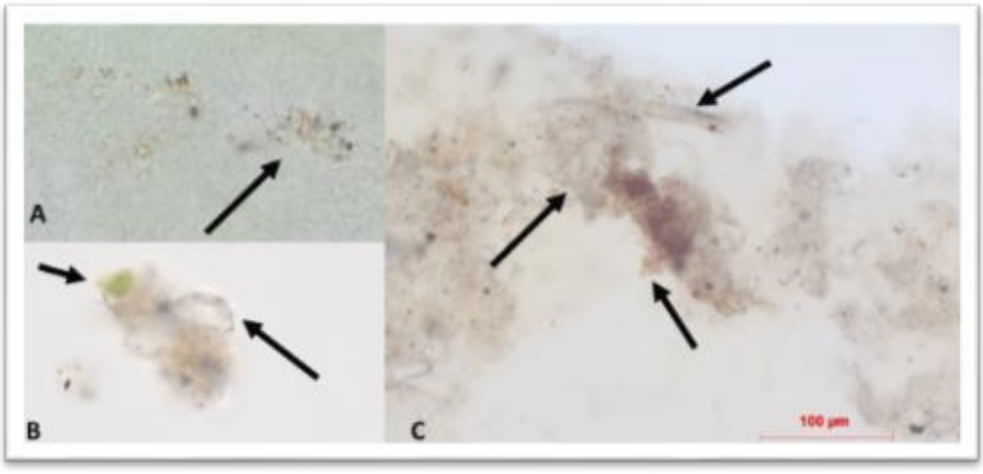

Figure 53. Examples of soil from ancient dental calculus. Note lumps of debris (A, B and C) of unsorted different particles as they are to be found in small lumps of soil.

7.7.3 Pathways to inclusion

The lithological evidence retrieved in dental calculus is of problematic interpretation as a number of human activities produce lithological dust, and pathways of inclusion vary from inhaled dust to dirt on food. The presence of lumps of soil in calculus makes quantification of the debris problematic as certain types of minerals found as single particles could have originated from small lumps of soil entombed in the calculus matrix, which were free to separate from one another during the preparation process. Particles such as carbonates will also be under-represented in the counting due to the effect of HCl in the process of extraction. Care therefore must be taken in quantifying such particles. It is also very likely that the large particles observed had come from other local sources, and they could represent for example milling stone grit. Milling stone grit incorporated to flour during milling was a known problem already in the Saxon period, due to the abrasive effect it had on teeth (Banham 2004). Although no confirmation is possible at this stage, the large amount of these fine particles, mostly in small flecks, maybe suggestive of the handling and inhalation of powdered lithological material, and could be the result of pottery making. However, this hypothesis cannot be confirmed by light microscopy only (see section on lithological evidence in future directions).

7.7.4 Comparison with the archaeological data

Comparison with archaeological data is not applicable in this case as soil and dirt would be ubiquitous in the environment. However, mineral grit of different types has been persistently found in human dental calculus across a variety of samples and periods of time (e.g., Hardy et al. 2015; Radini et al. 2016), suggesting material entering the mouth is not only food and crafts but also soil and dirt present in the environment or settled on material introduced into the mouth. Incidentally, small flecks of quartz and other debris similar to those found in indicated skeletons, were interpreted by Charlier et al. (2009) as potential occupational dust too. This offers potentially new and important insights into food hygiene (see ch. 9, for more details).

7.8 Unidentified and un-diagnostic remains

All the individuals analysed in this study had some debris that could not be identified or assigned to a particular Kingdom, including particles that could be either mineral or phytoliths. Very often this was due to the small size of the remains since such remains were often below 2 or 3 microns, or due to the lack of visible features to allow a better understanding of the remains. Finally, in some cases debris remained surrounded by calculus flecks that could not be further dissolved by adding HCl to the slides. However, all such remains were counted in order to provide a realistic picture of micro-remains quantity in dental calculus. For the scope of this study, quantification of non-diagnostic debris and unidentified debris was not reported as it cannot provide information on diet and living conditions, however it was recorded.

7.9 Summary and overview to the proceeding chapter

A variety of micro-remains that did not belong to the Kingdom Plantae were retrieved during this analysis. Although not as frequently and in the same quantities of plant remains, debris of fungal, animal and lithological origin was well represented in the assemblage. Spores, yeasts and mould remains were very common and they were attributed to yeast, moulds and plant pathogen. Animal debris included parasite ova, feather fragments from at least 3 different groups of birds, and wool. Debris of lithological origin such as minerals, mineral aggregates and soil flecks were present in the entire assemblage. Lack of reference collections, limited amount of debris with diagnostic features and the need of a microscope with more analytical power have limited the identification achieved. Ways of improving identification and the value of such lines of evidence is explored in Chapter 9 (see session future directions).

Having described the debris found, its potential pathways of inclusions and its archaeological visibility, the next chapter will examine how the debris found is distributed across the populations. The analysis of non-plant material revealed a high amount of fungal debris as well as other particles of non-dietary origin. However, the non-plant remains represented only a small portion of the remains entombed in the

calculus matrix. Finally, animal remains are very few, when compared with other lines of evidence.

CHAPTER 8

DATA ANALYSIS

8.1 Introduction

Having surveyed the types of evidence retrieved in dental calculus in the previous two chapters, this chapter will focus on the analysis of the data to investigate how various remains are distributed across the samples, with a focus on how diagnostic debris can potentially inform about differences in diet and living conditions and their changes. To this end, this chapter is organized in relation to the aims and objectives of this study, which were to better understand the usefulness of the micro-debris entrapped in the calculus matrix in providing new insights into past ways of life. Before presenting the results of the analyses, two pressing points need to be brought forward. First of all, so far there has been a very limited amount of published work on the remains in human dental calculus conducted by samples large enough to allow statistical analysis. To the knowledge of the author, this PhD is in fact only the third study to date where statistical analysis of dental calculus microdebris is possible to some extent. Dudgeon and Tromp (2014), were the first to achieve a data set large enough to be approached statistically, having worked on data from Rapa Nui (Easter Island) consisting of 104 individuals and dating to the 16th - early 18th century. The second study was conducted by Leonard et al. (2015) on a modern population: a dataset of 74 foragers-horticulturalists from Northwestern Namibia and Southwestern Angola. Although from two very different geographical and temporal contexts, these studies have influenced the conceptual approach of the current data and variables, as well as data preparation and analysis. These aspects are treated in more detail in the following paragraphs.

8.1.1 Some initial considerations

Before entering into the details of the analysis, including data preparation and selection of variables analysed, some important considerations need to be put forward. There are

three main important factors that affect the analysis of microdebris entrapped in dental calculus:

1) The level of identification achieved during the analysis on different types of debris 'captured' by the calculus matrix.
2) The extent to which the quantity of such typologies of debris is direct evidence of the original amount that entered the mouth and became captured by the forming calculus matrix.
3) The length of time taken to capture the debris in the calculus matrix.

Chapters 6 and 7 have shown that the variety of debris that becomes entrapped in the calculus matrix is of both dietary and non-dietary origin and that multiple pathways of inclusion are present, often for the same types of debris. Only debris identified to taxon/pathway can be used in the analysis in order to be comparable across populations, as pointed out by Leonard et al. (2015); therefore, the selection of variables to be analysed took into account the level of identification needed for the research questions this PhD aimed to answer.

Secondly, the amount of micro-debris captured by the calculus matrix cannot be securely linked to the original amount of debris to which the individual was exposed. For instance, 45 starch granules of the Triticeae tribe could equally come from 1 single grain of a species member of this tribe, or from 45 grains of these species, or any unknown mix of species of this tribe (e.g., 30 grains of barley and 15 grains of wheat).

Finally, as highlighted in Chapter 2, it is not possible to fully understand how long the calculus took to form; therefore, it is not clear what portion of the life of an individual is represented in the samples examined. For instance, a calculus that formed on an incisor (one of the first teeth to erupt) of an individual that died in adulthood could potentially have a large amount of debris not because that individual was exposed to a larger quantity of debris, but because he/she was exposed to it for a longer period of time. Equally, a calculus deposit that is larger than another one will potentially have more debris in total due to its size rather than to concentration.

In order to minimize such problems, the sampling strategy adopted targeted calculus samples on the 3rd molar of young adults, as explained in Chapter 3. This practice reduced the window of time represented by each calculus deposit. In addition, the dataset under study is large and a wide reference collection was used for the identification of the debris. Despite the above measures, the interpretation of any statistical results needs to be taken with caution for the reasons outlined above.

8.1.2 Data preparation and selection of variables

The first step was to decide if the data were going to be compared directly or by concentration. While sample volume and weight are variables regularly taken into account in other palaeo-environmental analyses (e.g., archaeobotany and palynology), this has not been the case in dental calculus research. Dudgeon and Tromp (2014) conducted their analysis without taking into consideration sample weight and, therefore, without really considering the potential link between the original amount of calculus and the amount of the debris found. Leonard et al. (2015, 454) however, demonstrated that the weight of the calculus sample clearly affects the quantity of remains in the analysis with direct consequences on their distribution across the population. In specific, when the weight of the calculus deposits was introduced in the analysis, significant differences between age and sex groups disappeared. In the current study, therefore, all analyses were conducted using the concentration of debris per milligram. In terms of selection of variables[13], all typologies of debris that could be identified to a taxon or a specific pathway of inclusion were analysed. Unlike previous works, such variables did not include only starch granules and phytoliths but all remains that were assigned to a taxon ('identified' starches and phytoliths) and that could be correctly quantified. Wherever this was not possible, pathways to inclusion were

[13] In this chapter the term 'variable' refers to each typology of debris within each broad category (e.g., wool in the broad category of animal remains/hair)

selected: for instance, many phytoliths could not be identified to a specific taxon; however, some phytoliths were burnt, very likely as a result of exposure to ash and smoke in fires. Unidentified debris was excluded from this analysis because they could not provide insights into how such remains could record diet and living conditions. The way each variable was analysed either on its own or combined with others will be specified in the relevant sections. In most statistical tests multiple categories of debris were used in the comparisons together, as their combination represented specific types of diet, occupation, and environment. Due to the biases pointed out at the beginning, data were considered not parametric. Therefore, multivariate tests were adopted, primarily nonparametric MANOVA. The comparisons conducted included: a) Medieval males vs. females, b) Anglo-Saxon males vs. females, c) Medieval groups vs. Anglo-Saxon groups, and d) St Michael's individuals vs. St Peter's individuals. Principal Component Analysis was also attempted; however, in all cases the percentage of total variance explained by the first two Principal Components was below 50%; thus, the PC1-PC2 scatterplots could not be used to accurately identify clusters of individuals or outliers. Even when the first three PCs were adopted, the cumulative variance did not raise above 60%, while in most cases it was notably lower than that. A representative example is provided in Table 6 where the first 3 PCs explain less than 35% of the original variance of the dataset. For this reason, the PCA results will not be reported here. All tests were run in PAST (Hammer et al. 2001).

8.1.3 Structure and depth of the analysis

The large dataset examined is suitable for a number of analyses, however due to the explorative nature of this the attention was focused on the main questions that need

Component	Initial Eigenvalues		
	Total	% of Variance	Cumulative %
1	2.956	14.782	14.782
2	2.252	11.261	26.043
3	1.684	8.418	34.461
4	1.311	6.555	41.016
5	1.142	5.710	46.725
6	1.066	5.330	52.055
7	1.025	5.127	57.182
8	1.014	5.072	62.254
9	.987	4.933	67.186
10	.936	4.681	71.867
11	.873	4.364	76.232
12	.826	4.128	80.359
13	.793	3.963	84.322
14	.694	3.471	87.793
15	.606	3.029	90.822
16	.597	2.985	93.807
17	.509	2.544	96.351
18	.379	1.896	98.247
19	.351	1.753	100.000
20	-1.521E-17	-7.606E-17	100.000

Table 6. Example of PCA results obtained from the overall built environment data set.

answering for the aims of this study:

1) Are there any patterns in the way in which dietary remains are distributed across time and sexes?
2) Can calculus provide information on the natural environment surrounding the studied individuals during life?
3) Are there diachronic changes between populations linked to the well-known climate change and deforestation known to have occurred in the period examined (Hoffmann 2014)?

4) What type of information can dental calculus provide in relation to the built environment?
5) Can we detect potential 'occupational' dust using debris entombed in the calculus matrix?

Finally, a short section explores the evidence of Fungi due to their potential as a palaeoenvironmental indicator.

8.2 Patterns associated to the dietary evidence

8.2.1 Dietary debris versus non dietary remains

One of the aims of this PhD was to establish what portion of the debris in dental calculus could be considered securely of dietary origin and how representative this is of plant foods in the diet. To begin to understand the role played by dietary debris, the concentration of debris that could be securely linked to diet was compared with debris where pathways of inclusion were not only or not at all of dietary origin. Secure dietary debris (and finds that can be considered mostly the result of accidental ingestion due to diet) show that **only a third of the remains can be securely interpreted as secure deliberate consumption**. The dietary remains are carefully explored in the following section.

8.2.2 Dietary remains – overall patterns

First, patterns were explored by combining all remains in calculus that are almost securely the outcome of deliberate food consumption, in order to test if any general differences were visible across the populations studied. The variables selected for this analysis were:

1) Staple food of plant origin represented by starch granules of cereals (Triticeae and Avenae) and legumes (Fabaceae)
2) Starch granules, phytoliths and plant tissues that are evidence of imported items
3) Plant tissues such as leafy greens (in specific, leek)

4) Plant tissues from weed seeds (identified as most likely the result of accidental ingestion of contaminated crop)
5) Non-arboreal pollen (due to the likelihood of being the result of deliberate ingestion of the flowering parts of species such as herbs or medicinal plants or as the pollen load of honey)
6) Fragments of tissues of hemp seeds (due to their mild medicinal and dietary uses)

While a number of yeasts are used for brewing and as a rising agent (as pointed out in ch 7), many are also naturally present in the microbial flora of the mouth. As it was not possible to identify them, they were omitted from the analysis.

One of the main research questions of this PhD was whether by combining multiple variables associated to diet, differences across populations could be identified. Whenever a statistically significant difference was identified among groups, the mean concentration of debris per group was given in the text to facilitate the interpretation of the results. If the frequency of the remains across the population was relevant and informative, it was reported too.

The results of the non-parametric MANOVA revealed a statistically significant difference between the Anglo-Saxon and the Medieval populations (**p-value = 0.010**). Regarding potential differences between male and female dietary patterns, no statistically significant difference was identified between sexes either in the Anglo-Saxon period (**p-value = 0.793**) or the Medieval one (**p-value = 0.100**). In contrast, a significant difference was traced in the overall diet between St Peter's and St Michael's (**p-value = 0.004**). This difference remained significant even when the doubtful data from oats were removed (**p-value = 0.004**) and is due to the lower number of finds of Legume starch granules in St Peter's males (fig. 52).

Since staple food crops, in particular legumes and cereals, were hugely represented in the dataset in part due to the amount of starch granules released in the mouth during a meal, all above analyses were repeated without them. The difference between Medieval and Anglo-Saxon groups was again statistically significant (**p-value = 0.002**). Similarly, no significant difference was found between sexes in the Anglo-Saxon (**p-**

value=0.724) and Medieval period **(p-value=0.444**), while now the difference between the two Late Medieval cemeteries also appeared non-significant (**p-value=0.408**).

The above results reveal the first important general patterns: there were significant differences between periods of time (Anglo-Saxon versus Medieval), but no significant differences could be traced between sexes in both periods, or between the two cemeteries. This is an important finding as it suggests that the plants consumed as or with food differed temporally but were available and accessed equally in the same period of time. In the following paragraphs, plant remains will be broken down to sub-categories in order to explore different dietary components.

8.2.3 Staple starchy food

This category used as variables the three main types of starchy food plants identified: Triticeae (e.g., wheat and barley); Fabeae (such as vetches and peas) and Aveneae (oats). A statistically significant difference was found between the Anglo-Saxon and Late Medieval groups (**p-value = 0.026**), as well as between the individuals from St Peter's and St Michael's (**p-value = 0.002**); however, the difference between Anglo-Saxon males and females (**p-value = 0.767**) and Late Medieval males and females (**p-value = 0.099**) did not appear significant.

Due to the fact that cereal crops release a very large amount of starch granules into the mouth, they were analysed separately from other food plants. Furthermore, the identification reached was tribe or family level, so it is not possible to separate different species of the same tribe and truly understand how different types of such food formed the record entombed in the calculus. Nevertheless, they were the most represented remains in the assemblages. As cereals were the main staple crop in the period, the concentration of starch granules in each category of starchy food is most likely the result of different parts of different species. Comparing concentrations therefore in this specific case was not very reliable. Here, frequency of individuals exhibiting each type of staple starchy food debris in each population was considered (fig. 54). The results highlighted the tribe Aveneae was underrepresented in the male individuals from St Peter's for reasons that will be explored in the discussion, but likely related to problems

of identification. In Chapter 6 it was stressed that it is very difficult to correctly quantify compound starch granules so the differences observed could be simply the result of more intact compound granules present during capturing such debris. For instance, differences in chewing or oral health could be causing certain people to chew less and, therefore, starch granules of compound nature would be present in larger lumps. In contrast, if individuals had to chew more, this could break up the lumps of compound granules, resulting in higher numbers of free smaller units of the compound granule itself. It also highlighted that legumes were found in more individuals buried in the cemetery of St Peter's and during the Anglo-Saxon period, probably reflecting a similar problem. The data altogether are consistent with what is known about the main staple crops through the Middle Ages, both in the region (Monckton 2006; Monckton and Radini 2009) and across Britain (e.g., Dyer 2002, Moffett 2006), as cereals and legumes were staple crops throughout the period under study. Such results point to the direction that if plants rich in starch granules are staple in the diet, calculus does record such information.

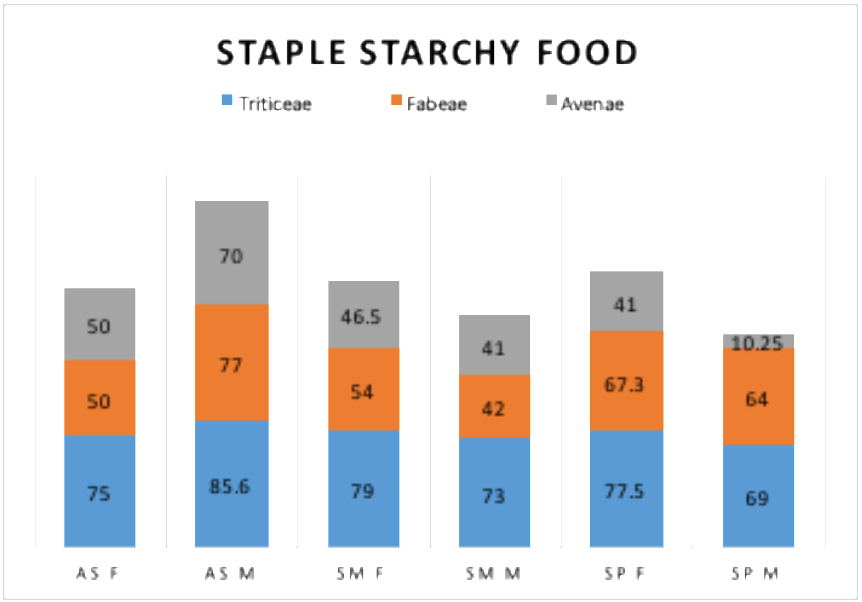

Figure 54. Number of individuals with evidence of starch granules belonging to staple food plants across the studied populations: AS =Anglo-Saxon; SM=St Michael's; SP= St Peter's; F=females; M=males.

8.2.4 Weeds

Although not completely the result of deliberate consumption, fragments of the weed seed coats were scattered across the populations. It was suggested previously that many seeds of weeds were in the past left in the eaten crops as they were edible, so here they are considered as accidental consumption associated to food and could provide direct evidence of how 'clean' the crops were. Here the variables considered were campions (*Silene* sp.) and corncockle (*Agrostemma githago*). Poppy (*Papaver* sp.) was removed as it was found only in two individuals. The application of nonparametric MANOVA identified a significant difference between the Medieval and the Anglo-Saxon period (**p-value=0.001**). In contrast, no significant difference could be traced between sexes in the Medieval period (**p-value=0.468**) or the Anglo-Saxon period (**p-value=0.092**), neither between the individuals buried at St Michael's and St Peter's (**p-value = 0.562**). Tissue fragments of *Silene* spp. (campions) seed coats were found only in the Anglo-Saxon period, in 14 individuals, suggesting already a difference in the presence of such weeds between the two periods. Poppies are very common in arable fields, while campions can grow in a variety of habitats depending on the species, and they have had a strong presence in the pollen record in correspondence with farming activities. Some species (e.g., *S. alba*) were introduced in the Neolithic (Godwin 1975, 143) and therefore are very likely to be a weed of crops, like poppy. Lack of species identification prevents a more secure ecological understanding, but work is in progress to improve this. Among the individuals that had diagnostic plant tissues, the most represented was *c*orncockle, aligning with evidence from macroscopic remains which places it as one of the most common weeds in the Medieval period (Hall 2003). Such finds were retrieved in 10 Anglo-Saxon and 72 Late Medieval individuals, roughly in one person out of three for each population. Corncockle remains had a similar concentration between the two periods (Anglo-Saxon mean=0.2/mg and Late Medieval mean=0.03/mg).

The temporal difference identified is potentially the result of different farming practices between the two periods and it will be explored in more detail in chapter 9.

8.2.5 Leafy crops

A number of plant tissues and undiagnostic tissue fragments were found that could belong to leafy crops, but only one typology had characteristics that could be securely identified. These were leek epidermis fragments. Mann-Whitney tests were conducted to compare *Allium* sp. between Anglo-Saxon and Medieval populations and the difference between them was statistically significant (**p-value=0.0001**). This difference is due to the higher concentration found in the Anglo-Saxon period when compared to the Later Medieval period (Anglo-Saxon mean=0.063/mg, Later Medieval mean = 0). Incidentally, the highest amount was found in an Anglo-Saxon individual (RT12, 0.1667/mg). The difference between males and females did not appear to be significant either in the Anglo-Saxon (**p-value = 0.250**) or the Late Medieval period (**p-value = 0.899**), as did the difference between the individuals buried at St Michael's and St Peter's (**p-value = 0.963**).

The frequency of such remains was also higher in the Anglo-Saxon period, since leek remains were found in 50% of the Anglo-Saxons, and in less than 8% of the Medieval samples, suggesting that such remains were "captured" more often across individuals of the Early Medieval period. This result is consistent with Moffet's (2006) observation that leeks were the most common greens in the Early Medieval period and were replaced by cabbages later on.

However, due to the small number of individuals exhibiting such debris, it is also possible that differences in the cooking practices between the two time periods may have affected the survival of such lines of evidence. Such issues will be discussed in detail in the 'preservation' section of Chapter 9.

8.2.6 Potential Herbs/Condiments and Medicinal plants

Of particular interest were the finds of a number of remains of both non-arboreal pollen and plant tissues, which potentially came from species or Families of plants used as herbs or for their medicinal properties. Most of such remains belonged to the non-arboreal pollen of species of plants also found commonly in the pollen load of honey (Sawyer 2006). Variables considered here are those of non-arboreal pollen and the

plant tissue of hemp seeds. It must be stressed that such variables could also be due to differences in the natural environment around the individuals, as two of them are weeds of crops (Chenopodiaceae and Polygonaceae); therefore, it was useful to investigate them. Non-arboreal pollen remains were found in a very small number of individuals and were more frequent across the Anglo-Saxon populations than they were in the Middle Ages (see Figure 8-3).

Statistically significant differences were traced between the Anglo-Saxon and Medieval groups (**p-value=0.0001**), but no significant difference appeared between males and females in the Anglo-Saxon population (**p-value=0.984**) or the Late Medieval one (**p-value=0.601**). Therefore, the overall average concentration of non-arboreal pollen was significantly higher in the Anglo-Saxon period when compared with the Later Medieval one (Anglo-Saxon mean=0.026/mg, Late Medieval mean=0.005/mg). Pollen of the Polygonaceae Family and Rosaceae type was found to be much higher in the Anglo-Saxon period (Anglo-Saxon period: Polygonacea mean=0.052/mg, Rosaceae type mean=0.064/mg; Late Medieval period: Poligonacea mean=0.008/mg, Rosaceae type mean=0.006/mg). The concentration of Asteraceae pollen is also higher in the Anglo-Saxon period (Anglo-Saxon mean=0.037/mg; Late Medieval mean=0.013/mg); All the remaining species were very poorly represented in the record, and their average concentration was much lower but still different: *Artemisia* was higher in the Anglo-Saxon period than the Later Medieval one (Anglo-Saxon mean=0.009/mg; Late Medieval mean=0.001), Chenopodiaceae followed the same pattern (Anglo-Saxon mean= 0.009/mg; Late Medieval mean= 0.003/mg). Finally, the less represented species were those of *Centaurea* type and *Borago/Anchusa* pollen, which were very rare in the assemblages. *Here Centaurea* pollen still has a slightly higher average concentration in the Anglo-Saxon period (Anglo-Saxon mean=0.003; Late Medieval mean=0.002), while *Borago/Anchusa* has a higher average concentration in the Later Medieval period (Anglo-Saxon mean= 0.002/mg, Late Medieval mean=0.005/mg), although the values are close.

Such differences are also clear in the frequency of the species and the lower occurrence of non-arboreal pollen in the Medieval population, clearly visible in figure 55. However,

the presence of potential medicinal and herbal plants very likely depends on personal taste as well as the health of the individuals in which such lines of evidence were found, and this aspect must have had some effect on the results obtained.

As evident from the aforementioned data, the discriminant factor appears to be the pollen of the Rosaceae type. It must be stressed again that the identification of such remains was doubtful due to poor status of preservation, so the above pattern needs to be taken with caution. However, relevant remains are currently subject to further examination to try to refine the identification to species level.

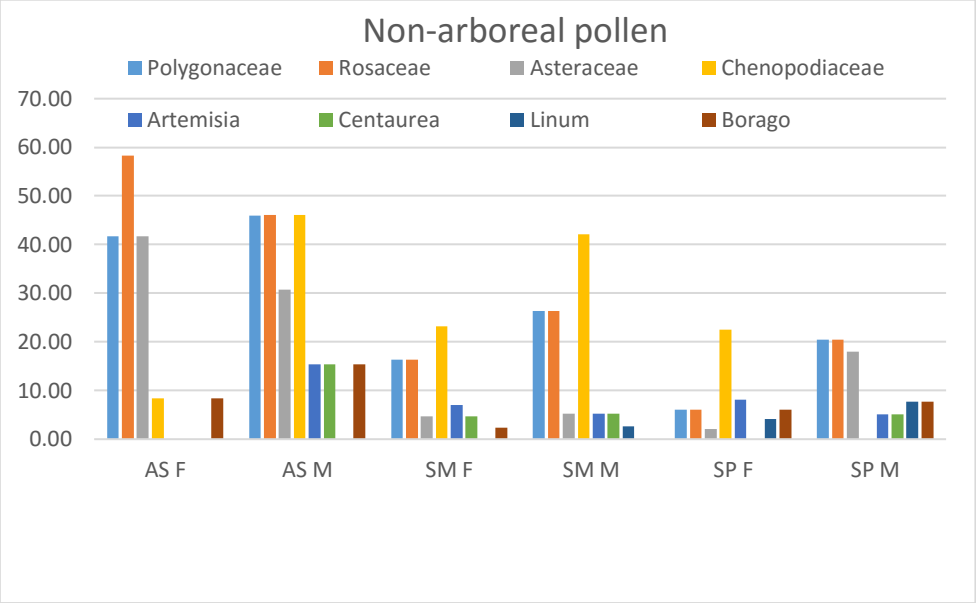

Figure 55. Percentage of individuals that had non-arboreal pollen across the population: note the clear difference between Medieval and Anglo-Saxon individuals and the parameter of Rosaceae type pollen. Note that *Borago* here indicates the type of Boraginaceae-*Borago-Anchusa*, while *Linum* indicate *Linum* type.

