DEPARTAMENTO DE CIÊNCIAS DA VIDA
FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE DE COIMBRA

Burned bones vs unburned bones: a pilot study about the impact of differential post-depositional taphonomy on bioanthropological research

Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Evolução e Biologia Humanas, realizada sob a orientação científica da Professora Doutora Eugénia Cunha (Universidade de Coimbra) e do Doutor David Gonçalves (Universidade de Coimbra)

Ana Isabel Cesário da Costa de Matos Amarante
2016

Dedicated to my Mom,

Who is always "present"

motivation, patience and tireless understanding. Thanks for taking my bad - temper. Thank you for everything!

Agradecimentos

Em primeiro lugar agradeço profundamente ao meu orientador, o Doutor David Gonçalves, pelo incansável apoio, auxílio e incentivo, pelos preciosos conselhos, pelas críticas construtivas e partilha de conhecimentos ao longo deste percurso árduo. Agradeço-lhe, também, por me ter incutido o gosto pelo estudo de ossos queimados. À Professora Doutora Eugénia Cunha por ter aceitado ser minha orientadora e por ter proporcionado a realização deste projecto.

Um agradecimento, igualmente especial, à Doutora Teresa Ferreira e ao Professor Doutor Luís Batista de Carvalho por todo o apoio, incentivo e conhecimento partilhado. Os seus conselhos ao longo da elaboração deste trabalho foram essenciais.

Agradeço, igualmente, ao Laboratório de Antropologia Forense do Departamento de Ciências da Vida da Universidade de Coimbra por permitirem o acesso aos esqueletos não-identificados provenientes do cemitério dos Capuchos, Santarém, e pelo espaço disponibilizado. À Unidade de I&D "Química-Física Molecular" da Universidade de Coimbra quero agradecer a disponibilidade do equipamento de Espectroscopia de Infravermelho com Transformadas de Fourier (FTIR), sem o qual não teria sido possível realizar as análises espetroscópicas e a todos os seus colaborados que sempre se mostraram disponíveis. Ao Centro de Investigação em Antropologia e Saúde (CIAS) pela cedência do forno, essencial para este projecto.

À Ines Buekenhout e ao Calil Makhoul agradeço todo o auxílio disponibilizado, motivação, paciência e amizade.

Não posso deixar de agradecer a todos os meus colegas de Mestrado, em especial à Andreia Perinha, à Andréa, à Ana Pelicano e à Mariana Rodrigues pelo companheirismo, amizade, apoio, compreensão e paciência demonstrados ao longo desta etapa académica, e por me fazerem sentir sempre em casa. Também aos meus colegas e amigos do Taekwondo (ACM), pela amizade e por todos os bons momentos proporcionados.

Aos meus Amigos de Lisboa, principalmente à Andreia Matela, Malwa, Nádia e Rute, que apesar da distância, sempre me apoiaram, motivaram e compreenderam o meu distanciamento. Obrigada por serem as pessoas que são e pela vossa amizade.

À Ana Paula pela amizade construída ao longo dos anos, por dizer sempre o que tem a dizer, pela sua motivação, os seus conselhos, a sua força e por nunca me deixar desistir.

Por fim, estou eternamente grata ao meu pai – Alberto Amarante – à minha irmã – Rita Amarante – e aos meus avós – João e Maria -, sem eles não teria sido possível a realização e conclusão de mais esta etapa da minha vida. Obrigada pelo apoio, força, motivação, paciência e compreensão incansáveis. Obrigada por aturarem o meu mau-feitio. Obrigada por tudo!

ABSTRACT

Archaeological and forensic contexts very often include skeletal remains that can result from accidental exposure to fire, mortuary practices, mass disasters or foul play. These burning events induce several changes on the morphology, dimensions and mass of skeletal remains which add to other changes that occur during burial such as surface and chemical alterations. These alterations may interfere with the reliability of bioanthropological methods based on metrics, mass, bioapatite crystallinity, C/P and CO_3^{-2}/P ratios. Thus it is very important to understand how post-depositional taphonomic processes affect burned bones and teeth over time.

Skeletal remains, both unburned and burned, were experimentally buried under controlled conditions to investigate this issue. This research is designed to last 10 years but the first 8 months were the focus of this Master's project. Unburned remains were used as a reference for comparison with the burned remains. The study sample was composed of 96 samples, 64 skeletal remains (32 cortical bones and 32 trabecular bones) and 32 teeth (third molars). Sixteen bones and 8 teeth remained unburned while the other 48 bones and 24 teeth were experimentally burned at three different temperatures (500° C, 900° C and 1050° C). The skeletal remains belonged to three un-reclaimed skeletons of unknown identity from the Capuchos Cemetery, in Santarém (Ferreira et al., 2014) while teeth were provided by different dental clinics and extracted from adult women, and were shallowly buried in containers with acidic soil and placed on the outdoors. Metrics, mass, bioapatite crystallinity and C/P and CO_3^{-2}/P ratios of 24 samples were documented before burial and during regular intervals (2 months) for the past 8 months. Another 24 samples were buried for 6 months and subjected to the same analyses.

So far, the investigation found some differences in post-depositional behaviour between unburned and burned bones. Generally, the former tended to increase more in mass during the first two months, especially for unburned trabecular bones and for both trabecular and cortical bones burned at 500 $^{\circ}$C. The remaining samples experienced less but still important mass increase. However, after eight months bone's mass decreased slightly for all samples of both trabecular and cortical bones. Metrically, no substantial changes were found for most of both burned and unburned specimens but episodic cases of large variations were recorded. Finally, the crystallinity revealed some stability for

trabecular and cortical bones and teeth, both unburned and burned at 500º C, contrary to trabecular and cortical bones and teeth burned at higher temperatures in which crystallinity index values are more unstable and higher. Both C/P and CO_3^{-2}/P remained quite stable during all observations.

Our preliminary results suggest that, when dealing with buried remains: (i) caution is needed when using skeletal mass as a basis for bioanthropological inferences (it is often used as an indicator of skeletal completeness and minimum number of individuals); (ii) osteometric examination is apparently not impaired but more research is needed to explain outlier cases; (iii) the infrared spectroscopic estimation of temperature at which remains have been subjected appears to be affected mainly above 500º C. Expectantly, the subsequent investigation that will last until 2025 will help consolidating these first results and better determine how burial can affect bioanthropological methods.

Key-words: forensic anthropology; bioanthropology; heat-induced modifications; burial FTIR-KBr; bone mass variations; bone metric variations

RESUMO

Contextos arqueológicos e forenses incluem muitas vezes restos de esqueletos que podem resultar de uma exposição acidental ao fogo, práticas mortuárias, desastres em massa ou tentativa de omissão de cadáver. O processo de queima pode provocar várias alterações na morfologia, dimensão e massa dos restos humanos, que se acrescem às outras mudanças que ocorrem durante o enterramento, tais como alterações ao nível da superfície do osso ou alterações químicas. Estas alterações podem interferir com a fiabilidade dos métodos bioantropológicos, com base nos métodos osteométricos, na massa, na cristalinidade, e razões de C/P e CO_3^{-2}/P. Assim, é muito importante entender como os processos tafonómicos pós-deposicionais afetam os ossos e os dentes queimados ao longo do tempo.

Os restos esqueléticos, quer não-queimados como queimados, foram experimentalmente enterrados sob condições controladas para investigar esta questão. Esta pesquisa está projetada para durar 10 anos, mas os primeiros 8 meses foram o foco do projeto desta tese. Os restos não-queimados foram usados como referência para comparação com os restos queimados. A amostra foi composta por 96 restos esqueléticos, 64 fragmentos ósseos (32 osso cortical e 32 ossos trabeculares) e 32 dentes (terceiros molares). Dezesseis ossos e 8 dentes permaneceram não-queimados, enquanto os outros 48 ossos e 24 dentes foram experimentalmente queimados a diferentes temperaturas (500 °C, 900 °C e 1050 °C). Os restos esqueléticos pertenciam a três esqueletos não reclamados e de identidade desconhecida do Cemitério dos Capuchos, em Santarém (Ferreira et al., 2014), enquanto os dentes foram fornecidos por diferentes clínicas dentárias e extraídos de mulheres adultas. Estes foram superficialmente enterrados em recipientes com solo ácido e colocados ao ar livre. Foram registadas as medidas, a massa, a cristalinidade e as razões de C/P e CO_3^{-2}/P de 24 amostras antes do enterro e durante intervalos regulares (2 meses) para os últimos 8 meses. Outras 24 amostras foram enterradas durante 6 meses e submetidas às mesmas análises.

Até agora, com este estudo foi possível verificar algumas diferenças no comportamento pós-deposicional entre os ossos não-queimados e queimados. De um modo geral, as primeiras amostras a serem exumadas tenderam a aumentar mais em massa

durante os dois primeiros meses, especialmente para os ossos trabeculares não-queimados e para os ossos trabeculares e corticais queimados a 500 ºC. As amostras restantes sofreram menos variações, mas ainda assim registou-se um importante aumento de massa. No entanto, após oito meses a massa óssea diminuiu ligeiramente em todas as amostras de ambos os ossos trabeculares e corticais. Quanto às medições efectuadas, não foram verificadas variações substanciais para a maioria das amostras, tanto queimadas como não-queimadas, no entanto foram registados casos esporádicos de grandes variações. Finalmente, a cristalinidade revelou alguma estabilidade para os ossos trabeculares e corticais e dentes, ambos não-queimados e queimados a 500 °C, contrariamente aos correspondentes queimados a elevadas temperaturas, em que os valores do índice de cristalinidade são mais instáveis e mais elevados. Ambas as razões C/P e CO_3^{-2}/P se mantiveram bastante estáveis durante todas as observações.

Os resultados preliminares sugerem que, ao lidar com restos esqueléticos enterrados: (i) é necessário algum cuidado ao utilizar a massa esquelética como referência em inferências bioantropológicas (muitas vezes é usado como um indicador da integridade do esqueleto e para determinação do número mínimo de indivíduos); (ii) aparentemente a análise osteométrica não é prejudicada, mas mais estudos são necessários para explicar os casos de valores atípicos; (iii) a estimativa da temperatura a que os restos esqueléticos foram submetidos através de espetroscopia de infravermelhos parece ser afetada principalmente a temperaturas acima de 500 °C. Espera-se que investigações futuras, que durarão até 2025, venham ajudar a consolidar estes primeiros resultados e a determinar melhor como é que os enterramentos podem afetar os métodos bioantropológicos.

Palavras-chave: antropologia forense; bioantropologia; alterações térmico-induzidas; FTIR-KBr; variações de massa óssea; variações métricas ósseas

Contents

List of Tables

List of Figures

List of Abbreviations

01: proximal end

02: distal end

ATR: Attenuated Total Reflectance

Ca: Calcaneus

CC_NI: Cemitério dos Capuchos, Não Identificado

CEJ: Cement-Enamel Junction

CI: Crystallinity Index

Cl: Clavicle

Cub: Cuboid

DSP: Diagnose Sexuelle Probabiliste

F: Femur

Fib: Fibula

FTIR: Fourier Transform Infrared Spectroscopy

H: Humerus

KBr: Potassium Bromide

Nav: Navicular

Pa: Patella

PMI: *Post-mortem* Interval

R: Radio

SF: Splitting Factor

T: Tibia

Ta: Talus

U: Ulna

Unb: Unburned

V: Vertebra

1. Introduction

Archaeological records very often include burned bones; this type of remains can be due to cooking, accidental exposure to fire, being used as fuel or mortuary practices (Stiner et al., 1995; Lebon et al., 2008; Snoeck et al., 2014). Also in forensic contexts there is the possibility to encounter burned remains, as a result of plane crashes or other mass disasters, natural disasters, wars, or even the disposal of cadavers in order to hide a crime, amongst other events (Grévin et al., 1998; Harbeck et al., 2011; Symes et al., 2012; Piga et al., 2009; Ellingham et al., 2015; Gonçalves et al., 2015; Piga et al., 2016). Furthermore, nowadays there are some other events that are becoming more and more frequent such as explosions, terrorist attacks and suicide bombers.

Heat-induction causes changes in a bone's features. Thus, the examination of a bone exposed to these conditions will be more challenging since conventional methods cannot be applied. To settle these problems it is crucial to understand how bone reacts to thermal alterations. For example, skeletal mass is one of the analyses applied to infer whether a skeleton is complete or not, by performing comparisons between archaeological remains and modern cremated skeletons (Gonçalves et al., 2013a). However, to do such inferences, one must not only understand heat-induced mass loss but also understand subsequent post-depositional mass loss. Several studies comprising the taphonomy of unburned skeletal remains have been carried out (Nicholson, 1993; Andrews, 1995; Bell et al., 1996; Lieverse et al., 2006; Munro et al., 2007; Ross and Cunningham, 2011; De Becdelievre et al., 2015; Aplin et al., *in press*). However, the possible differences between them, regarding post-depositional decomposition, have not been addressed before and thus constitute the main objective of this investigation: to understand how post-depositional taphonomy influence bone mass and metric measurements, as well as some molecular compounds on both unburned and burned bones, causing eventual differences on those features, aiding forensic scientists and archaeologists to solve some problems during investigations.

1.1 Why study taphonomy?

Taphonomy is known as the science that studies all the processes that occur from the time of death until the remains' discovery; it started with paleontology and Efremov

was the first to define it, in 1940 (Efremov, 1940; Behrensmeyer and Kidwell, 1985; Wilson, 1988; Martin, 1999; Smith, 2005; Dirkmaat et al., 2008; Lyman, 2010; Domínguez-Rodrigo et al., 2011; Pokines, 2014). It is essential to critically examine human remains from both archaeological and forensic settings (see Wilson, 1988; Martin, 1999; Dirkmaat et al., 2008; Lyman, 2010; Domínguez-Rodrigo et al., 2011; Ferreira, 2012) because they may tell a story and are essential to understand what occurred either on archaeological and forensic scenarios. Thus, the need to understand post-depositional taphonomic processes on burned bones sustained the necessity to proceed with the present research. By understanding these taphonomic processes and their effects on the burned skeleton we expect to be able to answer questions such as: do post-depositional agents influence burned and unburned skeletal remains in a different manner and in what degree? Is this dependent of the temperature at which the remains have been burned? Do post-depositional agents affect cortical and trabecular bone in the same way?

This investigation partly follows previous ones that focused on the effect of burial on human bone. Littleton (2000) studied taphonomic processes on deliberately buried bodies. Roberts et al. (2002) and Dal Sasso et al. (2014), on the other hand, studied the taphonomic and diagenetic processes of cooked bones and its relation to environmental conditions from an archaeological viewpoint. Also, Trueman et al. (2004) developed studies in order to understand the *post-mortem* changes on bones. The aims of these experiments are shared by the research being here presented. Besides these, several other investigators undertook taphonomic studies of remains from other species recovered from different time periods and environments (see Guarino et al., 2006; Forancelli Pacheco et al., 2012; Karr and Outram, 2012; Bertran et al., 2015).

Teeth can also suffer taphonomic modifications. Some processes are biological. For instance, roots can crush teeth; plants can stain teeth whilst lichens and mosses have the power to discolor the dental root (Schmidt, 2008). Factors such as wind, water, sun and soil, can also interfere with the preservation of human teeth (Schmidt, 2008). Contrary to the previous factors, these specifically, can influence and modify the preservation of human remains. However, the sample used in the present study was not affected by all of these biological factors since the experiment was performed in a controlled environment. For example, events such as the bone transport, i.e., bone place's alteration due to water or carnivores or other animals (Evans, 2014; Pokines, 2014), were not investigated.

All these different kinds of studies demonstrate the importance of understanding taphonomic processes that affect human remains. In addition, this research was focused on human bone fragments and on how taphonomy changes trabecular and cortical bone of both unburned and burned remains. The latter represents a type of remains which hasn't been studied often, especially by adopting a comparative approach with unburned counterparts thus demonstrating the relevance and timely nature of the present research. In order to achieve this, human remains burned under controlled conditions in a furnace were buried in acid soil alongside with unburned remains.

1.2 Taphonomic Agents

Smith (2005) and Lyman (2010) agree that taphonomy is divided into two main sub-fields: 1) biostratinomy, which refers to processes that occur until final burial (Smith, 2005; Lyman, 2010) and 2) diagenesis, which refers to interactions between biological, chemical and physical factors (Hedges and Millard, 1995; Hedges, 2002; Reiche et al., 2003; Stathopoulou et al., 2008). Whereas, according to Sorg and Haglund (2002) taphonomic processes are the result of symbioses between ecology, biology and physics, being observable in four different occasions: 1) *ante-mortem* period; 2) *peri-mortem* period; 3) *post-mortem* period; and 4) post-recovery period. Taking into account these two concepts of taphonomic processes, this thesis will focus on diagenesis – as it addresses the physical and chemical consequences of burial on bones (Smith, 2005) – during the post-mortem period – as it concerns the period from deposition to recovery of the remains, i.e., when remains produce changes in their microenvironment (Sorg and Haglund, 2002).

For a few decades now there has been an increasing interest in understanding what happens to the body after death, i.e., the decomposition processes and how taphonomic events affect skeletal remains after burial. To better understand these processes a number of studies have been conducted by several investigators, such as Ross and Cunningham (2011), Ferreira (2012), Ubelaker (2013), Wilson-Taylor (2013), Buekenhout (2014) and Cravo (2015). These authors focused mainly on the *post-mortem* interval (PMI). However, they concluded that it is one of the most difficult questions to answer in forensic taphonomy (Swift, 1998; Love and Marks, 2003; Pinheiro, 2006; Cattaneo, 2007; Rogers,

2010; Ferreira, 2012; Ferreira and Cunha, 2012; Ubelaker, 2013; Buekenhout, 2014). Nonetheless, this study has a deeper focus on other taphonomic affairs than, specifically, PMI. To fully understand taphonomic processes, it is crucial to be aware of the factors that are involved, that influence it, and that have traditionally been classified as: 1) primary or intrinsic factors, which include bone chemistry, size, structure, shape, density and mass which, for instance, can be dependent on individual features (e.g. age-at-death; sex) or be the result of pathological changes; or 2) secondary or extrinsic factors, concerning temperature, air, soil type, water, local fauna and flora as well as anthropogenic effects such as burial type and other human activities that can result, or not, in burned bone (Henderson, 1987; Galloway, 1997; Gill-King, 1997; Roberts et al., 2002; Pinheiro, 2006; Thompson and Chudek, 2007; Surabian, 2012; Buekenhout, 2014). Some of the primary factors, such as size, structure, shape, mass and bone chemistry can be destroyed or altered by heat-induction.

The Scientific Working Group for Forensic Anthropology (2013), however, classified these taphonomic processes into three different groups: 1) abiotic agents, like weathering and thermal events; 2) biotic agents such as decomposition processes and the intervention of animals/insects or roots; and 3) anthropogenic effects, i.e., human intervention in any stage following death. Environmental characteristics – climate, substratum and its pH, vegetation growth of molds, bacteria and fungi present in the soil and deposition of remains (open-air exposure vs. burial, for example) (Behrensmeyer, 1978) – must be taken into account when studying remains, as they can accelerate or retard the taphonomic processes (Ross and Cunningham, 2011).

1.3 Soil

The present research is based on buried human remains (bones and teeth), both unburned and burned. Bearing in mind that many bodies are buried in shallow graves, and thus in direct soil or substratum contact, it is crucial to understand how soil and its compounds act on the remains' surface. However, in a burial context soil is not the only factor affecting bone and teeth's integrity; the remains degradation will also depend on their composition (cortical or trabecular bone and organic content, for example), the influence of water, plant roots and pH. Thus, depending on the environment of burial, the

hypothesis here tested is that unburned and burned bone and teeth probably respond in different ways because they are intrinsically different. To investigate this research question, the documentation of chemical interactions between soil and bone and teeth was carried out in this study.

The soil is of extreme importance to understand both archaeological and forensic contexts (Surabian, 2012). However, it is arguably even more so in forensic cases as it can give clues and help to establish connections between a suspect, a victim and/or an object and the crime scene (Surabian, 2012; Woods et al., 2014a; 2014b). It can tell us about the type of inhumation – whether it is primary or secondary. Since soil is one of the basic agents of taphonomic processes, as it facilitates common ways of corpse deposition – on or under the surface – several researches have previously been focused on this feature.

There is a wide variety of soil types, with different compositions, properties and interactions with the remains, depending on the place they are buried (Fitzpatrick, 2008). This variety will depend on the following five factors: parent material, climate, organisms, topography and time (Soil Survey Division Staff, 1993; FAO, 2006; Fitzpatrick, 2008). Also, some properties can help with the identification of soil conditions; for instance, soil color – this feature is partly related with iron oxides and organic matter (FAO, 2006; Bigham and Ciolkosz, 1993 *in* Fitzpatrick, 2008) –, soil consistence – it can be loose, soft, firm, very hard or rigid (Soil Survey Division Staff, 1993; Fitzpatrick, 2008) –, soil texture – which depends on the proportion of sand, silt and clay in soil (Fitzpatrick, 2008) –, soil structure – concerning the way soil particles are organized and articulated amongst each other (Schoeneberger et al. 2002 *in* Fitzpatrick, 2008) –, and, finally, segregation fragments – or aggregation of different mineral particles (Fitzpatrick, 2008). Another very important factor to take into account is soil pH, which measures the acidity and the basicity of the soil (Surabian, 2012).

The type of soil and depth of burial are two elements that must be taken into account in forensic and archaeological investigations, as it will influence bone and teeth degradation (once a bone gets in contact with soil chemical reactions, accelerating decomposition takes place (Janaway, 1996)) and bone representativity. They influence the speed rate of body decomposition and, besides this, soil pressure is another important factor that can modify bone shape and increase the challenge of osteometric analysis (Henderson, 1987; Janaway, 1996; Pinheiro, 2006). For instance, in the presence of porous soils and near surface deposition, decomposition will be faster, in contrast to those

buried deeper or in coffins (Saukko and Knight, 2004; Pinheiro, 2006). When a body is buried, the low levels of air, difficulty of access by predators and insects and low temperatures will slow down decomposition rate (Henderson, 1987; Rodriguez, 1997; Turner and Wiltshire, 1999; Janaway, 1996; Fiedler and Graw, 2003; Pinheiro, 2006), mimicking refrigeration chamber conditions (Pinheiro, 2006). Adding to burial depth, other influent factors cannot be forgotten: the action of water will increase, quite a lot, the rate of decomposition and the dispersion of human remains (Saukko and Knight, 2004; Pinheiro, 2006; Ferreira, 2012). Water instigates microbial activity through chemical reactions affecting mineral compound's connections, aiding to an increase of bone degradation (Rogers, 2010; Buekenhout, 2014). Also, the interaction among soil and water is important; lighter soil will drain the water better than heavier ones (Saukko and Knight, 2004). Thus the lighter ones will accelerate decomposition due to the exchange of water and oxygen (Janaway, 1996). Also acidic soils are able to slow down cadaver decomposition (Carter and Tibbett, 2008). Contrary to what happens to cadavers with soft tissues, the action of acidic soils on dry bones increases its surface deterioration and decomposition, especially if they have a pH lower or equal to 5.3 (Gordon and Buikstra, 1981; Janaway, 2002; Surabian, 2012).

Soil is composed of organic and inorganic material (Woods et al., 2014a). However, the inorganic or mineral components have been much more studied than the organic ones (Dawson and Hillier, 2010; Woods et al., 2014a). It is known that acidic pH will break down the inorganic matrix of hydroxyapatite, leading to accelerated bone decomposition, as noted earlier on (Nafte, 2000 *in* Surabian, 2012).

Several studies involving soil and its influence on the decomposition process have already been conducted. However, the majority of these researches focused on cadavers or fresh bones such as the ones from Rodriguez and Bass (1985), Carter and Tibbett (2008) or, more recently, Chimutsa et al. (2015). Nonetheless, authors such as Gordon and Buikstra (1981) and Surabian (2012) developed experiments to understand bone preservation of skeletal remains buried in acidic soil, as is the case of the present research. However, those did not involve burned bones. Since many skeletal remains examined by biological anthropologists, especially forensic ones, have been subjected to high temperatures, it is critical to increase our knowledge about them and about burial-related taphonomy (Stiner et al., 1995; Bennett, 1999).

1.4 Bone and Teeth Composition

For the present research, analyses and observations were performed on cortical and trabecular bone, and also on teeth. As they are intrinsically different it is essential to describe their structure, shape, macro and microscopic composition for a better interpretation of the obtained results.

According to White and Folkens (2000), the human skeleton comprises three different types of bones which can be classified into: 1) long bones, 2) flat bones and 3) irregular bones. Van Wynsberghe et al. (1995), in turn, classified them into five categories, adding to the previous ones the short and sesamoid bones. However, not all authors classify sesamoid as a bone category; this is the case of Ross and Romrell (1989) that only classify bones into four categories: long, short, flat and irregular.

Despite the disagreement that still exists when it comes to the classification of bones according to their shape, microscopically they show some regularity (White and Folkens, 2000; White et al., 2012). It is possible to say that all bones share the same two essential structural components: cortical or compact bone and trabecular or spongy bone (Ross and Romrell, 1989; Van Wynsberghe et al., 1995; White and Folkens, 2000; White et al., 2012). Compact bone owes its name due to the fact that it presents a solid and dense appearance (Ross and Romrell, 1989; Van Wynsberghe et al., 1995; White and Folkens, 2000; White et al., 2012). Trabecular bone has a very porous structure resembling a sponge (Ross and Romrell, 1989; Van Wynsberghe et al., 1995; White and Folkens, 2000; White et al., 2012).

On the other hand, from a molecular viewpoint, bone is essentially composed of two intertwined components: collagen – that comprises most of the organic phase – and hydroxyapatite crystals (in mature bones) – the mineral or inorganic phase (Child, 1995; Van Wynsberghe et al. 1995; White and Folkens, 2000; Munro et al., 2007; Pijoan et al., 2007; Zazzo and Saliège, 2011; White et al., 2012). These properties are transversal to the bones of all mammal species and to cortical and trabecular bone (White et al., 2012). The organic phase is mainly composed (90%) of the protein molecule collagen that confers elasticity and flexibility to bone (White et al., 2012). On the other hand, the mineral phase provides bone with firmness and hardness (White et al., 2012).

Introduction

Teeth are composed of different components. A tooth is divided into two main parts: crown and root, being each of them covered with two different components (Turp and Alt, 1998; Lucas, 2004; Hilson, 2005). Whilst the crown is covered by enamel the root is covered by cement (Turp and Alt, 1998; Lucas, 2004; Hilson, 2005). Interiorly, the tooth is composed of dentine (Turp and Alt, 1998; Hilson, 2005).

Teeth also possess a molecular structure comprising organic and inorganic compounds. In dentine, cement and enamel (trace amounts) is also possible to find the most important fibrous protein that composes bone, collagen (Turp and Alt, 1998; Hilson, 2005). Dentin and cement also presents a big percentage of inorganic compounds (70%) in their composition whilst enamel is almost completely composed by inorganic compounds (95%) (Turp and Alt, 1998; Lucas, 2004; Hilson, 2005). The inorganic phase of teeth is mainly composed of calcium phosphate mineral present mainly in the form of apatite (Turp and Alt, 1998; Hilson, 2005). The reason why enamel is considered one of the hardest materials of the human body is due to its composition, which is almost entirely represented by mineral compounds (Von Koenigswald, 1982).

