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Reconstructing the Paleodiet of the Punic inhabitants at the site of Kerkouane (Tunisia)

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This Thesis document presented as a requirement to obtain the Academic Title of Erasmus Mundus Master in Archaeological Material Science. (ARCHMAT)

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Abstract

The proposed project intends to investigate the dietary system of the Punic population at Kerkouane, Tunisia. The cutting-edge approach of stable carbon and nitrogen isotopes analysis of human bones will allow us to assess the subsistence pattern of the past population. Subjected samples are obtained from the burials found at the necropolis of Arg El-Ghazouani; as the site is situated in the Northeast, the coastal rim of Tunisia will tend to show the consumption of mosaic of food resources of marine and terrestrial origin. It will be crucial to understand the dietary preferences of local habitats among these resources. Combining proxy data ($\delta^{13}C$ and $\delta^{15}N$) will throw light on the answers to the abovementioned questions. The $\delta^{13}C$ and $\delta^{15}N$ stable isotopes reported in this study for humans remain (=25) from the necropolis of Arg El-Ghazouani. The results suggest that the diet of the individuals of Arg El-Ghazouani was based on C3 terrestrial plants, with no significant indication of C4 plants consumption. In addition, to a variety of domestic animals like sheep, goats, and cattle. Although the $\delta^{15}N$ indicates a higher variability among the N values, it is still impossible to display a high consumption. It could be a contribution of marine resources in the individual's Arg El-Ghazouani. However, there were no significant diet differences among Arg El-Ghazouani concerning age, sex, and funerary features. The isotopic data of Kerkouane compared to the ancient roman site of Leptiminus, located on the Mediterranean coast of Lamta town, Tunisia. In this thesis study, there was a significant dietary difference observed in the inhabitants of Tunisia during the past time.

Keywords

Kerkouane, Punic, sepulchral, stable isotopes, carbon, nitrogen, paleodiet

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CHAPTER ONE

1 Introduction

1.1 background

Many historians' resources have pointed out the importance of the North African coast as an essential link between the eastern and western Mediterranean. Dexter Hoyos mentions that the Phoenicians, who were calling themselves the Canaans (Kn'nm) who had been established in the east part of the Mediterranean, Tyre, Byblos, and Sidon present-day Libanon; before they started to move toward the western part of the Mediterranean and begin to establish their new settlements, especially in North Africa what are known today (Libya, Tunisia, and Algeria). The critical settlement they established in north Africa is Carthage which became the capital of the Phoenicians- punic civilization in the Mediterranean region, among with other settlements in Tunisia such as Utica and kerkoune, the provenance of Tripolitania Libya such as Liptes Magna and Sebratha, and other crosses North Africa (Dexter Hoyos, 2010).

The Punic, on the other hand, are not those Phoenicians who left Tyre, Byblos, or Sidon to create cities like Utica and Liptes Magna on the North African coast and settled there, but those who were born out of that civilized encounter in its complete and expansive sense. In the western Mediterranean, the Punic civilization differs from the Phoenician culture. Nonetheless, it is a civilization that arose through the interaction of the Eastern Phoenician civilization with cultures discovered by the Phoenicians in locations overlooking the western Mediterranean's waters. Punic civilization arose in the west Mediterranean as a result of contact between Eastern Phoenician and African societies. (Camps, 1979; Courtois, 1950; Mh, Fantar,1972).

This thesis study will focus on one of the essential Punic cities in North Africa: Kerkouane. Essentially one of the city's four necropolises, two of which have been identified necropolises located close to the city, north, and the south. The other two necropolises were situated to the North and the west of the city. The majority of these graveyards were burial chambers with a staircase and interment in jars in simple pits, and they were constructed in the sandstone hills (Mh, Fantar 1972). The samples used in this study came from the necropolis known by the name Arg El-Ghazouani.

The excavations, and the discoveries spotted in kerkouane since its foundation in 1952 (Mh, Fntar, 2005), reveal a lot about the history, economic and social status of the city's inhabitants. However, the ancient diet is crucial to understanding the nutritious habits of the antique communities; therefore, the stable isotopes of both ancient human and animal remains can provide paleodietary reconstruction and information on the ancient diet. However, this thesis project emphasizes understanding the dietary habits and variations concerning sex, age, and funeral features among the individuals of kerkouane.

The human skeletal samples of this thesis came from the necropolis of Arg El-Ghazouani, city of kerkouane (Tunisia), and stored in the Biology of Ancient Population laboratory at the Department of Environmental Biology of the Sapienza University of Rome.

1.2 Problem statement

Current hypotheses talk about the presence of malty ethnic groups showing wider cultural diversity. The diver’s cultural system must have left a significant impact on dietary systems. It is also crucial to understand the proportion between a marines-based diet and a terrestrial diet. Lastly, dietary variability among the individual might infer individuals’ economic status.

1.3 Research objective

- To access the stable isotopic values of human skeletons remains of the necropolis of Arg el-Ghazouani.
- To understand the diet variability among the individuals
- To understand the diet variation concerning sex, age, tomb types, and funeral features.
- Establish an isotopic database for the punic period in Tunisia for future studies.

1.4 Limitation

The analytical technique is destructive. In addition, due to the pandemic situation, we couldn't reach the site for collecting animal bones or archaeobotanical remains to build the local isotopic signals. However, due to the lack of isotopic data about this site or any site related to the same period of the Punic city Kerkouane, the current work will incorporate previously published stable isotopic data (Keenleyside et al., 2009) to establish a local and extra-local context.

1.5 Significant

This study is highly significant as it will be the first attempt to analyse the dietary pattern of Punic habitants of Kerkouane. The study will also try to fill some part of the large void that exists due to the scarcity of paleodietary studies in the current region. Successful results would provide valuable data by providing insights into the local subsistence pattern at Kerkouane.

1.6 Methodology

I will aim to study archaeological bone assemblages to answer some of the crucial questions like paleo diet through isotope geochemistry. Twenty-five individuals will be selected from the skeletal materials of the necropolis Arg el-Ghazouani. Collected material will be further treated and prepared for collagen extraction. Compact bones will be preferred for collagen extraction. The weight of extracted collagen sample should be between 1 to 2 gm.

During this Work, all relevant protocols will be followed (Ambrose, 1990). Isotopic values of extracted collagen samples will be determined using EA-IRMS. Multiple statistical tools will be used to analyze the generated data for correlation and variability.

1.7 Overview of chapter

This thesis will include sex chapters starting with the introduction, background of the study. The second chapter will be the literature view where I will be talking about the phonics-Punic in north Africa and the site of Kerkouane, also the Phonics and Punic Funereal features in the Mediterranean area. In addition, I will be talking about dietary reconstruction using stable isotopes. The third chapter will be about the materials and the methodology. The fourth will be the study's results. The fifth is the discussion, the conclusion in the six-chapter, followed by all the references I used for this thesis.

CHAPTER TWO

2. literature review

2.1 Phoenicians and Punic in north Africa: An overview

The most North African area frequented by Phoenicians and conquered by the Punics includes the territory that extends from the western Libyan coasts to the fertile plains of eastern coastal Tunisia, behind the Atlas Mountains. Overall, the area is distinguished by a denser population as you go up towards Cape Bon. Starting from the southern collar, two settlements are included in the current Libyan territory: Leptis Magna and Sabratha. According to Sallust, the former was founded by the Sidons (*Bell. lug,.78,1*) while Silius Italicus (Pun., 3,265) and Plinius hold it derived from Tyre; the archaeological materials, in turn, dates the Phoenician phase of the site back to at least the seventh century (Carter 1965; De Miro - Polito 2005). The strong Levantine imprint of the place was maintained in more recent times. In Roman times, the polyads of Leptis Magna were two divinities of clear oriental origin: Milkashtart and Shadrafa (Di Vita 1968). Sabratha, on the other hand, dates to the 6th century BC (Di Vita 1969). With the coming of the full Punic age, the testimonies of *Leptis Magna* and *Sabratha* are joined, among others, by those of the current *Lamta* (formerly *Leptis Minor*; Ben Lazreg- Mattingly, 1992) and Tripoli: of the latter, Silius Italicus recalls the composition of the population, consisting of Sicilian elements and elements of African origin (III,257).

Two locations along the northern coast belong to the *Bizacene* region: *Acholla* (now *El-Alia*) and *Hadrumetum* (Sousse). Classical texts said the Phoenicians founded both (for *Acholla*: Stephan of Byzantium, Ethica, ed. Meineke, p.152,19-20; for *Hadrumetum*: Solinus, XXVII,9-1 0), which, in the case of the second center, is also suggested by the great Tophet structure that dates to the sixth century (Cintas 1947; Foucher 1964). Subsequently, the *Bizacene* was involved in the Carthaginian intervention in the settlements of Thapsus (Fantar 1978), *Monastir* (ancient Ruspina), and *Mahdia* (Ben Younes 1985).[1]

The fortresses and Necropolis of Cape Bon, the large peninsula of Cape Bon, marks the closest point of Africa to Sicily (for approximately 140 km) and therefore appears to be one of the most critical areas from a strategic point of view. While data regarding the Phoenician age remains relatively scarce, the Punic expansion, on the contrary, shows to be intensive and characterized both by the solid military commitment and by the deep-rooted exploitation of local resources.

First, Italian-Tunisian collaborations launched in the 1970s made it possible to detect the presence, after around 500, of a capillary network of fortresses (Acqyaro et al. ii 1973; Barreca-Fantar 1983). On the eastern side, this is evidenced by the remains of two important buildings: *Kelibia* and, further North, *Ras ed-Drek* (Fig. 2.1). In *Kelibia*, what remains of a defensive structure has been recognized as the basis of a Hispanic-Turkish installation. In the case of *Ras ed-Drekè*, on the other hand, there is a fortress consisting of two buildings, the largest of which must have included five cisterns. Between the two settlements, there is the crucial center of Kerkouane, built in the sixth century and equipped with robust wall structures (Fantar 1984-1986). Furthermore, as mentioned, Carthage's interest in this region also extended to the natural riches of the territory at the tip of the Cape; the sandstone quarries of *El-Haouaria* were used to construct Kerkouane and some buildings of the larger colony.[2]

[1] Sandro Filippo Bondi, *Fenicie Cartaginesi, Una civiltà mediterranea.* (Rome 2009). Pag-119-120.
[2] Sandro Filippo Bondi, Fenicie Cartaginesi, Una civiltà mediterranea. (Rome 2009). Pag-120.

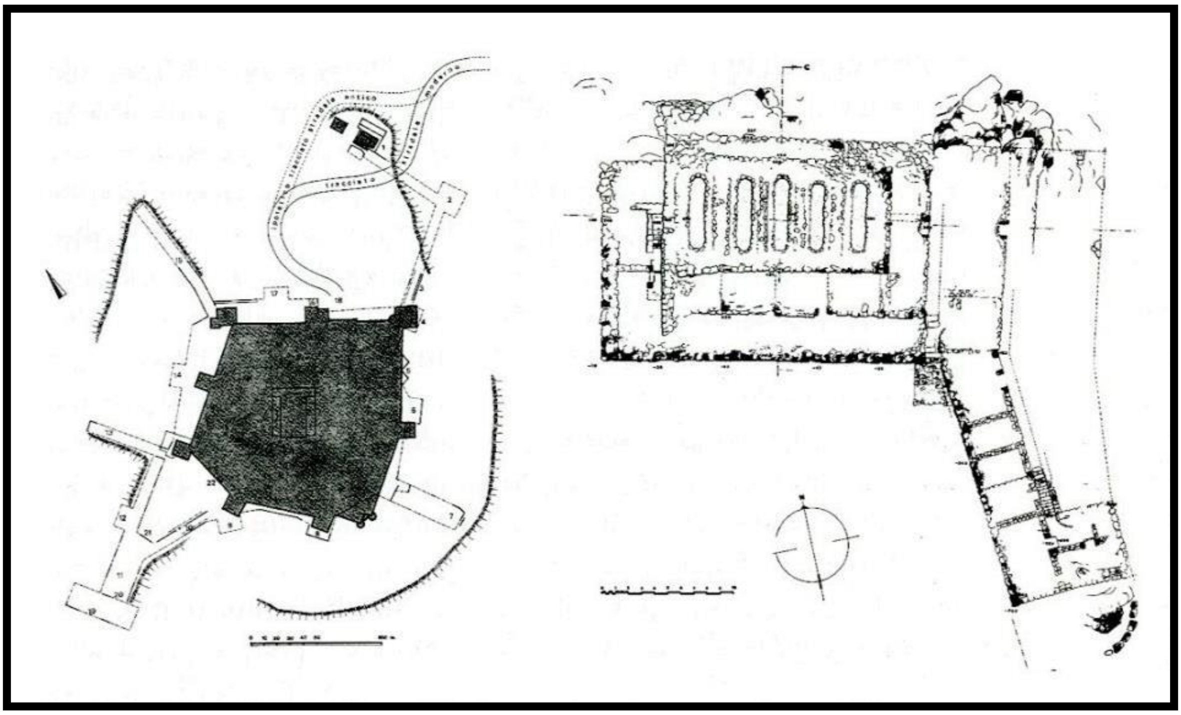


Fig 2.1. Left: *Kelibia*. Plan of the Panico-Turkish fortress and the rest of the Punic defense structures. Right: *Rãs ed-Drek.* Pinta of the defensive Punic building (*Bondì 2009*).

The same region provided significant evidence of funerary areas from the Punic age (Fantar 2002), located in the sector of the east coast, which extends from *Ras ed-Drek* to *Tazarka*. In addition, to the tombs of Kerkouane, some Necropolises are dated between the 4th and 2nd centuries BC (MH. Fantar,1984), mainly consisting of the "pit" and "chamber" typologies with access dromos, are worthy of note: *El Mansoura* in *Kelibia*, *Sidi Jamel Eddine*, *Sidi Salem Heman* in *Menzal Témime* (Ancient *Tafekhsit*) and *Kesar er-Saad* in Korba.

2.2 Kerkouane, The site

The city located on the eastern side of the same Cape was founded in the sixth century BC (MH. Fantar,1984) and is currently one of the most known Punic settlements (fig. 2.2), thanks to the research launched in 1953 to ensure its recovery. The absence of modern structures made it possible to investigate the city facilities as they must have looked when abandoned, namely in the third century BC. [3]

Fig 2.2 Map: Geographical location of the archaeological City of Kerkouane. (Found spot marks the locations) (modified from Natgeo).

The city, overall, seems the result of a rather particular planimetric, even if not always systematic (Fig 2.3; Fantar 1984-1986) project. Following an insulae pattern, the public and private environments line up on wide streets (which can reach almost 5 meters in width). However, blocks do not constantly follow the road layout. In some sections, the latter is divided into large trunks of different sizes; moreover, within regular insulae, delimited by streets that meet at right angles, neighborhoods alternate whose arrangement borders on chance. Three squares occupy the north-

[3] Mh. H. Fantar, *Kerkouane, cite punique du Cap Bone (Tunisia), Institute National d Archeologie et d Art, Tunis* 1984. P13.

eastern arch; their concentration in a relatively limited area interspersed only by an insulae gives the impression of a sector dedicated to the main social, economic, and administrative activities.[4]

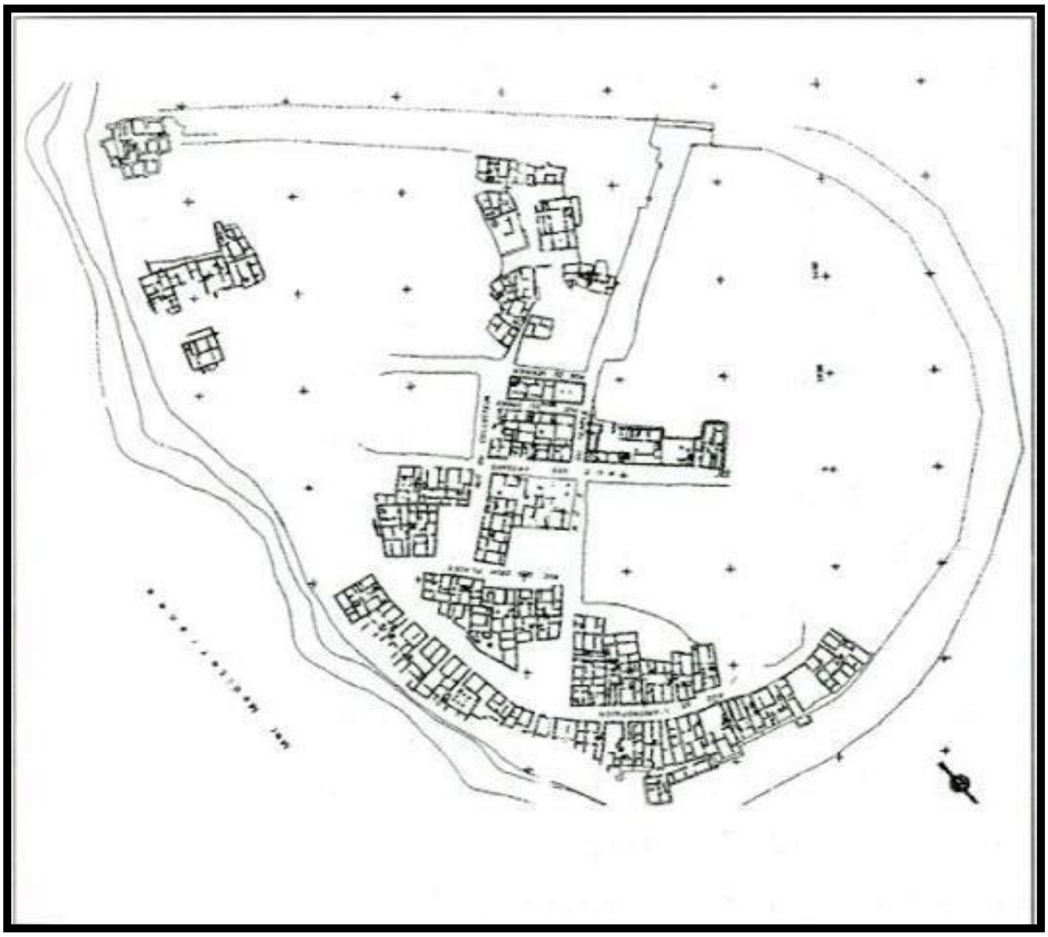


Fig 2.3. Kerkouane city map. (*Bondì 2009*).

The first structure encountered on the northern side are the walls. They are divided into two curtains that surround the settlement to the West, North to South. The eastern side facing the sea opens directly onto the rocky coast, where traces remain an avenue that must have followed the same coastline.

The oldest curtain is the inner one, which was probably built when the city was founded but destroyed, according to M.H. Fantar (1984), following the siege of *Agathocles* in 310 BC. The used technique, called "herringbone," is due to the Phoenician contribution and ensures the anteriority of this sector

4 Sandro Filippo Bondi, Fenicie Cartaginesi, Una civiltà mediterranea. (Rome 2009). Pag-121.

compared to the external one (Fig. 2.4); the same phase would include a bastion with a curvilinear profile located in the southern section. The second curtain, partly built-in *opus africanum*, was erected perhaps at the dawn of the first conflict with Rome about ten meters from the other, to the detriment of older artisanal districts. The internal walls were restored during this phase and partly modified by constructing the North Tower and transforming the southern sector, now equipped with towers, ramparts, and an access door. Also noteworthy is the construction technique of another door, the western one; it does not cross the walls but is staggered and parallel to the profile of the enclosure.

