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Scienze e Tecnologie per la Conservazione dei Beni Culturali

**Environmental archaeology from a Roman Villa at Spoletino (Viterbo, Italy)**

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## **Abstract:**

My thesis focuses on the reconstruction of environmental conditions and land use in the Tiber Valley during the 1<sup>st</sup> century AD, as highlighted by a combination of archaeological and archaeobotanical studies. The study site is a cistern connected with a Roman Villa at Spolefino, in the province of Viterbo, which was a very important rural site of central Italy during the Roman Imperial Age. The importance of the site lays in the exceptional richness of different kinds of materials, especially in a high quantity of pottery pertaining to various typologies (from domestic to storage use), recovered from a large cistern and studied within a collaboration between Sapienza Università di Roma (Dipartimento di Biologia Ambientale and Dipartimento di Scienze dell'Antichità) and the Dipartimento di Studi Umanistici of Università di Roma Tre. The discovery of the cistern, with its important artifacts, dates back to 2014 and the excavation works continued until 2018: the site retains a special attention as the region's organization in the Roman period is quite unknown, whereas it was of fundamental significance for its advantageous position within the Tiber valley granting a direct connection to Rome itself. Multiple methodological approaches were used to study plant micro- and macroremains, including pollen, diatoms, and charcoal, identified through light and stereomicroscope, as well as through Environmental Scanning Electron Microscope (ESEM). The results shed light on the natural conditions of the area surrounding the Roman Villa during the early Roman Imperial Age as indicated by pollen analysis, on the aquatic environment of the cistern reconstructed through diatoms, and on the human activity towards exploitation of the natural resources and cultivation of fruit trees, as provided by charcoal analysis, complemented by pollen data. The main floristic elements of the surrounding woodlands were deciduous and evergreen oaks, accompanied by other tree taxa, such as elms. My data suggest intentional plantation and management of *Olea*, *Juglans* and possibly *Prunus*, while herbaceous taxa indicate agropastoral activities in the Spolefino area. The variety and complementarity of plant remains provide new insights into the relation between man and landscape in the Roman times, in this strategic area in the Tiber Valley that was until now uninvestigated from both the archaeological and archaeobotanical points of view.

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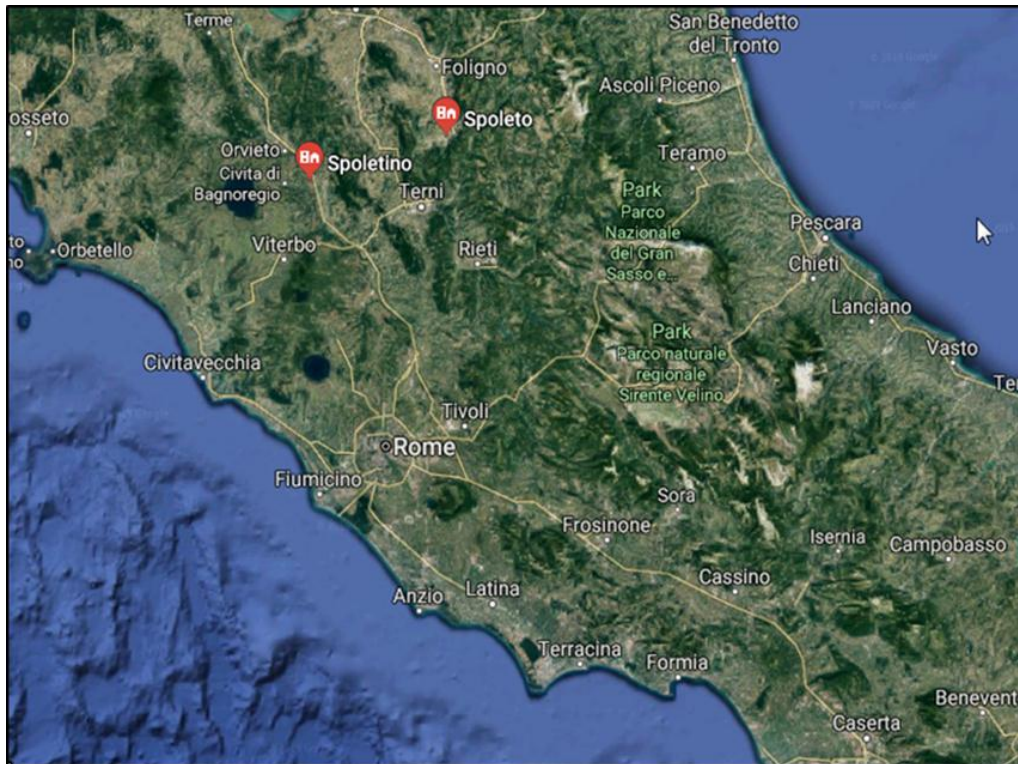
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## 1- INTRODUCTION

The importance of the Roman Villa at Spoletino lies in its location, in the Tiber Valley, and in the important findings discovered in a cistern during the excavations of 2014/2018. Through a series of archaeological excavations in the modern site of Spoletino (Civitella d'Agliano, VT) the cistern was completely excavated and revealed many archaeological objects coming from the villa. The excavations were carried out as part of a memorandum of understanding signed in 2014, between the Soprintendenza Archeologica, delle Belle Arti e del Paesaggio per l'area metropolitana di Roma, la provincia di Viterbo e l'Etruria Meridionale, the University of Roma Tre and the municipal administration of Civitella d'Agliano (VT). The cooperation aimed at carrying out intensive and extensive archaeological research in the municipality, with particular regard to the village of Spoletino-Torricella and the surrounding area. The results of the excavations are still unpublished and are property of the Director of the excavation, prof. Marcello Spanu, University of Roma Tre, and of the Soprintendenza (dott.ssa Maria Letizia Arancio): to both of them goes my biggest gratitude for giving me the opportunity of working on these materials (See figure 1).



*Figure 1: Google map of Spoletino site*



This site contains a wide range of archaeological information that were uncovered through multiple sessions of survey and exploration. The main building is a big cistern filled up in two different phases, one during the 1<sup>st</sup> century AD and the second one in the 4<sup>th</sup> or 5<sup>th</sup> century AD, revealing that in the nearby area a big Roman and Late Roman villa existed. The villa has not been identified yet, whereas the cistern was discovered in 2006 during cultivation activities.

The thesis project aims at studying the macroscopic and microscopic remains found in the filling of the cistern, in order to investigate and reconstruct the environmental situation of the Spoletino villa, during the 1<sup>st</sup> century AD.

Therefore, the work could be a valuable and important evidence in order to understand the overall situation when the villa was existing. Thanks to the analyses, it will be possible to understand if the villa was the center of agricultural production. The fact that is also confirmed by the important discoveries found within the cistern, a fundamental appendix to the villa itself. These data will be presented in the context of this work, depending and being rooted on the theories and assumptions of the archeologists working there.

Moreover, the record of the climate in that period and the promotion of the human cycle and activity in this context will be examined. The relationship between the presence of the cistern in this area and the process of agricultural production is based on samples taken from one of the stratigraphic layers within the reservoir. This work was carried out in collaboration between the Dipartimento di Biologia Ambientale and Scienze dell'Antichità, Sapienza University of Rome and the Dipartimento di Studi Umanistici, University of Roma Tre.

The results will be in combination with the hypothesis of the archaeologists, and the perspective about the life within the villa includes the correlation between the findings and the human activities, related to the study of archaeological layers. In addition, the importance and role of the cistern in the region will be analyzed also using archaeometry, to improve or support the archaeological hypothesis. Moreover, the most important aim of this thesis project is to investigate the archaeological remains for the study of estimated weather changes of human activities, using a multi-versatile systematic approach.

## 2- HISTORY OF THE SITE

The site under examination is located at Spoletino - Torricella, in the municipality of Civitella d'Agliano (VT). It is in the Tiber Valley, north of Rome, on the right bank of the river, within a very fertile and cultivated area. We know that in the Roman period this area was rich of villas, none of which unfortunately has been thoroughly investigated.

Roman villas organization:

While de-urbanization was taking place in the Roman world. Roman society, favored urban growth as a means to strengthen its political hold on the Mediterranean, although rural in its origins. Therefore, the Roman rural villa developed as a contrast to the urban village consequence was a rapid expansion of towns in the 1<sup>st</sup> century BC and the construction of rural settlements in their provinces (Matijasic, 1982)

The three main types of Roman rural, which are classified according to the functional similarities with urban villas in such elements as an architectural project.

1. The luxury design may have a farming establishment integrated into the architectural model, but the meanwhile represents a continuance of the urban lifestyle. It also expresses the survival of some of the basic and unchangeable principles of Roman life: individuality and enjoyment of the beauties of nature (Matijasic, 1982).
2. The second model of the Roman villa is "urban-rural" or working villa is a far more common category, which consists of a small residential section. Here the pars fructuaria is merged with the pars urbana; the most of villas in the Roman world fall into this group (Matijasic, 1982).
3. A third category of the Roman villa was serving as the pillar of the Roman economy in the Mediterranean basin. Which is called Rostovtzeff that depend on "an agricultural factory run by slaves," its activities consisting of the intensive processing of agricultural and other productions (Matijasic, 1982).

Generally, the Roman villas differ on each other in few differences such as the distribution the parts of the building or the rooms within the villa, or with the way of the decoration and decorative, but in the meanwhile, they have the main similarity of many characteristics and the essential architecture plan of buildings. Villas comprise three part the "winter and summer residences, which have wings, are opening into a portico around the courtyard probably are

residential, divided into smaller rooms. On the other side of the residential there where the second part quarter is a cowshed with storeroom, or perhaps a slaves' bedroom, some of the villas have a constructed of an upper, the villa perhaps are including a big olive-oil plant near it. In addition, part of the villa with the wheat storage as well. Usually the villa link with cistern, which is necessary for rainwater collecting and supplying the water to agricultural productions in the farm (Matijasic, 1982).

Usually, the best size for a farm (the third part) is a hundred hectare if it includes various types of soil it is noticeable that on the smallest and most awkward patch of ground the rows of plants or vines are always exactly placed so that each may obtain its due share of sunlight (Frayn, 1974).

According to some written accounts from the end of the last century, the existence of a villa in the area of Spoletino is confirmed<sup>1</sup>. Unfortunately, the documents containing the precise location of the villa were lost and its remains were not uncovered yet. Nevertheless, the memory of the villa existence has been preserved in the oral tradition and it attracted archaeologists who wished to identify its location.

In 2006, a preliminary archaeological survey was conducted in the area of interest, due to the planting of a new vineyard. At this time, the cistern location was recognized. Consequently, in 2014 it was decided to carry out a series of extensive excavations in the area of Spoletino-Torricella, which are still ongoing. The area of the cistern, located north of the intersection between the Strada Comunale di Pianucciole and the Strada Comunale dello Spoletino, was protected by a vincolo of the Soprintendenza Archeologica, delle Belle Arti e del Paesaggio per l'area Metropolitana di Roma, la provincia di Viterbo e l'Etruria Meridionale (see figure 2).

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<sup>1</sup> In the late Eighties a small bath building was excavated at some 300 m to the west of the cistern, and maybe it could be referred to the villa. Other information was obtained by the courtesy of prof. E. Borgia and prof. M. Spanu who are working at Spoletino, directing the pottery laboratory and the excavation.

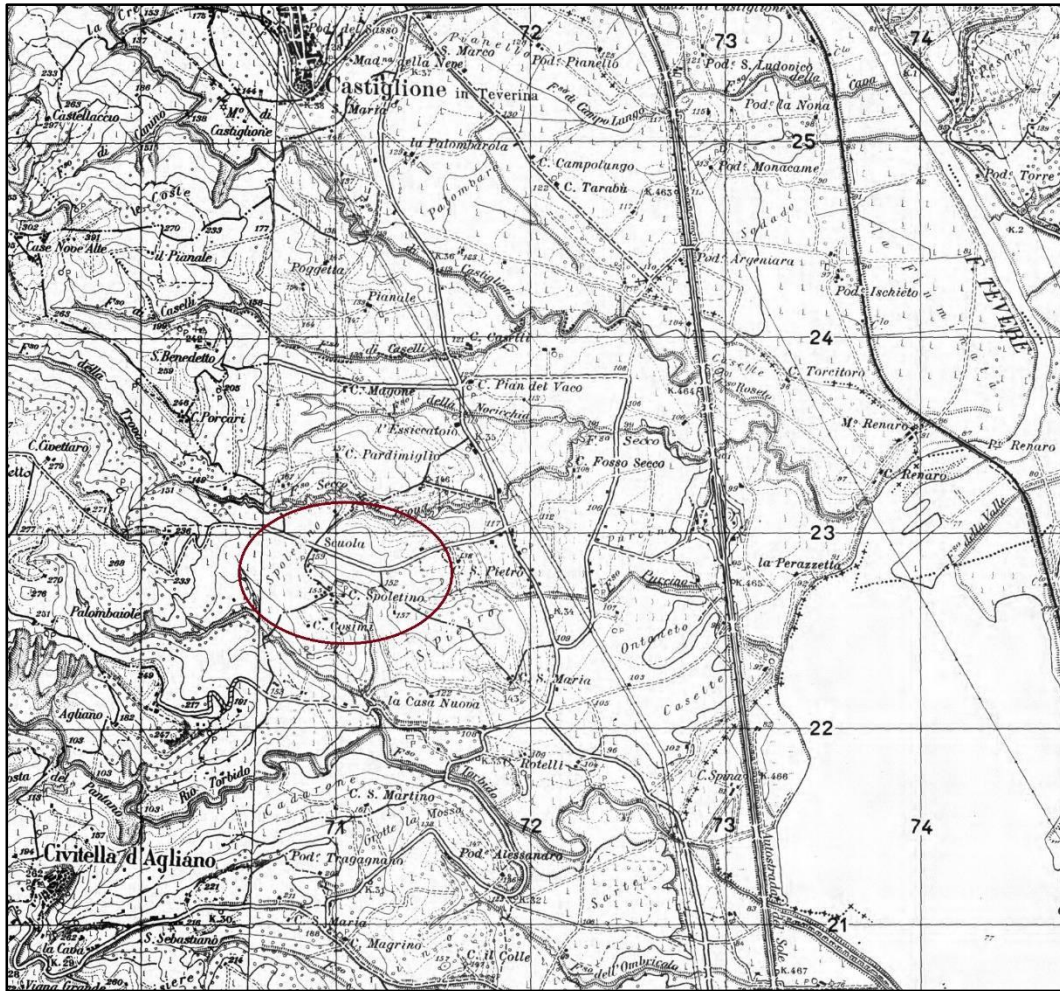


Figure 2: Location of Spoletino, detail. I. G. M.F. 137. NO Castiglione in Teverina and I. G. M. F. 137, I. SO Attigiano (from Mara Elefante thesis)

As preliminary operations to the archaeological investigations, in 2014 an initial topographic mesh was made with the aid of a total station, a prospecting session was carried out with georadar and the dimension of the campaign plan limited to the area under investigation. The data suggested the presence of more or less continuous masonry structures, circumscribing an area of about 40 x 15 m. In the southern sector, there were evident traces of masonry structures, concomitantly with a height of about 30 cm, which followed an oblique alignment from east to west.