Any of the chemical or structural compounds of bone and teeth can be modified after death due to diagenetic processes (Munro et al, 2007). Possibly, these processes present differences according to the type of skeletal remains (compact, trabecular, and teeth) and according to their condition (burned or unburned). Such hypothesis was here investigated through Fourier-transform infrared spectroscopy, which will be explained later on.

1.5 Burned Bones and Teeth

This research is focused on burned bones and their comparison with unburned bones. Thus, it is of extreme importance to understand the burning processes, its progression and which changes occurs at each temperature to have a better notion of the different "entities" that are being compared. Unburned bones are distinguishable from burned bones, but the latter are also different from one another depending on the burning intensity at which they have been burned (Baby, 1954; Buikstra and Swegle, 1989; Thompson, 2005; Asmussen, 2009; Squires et al., 2011; Snoeck et al., 2014; Ellingham et al., 2015).

Since the 1960s, there has been an increase in the amount of research that focuses on burned bones. These studies were often carried out to better understand the alterations that heat produces in the structure, size and morphology of the skeleton and their implications for the evaluation of the biological profile (Ellingham et al. 2016), as heat-induced alterations interfere with the reliability of analytical methods usually applied for biological profiling (Piontek, 1975; Thompson, 2002; Fairgrieve, 2008; Gonçalves, 2011). Some authors performed studies that, among other things, allowed 1) to better understand the action of heat and consequent alterations in the skeletal mass (e.g. Klein, 2006; Gonçalves et al., 2013a); 2) to interpret rites and funerary practice more thoroughly (e.g. Etxeberria, 1994; Gonçalves et al., 2011; May, 2011; Squires et al., 2011); 3) to help with the identification of individuals in forensic contexts (e.g. Grévin et al., 1998; Thompson, 2004); and 4) to document structural and dimensional changes (e.g. Thompson, 2005; Thompson and Chudek, 2007; Ubelaker, 2009; Coelho, 2015).

Mayne Correia (1997) proposed four stages to classify bone transformations due to heat-induction, later revised by Thompson (2004). The latter one indicated that the first stage, dehydration, occurs between 100 ºC and 600 ºC. At this stage it is possible to observe fracture patterns and mass loss, due to the evaporation of water. The second stage is decomposition, meaning the destruction of organic compounds between 300 ºC and 800 ºC. The third stage is inversion, between 500 ºC and 1100 ºC, where it is possible to observe the elimination of carbonates. When the bone is exposed to temperatures over 700 ºC the fusion stage will take place, where one can observe changes in size (Thompson, 2004; Thompson, 2005; Ubelaker, 2009; Gonçalves, 2011; Ellingham et al., 2015; Ellingham et al., 2016). Harbeck et al. (2011), on the other hand, reached different results, probably due to the different burning conditions.

Cortical and trabecular bones on the present study were subject to 500 ºC, 900 ºC and 1050 ºC. Thus, according to the classification given by Thompson (2004) is predictable to observe at 500 ºC dehydration, decomposition and eventually inversion and at 900 ºC and 1050 ºC is possible to record all the stages mentioned above: dehydration, decomposition, inversion and fusion.

As proposed by the authors mentioned above, shrinkage (Figure 1.1), which is the most common heat-induced dimensional change at high temperatures, is more likely to occur due to changes in bone crystal structure (Thompson, 2005; Ubelaker, 2009; Gonçalves, 2011) which can be correlated to bone type, proportion between cortical and spongy bone and inorganic content (Shipman et al., 1984; Thompson, 2005; Ubelaker,

2009; Gonçalves, 2011). However, not all authors agree on whether cortical or spongy bone is more liable to heat-inducted shrinkage (Thompson, 2005). Bone condition and its biological age are also very important for the interpretation of heat-induced patterns (Thompson, 2005; Gonçalves et al., 2011).

Also, teeth have been the object of heat-induction studies to verify the preservation of dental evidence (e.g. Hill et al., 2011) and its potential for osteoprofiling. For example, Santos (2015) and Gouveia (2015) used burned teeth of identified individuals to estimate age-at-death and sex, respectively.

Figure 1.1 – Anterior view of tibia's proximal half of diaphysis. Left: unburned; right: burned at 1050 ºC with visible shrinkage.

Despite the fact that these studies have been performed to better understand the effects of temperature on bones and teeth, none of them observed the effect that burial has on this kind of remains, even though that burial has an important effect as it can increase and incite additional bone modifications such as in color, mass, dimensions, chemical composition, and shape, among others alterations. That is at the root of the present investigation, to obtain a deeper knowledge about this subject is the aim of this study and thus answer some of the questions that remain unsolved in this field.

Previous investigation provided some information about inhumed burned bones. Stiner et al. (1995), performed an experiment on bones burned in a controlled fire to examine the relationship between some changes caused by heat-induction such as alterations in coloration, chemical compounds (mineral and organic), mechanical properties of bone and to understand which soils are better at protecting buried bones from fires. Some years later, Bennett (1999) executed a similar study, involving burned bones and burial processes. Even so, the experimental conditions were a little bit different, since the author initially buried the bones and only later burned them by starting a fire at the surface capable of inflicting heat damage to the buried remains. So, it is possible to state that with the current experiment the inverse will be done; in other words, the bones will be burned firstly and buried later. With her study, Bennett (1999), wanted to identify some features presented by bones burned during burial considering heat intensity, duration and sedimentary formation. Both Stiner et al. (1995) and Bennett (1999) studies are important to establish some parallelisms with the present study as they analyze chemical and structural modifications and the effects of soil on buried burned bones, respectively.

Spectroscopic analysis allows to approximately infer at which temperatures bones were burned (Thompson, 2005; Thompson et al., 2009; Thompson et al., 2013; Ellingham et al., 2015; Ellingham et al., 2016). However, experimental research has been exclusively done on burned bones that were not submitted to post-burning inhumation. Therefore, the impact of diagenesis on spectroscopic markers is unknown. The present research intends to help filling this gap.

1.6 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy is a method of vibrational spectroscopy. It is one of the most used techniques to analyze the structure and composition of bone material, since it is very effective and sensitive on the analyzes of both mineral and organic phases of bone (Ruppel et al., 2006; Boskey and Camacho, 2007; Lebon et al., 2008; Chadefaux et al., 2009; Lebon et al., 2010; Berzina-Cimdina and Borodajenko, 2012). FTIR analysis comprises two methods: Attenuated Total Reflectance (ATR) and potassium bromide (KBr) (Thompson et al., 2009; Thompson et al. 2013). While ATR focus an infrared beam

directly into the sample and read its reflection, KBr is mixed with the sample becoming a transparent pellet in which the infrared beam cross the pellet (Thompson et al., 2009). Thus, one advantage of FTIR-ATR is that it is a non-destructive method and gives very specific information, in a particular wave number range, about peak location, intensity and width (Chadefaux et al., 2009; Berzina-Cimdina and Borodajenko, 2012).

Research in this field has been developed to better understand the differences in bones and teeth components of burned samples and unburned samples, normal and diseased bone, and also bones and teeth being constrained to burial environments (e.g. Stiner et al., 1995; Surovell and Stiner, 2001; Lee-Thorp and Sponheimer, 2003; Oréfice et al., 2003; Morris and Finney, 2004; Munro et al., 2007; Lebon et al., 2008; Stathopoulou et al., 2008; Thompson et al., 2009; Lebon et al., 2010; Squires et al., 2011; Lebon et al., 2014; Snoeck et al., 2014; Toffolo et al., 2015; Ellingham et al., 2016). However, none included unburned bones and experimentally burned bones inhumed under controlled conditions. This approach allows for the comparison of spectroscopic markers on different taphonomic stages. On the other hand, although FTIR-ATR has been proving the best method to perform the analysis, we will use FTIR-KBr, mainly due to the fact of the laboratory does not own FTIR-ATR.

Amongst the broad number of chemical functional groups present on bones, phosphate and carbonate groups are the most studied in this field, in many of their conformations. Phosphate is represented by four different vibrational modes, which are ν_1 (960 cm^{-1}), ν_2 (between 430 cm^{-1} and 450 cm$^{-1)}$, ν_3 (between 1028 and 1100 cm^{-1}) and ν_4 (565 cm^{-1} and 605 cm^{-1}), all of them belonging to PO_4^{2-} and HPO_4^{2-} functional groups (Lebon et al., 2008; Lebon et al., 2010; Thompson et al., 2013; Pestle et al., 2014). Carbonate group, as well, has different vibrational modes such as $\nu_3\,CO_3$ at 1415 cm^{-1} and $\nu_2\,CO_3^{2-}$ group at 874 cm^{-1}, approximately (Lebon et al., 2010; Thompson et al., 2013; Pestle et al., 2014). Also, collagen is composed of different functional groups in which is located amide group that originate several vibrational structures as: amide I (ν(C=O) vibration) at 1660 cm^{-1}, which perform the collagen secondary structure, amide II (ν(C-N)) at 1550 cm^{-1} and amide III at 1250 cm^{-1} (Chadefaux et al., 2009; Ellingham et al., 2015) (Figure 1.2).
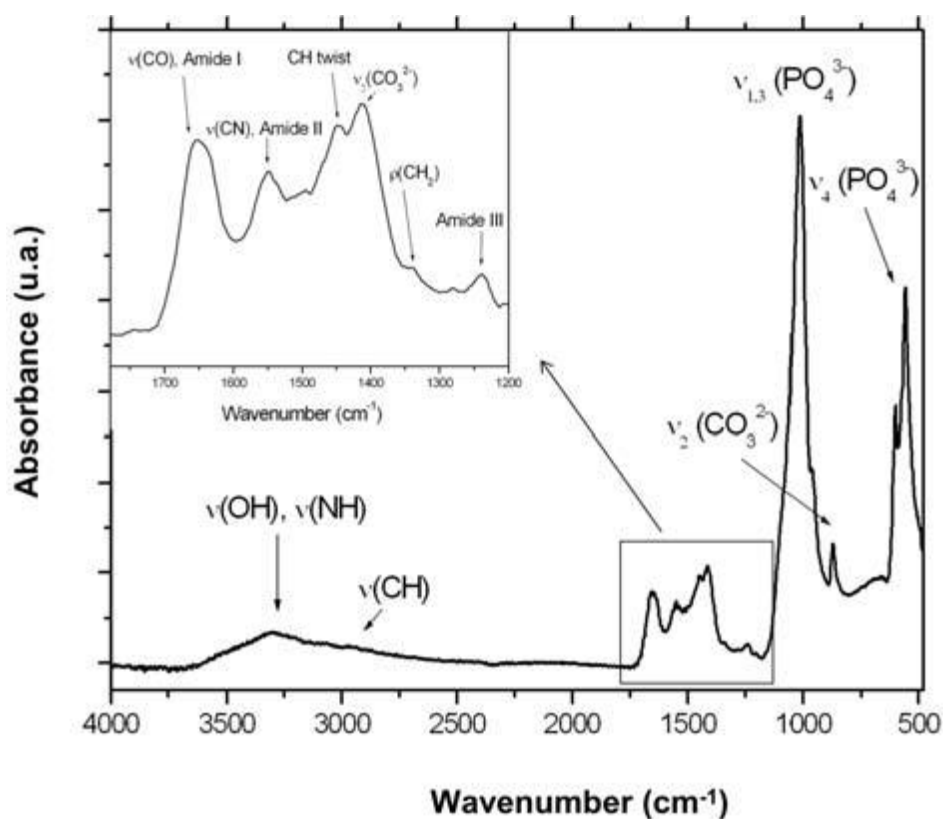
Figure 1.2 – Typical FTIR spectrum of bone material with bands assignment (Chadefaux et al., 2009).

However, due to the fact that heat induction and diagenetic or taphonomic alterations (caused by the burial environment) have a strong influence on bone and teeth mineral phases, on the organic matters, and on crystal rearrangements, some components and proteins are more interesting than others, regarding FTIR spectroscopy (Lebon et al., 2008; Chadefaux et al., 2009; Lebon et al., 2010; Reiche et al., 2010; Abraham et al., 2011; Lebon et al., 2011; Thompson et al., 2013). Thus, to this specific investigation, only v_3 and v_4 vibrational phosphate modes, $v_3\,CO_3$ and $v_2\,CO_3^{2-}$ carbonate groups are studied. More specifically, the analyses are focused on some ratios, calculated between the intensity of those vibrational modes, such as, C/P and CO_3^{-2}/P (adapted from Thompson et al., 2013). More, the splitting factor (SF) or crystallinity index (CI) - that represents the degree of order of a crystalline substance by separating the two antisymmetric bending phosphate bands (v_4PO_4) at 565 and 605 cm^{-1} - is another very important and significant factor to be studied (Lebon et al., 2008; Chadefaux et al., 2009; Lebon et al., 2010;

13

Abraham et al., 2011; Thompson et al., 2013). To calculate the CI, the following formula is used: CI = (Abs$_{565\ cm}$$^{-1}$ + Abs$_{605\ cm}$$^{-1}$) / Abs$_{595\ cm}$$^{-1}$ (Thompson et al., 2013).

1.7 Aims

Therefore, the main goal of this study is to investigate if there is differential post-depositional preservation between inhumed unburned bone and inhumed burned bone in function of time. Such hypothesis, which is suspected but not yet documented, may have major implications for the analysis of forensic and archaeological contexts.

Within that main objective, some specific secondary aims are inherent to this investigation:

1) Document mass loss or gain in inhumed unburned bone and inhumed burned bone;

2) Record eventual metrical modifications in inhumed unburned bone and inhumed burned bone;

3) Document chemical (organic and inorganic) differences through FTIR spectroscopy in inhumed unburned bone and inhumed burned bone.

All these aspects are observed in both trabecular and cortical bone and teeth to ascertain if there is some difference between them. Subsequently this investigation can be very useful to solve several problems inherent to burned bones and its modifications due to the effects of post-depositional agents as, for instance, the estimation of skeletal completeness and of the minimum number of individuals, interference with metric analysis and bone's chemical compounds.

2. Material and Methods

2.1 Sample Composition

The experiment was based on the burial of bone fragments and teeth and two different samples were used in this work. A control sample composed of unburned skeletal remains and a study sample composed of burned skeletal remains. In a total of 96 samples, 64 skeletal remains (32 cortical bones and 32 trabecular bones) and 32 teeth (third molars), in which 16 skeletal remains and 8 teeth remained unburned and the other 48 skeletal remains and 24 teeth were burned at three different temperatures (500 $^{\circ}$C, 900 $^{\circ}$C and 1050 $^{\circ}$C). Three different types of skeletal remains (cortical, trabecular and teeth) were used on this study because they are macroscopically organized in different forms and probably post-depositional taphonomy interacts with them differently. The cortical bones were represented by fragments of long bones (clavicle, humerus, ulna, radio, femur, tibia and fibula) while trabecular or mostly trabecular bones were represented by vertebrae fragments, patella, calcaneus, talus, navicular and cuboid. Long bones have in their composition cortical and trabecular bone. Since they were used to study cortical bone, they were sectioned into fragments, separating the epiphysis (mostly trabecular bone) from the diaphysis (mostly cortical bone). Another reason to divide long bones into fragments is the fact that the containers, where bones were buried, have a small spot for each fragment. None of the previous problems occurred with trabecular bones, except in vertebrae. Also, the division between trabecular and cortical bone was done mainly because this distinction is frequently used in most of the literature regarding burned bones. Concerning teeth, it was decided to use only third molars in order to homogenize the sample and also because it was easiest to obtain them.

Since biological profile information inherent to the identified skeletons, used for bioanthropological studies, was not available and not really critical to this investigation, and that the skeletons would be almost completely altered/destroyed by heat-induction and post-depositional taphonomy, we decided to use unidentified skeletons. The 64 skeletal remains belonged to three skeletons from the Capuchos Cemetery, Santarém (Ferreira et al., 2014), housed at the Laboratory of Forensic Anthropology in the Department of Life Sciences of the University of Coimbra. The studied teeth were provided by different dental clinics and extracted from adult women (see Gouveia, 2015 and Santos, 2015). The skeletons were female as well in order to ensure a homogeneous

sample as we wanted to avoid the possible existence of bone properties that may be inherently different between male and female individuals and consequently able to alter the results of the study, such as bone mass, bone composition and bone density. Since the skeletons were unidentified, a brief analysis regarding sex diagnosis and age at death was performed. For sex diagnosis, the DSP method (Murail et al.*,* 2005) was applied to the *os coxae*. To infer age at death, we used the Brooks and Suchey (1990) and Lovejoy et al. (1985) methods which are also applied to the *os coxae*.

2.2 Sample Treatment

The first thing to be done before observations can take place was the cleansing of the skeletons. For this procedure, a toothbrush and a stick were used in order to remove excess soil. After that, the remains were labeled with nail varnish and a transparency pen. As the cleansing procedure of the skeletons was taking place we also made the respective inventory by filling in a proper record sheet from the Laboratory of Forensic Anthropology. Bones from the right side of the skeleton were selected to proceed with the analysis since left bones were used on other investigations.

Four procedures were followed in this experiment: 1) weighing; 2) metric measurements; 3) photographic record; and 4) sample collection of bone powder. A more detailed description and relevance of these procedures is presented later in the text. These procedures were followed in four different moments: 1) the pre-sectioning stage; 2) the post-sectioning and pre-burning stage; 3) the post-burning stage; and 4) the post-burial stage. The four procedures were common to all four stages, but there were some exceptions; the pre-sectioning stage did not include the sample collection of bone powder; the teeth were not sectioned, thus being submitted only to burning and burial and their consequent procedures (all the stages are explained in detail below). Table 2.1 resumes the procedures undertaken for each type of bone and the respective stage.

Table 2.1 – Stages and procedures followed for each bone type and teeth. Tarsal bones, patella and teeth were not cut.

Stages	Procedures	Cortical	Trabecular Vertebrae	Trabecular Tarsal Bones and Patella	Teeth
Pre-sectioning	Weighing	✓	✓	✓	✓
	Metric measurements	✓	✓	✓	✓
	Photograpic records	✓	✓	✓	✓
	Sample collection of bone powder	✗	✗	✓	✓
Post-sectioning and Pre-burning	Weighing	✓	✓	✗	✗
	Metric measurements	✓	✓	✗	✗
	Photograpic records	✓	✓	✗	✗
	Sample collection of bone powder	✓	✓	✗	✗
Post-burning	Weighing	✓	✓	✓	✓
	Metric measurements	✓	✓	✓	✓
	Photograpic records	✓	✓	✓	✓
	Sample collection of bone powder	✓	✓	✓	✓
Post-burial	Weighing — Before sample	✓	✓	✓	✗
	Weighing — After sample	✓	✓	✓	✗
	Metric measurements	✓	✓	✓	✗
	Photograpic records	✓	✓	✓	✓
	Sample collection of bone powder	✓	✓	✓	✓

2.2.1 Pre-sectioning Stage

This first stage concerns three procedures: (i) metric measurements (mm) of the bones (Moore-Jansen et al., 1994) and teeth (adapted from Gouveia, 2015 and Santos, 2015), performed with an osteometric board, a measuring tape, a sliding caliper and, specifically in the case of teeth, a digital sliding caliper (Mitutoyo Digimatic, precision of 0.01mm); (ii) mass (g) of the bones was recorded with a digital balance (Kern EW600-2M, precision of 0.01g); (iii) photographic record, the pictures were taken with a Nikon COOLPIX P520 camera (lens Nikkor 42x wide optical zoom ED VR 4.3-180mm 1:3-

5:9) included a metric scale. Metric measurements were applied in order to record some eventual heat-induced and post-depositional metrical alterations. Also weighing was registered to interpret possible differences on bone mass due to heat-induction and post-depositional taphonomy. Photographic record is, equally, essential, since it makes possible future sample comparisons between inhumations.

For teeth, all the mentioned standardized procedures were followed, but only once, as these remains were not sectioned. The standard odontometric measurements were the following: the height (mm) from the cement-enamel junction (CEJ) to the apex; maximum tooth height (mm); root midpoint; bucco-lingual diameter at root midpoint (mm); mesio-distal diameter at root midpoint (mm); bucco-lingual diameter at CEJ (mm); mesio-distal diameter at CEJ (mm); bucco-lingual diameter of the crown (mm); and mesio-distal diameter of the crown (mm) (Appendix 8.1).

2.2.2   Post-sectioning and Pre-burning Stage

After the fulfillment of the first stage, it was possible to prepare bones for the second stage. Bone sectioning was carried out by using a hand saw. The long bones (cortical bone) were sectioned into four fragments: proximal epiphysis, distal epiphysis, proximal diaphysis and the distal diaphysis as the diaphysis was cut in the middle, leaving us with two fragments (Figure 2.1). Only the diaphysis fragments were used in the experiment. In the case of the clavicles, these were sectioned into two fragments, the acromial end and the sternal end. On the other hand, to trabecular bones, a different procedure was followed. Whilst the patella and the tarsals were not sectioned, the vertebrae were, at the pedicle point, being of use only the vertebral body (Figure 2.2).

This was done because vertebra is a "mixed" bone, it has both cortical and trabecular bone. To avoid difficulties in interpreting possible differences between cortical and trabecular bones was decided to remove neural arch. The trabecular bone traces remaining inside the long bones were removed with the support of a file tool. This procedure was done in order to avoid the risk of soil getting stuck in the trabecular bone and thus interfering with the bone's mass during analysis.

Figure 2.1 – Unburned femoral fragments from an unidentified skeleton of the Capuchos cemetery (CC_NI_16) used as representative of cortical bone. The femur was sectioned at mid-maximum length and at the metaphyseal region to remove the epiphyses. Top: diaphysis proximal half in posterior view; Bottom: diaphysis distal half in posterior view.

Figure 2.2 – Unburned vertebral body from an unidentified skeleton of the Capuchos cemetery (CC_NI_16) used as a representative of trabecular bone. It was sectioned in the pedicles region.

After sectioning the bones, the first three procedures, as already mentioned above, were applied once more (metric measurement, mass and photographic record). The metric measurements carried out on the fragments of the long bones (Appendix 8.1) were obviously different from the pre-sectioning stage and referred to: the maximum length; the proximal, medial and distal maximum diameters; and the proximal, medial and distal

maximum circumferences (adapted from Moore-Jansen et al., 1994) (Figure 2.3). It must be kept in mind that these measurements are only applicable to bone fragments and not standard measurements of complete bones. The measurements recorded on trabecular bones (Appendix 8.1) were: the maximum length and maximum breadth in tarsals (Figure 2.4); height, maximum breadth, maximum length and vertebral body length in vertebrae (Figure 2.4); and the maximum breadth, maximum length and maximum height in the patella (Figure 2.4). These measurements were selected since bone length, diameter and circumference can be easily repeated and replicated.

Moreover, further bone sampling for chemical analysis was carried out for Fourier transform infrared spectroscopy (FTIR) analysis. Samples from the most proximal extremity of the proximal diaphysis and the most distal extremity of the distal diaphysis of the long bone fragments were collected. As for the trabecular bones, the samples were taken from the articular surface region and cortical rim of the vertebral body. These samples were collected immediately below the bone surface, as this can be contaminated. To avoid contamination bone surface was scraped (this procedure only took place before the burn step). All the samples were collected with the aid of a scalpel blade and stored in a 1.5 ml microtube (approx. 0.5 ml).

Figure 2.3 – Fragment of femur's measurements. a: length; b: maximum distal diameter; c: maximum medial diameter; d: maximum proximal diameter.
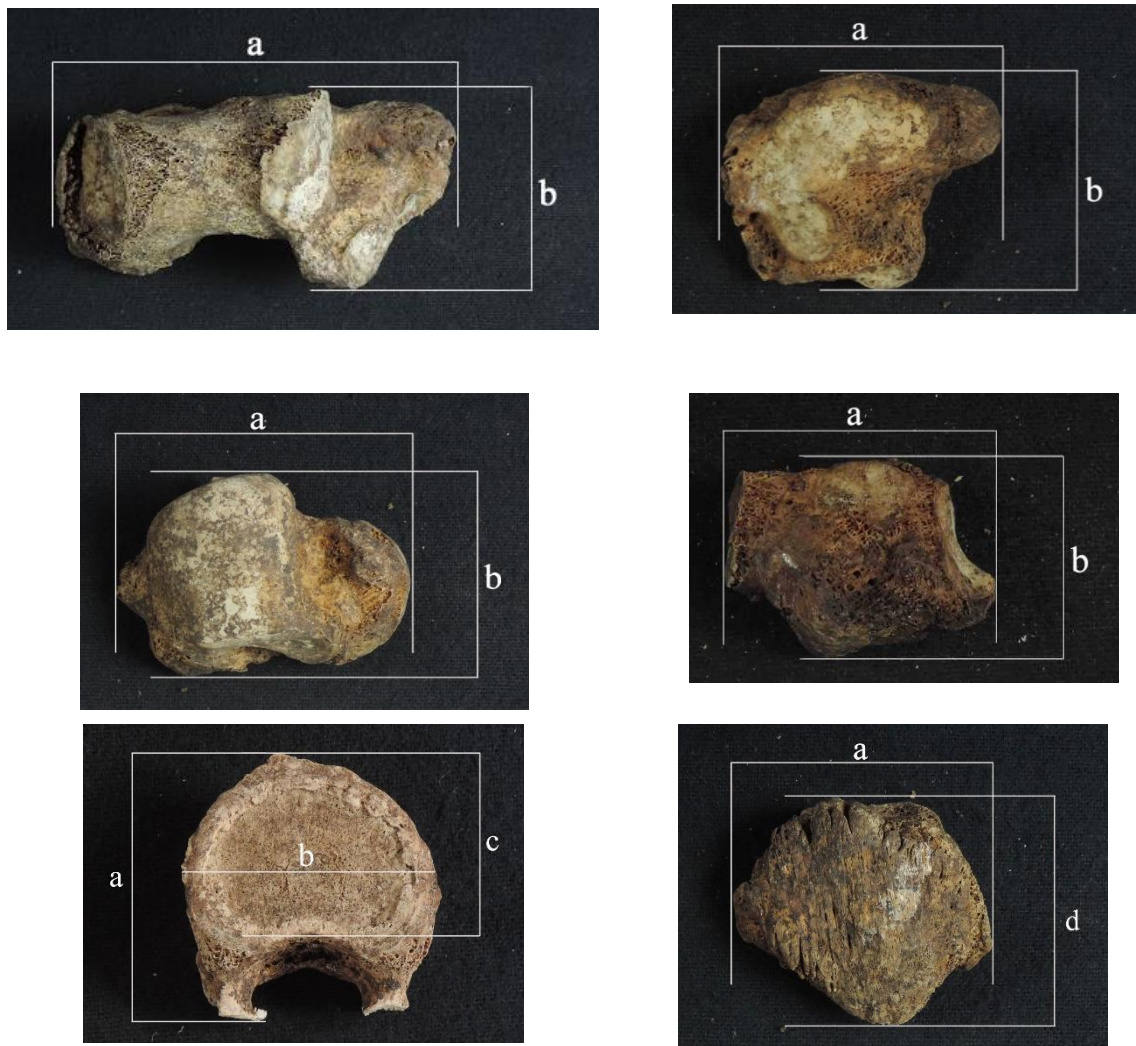
Figure 2.4 – Trabecular bone's measurements. Upper left: calcaneus; upper right: navicular; middle left: talus; middle right: cuboid; down left: vertebra; down right: patella. a: length; b: breadth; c: vertebral body length; d: height.

