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ERASMUS MUNDUS MASTER IN ARCHAEOLOGICAL MATERIALS SCIENCE (ERASMUS MUNDUS-ARCHMAT)

MESTERADO EM ARQUEOLOGIA E AMBIENTE (ERASMUS MUNDUS-ARCHMAT)

Diet of the Post-medieval population at Lagos, 14th-19th Portugal

Mushweshwe Tawanda (43703)

Anne-France Maurer

(Supervisor: Universidade de Evora)

Cristina Maria Barrocas Dias

(Co-supervisor: Universidade de Evora)

Maria João Valente

(Co-supervisor: Universidade do Algarve)

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Universidade de Évora

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Anne-France Maurer

(Orientador - Universidade de Évora)

Cristina Maria Barrocas Dias

(Co-orientador - Universidade de Évora)

Maria João Valente

(Co-orientador - Universidade de Évora)

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PANEL OF JURY

President: Professor Susana Gómez Martinez, Universidade de Évora

Examiner: Professor Maria Teresa Matos Fernandes, Universidade de Évora

Supervisor: Anne-France Maurer, Universidade de Évora

Partner Member: Laura Parducci, Università di Roma La Sapienza

ABSTRACT

Stable isotopic analysis of carbon and nitrogen ratios in bone collagen was done on twelve human skeletons selected from a batch excavated in Largo de Santa Maria da Graça from 2004-2005 (Lagos, Portugal). Elemental Analyser- Isotopic Ratio Mass Spectrometry (EA-IRMS) was used for stable isotope analysis of carbon and nitrogen to reconstruct the diet of post-Medieval population of Lagos between the 14th-19th centuries. Results of the analysis provided a basis for understanding dietary changes with time represented by the two chronological phases of the cemetery of Santa Maria da Graça. Distinction in phases of the cemetery may be due to new food resources from the colonial enterprises by the Portuguese Kingdom between the 15th-19th centuries. The correlation between carbon and nitrogen of the humans may have been from much consumption of marine resources with little terrestrial animal products. Little distinction of food uptake when considering gender and pathologies was reflected.

Keywords*: Diet, Paleodiet, Stable isotopes, Lagos, Santa Maria da Graça, Post-Medieval Age, Age of Discoveries.*

RESUMO

Titulo: Dieta da população de Lagos entre os séculos XIV-XIX, Portugal

Foi efectuada análise das razões isotópicas de carbono e azoto no colagénio de restos osteológicos de doze esqueletos humanos inumados no Largo de Santa Maria da Graça (Lagos, Portugal) entre 2004-2005, e que podem ser agrupados em dois períodos cronológicos. A análise foi efectuada com recurso a um Analisador Elementar acoplado a Espectrómetro de Massa de Razões Isotópicas (EA-IRMS), com o intuito de reconstruir a dieta dos habitantes de Lagos entre os séculos XIV e XIX. A diferença observada entre indivíduos originários das 2 fases do cemitério pode dever-se à introdução de novos alimentos no Reino de Portugal durante a Expansão Ultramarina. A correlação entre os valores das razões isotópicas de carbono e azoto observada nos indivíduos estudados pode dever-se ao grande consumo de recursos marinhos quando comparado com produtos de origem animal terrestre; não foram observadas grandes diferenças quando se considera o género e as patologias dos indivíduos.

Palavras-chave: *Dieta, Paleodieta, Isótopos estáveis, Lagos, Santa Maria da Graça, Expansão ultramarina*

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LIST OF ABBREVIATIONS

AIR	Ambient Inhalable Reservoir
ATR-FTIR	Attenuated Total Reflection Fourier-transform infrared spectroscopy
CAM	Crassulacean Acid Metabolism
CV	Cacela-a-Velha
DNA	Deoxyribonucleic acid
HCl	Hydrochloric acid
IRMS	Isotopic Ratio Mass Spectrometry
LAB	Laboratory of Biological Anthropology
NaOH	Sodium hydroxide
OSC	Oficina do Senhor Carrilho
PDB	PeeDee Belemnite Carbonate
SW-NE	South West-North East
W-E	West-East
XRD	X-ray Diffraction

CHAPTER 1: INTRODUCTION

Understanding ways of sustenance of past societies has been one of the most fascinating topics amongst archaeologists and related researchers because food consumption has, since the dawn of humanity, been a driving force in changing and defining our anatomical and cognitive development as well as the sociocultural ways of living. Various methods have been used to reconstruct paleodiets and these include archaeobotanical and zooachaeological studies in which both plant and faunal remains from archaeological sites have been studied, respectively. Direct methods which have been used by archaeologists include analysis of animal bones, macrobotanical plant remains, pollen and phytoliths in the soil, organic residues in pottery and coprolites. Indirect evidence has also been analysed from skeletal pathology, dental wear patterns, ethnographic observations, writings, and artistic depictions (Tykot, 2004).

However, results from both direct and indirect methods are a determination of the main menu, with animal foods being the only source of semi quantitative dietary estimates (Tykot, 2004). It should be noted that these techniques have a limitation of mainly pointing out the quantitative evidence and they rarely touch the qualitative data since they are mainly used as observable units. Nonetheless, a new era of research was developed in the late 1970s called bone chemistry and it expanded the understanding of human dietary practices (Vogel and Merwe, 1977). Since then, it has been understood that biochemically, bones, teeth and other tissues preserved in the archaeological record may provide direct information about the diet of individuals after being analysed (Tykot, 2004).

According to Tykot, (2004), the combination of stable carbon and nitrogen isotopes in bone collagen, as well as carbon and oxygen isotopes in bone apatite or tooth enamel may be used to reconstruct prehistoric diet. Thus, this has led to a plethora of researches on how diets may vary basing on age, sex, social and economic status, inter-site analysis as well as different time periods.

The use of stable isotopes, extracted from human and faunal bone collagen and teeth, in archaeological studies specifically in reconstructing diets of past societies have been increasingly common since the 1970s up to now. The use of stable isotopes in palaeodietary studies is now being preferred as it provides advantages which are mentioned in the third chapter. The Iberian Peninsula has had stable isotope analysis in studying past populations' diets recently and such studies include Alexander et al. (2015), Jiminez-Brobeil et al. (2016), Sarkic et al., (2018), MacKninnon et al. (2019), Saragoça et al., (2016). Studies mainly focusing on paleodiets of populations in Portugal are few and much more recent as compared to other areas in Iberia. These researches made use of stable isotope analysis and they include one by Luxton, (2015) on relationship between diet and osteoporosis in Medieval Portugal, (Curto, et al. 2018) which focused on understanding of how military orders influenced diets of the general populations with focus on Medieval Tomar, Portugal. Another research on Portugal on Medieval Lisbon's Islamic population is from São Jorge Castle by Toso et al. (2019) and the other is by MacRoberts et al (2020) which used multi-isotopic analysis in a bid to understand diet and mobility during the Christian conquest of Iberia of a 12th -13th century military order in Évora, Portugal. There are also some researches which include two which focused on the area of Algarve the first one which is on last Muslims in during the 12th - 13th AD by Aceves (2019). The other is by Gonzàlez (2019) on first Christians in Algarve during the 13th and 14th AD.

There are hardly any studies on palaeodietary reconstruction using stable isotope analysis on post medieval populations in Portugal and the Iberian Peninsula except for a few from Spain which include Lopez-Costas and Müldner, (2018), Sarkic et al., (2018) and Mackinnon et al., (2018). To fill the above-mentioned gap in knowledge, this study focuses on investigating the dietary cultural habits of the people who resided in the area of Lagos, Portugal during the Post-medieval period from the 14th to 19th centuries using stable isotope analysis.

In this study, twelve human skeleton samples were selected from a batch excavated during an archaeological intervention exercise between 2004 and 2005 which was carried out in Largo de Santa Maria da Graça (Lagos, Portugal) and surrounding area with the purpose of installing infrastructures. The selected skeletons represent the two phases which the burials were categorised chronologically: Phase 1 late 14th to mid-16th centuries and Phase 2 mid-16th to 19th century when the cemetery was completely closed for any more burials. It should be noted that due to the COVID-19 pandemic, it was not recommended to plan to analyse more human and fauna bone collagen. Nonetheless, this research was done to have preliminary results on the site that is included into a larger project.

Lagos is a coastal city located in Algarve, the southern region of Portugal (see Figure1.1). The region of Algarve, by its geographical position, stands out as a unique stratigraphic and morpho-tectonic region with its east-northeast to west southwest orientation (Cachão et al., 2006). This coastal city became important in the geopolitics of the Portuguese Kingdom and its expansion globally during the Age of Discovery. This resulted in mixed populations, from different regions within the Portuguese Kingdom and beyond the European continent, which may have impacted the way of sustenance of people of Lagos. Hence it is worthy studying the paleodiets of these people since the selected samples cover a large span of time, thus it may be noted if diet changed in time or not as a consequence of the expansion of the Empire.

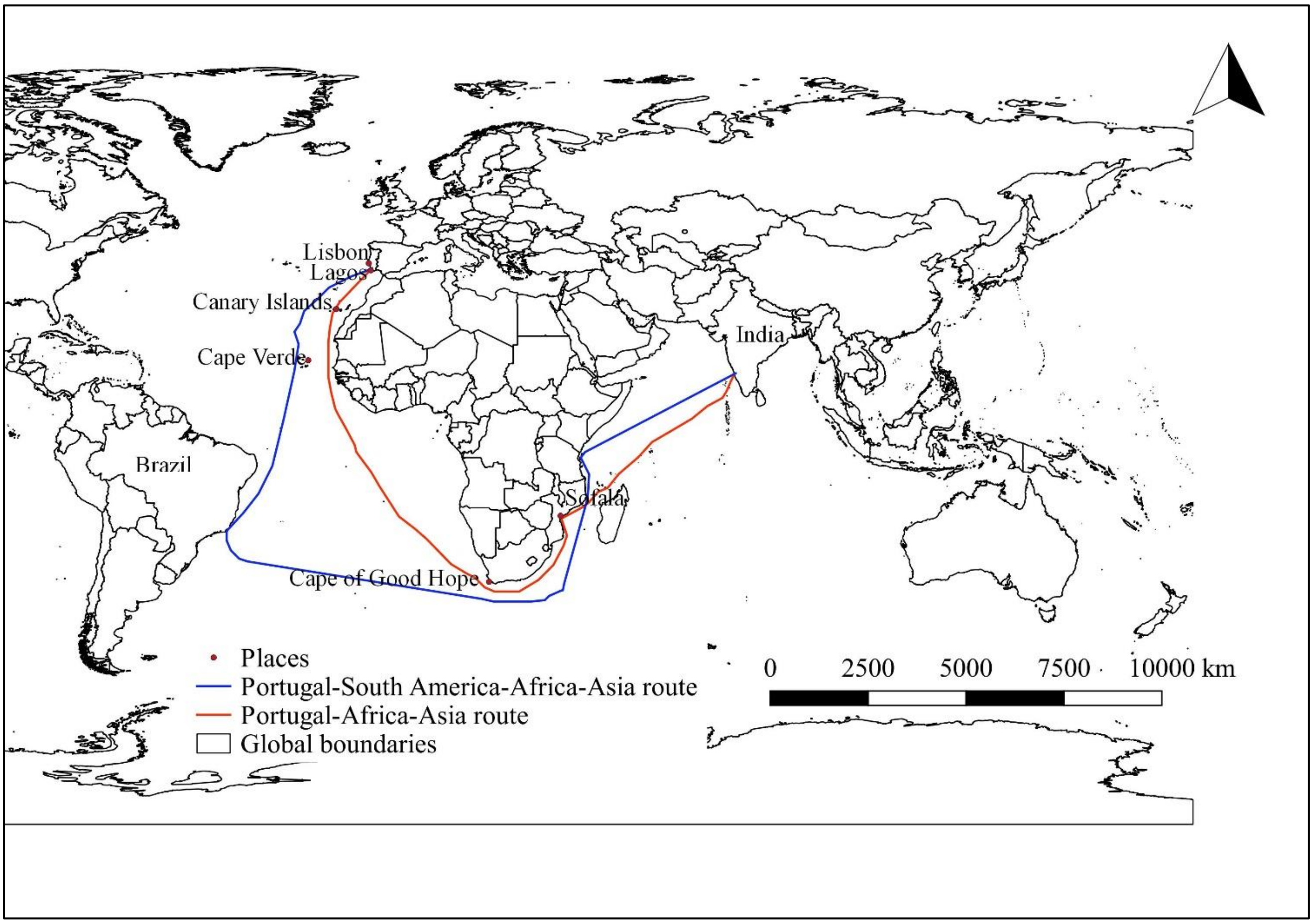

Figure 1.1 Approximated routes made by Portugal during the Age of Discovery in the 1500s (Map modified by author from www.livehistoryindia.com)

This research aims at investigating diet through two stable isotopic systems: carbon and nitrogen isotopes extracted from bone collagen from anthropological remains. These isotopes provide information on the type of plants ingested ($\delta^{13}C$), the consumption of marine resources and protein intake ($\delta^{15}N$). Stable isotope analysis studies are made possible because dietary habits are archived in the skeleton during the life of the individuals.

Using bone isotopic composition from humans buried in the necropolis of Santa Maria da Graça, this research provides an initial insight into the dietary resources the population of Lagos consumed. Another matter which is to be investigated in this study is to find out if there were any dietary changes in Lagos through a time span of around 500 years; which is from the time when the cemetery of Santa Maria da Graça was opened up to the time of its closure in the 19th century. Overall dietary comparison of different time frames; that is from medieval to post-medieval periods, will also be done in a bid to understand if there has been a general change in dietary practices in the region of Algarve.

CHAPTER 2: CONTEXTUALIZATION OF THE STUDY

2.1 Post-medieval period in Portugal

This section of the research will be dealing with giving a narrative of the Age of Discoveries in which Portugal was at the centre of it. The next sub-section details the historical background to the post-medieval period in Portugal.

2.1.1 Historical Background

Given the fact that city of Lagos, where the church of Santa Maria da Graça is located, played a major role in the era of discoveries, it is vital to include the historical background which links Lagos to the overall overseas and colonial enterprise by the Portuguese Kingdom between 15th and 19th centuries. These ventures transformed Portugal into a multicultural country in permanent contact with the rest of the European continent and the world at large (Casimiro, 2020). Thus, such cultural transformations impacted dietary practices of the Portuguese people, as will be discussed below, meaning that the population of Lagos was also impacted by these changes.

The navigations that started during the 15th century made Portugal to be among the first countries to be in contact with all the known world, and this led to an influx of new people and goods which rapidly transformed Portugal into a multicultural country (Casimiro, 2020). It has been argued that not all overseas Portuguese territories were exploited in the same way. From Africa, people were the most important "commodity" exhibited by forced movement of thousands of humans; from South America came precious metals, animals and plants such as potatoes, cocoa and corn of which these new foodstuffs were key in changing European food habits (Casimiro, 2020). It is further postulated that exotic animals were key targets by Portuguese sailors because they had a considerable impression on them and these include birds like turkeys which were imported to the Iberian Peninsula in the early 16th century from Central America (Casimiro, 2020).

From Asia, plants, mostly spices, ceramics and semi-precious stones featured strongly in European households from early 16th century influencing the production of tableware and dining habits (Casimiro, 2020). Plants, like pepper, cloves, cinnamon, which entered Portugal in early 16th century changed the way people consumed food just a few decades within their arrival because they became the staple of recipes and were used on a daily basis (Casimiro, 2020).

The global navigation done by Portugal made it become the first country in Early Morden Europe to receive large quantities of non-European products. All these contacts are said to have endured more than five centuries and they were the basis for the development of a multicultural country especially in the largest cities and those connected to overseas trade (Casimiro, 2020). The coastal cities which were connected to the international trade include Lisbon, Porto, Coimbra, Aveiro, Setúbal which are located on the Atlantic seafront, and they received a steady stream of commodities from the colonies (Casimiro, 2020). The city of Lagos also had a share in these mentioned activities; hence it is worthy discuss its importance in providing a diversified culture in Portugal hence dietary changes which would be brought about through several factors.

2.1.2 Historical development of Lagos

This section of the research will be dealing with giving a narrative of the city of Lagos where the church of Santa Maria da Graça is located, and on the church itself. Much of the history written in this paper has been adopted from two publications namely Diaz-Guardamino and Moran, (2008) and the other one is by Pereira (2012).

According to Pereira, (2012), Lagos was founded in the vicinity of Lacobriga, an ancient Roman city whose urban centre was located on Mount Molião, 2 kilometres from present day Lagos. The occupation of the area of Lagos took place in antiquity between the 1^{st} and 6^{th} centuries on a low hill on the right bank of Moleão River, situated between Tauros and Naus streams. It was a village of little political relevance but dedicated to fish production which seems to have been voted to be abandoned during the early years of the 7^{th} century (Arruda, 2007).

Archaeological excavations around the city have shown that there seems to be complete absence of the Islamic era though some authors have considered the hypothesis that the castle of Lagos, which is embedded in the walls of present Hospital da Misericórdia, is on the site of an ancient Islamic fortress. This second opinion on the identification of Lagos castle as a vestige of a local occupation by Islamic communities relies primarily on the fact that the same castle of *Lagus* could correspond to the one of those that King D. Sancho I conquered in 1189 (Martins, 2002). Pereira (2012) substantiated this opinion connecting the hypothesis of the Islamic presence to the Islamic settlement of *Zawiyya*, that the late-12^{th}-century Islamic geographer Al-Idris mentioned. Based on the proximity of the mentioned *Zawiyya* and the unidentified Islamic inhabitation, Pereira (2012) suggested that the Islamic Lagos was *Zawiyya*, on which was founded the later Portuguese village of S. João.

Lagos was the village and then city of the Kingdom of Algarve that was most developed between the 15th and 16th centuries. From all over Algarve, Lagos was the first village to be fully provided with extensive rammed walls fully suited to the firearms of the time. It has been historically proven that during the 13th and 14th centuries, Lagos was fortified by the Portuguese with a defensive device, that was concluded during the reign of Don Afonso IV. The Portuguese fortification of this period was built with local limestone and its design had a rectangular urban layout typically late medieval (Teixeira and Valla, 1999). This Portuguese alteration of the settlement modified the pre-existing Islamic models, as seem to show the *albarras* towers that flank the east of São Goncalo and the north (Gomez, 2002).

The city of Lagos' thriving with fishing and commercial activity meant that there was the possibility of building projects. There was building of the Hospital which was established in 1412 by Lourenco Estevens, squire of D. John 1 (Pereira, 2012). The prominent constructions were temples for Christian worship, and these include Santa Maria da Graça which is thought to have been built around 1478 in Vila Adentro and remodelled in 1420 at the initiative of Soeiro da Costa. The other construction was that of Nossa Senhora da Conceição which was built out of extramural and was surrounded by a consolidated suburb (Paula, 1992). It is between these two urban pillars along the bank of the Moleão River which a strip related to fishing and maritime trade emerged. This strip consolidated Lagos as an important port village.

Lagos was converted, after 1415, into the most important war square for the military support of the rear guard to the defence of Ceuta for the supply of this North African city and for military support to other Portuguese fortresses of Morocco. The mid-15th century saw Infante D. Henrique the Navigator establishing in Lagos the centre of its commercial emporium which was based on trafficking with West African coast (Russell, 2001). Pereira (2012) points out that the presence of Infante D. Henrique in Lagos represented one of the moments of greatest economic and demographic flourish in the village. The city of Lagos gained a reasonable political and economic splendour between the middle of the 15th century and the end of the 16th century. This was due to the city's prominent position in maritime enterprises of the discoveries and in national geopolitics for its neighbourhood with North Africa, and this led to a consequent demographic increase (Loureiro, 1991; 1984). Since then, the population has organized itself around the parishes of Santa Maria and São Sebastião (Rocha, 1991).

According to Stephens, (1891), Lagos became the centre of Portuguese maritime exploration as it helped in the discovering of a direct route to India and the introduction of cheap forced labour. The city made navigators like Lancarote who, in 1444 went upon a slave-taking expedition and brought home two hundred captives who were set to work on the domains of the Order of Christ in the Algarves. A year later, Lancarote sailed with a fleet of fourteen ships from Lagos and brought back a large body of unfortunate slaves, and this system made large profits to be made in slave trading which had its centre at Lagos (Stephens, 1891:149). Such activities led to the great estates of southern Portugal to be speedily brought under cultivation. In other words, Lagos became the first doorway for slaves from Africa into the post-medieval Europe.

The above notion is supported by several scholars who argue that some of the earliest non-Europeans were brought from North Africa in the early 15th century as Moorish slaves and that there are records from 1444 of first mass importation of black African slaves entering the city of Lagos, possibly the first time that Sub-Saharan African people were brought as cargo into Europe (Phillips, 2013; Stella, 2000; Henriques, 2011). Furthermore, an archaeological excavation in Lagos in the year 2009 made a discovery of 155 individuals in a mass grave with no sign of ritual inhumation; and this group has been studied using DNA analysis and physical anthropology, and the majority of them have been confirmed to be of African ancestry (Martiniano et al., 2014; Nevese et al., 2011; Wasterlain et al., 2016).

Stephens (1891) notes that:

……..The king, the nobles, and the military orders were, however, quite undisturbed by this extensive emigration and rapid depopulation, for their large estates were much more cheaply cultivated by African slaves, who had been imported in such numbers that the Algarves was almost entirely populated by them, and in Lisbon itself they outnumbered the freemen by the middle of the sixteenth century…..page 182.

The early years of the 16th century saw the Moorish and French piracy constantly threatening the Algarve coast (Pereira, 2012). This threat made the Portuguese crown to be afraid of an invasion hence it began to provide defensive means to the most vulnerable localities of which Lagos was included (Pereira, 2012). Because of its geographical location, Lagos was linked to various commercial fleets that anchored in the Lagos Bay for purposes which include supplying goods, depositing, and loading new goods. Such economic dynamism stimulated the interests of pirates who saw such circulation of goods as an excellent opportunity to enrich them (Pereira, 2012).

Construction projects happened in Lagos which include a new fortified parameter that covered two urban centres of São Sebastião and Vila Adentro. This was made possible due to increase in population distributed between the mentioned suburbs. Another necessary factor which made construction projects happening in Lagos was the development of the port and the location of the administrative buildings near the mouth of the Ribeira do Taurus and such projects helped in protecting the port area. Although the project for the construction of *Cerca Nova* (New Wall) may go to the end of the reign of King Manuel I, the construction was promoted from the reign of D. João III (Rocha, 1991; Guerreiro and Magalhães, 1983). Nonetheless, such a project was deemed to be time-consuming, as is written in the plans commissioned by D. João de Castro, governor of Algarve and detailed drawn by Alessandro Massay in 1617 and 1621, to the extent that the wall was still to be completed in the early 17th century (Rocha, 1991; Guedes, 1988).

Additionally, in the 16th century, the square of Lagos was reinforced with the construction of two fortresses namely the Solaria, to the south-eastern part of the city and situated near the place where much later came to rise, in Ponta da Bandeira protecting the walls; and this was executed during the reign of King D. João III (Callixto, 1992). With these fortresses, and with another built later in Ponta da Bandeira in the last quarter of the 17th century, it was intended to face the attacks of pirates and privateers, namely the Turks in the 16th century, and the Maghrebines in the 17th and 18th centuries (Callixto, 1991). These kinds of attacks put at risk the main economic activities like fishing and the movement of ships on the Route of the Indies, which, in fact, carried gold, silver, spices among others (Magellan, 1970; Megellan, 1991).

2.2 The site under investigation: Santa Maria da Graça

2.2.1 History of Santa Maria da Graça

The construction of the Church of Santa Maria da Graça, according to historical references, happened in 1378 AD and the removal and closure of the cemetery in 1893 AD. The Church of Santa Maria da Graça was the mother church and was built eccentrically at the highest point of the intramural hill (Pereira, 2012). It was the only parish seat until the mid-16th century when Hermitage of Our Lady of Conception was transformed into the Church of Sebastian and had its own parish cemetery. It is during this late stage of Middle Ages that there is the model of cemetery parish created around mother churches and such being entirely controlled by the ecclesiastical institutions was fully established in most parts of the Iberian Peninsula.

The foundation of the church of Santa Maria da Graça is said to presuppose the beginning of the use of its surrounding space as a parish cemetery. Written sources state that the closure of the cemetery took place in the 19th century on the 31st of January 1867 (Rocha, 1991) which is about 130 years after the destruction of the Mother Church by the 1755 earthquake. Thus, the archaeological intervention carried out in the Largo de Santa Maria de Graça and associated areas has a usage time span of almost 500 years.

Several written sources confirm the impact that the earthquake of 1775 left in Lagos (Rocha, 1991; Paula, 2006). The Mother Church of Santa Maria da Graça was ruined in addition to a widespread destruction of houses. Bishop Francisco Gomes Avelar took an initiative to the temple rebuilt soon after the earthquake, asking the *lacobrigenses* to contribute in tackling the expenses. Although assuming and reiterating the commitment, the *lacobrigenses* did not make any payment, so the prelate gave up his company and the walls were half raised (Rocha, 1991). The walls were ordered for demolition in 1893 due to the terrible state in which the place, until 1867 was being used as a cemetery, was continually being vandalised coupled with the little healthiness it presented. Generally, the reconstruction of Lagos after the 1775 earthquake lasted until the first two decades of the 19th century (Pereira, 2012).

2.2.2 Archaeological Background of Santa Maria da Graça, Lagos

The following archaeological narration has been adopted from the publication 'Entre *Muralhas e Templos: A intervenção arqueológica no Largo de Santa Maria da Graça Lagos (2004-2005)*' by Diaz-Guardamino and Moran (2008).

Excavations under POLIS de Lagos program, an intervention was carried out in Largo de Santa Maria da Graça and surrounding area with the purpose of installing infrastructures during the early years of the 21st century. Knowing the existence of ancient church of Santa Maria da Graça and its necropolis in this area, the then Office of the Historical Centre of the Municipality of Lagos, through archaeologist Elena Moran, contacted the Laboratory of Biological Anthropology (LAB) of the University of Évora for the recovery of human skeletons. The purpose or objective of this intervention was to safeguard and document all bone estate exhumed at the site, taking into account its archaeological context, in order to allow paleobiological and anthropological study from the funeral point of view so as to know a little more about the ancient population of Lagos.

During the opening of the ditch for the placement of the underground ecopoint, next to the wall adjacent to Porta da Vila, the first human bones were discovered, as well as the first graves and pits of inhumation of the 142 that would be identified and excavated in that area. The work began in November 2004, and the LAB personnel which include Catarina Costa, Pedro Almeida, Sónia Ferro and Sónia Santos, moved to the site under the direction of Teresa Matos Fernandes. The excavated area, which stretched between part of Largo de Santa Maria da Graça and the street that connects to Rua Miguel Bombarda, totalled $336.2m^2$.

Archaeologists used the methodology which consisted of the disassembly of the various stratigraphic layers super adjacent to the graves, which were identified sequentially, depending on the order of appearance. When the soils that filled and covered the pits were removed, the burials were delimited, followed by the photographic and graphical recording. For each burial, the altimetry was also recorded, and a field form was filled out which recorded various anthropological data.

Above all, the excavation was carried out manually and the strata were disassembled and recorded by registration units that were sought to correspond to stratigraphic units-as much as possible-or artificial layers-whenever and when the excavation process made it advisable-and this case subsequently allocated to the corresponding stratigraphic units, by conjugated reading of planes and profiles. There was also georeferencing of the area which was excavated (see Figure 2.1).