Fig 2.4. On the lift: detail of the internal count built with "herringbone" technique. On the right: the south gate and walls with patrol walkway (modified after *Bondì 2009*).

The houses, in turn, have differentiated plants. The most common type is the house with a central courtyard: access is provided by an opening located on the side of the road and often followed by a narrow corridor; inside, the rooms are arranged around an open space. Among the different examples, the so-called peristyle house should be noted, which is positioned on the northeast arch facing the sea and characterized, as the name indicates, by a small courtyard with an entrance.[5] Not infrequently, in the yard of the residences, there are wells functional to water supply and stepped accesses to the upper floors or the terraces. In many cases, these are homes that reflect the wealth of city society: they are

[5] Sandro Filippo Bondi, Fenicie Cartaginesi, Una civiltà mediterranea. (Rome 2009). Pag-123.

often equipped with *opus-signinum* flooring, on which the symbol of *Tanit* is sometimes depicted. The presence of well-finished bathrooms also attests to the social level of the inhabitants. In addition to the essential private buildings, Kerkouane has returned the largest Punic temple known to date (Fig 2.5). The plan is located in a central position, just southwest of the sector between the three squares. It is accessed thanks to an entrance that opens directly onto the street, whose threshold is delimited by the bases of two pillars. Straight to the right of the entrance, there is a quadrangular room; there, the presence of docks covered with hydraulic plaster made us think of a room for ablutions.

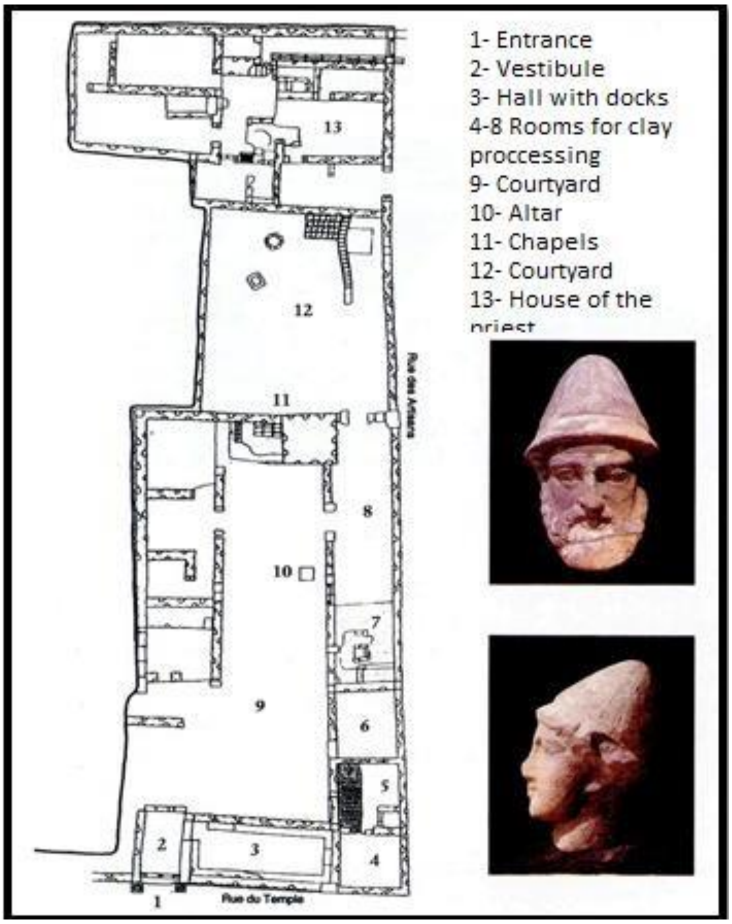


Fig 2.5. Kerkouane, the temple. Left: the plan of the building. Right: votive Clay heads (modified after, *Bondì 2009*).

Inside, the sanctuary has a rather complex plan. After the vestibule, located behind the entrance, there is a large courtyard with an adjoining small altar and surrounded by numerous rooms. On the right-wing, rooms belonging to a clay laboratory can be seen. Behind the large yard, there are the podiums of two small adjacent chapels raised above the level of the same courtyard. These separate the central front of the complex from a rear area, which is also open: the discovery of numerous traces of ashes from animal bones has allowed the place to be identified as a "courtyard for sacrifices." Finally, the complex is closed by a house made up of numerous rooms whose contiguity with the temple has led to the interpretation of the structure as a "priest's house."

However, the port's location, of which no trace has been found, remains unresolved. Moreover, the coast of Kerkouane does not seem suitable for the establishment of such a system: it is jagged and open to the winds, and it has shallow waters that do not facilitate the landing of ships. However, it is possible that, outside the walls, two small inlets constituted the landing point for fishermen's boats to the North.[6]

Regarding the funerary areas, the city boasted a system of Four Necropolises, all extra-urban as usual. The most important sector is Arg El-Ghazouani, northwest of the city, the only one systematically investigated and still being excavated (Fig 2.6; Acquaro et al. ii 1973; Fantar 2002).

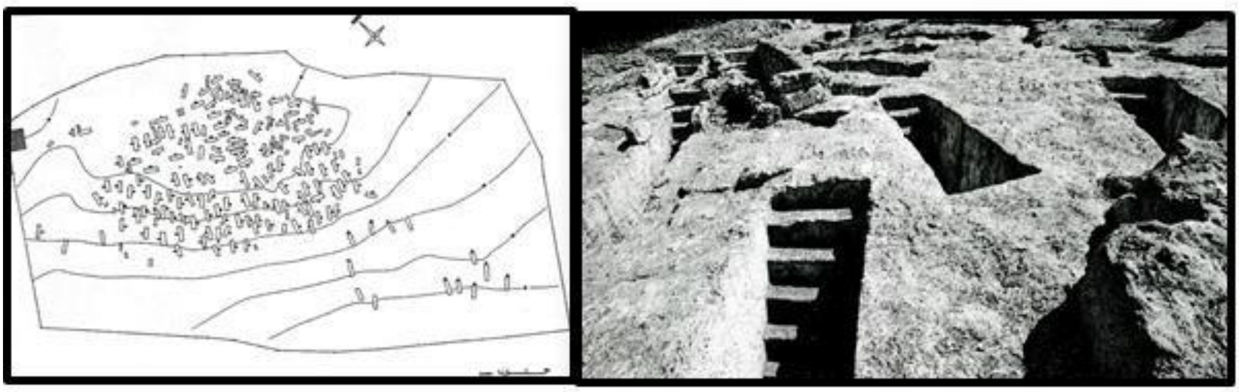


Fig 2.6. Kerkouane, Arg El-Ghazouani, Necropolis. Left: a plan of the funeral area. Right: partial view of the tombs (modified after, *Bondì 2009*).

The plant includes tombs dating from the sixth to the third century and is located on a hill overlooking the sea on the east and north-east sides; in the tombs, dug directly into the rocky bank, the burial ritual

[6] Sandro Filippo Bondi, Fenicie Cartaginesi, Una civiltà mediterranea. (Rome 2009). Pag-124.

is prevalent. About fifty grave burials and 150 specimens of the single-chamber type are known, with access via dromos. The latter occupies the entire corridor width; however, it is not uncommon for the steps to be set on both sides of the entrance or a single side. There is no shortage of cases where the stairs occupy the entire corridor space and then narrow downwards.

To the North, a short depression separates the Necropolis of Arg El-Ghazouani from *Gebel Mlezza.* The latter is known above all for having provided one of the most notable examples of Punic funerary painting (Tomb 8, fig 2.7). [7]

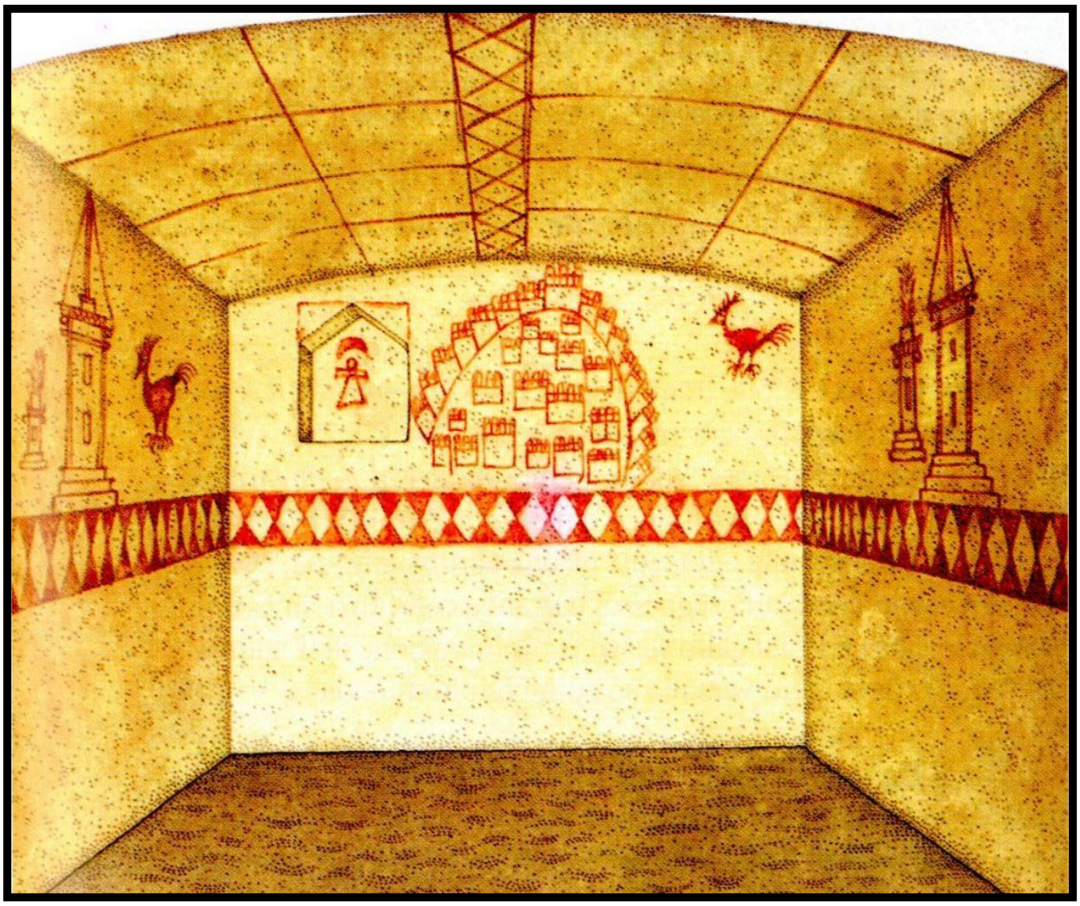


Fig 2.7A. Kerkouane, *Geble Mlezza*. Tomb 8: reconstructive drawing of the paintings (*Bondì 2009*).

[7] Sandro Filippo Bondi, Fenicie Cartaginesi, Una civiltà mediterranea. (Rome 2009). Pag. 126-127.

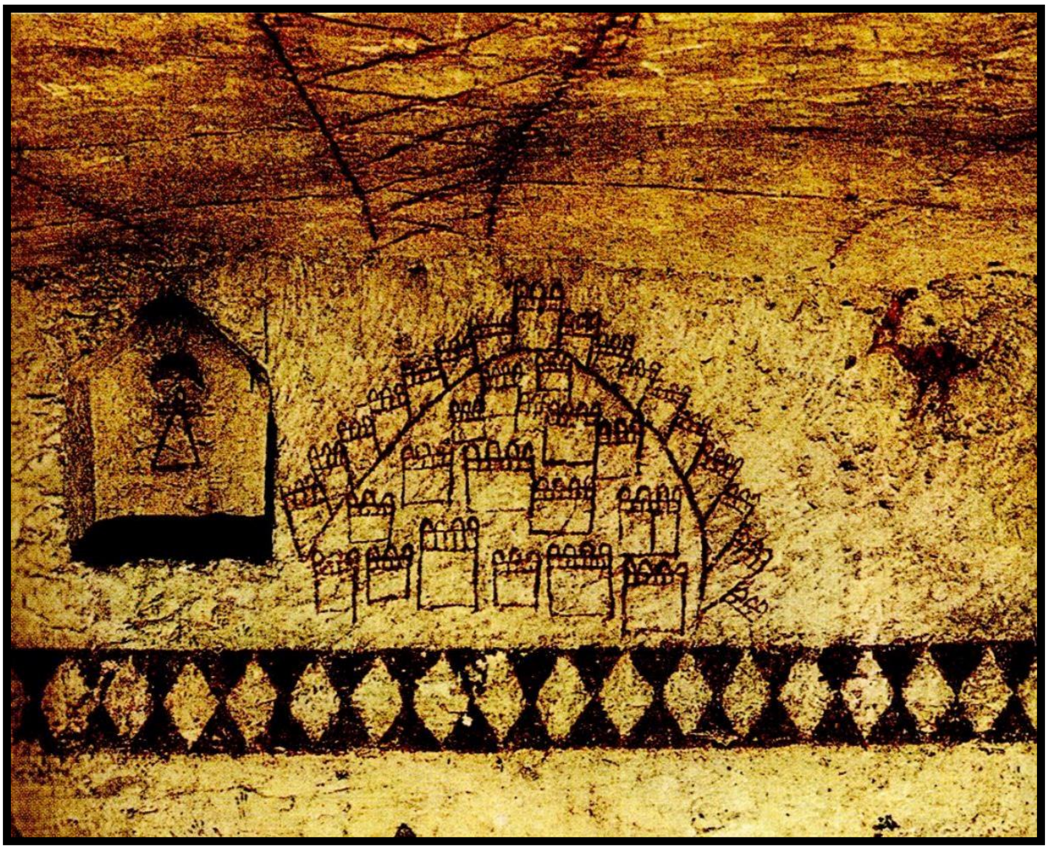


Fig 2.7B. the bottom part of the sepulchre, (*Bondì 2009*).

The burial chamber in question is accessible through a well, and inside, it has a red Ochre decoration made directly on the rock. The decorative apparatus, readable from right to left and centered on the figure of a rooster that seems to move from a sort of mausoleum to a city surrounded by a curtain wall, has been interpreted as a narrative sequence with eschatological functions, which can be seen in the rooster and the representation of the soul of the deceased traveling to its homeland.

Between Cape Bon and *Ras Zebib*:

The region between Cape Bon and the current north-eastern extremity of Algeria is one of the areas most affected by the Phoenician and Punic presence. In particular, the wide arc between Cape Bon and *Ras Zebib* hosts two of the most important Phoenician and Punic centers in the western Mediterranean, Carthage, and Utica: the first originated in the area of an enormous gulf (the present-day Tunis), with a highly fertile hinterland. In contrast, the second extends over a peninsula at the mouth of the *Megerda* river (Lèzine 1970).[8]

These primary settlements, which arose following the Phoenician colonization, were flanked in the Carthaginian age by numerous centers which, alongside those of Cape Bon, attest to the favor reserved by Carthage for the North African regions projected towards the Tyrrhenian and the Mediterranean.

The metropolis action once again concentrated on the arrangement of defense systems (Fantar 1983), is recognizable above all in *Ras Fortas*, at the eastern side of the Gulf of Tunis, where there is a fortress divided into two thick walls with towers and built far away from an indigenous Necropolis (Fig 2.8). Furthermore, two other fortifications have been recognized at the western end of the same gulf, in *Gebel Touchela* and *Gebel Fratas* (in the area of *Ras Zebib*): this way, since the 5th century, Cape Bon on one side and *Ras Zebib* on the other outlined a highly organized region from a defensive point of view.[9]

The action of Carthage is also recognizable thanks to some attestations from Tunis itself from the 4th and 3rd centuries BC, and testimonies from *Bizerte*, the ancient *Ippona Akra,* perhaps datable to the 5th century. Moreover, the internal territory controlled by the city must have been affected by occupation strategies, as documented by the subdivision of the area into districts (Manfredi 2003).

[8] Sandro Filippo Bondi, Fenicie Cartaginesi, Una civiltà mediterranea. (Rome 2009). Pag-128.
[9] Sandro Filippo Bondi, Fenicie Cartaginesi, Una civiltà mediterranea. (Rome 2009). Pag. 129-130.

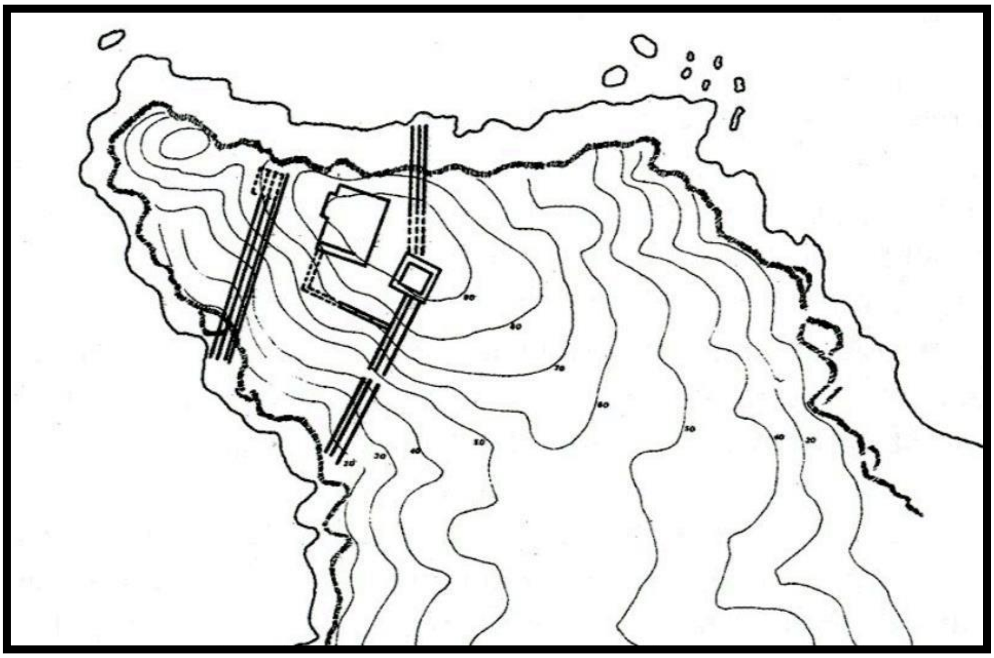


Fig 2.8. *Ras Fortas*. Plan of the Punic fortifications (*Bondì 2009*).

2.3 The Phonics Funereal Types In The Western Mediterranean

In the Western Mediterranean, eighteen sepulchral architectural types are of Phoenician origin, ranging from mass graves to tombs with dromos. The first significant difference occurs in the architectural complexity of the graves, which can be on the surface or in-depth. All types cannot be found in all Necropolises, but most of them are present in Carthage. Furthermore, for the Phoenician-Punic Necropolis, it is essential to remember the simultaneous presence of two types of rituals regarding the treatment of the dead: the burial and the cremation. The prevalence of one ritual typology over the other varies between the necropolises, according to the chronology, taken into consideration. And to highlight the presence of simple burials in jars and mass graves, which could contain burials, cremations, or mixed. In both cases, the tombs in the ground were at a shallow depth.