2.2.3   Post-burning Stage

After first and second stages had taken place for the unburned bones and teeth, the third stage followed up: burning. The maximum temperature reached is dependent on the burning conditions (e.g. fuel; oxygen availability; duration) (Shipman et al., 1984). In the present study, this procedure was performed under controlled conditions in an electric muffle furnace (Barracha, K-3 three-phased, 14A), that subjected bones and teeth to high temperatures that inevitably lead to heat-induced changes (Figure 2.5). However, different bone changes occur at different temperatures, regardless of the device. Shipman et al. (1984) investigated four features that are affected by heating: color, microscopic

21

morphology, crystalline structure and shrinkage. Adding to this, several years earlier Baby (1954) and Binford (1963) recorded another heat-induced feature: fracture patterns, also referred by Spennemann and Colley (1989), Mayne Correia (1997), Thompson (2004) and more recently Thompson and Chudek (2007). This research did not analyze all the possible alterations produced by heat-induction; the most important and of greater relevance to the present research are: bone mass loss, eventual metrical changes, crystalline structure and chemical compounds alterations.

Figure 2.5 – Muffle furnace, Barracha, K-3 three-phased, 14A, containing teeth (bottom) and some sectioned bones (top).

For 48 skeletal remains (24 cortical and 24 trabecular) and 24 teeth, burning was carried out at three different maximum temperatures 500 °C, 900 °C and 1050 °C. At each temperature, eight cortical and eight trabecular bones were burned as well as eight teeth. Thereafter, the procedures mentioned at the beginning of this chapter (metric and mass measurements, photographic record and samples collection) were repeated for the burned bones and teeth (Appendix 8.2). Concerning to weighing, it was done before and after the sample collection of bone powder. This is done because the comparisons of loss mass are between a certain inhumation (at T1, e.g.) and the previous one (at T0, e.g.) and occurs a mass loss after the sample collection, is essential to do both weighing.

2.2.4   Post-burial Stage

After all the observations have been performed it was possible to proceed to the fourth and last stage of this study. All the samples were buried in eight containers with an acid substrate (pH 4.0-4.5) (Annex 7.1). The container was divided into a grid with 8 spots, each of them labeled with relevant information about the sample, as can be seen in Figure 2.6. Thirty two cortical samples were buried into four containers (eight bones in each) while 32 trabecular samples were buried into the other four containers (eight bones in each); eight teeth per container were also buried alongside the trabecular bones.

Figure 2.6 – Bone burial into the containers filled up with acid substrate.

Twenty-four samples (8 cortical bones, 8 trabecular bones and 8 teeth) were exhumed and analyzed every 2 months, making it possible to recover four sets of data (Appendix 8.3). After 6 months, 24 other samples were analyzed for the first time, increasing the number of samples to a total of 48 (Appendix 8.3). Once again, all the procedures, previously described, were applied. However, considering the great fragmentation of teeth, only photographic record and sample collection of bone powder were performed.

Although this investigation has been projected to, at least, 10 years, due to master's timings limitations, we decided that a periodicity of 2 and 6 months was more suitable to start this study. The analyses performed every 2 months were completed in one day, i.e., the samples were exhumed and re-inhumed on the same day. This was possible because at this point only two samples (four in the case trabecular bones' container which include teeth) from each container were analyzed. One container at a

time was examined. After removing the substrate from each grid cell, skeletal remains and teeth were analyzed, repeating all the procedures. When the analysis was finished, skeletal remains returned to the spots which were refilled with the substrate removed before.

The containers were placed outdoor (third floor of the Department of Life Sciences), with a net covering from eventual insects, birds or other small animals' interference, under the seasonal weather conditions (Figure 2.7). Also, in the figure 2.7 it is possible to observe the growth of vegetation, probably due to the seeds included in the substrate and the favorable climacteric conditions.

Figure 2.7 – Eight containers with trabecular and cortical bone fragments and teeth filled up with acidic substrate and a net covering. Visible vegetation's growth.

2.3 Statistical Analysis

Due to the sample size, which is small, only descriptive statistics were used to calculate the average and frequency and thus make comparisons between the different groups of samples. These calculations were performed with Excel from Microsoft Office. The variation of mass and metric measurements were calculated applying the following formula: [(altered dimension – original dimension)/original dimension] x 100 (adapted from Shipman et al., 1984). Since sampling was done for each bone every two months,

their mass was consequently affected by that procedure. This meant that the variation from the first bi-monthly burial until the last bi-monthly exhumation could not be calculated directly. Therefore, a theoretical variation was calculated indirectly by adding (or subtracting, depending on each case) the percentage variation that was recorded for every bi-monthly inhumation to the original mass. For example, a bone weighing 10g in the first bi-monthly inhumation and weighing 12g at the end of it, increased its mass in 20%. After sampling, the very same bone would forcibly weigh less than those 12g when buried for the second bi-monthly inhumation, for instance 11g. In turn, the mass variation at the end of this second bi-monthly inhumation was then calculated in reference to the post-sampling mass, and so on for the next inhumations. Given this, these percentage mass variations were then used to estimate the mass of each bone if no sampling had been done.

2.4 FTIR Spectroscopy

The Fourier Transform Infrared (FTIR) spectra were recorded on a Bruker Optics Vertex 70 FTIR spectrometer, purged by CO_2-free dry air, in the 400-4000 cm^{-1} mid-IR range, using 7.0 mm diameter KBr disks (ca. 1% w/w). A KBr beam splitter and a liquid nitrogen cooled Mercury Cadmium Telluride (MCT) detector were used. The spectra were collected for *ca.* 2 minutes (128 scans), with a 2 cm^{-1} resolution. The error in wavenumbers was estimated to be less than 1 cm^{-1}.

The KBr disks for FTIR transmission analysis were obtained by mixing 100 mg of KBr and 1 mg of bone powder, approximately. After, these two compounds were well mixed, half of the mixture (50 mg) was compacted in a hand-press (Spectra-Tech) for 30 seconds (approx.) to assemble the final pellet.

3. Results

3.1 Trabecular and Cortical Bone's Mass Variation

As we can see in Table 3.1 after two months, an increase of trabecular bone's mass occurred, being more visible in unburned bones and bones burned at 500 °C. The same happened to the bones buried for six months, but with the addition of the bones burned at 900 °C, although to a lesser extent. Subsequent bi-monthly exhumations revealed a slight increase until the fourth exhumation (after eight months) in which it was possible to observe a decrease of bone's mass. When analyzing the last column of Table 3.1 (variation from the first bi-monthly burial until the last bi-monthly exhumation), one can perceive an increase in bone mass over time. Nonetheless, in general the vertebrae were the bones where it was possible to see a greater variation on bone's mass. In the particular case of 16V06, after the first observation a large decrease in bone's mass variation was recorded. This occurred due to a destruction of the fragment as can be seen in the Figure 3.1. Thus this vertebra was removed from the analysis.

Figure 3.1 – Destroyed vertebral body from individual 16 burned at 900 ºC.

On Figure 3.2, it is possible to observe an initial decrease due to the burning process followed by a slight increase of bone's mass after two months of burial.

Results

Generally, on the second and third exhumations, bone's mass increased steadily until the fourth observation in which a decrease was visible.

Table 3.1 – Bi-monthly and six-monthly descriptive analysis of the mass variation on trabecular bones.

Bone	T (°C)	m_0 oct2015 m_1 dec2015 $\Delta m/m_0$ (%)	m_1 dec2015 m_2 feb2016 $\Delta m/m_1$ (%)	m_2 feb2016 m_3 april2016 $\Delta m/m_0$ (%)	m_0 oct2015 m_3 april2016 $\Delta m/m_0$ (%)	m_3 april2016 m_4 jun2016 $\Delta m/m_3$ (%)	m_0 oct2015 m_4 jun2016 $\Delta m/m_0$ (%)
16Cub	Unb	40.6	3.5	7.3	-	-4.0	49.9
16V05	Unb	47.9	-2.0	14.2	-	-6.9	54.1
16V04	Unb	-	-	-	25.0	-	-
17Cub	Unb	-	-	-	17.1	-	-
16Pa	500	45.6	2.6	7.4	-	-1.3	58.4
16Nav	500	49.2	-0.2	3.6	-	-3.0	49.7
17V05	500	-	-	-	35.0	-	-
17V04	500	-	-	-	32.1	-	-
16Ta	900	12.3	1.7	1.4	-	-1.2	14.4
16V06[1]	900	28.3	-21.8	-11.0	-	-15.9	-24.9
17Pa	900	-	-	-	18.4	-	-
17V07	900	-	-	-	40.8	-	-
16Ca	1050	11.8	3.4	5.3	-	-3.2	17.8
16V07	1050	25.2	4.1	5.0	-	-5.6	29.2
17Ca	1050	-	-	-	9.7	-	-
17Ta	1050	-	-	-	6.5	-	-
Average		33.2	1.9	6.3	23.1	-3.6	35.2

[1] values corresponding to this sample were removed from the average calculations.

Figure 3.2 – The evolution of trabecular bone's mass variation from T0 (before burn) to T4 (fourth bi-monthly exhumation after sample collection) and from T0 (before burn) to T3 (after sample collection) to bones buried for six months. The values refer to absolute mass variations in grams.

In cortical bones, an increase in its mass after two and six months was noticeable, mainly in bones burned at 500 °C (Table 3.2). Generally, after eight months, bone's mass showed a slight decrease. However, after examining the last column of Table 3.2, concerning the variation from the first bi-monthly burial until the last bi-monthly exhumation, a mass increase over time is clear.

Table 3.2 – Bi-monthly and six-monthly descriptive analysis of the mass variation in cortical bones.

		m_0 oct2015 m_1 dec2015	m_1 dec2015 m_2 feb2016	m_2 feb2016 m_3 april2016	m_0 oct2015 m_3 april2016	m_3 april2016 m_4 jun2016	m_0 oct2015 m_4 jun2016
Bone	**T (ºC)**	$\Delta m/m_0$ (%)	$\Delta m/m_1$ (%)	$\Delta m/m_2$ (%)	$\Delta m/m_0$ (%)	$\Delta m/m_3$ (%)	$\Delta m/m_0$ (%)
16T02	**Unb**	3.9	3.4	2.2	-	-5.9	3.3
16R02	**Unb**	2.1	1.9	9.4	-	-4.3	9.0
17T02	**Unb**	-	-	-	7.3	-	-
17R01	**Unb**	-	-	-	9.0	-	-
16F02	**500**	35.2	2.0	1.7	-	-0.8	39.1
16H01	**500**	34.4	1.1	3.6	-	-0.8	39.6
16Cl02	**500**	-	-	-	25.7	-	-
17F02	**500**	-	-	-	35.0	-	-
16U01	**900**	4.6	2.4	2.0	-	0.3	9.6
16Fib01	**900**	3.6	0.0	3.8	-	0.1	7.6
16R01	**900**	-	-	-	3.4	-	-
17U01	**900**	-	-	-	2.9	-	-
16F01	**1050**	7.7	1.6	1.6	-	-1.0	10.0
16T01	**1050**	6.1	1.8	2.3	-	-1.5	8.8
16H02	**1050**	-	-	-	4.2	-	-
17F01	**1050**	-	-	-	8.0	-	-
Average		12.2	1.8	3.3	11.9	-1.7	15.1

As can be seen in Figure 3.3, after heat-induction, a loss on bone's mass was quite clear. However, in the first two months of burial, a slight increase of mass for some bones and a stabilization throughout the next six months could be observed. Also, the same slight increase could be seen on the bones exhumed after six months.
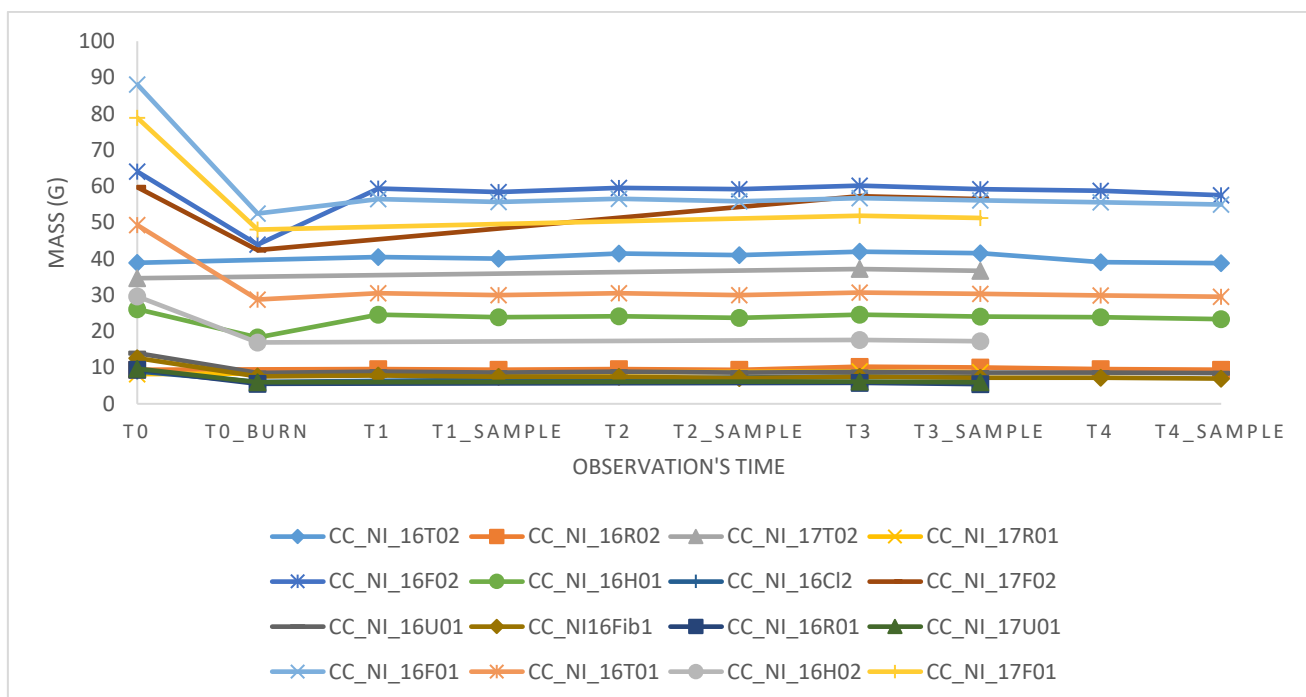
Figure 3.3 – The evolution of cortical bone's mass variation from T0 (before burn) to T4 (fourth bi-monthly exhumation after sample collection) and from T0 (before burn) to T3 (after sample collection) to bones buried for six months. The values refer to absolute mass variations in grams.

3.2 Trabecular Bone's Metrical Variation

3.2.1   Height

Concerning the height of trabecular bones, in general, the variations observed on both tarsals and vertebrae were not too large and this can most probably be attributed to measurement errors. The only exception may have been the navicular of individual 16 (burned at 500 ºC), which decreased 8.6% in height from month 2 to month 4 (Table 3.3). In the same individual, the unburned vertebra 16V05 increased substantially in size after the second month (22.2%) which suggests that this was not entirely due to measurement error, especially because subsequent measurements in months 4, 6, 8 and the variation from burial time to eighth month (28%) appear to confirm that result (Table 3.4). Although not as large, vertebra 16V04 also revealed an important size change (10.5%).

Table 3.3 – Bi-monthly and six-monthly descriptive analysis of the patella and tarsal bone's height variation.

Bone	T (°C)	h_0 oct2015 h_1 dec2015 $\Delta h/h_0$ (%)	h_1 dec2015 h_2 feb2016 $\Delta h/h_1$ (%)	h_2 feb2016 h_3 april2016 $\Delta h/h_2$ (%)	h_0 oct2015 h_3 april2016 $\Delta h/h_0$ (%)	h_3 april2016 h_4 jun2016 $\Delta h/h_3$ (%)	h_0 oct2015 h_4 jun2016 $\Delta h/h_0$ (%)
16Cub	Unb	2.9	-5.6	2.9	-	0.0	0.0
17Cub	Unb	-	-	-	0.0	-	-
16Pa	500	0.0	-2.4	-2.4	-	2.5	-2.0
16Nav	500	2.9	-8.6	0.0	-	0.0	-6.0
17Pa	900	-	-	-	-2.8	-	-
Average		1.9	-5.5	0.2	-1.4	0.8	-3.0

Table 3.4 – Bi-monthly and six-monthly descriptive analysis of the vertebrae bone's height variation.

Bone	T (°C)	vh_0 oct2015 vh_1 dec2015 $\Delta vh/vh_0$ (%)	vh_1 dec2015 vh_2 feb2016 $\Delta vh/vh_1$ (%)	vh_2 feb2016 vh_3 april2016 $\Delta vh/vh_2$ (%)	vh_0 oct2015 vh_3 april2016 $\Delta vh/vh_0$ (%)	vh_3 april2016 vh_4 jun2016 $\Delta vh/vh_3$ (%)	vh_0 oct2015 vh_4 jun2016 $\Delta vh/vh_0$ (%)
16V05	Unb	22.2	0.0	4.5	-	0.0	28.0
16V04	Unb	-	-	-	10.5	-	-
17V05	500	-	-	-	0.0	-	-
17V04	500	-	-	-	0.0	-	-
17V07	900	-	-	-	4.3	-	-
16V07	1050	-5.3	5.6	5.3	-	0.0	5.0
Average		8.5	2.8	4.9	3.7	0.0	17.0

Figure 3.4 reveals a decrease in the height of trabecular bones, mainly in 16Ta, 16Nav, 17V05 and 16V06 due to burning processes. In the following observations, after heat-induction, some minor fluctuations have been recorded. However, as mentione

above, most of these post-depositional changes cannot be safely attributed to other than measurement changes.
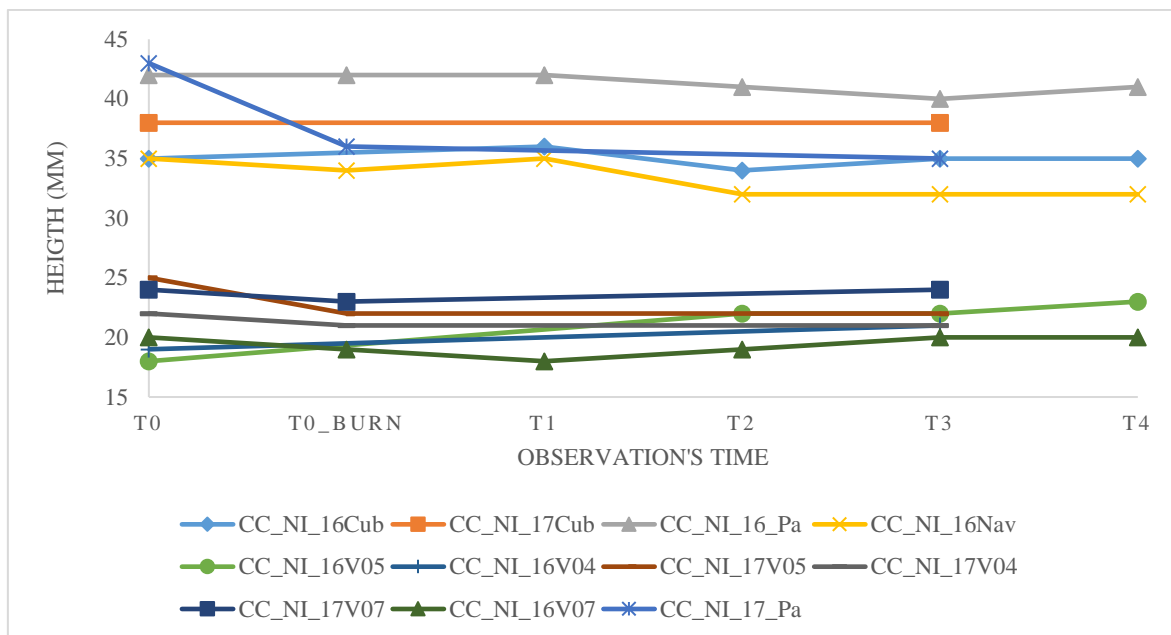
Figure 3.4 – The evolution of trabecular bone's height variation from T0 (before burn) to T4 (fourth bi-monthly exhumation after sample collection) and from T0 (before burn) to T3 (after sample collection) to bones buried for six months. The values refer to absolute size variations in mm.

3.2.2   Breadth

In table 3.5, an increase in the breadth of 16Ca of 12.9% after two months of burial was the largest recorded variation. Concerning variations from burial until the last bi-monthly observation, 16Nav (500 ºC) revealed a decrease of 13%. However, variations nearing 10% were also documented for several bones and several inhumation times. Also, the value of 17Ta is very discrepant from the others; it can be a real value or an error of measurement or excel insertion.

Table 3.5 – Bi-monthly and six-monthly descriptive analysis of the patella and tarsal bone's breadth variation.

Bone	T (ºC)	b_0 oct2015 b_1 dec2015 $\Delta b/b_0$ (%)	b_1 dec2015 b_2 feb2016 $\Delta b/b_1$ (%)	b_2 feb2016 b_3 april2016 $\Delta b/b_2$ (%)	b_0 oct2015 b_3 april2016 $\Delta b/b_0$ (%)	b_3 april2016 b_4 jun2016 $\Delta b/b_3$ (%)	b_0 oct2015 b_4 jun016 $\Delta b/b_0$ (%)
16Cub	Unb	0.0	4.5	-4.3	-	9.1	9.0
17Cub	Unb	-	-	-	4.8	-	-
16Pa	500	5.6	5.3	-10.0	-	0.0	0.0
16Nav	500	-8.7	-4.8	-5.0	-	5.3	-13.0
16Ta	900	0.0	-8.6	0.0	-	0.0	-9.0
17Pa	900	-	-	-	0.0	-	-
16Ca	1050	12.9	0.0	-5.7	-	-9.1	-3.0
17Ca	1050	-	-	-	5.7	-	-
17Ta[1]	1050	-	-	-	-31.3	-	-
Average		2.0	-0.7	-5.0	3.5	1.1	-3.0

[1] values corresponding to this sample were removed from the average calculations.

Once more, in Figure 3.5, a decrease on the breadth of trabecular bones due to heat-induction was visible. A more pronounced increase was seen in 16Ca during the first two months and decrease after four months of burial. Also in 17Ta, a decrease was perceptible after six months.
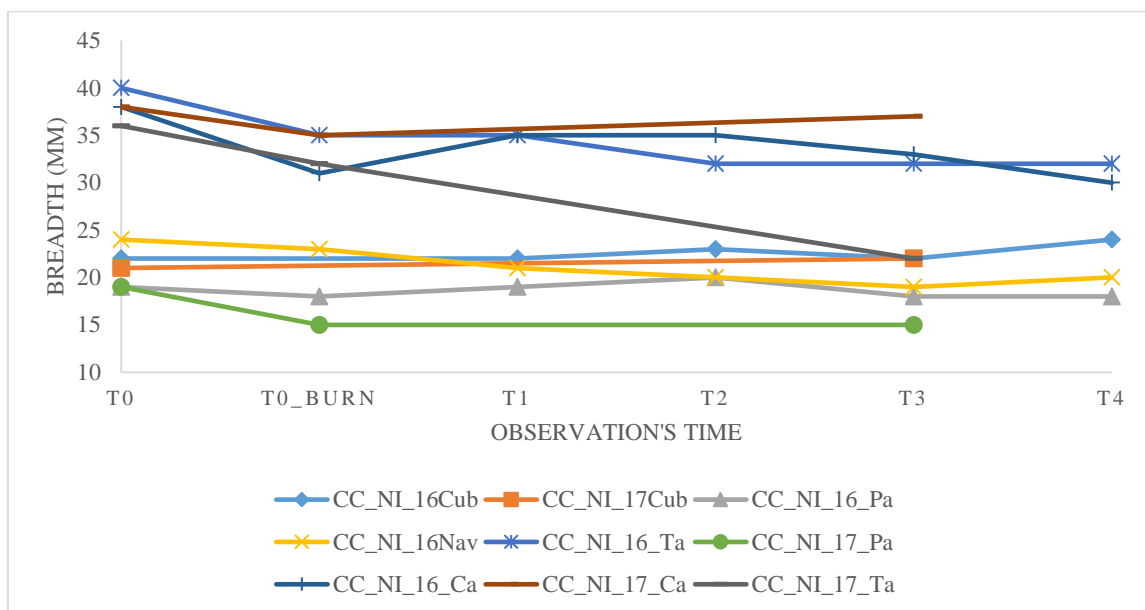
Figure 3.5 – The evolution of trabecular bone's breadth variation from Time 0 (before burning) to Time 4 (fourth bi-monthly exhumation after sample collection) and from T0 (before burn) to T3 (after sample collection) to bones buried for six months. The values refer to absolute size variations in mm.

3.2.3   Length

Table 3.6 and Figure 3.6 demonstrate that the post-depositional metric variations concerning tarsals and patella length were very slight. The possible exception were the cuboid from individual 16 and the navicular of individual 16 for which a 8.0% and a 7.3% changes were recorded after two and 6 months, respectively. However, the variation after eight months does not seem to show relevant changes that can be attributed to other than measurement error with certainty.

Table 3.6 – Bi-monthly and six-monthly descriptive analysis of the patella and tarsal bone's length variation.

Bone	T (°C)	$l_0$oct2015 $l_1$dec2015 $\Delta ml/ml_0$ (%)	$l_1$dec2015 $l_2$feb2016 $\Delta ml/ml_1$ (%)	$l_2$feb2016 $l_3$april2016 $\Delta ml/ml_2$ (%)	$l_0$oct2015 $l_3$april2016 $\Delta ml/ml_0$ (%)	$l_3$april2016 $l_4$jun2016 $\Delta ml/ml_3$ (%)	l_0 oct2015 l_4 jun016 $\Delta ml/ml_0$ (%)
16Cub	Unb	-8.0	4.3	-4.2	-	4.3	-4.0
17Cub	Unb	-	-	-	-4.0	-	-
16Pa	500	-2.3	-2.4	0.0	-	0.0	-5.0
16Nav	500	-2.4	0.0	-7.3	-	5.3	-5.0
16Ta	900	2.2	-4.3	0.0	-	0.0	-2.0
17Pa	900	-	-	-	-2.9	-	-
16Ca	1050	1.5	-1.4	-4.4	-	0.0	-4.0
17Ca	1050	-	-	-	0.0	-	-
17Ta	1050	-	-	-	-4.8	-	-
Average		-1.8	-0.8	-3.2	-2.9	1.9	-4.0
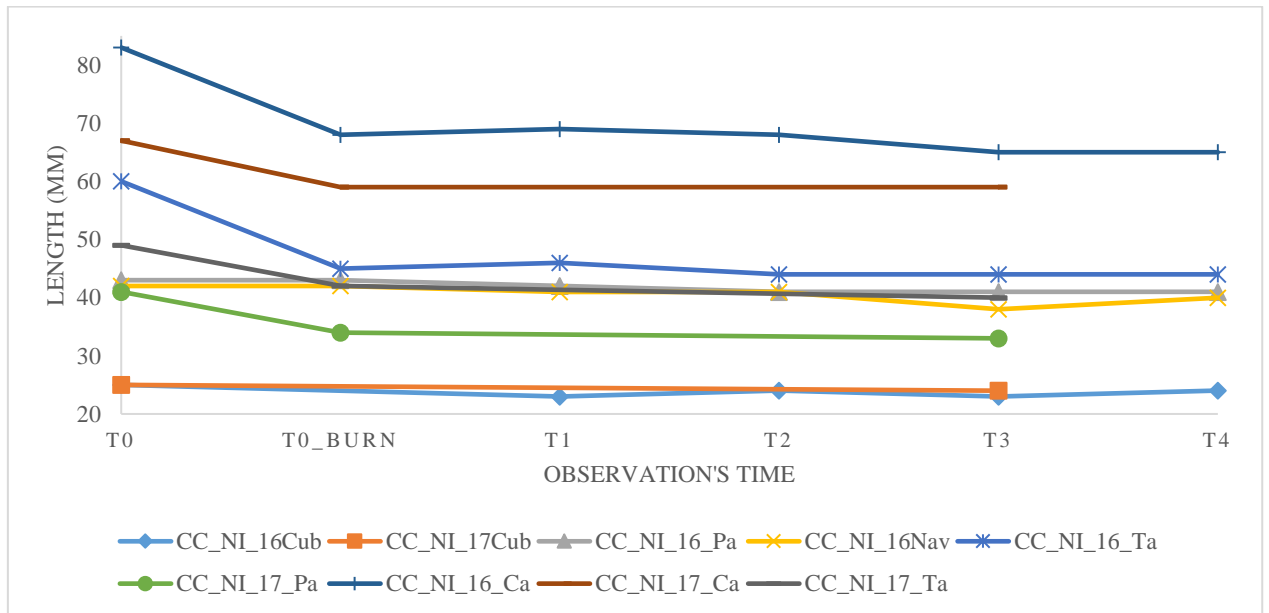
Figure 3.6 – The evolution of trabecular bone's length variation from Time 0 (before burn) to Time 4 (fourth bi-monthly exhumation after sample collection) and from T0 (before burn) to T3 (after sample collection) to bones buried for six months. The values refer to absolute size variations in mm.