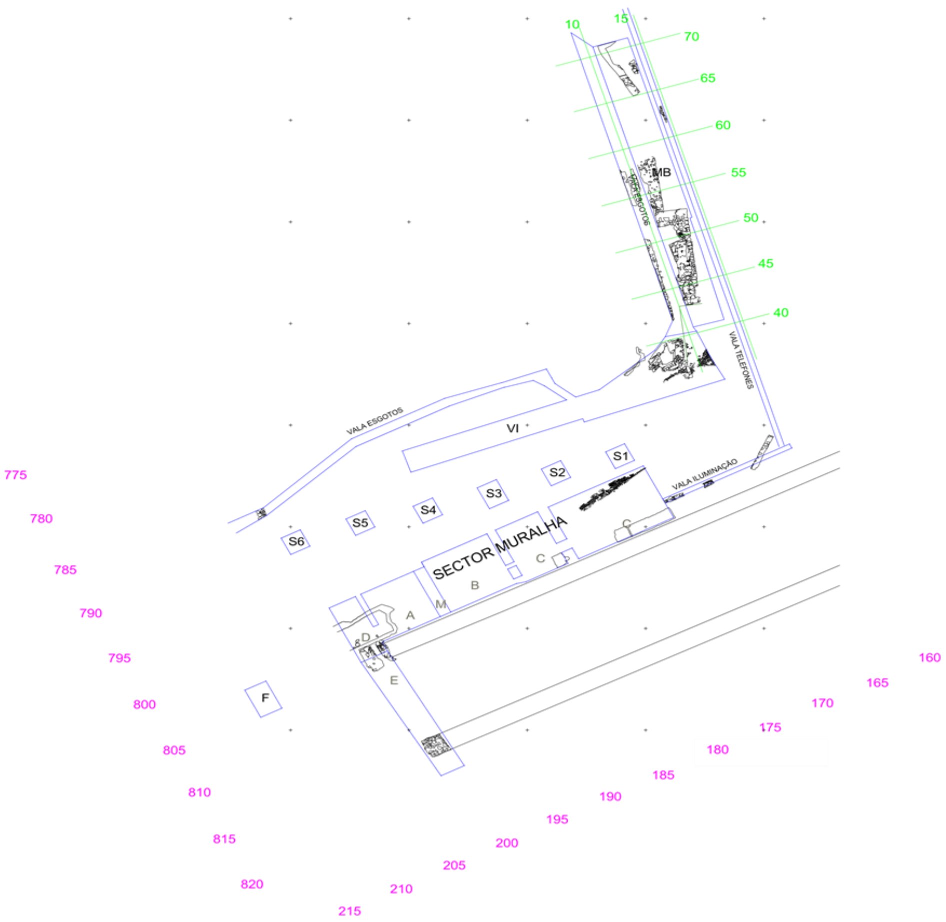

Figure 2.1: General cutting plant and local georeferencing systems/grids (Diaz-Guardamino and Moran, 2008)

After all the above processes, all the bones excavated were lifted, identified, properly packed in plastic bags, and packed in Styrofoam boxes to be transported to the LAB, where they were in storage.

The funerary place which was archaeologically intervened covers large sectors situated to the east and south of the site where the church was located. Chronology of the burial place was mainly deducted from the coins (637-1 and 637-2) which were originally associated with the burials and it was concluded that the burials were carried out between the end of the 15th and mid-16th centuries the moment immediately preceding the construction of the Cerca Nova. Interesting to note is the fact that these burials have a noteworthy detail: they present a West-East (W-E) guidance pits which denounces the orientation of the side walls of the former church.

Chronology was also deducted from vertical stratigraphic study which allowed establishing a general relative sequence in two phases for various groups of graves. The first group comprises two burials 119 and 134, and possibly also by burial 123 superimposed by two layers of very compact clay land, one of orange colour and the other of dark brown colour. These layers have been interpreted by archaeologists as the level of use of the cemetery prior to the construction of Cerca Nova. Relative chronology was also done from the analysis of the deposits of land which, with its reserves, was interpreted as a dump because of abundance of ceramic fragments and remnants of fauna. Such land deposits were against the structure associated with Cerca Nova and therefore were posterior to the Cerca Nova. The study of ceramic material provided the data that place temporally the "dumpster" between the 16^{th} and 17^{th} centuries.

The data provided by the monetary findings, vertical stratigraphy and horizontal stratigraphy highlighted a series of general norms which archaeologists characterised into two different forms in the use of the cemetery space which can be interpreted chronologically into two phases:

Phase 1- this phase is between late 14^{th} and mid-16^{th} centuries and the majority orientation of pits, 96.4% of the totality, generically W-E, corresponding to the orientation of the building of the former Mother Church (see Figure 2.2). There is low density and dispersion in the areas to the east and south of the site of the former Church. There is quiet a relevant number of anthropomorphic fossas at this stage excavated in the rock, 37.5% of the totality. Corresponding to this medieval phase of the cemetery, there were 56 burials in pits excavated mostly in the geological substrate (75%), which presented oval, anthropomorphic and pseudo-rectangular typology, mostly with W-E orientation (91.07%), probably following the orientation of the major axis of the primitive late-medieval temple. However, some burials have SW-NE orientation (1.79%).

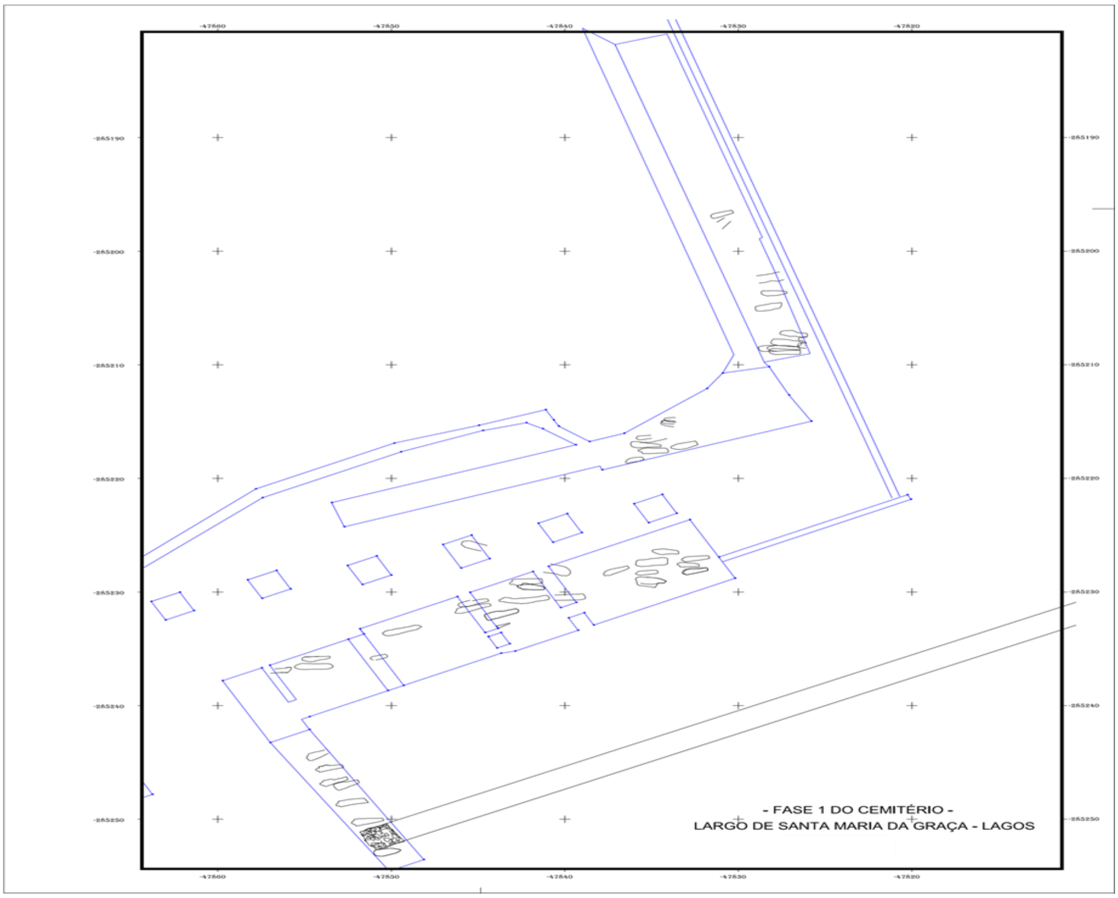

Figure 2.2: Sketch portraying Phase 1 of the cemetery of Santa Maria da Graça (Adapted from Diaz-Guardamino and Moran, 2008)

Phase 2- this phase is between mid-16th to 19th centuries and the majority of the pits , 83.6% of the totality, generically southwest-northeast (SW-NE), corresponding to the orientation of the Cerca Nova section that delimits the cemetery on the south side (Figure 2.3). The concentration of graves is in the area situated next to the wall of the Cerca Nova and a relevant number of pits, 39% presents an indeterminate morphology excavated over levels of use/burials. During the second phase, the 85 identified graves that date between the sixteenth and nineteenth centuries are concentrated almost entirely next to Cerca Nova. The most recent phases correspond to the open graves in the two dumps detected intra-walls, next to the modern wall, and which, judging by the diagnostic materials collected in them, correspond to two different moments: one of the sixteenth-seventeenth centuries and another, later, of the seventeenth-eighteenth century.

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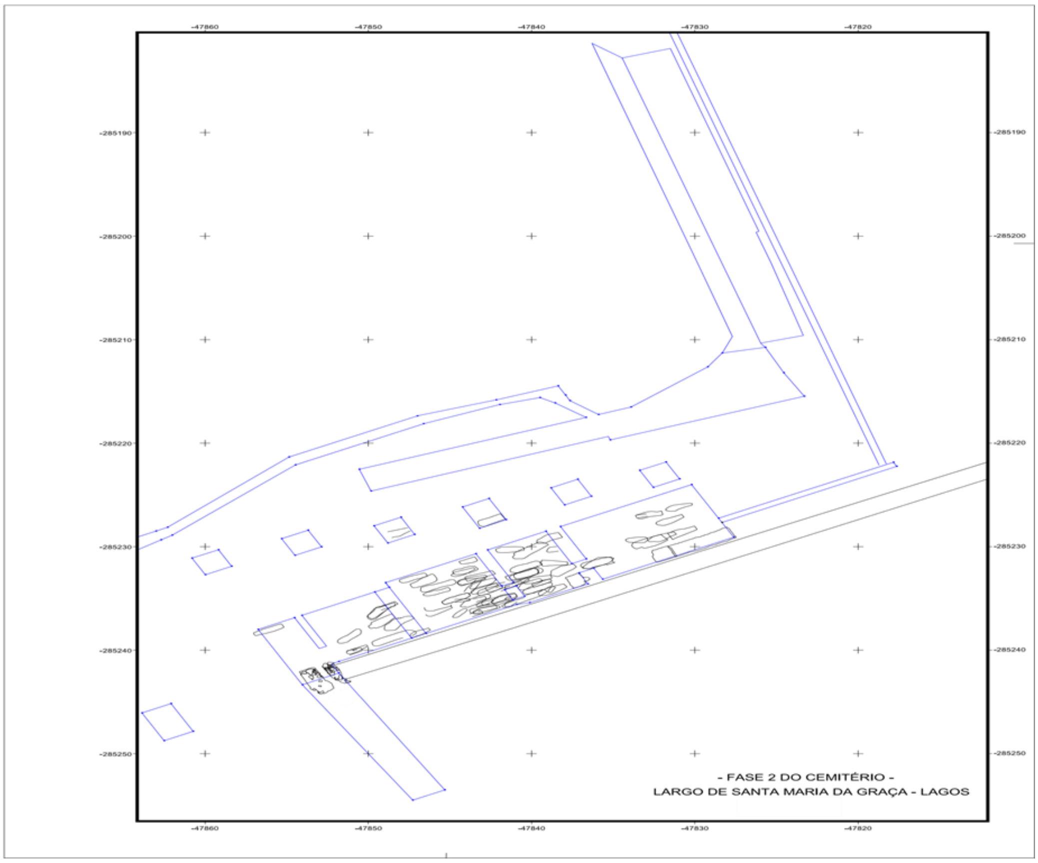

Figure 2.3: Sketch portraying Phase 2 of the cemetery of Santa Maria da Graça (Adapted from Diaz-Guardamino and Moran, 2008)

Nevertheless, the cases analysed represent less than 30% of all burials documented in the archaeological intervention, a statistically non-representative amount that precludes a safe chronological assignment for the rest of the burials based on these characteristics. The conclusions obtained in other studies dealing with in depth aspects of the spatial organization of medieval necropolises have allowed archaeologists to propose, as a hypothesis of interpretation, the attribution of the remaining burials to these phases, bases fundamentally, on the orientation of the pits. Thus, archaeologists proposed, proper reservations on the orientation of the pits and vertical stratigraphy, that it is possible to hypothesise for other burials of the parish cemetery of Santa Maria da Graça two different trends in the use of necropolis space that can be interpreted chronologically.

One important aspect worth mentioning in relation to the chronological development of the necropolis area is the typological aspect of the pits. Anthropomorphic typologies are predominant in the upper medieval rural necropolises of the Iberian Peninsula. As some studies have shown in the case of Portugal, there is recurrence of these typologies in the later times in the Middle Ages (Tente and Lourenco, 1998). In the case of Santa Maria da Graca, archaeologists noted the presence of pits excavated in the rock of anthropomorphic typology in the contexts dating from the end of the 14th century. The cemetery of Santa Maria da Graça are several types of pits: anthropomorphic, oval, rectangular/ sub-rectangular, a trapezoidal fossa, and a very large group of pits of indefinite morphology.

The important and most highlighted trend in burials is that during the 1st phase there is a more dispersed use in the cemetery space and there is a majority trend towards the use of new spaces and excavation in the rock. During the 2nd phase there is a tendency for a higher concentration and overlap of pits, which are mainly excavated in the soft lands which makes it difficult to diagnose morphologies.

2.2.2.1 Arrangement of the Necropolis

The old Mother Church of Santa Maria da Graça was built from scratch next to the city's west gate, inside the medieval fortified enclosure and significantly at its highest point. This location of the Church and cemetery associated with it could correspond structurally to the place that the dead occupied in the mentality of the time. The cemetery of Santa Maria da Graça had different intensities in the occupation of the necropolis. According to the analysis of available chronological parameters, there are many burials with probable but not secure attribution. Taking together the data collected in the archaeological interventions, it was highlighted the general presence of burials on the north, east and south sides of the old church, noting a greater concentration in the area located next to the Cerca Nova.

Table 2.1: Typology of the fossa according to the two differentiated phases (black numbers indicate known chronological assessment) (Adapted from Diaz-Guardamino and Moran, 2008)

	Anthropomorphic	**Oval**	**Rectangular**	**Undetermined**	**Trapezoidal**
Phase 1	3	2	2	8	0
Phase 1?	18	1	1	21	0
Phase 2	0	2	1	19	1
Phase 2?	16	21	5	20	0

Table 2.2: Summary of burials documented in the intervention of Largo de Santa Maria da Graça (Adapted and modified from Diaz-Guardamino and Moran, 2008).

Burials	**Phase 1**	**Phase 2**
Total	56	85
Secure chronological assessment	15	23
Probable chronological assessment	41	23
Excavated on geological substrate	42	62
Dug over used levels or burials	14	59
West to East Orientation	51	11
South-West to South-East Orientation	1	69
Undetermined	4	5

As sources document, the proximity of burials to the temple depended on social and economic resources of the deceased or his family (Pina, 1995). Hence it is possible that the context of the parish cemetery of Santa Maria da Graça the individuals buried in the areas closest to the church were, within their social context, people with greater social privileges or individuals and families who intended to acquire privileged positions. It is against this background that there is the possibility of the existence of social differences in the context of the cemetery itself. However, it is important to mention that from the 15th century, the Church of Santa Maria da Graça became the seat of the parish of the residents of Vila-Adentro, and that the individuals buried in this cemetery belonged, as a whole to a privileged social class. The residents of the suburb, belonging to the lower social group belonged to a different parish, the *Ermida de Nossa Senhora da Conceição* (Hermitage of Our Lady of Conception) and this hermitage was transformed into the *Igreja de São Sebastião* (Church of Sebastian) and had its own parish cemetery.

The funerary space is said to have radically changed from the 16th century when the Cerca Nova was supposedly built on this site. By that time, the burials carried outside the temple were now concentrated in almost all on the south side of the old church, more specifically in the area next to Cerca Nova. It is in this area where archaeologists recorded the largest number of burial overlaps, which were practiced directly on pre-existing burials which were also respected and maintained.

Pina (1995) points out that in the wills of the 14th and 15th centuries, there is expressed desire not to totally unlink the deceased from the world of the living. Thus, the proximity of relatives in the grave sought to ensure the permanence of the family structure beyond death. In the context of Santa Maria da Graça, men and women had significantly different behaviour. It was desirable that men be buried preferably by their lineage, firstly with the father, secondly to the child, third to the spouse, forth to the mother, and finally to the other family members. As for women, it would be preferred for them to be buried first with their spouse, secondly to their mother and thirdly to their daughters.

The reuse of pre-existing burial space in the cemetery of Santa Maria da Graça has been documented by archaeologists and there are two ways of reuse. On one hand, there is reuse of pits with pre-existing burials, but where these burials are disjointed, extracted and, when new inhumation is practiced, they are re-deposited in the form of ossuaries. Such behaviour is documented in 14 cases attributed to the first phase of the cemetery use, 7 of these with secure chronology. One of the reuses is related to the possible simultaneous inhumation of an adult woman with a baby (burials 104 and 107). There is one more case in which the new burial, burial 78 of child of an indeterminate gender, was practiced on a pre-existing burial, burial 84, but respecting it.

During the second phase of the use of cemetery of Santa Maria da Graça, the behaviour of respecting and maintaining pre-existing burials becomes more common. Archaeologists managed to document 13 cases, 11 of which offer a second in the sequence, while the remaining two (burials 61 and 56) offer a second and third moments, thus producing an overlap of three bodies buried successively. If these data are cross-referenced with the diagnosis of gender and age, it is of great interest to the recurrent overlap of male adults over male adults, documented on five occasions. In 3 cases, an indeterminate child is buried over a male adult, and finally in one case, a male adult is buried over an adult woman. However, there was no guarantee with absolute certainty whether there were family ties between overlapping individuals, since only DNA analysis could answer these questions.

Consequently, as a result, it is possible to enumerate a series of variables that probably influenced jointly and variably the spatial deposition of bodies: kinship space serving, purely functional topography, proximity to certain architectural spaces with symbolic charge.

In terms of its organization, the cemetery of Santa Maria da Graça was organised around the place where the Mother Church was built. Although the Church was destroyed by the earthquake of November 1, 1755, the necropolis space was continued to be used as a cemetery until its closure on January 31, 1867. As mentioned early on, two different forms in space management have been detected that can be interpreted chronologically, Phase 1 and Phase 2.

The use of funerary space of Santa Maria da Graça corresponds to two distinct moments. The variable with a more relevant role, in the spatial articulation, is the orientation of the pits that generically corresponds to two trends: A W-E and another SW-NE. It is important to mention that there are two reference buildings when it comes to the understanding of the cemetery of Santa Maria: the building of the Old Mother Church and the Cerca Nova, corresponding respectively to a 1st and a 2nd phases of the cemetery.

The data indicated that the graves safely dated in the 1st Phase present W-E orientation but among the structural remains exhumed in the excavation, archaeologists found nothing which correspond to this orientation. Also not detected in the works were structural remains in situ belonging to the original building of the church such as foundations or walls. But thorough architectural and historical researches led archaeologists to propose that the side walls of the primitive church, then situated in the place where the buildings of Largo exist today, had an orientation closer to the W-E axis.

The intervention, to the south and east of the site of the old church allowed to confirm the delimitation of the cemetery, on the south side, by *Cerca Velha*, Old Wall (between the end of the 14th century and the mid-16th century) and Cerca Nova (from the mid-16th to the end of the 19th centuries). On the east side, no structures were documented that could safely be interpreted as belonging to the cemetery fence. Few structural remains were documented, of little relevance, which both by the orientation they present and by the stratigraphic situation, were very damaged by the contemporary infrastructure ditches and did not provide definitive data in relation to the theme. On the northern side no structures related to the cemetery were identified, but it was only on the western side, on Rua da Porta da Vila, a wall was recorded.

These data indicate that only on the south side were reached the limits of cemetery and the funerary space originally extended beyond the limits established by the current building to the east, to the east, north and west of the streets of Porta da Vila, Adro and Santa Maria de Graça. As far as data indicate, the Church was located on the site of the current buildings of Largo, the southern boundaries (Cerca Velha and Cerca Nova) situate the boundary of the cemetery to the south of the church during the 1st Phase at 35 metres, and during the 2nd Phase at 24 metres.

2.2.2.2 Anthropological Information

Bone pathologies result from the imbalance between two bone cell populations, osteoblasts, responsible for the production of new tissue, and osteoclasts, which promote destruction. Of the 12 human samples which were analysed in this study, a third of them were reported to have some pathological conditions.

LNSG 7: This mature male adult has a bone callus in the anteromedial face of the lower extremity of the left ulna. The lesion may have been oblique and incomplete. The radial bone has no traumatic evidence.

LNSG 117: Observation was that the individual (male/young adult) must have had a severe and generalized infectious process in the left region of the skeleton (see Figure 2.16). There are signs of osteomyelitis along the diaphysis of the femur, radius, ulna, fibula, and lame. The healing status varied observing both active and remodelled cases.

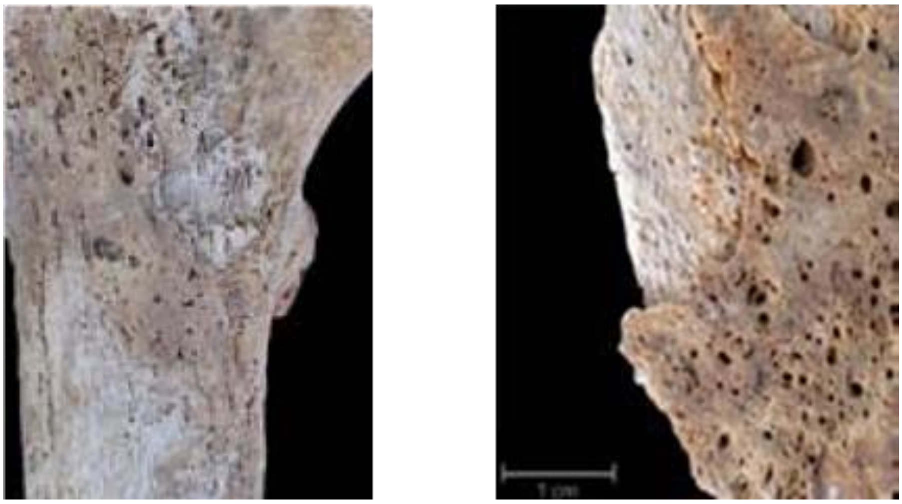

Figure 2.4: Pathological conditions on LNSG 117 (Adapted from Diaz-Guardamino and Moran, 2008)

LNSG 120: It was only possible to recover, from this female adult, the lower region of the knee. A generalised infection was observed, in the left tibia the lesions are remodelled with cloacas in the diaphysis but without evidence of any sequestrum/osteomyelitis. In the right bone, there is no affectation of the medullary cavity, and has been classified as periostitis. A severe case of periostitis was also recorded in a first metatarsal.

LNSG 134: This male has J-shaped depression in the occipital, above the nuchal line and next to the lamboid suture. The lesion has a maximum length of 41.86mm and is circumscribed to the outer plank. The lower limit is regular; however, the upper and lateral region forms a depression, which presents irregularities in bone texture. This traumatic event appears to have resulted from direct trauma probably with an object like the negative found in the skull. Lesions are in the thoracic and lumbar spine, being characterised by slight depressions on one of the faces of vertebral bodies.

CHAPTER 3: SCIENTIFIC BACKGROUND

3.1 Bone Description

3.1.1 Bone Structures

Bones of both humans and animals can provide information on dietary practices. Archaeological bones also provide further details on paleopathology, age, gender, and cultural practices including mortuary practices. All these are made possible mainly because bones (and teeth) survive much better in archaeological record than any other human or animal tissues (Childs, 1995), thanks to the nature and anatomy of bones. Despite a wide range of differences in their external form and shape, bones basically have a makeup which is remarkably constant at microscopic and gross level. The following passages, adapted from White and Folkens (2005), provide a description on what constitutes the bones.

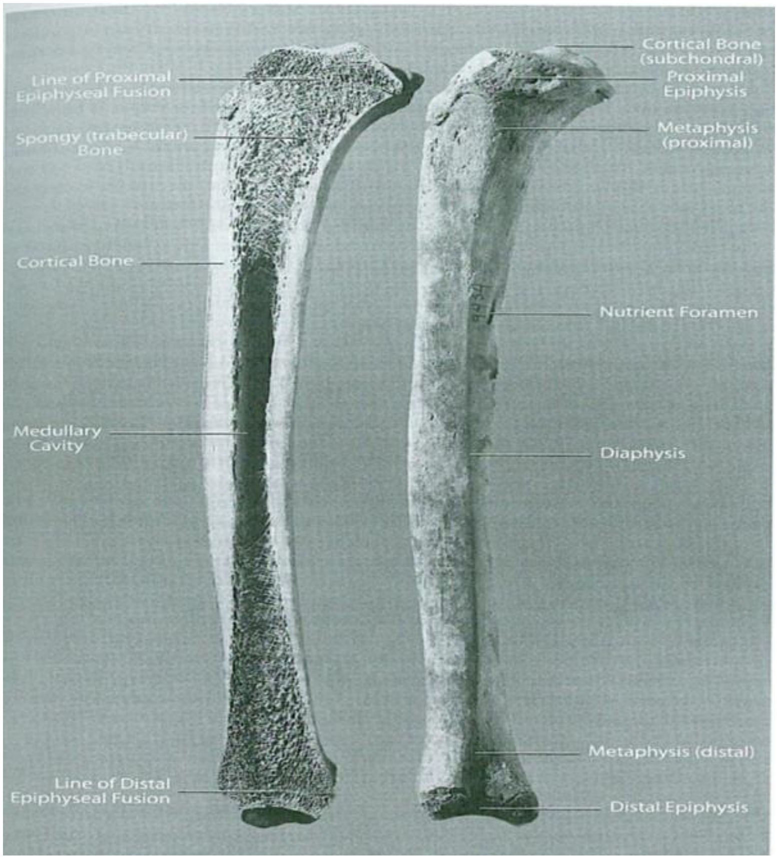

Figure 3.1: A left tibia sectioned to show the anatomy of human long bone (White and Folkens, 2005)

All bones in the adult skeleton have two basic structural components which are compact and spongy bone. The compact, also known as cortical, part of the bone is solid and dense and is found in the walls of bone shafts and also on external bone surfaces. At joints, compact bone covered by cartilage during life is called subchondral bone and this is recognized as smoother and shinier than non-articular compact bone. The spongy bone is porous, lightweight and has a honeycomb structure and is found under protuberances where tendons attach. It is also known as cancellous or trabecular bone, which is named after thin spicules, named trabeculae. In terms of molecular and cellular compositions, both the compact and spongy bone tissues are identical, and it is in porosity difference which separates these gross anatomical bone types.

It is in the spongy or trabecular bone areas where there are sites constituting red marrow, a blood-forming, or hematopoietic, tissue that produces red and white blood cells and platelets. Inside the medullary cavity of tubular bones are yellow marrow constituting mainly a reserve of fat cells, and these are all surrounded by compact bone. The red marrow is progressively replaced by yellow marrow in most of the bones.