As for the elementary funerary architectural typology in the western Phoenician-Punic graveyard, there is the burial in a simple pit (Fig 2.9), made by digging a pit on the surface of the rocky platform, able to house an inhumed individual or the urns containing the cremated remains of infants and adults.

Some variants of these tombs added lateral projections to be surmounted by roofing slabs, a variant found only in Carthage. They were rectangular pits paved with masonry in stone in the Punic capital in brick Utica. In Carthage, there are some examples of simple pill large pits, in a central partition to separate the tomb in equal parts, suitable for housing two bodies in the Necropolis of the Western Mediterranean. In North Africa, mainly in the necropolis of *Bir Massouda* near Carthage.

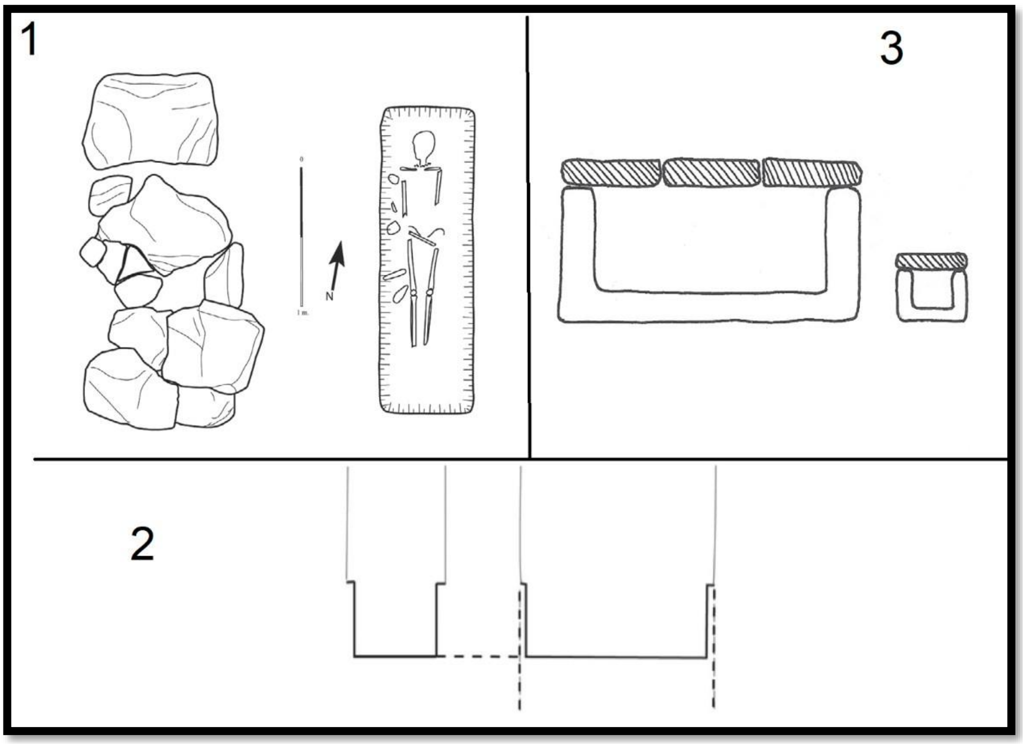


Fig 2.9. Example of a simple grave burial (modified after, Jonghe, 2018; Murciano, 2020)

There are *pozzies* and cylindrical wells, with an average depth of 1.20 m and a diameter between 0.50 and 0.80 m. The wells-tombs, also known as hypogea, consist of chambers dug into the rocky ground, with simple access to the well. (Fig 2.10)

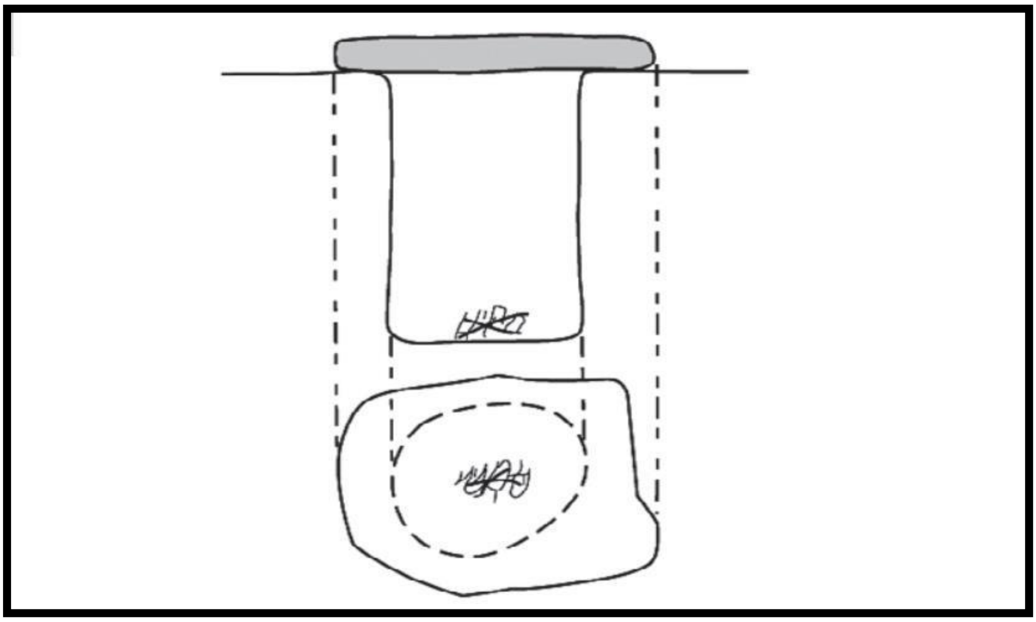


Fig 2.10. Pozzi (Jonghe, 2018).

The tombs built based on a small dwelling with a rectangular base with a vertical well entrance are variants of the wells-tomb. They are documented in Carthage in the excavations of (P. Gauckler and RP Delattre), which took place in the second decade of the twentieth century. At Utica, in the 1924 excavations supervised by Jacques Moulard. (Jonghe, 2018). In place of the access to the well-tomb (fig 2.11), there is an entrance dromos to the tomb, where through a ramp, a staircase, or a combination of the two, one descends into one or more burial chambers in which benches are arranged for the deposition of bodies or funeral equipment. In some Necropolises, there are also cist tombs, a stone box burial formed by a shallow pit in the ground, rectangular in shape, sometimes with a floor, with four vertical stone slabs positioned one on each side as walls, and a horizontal one the covers and closes the burial.

The necropolis of Arg El-Ghazouani, in Kerkouane, offers a relatively limited variety of sepulchral types. It presents the three main Phoenician-Punic architectural types, albeit with numerous variations inside the tombs. The types of presents are the already mentioned chamber tombs with dromos, the pit tombs, and some chamber tombs with wells Inside. Especially in the chambers with dromos, there are numerous variations in the access modality and the type of deposition.

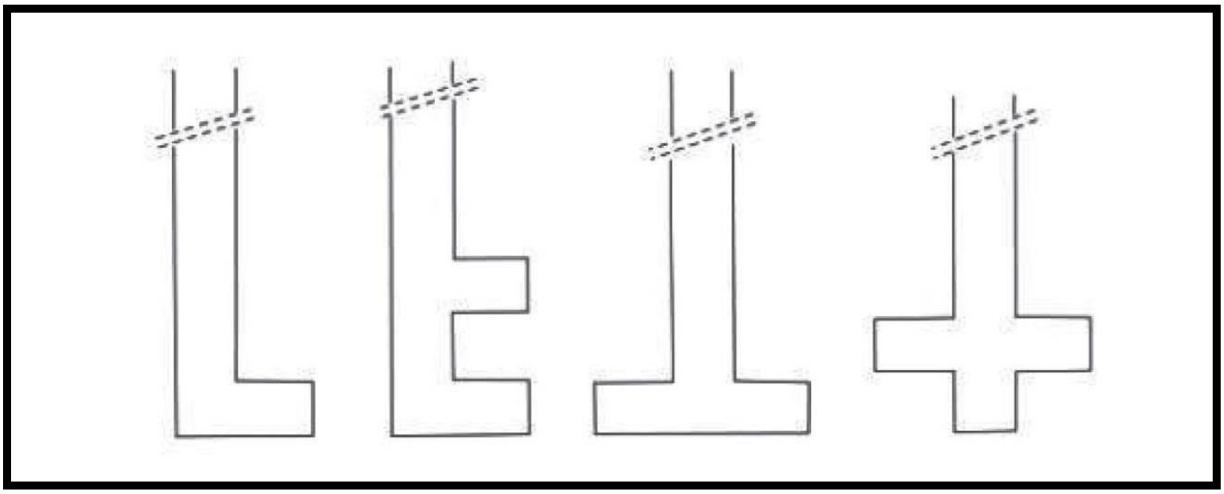


Fig 2.11. Example of a well-tomb and its multi-chamber variants (Jonghe, 2018).

Chamber tombs with dromos can have full or partial step access, occupying the entire chamber's entire descent or only one of the two side parts with a narrow staircase. There are also quarries for offerings for funeral rites. The chambers were sealed by a large stone slab with a quadrangular plan and were built with a flat or double sloping ceiling. The burials body is placed in lateral decubitus on the ground, on beds with plant motifs, or inside wooden sarcophagi (fig 2.12). The only wooden coffin of Punic origin that has survived was carved on the figure of a female subject, known as the "Lady or Princess of Kerkouane," with the face of *Astarte*, goddess of fertility, death, and war Of Semitic origin. The cremated bones were instead deposited in amphorae inserted in niches in the wall or the ground.

As already mentioned by way of the existence of Four Necropolises around the City, an explanation for the simultaneous presence of the practice of burial, which is prevalent in the site, and cremation, could perhaps also be found both in the company of geniuses of other origins, mainly Greek-Italic, or in a different funerary rituality based on the social class of belonging. In some hypogea, some epitaphs show the name of the deceased. [10]

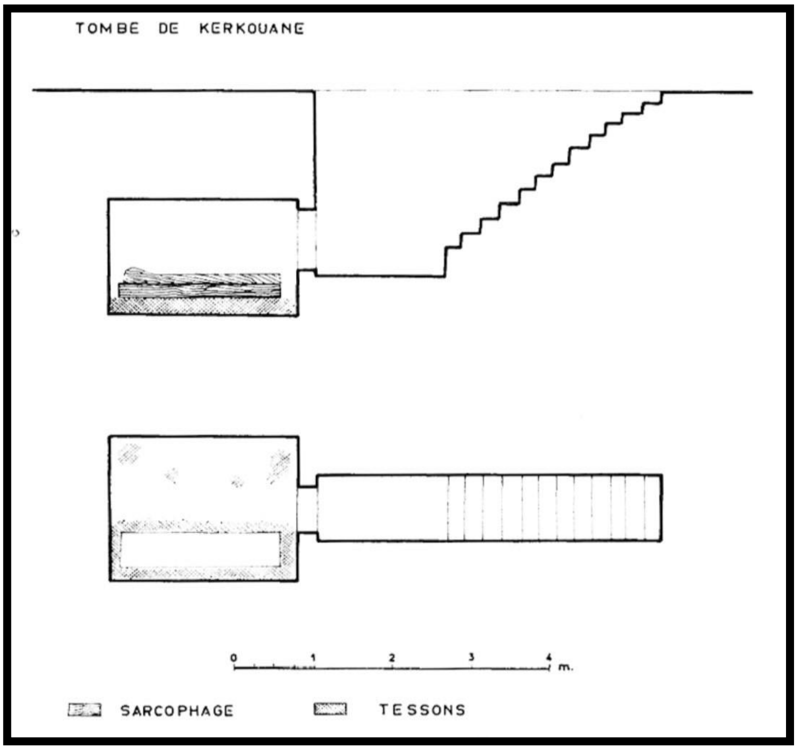


Fig 2.12. Hypogeum of the lady of Kerkouane, note the access stairway to the sepulchral chamber (Mh, Fantar, 1972).

2.4 Paleodiet

The palaeodietary analysis is essential for the archaeological investigation of lifeways and subsistence practices within ancient societies (Johnston, 2010). The use of stable isotopes analysis has become more and more popular for understanding the composition of paleodiets, among the other research methods (Ambrose & Sandford, 1993). Several studies (e.g., (Johnston, 2010; Katzenberg & Harrison, 1997; Keegan & Deniro, 1988) acknowledged the ability of the analysis of the stable

[10] Matteo Murciano, *Analisi Antropologica Della Necropoli Punica Di Kerkouane, Dipartimento di storia Antropologia Religioni Arte Spettacolo Corso di Laurea in Storia, Antropologia, Religioni*. (2020). page 9-12.

isotopes to directly recognize multiple types of diets through the examination of stable carbon and nitrogen isotopes. Therefore, the study of these stable isotopes in bone and enamel displays information about the ancient dietary history of the analyzed individual. However, the stable isotopes of archaeological remains, mainly bones, are not the only method providing palaeodietary reconstruction; teeth' chemical signature can also give information on the ancient diet (Pate, 1994).

The essential concept behind the paleodiet is "*we are what we eat*" (Pate, 1994). The method of measuring stable isotopes has been widely used and accepted to reconstruct ancient diets, ecologies, and environments. Palaeodietary reconstruction is based on analyzing the isotopic composition of food nutrient sources such as dietary proteins, carbohydrates, and lipids, transformed through the body and preserved in human tissues (Keegan & Deniro, 1988).

Precisely, the isotopes of specific elements (like carbon, nitrogen, and oxygen) reveal the types of plants and animal resources consumed by ancient populations and the exploitation of terrestrial versus marine resources. Therefore, the isotopic composition of human and animal tissues reverberates the food consumed during their lifetime (Deniro & Schoeniger, 1983; Keegan & Deniro, 1988).

2.5 Stable isotopes

The number of protons in an element's nucleus defines its isotope: for instance, carbon boasts six protons, whereas nitrogen and oxygen do seven and eight, respectively. Isotopes are components differing in the number of neutrons contained in their nucleus (fig 2.13). Usually, elements at the low end of the periodic table have an equal number of neutrons and protons (nucleons). Said nucleons constitute the majority of the atom's mass. Superscript numbers to the left of the element's symbol, such as ^{12}C, ^{14}N, and ^{16}O, stand for the isotopic mass of the common forms of these elements. Non-common conditions present additional neutrons (Ambrose & Sandford, 1993).

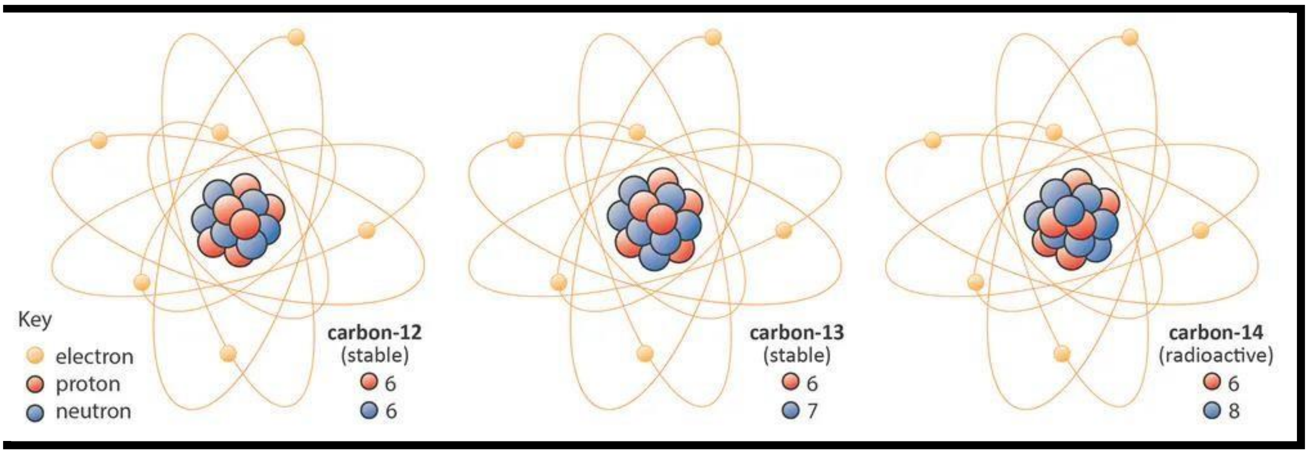


Fig 2.13. Three naturally occurring carbon isotopes. While each has the same number of protons and electrons, they differ in the number of neutrons. (Williams 2013). https://theconversation.com.

When it comes to diet reconstruction, the essential chemical elements are carbon, nitrogen, and sulfur; the analysis of isotopes has been chiefly used in archaeological contexts for diets to be reconstructed using the analysis of the carbon and nitrogen isotope composition of bone collagen, the carbon isotope composition of prehistoric humans' bone apatite, and the carbon and nitrogen isotope composition of encrustations on potsherds (Ambrose & Sandford, 1993).

Table 2.1 features some of the chemical elements that have several isotopes, as well as the abundances of those isotopes. Stable isotopes do not decay over time, as opposed to unstable (radioactive) isotopes. For instance, in a dead organism, ^{14}C decays to ^{14}N, while the amounts of ^{12}C and ^{13}C in that same organism remain constant.

Element	Isotope	Fractional abundance	1‰ change	International standard
Hydrogen	^{1}H	0·999844		Standard Mean Ocean
	^{2}H	0·000156	0·000000156	Water (SMOW)
Carbon	^{12}C	0·98889		PeeDee Belemnite
	^{13}C	0·01111	0·00001123	limestone (PDB)
Nitrogen	^{14}N	0·99634		Air
	^{15}N	0·00366	0·00000367	
Oxygen	^{16}O	0·99755		
	^{17}O	0·00039	0·00000039	Standard Mean Ocean
	^{18}O	0·00206	0·00000207	Water (SMOW)
Sulfur	^{32}S	0.9502		Canyon Diablo
	^{33}S	0.0075		Iron meteorite
	^{34}S	0.0421	0.0000450045	(CDT)
	^{36}S	0.0002		

Table 2.1 average terrestrial abundances of stable isotopes of elements used in ancient human tissues analysis (modified after Hoefs1996; Schoeller 1999).

When it comes to chemical reactions (e.g., the conversion of atmospheric CO2 into glucose on the part of plants), the relative amounts of ^{12}C and ^{13}C differ in plant tissue relative to that of atmospheric CO2, owing to the fact that isotopes vary in mass and thus possess slightly different chemical and physical properties. Unusually, isotopes with higher mass (heavier isotopes, e.g., ^{13}C) react slightly more slowly than lighter isotopes (e.g., ^{12}C). The term isotope effect refers to physical phenomena that take place during chemical reactions owing to the mass differences in isotopes. In contrast, the term fractionation refers to the resulting difference in the isotope ratio of plant tissue carbon compared with the atmospheric CO2 carbon caused by isotope effects. Fractionation serves as the basis for stable isotope variation in biological and geochemical systems; consequently, understanding the chemical reactions yielding stable isotope variation enables physical anthropologists, archaeologists, geochemists, and ecologists to solve a wide range of exciting problems using stable isotope analysis (Ambrose & Sandford, 1993).