It must be stressed that with the exception of *Borago/Anchusa* type pollen, very likely here result of deliberate consumption, all other Family/species are occasionally airborne, found in the pollen load of honey and also found in arable fields (Hyde and Adams 1958). Therefore, alternative pathways to inclusion are possible. Nevertheless,

they do indicate changes in uses/exposure to plants that require a closer proximity to the flowering part than arboreal pollen.

The last category of debris examined here is *Cannabis sativa* (hemp), which is known either as a fibre or today for its drug properties. Hemp seeds are considered here as potentially medicinal, but they are nutritious and could therefore have been eaten as an addition to the staple food. The surprising factor here is that such remains have a much higher average concentration (Anglo-Saxon mean=0.037/mg; Late Medieval mean=0.005/mg) and frequency (40% individuals in the Anglo-Saxon period and 15% in the Late Medieval period) in the Anglo-Saxon period. Such remains are explored in more detail in the Discussion, but they are likely to represent a change in the uses of the species.

8.2.6 Imports

The analysis of micro-debris generated by the consumption of imported items, with otherwise low archaeological visibility, was one of the most interesting aspects of this study. As the Medieval period saw an increase in trade and the movement of people, the distribution of imported items across populations and between sexes may provide interesting information regarding access to food items acquired through long distance trade and relevant diachronic changes. In particular, two patterns were investigated:

1) Do imports occur more in the Later Medieval period, where trade and routes would facilitate the exchange of food and overall travelling?
2) Are imports equally distributed among sexes or are there statistically significant differences?

The most common species present were those of the family Zingiberaceae, which included the imports ginger and galangal found in the Medieval period in the historical record of Leicester (Browning et al. forthcoming). Other imports were very rare, such as millets, fenugreek and palm, which were found in only one or two individuals. Non parametric MANOVA tests were conducted using all types of imports as variables simultaneously. The comparison between the Anglo-Saxon and Medieval groups gave a **p-value = 0.078**, indicating that differences in access to food imports was not

statistically significant but nevertheless rather close to the level of statistical significance (p=0.05). These results are very important in terms of food trade as they show that there was some access to imported species in the Anglo-Saxon period, although they need to be taken with caution due to the small dataset for the period. It must be stressed that at least for the pagan Anglo-Saxon cemetery of Empingham, grave goods are suggestive of long distance trade for the population and are known for the period (Timby 1996).

Differences between sexes were tested in each period, but they did not appear to be statistically significant (**p-value=0.402** for the Medieval period, **p-value = 0.415** for the Anglo-Saxon period). Similarly, the comparison between the two Later Medieval populations of St Peter's and St Michael's did not identify a significant difference (**p-value=0.347**), suggesting the two populations had similar access to imports. It must be noted (and will be discussed further in the relevant section of Chapter 9) that the very rare imports (the potential evidence of sorghum and palm-date) are only present in males in the Medieval period, while millet is found in a female in the early Anglo-Saxon sample.

8.3 Patterns related to the natural environment

As seen in Chapter 4, major changes are known to have occurred during the Medieval period, associated to deforestation as well as climate change. In order to explore the potential of ancient human dental calculus to capture changes in the natural environment, both plant and fungal remains likely to be plant-pathogens, and known to grow in wet conditions (see Chapter 7) were considered. While arboreal pollen, wind pollinated, has a secure identification, the variables of the fungal debris were a bit more difficult to select. In the context of answering questions related to the natural environment, the only secure variables selected were fungal spores belonging to the genera *Alternaria*, *Curvularia* and *Fusarium*, where the identification had a degree of security and the species of such genera include only plant pathogens.

8.3.1 Natural environment: the evidence analysed as a whole

Initially, all variables that could be related to the natural environment were analysed together. A significant difference was identified by means of nonparametric MANOVA between the Anglo-Saxon and the Medieval period (**p-value=0.0002**) (Anglo-Saxon mean=0.046/mg; Late Medieval mean=0.008/mg), but no difference could be detected between males and females in the Anglo Saxon (**p-value = 0.281**) and Medieval period (**p-value = 0.153**). However, a significant difference was found between St Michael's and St Peter's (**p=0.049**) (St Michael's mean=0.006; St Peter's mean=0.010). These differences appear to be the outcome of both pollen and fungal remains and are explained below. Finally, as the identification of the arboreal pollen was very reliable, while that of fungal spores needs to be confirmed, these two broad categories were also analyzed independently.

8.3.2 Arboreal Pollen

All species of arboreal pollen, including shrubs, which are considered to be wind pollinated and very likely airborne in the environment, were considered for this analysis. A statistically significant difference was found between the Anglo-Saxon and the Medieval period (**p-value=0.0003**) (Anglo-Saxon mean=0.053; Late Medieval mean=0.007), but no significant difference to arboreal pollen exposure could be traced within individuals of the same period of time in both the Anglo-Saxon (**p-value=0.245**) and the Medieval period (**p-value=0.167**). This pattern is clearly visible in Figure 8-6 where the number of individuals with specific types of pollen is plotted. Similarly, the difference between the Medieval Parishes of St Michael's and St Peter's did not appear to be significant (**p-value=0.139**). Of interest is oak (Anglo-Saxon mean=0.080; Late Medieval mean=0.005) and ash (Anglo-Saxon mean=0.055; Medieval mean=0.003), which are known to have been common in Anglo-Saxon wood pastures and then declined in the Later Medieval period (Hooke 1998, 2010), while oak pollen and hazel type pollen are those with the highest frequency (see Figure 8-3). The data therefore potentially reveal a difference of the natural environment between the Anglo-Saxon population and the later Medieval one and will be discussed in Chapter 9.

8.3.3 Plant Pathogens

The distribution of fungal debris was analyzed in order to assess if significant differences existed between and within the Anglo-Saxon and the Medieval populations. The three categories of debris analyzed are the debris attributed to genus *Alternaria sp.*, *Curvularia* sp. and *Fusarium* sp. Fungal remains were almost ubiquitous in the Late Medieval period (**99% of the individuals had such remains**) and less frequent in the Anglo-Saxon period (**60% of the individuals had fungal spores**), although they appear to be in higher concentration in the Anglo-Saxon period. It is striking that no remains of *Fusarium* sp. spores were found in the Anglo-Saxon period. It must be stressed that none of the remains of fungal debris were positively identified to species level and the results need to be taken with caution.

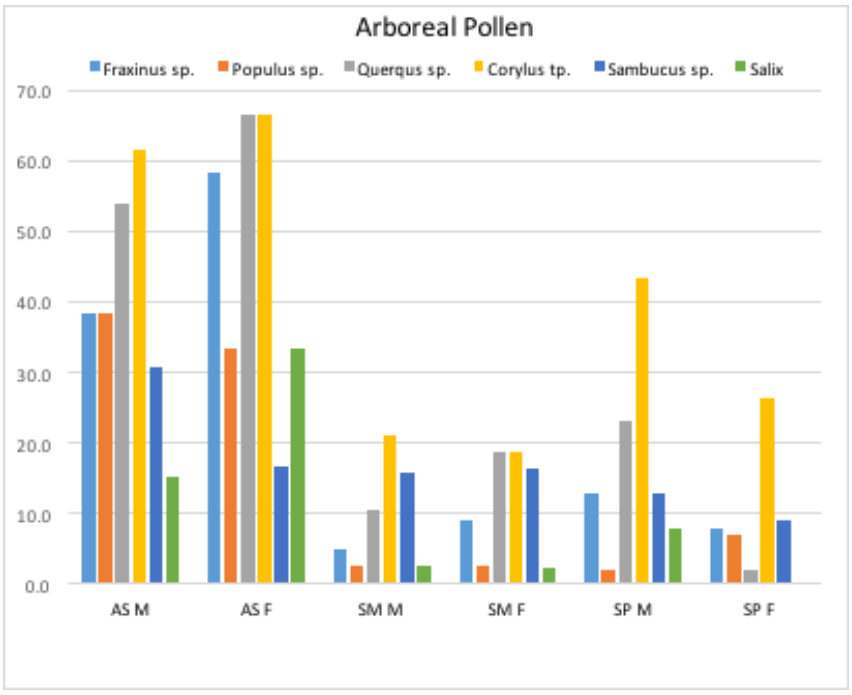

Figure 56. Percentage of individuals with different pollen types. Note the clear difference between the Anglo-Saxon (AS) and the Medieval populations (SM and SP), especially for what concerns long lived species such as *Quercus* sp. (oak). In general, the pollen of all other trees was also found in lower amount and with low frequency in the Medieval period. M=males; F=females

The statistical difference between the Anglo-Saxon and the Late Medieval population was significant (**p-value=0.0001**) (Anglo-Saxon mean=0.024; Late Medieval mean=0.011; *Alternaria*: Anglo-Saxon mean=0.045; Late Medieval mean= 0.015; *Curvularia* sp.: Anglo-Saxon mean=0.260, Late Medieval mean=0.011; *Fusarium* sp.: Anglo-Saxon mean=0, Medieval mean=0.012). No difference appeared to be present between sexes in both the Medieval (**p-value = 0.246**) and the Anglo-Saxon period (**p-value = 0.998**). This is suggestive that within the same population both sexes were equally exposed to such pathogens, therefore, the debris was very likely present uniformly in the environment). The data suggest great potential for future analysis due to their ubiquity across the assemblage, but a reference collection will need to be built for this purpose.

8.4 Patterns linked to the built environment

A further goal of this survey was to address the value of non-dietary debris and the information that can be obtained when the quantified data that are likely not of dietary remains are analyzed together. Here I will look at those remains that were found in dental calculus that were very likely present in the environment both in the form of "dust" in the air or as a result of activities that may produce such debris during the practicing of crafts.

This section focuses on 2main aspects of interest to this PhD:

1) Debris that can provide information on housing conditions, consistent with "dust", including occupational dust
2) Potential evidence of smoke/cooking practices (this is separated from the line of evidence of point 1 for reasons that will be detailed further on in the text)

The approach followed here looks into patterns between periods of time and sexes, but also focuses on "anomalies" in the concentration of certain typologies of debris that could be considered "occupational dust". For instance, plant fibers belonging to flax/hemp or wool would be naturally present in the "human environment" experienced by people due to the ubiquity of clothing and other useful material made with such fibres. Some amount of debris belonging to such variables is expected to be found in the

analysed population. However, those who may have been involved in the production of clothing or items made with such materials are expected to be exposed to much higher concentrations of such debris for prolonged periods of time.

8.4.1 Patterns potentially related to an indoor environment and occupation

During this study a number of particles were found across the populations that were very unlikely the result of deliberate consumption as food, and which today are often retrieved in dust traps both indoor and outdoor (Lacey and West 2007), such as burnt debris, small insect parts, and fibers of different nature. Here an attempt was made to analyse such particles all together to assess if it is possible to gain some insight into overall exposure to dust particles. The variables included in the analyses were:

- All burnt debris: This includes burnt phytoliths, as all this debris had entered the mouth after having been burnt and therefore is the result of exposure either to ash or smoke.
- All fibers of both animal (feathers and wool) and plant origin (bast fibers) that could come from activities such as textile work and the plucking of birds
- Unburnt phytoliths: This includes typologies that did not have any evidence of charring. As seen in Chapter 6, the phytoliths found in the calculus represent parts of the plants that are not deliberately eaten, such as crop processing by-products, stems and leaves of Families of plants that are not known to be edible.
- Small particles of animal origin, such as insect remains (here insect remains are combined together)

For what concerns the variables mentioned above, which all together can be considered exposure to dust in the environment, there was a significant difference between the Anglo-Saxon (mean=0.74/mg) and Late Medieval populations (mean=0.023/mg) (**p-value=0.0001**) but no significant difference between the Parish of St Michael's and St Peter's (**p-value=0.805**). While a significant difference was identified between males and females during the Late Medieval period (**p-value=0.0005**) (St Michael's mean=0.028; St Peter's mean=0.022); the difference between sexes in the Anglo-Saxon period did not appear to be significant (**p-value=0.939**). It must be noted that the

significant difference between Late Medieval males and females means are due to the presence of outliers for a number of variables, such as the high concentration of wool or bast fibers in some females. The particularly high concentration of specific variables as the one mentioned, will be examined later on in this chapter as potential evidence of occupation.

8.4.2 Burnt debris

The evidence of microcharcoal and soot is considered as a potential indicator of exposure to smoke. As stated in Chapter 6, it must be stressed that burnt debris can have originated by exposure to smoke, but also by accidental ingestion on smoked or roasted food as well as accidentally ingested as microcharcoal from the fire where the food was cooked. Therefore, variation in the occurrence of burnt debris between individuals and populations may offer insights regarding changes in the exposure to potential respiratory irritants. The distribution of burnt debris showed a significant difference between Anglo-Saxon (overall mean=0.224: ash and soot mean=0.064/mg, burnt fine particles mean=0.261/mg, large fine particles mean 0.348/mg) and Late Medieval (overall mean=0.076/mg: ash and soot mean=0.036/mg, burnt fine particles mean=0.086/mg, burnt large particles mean=0.036/mg) populations (**p-value=0.0001**), but no significant difference could be detected between the groups of St Michael's and St Peter's (**p=0.576**). The difference between males and females was statistically significant in the Medieval period **(p-value=0.005**) (male mean=0.092/mg; female mean=0.63/mg) but no significant difference was detected between sexes in the Anglo-Saxon era (**p-value=0.913**). It has been pointed out in Chapter 6, that such debris can originate from different sources so such results are to be taken with caution. The above results, combined with the general dust analysis conducted in the previous sections have potential important implications on the use of dental calculus as an indicator of living conditions, and will be discussed in detail in chapter 9.

8.4.3 Occupational dust

Debris very likely generated by occupational dust was found scattered across the population and may represent one of the most important contributions ancient dental calculus research has to offer to the discipline of bioarchaeology.

The variables analyzed here are fibres of both plant and animal origin, feathers, and wood particles. Due to the use of Cyperaceae in roofing and basketry, remains of phytoliths securely identified as belonging to this family were included in the dataset. A final mention of the lithological evidence is provided at the end of this section, although such a line of evidence was excluded from the analysis due to the many problems encountered with the identification and quantification of debris (see Chapter 9). While statistical analysis was conducted on the combination of the variables above, for each variable the individuals with higher concentration are mentioned as potential cases of occupational dust.

An overview For what concerns occupational dust, no difference appeared to exist between Medieval and Anglo-Saxon populations as a whole (**p-value=0.114)**. Equally, no significant difference was traced between the two Medieval cemeteries (**p-value=0.669**). However, significant differences were found between males and females in the Medieval period (**p-value=0.0003**) (female mean=0.026; male mean=0.013), but the difference between males and females in the Anglo-Saxon period did not appear to be significant (**p-value=0.074**).

Fibers of plant origin Microremains generated during the use of fibers in craft and the dust generated by practicing activities such as textile work and spinning can be potentially detected by the presence of high concentrations of plant fiber, tissues and phytoliths that are the result of the break-down of such material. In this section I will be focusing on those individuals which showed very high concentrations of such debris when compared with others. Bast fibers in very high concentration were found in two females: **SP61 = 0.1481/mg**, and **SM17 = 1.00/mg**. Plant tissue potentially related to occupational dust and consisting of flax capsules (the part of the plant that needs to be removed before carding) were found in two individuals of the Anglo-Saxon period: **EMP**

13 = 0.0667/mg, and **EMP 4 = 0.0833/mg**, as well as one Medieval male: **SP29 = 0.0293/mg** and one Medieval female: **SP69 = 0.037/mg**. Finally, three individuals were found to have high concentrations of phytoliths of the Cyperaceae Family, these were male **SP 36 = 0.74/mg,** and females **EMP13 = 0.80/mg** and **EMP 4 = 0.75/mg.** These individuals had almost twice the quantity of this debris compared to the highest amount of the remaining individuals. Furthermore, the two females, EMP13 and EMP4, were found to have high concentrations of plant remains of both flax fibers and flax capsule, suggesting that they were likely engaged in crafts that involved plant matter.

Wool Seventeen individuals of the Anglo-Saxon period had wool debris and this was almost equally distributed between males and females, while 52 individuals (28 females, 20 males and 4 of unidentified sex) had wool in the Medieval sample. The highest amount was found in the Anglo-Saxon **female RT9,** with a concentration of **1/mg**, and the lowest amount in **male SM73 (0.025/mg),** with the highest amount of remains found in four females, two Anglo Saxon (**RT5 = 0.8/mg**; **RT9 = 1/mg**) and two Later Medieval (**SM22 = 0.851/mg**; **SM76 = 0.7880/mg**). This could be the result of women being more involved in the crafts related to dust stages of the textile production such as wool spinning/carding, which were a type of work women could do at home, even if it did not pay well (Ward 2016, 95). The higher amount of individuals exposed to wool in the Anglo-Saxon period may be attributed to women conducting all stages of textile work, from wool carding to weaving in the house. Indeed, spindles are often found associated to female graves (Timby 1996). However, practicing such activities in the house would not only expose women to higher amounts of wool but also every member of the household. As this is important evidence for the Anglo-Saxon period, there is need to investigate this further but this goes beyond the scope of this PhD. An expert in the history of Anglo-Saxon clothing and wool, Penelope Rogers, will be examining the evidence and contribute with the contextualisation of such data in the near future.

Wood Wood remains were very rare in the population under study. They were only found in one individual dating to the Anglo-Saxon period and 11 in the Later Medieval one (fig 8.3). The concentration of debris found in them shows clearly two male

individuals in the Medieval period being outliers: **SM55 (St Michael's)** and **SP 18 (St Peter's)**. The presence of wood may have different pathways to inclusion, as suggested in Chapter 6, however the concentration of debris in SM55 and SR18 suggests higher/prolonged exposure to wood dust, which may represent occupational dust. Exposure to wood dust could be generated by a variety of daily activities, such as the break-down of wood for fire and kindling, and this maybe result in the build-up in the calculus of particles of wood that comes from different sources. However, the presence of such remains mainly in men is indicative of some sort of closer or prolonged contact with wood dust, and therefore could be occupational.

Feathers Feather debris was an interesting line of evidence retrieved from this study. The presence of bird feathers is likely the result of their importance in diet and the associated activity of plucking birds. No significant difference could be detected between the Anglo-Saxon and the Medieval period (**p-value=0.280**) or between St Michael's and St Peter's populations (**p=0.156**). A significant difference was found between males and females during the Late Medieval period (**p-value=0.001**), where a higher amount of feather barbule fragments occurred in women (female mean= 0.019, male mean= 0.006). This finding becomes even more notable when compared to the Anglo-Saxon period, where no significant difference could be traced between the sexes (**p-value=0.949**)**.** This evidence is currently under examination in a collaborative project with Dr. Richard Thomas and his team, who also provided feathers for comparison. The results will potentially offer interesting insights into poultry keeping in the Middle Ages (Richard Thomas' personal comment).

Lithological elements Although it was not possible to fully quantify the lithological elements for reasons stated in Chapter 7, two male individuals appeared very rich in mineral grit in their calculus. It was, therefore, decided to mention them in this section since lime and pottery work as well as the construction of ceramic material would generate large amounts of mineral dust. As a result, it is possible that the calculus of these two individuals captured occupational dust generated by practicing the above crafts.

It must be stressed that the high concentration of potential occupational remains mentioned above, is not reflected in other lines of evidence in the same calculus. While biases may be present in the formation process of calculus between individuals, the high concentration of only specific type or remains, such as fibers (e.g. wool) is suggestive of such concentration to be the result of higher exposure to such debris. If such high concentrations were due to biases in the formation process, this would affect all categories of debris, not only one.

8.5 Summary of the analyses

Although statistical analysis on the remains needs to be taken with caution in dental calculus research, some interesting patterns were traced:

- Staple cereal food was significantly different between the Anglo-Saxon and Later Medieval period, as well as between the two Late Medieval cemeteries.
- Food access was investigated using imports, and surprisingly enough, no significant variations were seen in access to imports, between sexes and across populations. This analysis highlighted the presence of imports in the Anglo-Saxon cemetery.
- The two Medieval cemeteries did not differ much in the debris generated by diet, suggesting food access was similar in both of them.
- A significant dietary pattern was found in the microremains of the leafy crop leek, which was more common and higher in concentration in the Early Medieval population that in the Later Medieval period, a result in accordance with what is currently known about the consumption of such crops in the Medieval period.
- Medicinal plants and herbs were found to be considerably different between the Anglo-Saxon and Late Medieval period, while no patterns were visible within the same period of time, and in the Medieval period between the populations. Differences in the natural environment, uses of such species, as well as cooking practices may underline such differences. Pollen of the Rosaceae Family was found to be the main variable producing the above patterns.

- Overall, statistically significant differences were found between the Anglo-Saxon and the Medieval populations for variables that are very likely direct evidence of food consumption. Statistically significant differences were also found for both the natural and built environment, with regard to burnt and unburnt debris consistent with dust, suggesting that the difference existing in housing between the Early and the Later Medieval period was “captured” by the calculus matrix.
- Finally, it is also interesting that many variables that were different between the Anglo-Saxon and Late Medieval periods were not different between the sexes within periods, suggesting the diet and living conditions for males and females were similar. This may reinforce the hypothesis that calculus does indeed capture a number of aspects of daily life that go beyond diet.

The results suggest overall that a variety of patterns can be identified using debris entombed in human dental calculus. Further work should however focus on those variables that are somewhat diagnostic and comparable. In the following chapter the results of the analysis will be discussed in relation to broad patterns that may contribute to their interpretation within the broader field of Environmental Archaeology.

CHAPTER 9

DISCUSSION AND CONCLUSIONS

9.1 Introduction

The aim of this study was to test the potential of dental calculus for providing meaningful evidence about diet, lifestyle and living conditions in the past and exploring the range of material it is possible to identify and record, besides starch granules and phytoliths, which have been the main focus of the work so far. The level of identification achieved for each typology of debris varied hugely across the studied individuals, while multiple pathways to the inclusion of remains complicated the interpretation. Despite this, differences were detected in the debris that could be identified, not only between populations of different periods of time but sometimes even in the same populations between sexes and cemeteries. In order to evaluate the contribution of dental calculus finds to aspects of diet and living conditions it is necessary to contextualize the results in a variety of research topics (diet, food access, hygiene) in different disciplines (e.g., archaeobotany, archaeozoology palaeoecology). This discussion chapter therefore is aimed at the following points:

- Firstly, I will assess the role of debris retrieved within the established disciplines of Archaeobotany and Archaeozoology and the study of Fungi, providing a comparison and highlighting both the potential and limitations of the dataset obtained.
- Secondly, the information gained from the remains in dental calculus in relation to the topic of food access, living conditions and social structure will be explored.

As with previous chapters, some observations provided here have also been made in work authored and co-authored by the author of this PhD and recently published (e.g., Cristiani et al. 2016; Radini et al. 2016a, 2016b).

9.2 Calculus as Archaeobotanical evidence

This section focuses on the archaeological visibility of plant remains in dental calculus, while aspects related to their social access and distribution across populations are discussed in the section on living conditions. In order to meet the aims of this PhD, it is necessary to address the contribution that plant remains in calculus can make as archaeobotanical evidence in order to offer a better understanding of the role of plant micro-debris in diet and non-dietary contexts. As seen in Chapters 6 and 8, several pathways to inclusion of micro-debris of plant origin in dental calculus were found, and the remains retrieved during this analysis represented various aspects concerning the interaction between people and plants: dietary, artefactual and environmental. In order to facilitate the discussion on different subjects, this section is structured as follows:

- First, dental calculus remains are examined in relation to dietary aspects and divided into staple food crops and other dietary items.
- The focus then moves to the role that dental calculus finds may have in providing new insights into plants in crafts.
- Finally, the information provided regarding the natural environment experienced by individuals during life is addressed.

9.2.1 Dental calculus and plants in the diet

This section offers a perspective on plants in the diet following the structure used by others (e.g., Moffett 2011), by presenting the evidence of different food plants and contextualizing them within the discipline of archaeobotany.

Preservation and taphonomic processes Before entering the interpretation of plant micro-debris in dental calculus, it is necessary to clearly define known biases occurring in the process of preservation of dental calculus debris. As the majority of remains belonged to plants, such issues are dealt with in this section. As pointed out by Moffett

(2011, 347), human activities and means of preservation directly affect the record visible in archaeological sites. These considerations are true for the calculus matrix as well. Some very fragile remains, such as leek epidermis and very small plant fibers and even feather barbule, are a very good example of the ability of dental calculus to preserve lines of evidence very rarely found in the soil. The preservation in dental calculus is clearly due to its mineralized matrix, which is very stable through time, as plant remains preserved in a variety of contexts and periods of time clearly show (e.g., Buckley et al. 2015; Hardy et al. 2012). Therefore, it is possible to consider the calculus matrix as a form of mineralization, however, the extent of mineral replacement in the remains is unknown and will remain unknown until a better understanding is gained of the calculus formation processes. Although such processes are not fully understood, debris entering the calculus is likely to preserve well in its matrix. The major biases in the data are, therefore, due to what is the calculus able to 'capture' during its formation process.

First of all, the results of this PhD show that for the most part the entrapped remains (starch granules, small fibers, phytoliths, fine particles of microcharcoal) had a diameter below 20 microns. While some debris was found to be between 20 and 60 microns, only very few remains were around 200 microns and none were larger than this (see Table 1, Appendix 3). This suggests that debris entombed in the calculus matrix, at least for the Medieval period in the area, is subject to particle size, potentially due to micro-topography of the calculus surface (which may affect the capacity of the calculus to "lodge" micro-particles within specific sizes). The size of the particles affected the analysis in two ways:

1) In order to enter the calculus matrix, the debris of plants/animals needs to be broken down into fragments small enough to become entrapped
2) Such fragments need to retain features useful for their identification

Different plant and animal remains respond differently to the mechanical processes that result in their breaking up. For instance, fragments of meat may become present in the mouth as a result of chewing fibrous material, but it is very unlikely that their quantity

could be comparable to the quantity of small starch granules released in the mouth by a single seed of wheat, which can be easily broken down to its starch granules that are consistently below 30 microns. Equally, debris in the environment (both natural and built) contains particulate matter (see Chapter 3), which is in the right size to become entrapped in the calculus matrix. Therefore, particle size and the diagnostic features visible within that range of size appear to produce a bias in the record entombed in the calculus matrix. Mention of the above processes and biases will be made where necessary in the following sections.

Staple starchy food: cereals, legumes and their weeds The presence of starch granules belonging to staple food of the Triticeae tribe (e.g., wheat, rye and barley), Aveneae (oats) and Fabeae (legumes), indicate that at least for the studied populations, staple crops in the diet were visible at the tribe level of identification. There is extensive evidence of cereal crops of the tribe Triticeae and Avenae in Leicester and across all England in both the archaeological (e.g., Monckton 2015, Moffett 2011, Radini 2009) as well as the historical record (e.g., Dyer 2006) suggesting that these were staple foods in Medieval diet. The presence of such remains is therefore in accordance with what is known for the period. However, the remains lacked diagnostic features to provide identification to the species or even the genus level and, therefore, they would not be useful in diachronic studies of the consumption and proportion of different cereals.