The most important discovery is a very big Roman cistern (see figure 3), which was filled up with pottery and objects coming from the neighboring villa. The excavation works demonstrated that the cistern had exceptional dimensions of 42 m x 14 m, being the biggest known example from the rural area of Etruria at the time. The cistern was built maybe in the Late Republican age in very



strong *opus caementicium* according to Vitruvian rules, but its walls are preserved to a maximum height of 1.80 m, having been partially destroyed during the time, mainly by recent agricultural activities. The longer walls were provided of two buttresses on each side, to make its structure stronger. It was not covered, and the water supply was certainly used for irrigation and therefore the presence of the cistern suggests that the surrounding area was intensively cultivated. The cistern was filled mainly with rainwater but had a channel in the southeastern wall to take out the water in excess.



In the mid-1<sup>st</sup> century AD the cistern was divided into two partitions by a transversal wall oriented east/west, built in *opus caementicium* with facings in small stones. The northern portion of the cistern, wider than the other, came out of use and was at that time filled with soil and pottery coming from the villa, which was in this moment completed renovated.

Figure 3: Aerial photo of Spoletino cistern 2015. © Spoletino archive (University of Roma Tre).

The samples analyzed in this research come from this filling, datable to the age of Nero. The southern portion of the cistern continued to be used until the 4<sup>th</sup> century; it was provided of hydraulic mortar and had a small staircase in its southwestern corner. This means that the

agricultural purposes changed in this period (as the water collected was in a minor quantity), but that the villa was still existing and connected with cultivation. The villa and the cistern were completely abandoned in the 4<sup>th</sup> century AD, as demonstrates the filling of the southern half of the cistern, in which many building materials from the destroyed villa were uncovered (such as bricks, mortar, fragments of frescoes, etc.). Even if the research has not been completely finished, the botanical material recovered in the northern portion of cistern is in sufficient quantity to be studied and it is characterized by remarkable state of preservation as well, which allows this thesis project to be carried out. The archaeometrical investigation can serve as supplementary method to the archaeological research, which in turn helps to better understand past events and reality.

### 3- SELECTION OF THE SAMPLES

According to stratigraphic sequence study the samples studied in this project were selected from two-layers US (201), US (202), both of which filling the northern portion of the cistern to the 1th century AD, more precisely to the period of Emperor Nero. The samples were chosen due to the importance of the context and to their good preservation state. According to the features of the findings, in the layers with household finds (such as pottery, glass vessels, metal objects, etc.), were thrown and well preserved due to the appropriate wet conditions of the soil. The excavation work in this part of the cistern was carried out by different trenches, in various years, all of which were filled up after the work to better preserve the building. The US 201 and 202 were excavated in 2015 when a large L-shaped sounding was opened (see figure 4). It runs for 13.55 m on the entire north side of the cistern and for 12 m along the western side and was unified with the test named C. Under the humus layers (UUSS 200 and 300), characterized by heterogeneous archaeological material scattered because of agricultural use of the land, the US 201 was identified, extended over the entire area distinguishable above all by the presence of a large amount of pottery and other materials of big size.

The stratigraphic unity 201 was very compact due not only to its natural clayey matrix but also to the high concentration of crumbled mortar. As already mentioned, it was characterized by a considerable amount of different types of archaeological findings, in very high concentration and in quite good preservation, such as: amphorae, common ware, *dolia*, *mortaria*, large containers, Italic *sigillata*, oil lamps, vitreous finds, worked bones, loom weights, bronze findings, iron nails and clamps, tiles, ashlar of travertine, mosaic *tesserae*, and fragments of plaster (see figure 4).

The materials were not equally distributed within the layer, as they were more concentrated in the western sector up to about 2 m from the cistern wall, and then thinned out to extreme sporadicity, proceeding towards the eastern limit.

The stratigraphic unit US 202 was identified and distinguished because of its slightly different composition and by the smaller quantity of archaeological materials.

The samples of sediment study were obtained in two groups from various sections in both of the layers and in different periods. The first group was selected in 2015 during the excavation campaign from the remains of the sediments within the Amphoras and from the sediments

surrounding the materials as well. The second the group was obtained thanks to a specific sounding carried out on the eastern portion of the northern area of the cistern in June 2018, in order to complete the prediction results of the preliminary study of layer US 201. It was 60 x 60 cm wide and the samples were taken each 5 cm going down until the bottom of the cistern, for a total of 11 bags.

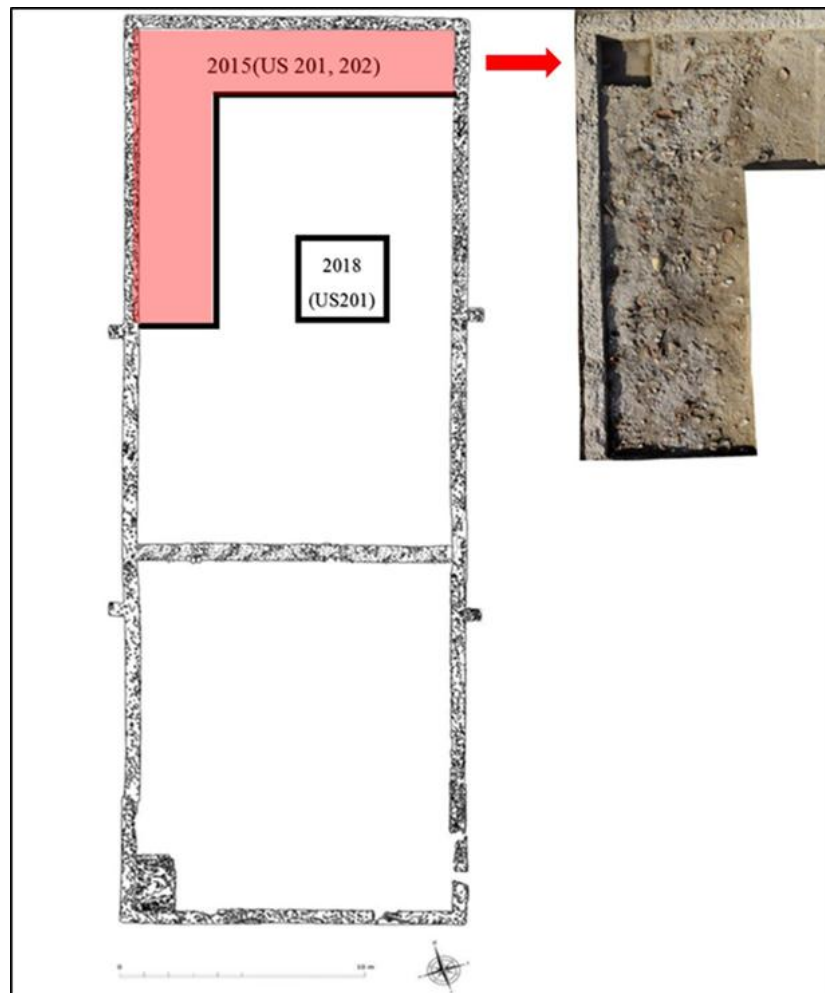
In the following table all the information relating to the samples was collected, indicating the date of excavation, the layer of origin, the type of sample, its provenance (if it was found within a vessel, the term was reported in Italian as it was indicated from the archaeologists), and the results of the present investigation.



Site	Date	Layer US	Type of the sample	Provenance	Weight of the sediments
SP	2015	201	Sediments with charcoal		80 g
SP	2015	201	Sample of soil	Amphora 12	57 g
SP	2015	201	Sample of soil	Amphora 53BIS	30 g
SP	2015	201	Sample of soil	Amphora 19	32 g
SP	2015	201	Sample of soil	Amphora 18	20 g
SP	2015	201	Sample of soil	Amphora 66	11 g
SP	2015	201	Sample of soil	Amphora 16	27 g
SP	2015	201	Sample of soil	Amphora INTEGRA	42 g
SP	2015	201	Sample of soil	olla da giardino n-1	52 g
SP	2015	201	Sample of soil	olla da giardino n-2	66 g
SP	2015	201	Sample of soil	olla da giardino n-3	51 g
SP	2015	201	Sample of soil	vasetto ovoide	35 g
SP	2015	201	Sample of soil	Amphora iberica	58 g
SP	2015	201	Charcoal		54 g
SP	2015	201	Charcoal		20 g
SP	2015	201	Charcoal		63 g
SP	2015	201	Charcoal		12 g
SP	2015	201	Charcoal		25 g
SP	2015	201		Under the wall	290 g
SP	2015	201		From the west	379 g
SP	2015	201		From the west	272 g
SP	2015	201		From the west	410 g
SP	2018	201	Sample of soil	0-5 cm	3800 g
SP	2018	201		5-10 cm	2400 g
SP	2018	201		10-15 cm	3300 g
SP	2018	201		15-20 cm	---
SP	2018	201		20-25 cm	1728 g

SP	2018	201		25-30 cm	2800 g
SP	2018	201		30-35 cm	1554 g
SP	2018	201		35-40 cm	1846 g
SP	2018	201		40-45 cm	1867 g
SP	2018	201		45-50 cm	2000 g
SP	2018	201		50-55 cm	1903 g

*Table 1: The sample sediments analyzed in this thesis*



*Figure 4: The plane of the cistern with the location of sediments samples, samples group 1 (layer US 201, 202) 2015 and sample group 2 (layer US 201) 2018.*

## 4- Methodologies and Experimental Analysis

### 4 - 1 Materials

In general, the archaeological materials are classified according two standards:

Macrofossils	>0.2 mm	Wood, roots, seeds, fruits, flowers, leaves, fibres, bones, shells
Microfossils	<0.2 mm	Pollen and spores (missing if there was combustion or oxidation) phytoliths, diatoms, micro-charcoal

After the sample preparation, I obtained three types of materials, which I will use for the palaeoenvironmental interpretation of the archaeological site:

A- Charcoal

B- Pollen

C- Diatoms

Plant remains	Aims
Charcoal	A partial representation of woody plants, cultivated trees, wood technology
Pollen	Reconstruction of natural or human activities, Human induced vegetation changes through time
Diatoms	Reconstruction of the water ecology system

#### A- Charcoal

Charcoal (anthracological remains) is charred wood, due incomplete combustion by fire. It is chemically highly inert, so it is not subject to degradation by the action of microorganisms. It can be preserved for a long time in non-oxidizing environment. Generally, wood undergoes to the reduction in its volume when it is carbonized and is transformed to a friable material. Thus, it is recommended to store it in dry and rigid containers, in order to avoid crumbling and fragmentation. Anthracological remains usually recovered from the archaeological contexts and soil horizons, so they may present well-preserved anatomical structures that allow their easy identification (Celant & Coccolini, 2015).

In order to examine and identify the fragments of charred wood, three anatomical sections are studied by stereomicroscope and ESEM.

### **1. Transversal (or cross) section**

In this section, it is possible to identify what type of wood it is (softwood/hardwood). The section of tracheids is generally polygonal (square, angular) while vessels are roundish. In the wood formed in spring, tracheids, which have a conduction function, are rather large (early wood) while in summer they are smaller (late wood). This seasonally different size of cells results in the succession of annual rings. In conifer wood there can be also see resin canals (they may be present or not) and they are diagnostic for the identification of wood remains.

### **2. Tangential section** (in the direction of the rings)

Vertical elements may be tracheids and vessels, as well as fibres and parenchyma cells in angiosperm wood. In this section is possible to see the real height of the rays and elements composing it (See Figure 5). Rays are mostly formed of parenchyma cells but in some conifers, there may be also tracheids and resin canals in rays.

### **3. Radial section** (along the rays from the centre to outside, crossing the rings)

Vertical elements may be tracheids and vessels, as well as fibres and parenchyma cells in angiosperm wood. Horizontal elements are rays. In hardwood, perforation plates can be observed in radial section (Celant & Coccolini, 2015).

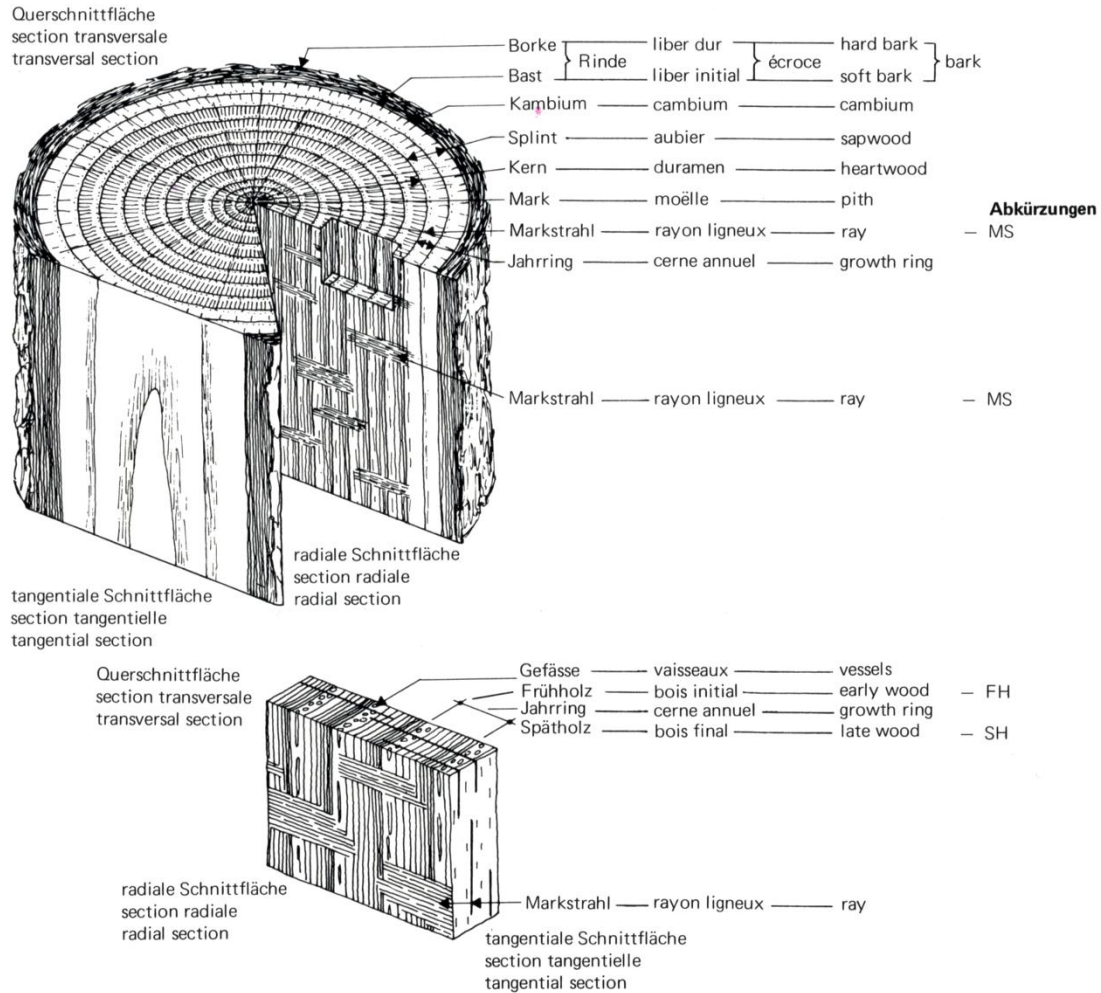


Figure 5: Three anatomical sections for wood analysis (Transversal, Tangential, and Radial), with visible elements (vessels, tracheids, rays, and parenchyma) (Schweingruber, 1990).