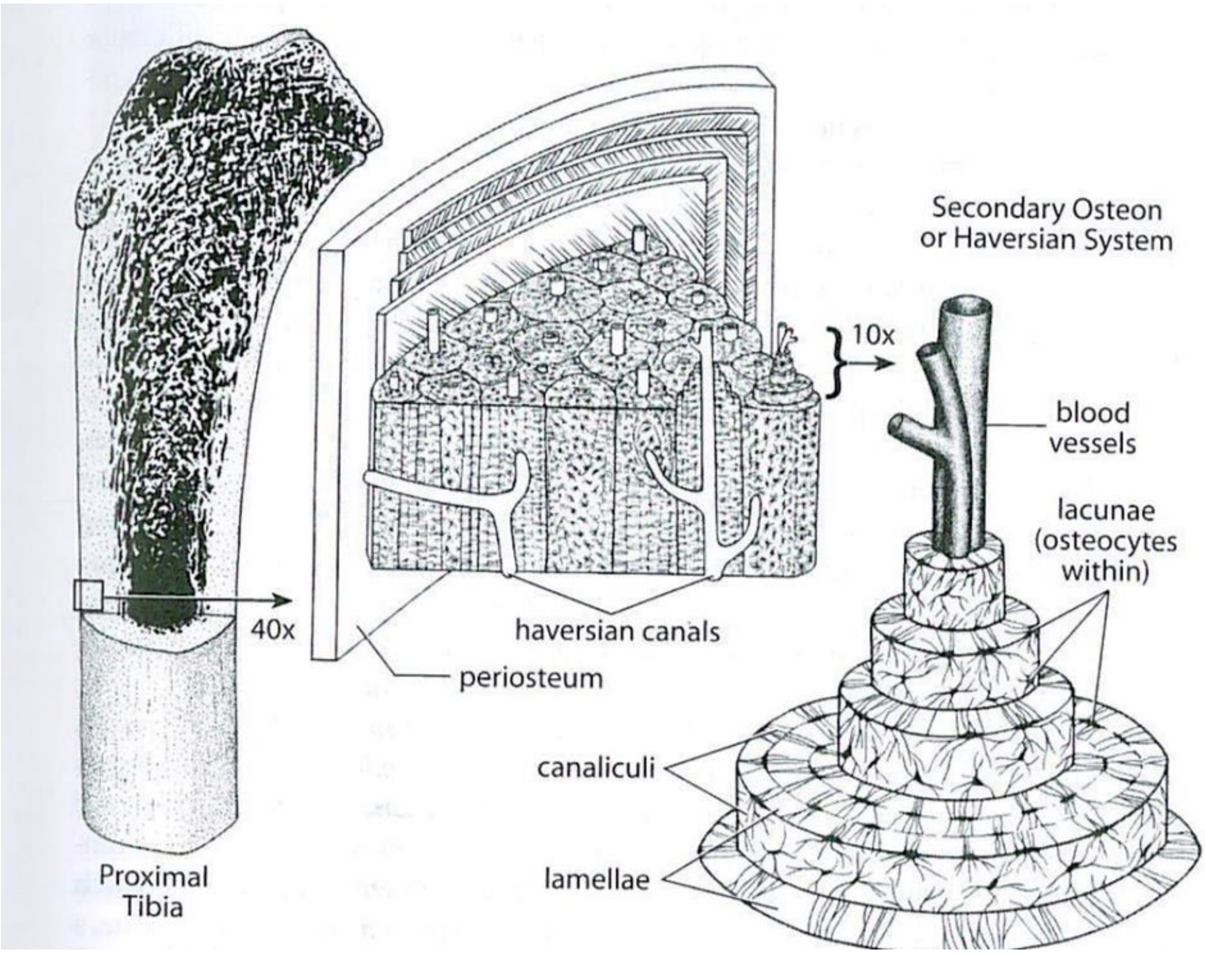

Figure 3.2: Microscopic structure of bone (Adapted from White and Folkens, 2005)

The ends of long bones are called the epiphyses because they develop from the secondary ossification centres of the bone. Diaphysis is the shaft of a long bone and it is the result of the primary ossification centre of the bone. The expanded, flared ends of the shaft are called metaphysis, and a good example is where the epiphysis at the knee end of the femur fuses to the metaphysis of the shaft when the bone is complete. For paleodietary studies, it the compact part of the diaphysis of long bones that is analysed.

3.1.2 Molecular Structure of Bone

At molecular level, all bone tissues are basically the same in all mammals despite shapes or size. Bone tissue is a composite material and is comprised of two kinds of materials. The first one is called collagen which is a large protein molecule and constitutes about 90% of the organic content of the bone. Collagen (collagen 1) molecules intertwine to form flexible, slightly elastic fibres in bone. Childs (1995) notes that collagen's structure is triple helix that assemble themselves into highly organized fibrils, and these are found in many vertebrate tissues in various forms. This helix incorporates an amino acid sequence glycine-proline-hydroxyproline.

In a way, collagen from bones, which is composed of amino acids, is the main constituent of organic part of the bones; and this collagen is thought to survive through time depending mainly on burial context, type of soil, soil pH among others. The bone collagen is protected by the bone mineral phase called apatite against enzyme attacks (Child, 1995). It is this collagen and the mineral part from ancient bones that is extracted and analysed; and the isotopic ratios into the bone collagen derive mainly from dietary proteins, while apatite reflects the isotopic composition of the complete diet including carbohydrates and lipids (Saragoça et al., 2016).

The second one is hydroxyapatite, a form of calcium phosphate [$Ca_{10}(PO_4)_6(OH)_2$] which is a dense inorganic filling and is responsible for the stiffening of collagen in mature bones. It is this calcium phosphate that impregnate the collagen matrix and this weave of protein and minerals gives bone its amazing properties (White and Folkens, 2005). Under experimental conditions, the mineral component gives bone its hardness and rigidity but when soaked in acid the minerals dissolve and bone become a rubber like flexible structure. When a bone is heated to combust, the organic collagen becomes extremely brittle and crumbles because of loss of collagen. Such crumbling and rigidity are mainly common in bones from archaeological contexts (White and Folkens, 2005). Thus, when exposed to heat, the bone becomes brittle because the collagen is combusted and leaves the mineral part alone whose properties are changed thereby reaching stability level.

Bones remineralise through nutritional processes and this happens throughout the life of an individual since they are bio-mineral materials. Thus, bones tend to have the ability of growing during the lifetime of an individual. Bones follow growth patterns in which the bone increases in size by increasing the number of cells and the intercellular material between them. The main phase is completed at adulthood, however bones continue to remodel through the combined resorption of older bone and formation of new bone that occurs during normal life (Fahy, et al., 2017). This turnover is important in stable isotopic analysis since it helps in determining the period of dietary history as new bone forms and incorporates the isotopic composition of the individual's diet. However, remodelling rate vary according to bone type and age, and several researchers (Mays, 1998; Cox and Sealy, 1997) have studied the annual turnover rate of the human skeleton which has been calculated. In young children, there is high rate of turnover ranging from 100-200% at 1 year, 10 % between 3-7 years, and 1% at 8 years. During puberty, turnover is small, and values of turnover for adults, between 20 and 60, range from 0.3 to 3%.

In adults, collagen turnover is slow, and it requires at least 10-30 years for complete replacement; and in terms of bone type, trabecular bone turns over 3-10 times faster than cortical bone with trabecular at 10% per year and cortical at 2.5% per year (Mays, 1998; Cox and Sealy, 1997). Ribs, metacarpals and humeri have the highest rate of bone remodelling with complete remodelling happening every 5-10 years whereas the femur and tibia are thought to remodel slower and they complete remodelling every 10 to 25 years (MacKinnon, 2015; Fay, et al., 2017).

Thus, the state of preservation of both bones and teeth in the archaeological record is important to discuss, that is diagenetic alterations of bones and teeth which may have modified the biological signal looked for. Typically, the most influential factors affecting the state of preservation of archaeological bones depends on the environment in which they were buried and of the age of burial, soil chemistry and microbial activities on the bones (Child, 1995). Bones from archaeological records undergo several processes from the time they are buried, to the excavation, and storage. Such processes, be they natural or human induced, have the potential to alter the bones mechanically or chemically or even introducing humic acids or fungi (Schoeninger and Moore, 1992). The organic component of the bone is susceptible to physical, biological, and chemical alterations. Even though an archaeological bone may appear to be poorly preserved, most of the time, this is not an indicator that there is no organic component preserved in the bone. Child, (1995) notes that the carbon to nitrogen ratio in archaeological bone collagen can in fact remain the same as that of modern bone until almost all the collagen has been lost, around 97% of it.

As mentioned earlier on the section of bone composition, collagen is well protected by the mineral component of the bone. This has led to the hypothesis that bone collagen could survive well in the archaeological record (Child, 1995). It is worthy to point out that bones are best preserved in soils with pH which is neutral or slightly alkaline because the hydroxyapatite protects the organic part, while unfortunately, hydroxyapatite dissolves in acidic environments. Extreme humidity and temperature changes also impact greatly bone crystal and organic structure, and so are soluble salts in soils which also stresses the bone.

Because of the diagenic processed mentioned above, there is required to carefully remove and clean contaminants from bones recovered from archaeological contexts, and also measuring the extent of diagenesis and viability of preserved collagen (Schoeninger and Moore, 1992). Post-mortem alteration of bone collagen is estimated according to the amount of C, N, C/N and collagen yield. Changes in crystallinity of the bone mineral occurring usually during diagenesis, are measured by using techniques such as Attenuated Total Reflection Fourier-Transform Infrared Spectroscopy (ATR-FTIR) and X-ray Diffraction (XRD) (Saragoça et al., 2016).

3.2 Stable isotopes in palaeodietary studies.

All living organisms comprise common elements such as hydrogen, carbon, oxygen, nitrogen, calcium, and other trace elements such as strontium. These elements, like others found elsewhere, come in different isotopes in which they differ in how many neutrons they possess. In short, isotopes are atoms of an element with different masses, each have the same number of electrons and protons but differ in the number of neutrons; but in terms of chemistry they behave like other isotopes of the same element though at a fine level and at slightly different ways (DeNiro, 1987). Some isotopes are unstable and radioactive, and by measuring their decay, it is possible to determine the age of many materials. A good case is the use of carbon, specifically ^{14}C, in radiocarbon dating. Some isotopes are stable, and they also vary in their mass. Heavier isotopes have many neutrons in their nuclei as compared to lighter ones. An example is ^{12}C lighter than ^{13}C, which leads to ^{12}C breaking and forming chemical bonds more rapidly than ^{13}C.

Ambrose, (1993) notes that the difference of neutrons of a particular element add to the mass of the atoms but not the chemical properties. This in turn slows the atoms' rate of movement, chemical reaction and state transition leading to fractionation of the isotopes, usually the lighter against the heavier isotopes of an element (Ambrose, 1993). The resulting difference from fractionation in the natural proportion of stable isotopes are very small on the order of a few thousandths of a percent (‰) (Ambrose, 1993). Thus, with the development of more sensitive analytical equipment, trace elements can be measured and presented in parts per million (ppm) (Katzenberg, 2008).

Fractionation comes in two forms, equilibrium and kinetic, and the former results from the differential exchange of isotopes between two physical phases that are equilibrium with one another (Brown and Brown, 2011). A good example is evaporation and condensation of water where isotopically heavy water, with $^{1}H_2{}^{18}O$, would evaporate more slowly than lighter one with $^{1}H_2{}^{16}O$. Since isotopically light water molecules evaporate at a rate which is faster than isotopically heavy water molecules, the remaining liquid water becomes enriched in heavy isotopes (Ambrose, 1993). In kinetic fractionation, such as photosynthetic processes, reactions occur during a unidirectional physical or chemical phenomenon which involves usually preferential reaction of lighter isotope against heavier ones.

Kinetic variability allows plants and animals to assimilate one isotope over the other and this happens during biochemical reactions associated with photosynthesis and metabolism of food by animals (Pate, 1994). The resultant product becomes enriched in lighter isotopes (Brown and Brown, 2011). Fractionation enriches or depletes the stable isotopic composition of plant and animal tissues relative to atmospheric, seawater bicarbonate, or dietary ratios (Pate, 1994).

In nature, for most elements one isotopic form predominates with very minor quantities of other isotopes like for example 99% of all carbon occurs as ^{12}C with 1% ^{13}C, nitrogen occurs as 99% ^{14}N and 0.4% ^{15}N (Fry 2006). More details on nature of the main stable isotopes used in palaeodietary reconstruction are below. To express the ratios of these stable isotopes including oxygen and sulphur, a delta terminology is usually used as is expressed in the equation below adapted from Katzenberg (2008):

$$\delta \text{‰} = \frac{R_{(sample)} - R_{(standard)}}{R_{(standard)}} \times 1000$$

The advent of using stable isotopes in exploring and reconstructing diets of past populations was during the 1970s with the works of DeNiro and Epstein (1976) who made the assumption that tissues of a consumer tend to reflect the isotopic array of ingested food. Early analysis of $\delta^{15}N$ and $\delta^{13}C$ from archaeological human bone collagen, such as the works of Schoeninger and DeNiro (1984); Merwe (1982); used modern animal tissues and bone collagen as baseline for establishing dietary differences between marine/ terrestrial and C3/ C4 plant-based diets in ancient human populations (Birch, 2013).

Essential to isotopic studies of diet and mobility is the understanding of how isotopic signals are transferred from the environment, like water or food, towards consumer tissues (Fernandes and Jaouen (2017). Thus, isotope analysis is based on the idea that human and animal body tissues reflect the isotopic composition of the food stuffs and water ingested (Müldner and Richards, 2007). The techniques which were used back then appeared to provide direct quantitative means for reconstructing diet (Sandford, 1993).

Stable isotope studies have given the possibility to make specific understanding of past diets through its ability to focus on a more reduced population up to an individual. This has given researchers tools to understand characteristics that occur at social and cultural levels such as dietary differences in between statuses, sex, and age, that is intra site analysis. It is also possible to observe variations between diverse populations even between nearby sites (inter-site analysis), and dietary variations in short period of time (Tykot, 2004).

Carbon (^{12}C and ^{13}C) and nitrogen (^{14}N and ^{15}N) stable isotopes from bone collagen are the main isotopes commonly studied in human remains and they can provide information about plant and meat food of ancient humans, domesticated process of plants and animals (Wang et al., 2014). Stable isotopes of oxygen (^{16}O and ^{18}O) and strontium (^{86}Sr and ^{87}Sr) have also been studied in bone and tooth enamel to gain information on residence and mobility whilst stable isotopes of sulphur (^{32}S and ^{34}S) are useful in in distinguishing marine, freshwater and terrestrial foods. It is the isotopic abundance of an element that can be measured using a spectrometer whereby bone collagen, is combusted to release gases such as N_2 or CO_2 (Schoeninger and Moore, 1992). This combustion usually takes place in an Elemental Analyser and gases produced are analysed using an Isotopic Ratio Mass Spectrometry (IRMS) where the electronic detector can differentiate between different stable isotopes of carbon and nitrogen within the gases produced and separate them according to mass. The produced gas from the sample is then compared to a lab standard which is in turn calibrated to internationally accepted standards (Schoeninger and Moore, 1992).

During the process of measurement, the gas from the sample is compared with that from a laboratory standard and these lab standards must be calibrated relative to international standards. For carbon, the internationally recognised standard is PeeDee Belemnite Carbonate (PDB), a marine carbonate; and for nitrogen the sample ratio is relative to AIR (Ambient Inhalable Reservoir) which became the internationally recognised standard following the demonstration that the isotope ratio of N_2 in the atmosphere is constant across the globe (Shoeninger and Moore, 1992). Since most biological materials have less ^{13}C relative to ^{12}C than does PDP, most biological samples have negative $\delta^{13}C$ values. On the other hand, most biological materials have larger $^{15}N/^{14}N$ ratios than the abundance ratio in the atmosphere, thus most biological samples have positive $\delta^{15}N$ values (Shoeninger and Moore, 1992).

Carbon and nitrogen isotope analysis are usually done together and they complement each other in giving a clear vision of diet hence making it possible to infer the source of protein consumed and the difference in contribution between marine and terrestrial resources (Ambrose and Norr, 1993).

It is important to note that environmental conditions such as temperature and humidity, cause variations in the activity of nitrifying the bacteria prevalent in soils or in how plants take up their carbon dioxide. Moreover, there are also variations in the isotopic baseline depending on altitude, latitude, and climate (Goude and Fontuge, 2016).

It is vital to make an establishment of a site-relevant isotopic baseline for interpreting human skeletal data thus the analysis of faunal remains provide a good estimate both animals and the plants they consumed (Tyko, 2004). Creating an isotopic baseline is mainly important in coastal areas where marine and riverine resources; and C4 and /or CAM plants may have been available for direct or indirect consumption by humans (Tyko, 2004).

3.2.1 Carbon Stable Isotopes

The principles behind the use of stable isotopes to study diet are illustrated by the use of $\delta^{13}C$ measurements taken from human skeletons to determine when maize was first used as a foodstuff in parts of the New World (Brown and Brown, 2011). The $\delta^{13}C$ isotope values are impacted by food webs because of the correlation between diet and animal tissue carbon values ($\delta^{13}C$); and there is enrichment in $\delta^{13}C$ in animal's body tissues relative to its diet due to fractionation that occurs during formation of tissues (DeNiro and Epstein 1978; Teeri and Schoeller 1979; van der Merwe and Vogel 1978).

During the photosynthetic process of plants, fractionation of stable carbon isotopes occurs, and the resulting $\delta^{13}C$ values are characteristic for terrestrial plants that use photosynthetic pathways of C3, C4 or Crassulacean Acid Metabolism (CAM). C3 plants are those that undergo C3 photosynthesis, so named because the first product contains three carbon atoms; and at the same time, C4 undergo C4 photosynthesis, in which the first product is a compound containing four carbon atoms (Shoeninger and Moore, 1992). C3 plants, Calvin-Benson pathway (Tyko, 2004), have ^{13}C values that range typically from -22‰ to -30‰ with an average of -27‰; and they include plants like wheat, rice, barley, and legumes (O'Leary, 1988; Malainey, 2010). This is mainly because carbon dioxide molecules containing ^{12}C are converted more readily into glucose thereby enriching the plant tissues with more ^{12}C than ^{13}C.

Desert plants and tropical grasses, adapted to hot arid environments, like millet, maize, sorghum, and sugarcane have a photosynthetic pathway of C4, the Hatch-Slack pathway (Tyko, 2004). They generally have ^{13}C between -9‰ and -19‰ with an average of around -13‰ (O'Leary, 1988; Loftus and Sealy, 2012; Rao et al., 2012). The reason behind the enrichment of C4 plants in ^{13}C is that most of all the absorbed carbon dioxide is eventually converted into sugar (see figure 3.3). Canopy effect occurs in some forested areas resulting in more negative carbon isotope values due to incomplete atmospheric mixing (Van de Merwe and Medina, 1989).

The concept here worth mentioning is that the consumption of C3 and C4 plants influences the ratios of $^{13}C/^{12}C$ in the bodies of consumers, and the extent of fractionation depends on the tissues like bones or teeth which all vary in terms of the ratios (DeNiro and Epstein, 1978; Schoeninger and Moore, 1992). In each trophic level of food chain, from prey to predator collagen, the $\delta^{13}C$ value is enriched by +1‰, whereas the fractionation factor between plants and bone collagen is 5‰ (Redfern et al. 2012).

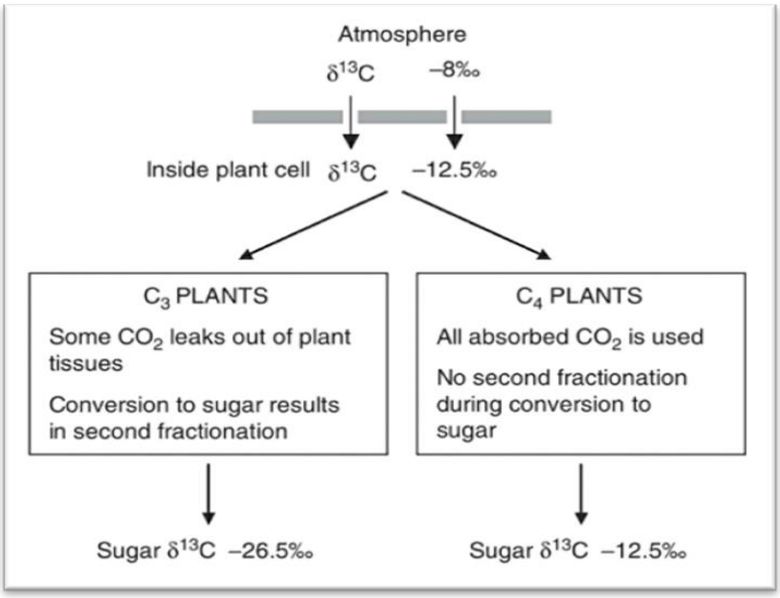

Figure 3.3: The differences between the carbon isotope fractionations occurring during photosynthesis in C3 and C4 plants (Adapted from Brown and Brown, 2013).

Fresh water aquatic plants have a $\delta^{13}C$ values which are similar to those of terrestrial C_3 plants (Pate, 1994), but photosynthesis in the oceans is different from terrestrial one in the sense that atmospheric carbon dioxide is mainly substituted by dissolved bicarbonate which is enriched in ^{13}C compared with the atmosphere and so has a more positive $\delta^{13}C$ value. According to Schoeninger and Moore (1992), marine organisms use several carbon sources including terrestrial detritus washed into the oceans by rivers, with $\delta^{13}C$ representative of a mixture of local terrestrial plants; dissolved CO_2 with $\delta^{13}C$ values of atmospheric CO_2 (-7.0‰) and dissolved carbonic acid with $\delta^{13}C$ values close to zero (0.0‰).

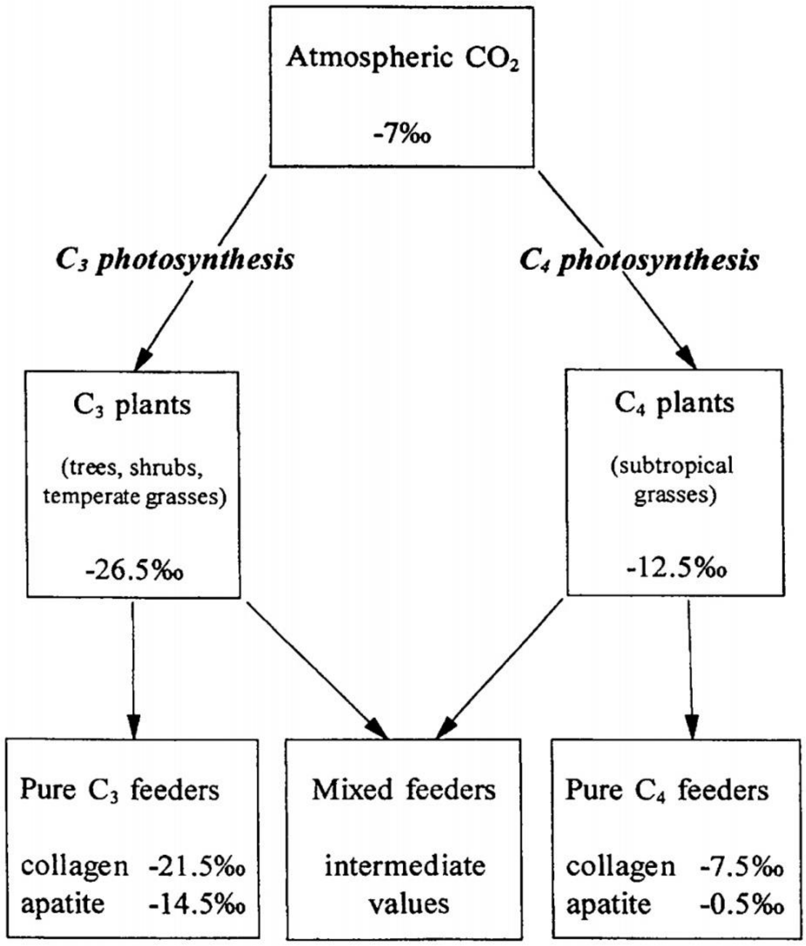

Figure 3.4: Carbon isotope fractionation in terrestrial food webs (Adapted from Tyko, 2004).

The tissues of marine photosynthesizers therefore have higher $\delta^{13}C$ values than are found in terrestrial C3 plants, with the mean being approximately -20‰. Human do not eat marine algae or planktons, other than seaweed, but they eat fish and other marine invertebrates extensively especially those populations living close to the sea. Since many of these marine animals tend to consume directly on the photosynthesizers like planktons and algae, so their tissues take on $\delta^{13}C$ values close to those of the primary marine producers which is usually around -16‰. As a result, the $\delta^{13}C$ values of humans whose diet has substantial marine input reflects the values found in fish and shellfish rather than those present in terrestrial vegetation and the animals that fed on the vegetation, thus a marine diet leaves a distinctive $\delta^{13}C$ signature in a preserved skeleton.

The non-overlapping ranges of C_3 and C_4 plants provide the basis for using stable isotopes of carbon in preserved human tissue for revealing diet (Katzenberg and Waters-Rist, 2019). The overall point is that δ ^{13}C values can be used to distinguish marine feeders from terrestrial feeders only when C_4 plants are absent from terrestrial diets. At the same time, the relative importance of C_3 vs C_4 plant foods can be determined only if marine dietary components are discounted (Pate, 1994).

Pate, (1994) reports that there are environmental variations in δ ^{13}C values and they are caused by a number of factors which include the production of depleted ^{13}C-depleted CO_2 by combustion of fossil fuels (δ ^{13}C = -27‰) and the burning of biomass and oxidation of decomposing terrestrial organic matter (δ ^{13}C = -28‰), which have caused a decrease in the δ ^{13}C values of atmospheric CO_2. Another factor is that the composition of atmospheric CO_2 varies with latitude, season, and altitude and this means that mean δ^{13}C values are approximately 0.2 ‰ more negative in the northern part of the northern hemisphere than in equatorial and southern latitudes. Seasonal variability is negligible in equatorial and southern latitudes, while differences of 0.5‰ are reported for the northern temperate zone. The most negative values in the northern hemisphere occur during winter and early spring; thus, differences between the two hemispheres are reduced during the plant growing season (Fraser et al., 1983; Mook et al., 1983; Korner et al., 1988).

The mean δ ^{13}C value of -7 to -8‰ for atmospheric CO_2 pertains to open environments, but the atmospheric CO_2 in forest habitats is depleted in ^{13}C relative to open habitats (Pate, 1994). These more negative values result from the injection of ^{13}C-depleted CO_2 produced by the decomposition of plant litter on the floor and CO_2 δ ^{13}C values vary little from the ground level to the upper canopy (Pate, 1994). In other words, there is some recycling of CO_2, the atmosphere gets less enriched in ($^{12}CO_2$) in a close forest the CO_2 cannot circulate as it circulates and mixes in an open area. The upper canopy values are different from those near the soil. Concerning the gradient of leaf δ ^{13}C values from ground level to upper canopy is produced by additional factors which are fractionation which is caused by increased diffusional resistance in leaves under low-light photosynthetic conditions; and other physiological factors (Pate, 1994). Ambrose and DeNiro, (1986) note the above mentioned processes result in an additional 4-5‰ δ^{13}C depletion in plants growing near the forest floor; and these depleted δ ^{13}C values will be passed on to animals feeding in forest habitats.

Soil moisture stress in arid land habitats leads to a reduced discrimination against ^{13}C during C_3 plant photosynthesis. Coastal westlands offer a variety of habitats with a wide range of plant δ ^{13}C values and these coastal zones show a salinity gradient from inland fresh-water wetlands to estuarine salt mashies, accompanied by a vegetational gradient of C_3 species dominating in freshwater wetlands to C_4 species dominating in the more saline marshes (Pate, 1994).