While it is possible in some archaeological contexts that preserve very large amounts of starch granules to separate species of *Aegylops* spp., *Triticum* spp. and *Hordeum* spp. upon morphology and palaeogeography of their distribution (Cristiani et al. 2016; Piperno et al. 2004), this was not possible in this study. In terms of species of cereals, the identification only to tribe Triticeae is very limiting for the region and the period, since a number of staple foods, such as different species of wheat, barley and rye, all belong to the same tribe. In recent years there has been increasing evidence and

interest on the typologies of wheat eaten from the Anglo-Saxon period onwards. Moffet (2012, 349) has pointed out how emmer (*T. dicoccum*) and spelt wheat (*T. spelta*), known as glume wheat[14], had been seen for many decades as the staple food cereals in the pre-Anglo-Saxon society and replaced by species of free-threshing wheat[15] (e.g., *T. aestivum* and *T. durum*). It has now been known for a while that the situation is more complex than previously thought. Moffet (2011) has provided evidence of emmer wheat in the Anglo-Saxon period in Oxfordshire; a fact recently confirmed by the author of this PhD in archaeological sites in Wallingford, Oxfordshire, where remains of emmer and spelt wheat were found (Radini 2013), dating to the Later Anglo-Saxon period. So, the identification of starch granules to Tribe level achieved during this PhD is somewhat limited in comparison to our understanding of consumption of such species during this period. For Leicestershire and the East Midlands, this research cannot contribute to the target of understanding the history of rivet wheat, for example. The presence of starch granules consisting of oats could suggest that such plants were eaten more commonly than thought. Moffett has pointed out that charred remains of oats are difficult to identify (2011, 352), however identification to the genus level (*Avena* sp.) is reliable. The identification to the tribe level in starch analysis includes different genera and species, as mentioned in Chapter 6, therefore, it is not possible to fully determine if such remains represent cultivated species or weeds of crops. Mixed crops (including oats, wheat, barley and rye) were common in the Middle Ages (Moffett 2006, 50-51) and therefore the remains allocated to the tribe Triticeae and Aveneae could be from one single species in each tribe or a variety of them in combination. What is very

[14] With the term glume wheat archaeobotanists indicate species of the genus *Triticum* that have grains enclosed in rigid and tough husks. These need to be removed before consumption, requiring more labour.

[15] In free-threshing wheat, the husks break up very easily freeing the grain, and therefore they require less labour.

interesting in this analysis, is the occurrence in many individuals of starch granules belonging to the tribe of Fabeae. While the traditional archaeobotanical record can sometimes identify legumes to the species level and even variety (Hall 2001), this is again not possible with starch granules in many cases. The contribution of dental calculus to our knowledge of the consumption of legumes is mainly due to the fact that this category of remains has low archaeological visibility. Moffett (2011) and Hall (2001) have suggested that remains of legumes may have come less often in contact with fire when compared to cereals. Legumes are rarely found in the archaeological record of Leicestershire, when compared to cereal remains, but nevertheless they have indeed been found (Monckton 2015; Radini 2009, 2016). So, for the category of legumes, data from this PhD have shown that they were very likely eaten more often, probably as a garden produce, than what can be inferred from the archaeobotanical record available so far. However, how different species could contribute to such a record, cannot be addressed currently by starch analysis only. Although not linked to deliberate consumption, but strictly associated to the finds of cereals and legumes, was the seed epidermis of weeds. The remains were limited in terms of number of species when compared to the archaeological finds in Leicester (e.g., Browning et al. forthcoming; Monckton and Radini 2009, Radini 2009). However, corncockle, one of the most common species in the archaeobotanical record, was found in one individual out of four[16]. Weed ecology can provide valuable information on the way crops were grown and processed, and weeds have been routinely found in deposits in Leicester (e.g., Radini 2009). The limited evidence of weeds therefore prevents this study from gaining information on agricultural husbandry practices; however, it does provide the information that weed seeds were left in the crops to some extent and eaten with

[16] For a detailed history of the plant, see Allan Hall's essay on the subject: http://www.yorkarchaeology.co.uk/wp-content/uploads/2015/08/The-Corncockle.pdf

them. There is archaeological evidence of their ingestion in cess-pits across England (Hall and Kenward 1989). The other two species, campion and poppy, were rare but they too were found in the archaeological record of the region (Monckton 2015).

For what concerns the field of dental calculus research, this study shows that staple starchy food eaten by the populations has consistently appeared in the calculus matrix, suggesting that at least at population level, is possible to infer the consumption of staple starchy food. This could appear initially to contrast with the data found by Leonard et al. (2015), where the staple starchy food, maize, eaten once a day at least by the individuals who took part in the study, was only found in 30% of the calculus samples. Leonard et al. (2015, 456) pointed out that many domesticated plants are selected for the high quantity of starch granules in them, increasing their chances of become entrapped in the calculus matrix, and therefore the low quantity of maize was due to processing. The consumption of domesticated plants (cereals and legumes) was predominant in the Middle Ages and it is here clearly reflected by the ubiquity of staple domesticated cereal but also legumes in the dental calculus record. There is however another explanation. In general, the large calculus samples available for the Medieval populations (normally above 15 mg) may generate higher chances to retrieve starch granules in a status of preservation to be identified, so the visibility is not only due to the survival/preservation of starch granules but also due to the original sample size, which potentially affects the visibility of plants in the diet of prehistoric populations where calculus samples are smaller. Incidentally, the choice of providing concentration of remains/mg adopted in this study will allow comparing data in an easier way in future studies and confirming such a hypothesis. It is also possible that the generally higher amount of starch granules found in the populations is due to not only the role of the plants but to oral microbioma and oral hygiene, directly linked to a higher quantity of calculus deposits on the teeth of Medieval people. The way different types of "microbiota" in the mouth can affect the survival of microdebris, in particular starch granules which offer a source of sugars to the plaque-forming bacteria, is unknown.

Altogether, this study is a positive step in the visibility of domesticated starchy plant food in the diet using dental calculus. However, unless starch analysis develops

techniques to identify such remains to the species level, traditional archaeobotanical evidence will be the only way to address diversity in the production and consumption of cereal remains and culture shifts of their use, as is the case of emmer in the Anglo-Saxon period.

Herbs and green vegetables Green vegetables are very rare in the archaeological record; either they are eaten fresh and they do not come in contact with fire, or if they do get exposed to firing conditions they don't survive. Moffett (2006) also points out that many vegetable plants have seeds too similar compared to their wild relatives, and finally many species of plants that can be eaten as vegetables (such a sorrels and goosefoots) are also weeds of crops so they cannot be securely linked to the deliberate consumption of such plants even when found (Radini 2009). In what concerns the visibility of leafy crops in dental calculus, cooking and chewing leafy parts results in damaging them to such extent that in the majority of the cases they lose any diagnostic features. There is however, a wide historical record, throughout the Medieval period, of their consumption in a variety of written records, including medical scripts and recipe books (Banham 2004) providing clear evidence of their presence and use in the diet.

A potential useful part of leafy crops are phytoliths and calcium oxalate inclusions, often found in leafy crops such as beet, spinach and asparagus. There is very limited evidence of such cellular inclusions in the dental calculus record apart from the current study and that of Power et al. (2014). This is very likely due to the fact that such cellular inclusions dissolve in a weak solution of HCl, and therefore their retrieval may be difficult given that current methodologies of extraction use in most cases HCl to dissolve the calculus mineral matrix. Although calcium oxalate and sclereids were retrieved in very few cases in this study, they are found in many parts of the plant (including wood), and therefore cannot be securely linked to the consumption of leafy greens. Furthermore, undiagnostic plant tissues, too fragmented or damaged to be identified, are ubiquitous among the micro-remains recovered during this study, and could easily be the result of leafy crops and other vegetables.

In light of the above issues, the presence of leek epidermis in the results of this study becomes clearly a very important find. It is believed that leeks were a normal item in the Anglo-Saxon period (Banham 2004). Despite the fact that the leek is not native to this country and needs human care in order to be cultivated in England (Moffett 2012, 356), it can withstand cold weather, spend the winter in the ground and be lifted when needed. According to Hall (2001), it was often replaced by cabbages in the Later Medieval period, as its seeds and plant tissues decline in the archaeological record. The rarity of the remains is even more striking when combined with the statistically significant diachronic changes seen in this study (see Chapter 8) and could be seen as evidence of a change of tastes between periods. However, the lower number of finds of leek in the later Medieval period could result from the way the plant was cooked, although this will need to be investigated further outside this context. Cooking practices as a bias in the debris record cannot be underestimated.

Although the epidermis of leek is the only secure find of the consumption of leafy greens in this study, a few other remains could be interpreted as potential evidence of leafy crops. The presence of daisy family (Asterceae), sorrels (Polygonaceae) and goosefoot (Chenopodiaceae) pollen could point to the consumption of such species as leafy greens. All such families of plants have several species that have edible leaves (Vaughan and Geissler 1997, 160, 170, 172) and to some extent the leaves of borage are also edible. Despite the finds of leek epidermis, however, the archaeobotanical visibility of vegetables in the record entombed in the calculus matrix remains poor, mainly due to lack of features useful for identification in plant tissues in this study.

Fruits and nuts This research has not retrieved any secure evidence of the consumption of parts of fruits and nuts, even though these are a common find in the archaeobotanical and historical record in Britain (Dyer 2000, 113-131; Moffett 2006, 46) and cess-pit evidence from the region (e.g., Monckton 1994 and 1999; Monckton and Radini 2009; Radini 2009). There are a number of potential reasons for their low visibility in calculus, ranging from the lack of diagnostic remains to the fact that maybe the fruits and nuts do not break down in parts sufficiently small when eaten. It is also possible that the abrasive nature of many hard nuts and some fruits and their acidity,

could actually prevent calculus formation (in the case of fruit acids), and even remove the calculus carbonate deposit. Such aspects have not been explored so far in the field of ancient dental calculus research. The presence of pollen of elder and hazel in the dental calculus shows that such trees/shrubs were present in the environment, and this could be considered as indirect evidence of their consumption. The evidence of phytoliths from date palm is considered below.

Luxury food plants One of the most important contributions of this study to the discipline of archaeobotany is represented by the presence of starch granules and phytoliths from plants that can be considered luxury items and have very low archaeobotanical visibility. These were mostly species of plants that do not grow in Britain, which are the result of long distance trade and they are normally approached as an indicator of high status because of their rarity and cost, which makes them "out of the reach of mass consumption" (Van der Veen 2003, 409). Their low archaeological visibility is due to a number of factors, which range from biases in the preservation of the parts eaten, as pointed out by Livarda (2008), but also due to the fact that they are eaten by a smaller number of people, and they are very often not eaten daily. Their value as a social indicator has been pointed out by Van der Veen (2003) who also stressed the increasing interest of archaeologists in the role that food played in shaping social status and identity (2003, 405). In the Later Medieval period gifts of food were a very important cultural part (Wooglar 2011), and the role of luxury food as a gift shall not be underestimated, even if not addressable in this study. All remains found in this PhD belong to items that are very little known in the archaeological record not only in Britain but in North West Europe in general (Livarda 2008). These were mainly represented by species of the Zingiberaceae Family (ginger/galangal), which is not native to the region. Such species are aromatics, used in food, festivities and medicine: natural products, traditionally prepared for long-term storage and distant travel, they have figured in legend and history for thousands of years (Dalby 2000). The eaten part of such plants is the root, very often grounded to powder, so very unlikely to survive in the soil. The other remains belonged to sorghum, millet, fenugreek and buckwheat and were very infrequent in the populations, suggesting their access was rarer. Studies have

shown that in general wheat and barley grains have a higher rate of survival from charring than millets (Märkle and Rösch 2008). The author has worked on Northern African material and date palm survives well the charring process, while survival from the charring process of the other crops compared to wheat and barley is unknown to the author. Therefore, at least for millet and sorghum, charring can reduce their visibility where they are not consumed as staple crops. It can be stated that the archaeological visibility of such imported species in calculus is due to the very different nature of the "environment" capturing the debris: the human mouth. All the species found have the following in common: the consumption of such species of plants breaks down the plant matter/seeds/tuber chewed into small diagnostic elements: starch granules (ginger/galangal, millet, buckwheat, sorghum), phytoliths (palm date) and small fragments of the palisade tissues (fenugreek). The mineralized nature of the calculus preserves such remains in situ and very well, so that the deterioration that would normally occur in the soil or fire does not happen. Once the remains are retrieved, their identification is reliable as they do not belong to species of plants and in the case of ginger/galangal even families of plants that are native to the location where they are considered luxury items. A large reference collection of native species will then confirm their identification, which, in the absence of close relatives, can be narrowed down to the species level. Therefore, dental calculus holds great potential in the detection of trade and the movement of plant food. Recent work conducted by the author in the Balkans (Cristiani et al. 2016) showed that such an assumption can also be extended to staple crops and provide new insights into the spread of crops from their area of origin. To conclude, Madella et al. (2013) suggested microfossil content of dental calculus as a valuable source of information on the consumption of millet species and other plants with low visibility and the current study supports this suggestion.

9.2.2 Potential medicinal plants

The detection of medicinal plants in the archaeological record is challenging. Many species that are medicinal have medicinal properties only if eaten in large quantities and only if the specific medicinal part of the plants is consumed, while the vegetative

part used often does not survive well. Another problematic aspect of medicinal plants is that they are not routinely consumed by large numbers of people or often.

The challenges encountered in archaeobotany to identify such evidence are many and for a detailed review on the subject, the work by Chaves and Reinhard (2006) is recommended. However, an example of the relevant challenges is the following. Sorrel, a member of the dock Family, was thought to have medicinal properties against plague (Hatfield 2007, 321). Seeds of sorrel are very common in the archaeological record, but their presence in the archaeobotanical record can be for the most part explained by the fact that sorrel grows easily in disturbed soil and it is a very common weed of crops, while it is equally likely that dock leaves and seeds were consumed on a regular base (Radini 2009). Finding such species in an archaeological sample in the Later Medieval period, when pandemic episodes of plague spread across Europe is unlikely to be linked to their use as a remedy but due to their wide distribution in the environment.

Here the presence of pollen of species of plants that produce pollen in low amounts is suggestive of the consumption of such species in the form of flowering parts. The most secure herb/medicinal species retrieved is that of Borage. However, all taxa of pollen identified have species where the flower has some uses as a medicine and herb. Among them, most striking is the retrieval of fragments hemp seeds (*Cannabis sativa*), which has both nutritional and medicinal properties. Currently work is being conducted in collaboration with an historian, Dr Deborah Banham, to trace the historic record of its use aside the archaeological. The reason behind these finds in calculus is very similar to the observations made for the visibility of imported luxury plant foods. The visibility of seeds of hemp is also due to the fact that the surface of the seed is diagnostic even in very small fragments, as pointed out by Hall (2003, 107). Again the diachronic changes observed have potential of their own to suggest changes in the use of medicinal plants and herbs as well as changes in taste between different periods of time. In support of the hypothesis that dental calculus data can help with inferring potential changes in taste, the work by Buckley et al. (2015), showed that plants of bitter tiger-nut (*Cyperus rotundus* L.) were eaten by the early Mesolithic people in Sudan. Today the plant is no

longer considered a food in the region but is seen as a weed due to its bitter taste. Therefore, calculus holds potential on the subject of changes of taste through time.

9.2.3 Non dietary plants and crafts

A further line of evidence of low archaeological visibility is represented by the retrieval of plant fibers (bast fibers of hemp, flax and nettle) as well as the phytoliths of sedges (Cyeperaceae) which are used in crafts and textile, as well as roofing material. The visibility of plant fibers used in material culture is normally limited in the archaeological context where material culture made with plants, from textile to rope, is preserved mainly in anoxic waterlogged conditions, arid environments or in permafrost and ice, such as shipwrecks, graves and bogs (Gale and Cutler 2000). In all cases it is the artefact that is visible not the individual who potentially made it, a fact that may be possible to see with the evidence entombed in the calculus. The evidence of plants being processed for fibres is that of retting pits, found in many locations in Leicestershire (Monckton 2006). There is in general the tendency of thinking at a large-scale processing of such fibers, however, there is strong historical evidence for crops, such as hemp and flax, being cultivated as horticultural products in both rural peasant and even urban gardens which were likely much smaller than those available in the countryside (Dyer 2000, 121-124) and therefore they would be easily accessible both by the Anglo-Saxon and the Medieval urban population. Diachronic changes in the distribution of such finds as well as the identification of outliers has social implications that will be discussed later on.

9.2.4 Palaeoenvironment

The environmental remains are those belonging to pollen of arboreal species. The composition and cover of woodland in the Anglo-Saxon period is the subject of great interest in the discipline of archaeobotany and it is an open debate. In contrast, the effect of climate change and deforestation in the Later Medieval period are well known from a number of sources, predominantly by the analysis of pollen and charcoal. Here the evidence of dental calculus can be approached as palaoenvironmental for the retrieval of pollen grains of arboreal species. It must be stressed that such information can only be detected at population level. The data show significant changes in the

occurrence and composition of tree flora to which the individuals studied where exposed. Incidentally, the high presence of pollen of the Rosaceae type that could not be identified may be indicative of the presence of thorn, a member of the family normally known to be a component of such pastures (Hooke 2010, 192). Here the pattern shows the strong presence of oak and ash in the Anglo-Saxon period, two elements of wood pasture, that decline sharply in the Later Medieval period. The data therefore suggests if a sufficient number of individuals is analysed, changes in the composition of woodland can be detected using pollen remains in dental calculus. Such information could be of great value also because it can provide information on the local environment by analysing the distribution of such remains between sexes. For instance, in this study, the Anglo-Saxon and Medieval populations show a clear difference in the arboreal pollen, with tree species being more common in the Anglo-Saxon period. There were no differences between the sexes in each period suggesting sexes were exposed to the same typologies of trees in the surroundings, indicating that dental calculus can be potentially used at a population level to reconstruct the local environment a population was exposed too. Having stressed the above, it must also be take in consideration that the Anglo-Saxon populations were chosen as a 'rural' comparison with the urban setting. Therefore, it is equally possible the differences observed in the pollen found in calculus, could be the result of the urban environment being and its surroundings being less forested than the more rural environment in the same period of time. Only future research on rural Later Medieval populations will provide clarification regarding the nature of the changes observed in the pollen record. Nevertheless, the change in species composition and the disappearance of oak cannot be underestimated.

9.2.5 Other remains of plant origin

A variety of other remains were present apart from those mentioned above. This included wood debris that could only be identified securely in a few cases as conifer wood, as well as undiagnostic phytoliths and plant tissue. While these cannot provide much information on their own, their particle size and origin may hold potential for assessing aspects related to occupation and lifestyle, and will be discussed in the

relevant section of this chapter that addresses the value of dental calculus debris as a window into people's lifestyle and social structure.

9.3 Calculus as Zooarchaeological evidence

Firstly, it is interesting to notice that zooarchaeological evidence is underrepresented in the dental calculus matrix. The only studies to date apart from the current one where animal evidence was found are those of Buckley et al. (2015), Hardy et al. (2015), Radini et al. (2016), and Warinner et al. (2014), in all of which the animal debris was identified by the author of this PhD. Due to the lack of reporting evidence not consistent with starch granules and phytoliths, it is difficult to compare the data from this PhD to that of other studies. The low amount of remains of animal origin, compared to plants in this study, is potentially due to the fact that fragmentation of remains in diagnostic fragments occurs more naturally and more often in plants than animals. Plant cells have a number of inclusions or secretions, such as starch, phytoliths and calcium oxalate, of small size that are likely released into the mouth with chewing or are broken down and freed during plant processing and ash making. Animal remains instead do not tend to produce large amounts of debris. Therefore, it is no surprise that the majority of the debris retrieved represents non-edible animal parts, sometimes very small. Incidentally, the zooarchaeological remains retrieved are normally found as particulate matter in the dust samples (Lacey and West 2007). Nevertheless, the remains found provided important insights into a number of aspects of diet and living conditions. Their value as zooarchaeological evidence is assessed in the following paragraphs.

9.3.1 Feather fragments

The identification achieved here is to Order/family, while the zooarchaeological evidence of bird bones provide a more secure identification as species, as well as sex, pathology and butchery. While interesting for a number of purposes, the bird evidence from dental calculus cannot replace for now the information that can be achieved by zooarchaeology. The remains of fragments of feather barbules could represent the deliberate consumption of animal meat. Birds played an important role in Medieval

diet, their eggs and meat were consumed, but they were also used as a form of currency for rent or other types of payments (Richard Thomas' personal comment). The evidence therefore is suggestive of the presence of birds in the diet and it is a useful dataset to consider. However, the presence of significant differences in the distribution of such debris across the population is suggestive of females being more exposed to the dust of such birds, and could also suggest that the birds were tended locally, potentially in the "back yard". There is zoo-archaeological evidence of pig keeping in the North East corner of Medieval Leicester (Browning 2009). Currently there is great attention paid to the role of close contact between humans, birds and pigs, as in pig throat swine and bird flu can intermingle generating new types of the disease wherever pigs and birds are kept in close proximity (e.g., Herring 2010). Such line of evidence could therefore provide important information related to hygiene. Finally, while at this stage the secure identification of feather dust to species level is not possible, the evaluation of sex differences in the exposure to feather dust could contribute to a better understanding of gender differences in poultry keeping and processing in past societies, an aspect difficult to address in the archaeological record.

9.3.2 The evidence of wool

This study demonstrates the contribution of dental calculus in providing evidence of wool fibers across populations. Such remains are very rare in the archaeological record and when associated to sex and social status, they can provide important information regarding the production of textiles (see section on occupational dust, later in this chapter). Again, this record provides evidence of the presence of wool, indicating craft uses of the animals rather than aspects of consumption.

9.3.3 Parasite ova

Probably one of the most interesting contributions of the archaeozoological evidence is the indirect information provided on hygiene in the form of parasites. The parasite ova in teeth can be the result of the consumption of contaminated meat as well as dirty vegetables if manure was used to grow them. Studies have shown that cultural perceptions of faecal material and the manner of disposal of such material highly

influence exposure to parasites and diseases that use faecal material as a vector (e.g., Herring 2009). In settings where human and animal faeces are routinely used as manure (e.g., in many rural areas of developing countries), and not perceived as potentially harmful, behavioural barriers that can prevent or reduce contact and contamination drop. As a consequence, individuals culturally less 'worried' about 'faeces' will be more exposed to the diseases these may cause. Manure used to fertilize garden vegetables is equally likely to carry diseases as contaminated meat, but likely less "repulsive" to Medieval people than animal intestines. Individuals affected by intestinal parasites suffer several nutritional deficiencies as the parasites compete with the host for nutrients; these in turn can make the individuals more vulnerable to other diseases, for example influenza, and affect all age groups, and even their development, as several studies have shown (May 1959).

9.3.4 Entomological remains

Entomological remains provide evidence for the natural environment. In particular, butterfly and moth scales have been retrieved in the calculus matrix and they could come from a number of species in the environment as well as from indoors. While little can be said about them without secure identification, in this PhD they were interpreted as "dust". It has already been pointed out in Chapter 7 that the only evidence of insect remains from Leicester is that of fly puparia from cess pits. The data from the calculus are novel. As has been pointed out by Nazari (2014)[17], Medieval manuscripts (see fig. 57) reveal a variety of methods to catch butterflies and moths, while of course the majority are not realistic, it could potentially suggest they were more common in the environment than today. Many species of butterfly and moth are in current decline due to human impact on their environment and food sources. Therefore, such a line of

[17] http://www.medievalists.net/2014/12/06/chasing-butterflies-medieval-europe/

evidence from dental calculus holds some interesting potential, if techniques are developed to allow the identification of such remains. As for previous finds, this will be subject to further investigation in the future.

Figure 57. Example of butterfly catching devices from Medieval manuscript (from http://www.medievalists.net/2014/12/06/chasing-butterflies-medieval-europe/)

9.4 Fungi: the great potential of a neglected line of evidence

Particularly interesting is the survival of fungal debris of various types in the dental calculus. Fungi include species that are edible but also species that are plant pathogens and indoor microorganisms responsible for mould and food spoilage (Li et al. 2007), while several species can act as opportunistic pathogens for humans (Rippon 1982). This research has therefore retrieved an important line of evidence although problems with identification greatly reduced the information that could be deduced from them. That stated, one important trend has been detected: the number of individuals and the overall quantity of fungi almost doubled from the Anglo-Saxon to the Later Medieval period, suggesting major changes in the exposure to such typologies of biological "particles". Plant diseases of fungal origin, such as rots and blights, can cause crop loss

and failure both before and after harvest. According to The American Mycological Society, mycotoxins released on the infected crop are a risk to people's and animals' health if inhaled or ingested in high quantity[18]. Large-scale episodes of plant diseases have, and have had, a devastating influence on people's life and economy (Fry and Goodwin 1997) for crop failures can cause mass mortality events as well as affect the cost of crops and increase the inequality of people's access to staple foods. Plant diseases can make crops more vulnerable to weeds, affecting the overall harvest. Abrupt climate change dramatically affects both the natural and the built environment. The effect on food security of fungal plant pathogens is an aspect to take in consideration when approaching the subject of climate change and famines, especially in past societies. It must also be stressed that famine years were common in the Medieval periods, and some events were particularly dramatic and well documented in Leicester and elsewhere, for example the Great Famines 1315-17 were very severe episodes in Leicester (Morris et al. 2011).

However, while tempting, the direct correlation between the visible patterns in the distribution of fungal debris and climate deterioration may not be the only reason for such a trend. First of all, without secure identification, it is not possible to assess what portion of fungal material was deliberate (mushrooms) or accidental consumption (as moulds on food, or in soil and grit from the environment). Secondly, the Medieval period witnessed a number of changes in the manner of food production, such as farming husbandry practices (Banham 2004; Thomas et al. 2016), a shift from glume to free threshing wheat mentioned above (Moffett 2011), introduction of different ways of ploughing and manuring (Monckton 2006), as well as increased complexity in the way the food was distributed and stored (Dyer 2000, 257-281). All these variables may have contributed to the observed pattern: free threshing wheat, rye and oats are vulnerable to fungal attack (Richardson and Richardson, 2015), manuring can enrich crops of fungal

[18] http://www.apsnet.org/edcenter/intropp/topics/mycotoxins/Pages/default.aspx

debris that naturally develop on dung matter (e.g., Bell 1983) and the transport and storage of food can also result in mould. Climate deterioration may well be a complicating factor to an existing way of life that could be potentially more prone to fungal growth on crops and food than in preceding periods. There is wide archaeological evidence of drying kilns, including in Leicester (e.g., Radini 2009). There is also historical evidence that harvesters were given an "extra tip" to speed up the harvest in fear of rain (Dyer 2000, 78). Such practices show that Medieval people were aware of the problems caused by crop spoilage and cultural buffering mechanisms existed to prevent it. Positive identification of these remains in the future may allow a better understanding of climate change, indoor conditions, food spoilage, and storage, from a completely different point of view and from a line of evidence almost invisible archaeologically so far.

9.5 Calculus as a window on people's everyday life

The retrieval of a number of finds due to deliberate consumption of food and drink, but also likely due to the exposure to high concentrations of non-dietary debris, opens the interesting possibility of calculus as a line of evidence to assess food access (as in the case of imports) and the exposure to dust and debris with potential consequences for human health. In addition, it points towards occupations and craft activities practiced by those individuals. At the beginning of this thesis it was stated that the interaction of diet, occupation, natural and built environment directly affects human wellbeing. Forming in the human mouth, dental calculus offers the possibility of looking more directly into the individual and population "experience" of such complex interactions. Aspects of living conditions relevant to the subject are discussed here.

9.5.1 Food access and food hygiene

Food can be approached from many standpoints, such as nutritional, economic, social and even ecological. It is a very complex subject and of course it is not possible to enter into details here, however for a more detailed discussion on the subject of the role of food in shaping past societies, the work of a number of authors is suggested, among

others, Goody (1982), Van der Veen (2003), Livarda (2008, 2011) and Twiss (2012). However, a number of finds in this thesis should be evaluated under a wider perspective rather than their visibility in the archaeological context. The fact that dietary finds with lower archaeological visibility, including imports, are found in skeletal remains where sex, age and potentially social status are known, can be used to assess a number of social aspects in relation to cultural and social practices in the consumption of food as material culture. The lines of evidence retrieved in this PhD touch two main points: food access and food hygiene.