## B- POLLEN

Pollen grains represent the microgametophytes of a seed plant that is the reproductive “male” structure of plants. They usually have spherical or elliptical form and their size range is 0.01 mm-0.2 mm. The external wall, called “exina” is made by sporopollenin that is a strong and chemical inert polymer. The presence of sporopollenin allows the preservation of the grains to chemical and physical processes through time. However, this biological polymer is subject to rapid oxidation, so the most important factor to preserve pollen grains is an anaerobic context that does not permit the degradation of the exina. Natural anoxic environment are peat bogs, swamps, the bottom of the lakes, river and stream sediments.

Pollen can be dispersed by wind and precipitations in function of the shape, size and type of pollination, in this way it can be found at considerable distances from the parent plant. The good preservation and the good dispersion make it the most abundant fossil in many sediments, so to allow quantitative evaluations and statistic representations of the data even from small amounts of sediment. With pollen analysis, it is possible to reconstruct past environment conditions and relate them with past climate and human impact. Therefore, the study of pollen grains is useful to a comprehensive understanding of past human, vegetation dynamics and to determine relative proportions of woodland and open vegetation in past landscapes (Smol et al., 2002).

## IDENTIFICATION OF POLLEN GRAINS

### Number and disposition of pollen grains:

The first taxonomic character to be taken into account for a sound identification is the number and the disposition of pollen grains. We can recognize single pollen grains and aggregate pollen grains (Figure 6), which can have different numbers (monad, dyads, tetrads and polyads) and disposition (tetrahedral, tetragonal, rhomboidal, linear, etc.) or be united in a unique structure called “pollinia” (typical of Orchidaceae) (Moore et al., 1991).

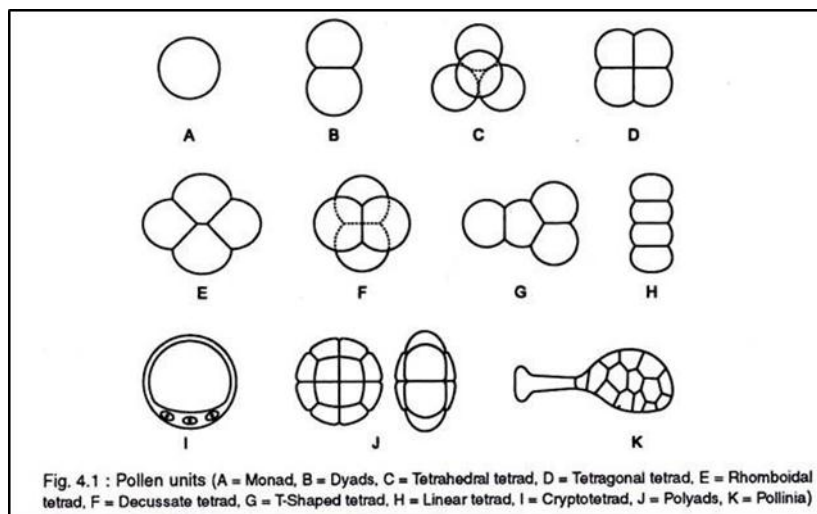


Figure 6: The number and the disposition of pollen grains in dyads, tetrads and polyads

<http://www.biologydiscussion.com/palynology/morphological-characteristics-of-pollen-grains>

The size and shape (e.g., oblate, spherical or prolate) of the grains are very important selective characters to be considered for the identification (see Figure 7).

Pollen size class	Length of longest axis
1. Very small grains ( <i>Sporae perminutae</i> )	<10µm
2. Small grains ( <i>Minutae</i> )	10 - 25 µm
3. Medium sized grains ( <i>Mediae</i> )	25 - 50 µm
4. Large grains ( <i>Magnae</i> )	50 - 100 µm
5. Very large grains ( <i>Permagnae</i> )	100 - 200 µm
6. Gigantic grains ( <i>Giganteae</i> )	> 200 µm

Figure 7: The size and shape of pollen grain

<http://www.biologydiscussion.com/palynology/morphological-characteristics-of-pollen-grains>

#### Apertures of pollen grains:

On the pollen surface, two different types of apertures can be found: Colpus, a furrow long boat-shaped aperture with pointed ends, and Porus, that is simple pore on the exine (Moore et al., 1991). Based on the presence/absence of these elements, we can divide pollen types in (See Figure 8):

- Inaperturate pollen grains: without apertures
- Colpate pollen grains: with only colpus
- Porate pollen grains: with only pores
- Colporate pollen grains: with both colpus and porus.

Moreover, the type, position and number of apertures determine an accurate taxonomic classification. An equatorial distribution of the apertures is identified by the prefix “zono-“, while a diffuse position of the apertures is identified by the prefix “panto-“. In the same way with the prefixes “di-“, “tri-“, etc. it is possible to categorize different pollen grains. The combination of these taxonomic characters identifies several classes. (Moore, et al., 1991) (See Figure 8).

## Ornamentation of pollen grains:

Other useful characters for the identification of pollen taxa reside in the exine ornamentation that is determined by the presence/absence of tectum and by sculpture elements (Moore et al., 1991). See Figure 9:

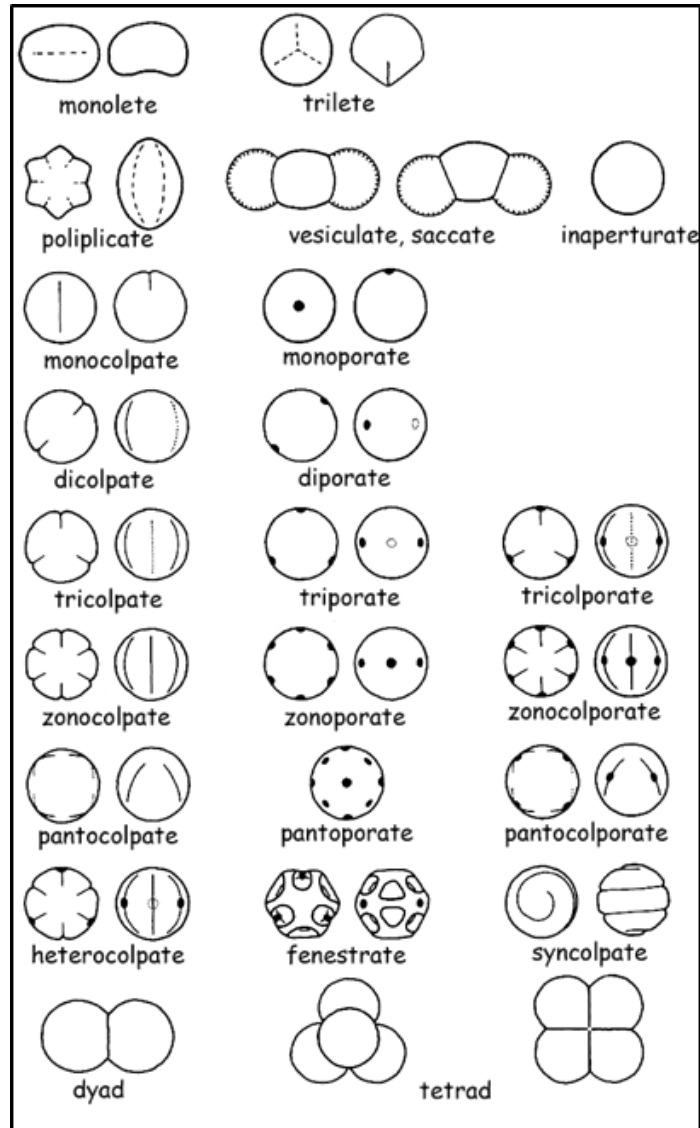


Figure 8: The different types of aperture, and the number of apertures

<http://www.biologia.edu.ar/botanica/tema22/tema22-9polen.htm>



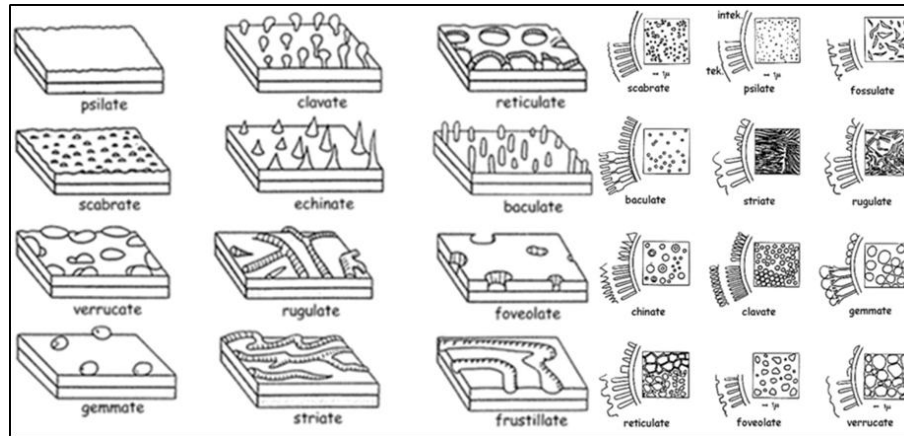


Figure 9: The different ornamentation of pollen grains

<http://www.biologydiscussion.com/plant-anatomy/pollen-and-spore-walls>

### C- DIATOMS

Diatoms are unicellular algae, Division Bacillariophyta (See Figure 10). Most diatoms are planktonic, but some are bottom dwellers or grow on other algae or plants. Diatoms are eukaryotic organisms characterized by siliceous cell walls and yellow-brown pigmentation (Smol et al., 2002). It is possible to divide diatoms into two big groups: Pennales, which have bilateral symmetry, and Centrales, which have radial symmetry. The most important diversity in shape is the division between

- Elongated diatoms (mostly benthonic)
- Roundish diatoms (mostly planktonic, floating in water).

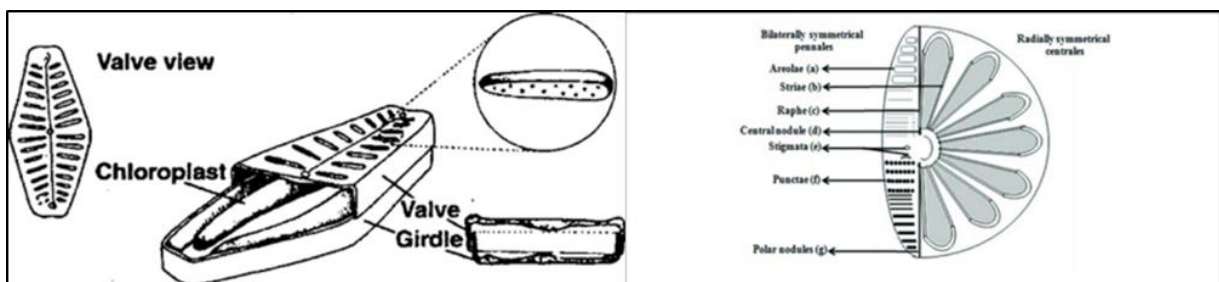


Figure 10: Division Bacillariophyta (Diatoms)

Diatom cell consists of two more or less identical (thecae) cell enclosing an organ, one slightly larger than the other. The thecae have two main portions that are the valve and the

cingulum. The valves can be identified in microscope preparations, as they hold most of the taxonomic features used in standard floras. The cingulum includes a single or sometimes multiple series of chains that are created during the process of cell division, permitting the internal formation of daughter cells. Moreover, Diatoms have very fine and characteristic ornamentation. Shape and ornamentation are typical for each species so that we can identify diatoms at the species level. Dots and striae on the valve surface are diagnostic characters. Other diagnostic characters include the raphe, in Pennales. The raphe is a canal; it can be central or at the margin of the frustule. Therefore, its presence or absence is a diagnostic character like the dimension, number, density and shape of striae and points (Smol et al., 2002) (See Figure 11).

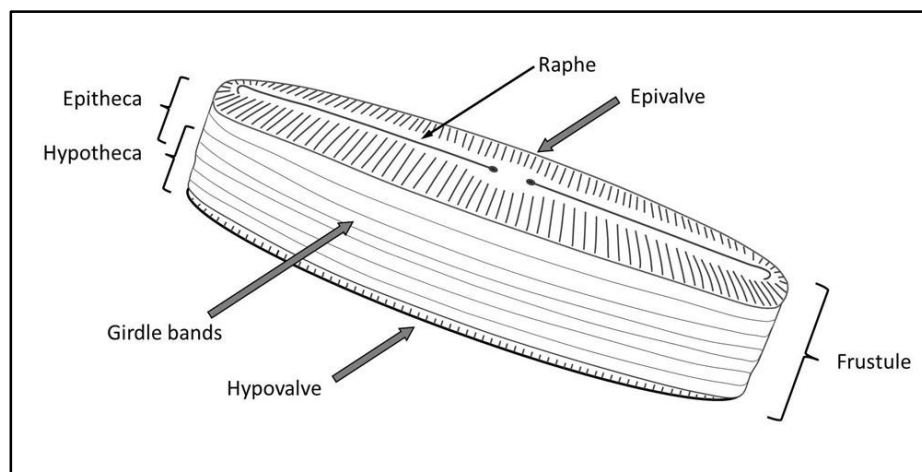


Figure 11: the general features of Diatoms (<https://www.diatoms.de>)

Diatoms are major players in global biogeochemical cycles. They are found in most aquatic environments, including marine sediments, brackish and fresh waters, as well as in damp sub-aerial habitats. In lakes, diatoms are found in abundance in both planktonic and benthic habitats, and together form the source communities for the sediment record. Mostly, the species found in different habitats within a lake are characteristic of those habitats, although many species can be in more than one habitat (indifferent species) (Flower, 1993).