3.2.2 Nitrogen Stable Isotopes

Nitrogen has two stable isotopes which are ^{14}N and ^{15}N, with ^{14}N being predominant in the biosphere making up to 99.64% of all nitrogen atoms. The atmospheric nitrogen reservoir has a mixed and uniform isotope composition with an approximation of 0‰ and dissolved N_2 in the ocean has a $\delta^{15}N$ of about +1.0‰ (Pate, 1994). Two major processes account for the transfer of nitrogen into the biological realm, and the first depends on nitrogen-fixing organisms such as blue or green algae in aqueous situations and bacterial nodules on terrestrial plant roots (Shoeninger and Moore, 1992). This nitrogen fixing results in synthesized tissues with $\delta^{15}N$ values like that of atmospheric N_2 which is closer to zero. The second process encompasses bacterial breakdown of complex nitrogen-containing molecules in organic matter following death of organisms; nitrates are produced which can be used directly by vascular plants; and these nitrates have the $^{15}N/^{14}N$ ratios which contain more ^{15}N relative to ^{14}N than is true in the atmosphere (Shoeninger and Moore, 1992).

Legumes have symbiotic relationship with the bacteria of the genus *Rhizobium* and this bacteria live in the roots and are able to fix nitrogen, combining it with other elements such as hydrogen or oxygen, thereby making it available to the plant (Katzenberg and Waters-Rist, 2019). As mentioned above, other plants get their nitrogen from decomposed organic matter which breaks down to compounds such as ammonia or nitrate. Thus, legumes tend to have $\delta^{15}N$ values closer to that of atmospheric nitrogen while non-leguminous plants are more enriched in $\delta^{15}N$ and therefore haver higher $\delta^{15}N$ values (Katzenberg and Waters-Rist, 2019).

Nitrogen isotope fractionation in the biosphere is influenced by the balance between the microbial fixation of atmospheric nitrogen into organic and inorganic compounds and is released again by denitrification. The $\delta^{15}N$ values in marine environments are slightly more positive than those in terrestrial soils. This is due to greater amount of denitrification happening in oceans; therefore, coastal marine regions have $\delta^{15}N$ values in the range 5-6‰ as compared to 1-4‰ for terrestrial soils.

C_3 and C_4 plants tend to be indistinguishable when $\delta^{15}N$ is measured mainly because all plants which are grown in a single area acquire similar $\delta^{15}N$ values thereby reflecting the value of the soil from which they acquire their fixed nitrogen. The $\delta^{15}N$ in bone collagen values can provide information about trophic level that a specific organism holds in the food chain (Brocherens and Drucker, 2003). These trophic levels are generally considered to increase between 3 and 5‰ with each trophic level up inside the food web due to biological fractionation (Brocherens and Drucker, 2003; Schwarez and Shoeninger, 1991).

In short, nitrogen isotopes vary depending on trophic level (Katzenberg and Waters-Rist, 2019), and terrestrial plants have generally 4‰ lower $\delta^{15}N$ values than marine plants, and marine animals have higher $\delta^{15}N$ values than terrestrial animals due to the trophic level effect with more trophic levels in marine than terrestrial ecosystems (Morghaddam et al. 2016). Thus, nitrogen isotopes can be used to analyse the relative contribution of animal and plant food, and in distinguishing terrestrial diets from marine ones (Schoeninger, 1984). Individuals consuming mainly vegetarian diet tend to have $\delta^{15}N$ values ranging from 3 to 9 ‰, while individuals consuming meat of terrestrial herbivores will have $\delta^{15}N$ ranging from 9 to 12‰ (DeNiro and Epstein, 1981; Hedges and Reynard, 2007). Adding to that, meat protein dominates the $\delta^{15}N$ values of bone collagen over plants in individuals with an omnivorous diet (Guede et al., 2018).

Ambrose, (1991) indicates that species from different ecosystems cannot be compared without considering the isotopic composition of the local food web. Plants vary in their nitrogen isotope ratios depending on the source of nitrogen in the soil; and continuous occupation of a region with deposition of animal waste including use of manure as fertilizer may result in higher nitrogen ratios in plants (Borgaard et al 2013).

Above all, bone collagen $\delta^{15}N$ values provide a general means to distinguish diets based primarily on marine foods and terrestrial foods. This is mainly because food chains in marine ecosystems are longer than terrestrial food chains and there are more ^{15}N enrichment steps (Pate, 1994) (see figure 3.5 for more details). Mean collagen $\delta^{15}N$ values of +5.7‰ ± 2.2‰ are for terrestrial mammals and +15.6 ± 2.2‰ for marine mammals (Shoeninger and DeNiro, 1984). Pate notes that the terrestrial range, +1.9 to +10.0‰, and marine range, +11.7 to +22.9‰, values do not overlap. But there are some cases where ^{15}N enrichment in terrestrial environments results in mammal bone collagen $\delta^{15}N$ values that overlap with those for marine mammals; and significant ^{15}N enrichments are reported for both arid-land environments and costal or saline environments (Pate, 1994).

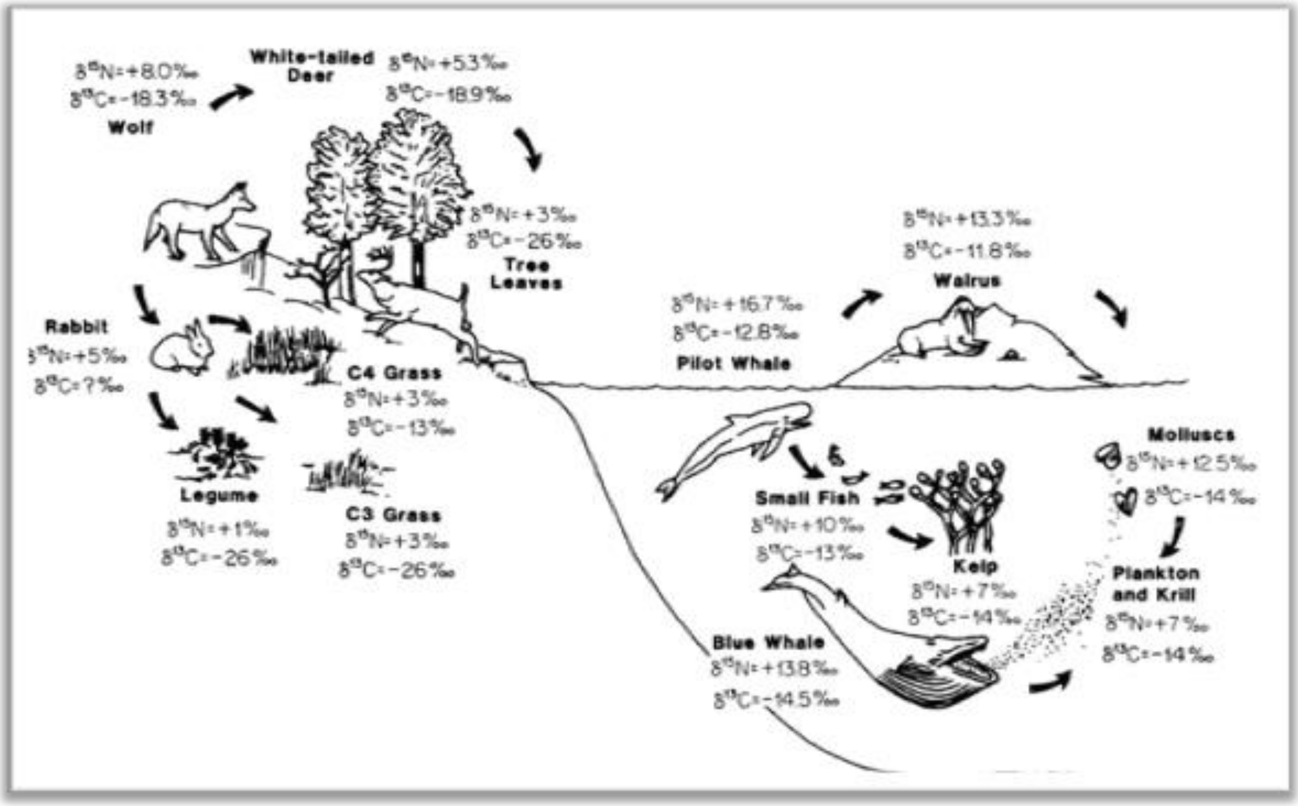

Figure 3.5: Distribution of stable isotope ratios of carbon and nitrogen in the biosphere (Adapted from Schoeninger and Moore, 1992).

CHAPTER 4: MATERIALS AND METHODS

4.1 Sample Description

With regards to the sanitary situation, and the lab procedures involved, a representative set of twelve individuals were carefully sampled out the 142 individual skeletons of which were excavated from graves and pits of inhumation during the POLIS de Lagos program, where an archaeological intervention was carried out in Largo de Santa Maria da Graça and its surrounding necropolis with the purpose of installing infrastructures. The samples were chosen according to 2 phases, and this was done to at least have sample representation from the late 14th century to late 19th century. There was equal representation according to sex though all the samples analysed in this study were adults and no children were analysed.

Four bone types were used for analysis: femur, tibia, fibular and ulna. 7 human samples had their femur taken for collagen extraction whilst 3 samples (LNSG 29, LNSG 94, LNSG 120) had their tibias selected for analysis. Samples LNSG 134 and LNSG 36 had their fibula and ulna sampled respectively for collagen extraction. Table 1 provides an illustration of the human samples and types of bones which were chosen for analysis of ^{13}C and ^{15}N.

Table 4.1: Information on human samples selected for this study

Sample Name	Sex	Element	Sample Description	Chronology/Phases
LNSG 7	Male	Femur	hard bone and compact, glue and marker present	Phase 2
LNSG 29	Female	Tibia (right)	marker present with glue, compact	Phase 2
LNSG 36	Male	Ulna (right)	marker and glue present, fragile and brittle	Phase 1
LNSG 40	Male	Femur (left)	marker and glue present, some black spots inside	Phase 2
LNSG 90	Female	Femur (left)	glue present, compact	Phase 2
LNSG 94	Male	Tibia (right)	soft bone and easy to clean	Phase 2
LNSG 102	Male	Femur (left)	some sections are fragile	Phase 1
LNSG 116	Female	Femur (right)	hard bone difficult to clean, marker and glue present	Phase 1
LNSG 117	Male	Femur (left)	brittle outside	Phase 1
LNSG 120	Female	Tibia (left)	glue present, evidence of pathology, brittle and fragile	Phase 1
LNSG 124	Female	Femur (left)	has a black spot and soft to cut	Phase 1
LNSG 134	Male	Fibula (left)	glue present, compact	Phase 1

4.2 Collagen Extraction

Samples were first physically examined before they were photographed, and notes were made on exclusive features on the surfaces of the bones. After photographing, each individual bone sample was cut to get a piece weighing between 500-700mg.The cutting was done using a DREMEL™ drill and cutting was done on more compact areas which have the potential yielding collagen as compared to other sections. The pieces were then cleaned using a drill to remove the outer layers of the bone which would have been contaminated by the soils and also to remove glue which was present on many samples. Cleaning was also done to remove the trabecular bone which is not only susceptible to diagenetic process, but may also yield a different isotopic composition. This was done to reduce any contamination that may affect carbon and nitrogen isotopic ratios of the collagen, and in turn, provide biased estimates of dietary habits of these individuals.

Figure 4.1: DREMEL™ drill used for cutting and cleaning of bone samples

The cleaned pieces were weighed and broken into smaller pieces; and they were put separately inside 16mm Ezze tubes™ (Elkay Laboratory Products) whose lids were perforated to allow escape of gases when bone reacts with acid. The tubes were tightly closed to avoid loss or contamination of the bone samples and were then labelled according to sample name.

The Longin method (Longin, 1971) modified by Brown et al, (1988) was used for collagen extraction and the following procedures were done:

10ml of 0.5M of hydrochloric acid (HCl) was poured into tubes containing the broken bones and vortexed before they were put in a fridge. The samples were vortexed once per day for one week except weekends when the Lab was closed.

After one week, the acid was removed and replaced with fresh acid and the same procedure of vortex was done for another week. This process was done to demineralise the bone. After two weeks the samples were rinsed with ultrapure water between 5-7 times and between each rinsing they were centrifuged for 5 minutes at a speed of 6 until the samples were neutral. The samples were then placed in into 0.125M of sodium hydroxide (NaOH) and were left for 20 hours at room temperature. Applying NaOH helps in removing base soluble/ organic contaminants like humic acids. After that NaOH was removed and the samples were rinsed between 3 to 5 times with ultrapure water, and they were vortexed and centrifuged for 5 minutes each at 6000rpm until they became neutral.

A weak acid solution of 10ml of 0.01M HCl was then added to the samples and were placed in the oven for 48 hours at a temperature of 70°C, the process of gelatinisation. Once gelatinisation was done, sample filtration occurred using 9ml Ezee-filter™ separators (Elkay Laboratory Product). Filtration was done in labelled pre-weighed vials to remove any insoluble particles and when filtration was done, the collagen was freeze-dried using liquid nitrogen and then lyophilised for 48 hours to remove humidity using a Telstar LyQuest freeze dryer. After 48 hours in the lyoph, the vials were then weighed to obtain the collagen yield for each sample. The next process was to weigh collagen into tin capsules using microbalance and standards were also weighed. Sample weight was around 0.7mg and for standards the weight was between 0.2-0.3mg. After that, the samples were then stored in a desiccator with silica gel until they were analysed by EA-IRMS.

Figure 4.2: Microbalance to weigh samples for IRMS analysis

4.3 EA-IRMS Analysis

The carbon and nitrogen analysis were conducted using an Elemental Analyser (EA Flash 2000 HT, Thermo Fisher Scientific®) coupled with a Conflo IV to a Delta V Advantage Isotope Ratio Mass Spectrometer. Helium was used as a carrier gas at a flow rate of 95mL/min (Figure 4.3). Before tin capsules containing collagen and standards were flush combusted, blank capsules were fed into the analyser to monitor background noise and checking for contamination.

International reference materials were also included in the analysis. These reference materials have known nitrogen and carbon isotopic values. For carbon, IAEA-CH-6, sucrose, was used with known $\delta^{13}C$ = -10.499 ‰; and IAEA-600 (caffeine, $\delta^{13}C$ = -27.771‰ and $\delta^{15}N$ = +1.0 ‰). IAEA-N-2 (ammonium sulphate, $\delta^{15}N$ = +20.3‰) was also measured. The purpose for using these standards was for chemical calibration and determination of isotopic composition of carbon and nitrogen in bone collagen extracted from the samples. In-house standards were also weighed and combusted together with tin capsules containing collagen and international reference materials. These in-house standards are L-alanine, with $\delta^{13}C$= -19.17‰ and $\delta^{15}N$= +4.26‰.

Figure 4.3: EA-IRMS equipment which was used for nitrogen and carbon stable isotope analysis at HERCULES Laboratory.

Carbon and nitrogen stable isotope analysis were performed in the HERCULES Laboratory, Evora, Portugal.

CHAPTER 5: RESULT PRESENTATION AND INTERPRETATION

5.1 Preservation of Bone Collagen of humans buried at Lagos

Collagen yield was calculated after the collagen was extracted and before IRMS analysis. This parameter is important in assessing collagen preservation in archaeological bones and to find the extent to which diagenetic processes could have impacted preservation of collagen. Collagen yield is calculated as a percentage of the weight of raw bone. It has been proposed by DeNiro and Weiner (1988) that collagen yields which are under 2% could signify potential problems on the samples themselves due to collagen alteration when the bones were buried, hence those samples should be discarded. In this study, the collagen yield ranged from 2.1-17.9 % with the mean value of 7.6%. 2 samples, LNSG 36 and LNSG 94, were not analysed. LNSG 36 completely dissolved during the alternating processes of demineralisation and removal of organic contaminants. LNSG 94 did not have enough collagen for microbalance weighing hence no collagen from that sample was analysed.

The percentage of carbon and nitrogen (C% and N% respectively) for bone collagen was obtained using the Elemental Analyser. Calculating the C% and N% helps to determine the bone collagen quality. The range which the percentage should fit was established by Ambrose (1990). For carbon, the range is between 15.3- 47% and between 5.5-17.3% for nitrogen. The samples analysed in this study had the C% and N% within the established ranges, with the C% mean value of 38.3% and N% mean value of 15.5 %, except for sample LNSG 116 with a higher N amount of 26%.

Another important indicator of collagen quality is the carbon-nitrogen ratio (C/N ratio). The reason why C/N ratio is important in the stable isotope analysis of bone collagen is because it provides information concerning sample contamination which would have happened during diagenesis. Such contaminants include humic acids and lipids. C/N ratio is derived from the Elemental Analyser which provides percentage of both carbon and nitrogen of each sample. DeNiro and Weiner, (1988) note that the material extracted with a C/N ratio in the range of 2.9 and 3.6 ought to preserve the in vivo stable isotope ratios from when the organism, under analysis, was alive. Except for sample LNSG 116 which had C/N ratio of 1.8, all other samples had values which are inside the range mentioned in DeNiro and Weiner, (1988). This sample was therefore discarded from isotopic interpretation. Samples LNSG 36 and LNSG 94 were not analysed due to the reasons mentioned above (See Table 5.1 for the summary).

Table 5.1: Statistical presentation of C/N ratio and summary of Carbon and Nitrogen % of human samples from Santa Maria da Graça necropolis

Sample Name	**Sample Weight (grams)**	**Weight of Vials with Collagen**	**Collagen**	**% Collagen**	**%N**	**%C**	**C/N**	**δ^{15}N**	**δ^{13}C**
LNSG 7	0.469	6.2	0.1	17.9	15.5	41.5	3.1	12.4	-16.3
LNSG 29	0.468	6.2	0.0	7.3	15.1	40.2	3.1	13.2	-15.9
LNSG 36	0.460	-	-	-	-	-	-	-	-
LNSG 40	0.536	6.2	0.1	10.3	15.0	40.0	3.1	12.0	-16.3
LNSG 90	0.606	6.2	0.1	9.3	7.3	18.7	3.0	11.1	-17.5
LNSG 94	0.523	6.1	0.0	1.4	-	-	-	-	-
LNSG 102	0.587	6.2	0.0	2.3	15.3	40.7	3.1	12.3	-16.7
LNSG 116	0.546	6.1	0.1	10.8	26.2	41.1	1.8	15.1	-17.1
LNSG 117	0.585	6.1	0.0	2.1	14.3	38.2	3.1	11.8	-17.4
LNSG 120	0.565	6.2	0.1	12.0	15.4	41.0	3.1	11.1	-18.1
LNSG 124	0.546	6.2	0.0	4.9	15.5	41.3	3.1	10.8	-17.9
LNSG 134	0.402	6.2	0.0	6.3	15.1	40.4	3.1	12.6	-16.7
mean					15.5	38.3	3.0	12.2	-17.0
Standard deviation								1.4	0.6

5.2 Dietary investigation of Santa Maria da Graça

5.2.1 Using faunal bone collagen from previous studies in Algarve region as a baseline for interpreting dietary habits of the human population at Lagos

Since this research could not include faunal remains from Santa Maria da Graça, it was necessary to utilise the data which was generated by previous studies on the region of Algarve specifically faunal bone collagen from studies by Aceves (2019) and Gonzàlez (2019). The faunal bone collagen data from the mentioned researches were used as baseline for human dietary investigation. Only carbon and nitrogen stable isotope data values were used for this study. It is important to note that the faunal data to be used here do not necessarily correspond to the period and actual location of the studied necropolis of Santa Maria da Graça in Lagos.

In the case of faunal samples investigated by Aceves (2019), the bones were excavated from trash pits in Oficina do Senhor Carrilho (OSC), Loulé and dated from the Almohad period (c. 12^{th}- 13^{th} AD). The $\delta^{13}C$ values for faunal bone collagen from OSC ranged between -22.8‰ and -18.7‰ with the mean value of -20.8‰ ± 1.08‰. The $\delta^{15}N$ values ranged between 3.1‰ and 11.7‰ with a mean value of 6.3‰ ± 2.56‰. In terms of the faunal samples from Cacela-a-Velha (CV), examined by Gonzàlez (2019), 28 faunal samples show $\delta^{13}C$ values that range from -22.3‰ to -18‰ with a mean value of -20.2‰ ± 0.93‰; and $\delta^{15}N$ values that range from 2.5‰ to 12.1‰ with a mean value of 7.9 ± 2.49‰.

Table 5.2: Comparative depiction of δ13C and δ15N mean values of faunal samples from Oficina do Senhor Carrilha (Loulé) and Cacela-a-Velha

Species	13C (Min)	13C (Max)	13C (Mean)	15N (Min)	15N (Max)	15N (Mean)
Rabbits (Oficina do Senhor Carrilho)	-22.8	-21.5	-22.1	3.1	5.2	4.3
Rabbits (Cacela-a-Velha)	-22.3	-20.4	-19.7	2.5	6.3	4.7
Red deer (Oficina do Senhor Carrilho)	-20.7	-20.4	-20.6	3.9	8.7	7.0
Red deer (Cacela-a-Velha)	-20.4	-20.0	-20.2	4.9	6.3	5.4
Ovicaprids (Oficina do Senhor Carrilho)	-20.9	-19.9	-20.4	6.5	7.2	6.9
Ovicaprids (Cacela-a-Velha)	-20.8	-19.1	-20.2	6.3	12.1	9.0
***Sus* sp. (Oficina do Senhor Carrilho)**			-20.3			4.4
***Sus* sp. (Cacela-a-Velhha)**	-20.7	-19.0	-20.3	6.6	10.0	8.6
Equus sp (Oficina do Senhor Carrilha	-21.2	-21.1	-21.2	4.1	4.2	4.2
Chicken (Oficina do Senhor Carrilha	-19.2	-18.7	-19.0	10.4	11.7	10.9
Chicken (Cacela-a-Velhha)	-19.5	-18.0	-18.8	6.7	11.0	9.5
Cattle (Cacela-a-Velhha)	-21.4	-20.0	-20.4	7.3	11.7	9.1
Fox (Cacela-a-Velhha)			-19.7			9.5

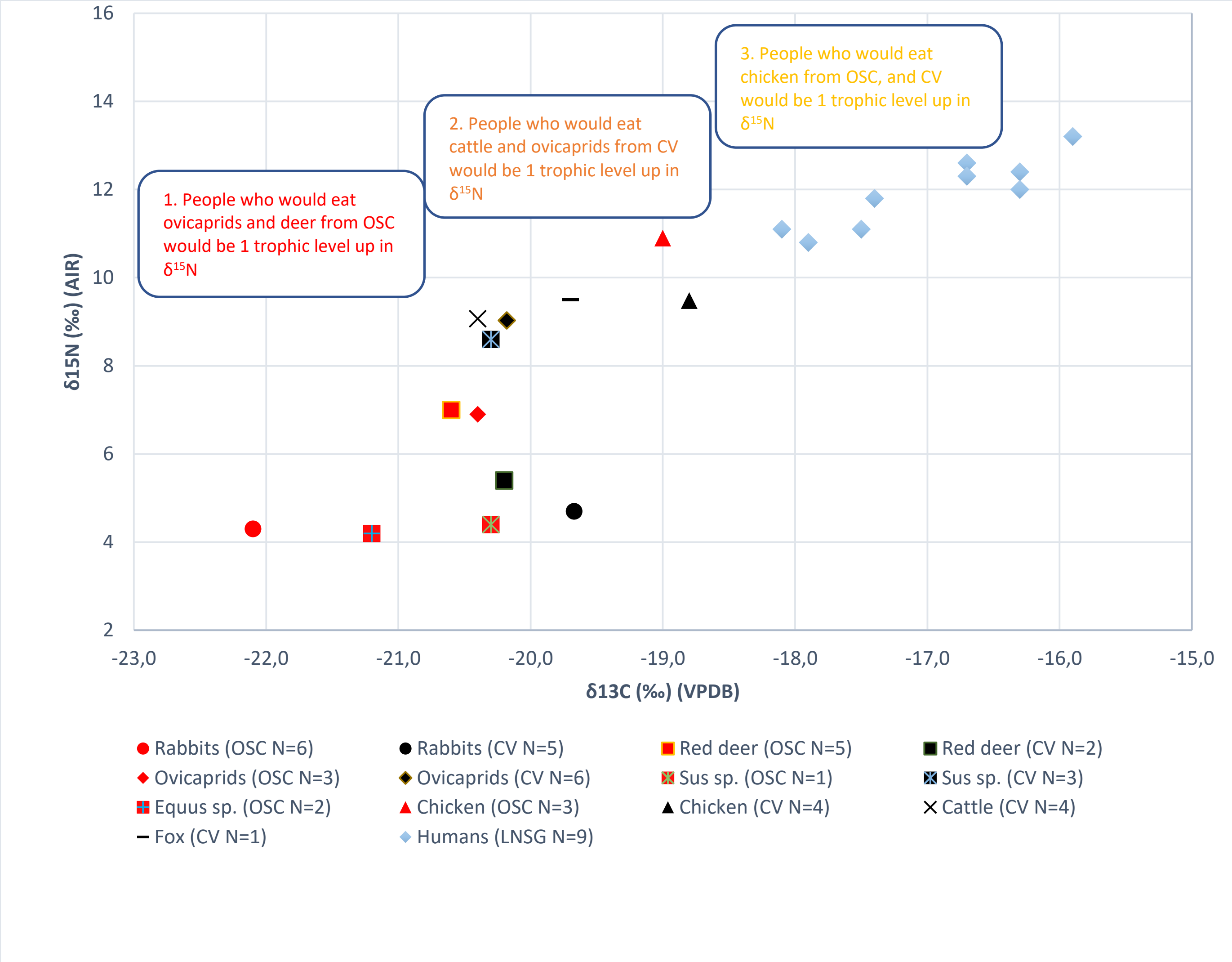

Figure 5.1: Overall comparison $\delta^{13}C$ / $\delta^{15}N$ from human samples from Santa Maria da Graça (LNSG) with faunal mean $\delta^{13}C$ / $\delta^{15}N$ values of samples from Oficina do Senhor Carrilho (OSC) adapted from Aceves, 2019 and faunal samples from Cacela-a-Velha (CV) (Adapted from Gonzàlez, 2019).

The $\delta^{13}C$ values of red deer from both OSC and CV indicate the consumption of C3 plants. This may be because deer favourably inhabits in meadows, trees or bushes which are often far away from human residences. Deer mostly feed on twigs or leaves of herbs and trees which are usually C3 plants. There is a difference in $\delta^{13}C$ values of rabbits from both sites though they are in the same range of $\delta^{15}N$ values between 4‰ and 5‰. Rabbits from OSC have a more negative $\delta^{13}C$ as compared to the ones from CV. This may be due to differences in habitats which in turn affected their dietary intakes: rabbits from OSC could have fed closer to wooded areas which are susceptible to canopy effect, that is the ^{13}C values of plants, like grasses, which grow below trees are lower than those which are high enough to receive much sunlight. Plants which are exposed less to sunlight day in day out tend to have more negative $\delta^{13}C$ values.

On the other hand, rabbits from CV have a more positive mean value of $\delta^{13}C$ and slightly higher $\delta^{15}N$ which can be explained by the fact of environmental conditions. CV rabbits could have fed in wider areas or areas which are closer to seacoast with some C4 plants native to Portugal grow, cordgrass (*Spartina maritima*) and *Moricandia arvensis* (Gonzàlez, 2019). The other reason for a slightly higher $\delta^{15}N$ of rabbits from CV as compared to rabbits from OSC may be due to habitat proximity which would have been closer to those of humans where soils would be manured and in turn have enrichment of ^{15}N due to various reasons which include agricultural practices.

There is a high contrast between the *Sus sp.* species from OSC and CV in terms of their $\delta^{15}N$ values, but they are within the same range of $\delta^{13}C$ values. This contrast maybe due to two main reasons the first being that the pig from OSC may have been a wild one due to its lower $\delta^{15}N$ value and may have been hunted. This is also supported by the fact that the period in which the pig falls into is the Islamic era and pigs were not, and are even during this time, not a food source for Muslims and are considered *haram* and would only be eaten when it is extremely the need to have something to eat like during food shortages (Aceves, 2019).