3.2.4 Vertebral Breadth

Both unburned vertebrae, 16V05 and 16V04, exhumed after two and six months, respectively, stands out from the other vertebrae with a larger positive variation of the vertebral breadth (Table 3.7). These vertebrae had already shown major changes regarding their height thus supporting the previous assessment that this change may not be entirely due to measurement error.

Table 3.7 – Bi-monthly and six-monthly descriptive analysis of the vertebral bone's breadth variation.

Bone	T (ºC)	vb_0 oct2015 vb_1 dec2015 $\Delta vb/vb_0$ (%)	vb_1 dec2015 vb_2 feb2016 $\Delta vb/vb_1$ (%)	vb_2 feb2016 vb_3 april2016 $\Delta vb/vb_2$ (%)	vb_0 oct2015 vb_3 april2016 $\Delta vb/vb_0$ (%)	vb_3 april2016 vb_4 jun2016 $\Delta vb/vb_3$ (%)	vb_0 oct2015 vb_4 jun2016 $\Delta vb/vb_0$ (%)
16V05	Unb	10.0	0.0	4.5	-	0.0%	15.0
16V04	Unb	-	-	-	18.8	-	-
17V05	500	-	-	-	-2.5	-	-
17V04	500	-	-	-	-9.1	-	-
17V07	900	-	-	-	0.0	-	--
16V07	1050	3.4	-3.3	0.0	-	-3.4	-3.0
Average		6.7	-1.7	2.3	1.8	-1.7	6.0

Besides the decrease in the vertebral breadth of some vertebrae after burning process, metrical variations over the four exhumations appear not be so large as Table 3.7 seems to expose (Figure 3.7).
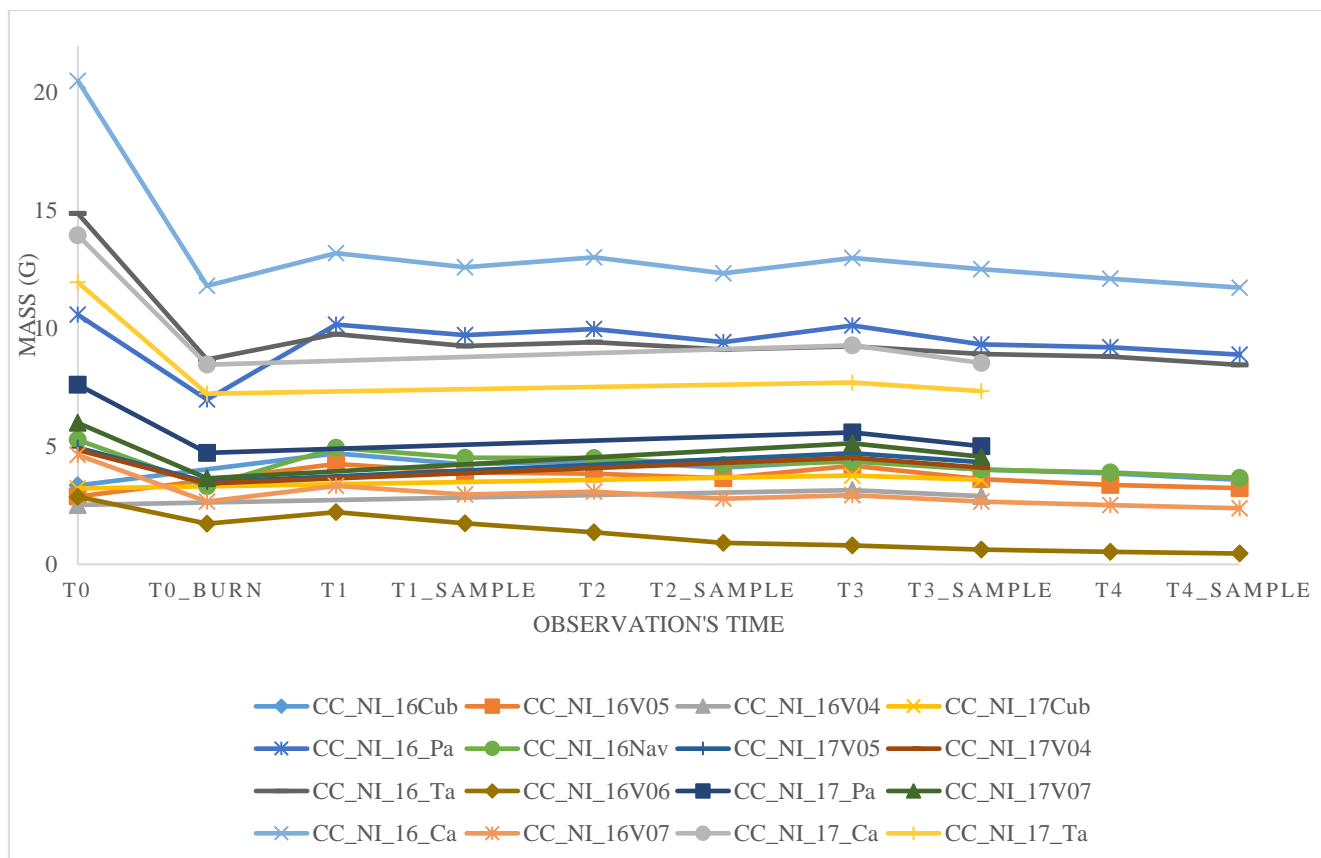
37
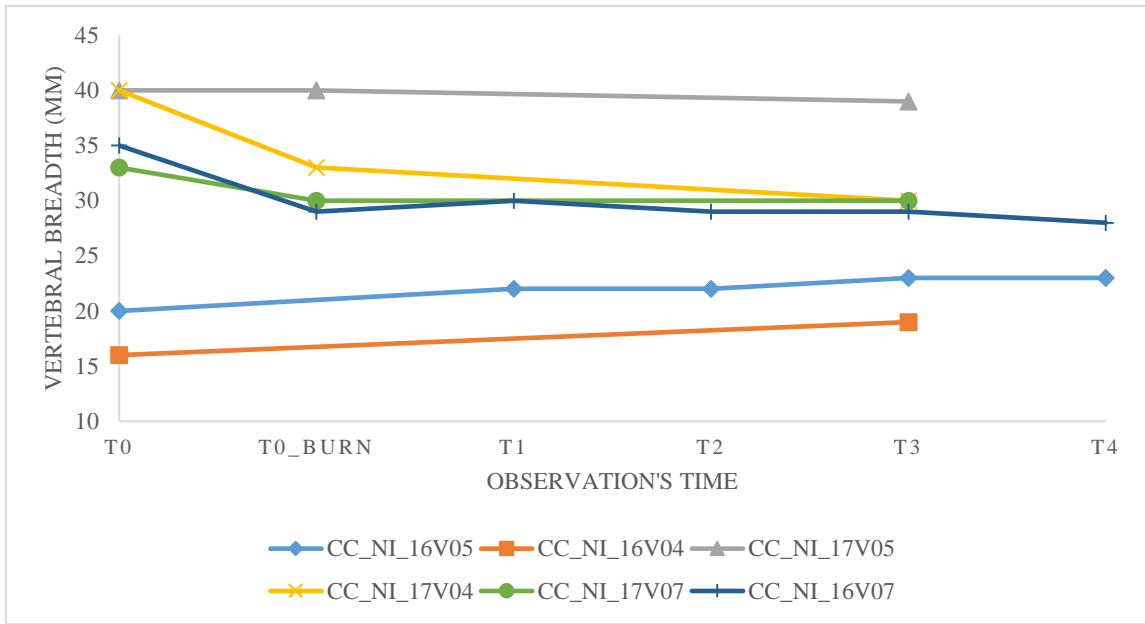
Figure 3.7 – The evolution of vertebral bone's breadth variation from Time 0 (before burn) to Time 4 (fourth exhumation after sample collection) and from T0 (before burn) to T3 (after sample collection) to bones buried for six months. The values refer to absolute size variations in mm.

3.2.5   Vertebral Length

In the Table 3.8, it is shown that 17V05, burned at 500 °C, was the fragment whose length shrunk the most after six months of inhumation. In the case of the unburned vertebrae from individual 16, none appeared to present the same size variations that have been recorded for both height and breadth. The 17V05 vertebra increased its length after heat-induction, but contrary to other vertebrae, revealed a continuous post-depositional length decrease (Figure 3.8).

Table 3.8 – Bi-monthly and six-monthly descriptive analysis of the vertebral bone's length variation.

| Bone	T (ºC)	vl_0 oct2015 vl_1 dec2015 $\Delta vl/vl_0$ (%)	vl_1 dec2015 vl_2 feb2016 $\Delta vl/vl_1$ (%)	vl_2 feb2016 vl_3 april2016 $\Delta vl/vl_2$ (%)	vl_0 oct2015 vl_3 april2016 $\Delta vl/vl_0$ (%)	vl_3 april2016 vl_4 jun2016 $\Delta vl/vl_3$ (%)	vl_0 oct2015 vl_4 jun2016 $\Delta vl/vl_0$ (%)
16V05	Unb	6.1	2.9	2.8	-	-2.7	9.0
16V04	Unb	-	-	-	6.3	-	-
17V05	500	-	-	-	-16.2	-	-
17V04	500	-	-	-	0.0	-	-
17V07	900	-	-	-	5.4	-	-
16V07	1050	3.3	3.2	-6.3	-	3.3	3.0
Average		4.7	3.0	-1.7	-1.1	0.3	6.0
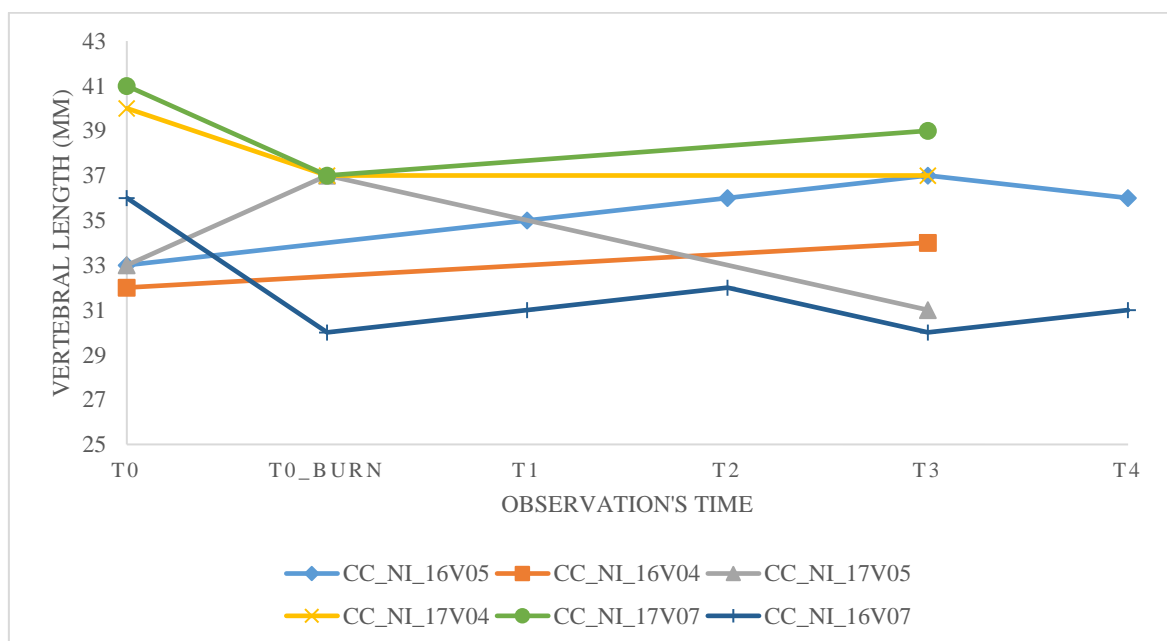
Figure 3.8 – The evolution of vertebral bone's length variation from Time 0 (before burn) to Time 4 (fourth bi-monthly exhumation after sample collection) and from T0 (before burn) to T3 (after sample collection) to bones buried for six months. The values refer to absolute size variations in mm.

3.2.6 Vertebral Body Length

After six months of inhumation, 17V05 (500 °C) and 17V04 (500 °C), slightly reduced their vertebral body length (Table 3.9) although it is not clear if this can be attributed to measurement error alone. In general, no important changes can be identified with certainty. A decrease after heat-induction followed by a stabilization was documented in all cases regarding the vertebral body length (Figure 3.9).

Table 3.9 – Bi-monthly and six-monthly descriptive analysis of the vertebral body length variation.

Bone	T (ºC)	vbl_0 oct2015 vbl_1 dec2015 $\Delta vbl/vbl_0$ (%)	vbl_1 dec2015 vbl_2 feb2016 $\Delta vbl/vbl_1$ (%)	vbl_2 feb2016 vbl_3 april2016 $\Delta vbl/vbl_2$ (%)	vbl_0 oct2015 vbl_3 april2016 $\Delta vbl/vbl_0$ (%)	vbl_3 april2016 vbl_4 jun2016 $\Delta vbl/vbl_3$ (%)	vbl_0 oct2015 vbl_4 jun2016 $\Delta vbl/vbl_0$ (%)
16V05	Unb	5.9	0.0	0.0	-	0.0	6.0
16V04	Unb	-	-	-	6.3	-	-
17V05	500	-	-	-	-8.3	-	-
17V04	500	-	-	-	-8.0	-	-
17V07	900	-	-	-	-4.0	-	-
16V07	1050	-4.3	0.0	0.0	-	0.0	-4.0
Average		0.8	0.0	0.0	-3.5	0.0	1.0
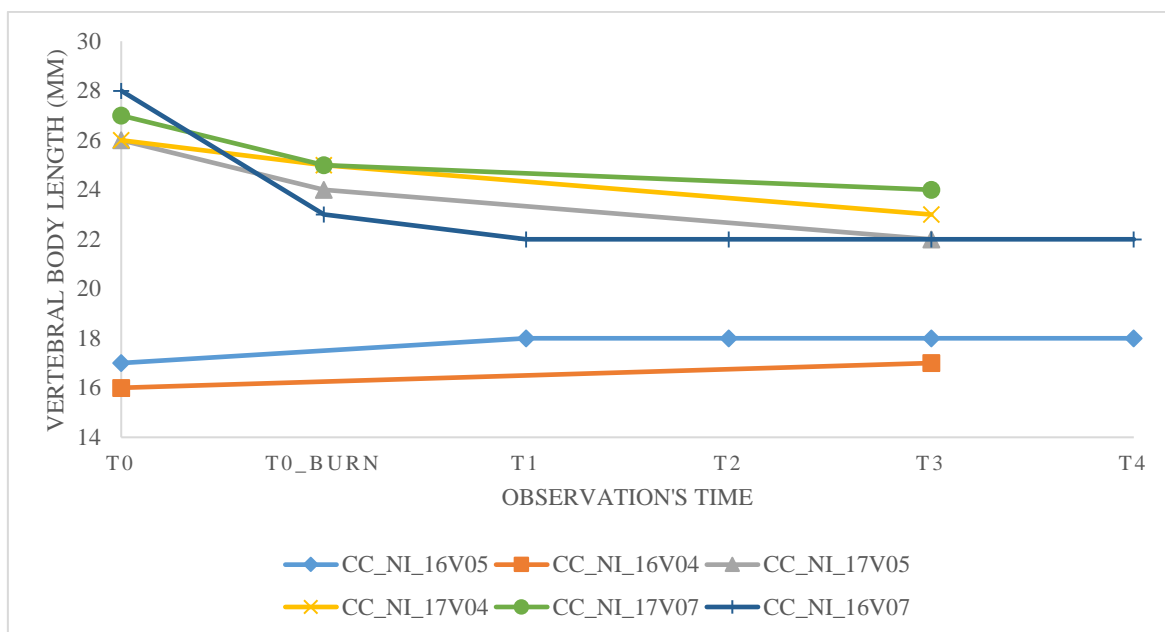
Figure 3.9 – The evolution of vertebral body length variation from Time 0 (before burn) to Time 4 (fourth exhumation after sample collection) and from T0 (before burn) to T3 (after sample collection) to bones buried for six months. The values refer to absolute size variations in mm.

3.3   Cortical Bone's Metrical Variation

Regarding the cortical bones and their measurements (length, proximal diameter, medial diameter, distal diameter, proximal circumference, medial circumference and distal circumference), a decrease in all measurements after the burning process was recorded. After all scheduled exhumations, no significant variation on cortical bone measurements has been detected (varying from -9.1% to 22.7%, although most of the values are around 0.0%), exception to 17T02 (unburned) and 16Cl02 (500 ºC), with an increase of 22.7% and 12.0% at the end of its first biannual exhumation in the medial diameter and distal diameter, respectively (due to space constraints, tables and figures were included in Appendix 8.4).

3.4 Post-depositional Chemometric Variation

3.4.1   Crystallinity Index
3.4.1.1 Trabecular Bones

Table 3.10 reveals greater CI values in fragments burned at 900 ºC and 1050 ºC than in unburned fragments and fragments burned at 500 ºC both after heat-induction and post-deposition. Unburned bones and bones burned at 500 ºC showed more stable crystallinity indices. In general, vertebrae were the bones showing greater variations of CI values. Regarding exhumations performed at the sixth month, bi-monthly exhumed bones decrease their CI while bones exhumed only after six months increase the CI. Except in the cases of the bones exhumed after six months burned 1050 ºC that maintain CI values. Also to CI, vertebra 06 of individual 16 and 16Cub (at the second exhumation) revealed completely different values.

Table 3.10 – Values of crystallinity index to unburned trabecular bones and bones burned from 500 ºC to 1050 ºC (from pre-burning to the fourth bi-monthly exhumation).

Bone	T (ºC)	Before burn	After burn	Two months	Four months	Six months	Eight months
16Cub	Unb	3.6	-	3.4	12.5	3.3	3.8
16V05	Unb	2.5	-	3.6	3.8	3.3	4.0
16V04	Unb	3.3	-	-	-	3.3	-
17Cub	Unb	3.6	-	-	-	3.9	-
16Pa	500	3.3	3.8	3.8	4.0	3.9	3.9
16Nav	500	3.2	3.6	3.8	4.0	3.7	4.0
17V05	500	3.5	4.0	-	-	4.5	-
17V04	500	3.3	4.2	-	-	4.5	-
16Ta	900	3.3	6.1	6.4	5.6	5.0	4.9
16V06	900	3.4	10.2	10.6	12.4	11.2	12.8
17Pa	900	3.1	6.8	-	-	7.6	-
17V07	900	3.5	7.5	-	-	8.4	-
16Ca	1050	3.3	5.6	5.5	6.9	5.8	5.4
16V07	1050	3.3	6.3	6.9	10.0	7.0	7.7
17Ca	1050	3.3	6.2	-	-	6.1	-
17Ta	1050	3.3	5.6	-	-	5.7	-

3.4.1.2 Cortical Bone

Regarding the CI of cortical bones (Table 3.11), values increased with burning temperature increase, until 900 ºC, decreasing at 1050 ºC. During the observations, CI values seemed to be quite stable up to 500 ºC. However, unburned bones exhumed only at the end of six months revealed a slightly increase of the CI values. Greater variations were verified during exhumations for bones burned at higher temperatures, mainly after the first exhumation (two months) where marked differences were recorded. For instances, the fragment of proximal ulna of the individual 16 (16U01) had CI fluctuations between 5.8 and 8.6 (excluding the before burning value), on the other hand, a proximal fragment of the femur of the individual 16 (16F01) showed a constant decrease of CI from 7.6 to 6.0.

Table 3.11 – Values of crystallinity index to unburned cortical bones and bones burned from 500 ºC to 1050 ºC (from pre-burning to the fourth bi-monthly exhumation).

Bone	T (ºC)	Before burn	After burn	Two months	Four months	Six months	Eight months
16T02	Unb	3.1	-	3.2	3.2	3.2	3.5
16R02	Unb	3.1	-	3.1	3.3	3.1	3.1
17T02	Unb	3.0	-	-	-	3.3	-
17R01	Unb	3.1	-	-	-	3.5	-
16F02	500	3.1	3.6	3.5	3.8	3.5	3.6
16H01	500	3.2	3.5	3.7	3.6	3.6	3.7
16Cl02	500	3.0	3.5	-	-	3.5	-
17F02	500	3.0	3.7	-	-	3.8	-
16U01	900	3.2	6.2	5.8	8.0	6.0	8.6
16Fib01	900	3.2	7.0	6.7	7.4	6.0	7.8
16R01	900	3.1	7.0	-	-	9.3	-
17U01	900	3.1	7.6	-	-	7.6	-
16F01	1050	3.1	7.6	7.3	6.9	6.9	6.0
16T01	1050	3.1	5.1	6.5	6.3	7.7	6.2
16H02	1050	3.1	5.8	-	-	6.5	-
17F01	1050	3.2	6.2	-	-	7.4	-

3.4.1.3 Teeth

As can be seen in Table 3.12, also teeth increased CI values with heat-induction increase up to 900 ºC and a slightly decrease at 1050 ºC. Unburned teeth and teeth burned at 500 ºC showed more stable CI values than teeth burned at 900 ºC and 1050 ºC. These last ones revealed greater fluctuations during the four bi-monthly exhumations, except for tooth O4 that increased after two months of burial and then decreased continuously over six months, from 9.2 to 7.8.

Table 3.12 – Values of crystallinity index to unburned teeth and teeth burned from 500 ºC to 1050 ºC (from pre-burning to the fourth bi-monthly exhumation).

Tooth	T (ºC)	Before burn	After burn	Two months	Four months	Six months	Eight motnhs
AC8a	Unb	3.1	-	3.1	3.2	3.0	3.1
MD89	Unb	3.4	-	3.0	3.1	3.1	3.1
D4	Unb	3.1	-	-	-	3.1	-
H441	Unb	3.1	-	-	-	3.2	-
T9	500	3.1	3.2	3.4	3.5	3.4	4.1
AE26	500	3.1	3.3	3.5	3.5	3.6	3.7
M2	500	3.1	3.4	-	-	3.6	-
AI54	500	2.9	3.3	-	-	3.5	-
O4	900	3.1	6.3	9.2	8.3	8.1	7.8
AH3	900	3.1	6.9	8.7	7.5	6.6	6.8
AH10	900	3.0	6.7	-	-	8.2	-
M10	900	3.0	8.2	-	-	7.7	-
AC10b	1050	3.2	7.6	7.2	6.8	6.2	6.5
J25	1050	3.2	6.4	6.5	7.1	6.2	6.5
V2	1050	3.0	4.1	-	-	7.4	-
N17	1050	3.0	6.1	-	-	8.3	-

3.4.2   C/P ratio

3.4.2.1 Trabecular Bone

In Table 3.13, a decrease of C/P ratio with burning temperature increase is perceptible. In several bones (except 16Ca) it was visible that C/P ratio decreased to zero with temperature increase (mainly to 900 ºC and 1050 ºC). In general, those values maintained after burial. Also C/P ratio values of unburned bones and bones burned at 500 ºC seemed to remain stable.

Table 3.13 – Values of C/P ratio of unburned trabecular bones and bones burned from 500 ºC to 1050 ºC (from pre-burning to the fourth bi-monthly exhumation).

Bone	T (ºC)	Before burn	After burn	Two months	Four months	Six months	Eight months
16Cub	Unb	0.2	-	0.3	0.1	0.2	0.1
16V05	Unb	0.5	-	0.3	0.3	0.2	0.2
16V04	Unb	0.2	-	-	-	0.3	-
17Cub	Unb	0.2	-	-	-	0.2	-
16Pa	500	0.3	0.2	0.2	0.2	0.1	0.2
16Nav	500	0.3	0.2	0.2	0.2	0.2	0.2
17V05	500	0.2	0.1	-	-	0.1	-
17V04	500	0.1	0.1	-	-	0.1	-
16Ta	900	0.2	0.0	0.0	0.1	0.1	0.1
16V06	900	0.3	0.0	0.0	0.0	0.0	0.0
17Pa	900	0.3	0.0	-	-	0.0	-
17V07	900	0.2	0.0	-	-	0.0	-
16Ca	1050	0.3	0.2	0.2	0.0	0.1	0.1
16V07	1050	0.3	0.0	0.1	0.0	0.1	0.0
17Ca	1050	0.2	0.0	-	-	0.0	-
17Ta	1050	0.2	0.0	-	-	0.0	-

3.4.2.2 Cortical Bone

We can see in Table 3.14 that for cortical bone, samples decreased their C/P ratio values when temperature increased. Both unburned bones and bones burned at 500 ºC showed very regular C/P ratio values. Only 16R02 increased slightly its C/P value in the third observation (sixth month). Also bones burned at 900 ºC and 1050 ºC revealed quite stable values with small variations.

Table 3.14 – Values of C/P ratio to unburned cortical bones and bones burned from 500 ºC to 1050 ºC (from pre-burning to the fourth bi-monthly exhumation).

Bone	T (ºC)	Before burn	After burn	Two months	Four months	Six months	Eight months
16T02	Unb	0.3	-	0.3	0.3	0.3	0.3
16R02	Unb	0.3	-	0.3	0.3	0.4	0.3
17T02	Unb	0.3	-	-	-	0.3	-
17R01	Unb	0.3	-	-	-	0.3	-
16F02	500	0.3	0.2	0.2	0.2	0.2	0.2
16H01	500	0.3	0.2	0.2	0.2	0.2	0.2
16Cl02	500	0.3	0.2	-	-	0.2	-
17F02	500	0.3	0.2	-	-	0.2	-
16U01	900	0.3	0.1	0.1	0.0	0.1	0.1
16Fib01	900	0.3	0.1	0.0	0.0	0.0	0.0
16R01	900	0.3	0.1	-	-	0.0	-
17U01	900	0.3	0.1	-	-	0.0	-
16F01	1050	0.3	0.0	0.0	0.1	0.1	0.1
16T01	1050	0.3	0.1	0.1	0.0	0.1	0.1
16H02	1050	0.3	0.2	-	-	0.0	-
17F01	1050	0.3	0.0	-	-	0.0	-

3.4.2.3 Teeth

In Table 3.15, a decrease of C/P with heat-induction temperature increase is visible. Once again, C/P values decreased to zero when samples were burned at 900 ºC and 1050 ºC. All the samples remained very stable during the four bi-monthly exhumations. However, a slight variation in tooth AC8a in the second bi-monthly exhumation was visible.