Their most distinctive characteristic is the elaborate, and very resistant, siliceous cell wall (features used to define and classify species). Diatoms are included within the microfossil remains <0.2 mm. The study of diatoms can be an important goal for community ecology dealing with the characterization and prediction of changes in community patterns along environmental gradients. The major environmental factor affecting diatom distribution patterns is the water

level, determining the abundance of planktonic versus benthonic species. Some species are typical of running water and damp soils. Many diatoms live in a specific pH range (each species has a suitable range of alkalinity, e.g., acidophilus versus acidobiontic species), so the diatom record may be used as a proxy for changes in water chemistry. Diatoms may be very selective with respect to salinity, being in some cases adapted to hypersaline environments, saline or brackish water or they may be even halophobous and live in the environment with only minimal concentrations of salts (Smol et al., 2002).

#### **4 - 2 Samples preparation**

Different methodologies have been used to process the study material and to extract the macroscopic and microscopic remains from sediments. The following extraction and preparation techniques have been used to process the sediment from layer US 201:

- a- Water separation and dry picking in order to separate plant macroremains
- b- Preparation of anthracological remains (charred) for microscopically observation
- c- Chemical treatment for pollen analysis
- d- Chemical treatment for diatom analysis.

##### **A- Water separation and dry picking**

The water separation methods were applied to twelve samples of sediment within amphoras from layer US (201) belonging to the excavation works of 2015. The same process was applied to 10 samples obtained from the same layer in July 2018. Those sediments were collected at 5 cm interval along a vertical sequence, starting from 0-5 cm to 55-60 cm, in order to check whether they contained seeds.

From each sample, an amount of 2/3 of the available sediment was processed, according to the following steps.

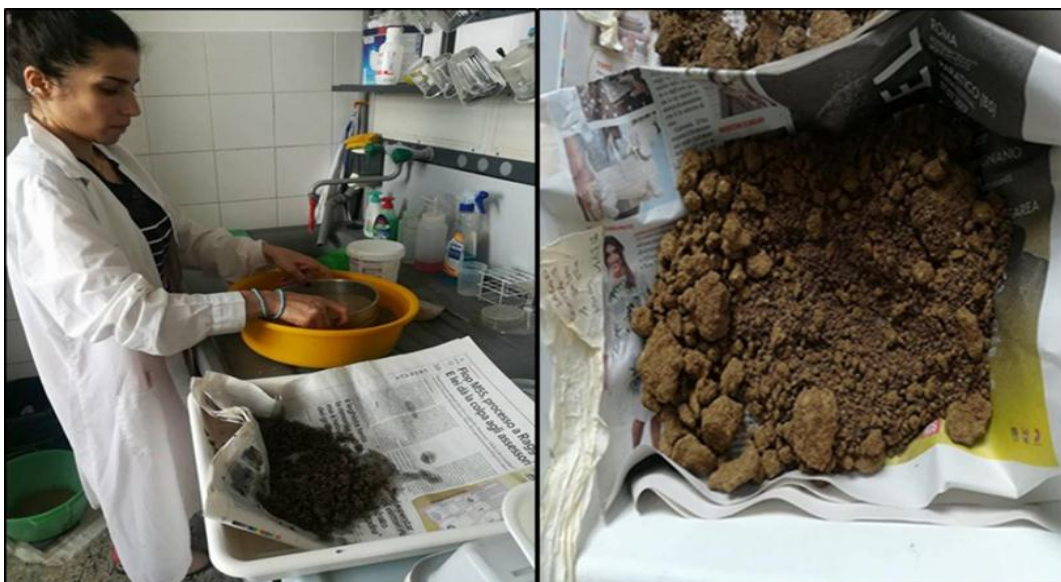
- 1- Soaking the sample in warm water. In the samples from Spoletino it was necessary to add three or more drops of hydrogen peroxide ( $H_2O_2$ ), in order to help plant remains to detach from the sediment and float on the water surface.
- 2- Gently stirring the sediment soaked in water to let the macroremains float on the water. Macroremains were picked up by means of a small brush (See Figure 12).

- 3- Wet sieving process, by means of sieves with decreasing mesh (2.0, 1.0 and 0.5 mm), in order to avoid any loss of the macroremains (See Figure 13).
- 4- Drying of the sieved material in a container far from heat sources (See Figure 13).



*Figure 12: The treatment of the samples with water and oxygen peroxide (H<sub>2</sub>O<sub>2</sub>), and wet sieving*

A further check of plant remains in the sediments was done in the attempt to pick up whatever could not be obtained from the water separation process, to avoid the loss of plant remains that are not able to float on the surface. In this way, I could collect some fragments of charcoal related to the layer US 201. These fragments were added to the amount of the charcoal remains I selected by the sieving process (See figure 13).



*Figure 13: Sieving and drying process of the soil*

## **B- Preparation of anthracological remains**

The preparation of charred samples from the archaeological contexts depends on the chemical and physical preservation of the materials, which varies from near normal to highly degraded. In order to prepare and observe the charred materials, the following tools and instruments are needed:

- 1- A soft brush, a scalpel, hard forceps with flexible tips and a small hammer, in case of very hard samples
- 2- Light reflected microscope with a magnification range between 10-80x. Charred wood is observed on clean surfaces, after fracturing the materials.
- 3- Metallographic microscope or SEM, in order to detect the smaller diagnostic details of charred wood.

The work stages start by cleaning the surface of the sample, using a soft brush. In some cases, a scalpel may be used to eliminate the embedding sediment. These steps are necessary to prepare the surface of the charcoal samples and examining them under the stereomicroscope, in order to obtain the anatomical orientation, which starts by the recognition of the transversal surface of the samples.

Normally, clean surfaces in charcoal samples are obtained by fracturing them using the thumbs and the index finger of both hands. However, some of my samples were so small and delicate that I had to observe them under the microscope without fracturing and by using forceps with flexible tips to transfer the prepared fragment to microscope slides (Celant & Coccolini, 2015).

The charcoal study of my thesis included the charcoals scattered within layer 201 of the cistern. Besides some charcoal fragments were found in the Amphoras.

The total amount of charcoal was about 115 g. A selection of 50 charcoal fragments was chosen because of their good condition and preservation, in order to examine them under the stereo microscope. The size of the charcoal fragments mostly ranged from 50 mm to 3 cm.

### **C- Chemical treatment for pollen analysis**

#### **Preparation Techniques:**

There are many possible processes used to the prepare pollen samples and making the grains visible under a microscope, depending on the nature of the sample. The treatment generally consists of removal of the organic and inorganic matter from the sample in order to facilitate the identification of pollen grains under the light microscopy. These preparation methods are based on chemical separation, based on the chemically resistant properties of the pollen grains, whose outer wall is made of sporopollenin. Centrifugation procedures are used in order to separate pollen from chemicals and water. In some cases, sieving the fraction larger than 200  $\mu\text{m}$  and finer than 8  $\mu\text{m}$  may considerably improve the result of pollen processing (Magri & Di Rita, 2015).

The pollen preparation technique largely depends on the attributes of the sediment that contains the pollen grains. However, to avoid any effect on the final interpretation of the pollen data due to different preparation techniques, it is always better to use a standard preparation method. Generally, there are a number of steps required to extract the pollen grains from archaeological sediment (Magri & Di Rita, 2015):

- A- Standard procedure
- B- Sieving
- C- Deflocculation
- D- Gravity separation
- E- Acetolysis.

### **A- Standard procedure**

I used the standard procedure as a minimum treatment to extract pollen grains. It can be complemented by sieving, flocculation, gravity separation, and acetolysis (See Figure 14).

- 1- To avoid pollution of the pollen sample, the glassware and the laboratory benches were carefully cleaned, as well as all the tools used to take subsamples. A known amount of sediment was put in a Falcon tube (50 mL);
- 2- To remove the carbonate fraction of the sediment, 30 mL of 37% HCl were added to the sample, until effervescence stopped; then the sample was centrifuged (3500 RPM, 5 minutes).
- 3- To calculate the pollen concentration, one tablet containing the estimated number of 13.911 *Lycopodium* spores was added to the sample.
- 4- To remove silica and silicates from the sediment 30 mL of 50 % HF were added. The sample was left in HF overnight; then the sample was centrifuged (3500 RPM, 5 minutes).
- 5- To remove colloidal silica and silicofluorides 30 mL of 37% HCl were added; then the sample was centrifuged (3500 RPM, 5 minutes).
- 6- To enhance the sediment disaggregation and facilitate the dissolution of humic acids, 25 mL of 10 % NaOH were added to the sample. The sample was put in a hot waterbath for 10 minutes, then it was centrifuged (3500 RPM, 5 minutes).
- 7- The sample was finally washed in distilled water and centrifuged several times, until chemically neutral.
- 8- The sample was stored in glycerol.

## **B- Sieving**

In order to remove or eliminate the coarse particles, the sample may be poured through 180  $\mu\text{m}$  mesh sieves. This sieving procedure is useful to eliminate vegetal fragments when the treated sediment is a peat.

## **C- Deflocculation**

This process requires to remove clay particles by adding 20 mL of 10%  $\text{Na}_4\text{P}_2\text{O}_7$  before sieving through an 8  $\mu\text{m}$  mesh sieve.

## **D- Gravity separation**

A saturated solution of  $\text{ZnCl}_2$  (specific gravity: 1.96) may be added to the sample. The supernatant is then decanted into a solution of distilled water with a few drops of  $\text{HCl}$  in order to avoid precipitation of zinc hydroxide.

## **E- Acetolysis**

The acetolysis mixture (composed of nine parts of  $(\text{CH}_3\text{CO})_2\text{O}$  and one part of concentrated (95–98 %)  $\text{H}_2\text{SO}_4$ ) is added to the sample, which is then put into a boiling water bath (90 °C) and left for 3 min.  $\text{CH}_3\text{CO}_2\text{H}$  is rapidly added to the sample, which is then washed in distilled water several times until it is neutral.

One sample from the Spoletino cistern was processed. It was collected from the layer US 202, which is in contact with the layer US 201. The minimum standard procedure, as described above, was followed.



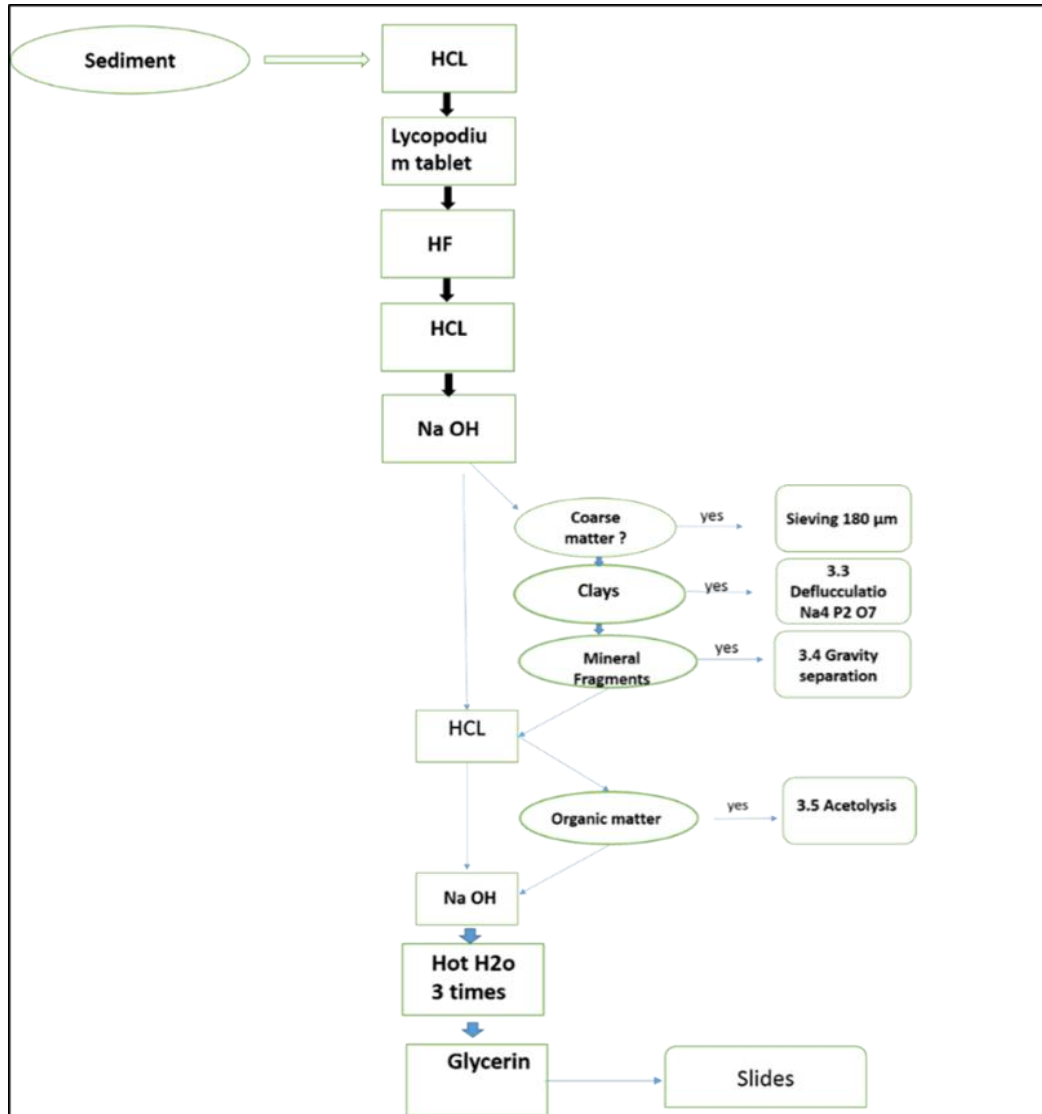


Figure 14: Steps of preparation of pollen samples.

#### D- Chemical treatment of diatoms

The ways of preparing the sample for diatom analysis are various, depending on the characteristics of the sediment. In any case, it is important to prevent any damage and crumble of the sample being analyzed, to avoid the loss or damaging of diatom valves, as delicate spines and processes are easily destroyed by vigorous stirring and rapid centrifugation. In order to choose the appropriate study samples, it is important to pay attention to safety issues, especially the proper use of safety disposals and fume cupboards (Flower, 1993).