The mean $\delta^{15}N$ value of the pigs from CV show a much more enriched level of ^{15}N as compared to the one from OSC. This may be due to domestication of these pigs which would likely have been eating food resources eaten by the Christian population of CV; and would also eat discarded foodstuffs and human waste since domesticated pigs would usually roam around rather than being confined in pigsties. Directly or indirectly, these foodstuffs produce higher $\delta^{15}N$ values in pig bone collagen hence a speculation that the pigs from CV would have been domesticated. This issue of domestication is also supported by the clustering of the value points of cattle, ovicaprids and *Sus sp*. from CV. However, both pig samples, from OSC and CV, lay in the same range of $\delta^{13}C$ values despite their differences in $\delta^{15}N$. This may be because pigs in general are omnivorous and may have eaten C3 plant resources despite the differences in setting of the sites they originate from.

Figure 5.1 shows that the $\delta^{15}N$ values of ovicaprids and cattle from CV have higher mean values between 8 to 10‰ as compared to the rest of the faunal samples, but close to *Sus sp*. from CV. The reason behind high levels of $\delta^{15}N$ in cattle, goats and sheep samples from CV is probably because of their domestication and may also be due to agricultural practices such as manuring of agricultural land. The practice of fertilising agricultural land with seaweed increases $\delta^{15}N$ values in the food chain especially given that these domesticated animals would have been fed, as fodder, with agricultural plants which may have been enriched in $\delta^{15}N$. If compared to some $\delta^{15}N$ values of humans from Santa Maria da Graça, a clear increase in trophic level and enrichment of 3‰ can be observed while there would be around 5‰ and more if compared to the domestic herbivores from Loulé.

From figure 5.1, the mean values of chickens from OSC are in the same range of $\delta^{15}N$ values with some of the humans from Santa Maria da Graça (Lagos) whose $\delta^{15}N$ values are within 10‰ and 12‰ range. On the other hand, chickens from CV have $\delta^{15}N$ mean value which is below the range of OSC chickens as well as humans from Santa Maria da Graça. The reason behind such lower $\delta^{15}N$ may be due to different foodstuff the omnivorous chickens ate which may have been lower in ^{15}N isotopes and environments in which these chickens were bred and raised. With the exception of the chickens from Loulé, the faunal samples from Loulé and Cacela-a-Velha yield lower $\delta^{13}C$ and $\delta^{15}N$ values as compared to the human samples from Santa Maria da Graça in Lagos. The trophic levels are generally considered to increase their $\delta^{15}N$ between 3‰ and 5‰ with each trophic level up inside the food web due to biological fractionation (Brocherens and Drucker, 2003; Schwarez and Shoeninger, 1991). Individuals consuming meat of terrestrial herbivores have generally $\delta^{15}N$ ranging from 9‰ to 12‰ (DeNiro and Epstein, 1981, Hedges and Reynard, 2007) although this depends on numerous factors (environment, agricultural practices).

The 3 squares in Figure 5.1 show what would be the values of consumers who would eat some of the animals from OSC and CV. Thus, people who would eat ovicaprids and deer from OSC, as shown in box 1, would be 1 trophic level up in $\delta^{15}N$ (+3‰) as compared to the animals they consume. At the same time, people who would eat cattle and ovicaprids from CV (box 2), would be 1 trophic level enriched in $\delta^{15}N$ whilst those people who would chicken from OSC, and CV (box 3) usually are 1 trophic level up in $\delta^{15}N$.

All human samples depict a higher trophic level, when observing the $\delta^{15}N$ values ranging between 10‰ and 14‰, than the rest of the faunal samples. This is to show that the values for the humans go beyond the expected trophic level at least in ^{13}C and at least for people from Phase 2 of the necropolis of Santa Maria da Graca (see information below). However, these people can have higher nitrogen values when compared to the animals they would have consumed but they have higher carbon levels that do not seem to come from consumption of the animals. When observing the depiction in Figure 5.1, the humans with values higher $\delta^{13}C$ could be millet consumers or later maize. The correlation between carbon and nitrogen of the humans from Santa Maria da Graça may be from fish and other marine resources where they derived much of their protein from, but the humans from Santa Maria da Graça may have consumed animal products from terrestrial animals.

5.2.2 Overall diet in Lagos

All samples from Santa Maria da Graça show high levels of $\delta^{15}N$. As seen from Table 5.4 and Figure 5.2, high levels of $\delta^{15}N$ (>12pm) in samples LNSG 7, LNSG 29, LNSG 102, LNSG 117 and LNSG 134 could mean that the individuals may have relied on marine resources as food. This may be true because logically speaking it is possible that the fishing activities in Lagos were mainly meant to sustain the local population and for trading with other places. Also, if considering that individuals consuming exclusively meat of terrestrial herbivores will have $\delta^{15}N$ ranging from 9 to 12‰ (DeNiro and Epstein, 1981; Hedges and Reynard, (2007), the other samples whose $\delta^{15}N$ range is between 10.8 and 12‰, may have consumed more meat from terrestrial herbivores than marine resources.

Because of Lagos' prominent position in maritime enterprises of the Discoveries and in national geopolitics for its relations with North Africa, sub-Saharan Africa, Asia and the Americas (Diaz-Guardamino and Moran, 2008; Casimiro, 2020), it is highly probable that there were changes in dietary practices of the people of Lagos which was influenced by the introduction of C4 plants from tropical areas of Africa and South America. These voyages beyond the European continent brought in new foodstuffs which include maize from the Americas and some spices from Asia. These products could have altered the dietary intake of the population of Lagos, as it did for other areas within Iberian Peninsula and the rest of Europe since it would have created more dietary diversity.

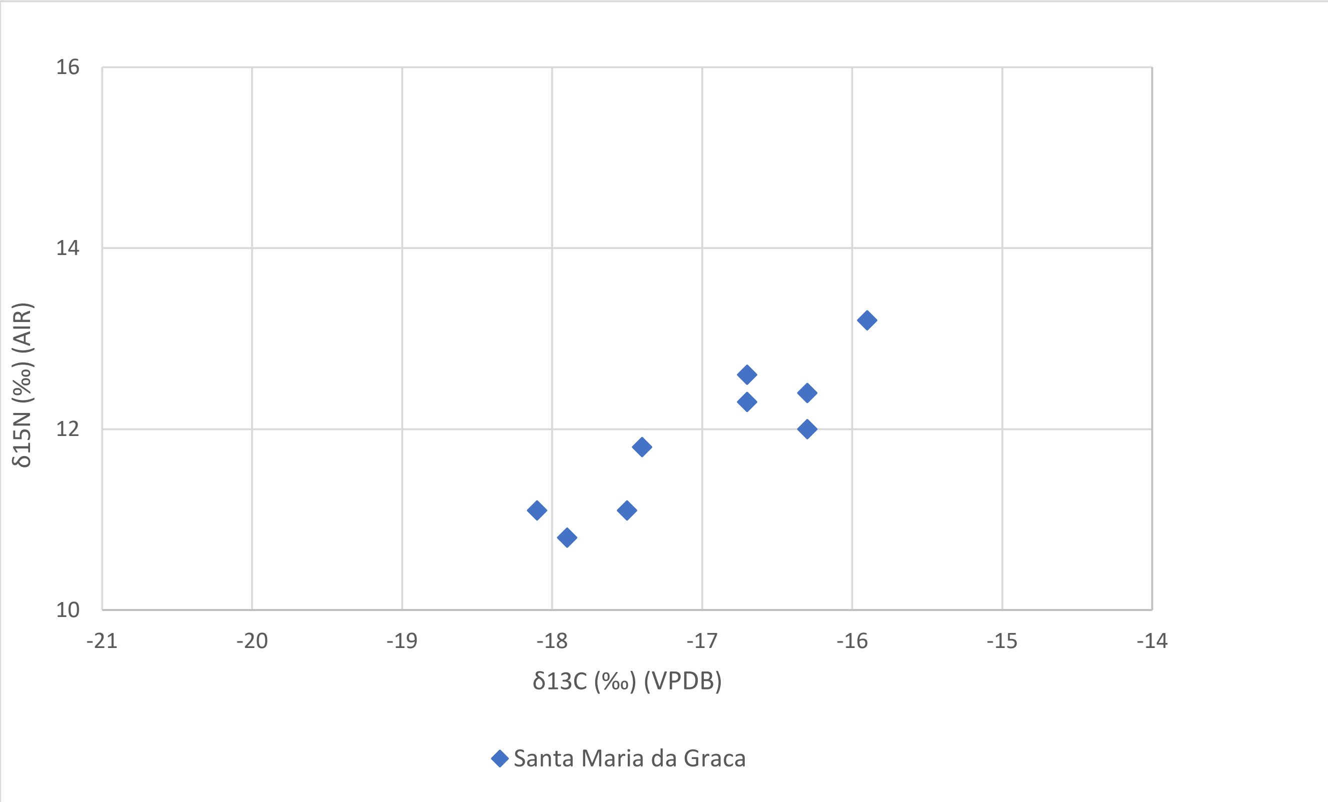

Figure 5.2: Scatter graph of $\delta^{13}C$ and $\delta^{15}N$ values of individuals from Santa Maria da Graça necropolis.

Since many marine animals tend to feed directly on the photosynthisizers like planktons and algae, their tissues take on $\delta\ ^{13}C$ values close to those of the primary marine producers which is usually around -16‰. As a result, the $\delta^{13}C$ values of humans whose diet has substantial marine input reflects the values found in fish and shellfish. This may be the case for the population of Lagos, as seen from Table 5.1, whose mean $\delta^{13}C$ value is -17‰ which goes beyond (more than 2‰) one trophic level enrichment in ^{13}C as compare to the domesticated herbivores from Loule and Cacela-a-Velha. This notion is supported by a higher mean value of $\delta^{15}N$ which is 12.2‰. In addition, there is a correlation between ^{13}C values and ^{15}N values recorded in the human bone collagen thus such correlation advocates for more seafood consumption by the people of Lagos.

It is important to mention that during the 16^{th} especially during the 17^{th} century, the second half of the Little Ice Age climatic downturn occurred (Oliva et al., 2018) and this led to a decline in fish stocks. Moreover, this Little Ice Age caused the environmental crises resulting in famines leading to dependence on maize elsewhere in the Iberian Peninsula (Lopez-Costas and

Muldner, 2018). Thus, such changes in climate could also have impacted the way of life of the population of Lagos as it did elsewhere in the Iberian Peninsula.

5.2.3 Variability of the Data: Time and Gender?

It is interesting to note from Figure 5.3 that there seem to be a clear distinction of the samples which is roughly around $\delta^{13}C$ -17‰ range. If this distinction is interpreted in terms of the 2 Phases in which the cemetery of Santa Maria da Graça has been divided into, it can be observed that the samples on the left side of the graph (^{13}C lower than -17‰) correspond to the majority of the 1st Phase, late 14th to mid-16th centuries (Diaz-Guardamino and Moran, 2008) with the exception of one sample LNSG 90 which is from the 2nd Phase. Similarly, samples clustered on the less negative side of -17‰ $\delta^{13}C$ value, correspond to the 2nd Phase of the cemetery of Santa Maria da Graça, except LNSG 102 and LNSG 117 which relate to the 1st Phase.

The clustering on the left side of the graph (lower than -17‰) shown in Figure 5.3 may have been due to several reasons : less negative $\delta^{13}C$ values and a $\delta^{15}N$ range between 11‰ and 12‰ (majority of samples from Phase 1) could mean intake of marine fish in the lower tropic level. Mixing C4 and C3 plant resources could also be the reason behind less negative $\delta^{13}C$ values ranging between -18.5‰ and -17.5‰. Sorghum and millet were consumed prior to Post medieval period during which the cemetery of Santa Maria was used for burials, hence usage of sorghum and millet could also have continued post medieval times.

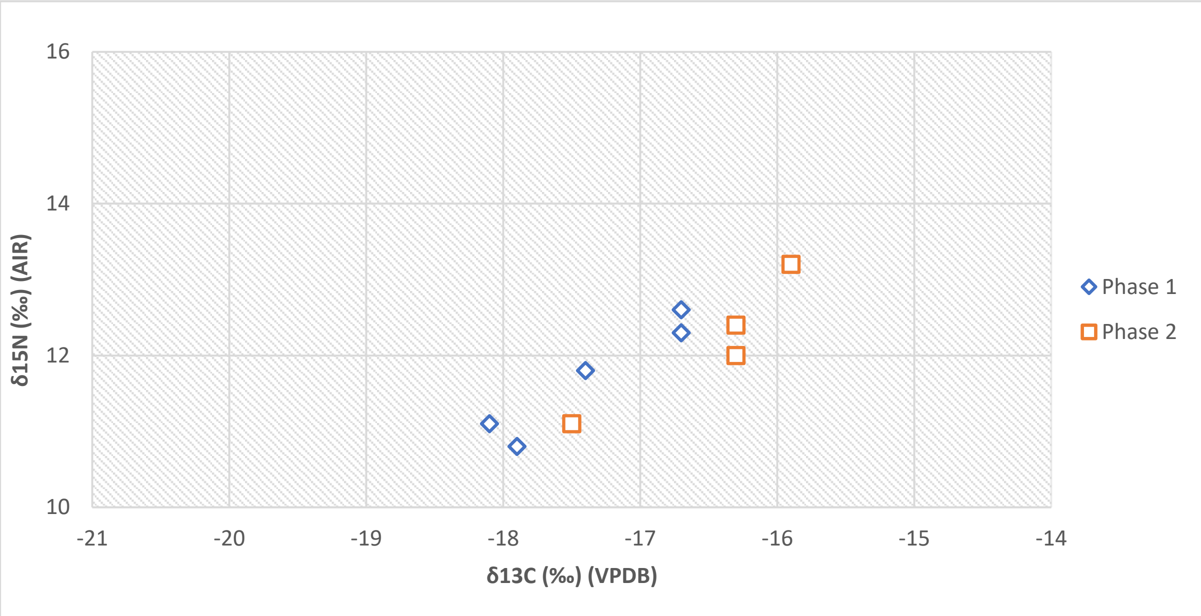

Figure 5.3: Dietary comparison by Phases.

To explain the reason behind the cluster which, in this research has been marked as representing the 2nd Phase of Santa Maria da Graça, mid-16th to 19th centuries (Diaz-Guardamino and Moran, 2008) there are several plausible reasons. The first one is that it is during the 16th century that there was full introduction of maize in the European continent. Lagos, because of its geographical location, was linked to various commercial fleets that anchored in the Lagos Bay for purposes which include supplying goods, depositing, and loading new goods (Pereira, 2012). As one of the cities in the core of maritime enterprises of the Portuguese kingdom, it is possible that maize crop and sugarcane could have been significant food sources to the people who resided there. The other reason can be that the individuals under study that displayed a less negative shift in the $\delta^{13}C$ values in the second cluster may have consumed a mixed diet of more C4 plants like maize and some C3 plants; such as wheat, barley among others, which had been cultivated since the early years of Lagos.

As seen from Figure 5.3, the mean values of Phase 1 (11.7‰ for $\delta^{15}N$ and -17.4‰ for $\delta^{13}C$) and Phase 2 (12.2‰ for $\delta^{15}N$ and -16.5‰ for $\delta^{13}C$) indicates a less negative shift in $\delta^{13}C$ and also a slight shift in $\delta^{15}N$ over time. History also mentions that the maritime enterprises of the Portuguese kingdom of early 16th also brought in new exotic plants such as maize, potatoes and cocoa from the Americas; and pepper, cloves, cinnamon, from Asia. These plant resources entered Portugal and changed the way people consumed food just a few decades within their arrival because they became the staple of recipes and were used on a daily basis (Casimiro, 2020). Thus, the people of Lagos would have eventually altered their dietary practices due to the introduction of these crops and spices.

But the introduction of maize alone, does not explain the increase in ^{15}N values. Thus the reason why there is an increase in the δ^{15}N values maybe that the people may have consumed marine resources especially fish in higher trophic levels which explains why they have an increase in both δ^{13}C values and a δ^{15}N. Another reason maybe that the people from the second phase may have consumed meat and/or dairy products from domesticated animals which would have lived in confined areas, which would in turn continuously enrich the earth of the limited space in ^{15}N found in animal dung. The consumption foodstuff enriched in ^{15}N, such as chickens and eggs, could be the reason why the people from Lagos, mainly from the second phase have high δ^{15}N.

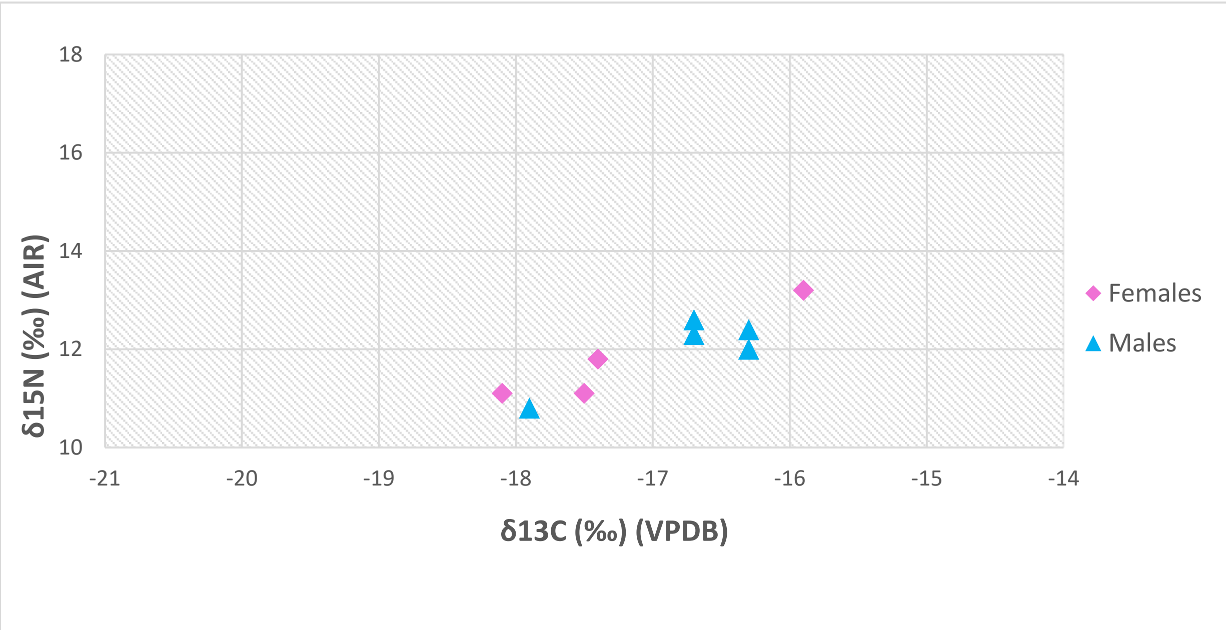

Figure 5.4: Illustration of δ^{13}C and δ^{15}N values distinguishing females from males

If there is a ruling out of direct consumption of C4 plants like sorghum and millet by the people who were buried during the later phase of the necropolis of Santa Maria da Graça, a new perception can be put into argument concerning consumption of C4 plants like maize. This is so because the Church of Santa Maria da Graça became the seat of the parish of the residents of Vila-Adentro, and that the individuals buried in this cemetery belonged, as a whole to a privileged social class (Diaz-Guardamino and Moran, 2008). It is known that millet and sorghum were considered as food sources for the poor though it was consumed by the rich and privileged during scarcity times when other carbohydrate sources would have dwindled like during droughts or famines. Thus, the consumption of sorghum and millet by the privileged class who were buried in the cemetery of Santa Maria da Graça during the later phase of cemetery may not be likely unless it was indirect consumption through edible domesticated animals which were fed with these cereals.

Upon observation of the data as presented in Figure 5.4, there is a general separation between females and males, which also has the same resemblance to the difference in phases which are discussed above. One male, LNSG 124, has the lowest $\delta^{15}N$ value of 10.8‰ as compared to all the samples but is in the same range with the most females who have $\delta^{15}N$ values within 10‰ and 12‰. This cluster also share the same range of $\delta^{13}C$ which is more negative as compared to the other cluster, between -18.1‰ and -17.4‰. Apparently, these individuals are within the time frame of Phase One of the cemetery of Santa Maria da Graça except for LNSG 90 which is from Phase Two.

On the other hand, the cluster of less negative $\delta^{13}C$ values, above -17 ‰ comprises 4 males who seem to share the same range of $\delta^{15}N$ between 12 and 12.6‰. One female has the most positive $\delta^{15}N$ and $\delta^{13}C$ values compared to the rest of the samples of Santa Maria da Graça, and this female corresponds to the second phase of the cemetery. Two of the males, LNSG 134 and LNSG 102 are from Phase 1 of the cemetery whilst the rest are from the second phase of the cemetery. The most plausible reason to why there is such a distinction between males and females from Santa Maria da Graça may be related to the different time frames which the individuals are from, rather than cultural norms and values in terms of food consumption, but this can only be confirmed with the future analysis of additional samples.

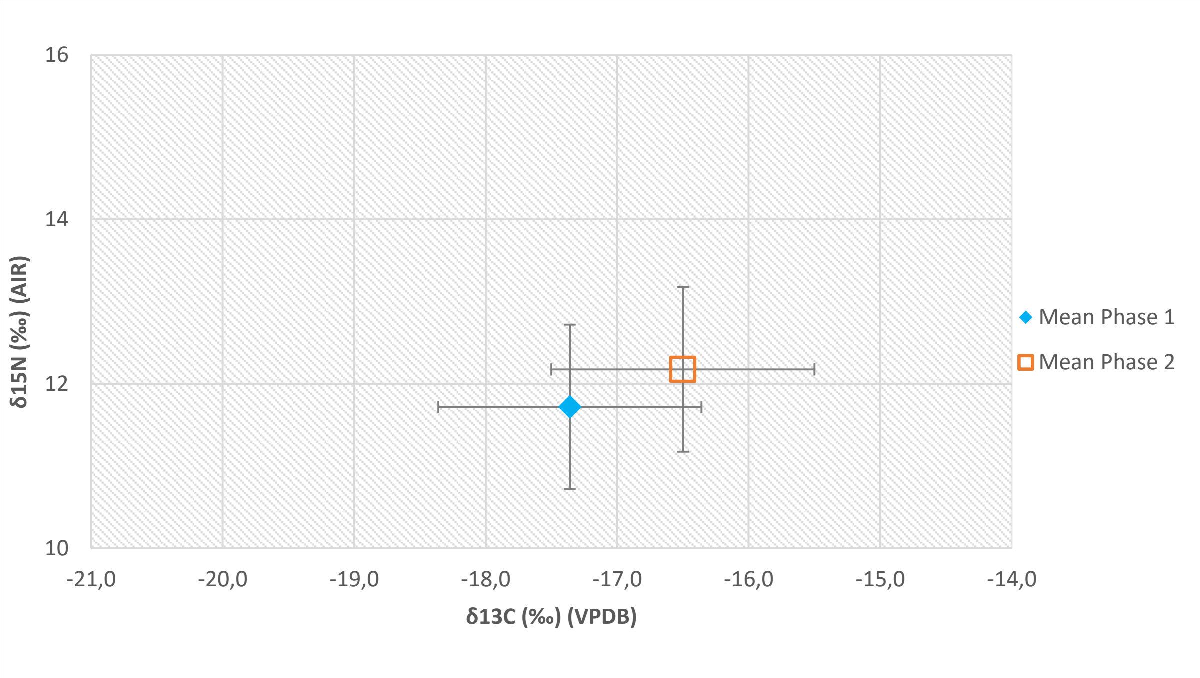

Figure 5.5: Comparison of Phases of the Necropolis of Santa Maria da Graça by outlining their $\delta^{13}C$ and $\delta^{15}N$ mean values

The introduction of new food stuffs from the Portuguese maritime expeditions could be the only reason why there is such a distinction. The probable reason why the female LNSG 29 has such high $\delta^{13}C$ and $\delta^{15}N$ values is because she might have had a diet that included the intake of large proportions of marine organisms which, in turn would increase the $\delta^{13}C$ and $\delta^{15}N$ values of bone collagen. The other reason LNSG 29 has high $\delta^{13}C$ and $\delta^{15}N$ values may result from a diet with a combination of more C4 plants, probably corn and/sugar, and animal protein fed with C4. On the other hand, the male individual LNSG 124, could have had a mixed diet of both C4 and C3 plants with an uptake of terrestrial animal protein.

In as much as there is a distinction between some males and females from the batch of samples studied here, it can be observed that the probable reason to such distinction may be because of individual choices, food preferences and availability of certain food resources rather than cultural norms. It is known however that religion did play a role in influencing the way of sustenance of the people during the post-medieval era. Thus, Christianity may have impacted the choice of food preferences especially in terms of flesh-based diet whereby whiter meats such as from chicken and fish were associated with purity hence the promotion by the Church to consume such food.

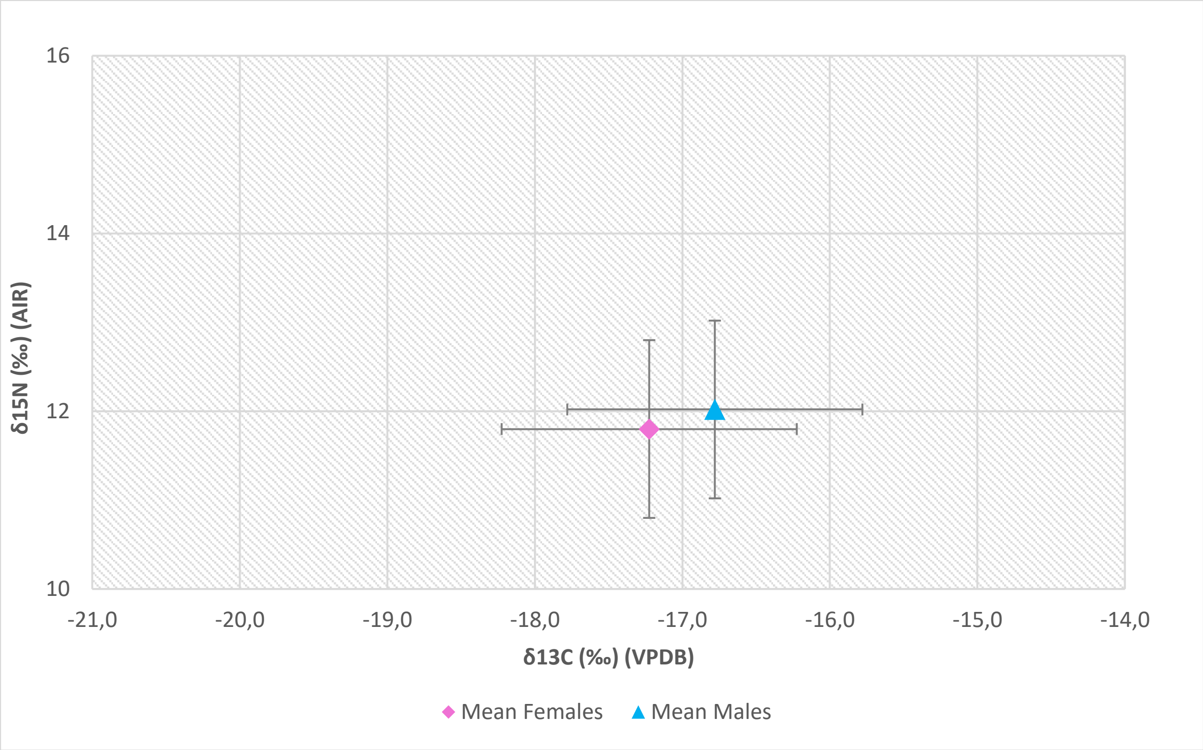

Figure 5.6: Gender comparison of the samples from the Necropolis of Santa Maria da Graça through outlining their $\delta^{13}C$ and $\delta^{15}N$ mean values.