In the seventies, several scientists such as DeNiro & Epstein, 1978; Van Der Merwe & Vogel (1977); and Van Der Merwe & Vogel (1978,1981), have conducted one of the earliest studies on stable isotope; however, earlier discoveries of stable isotope have been recorded in 1913 and were carried out until the 1930s when the most stable isotope has been identified. From the 1940s to the 1960s, commercial mass spectrometers have been developed and have advanced substantially (Gross,1979). In the 1980s, isotope analysis made a huge leap in shrinking the time, cost, and sample processing capacity; the complete process became simpler, quicker, and less expensive. These advancements made the scientists increase the samples they wished to analyze and expanded the application of stable isotopes. By the 1990s, researchers extended their sample selections to other than human remains to include non-human faunal bone and prehistoric plants (Ambrose & Sandford, 1993; Hedges & Reynard, 2008).

Carbon was the first element for which stable isotope variation was used in archaeology, which follows archaeologists' familiarity with radiocarbon. Interest in other elements such as nitrogen, oxygen, and sulfur, flourished once the potential of studying stable carbon isotopes in preserved protein was understood. Each of these elements and their stable isotopes has been studied extensively in geological and ecological systems. Archaeologists are relative latecomers to studying the elements' stable isotope variation, apart from carbon (Ambrose & Sandford, 1993).

The second element to be used in paleodiet research was nitrogen. DeNiro & Epstein, 1978,1981) carried out controlled feeding experiments on several species to study the relationship between the stable carbon and stable nitrogen isotope ratios in diet and animal tissues. Shortly after that, while working with two postdoctoral researchers, DeNiro explored trophic levels and regional variation in nitrogen isotopes (Deniro & Schoeniger, 1983) and trophic level variation and dietary differences in east Africa (Ambrose & DeNiro, 1986).

Stable carbon isotope analysis has become a vital method used on evidence found in archaeological sites. This technical isotope specialty has expanded to include studying nitrogen, oxygen, strontium of teeth and bones (Ambrose & Sandford, 1993). However, Stable isotopes were not applied only to research and understand the ancient diet. Still, likewise is used to understand the habitation patterns of the ancient populations, paleoclimate, prehistoric health status, diseases, and social life history (Katzenberg & Krouse, 1989; Katzenberg & Harrison, 1997).

2.5.1 Carbon

Carbon, the fundamental element of all organic life forms, is the most common in nature. Its biogeochemical cycle takes place between the geosphere, hydrosphere, biosphere, and atmosphere; carbon dioxide (CO2), which has a $\delta^{13}C$ value of -7 ‰ca, is the carbon produced in the atmosphere (Sharp, Z.D. 2016), carbon has two stable isotopes, namely ^{13}C and ^{12}C, with around 1.1 and 98.9% of neutral abundance (Deniro & Schoeniger, 1983). As a general rule, the ratio between the two stable isotopes of carbon ($^{13}C/^{12}C$), as expressed with delta notation $\delta^{13}C$, is calculated against Vienna Pee Dee Belemnite (VPDB) international standards, using the formula:

$$\boldsymbol{\delta}^{13}\mathrm{C} = [\{(\frac{sample\ 13C}{12C})/(\frac{standard\ 13C}{12C})\}\text{-}1] \times 1000$$

The original VPDB, which was collected from the banks of the Pee Dee River in South Carolina and had a high $^{13}C/^{12}C$ ratio, resulting in usually negative $\boldsymbol{\delta}^{13}C$ values, was a fossilized shell sample of a belemnite; as said sample cannot be used in every isotopic study, other reference standards are being calibrated based on it. Therefore, carbon isotope values relative to "VPDB" are commonly used to show that the data is based on the values of the standard material (Ambrosea, 1990; Buikstra & Ubelaker, 1996; Katzenberg & Krouse, 1989; Noche-Dowdy, 2015).

The photosynthesis process enables terrestrial plants to absorb the carbon found in the atmosphere; the model produces a typical isotope fractionation based on the photosynthetic pathways, resulting in diverse $\delta^{13}C$ values.

In temperate climates, most plants like wheat, barley, rice, and legumes represent 80% of the terrestrial plants, synthesized by the Calvin-Benson cycle or C3 photosynthesis pathway, resulting in a $\delta^{13}C$ mean value of -26.5 ‰. Tropical plants such as maize, sorghum, millet, and sugar cane, which represent 15% of the terrestrial plants, synthesize based on the Hatch-Slack cycle or C4 photosynthesis pathways, which have an average $\delta^{13}C$ value of - 12.5 ‰ (Katzenberg & Krouse, 1989). However, plants such as euphorbias, agaves, and cacti use another photosynthetic pathway known as CAM (Crassulacean acid metabolism), which yields $\delta^{13}C$ values that are intermediate to those of C3 and C4 plants, with a range between -10 and -20 ‰ (Fig 2.14), since some plants alternate the C3 and C4 cycle between day and night (van der Merwe, 1982).

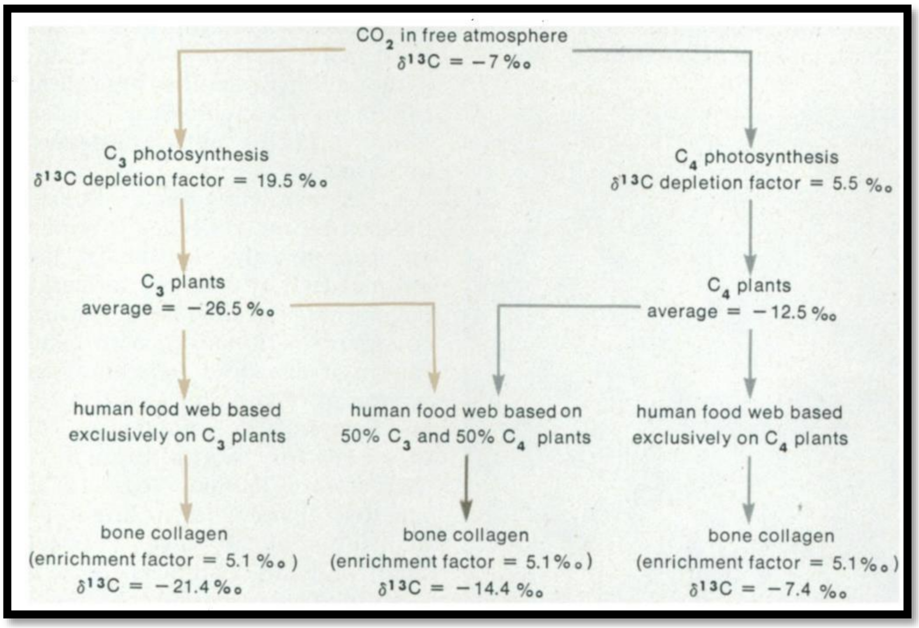


Fig 2.14. A Diagram shows three different human diets based on C3, C4, and CAM plants; each pathway leaves a signature in the $\delta^{13}C$ value of human bone collagen (van der Merwe, 1982).

In marine organisms, carbon is derived mainly from bicarbonates dissolved in the ocean surface, with an isotopic value of about 0 ‰, richer in $\delta\,^{13}C$ about 7% compared to atmospheric carbon dioxide. Consequently, the marine resources show values of $\delta^{13}C$ higher than those of terrestrial food sources, with the C3 and C4 terrestrial source plants that might have overlapping ^{13}C values with the marine source plants (Schwarcz & Schoeninger, 1991).

2.5.2 Nitrogen

When it comes to the food chain, nitrogen can be introduced from the soil or the atmosphere (Deniro, 1980; Katzenberg & Krouse, 1989). The isotope values of nitrogen are described using the delta (δ) notation in parts per thousand (‰) relative to atmospheric Nitrogen (AIR) (Ambrose, 1991). The Nitrogen stable isotope ratio of carbon can be calculated using the following formula:

$$\delta\ ^{15}N = [\{(\frac{sample\ 15N}{14N})/(\frac{standard\ 15N}{14N})\}\text{-}1]\ x\ 1000$$

The nitrogen isotopes are measured to identify the trophic level of animals and plants in the food chain (Ambrose, 1991; Deniro, 1980). According to literature, the isotopic value of nitrogen increases by 3 to 5‰ throughout every trophic level present in the food chain (Deniro, 1980; Katzenberg & Krouse, 1989). For instance, the nitrogen isotope value of animals is 3% higher in ^{15}N than the plants and animals that said animals consume (Ambrose, 2000; Deniro & Schoeniger, 1983). The analysis of nitrogen isotope also enables the identification of legume versus non-legume consumption. Many legumes have a symbiotic relationship with bacteria, which makes them nitrogen-fixing plants; in turn, this results in specific lower N values (Virginia & Delwiche, 1982). The majority of non-legume plants that obtain nitrogen from the soil have a higher ^{15}N content than legume plants, which leads to distinctive N values (Virginia & Delwiche, 1982). Legumes and blue-green algae, nitrogen-fixing plants, have lower ^{15}N values than non-nitrogen fixation plants (Deniro, 1980).

The stable isotopes of nitrogen are also studied to differentiate between ancient marine and terrestrial dietary sources. In 1984 Schoeninger and DeNiro published research about the isotope values of different birds, plants, and mammals. The study highlighted those marine animals had ^{15}N values generally higher than terrestrial animals. Consequently, it has been observed that the ^{15}N values of vertebrates are 6-8% higher than animals found at similar trophic levels in the majority of terrestrial environments (Deniro & Schoeniger, 1983). Indeed, it is possible to overlap ^{13}C values with quite different ^{15}N ratios while reconstructing the dietary inputs of ancient marine animals, including mammals, fish, mollusks, algae, cultigens, and others (Keegan & Deniro, 1988).

Variations in the isotope value of nitrogen within and between different ecosystems have been proved by Ambrose (1990). In the same ecosystem, the isotope value of nitrogen in a wet, cold environment is lower than that detected in an arid, hot climate. Similarly, the obligate drinker nitrogen isotope value is higher than that of non-obligate drinker mammalian herbivores. Therefore, mammalian herbivores' different habitats, ecosystems, and water conception behaviors require consideration during nitrogen stable isotope analysis. Consequently, ecosystem variability is one of the critical issues in the study of stable isotopes. According to scholars (e.g., Bocherens & Drucker, 2003; Katzenberg & Krouse, 1989), application of the nitrogen stable isotope approach in the modern ecosystem may be limited, as the distribution of $\delta^{15}N$ values in food sources may be impacted by modern chemical fertilizers (Deniro, 1980).

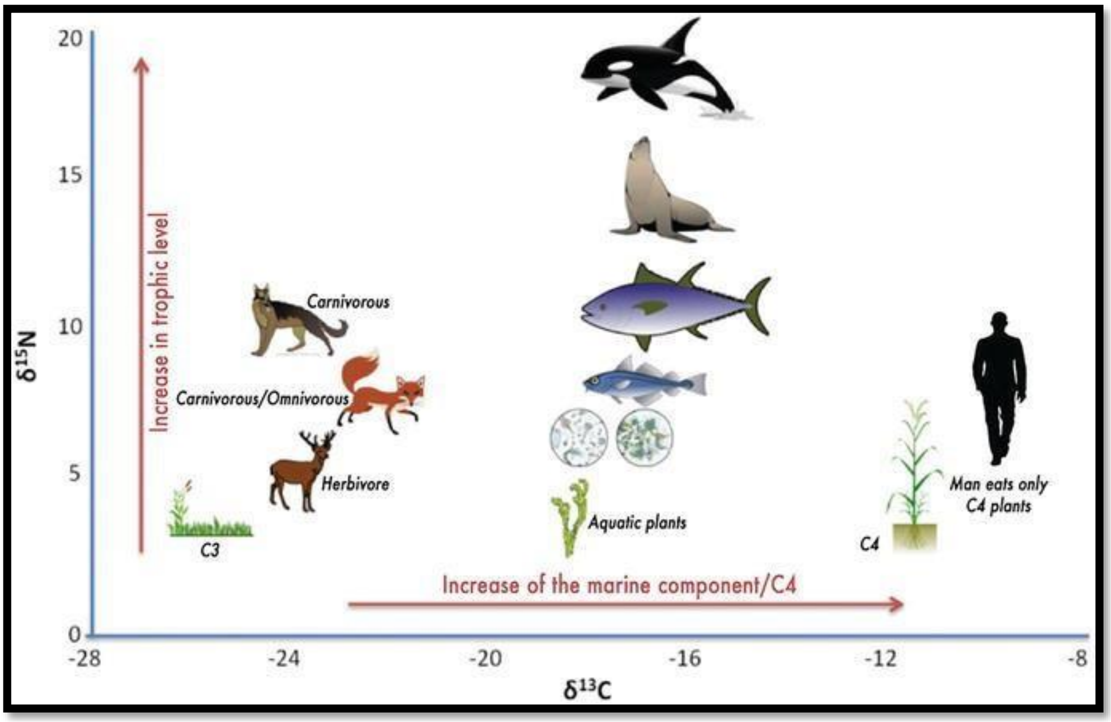


Fig 2.15. A simplified summary of C and N value of terrestrial versus marines' resources (after Berto, D. et al., 2019)

2.6 The Chemistry Of Ancient Human Bone

The application of chemistry in archaeology is expressed at its best in the analysis of human bones. Paleodiet reconstruction based on stable C and N isotope analysis is mainly undertaken on bone collagen. Using the human body as a "biological archive" (Borgognini Tarli e Pacciani, 1993) identifies past biographies. If determining past demography or health conditions is a matter of primary importance, understanding past diet and subsistence remains is a frequently approached line of investigation. Bone chemistry is the most direct and reliable method to determine paleo nutrition, according to the principle: "*the reading of chemical signatures passed from the foods eaten to the consumer allows diet documentation*" (Tafuri, 2015).

The human bone is composed of an organic and an inorganic fraction (Table 2.2). The organic matrix forms up to 30% of the dry weight of bone and is primarily constituted by Type I collagen. On the other hand, bone ash is a mineral combination of calcium phosphate that forms hydroxyapatite and phosphorus (Price, 1989). Collagen constitutes 90% of the organic matrix of bone and is the main protein in the human organism. Its function is related to maintaining the structural integrity of various tissues and organs of which it is part. It can be present in different quantities, combined with other components, and have different molecular sequences, sizes, and tissues that classify it into different types.

COMPOSITION OF MINERALISED TISSUES				
	Bone	**Developing enamel**		**Mature enamel**
	Weight	*Volume*	*Weight*	*Volume*
Inorganic (%)	88 (80-100) 70	37	16	≥96
Organic (%)	30	19	20	<0.2->0.6
Density (g/cm^3)	2-2.05	1.45		2.9-3
Calcium (%)	24			34-40
Phosphorus (%)	11.2	16-18		
Ca/P ratio (weight)	2.15	1.92-2.17		
CO_2 present as	3.9	1.95-3.66		
carbonate (%)	0.5	0.25-0.9		
Sodium (%)	0.3	0.25-0.56		
Magnesium %	5000	<25->5000		

Table 2.2. Chemical composition of human enamel and bone (modified after Hillson, 1996).

On the other hand, the inorganic matrix of bone is formed by a microcrystalline structure of carbonate apatite, tricalcium phosphate hydrate, and hydroxyapatite (Carlstrom and Engstrom, 1956). The hydroxyapatite (Ca5(PO4)3(OH) compound makes up 70% of the bone composition; in addition, hydroxyapatite is the most unsolvable inorganic part of the human skeletal component (Katzenberg, 2007). At the microscopic level, two types of bone are recognized; the first is compact or cortical bone, which comprises parallel bundles of osteons and has excellent strength. The second is cancellous or trabecular bone, commonly called "spongy," which has a much less rigid organization, being less dense and permeated with channels (Katzenberg & Harrison, 1997; Katzenberg, 2007).

The ancient human bone's remains give us insight into the ancient dietary systems and ecology through its chemical compositions. By analyzing the bone collagen as well as the apatite, we understand the food sources of ancient populations. Bone is continuously remodeled over time; thus, the isotopic composition of bone tissue, collagen, and structural carbonate, indicates an average diet from 10 to 25 years (Deniro & Schoeniger, 1983; Katzenberg, 2007).

CHAPTER THREE

3. Materials and methods

3.1 Materials

The human remains objects of this thesis project are stored in the Biology of Ancient Population laboratory at the Department of Environmental Biology of the Sapienza University of Rome. The human skeletal samples came from the necropolis of Arg El-Ghazouani, at the Punic city of Kerkouane in Tunisia. This chapter will briefly describe the site and the human skeletal remains, particularly the samples analyzed in this project. Due to the Covid-19 pandemic, it was impossible to visit the site and collect animal bones samples as previously planned. Therefore, the human isotopic data will be compared to associable animal data available in the literature.

3.1.1 Necropolis of Arg El-Ghazouani

A total of four necropolises have been identified in Kerkouane, located according to the Punic burial ritual at a certain distance from the urban agglomeration, but close enough to guarantee citizens the possibility of frequenting them assiduously. The few available data refer to a current occupation of the four necropolises, dated between the 6th and 3rd centuries B.C. (MH. Fantar, 1984). All were attesting their simultaneous attendance by the population and indicating asynchrony with the City of Kerkouane, in their development and abandonment, due to the destruction of the city.

All the necropolises around Kerkouane have in common the proximity to the rock in the burials, a characteristic feature of the Punic funerary culture, using the rocky platforms of the calcareous soil malleable material available on-site to sculpt the funeral structures. The two necropolises to the North and South of the settlement are the most proximal to the city, both located on the cliffs that make up the region's coast.

About 500 m from the urban settlement, to the northwest of the city, has situated the necropolis of Arg el-Ghazouani, the most documented and excavated of the four, which houses many burials, mainly with chamber tombs with *dromos* and fossa. Although the place was already known thanks to incidental findings over time, the discovery date is set for 1929. The excavations were initially entrusted to the judicial police brigadier who first ascertained the discovery, J.Combres, which dealt mainly with the Necropolis of Arg El-Ghazouani (H. Gallet & L. Slim, 1983).

Many tombs had already been looted starting from the same ancient period, probably due to the ease in identifying the burials and the presence of the steles and stone monuments located outside them to signal their presence. However, some excavation campaigns, especially in the last century, have brought to light some intact tombs with preserved skeletal remains and the entire funerary equipment (H. Gallet & L. Slim, 1983).

The necropolis covers a longitudinal space of about 1 km (Fig 3.1) and is located according to a north-south orientation, maintaining a direction parallel to the coast. The rocky complex that houses this necropolis is almost entirely occupied by the hypogea excavated inside it, to the point that, given the lack of space, the various cavities almost lean on each other. Nevertheless, there are sometimes some tiny squares, generally equipped with seats, obtained between the graves and perhaps intended for frequentation by the relatives of the deceased. The objects found are primarily made up of Punic ceramic material, alongside various imported ceramics, especially Attic and Greek-Italic, various amulets, coins, terracotta statues, scarabs, bronze mirrors, and jewels (MH. Fantar, 1984) (Fig 3.2).