It is very important to avoid contamination of the samples, when diatoms from different sites are being prepared simultaneously. It is essential that the laboratory glassware is clean. It should be cleaned with hot 10%  $\text{Na}_2\text{CO}_3$  in case the glassware is to be reused. Where it is necessary to prepare sediment samples, a waterbath using disposable test tubes rather than beakers may be preferable (Renberg, 1990). During the work, it is necessary to pay attention to avoid the dangers, which occur due the use of the chemical material such as hydrogen peroxide, which is a powerful oxidizing agent and may cause damages to the skin (Smol et al., 2002). The main steps to preparing the slides of diatoms samples follows the chart in Figure 15.

One sample from the Spoletino cistern was processed. It was collected from the layer US 202, which is in contact with the layer US 201, and was also the subject of pollen analysis.

The sediment treated corresponded to around 3.00 g, subdivided into three samples.

The following steps were used:

- Removing soluble salts in hydrochloric acid: carbonate and many metal salts and oxides can be removed by diluted hydrochloric acid. In this case, I have added HCl (7%) to the sample and I have left it reacting for a night.
- Repeated washing in distilled water every two hours to get rid of the finest material with the water.
- Removing organic matter by using oxidation by hydrogen peroxide (Smol et al., 2002). Hydrogen peroxide was used in a wide heatproof beaker in a fume cupboard, in a water bath (See Figure 16).
- Repeated washing in distilled water every two hours to get rid of the finest material with the water (see Figure 17).

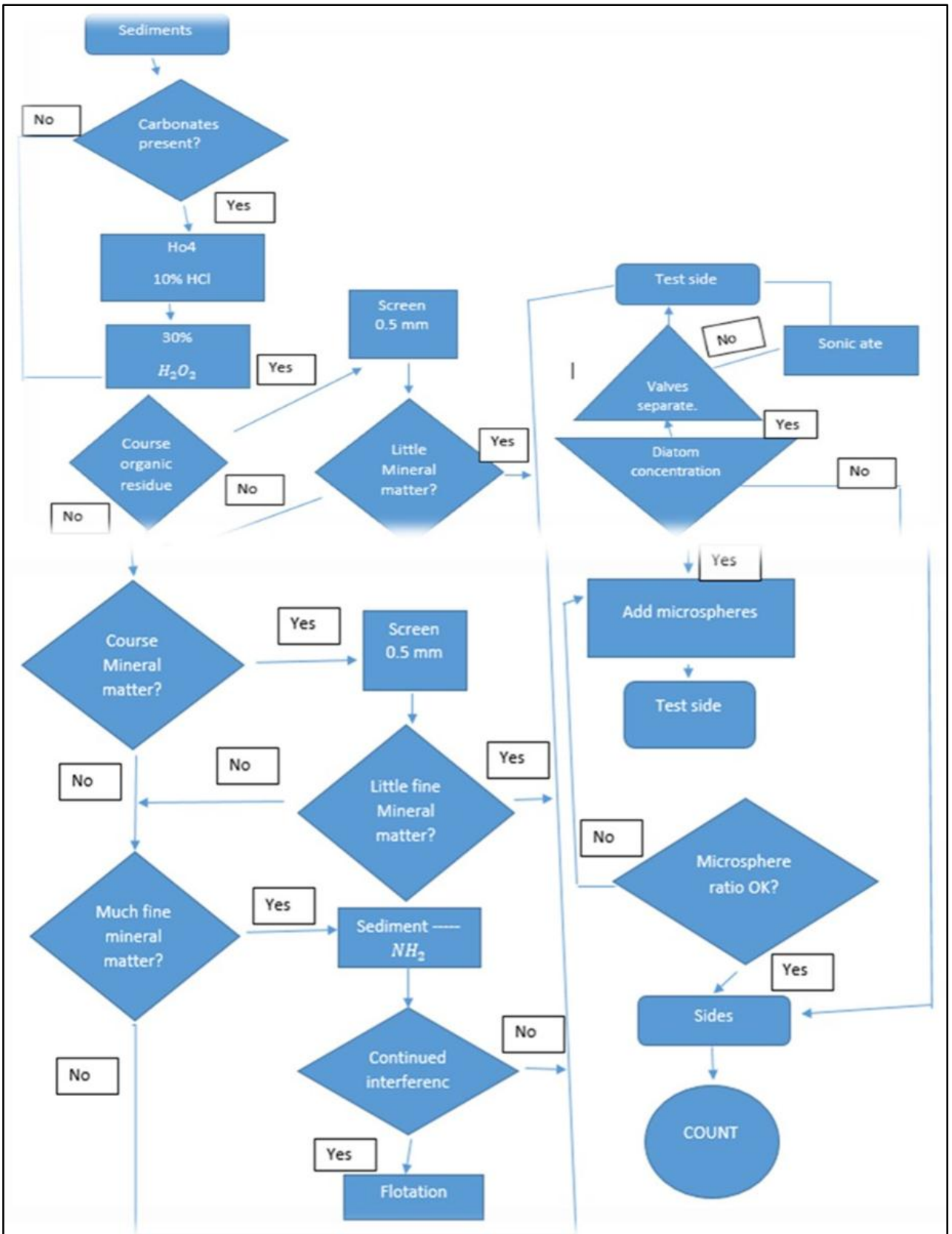


Figure 15: Chart explaining the preparation of diatom samples.



*Figure 16: Processes of removing salts soluble in hydrochloric acid (HCl, 7%), and samples in water bath.*



*Figure 17: The samples before and after purification with distilled water.*

### **Slide preparation**

Slide preparation should consider the amount of material to be dropped within a perfectly clean slide: approx. 2.0 ml of diatom suspension is dropped by pipette on to a coverslip, the diatoms can settle, and the water evaporates at room temperature. Care needs to be taken not to disturb the coverslip. When dry, the coverslip is mounted using a resin with a high refractive index such as Naphrax (refractive index = 1.65). One drop of Naphrax is placed on a glass slide and the coverslip is inverted with the dried diatoms over the drop. The slide is heated on a hotplate at about 130°C for few minutes to drive off the toluene in the Naphrax. The slide is then cooled, to check that the coverslip does not move when pushed with a fingernail. If it does, the slide will need to be re-heated. All slides need to be checked to ensure that the concentration of diatoms on the coverslip is appropriate for counting (Smol et al., 2002).

## **4 -3 Instruments**

### **A- Optical Microscopy**

Optical Microscopy was used to examine the various samples of this project in order to identify the taxa of the charred wood, pollen, and diatoms. I used two types of microscopes:

#### **- Stereomicroscope:**

I used stereomicroscope Stemi SV11 ZEISS. Oculars 10x, at 4x magnification to examine the anatomy of 50 fragments of charcoal. Each fragment of charcoal was broken along the transverse section.

#### **- Light microscope**

Was used to identify pollen and diatom taxa. A Zeiss Axioscope microscope at 400x and 630x magnifications was used for pollen analysis, and at 1000x magnifications for diatom analysis. The microscope was also provided with Nomarski Differential Interferential Contrast (DIC).

### **B- Environmental Scanning Electron Microscopy (ESEM)**

Scanning Electron Microscopy coupled with Energy Dispersive Spectroscopy is a non-destructive technique, widely used in many material analyses for obtaining multiple data information. It gives clear images of the surface of the sample and it allows identifying the texture and deep details. A Hitachi TM-3000 Tabletop Scanning Electron Microscope was used for detailed analysis. No sample preparation was needed as the TM-3000 allows for non-conductive sampling to be imaged without the need for coating. The images were captured using secondary electron imaged as opposed to backscattered electrons at magnifications varying from 150x to 1.5Kx. The TM-3000 was operated in environmental mode (ESEM) at a voltage of 15Kv. The SEM was used solely for imaging and no point analysis was undertaken.

## 5- RESULTS

### 5 – 1 - Charcoal

Charcoal fragments were scattered throughout the accumulation sediments. Some of them were obtained from a sieving process during the excavation works. Another fraction of charcoals was picked up from the sediment and washed in the lab, after water separation of the sediment. The recovered specimens have a size ranging from 0.2 to 3 cm. Additional charcoal pieces below these sizes were found but considered too fragmented to be able to be properly identified. A total number of 50 fragments of charcoal were identified. Besides, 22 fragments were too small and badly preserved for identification. The samples, examined under the stereomicroscope at different magnification were separated into five categories, based on the observed features (see Table 2). Subsequently the five groups were identified by observation of the diagnostic features by the Hitachi TM3000 ESEM.

The largest category of sorted charcoal fragments is represented by the genus *Quercus*, which is known in English as Oaks, from the family Fagaceae. It includes 29 fragments of *Quercus* charcoal, recovered and identified from the assemblage. These *Quercus* fragments were divided into two types: *Quercus* sp. evergreen group (11 fragments, including 5 fragments of trunks and 6 possible branches) and *Quercus* sp. deciduous group (18 fragments, including 15 fragments of trunks and 3 possible branches). A description of the anthracological remains is reported below.

A second identified taxon is *Ulmus* (family Ulmaceae), known in English as Elm, represented by 6 fragments. *Ulmus* wood cannot be recognized to the species level.

The third type of wood belongs to the genus *Prunus* (family Rosaceae). It is represented by 9 fragments (See Figures 21-23).

The fourth group of charcoal includes 6 fragments of *Olea europaea*, known in English as Olive tree.

A number of 22 fragile fragments were not identified because of the lack of diagnostic features and their tiny sizes.

TAXA	Number	Trunk	Possible Branch
<i>Quercus</i> deciduous	18	15	3
<i>Quercus</i> evergreen	11	5	6
<i>Ulmus</i> sp.	6		
<i>Prunus</i> cf <i>avium</i>	9		
<i>Olea europaea</i>	6		
Damaged fragments	22		

Table 2. The five categories of retrieved charcoal

### ***Quercus* sp. deciduous group**

Heartwood distinct. Ring porous. Early wood porous ring with one to many rows of pores more or less compact. Latewood pores solitary or in more or less radially oriented groups (flame-like groupings), in wide growth rings pore arrangement is radial to dendritic. Apotracheal parenchyma either diffuse and sometimes in uniseriate diagonal and tangential bands, frequency variable. Broad rays visible to the naked eye (Schweingruber, 1990) (See Figure 18).

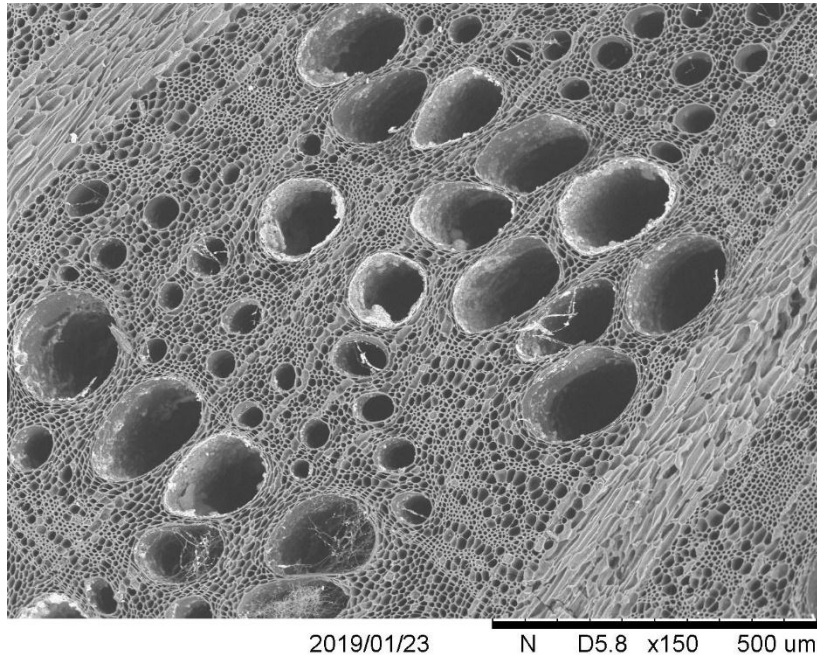
Tangential section: rays uniseriate and multiseriate, up to 1 mm wide (up to 30 cell) and 1-5 cm high, frequently absent in young shoots.

Radial section: the rays are homogeneous, sometimes with square cells in uniseriate rays. Libriform fibers and vasicentric tracheids. Simple perforation plates (Schweingruber, 1990).

### ***Quercus* sp. evergreen group**

I could observe 11 trunk fragments of this genus of *Quercus* evergreen group, in addition to 5 pieces belonging to branches. The transversal section is characterized by diffuse porosity, growth ring border indistinct, and transition from early wood to latewood indistinct. In the

tangential section, two distinct sizes of rays are visible (uniseriate and multiseriate). Ray height in multiseriate rays > 1 mm. Vessel-ray pits vertical (Akkemik et al., 2012).



*Figure 18: Transversal section of deciduous Quercus from Spoletino. Visible rays and porous ring.*

### ***Ulmus* sp.**

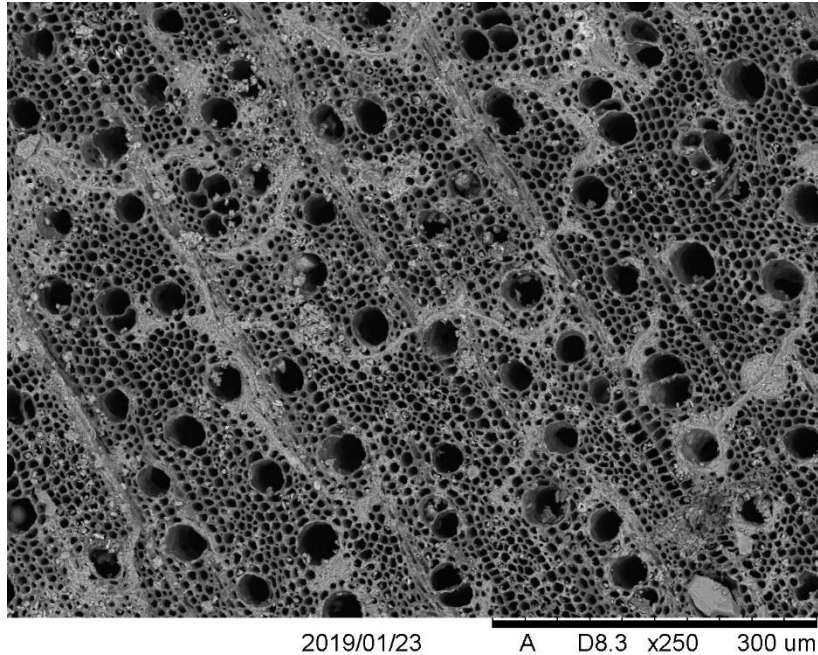
The common English name is Elm. In the transversal section, *Ulmus* appears as a ring-porous wood, with groups of 2-4 pores forming more or less tangential bands in the latewood. It is also characterized by abundant paratracheal parenchyma in early wood and tangential bands in latewood. In the tangential section, multiseriate rays (4-5 cells wide) are visible, 30-60 cell high. In the radial section, simple perforation plates and homogeneous to heterogeneous rays are visible. Vessels present distinct spiral thickenings.