Table. 3.15 – Values of C/P ratio to unburned teeth and teeth burned from 500 ºC to 1050 ºC (from pre-burning to the fourth bi-monthly exhumation).

Tooth	T (ºC)	Before burn	After burn	Two months	Four months	Six months	Eight months
AC8a	Unb	0.3	-	0.3	0.1	0.3	0.3
MD89	Unb	0.3	-	0.3	0.3	0.3	0.3
D4	Unb	0.3	-	-	-	0.3	-
H441	Unb	0.3	-	-	-	0.3	-
T9	500	0.3	0.2	0.1	0.1	0.1	0.1
AE26	500	0.3	0.2	0.1	0.1	0.1	0.1
M2	500	0.3	0.2	-	-	0.1	-
AI54	500	0.3	0.2	-	-	0.2	-
O4	900	0.3	0.0	0.0	0.0	0.0	0.0
AH3	900	0.3	0.0	0.0	0.0	0.0	0.0
AH10	900	0.3	0.0	-	-	0.0	-
M10	900	0.3	0.0	-	-	0.0	-
AC10b	1050	0.3	0.0	0.0	0.0	0.0	0.0
J25	1050	0.3	0.0	0.0	0.0	0.0	0.0
V2	1050	0.3	0.0	-	-	0.0	-
N17	1050	0.3	0.0	-	-	0.0	-

3.4.3 CO_3^{-2}/P ratio

3.4.3.1 Trabecular Bone

In Table 3.16, the difference between unburned and burned bones is not so clear. However, a slight decrease of CO_3^{-2}/P value with heat-induction temperature increase is visible. During the exhumations any relevant variation of the CO_3^{-2}/P was recorded.

Table 3.16 – Values of CO_3^{-2}/P ratio to unburned trabecular bones and bones burned from 500 ºC to 1050 ºC (from pre-burning to the fourth bi-monthly exhumation).

Bone	T (ºC)	Before burn	After burn	Two months	Four months	Six months	Eight months
16Cub	Unb	0.1	-	0.1	0.0	0.1	-
16V05	Unb	0.3	-	0.1	0.1	0.1	0.1
16V04	Unb	0.1	-	-	-	0.2	-
17Cub	Unb	0.1	-	-	-	0.1	-
16Pa	500	0.1	0.1	0.1	0.1	0.1	0.1
16Nav	500	0.1	0.1	0.1	0.1	0.1	0.1
17V05	500	0.1	0.1	-	-	0.0	-
17V04	500	0.1	0.0	-	-	0.0	-
16Ta	900	0.1	0.0	0.0	0,0	0.0	0.0
16V06	900	0.1	0.0	0.0	0.0	0.0	0.0
17Pa	900	0.1	0.0	-	-	0.0	-
17V07	900	0.1	0.0	-	-	0.0	-
16Ca	1050	0.1	0.1	0.1	0.0	0.0	0.0
16V07	1050	0.1	0.0	0.0	0.0	0.0	0.0
17Ca	1050	0.1	0.0	-	-	0.0	-
17Ta	1050	0.1	0.0	-	-	0.0	-

3.4.3.2 Cortical Bone

The same trend occurred in cortical bones, showing a CO_3^{-2}/P ratio decrease with burning temperature, although not being so evident (Table 3.17). CO_3^{-2}/P ratio kept stable over all the exhumations.

Table 3.17 – Values of CO_3^{-2}/P ratio to unburned cortical bones and bones burned from 500 ºC to 1050 ºC (from pre-burning to the fourth bi-monthly exhumation).

Bone	T (ºC)	Before burn	After burn	Two months	Four months	Six months	Eight months
16T02	Unb	0.2	-	0.1	0.2	0.2	0.1
16R02	Unb	0.2	-	0.2	0.2	0.2	0.2
17T02	Unb	0.2	-	-	-	0.2	-
17R01	Unb	0.2	-	-	-	0.2	-
16F02	500	0.1	0.1	0.1	0.1	0.1	0.1
16H01	500	0.1	0.1	0.1	0.1	0.1	0.1
16Cl02	500	0.2	0.1	-	-	0.1	-
17F02	500	0.2	0.1	-	-	0.1	-
16U01	900	0.1	0.0	0.0	0.0	0.0	0.0
16Fib01	900	0.2	0.0	0.0	0.0	0.0	0.0
16R01	900	0.2	0.0	-	-	0.0	-
17U01	900	0.2	0.0	-	-	0.0	-
16F01	1050	0.2	0.0	0.0	0.0	0.0	0.0
16T01	1050	0.1	0.0	0.0	0.0	0.0	0.0
16H02	1050	0.2	0.1	-	-	0.0	-
17F01	1050	0.1	0.0	-	-	0.0	-

3.4.3.3 Teeth

In teeth, the decrease of CO_3^{-2}/P ratio followed the same pattern of the CO_3^{-2}/P ratio of trabecular and cortical bones. Tooth AC8a presented some fluctuations of CO_3^{-2}/P during exhumations. CO_3^{-2}/P ratio values of the other samples revealed to be very constant over bi-monthly observations (Table 3.18).

Table 3.18 – Values of CO_3^{-2}/P ratio to unburned teeth and teeth burned from 500 ºC to 1050 ºC (from pre-burning to the fourth bi-monthly exhumation).

Tooth	T (ºC)	Before burn	After burn	Two months	Four months	Six months	Eight months
AC8a	Unb	0.2	-	0.1	0.3	0.2	0.1
MD89	Unb	0.1	-	0.1	0.1	0.2	0.1
D4	Unb	0.1	-	-	-	0.2	-
H441	Unb	0.2	-	-	-	0.2	-
T9	500	0.1	0.1	0.1	0.1	0.1	0.0
AE26	500	0.1	0.1	0.1	0.1	0.1	0.1
M2	500	0.1	0.1	-	-	0.1	-
AI54	500	0.2	0.1	-	-	0.1	-
O4	900	0.1	0.0	0.0	0.0	0.0	0.0
AH3	900	0.1	0.0	0.0	0.0	0.0	0.0
AH10	900	0.1	0.0	-	-	0.0	-
M10	900	0.1	0.0	-	-	0.0	-
AC10b	1050	0.1	0.0	0.0	0.0	0.0	0.0
J25	1050	0.1	0.0	0.0	0.0	0.0	0.0
V2	1050	0.2	0.0	-	-	0.0	-
N17	1050	0.2	0.0	-	-	0.0	-

4. Discussion

4.1 Post-depositional Mass Variation

Investigations performed by Person et al. (1996), Thompson (2004), Enzo et al. (2007), amongst others, show that heat-induction treatment on bone promotes mass loss. This occurs mainly due to water and organic components loss caused by dehydration and decomposition (Shipman et al., 1984; Thompson, 2004; Kalsbeek and Richter, 2006; Gonçalves et al., 2013a). In the present study mass loss after burning was also evident, thus agreeing with the conclusions reached by the mentioned investigators. Nonetheless, the samples used in other studies were not subjected to burial. One of the questions we wanted to answer was if additional mass loss or gain occurs in inhumed unburned bones and inhumed burned bones. This research confirmed that inhumed bones are indeed affected by mass changes. Although trends (mass increase vs mass decrease) tended to be similar among all bones, differences were found according to the type of bone (trabecular vs compact) and to the degree of heat treatment (or its absence).

A similar pattern was observed for the first exhumations taking place after two and six months - a clear trend for an increase of bone's mass in both trabecular and cortical bone. However, from the sixth to the eighth months of inhumation, a decrease in bone's mass was visible. The fourth bi-monthly exhumation was done in June, when the weather was not so rainy. Therefore, water must have played an important role regarding mass variation, increasing it during rainy periods and decreasing it during dry periods. Those variations can be explained by hydration and dehydration due to weathering. Delannoy et al. (2016) also concluded that the environment has a great influence in bone's mass variation, suggesting that more humid and colder environment will inhibit bone's mass loss. On the other hand, factors as bone micro-fragmentation and bio erosion due to action of decomposer microorganisms may also affect bone's mass loss. Furthermore, plants promote the absortion of water from soil which, in some way, may produce alterations in bone's mass by removing some of its water.

As mentioned above, trends were similar but the magnitude of the changes varied a lot among inhumed bones. After two months, a large difference in bone's mass increase between unburned trabecular bone (41-48%) and unburned cortical bone (2-4%) was notorious. Indeed, trabecular unburned bones increased as much in mass as trabecular

bones burned at 500 ºC (46-49%), in contrast to cortical unburned bones which had a much smaller mass increase than cortical bones burned at 500 ºC (34-35%). At 900 ºC and 1050 ºC, both trabecular (12%, if only non-vertebral bones are taken into consideration) and cortical bones (4-5% and 6-8%, respectively) had slight increase in masses. So, trabecular bones had considerable mass increases if unburned or burned at 500 ºC. Vertebrae revealed not to be the best and most reliable specimens to use in this study since they present very different results in comparison to the other trabecular fragments. This can mainly be due to the treatment applied to the vertebrae (pedicles removal) and its intrinsic fragility which could lead to an increase of the vulnerability to the intrusion of exogenous material worsening, even more, their preservation. On the other hand, only cortical bones burned at 500 ºC had a considerable mass increase.

When we analyzed the values of mass variation from the first bi-monthly burial until the last bi-monthly exhumation (after eight months) we recorded a similar trend. Only 16V06 revealed a decrease in bone mass between the first and last exhumation, but this can be explained by the bone's destruction. Comparisons with other researches can be made only with one concerning unburned bones, by Delannoy et al. (2016) who reached different results from the present study. On their study, they buried some unburned ribs in a content with a clay soil at a pH of 6.8 under cover, protecting the samples from rain but not from the high moisture, and others under a hood within controlled temperature and moisture. They verified that, in general, bones lost mass during the first days, stabilizing or decreasing slightly their mass later. Bone's mass was recorded every day during 90 days contrary to the present research that recorded bone's mass every two months during eight months. All these different parameters lead to different results which are not easy to explain. Nonetheless, Child (1995) and Hedges and Millard (1995) agree that bone's porosity, microstructure and chemical composition will affect their interaction with water and microorganisms on soil.

Bones burned at 500 ºC have been subjected to dehydration and decomposition of the organic phase (Thompson, 2004) leaving plenty of room for the intrusion of exogenous material during inhumation that therefore adds to the bone's mass. Also, Thompson (2003) verified that bones submitted to 500 °C show a modest increase in porosity. This can also partly explain the greater mass increase observed in both trabecular and cortical bones burned at 500 ºC, when compared to bones burned at higher temperatures that lead to less porosity (Thompson, 2004), as it can facilitate the entrance of water and other microorganisms that will influence bone mass. In the case of unburned

bones, no dehydration and decomposition took place previous to inhumation so, possibly, there wasn't as much room for exogenous intrusions to start with. However, trabecular unburned bones were also dramatically affected by a mass increase, which can partly be due to its characteristic structure which is more permissive to exogenous intrusions. Cortical bones are not so porous and were probably too saturated with water and organic compounds to allow for considerable exogenous intrusions.

As for the 900 ℃ and 1050 ℃ bones, high temperatures promote recrystallization, which will change the organization of bone crystals (Shipman et al., 1984; Stiner et al., 1995). Bones subjected to heat-induction treatment (>800 ℃) are less affected by weathering, present a better preservation and are proportionally more mineralized, as there occurs a reorganization of the inorganic phase which fills the pores (Littleton, 2000; Thompson, 2005). This can partly explain why both trabecular and cortical bones burned at 900 ℃ and 1050 ℃ did not suffer as much mass alterations. As the recrystallization of the inorganic phase takes place, pores are filled up due to the coalescence of the inorganic phase, leaving less room for exogenous materials such as water and microorganisms - at least in such a short inhumation time. Potentially, this explains the reduced mass increase observed for bones heated at very high temperatures.

After 8 months of inhumation, and coinciding with the starting of summer, most of all bones, regardless of type or heating protocol, reverted the trend of mass increase. The loss of mass was relatively similar and generally ranged between 1 and 7%. Also, pH influences bone's mass variation. Kalsbeek and Richter (2006) performed a study in which they left unburned and burned (100 ℃ – 1000 ℃) bones immersed in an acidic buffer (pH 3, pH 5) and in a basic buffer (pH 10) during 28 days. They concluded that pH 3 and pH 5 promote a larger increase in bone's mass loss, which is mainly caused by the dissolution of hydroxyapatite and collagen. Hence we can extrapolate that an acidic soil probably will also decrease bone's mass. However that did not occur within the first six months due to climacteric conditions, mostly rain.

Thus, the results reached, so far, by this study will interfere and may invalidate the impact of the methodologies accepted based on mass to estimate the skeletal completeness and the minimal number of individuals. For example, the recent research performed by Gonçalves et al. (2016) that validated mass regression equations to estimate skeletal completeness may not be applicable to bones from contexts similar to these ones or may be applicable only under specific circumstances (e.g. depending on the type of

weather and on the humidity affecting the burial context). The same occurs with other methodologies based on mass and that establish comparisons with skeletal mass references such as the ones from Malinowski and Porawski (1969), Bass and Jantz (2004), May (2011) or Gonçalves et al. (2013a). More research must be done in order to understand how mass really varies over time under burial conditions.

4.2 Post-depositional Measurements Variation

Dimensional changes are due to the loss of water and organic phase and the rearrangement of the mineral phase (Thompson, 2005). Thus, bones with less quantity of mineral content will shrink further (Lange et al., 1987 In: McKinley, 1994). Considering the metrical measurements taken in fragments of cortical bone, in general these did not reveal large variations (i.e. >10%) in such a short time of inhumation, as was expected. Nonetheless, in the present study slight fluctuations occurred (both increases and decreases) during the four bi-monthly exhumations. Yet, they do not appear to be too relevant since none, or almost none alterations were perceptible. Large variations were not expected in such a short period of time and the variations in measurements may have occurred due to intra-observer errors or issues regarding the preservation of the bones that interfered with measurements. Given our results, during the exhumations of trabecular and cortical bones we did not identify a pattern to explain metric variation according to burned temperature, bone type, inhumation time, and metrical measurement. However, despite changes being unimportant in most cases, comparing to cortical bones, trabecular ones showed some higher average values of metric variation, mainly in vertebral height.

Thus, in cortical bones the larger variations were seen in 17T02 (unburned) and 16Cl02 (500 ℃), with an increase of 22.7% and 12.0% at the end of its first biannual exhumation in the medial diameter and distal diameter, respectively, which cannot for certain be discarded as being due to a possible expansion of bone. This was supported by their mass increase of 7.3% and 25.7%, respectively. In trabecular bones, 16Ca (1050 ℃), 16V05 (unburned) and 16V04 (unburned) increased their breadth in 12.9%, vertebral height in 22.2% and vertebral breadth in 18.8%, respectively, during the first observation. The results were also confirmed by large mass increases (11.8%, 47.9% and 25.0%, respectively). However, it must be stated that large mass increases were not always

followed by metric increases. That was the case for 16Cub (unburned), 16V07 (1050 ℃) and 17V07 (900 ℃).

Looking at the average of each measurement in cortical bones, they still presented very low percentages in comparison with trabecular bones. Possibly, that occurred due to the easier intrusion of soil and some roots of plants in the latter leading to a more important inflation but this does not entirely explain all situations. Beside the previous explanation, also bone structure can justify bone expansion. Trabecular bones have a more spread collagen orientation, have a weaker structure and are more vulnerable, comparing to cortical bones (Thompson, 2005).

To add some confusion to the results, instead of expansion, 17Ta (1050 ℃) apparently shrank in breadth 31.3%, and 17V05 (500 ℃) reduced its vertebral length in 16.2%. However, these considerable reductions can result from bone fragility and consequential bone destruction during their handling. For instance, no measurements were taken from 16V06 during exhumations due to bone destruction.

So far, no conclusive evidence of relevant metric variation was reached. However, the few metric variations found in some samples seem to reveal a possible existence of metric variations. As such, further investigation must be done to clarify this question.

4.3 Post-depositional Chemometric Variation

4.3.1 Crystallinity Index

Regarding crystallinity index (CI), the results reached in this study for heat-induced changes are in accordance with the results recorded by other authors. Thompson et al. (2013) and Ellingham et al. (2016) recorded a CI increase proportional to temperature increase, although it decreases slightly with higher temperatures (>800 ℃). In the present study we verified a similar phenomenon. CI increased in bones that were burned at temperatures up to 900 ℃, and decreased in burnings of 1050 ℃ and higher.

During the four bi-monthly exhumations, unburned bones and teeth and bones and teeth burned at 500 ℃ revealed very constant CI values. However, the bi-monthly absolute values for fragments burned at 900 ℃ and 1050 ℃ presented important

Discussion

variations that are more difficult to explain. Also, trabecular bones, especially vertebrae specimens, revealed greater oscillations (16Cub, 16V05, 16V06 and 16V07). In general, the fluctuations verified in all bones are more pronounced at the second exhumation (which took place after four months). When comparing CI values of trabecular bone, cortical bone and teeth, some differences were observed. Concerning unburned bones, trabecular bones were the ones that showed larger CI value variations (from 3.0 to 4.0). However, in general they revealed quite stable CI values; when heated up to 500 ℃ none of the elements revealed major variations, only tooth T9 presented a larger variation over the last two months. On the other hand, bones and teeth burned at 900 ℃ revealed very large fluctuations; trabecular bones and teeth increased CI values, while cortical bones decreased after the first exhumation, not presenting a pattern of decrease or increase during the other exhumations. Also, in samples burned at 1050 ℃, the same random fluctuations occurred; there was not a constant pattern of decrease or increase visible. We verified that unburned bones and teeth, and bones and teeth burned at 500 ℃ seemed to be chemically more stable during burial than samples burned at higher temperatures. These results suggest that the use of CI as a variable to estimate maximum temperature at which buried skeletal remains were subjected may not be reliable, at least, not for short-term burials.

Lebon et al. (2008) verified that CI values were quite stable until 600 ℃ and that between 700 ℃ and 900 ℃ those values increased to 8-10. A couple of years later, in another experimental study from Lebon et al. in 2010, they recorded CI values of 3.2 in unburned bones, values from 3.0 to 3.6 in bones burned up to 500 ℃ and from 3.3 to 9.9 in bones from a modern sample burned up to 900 ℃. It is curious that, in their experiment, unburned bone and bones burned at 500 ℃ revealed the same CI values, contrary to the present study that shows higher CI values for bones and teeth burned at 500 ℃. On the other hand, they also analyzed archaeological samples in which they observed somewhat different results that varied from 3.2 to 4.5 in unburned bones and from 3.5 to 10.5 in burned bones (500 ℃ – 900 ℃), classified by them as calcined). However, it is important to stress that the classifications of certain specific temperatures assigned by them to archaeological samples are forcibly untrustworthy, leading to less reliable results.

In the present study, CI values of unburned bones and teeth varied between 2.5 and 3.6 before burial and from 3.0 and 4.0 over bi-monthly exhumations. Therefore, our

results were quite similar to those from Lebon et al. (2010). The same occurred for bones and teeth burned at 500 ºC, since we obtained CI values between 3.2 and 4.2, although we presented slightly higher values. During bi-monthly exhumations, the CI values for bones and teeth burned at 500 ºC did not change much and varied from 3.4 and 4.5. On the other hand, bones and teeth burned at 900 ºC revealed CI values between 6.1 and 8.2 before burial and between 4.9 and 9.3 after burial during the following bi-monthly exhumations. Therefore, a larger range was observed after burial. Our results show more similarities with the archaeological samples used by Lebon et al. (2010) confirming that inhumation has an important effect on CI evolution. Again, their samples might not be completely reliable though since they may not fully correspond to the temperatures examined by them.

It is known that crystallinity index may change over years but the dramatic variations recorded in this short-term investigation were not expected. Authors as Stiner et al. (1995), Olsen et al. (2008) and Thompson et al. (2009) argue that diagenetic processes, weather and the environment may influence crystallinity, affecting in some way the CI values. Other hypothesis to explain these unexpected values is related to the presence of some plants in the containers, which may have interfered with bone's properties or even due to the considerably amounts of phosphate (200-400 mg/l) and organic matter (>70%) intrinsic to the soil that may have led to ionic exchanges between bones and soil or plants. Also, we cannot discard eventual problems with sample collection which may have led to the CI variations observed during the bi-monthly exhumations. Although we tried to make a comprehensive cleaning of the bone regions used for sampling, soil aggregated to bones may have contaminated the samples. Also, according to Surovell and Stiner (2001) and Stathopoulou et al. (2008), sample preparation and KBr matrix in contact to bone powder may lead to chemical modifications thus leading to the CI variations. However, these hypotheses can hardly explain the fact that such variations were not observed for both unburned bones and 500 ºC burned bones. It would be a huge coincidence that those problems happened to affect only the bones burned at higher temperatures.

Thompson et al. (2009), in their experiment to predict burning temperature through CI, argue that the results of CI reached by FTIR-KBr (used in this study) are less

precise and accurate comparing to FTIR-ATR. So, it is also possible that our results were affected by this problem. However, this could be true if CI variations were transversal to all bones. Instead, larger variations occurred mainly for bones burned at higher temperatures. Thus, it is possible that the observed CI variations are real. Therefore, further studies must be done in order to verify the results reached so far.

4.3.2   C/P and CO_3^{-2}/P ratios

Person et al. (1996), Olsen et al. (2008), Thompson et al. (2009, 2013) and Squires et al. (2011) verified that C/P and CO_3^{-2}/P ratios decrease when temperature increases, i.e., a reduction of carbon occurs with heat-induction process. The same was verified in the present samples, being that reduction more perceptible in teeth. They argue that this ratio decrease is due to the decomposition of carbonate bonds or organic compounds caused by increments in heat-induction. Consequently, an increase of the inorganic fraction can be seen.

For the samples burned at 500 ºC a slight decrease or a maintenance of C/P and CO_3^{-2}/P ratio values was verified, which is consistent with the reduction of carbonates and organic compounds documented by Person et al. (1996), Olsen et al. (2008), Thompson et al. (2009, 2013) and Squires et al. (2011). On the other hand, burnings performed at 900 ºC and 1050 ºC presented very low C/P and CO_3^{-2}/P ratio values (0.1) or even null values (0.0). This is due to the absence or vestigial values of the peak at 1415 cm^{-1} or 870 cm^{-1}, that corresponds to vibrational modes of CO_3^{-2} and its intensity is proportional to the carbonate amount present in bone sample. When temperature increases, organic and some inorganic compounds as carbonate will be eliminated. Only a few fluctuations were observed during exhumations and they occurred randomly to some bones and teeth. For C/P ratio, trabecular bones showed some slight variations for 16V05 (unburned) and for 16Ca; all the other samples seemed to maintain these values throughout the exhumations. As for cortical bones, with exception of 16H02, all the bones showed very stable values. The most stable group of samples comprised teeth; only AC8a revealed a greater fluctuation after four months that can be explained, perhaps, by an error

during sample preparation. Regarding CO_3^{-2}/P ratio, with the exception of 16V05 that showed a decrease in CO_3^{-2}/P ratio, and AC8a which showed several oscillations during exhumations, all the other samples revealed quite constant CO_3^{-2}/P ratio values. Possibly short-time contacts with soil and environment do not promote relevant changes in bone in CO_3^{-2}/P ratio. Thus, these parameters seem stable and may continue to be used to determine burning temperature. However, more investigations are necessary in order to understand if the outliers will stabilize over time or if they really represent different values. Further investigations are needed to understand what led to these values, possibly focused on plant activity.

Besides CI, C/P and CO_3^{-2}/P, there are other important parameters to be studied, such as phosphate high temperature (PHT), C/C, line width, CO/P and CO/ CO_3^{-2}. Regrettably, due to time constraints, only a few were taken into consideration in this study. Hopefully they will be addressed in the future, since this is an ongoing project.

5. Conclusion

It is not well known how all variables related to post-deposition interact with each bone, its mass and dimensions. For instance, the interaction between soil, plants organic matter and phosphates, bone element (trabecular and cortical) and teeth is not well known. That is also the case of how a soil's pH can affect teeth, trabecular and cortical bones and the effect of weather conditions, specifically rain, temperature and moisture on each bone element and teeth. In this manner, it will be interesting to investigate how each of those burial and bone properties interact with each other. Perhaps, further chemical analysis of soil compounds may allow a better comprehension of their interaction and a better understanding of the post-depositional variations recorded so far. This study is only in its beginnings, making the results reached until now very preliminary. The trends here documented may change with further analysis based on a longer diachrony.

Looking back, we conclude that the research design can be improved. For example, vertebrae revealed to be a poor representative of the trabecular bone type to perform this study. It is very fragile and, maybe, the removal of the pedicles that we carried out accelerated their deterioration since it facilitated the intrusion of dirt inside the bone. So, removing vertebrae pedicles was not, in retrospective, a good decision and we advise against it if other researchers intend to proceed with investigations similar to this one. Also, burned teeth suffered severe destruction hindering the study of mass and metrical variations. To avoid, at least, the inability of studying mass variation, maybe the use of protective nets could have helped to prevent the loss of small dental pieces. Yet, we performed bone powder collection to perform chemical analysis on the same samples in which we observed mass variation; this was probably not the best option. To improve data interpretation, we suggest to, additionally to the approach we followed here, to perform these procedures separately, i.e. to record mass variation and bone powder collection in different samples. This way, mass analysis would not be affected by periodical samplings. Also, bone sampling will, probably, increase bone deterioration. Teeth also became damaged due to the handling required to sample them. It could be interesting, as well, to observe samples subjected to the same treatment (e.g.: burning temperature) buried in a different environment (e.g.: soil with a different pH, indoor), in order to compare to the present study.

Conclusion

Regarding the performed chemical analysis, FTIR technique presents advantages and disadvantages. FTIR-KBr is more destructive than FTIR-ATR. Regrettably, the FTIR-KBr results may not be very accurate due to sample preparation problems. However, this does not explain the chemometric variations observed in the study since they referred systematically to samples burned at higher temperatures, while no considerable variation was found for the other samples. The KBr technique is not often used nowadays. In fact, FTIR-ATR is often pointed out as first choice. However, the laboratory where the analyses were performed was not equipped with an ATR module, so it was never an option. Nevertheless, the analysis can be repeated on FTIR-ATR with the remaining bone powder since samples were not completely used.