Food access Moffett (2006, 55) stressed the lack of synthetic work regarding different plant foodstuffs in their archaeological and social context, and that it is difficult to understand who could access what based on the traditional archaeobotanical record. Dental calculus can provide at least some insights into the distribution of certain types of plant food per capita and its change across sexes, and other parameters if available, within the same population or between different groups. In what concerns staple food, some statistically significant differences were found in relation to the consumption of oats and legumes, however, they are attributed also to problems with identification and will not be discussed further.

In terms of food access across individuals, the most relevant category is that of food imports. There are three main points that are interesting here. First of all, one individual out of four in each population consumed imported items, most commonly ginger or galangal. This is interesting as the social status of many individuals under study is thought not to be high and, in the case of St Michael's, the Parish is meant to have served the lowest status individuals of the Medieval city (Jacklin 2009). While there is evidence of both ginger and galangal in the historical record of Leicester (Browning et al. forthcoming), all imports had no previous record in the region.

There is the general tendency to assume that expensive food imports in the period considered were eaten by the wealthiest members of the society. As pointed out by Livarda (2011, 160), Dyer (1992) has suggested that even for the elite, exotic food may have been consumed on restricted occasions. Dyer (1992) has pointed out the existence

of a number of markets and trades that are almost unknown to historians. Tracking the true access to foodstuffs is therefore challenging not only for archaeologists but also for historians. As there is a historical record for the presence of both ginger and galangal, at least these species were available in the urban environment and may have been occasionally used as spices or for their medicinal properties by the lower members of the society. Therefore, the access visible here by Medieval lower status people in Leicester to such products is novel. Several hypotheses are possible to explain access to such imports, among them fluctuations of prices, generated by changes in their availability could have made them more at reach to people at times. Some of such species, for example ginger, were medicinal and maybe have sought after for that reason and worth the effort put in buying them. There is however the need to consider here an important factor that is specific with the context of the retrieval (dental calculus): where were such imports being consumed? Unlike the archaeobotanical record, the record of micro-debris in teeth is not direct evidence of the import in the city, which is actually something true for the entire line of evidence. For instance, a cess-pit in Medieval Leicester will contain most likely remains brought in and consumed in the city. Remains in teeth are those of plants that could have been consumed away from the city. In the case of ginger/galangal, the presence of them in a number of individuals across the population and equally between males and females is suggestive that such species were at reach to all individuals in Leicester. However, the presence of very rare imports, in specific the potential finds of sorghum and date palm, are only found in males. Although it is only in two individuals, this is a potential indication that such rare food items were eaten or accessed more often by men in the Later Medieval period, and could suggest gender differentiation in diet. The dataset is too small to be statistically significant, but the well known people mobility must be taken in consideration here (Dyer 2000). It is possible that such foodstuffs were eaten elsewhere by certain individuals, potentially during travel that saw male traders traveling more often than women in the Later Medieval period; it is in fact known that women were less involved in long distance trade than men (Ward 2016, 91). It is very interesting that such differences between sexes are not seen in the Anglo-Saxon period,

in accordance with what is known in terms of gender equality in the access to food in the Anglo-Saxon period (Banham 2004).

A third aspect in terms of appearance of imported food items is the following: no significant difference was seen between the Anglo-Saxon and Medieval periods. Little is known regarding luxury food items that were available in the Anglo-Saxon period, and therefore the number of species of plants found is an indication of access to imported foodstuff that is beyond what was known so far at least for the region. The finds of introduced species such as leek, hemp and borage, confirms the diversity of eaten plant food during this little known period. There is archaeological evidence of millets, cannabis and buckwheat grown in Northern France for the early medieval period (Backles 2005) and therefore the trade of such items or their consumption during trips is possible. It must be stressed that these findings require a thorough comparison with the written evidence and it is therefore a future direction of the work.

Food hygiene A brief mention is made here of the subject of food hygiene. The presence of parasite ova in dental calculus and its implications have been discussed before, however in this context it is worth mentioning the ubiquity of soil flecks and mineral grit. While pathways to inclusion for such lines of evidence are multiple and cannot be addressed without correct identification, the presence of the lithological and soil element is proof of the introduction into the mouth of debris that would carry its own specific bacterial load and be potentially harmful to health. Grit alone would cause dental wear seen in the populations examined (Jacklin 2009a, 2009b; Mays 1996). Soil and grit, just like the parasite ova, could enter the calculus associated with poorly cleaned vegetables and fruits or be the result of grit in grinding stones. Further identification of this line of evidence may help to understand how clean was the food ingested in different populations and periods of time, and help the interpretation of osteoarchaeological aspects such as dental wear. The recording of such lines of evidence in calculus should not be neglected, as it has been so far in most studies. The finding of yeast and mould could point to mould on food, as seen in previous paragraphs on Fungi. Changes in storage practices as well as increased complexity of food networks in the Later Medieval period may have influenced the frequency and

manner of food spoilage as well as the movement of other micro-organisms. Only the secure identification of such remains can provide true insights into the subject, nevertheless evidence of fungi consistent with mould and yeast has been retrieved in this study.

9.5.2 Dental calculus, "indoor pollution" and occupational dust

When the word "pollution" is mentioned among scholars that work in the Middle Ages, one of the first things that come to mind is the concept that Medieval people lived in dirty and filthy conditions (e.g. Zupko and Laures 1996). This concept has been challenged by many scholars (e.g., Dyer 2006). Monkton (2006) stressed that when the archaeological evidence from Leicester is examined, for example from floor layers, the archaeobotanical evidence of plant and other remains is normally very poor, indicating that rubbish from floor layers was regularly removed, and there is archaeological evidence for cess and rubbish pits to be regularly cleaned and treated with lime (Coward and Speed 2009). In their study of Medieval York, King and Henderson (2013) successfully integrated archaeological and historical evidence to create a better understanding of the palaeoecology of diseases observed in the skeletal remains of individuals: their study showed that attempts were made to improve the "cleanness" of the city under the pressure of disposal of waste generated by craft and households as well as pollution, but socio-environmental conditions were still likely to contribute to morbidity in the urban environment. All the debris consistent with non-dietary activities in this study can be considered within the respirable fraction of particulate matter therefore it could be present in the environment in high quantity and be airborne. As such, the remains represent a form of dirt that is not necessarily visible by the naked eye. The high presence of soil and grit is also suggestive of some dirt potentially entering the mouth due to ingestion of contaminated food. The presence of non dietary evidence in dental calculus opens the possibility of contributing to a better understanding of living conditions experienced by past people. Aspects relating to living conditions not linked to diet but to pollution and crafts will be explored below.

Potential evidence of indoor pollution: smoke and dirt Direct evidence of exposure to pollutants and dirt in the environment has been examined in Chapter 3 and shows how the osteoarchaeolgical evidence is very often multi-causal, preventing a secure link between pathology on bones and specific pollutants. Here a number of patterns show that dental calculus can provide insights into differential exposure to potential pollutants and smoke between sexes, populations and periods of time.

Firstly, Anglo-Saxon populations had almost double the concentration of remains of non-dietary origin when compared with Late Medieval populations. When all debris that falls into the category of dust was examined in Chapter 8, there is evidence of a higher exposure to a variety of particles, including smoke, that can be considered respiratory irritants. The reason behind this could be the nature of housing conditions and the location of crafts: in the Anglo-Saxon period a number of activities were very likely conducted in poorly ventilated buildings and in spaces shared between women and men, a fact here confirmed by the lack of differences between females and males seen in the analysis. A body of archaeological evidence is found for instance for the production of textiles in the Anglo-Saxon period, in the form of loom weights, which are almost ubiquitous in Anglo-Saxon sites in Britain (Leahy 2011, 445) as well as Leicestershire (Morris et al. 2011). Grave goods show the strict association between the craft of textile and women, as spindles are often found in female burials, as in Anglo-Saxon Empingham (Timby 1996, 61-62)**.** There is also strong archaeological and historical evidence that many aspects of textile work were carried out at home (Gilchrist 2012, 145) even in the Later Medieval period. During the Medieval period many crafts were moved to different locations and some even away from the urban environment (e.g., shops and workshops) and practiced by specialized craftspeople, resulting in the differential exposure between people and potentially between the sexes: a pattern also visible here when all potential indoor pollutants retrieved during analysis, including occupational dust, are observed together. In fact, in the Later Medieval populations there were significant differences both between sexes and cemeteries. Interestingly, however, there was no difference between Anglo-Saxon and Medieval populations, as the majority of the crafts would be present across periods. There were significant

differences seen in the exposure to specific types of dust, such as particulate matter from wool, bast fibers and sedges between males and females in the examined populations. Such patterns confirmed women were more exposed than men to respiratory irritants of this kind and likely captured aspects of the division of labour during the period.

Dust generated by the breakdown of building, flooring and roofing material, in this case potentially plant remains such as grasses and sedges would also be commonly found indoors. Many of the remains found (wool, feathers, plant fibers) were valuable as roofing and bedding material, such as mattresses (Pritchard 2003) and recently Dove and Wickler (2016) found archaeological evidence of a feather stuffed Viking pillow. Here evidence of unburnt phytoliths from plants that have been used as building, roofing and flooring material have been found. Gilchrist (2012, 143) has pointed out that the historical record suggests that a 50% of the recorded deaths in females in the later Medieval period were the consequence of incidents at home, whereas the figure was down to 20% in males. This is suggestive of women spending more time indoors, and is potentially supported by the data in this thesis. The dental calculus record therefore provided a rare window into exposure to particulate matter and dust present in the environment that could result in respiratory problems.

There is potentially evidence of a more diversified exposure to debris generated by craft activities in the Later Medieval period. From the 14th century onwards, especially when the population decreased during epidemics, labour increased in demand and both males and females would be involved in a variety of activities that may have been different between males and females (Dyer 2000). However, it must be stressed that the majority of debris of non-dietary origin found in this study is generated by material, such as flax, wool and wood that would have been very common in the environment. The production of textile involves many steps, all of which can produce dust, and many would actually be carried out in the house.

Micro-charcoal, soot and burnt debris, were included together in the analysis, as potential evidence of higher exposure to smoky indoor and outdoor environments and

occupations. However, smoke had several uses in the past including food preservation (Pennacchio, Jefferson and Hevens, 2010), so pathways to its inclusion are many. Care therefore must be taken in interpreting such evidence as direct exposure to smoke. However, significant differences were detected on such lines of evidence, suggesting differential exposure to such debris occurring between time periods, and between males and females in the Late Medieval population. If such remains were only the consequence of the ingestion of burnt particles of food or micro-charcoal transferred from the pot, there should be no difference between sexes in the Late Medieval period, as cooking practices would be similar within the same period. It is more likely that as women would be spending more time near the fire, they would be more likely to encapsulate such remains in the calculus matrix.

The non-dietary evidence found in this PhD overall suggests that dental calculus samples entrap a variety of remains generated by crafts, by the natural breakdown of building material and by the use of fire for heating and cooking. The exposure to such remains varied across time and between sexes in many cases, suggesting dental calculus may be useful in better understanding differential exposure to pollutants. While of course pathways to inclusion of debris are multiple and complicate the interpretation, the patterns found in this study are suggestive of dental calculus entombing the record of differential exposure to a variety for indoor particulate matter across periods of time and sexes. Only further study will confirm if this is only one case or if this information can be retrieved consistently in ancient populations.

Can we detect professions/crafts from calculus debris? So far we have talked about non dietary remains and exposure to debris that could be generated by crafts. However, exposure to the pollutants generated by crafts and practicing the craft is clearly not the same thing. Whether we can separate people that practiced those skills from those who were simply exposed to them is explored here. A number of individuals in this study had a very high concentration of micro debris such as wood dust, wool and plant fibers, and can provide a means of understanding if an individual was practicing crafts that could generate such debris. While of course there are limitations in the association between the amount of debris found in calculus and the amount of such debris in the

environment and for how long the exposure lasted, osteoarchaeological parameters may help. A range of osteological markers of activity have been recorded on the Anglo-Saxon and Late Medieval skeletons included in this study by the author of this PhD in collaboration with Dr. Efthymia Nikita. These markers include dental wear data from the anterior and posterior dentition, entheseal changes on the upper and lower limbs, cross-sectional geometric properties on all long bones, and degree of osteoarthritis in the joints of the appendicular skeleton and the spine. These markers are currently being processed and they will soon be examined in association with dental calculus microdebris evidence of specific occupations. The integration of osteoarchaeological parameters with dental calculus data can potentially help in understanding if an individual was systematically practicing a craft.

The main limitation that such an approach has is that it is mostly relevant in cases where a particularly high concentration of micro-debris has been identified in the calculus matrix. Such anomalies can only be detected if there is a dataset that is sufficiently large to be statistically significant in order to:

1) Establish a baseline to evaluate the occurrence and concentration of the supposed occupational pollutant across the population
2) Be able to compare if, in a given individual, dietary debris is in anomalous concentration. The presence of other anomalies in the concentration of multiple finds in the same individual could be suggestive of a bias in the calculus formation process.

It is therefore remarkable that the individuals in this study who had a very high concentration of a specific non-dietary debris were outliers only for one category of debris apart from two cases. An interesting aspect is that the two females who had a high concentration of fibers were outliers for two or more fibers of different nature, reinforcing the hypothesis that they were involved in textile crafts. In all cases but one, the dust generated by the “craft” is not indicative of a specific stage of the work or product. Such aspects of work may not be addressable using micro-debris in calculus apart from rare cases. Only one individual had remains of flax capsules. This part of the

plant would need to be removed before the fiber is processed and therefore, such debris could be the result of the stage of flax preparation just before the fiber is carded. The only way in which different stages in the production of material culture can be detected is therefore when the dust produced is specific to a stage. This was not possible in any other case in this study.

Finally, it must be pointed out that the Later Medieval period was characterized by high mobility of work, and in many cases many dusty crafts such pottery making, roofing and textile work, would be practiced only for a few months per year, giving the labour force a certain "occupational flexibility" (Dyer 2000, 73). Therefore, many of the individuals in this study could have been exposed to occupational dust for short periods of time, reducing the chances to detect it, and care must be taken in interpreting the sex differences observed as the direct result of division of labour. The variety of professions and skills that women demonstrated in the Later Medieval period is a complex subject (Ward 2002) and cannot be exhausted in this chapter. However, this study shows that using dental calculus it is possible to detect anomalies in a variety of pollutants potentially linked to occupations, providing a very important palaeoecological perspective, at a human scale, in ancient populations not available so far.

9.5.3 Dental calculus: Medieval Leicestershire and the Archaeology Research Agenda of the East Midlands

While the micro-remains in this study were contrasted in Chapters 6 and 7 with the record from Leicestershire, it may be useful to summarise here as a whole the contribution that this study has made towards Medieval life style in Leicester and its surroundings, with a particular reference to the research target identified for the period in the Archaeological Research Agenda.

In relation to diet, the current study indicates that a variety of plant remains were consumed during the Anglo-Saxon period. While archaeological data already existed for the staple cereals and legumes, from both Leicester (Monckton 2015, Radini 2009) and the county (Monckton 2006), the presence of leafy greens such as leek, and a number of potential medicinal plants including hemp were present in the diet. The imported

items suggested the population was engaged in a number of long distance trade activities, a fact already visible from the grave goods in Empingham (Timby 1996, 86-93), and therefore it is interesting to see that such trades and contacts did not involve only objects but also food. Although the dataset is small, it is possible to infer that no difference apparently existed in the variety of food accessed by sexes; such information was also not available before. For what concerns living conditions, it appears that the Anglo-Saxon populations were exposed to a higher amount of dust when compared to the later Medieval period, potentially due to the housing conditions. Apart from wool, there were no significant differences in the exposure to potential respiratory irritants between males and females, again suggesting equality between sexes in the use of space. However, females appear to have been exposed more to the dust of fibers such as wool and flax, and this was probably due to the fact that women were involved in all stages of textile work, including carding and weaving. Important information was also provided by the higher and almost ubiquitous arboreal pollen across the Anglo-Saxon individuals.

The Later Medieval samples have provided new insights into the consumption of leafy greens, medicinal plants/herbs as well as novel information regarding the consumption of luxury food plants such as ginger and galangal, millets, sorghum, fenugreek and buckwheat and the very rare finds of date palm phytoliths. Social access of such imported food seemed to be equal between sexes, however the very rare items were found only in men. Such aspects meet the research priority for the region to identify the consumption of leafy crops and imports in urban contexts. Differential exposure to a variety of debris showed potential gender differentiation in tasks. This was particularly evident in the higher amount of feather dust finds amongst women. Such data contribute to the research priority for understanding living conditions in the urban area. Finally, the pollen remains showed a deep decline in the occurrence of arboreal pollen and an increase in plant pathogens, confirming the dramatic changes in the environment due to human impact and climate change that are known for the period (Hoffmann 2014).

9.6 Limitations and future directions

This study has highlighted a number of limitations within dental calculus research:

1) The uncertainties related to the formation process of the calculus deposit are well known and need to be addressed
2) The variety of debris found, as well as its overall small size, is challenging and requires specialists in each field and specialised reference collections for its identification
3) In many cases such debris has multiple pathways to inclusions that further limit interpretation
4) The lithological element cannot be properly recorded at the moment; however, it is ubiquitous

These limitations are discussed below with reference to possible future directions.

The following sections were recently published in a peered reviewed book chapter (Radini et al. 2016) that was a proof of concept of this study, and are here proposed in an expanded version.

9.6.1 Limitations relating to typology, size and quantity of the remains

Despite the overall results of the survey being very positive, some limitations need to be pointed out. This is mainly due to the fact that the extraction procedures, but most of all the scanning of obtained slides, is a very time consuming process. Painstaking hours of microscopy work were conducted to generate such large data set. Therefore, it is most important to clarify the limitations of this approach.

In relation to typology of remains and their identification the following aspects are considered the major limits:

- This results of this study shows that the most represented categories of remains (starch granules, phytolith, fungal debris and charcoal) are challenging to identify. The lack of specific identification reduces the possibility of secure interpretation

- The size of the remains is also challenging with traditional light microscopy, therefore in many cases identification is not possible. This affects the results as the quantification of the remains identified cannot be considered secure, as the proportion of unidentified debris impacts the figures

In relation to debris linked to occupation and indicative of potential respiratory irritants the following can be said:

- Individuals with high amounts of potential occupational pollutants were very few in this analysis, especially considering the sample size here studied. This suggests that in order to be able to detect such individuals a very large data set is needed. Detecting such individuals clearly becomes a very time consuming task.
- The identification of individuals that are “anomalous” for concentrations of such debris may be difficult to evaluate if the sample of calculus is very small.
- Finally, the potential occupational debris found in this analysis is not specific to a single activity, which reduces its potential for interpretation

However, the implementation of a reference collection and the integration of osteoarchaeological parameters may help to overcome such problems.

It is therefore clear that studies that aim to use dental calculus samples to gain insights into potential occupational health need large data sets.

9.6.2 Limitations relating to the etiology of the calculus

Dental calculus takes time to form. Therefore, prolonged exposure to the same single type of particulate matter and/or high concentrations of it, increases the chances of it entering the calculus matrix. Nevertheless, it is not currently possible to know how long the calculus took to form and at what point in the life of an individual the formation took place. With the mobility that characterized people in the Middle Ages (Dyer 2006) and generally in the past, it is possible that some of the debris entered the calculus outside the area where the individual was buried. Therefore, care must be taken in the interpretation of the results.

9.6.3 The challenges of identification, quantification and pathways to inclusion

If calculus is to be approached as a line of evidence for non-dietary studies, a threshold needs to be set in order to distinguish between normal and abnormal levels of 'dust'. This first requires the correct identification of the debris, through appropriate reference collections. Implementing reference collections to include debris of non dietary origin appears now a necessity, particularly in relation to evidence from fungi, which could offer new insights into environmental changes and living conditions. In addition, it is necessary to establish a 'norm' across a large number of individuals in whom dust of the same type may appear so that extreme quantities can be detected, as shown by the preliminary scan undertaken here. This should become possible for the city of Leicester and for many other Medieval towns as large skeletal assemblages have recently been unearthed.

Finally, the pathways for particles into the mouth are many and encompass dietary, occupational and random processes, so these also need to be assessed before we can proceed to the interpretation of the data. Comparative material from other archaeological sites, from urban and rural populations, but also from other periods of time will allow a better understanding of how the debris is differentiated temporally and spatially. Note that, in order for such comparisons to take place, debris should be recorded and quantified in a standard way in all studies.

9.6.4 Unlocking the Lithological Element

Mineral debris found in the remains has not been fully explored so far; debris generated by the break-down of local crustal material and also occupational dust generated by stone carving, lime industries, pottery making, ingestion of particles from mortars and other activities can become entrapped in the calculus. If this debris is correctly identified and quantified, it may enable the detection of 'professions' in the past, providing interesting potential for forensic sciences, as suggested by Charlier et al. (2010). Soil and mineral debris possibly entering the mouth from food can also provide information on food hygiene, while geological debris may also offer a better understanding of dental wear (as flour tended to contain lithological debris left from

grinding). Finally, the identification of the origin of the debris may allow the understanding of trade routes and population movements, as archaeological provenance studies based on minerals and fossils have been proven successful. Isotope analysis may help to clarify this aspect and with reconstructing past population movements. In addition, the exploration of sex-related differences in mobility patterns could be explored and may offer insights to the underlying reasons for this phenomenon, e.g. patrilocal or matrilocal marriage, trade networks, and others. Archaeological interest in the inorganic material found embedded in dental calculus is focused on its potential for identifying particular activities in which the individual or population might have engaged. The hypothesis is that such exogenous material may have derived from carrying out practices or handling substances associated with particular industrial or craft activities. Pottery, for instance, involves the handling of grog, slip and clays; wall painting or manuscript illumination requires the grinding, preparation and use of mineral pigments; stone carving involves the production of dust, and metalworking the use of carbon sources and metallic ores. All of these activities might be expected to leave some trace in dental calculus, if oral entrapment of air-borne particles present at the place of work occurred. In addition, soil and mineral debris entering the mouth on dirty food can also provide information of food preparation and hygiene practices. In principle, it is possible to identify the inorganic material found in dental calculus using a scanning electron microscope (SEM) equipped with an energy-dispersive X-ray analysis (EDX) system. Single crystals embedded in the sub-gingival calculus on a tooth are identified using the SEM at high magnification and EDX is then used as a tool to 'spot-sample' a number of points on each crystal in order to provide data on its elemental composition. Identification of such inorganic materials is clearly of interest to archaeologists and anthropologists because of the wider implications that can be drawn concerning the lifestyle of a population. It may also allow the origin of the materials to be determined and so suggest possible routes for the movement of materials or even of populations, an approach that has already proven successful in the study of ceramics. In addition, a useful overview of this microscopic

examination approach and its potential for generating valuable forensic data appears in Charlier et al. (2010).

9.6.5 Integrating approaches

At the time in which this PhD is submitted the only study available that contrasts Ancient DNA and Proteomics with microscopy is that co-authored with Dr. Tina Warinner et al. (2014). This study highlighted that microscopy is complementary to DNA studies with in plant remains. Another interesting combination of data is that between microscopy and chemical analysis, which are again complementary to one another. Such an approach has only been tried to date by Hardy et al (2014), Buckley et al. (2015) and Radini et al (2016), but it has shown that results obtained from chromatography can provide evidence of inhaled debris, as well as strengthen the identification of microremains. Finally, although already mentioned in Chapter 2, isotopic analysis may be very useful in assisting the interpretation of how common certain types of plants were in diet. Recent work has used isotopic data from bone collagen in combination with starch granules analysis in order to provide a more complete picture of diet in past populations. The study conducted by Wang et al. (2015) obtained isotopic data from bone collagen and identified the presence of C3 and C4 plants in the diet. Having found evidence of both C3 and C4 plants in the skeletal remains, Wang et al. (2015) compared such results with those from starch granules retrieved from dental calculus, as it is possible to distinguish Triticeae and Paniceae starch upon morphology. While, as stressed by Leonard et al. (2015), we cannot correctly address the role of plants in the diet from starch granules embedded in the calculus, the isotopic signature may help. The successful approach of combining isotopic data and starch granules, may eventually help to address the proportion of items with different isotopic signature, once the dietary nature of the debris in the calculus has been confirmed.

9.6.6 Collaborative work in progress on the material

This is the first study to date on a large historical population aimed at assessing all categories of debris, identifying and quantifying them. It has resulted in a very large dataset that encompasses remains from all the Kingdoms of life. The variety of material

and the retrieval of very fragile remains testify not only the extraordinary preservation in the calculus matrix, but also that the new protocol developed for this study, although very time consuming, can extract the entire record entombed without damage. The analysis conducted in the context of the current study on micro-debris in human dental calculus has confirmed that a variety of remains apart from starch granules and phytoliths can be retrieved from its mineralised matrix but never explored in a large dataset and to this level of identification and quantification.

9.7 Summary of the results and conclusive remarks

In respect to **the aims and objectives** of this PhD, the following results were achieved:

- A review of the current state of microdebris and calculus research (Chapters 2 and 3) highlighted the lack of systematic research of the full spectrum of debris entrapped in the calculus matrix. A conceptual framework was developed that proposed evidence for the pathways through which a variety of particles may enter the human mouth, beyond those that are attributed to diet. This framework generated two papers (Radini et al. 2017, Radini et al. 2016a)
- In light of the need to extract all micro remains from calculus under controlled conditions whist preserving the integrity of the debris, a new method of extraction was developed. This method, although time consuming, incurs minimal damage whilst enabling the variety of remains entombed in the calculus to be recovered and allowing the retrieval of debris with different physical properties pseudo in-situ in the calculus matrix (as shown in many micrographs). Such an approach leaves no doubt as to the archaeological integrity of the finds. The new extraction protocol developed as a result of this project has been published during collaborative work led by Dr. Tina Warinner (Warinner et al. 2014) and is currently the extraction method adopted at the Department of Archaeology at the University of York.
- After extraction, debris from all kingdoms of life was recorded and quantified, with the only exception being the lithological evidence that could not be correctly quantified.

- Identification was attempted for every type of material found. The level of identification that was achieved varied depending on the diagnostic nature of the debris and was often linked to the size of the remains. All debris found was grouped into recurring types in order to be analysed. All the identifications proposed were supported by published work, online material and wherever needed, a micrograph was provided of modern origin to allow comparison. Problems with the identification and ways of overcoming these were also clearly stated. The identification criteria and reference collection used for this study were successfully used by the author in collaborative work (fully referenced in the text), allowing them to be peer reviewed before the final analysis took place.
- In order to carry out the identification, a large reference collection was built. While for reasons of space and time, it was not possible to take pictures of all species in the reference collection during this study, parts of it have been published in collaborative work with other authors (e.g., Buckley et al. 2015; Cristiani et al. in press; Hardy et al. 2015; Lucarini et al 2016; Radini et al. 2016). The reference collection has been placed at the disposition of a number of projects in UK and abroad and currently two PhD students are using it at the University of York.
- After identification, it was possible to determine multiple pathways of inclusion for many finds and some of the most likely pathways were proposed. This approach highlighted the fact that among the identified debris, remains were both of dietary and non dietary origin.
- The visibility of plant remains in calculus appears to be linked to the ability of calculus to capture debris that is generally below 60 microns in size. This results in size selection so the plants that are detected in the calculus matrix are those used in food and crafts that can break down to diagnostic fragments of that size. This limits the variability of the remains of plants that are entombed in the matrix when compared with those found in the archaeological and, in general, the historical record. However, the different pathways to inclusion (direct ingestion or inhalation) allow the calculus to entomb debris that otherwise has

low archaeological visibility, such as luxury food imports, leafy greens, potential medicinal plants as well as fibers and wood fragments.