### ***Prunus cf avium* L.**

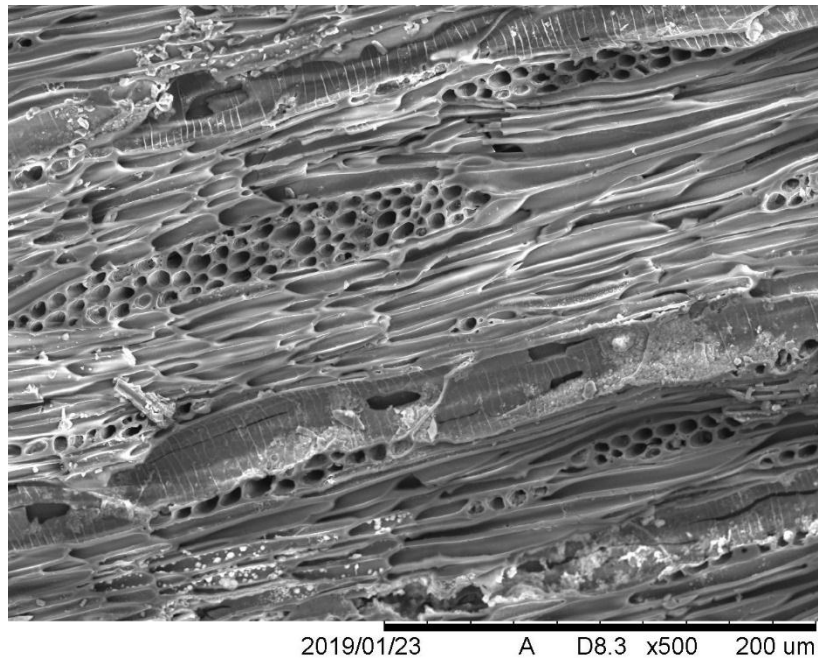
In the transversal section, heartwood is present. Semi-ring porous wood. Radial pore file, sometimes in clusters (See Figure 19). Gum deposits in tyloses are visible in the heartwood.



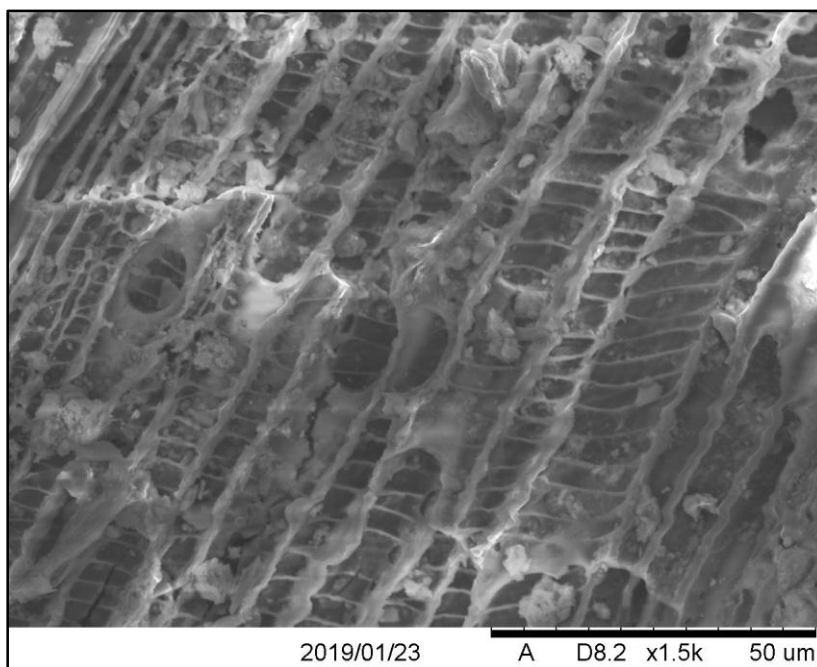
Parenchyma is apotracheal diffuse. In tangential section (See Figure 20), rays are 2-4 seriate, often uniseriate, average height is 15-30 cells. In radial section (See Figure 21), simple perforation plates are visible, as well as distinct spiral thickenings in vessels.



*Figure 19: Transversal section of Prunus sp. from Spoletino.*



*Figure 20: Tangential section of Prunus sp. from Spoletino.*



*Figure 21: Radial section of Prunus sp. from Spoletino.*

### ***Olea europaea* L.**

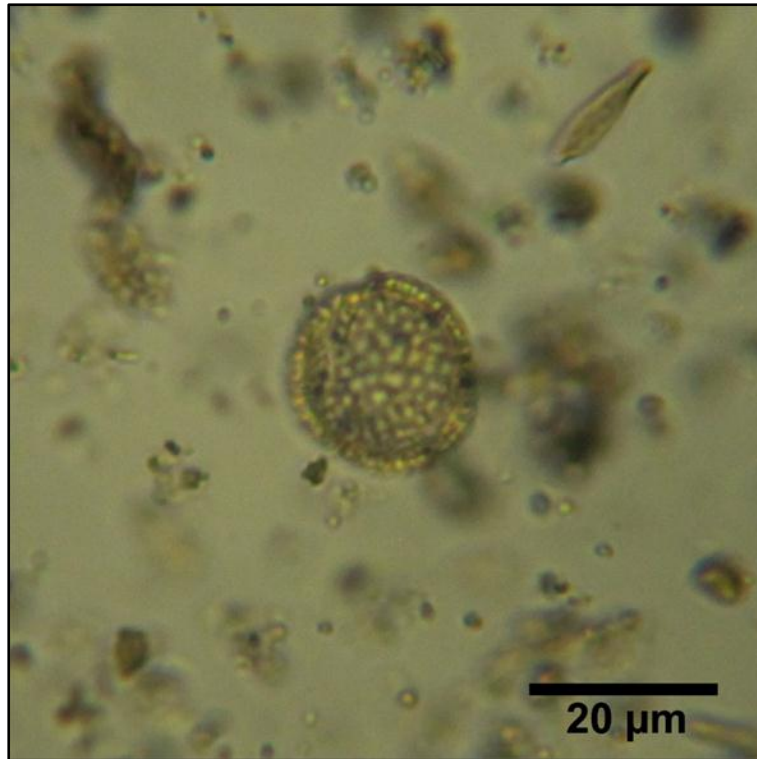
In transversal section, diffuse porosity is visible, with vessels arranged in no specific pattern, mostly in radial multiples or in clusters, with variable proportion of solitary vessels (Akkemik et al., 2012). Solitary vessels have circular to oval outline. In tangential section, uni- and biseriate etherocellular rays are present. Simple perforations of vessels are visible in radial section. Sometimes acicular crystals are present in ray cells.

### **5 – 2 - Pollen**

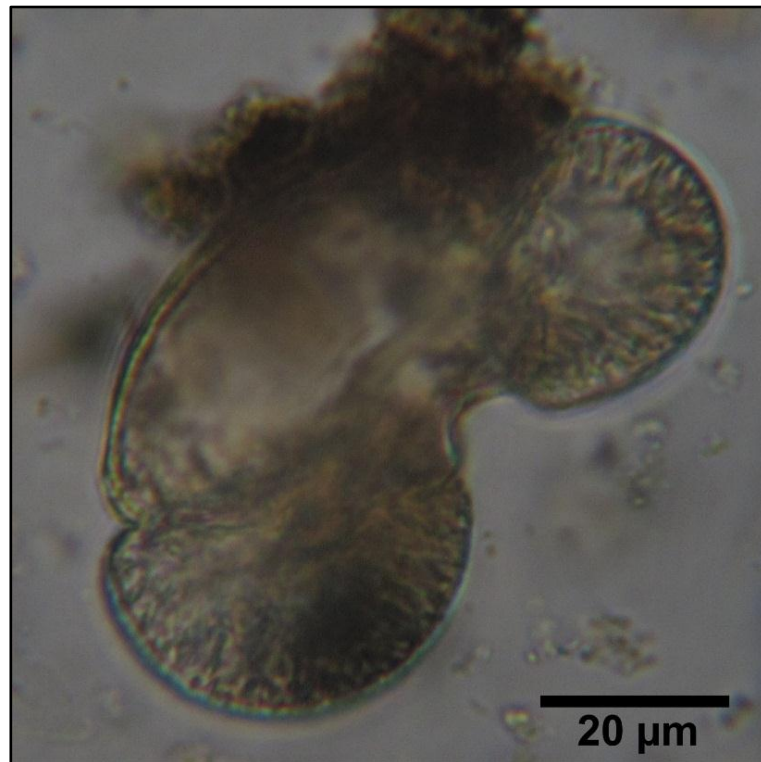
Upon examination of the pollen samples under the light microscopic the study identified over 18 taxa belonging to a number of 412 pollen grains. Besides, 242 *Lycopodium* spores from the added tablet of *Lycopodium* markers, and 9 *Glomus* were found. The results of pollen analyses are presented following the two categories of Arboreal pollen and Non Arboreal pollen (Table 3). A selection of pictures of pollen grains is presented in Figures 22- 23 -24- 25.

Date of laboratory treatment	2/06/2018 – 5/06/2018				
Quantity of sediment treated	2.58 g				
Date of microscope analysis	6-25/06/2018				
No. pollen grains counted	412				
No. <i>Lycopodium</i> added	13911				
No. <i>Lycopodium</i> counted	242				
Pollen concentration (grain/gram)	9179				
taxa	No.	%	taxa	No.	%
<b>Arboreal pollen</b>			<b>Non Arboreal pollen</b>		
PINUS	44	10.68	ASPHODELUS	4	0.97
ABIES	1	0.24	CEREAL TYPE	1	0.24
JUNIPERUS	4	0.97	CHENOPODIACEAE	7	1.70
ALNUS	22	5.34	CICHORIOIDEAE	1	0.24
CORYLUS	1	0.24	BRASSICACEAE	7	1.70
ERICACEAE	1	0.24	CYPERACEAE/JUNC.	2	0.49
FAGUS	10	2.43	FABACEAE	1	0.24
OLEA	71	17.23	LILIACEAE	2	0.49
OSTRYA/CARPINUS OR.	4	0.97	PLANTAGO	3	0.73
JUGLANS	6	1.46	POACEAE	22	5.34
QUERCUS deciduous	108	26.21			
QUERCUS evergreen	81	19.66			
QUERCUS suber/cerris	6	1.46			
ULMUS	3	0.73			
<b>NPPs</b>			<b>Spores</b>		
GLOMUS type	9	2.14	Trilete spores	3	0.96

Table 3: Results of pollen analysis of sample US 201



*Figure 22: Pollen grain of Olea*

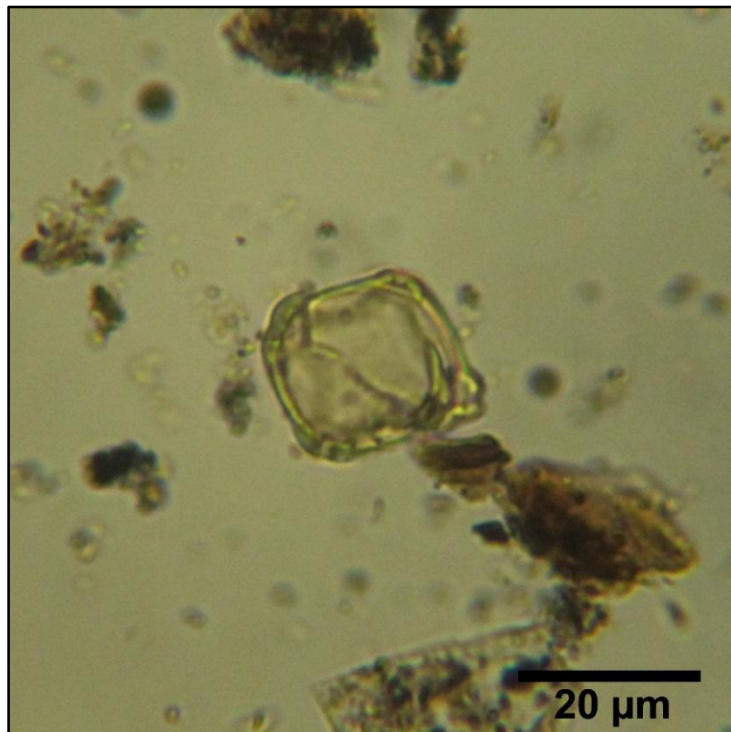


*Figure 23: Pollen grain of Pinus*





*Figure 24: Pollen grain of Amaranthaceae*



*Figure 25: Pollen grain of Alnus*

### 5 - 3- Diatoms

The results of diatom analysis are presented in Tables 4 and 5. A total of 680 valves were counted, belonging to 16 species. Two species were particularly abundant: *Achnanthes* cf *delicatula* (310 valves; 45.6%) and *A. hungarica* (220 valves; 32.4%). In addition, *Amphora montana* (55 valves; 8.1%), *A. veneta* (30 valves; 4.4%) and *Hantzschia amphioxys* (21 valves; 3.1%) were also well represented. The ecological requirements of the diatom species found at Spoletino are shortly described in Table 4.