Finally, as we already mentioned, methods to estimate the skeletal completeness and the minimal number of individuals based on mass (e.g. Bass and Jantz (2004), May (2011), Gonçalves et al. (2013a) or Gonçalves et al. (2016)) must be adjusted and improved in order to enable its application to bones under this type of conditions, but to achieve that more studies similar to this one are required. On the other hand, regarding osteometric methods, we are not able to say if standard methods (e.g. Silva (1995), Harris (2009), Gonçalves et al. (2013b)) are reliable. Some important metric changes were indeed observed, but variations in both trabecular and cortical bones, seemed to occur randomly. No specific trend was detected. Thus, burial can dramatically hamper anthropological work, since some of the reliability of commonly used methods can dramatically be jeopardized. Regarding chemometric methods (e.g. Thompson et al. (2009), Ellingham et al. (2015), Marques et al (2016)), they must be carefully applied to estimate bone burning temperature through CI, since, so far, post-depositional values revealed larger fluctuations, mainly for bones burned at higher temperatures.

6. References

Abraham, J.A.; Sánchez, H.J.; Marcelli, C.A.; Grenón, M.; Guidi, M.C.; Piccinini, M. 2011. A crystallinity study of dental tissues and tartar by infrared spectroscopy. *Analytical and Bioanalytical Chemistry*, 399: 1699-1704.

Andrews, P. 1995. Experiments in taphonomy. *Journal of Archaeological Science*, 22: 147-153.

Aplin, K.; Manne, T.; Attenbrow, V. *in press.* Using a 3-stage burning categorization to assess post-depositional degradation of archaeofaunal assemblages: some observations based on multiple prehistoric sites in Australasia. *Journal of Archaeological Science: Reports.* [Published online: 2015]. http://dx.doi.org/10.1016/j.jasrep.2015.11.029.

Asmussen, B. 2009. Intentional or incidental thermal modification? Analyzing site occupation via burned bone. *Journal of Archaeological Science,* 36: 528-536.

Baby, R.S. 1954. Hopewell cremation practices. *Papers in Archaeology of the Ohio Historical Society*, 1: 1-7.

Bass, W.M.; Jantz, R.L. 2004. Cremation weights in East Tennessee. *Journal of Forensic Science,* 49(5): 1-4.

Behrensmeyer, A.K. 1978. Taphonomic and ecologic information from bone weathering. *Palaeobiology,* 4(2): 150-162.

Behrensmeyer, A.K.; Kidwell, S.M. 1985. Taphonomy's contribution to paleobiology. *Paleobiology,* 11(1): 105-119.

Bell, L.S.; Skinner, M.F.; Jones, S.J. 1996. The speed of post mortem change to the human skeleton and its taphonomic significance. *Forensic Science International*, 82: 129-140.

Bennett, J.L. 1999. Thermal alteration of buried bone. *Journal of Archaeological Science,* 26: 1-8.

References

Bertran, P.; Beauval, C.; Boulogne, S.; Brenet, M.; Costamagno, S.; Feuillet, T.; Laroulandie, V.; Lenoble, A.; Malaurent, P.; Mallye, J-B. 2015. Experimental archaeology in a mid-latitude periglacial context: insight into site formation and taphonomic processes. *Journal of Archaeological Science,* 57: 283-301.

Berzina-Cimdina, L.; Borodajenko, N. 2012. Research of calcium phosphates using Fourier transform infrared spectroscopy. *INTECH Open Access Publisher, 123-148.*

Binford, L.R. 1963. An analysis of cremations from three Michigan sites. *Wisconsin Archaeologist,* 44(2): 98-110.

Boskey, A.; Camacho, N.P. 2007. FT-IR imaging of native and tissue-engineered bone and cartilage. *Biomaterias,* 28(15): 2465-2478.

Brooks, S.; Suchey, J.M. 1990. Skeletal age determination based on the os pubis: a comparison of the Acsádi-Nemeskéri and Suchey-Brooks methods. *Human Evolution,* 5(3): 227-238.

Buekenhout, I. 2014. *Quando só restam ossos: estudo da degradação e alteração óssea para estimativa do intervalo post-mortem.* Dissertação de Mestrado em Medicina Legal e Ciências Forenses, Faculdade de Medicina, Universidade de Coimbra.

Buikstra, J.E.; Swegle, M. 1989. Bone modification due to burning: experimental evidence. *In:* Bonnichsen, R.; Sorg, H. (eds.) *Bone modification.* Orono, Center for the Study of the First Americans: 247-258.

Carter, D.O.; Tibbett, M. 2008. Cadaver decomposition and soil: processes. *In:* Tibbett, M.; Carter, D.O. (eds.) *Soil analysis in forensic taphonomy: chemical and biological effects of buried human remains.* Boca Raton, CRC Press: 29-52.

Cattaneo, C. 2007. Forensic Anthropology: developments of a classical discipline in the new millennium. *Forensic Science International,* 165: 185-193.

Chadefaux, C.; Le Hô, A-S.; Bellot-Gurlet, L.; Reiche, I. 2009. Curve-fitting micro-ATR-FTIR studies of the amide I and II bands of type I collagen in archaeological bone materials. *e-Preservation Science,* 6: 129-137.

Child, A.M. 1995. Towards an understanding of the microbial decomposition of archaeological bone in the burial environment. *Journal of Archaeological Science,* 22: 165-174.

Chimutsa, M.; Olakanye, A.O.; Thompson, T.J.U.; Ralebitsu-Senior, T.K. 2015. Soil fungal community shift evaluation as a potential cadaver decomposition indicator. *Forensic Science International,* 257: 155-159.

Coelho, J. 2015. Unwarping heated bones: a quantitative analysis of heat-induced skeletal deformations using 3D geometric morphometrics. Dissertação de Mestrado em Evolução e Biologia Humanas, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologias, Universidade de Coimbra.

Cravo, L. 2015. *Estudo experimmental sobre decomposição cadavérica usando carcaças de Sus scrofa domestica.* Dissertação de Mestrado em Evolução e Biologia Humanas, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologias, Universidade de Coimbra.

Dal Sasso, G.; Maritan, L.; Usai, D.; Angelini, I.; Artioli, G. 2014. Bone diagenesis at the micro-scale: bone alteration patterns during multiple burial phases at Al Khiday (Khartoum, Sudan) between the Early Holocene and the II century AD. *Palaeogeography, Palaeoclimatology, Palaeoecology,* 416: 30-42.

Dawson, L.A.; Hillier, S. 2010. Measurements of soil characteristics for forensic applications. *Surface and Interface Analysis,* 42: 363-377.

De Becdelievre, C.; Thiol, S.; Santos, F.; Rottier, S. 2015. From fire-induced alterations on human bones to the original circumstances of the fire: an integrated approach of human cremains drawn from a Neolithic collective burial. *Journal of Archaeological Science: Reports*, 4: 210-225.

References

Delannoy, Y.; Colard, T.; Le Garff, E.; Mesli, V.; Aubernon, C.; Penel, G.; Hedouin, V.; Gosset, D. 2016. Effects of the environment on bone mass: A human taphonomic study. *Legal Medicine,* 20: 61-67.

Dirkmaat, D.C.; Cabo, L.L.; Ousley, S.D.; Symes, S.A. 2008. New perspectives in forensic anthropology. *Yearbook of Physical Anthropology,* 51: 33-52.

Domínguez-Rodrigo, M.; Fernández-López, S.; Alcalá, L. 2011. How can taphonomy be defined in the XXI century? *Journal of Taphonomy,* 9(1): 1-13.

Efremov, I.A. 1940. Taphonomy: a new branch of paleontology. *The Pan-American Geologist*, 74: 81-93.

Ellingham, S.T.D.; Thompson, T.J.U.; Islam, M.; Taylor, G. 2015. Estimating temperature exposure of burnt bone – a methodological review. *Science and Justice,* 55: 181-188.

Ellingham, S.T.D.; Thompson, T.J.U.; Islam, M. 2016. The effect of soft tissue on temperature estimation from burnt bone using Fourier Transform Infrared Spectroscopy. *Journal of Forensic Sciences,* 61(1): 153-159.

Enzo, S.; Bazzoni, M.; Mazzarello, V.; Piga, G.; Bandiera,P.; Melis, P. 2007. Astudy by thermal treatment and X-ray powder diffraction on burnt fragmented bones from tombs II, IV and IX belonging to the hypogeic necropolis of "Sa Figu" near Itirri, Sassari (Sardinia, Italy). *Journal of Archaeological Science,* 34: 1731-1737.

Etxeberria, F. 1994. Aspectos macroscópicos del hueso sometido al fuego. Revisión de las cremaciones desccritas en el País Vasco desde la Arqueología. *Munibe (Antropologia-Arkeologia),* 46: 111-116.

Evans, T. 2014. Fluvial taphonomy. *In:* Pokines, J.T:; Symes, S.A. (eds.) Manual of forensic taphonomy. Boca Raton, CRC Press: 115-142.

Fairgrieve, S.I. 2008. *Forensic cremation recovery and analysis.* Boca Raton, CRC Press.

FAO. 2006. *Guidelines for soil description.* 4th edition. Rome, FAO.

Ferreira, M.T. 2012. *Para lá da morte: estudo tafonómico da decomposição cadavérica e da degradação óssea e implicações na estimativa do intervalo pós-morte.* Tese de Doutoramento em Antropologia Forense, Departamento de Ciiências da Vida, Faculdade de Ciências e Tecnologia, Universidade de Coimbra.

Ferreira, M.T.; Cunha, E. 2012. Será credível estimar o PMI em restos cadavéricos em avançado estado de decomposição? *Cadernos do GEEvH,* 1(2): 7-20.

Ferreira, M.T.; Vicente, R.; Navega, D.; Gonçalves, D.; Curate, F.; Cunha, E. 2014. A new forensic collection housed at the University of Coimbra, Portugal: The 21st century identified skeletal collection. *Forensic Science International,* 245: 202.e1-202.e5.

Fiedler, S.; Graw, M. 2003. Decomposition of buried corpses, with special reference to the formation of adipocere. *Naturwissenschaften*, 90: 291-300.

Fitzpatrick, R.W. 2008. Nature, distribution, and origin of soil materials in the forensic comparison of soils. *In:* Tibbett, M.; Carter, D.O. (eds.) *Soil analysis in forensic taphonomy: chemical and biological effects of buried human remains.* Boca Raton, CRC Press: 1-28.

Forancelli Pacheco, M.L.A.; Villagran, X.S.; Martins, G.R. 2012. Macroscopic and microbiological alterations of bird and small mammal bones buried in a *Cerrado* biome (south western Brazil). *Journal of Archaeological Science,* 39: 1394-1400.

Galloway, A. 1997. The process of decomposition: a model from the Arizona-Sonoran Desert. *In:* Haglund, W.D.; Sorg, M.H. (eds.) *Forensic taphonomy: the postmortem fate of human remains.* Boca Raton, CRC Press: 139-150.

Gill-King, H. 1997. Chemical and ultrastructural aspects of decomposition. *In:* Haglund, W.D.; Sorg, M.H. (eds.) *Forensic taphonomy: the postmortem fate of human remains.* Boca Raton, CRC Press: 93-108.

References

Gonçalves, D. 2011. The reliability of osteometric techniques for the sex determination of burned human skeletal remains. *HOMO – Journal of Comparative Human Biology,* 62: 351-358.

Gonçalves, D.; Thompson, T.J.U.; Cunha, E. 2011. Implications of heat-induced changes in bone on the interpretation of funerary behaviour and practice. *Journal of Archaeological Science,* 38: 1308-1313.

Gonçalves, D.; Cunha, E.; Thompson, T.J.U. 2013a. Weight references for burned human skeletal remains from Portuguese samples. *Journal of Forensic Sciences,* 58(5): 1134-1140.

Gonçalves, D.; Thompson, T.J.U.; Cunha, E. 2013b. Osteometric sex determination of burned human skeletal remains. *Journal of Forensic and Legal Medicine,* 20: 906-911.

Gonçalves, D.; Cunha, E.; Thompson, T.J.U. 2015. Estimation of the pre-burning condition of human remains in forensic contexts. *International Journal of Legal Medicine,* 129: 1137-1143.

Gonçalves, D.; d'Oliveira Coelho, J.; Acosta, M.A.; Coelho, C.; Curate, F.; Ferreira, M.T.; Gouveia, M.; Makhoul, C.; Pinto, D.; Oliveira-Santos, I.; Vassalo, A.R.; Navega, D.; Cunha, E. 2016. One for all and all for one: linear regression from the mass of isolated bones to assess human skeletal mass completeness. *American Journal of Physical Anthropology*. [Published online: 2016]. DOI: 10.1002/ajpa.22979.

Gordon, C.C.; Buikstra, J.E. 1981. Soil pH, bone preservation, and sampling bias at mortuary sites. *Society for American Archaeology*, 46(3): 566-571.

Gouveia, M. 2015. *Avaliação do potencial da odontometria para a diagnose sexual em vestígios humanos queimados*. Dissertação de Mestrado em Evolução e Biologia Humanas, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade de Coimbra.

Grévin, G.; Bailet, P.; Quatrehomme, G.; Ollier, A. 1998. Anatomical reconstruction of fragments of burned human bones: a necessary means for forensic identification. *Forensic Science International*, 96: 129-134.

Guarino, F.M.; Angelini, F.; Vollono, C.; Orefice, C. 2006. Bone preservation in human remains from the Terme del Sarno at Pompeii using light microscopy and scanning electron microscopy. *Journal of Archaeological Science*, 33: 513-520.

Harbeck, M.; Schleuder, R.; Schneider, J.; Wiechmann, I.; Schmahl, W.W.; Grupe, G. 2011. Research potential and limitations of trace analyses of cremated remains. *Forensic Science International*, 204: 191-200.

Harris, S.M. 2009. *Sexual dimorphism in the tarsals: Implications for sex determination.* Master thesis, North Carolina State University.

Hedges, R.E.M; Millard, A.R. 1995. Bones and Groundwater: towards the modelling of diagenetic processes. *Journal of Archaeological Science,* 22: 155-164.

Hedges, R.E.M. 2002. Bone diagenesis: an overview of processes. *Archaeometry,* 44 (3): 319-328.

Henderson, J. 1987. Factors determining the state of preservation of human remains. *In:* Boddington, A.; Garland, A.N.; Janaway, R.C. (eds.) *Death, decay and reconstruction: approaches to archaeology and forensic science.* Manchester, Manchester University Press: 43-54.

Hill, A.J.; Lain, R.; Hewson, I. 2011. Preservation of dental evidence following exposure to high temperatures. *Forensic Science International,* 205: 40-43.

Hilson, S. 2005. *Teeth.* 2nd edition. Cambridge, Cambridge University Press.

Janaway, R.C. 1996. The decay of buried human remains and their associated materials. *In:* Hunter, J.; Roberts, C.; Martin, A. (eds.) Studies in crime: an introduction to Forensic Archaeology. London, B.T. Batsford: 58-85.

References

Janway, R.C. 2002. Degradation of clothing and other dress materials associated with buried bodies of archaeological and forensic interest. *In:* Haglund, W.D.; Sorg, M.H. (eds.) *Advances in forensic taphonomy: method, theory, and archaeological perspectives*. Boca Raton, CRC Press: 379-402.

Kalsbeek, N.; Richter, J. 2006. Preservation of burned bones: an investigation of the effects of temperature and pH on hardness. *Studies in Conservation*, 51(2): 123-138.

Karr, L.P.; Outram, A.K. 2012. Tracking changes in bone fracture morphology over time: environment, taphonomy and the archaeological record. *Journal of Archaeological Science,* 39: 555-559.

Klein, G.L. 2006. Burn-induced bone loss: importance, mechanisms, and management. *Journal of Burns and Wounds,* 5: 32-38.

Lebon, M.; Reiche, I.; Fröhlich, F.; Bahain, J.-J.; Falguères, C. 2008. Characterization of archaeological burnt bones: contribution of a new analytical protocol based on derivative FTIR spectroscopy and curve fitting of the v_1v_3 PO4 domain. *Analytical and Bioanalytical Chemistry,* 392: 1479-1488.

Lebon, M.; Reiche, I.; Bahain, J.-J.; Chadefaux, C.; Moigne, A.-M.; Fröhlich, F.; Sémah, F.; Schwarcz, H.P.; Falguères, C. 2010. New parameters for the characterization of diagenetic alterations and heat-induced changes of fossil bone mineral using Fourier transform infrared spectrometry. *Journal of Archaeological Science,* 37: 2265-2276.

Lebon, M.; Müller, K.; Bahain, J-J.; Fröhlich, F.; Falguères, C.; Bertrand, L.; Sandt, C.; Reiche, I. 2011. Imaging fossil bone alterations at the microscale by SR-FTIR microspectroscopy. *Journal of Analytical Atomic Spectrometry,* 26: 922-929.

Lebon, M.; Zazzo, A.; Reiche, I. 2014. Screening in situ bone and teeth preservation by ATR-FTIR mapping. *Palaeogeography, Palaeoclimatology, Palaeoecology,* 416: 110-119.

Lee-Thorp, J.; Sponheimer, M. 2003. Three case studies used to reassess the reliability of fossil bone and enamel isotope signals for paleodietary studies. *Journal of Anthropological Archaeology*, 22: 208-216.

Lieverse, A.R.; Weber, A.W.; Goriunova, O.I. 2006. Human taphonomy at Khuzhir-Nuge XIV, Siberia: a new method for documenting skeletal condition. *Journal of Archaeological Science*, 33: 1141-1151.

Littleton, J. 2000. Taphonomic effects of erosion on deliberately buried bodies. *Journal of Archaeological Science,* 27: 5-18.

Love, J.C.; Marks, M.K. 2003. Taphonomy and time: estimating the postmortem interval. *In:* Steadman, D.W. (ed.) *Hard Evidence: case studies in forensic anthropology.* Upper Saddle River, Prentice Hall: 160-175.

Lovejoy, C.O.; Meindl, R.S.; Pryzbeck, T.R.; Mensforth, R.P. 1985. Chronological metamorphosis of the auricular surface of the illium: a new method for the determination of adult skeletal age at death. *American Journal of Physical Anthropology,* 68: 15-28.

Lucas, P.W. 2004. *Dental functional morphology.* Cambridge, Cambridge University Press.

Lyman, R.L. 2010. What taphonomy is, what it isn't, and why taphonomists should care about the difference. *Journal of Taphonomy,* 8(1): 1-16.

Malinowski, A.; Porawski, R. 1969. Identifikationsmöglichkeiten menschlicher Brandknochen mit besonderer Berücksichtigung ihres Gewichts. *Zacchia,* 5: 392-410.

Marques, M.P.M.; Gonçalves, D.; Amarante, A.I.C.; Makhoul, C.I.; Parker, S.F.; Batista de Carvalho, L.A.E. 2016. Osteometrics in burned human skeletal remains by neutron and optical vibrational spectroscopy. *Royal Society of Chemistry Advances,* 6: 68638-68641.

References

Martin, R.E. 1999. *Taphonomy: a process approach.* Cambridge, Cambridge University Press.

May, S.E. 2011. The effects of body mass on cremation weight. *Journal of Forensic Science,* 56(1): 3-9.

Mayne Correia, P.M. 1997. Fire modification of bone: a review of the literature. *In:* Haglund, W.D.; Sorg, M.H. (eds.) *Forensic taphonomy: the postmortem fate of human remains.* Boca Raton, CRC Press: 275-293.

McKinley, J.I. 1994. *The Anglo-Saxon cemetery at Spong Hill, North Elmham part VIII: the cremations (East Anglian Archaeology 69).* Dereham: Norfolk Museums Service.

Moore-Jansen, P.M.; Ousley, S.D.; Jantz, R.L. 1994. *Data collection procedures for forensic skeletal material.* 3rd edition. Knoxville, The University of Tennessee.

Morris, M.D.; Finney, W.F. 2004. Recent developments in Raman and infrared spectroscopy and imaging of bone tissue. *Spectroscopy,* 18(2): 155-159.

Munro, L.E.; Longstaffe, F.J.; White, C.D. 2007. Burning and boiling of modern deer bone: effects on crystallinity and oxygen isotope composition of bioapatite phosphate. *Palaeogeography, Palaeoclimatology, Palaeoecology,* 249: 90-102.

Murail, P.; Bruzek, J.; Houël, F.; Cunha, E. 2005. DSP: a tool for probabilistic sex diagnosis using worldwide variability in hip-bone measurements. *Bulletins et mémoires de la Société d'Anthropologie de Paris,* 17(3-4): 167-176.

Nicholson, R.A. 1993. A morphological investigation of burnt animal bone and an evaluation of its utility in Archaeology. *Journal of Archaeological Science,* 20 : 411-428.

Olsen, J.; Heinemeier, J.; Bennike, P.; Krause, C.; Hornstrup, K.M.; Thrane, H. 2008. Characterisation and blind testing of radiocarbon dating of cremated bone. *Journal of Archaeological Science,* 35: 791-800.

Oréfice, R.L.; Discacciati, J.A.C.; Neves, A.D.; Mansur, H.S.; Jansen, W.C. 2003. In situ evaluation of the polymerization kinetics and corresponding evolution of the mechanical properties of dental composites. *Polymer Testing*, 22: 77-81.

Person, A.; Bocherens, H.; Mariotti, A.; Renard, M. 1996. Diagenetic evolution and experimental heating of bone phosphate. *Palaeogeography, Palaeoclimatology, Palaeoecology,* 126: 135-149.

Pestle, W.J.; Ahmad, F.; Vesper, B.J.; Cordell, G.A.; Colvard, M.D. 2014. Ancient bone collagen assessment by hand-held vibrational spectroscopy. *Journal of Archaeological Science,* 42: 381-389.

Piga, G.; Thompson, T.J.U.; Malgosa, A.; Enzo, S. 2009. The potential of X-ray diffraction in the analysis of burned remains from Forensic contexts. *Journal of Forensic Sciences,* 54(3): 534-539.

Piga, G.; Gonçalves, D.; Thompson, T.J.U.; Brunetti, A.; Malgosa, A.; Enzo, S. 2016. Understanding the crystallinity indices behavior of burned bones and teeth by ATR-IR and XRD in the presence of bioapatite mixed with other phosphate and carbonate phases. *International Journal of Spectroscopy.* [Published online: 2016]. DOI: http://dx.doi.org/10.1155/2016/481014.

Pijoan, C.MA. Mansilla, J.; Leboreiro, I.; Lara, V.H.; Bosch, P. 2007. Thermal alterations in archaeological bones. *Archaeometry,* 49(4): 713-727.

Pinheiro, J. 2006. Decay process of a cadaver. *In:* Schmitt, A.; Cunha, E.; Pinheiro, J. (eds.) *Forensic Anthropology and Medicine: Complementary Sciences From Recovery to Cause of Death.* Totowa, Human Press: 85-116.

Piontek, J. 1975. *Polish methods and results of investigations of cremated bones from praehistoric cemeteries.* Department of Anthropology, Adam Mickiewicz University, Poznan.

References

Pokines, J.T. 2014. Introduction: collection of macroscopic osseous taphonomic data and the recognition of taphonomic suits of characteristics. *In:* Pokines, J.T.; Symes, S.A. (eds.) *Manual of forensic taphonomy.* Boca Raton, CRC Press: 1-18.

Pokines, J.T. 2014. Faunal dispersal, reconstruction, and gnawing damage to bone in terrestrial environments. *In:* Pokines, J.T.; Symes, S.A. (eds.) *Manual of forensic taphonomy*, Boca Raton, CRC Press : 201-248.

Reiche, I.; Favre-Quattropani, L.; Vignaud, C.; Bocherens, H.; Charlet, L.; Menu, M. 2003. A multi-analytical study of bone diagenesis: the Neolithic site of Bercy (Paris, France). *Measurement Science and Technology,* 14: 1-12.

Reiche, I.; Lebon, M.; Chadefaux, C.; Müller, K.; Le Hô, A-S.; Gensch, M.; Schade, U. 2010. Microscale imaging of the preservation state of 5,000-year-old archaeological bones by synchrotron infrared microspectroscopy. *Analytical and Bioanalytical Chemistry,* 397: 2491-2499.

Roberts, S.J.; Smith, C.I.; Millard, A.; Collins, M.J. 2002. The taphonomy of cooked bone: characterizing boiling and its physico-chemical effects. *Archaeometry,* 44(3): 485-494.

Rodriguez, W.C.3rd; Bass, W.M. 1985. Decomposition of buried bodies and methods that may aid in their location. *Journal of Forensic Sciences*, 30(3): 836-852.

Rodriguez, W.C. 1997. Decomposition of buried and submerged bodies. *In:* Haglund, W.D.; Sorg, M.H. (eds.) *Forensic taphonomy: the postmortem fate of human remains.* Boca Raton, CRC Press: 459-467.

Rogers, C.J. 2010. *Dating death: forensic taphonomy and the postmortem interval.* Doctoral thesis in Philosophy, University of Wolverhampton.

Ross, M.H.; Romrell, L.J. 1989. *Histology: a text and atlas.* 2nd edition. Baltimore, Williams & Wilkins.

Ross, A.H.; Cunningham, S.L. 2011. Time-since-death and bone weathering in a tropical environment. *Forensic Science International,* 204: 126-133.

Ruppel, M.E.; Burr, D.B.; Miller, L.M. 2006. Chemical makeup of microdamaged bone differs from undamaged bone. *Bone*, 39: 318-324.

Santos, I. 2015. *Cimentocronologia: estimativa de idade-à-morte numa amostra de dentes queimados identificados de indivíduos portugueses.* Dissertação de Mestrado em Evolução e Biologia Humanas, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade de Coimbra.

Saukko, P.; Knight, B. 2004. *Knight's Forensic Pathology*. 3rd edition. London, Edward Arnold.

Schmidt, C.W. 2008. Forensic dental anthropology: issues and guidelines. *In*: Irish, J.D.; Nelson, G.C. (eds.) *Technique and application in dental anthropology.* Cambridge, Cambridge University Press: 266-292.

Shipman, P.; Foster, G.; Schoeninger, M. 1984. Burnt bones and teeth: an experiment study of color, morphology, crystal structure and shrinkage. *Journal of Archaeological Science,* 11: 307-325.

Silva, A.M. 1995. Sex assessment using the calcaneus and talus. *Antropologia Portuguesa,* 13: 107-119.

Smith, C. 2005. *Taphonomy: a resource guide*. Fall, Info 674 – resources in science/tech. [Document online]: carolsmith.us/downloads/674**taphonomy**.pdf.

Snoeck, C.; Lee-Thorp, J.A.; Schulting, R.J. 2014. From bone to ash: Compositional and structural changes in burned modern and archeological bone. *Palaeogeography, Palaeoclimatology, Palaeoecology,* 416: 55-68.

Soil Survey Division Staff. 1993. *Soil survey manual.* Soil Conservation Service. U.S. Department of Agriculture Handbook 18.

References

Sorg, M. H.; Haglund, W. D. 2002. Advancing Forensic taphonomy: purpose, theory, and practice. *In:* Haglund, W. D. and Sorg, M. H. (eds.) *Advances in Forensic Taphonomy: Method, Theory, and Archaeological Perspectives.* Boca Raton, CRC Press: 3-29.

Spennemann, D.H.R.; Colley, S.M. 1989. Fire in a pit: the effects of burning on faunal remains. *Archaeozoologia,* 3(1.2.): 51-64.

Squires, K.E.; Thompson, T.J.U.; Islam, M.; Chamberlain, A. 2011. The application of histomorphometry and Fourier Transform Infrared Spectroscopy to the analysis of early Anglo-Saxon burned bone. *Journal of Archaeological Science,* 38: 2399-2409.

Stathopoulou, E.T.; Psycharis, V.; Chryssikos, G.D.; Gionis, V.; Theodorou, G. 2008. Bone diagenesis: new data from infrared spectroscopy and x-ray diffraction. *Palaeogeography, Palaeoclimatology, Palaeoecology,* 266: 168-174.