- Several trends were identified at the population level as the debris showed major changes in indoor and outdoor environments, in line with existing broad knowledge of living conditions and the natural environment for the periods examined. This indicates that a large dataset of dental calculus samples register environmental and cultural changes and can therefore complement the traditional disciplines of archaeology, zooarchaoelgy and paleoenvironmental studies.
- This analysis also showed not only the potential of micro-debris to retrieve a variety of information related to the presence and absence of remains with low archaeological visibility, but also the potential of this deposit when combined with osteoarchaeological information (sex), and the archaeological context (e.g. cemetery, period, social status) to provide a novel "social" perspective of lifestyle. While so far dental calculus has been approached only as a source of information for the consumption of plants in prehistoric societies, its archaeological value in more recent populations as indicated here is far beyond expected. When all the remains entombed are considered together with osteoarchaeological parameters, they can become a way of bringing environmental archaeology to a human scale, potentially very deep in the human past. A preliminary proof of concept has already been published to promote the approach in the Medievalist community (Radini et al. 2016b)

This PhD has scratched the surface of the potential of this methodology and at the time in which this PhD is submitted, steps have been taken to set up collaborations to fully "exploit" the results of this study. Work on the material is currently progressing in the following main directions:

- The identification of micro-debris is very challenging and large reference collections are needed. The reference collection built for the purpose of this study is currently improving in order to make the identification of the imported items more solid and potentially expand the identification of the portion of the

debris that remains unidentified. A large portion of the remains has now been mounted on slides to be studied under the microscope with more analytical power, such as Raman Microscopy and SED-EDX, with the scope of unlocking lithological debris in calculus.

- In order to facilitate other researchers in the time consuming process of identification, steps are being taken to digitize the reference collection and ensure free access to it
- In order to contextualise the finds with aspects of living conditions and health to produce a more detailed picture of diet and living conditions, osteoarchaeological work is currently be conducted with Dr. Efthymia Nikita. Such work will be complemented by isotopic studies led by Dr. Michelle Alexander.
- In order to fully contextualise the finds and any future data in the historical and archaeological record, contact has been made with experts in the disciplines touched in this study.

A full publication plan has also been set with the collaborators, aiming to share the results of this study with others in the very near future. Finally, the variety of micro-remains found at the individual and population level can provide new insights into diet and living conditions that encompass all disciplines in the field of environmental archaeology, but also Medieval archaeology, archaeology of gender, and potentially provide new insights into the history of the complex interaction between people, diet living conditions and health. These aspects clearly highlight the complexity of the archaeological record entombed into the calculus matrix, originally postulated in the introduction of this study. Improving our understating of the calculus formation process and the analytical methods for the identification of remains that are challenging due to their size and typology appear to be the major important steps to be taken in the near future. Finally, due to clarity in the recording procedures and the very large dataset, this PhD provides a dataset that can be considered a solid comparative “baseline” for future studies in proceeding and preceding periods in Britain and Northern Europe.

APPENDIX I

COMMON FOOD PLANTS AND FIBERS BY SITE

Table 7. Food plants found in the archaeological record in Leicester and surroundings, by site and by period); AP Leicester Abbey (Buckley 2006); BL = Bonners Lane (Monckton 2004); CL = Causeway Lane (Monckton 1996a, 1999a); CS = Castle Street (Monckton 2006); DM = DMU Sites (Radini 2010); EB, East Bond St (Mockton 2009); EK, Eye Kettleby (Monckton, 2006); FS, Site 3 Freeschool Lane (Radini 2009), GL = Grange Lane, medieval (Monckton and Radini 2010); NP = St Nicholas Place (Monckton 2009); NS = Newarke Street (Monckton 1996); NU = St Nicholas Place Undercroft (Monckton 2009); OX = Oxford St RCP (Monckton 1999b); SL = Shires, Little Lane (Moffett 1993); SP = Shires, St Peters Lane (Moffett 1993); VS Vine Street (Monckton and Radini 2009), ; VW Site 2, Vaughan Way; YR = York Rd (Monckton 1999c).

	Anglo-Saxon	**Later Medieval**
CEREALS		
Wheat, grain, *Triticum* sp. free-threshing	CL,BL, FS	SL, SP, CL, BL, NU, FS, VS, VW, EB, DM, YR
Bread wheat, chaff, *Triticum aestivum* s.l. rachis		EB SL, SP, CL, NU, FS, VS, DM
Rivet wheat, chaff, *Triticum turgidum* type rachis		SL, SP, CL, NU, VS
Barley, grain, *Hordeum vulgare* L.	CL,BL, FS	SL, SP, CL, BL, NU, FS, VS, GL, DM, OX
Barley, chaff, *Hordeum vulgare* L. rachis		CL, BL, NU, FS, DM, SP
Rye grain, *Secale cereale* L	CL, FS	SL, SP, CL, BL, NP, NU, FS, VS, VW, DM, OX, YR
Oat grains (small, wild? type) *Avena* sp.	FS	SL, SP, CL, BL, NU, FS, VS, VW, DM, YR

Table 7 continued **CEREALS**	**Anglo-Saxon**	**Later Medieval**
Spelt, chaff, *Triticum spelta* L.		SL, FS
LEGUME CROPS		
Beans or Peas *Vicia/Pisum*	BL	SL, SP, CL, BL, NP, NU, EB, FS, VS, VW, GL, DM, OX, YR
Beans *Vicia faba* L.		SL, SP, CL, BL, NP, NU, FS, GL, DM
Peas *Pisum sativum* L.		CL, SL, SP, NP, NU, FS, BL
Cultivated Vetch, *Vicia sativa* ssp *sativa* (L.) B.		SL, SP, BL
IMPORTS		
Fig, *Ficus carica* L.		FS, SL, SP, NP, BL, NU, DM
Grape, *Vitis vinifera* L.		FS, DM
FRUITS, NUTS		
Hazel nut shell, *Corylus avellana* L.	FS	SL, SP, EB, CL, BL, NU, VS, DM, YR
Sloe fruit stones, *Prunus spinosa* L.		FS, DM, YR
Plums, Bullace, *Prunus domestica* L.		CL, NU, DM, SL, NP, FS, YR
Cherry type, *Prunus* sp.		FS, DM
Apple, *Malus* sp.		SL, CL, NU, NP, FS, DM
Raspberry, *Rubus idaeus* L.		SL
Blackberry, *Rubus fruticosus* agg.		NU, SL, NP, FS, YR
Elder, *Sambucus nigra* L.	BL, FS,	SL, SP. CL, BL, NP, NU, FS, VS, VW, NU, DM
GARDEN PLANTS		
Opium Poppy, (seed *Papaver somniferum* L.		SL, CL, NP,NU
Fennel, *Foeniculum vulgare* Miller.		DM
Borage (pollen), *Borago officinalis* L.		
Violet, *Viola odorata* L.		SL

Table 7 continued	**Anglo-Saxon**	**Later Medieval**
GARDEN PLANTS		
Flax, *Linum usitatissimum* (pollen) Hemp *Cannabis sativa* L. (pollen)	EK (hemp)	SL, SP (flax)
Wood Strawberry, *Fragaria vesca*		NP
Leeks, *Allium porrum* L.		SL
Mustards, *Brassica/Sinapis*		BL, FS, VS, DM
Brassicas, Brassicaceae	BL	NU, FS, VS
CROP WEEDS		
Black-bindweed, *Fallopia convolvulus* (L.) .		SL, SP, NP
Cleavers, *Galium aparine* L.		FS, SL, SP, BL, NU
Corn cockle, *Agrostemma githago* L.		SL, SP, NU
Stinking Mayweed, *Anthemis cotula* L.		SL, SP, CL, BL, NU, FS, SL, SP, NP, OX, YR
Wild carrot, *Daucus carota* L.		SL
Vetches/Tares ++ *Vicia/Lathyrus* type.		BL, NU

APPENDIX II

SAMPLE-SKELETON CONCORDANCE AND WEIGHT

Table 8. Sample concordances and sample weight for the Anglo-Saxon Cemeteries of Empigham and Rothley La Grange

EMP= Empingham 500-700 AD; RT=Rothley La Grance, 700-900 AD

Sample Id	SK ID Number	Weight in mg.
EMP1	EMP 69 GRAVE 1	23
EMP11	EMP 67 sk 6	12
EMP12	EMP 69 Grave13	12
EMP13	EMP 67 Grave6	14
EMP14	EMP II GRAVE 85	18
EMP15	EMP II GRAVE 91	21
EMP16	EMP II GRAVE 129	21
EMP17	GRAVE 63 BOX 1646	17
EMP18	EMP II grave 94	19
RT11	RT11	20
RT12	RT12	16
RT13	RT13	15
RT5	RT5	12
EMP10	Emp 67 sk 5	15
EMP2	Eps 69, skeleton 12	15
EMP3	GRAVE 61, LAB NO. 760448, BOX 1637, 862051	17
EMP4	EMP II, 1974, GRAVE 92	15
EMP5	EMP II, 1974, GRAVE 92	13
EMP6	EMP II, GRAVE 107, 1974	18
EMP7	EMP II GRAVE 119B	15
EMP8	EMP II GRAVE 131	15
EMP9	EMP II GRAVE 104B	16
RT2	Rothley La Grange RT2	14

Table 8 continued Sample Id	SK ID Number	Weight in mg.
RT3	Rothley La Grange RT3	14
RT4	Rothley La GrangeRT4	12
RT6	Rothley La GrangeRT6	16
RT7	Rothley La GrangeRT7	18
RT8	Rothley La GrangeRT8	18
RT9	Rothley La GrangeRT9	15
RT1	Rothley La Grange RT1	15
RT10	Rothley La Grange Rt10	15

Table 9. Sample concordances and sample weight for the Medieval Cemetery of St Peter's (all skeletons date between 1200-1450 AD). SK1 to Sk45 are males, Sk46 to 90 are females

Sample Id	Skeleton N.	Weight in mg.
SP1	91	32
SP2	116	30
SP3	41	29
SP4	42	28
SP5	60	30
SP6	60	34
SP7	63	26
SP8	226	32
SP9	436	23
SP10	473	34
SP11	511	32
SP12	654	27
SP13	700	32
SP14	1058	29
SP15	1189	27
SP16	1202	28
SP17	732	28
SP18	1374	29
SP19	1384	25
SP20	1478	26

Table 9 continued Sample Id	Skeleton N.	Weight in mg.
SP21	1494	32
SP22	1520	28
SP23	1522	31
SP24	1544	33
SP25	1592	28
SP26	1607	27
SP27	51	23
SP28	120	27
SP29	238	34
SP30	35	31
SP31	1399	29
SP32	25	23
SP33	57	30
SP34	116	23
SP35	571	28
SP36	551	27
SP37	663	26
SP38	373	25
SP39	643	27
SP40	695	28
SP41	108	26
SP42	256	25
SP43	295	32
SP44	299	31
SP45	421	35
SP46	433	27
SP47	509	29
SP48	552	34
SP49	1045	32
SP50	1165	33
SP51	1229	35
SP52	1267	32
SP53	1314	26
SP54	1356	27
SP55	1364	28
SP56	1473	29

Table 9 continued Sample Id	Skeleton N.	Weight in mg.
SP57	1475	30
SP58	1490	33
SP59	1494	37
SP60	1572	23
SP61	1580	27
SP62	1586	29
SP63	1597	36
SP64	1598	38
SP65	1601	34
SP66	1604	27
SP67	123	23
SP68	130	23
SP69	221	28
SP70	370	33
SP71	389	34
SP73	460	28
SP74	675	31
SP75	689	23
SP76	1008	41
SP77	1063	23
SP78	1151	25
SP79	1194	27
SP80	196	23
SP81	366	23
SP82	1505	28
SP83	601	27
SP84	508	35
SP85	696	32
SP86	712	24
SP87	722	23
SP88	377	20
SP89	500	21
SP90	517	23

Table 10. Sample concordances and weight for the Medieval Cemetery of St Michael's (all skeletons date between 1200-1450 AD). SK1 to Sk45 are males, Sk46 to 90 are females.

Sample Id	Skeleton N.	Weight in mg.
SM1	16	**29**
SM2	21	**32**
SM3	22	**32**
SM4	38	**31**
SM5	57	**30**
SM6	73	**29**
SM7	89	**33**
SM8	104	**23**
SM9	118	**34**
SM10	119	**28**
SM11	142	**27**
SM12	150	**28**
SM14	321	**27**
SM15	152	**28**
SM16	170	**26**
SM17	176	**42**
SM18	178	**39**
SM19	180	**31**
SM20	192	**28**
SM21	321	**28**
SM22	200	**27**
SM23	202	**23**
SM24	210	**26**
SM25	212	**28**
SM26	217	**29**
SM27	241	**30**
SM28	17	**21**
SM29	245	**28**
SM30	247	**29**
SM31	83	**32**
SM32	257	**34**
SM33	259	**30**
SM34	267	**23**

Table 10 continued Sample Id	Skeleton N.	Weight in mg.
SM35	268	**27**
SM36	273	**28**
SM37	281	**30**
SM38	287	**28**
SM39	293	**27**
SM40	34	**26**
SM41	296	**24**
SM42	299	**25**
SM43	158	**27**
SM44	313	**23**
SM45	331	**34**
SM46	31	**31**
SM47	35	**32**
SM48	44	**26**
SM49	53	**29**
SM50	76	**24**
SM51	81	**22**
SM52	86	**26**
SM53	92	**21**
SM54	94	**31**
SM55	97	**30**
SM56	107	**25**
SM57	256	**18**
SM58	282	**27**
SM59	129	**23**
SM60	134	**29**
SM61	267	**30**
SM62	144	**25**
SM63	147	**26**
SM64	160	**19**
SM65	280	**26**
SM66	190	**25**
SM67	216	**22**
SM68	218	**22**
SM69	221	**35**
SM70	222	**38**
SM71	303	**42**

Table 10 continued Sample Id	Skeleton N.	Weight in mg.
SM73	234	**40**
SM74	302	**22**
SM75	248	**28**
SM76	250	**36**
SM77	270	**38**
SM78	271	**39**
SM79	311	**18**
SM80	283	**35**
SM81	286	**22**
SM82	301	**15**
SM83	309	**18**
SM84	318	**17**
SM85	10	**15**
SM86	108	**13**
SM87	135	**18**
SM88	141	**21**
SM89	143	**22**
SM90	213	**24**

APPENDIX III

RESULTS KINGDOM PLANTAE

Table 11. Starch Granule distribution across the populations

Sample Id	**Sex**	Tirticeae	Fabeae	Aveneae
EMP11	**F**	0.733	0.000	0.533
EMP12	**F**	0.000	0.059	0.706
EMP13	**F**	0.200	0.000	0.200
EMP14	**F**	0.077	0.846	0.000
EMP15	**F**	0.778	0.167	0.611
EMP16	**F**	0.800	0.000	0.000
EMP17	**F**	0.200	0.267	0.267
EMP18	**F**	0.813	0.938	0.000
RT11	**F**	0.667	0.000	0.111
RT12	**F**	0.000	0.111	0.667
RT13	**F**	0.200	0.000	0.000
RT5	**F**	0.000	0.000	0.400
EMP10	**M**	0.632	0.632	0.316
EMP2	**M**	0.200	0.067	0.067
EMP3	**M**	0.417	0.250	0.250
EMP4	**M**	0.250	0.167	0.000
EMP5	**M**	0.857	0.000	1.643
EMP6	**M**	1.500	0.056	0.000
EMP7	**M**	0.238	0.000	0.048
EMP8	**M**	0.000	0.048	0.667
EMP9	**M**	0.294	0.176	0.412
RT2	**M**	0.267	0.067	0.000
RT3	**M**	0.000	0.000	1.600
RT4	**M**	0.750	0.188	0.000
RT6	**M**	0.214	0.214	0.571
EMP1	**USA**	0.043	0.000	0.087
RT7	**USA**	0.929	0.000	0.214
RT8	**USA**	0.417	0.083	0.000
RT9	**USA**	1.750	0.000	0.167
Table 11	**Sex**	**Tirticeae**	**Fabeae**	**Aveneae**

continued Sample Id				
RT1	**USY**	0.200	0.667	0.467
RT10	**USY**	0.438	0.500	0.000
SM1	**F**	0.000	0.517	0.069
SM10	**F**	0.000	0.179	0.000
SM11	**F**	0.000	0.000	0.667
SM12	**F**	0.500	0.071	0.607
SM14	**F**	0.074	0.037	0.000
SM15	**F**	0.107	0.536	0.821
SM16	**F**	0.038	0.154	0.962
SM17	**F**	0.310	0.000	0.024
SM18	**F**	0.051	0.103	0.179
SM19	**F**	1.032	0.097	0.097
SM2	**F**	0.031	0.000	0.000
SM21	**F**	0.429	0.107	0.000
SM22	**F**	0.111	0.222	0.000
SM23	**F**	1.522	0.000	0.087
SM24	**F**	0.115	0.038	0.000
SM25	**F**	0.821	0.000	0.000
SM26	**F**	0.034	0.034	0.448
SM28	**F**	1.667	0.000	0.000
SM29	**F**	0.107	0.036	0.000
SM3	**F**	0.250	0.094	0.000
SM31	**F**	0.375	0.000	0.719
SM32	**F**	0.441	0.353	0.000
SM33	**F**	0.467	0.000	0.033
SM34	**F**	0.783	0.000	0.261
SM35	**F**	0.852	0.148	0.000
SM36	**F**	0.000	0.000	0.179
SM37	**F**	0.833	0.000	0.000
SM38	**F**	1.143	0.429	0.036
SM4	**F**	0.516	0.129	0.226
SM40	**F**	0.692	0.000	0.038
SM41	**F**	0.125	0.000	0.000
SM43	**F**	0.296	0.185	0.111
SM44	**F**	0.913	0.478	0.000
SM5	**F**	2.433	0.567	0.000
Table 11 continued	**Sex**	**Tirticeae**	**Fabeae**	**Aveneae**

Sample Id				
SM6	**F**	0.034	0.552	0.276
SM7	**F**	0.091	0.000	0.394
SM8	**F**	0.043	0.130	0.000
SM9	**F**	0.382	0.000	0.000
SM45	**M**	0.088	0.000	0.353
SM46	**M**	0.000	0.000	0.129
SM47	**M**	0.563	0.000	0.094
SM48	**M**	1.423	0.154	0.000
SM49	**M**	0.000	0.000	0.103
SM50	**M**	0.958	0.000	0.000
SM51	**M**	0.000	0.227	0.000
SM52	**M**	0.538	0.000	0.000
SM53	**M**	1.143	0.714	0.048
SM54	**M**	0.774	0.097	0.000
SM55	**M**	0.500	0.567	0.000
SM58	**M**	0.037	0.111	0.296
SM59	**M**	1.043	0.000	0.391
SM61	**M**	0.400	0.000	0.300
SM62	**M**	0.000	0.960	0.000
SM63	**M**	0.538	0.038	0.038
SM65	**M**	0.692	0.000	0.000
SM66	**M**	0.960	0.000	0.840
SM67	**M**	0.818	0.000	0.000
SM68	**M**	0.364	0.136	0.091
SM69	**M**	0.057	0.000	0.029
SM71	**M**	0.571	0.286	0.167
SM73	**M**	0.075	0.425	0.000
SM74	**M**	0.591	0.136	0.045
SM75	**M**	0.036	0.036	0.000
SM76	**M**	0.944	0.000	0.194
SM78	**M**	0.026	0.308	0.000
SM79	**M**	0.111	0.000	0.000
SM80	**M**	0.086	0.114	0.000
SM81	**M**	0.455	0.000	0.182
SM82	**M**	0.400	0.333	0.000
SM83	**M**	0.222	0.000	0.000
Table 11 continued	**Sex**	**Tirticeae**	**Fabeae**	**Aveneae**

Sample Id				
SP41	**F**	1.308	0.500	0.000
SP42	**F**	1.640	0.000	0.000
SP43	**F**	0.000	0.000	0.125
SP44	**F**	0.000	0.419	0.000
SP45	**F**	0.657	0.400	0.000
SP46	**F**	0.667	0.630	0.000
SP47	**F**	0.793	0.793	0.000
SP48	**F**	0.500	0.059	0.147
SP49	**F**	0.594	0.219	0.000
SP50	**F**	0.000	0.848	0.000
SP51	**F**	0.914	0.000	0.000
SP52	**F**	0.344	0.125	0.000
SP53	**F**	0.577	0.000	0.000
SP54	**F**	0.889	0.259	0.148
SP55	**F**	0.643	0.000	0.000
SP56	**F**	1.207	0.483	0.000
SP57	**F**	0.000	0.100	0.400
SP58	**F**	0.697	0.000	0.000
SP59	**F**	0.676	0.000	0.081
SP60	**F**	0.478	0.130	0.000
SP61	**F**	0.667	0.222	0.185
SP62	**F**	0.241	0.069	0.000
SP63	**F**	0.444	0.000	0.000
SP64	**F**	0.079	0.079	0.316
SP65	**F**	0.500	0.147	0.441
SP66	**F**	0.111	0.222	0.593
SP67	**F**	0.739	0.304	0.000
SP68	**F**	1.304	0.087	0.043
SP69	**F**	1.214	0.143	0.071
SP70	**F**	0.000	0.000	0.000
SP71	**F**	0.676	0.147	0.029
SP73	**F**	0.643	0.107	0.000
SP74	**F**	0.419	0.000	0.032
SP75	**F**	0.000	0.130	0.000
SP76	**F**	0.537	0.268	0.000
SP77	**F**	0.000	0.000	0.565
Table 11 continued	**Sex**	**Tirticeae**	**Fabeae**	**Aveneae**

Sample Id				
SP78	**F**	0.000	0.720	0.040
SP79	**F**	0.444	0.000	0.000
SP80	**F**	1.000	0.522	0.217
SP81	**F**	0.000	0.565	0.000
SP82	**F**	0.036	0.536	0.000
SP83	**F**	0.000	0.667	0.000
SP84	**F**	0.600	0.000	0.000
SP85	**F**	0.844	0.656	0.031
SP86	**F**	1.167	0.833	0.000
SP87	**F**	0.565	0.000	0.565
SP88	**F**	0.000	0.000	1.600
SP89	**F**	1.524	1.095	0.571
SP90	**F**	0.043	1.174	0.000
SP10	**M**	1.265	0.412	0.000
SP11	**M**	0.219	0.781	0.000
SP13	**M**	0.719	0.000	0.000
SP14	**M**	0.034	1.172	0.000
SP15	**M**	0.037	0.000	0.000
SP16	**M**	0.000	0.964	0.000
SP18	**M**	0.379	0.931	0.000
SP19	**M**	0.000	0.520	0.000
SP2	**M**	0.000	0.100	0.000
SP20	**M**	0.462	0.000	0.000
SP21	**M**	0.438	0.375	0.000
SP22	**M**	0.571	0.000	0.000
SP23	**M**	1.032	0.581	0.000
SP24	**M**	0.970	0.727	0.000
SP25	**M**	0.429	0.893	0.000
SP26	**M**	0.407	0.074	0.000
SP27	**M**	0.000	0.652	0.000
SP28	**M**	0.148	0.148	0.407
SP29	**M**	0.088	0.000	0.000
SP3	**M**	1.483	0.517	0.000
SP31	**M**	0.103	0.655	0.000
SP32	**M**	0.217	0.000	0.000
SP33	**M**	0.000	1.067	0.000
Table 11 continued	**Sex**	**Tirticeae**	**Fabeae**	**Aveneae**

Sample Id				
SP34	**M**	0.261	0.000	0.000
SP35	**M**	0.143	0.000	0.000
SP36	**M**	0.852	0.444	0.000
SP37	**M**	0.885	0.000	0.000
SP38	**M**	0.000	0.920	0.080
SP39	**M**	0.000	0.667	0.000
SP4	**M**	0.036	0.000	0.786
SP40	**M**	0.786	0.571	0.107
SP5	**M**	0.033	0.433	0.000
SP6	**M**	0.000	0.353	0.000
SP7	**M**	1.231	0.038	0.000
SP8	**M**	0.000	0.250	0.000
SP9	**M**	1.000	0.000	0.000

Table 12. Distribution of Starch Granules from exotic plants

Type 1: Andropogoneae; type 2: Paniceae; type 3: Zingiberaceae, ginger type; type 4: Zingiberaceae, galangal type; type 5: cf. *Fagopyrum esculentum* L.