Date of laboratory treatment	<b>2-6-2018</b>		
Quantity of sediment treated	<b>3.00 g</b>		
Date of microscope analysis	<b>June-July 2018</b>		
No. Diatoms counted	<b>680</b>		
<b>Taxon</b>	<b>Dimension</b>	<b>Number</b>	<b>Ecology</b>
<i>Achnanthes hungarica</i>	Length 6-45 µm, width 4-8 µm	220	Spreading often in weakly alkaline, with medium to high electrolyte content
<i>Achnanthes</i> cf <i>delicatula</i>	Length 7-20 µm, width 4-8 µm	310	Cosmopolite, from brackish water to freshwater with moderately high electrolyte content
<i>Epithemia turgida</i>	Length 5-200 µm, width 13-35 µm.	2	Waters with medium, higher electrolyte content, relatively common in many fossil freshwater deposits.
<i>Navicula</i> cf <i>concentrica</i>	Length.40-75 µm, width 9-12 µm.	2	Scattered in Europe with mostly low-population, mainly in oligotrophic lakes
<i>Nitzschia</i> sp.	Length 1-57 µm long and 1.3-2.0 µm wide.	1	In the brackish water on the coasts of the sea. In some cases, it exists in spring water
<i>Gomphonema clavatum</i>	Lengths.20-95 µm, width 6-14 µm	8	Occurring in lakes with very high electrolyte content
<i>Pinnularia microstauron</i>	Length 20- 90 µm, width 7-11 µm	6	Widespread in all oligosaprotrophic waters and very common in low-electrolyte

			waters, more rarely with medium and higher electrolyte content
<i>Pinnularia borealis</i>	Length 24-110 $\mu\text{m}$ , width 5-18 $\mu\text{m}$ .	9	They are common in standing and flowing waters from the plain to the mountains, prefers aerial sites and is common in dry mosses, on walls and in moist soil.
<i>Aulacoseira italica</i>	Diameter 3-23 $\mu\text{m}$ , high 8-20 $\mu\text{m}$	2	They are relatively rare cosmopolitan littoral forms in more or less eutrophic trenches, ponds, rivers and lakes, but also from humid aerobic sites, but they are found in many fossil materials.
<i>Amphora veneta</i>	Length 5-60 $\mu\text{m}$ , width 7-18 $\mu\text{m}$	30	It is an ecological form and has been found in sub-alpine lakes and other medium-electrolyte waters.
<i>Amphora montana</i>	Length 9-25 $\mu\text{m}$ , width 7-10 $\mu\text{m}$	55	Widespread in the area from the plain to the mountains, but always sporadic and seldom forming denser populations
<i>Hantzschia amphioxys</i>	Length 20-210 (300) $\mu\text{m}$ , width 5-15 (25) $\mu\text{m}$	21	Distribution in a wide variety of inland waters, rarely brackish water. Not suitable as an indicator of water pollution degree
<i>Epithemia adnata</i>	Length 15-150 $\mu\text{m}$ , width 7-14 $\mu\text{m}$	5	Distribution in some ancient lakes of Europe. Materials from lakes and rivers. Widespread freshwater. A high abundance was recorded in lakes as well as in fossil
<i>Cyclotella ocellata</i>	Diameter 6-25 $\mu\text{m}$	1	Preferring waters with medium and higher electrolyte content, are widespread in the brackish waters of the North and Baltic Seas.
<i>Neidium septentrionale</i>	Length 20-40 $\mu\text{m}$ , width 5 -6.7 $\mu\text{m}$	7	Widespread in mountainous areas.
<i>Surirella brebissonii</i>	Length 8-70 $\mu\text{m}$ , width 8-30 $\mu\text{m}$	1	They prefer the lake water and small water bodies, usually they occur in the early and late spring

Table 4: Characteristics of the taxa of diatoms from Spoletino and their environmental distribution

The diatom taxa were divided into two categories according of freshwater / slightly brackish ecological requirements.

<b>Freshwater</b>	No.	%	<b>Slightly brackish</b>	No.	%
<i>Achnanthes hungarica</i>	220	32.35	<i>Epithemia turgida</i>	2	0.29
<i>Achnanthes cf delicatula</i>	310	45.59	<i>Nitzschia</i> sp.	1	0.15
<i>Navicula cf concentrica</i>	2	0.29	<i>Gomphonema clavatum</i>	8	1.18
<i>Pinnularia microstauron</i>	6	0.88	<i>Alaucoseira italica</i>	2	0.29
<i>Pinnularia borealis</i>	9	1.32	<i>Hantzschia amphioxys</i>	21	3.09
<i>Amphora veneta</i>	30	4.41	<i>Cyclotella ocellata</i>	1	0.15
<i>Amphora montana</i>	55	8.09			
<i>Epithemia adnata</i>	5	0.74			
<i>Neidium septentrionale</i>	7	1.03			
<i>Surirella brebissonii</i>	1	0.15			

*Table 5: Classification of the diatom taxa according to the Freshwater/Brackish environment*

### ***Achnanthes hungarica* (Grunow) Grunow**

This species is characterized by a valve linear--elliptical in shape, with broadly rounded to acutely rounded ends. The raphe is located in the axial area; it is narrow to broad, with linear form, in some cases slightly widened proximally. Linear-elliptic ends, length 6-45  $\mu\text{m}$ , width 4-8  $\mu\text{m}$ . The axial area is narrow lanceolate with wedge-shaped to broadly rounded ends, the central area is narrow, but usually slightly wider than the axial area, often asymmetrically extended and forming a transverse band, which usually reaches at least one side of the shell edge (Ettl et al., 1986).

Cosmopolitan, often in weakly alkaline areas, with medium to high electrolyte. Mostly epiphytic, growing on submerged plants; the association with duckweed plants (genus *Lemna*) is



particularly noticeable. *A. hungarica* is hardly confused with other species, but the association of the smallest and largest cells of the developmental cycle is not always easy to address (Round & Basson, 1997) (See figure 26-27).



Figure 26: *Achnanthes hungarica* (Grunow) Grunow in Cleve & Grunow 1880: the valve with the raphe. Scale bar is 10  $\mu$ m.

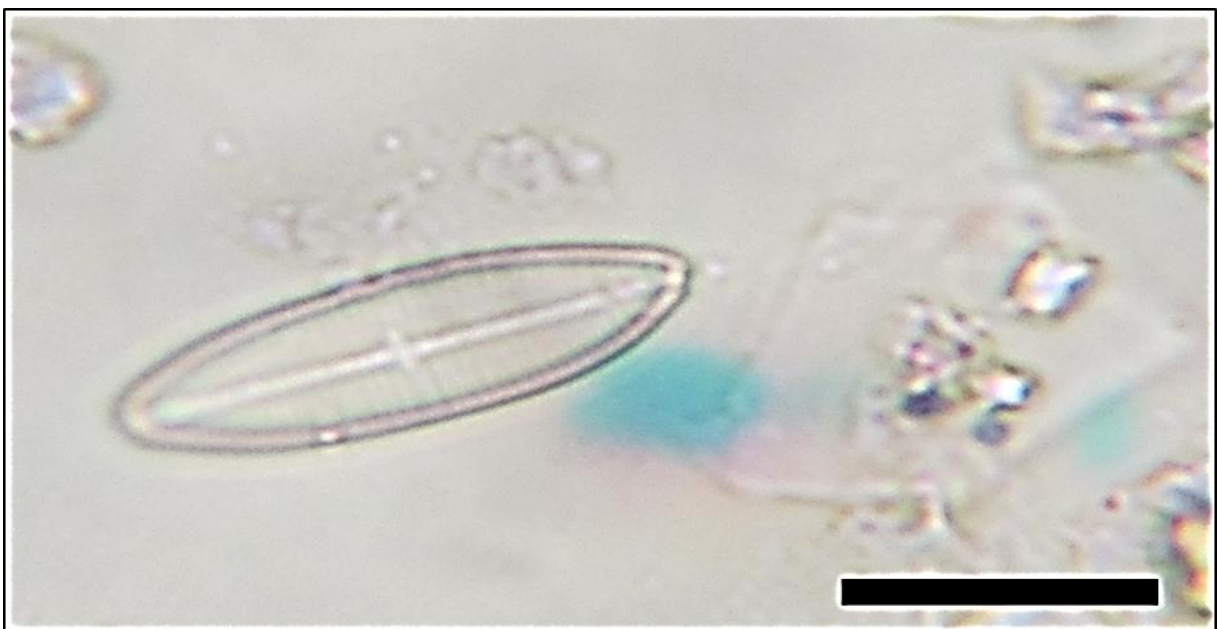


Figure 27: *Achnanthes hungarica* (Grunow) Grunow in Cleve & Grunow 1880: the valve without the raphe. Scale bar is 10  $\mu$ m.

***Achnanthes cf delicatula* (Kutzing) Grunow**

This species has valves with broadly lanceolate to elliptical outlines of very different sizes. Raphe distally bent on the same side. Strips on both valves form several areola rows. Length about 7-20  $\mu\text{m}$ , width 4-8  $\mu\text{m}$  (Ettl et al., 1986) (See figure 28-29)

Ecological amplitudes are limited to more electrolyte-rich waters, from calcareous freshwater sources with moderately elevated levels of electrolytes to brackish and seawater, therefore it can be considered cosmopolitan from brackish water to fresh water with moderately high electrolyte content. Some populations of this species are spread in brackish waters on seacoasts (Ettl et al., 1986).



*Figure 28: Achnanthes cf delicatula* (Kutzing) Grunow. Scale bar is 10  $\mu\text{m}$ .



*Figure 29: Achnanthes cf delicatula* (Kutzing) Grunow. Scale bar is 10  $\mu\text{m}$ .

***Epithemia turgida* (Ehrenberg) Kützing 1844**

A coarse texture and the shell outline, along with the location of the raphe on the shell, are characteristic features of this diatom. The valve is bent in valvar view, rectangular to elliptic in girdle view, with broad and flat truncated ends (Ettl et al., 1986).

Length: 5-200  $\mu\text{m}$ , width 13-35  $\mu\text{m}$ . The raphe branches curve either only in the middle of the valve towards the center of the valve (in long, linear forms), or they curve along their entire length and rise dorsally at the valve end (in shorter and broader forms) (Ettl et al., 1986).

Ecological amplitudes: all varieties are widespread. Often, as nursery plants in the littoral, they prefer meso- to eutrophic waters with medium to higher electrolyte content. They are also found in slightly brackish waters. Several varieties are relatively common in many fossil freshwater deposits (Ettl et al., 1986) (See figure 30).



*Figure 30: Epithemia turgida* (Ehrenberg) Kützing 1844. Scale bar is 10  $\mu\text{m}$ .



### ***Navicula cf concentrica* Carter 1981**

This species of diatom is characterized by lanceolate shape with pointed rounded ends; length 40-75  $\mu\text{m}$ , width 9-12  $\mu\text{m}$ . Raphe weakly lateral. Axial area distally rather narrow, lancet-shaped to the moderately large central area, which appears strongly asymmetric (Ettl et al., 1986) (See figure 31).

Environmental distribution: scattered in Europe with mostly small populations, mainly in oligotrophic lakes, relatively low in plant nutrients and containing abundant oxygen in the deeper parts (Ettl et al., 1986).



*Figure 31: Navicula cf. concentrica* Carter.1981. Scale bar is 10  $\mu\text{m}$ .

### ***Nitzschia* sp.**

Generally, many *Nitzschia* species have scalpel form. Cells symmetrical in valve view, very narrow, tapering from the middle part towards the cell apices. In girdle view, cells are linear to slightly bent bended and the ends are cut off straight. Cells form chains by overlapping of the tips, the overlap measuring c. 1/9 of the total cell length. The dimension of the cells are 1-57  $\mu\text{m}$  long and 1.3-2  $\mu\text{m}$  wide (Ettl et al., 1986) (See figure 32).

Ecological amplitudes: it concentrates in brackish waters along the coasts of the sea; otherwise, it is less frequent in inland salt water. It is not present in acid environments. In some cases, it is found in spring water (Priisholm, 2002).



*Figure 32: Nitzschia sp. Scale bar is 10  $\mu$ m.*

### ***Gomphonema clavatum* Ehrenberg 1832**

Characterized by striae formed by loose rows of few points. Valves very variable in size and proportion length/width, but always with a distinct keel form with narrow ends. Length 20-95  $\mu$ m, width 6-14  $\mu$ m. (Ettl et al., 1986) (See figure 27). The raphe is strongly wavy in large specimens, the axial area moderate to fairly wide, linear, the central region variable from very small to quite large by the occasional shortening of several central striae (Ettl et al., 1986) (See figure 33).

Ecological amplitudes: it usually occurs within lakes with silicate sediments and very high electrolyte content. It is sensitive to organic pollution (Ettl et al., 1986).



Figure 33: *Gomphonema clavatum* Ehrenberg 1832. Scale bar is 10  $\mu\text{m}$ .

#### ***Pinnularia microstauron* (Ehrenberg) Cleve 1890**

*Pinnularia* is one of the most species-rich genera of raphid pinnate diatoms. Members of *Pinnularia* and the closely related genus *Caloneis* have linear-lanceolate, blunt-ended or occasionally capitate valves with a central raphe system that terminates in helictoglossae at the poles (Souffreau et al., 2001) (See figure 34).

Valves linear to linear-lanceolate or linear-elliptic with parallel, slightly undulating or slightly convex sides, ends slightly wedge-shaped, rounded or broadly anteriorly rounded and flat to dull-shaped, length 20- 90  $\mu\text{m}$ , width 7-11  $\mu\text{m}$ . The outer striae slightly curved or slightly wavy, the raphe is weakly lateral or fusiform proximal with distinct, slightly sideways bent, drop-shaped central pores. The axial area is narrow, linear or slightly lanceolate towards the middle; central region very variable, small to large rhombic or a belt, which, however, does not always reach the edge of the rim or is asymmetrical (Ettl et al., 1986) (See figure 34).

Widespread in all oligotrophic waters and very frequently in low-electrolyte waters, more rarely with medium and higher electrolyte content (Ettl et al., 1986).



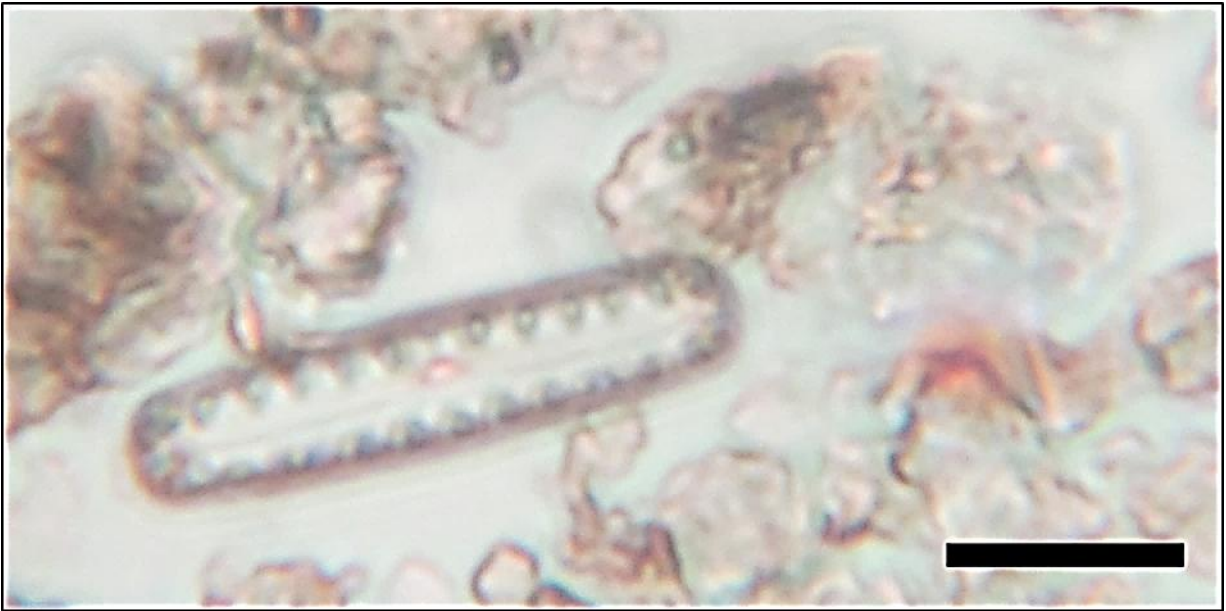
Figure 34: *Pinnularia microstauron* (Ehrenberg) Cleve 1890. Scale bar is 10  $\mu\text{m}$ .