It may become clearer to understand if there are clear distinctions and more variability amongst the individuals of in terms of phases and gender, if future stable isotope analysis would include a larger number of individuals from Santa Maria da Graça. The additional analysis of stable isotopes of sulphur on the skeletal remains from Santa Maria could provide a much clearer picture in future research. But as mentioned by Lopez-Costas and Alexander, (2019), distinguishing between consumption of C4 plants either directly or indirectly, and the consumption of aquatic resources is difficult when using $\delta^{13}C$ and $\delta^{15}N$ even when combined with $\delta^{34}S$; and this is also confirmed by Curto et al. (2018).

5.2.4 Pathologies and Diet

It should be noted that pathologies are evidence of health conditions on bones and do not influence diet, but they may be influenced by diet somehow. Thus, diet can affect health conditions of an individual hence pathologies themselves do not influence the diet, but a disease could influence the dietary behaviour. From Figure 5.7 it can be observed that 3 out of four individuals with reported pathologies have lower $\delta^{13}C$ values with mean value of -17.4‰ and standard deviation of 0.7‰. Only individual LNSG 7 has a less negative $\delta^{13}C$ value of -16.3‰.

All the unhealthy individuals lie within the same range of δ^{15}N values as with the healthy ones. Nevertheless, 3 individuals, LNSG 117, LNSG 120 and LNSG 134, have been reported to be from the 1st phase of the necropolis of Santa Maria da Graça with only LNSG 7 being from the 2nd phase.

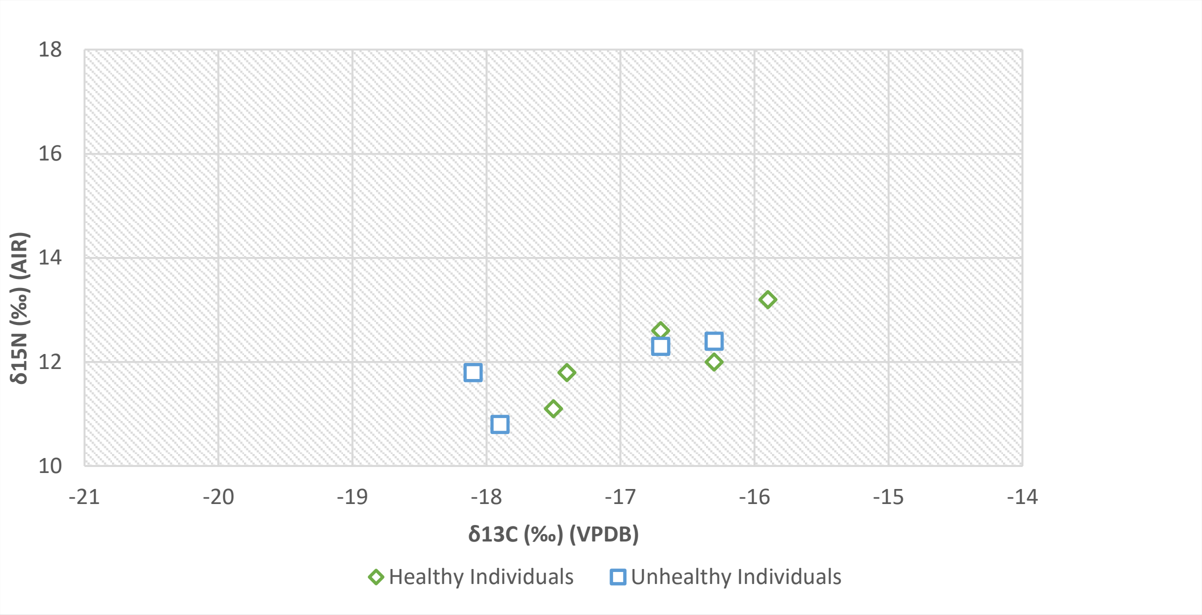

Figure 5.7: δ^{15}N and δ^{13}C values between health individuals and unhealth individuals from the cemetery of Santa Maria da Graça

Thus it may be possible though with limited assurance that the individuals who lived and were buried in the 1st phase of the necropolis would have had some serious nutritional related diseases which would impact their diets as evidenced from LNSG 117, who is a young adult male with signs of osteomyelitis along the diaphysis of the femur, radius, ulna, fibula and lame. Osteomyelitis causing bacteria can infect bones through a process of atherosclerosis when a bone is infected as the blood supply to that area of the bone becomes disrupted, and this can happen in association with diabetes (Bhowmik et al., 2018). LNSG 120, an adult female, had evidence of periostitis which according to The Health Jade Team, (2019) usually develops in people with chronic ulcers such as those with diabetes.

However, it is important to note that maize was considered a traditional remedy for some diseases (Gonzalez-Hernadez, 2002; Vallejo and Gonzalez, 2013). If maize's usage was extensive as a medicine in Lagos, the individuals with pathologies, especially those in the second phase of the cemetery, would be expected to have much higher δ^{13}C values. But the reason to why the diseased individuals record such δ^{13}C values may be because they may have had a mixed diet comprising both C3 and C4 plants.

5.3 Evolution of Diet in Algarve Region from Islamic to Post-Medieval Periods

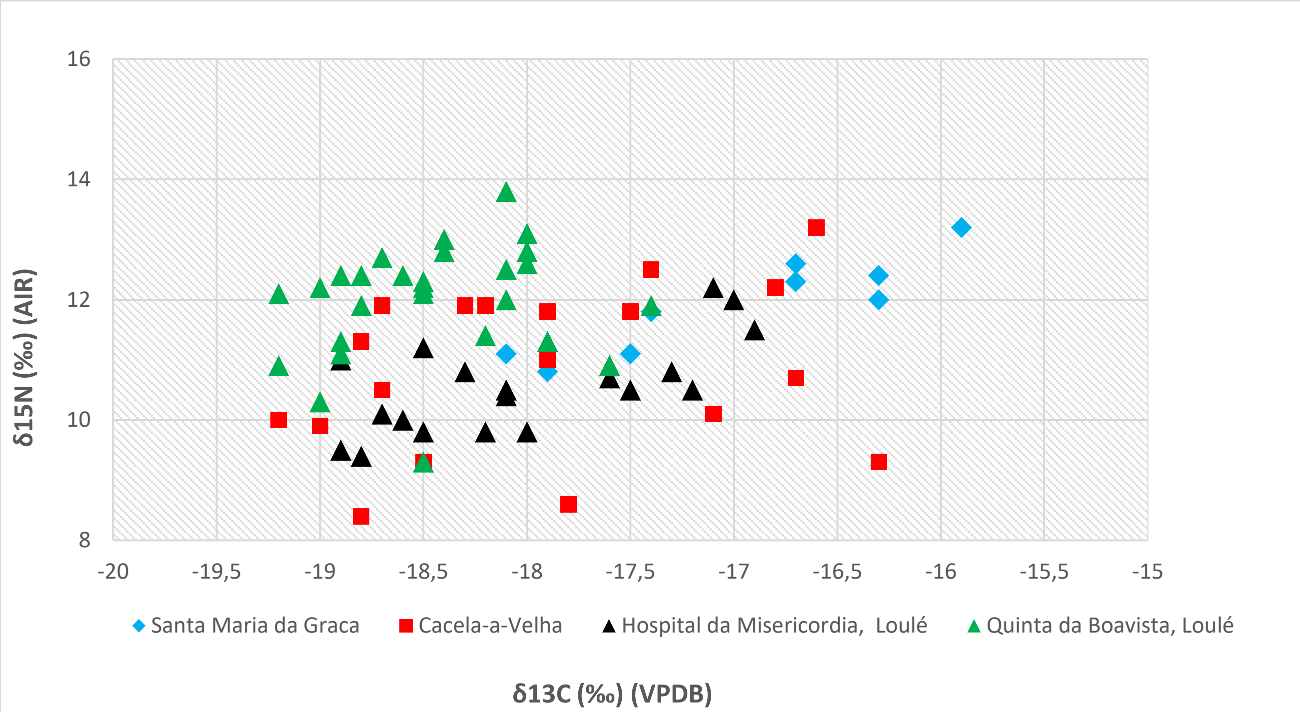

Figure 5.8: Merged data representing three different periods in Algarve Region: the Islamic period (Loulè), early Christian period (Cacela-a-Velhha) and Post-Medieval period (Santa Maria da Graça).

Using the data generated from this study (Post-medieval period) and also by Aceves (2019) and Gonzàlez (2019) from the Islamic period and the early Christian period respectively, it is possible to have a glimpse into how there may have been some dietary changes over time in the region of Algarve. A comparative analysis of the above-mentioned datasets outlined in Figure 5.8 helps to depict an evolution of diet over a large period in Algarve. But it is critical to mention that the discussion here does not entirely and fully represent overall dietary changes in the region of Algarve because the areas which were studied are different in terms of spatial distribution within the region.

As seen from Figure 5.8 generally, human samples from Loulé particularly those from Quinta da Boavista, have lower $\delta^{13}C$ values as compared to other sites. These human samples are argued to be from the Islamic period and their human bone collagen stable isotope results indicate a mainly C3 plant-based diet with the occasional input of C4 plants like millet and sorghum (Aceves, 2019). Aceves, (2019) further postulates that the presence of C3 and minor

C4 plant remains in medieval Islamic sites, along with the $\delta^{13}C$ values of Hospital da Misericordia and Quinta da Boavista, pave way for the assumption that plant intake was mainly C3 plants with occasional inputs of C4 plants,

Thus this clearly provides the reason why a lot of individuals from Loulé have more negative $\delta^{13}C$ values, which would indicate more consumption of C3 plants, because if they would have eaten diets consisting of one hundred percent C3 plants, they would have produced mean bone collagen $\delta^{13}C$ values of about -22‰ (Pate, 1994). However, if these individuals were only eating C3 plants and some animals which were C3 fed, there would be a trophic shift and their values would not be so negative.

Most individuals from Hospital da Misericordia, Loulé display $\delta^{13}C$ values which are in the same ranges as individuals from both Cacela-a-Velhha and Santa Maria da Graça. This may mean that these individuals from the Islamic period may have been consuming more C4 plants such as sorghum and millet which are known to have been consumed during this time. Aceves, (2019) points out that the consumption of C4 plants in Hospital da Misericordia may have been a little bit more constant than in Quinta da Boavista.

The human stable isotope values of the individuals from the early Christian period from Cacela-a-Velha suggest a diet that varies among individuals (Gonzàlez, 2019). Thus, as depicted in Figure 5.8, the humans from Cacela-a-Velha have wider ranges of both $\delta^{13}C$ and $\delta^{15}N$ values as compared to individuals from Loulé and Santa Maria da Graça. Human isotope values, as seen from Figure 5.8, from Cacela-a-Velha show a wide spectrum of dietary resources which overlaps with the Islamic period and Post-medieval period. This may perhaps suggest that there was overlap of dietary resources which were mainly present in the Islamic Period and those which were mainly present in the Christian Period.

It is also a possibility that part of the studied population from Cacela-a-Velha could have been converts or new Christians since the human samples studied dated to the first years of the conquest of the Algarve (Gonzàlez, 2019). Hence, the diversity of food resources, due to religious and cultural amalgamation, could be the explanation for why the values of the individuals from Cacela-a-Velha are scattered and overlap with samples from both Islamic and Post-medieval eras.

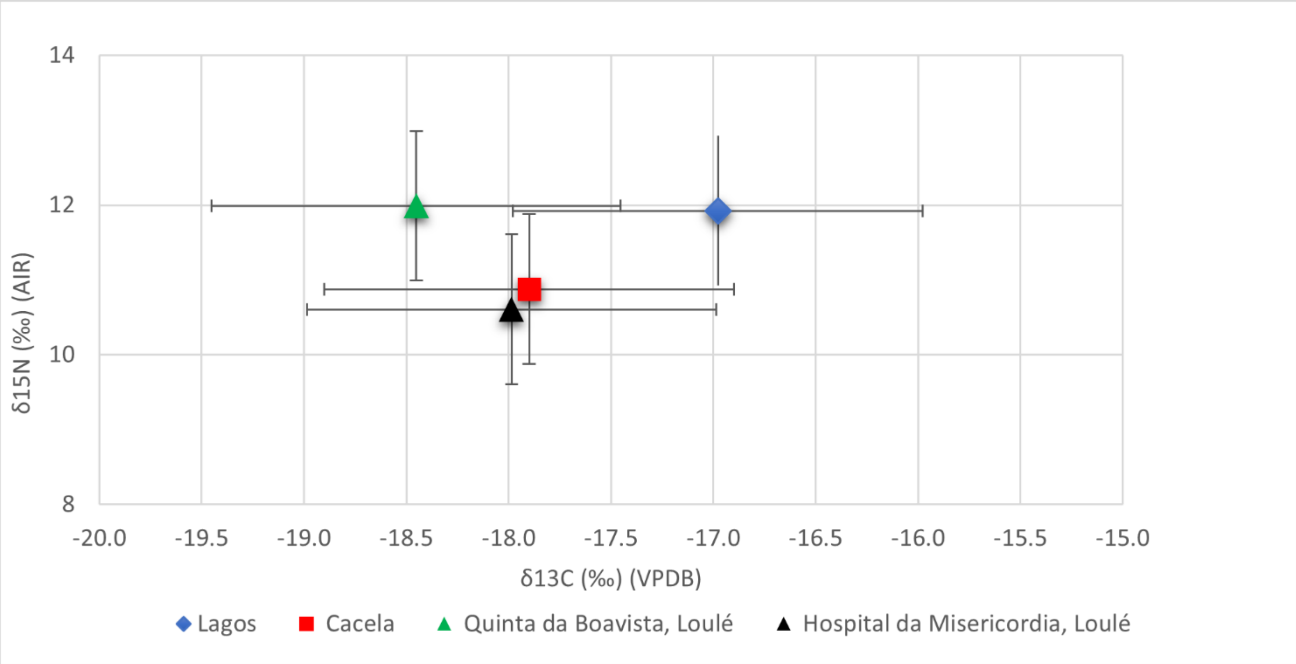

Figure 5.9 Comparative depiction of $\delta^{13}C$ and $\delta^{15}N$ mean values from Loulè, Cacela and Lagos

As seen from Figure 5.9, the mean $\delta^{13}C$ value of Lagos is less negative, around -17‰, as compared to Cacela whose $\delta^{13}C$ mean value is around -18‰. Individuals from Hospital da Misericordia, Loulé which have the $\delta^{13}C$ mean value of around -18‰ and the least $\delta^{13}C$ mean value is around -18.5‰ from Quinta da Boavista. A less negative $\delta^{13}C$ value from Lagos could be because the people of Lagos during the Post-medieval period could have had more C4 plants being introduced to their diets as a result of the Portuguese maritime expeditions around the world which began in the 16th century (Casimiro, 2020). As mentioned earlier in the section of dietary comparisons by phases, the maritime enterprises of the Portuguese kingdom of early 16th also brought in new exotic plants such as such as maize, potatoes and cocoa from the Americas; and pepper, cloves, cinnamon, from Asia. These new foodstuffs changed the way of life of production of tableware and dining habits (Casimiro, 2020).

Thus, having $\delta^{13}C$ and $\delta^{15}N$ values from Cacela overlapping the samples from both Loulé and Lagos may mean that there was continuous usage of some dietary resources from the Islamic period up to Post-medieval era in the Algarve region. This notion is also supported by the overlapping $\delta^{15}N$ values from all three sites, as illustrated in Figure 5.8, which could mean continuous consumption of similar protein sources from the Islamic age to the Post-medieval period, though during the post-medieval there was the introduction of new sources of meat, especially from birds like turkeys from Central America through Portuguese maritime enterprise.

However, it has been reported that the people of Loulè do not appear to have consumed fish as a major food source in their diets though Loulè is close to the Littoral sub-region in the Algarve region (Aceves, 2019). This trend has been argued to be mainly influenced more by cultural or socio-economical aspects rather than availability of fish (Aceves, 2019). But it is important to note that the mean $\delta^{15}N$ values from samples from Hospital da Misericordia depict a much more enriched ^{15}N based diet which probably means that those individuals may have consumed proteins from eggs and chickens in addition to domestic herbivore protein. If the individuals from Hospital da Misericordia would have consumed marine resources such as fish, their $\delta^{13}C$ would have been less negative yet that is not the case depicted in Figure 5.9.

Though from the data which is presented in Figures 5.8 and 5.9, it is difficult to pinpoint exactly which food components significantly influence the less negative $\delta^{13}C$ values, it is logical to assume that this may have been due to an increase in C4 plant intake especially during the later phase of post-medieval period. It may also be possible that the shift to the less negative $\delta^{13}C$ values indicated in the graphs depicted in Figures 5.8 and 5.9, of the samples from Santa Maria, was due to an increased intake of marine organisms which have longer food chains which result in higher $\delta^{15}N$ values, hence the increase in $\delta^{15}N$ mean values of the humans as compared to those from Loulè and Cacela.

Another reason may also be due to introduction of exotic animals for consumption like turkeys from Central America. There is an apparent trend in terms of dietary changes in time from the Islamic period up to Post-medieval times, but this trend may also be due to comparison of the rich with poor individuals across time and space. This may be true because here there is a comparison of different cultures and religions which define various aspects of life differently hence the trends depicted in Figures 5.8 and 5.9.

Important to note is the fact that it was impossible to analyse more samples from Lagos, as mentioned above, but with this preliminary data, it can be seen that there is a trend which points to an evolution of diet in the Algarve region. Nonetheless, the findings discussed here have potential bias due to the limited number of samples from Santa Maria but the data presented here offers the basis for further studies in understanding the dietary trends of the people of Lagos and Algarve region as a whole.

5.4 Dietary Comparison of Post-medieval Lagos with the Post-medieval Iberian Peninsula

The Iberian Peninsula saw drastic socio-economic and geopolitical changes from the 15th century onwards, and these changes influenced and transformed the way of life of the population from various regions of the Peninsula. This section deals with comparison and contextualising dietary practices of the city of Lagos with other regions of the Iberian Peninsula during the late medieval and Post-medieval eras.

Several datasets from sites, mainly from Spain were comparable studies in this discussion. The datasets are from Lopez-Costas and Müldner, (2018), Sarkic et al., (2018) and Mackinnon et al., (2018). The research by Sarkic et al., (2018) is on dietary reconstruction using $\delta^{13}C$ and $\delta^{15}N$ from bone collagen of Dominican nuns (16th-20th centuries) from the former Convent of Santa Catalina de Siena in Belmont, Cuenca, Central Spain.

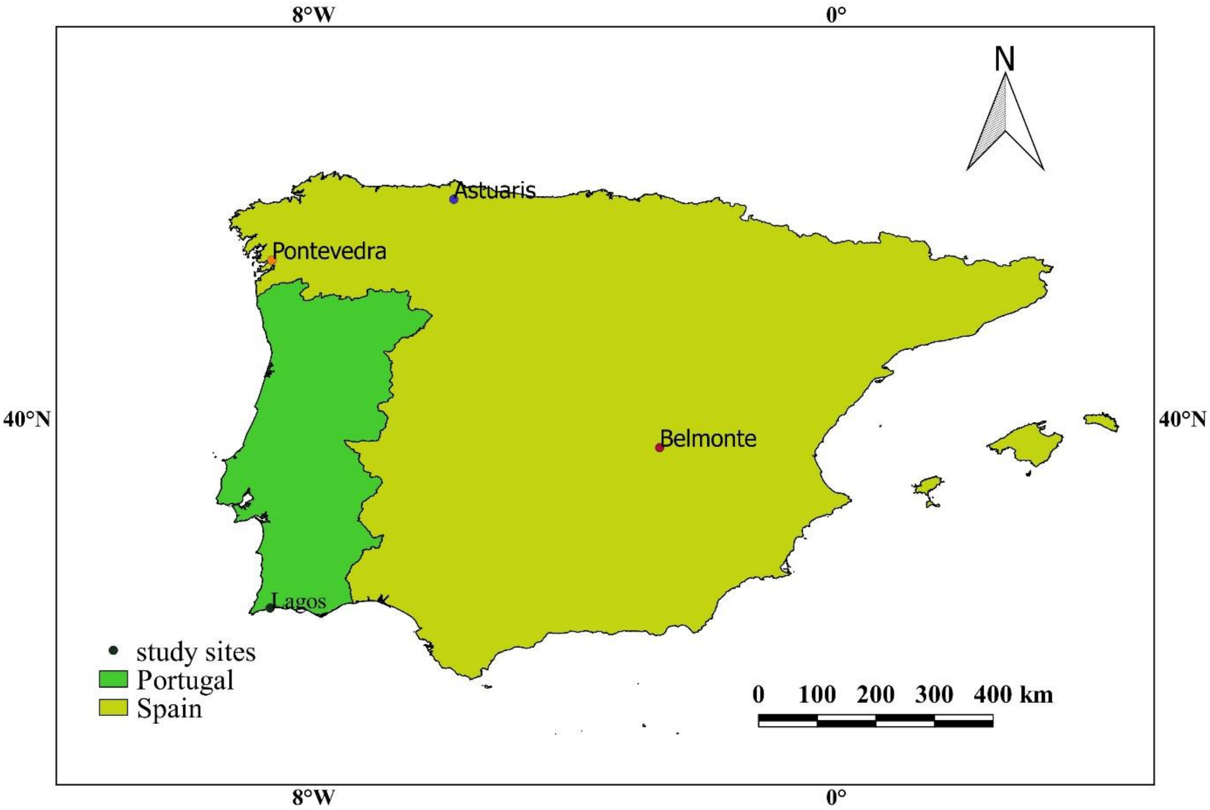

Figure 5.10: Map post-medieval sites in the Iberian Peninsula which were selected for dietary comparisons within this study. (Map generated by author using QGIS version 2.18. Las Palmas).

The research by Lopez-Costas and Muldner (2018), focused on dietary reconstruction using stable isotopes from bone collagen on human remains recovered from buried in the cemetery of Santa Maria (dating from the 13th to 17th centuries AD), and San Bartolomé (dating from the 13th to 15th centuries AD). MacKinnon et al., (2018) focused on exploring diet and status using stable isotopes from bone collagen in the Medieval and Modern periods of Asturias, Spain.

Table 5.3: Comparison of δ13C and δ15N mean values of human samples from Santa Catalina de Siena, Santa Maria, Pontevedra, Asturias and Santa Maria da Graça

Site Name	**Period**	**δ^{13}C Mean Value (‰)**	**δ^{15}N Mean Value (‰)**	**Reference**	**Number of Samples**
Santa Catalina de Siena (POB MON 1)	16th-17th century	-18.0 ± 0.4	11.4 ± 0.8	Sarkic et al., (2018)	47
Santa Catalina de Siena (POB MON 2) (Belmonte, Spain)	19th-20th century	-17.5 ± 1.3	11.9 ± 0.8	Sarkic et al., (2018)	9
Santa Catalina de Siena (POB CIV 2) (Belmonte, Spain)	19th-20th century	-18.1	12.3	Sarkic et al., (2018)	1
San Bartolomé (Pontevedra, Spain)	13th-15th century	-16.9 ± 1.0	11.7 ± 1.0	Lopez-Costas and Müldner, (2018)	16
Santa Maria (Pontevedra, Spain)	13th-17th century	-16.1 ± 2.0	12.9 ± 1.1	Lopez-Costas and Müldner, (2018)	47
Asturias, Spain	16th-19th century	-17.6 ± 2.2	9.9 ± 0.9	MacKinnon et al., (2018)	19
Santa Maria da Graça (Phase 1) (Lagos, Portugal)	14th-16th century	-17.4	11.7	This study	5
Santa Maria da Graça (Phase 2) (Lagos, Portugal)	16th -19th century	-16.5	12.2	This study	4

As seen from Table 5.3, the samples with highest δ^{13}C value are from Santa Maria, Pontevedra with the mean δ^{13}C value of -16.1 ‰. The lowest δ^{13}C value is from an individual from Santa Catalina de Siena (POB CIV 2) with the value of -18.1‰. Human samples from Asturias depict the lowest mean δ^{15}N values as depicted in Figure 5.10 as compared to the rest of the sites, but their mean δ^{13}C values are within same range as some other sites.

The differences between δ^{13}C and δ^{15}N values from humans buried in the cemeteries of San Bartolome and Santa Maria indicate that individuals from the cemetery of Santa Maria consumed more ^{15}N enriched foodstuffs than those from San Bartolomé (Lopez-Costas and

Müldner, 2018). This notion may be true if the mean $\delta^{13}C$ and $\delta^{15}N$ values are compared to the datasets from other sites which are depicted in Table 5.3 and Figure 5.10 which show that the individuals from Santa Maria have the highest mean $\delta^{13}C$ and $\delta^{15}N$ values. The $\delta^{13}C$ and $\delta^{15}N$ mean values from Santa Maria , who have been reported to have been fishermen (Lopez-Costas and Müldner, 2018), are almost similar to those from Santa Maria da Graça Phase 2 which also show high $\delta^{13}C$ and $\delta^{15}N$ values compared to the rest of the sites (Figure 5.10).

When focusing on populations buried in the cemeteries of Santa Maria and Santa Maria da Graça Phase 2, the most probable explanation of the trend depicted in Figure 5.10 is that there seem to be a greater consumption of C4 plants and more marine animals in higher trophic levels. Both Santa Maria and Santa Maria da Graça Phase 2 cemeteries correspond to the time when there was full introduction of new C4 crops in Iberia, from maritime enterprises which the kingdoms of Spain and Portugal were heavily involved in.

Though there is a great difference in timeframes of the two necropolises, the mean $\delta^{13}C$ and $\delta^{15}N$ mean values from Santa Maria da Graça Phase1 are close to the values from Santa Catalina de Siena (POB MON 2, 19^{th}-20^{th} cent.) (Figure 5.10). The probable explanation to this similarity is that there is a great difference between monastic dietary practices as compared to the secular population. Therefore, the nuns from Santa Catalina de Siena (POB MON 2) followed a strict and regulated diet which may have been mirrored in that of the individuals buried during the Phase 1 of the cemetery of Santa Maria da Graça. Probably the mean $\delta^{13}C$ and $\delta^{15}N$ values for the nuns, which although high, do not show a uniform diet as could be assumed, implying differences in proportions of food from animal origin (Sarkic et al., 2018). Thus, such variation in foodstuff may be the reason why there are some similarities in mean $\delta^{13}C$ and $\delta^{15}N$ values of the two cemeteries of Santa Maria da Graça Phase 1 and Santa Catalina de Siena (POB MON 2).