Stiner, M.C.; Kuhn, S.L.; Weiner, S.; Bar-Yosef, O. 1995. Differential burning, recrystallization, and fragmentation of archaeological bone. *Journal of Archaeological Science*, 22: 223-237.

Surabian, D. 2012. *Preservation of buried human remains in soil.* Natural Resources Conservation Service, U.S. Department of Agriculture, Connecticut.

Surovell, T.A.; Stiner, M.C. 2001. Standardizing infra-red measures of bone mineral crystallinity: an experiment approach. *Journal of Archaeological Science,* 28: 633-642.

SWGANTH, Scientific Working Group for Forensic Anthropology. 2013. Taphonomic observations in the postmortem interval. [Document online]: http://swganth.startlogic.com/Taphonomic%20Observations%20Rev0.pdf

Swift, B. 1998. Dating human skeletal remains: investigating the viability of measuring the equilibrium between [210]PO and [210]PBas a means of estimating the post-mortem interval. *Forensic Science International,* 98: 119-126.

Symes, S.A.; Dirkmaat, D.C.; Ousley, S.D.; Chapman, E.; Cabo, L. 2012. *Recovery and interpretation of burned human remains*. US Department of Justice Office of Justice Programs United States of America. [Online]. Rockville. [Access in 25-11-2015]. Available at: https://www.ncjrs.gov/pdffiles1/nij/grants/237966.pdf.

Thompson, T.J.U. 2002. The assessment of sex in cremated individuals: some cautionary notes. *Canadian Society of Forensic Science Journal,* 35(2): 49-56.

Thompson TJU. 2003. *An Experimental Study of the Effects of Heating and Burning on the Hard Tissues of the Human Body, and its Implications for Anthropology and Forensic Science*. PhD thesis. Sheffield: University of Sheffield.

Thompson, T.J.U. 2004. Recent advances in the study of burned bone and their implications for forensic anthropology. *Forensic Science International,* 146S: S203-S205.

Thompson, T.J.U. 2005. Heat-induced dimensional changes in bone and their consequences for forensic anthropology. *Journal of Forensic Sciences,* 50(5): 1008-1015.

Thompson, T.J.U.; Chudek, J.A. 2007. A novel approach to the visualization of heat-induced structural change in bone. *Science and Justice,* 47: 99-104.

Thompson, T.J.U.; Gauthier, M.; Islam, M. 2009. The application of a new method of Fourier Transform Infrared Spectroscopy to the analysis of burned bone. *Journal of Archaeological Science,* 36: 910-914.

Thompson, T.J.U.; Islam, M.; Bonniere, M. 2013. A new statistical approach for determining the crystallinity of heat-altered bone mineral from FTIR spectra. *Journal of Archaeological Science,* 40: 416-422.

Toffolo, M.B.; Brink, J.S.; Berna, F. 2015. Bone diagenesis at the Florisbad spring site, Free State Province (South Africa): implications for the taphonomy of the Middle and Late Pleistocene faunal assemblages. *Journal of Archaeological Science: Reports,* 4: 152-163.

References

Trueman, C.N.G.; Behrensmeyer, A.K.; Tuross, N.; Weiner, S. 2004. Mineralogical and compositional changes in bones exposed on soil surfaces in Amboseli National Park, Kenya: diagenetic mechanisms and the role of sediment pore fluids. *Journal of Archaeological Science*, 31: 721-739.

Turner, B.; Wiltshire, P. 1999. Experimental validation of forensic evidence: a study of the decomposition of buried pigs in a heavy clay soil. *Forensic Science International*, 101: 113-122.

Turp, J.C.; Alt, K.W. 1998. Anatomy and morphology of human teeth. *In*: Alt, K.W.; Rosing, F.W.; Teschler-Nicola, M. (eds.) *Dental Anthropology: fundamentals, limits and prospects*. Wien, SpringerWienNewYork: 71-94.

Ubelaker, D.H. 2009. The forensic evaluation of burned skeletal remains: a synthesis. *Forensic Science International,* 183: 1-5.

Ubelaker, D.H. 2013. Postmortem interval. *In:* Siegel, J.A.; Pekka, J. (eds.) *Encyclopedia of forensic sciences.* 2nd edition. Waltham: 24-27.

Van Wynsberghe, D.; Noback, C.R.; Carola, R. 1995. *Human anatomy and physiology.* 3rd edition. New York, WCB McGraw-Hill.

Von Koenigswald, W. 1982. Enamel structure in the molars of arvicolidae (rodentia, mammalia), a key to functional morphology and phylogeny. *In*: Kurtén, B. (ed.) *Teeth: form, function and evolution.* New York, Columbia University Press: 109-122.

White, T.D.; Folkens, P.A. 2000. *Human osteology.* 2nd edition. London, Academic Press.

White, T.D.; Black, M.T.; Folkens, P.A. 2012. *Human Osteology.* 3rd edition. Oxford, Academic Press.

Wilson, M.V.H. 1988. Taphonomic processes: information loss and information gain. *Geoscience Canada*, 15(2): 131-148.

Wilson-Taylor, R.J. 2013. Time since Death Estimation and Bone Weathering: The Postmortem Interval. *In*: Tersigni-Tarrant, M.A.; Shirley, N.R. (eds.) *Forensic Anthropology: An Introduction.* Boca Raton, CRC Press: 339-380.

Woods, B.; Lennard, C.; Kirkbride, K.P.; Robertson, J. 2014a. Soil examination for a forensic trace evidence laboratory – part 1: spectroscopic techniques. *Forensic Science International,* 245: 187-194.

Woods, B.; Kirkbride, K.P.; Lennard, C.; Robertson, J. 2014b. Soil examination for a forensic trace evidence laboratory – part 2: elemental analysis. *Forensic Science International,* 245: 195-201.

Zazzo, A.; Saliège, J.-F. 2011. Radiocarbon dating of biological apatites: a refiew. *Palaeogeography, Palaeoclimatology, Palaeoecology,* 310: 52-61.

7. Annex

Table 7.1 – Acidic special substratum datasheet from *Siro® Profissionais em Substratos*.

Characteristics	Values
pH in $CaCl_2$	4.0-4.5
Moisture	50-60%
Conductivity	0.6-1.0 CE
Nitrogen (N)	100-200 mg/l
Phosphorous (P_2O_5)	100-200 mg/l
Potassium (K_2O)	200-400 mg/l
Organic Matter (O.M.)	>70%
Producing Filling Volume	20L CEN 12580

8. Appendix

Appendix 8.1

Table 8.1 – Bone and Teeth Measurements Before Burning.

Bone	Values (mm)					
	Length	Breadth	Height	Vertebral Breath	Vertebral Length	Vertebral body length
16Cub	25	22	35	-	-	-
16V05	-	-	18	20	33	17
16Pa	43	19	42	-	-	-
16Nav	42	24	35	-	-	-
16Ta	60	40	-	-	-	-
16V06	-	-	22	29	30	23
16Ca	83	38	-	-	-	-
16V07	-	-	20	35	36	28
16V04	-	-	19	16	32	16
17Cub	25	21	38	-	-	-
17V05	-	-	25	40	33	26
17V04	-	-	22	40	40	26
17Pa	41	19	43	-	-	-
17V07	-	-	24	33	41	27
17Ca	67	38	-	-	-	-
17Ta	49	36	-	-	-	-

(Bone group label: **Trabecular**)

Table 8.1 – Bone and Teeth Measurements Before Burning (cont.)

Bone		Values (mm)						
		Length	Proximal diameter	Medial diameter	Distal diameter	Proximal circumference	Medial circumference	Distal circumference
Cortical	**16T02**	109	26	24	26	70	68	75
	16R02	85	15	14	17	41	40	43
	16F02	128	28	27	33	85	86	90
	16H01	110	23	21	24	68	66	68
	16U01	21	17	15	15	48	45	45
	16Fib01	128	13	14	14	38	41	43
	16F01	121	33	28	28	94	87	86
	16T01	114	36	32	27	94	84	75
	17T02	110	26	22	24	68	63	73
	17R01	84	14	12	14	44	38	40
	16Cl02	72	13	14	26	39	43	59
	17F02	134	27	27	35	79	83	98
	16R01	87	16	13	15	51	41	42
	17U01	76	15	13	13	43	40	40
	16H02	111	22	20	25	62	59	66
	17F01	135	30	26	26	83	80	78

Tooth reference	Height from the CEJ to the apex (mm)	Maximum tooth height (mm)	Root midpoint (mm)	Bucco-lingual diameter at root midpoint (mm)	Mesio-distal diameter at root midpoint (mm)	Bucco-lingual diameter at CEJ (mm)	Mesio-distal diameter at CEJ (mm)	Bucco-lingual diameter of crown (mm)	Mesio-distal diameter of crown (mm)
AC8a	15.90	21.61	7.95	9.46	7.11	10.28	7.03	10.20	8.82
MD89	13.51	19.96	6.76	6.89	3.42	9.35	6.74	10.10	9.98
T9	11.48	19.14	5.74	9.32	6.01	9.23	7.04	10.50	9.28
AE26	12.77	19.15	6.38	8.89	5.45	10.37	7.54	10.77	9.67
O4	11.19	17.43	5.59	4.06	4.17	9.01	6.55	9.92	9.31
AH3	10.22	17.03	5.11	5.80	4.01	8.64	6.38	10.15	8.76
AC10b	8.08	18.75	4.04	3.16	8.54	7.50	8.68	8.83	9.80
J25	10.76	19.70	5.38	4.43	7.40	7.55	8.38	8.80	9.99
D4	9.25	15.57	4.63	6.05	7.47	8.64	8.58	10.33	12.16
H441	11.34	19.40	5.67	8.52	3.51	10.88	5.54	10.51	9.16
M2	6.24	13.81	3.12	7.27	3.53	9.36	6.67	10.40	9.92
AI54	12.79	18.72	6.39	10.57	4.75	12.06	8.59	13.23	9.56
AH10	12.12	18.76	6.06	7.12	3.24	8.23	7.48	10.40	9.18
M10	10.11	17.06	5.55	7.54	3.22	9.89	6.78	10.80	9.10
V2	12.76	18.81	6.38	5.17	7.71	6.63	11.39	9.38	12.19
N17	11.21	20.06	5.60	9.32	5.30	9.33	5.98	10.72	8.59

Appendix 8.2

Table 8.2 – Bone and Teeth Measurements After Burning.

Bone		Values (mm)					
		Maximum length	Maximum breadth	Height	Vertebral Breath	Vertebral Length	Vertebral Body Measurment
Trabecular	16Cub	25	22	35	-	-	-
	16V05	-	-	18	20	33	17
	16Pa	43	18	42	-	-	-
	16Nav	42	23	34	-	-	-
	16Ta	45	35	-	-	-	-
	16V06	-	-	16	27	28	21
	16Ca	68	31	-	-	-	-
	16V07	-	-	19	29	30	23
	16V04	-	-	19	16	32	16
	17Cub	25	21	38	-	-	-
	17V05	-	-	22	40	37	24
	17V04	-	-	21	33	37	25
	17Pa	34	15	36	-	-	-
	17V07	-	-	23	30	37	25
	17Ca	59	35	-	-	-	-
	17Ta	42	32	-	-	-	-

Bone		Values (mm)						
		Length	Proximal diameter	Medial diameter	Distal diameter	Proximal circumference	Medial circumference	Distal circumference
Cortical	16T02	109	26	24	26	70	68	75
	16R02	85	15	14	17	41	40	43
	16F02	121	27	28	32	84	85	93
	16H01	110	22	21	24	66	63	67
	16U01	73	13	11	12	37	36	36
	16Fib01	110	10	11	11	31	32	35
	16F01	118	-	-	-	-	-	-
	16T01	101	25	26	22	69	66	60
	17T02	110	26	22	24	68	63	73
	17R01	84	14	12	14	44	38	40
	16Cl02	71	13	14	25	39	43	68
	17F02	133	26	26	34	77	81	95
	16R01	74	13	11	12	40	32	33
	17U01	67	11	10	10	34	31	31
	16H02	95	17	15	19	47	46	51
	17F01	122	25	21	20	67	62	62

Table 8.2 – Bone and Teeth Measurements After Burning (cont.)

Tooth reference	Height from the CEJ to the apex (mm)	Maximum tooth height (mm)	Root midpoint (mm)	Bucco-lingual diameter at root midpoint (mm)	Mesio-distal diameter at root midpoint (mm)	Bucco-lingual diameter at CEJ (mm)	Mesio-distal diameter at CEJ (mm)	Bucco-lingual diameter of crown (mm)	Mesio-distal diameter of crown (mm)
AC8a	15.9	21.61	7.95	9.46	7.11	10.28	7.03	10.2	8.82
MD89	13.51	19.96	6.76	6.89	3.42	9.35	6.74	10.1	9.98
T9	10.28	18.27	5.14	8.07	5.39	9.2	6.53	11.33	9.51
AE26	12.62	19.39	6.31	8.87	5.76	10.27	7.37	11.28	10.15
O4	-	-	-	-	-	-	-	-	-
AH3	8.66	-	4.33	4.63	3.31	8.83	5.76	-	-
AC10b	11.57	-	5.79	2.95	7.09	6.65	7.16	-	-
J25	8.96	17.66	4.48	4.75	5.94	6.29	6.98	9.12	10.58
D4	9.25	15.57	4.63	6.05	7.47	8.64	8.58	10.33	12.16
H441	11.34	19.40	5.67	8.52	3.51	10.88	5.54	10.51	9.16
M2	5.91	13.59	2.96	6.82	4.31	10.18	6.75	11.57	9.92
AI54	12.41	19.83	6.21	10.81	6.06	13.59	9.31	14.30	10.64
AH10	9.69	17.27	4.85	6.27	2.84	9.36	6.53	10.93	9.87
M10	8.25	-	4.13	7.11	3.93	9.14	5.37	-	-
V2	9.65	16.65	4.83	7.41	4.86	9.81	5.95	10.98	9.40
N17	11.69	-	5.85	8.21	3.69	8.49	4.98	-	-

Appendix 8.3

Table 8.3 – Burial Chronogram.

Treatment	Sample	Months			
		2	4	6	8
Unburned	16Cub	x	x	x	x
	16V05	x	x	x	x
	16V04			x	
	17Cub			x	
	AC8a	x	x	x	x
	MD89	x	x	x	x
	D4			x	
	H441			x	
500 °C	16Pa	x	x	x	x
	16Nav	x	x	x	x
	17V05			x	
	17V04			x	
	T9	x	x	x	x
	AE26	x	x	x	x
	M2			x	
	AI54			x	
900 °C	16Ta	x	x	x	x
	16V06	x	x	x	x
	17Pa			x	
	17V07			x	
	O4	x	x	x	x
	AH3	x	x	x	x
	AH10			x	
	M10			x	
1050 °C	16Ca	x	x	x	x
	16V07	x	x	x	x
	17Ca			x	
	17Ta			x	
	AC10b	x	x	x	x
	J25	x	x	x	x
	V2			x	
	N17			x	

Table 8.3 – Burial Chronogram (cont.).

Treatment	Sample	Months			
		2	4	6	8
Unburned	16T02	x	x	x	x
	16R02	x	x	x	x
	17T02			x	
	17R01			x	
500 ^{o}C	16F02	x	x	x	x
	16H01	x	x	x	x
	16Cl02			x	
	17F02			x	
900 ^{o}C	16U01	x	x	x	x
	16Fib01	x	x	x	x
	16R01			x	
	17U01			x	
1050 ^{o}C	16F01	x	x	x	x
	16T01	x	x	x	x
	16H02			x	
	17F01			x	

Appendix 8.4

Table 8.4 – Bi-monthly and six-monthly descriptive analysis of cortical bone's length variation.

| Bone	T (ºC)	l_0 oct2015 l_1 dec2015 $\Delta l/l_0$ (%)	l_1 dec2015 l_2 feb2016 $\Delta l/l_1$ (%)	l_2 feb2016 l_3 april2016 $\Delta l/l_2$ (%)	l_0 oct2015 l_3 april2016 $\Delta l/l_0$ (%)	l_3 april2016 l_4 jun2016 $\Delta l/l_3$ (%)	l_0 oct2015 l_4 jun2016 $\Delta l/l_0$ (%)
16T02	Unb	0.0	0.0	0.9	-	0.0	0.9
16R02	Unb	1.2	-1.2	0.0	-	0.0	0.0
17T02	Unb	-	-	-	0.0	-	-
17R01	Unb	-	-	-	1.2	-	-
16F02	500	0.0	0.8	-0.8	-	0.0	0.0
16H01	500	0.0	0.0	-0.9	-	0.0	-0.9
16Cl02	500	-	-	-	2.8	-	-
17F02	500	-	-	-	0.0	-	-
16U01	900	1.4	1.4	-1.3	-	0.0	1.4
16Fib01	900	-2.7	-6.5	0.0	-	0.0	-9.1
16R01	900	-	-	-	2.7	-	-
17U01	900	-	-	-	0.0	-	-
16F01	1050	0.8	0.0	0.8	-	-0.8	0.8
16T01	1050	7.9	-5.5	0.0	-	0.0	2.0
16H02	1050	-	-	-	3.2	-	-
17F01	1050	-	-	-	0.0	-	-
Average		1.1	-1.4	-0.2	1.2	-0.1	-0.6
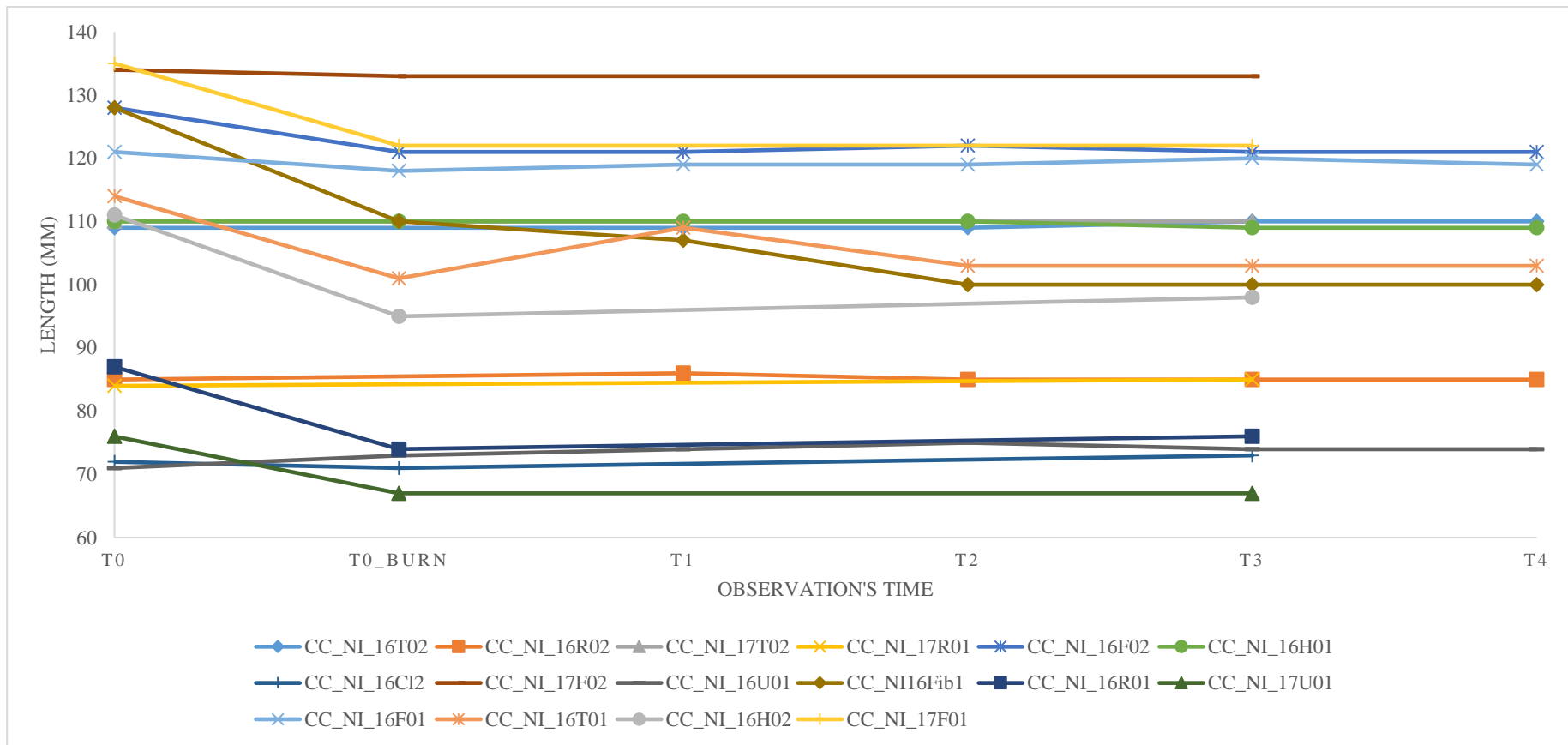
Figure 8.1 – The evolution of cortical bone's length variation from Time 0 (before burning) to Time 4 (fourth bi-monthly exhumation after sample collection) and from T0 (before burn) to T3 (after sample collection) to bones buried for six months. The values refer to absolute size variations in mm.

Table 8.5 – Bi-monthly and six-monthly descriptive analysis of cortical bone's proximal diameter variation.

| Bone	T (ºC)	pd_0 oct2015 pd_1 dec015 $\Delta pd/pd_0$ (%)	pd_1 dec2015 pd_2 feb2016 $\Delta pd/pd_1$ (%)	pd_2 feb2016 pd_3 april2016 $\Delta pd/pd_2$ (%)	pd_0 oct2015 pd_3 april2016 $\Delta pd/pd_0$ (%)	pd_3 april2016 pd_4 jun2016 $\Delta pd/pd_3$ (%)	pd_0 oct2015 pd_4 jun2016 $\Delta pd/pd_0$ (%)
16T02	Unb	0.0	0.0	0.0	-	0.0	0.0
16R02	Unb	0.0	0.0	0.0	-	0.0	0.0
17T02	Unb	-	-	-	0.0	-	-
17R01	Unb	-	-	-	7.1	-	-
16F02	500	3.7	0.0	-3.6	-	0.0	0.0
16H01	500	4.5	-4.3	0.0	-	0.0	0.0
16Cl02	500	-	-	-	0.0	-	-
17F02	500	-	-	-	0.0	-	-
16U01	900	-7.7	0.0	0.0	-	0.0	-7.7
16Fib01	900	0.0	0.0	0.0	-	0.0	0.0
16R01	900	-	-	-	0.0	-	-
17U01	900	-	-	-	0.0	-	-
16T01	1050	0.0	0.0	0.0	-	0.0	0.0
16H02	1050	-	-	-	0.0	-	-
17F01	1050	-	-	-	-4.0	-	-
Average		0.1	-0.6	-0.5	0.4	0.0	-1.1
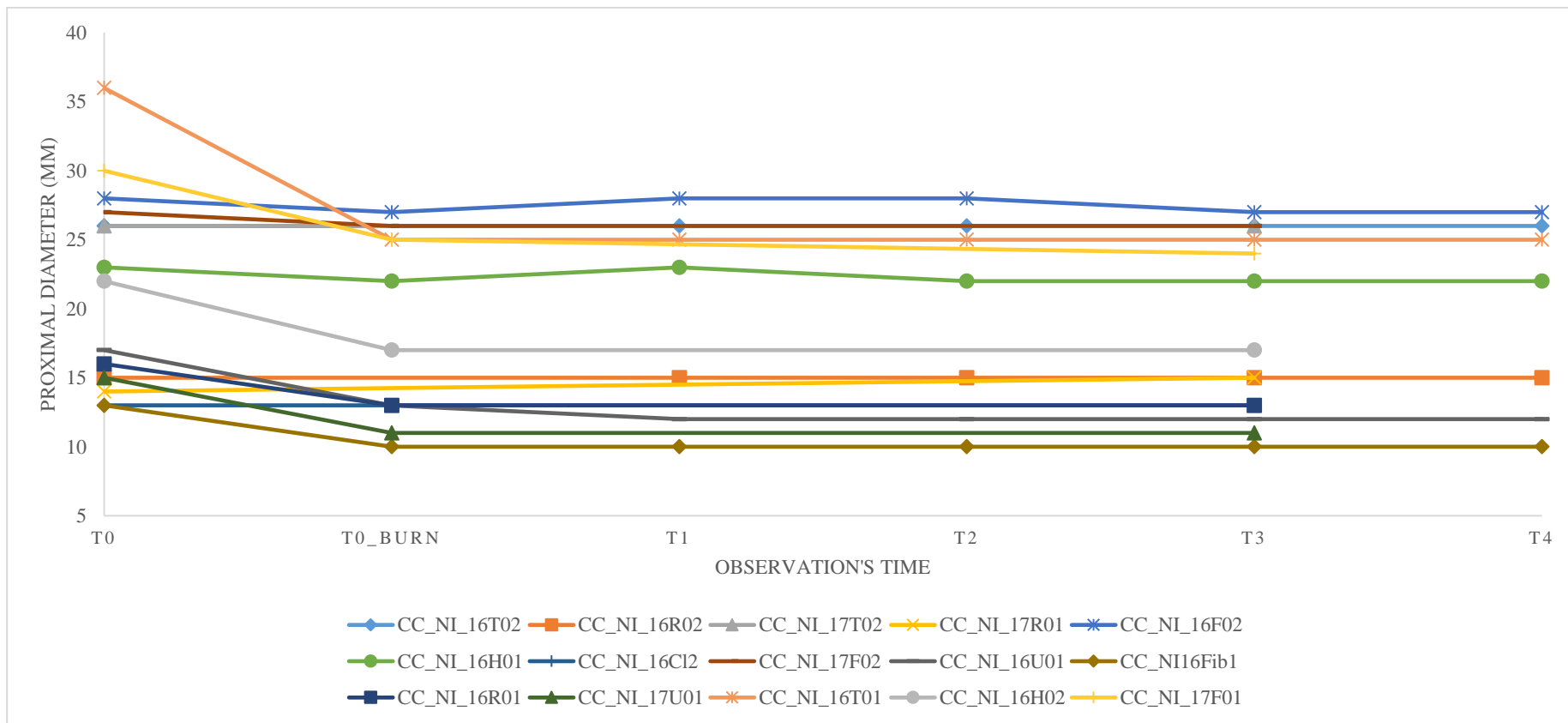
Figure 8.2 – The evolution cortical bone's proximal diameter variation from Time 0 (before burning) to Time 4 (fourth bi-monthly exhumation after sample collection) and from T0 (before burn) to T3 (after sample collection) to bones buried for six months. The values refer to absolute size variations in mm.

Table 8.6 – Bi-monthly and six-monthly descriptive analysis of cortical bone's medial diameter variation.