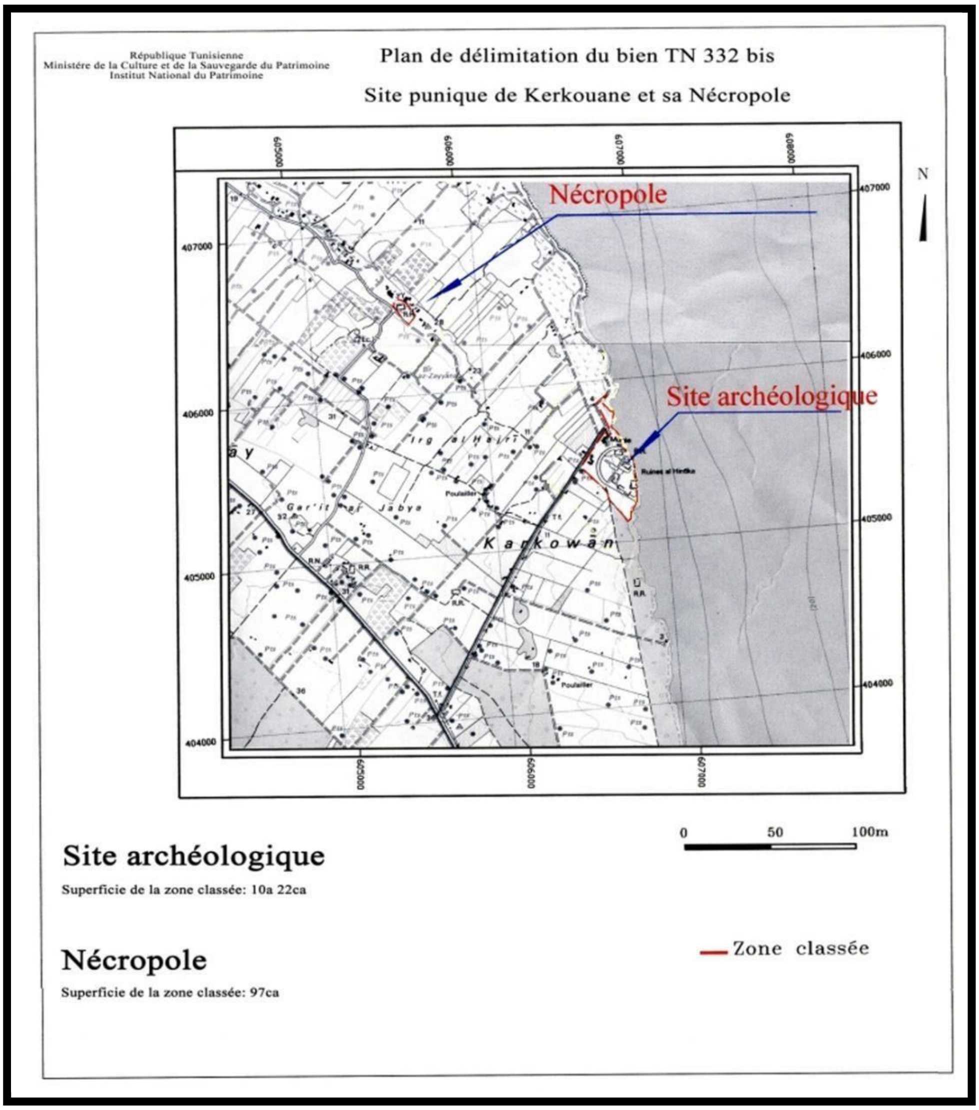


Fig 3.16 Map of the position of the archaeological site of Kerkouane and the Necropolis of Arg El-Ghazouani (from the archive of the Ministry of culture and Saving cultural Heritage – Tunisia).

Fig 17 Archaeological findings in the necropolis of Arg El-Ghazouani. (M.H. Fantar, 1984)

3.1.2 The human remains of Arg El-Ghazouani

This paragraph reports the study of all the known human remains from Arg El-Ghazouani necropolis in Kerkouane, for a total number of 113 individuals (Murciano, 2020), including the 25 samples analysed for stable carbon and nitrogen isotopes in this thesis project. However, this was a preliminary analysis. The material analysed is almost exclusively dental elements, which was beneficial to determine an estimate of the age at death of the various individuals (Graph 3.3), undertaken by M. Murciano for his recent Master thesis (Murciano, 2020).

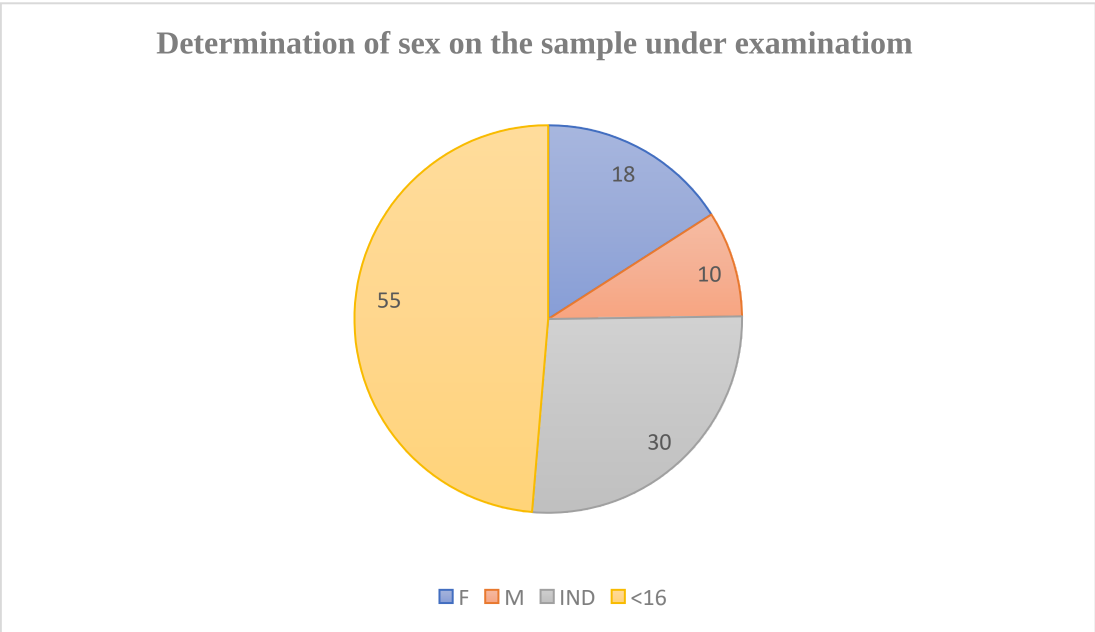
Graph 18 Mortality rate by age group (from Murciano, 2020).

In creating the mortality rate graph, Murciano (2020) collected the individuals according to different age groups, trying not to make too large groups, especially for sub-adults, to show a complete trend in mortality. In reading the data and analyses that will follow, it should be kept in mind that the total number of individuals examined is 113. First, we can note an essential division between adults and sub-adults. The sub-adults are 55 individuals (48.7%), while the adults are 57 (50.4%), not showing a clear majority in the presence of one group compared to the other. There is also one individual (0.9%) who is found to be undetectable. As can be seen from the trend of Graph 1, adults between 21 and 35 and those between 36 and 50 are both represented by 22 individuals (19.5%).

Children over the age of 5 are always numerous compared to the data taken into consideration (Murciano, 2020). Still, there is a sharp decline in the number of individuals with increasing age, starting from 15 individuals between 6 and 8 years (13.3%) to 12 individuals between 9 and 12 years (10.6%), up to the minimum in a range between 13 and 15 years (2.6%). After this age group, the graph rises again, with nine individuals between 16 and 20 years old (8%) and the chart trend already described.

In the end, there is a new minimum among the four individuals of > 50 years old (3.5%). It, therefore, appears evident that the highest mortality range is in the years immediately after birth, the first 2-3 years, and immediately after puberty, in the adult phase. The decrease in the number of individuals after five years can perhaps be explained by the greater probability of survival than children generally acquire after the dangers of the first years of life, especially given the high mortality due to infections and diseases that are now easily treatable (Murciano, 2020). Even the scarce data relating to adults> 50 years can perhaps be explained by the few individuals who managed to reach that age at the time, given the high mortality recorded in the immediately preceding bands (Murciano, 2020).

The second graph analyses the proportions obtained by determining the sex of the individuals belonging to the sample examined (Graph 3.4). As with any statistical analysis, the increase in data usually corresponds to a stabilisation of the data, and the presence of more data reduces the variability of the data itself.



Graph 19 determination of sex of the sample examined from Arg-El-Ghazouani (modified after Murciano, 2020)

For some individuals of the available sample, it was impossible to accurately determine the age at the death due to the fragmentation of the finds. However, it was possible to assess adult age even with a vast range (30-50), and for some of these, it was possible to hypothesise their sex. As mentioned above thus we have 57 adult individuals, which makes up the total for our subsequent analysis. Female individuals on the illustrated sample are 31.1%, while male individuals are 17.2%. The indeterminates are 51.7%, but the partial analysis of the sample could lead to a variation in the preliminary data obtained. At present, there is a more significant presence of female individuals. One explanation of the more influential female presence in the necropolis could be connected to the numerous depositions of infants, which allow us to hypothesise the existence of a maternal-infantile context within site (Murciano, 2020). However, it is necessary to expand the sample under analysis to define this hypothesis better. The data obtained could vary considering future analysis conducted on the skeletal remains currently still in Tunisia, as they are difficult to access and transport due to the Covid-19 emergency. It was impossible to visit the site to achieve the necessary studies on the material placed there, which were therefore limited to the material present in Italy (Murciano, 2020)

Here is a table of the thesis project samples (Tab.2). Among the 113 individuals present at the Biology of Ancient Population laboratory at the Department of Environmental Biology of the Sapienza University of Rome, 25 individuals were sampled for stable carbon and nitrogen analysis to understand the ancient dietary practices of inhabitants of Kerkouane. The 25 samples consist of seven females, six males, and seven individuals not determined; they were selected because of their better preservation among those available in the laboratory, excluding the remaining, which are currently understudies.

ID	Tomb N	Tage name	Age	Sex	Bone portion
Arg - 1	1	T.1/05 C1	20–24 yo	Female	Mandible
Arg - 2	20	T.20	30_ 40 yo	Female	Mandible
Arg - 4	1	T.1/97. Ind D	27–33 yo	Male	Mandible
Arg – 5	4	T.4/98 XD	35-50 yo	Female	Mandible
Arg – 7	3	T.3/98 Busta E	30–35 yo	Indeterminate	Mandible
Arg– 8	No indication	Kerkouane ARG No Indication	20–25 yo	Male	Mandible
Arg– 9	1	T.1/10 Ind 2	4-5 yo	Male (?)	N. D
Arg – 10	1	T.1/06 Ind A	20-25 yo	Female	Mandible
Arg – 12	1	T.1/97. Ind B	50 yo	Male	Mandible
Arg – 13	5	T.5/08	30 – 35 yo	Indeterminate	Maxilla
Arg – 14	2	T.2/00 Crania 4B	6-7 yo	Male (DNA)	Mandible
Arg – 16	4	T.4/98 XF	30 – 50 yo	Female	Mandible
Arg – 18	3	T.3/98 XJ	25 – 50 yo	Indeterminate	Mandible
Arg – 19	1	T.1/97 Ind A	30-35 yo	Male	Maxilla
Arg – 20	1	T.1/04 Ind B (Info)	5-6 yo	Indeterminate	Mandible
Arg – 21	1	T.1/97 Ind XH	20-25 yo	Indeterminate	Mandible
Arg – 22	2	T.2/08 Ind A	9-11 yo	Indeterminate	Mandible
Arg – 23	1	T.1/04 Ind A	16 – 20 yo	Indeterminate	Maxilla
Arg – 24	3	T.3/98 XI	40 – 50 yo	Female	Mandible
Arg – 25	2	T.2/98 Ind D	20 – 30 yo	Female	Mandible

Table 3. Details of the human remains were sampled from the necropolis of Arg El-Ghazouani. N.D. = not determined.

3.2 Methodological procedure

3.2.1 Sample collections and preparation

Twenty-five human bone samples from the Necropolis of Arg El-Ghazouani site, dated between the 4th - 6th centuries B.C. (MH. Fantar,1984), underwent stable carbon and nitrogen isotopes analysis. The sample collection is housed in the Biology of Ancient Population laboratory in the Department of Environmental Biology at the Sapienza University of Rome. The samples were cleaned and polished by a Dremel multitool to remove any dirt or sediment on the bone's surfaces before sampling approximately 3.5g to 5g of bone from the mandible and maxillary portion (fig 3.5).

Fig 20. Cleaned and polished bone (left), bone sampling (right) (Photo by Abobaker Afat).

3.2.2 Collagen extraction

The collagen was extracted using a protocol on bone chunks modified from Longin, (1971). The first step is the demineralisation: after the samples are cleaned, cut, and weighed for 0.5-1g, the bone chunk is placed into 12 ml glass test tubes, to which are added approximately 8ml of 0.5M HCl solution, marking the tube with the given sample I.D. by using a permanent marker (fig 3.6).

All the tubes were covered with aluminum foil marked with the sample name and the processing date. The samples are left at 4ºC for several days, with acid changes each 2-4 days by decanting off acid with Pasteur pipettes into a waste beaker. If the sample were fragmented, we would decant by using an Ezee filter to avoid sample loss. When soft and floating, the samples are demineralised, then neutralised by distilled water rinsing them at least three times.

Fig 21. The process of changing acid and neutralising the samples (right) the sample placed in the tubes with HCl solution (left), (Photo by Abobaker Afat).

The samples were gelatinised in pH 3 HCl at 75 °C for 48 hours (Bocherens & Drucker, 2003; Longin, 1971). The collagen was then dissolved, and the samples were filtered off with 5-8µm Ezee filters into pre-weighed plastic tubes.

The final step of collagen extraction is Freeze-drying. The collagen was frozen overnight at -22 °C and transferred to -80 °C, and then freeze-dried for at least two days until dry. Each sample was weighed to calculate the collagen yield if >0.5% (Van Klinken, 1999) sampled (ca. 1 mg) into tin capsules and analysed by Elemental Analysis Isotope Ratio Mass Spectrometry (EA-IRMS).

3.2.3 Analytical procedure

Stable carbon and nitrogen isotope ratio analysis of pre-weighed collagen samples were undertaken by Elemental Analysis - Isotope Ratio Mass Spectrometry (EA-IRMS) at Iso-Analytical Limited (United Kingdom); the samples and references were weighted in tin capsules sealed and loaded in autosampler on a Europa scientific elemental analyser. The samples are dropped into a heater at 1000 °C and combusted in the presence of oxygen, reaching a rising temperature to ~1700 °C. The combusted gases are swept in a helium stream over a combustion catalyst (Cr2O3), copper oxide wires to oxidise hydrocarbons, and silver wool to remove sulfur and halides. The resultant gases N2, NO*x*, H2O, O2, and CO2, were swept at 600 °C through a reduction stage of pure copper wires, extracting any oxygen and converting NOx species to N2.

The nitrogen and carbon dioxide are separated using a packed column gas chromatography at a stable temperature of 65 °C; the nitrogen peak enters the ion source of the Europa Scientific 20-20 IRMS, where it is ionised and accelerated. The Nitrogen and carbon dioxide gas species of different masses are separated in a magnetic field then measured using a Faraday cup collector array to measure the isotopomers of N2 and CO2 (from Iso-A Laboratory protocol).

Both samples and references were converted to N2, CO2 and analysed in a batch process, in which the samples are analysed interspersed with the references. Iso-Analytical references for quality control check are soy protein, L-alanine, tuna protein, oxalic acid, and ammonium sulphate. The measurements of all isotopes refer to the ratio between heavy and light isotope ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) measured as δ values in parts per mil (‰), they are calibrated against and traceable to sucrose (IAEA-CH-6) and ammonium sulphate (IAEA-N-1) inter-laboratory comparison standards distributed by the International Atomic Energy Agency, Vienna.

CHAPTER FOUR

4. Results

4.1 Evaluation of Sample integrity

For this study, we prepared 25 human bone samples for collagen extraction. All the samples were individuals from the necropolis Arg El-Ghazouani (ARG). A total of 20 samples contained well-preserved collagen; in the other five, collagen did not meet quality indicators (Tab. 4.1). The collagen yield of the samples varies between 0.65% and 16.51% (table 4.1). The collagen yield of the excluded five samples was 0.26%, 0.38%, 0.27%, 0.31%, and 0.25%, as Van Klinken (1999) reported the excellent quality of collagen yield must be at least 0.5%, that's why these five samples were excluded from the carbon and nitrogen analysis, the evaluation of the collagen content in the samples is very fundamental to understand the quality of the samples.

ID	Tag name	Bone sample Weight(g)	Empty tube(g)	Tube with collagen (g)	Collagen (g)	Yield (%)
ARG - 1	T.1/05 C1	1.0031	4.3366	4.3538	0.0172	1.71
ARG - 2	T.20	0.9136	4.3115	4.3841	0.0726	7.95
ARG - 3	T.1/06 Ind B	0.9357	4.3655	4.3679	0.0024	0.26
ARG - 4	T.1/97. Ind D	0.9984	4.3019	4.4667	0.1648	16.51
ARG - 5	T.4/98 XD	1.0234	4.2888	4.3139	0.0251	2.45
ARG - 6	T.2/06 Ind A	1.0372	4.2696	4.2735	0.0039	0.38
ARG - 7	T.3/98 Busta E	1.3579	4.3054	4.3648	0.0594	4.37
ARG - 8	Kerkouane ARG No Indication	1.3132	4.3081	4.3166	0.0085	0.65
ARG - 9	T.1/10 Ind 2	0.9845	4.273	4.3188	0.0458	4.65
ARG - 10	T.1/06 Ind A	1.5924	4.313	4.3388	0.0258	1.62
ARG - 11	ARG Crania N°6	1.2237	4.3064	4.3097	0.0033	0.27
ARG - 12	T.1/97. Ind B	1.3261	4.3057	4.4197	0.114	8.60
ARG - 13	T.5/08	1.0938	4.292	4.3826	0.0906	8.28
ARG - 14	T.2/00 Crania 4B	1.0475	4.3595	4.5055	0.146	13.94
ARG - 15	T.2/00 Crania 4A	1.0769	4.3104	4.3137	0.0033	0.31
ARG - 16	T.4/98 XF	1.0615	4.3165	4.3276	0.0111	1.05

ARG - 17	T.3/98 Busta B, XD	1.2139	4.3108	4.3138	0.003	0.25
ARG - 18	T.3/98 XJ	1.094	4.2848	4.3021	0.0173	1.58
ARG - 19	T.1/97 Ind A	1.288	4.3419	4.3558	0.0139	1.08
ARG - 20	T.1/04 Ind B (Info)	1.0762	4.311	4.3569	0.0459	4.27
ARG - 21	T.1/97 Ind XH	1.0788	4.3022	4.3229	0.0207	1.92
ARG - 22	T.2/08 Ind A	1.1479	4.2862	4.3293	0.0431	3.75
ARG - 23	T.1/04 Ind A	1.0614	4.2973	4.3616	0.0643	6.06
ARG - 24	T.3/98 XI	1.0103	4.2962	4.3193	0.0231	2.29
ARG - 25	T.2/98 Ind D	1.0422	4.3142	4.3615	0.0473	4.54

Table 3.1. collagen extraction process weights and the percentage of collagen extracted from ARG samples. In red, the samples excluded from the analysis.

Nonetheless, Van Klinken (1999) mentioned that it was essential to recognise the integrity of the sample in order to see how qualified the data from the mass spectrometry for the isotopic analysis are afterward. Thus, one shall observe the indicators before we rely on the analysis of the samples; the first is making sure the mass spectrometry works appropriately. For doing that, the mean and standard deviation value of the laboratory standard and the samples materials should be close to ±0.1‰; so, in this case, the measurement of our samples is reliable.