Sample Id	**SEX**	Type 1	Type 2	Type 3	Type 4	Type 5
EMP11	**F**	0.000	0.800	0.000	0.267	0.000
RT11	**F**	0.000	0.000	0.000	0.000	0.056
EMP5	**M**	0.000	0.000	0.000	0.000	0.143
SM1	**F**	0.000	0.000	0.069	0.092	0.000
SM21	**F**	0.000	0.000	0.286	0.381	0.000
SM26	**F**	0.000	0.000	0.379	0.506	0.000
SM29	**F**	0.000	0.000	0.071	0.095	0.000
SM30	**F**	0.000	0.000	0.034	0.046	0.000
SM35	**F**	0.000	0.000	0.148	0.198	0.000
SM37	**F**	0.000	0.000	0.000	0.000	0.467
SM39	**F**	0.000	0.000	0.852	1.136	0.000
SM5	**F**	0.000	0.000	0.400	0.533	0.000
SM49	**M**	0.000	0.000	0.414	0.552	0.000
SM51	**M**	0.000	0.000	0.227	0.303	0.000
SM59	**M**	0.000	0.000	0.304	0.406	0.000
SM68	**M**	0.000	0.000	0.273	0.364	0.000
SM69	**M**	0.000	0.000	0.000	0.000	0.171
SM73	**M**	0.000	0.000	0.050	0.067	0.525
SM76	**M**	0.500	0.000	0.056	0.074	0.000
SM78	**M**	0.000	0.000	0.205	0.274	0.000
SM81	**M**	0.000	0.000	0.273	0.364	0.000
SP57	**F**	0.000	0.000	0.167	0.222	0.000
SP63	**F**	0.000	0.000	0.000	0.333	0.000
SP66	**F**	0.000	0.000	0.000	0.000	0.037
SP67	**F**	0.000	0.000	0.043	0.058	0.000
SP70	**F**	0.000	0.182	0.000	0.061	0.000
SP71	**F**	0.000	0.000	0.000	0.000	0.206
SP76	**F**	0.000	0.000	0.171	0.228	0.000
SP79	**F**	0.000	0.000	0.185	0.247	0.000

Table 12 continued Sample Id	SEX	Type 1	Type 2	Type 3	Type 4	Type 5
SP87	**F**	0.000	0.000	0.304	0.406	0.000
SP89	**F**	0.000	0.000	0.095	0.127	0.000
SP90	**F**	0.000	0.000	0.043	0.058	0.000
SP10	**M**	0.000	0.000	0.088	0.118	0.000
SP11	**M**	0.656	0.000	0.000	0.438	0.000
SP14	**M**	0.000	0.000	0.103	0.138	0.000
SP16	**M**	0.000	0.000	0.179	0.238	0.000
SP18	**M**	0.000	0.000	0.310	0.414	0.621
SP22	**M**	0.000	0.000	0.214	0.286	0.000
SP25	**M**	0.000	0.000	0.071	0.095	0.000
SP26	**M**	0.000	0.000	0.185	0.247	0.000
SP3	**M**	0.000	0.586	0.000	0.195	0.000
SP30	**M**	0.000	0.000	0.419	0.559	0.000
SP33	**M**	0.000	0.000	0.167	0.222	0.000
SP35	**M**	0.000	0.000	0.250	0.333	0.000
SP5	**M**	0.000	0.000	0.000	0.000	0.100
SP7	**M**	0.000	0.000	0.192	0.256	0.000

Table 13. Distribution of Phytoliths

Type 1: unburnt phytoliths; type 2: burnt phytoliths; type 3: phytoliths consistent with those from the Cyperaceae Family; type 4: phytoliths consistent with those from the Cyperaceae Family, showing evidence of burning;

Sample Id	Sex	Type 1	Type 2	Type 3	Type 4
EMP11	**F**	0.467	0.133	0.067	0.067
EMP12	**F**	0.176	0.000	0.176	0.118
EMP13	**F**	0.133	0.067	0.800	0.000
EMP14	**F**	0.154	0.000	0.231	0.000
EMP15	**F**	0.111	0.000	0.000	0.056
EMP16	**F**	0.267	0.133	0.067	0.000
EMP17	**F**	0.267	0.000	0.133	0.000
EMP18	**F**	0.125	0.125	0.063	0.063
RT11	**F**	0.000	0.167	0.000	0.056
RT12	**F**	0.167	0.000	0.000	0.000
RT13	**F**	0.133	0.000	0.000	0.067
RT5	**F**	0.000	0.000	0.133	0.000
EMP10	**M**	0.105	0.000	0.421	0.000
EMP2	**M**	0.400	0.133	0.200	0.000
EMP3	**M**	0.000	0.083	0.000	0.000
EMP4	**M**	0.917	0.417	0.333	0.167
EMP5	**M**	0.357	0.000	0.000	0.000
EMP6	**M**	0.389	0.222	0.056	0.111
EMP7	**M**	0.143	0.048	0.095	0.000
EMP8	**M**	0.143	0.048	0.238	0.000
EMP9	**M**	0.294	0.235	0.000	0.059
RT2	**M**	0.200	0.067	0.067	0.133
RT3	**M**	0.100	0.000	0.000	0.000
RT4	**M**	0.188	0.000	0.125	0.000
RT6	**M**	0.286	0.071	0.000	0.000
SM1	**F**	0.103	0.000	0.138	0.000
SM10	**F**	0.036	0.000	0.000	0.000
SM11	**F**	0.074	0.000	0.000	0.000
SM14	**F**	0.481	0.037	0.074	0.000

Table 13 continued Sample Id	Sex	Type 1	Type 2	Type 3	Type 4
SM15	**F**	0.250	0.000	0.000	0.000
SM16	**F**	0.077	0.000	0.000	0.038
SM17	**F**	0.357	0.048	0.048	0.000
SM18	**F**	0.103	0.026	0.051	0.051
SM19	**F**	0.129	0.000	0.194	0.000
SM20	**F**	0.750	0.036	0.107	0.107
SM21	**F**	0.250	0.000	0.000	0.000
SM22	**F**	0.444	0.074	0.333	0.037
SM23	**F**	0.000	0.043	0.000	0.000
SM24	**F**	0.538	0.000	0.000	0.038
SM25	**F**	0.500	0.107	0.143	0.000
SM26	**F**	0.621	0.138	0.138	0.034
SM27	**F**	0.100	0.000	0.067	0.000
SM28	**F**	0.143	0.000	0.048	0.143
SM29	**F**	0.429	0.000	0.000	0.000
SM3	**F**	0.000	0.000	0.031	0.000
SM30	**F**	0.000	0.000	0.034	0.000
SM31	**F**	0.031	0.000	0.000	0.000
SM34	**F**	0.000	0.000	0.087	0.000
SM36	**F**	0.036	0.000	0.000	0.000
SM41	**F**	0.042	0.000	0.000	0.000
SM44	**F**	0.174	0.000	0.087	0.000
SM5	**F**	0.133	0.000	0.067	0.000
SM6	**F**	0.000	0.000	0.103	0.000
SM7	**F**	0.061	0.000	0.000	0.000
SM8	**F**	0.043	0.000	0.000	0.000
SM9	**F**	0.147	0.000	0.088	0.000
SM45	**M**	0.147	0.029	0.029	0.029
SM46	**M**	0.032	0.000	0.387	0.000
SM52	**M**	0.038	0.000	0.269	0.000
SM53	**M**	0.048	0.000	0.000	0.000
SM54	**M**	0.032	0.000	0.000	0.000
SM57	**M**	0.056	0.000	0.000	0.000
SM59	**M**	0.043	0.000	0.000	0.000

Table 13 continued Sample Id	Sex	Type 1	Type 2	Type 3	Type 4
SM60	**M**	0.034	0.000	0.000	0.000
SM63	**M**	0.038	0.000	0.077	0.000
SM68	**M**	0.273	0.000	0.000	0.000
SM69	**M**	0.086	0.000	0.029	0.000
SM70	**M**	0.158	0.026	0.000	0.000
SM71	**M**	0.024	0.000	0.024	0.000
SM73	**M**	0.075	0.050	0.000	0.000
SM74	**M**	0.091	0.000	0.091	0.000
SM75	**M**	0.107	0.000	0.000	0.036
SM76	**M**	0.111	0.000	0.083	0.000
SM78	**M**	0.103	0.000	0.051	0.000
SM79	**M**	0.167	0.000	0.056	0.000
SM80	**M**	0.029	0.000	0.000	0.000
SM81	**M**	0.182	0.000	0.045	0.000
SM82	**M**	0.000	0.133	0.000	0.000
SM83	**M**	0.222	0.000	0.000	0.056
SP41	**F**	0.115	0.000	0.000	0.000
SP42	**F**	0.080	0.000	0.000	0.000
SP45	**F**	0.029	0.000	0.000	0.000
SP47	**F**	0.034	0.000	0.000	0.000
SP49	**F**	0.031	0.000	0.031	0.000
SP50	**F**	0.000	0.000	0.030	0.000
SP51	**F**	0.029	0.000	0.000	0.000
SP52	**F**	0.063	0.000	0.000	0.000
SP54	**F**	0.370	0.074	0.000	0.000
SP55	**F**	0.071	0.000	0.000	0.000
SP58	**F**	0.061	0.000	0.000	0.000
SP59	**F**	0.054	0.000	0.000	0.000
SP61	**F**	0.037	0.000	0.000	0.000
SP64	**F**	0.211	0.026	0.000	0.026
SP65	**F**	0.294	0.000	0.088	0.088
SP66	**F**	0.778	0.037	0.000	0.000
SP67	**F**	0.130	0.000	0.478	0.217
SP68	**F**	0.522	0.043	0.043	0.043
SP69	**F**	0.250	0.036	0.214	0.000
SP70	**F**	0.364	0.000	0.091	0.030

Table 13 continued Sample Id	Sex	Type 1	Type 2	Type 3	Type 4
SP71	**F**	0.059	0.000	0.118	0.000
SP73	**F**	0.214	0.036	0.000	0.000
SP74	**F**	0.516	0.000	0.000	0.032
SP75	**F**	0.348	0.043	0.174	0.000
SP76	**F**	0.098	0.000	0.049	0.024
SP77	**F**	0.000	0.000	0.130	0.000
SP78	**F**	0.040	0.000	0.000	0.000
SP80	**F**	0.043	0.000	0.043	0.000
SP82	**F**	0.036	0.000	0.000	0.000
SP83	**F**	0.074	0.000	0.074	0.000
SP85	**F**	0.000	0.000	0.094	0.000
SP86	**F**	0.083	0.000	0.000	0.000
SP88	**F**	0.000	0.000	0.200	0.000
SP89	**F**	0.048	0.000	0.000	0.000
SP16	**M**	0.071	0.000	0.000	0.000
SP17	**M**	0.036	0.000	0.000	0.000
SP2	**M**	0.100	0.000	0.033	0.033
SP20	**M**	0.000	0.000	0.038	0.000
SP22	**M**	0.107	0.000	0.000	0.000
SP23	**M**	0.419	0.000	0.097	0.000
SP24	**M**	0.182	0.030	0.061	0.030
SP25	**M**	0.143	0.000	0.036	0.000
SP26	**M**	0.259	0.000	0.000	0.000
SP27	**M**	0.217	0.043	0.130	0.043
SP28	**M**	0.222	0.000	0.074	0.000
SP29	**M**	0.235	0.029	0.029	0.000
SP3	**M**	0.241	0.069	0.069	0.000
SP30	**M**	0.000	0.000	0.226	0.000
SP32	**M**	0.652	0.000	0.087	0.043
SP35	**M**	0.071	0.000	0.071	0.000
SP36	**M**	0.519	0.000	0.741	0.000
SP38	**M**	0.040	0.000	0.040	0.000
SP4	**M**	0.500	0.036	0.000	0.000
SP40	**M**	1.000	0.000	0.000	0.000

Table 13 continued Sample Id	Sex	Type 1	Type 2	Type 3	Type 4
SP5	**M**	0.200	0.000	0.367	0.000
SP7	**M**	0.077	0.000	0.000	0.000
SP9	**M**	0.043	0.000	0.000	0.000

Table 14. Distribution of Arboreal Pollen

Sample Id	Sex	*Alnus*	*Betula*	*Fraxinus*	*Populus*	*Quercus*
EMP11	**F**	0.0000	0.0000	0.0000	0.1333	0.0667
EMP12	**F**	0.0588	0.0588	0.1176	0.0000	0.1176
EMP13	**F**	0.0667	0.0000	0.0667	0.1333	0.2000
EMP14	**F**	0.0000	0.0769	0.0000	0.0000	0.0769
EMP15	**F**	0.0000	0.0556	0.1667	0.0000	0.0556
EMP16	**F**	0.0667	0.0000	0.0000	0.4000	0.0000
EMP17	**F**	0.0000	0.0667	0.2667	0.0000	0.3333
EMP18	**F**	0.0000	0.0000	0.0625	0.0000	0.0000
RT11	**F**	0.0556	0.0000	0.0000	0.0000	0.0556
RT12	**F**	0.0000	0.0000	0.0000	0.2778	0.0000
RT13	**F**	0.0000	0.0000	0.2000	0.0000	0.1333
RT5	**F**	0.0000	0.0000	0.1333	0.0000	0.0000
EMP10	**M**	0.0526	0.0000	0.1053	0.0000	0.0000
EMP2	**M**	0.0000	0.0667	0.0000	0.0667	0.0667
EMP3	**M**	0.0000	0.0000	0.0833	0.0000	0.0000
EMP4	**M**	0.2500	0.0833	0.0000	0.0000	0.0000
EMP5	**M**	0.0000	0.0000	0.0714	0.0714	0.1429
EMP6	**M**	0.0000	0.0000	0.0000	0.0000	0.0556
EMP7	**M**	0.1429	0.0000	0.0000	0.1429	0.1429
EMP8	**M**	0.0000	0.0476	0.0952	0.0000	0.0000
EMP9	**M**	0.0000	0.0000	0.0000	0.0588	0.2353
RT2	**M**	0.0000	0.0000	0.0667	0.0000	0.0000
RT3	**M**	0.0000	0.0000	0.0000	0.2000	0.1500
RT6	**M**	0.0000	0.1429	0.0000	0.0000	0.0714
SM14	**F**	0.0370	0.0000	0.0000	0.0000	0.0370
SM15	**F**	0.0000	0.0357	0.0000	0.0000	0.0000
SM18	**F**	0.0256	0.0000	0.0000	0.0000	0.0000
SM19	**F**	0.0000	0.0323	0.0000	0.0000	0.0323
SM22	**F**	0.0370	0.0000	0.0370	0.0000	0.0370
SM24	**F**	0.0769	0.0385	0.0000	0.0000	0.0000
SM25	**F**	0.0000	0.0357	0.0000	0.0000	0.0000
SM26	**F**	0.0000	0.0000	0.0000	0.0000	0.0345
SM27	**F**	0.0000	0.0000	0.0000	0.0000	0.0000
SM29	**F**	0.0000	0.0357	0.0000	0.0000	0.0357
SM30	**F**	0.0000	0.0000	0.0000	0.0000	0.0345

Table 14 continued Sample Id	Sex	*Alnus*	*Betula*	*Fraxinus*	*Populus*	*Quercus*
SM33	**F**	0.0000	0.0000	0.0333	0.0000	0.0000
SM34	**F**	0.0000	0.0435	0.0000	0.0000	0.0000
SM36	**F**	0.0000	0.0000	0.0000	0.0000	0.0357
SM38	**F**	0.0000	0.0714	0.0000	0.0000	0.0000
SM41	**F**	0.0000	0.0000	0.0000	0.0417	0.0000
SM44	**F**	0.0435	0.0000	0.0000	0.0000	0.0000
SM45	**M**	0.0000	0.0294	0.0000	0.0000	0.0000
SM51	**M**	0.0000	0.1364	0.0000	0.0000	0.0000
SM54	**M**	0.0000	0.0323	0.0000	0.0000	0.0000
SM57	**M**	0.0000	0.0000	0.0556	0.0000	0.0556
SM69	**M**	0.0000	0.0286	0.0000	0.0000	0.0000
SM7	**F**	0.0000	0.0000	0.0000	0.0000	0.0303
SM71	**M**	0.0238	0.0000	0.0000	0.0000	0.0000
SM73	**M**	0.0000	0.0250	0.0250	0.0000	0.0000
SM74	**M**	0.0000	0.0000	0.0000	0.0000	0.0455
SM75	**M**	0.0000	0.0000	0.0000	0.0000	0.0000
SM76	**M**	0.0278	0.0000	0.0000	0.0000	0.0000
SM77	**M**	0.0000	0.0000	0.0000	0.0000	0.0263
SM78	**M**	0.0000	0.0000	0.0256	0.0000	0.0000
SM79	**M**	0.0000	0.0556	0.0000	0.0556	0.0000
SM80	**M**	0.0286	0.0000	0.0000	0.0000	0.0000
SM82	**M**	0.0000	0.0667	0.0667	0.0000	0.0667
SP48	**F**	0.0000	0.0000	0.0000	0.0294	0.0000
SP52	**F**	0.0000	0.0000	0.0313	0.0000	0.0000
SP56	**F**	0.0000	0.0000	0.0345	0.0000	0.0000
SP64	**F**	0.0000	0.0263	0.0000	0.0000	0.0000
SP66	**F**	0.0370	0.0000	0.0000	0.0000	0.0370
SP67	**F**	0.0000	0.0435	0.0000	0.0000	0.0000
SP68	**F**	0.0000	0.0000	0.0000	0.0435	0.0000
SP69	**F**	0.0357	0.0000	0.0000	0.0000	0.0357
SP70	**F**	0.0000	0.0303	0.0303	0.0000	0.0000
SP73	**F**	0.0357	0.0000	0.0000	0.0000	0.0000
SP74	**F**	0.0000	0.0323	0.0000	0.0000	0.0323
SP83	**F**	0.0000	0.0000	0.0000	0.0370	0.0000
SP89	**F**	0.0000	0.0000	0.0000	0.0952	0.0000
SP11	**M**	0.0000	0.0000	0.0313	0.0000	0.0000

Table 14 continued Sample Id	Sex	*Alnus*	*Betula*	*Fraxinus*	*Populus*	*Quercus*
SP12	**M**	0.0000	0.0000	0.0000	0.0000	0.0370
SP13	**M**	0.0000	0.0000	0.0000	0.0313	0.0000
SP15	**M**	0.0000	0.0000	0.0000	0.0000	0.0370
SP20	**M**	0.0000	0.0000	0.0385	0.0000	0.0000
SP23	**M**	0.0323	0.0000	0.0000	0.0000	0.0323
SP24	**M**	0.0000	0.0000	0.0000	0.0000	0.0303
SP25	**M**	0.0000	0.0357	0.0357	0.0000	0.0357
SP26	**M**	0.0000	0.0370	0.0000	0.0000	0.0370
SP27	**M**	0.0000	0.0435	0.0000	0.0000	0.0000
SP28	**M**	0.0370	0.0000	0.0370	0.0370	0.0000
SP29	**M**	0.0000	0.0294	0.0000	0.0000	0.0294
SP3	**M**	0.0345	0.0345	0.0000	0.0000	0.0345
SP30	**M**	0.0000	0.0000	0.0000	0.0000	0.0323
SP32	**M**	0.0000	0.0435	0.0000	0.0000	0.0000
SP4	**M**	0.0000	0.0000	0.0357	0.0000	0.0000
SP5	**M**	0.0000	0.0333	0.0000	0.0000	0.0333
SP7	**M**	0.0000	0.0000	0.0000	0.0385	0.0000

Table 15. Distribution of Non-Arboreal Pollen A-B

Sample Id	Sex	*Aretmsia* type	Asteraceae	*Rosaceae* type	*Borago-Anchusa*
EMP11	**F**	0.0000	0.2308	0.0000	0.0000
EMP12	**F**	0.0000	0.0000	0.0833	0.0000
EMP13	**F**	0.0000	0.1667	0.0833	0.0000
EMP14	**F**	0.0000	0.0000	0.4286	0.0000
EMP15	**F**	0.0000	0.0500	0.0000	0.0000
EMP16	**F**	0.0000	0.0667	0.0667	0.0000
EMP17	**F**	0.0833	0.1667	0.0000	0.0000
EMP18	**F**	0.0357	0.0000	0.0000	0.0000
RT12	**F**	0.0000	0.0000	0.0952	0.0000
RT13	**F**	0.0000	0.0667	0.1333	0.0667
RT5	**F**	0.0000	0.0000	0.0741	0.0000
EMP10	**M**	0.0000	0.0667	0.0000	0.0000
EMP2	**M**	0.0000	0.1429	0.0714	0.0000
EMP3	**M**	0.0000	0.0000	0.0526	0.0000
EMP4	**M**	0.0000	0.1176	0.0000	0.0000
EMP5	**M**	0.0625	0.1250	0.0625	0.0000
EMP6	**M**	0.0000	0.0476	0.0000	0.0000
EMP7	**M**	0.0000	0.0000	0.0667	0.0000
EMP8	**M**	0.0000	0.0000	0.0435	0.0000
RT2	**M**	0.0000	0.0370	0.0000	0.0000
RT3	**M**	0.0000	0.0667	0.0000	0.0000
RT6	**M**	0.0000	0.0000	0.0000	0.0370
SM12	**F**	0.0476	0.0000	0.0000	0.0000
SM15	**F**	0.0238	0.0000	0.0476	0.0000
SM18	**F**	0.0000	0.0000	0.0333	0.0000
SM19	**F**	0.0000	0.0357	0.0357	0.0000
SM21	**F**	0.0000	0.0000	0.0667	0.0000
SM24	**F**	0.0000	0.2609	0.0000	0.0000
SM26	**F**	0.0000	0.0000	0.0690	0.0000
SM27	**F**	0.0000	0.0256	0.0256	0.0000
SM29	**F**	0.0000	0.0000	0.0968	0.0000
SM33	**F**	0.0000	0.0000	0.0345	0.0000
SM35	**F**	0.0000	0.0000	0.0588	0.0000
SM41	**F**	0.0000	0.0000	0.0435	0.0000
SM44	**F**	0.0000	0.0000	0.1071	0.0000

Table 15 continued Sample Id	Sex	*Aretmsia* type	Asteraceae	*Rosaceae* type	*Borago-Anchusa*
SM54	**M**	0.0000	0.0333	0.0000	0.0000
SM57	**M**	0.0000	0.0000	0.0625	0.0000
SM59	**M**	0.0000	0.0000	0.0556	0.0000
SM61	**M**	0.0000	0.0000	0.0435	0.0000
SM74	**M**	0.0000	0.0000	0.0370	0.1111
SM75	**M**	0.0000	0.0000	0.0000	0.1154
SM77	**M**	0.0800	0.0000	0.0000	0.0000
SM79	**M**	0.0000	0.2500	0.0000	0.0000
SM82	**M**	0.1333	0.0000	0.0667	0.0000
SM83	**M**	0.0000	0.0000	0.0417	0.0000
SP52	**F**	0.0000	0.0435	0.0000	0.0000
SP56	**F**	0.0000	0.0000	0.0263	0.0000
SP60	**F**	0.0000	0.0000	0.0263	0.0000
SP66	**F**	0.0000	0.0000	0.0385	0.0000
SP68	**F**	0.0000	0.0000	0.0476	0.0000
SP70	**F**	0.0000	0.0000	0.0000	0.0294
SP73	**F**	0.0000	0.0000	0.0357	0.0000
SP74	**F**	0.0000	0.0000	0.0714	0.0000
SP76	**F**	0.0000	0.0333	0.0000	0.0000
SP80	**F**	0.0000	0.0000	0.0541	0.0000
SP84	**F**	0.0000	0.0000	0.0286	0.0000
SP87	**F**	0.0357	0.0000	0.0000	0.0000
SP13	**M**	0.0000	0.0000	0.0800	0.0000
SP2	**M**	0.0000	0.0000	0.0435	0.0000
SP23	**M**	0.0000	0.0667	0.0000	0.0000
SP25	**M**	0.0000	0.0000	0.0556	0.0000
SP3	**M**	0.0000	0.0976	0.0000	0.0000
SP30	**M**	0.0000	0.0000	0.0323	0.0000
SP32	**M**	0.0000	0.0000	0.0370	0.0000
SP37	**M**	0.0000	0.0455	0.0000	0.0000
SP4	**M**	0.0000	0.0000	0.0294	0.0294
SP7	**M**	0.0000	0.0000	0.0870	0.0000

Table 16. Non-arboreal pollen C-P

Type 1: Chenopodiaceae; type 2: *Linum* sp. type; type 3: *Centaurea* type; type 4: Corylaceae type; type 5: Polygonaceae

Sample Id	Sex	Type 1	Type 2	Type 3	Type 4	Type 5
EMP12	**F**	0.0000	0.0833	0.0000	0.0833	0.0000
EMP13	**F**	0.0000	0.0000	0.0000	0.0833	0.0833
EMP14	**F**	0.0357	0.0000	0.0000	0.0357	0.0000
EMP15	**F**	0.0000	0.0000	0.0000	0.0000	0.1500
EMP16	**F**	0.0667	0.0000	0.0000	0.0000	0.1333
EMP17	**F**	0.1667	0.0000	0.0000	0.2500	0.1667
EMP18	**F**	0.0000	0.0000	0.0000	0.0000	0.0357
RT12	**F**	0.0000	0.0476	0.0000	0.0000	0.0000
RT13	**F**	0.0000	0.0000	0.0000	0.0667	0.0000
RT5	**F**	0.0000	0.0000	0.0000	0.1852	0.0370
EMP10	**M**	0.0000	0.0000	0.0000	0.0667	0.1333
EMP3	**M**	0.0526	0.0000	0.0000	0.1579	0.1053
EMP4	**M**	0.0000	0.0000	0.0000	0.1176	0.0000
EMP5	**M**	0.0000	0.0000	0.0000	0.0000	0.0625
EMP6	**M**	0.0000	0.0000	0.0000	0.0952	0.0476
EMP7	**M**	0.0000	0.0000	0.0000	0.0667	0.2000
EMP8	**M**	0.0000	0.0000	0.0000	0.1739	0.0000
EMP9	**M**	0.0000	0.0000	0.0000	0.0714	0.2143
RT2	**M**	0.0370	0.0000	0.0000	0.0370	0.0000
RT6	**M**	0.0000	0.0000	0.0370	0.1111	0.1111
SM14	**F**	0.0313	0.0313	0.0313	0.0313	0.0000
SM15	**F**	0.0000	0.0000	0.0000	0.0238	0.0000
SM17	**F**	0.0000	0.0000	0.0000	0.0238	0.0000
SM18	**F**	0.0000	0.0000	0.0000	0.0000	0.0333
SM19	**F**	0.0000	0.0000	0.0000	0.0000	0.0357
SM20	**F**	0.0000	0.0000	0.0000	0.0323	0.0000
SM24	**F**	0.0000	0.0000	0.0000	0.0000	0.0435
SM25	**F**	0.0000	0.0000	0.0741	0.0000	0.0000
SM27	**F**	0.0256	0.0000	0.0000	0.0000	0.0000
SM28	**F**	0.0000	0.0000	0.0000	0.0385	0.0000
SM29	**F**	0.0000	0.0000	0.0000	0.0323	0.0000
SM30	**F**	0.0000	0.0000	0.0000	0.0357	0.0000

Table 16 continued Sample Id	Sex	Type 1	Type 2	Type 3	Type 4	Type 5
SM31	**F**	0.0000	0.0000	0.0000	0.0000	0.0294
SM33	**F**	0.0345	0.0000	0.0000	0.0000	0.0000
SM34	**F**	0.0000	0.0000	0.0000	0.0345	0.0000
SM41	**F**	0.0000	0.0000	0.0000	0.0435	0.0435
SM44	**F**	0.0000	0.0000	0.0000	0.0357	0.0000
SM5	**F**	0.0000	0.0000	0.0435	0.0000	0.0000
SM7	**F**	0.0000	0.0000	0.0000	0.0000	0.0250
SM50	**M**	0.0000	0.0000	0.0000	0.0357	0.0000
SM51	**M**	0.0000	0.0000	0.0000	0.1111	0.0000
SM55	**M**	0.0000	0.0000	0.0000	0.0313	0.0000
SM57	**M**	0.0000	0.0000	0.0000	0.0625	0.1250
SM65	**M**	0.0000	0.0000	0.0000	0.0000	0.0333
SM66	**M**	0.0000	0.0000	0.0000	0.0625	0.0000
SM70	**M**	0.0000	0.0000	0.0000	0.0400	0.0000
SM71	**M**	0.0000	0.0000	0.0000	0.0000	0.0333
SM73	**M**	0.0000	0.0000	0.0769	0.0000	0.0769
SM76	**M**	0.0000	0.0000	0.0000	0.0714	0.0000
SM77	**M**	0.0000	0.0000	0.0000	0.0400	0.0400
SM78	**M**	0.0000	0.0000	0.0000	0.0690	0.0000
SM80	**M**	0.0000	0.0000	0.0000	0.0000	0.0357
SP41	**F**	0.0000	0.0000	0.0000	0.0345	0.0000
SP45	**F**	0.0000	0.0000	0.0000	0.0286	0.0000
SP48	**F**	0.0000	0.0000	0.0000	0.0357	0.0000
SP52	**F**	0.0000	0.0000	0.0000	0.0435	0.0000
SP54	**F**	0.0000	0.0000	0.0000	0.0286	0.0000
SP56	**F**	0.0000	0.0263	0.0000	0.0000	0.0000
SP60	**F**	0.0000	0.0000	0.0000	0.0000	0.0263
SP64	**F**	0.0000	0.0000	0.0000	0.0345	0.0345
SP66	**F**	0.0385	0.0000	0.0000	0.0385	0.0000
SP67	**F**	0.0000	0.0000	0.0000	0.0263	0.0263
SP69	**F**	0.0000	0.0000	0.0323	0.0323	0.0000
SP70	**F**	0.0000	0.0000	0.0000	0.0294	0.0588
SP74	**F**	0.0000	0.0000	0.0000	0.0000	0.0357
SP75	**F**	0.0000	0.0000	0.0000	0.0000	0.0256
SP76	**F**	0.0000	0.0000	0.0000	0.0000	0.0333
SP83	**F**	0.0435	0.0435	0.0000	0.0000	0.0000

Table 16 continued Sample Id	Sex	Type 1	Type 2	Type 3	Type 4	Type 5
SP85	**F**	0.0294	0.0000	0.0000	0.0000	0.0000
SP89	**F**	0.0000	0.0000	0.0833	0.0000	0.0833
SP10	**M**	0.0000	0.0000	0.0000	0.0000	0.0417
SP11	**M**	0.0000	0.0000	0.0000	0.0435	0.0000
SP12	**M**	0.0000	0.0323	0.0000	0.0000	0.0000
SP15	**M**	0.0000	0.0000	0.0435	0.0000	0.0435
SP16	**M**	0.0000	0.0000	0.0000	0.0000	0.0278
SP17	**M**	0.0000	0.0000	0.0000	0.0000	0.0455
SP20	**M**	0.0000	0.0000	0.0000	0.1304	0.0000
SP22	**M**	0.0000	0.0000	0.0000	0.0323	0.0000
SP23	**M**	0.0000	0.0000	0.0000	0.0667	0.1333
SP24	**M**	0.0000	0.0000	0.0000	0.0769	0.0000
SP25	**M**	0.0000	0.0000	0.0000	0.0556	0.0556
SP26	**M**	0.0000	0.0000	0.0000	0.0556	0.0000
SP27	**M**	0.0000	0.0000	0.0000	0.0370	0.0000
SP28	**M**	0.0000	0.0000	0.0000	0.0667	0.0000
SP29	**M**	0.0556	0.0000	0.0000	0.0000	0.0556
SP3	**M**	0.0000	0.0732	0.0000	0.0244	0.0000
SP30	**M**	0.0000	0.0000	0.0000	0.0323	0.0000
SP32	**M**	0.0000	0.0000	0.0000	0.1111	0.0000
SP34	**M**	0.0000	0.0000	0.0000	0.0455	0.0000
SP35	**M**	0.0000	0.0000	0.0000	0.0000	0.0313
SP4	**M**	0.0294	0.0000	0.0294	0.0294	0.0294
SP5	**M**	0.0000	0.0000	0.0000	0.0455	0.0000
SP7	**M**	0.0000	0.0000	0.0000	0.0435	0.0000