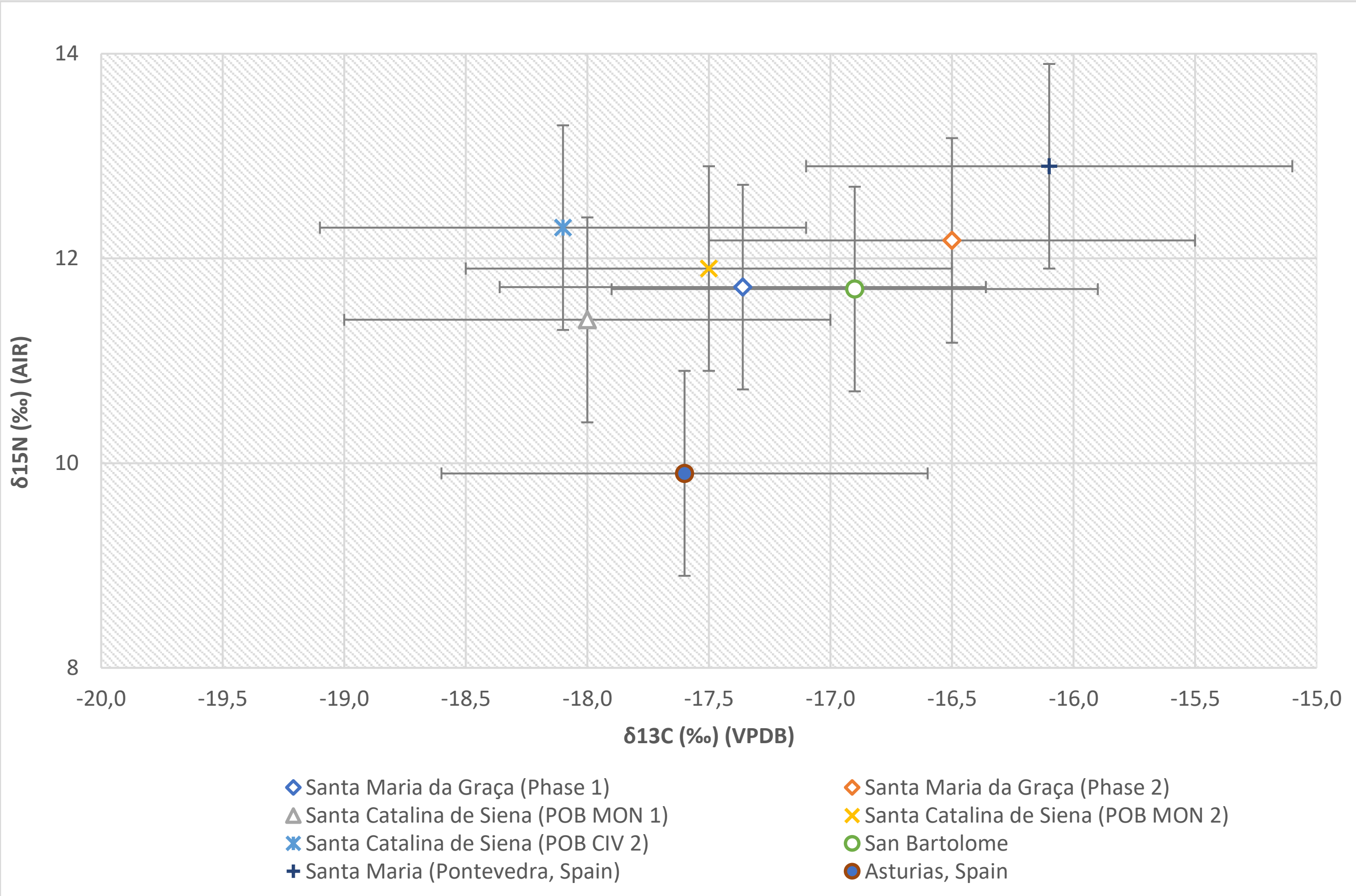

Figure 5.11: Scatterplot depicting δ13C and δ15N mean values from Santa Maria da Graça (Lagos, Portugal), Santa Catalina de Siena (Belmont, Spain), Santa Maria (Pontevedra, Spain) and Asturias (Spain)

An intra-population comparison of people from San Bartolomé and Santa Maria show that the main sources of dietary protein did not vary between males and females or different age groups. This indicates that families shared the same diet and possibly similar lifestyles (Lopez-Costas and Müldner, 2018). If this notion on dietary variability by gender, age and lifestyle, is applied to individuals buried in the necropolis of Santa Maria da Graça; it can be seen that there are some kind of similarities when compared to San Bartolomé and Santa Maria in Spain. It is possible that this kind of lifestyle is rooted in Christianity religion since all the sites mentioned are from the late Christian era.

CHAPTER 6: CONCLUSION

This research focused on using stable isotopes of carbon and nitrogen, extracted from human bone collagen, to investigate the dietary cultural habits of the people who resided in the area of Lagos, Portugal during the Post-medieval period from the 14th to 19th centuries. The individuals under study were buried in the necropolis of Santa Maria da Graça and twelve individuals were selected; and these individuals were carefully selected to represent the two phases which archaeologists categorised the cemetery chronologically. These points in time were named Phase 1 which was from late 14th to mid-16th centuries and Phase 2 which was mid-16th to 19th century when the cemetery was completely closed for any more burials.

As mentioned in earlier chapters, the coastal city of Lagos was among other major coastal cities of the Portuguese Kingdom that played a critical role in the era of Discoveries during the 16th century which saw a radical changes in Portugal and the European continent at large. Such changes include new food resources from other continents as well as new cultural norms which changed dietary habits of the Portuguese and the rest of the European continent. Because of the just mentioned reasons, this research came to be as a preliminary study of as to explore if the mentioned changes would have influenced the dietary intake of the population of Lagos during the 14th to the 19th centuries.

After the collagen extraction processes and EA-IRMS analysis, interesting results were acquired. Several aspects were considered during the interpretation of the results and they include overall dietary investigation of the population of Lagos. As mentioned in earlier chapters, the human samples selected for this research cover a long period which ranges from the late 14th century up until the late 19th century when the cemetery of Santa Maria da Graça was closed. Thus, there seem to be some changes in dietary intake for a period of about 500 years, that is counting from the time when the cemetery was first used until when it was shut down.

In terms of dietary practices, it is possible that the people of Lagos relied on several food resources from marine environments. This is a possibility since Lagos is located by the shores of the Atlantic Ocean, and that the city thrived on fishing and commercial activity (Pereira, 2012). High levels of $\delta^{15}N$ in samples LNSG 7, LNSG 29, LNSG 102, LNSG 117 and LNSG 134 could mean that the individuals may have relied on marine resources as food. However, the rest of the individuals studied depict the mentioned range of $\delta^{15}N$ between 9 to 12‰ which has been noted to be as a result of exclusively consuming meat of terrestrial herbivores (DeNiro and Epstein, 1981; Hedges and Reynard, 2007). Thus, it is highly possible that the people of Lagos relied on both marine and terrestrial animals as their sources of protein.

This research also investigated the variability of data to find out if the variation noted was due to time and/or gender. Dietary comparison by Phases provided some distinctions between Phase 1 and Phase 2 of the cemetery of Santa Maria da Graça. The second phase of the cemetery depicted less negative $\delta^{13}C$ values and this may have been due to full introduction of the maize and other potatoes and cocoa from the Americas; and pepper, cloves, cinnamon, from Asia. These plant resources entered Portugal and changed the way people consumed food as they became the staple of recipes and were used daily (Casimiro, 2020). This could also be associated with increased consumption of marine products.

The general separation between males and females resembled the 2 Phases which the necropolis of Santa Maria da Graça is divided into. The clustering of the datapoints relating to gender may have been due to different time frames which the individuals are from rather than cultural norms and values in terms of food consumption. Additionally, factors such as individual choices, food preferences and availability of certain food resources; could be the reasons behind such distinction between females and males. Christianity would have played a significant role in influencing dietary behaviours of the people due to Church's food preferences over others and through some practices such as fasting.

To understand dietary evolution in the Algarve region, datasets from previous studies: Islamic period (Loulé) by Aceves (2019) and early Christian period (Cacela-a-Velha) by Gonzalez (2019); were utilised and were compared to the data generated from this study. A trend was noticed upon comparing the $\delta^{13}C$ and $\delta^{15}N$ mean values from Loulé, Cacela and Lagos. This trend showed that the three phases, Islamic, Early Christian and Post-medieval periods, can easily be distinguished from each other.

The most significant point worthy mention is that the mean $\delta^{13}C$ and $\delta^{15}N$ values of Cacela may mean that there were the diverse food resources due to religious and cultural amalgam, hence an explanation to why the individuals from Cacela-a-Velha are scattered and overlap with samples from both Islamic and Post-medieval eras. There seem to be continuous usage of some dietary resources from the Islamic period up to Post-medieval era in the Algarve region.

Nevertheless, if future stable isotope analysis of the individuals from Santa Maria da Graça is done in a large batch, there will be clearer understanding concerning dietary distinctions and variability among the individuals in terms of phases and gender. If there will be inclusion of sulphur stable isotope analysis, a clearer picture, of dietary practices of the population buried in the cemetery of Santa Maria da Graça, would be attainable. There is a trend in terms of dietary changes in time from the Islamic period up to Post-medieval times, but this trend may also be due to comparison of the rich with poor individuals across time and space. It is also not clear if the data is that much valid due to limited number of samples from Santa Maria da Graça but the data presented here offers the basis for further studies in understanding the dietary trends of the people of Lagos and the Post-medieval societies in Portugal.

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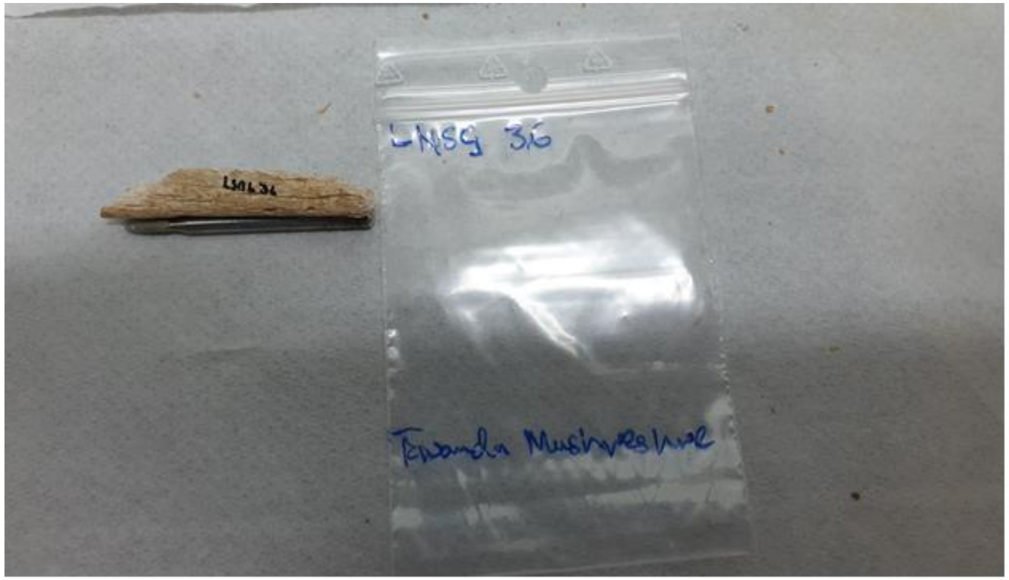
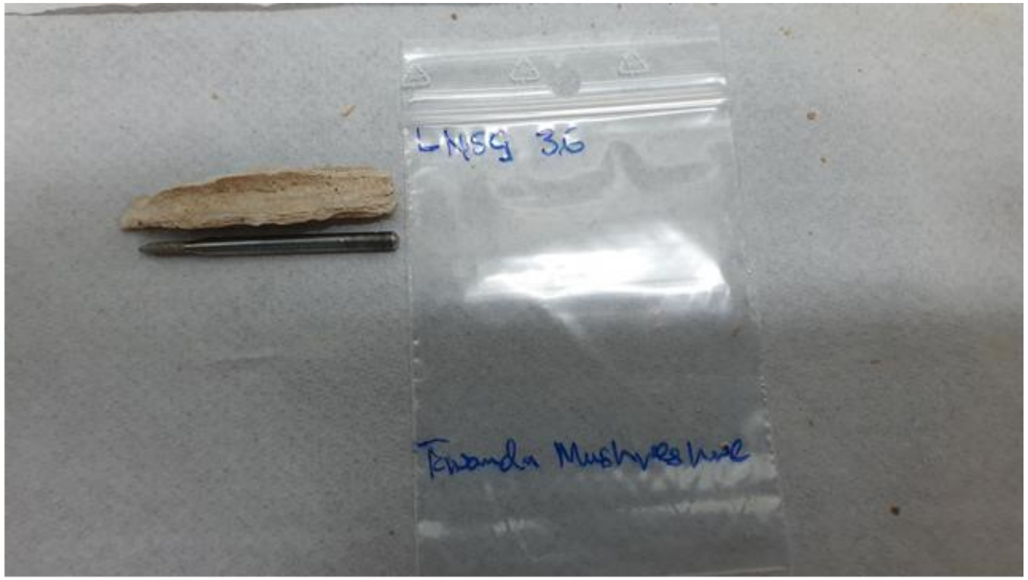
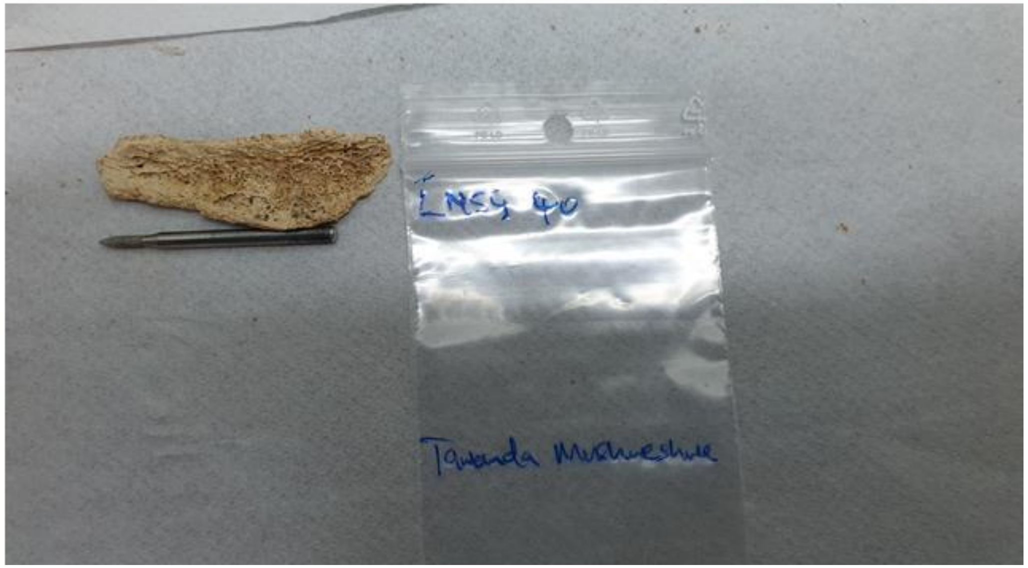
APPENDICES

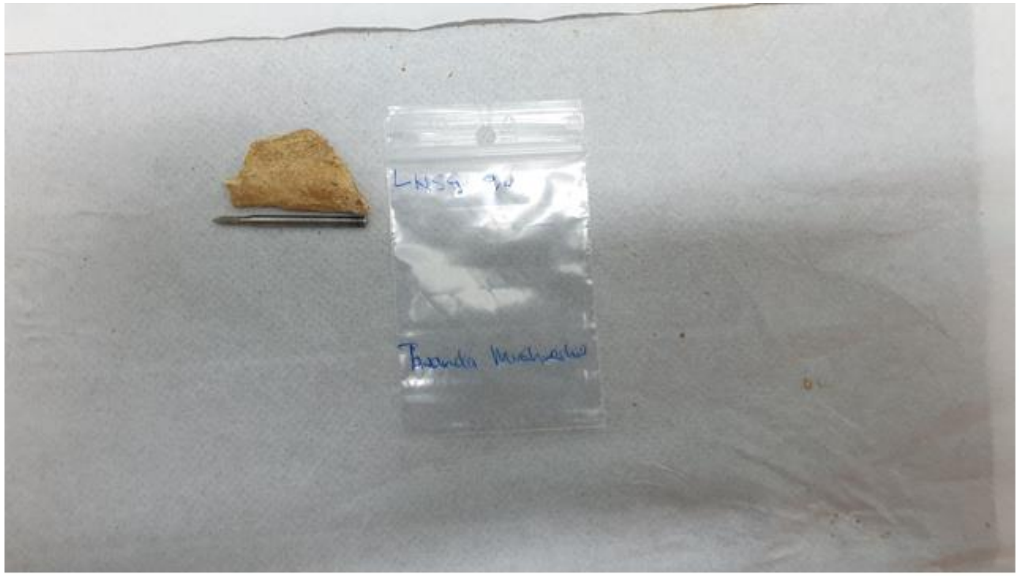
Appendices 1 Samples used in the stud

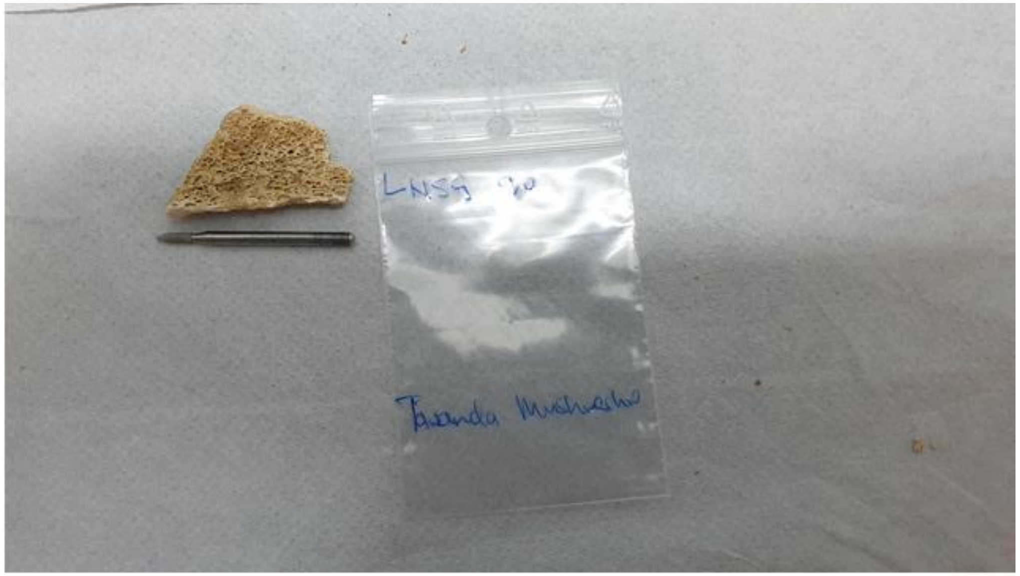

LNSG 36
Tawanda Mushweshwe

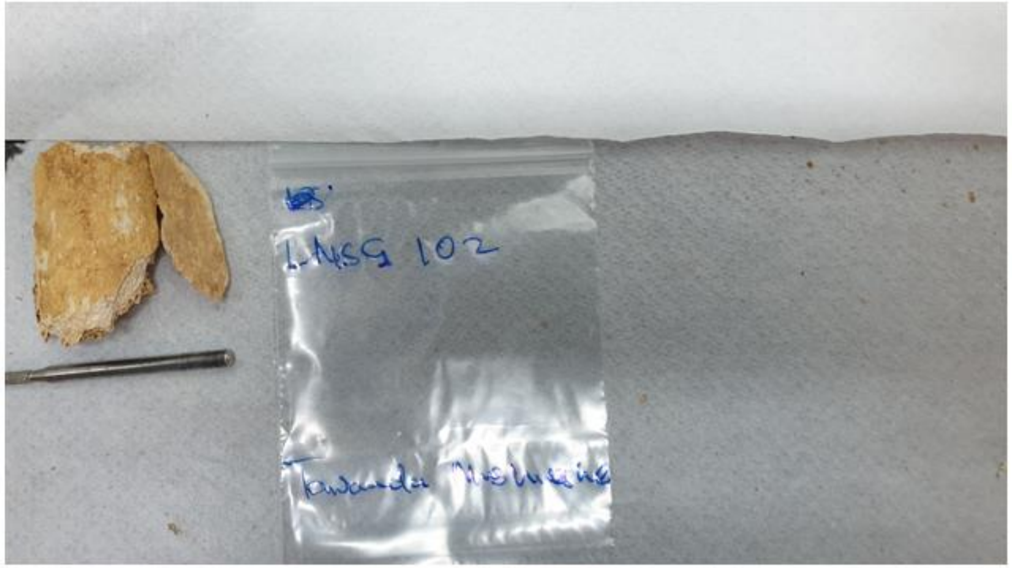

LNSG 36
Tawanda Mushweshwe

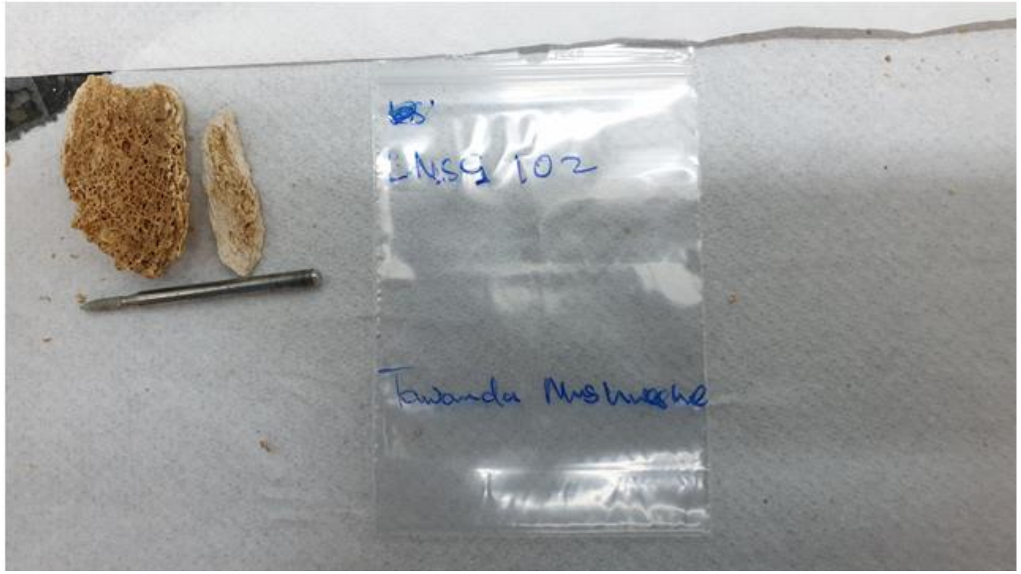

LNSG 40

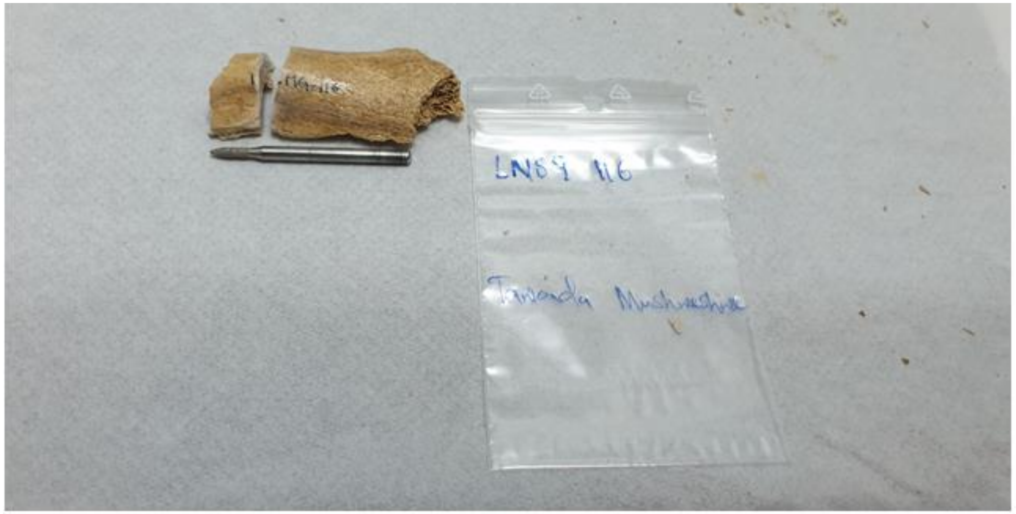
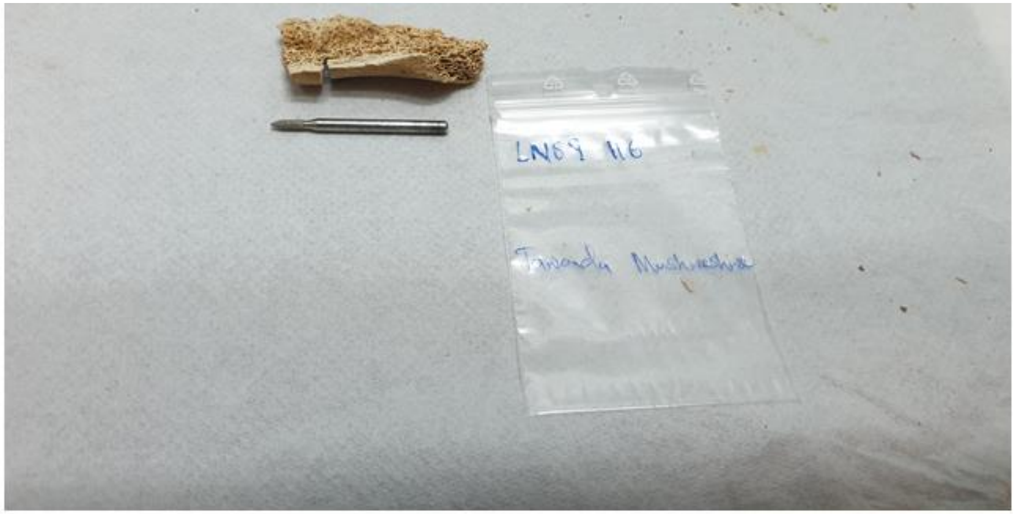
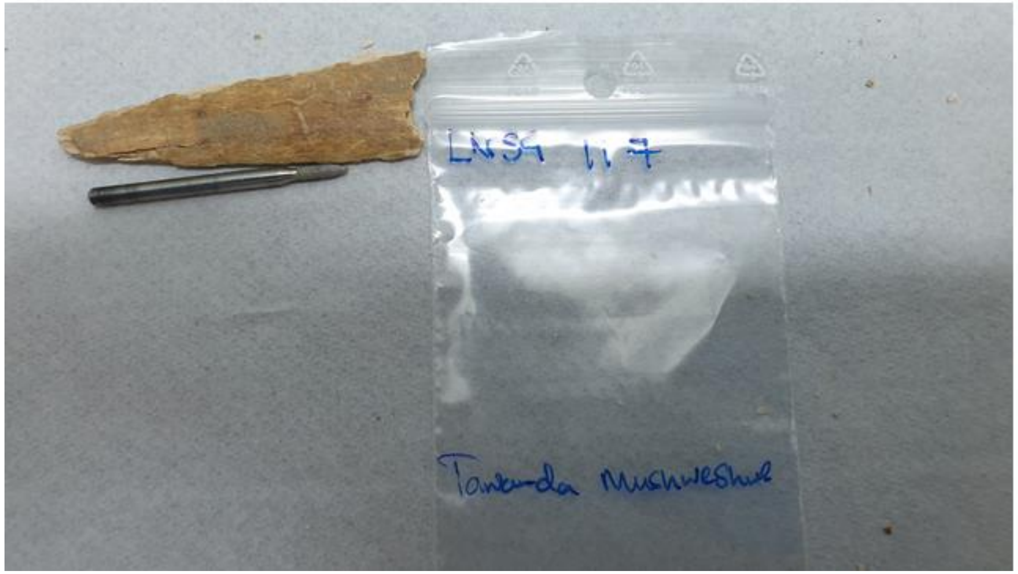
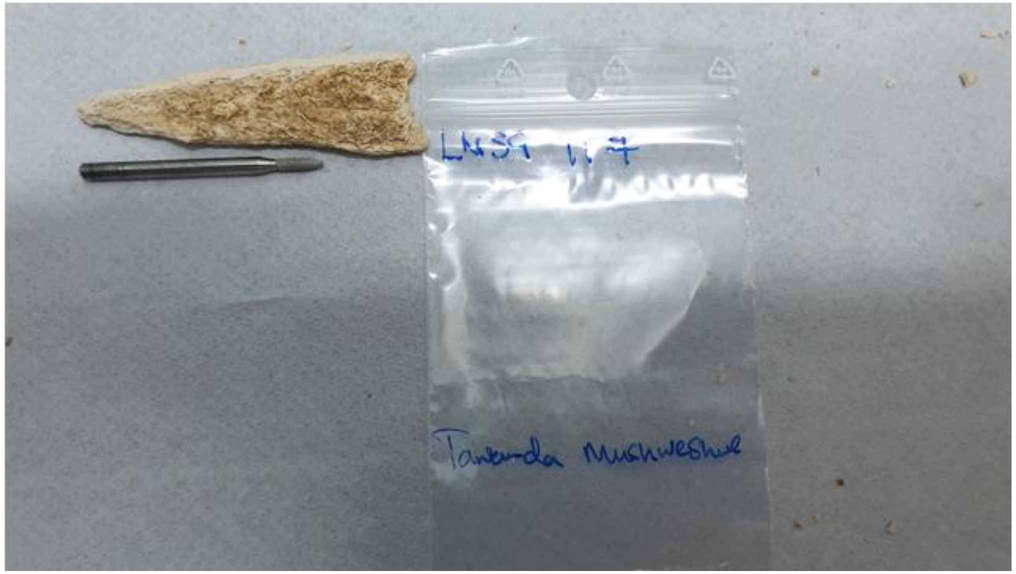