The other indicator is the preservation of the bone collagen (protein composition) in our samples, assessing the C/N ratio; it can be seen in table 4.2 the C: N ratio of the ARG samples. The C:N values should be between 3.1 to 3.5 (Van Klinken, 1999). The ratio is calculated as the percentage of C by the percentage of N and multiplied by 14/12, which gives us an atomic ratio (ratio of the number of C atoms to the number of N atoms in the sample), with the following formula (Amt % C / Amt % N) × (14/12). As can be seen in table 4.2, all the 20 samples show a C/N ratio from 3.2 to 3.5, so all the samples' values are within the accepted range.

Sample code	δ^{13}CV-PDB	δ^{15}NAIR	C: N
ARG – 1	-18.87	12.13	3.5
ARG - 2	-19.91	13.73	3.3
ARG – 4	-19.4	11.76	3.2
ARG – 5	-18.94	11.51	3.3
ARG – 7	-18.95	12.09	3.2
ARG – 8	-18.86	12.69	3.5
ARG – 9	-19.64	11.05	3.3
ARG – 10	-19.4	12.19	3.4
ARG – 12	-18.71	11.96	3.3
ARG – 13	-18.95	11.67	3.3
ARG – 14	-19.53	11.46	3.2
ARG – 16	-19.16	11.9	3.4
ARG – 18	-19.09	12.5	3.3
ARG – 19	-19.17	13.47	3.4
ARG – 20	-19.32	11.38	3.3
ARG – 21	-19.26	12.43	3.4
ARG – 22	-19.35	11.28	3.3
ARG – 23	-19.28	12.13	3.3
ARG – 24	-18.72	11.86	3.3
ARG – 25	-18.52	11.86	3.2

Table 4.2. the stable carbon and nitrogen isotope composition and related C: N ratio of ARG samples.

4.1. The Carbon and Nitrogen Content (%)

A total of 20 samples out of 25 were analysed in this thesis; five samples were excluded due to the poor collagen quality yield. Another quality indicator to evaluate is the carbon and nitrogen content. Since as Van Klinken, (1999) mentioned, the carbon content value with preserved collagen should be >35%. The carbon content percentage ranges from 33.12% (ARG-1) to 47.99% (ARG-23), with a mean value of 46.35%. Therefore, all the samples for this study were almost >35%. Since the carbon percentage content is used as a signal of sample integrity, all the samples in this thesis should be reliable.

The nitrogen content, according to Van Klinken (1999), for archaeological bone collagen should be considered when the values are between 11 and 16 wt%. The nitrogen content percentage values of the Arg El - Ghazouani samples are between 11.15% (ARG-1) and 17.19% (ARG-23), with a mean value of 13.95%. In this manner, the nitrogen content of our samples is reliable too. Both carbon and nitrogen content percentages are shown in table 4.3.

Sample code	**Carbon Content %**	**$\delta^{13}C$V-PDB ‰**	**Nitrogen Content %**	**$\delta^{15}N$AIR ‰**	**C: N**
ARG – 1	33.12	-18.87	11.15	12.13	3.5
ARG - 2	47.68	-19.91	17.02	13.73	3.3
ARG – 4	43.62	-19.4	16.09	11.76	3.2
ARG – 5	36.39	-18.94	12.87	11.51	3.3
ARG – 7	47.04	-18.95	17.02	12.09	3.2
ARG – 8	43.77	-18.86	14.79	12.69	3.5
ARG – 9	41.77	-19.64	14.90	11.05	3.3
ARG – 10	39.24	-19.4	13.51	12.19	3.4
ARG – 12	37.55	-18,71	13.44	11.96	3.3
ARG – 13	35.62	-18.95	12.78	11.67	3.3
ARG – 14	43.16	-19.53	15.60	11.46	3.2
ARG – 16	40.29	-19.16	13.95	11.90	3.4
ARG – 18	47.68	-19.09	16.92	12.50	3.3
ARG – 19	34.91	-19.17	11.85	13.47	3.4
ARG – 20	46.62	-19.32	16.35	11.38	3.3
ARG – 21	43.28	-19.26	14.79	12.43	3.4
ARG – 22	41.45	-19.35	14.79	11.28	3.3
ARG – 23	47.99	-19.28	17.19	12.13	3.3
ARG – 24	39.33	-18.72	14.08	11.86	3.3
ARG – 25	45.25	-18.52	16.35	11.86	3.2

Table *0*. the carbon and nitrogen content percentage of Arg El-Ghazouani

4.2 δ13C and δ15N results of Arg El-Ghazouani samples

The $\delta^{13}C$ isotopes values range between -19.91‰ (ARG-2) and -18.52‰ (ARG-25) with a mean value of -19.15‰, from all the entire samples ARG- 2 shows the lowest $\delta^{13}C$ value recorded (-19.91‰) (Tab. 4.4). The $\delta^{15}N$ values ranged between 11.05‰ (ARG-9) and 13.73‰ (ARG-2) with a mean value of 12.05‰, as is shown in table 4.4. The $\delta^{13}C$ and $\delta^{15}N$ range values are respectively 1.39‰ and 2.86 ‰, indicating a higher variability among N values.

Sample code	$\delta^{15}N_{AIR}$	$\delta^{13}C_{V-PDB}$	C: N ratio	AGE	TOMB	SEX
ARG – 1	12.13	-18.87	3.5	20-24 yo	1	Female
ARG - 2	13.73	-19.91	3.3	30-34 yo	20	Female
ARG – 4	11.76	-19.4	3.2	27-33 yo	1	Male
ARG – 5	11.51	-18.94	3.3	35-50 yo	4	Female
ARG – 7	12.09	-18.95	3.2	30-35 yo	3	N.D
ARG – 8	12.69	-18.86	3.5	20-25 yo	No indication	Male
ARG – 9	11.05	-19.64	3.3	4-5 yo	1	Male (?)
ARG – 10	12.19	-19.4	3.4	20-25 yo	1	Female
ARG – 12	11.96	-18,71	3.3	50 yo	1	Male
ARG – 13	11.67	-18.95	3.3	30-35 yo	5	N.D
ARG – 14	11.46	-19.53	3.2	6-7 yo	2	Male (DNA)
ARG – 16	11.90	-19.16	3.4	30-50 yo	4	Female
ARG – 18	12.50	-19.09	3.3	25-50 yo	3	N.D
ARG – 19	13.47	-19.17	3.4	30-35 yo	1	Male
ARG – 20	11.38	-19.32	3.3	5-6 yo	1	N.D
ARG – 21	12.43	-19.26	3.4	20-25 yo	1	N.D
ARG – 22	11.28	-19.35	3.3	9-11 yo	2	N.D
ARG – 23	12.13	-19.28	3.3	16-20 yo	1	N.D
ARG – 24	11.86	-18.72	3.3	40-50 yo	3	Female
ARG – 25	11.86	-18.52	3.2	20-30 yo	2	Female
Mean value	12.05	-19.15	3.2			
Standard Deviation	0.67	0.34	0.1			

Range value	2.86	1.39

Table *0*stable carbon and nitrogen values of necropolis Arg El-Ghazouani with osteological and archaeological information. N.D= not determined.

The following graph (Fig 4.1) shows the $\delta^{13}C$ and $\delta^{15}N$ bone collagen stable isotopes values of necropolis Arg El-Ghazouani human bone samples.

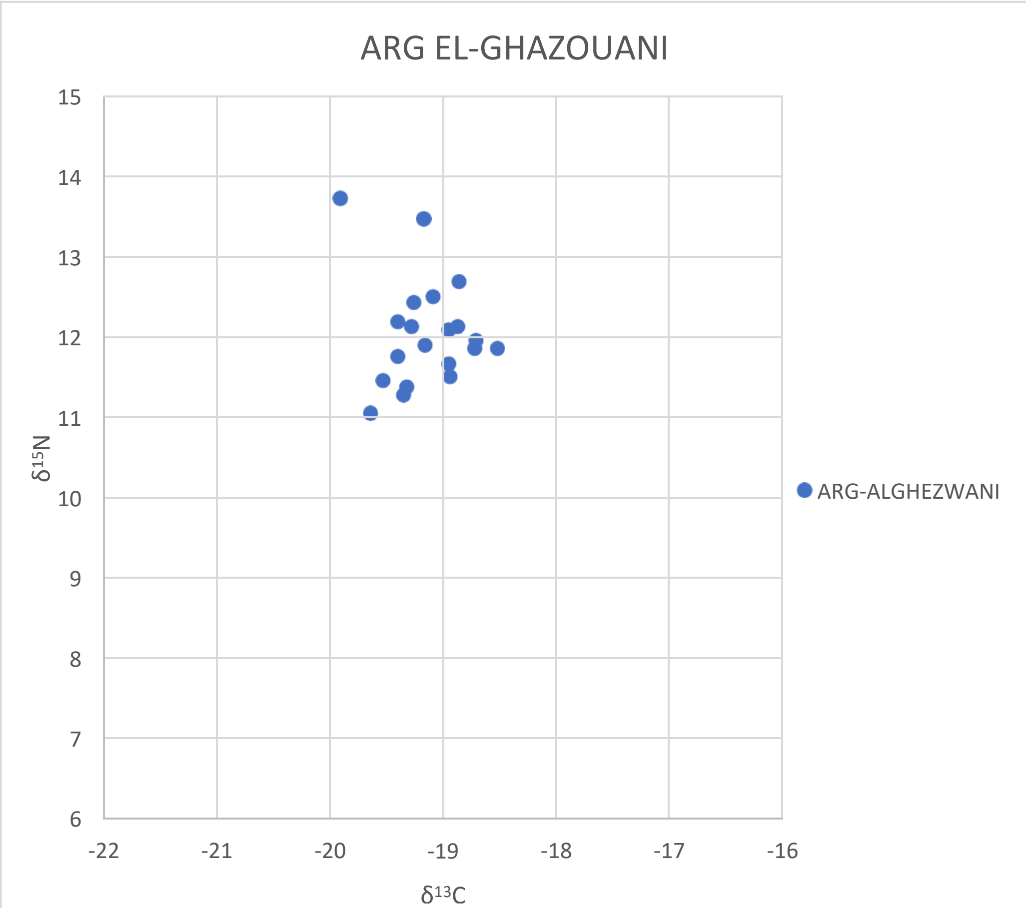


Fig 22. the $\delta^{13}C$ and $\delta^{15}N$ measured in bone collagen at Arg El-Ghazouani.

4.3 Isotopic variation in sex, age, funerary features

Among the 20 analysed individuals, there are six males, seven females, and seven not determined individuals. In the sample ARG-14, the sex is determined by a DNA study by the Laboratory of the biology of the ancient population by prof. Alfredo Coppa (Unpublished) (Tab. 4.4). The $\delta^{13}C$ isotope values of male individuals' range between -19.64‰ to -18.71‰ with a mean value of -19.17‰, and the female isotope value range between -19.91‰ to -18.52‰ with a mean value of -18.94‰, and for the not determined samples, the $\delta^{13}C$ isotope values range between -19.35‰ to -18.95‰ with a mean value -19.27‰. Likewise, the $\delta^{15}N$ isotope values of females range between 11.51‰ to 13.73‰ with a mean value of 11.90‰, the N male isotope values range between 11.05‰ to 13.47‰ with a mean value of 11.96‰, and lastly for the not determined individuals, the $\delta^{15}N$ isotope value range between 11.28‰ to 12.43‰ with a mean value of 11.88‰. The $\delta^{13}C$ and $\delta^{15}N$ isotope values of male, female, and the not determined are shown in figure 4.2

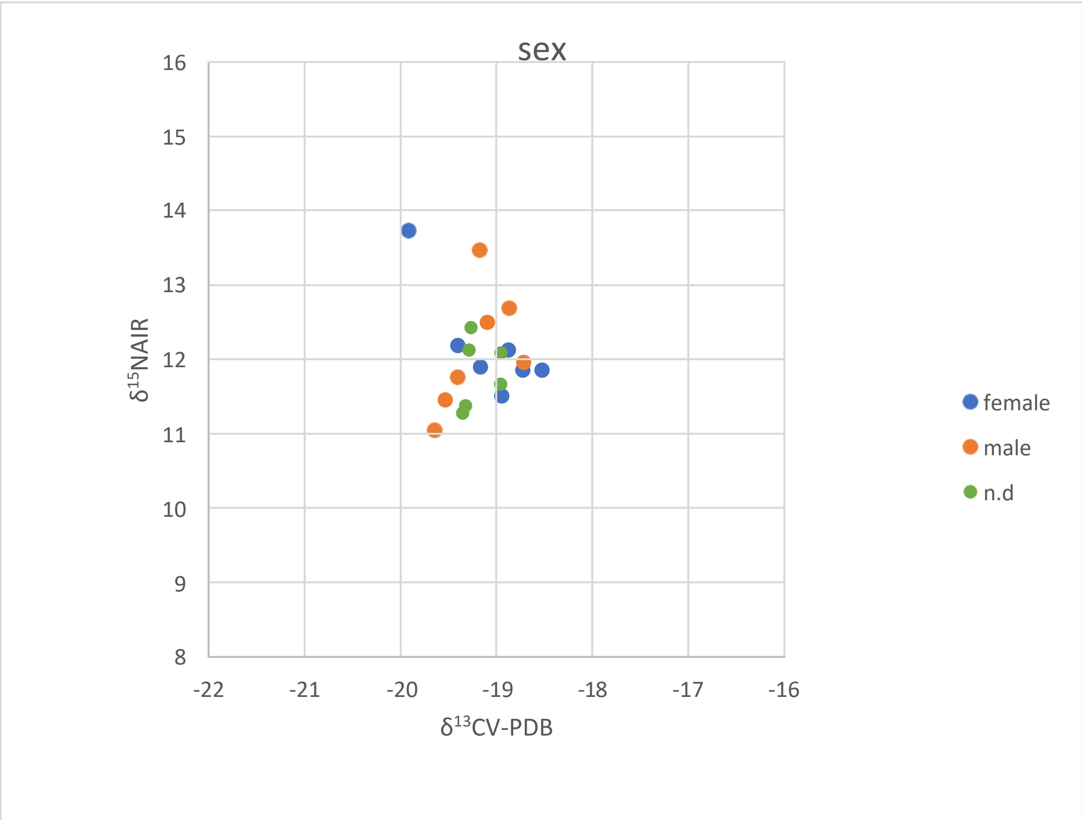


Fig 4.23. The $\delta^{13}C$ and $\delta^{15}N$ isotope values of male (orange), female (blue), and not determined (green).

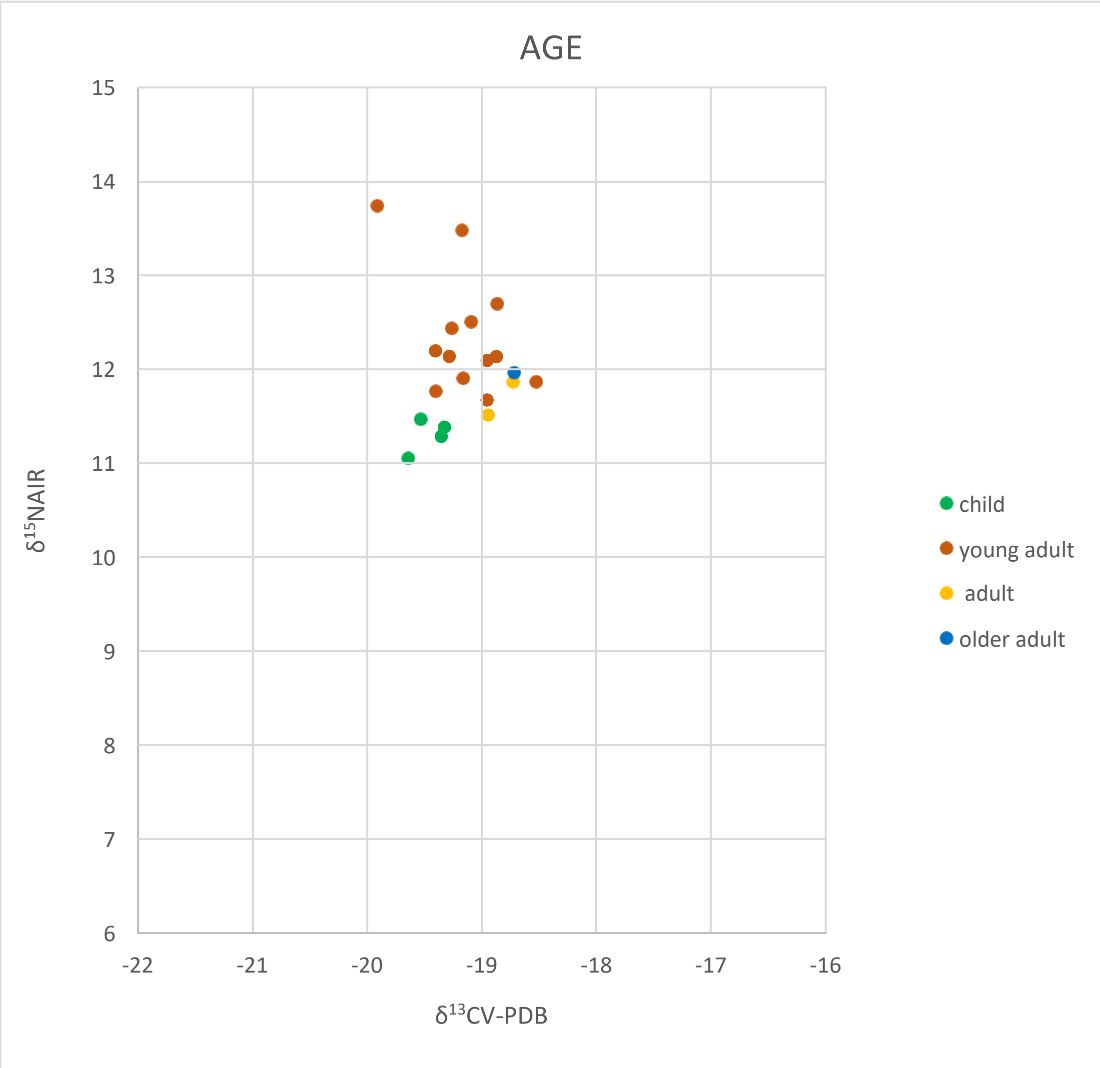


Fig 24 The δ^{13}C and δ^{15}N isotope values of individuals according to age classification.

The classification of the age group samples is arranged into a child (0-11 years old), young adult (16-35 years old), adults (35-50 years old), and finally older adults (50+ years old) (Fig. 4.3). The δ^{13}C isotope values of the child ranged from -19.64‰ to -19.32‰, with a mean value of -19.35‰. For the young adults, the values ranged between -19.91‰ to -18.52‰, with a mean value of -19.16‰. And the adults are -18.94‰ to -18.72‰ with a mean value of -18.83‰; finally, the only older adults are -18.71‰. Likewise, the δ^{15}N isotope values of the child are ranged from 11.05‰ to 11.46‰, with a mean value of 11.38‰; and the young adults with values ranging from 11.90‰ to 12.96‰ with a mean value of 12.13‰. The adults show δ^{15}N values range from 11.51‰ to 11.86‰ with a mean value of 11.69‰, and the only older adult δ^{15}N is 11.96‰.