Table 17. Distribution of cereal bran across the populations

Sample Id	Sex	Tirticeae	Cereal bran unid.
EMP13	**F**	0.2000	0.3333
EMP14	**F**	0.0000	0.3846
EMP17	**F**	0.0000	0.2000
RT12	**F**	0.0000	0.0556
RT5	**F**	0.0000	0.2000
EMP10	**M**	0.0000	0.2105
EMP2	**M**	0.0000	0.3333
EMP5	**M**	0.0000	0.4286
EMP6	**M**	0.1111	0.0000
EMP8	**M**	0.0000	0.1905
SM20	**F**	0.0714	0.0000
SM26	**F**	0.0000	0.1034
SM31	**F**	0.0938	0.0000
SM33	**F**	0.0000	0.1000
SM36	**F**	0.0000	0.0714
SM39	**F**	0.1852	0.0000
SM43	**F**	0.0000	0.0370
SP60	**F**	0.0870	0.0000
SP66	**F**	0.1852	0.0000
SP73	**F**	0.2143	0.0000
SP75	**F**	0.0870	0.0000
SM55	**M**	0.0667	0.0000
SM61	**M**	0.0000	0.0667
SM68	**M**	0.0000	0.0455
SM70	**M**	0.0526	0.0000
SM77	**M**	0.0000	0.0789
SM82	**M**	0.0000	0.1333
SP18	**M**	0.1034	0.0000
SP36	**M**	0.0741	0.0000
SP4	**M**	0.1071	0.0000

Table 18. Distribution of Plant Tissues A-C

Sample Id	Sex	*Agrostemma githago*	*Allium cf. porrum*	*Cannabis sativa*
EMP11	**F**	0.067	0.067	0.067
EMP14	**F**	0.000	0.077	0.077
EMP15	**F**	0.056	0.000	0.111
EMP16	**F**	0.000	0.067	0.000
EMP18	**F**	0.063	0.063	0.000
RT11	**F**	0.056	0.000	0.056
RT12	**F**	0.000	0.167	0.000
RT13	**F**	0.000	0.000	0.067
EMP10	**M**	0.000	0.105	0.053
EMP4	**M**	0.083	0.083	0.250
EMP5	**M**	0.071	0.000	0.000
EMP6	**M**	0.000	0.000	0.111
EMP7	**M**	0.048	0.095	0.000
EMP8	**M**	0.000	0.143	0.238
EMP9	**M**	0.059	0.000	0.000
RT2	**M**	0.000	0.133	0.000
RT4	**M**	0.000	0.125	0.000
RT6	**M**	0.071	0.143	0.000
SM11	**F**	0.037	0.000	0.000
SM15	**F**	0.036	0.000	0.000
SM18	**F**	0.051	0.000	0.000
SM2	**F**	0.000	0.063	0.000
SM21	**F**	0.036	0.000	0.000
SM22	**F**	0.037	0.000	0.000
SM24	**F**	0.038	0.000	0.000
SM27	**F**	0.000	0.000	0.033
SM28	**F**	0.048	0.000	0.000
SM29	**F**	0.000	0.000	0.393
SM3	**F**	0.031	0.000	0.000
SM31	**F**	0.031	0.000	0.000
SM33	**F**	0.067	0.000	0.000
SM36	**F**	0.143	0.000	0.000
SM38	**F**	0.071	0.000	0.000
SM40	**F**	0.308	0.000	0.000
SM42	**F**	0.120	0.080	0.000

Table 18 continued Sample Id	**Sex**	*Agrostemma githago*	*Allium cf. porrum*	*Cannabis sativa*
SM6	**F**	0.034	0.000	0.000
SM45	**M**	0.206	0.000	0.000
SM48	**M**	0.192	0.000	0.000
SM50	**M**	0.167	0.000	0.000
SM53	**M**	0.095	0.000	0.000
SM54	**M**	0.000	0.032	0.000
SM55	**M**	0.067	0.000	0.000
SM57	**M**	0.056	0.000	0.000
SM59	**M**	0.043	0.000	0.000
SM61	**M**	0.033	0.000	0.000
SM62	**M**	0.000	0.080	0.000
SM64	**M**	0.053	0.000	0.000
SM67	**M**	0.091	0.000	0.000
SM69	**M**	0.000	0.000	0.029
SM70	**M**	0.079	0.000	0.000
SM74	**M**	0.091	0.045	0.000
SM78	**M**	0.026	0.000	0.000
SM80	**M**	0.029	0.000	0.000
SM82	**M**	0.067	0.000	0.000
SP47	**F**	0.034	0.000	0.000
SP48	**F**	0.029	0.059	0.000
SP51	**F**	0.086	0.000	0.000
SP53	**F**	0.000	0.038	0.000
SP54	**F**	0.185	0.000	0.000
SP56	**F**	0.034	0.000	0.000
SP57	**F**	0.000	0.067	0.000
SP59	**F**	0.027	0.000	0.000
SP62	**F**	0.034	0.000	0.000
SP64	**F**	0.026	0.000	0.000
SP65	**F**	0.000	0.029	0.000
SP67	**F**	0.043	0.000	0.000
SP68	**F**	0.043	0.000	0.000
SP69	**F**	0.000	0.000	0.036
SP70	**F**	0.030	0.000	0.000
SP73	**F**	0.036	0.000	0.000
SP74	**F**	0.355	0.000	0.000

Table 18 continued Sample Id	**Sex**	*Agrostemma githago*	*Allium cf. porrum*	*Cannabis sativa*
SP75	**F**	0.087	0.000	0.000
SP76	**F**	0.098	0.000	0.049
SP78	**F**	0.320	0.000	0.000
SP81	**F**	0.304	0.000	0.000
SP83	**F**	0.074	0.000	0.000
SP84	**F**	0.057	0.000	0.000
SP86	**F**	0.083	0.000	0.000
SP90	**F**	0.174	0.000	0.000
SP12	**M**	0.037	0.000	0.000
SP15	**M**	0.037	0.000	0.000
SP17	**M**	0.036	0.000	0.000
SP19	**M**	0.040	0.000	0.000
SP23	**M**	0.032	0.000	0.065
SP24	**M**	0.030	0.000	0.000
SP25	**M**	0.071	0.071	0.000
SP27	**M**	0.174	0.000	0.043
SP28	**M**	0.037	0.000	0.037
SP29	**M**	0.059	0.000	0.029
SP3	**M**	0.069	0.000	0.034
SP30	**M**	0.065	0.000	0.000
SP32	**M**	0.043	0.000	0.043
SP33	**M**	0.033	0.100	0.000
SP36	**M**	0.037	0.000	0.000
SP4	**M**	0.000	0.000	0.036
SP5	**M**	0.033	0.000	0.000
SP9	**M**	0.043	0.000	0.000

Table 19. Results Plant Tissues 2

Type 1: *Linum usitatissimum*; Type 2: *Papaver* sp.; Type 3: *Silene* sp.; Type 4: *Trigonella foenum-graecum*

Sk	**Sex**	Type 1	Type 2	Type 3	Type 4
EMP12	**F**	0.000	0.000	0.059	0.000
EMP13	**F**	0.067	0.000	0.000	0.000
EMP16	**F**	0.000	0.000	0.067	0.000
EMP2	**M**	0.000	0.000	0.133	0.000
EMP4	**M**	0.083	0.000	0.333	0.000
EMP6	**M**	0.000	0.000	0.111	0.000
EMP9	**M**	0.000	0.000	0.059	0.000
RT4	**M**	0.000	0.000	0.188	0.000
SM17	**F**	1.000	0.000	0.000	0.000
SM18	**F**	0.000	0.026	0.000	0.000
SM25	**F**	0.000	0.000	0.036	0.000
SM45	**M**	0.000	0.029	0.000	0.000
SM77	**M**	0.000	0.000	0.026	0.000
SP61	**F**	0.148	0.000	0.000	0.000
SP68	**F**	0.000	0.000	0.043	0.000
SP69	**F**	0.036	0.036	0.000	0.000
SP24	**M**	0.000	0.000	0.000	0.364
SP29	**M**	0.029	0.029	0.000	0.000
SP3	**M**	0.000	0.000	0.034	0.000

Table 20. Distribution of Plant Fibers

Sample Id	**Sex**	Bast fibers *Linum/Cannabis*	cf. *Linum usitatissimum*	Undiagnostic fibers
EMP11	**F**	0.0667	0.0000	0.0000
EMP14	**F**	0.0000	0.0000	0.1538
EMP16	**F**	0.0667	0.0000	0.2000
EMP18	**F**	0.0625	0.0000	0.1875
RT11	**F**	0.0000	0.0000	0.3333
RT12	**F**	0.1111	0.0000	0.3333
RT13	**F**	0.0000	0.0000	0.4667
RT5	**F**	0.0000	0.0000	0.3333
EMP4	**M**	0.0833	0.0000	0.0000
EMP5	**M**	0.1429	0.0000	0.0000
EMP6	**M**	0.0556	0.0556	0.0000
EMP9	**M**	0.0588	0.0000	0.0000
RT2	**M**	0.0000	0.0000	0.2667
RT3	**M**	0.0500	0.0000	0.0000
RT6	**M**	0.0714	0.0000	0.0000
SM11	**F**	0.0000	0.0000	0.1111
SM14	**F**	0.0370	0.0000	0.1481
SM15	**F**	0.0000	0.0000	0.0357
SM17	**F**	0.3333	0.0000	0.0238
SM18	**F**	0.0000	0.0000	0.0769
SM19	**F**	0.0645	0.0000	0.0323
SM2	**F**	0.0000	0.0000	0.0938
SM21	**F**	0.0357	0.0000	0.0000
SM22	**F**	0.0000	0.0000	0.1111
SM24	**F**	0.0000	0.0000	0.0769
SM25	**F**	0.1071	0.0000	0.1071
SM26	**F**	0.0000	0.0000	0.1034
SM27	**F**	0.0000	0.0000	0.0333
SM28	**F**	0.0952	0.0000	0.1905
SM29	**F**	0.8214	0.0000	0.0357
SM34	**F**	0.0000	0.0000	0.1304
SM37	**F**	0.0000	0.0000	0.1333
SM42	**F**	0.0000	0.0000	0.1600
SM5	**F**	0.0000	0.0000	0.1333

Table 20 continued Sample Id	**Sex**	Bast fibers *Linum/Cannabis*	cf. *Linum usitatissimum*	Undiagnostic fibers
SM8	**F**	0.0000	0.0000	0.2174
SM45	**M**	0.0294	0.0000	0.0882
SM48	**M**	0.0000	0.0000	0.1538
SM50	**M**	0.0000	0.0000	0.1667
SM53	**M**	0.0000	0.0000	0.1905
SM55	**M**	0.0000	0.0000	0.1000
SM58	**M**	0.0000	0.0000	0.1111
SM61	**M**	0.0000	0.0000	0.1333
SM64	**M**	0.0000	0.0000	0.1579
SM65	**M**	0.0000	0.0000	0.1538
SM68	**M**	0.0000	0.0000	0.1364
SM71	**M**	0.0238	0.0000	0.0952
SM74	**M**	0.0000	0.0000	0.0455
SM75	**M**	0.0357	0.0357	0.0000
SM76	**M**	0.0000	0.0000	0.0278
SM78	**M**	0.0256	0.0000	0.0256
SM83	**M**	0.0000	0.0000	0.0556
SP41	**F**	0.0000	0.0000	0.1154
SP43	**F**	0.0000	0.0000	0.0625
SP44	**F**	0.0000	0.0000	0.0645
SP45	**F**	0.0000	0.0000	0.0571
SP46	**F**	0.0000	0.0000	0.0741
SP47	**F**	0.0345	0.0000	0.0690
SP49	**F**	0.0000	0.5625	0.0313
SP50	**F**	0.0000	0.0000	0.0606
SP51	**F**	1.9143	0.0000	0.0000
SP52	**F**	0.0000	0.0000	0.0313
SP53	**F**	0.0385	0.0000	0.0000
SP57	**F**	0.0333	0.0000	0.0000
SP60	**F**	0.0870	0.0000	0.0000
SP63	**F**	0.0833	0.0000	0.0000
SP64	**F**	0.0526	0.0000	0.0000
SP66	**F**	0.1111	0.0370	0.0741
SP67	**F**	0.0870	0.0000	0.1304
SP68	**F**	0.0000	0.0000	0.2174
SP69	**F**	0.0357	0.0000	0.2143

Table 20 continued Sample Id	**Sex**	Bast fibers *Linum/Cannabis*	cf. *Linum usitatissimum*	Undiagnostic fibers
SP70	**F**	0.0000	0.0000	0.2121
SP71	**F**	0.0000	0.0000	0.0294
SP73	**F**	0.0000	0.0000	0.0714
SP74	**F**	0.0323	0.0000	0.0968
SP75	**F**	0.0000	0.0000	0.2174
SP76	**F**	1.8293	0.0000	0.0000
SP79	**F**	0.0000	0.0000	0.1481
SP81	**F**	0.0000	0.0000	0.2609
SP82	**F**	0.0000	0.0000	0.2500
SP84	**F**	0.0000	0.0000	0.2286
SP87	**F**	0.0000	0.0000	0.3913
SP90	**F**	0.0000	0.0000	0.0870
SP11	**M**	0.0000	0.0000	0.0625
SP13	**M**	0.0000	0.0000	0.0938
SP16	**M**	0.0000	0.0000	0.1071
SP2	**M**	0.0000	0.0333	0.0000
SP20	**M**	0.0000	0.0000	0.0769
SP21	**M**	0.0000	0.0000	0.0938
SP23	**M**	0.0000	0.0323	0.0000
SP24	**M**	0.0000	0.0000	0.0606
SP25	**M**	0.0714	0.0000	0.0357
SP26	**M**	0.0000	0.0000	0.0741
SP27	**M**	0.0000	0.0000	0.0435
SP28	**M**	0.0000	0.0370	0.0000
SP29	**M**	0.0294	0.0000	0.0294
SP3	**M**	0.1034	0.0000	0.0345
SP30	**M**	0.0000	0.3871	0.3871
SP31	**M**	0.0000	0.0000	0.0690
SP32	**M**	0.0000	0.0000	0.0435
SP33	**M**	0.0000	0.0000	0.0667
SP34	**M**	0.0000	0.0000	0.0870
SP35	**M**	0.0000	0.0000	0.0357
SP36	**M**	0.0000	0.0000	0.1481
SP38	**M**	0.0000	0.0000	0.1600
SP39	**M**	0.0000	0.0000	0.1111
SP4	**M**	0.0000	0.0000	0.1429

Table 20 continued Sample Id	**Sex**	Bast fibers *Linum/Cannabis*	cf. *Linum usitatissimum*	Undiagnostic fibers
SP5	**M**	0.0333	0.0000	0.0333
SP9	**M**	0.0000	0.0000	0.0435

Table 21. Distribution of Wood debris

Sample id	**Sex**	Conifer wood	Wood	Wood elements
EMP10	**M**	0.053	0.000	0.000
SM55	**M**	1.133	0.400	2.500
SP63	**F**	0.028	0.000	0.000
SP18	**M**	0.483	1.931	1.103
SP23	**M**	0.000	0.000	0.032
SP24	**M**	0.333	0.000	0.000
SP25	**M**	0.036	0.000	0.000
SP27	**M**	0.000	0.000	0.043
SP28	**M**	0.037	0.000	0.000
SP29	**M**	0.000	0.000	0.029
SP4	**M**	0.036	0.000	0.036
SP1	**USC**	0.000	0.000	0.031

Table 22. Distribution of charcoal and soot

Sample id	**Sex**	Ash-soot	Fine Particles	Large particles
EMP11	**F**	0.067	0.200	0.733
EMP12	**F**	0.059	0.235	0.765
EMP13	**F**	0.067	0.133	0.333
EMP14	**F**	0.077	1.000	0.231
EMP15	**F**	0.056	0.056	0.056
EMP16	**F**	0.067	0.067	0.733
EMP17	**F**	0.067	0.133	0.733
EMP18	**F**	0.063	0.688	0.313
RT11	**F**	0.056	0.056	0.056
RT12	**F**	0.056	0.722	0.167
RT13	**F**	0.067	0.267	0.267
RT5	**F**	0.067	0.000	0.000
EMP10	**M**	0.053	0.158	0.158
EMP2	**M**	0.067	0.067	0.067
EMP3	**M**	0.083	0.083	0.250
EMP4	**M**	0.083	0.833	0.250
EMP5	**M**	0.071	0.500	0.929
EMP6	**M**	0.056	0.167	0.667
EMP7	**M**	0.048	0.238	0.524
EMP8	**M**	0.048	0.095	0.333
EMP9	**M**	0.059	0.059	0.176
RT2	**M**	0.067	0.133	0.467
RT3	**M**	0.050	0.050	0.150
RT4	**M**	0.063	0.188	0.313
RT6	**M**	0.071	0.786	0.071
SM1	**F**	0.034	0.000	0.000
SM10	**F**	0.036	0.214	0.107
SM11	**F**	0.037	0.222	0.222
SM12	**F**	0.036	0.036	0.000
SM14	**F**	0.037	0.074	0.037
SM15	**F**	0.036	0.036	0.000
SM16	**F**	0.038	0.038	0.038
SM17	**F**	0.024	0.024	0.071
SM18	**F**	0.026	0.051	0.077
SM19	**F**	0.032	0.484	0.161

Table 22 continued Sample id	Sex	Ash-soot	Fine Particles	Large particles
SM2	**F**	0.031	0.000	0.000
SM20	**F**	0.036	0.393	0.143
SM21	**F**	0.036	0.036	0.107
SM22	**F**	0.037	0.000	0.074
SM23	**F**	0.043	0.043	0.043
SM24	**F**	0.038	0.077	0.423
SM25	**F**	0.036	0.143	0.071
SM26	**F**	0.034	0.103	0.000
SM27	**F**	0.033	0.033	0.033
SM28	**F**	0.048	0.048	0.000
SM29	**F**	0.036	0.393	0.036
SM3	**F**	0.031	0.219	0.000
SM30	**F**	0.034	0.103	0.000
SM31	**F**	0.031	0.031	0.000
SM32	**F**	0.029	0.235	0.000
SM33	**F**	0.033	0.233	0.000
SM34	**F**	0.043	0.217	0.000
SM35	**F**	0.037	0.074	0.185
SM36	**F**	0.036	0.071	0.000
SM37	**F**	0.033	0.067	0.167
SM38	**F**	0.036	0.000	0.000
SM39	**F**	0.037	0.074	0.000
SM4	**F**	0.032	0.000	0.097
SM40	**F**	0.038	0.154	0.231
SM41	**F**	0.042	0.000	0.000
SM42	**F**	0.040	0.080	0.280
SM43	**F**	0.037	0.000	0.000
SM44	**F**	0.043	0.130	0.043
SM5	**F**	0.033	0.000	0.133
SM6	**F**	0.034	0.241	0.000
SM7	**F**	0.030	0.000	0.091
SM8	**F**	0.043	0.304	0.174
SM9	**F**	0.029	0.118	0.176
SM45	**M**	0.029	0.118	0.324
SM46	**M**	0.032	0.161	0.000
SM47	**M**	0.031	0.125	0.250

Table 22 continued Sample id	Sex	Ash-soot	Fine Particles	Large particles
SM48	**M**	0.038	0.000	0.462
SM49	**M**	0.034	0.276	0.000
SM50	**M**	0.042	0.083	0.000
SM51	**M**	0.045	0.000	0.273
SM52	**M**	0.038	0.231	0.154
SM53	**M**	0.048	0.143	0.000
SM54	**M**	0.032	0.226	0.000
SM55	**M**	0.033	0.167	0.000
SM56	**M**	0.040	0.120	0.160
SM57	**M**	0.056	0.167	0.000
SM58	**M**	0.037	0.111	0.000
SM59	**M**	0.043	0.087	0.000
SM60	**M**	0.034	0.000	0.000
SM61	**M**	0.033	0.067	0.000
SM62	**M**	0.040	0.000	0.000
SM63	**M**	0.038	0.038	0.000
SM64	**M**	0.053	0.105	0.000
SM65	**M**	0.038	0.154	0.000
SM66	**M**	0.040	0.000	0.000
SM67	**M**	0.045	0.091	0.000
SM68	**M**	0.045	0.045	0.091
SM69	**M**	0.029	0.086	0.000
SM70	**M**	0.026	0.053	0.132
SM71	**M**	0.024	0.024	0.000
SM73	**M**	0.025	0.275	0.075
SM74	**M**	0.045	0.091	0.000
SM75	**M**	0.036	0.107	0.000
SM76	**M**	0.028	0.139	0.000
SM77	**M**	0.026	0.079	0.053
SM78	**M**	0.026	0.154	0.000
SM79	**M**	0.056	0.056	0.111
SM80	**M**	0.029	0.029	0.057
SM81	**M**	0.045	0.000	0.136
SM82	**M**	0.067	0.067	0.133
SM83	**M**	0.056	0.167	0.222
SM84	**USY**	0.059	0.235	0.000

Table 22 continued Sample id	Sex	Ash-soot	Fine Particles	Large particles
SM85	**USY**	0.067	0.667	0.333
SM86	**USY**	0.077	0.231	0.154
SP41	**F**	0.038	0.000	0.077
SP42	**F**	0.040	0.000	0.000
SP43	**F**	0.031	0.031	0.094
SP44	**F**	0.032	0.000	0.000
SP45	**F**	0.029	0.000	0.114
SP46	**F**	0.037	0.037	0.000
SP47	**F**	0.034	0.000	0.000
SP48	**F**	0.029	0.029	0.118
SP49	**F**	0.031	0.063	0.000
SP50	**F**	0.030	0.091	0.000
SP51	**F**	0.029	0.114	0.000
SP52	**F**	0.031	0.000	0.125
SP53	**F**	0.038	0.000	0.000
SP54	**F**	0.037	0.148	0.000
SP55	**F**	0.036	0.000	0.000
SP56	**F**	0.034	0.172	0.000
SP57	**F**	0.033	0.000	0.133
SP58	**F**	0.030	0.030	0.000
SP59	**F**	0.027	0.054	0.000
SP60	**F**	0.043	0.087	0.000
SP61	**F**	0.037	0.037	0.407
SP62	**F**	0.034	0.000	0.207
SP63	**F**	0.028	0.028	0.111
SP64	**F**	0.026	0.026	0.053
SP65	**F**	0.029	0.059	0.059
SP66	**F**	0.037	0.111	0.074
SP67	**F**	0.043	0.261	0.000
SP68	**F**	0.043	0.000	0.087
SP69	**F**	0.036	0.107	0.000
SP70	**F**	0.030	0.030	0.061
SP71	**F**	0.029	0.000	0.029
SP73	**F**	0.036	0.071	0.000
SP74	**F**	0.032	0.129	0.032
SP75	**F**	0.043	0.304	0.043

Table 22 continued Sample id	Sex	Ash-soot	Fine Particles	Large particles
SP76	**F**	0.024	0.268	0.049
SP77	**F**	0.043	0.087	0.217
SP78	**F**	0.040	0.000	0.160
SP79	**F**	0.037	0.074	0.000
SP80	**F**	0.043	0.087	0.000
SP81	**F**	0.043	0.087	0.000
SP82	**F**	0.036	0.000	0.036
SP83	**F**	0.037	0.037	0.037
SP84	**F**	0.029	0.000	0.029
SP85	**F**	0.031	0.031	0.000
SP86	**F**	0.042	0.000	0.042
SP87	**F**	0.043	0.043	0.000
SP88	**F**	0.050	0.000	0.100
SP89	**F**	0.048	0.095	0.000
SP90	**F**	0.043	0.130	0.087
SP10	**M**	0.029	0.000	0.059
SP11	**M**	0.031	0.094	0.188
SP12	**M**	0.037	0.037	0.000
SP13	**M**	0.031	0.375	0.125
SP14	**M**	0.034	0.000	0.000
SP15	**M**	0.037	0.074	0.111
SP16	**M**	0.036	0.036	0.107
SP17	**M**	0.036	0.250	0.250
SP18	**M**	0.034	0.000	0.034
SP19	**M**	0.040	0.120	0.000
SP2	**M**	0.033	0.133	0.033
SP20	**M**	0.038	0.115	0.000
SP21	**M**	0.031	0.000	0.000
SP22	**M**	0.036	0.000	0.107
SP23	**M**	0.032	0.032	0.355
SP24	**M**	0.030	0.091	0.091
SP25	**M**	0.036	0.286	0.071
SP26	**M**	0.037	0.444	0.037
SP27	**M**	0.043	0.652	0.130
SP28	**M**	0.037	0.148	0.037
SP29	**M**	0.029	0.029	0.088

Table 22 continued Sample id	Sex	Ash-soot	Fine Particles	Large particles
SP3	**M**	0.034	0.103	0.379
SP30	**M**	0.032	0.000	0.097
SP31	**M**	0.034	0.414	0.000
SP32	**M**	0.043	0.217	0.130
SP33	**M**	0.033	0.133	0.100
SP34	**M**	0.043	0.304	0.174
SP35	**M**	0.036	0.000	0.250
SP36	**M**	0.037	0.444	0.000
SP37	**M**	0.038	0.038	0.462
SP38	**M**	0.040	0.280	0.000
SP39	**M**	0.037	0.222	0.037
SP4	**M**	0.036	0.500	0.107
SP40	**M**	0.036	0.143	0.000
SP5	**M**	0.033	0.067	0.133
SP6	**M**	0.029	0.059	0.000
SP7	**M**	0.038	0.269	0.154
SP8	**M**	0.031	0.188	0.188
SP9	**M**	0.043	0.130	0.000
SP1	**USC**	0.031	0.063	0.031

Table 23. Distribution of non-starchy cellular inclusions

Sample Id	Sex	Sclereids	Raphids	Druse
EMP1	**USA**	0.0435	0.0000	0.0000
EMP11	**F**	0.0000	0.0000	0.0667
EMP12	**F**	0.0000	0.0000	0.0000
EMP13	**F**	0.0000	0.0667	0.0000
EMP14	**F**	0.0769	0.0769	0.0000
EMP15	**F**	0.0000	0.0000	0.0000
EMP16	**F**	0.0667	0.0000	0.0667
EMP17	**F**	0.0000	0.0667	0.0000
EMP18	**F**	0.0000	0.0000	0.0000
RT11	**F**	0.0000	0.0000	0.0000
RT12	**F**	0.0000	0.0000	0.0000
RT13	**F**	0.0667	0.0000	0.0000
RT5	**F**	0.0000	0.0000	0.0000
EMP10	**M**	0.1053	0.0000	0.0000
EMP2	**M**	0.0000	0.0000	0.0000
EMP3	**M**	0.0000	0.0000	0.0000
EMP4	**M**	0.0833	0.0000	0.0000
EMP5	**M**	0.0000	0.0714	0.0000
EMP6	**M**	0.0000	0.0000	0.0556
EMP7	**M**	0.0952	0.0000	0.0000
EMP8	**M**	0.0000	0.0476	0.0000
EMP9	**M**	0.0000	0.0000	0.0000
RT2	**M**	0.0000	0.0667	0.0667
RT3	**M**	0.0000	0.0000	0.0000
RT4	**M**	0.0000	0.0625	0.0000
RT6	**M**	0.0714	0.0000	0.0000
SM11	**F**	0.0370	0.0370	0.0370
SM15	**F**	0.0000	0.0357	0.0000
SM16	**F**	0.0000	0.0000	0.0769
SM17	**F**	0.0238	0.0000	0.0000
SM19	**F**	0.0000	0.0000	0.0323
SM21	**F**	0.0357	0.0000	0.0000
SM22	**F**	0.0000	0.0000	0.0370
SM23	**F**	0.0000	0.0435	0.0000
SM26	**F**	0.0000	0.0000	0.0345