LNSG 40
Tawanda Mushweshwe

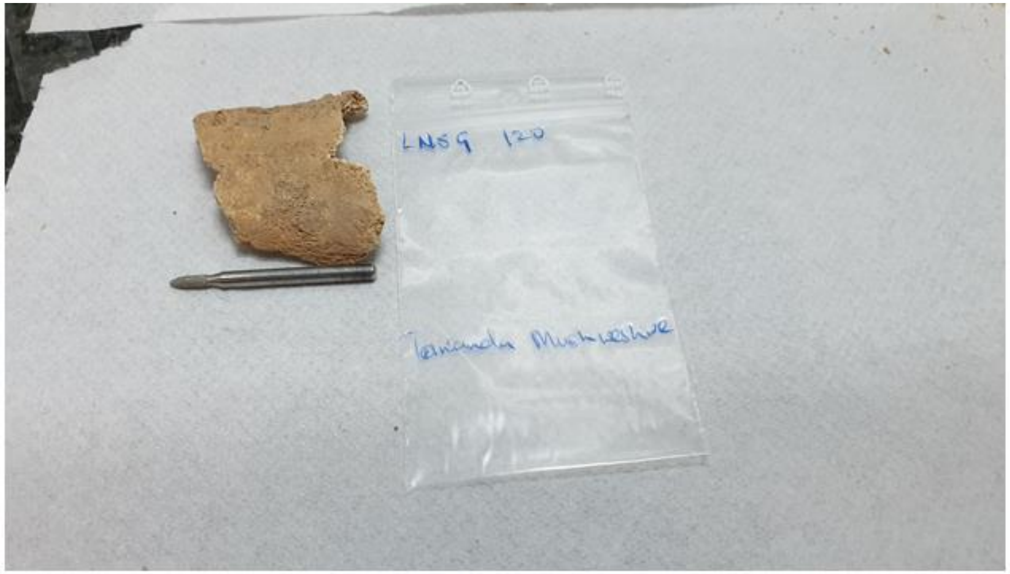


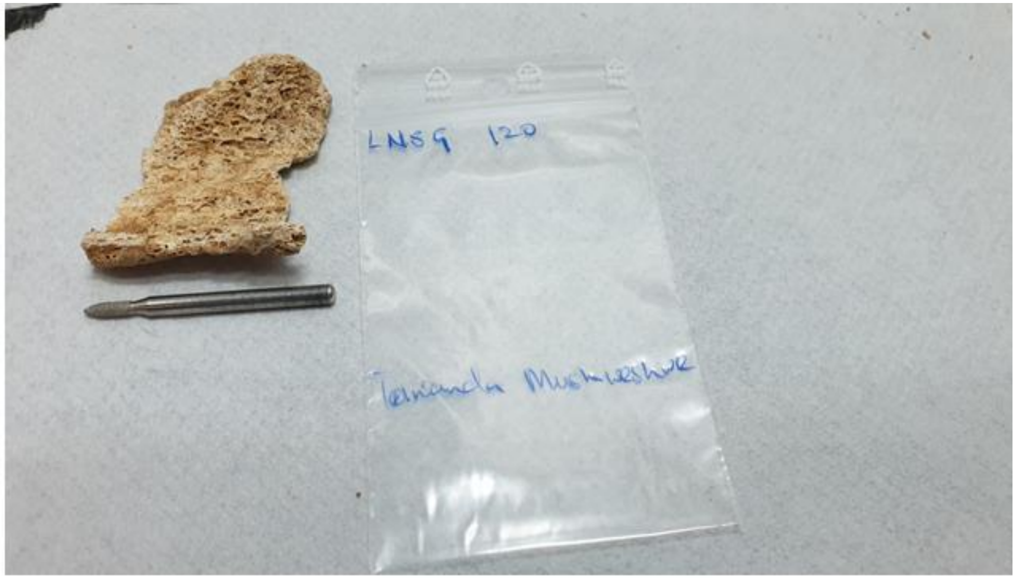



LNSG 102


LNSG 102


LN89 116
Tawanda Mushweshwe

LN89 116
Tawanda Mushweshwe

LN59 117
Tawanda Mushweshwe

LN59 117
Tawanda Mushweshwe

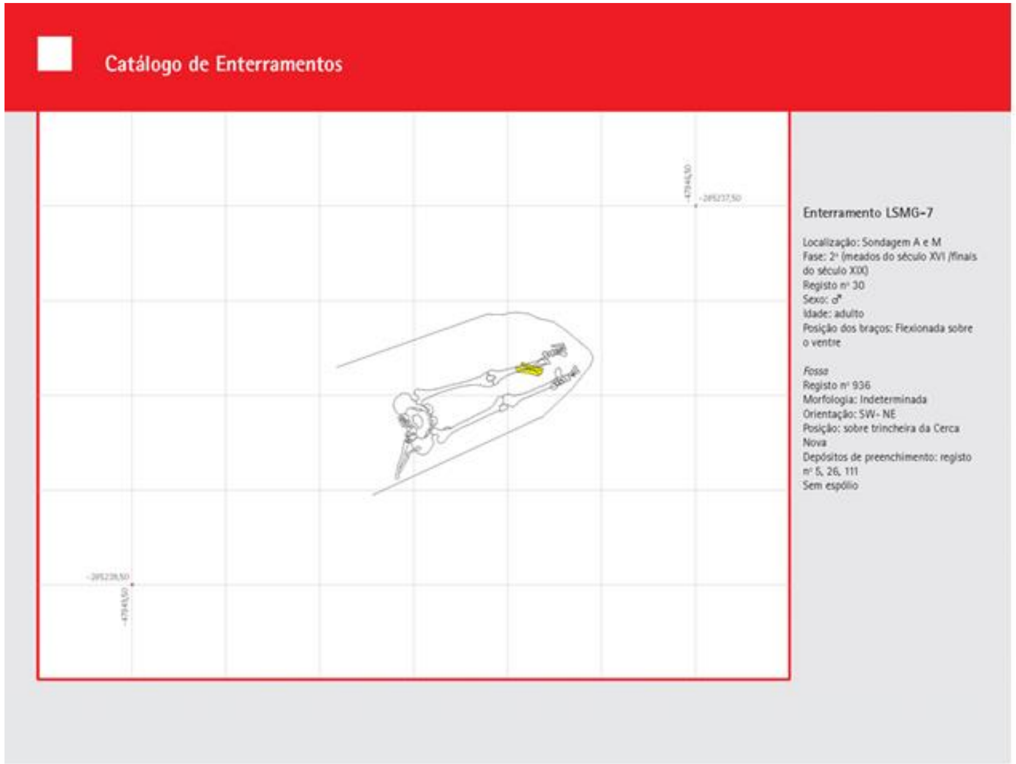

LNSG 120

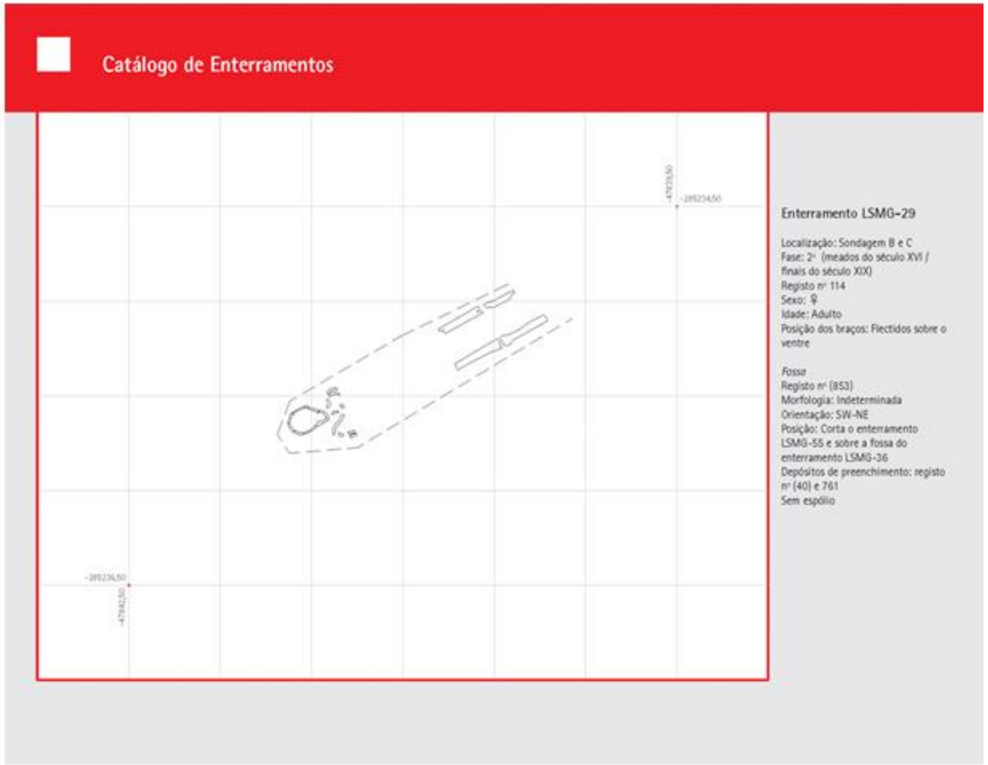

LNSG 120

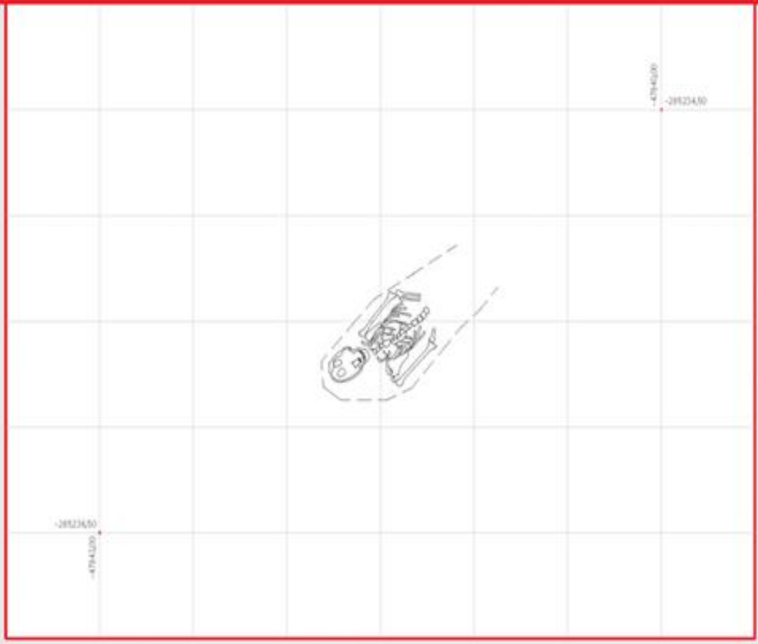

LNSG 124


LNSG 124

Appendices 2: Catalogue of burials from Diaz-Guardamino and Moran (2008).







Enterramento LSMG-36

Localização: Sondagem B
Fase: 2ª (meados do século XVI /finais do século XIX)
Registo nº 142
Sexo: ♂
Idade: Adulto
Posição dos braços: Flectidos sobre o ventre

Fosso
Registo nº 870
Morfologia: Indeterminada
Orientação: SW-NE
Posição: Parcialmente sobre o enterramento LSMG-47
Depósitos de preenchimento: registo nº 138 e 212
Sem espólio

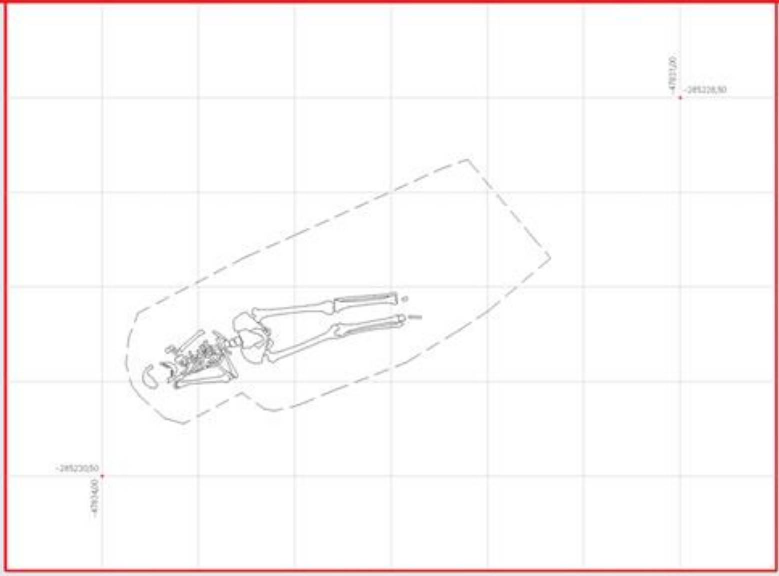
Catálogo de Enterramentos

Enterramento LSMG-40

Localização: Sondagem B
Fase: 2ª (meados do século XVI /finais do século XIX)
Registo nº 156
Sexo:♂
Idade: Adulto
Posição dos braços: Indeterminada

Fosso
Registo nº 872
Morfologia: Indeterminada
Orientação: SW-NE?
Posição: Sobre a trincheira de fundação da muralha moderna ?
Depósitos de preenchimento: registo nº 149
Sem espólio

Catálogo de Enterramentos

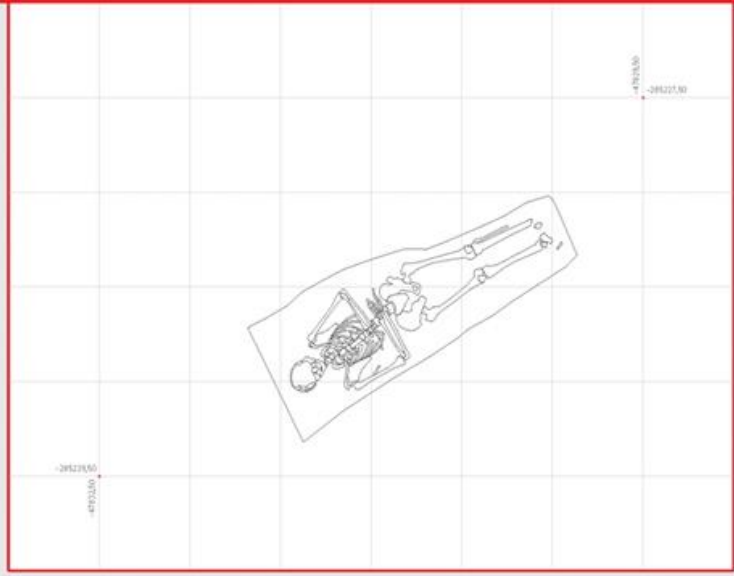


Enterramento LSMG-90

Localização: Sondagem C
Fase: 2ª (meados do século XVI /finais do século XIX)
Registo nº 427
Sexo: ♀
Idade: Adulto
Posição dos braços: Flectidos sobre o tórax

Fossa
Registo nº 919
Morfologia: Oval
Orientação: W-E
Posição: Sobre os depósitos da lixeira moderna contra estrutura moderna encostada à Cerca Nova.
Depósitos de preenchimento: registo nº 425
Sem espólio

Catálogo de Enterramentos

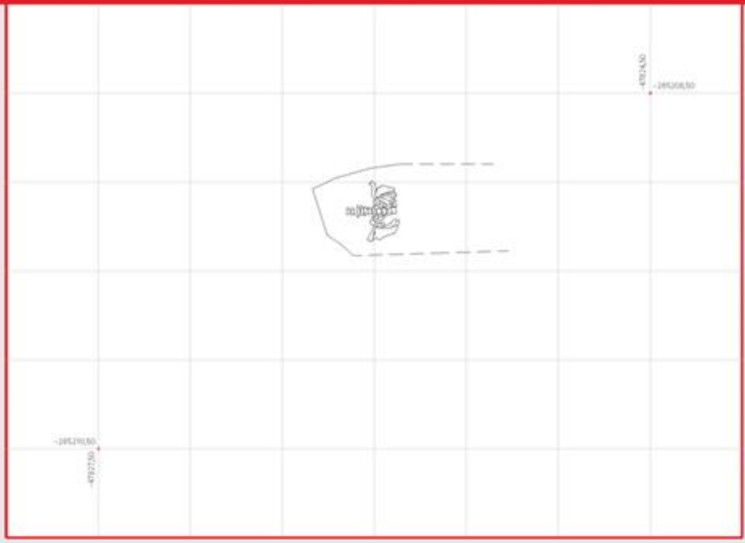


Enterramento LSMG-94

Localização: Sondagem C
Fase: 2ª (meados do século XVI /finais do século XIX)
Registo nº 451
Sexo: ♂
Idade: Adulto
Posição dos braços: Flectidos sobre o ventre

Fossa
Registo nº (926)
Morfologia: Rectangular?
Orientação: SW-NE
Posição: sobre os depósitos da lixeira moderna
Depósitos de preenchimento: registo nº (443)
Espólio: Pregos (do caixão)

Catálogo de Enterramentos

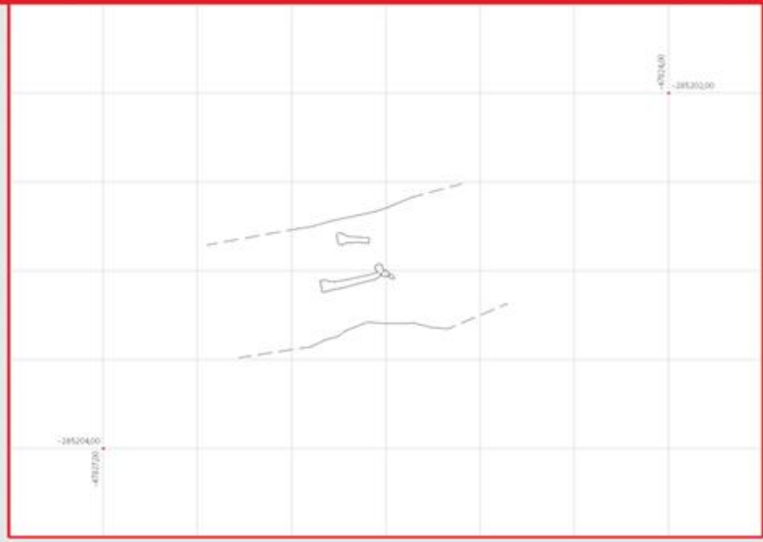


Enterramento LSMG-117

Localização: Sondagem MB
Fase: 1ª (séculos XIV /XVI)
Registo nº 662
Sexo: ♂
Idade: Adulto
Posição dos braços: Indeterminada

Fossa
Registo nº 982
Morfologia: Indeterminada
Orientação: W-E
Posição: Sobre a rocha
Depósitos de preenchimento: registo nº (638)
Espólio: a terra de enchimento é partilhada por três enterramentos, e nela foi recolhido um botão

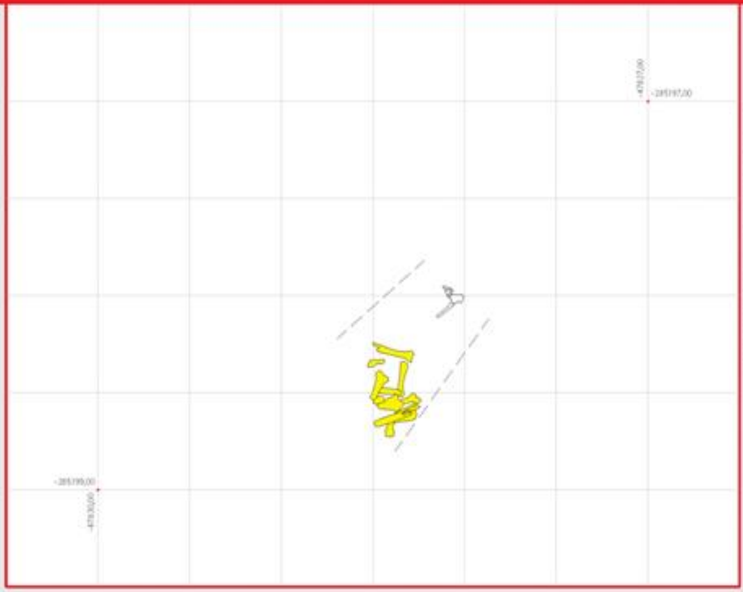
Catálogo de Enterramentos



Enterramento LSMG-120

Localização: Sondagem MB
Fase: 1ª (séculos XIV /XVI)
Registo nº 663
Sexo: ♀
Idade: Adulto
Posição dos braços: Indeterminada

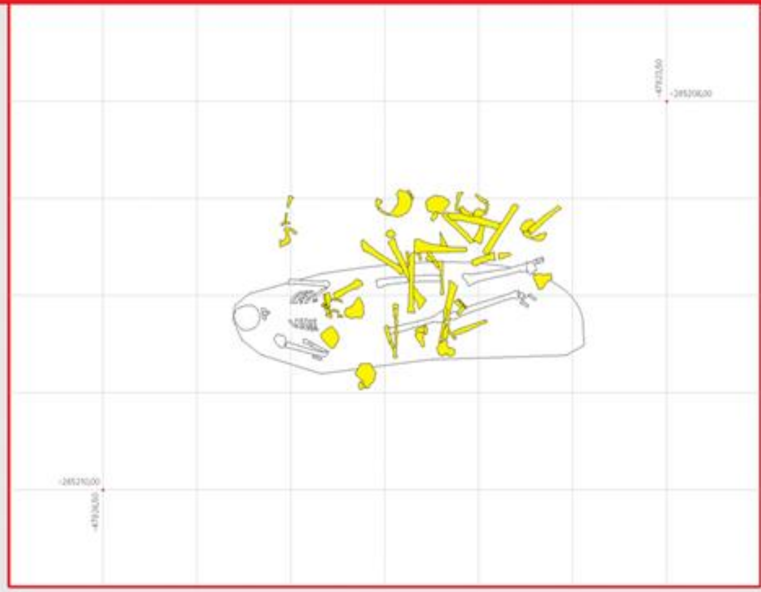
Fossa
Registo nº 987
Morfologia: Indeterminada
Orientação: W-E
Posição: Sobre a rocha
Depósitos de preenchimento: registo nº 690
Sem espólio



Enterramento LSMG-102

Localização: Sondagem MB
Fase: 1ª (séculos XIV /XVI)
Registo nº 521
Ossário: registo nº 757
Sexo: ♂
Idade: Adulto
Posição dos braços: Indeterminada

Fossa
Registo nº 979
Morfologia: Indeterminada
Orientação: W-E ?
Posição: Sobre a rocha
Depósitos de preenchimento: registo nº 520
Sem espólio

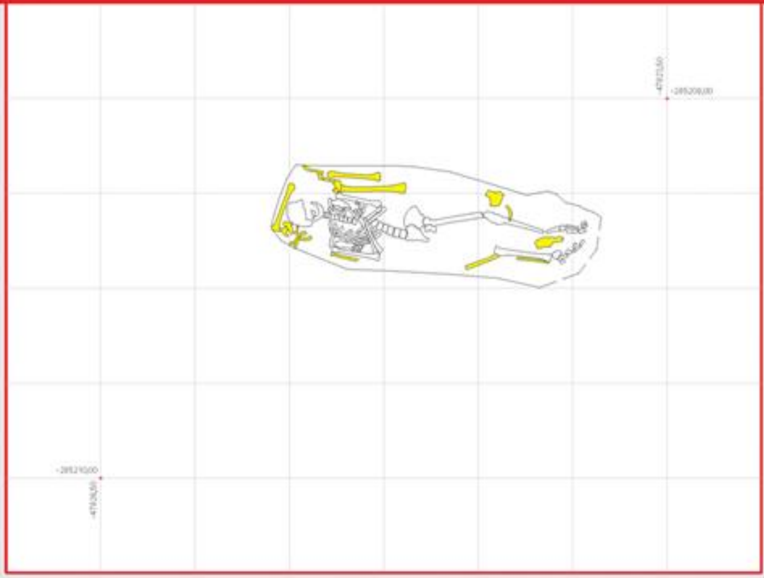


Enterramento LSMG-116

Localização: Sondagem MB
Fase: 1ª (séculos XIV /XVI)
Registo nº 644
Sexo: ♀
Idade: Adulto
Posição dos braços: Indeterminada

Fossa
Registo nº 980
Morfologia: Pseudorectangular
Orientação: W-E
Posição: Corta as fossas dos enterramentos LSMG 115 e LSMG 117. Sobrepõe, parcialmente à fossa do enterramento LSMG-124
Depósitos de preenchimento: registo nº (638)
Espólio: a terra de enchimento é partilhada por três enterramentos, e nela foi recolhido um botão

Catálogo de Enterramentos

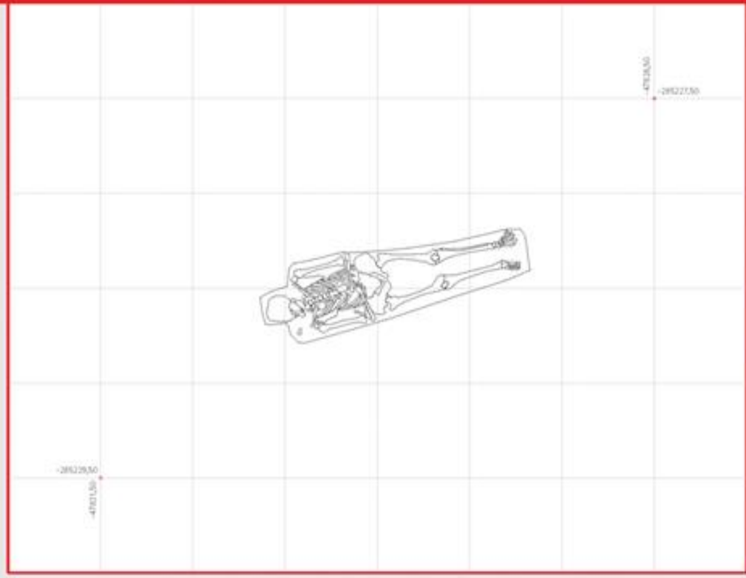


Enterramento LSMG-124

Localização: Sondagem MB
Fase: 1ª (séculos XIV /XVI)
Registo nº 692
Sexo: ♀
Idade: Adulto
Posição dos braços: Cruzados sobre o tórax

Fossa
Registo nº 983
Morfologia: Pseudorectangular
Orientação: W-E
Posição: Corta parcialmente o enterramento LSMG-125 e sobrepõe o enterramento LSMG-125 e a rocha
Depósitos de preenchimento: registo nº 691
Sem espólio

Catálogo de Enterramentos



Enterramento LSMG-134

Localização: Sondagem C
Fase: 1ª (séculos XIV /XVI)
Registo nº 732
Sexo: ♂
Idade: Adulto
Posição dos braços: Flectidos sobre o ventre

Fossa
Registo nº 930
Morfologia: Antropomórfica
Orientação: W-E
Posição: Sobre a rocha
Depósitos de preenchimento: registo nº 731
Sem espólio