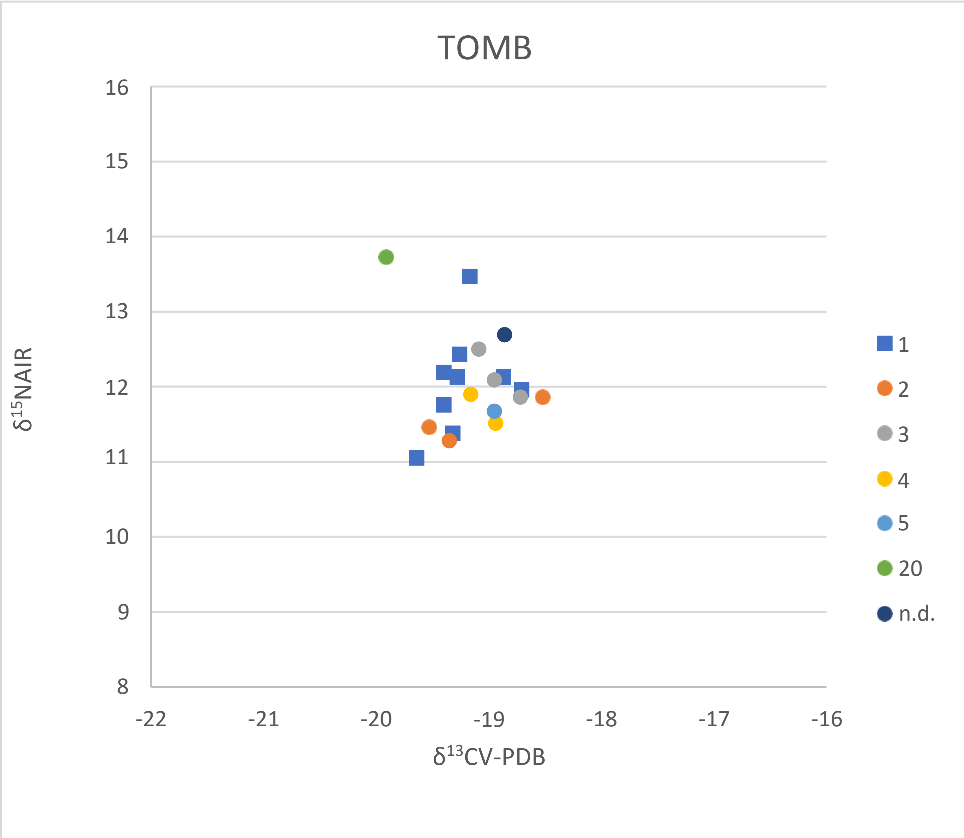


Fig 25 the $\delta^{13}C$ and $\delta^{15}N$ isotope values of individuals according to the tombs of provenience.

To see if there were any isotopic differences according to the funerary ritual, the samples are represented in figure 4.4, highlighting the tomb of provenience.

The $\delta^{13}C$ isotope values of the individuals from tomb one (n= 9) range between -19.64‰ to -18.71‰, with a mean value of -19.30‰. The values from samples of the tomb two (n=3) range between -19.53‰ to -18.52‰, with a mean value of -19.00‰. The C values of individuals from tomb three (n= 3) range between -19.09‰ to -18.72‰ with a mean value -18.95‰. The values from tomb four (n= 2) range -19.16‰ to -18.94‰ with a mean value -19.05‰, while the individuals from tomb five (n=1), twenty (n=1), and the not determined tomb (n=1) are respectively -18.95‰, -19.91‰ and -18.86‰. Likewise, the $\delta^{15}N$ isotope values of tomb one (n=9) are 11.05‰ to 13.47‰, with a mean value of 12.13‰. Tomb two (n=3) show N values from 11.28‰ to 11.86‰ with a mean value of

11.03‰, individuals from tomb three (n=3) N values range from 11.86‰ to 12.50‰ with a mean value of 12.09‰. Tomb four (n=2) individuals show N values from 11.51‰ to 11.90‰ with a mean value of 11.71‰. From tomb five and tomb twenty, we had only one individual to analyse, as for the not determined tomb, their $\delta^{15}N$ isotope values are respectively 11.67‰, 13.73‰, and 12.69‰.

The statistics analysis was not undertaken because the sample is not numerous enough for a reliable statistical evaluation.

4.4 the δ13C and δ15N isotopes values from another site in Tunisia

Due to covid 19 restrictions, we could not visit the main site in Kerkouane to collect the available fauna, necessary to discuss and frame the human isotopes values above reported. So, we took into consideration the available comparable data in the literature.

The comparison is made between Kerkouane and another site in Tunisia called Leptiminus; for understanding the diets in the necropolis of Arg El-Ghazouani, the available isotopic data used came from the ancient Roman city of Leptiminus, which is located on the Mediterranean coast of Lamta town, Tunisia (Keenleyside et al., 2009). since we could not collect the animal bones samples from the Arg El-Ghazouani site; additionally, due to the lack of isotopic data from Kerkouane surrounding and contemporary sites, the data was used in this thesis for comparison came from the Leptiminus site instead of the other isotopic study available for Tunisian coast from Theodosia wall cemetery site in Carthage, dated to the vandalic period (5th-6th centuries A.D.) (Ma et al., 2021). Thus, Leptiminus was the more comparable isotopic study and the closest site in terms of temporal proximity to Kerkouane, making the comparison almost acceptable if considering all the chronological and geographical differences between the two sites.

In fact, comparative data means similar ecology and chronology. Still, the chronological period is slightly different from the Punic city of Kerkouane, dated between the 4th-6th century B.C. (MH.

Fantar, 1984), while Leptiminus is a Roman city dated primarily from the 2nd and 3rd centuries A.D. (Keenleyside et al., 2009), with a second occupation from late 4th to 5th centuries A.D. We chose the data from the first phase because of the minor period difference between the two sites; they have about seven to nine centuries of difference; the chronological difference between the two periods make the comparison not entirely reliable, but in the absence of contemporary contexts, it can give us an overview of the sites because they are both situated on the same coastal site, and in similar environments; however, the distance between Leptiminus and Kerkouane is approximately 250km (Fig. 4.5). All these aspects will be taken into account in the following data comparison.

The samples chosen for the comparison came from three separate cemeteries from the same city of Leptiminus dated from the 2nd and 3rd centuries A.D., identified as sites number 10, 200, and 304. The excavations of site 10 began in 1989 and 1990; further excavation took place in 1991. While Site 200 was excavated in 1992, and Site 304 was excavated from 2004 to 2006 (Keenleyside et al., 2009). Leptiminus also presents isotopic data from different species, primarily domestic, which will be essential for interpreting our human data from Kerkouane.

Fig 26 The geographical location of the city of Kerkouane (blue dot) and the location of the Leptiminus site in the town of *Lamta* (yellow dot). (modified from google earth).

The $\delta^{13}C$ isotope values of the fifty-two individuals from the comparable chronological phase from Leptiminus ranged between -19.02‰ to -16.09‰, with a mean value of -17.85‰, and the $\delta^{15}N$ isotope values ranges between 8.40‰ to 16.09‰, with a mean value of 12.83‰ (Tab. 4.5). The range values of the $\delta^{13}C$ and $\delta^{15}N$ are respectively 2.70‰ and 8.50‰.

The $\delta^{13}C$ isotope values of the eight faunal samples range between -21.10‰ to -18.20‰, with a mean value of -19.41‰, and the $\delta^{15}N$ isotope values ranged between 6.00‰ to 12.90‰, with a mean value of 8.54‰ (Tab. 4.6). The range value of $\delta^{13}C$ and $\delta^{15}N$ of the fauna is of 2.90‰ and 6.90‰, respectively.

Graph 4.6 shows the $\delta^{13}C$ and $\delta^{15}N$ of the human individuals from Arg El-Ghazouani and the faunal from Leptiminus, which will be discussed in the following chapter.

I.D. no.	**Site**	**$\delta^{13}C_{co}$‰**	**$\delta^{15}N_{co}$‰**
139	10	-18.3	11.9
766	10	-18.1	13.7
838	10	-18.0	12.6
1020	10	-18.2	14.4
1033	10	-18.8	11.5
1222	304	-18.4	14.7
1312	304	-17.6	11.7
1315	304	-17.2	14.8
1327	304	-17.2	16.9
1335	304	-17.4	11.3
1349	304	-18.4	13.5
1440	304	-17.1	13.0
1472	304	-18.1	11.4
1537	304	-17.8	11.7
1538	304	-17.3	15.0
1564	304	-17.2	15.7
1652	304	-17.6	12.9
1748	304	-18.9	14.0
1759	304	-18.6	11.5
1830	304	-18.6	12.2
1836	304	-18.1	15.1
1901	304	-16.5	13.4
2027	304	-16.5	13.1
2034	304	-17.9	12.6
2046	304	-17.9	12.0
2135	304	-19.0	10.7

2155	304	-17.4	13.0
3067	200	-17.9	13.4
3073	200	-18.8	10.5
3089	200	-18.0	13.7
3098	200	-17.1	12.5
3115	200	-17.9	15.5
3117	200	-17.5	12.9
3178	200	-18.5	115
3193°	200	-17.6	12.7
3199	200	-18.1	10.4
3211°	200	-17.8	12.4
3231	200	-16.7	13.3
3247	200	-19.2	8.4
3270	200	-17.7	10.0
3315	200	-16.9	12.2
3341	200	-18.0	11.5
3342	200	-18.6	13.7
3345	200	-18.4	12.8
3392	200	-18.2	12.1
3408	200	-18.0	13.1
3421°	200	-17.0	12.6
3421b	200	-17.7	13.7
Amph. K	200	-17.0	15.6
Average		-17.85	12.83
SD		0.66	1.62
Range Value		2.70	8.50

Table 0isotope values of human samples (%) dated from the 2nd and 3rd centuries A.D., from Leptiminus, Tunisia (Keenleyside et al., 2009).

I.D. no.	Species	$\delta^{13}C_{co}$	$\delta^{15}N_{co}$
5	Cow	-18.8	10.3
6	goat	-18.3	6.2
2	hare	-18.2	6.0
3	sheep	-21.10	10.4
7	sheep	-19.8	12.9
1	sheep/Goat	-19.1	6.0
8	equid	-21.00	6.90
4	pig	-19.00	9.60
average		-19.41	8.54
SD		1.13	2.61
Range Value		2.90	6.90

Table *0* Isotope values of faunal samples (‰) in Leptiminus (Keenleyside et al., 2009).

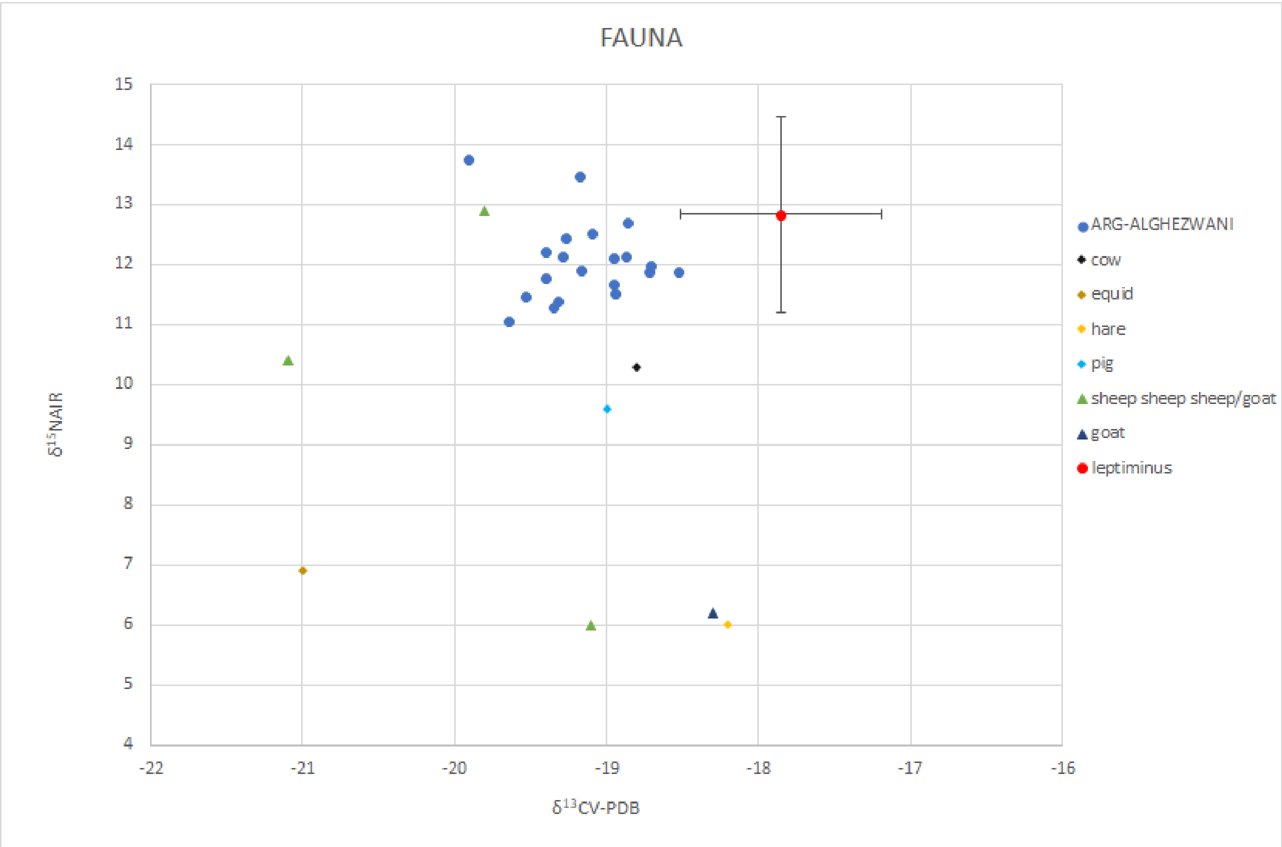


Fig 27 the $\delta^{13}C$ and $\delta^{15}N$ isotope values of the human individuals from Arg El-Ghazouani and the faunal from Leptiminus.

CHAPTER FIVE

5. discussions

5.1 Diet in Kerkouane

Ambrose (1990) holds $\delta^{13}C$ isotope values are used to draw a distinction between C3 and C4 plants consumption and a marine versus terrestrial diet. The $\delta^{13}C$ values of C3 plants range from -36 ‰ to -19 ‰ (Smith & Epstein, 1971). This means that a C3 consumption can be assumed with a $\delta^{13}C$ mean value of -19.15‰ (see table 4.4, chapt. 4). A botanical study carried out on samples found in the Punic harbor in Carthage, which dates back to the middle of the 4th century BC (Zeist, 2001), suggest consumption of the following of C3 plants: emmer wheat (*Triticum dicoccum*) and barley (*Hordeum*) (Zeist, 2001). In contrast, the $\delta^{13}C$ values of C4 plants range from -17‰ to -9‰(Smith & Epstein, 1971). No significant indication of C4 plants consumption (such as millet) has been found in the ARG samples; most importantly, there is no data about C4 plant consumption among Punic people in Kerkouane so far.

The $\delta^{15}N$ isotope values of Kerkouane ARG samples have a range of 2.86 ‰, which indicates a higher variability among N values. Based on these values, it is not possible to hypothesize a high consumption of marine resources, though as shown in fig. 5.1, the values of the three samples ARG 12 (-18.71‰, 11.96‰), ARG 24 (-18.72‰,11.86‰) and ARG 25 (-18.52‰, 11.86‰) suggest that said resources may have been consumed more than occasionally. From a zooarchaeological perspective, evidence has been found of marine resources among the Punic population; said resources include fish, namely gilt-head sea bream (*Sparus aurata*), tuna (*Thunnus sp*.,), probable grouper *(Serranidae*), and a European sea bass (*Dicentrarchus labrax).* Mollusks have been found as well (Moses et al., 2019). Clear evidence on the consumption of marine resources in Kerkouane can be obtained from a more in-depth analysis of local faunal remains, also considering its proximity to the coast.

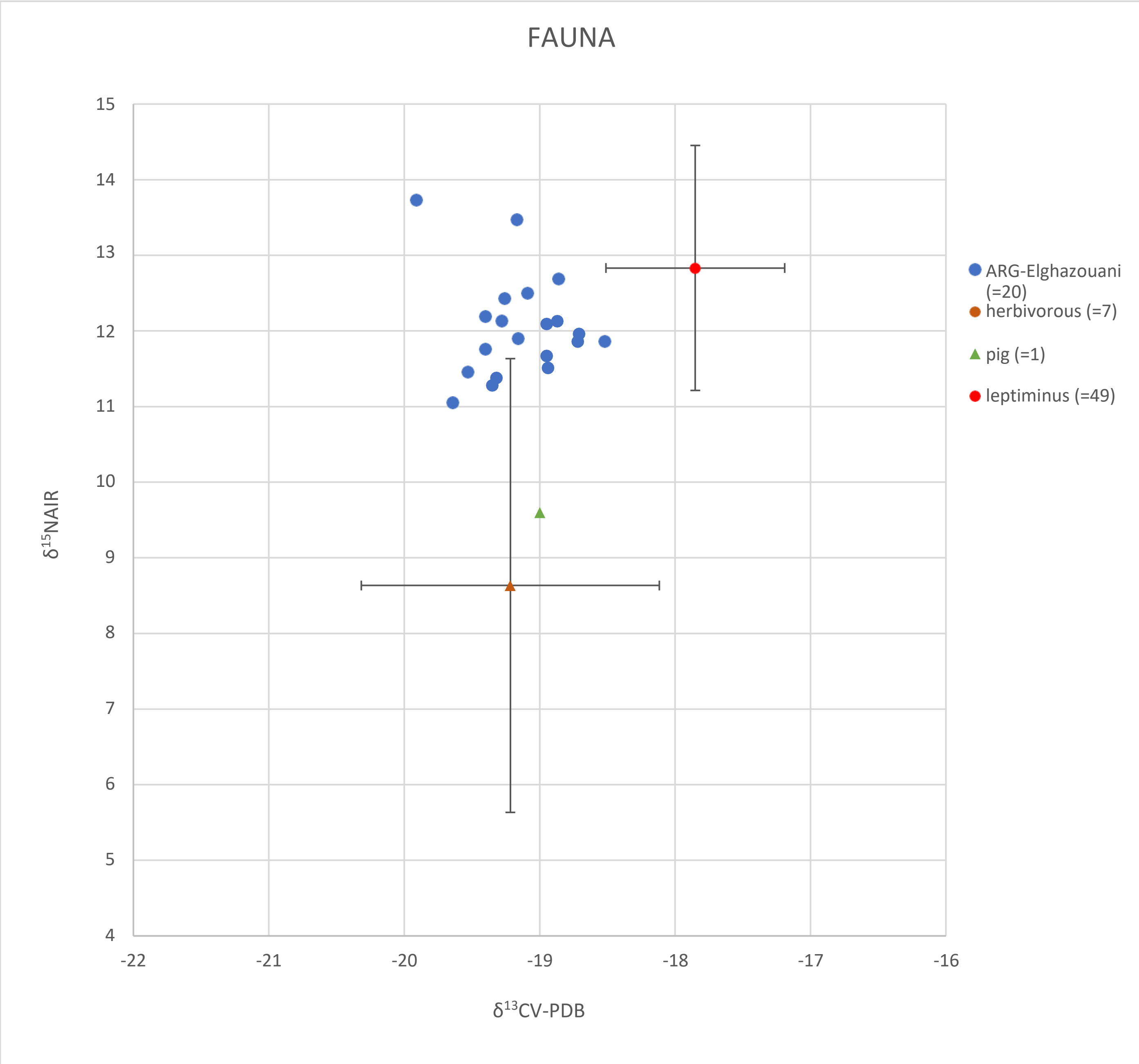


Fig 28. δ^{13}C and δ^{15}N values of Arg El-Ghazouani human samples with the comparative human and faunal data from the Leptiminus site (Keenleyside et al., 2009).