Table 23 continued Sample Id	Sex	Sclereids	Raphids	Druse
SM28	**F**	0.0000	0.0000	0.0476
SM29	**F**	0.0000	0.0357	0.0000
SM3	**F**	0.0313	0.0000	0.0000
SM44	**F**	0.0435	0.0000	0.0000
SM5	**F**	0.0000	0.0333	0.0000
SM7	**F**	0.0303	0.0000	0.0303
SM67	**M**	0.0455	0.0000	0.0000
SM71	**M**	0.0000	0.0238	0.0000
SM73	**M**	0.0250	0.0000	0.0000
SM76	**M**	0.0278	0.0000	0.0000
SM78	**M**	0.0256	0.0256	0.0000
SM81	**M**	0.0455	0.0000	0.0000
SM83	**M**	0.0556	0.0000	0.0000
SP45	**F**	0.0286	0.0000	0.0000
SP48	**F**	0.0294	0.0000	0.0000
SP53	**F**	0.0385	0.0000	0.0000
SP63	**F**	0.0000	0.0278	0.0000
SP65	**F**	0.0294	0.0000	0.0294
SP66	**F**	0.0000	0.0000	0.0741
SP68	**F**	0.0000	0.0000	0.0435
SP69	**F**	0.0714	0.0000	0.0000
SP70	**F**	0.0000	0.0000	0.0303
SP74	**F**	0.0323	0.0000	0.0000
SP75	**F**	0.0000	0.0000	0.0870
SP83	**F**	0.0370	0.0000	0.0000
SP89	**F**	0.0476	0.0000	0.0000
SP16	**M**	0.0357	0.0000	0.0000
SP2	**M**	0.0000	0.0000	0.0333
SP20	**M**	0.0385	0.0000	0.0000
SP23	**M**	0.0000	0.0323	0.0000
SP24	**M**	0.0303	0.0000	0.0000
SP25	**M**	0.0000	0.0000	0.0357
SP26	**M**	0.0000	0.0370	0.0000
SP27	**M**	0.0000	0.0000	0.0435
SP28	**M**	0.0370	0.0000	0.0000
SP29	**M**	0.0294	0.0000	0.0294

Table 23 continued Sample Id	Sex	Sclereids	Raphids	Druse
SP36	**M**	0.0370	0.0000	0.0000
SP4	**M**	0.0000	0.0000	0.0357
SP40	**M**	0.0357	0.0000	0.0000
SP5	**M**	0.0000	0.0333	0.0000
SP9	**M**	0.0435	0.0000	0.0000

APPENDIX IV: RESULTS KINGDOM FUNGI

Table 24. Distribution of Fungal remains

Sample Id	Sex	Cf. *Alteranria* sp.	Cf. *Curvularia* sp.	*Cf. Fusarium* sp.	Other spores
EMP11	**F**	0.133	0.000	0.000	0.133
EMP12	**F**	0.000	0.000	0.000	0.059
EMP13	**F**	0.000	0.067	0.000	0.067
EMP14	**F**	0.077	0.000	0.000	0.231
EMP15	**F**	0.000	0.000	0.000	0.222
EMP16	**F**	0.000	0.000	0.000	0.200
EMP17	**F**	0.000	0.000	0.000	0.000
EMP18	**F**	0.063	0.000	0.000	0.188
RT11	**F**	0.000	0.111	0.000	0.111
RT13	**F**	0.267	0.133	0.000	0.000
RT5	**F**	0.000	0.000	0.000	0.000
EMP10	**M**	0.000	0.053	0.000	0.053
EMP2	**M**	0.000	0.000	0.000	0.067
EMP3	**M**	0.083	0.083	0.000	0.083
EMP4	**M**	0.083	0.083	0.000	0.833
EMP5	**M**	0.000	0.143	0.000	0.357
EMP6	**M**	0.000	0.000	0.000	0.222
EMP7	**M**	0.048	0.000	0.000	0.286
EMP8	**M**	0.000	0.000	0.000	0.000
EMP9	**M**	0.118	0.000	0.000	0.059
RT2	**M**	0.000	0.000	0.000	0.133
RT3	**M**	0.050	0.000	0.000	0.050
RT4	**M**	0.000	0.000	0.000	0.000
RT6	**M**	0.214	0.000	0.000	1.357
SM1	**F**	0.000	0.000	0.000	0.517
SM10	**F**	0.000	0.000	0.000	0.000
SM11	**F**	0.000	0.000	0.000	0.000
SM14	**F**	0.037	0.000	0.000	0.333
SM15	**F**	0.000	0.000	0.000	0.286
SM16	**F**	0.038	0.000	0.000	0.038

Table 24 continued Sample Id	Sex	Cf. *Alteranria* sp.	Cf. *Curvularia* sp.	*Cf. Fusarium* sp.	Other spores
SM17	**F**	0.000	0.000	0.000	0.429
SM18	**F**	0.000	0.000	0.000	0.410
SM19	**F**	0.065	0.000	0.000	0.548
SM2	**F**	0.031	0.000	0.000	0.156
SM20	**F**	0.036	0.000	0.000	0.214
SM21	**F**	0.000	0.071	0.000	0.929
SM22	**F**	0.037	0.000	0.000	0.852
SM23	**F**	0.000	0.000	0.000	0.435
SM24	**F**	0.000	0.000	0.000	0.154
SM25	**F**	0.036	0.000	0.000	0.143
SM26	**F**	0.000	0.000	0.000	0.241
SM27	**F**	0.000	0.000	0.000	0.167
SM28	**F**	0.048	0.000	0.000	0.095
SM29	**F**	0.000	0.000	0.000	0.464
SM30	**F**	0.034	0.000	0.000	0.103
SM32	**F**	0.000	0.000	0.000	0.029
SM34	**F**	0.043	0.000	0.000	0.043
SM35	**F**	0.000	0.000	0.000	0.222
SM36	**F**	0.000	0.000	0.000	0.071
SM37	**F**	0.000	0.000	0.000	0.167
SM38	**F**	0.036	0.000	0.107	0.179
SM39	**F**	0.000	0.000	0.000	0.296
SM4	**F**	0.000	0.000	0.000	0.129
SM40	**F**	0.000	0.000	0.000	0.269
SM41	**F**	0.000	0.000	0.000	0.083
SM42	**F**	0.040	0.000	0.000	0.160
SM43	**F**	0.000	0.000	0.000	0.000
SM44	**F**	0.043	0.000	0.000	0.174
SM5	**F**	0.000	0.000	0.000	0.000
SM6	**F**	0.000	0.000	0.000	0.069
SM7	**F**	0.061	0.000	0.000	0.091
SM8	**F**	0.000	0.000	0.000	0.000
SM9	**F**	0.000	0.000	0.000	0.059
SM45	**M**	0.059	0.000	0.324	0.176
SM46	**M**	0.000	0.000	0.000	0.129
SM48	**M**	0.000	0.000	0.000	0.038

Table 24 continued Sample Id	Sex	Cf. *Alteranria* sp.	Cf. *Curvularia* sp.	*Cf. Fusarium* sp.	Other spores
SM49	**M**	0.069	0.000	0.000	0.069
SM50	**M**	0.000	0.000	0.000	0.083
SM51	**M**	0.000	0.000	0.000	0.182
SM52	**M**	0.000	0.000	0.077	0.000
SM53	**M**	0.095	0.000	0.000	0.000
SM54	**M**	0.000	0.000	0.000	0.097
SM55	**M**	0.000	0.000	0.067	0.000
SM56	**M**	0.000	0.000	0.000	0.040
SM57	**M**	0.056	0.000	0.000	0.000
SM58	**M**	0.000	0.000	0.037	0.000
SM60	**M**	0.034	0.000	0.000	0.034
SM61	**M**	0.000	0.000	0.000	0.000
SM62	**M**	0.000	0.000	0.040	0.080
SM63	**M**	0.038	0.000	0.000	0.000
SM65	**M**	0.038	0.000	0.000	0.423
SM66	**M**	0.000	0.000	0.080	0.000
SM67	**M**	0.091	0.000	0.000	0.000
SM68	**M**	0.045	0.000	0.000	0.227
SM69	**M**	0.000	0.057	0.000	0.171
SM70	**M**	0.053	0.000	0.000	0.132
SM71	**M**	0.024	0.000	0.000	0.071
SM73	**M**	0.000	0.000	0.050	0.050
SM74	**M**	0.000	0.091	0.000	0.136
SM75	**M**	0.000	0.000	0.000	0.179
SM76	**M**	0.000	0.028	0.000	0.000
SM77	**M**	0.000	0.000	0.053	0.053
SM78	**M**	0.000	0.000	0.000	0.128
SM79	**M**	0.000	0.056	0.000	0.167
SM80	**M**	0.000	0.000	0.000	0.000
SM81	**M**	0.000	0.000	0.000	0.045
SM82	**M**	0.000	0.067	0.000	0.000
SM83	**M**	0.056	0.000	0.000	0.111
SP42	**F**	0.000	0.040	0.040	0.000
SP43	**F**	0.031	0.000	0.000	0.125
SP44	**F**	0.000	0.000	0.000	0.000
SP45	**F**	0.000	0.000	0.029	0.000

Table 24 continued Sample Id	Sex	Cf. *Alteranria* sp.	Cf. *Curvularia* sp.	*Cf. Fusarium* sp.	Other spores
SP46	**F**	0.000	0.074	0.074	0.074
SP47	**F**	0.000	0.000	0.000	0.000
SP48	**F**	0.059	0.000	0.000	0.147
SP49	**F**	0.000	0.000	0.000	0.000
SP50	**F**	0.000	0.030	0.000	0.000
SP51	**F**	0.000	0.000	0.086	0.057
SP52	**F**	0.063	0.000	0.000	0.000
SP53	**F**	0.000	0.000	0.000	0.000
SP54	**F**	0.000	0.037	0.111	1.704
SP56	**F**	0.069	0.000	0.000	0.034
SP57	**F**	0.000	0.067	0.133	0.000
SP58	**F**	0.000	0.000	0.000	0.061
SP59	**F**	0.000	0.000	0.000	0.000
SP60	**F**	0.087	0.000	0.087	0.000
SP61	**F**	0.000	0.111	0.000	0.000
SP62	**F**	0.000	0.000	0.000	0.000
SP63	**F**	0.000	0.000	0.028	0.083
SP64	**F**	0.026	0.000	0.079	0.605
SP65	**F**	0.000	0.118	0.000	0.235
SP66	**F**	0.000	0.037	0.000	0.222
SP67	**F**	0.000	0.000	0.000	0.652
SP68	**F**	0.043	0.043	0.000	0.739
SP69	**F**	0.000	0.071	0.000	0.500
SP70	**F**	0.000	0.030	0.000	0.091
SP71	**F**	0.029	0.000	0.000	0.088
SP73	**F**	0.000	0.036	0.000	1.107
SP74	**F**	0.000	0.032	0.000	0.903
SP75	**F**	0.087	0.000	0.000	0.565
SP76	**F**	0.000	0.000	0.000	0.439
SP78	**F**	0.000	0.000	0.000	0.040
SP80	**F**	0.000	0.000	0.000	0.087
SP82	**F**	0.000	0.000	0.000	0.000
SP83	**F**	0.000	0.000	0.000	0.074
SP84	**F**	0.000	0.000	0.000	0.114
SP85	**F**	0.063	0.000	0.000	0.063
SP86	**F**	0.000	0.000	0.000	0.000

Table 24 continued Sample Id	Sex	Cf. *Alteranria* sp.	Cf. *Curvularia* sp.	*Cf. Fusarium* sp.	Other spores
SP87	**F**	0.000	0.000	0.000	0.000
SP88	**F**	0.050	0.000	0.000	0.200
SP89	**F**	0.000	0.000	0.000	0.143
SP10	**M**	0.000	0.088	0.000	0.000
SP11	**M**	0.000	0.000	0.000	0.063
SP12	**M**	0.000	0.000	0.000	0.000
SP13	**M**	0.000	0.156	0.000	0.000
SP14	**M**	0.000	0.000	0.000	0.207
SP15	**M**	0.000	0.000	0.000	0.000
SP16	**M**	0.000	0.000	0.000	0.000
SP17	**M**	0.000	0.071	0.000	0.071
SP18	**M**	0.000	0.000	0.000	0.103
SP19	**M**	0.000	0.000	0.000	0.000
SP20	**M**	0.000	0.154	0.115	0.115
SP21	**M**	0.000	0.000	0.000	0.063
SP23	**M**	0.032	0.032	0.000	0.065
SP24	**M**	0.030	0.030	0.030	0.182
SP25	**M**	0.036	0.000	0.000	0.107
SP26	**M**	0.000	0.000	0.074	0.148
SP27	**M**	0.000	0.000	0.000	0.174
SP28	**M**	0.037	0.037	0.000	0.185
SP29	**M**	0.029	0.029	0.000	0.441
SP3	**M**	0.034	0.034	0.000	0.103
SP30	**M**	0.032	0.000	0.000	0.000
SP31	**M**	0.000	0.000	0.069	0.207
SP32	**M**	0.000	0.043	0.000	0.217
SP33	**M**	0.000	0.000	0.000	0.000
SP34	**M**	0.043	0.000	0.043	0.217
SP35	**M**	0.000	0.000	0.000	0.000
SP36	**M**	0.000	0.074	0.000	0.000
SP37	**M**	0.000	0.000	0.000	0.115
SP38	**M**	0.040	0.000	0.000	0.000
SP39	**M**	0.000	0.111	0.000	0.000
SP4	**M**	0.000	0.000	0.071	0.107
SP40	**M**	0.000	0.000	0.000	0.000
SP5	**M**	0.067	0.000	0.000	0.167

Table 24 continued Sample Id	Sex	Cf. *Alteranria* sp.	Cf. *Curvularia* sp.	*Cf. Fusarium* sp.	Other spores
SP7	**M**	0.000	0.000	0.038	0.077
SP8	**M**	0.000	0.000	0.000	0.125

APPENDIX V: RESULTS KINGDOM ANIMALIA

Table 25. Distribution of insect remains

Sample id.	Sex	Undiagnostic	Lepidoptera (small)	Lepidoptera (Large)
EMP11	**F**	0.0667	0.0000	0.0667
EMP12	**F**	0.0588	0.0000	0.0000
EMP14	**F**	0.0769	0.0769	0.0000
EMP16	**F**	0.0667	0.0000	0.0000
RT12	**F**	0.0000	0.0556	0.0000
RT13	**F**	0.0667	0.0000	0.0000
EMP2	**M**	0.0667	0.0667	0.0000
EMP4	**M**	0.0833	0.0000	0.0000
EMP5	**M**	0.0000	0.0714	0.0000
EMP6	**M**	0.0556	0.0000	0.0000
EMP8	**M**	0.0476	0.0476	0.0000
EMP9	**M**	0.0000	0.0588	0.0000
RT3	**M**	0.0500	0.0000	0.0000
RT6	**M**	0.0714	0.0714	0.0000
SM11	**F**	0.0370	0.0000	0.0000
SM14	**F**	0.0370	0.0000	0.0000
SM15	**F**	0.0357	0.0714	0.0000
SM17	**F**	0.0000	0.0238	0.0000
SM18	**F**	0.0256	0.0000	0.0000
SM19	**F**	0.0323	0.0000	0.0000
SM20	**F**	0.0357	0.0000	0.0357
SM22	**F**	0.0370	0.0370	0.0000
SM23	**F**	0.0435	0.0000	0.0000
SM25	**F**	0.0357	0.0357	0.0000
SM26	**F**	0.0345	0.0000	0.0000
SM28	**F**	0.0476	0.0000	0.0000
SM29	**F**	0.0357	0.0357	0.0000
SM35	**F**	0.0370	0.0000	0.0000
SM4	**F**	0.0000	0.0323	0.0000
SM40	**F**	0.0385	0.0000	0.0000
SM44	**F**	0.0435	0.0435	0.0000
SM5	**F**	0.0333	0.0000	0.0000

Table 25 continued Sample id.	**Sex**	Undiagnostic	Lepidoptera (small)	Lepidoptera (Large)
SM48	**M**	0.0769	0.0000	0.0000
SM50	**M**	0.0000	0.0417	0.0000
SM55	**M**	0.0000	0.0333	0.0000
SM69	**M**	0.0571	0.0000	0.0000
SM70	**M**	0.0000	0.0000	0.0263
SM74	**M**	0.0455	0.0455	0.0000
SM75	**M**	0.0357	0.0000	0.0000
SM76	**M**	0.0278	0.0000	0.0000
SM80	**M**	0.0286	0.0286	0.0000
SM83	**M**	0.0556	0.0000	0.0000
SP56	**F**	0.0000	0.0000	0.0345
SP60	**F**	0.0000	0.0000	0.0435
SP64	**F**	0.0263	0.0263	0.0263
SP65	**F**	0.0294	0.0294	0.0000
SP66	**F**	0.0370	0.0000	0.0000
SP67	**F**	0.0435	0.0435	0.0000
SP68	**F**	0.0435	0.0435	0.0000
SP69	**F**	0.0357	0.0000	0.0357
SP70	**F**	0.0303	0.0303	0.0000
SP74	**F**	0.0323	0.0968	0.0000
SP75	**F**	0.0870	0.0435	0.0000
SP76	**F**	0.0244	0.0000	0.0000
SP77	**F**	0.0000	0.0435	0.0000
SP81	**F**	0.0000	0.0435	0.0000
SP82	**F**	0.0714	0.0000	0.0000
SP86	**F**	0.0000	0.0833	0.0000
SP89	**F**	0.0476	0.0000	0.0000
SP18	**M**	0.0345	0.0000	0.0000
SP2	**M**	0.0000	0.0333	0.0000
SP22	**M**	0.1071	0.0000	0.0000
SP23	**M**	0.0645	0.0000	0.0000
SP24	**M**	0.0303	0.0000	0.0303
SP25	**M**	0.0000	0.0357	0.0000
SP27	**M**	0.0435	0.0435	0.0000
SP28	**M**	0.0370	0.0000	0.0370
SP29	**M**	0.0882	0.0294	0.0000

Table 25 continued Sample id.	**Sex**	Undiagnostic	Lepidoptera (small)	Lepidoptera (Large)
SP32	**M**	0.0435	0.0000	0.0000
SP4	**M**	0.1071	0.0000	0.0357
SP5	**M**	0.0000	0.0333	0.0000

Table 26. Distribution of Animal Hair

Sample id.	Sex	*Sheep wool*	Undiag.
EMP11	**F**	0.2000	0.0000
EMP13	**F**	0.0667	0.0000
EMP15	**F**	0.1667	0.0556
EMP16	**F**	0.0667	0.0000
EMP18	**F**	0.0625	0.0000
RT11	**F**	0.0556	0.0000
RT12	**F**	0.1667	0.0000
RT5	**F**	0.8000	0.0000
EMP10	**M**	0.0526	0.0000
EMP2	**M**	0.0667	0.0000
EMP4	**M**	0.0833	0.0000
EMP5	**M**	0.5000	0.0714
EMP7	**M**	0.1429	0.0000
EMP8	**M**	0.0000	0.0476
RT2	**M**	0.0000	0.0667
RT3	**M**	0.0500	0.0000
SM12	**F**	0.1071	0.0000
SM15	**F**	0.0357	0.0000
SM17	**F**	0.6429	0.0000
SM21	**F**	0.0357	0.0000
SM22	**F**	0.8519	0.0370
SM3	**F**	0.0625	0.0000
SM31	**F**	0.1250	0.0000
SM35	**F**	0.1852	0.0000
SM4	**F**	0.0323	0.0000
SM40	**F**	0.2692	0.0000
SM5	**F**	0.1333	0.0000
SM8	**F**	0.2174	0.0435
SM47	**M**	0.0313	0.0000
SM52	**M**	0.0385	0.0000
SM55	**M**	0.0333	0.0000
SM57	**M**	0.1111	0.0000
SM61	**M**	0.4333	0.0000
SM68	**M**	0.0455	0.0000
SM70	**M**	0.0526	0.0000

Table 26 continued Sample id.	Sex	*Sheep wool*	Undiag.
SM73	**M**	0.0250	0.0000
SM74	**M**	0.0455	0.0000
SM77	**M**	0.0000	0.0263
SM81	**M**	0.0455	0.0000
SP42	**F**	0.0400	0.0000
SP46	**F**	0.0370	0.0000
SP49	**F**	0.0313	0.0000
SP52	**F**	0.0313	0.0000
SP57	**F**	0.0333	0.0000
SP61	**F**	0.0370	0.0000
SP63	**F**	0.1389	0.0000
SP64	**F**	0.0263	0.0000
SP65	**F**	0.0294	0.0000
SP67	**F**	0.0435	0.0000
SP69	**F**	0.0357	0.0000
SP75	**F**	0.0435	0.0000
SP76	**F**	0.7805	0.0000
SP81	**F**	0.1739	0.0000
SP86	**F**	0.0833	0.0000
SP89	**F**	0.0000	0.0476
SP90	**F**	0.0435	0.0000
SP11	**M**	0.0313	0.0000
SP17	**M**	0.0357	0.0000
SP2	**M**	0.0333	0.0000
SP22	**M**	0.0357	0.0000
SP26	**M**	0.0370	0.0000
SP27	**M**	0.0435	0.0000
SP29	**M**	0.0294	0.0000
SP35	**M**	0.0357	0.0000
SP39	**M**	0.0370	0.0000
SP4	**M**	0.0357	0.0000

Table 27. Distribution of feather barbules

Sample id.	Sex	Galliformes	Anseriformes	Strigiformes	Unknown
EMP11	**F**	0.0000	0.0667	0.0000	0.0000
EMP12	**F**	0.0000	0.0588	0.0000	0.0000
EMP14	**F**	0.0000	0.0769	0.0000	0.0000
EMP15	**F**	0.0000	0.0000	0.0000	0.0556
EMP17	**F**	0.0000	0.0667	0.0000	0.0667
RT11	**F**	0.0000	0.0556	0.0000	0.0000
EMP2	**M**	0.0000	0.0667	0.0000	0.0000
EMP4	**M**	0.0000	0.0833	0.0000	0.0000
EMP6	**M**	0.0000	0.0556	0.0000	0.0556
EMP8	**M**	0.0000	0.0476	0.0000	0.0000
EMP9	**M**	0.0000	0.0000	0.0000	0.0588
RT2	**M**	0.0000	0.0667	0.0000	0.0000
RT4	**M**	0.0000	0.0625	0.0000	0.0000
SM1	**F**	0.0000	0.0000	0.0000	0.0345
SM10	**F**	0.0000	0.0000	0.0000	0.0357
SM11	**F**	0.0370	0.1852	0.0000	0.0000
SM16	**F**	0.0000	0.0000	0.0000	0.0385
SM17	**F**	0.0238	0.0000	0.0000	0.0000
SM18	**F**	0.1026	0.1538	0.0000	0.3333
SM2	**F**	0.1875	0.2188	0.0000	0.0000
SM21	**F**	0.1786	0.0000	0.0000	0.5357
SM22	**F**	0.0000	0.0000	0.0000	0.0370
SM24	**F**	0.0000	0.0000	0.0385	0.0000
SM26	**F**	0.2414	0.0000	0.0000	0.0000
SM30	**F**	0.4138	0.0000	0.0000	0.0000
SM36	**F**	0.1429	0.0000	0.0000	0.0000
SM40	**F**	0.4231	0.0000	0.0000	0.0000
SM43	**F**	0.1852	0.0000	0.0000	0.0000
SM44	**F**	0.0000	0.0000	0.0000	0.0435
SM7	**F**	0.0000	0.0000	0.0000	0.0303
SM8	**F**	0.1304	0.0000	0.0000	0.0000
SM9	**F**	0.0000	0.1765	0.0000	0.0000
SM45	**M**	0.1765	0.0294	0.0000	0.0000
SM50	**M**	0.0000	0.0000	0.2083	0.0000
SM65	**M**	0.0385	0.0000	0.0000	0.0000

Table 27 continued Sample id.	**Sex**	Galliformes	Anseriformes	Strigiformes	Unknown
SM68	**M**	0.0000	0.0000	0.0455	0.0000
SM70	**M**	0.0263	0.0000	0.0000	0.0263
SM71	**M**	0.0000	0.0238	0.0000	0.0000
SM75	**M**	0.0357	0.0000	0.0000	0.0000
SM76	**M**	0.0000	0.0278	0.0000	0.0000
SM77	**M**	0.0000	0.0263	0.0000	0.0263
SM82	**M**	0.0000	0.0000	0.0000	0.0667
SM83	**M**	0.0000	0.0556	0.0000	0.0000
SP44	**F**	0.0323	0.0000	0.0000	0.0000
SP47	**F**	0.0000	0.5172	0.0000	0.0000
SP50	**F**	0.1818	0.0000	0.0000	0.0000
SP56	**F**	0.1034	0.0345	0.0000	0.0000
SP58	**F**	0.0000	0.0000	0.0303	0.0000
SP60	**F**	0.0000	0.0435	0.0435	0.0000
SP61	**F**	0.2222	0.0000	0.0000	0.0000
SP62	**F**	0.0000	0.4483	0.0000	0.0000
SP64	**F**	0.1316	0.0526	0.0000	0.0263
SP66	**F**	0.0000	0.0741	0.0000	0.0000
SP67	**F**	0.0000	0.0435	0.0000	0.0000
SP68	**F**	0.2174	0.0870	0.0000	0.0435
SP70	**F**	0.0000	0.0303	0.0000	0.0000
SP71	**F**	0.0294	0.0000	0.0000	0.0000
SP74	**F**	0.0000	0.0000	0.0000	0.0323
SP76	**F**	0.0244	0.0000	0.0000	0.0000
SP81	**F**	0.0435	0.0000	0.0000	0.0000
SP87	**F**	0.0435	0.0000	0.0000	0.0000
SP15	**M**	0.0370	0.0000	0.0000	0.0000
SP2	**M**	0.0000	0.0333	0.0000	0.0000
SP23	**M**	0.0000	0.0323	0.0000	0.0000
SP24	**M**	0.0303	0.0000	0.0303	0.0303
SP25	**M**	0.0000	0.0357	0.0000	0.0000
SP26	**M**	0.0000	0.0370	0.0370	0.0000
SP27	**M**	0.0435	0.0000	0.0000	0.0000
SP28	**M**	0.0000	0.0370	0.0000	0.0000
SP29	**M**	0.0000	0.0294	0.0294	0.0000
SP3	**M**	0.0000	0.0000	0.0000	0.0345

Table 27 continued Sample id.	**Sex**	Galliformes	Anseriformes	Strigiformes	Unknown
SP32	**M**	0.0000	0.0435	0.0000	0.0000
SP34	**M**	0.0000	0.0435	0.0000	0.0000
SP4	**M**	0.0000	0.0357	0.0000	0.0357
SP5	**M**	0.0333	0.0000	0.0000	0.0333
SP8	**M**	0.0313	0.0000	0.0000	0.0000
SP1	**USC**	0.0000	0.0938	0.0000	0.0000

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