Bone	T (ºC)	md_0 oct2015 md_1 dec2015 $\Delta md/md_0$ (%)	md_1 dec2015 md_2 feb2016 $\Delta md/md_1$ (%)	md_2 feb2016 md_3 april2016 $\Delta md/md_2$ (%)	md_0 oct2015 md_3 april2016 $\Delta md/md_0$ (%)	md_3 april2016 md_4 jun2016 $\Delta md/md_3$ (%)	md_0 oct2015 md_4 jun2016 $\Delta md/md_0$ (%)
16T02	Unb	4.2	0.0	0.0	-	0.0	4.2
16R02	Unb	0.0	0.0	7.1	-	-6.7	0.0
17T02	Unb	-	-	-	22.7	-	-
17R01	Unb	-	-	-	8.3	-	-
16F02	500	-3.6	3.7	0.0	-	0.0	0.0
16H01	500	0.0	0.0	0.0	-	0.0	0.0
16Cl02	500	-	-	-	0.0	-	-
17F02	500	-	-	-	3.8	-	-
16U01	900	0.0	9.1	-8.3	-	0.0	0.0
16Fib01	900	-9.1	0.0	0.0	-	0.0	-9.1
16R01	900	-	-	-	-9.1	-	-
17U01	900	-	-	-	0.0	-	-
16T01	1050	0.0	0.0	0.0	-	3.8	3.8
16H02	1050	-	-	-	0.0	-	-
17F01	1050	-	-	-	0.0	-	-
Average		-1.2	1.8	-0.2	3.2	-0.4	-0.2

Figure 8.3 – The evolution of cortical bone's medial diameter variation from Time 0 (before burning) to Time 4 (fourth bi-monthly exhumation after sample collection) and from T0 (before burn) to T3 (after sample collection) to bones buried for six months. The values refer to absolute size variations in mm.

Table 8.7 – Bi-monthly and six-monthly descriptive analysis of cortical bone's distal diameter variation.

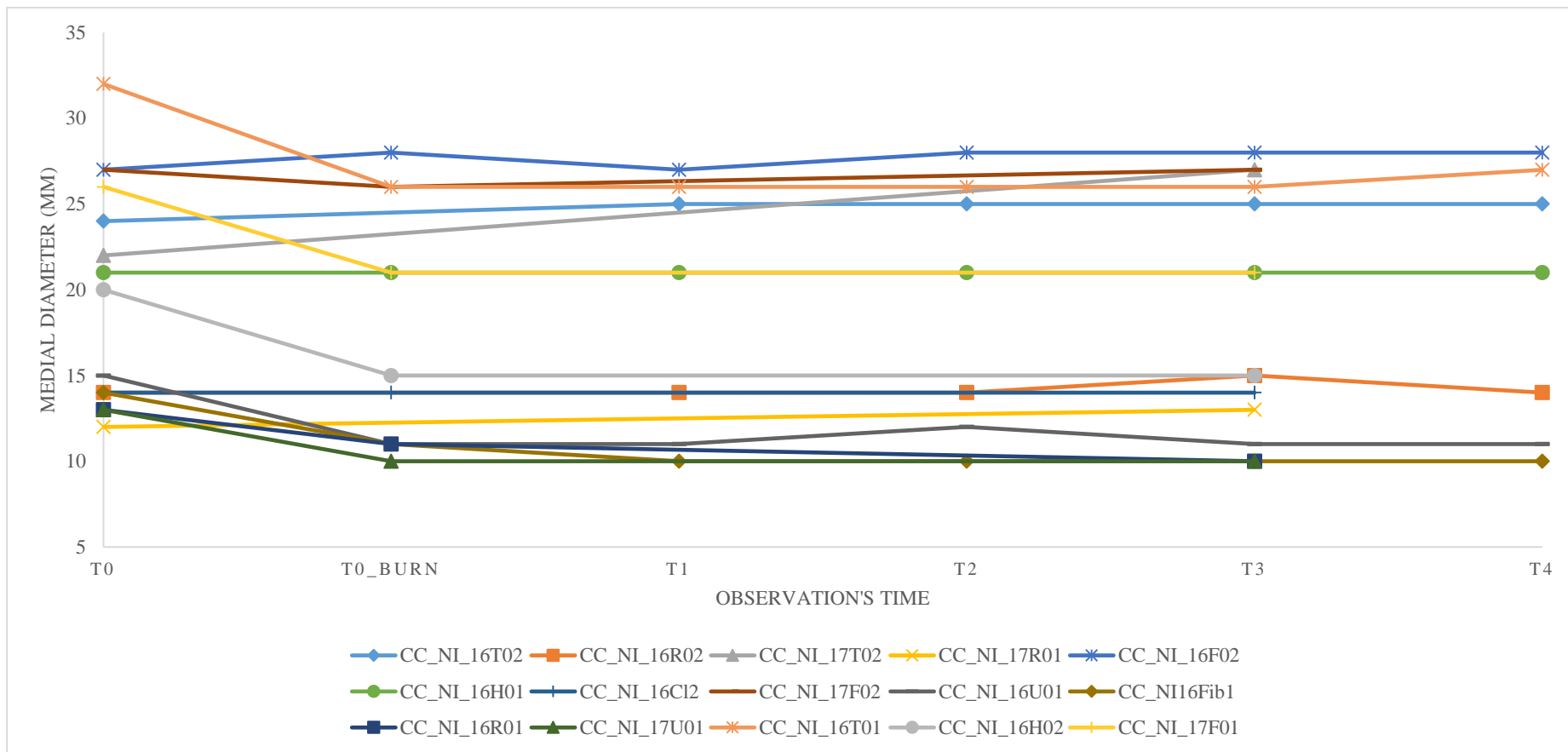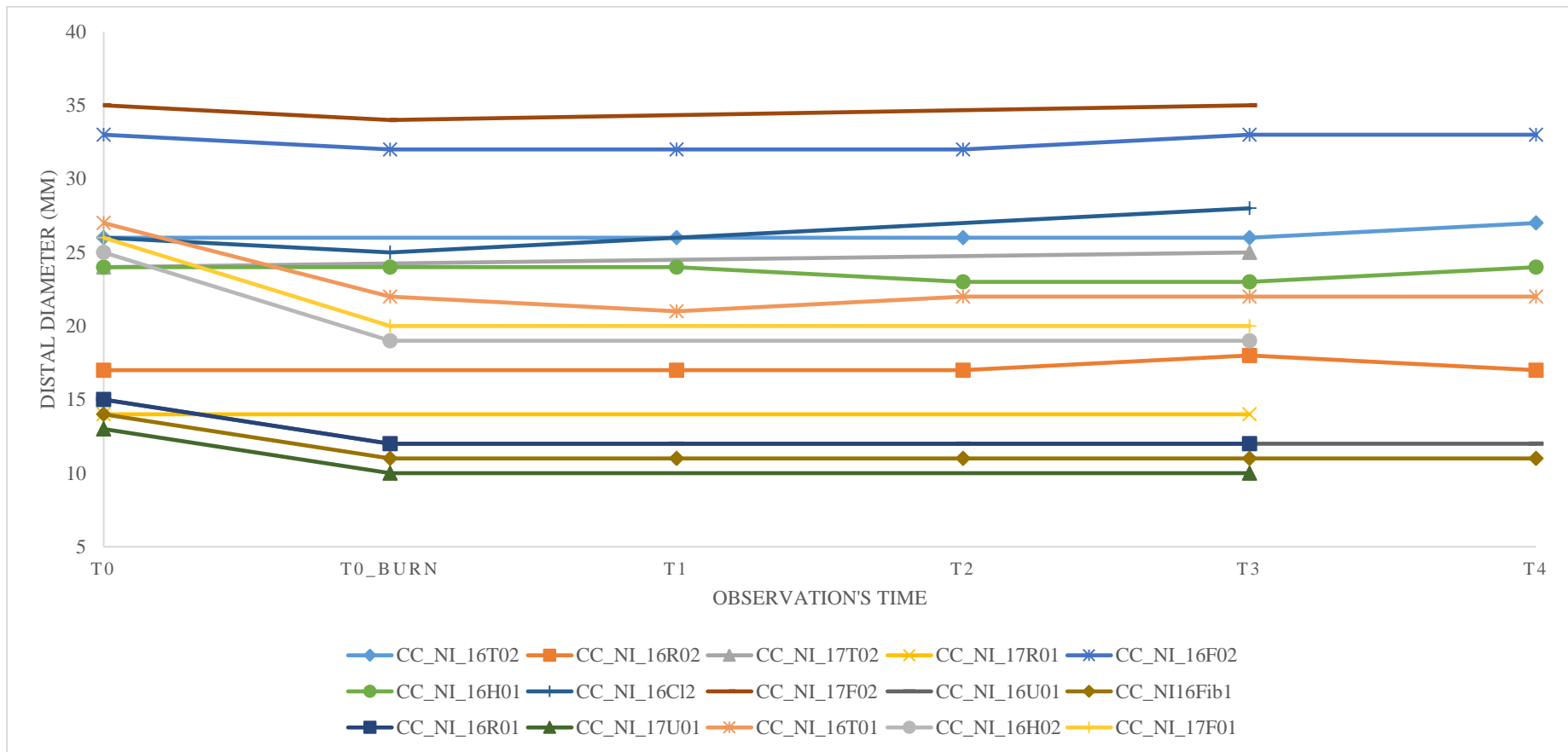| Bone	T (ºC)	dd_0 oct2015 dd_1 dec2015 $\Delta dd/dd_0$ (%)	dd_1 dec2015 dd_2 feb2016 $\Delta dd/dd_1$ (%)	dd_2 feb2016 dd_3 april2016 $\Delta dd/dd_2$ (%)	dd_0 oct2015 dd_3 april2016 $\Delta dd/dd_0$ (%)	dd_3 april2016 dd_4 jun2016 $\Delta dd/dd_3$ (%)	dd_0 oct2015 dd_4 jun2016 $\Delta dd/dd_0$ (%)
16T02	Unb	0.0	0.0	0.0	-	3.8	3.8
16R02	Unb	0.0	0.0	5.9	-	-5.6	0.0
17T02	Unb	-	-	-	4.2	-	-
17R01	Unb	-	-	-	0.0	-	-
16F02	500	0.0	0.0	3.1	-	0.0	3.1
16H01	500	0.0	-4.2	0.0	-	4.3	0.0
16Cl02	500	-	-	-	12.0	-	-
17F02	500	-	-	-	2.9	-	-
16U01	900	0.0	0.0	0.0	-	0.0	0.0
16Fib01	900	0.0	0.0	0.0	-	0.0	0.0
16R01	900	-	-	-	0.0	-	-
17U01	900	-	-	-	0.0	-	-
16T01	1050	-4.5	4.8	0.0	-	0.0	0.0
16H02	1050	-	-	-	0.0	-	-
17F01	1050	-	-	-	0.0	-	-
Average		-0.6	0.1	1.3	2.4	0.4	1.0

Figure 8.4 – The evolution of cortical bone's distal diameter variation from Time 0 (before burning) to Time 4 (fourth bi-monthly exhumation after sample collection) and from T0 (before burn) to T3 (after sample collection) to bones buried for six months. The values refer to absolute size variations in mm.

Table 8.8 – Bi-monthly and six-monthly descriptive analysis of cortical bone's proximal circumference variation.

| Bone	T (ºC)	pc_0 oct2015 pc_1 dec2015 $\Delta pc/pc_0$ (%)	pc_1 dec2015 pc_2 feb2016 $\Delta pc/pc_1$ (%)	pc_2 feb2016 pc_3 april2016 $\Delta pc/pc_2$ (%)	pc_0 oct2015 pc_3 april2016 $\Delta pc/pc_0$ (%)	pc_3 april2016 pc_4 jun2016 $\Delta pc/pc_3$ (%)	pc_0 oct2015 pc_4 jun2016 $\Delta pc/pc_0$ (%)
16T02	Unb	2.9	0.0	0.0	-	-1.4	1.4
16R02	Unb	2.4	2.4	2.3	-	0.0	7.3
17T02	Unb	-	-	-	1.5	-	-
17R01	Unb	-	-	-	4.5	-	-
16F02	500	0.0	0.0	0.0	-	-1.2	-1.2
16H01	500	-1.5	1.5	-1.5	-	0.0	-1.5
16Cl02	500	-	-	-	-2.6	-	-
17F02	500	-	-	-	0.0	-	-
16U01	900	2.7	-2.6	2.7	-	0.0	2.7
16Fib01	900	3.2	0.0	0.0	-	0.0	3.2
16R01	900	-	-	-	5.0	-	-
17U01	900	-	-	-	0.0	-	-
16T01	1050	1.4	-2.9	2.9	-	-1.4	0.0
16H02	1050	-	-	-	2.1	-	-
17F01	1050	-	-	-	0.0	-	-
Average		1.6	-0.2	0.9	1.3	-0.6	1.7
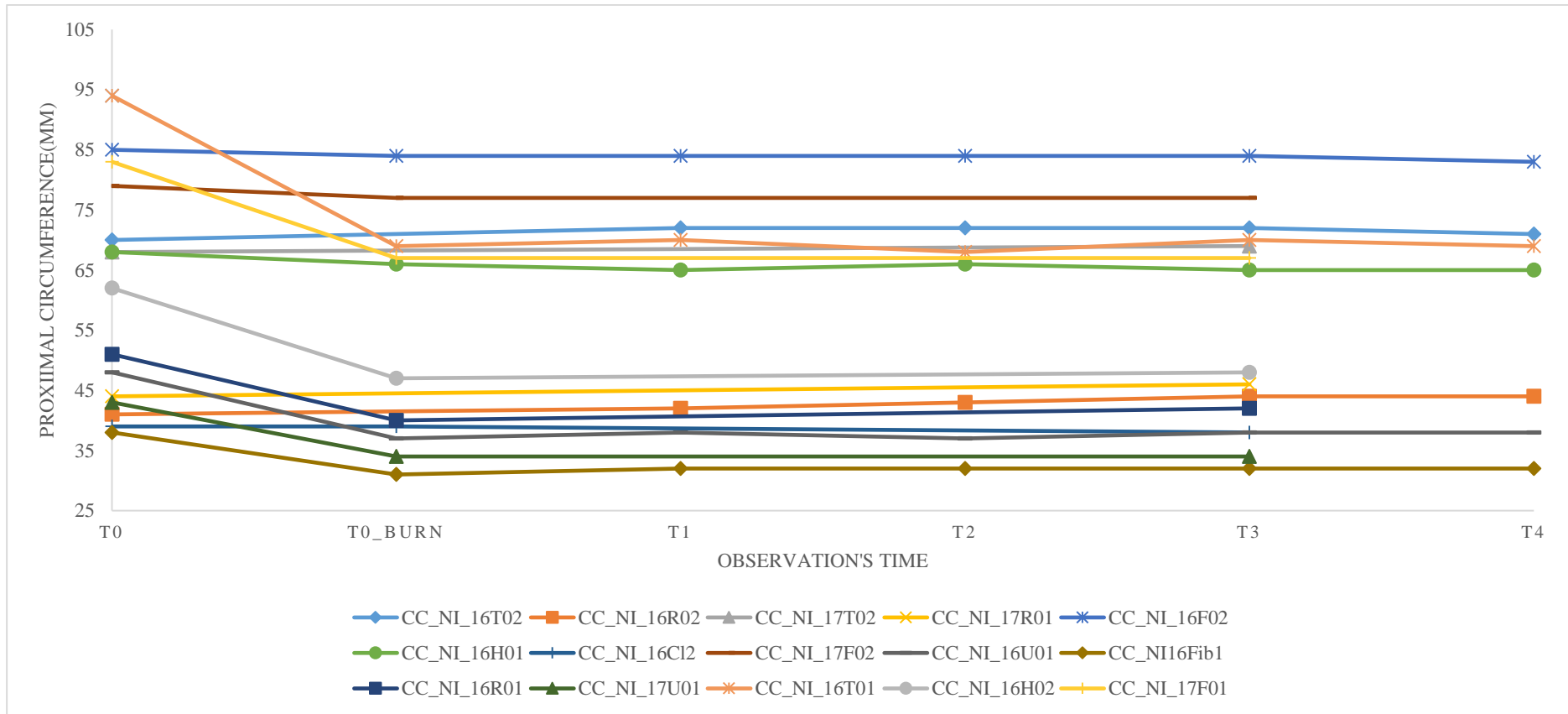
Figure 8.5 – The evolution of cortical bone's proximal circumference variation from Time 0 (before burning) to Time 4 (fourth bi-monthly exhumation after sample collection) and from T0 (before burn) to T3 (after sample collection) to bones buried for six months. The values refer to absolute size variations in mm.

Table 8.9 – Bi-monthly and six-monthly descriptive analysis of cortical bone's medial circumference variation.

| Bone	T (ºC)	mc_0 oct2015 mc_1 dec2015 $\Delta mc/mc_0$ (%)	mc_1 dec2015 mc_2 feb2016 $\Delta mc/mc_1$ (%)	mc_2 feb2016 mc_3 april2016 $\Delta mc/mc_2$ (%)	mc_0 oct2015 mc_3 april2016 $\Delta mc/mc_0$ (%)	mc_3 april2016 mc_4 jun2016 $\Delta mc/mc_3$ (%)	mc_0 oct2015 mc_4 jun2016 $\Delta mc/mc_0$ (%)
16T02	Unb	2.9	0.0	-1.4	-	1.4	2.9%
16R02	Unb	0.0	0.0	0.0	-	0.0	0.0
17T02	Unb	-	-	-	1.6	-	-
17R01	Unb	-	-	-	2.6	-	-
16F02	500	-1.2	0.0	0.0	-	1.2	0.0
16H01	500	0.0	1.6	-1.6	-	1.6	1.6
16Cl02	500	-	-	-	-2.3	-	-
17F02	500	-	-	-	0.0	-	-
16U01	900	0.0	0.0	2.8	-	0.0	2.8
16Fib01	900	0.0	0.0	0.0	-	0.0	0.0
16R01	900	-	-	-	3.1	-	-
17U01	900	-	-	-	3.2	-	-
16T01	1050	0.0	0.0	1.5	-	0.0	1.5
16H02	1050	-	-	-	0.0	-	-
17F01	1050	-	-	-	1.6	-	-
Average		0.3	0.2	0.2	1.2	0.6	1.3
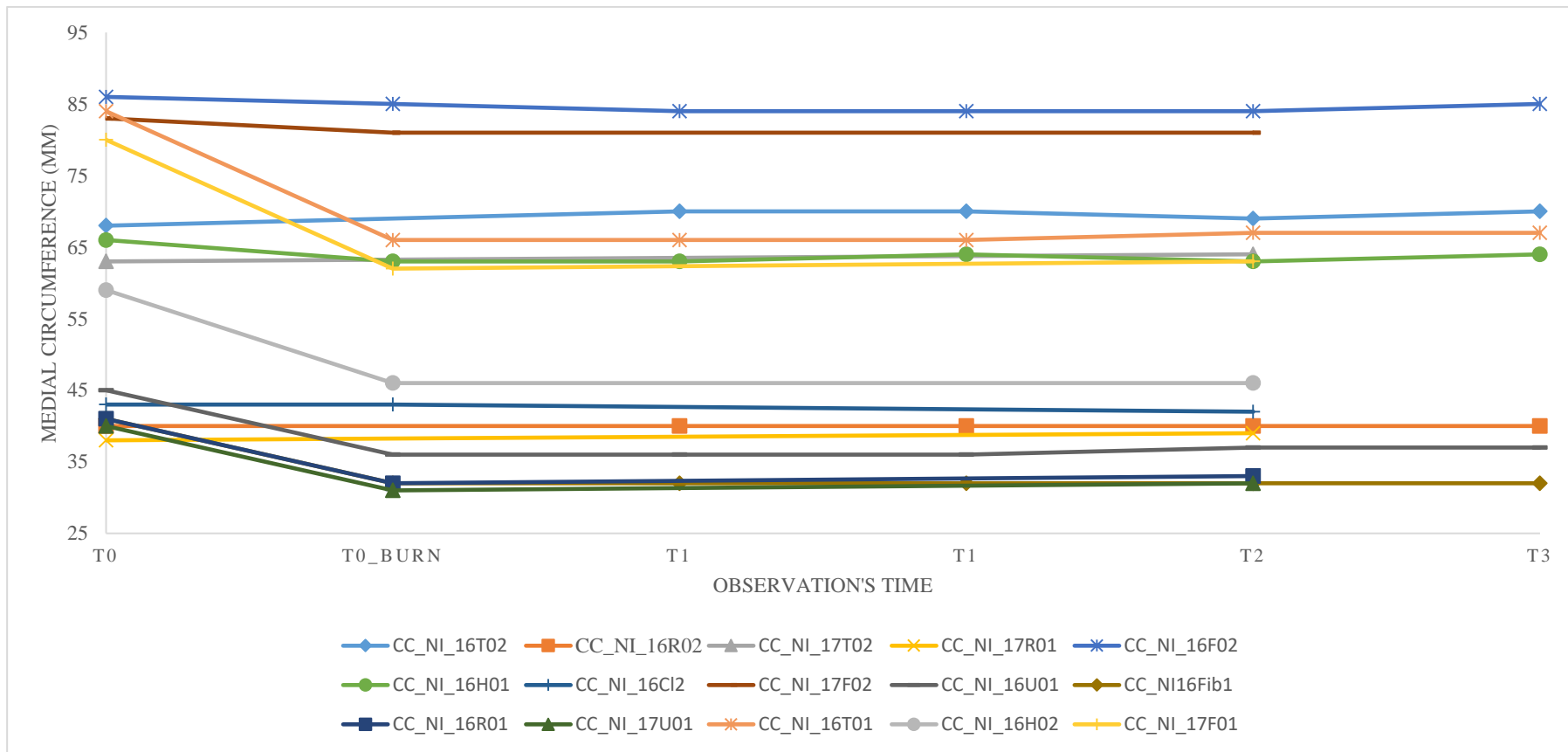
Figure 8.6 – The evolution of cortical bone's medial circumference variation from Time 0 (before burning) to Time 4 (fourth bi-monthly exhumation after sample collection) and from T0 (before burn) to T3 (after sample collection) to bones buried for six months. The values refer to absolute size variations in mm.

Table 8.10 – Bi-monthly and six-monthly descriptive analysis of cortical bone's distal circumference variation.

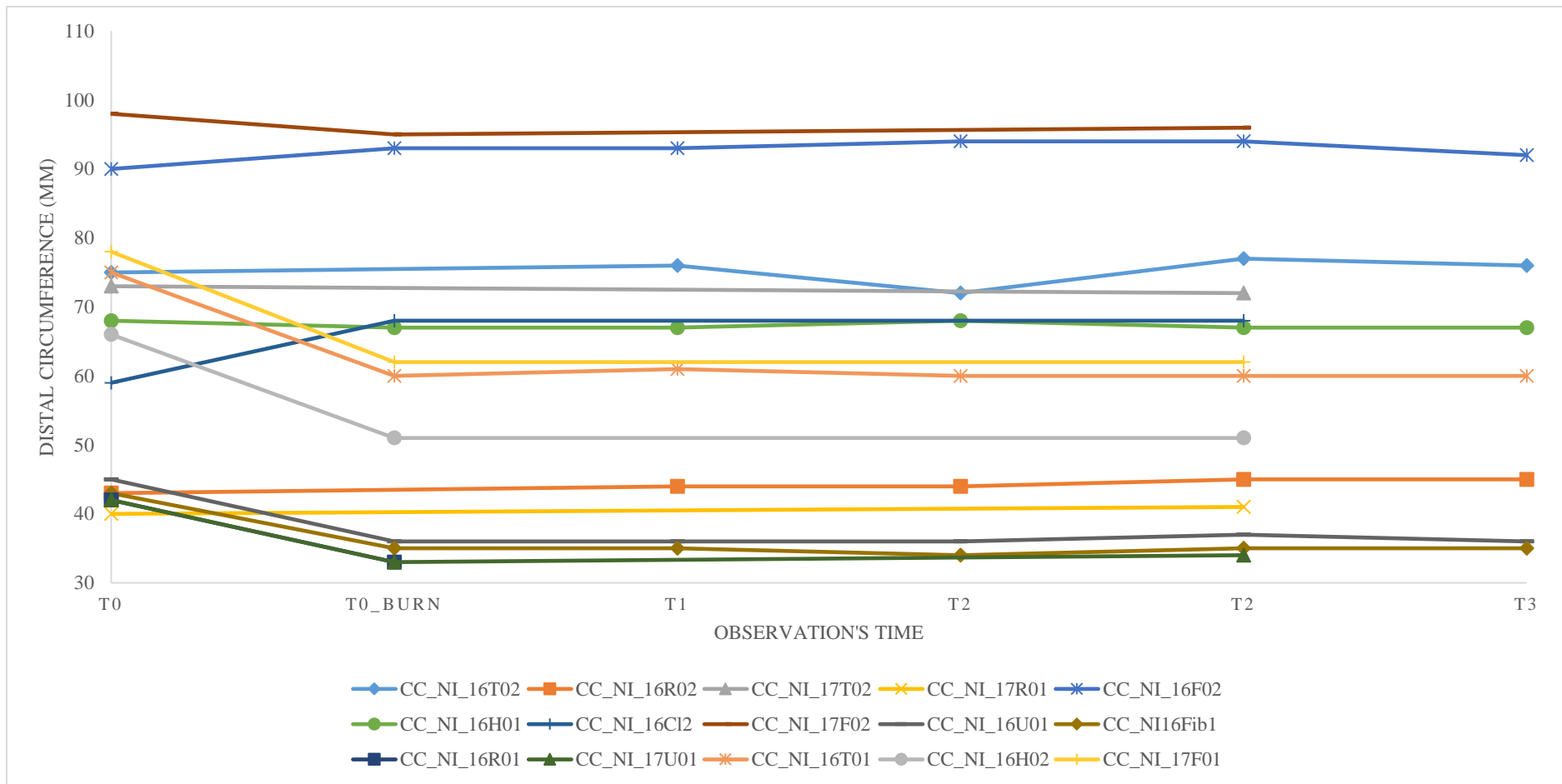| Bone	T (ºC)	dc_0 oct2015 dc_1 dec2015 $\Delta dc/dc_0$ (%)	dc_1 dec2015 dc_2 feb2016 $\Delta dc/dc_1$ (%)	dc_2 feb2016 dc_3 april2016 $\Delta dc/dc_2$ (%)	dc_0 oct2015 dc_3 april2016 $\Delta dc/dc_0$ (%)	dc_3 april2016 dc_4 jun2016 $\Delta dc/dc_3$ (%)	dc_0 oct2015 dc_4 jun2016 $\Delta dc/dc_0$ (%)
16T02	Unb	1.3	-5.3	6.9	–	-1.3	1.3
16R02	Unb	2.3	0.0	2.3	–	0.0	4.7
17T02	Unb	–	–	–	-1.4	–	-
17R01	Unb	–	–	–	2.5	–	-
16F02	500	0.0	1.1	0.0	–	-2.1	-1.1
16H01	500	0.0	1.5	-1.5	–	0.0	0.0
16Cl02	500	–	–	–	0.0	–	-
17F02	500	–	–	–	1.1	–	-
16U01	900	0.0	0.0	2.8	–	-2.7	0.0
16Fib01	900	0.0	-2.9	2.9	–	0.0	0.0
16R01	900	–	–	–	3.0	–	-
17U01	900	–	–	–	0.0	–	-
16T01	1050	1.7	-1.6	0.0	–	0.0	0.0
16H02	1050	–	–	–	0.0	–	-
17F01	1050	–	–	–	0.0	–	-
Average		0.8	-1.0	1.9	0.7	-0.9	0.7

Figure 8.7 – The evolution of cortical bone's distal circumference variation from Time 0 (before burning) to Time 4 (fourth bi-monthly exhumation after sample collection) and from T0 (before burn) to T3 (after sample collection) to bones buried for six months. The values refer to absolute size variations in mm.