Given the absence of faunal remains or previous isotopic data from Kerkouane, the isotopic data used for analyzing animal consumption in Kerkouane comes from the ancient Roman site of Leptiminus, which is located on the Mediterranean coast of Lamta town, Tunisia (Keenleyside et al., 2009), though not contemporaneous or close to Kerkoune (see chap. 4).

A known trophic enrichment shift (+3-5‰ for N and +1‰ for C) should be detected in humans (DeNiro & Epstein, 1978) when compared to the values of animal species ideally consumed. Indeed, the comparison between the Arg El-Ghazouani and Leptiminus sites revealed said trophic enrichment shift: the animal species eaten in Leptiminus were sheep, goats, cows, pigs, hares, and equids (see table 4.6, chapt.4).

The C and N human-herbivore offset (sheep, goats, hares, and cows) in Arg samples are 0.07‰ and 3.42‰, respectively, which is typical of mixed terrestrial resources consumers (as suggested by the enrichment). According to several zooarchaeological studies (Cardoso et al., 2016; Moses et al., 2019), herbivores (sheep, goats, and cows) were the most common food source during the Punic time in Tunisia. The C and N human-omnivore offset (pig) is 0.15‰ and 2.45‰, respectively; in this case, the enrichment suggests that pigs were not highly consumed among the analysed individual from Arg El-Ghazouani, zooarcheological evidence from two other punic sites (Zita and Utica) suggest that the pig was not highly consumed during the punic time (Cardoso et al., 2016; Moses et al., 2019). Unlike during Roman time, where consuming pigs became more common (Keenleyside et al., 2009).

5.2 The Isotopic variation in relation to sex, age, funerary features

5.2.1 Variation by Sex

The mean $\delta^{13}C$ and $\delta^{15}N$ isotope values of females (=7) are -18.94 ± 0.47‰and 11.90 ± 0.72‰, respectively, whilst for males (=7) they are -19.17 ± 0.31‰, and 11.96 ± 0.82‰, respectively. When it comes to not determined individuals (=6), the $\delta^{13}C$ and $\delta^{15}N$ values are -19.27 ± 0.18‰, and 11.88 ±0.46‰, respectively. Fig 4.2 shows the $\delta^{13}C$ and $\delta^{15}N$ isotope values of males, females, and the not determined. Nevertheless, the difference in N values between males and females is very low (0.06‰), while in C, it amounts to 0.23‰. There are no significant sex differences in the $\delta^{13}C$ and the $\delta^{15}N$

values; this could mean that females and males consumed almost the same food or that differences in their diets could not be detected isotopically.

5.2.2 Variation by age

The mean $\delta^{13}C$ and $\delta^{15}N$ isotope values of children (=4) are -19.44 ± 0.15‰, and 11.33 ±0.18‰, respectively. When it comes to young adults (=13), the values are -19.16 ± 0.34‰, and 12.13 ±0.63‰, respectively. The isotope mean values of adults (=2) are -18.83±0.16‰ and 11.69 ±0.25‰. Nevertheless, the difference in N values between young adults and adults amounts to 0.44‰, while that in C values amounts to 0.33‰. Therefore we can say that no significant differences in the diet of young adults and adults were detected in ARG samples. Moreover, the fact that only one sample has been collected for older adults (+50 years old) prevents the evaluation of dietary changes between younger adults and adults. Fig. 4.3 shows the $\delta^{13}C$ and $\delta^{15}N$ isotope values of individuals according to age.

The difference in N values between children and young adults is 0.80‰, while that in C values is 0.28‰; moreover, the difference between the N and C values of children and adults is 0.36‰ and 0.61‰, respectively. Indeed, young adults show higher values than children (see fig, 4.3). The decrease in the $\delta^{15}N$ value of children down to levels similar to those of young adults and adults could represent a massive change in diet habits, in fact, it has been seen that the $\delta^{15}N$ values will decrease when infants are introduced to a new dietary system after weaning (Fogel et al., 1997). However, the small number of child samples compared to young adults will not produce significant diet differences.

5.2.3 Variation by the tomb of provenience

The mean $\delta^{13}C$ and $\delta^{15}N$ isotope values of individuals from tomb one (=9) are -19.28 ±0.28‰ and 12.13 ±0.68‰, respectively. Those from tomb two (=3) are -19.35±0.54‰ and 11.46±0.30‰, while those of tomb three (=2) and four (=2) are -18.95±0.19‰,12.09 ±0.32‰, and -19.05 ±0.16‰, 11.71± 0.28‰, respectively. Nevertheless, the difference in N between tomb one (the most numerous) and tomb two amounts to 0.67‰, while it is 0.07‰ when it comes to C. When it comes to a comparison with tombs three and four, the difference is 0.38‰ in terms of N and 0.1‰ in terms of C.

According to a tomb-based criterion, no significant diet differences have been found among the individuals of Arg El-Ghazouani samples. Nevertheless, the presence of only one sample in tombs five and twenty will make it difficult to evaluate the dietary changes among the individuals in the other tombs. Further studies and analyses on an adequate number of samples will provide a clearer picture of variation in diet among all the individuals in Kerkoune. Fig. 4.4 shows the $\delta^{13}C$ and $\delta^{15}N$ isotope values of individuals according to a tomb-based criterion.

In conclusion, the data in this thesis suggests the absence of significant diet differences among the males and females of the Kerkoune community. People were likely consuming or having access almost to the same food resources. Also, no age-based differences have been found in this study; although, the small number of samples we have (especially concerning children) may make the construction of a theory about the diet habits inside the community of Kerkoune complex.

No considerable differences in dietary sources were found at a tomb level either. However, more samples would be needed to see if any diet differences can be detected among the individuals and to try and understand who had a higher social status inside the society together with the archeological finds inside the tombs; this isotopic data could support the hypotheses and theories about social status in Kerkoune (Mh Fantar, 1986). However, the small number of samples included in this thesis project cannot produce a reliable overview of the social life of the population of Kerkoune.

5.3 Comparison with other site

As stated before, faunal remains in Kerkouane could not be sampled due to Covid-19 restrictions, owing to the lack of comparable isotopic data from sites surrounding Kerkouane, previously published isotopic data from Leptiminus (Keenleyside et al., 2009) was used (see table 4.6). Although they are separated by centuries, Leptiminus is the closest site in terms of temporal proximity to Kerkouane, of which we have available isotopic data. Nevertheless, a direct comparison was difficult to make, as no other site has isotopic data recorded from the same period as Kerkouane. However, the isotopic data used for comparisons between two sites divided by this range of space and time can entail significant problems with regard to the natural variability related to the changes in the isotopic baseline, which can lead to obstacles in identifying dietary variations between different populations.

The mean $\delta^{13}C$ values of the Leptiminus site (see graph 5.2) suggest that people consumed C3 plants. No significant presence of C4 plants consumption was found. Based on the archeological evidence that has been found at the site, C3 plants and products such as bread, wine, cheese, eggs, and olive oils formed part of the Leptiminus population diet (Keenleyside et al., 2009). In addition, there is no telling whether legumes or C4 plants were an essential dietary source for humans or the fauna of Leptiminus; in fact, six legume seeds have been discovered, which cannot suggest legumes being a dietary source. Spurr (1983) said that C4 plants (millet) were a source of animal fodder in many sites in Northern Africa, but this does not seem to apply to Tunisia (Keenleyside et al., 2009).

The mean $\delta^{15}N$ values (see graph 5.2) suggest that a significant part of the local diet in Leptiminus comes from marine resources. Indeed, the tanks that were used for fish processing in Leptiminus (Mattingly et al., 2000) and the site's coastal position could entail that residents of Leptiminus included marine resources in their diet(Keenleyside et al., 2009).

Graph 5.2 shows that the human samples of Leptiminus have higher N values than those of Kerkoune. People from Leptiminus could have had a higher protein consumption than those from Kerkoune.

Though Kerkoune is also located on the coast, it was not possible to theorize a high consumption of marine resources in this study. Still, the inhabitants of Kerkoune might have had different food consumption approaches; maybe, dietary differences may have been due to environmental and ecological changes that occurred during the centuries. Furthermore, the analysis of marine remains should provide us with a better overview of the dietary system of the inhabitants of Kerkoune.

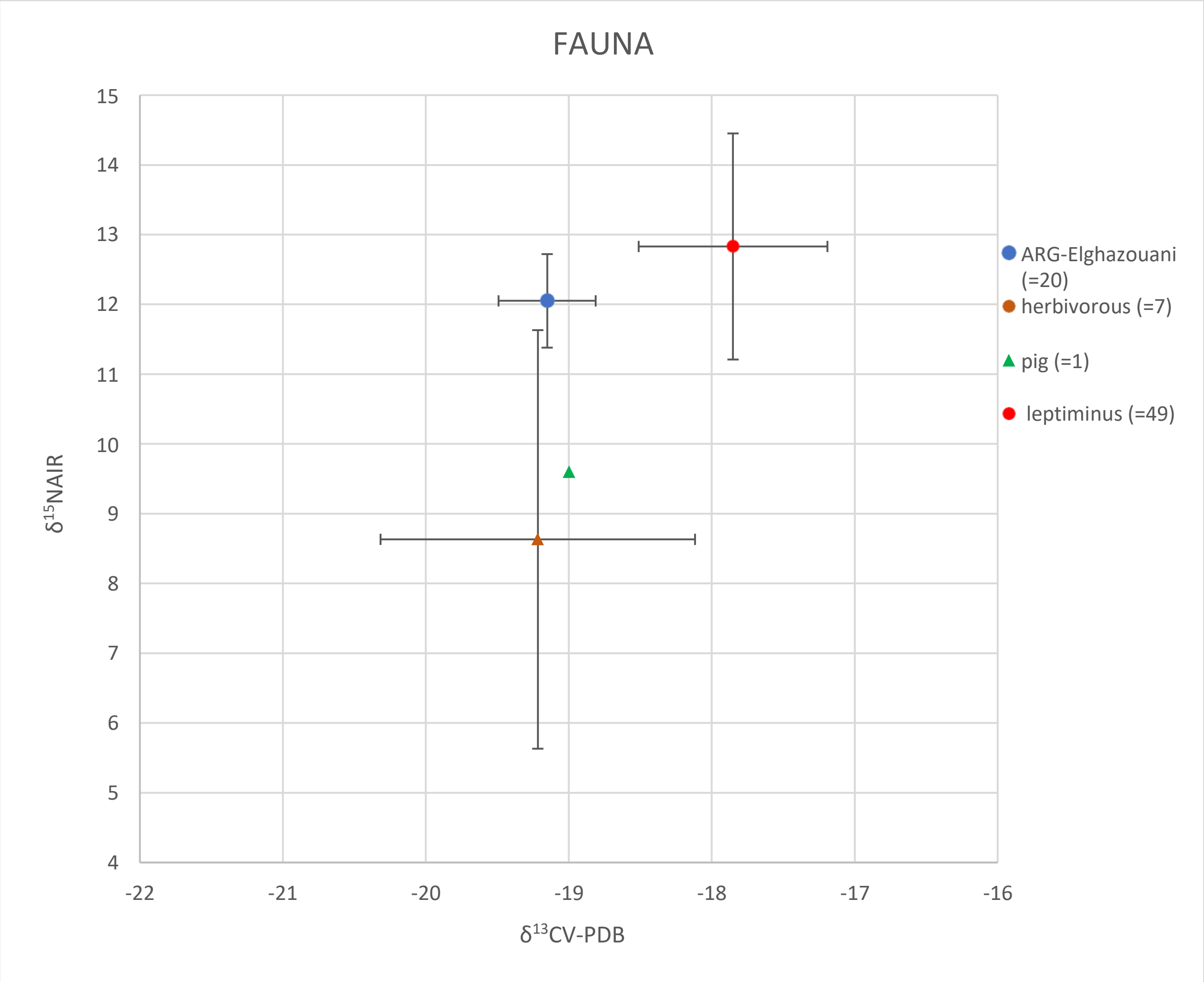


Fig 5.29. $\delta^{13}C$ and $\delta^{15}N$ values of Arg El-Ghazouani human samples with the comparative human and faunal data from the Leptiminus site (Keenleyside et al., 2009).

5.4 paleo diet of Tunisian communities during ancient Phoenician-Punic times

Knowledge of the diet of ancient Tunisian communities has been acquired from historical texts, archaeological evidence, and zooarchaeological and botanical remains. Though what food was available at that time can be inferred from said sources, these cannot yet precisely indicate the kind or quantity of food that was consumed at that time; in addition to these resources, the analysis of the stable isotope of human and animal remains has become a popular method for directly identifying the source of food consumed in ancient times.

However, several Tunisia-based studies have provided a great wealth of knowledge about ancient diet: witness the zooarchaeological evidence collected from the Punic and Roman site in the urban mound of Zita, south-east Tunisia (Moses et al., 2019). This archaeological site was occupied from around 500 BCE to the Roman period, dating back to the mid-5th century CE (Moses et al., 2019). Zita was a Carthaginian industrial colony founded in the early 6th century BCE that became even more industrial during the Roman period around 50 CE (Kaufman, Drine, Barnard, & Khedher, 2015; Moses et al., 2019). According to the evidence collected there, meat was a crucial part of the Zita community diet, just like the other ancient communities in Tunisia and North Africa; based on faunal remains, sheep and goat were the primary sources of meat, together with mollusks, fish, cattle, chicken, and pig. According to King (1999) and Mackinnon (2010, 2017), pigs were more common in the Roman period than sheep/goats were in Carthage and other North African sites during the Punic period. However, wild animals like hares and birds were also part of the diet. As Zita was on the coast, marine sources were likely to be the most consumed. Still, this area's evidence and faunal remains suggest that terrestrial sources were also an essential part of their diet. Unlike pigs and cattle, livestock such as sheep and goats require less water, which is ideal in a semi-arid climate.

Nevertheless, in spite of the many obstacles posed by the environment, raising pigs was a common choice in Carthage during the Roman period; even though Carthage is wetter and probably used to be wetter than Zita, breeding pigs was not ideal in either site. It shows that culture is more influential than the environment when it comes to meat consumption. Furthermore, the faunal samples found in Zita indicate that meat consumption habits persisted over time despite political changes(Moses et al., 2019).

Additional zooarchaeological studies carried out at the Phoenician settlement of Utica, northern Tunisia, in the 19th and early 20th century, brought to light a Phoenician-Punic necropolis (Cardoso et al., 2016); additional studies conducted in Utica in 2003 (Ben Jerbania and Redissi, 2014) and 2010 (López Castro et al., 2012, 2014, 2015, 2016) unearthed an early Phoenician settlement. However, the faunal remains found in Utica suggest that meat consumed came from *Bos taurus* (domestic oxen), *Ovis/Capra* (sheep/goat), *Sus sp* (Suidae, essentially domestic), *Equus caballus* (horse), and *Canis familiaris* (domestic dog). Nevertheless, domestic cattle (adult bovids) remained the main component of the Utica diet; according to Antunes (1991), these weighed between seven and nine times more than adult sheep or goats(Cardoso et al., 2016).

Indeed, these species were a common source of protein and milk; cattle could also pull carts and ploughs, which is a testament to these animals' multiple uses and paramount importance. However, the Utica diet was also based on domestic sheep and goats, which were a primary food source, just like cattle. On the other hand, swine were rarely domesticated, as shown by the tusks discovered in the site, the size and morphology of which suggest the presence of wild boar. These three groups of domestic animals (sheep/goats, adult bovines, and swine) were the most common meat sources in the Utica diet. Nevertheless, the fact that the presence of these species can reflect the economic conditions and social and religious state of this community is worth highlighting.

In fact, the isotopic results from this thesis project support the zooarchaeological perspective from those two sites in Tunisia; this evidence suggests the consumption of domestic animals like sheep, goats, and cattle in Kerkoune and having almost the same dietary habits in these communities during the punic period. However, further analysis with more directly associable animal data from Kerkouane is essential.

CHAPTER SIX

6. Conclusion

This thesis is the first study to report isotopic results from one of the four necropolises in the best-known Punic settlements in North Africa, namely the city of Kerkouane. The city's being located at Cape Bone in North Africa makes it very close to Europe (and more specifically Sicily, given the 140km distance); therefore, this area is crucial to connect and understand the phonic-Punic expansion through the Mediterranean region. The reconstruction of the diet of the inhabitants of the Arg El-Ghazouaani necropolis suggests that it was mainly based on C3 plants food, and domestic animals; nevertheless, no significant trace of C4 plants consumption was noted in the Arg El-Ghazouani sample. The variability among the N isotope values of Kerkouane indicates a contribution of marine resources but not a high consumption thereof.

No significant dietary differences have been detected concerning age, sex, and funerary features among the individuals of Kerkoune. Given the small number of samples used in this study, future studies will give us a clearer picture of social status in Kerkoune.

The isotopic results of this thesis project were compared with those of the ancient Roman site Leptiminus, which is located on the Mediterranean coast of Lamta town, Tunisia. Despite the chronological and geographical differences between the two sites, the inhabitants of Kerkoune might have had dietary habits similar to those of Leptiminus. For instance, C3 terrestrial plant consumption was noted in both locations, and evidence of consumption of domestic animals like sheep, goats, and cattle was found as well. Still, although Leptiminus and Kerkoune are both located on the coast, only the inhabitants of Leptiminus consumed a significant amount of marine protein. The isotopic data shows that marine protein may have contributed to the diet of the Punic inhabitants of Kerkoune; however, no high consumption can be theorized. Subsequently, this might indicate the presence of different dietary habits among the ancient inhabitants of Tunisia over centuries.

Nevertheless, additional studies and investigations about ancient paleo diet ($^{13}C/^{12}C$ $^{15}N/^{14}N$), paleoclimate ($^{18}O/^{16}O$), and mobility ($^{87}Sr/^{86}Sr$) in the other necropolis of Kerkoune, as well as other punic settlements in Tunisia and North Africa, will provide us a with more precise, more complete picture about the ancient Punic past in the Mediterranean.

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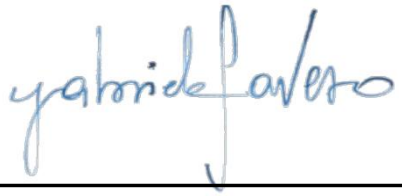

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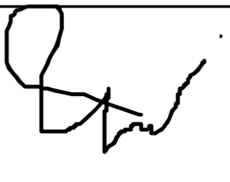

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ABOBKER AFAT