### ***Pinnularia borealis* Ehrenberg 1843**

Almost rectangular form with the outline of the valves variable from narrow-linear to broadly linear, to linearly elliptical with parallel, slightly convex, concave or triangular sides. Length 24-110  $\mu\text{m}$ , width 5-18  $\mu\text{m}$ . The outer end of the raphe branch is slightly bent in the middle region and, especially in the case of larger shapes, is significantly laterally bent proximally to one side and terminating in a moderately large central pore, forming end gaps (Ettl et al., 1986) (See figure 35).

Environmental distribution: they are common in standing and flowing waters from the plain to the mountains, preferring aerial sites. They are common on dry mosses, on walls, and in moist soil. Moreover, the genus occurs globally in freshwater habitats of varying pH and trophic status, and in moist soils, peatlands, springs and marine coastal environments (Souffreau et al., 2001).





*Figure 35: Pinnularia borealis* Ehrenberg 1843. Scale bar is 10  $\mu\text{m}$ .

***Aulacoseira italica* (Ehrenberg) Simonsen 1979:**

The genus named *Aulacoseira* was first studied by Thwaites in 1848. He based the genus on *Melosira crenulata* Kutzing 1844 which Kutzing had presented, presumably as a form of *M. italica*, even though he reported the former (living) from flowing waters and the latter from diatomaceous earth (Crawford et al., 2011).

Characterized by cylindrical cells, the great majority of the cells are united in pairs as siblings, with flat to very slightly convex end faces, joined to long, closed chains, which may be easily loosened, diameter 3-23  $\mu\text{m}$ , height 8-20  $\mu\text{m}$  (See figure 36).

Ecological distribution: cosmopolitan littoral forms in more or less eutrophic ponds, rivers and lakes, but also from humid aerobic sites, they are found in many fossil materials (Crawford & Likhoshway, 1999).





*Figure 36: Aulacoseira italica (Ehrenberg) Simonsen 1979. Scale bar is 10  $\mu\text{m}$ .*

### ***Amphora veneta* Kützing 1844**

Cells elliptic with blunt rounded, sometimes slightly advanced ends, length 5-60  $\mu\text{m}$ , width 7-18  $\mu\text{m}$ , striae numerous, about 26-32 str./10, more or less delicately lined. Valves strongly dorsiventral with strongly convex dorsal side, ventrally straight or slightly concave, in the middle often a little bulbous, ends dull rounded, slightly ventrally pulled down (Ettl et al., 1986).

Raphe straight or slightly bent, filiform, very strongly displaced to the ventral margin, proximal ends almost straight or slightly dorsally curved, central pores often far apart, especially on longer specimens. However, this characteristic is very variable, and many populations and small specimens have quite close central pore. Distal raphe ends usually difficult to see and dorsally curved axial area on the dorsal side very narrow, on the ventral side wider, a delimited central area is missing, lateral area not visible in the LM (Ettl et al., 1986) (See figures 37-38) .

Environmental distribution: this taxon is an ecological form and has been found in sub-alpine lakes and other medium-electrolyte waters (Ettl et al., 1986).



Figure 37: *Amphora veneta* Kützing 1844. Scale bar is 10  $\mu\text{m}$



Figure 38: *Amphora veneta* Kützing 1844. Scale bar is 10  $\mu\text{m}$ .

### ***Amphora montana* Krasske 1932**

*Amphora montana* is characterized by convex sides and more or less drawn, flat rounded ends, length 9-25  $\mu\text{m}$ , width 7-10  $\mu\text{m}$ . The shells are slightly dorsiventral, dorsally and ventrally slightly convex, the ends long and slightly angled ventrally (Ettl et al., 1986) Raphe straight or slightly curved, proximally and distally dorsally distended, filiform. Axial area very narrow, central region uneven, developed only on the ventral side as semicircular to a semi-acicular surface (Ettl et al., 1986) (See figure 39).

Environmental distribution: this taxon is an aerobic form, widespread in the area from the plain to the mountains, but always sporadic and seldom forming denser populations (Ettl et al., 1986).



*Figure 39: Amphora montana Krasske 1932.*

### ***Hantzschia amphioxys* (Ehrenberg) W. Smith 1880**

*Hantzschia amphioxys* is a kind of diatoms with extremely variable length/width proportions, length 20-210 (300)  $\mu\text{m}$ , width 5-15 (25)  $\mu\text{m}$  (Ettl et al., 1986). Raphe with 4-11 fibulae / 10  $\mu\text{m}$ , the 2 middle farther apart from the neighboring ones, str. 11-28 / 10  $\mu\text{m}$ , the middle often spread slightly (Ettl et al., 1986) (See figure 40).

Environmental distribution: this diatom is cosmopolitan, being the most frequent diatom in the air-plank clay and in long-term dry, briefly creeping biotopes such as rock formations and soil. On the other hand, with a hard-to-determine ecological focus in a wide variety of inland waters, rarely brackish water. Not suitable as an indicator of water pollution degree (Ettl et al., 1986).



Figure 40: *Hantzschia amphioxys* (Ehrenberg) W. Smith 1880. Scale bar is 10  $\mu\text{m}$ .

### ***Epithemia adnata* (Kutzing) Brebisson**

The main diagnostic features differentiating the species of the genus *Epithemia* is the shape and sizes of valves, the number of ribs and rows of areolae forming striae (in 10  $\mu\text{m}$ ), and the position of branches of the canal-raphe (Vishnyakov et al., 2014). Morphologically, *Epithemia adnata* is characterized by a V-shaped raphe. It is differentiated from other *Epithemia* by characters like cell shape and size, apex shape, the extent of raphe curvature and striae/costae density (Ettl et al., 1986). (See figure 41). Length 15-150  $\mu\text{m}$ , width 7-14  $\mu\text{m}$  (See figure 41).

The environmental distribution of species of the genus *Epithemia*, as of many other groups of diatoms, is considered cosmopolitan. They are widespread in freshwater environments; a high abundance was recorded in extant lakes as well as in fossil (Vishnyakov et al., 2014).



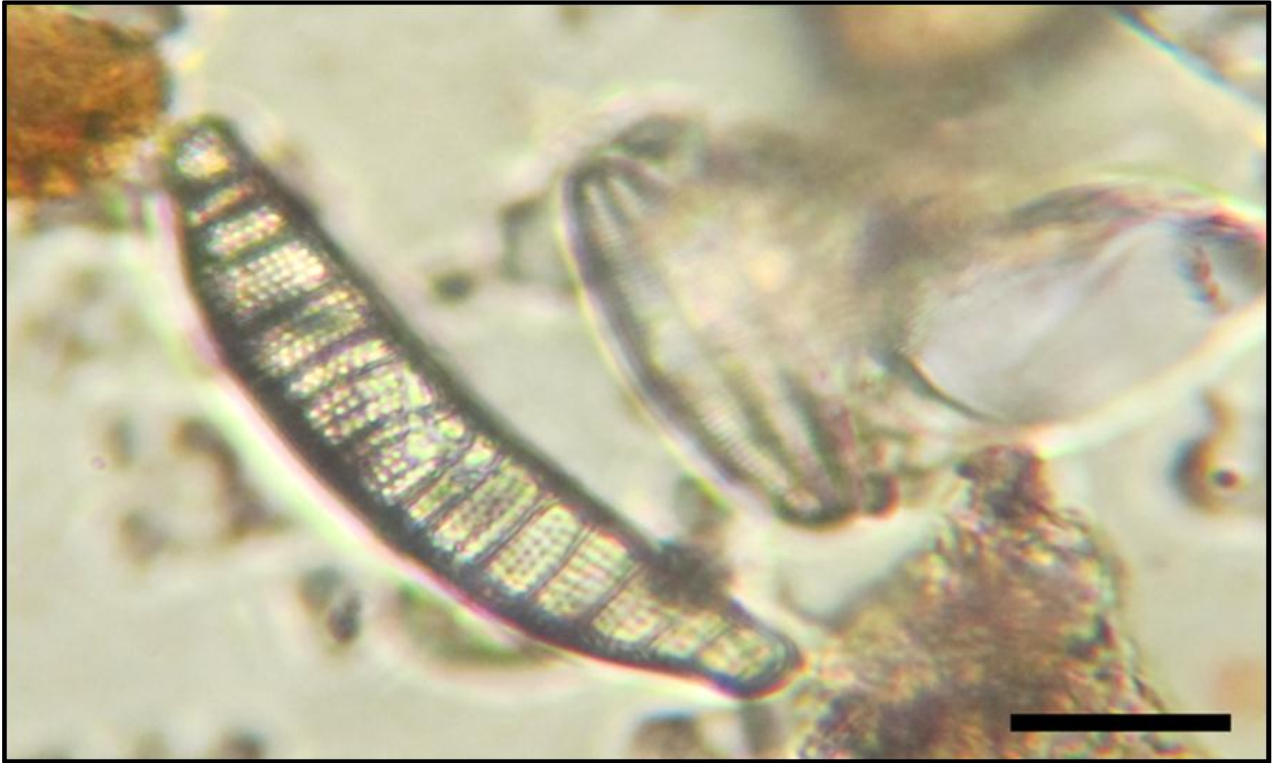


Figure 41: *Epithemia adnata* (Kutzing) Brebisson. Scale bar is 10  $\mu\text{m}$ .

### ***Cyclotella ocellate* Pntocsek 1901**

It has discus-shaped cells with almost flat shapes, diameter 6-25  $\mu\text{m}$ . The edge zone is striped, about 13-15 radial stripes / 10  $\mu\text{m}$ . Midfield with two to five (but mostly three) ocelli, which often have a greenish glow in the LM, and corresponding papillae. In the center a single clear point (Ettl et al., 1986) (See figure 42).

Environmental distribution: this species of *Cyclotella* prefers waters with medium to high electrolyte content, it is widespread in the brackish waters of the North and Baltic Seas (Ettl et al. 1986).



Figure 42: *Cyclotella ocellata* Pantocsek 1901. Scale bar is 10  $\mu\text{m}$ .

***Neidium septentrionale* Clever-Euler 1939.**

It is characterized by shells linear with concave or triangular edges, the middle area is always narrow, rounded ends or weakly wedge-shaped, length 20-40  $\mu\text{m}$ , width 5 -7  $\mu\text{m}$ . (Ettl et al., 1986). Raphe filiform, distally bifurcate proximally with thin central ends bent to opposite sides. Axial area narrow, linear, with marginal longitudinal lines, a rectangular fascia, often in the middle rhombic, in the central area. Very delicately dotted, parallel lines marginal, often oblique to the midline, parallel to convergent at the ends, 30-35 / 10  $\mu\text{m}$ , points approximately the same distance (Ettl et al., 1986) (See figure 43). Environmental distribution: widespread, it is found in mountainous areas (Ettl et al., 1986).



Figure 43: *Neidium septentrionale* Clever-Euler 1939. Scale bar is 10  $\mu\text{m}$ .

***Surirella brebissonii* Krammer & Lange- Bertalot**

It is characterized by round cells, moderately wedge-shaped. Large valves oblong-oval, medium oval, smallest broad-elliptic to almost round, length 8-70  $\mu\text{m}$ , width 8-30  $\mu\text{m}$  (Ettl et al., 1986) (See figure 44).

Environmental distribution: *Surirella brebissonii* prefers lake water and small water bodies. It usually occurs in the early and late spring (Dalu et al., 2016).



*Figure 44: Surirella brebissonii* Krammer & Lange-Bertalot. Scale bar is 10  $\mu\text{m}$ .

## 6- DISCUSSION

The results obtained from charcoal, pollen and diatom analysis from Spoletino have been discussed separately in the following sections.

### 6 – 1- Charcoal

The charcoal fragments retrieved from the cistern in the Spoletino Villa are not abundant, but they are very interesting for the insights they provide into the land use. In spite of the reduced number of identified fragments (50), a high diversity of taxa is recorded (5). All the largest wood fragments picked by archaeologists during the excavation belong to trunk fragments of deciduous and evergreen oaks (Table 2). They were possibly used for wooden structures. Oaks and elms are common components of the natural vegetation at hilly elevations and may represent the most widespread trees, whose wood was commonly used for daily activities in the villa.

Determining woods of the genus *Prunus* to the species level is very difficult (Schweingruber, 1990), in the absence of carpological remains. For this reason, although the anatomical characters of the retrieved *Prunus* charcoal point to *Prunus avium*, we leave this identification as tentative.

The presence of a significant number of fragments of *Olea europaea* (12%) and of *Prunus* cf. *avium* (18%) confirms the local presence of cultivated taxa, suggesting intentional plantation, although these trees may also be part of the natural vegetation of the Spoletino area, although in smaller amounts.

### 6 – 2 - Pollen

The results of the pollen analysis carried out in the site of Spoletino point to a forested environment (Arboreal Pollen percentages 87%), whose main natural trees were represented by deciduous *Quercus* (26%) and evergreen *Quercus* (20%). Oaks formed widespread woodlands in many parts of Lazio during Roman Times, as testified by their dominance in almost all the regional coastal and inland pollen records (Magri and Sadori, 1999; Di Rita et al., 2010; Bellotti et al., 2011; Di Rita et al., 2018; Sadori, 2018). The local woodlands were also composed of other



trees and shrubs never exceeding 2%, such as *Quercus cerris*, *Juniperus*, *Ostrya*, *Ulmus* and *Corylus*, which are typical floristic elements of the hilly and lowland stands of the *Quercetum mixtum* vegetation. The record of *Alnus* (5%), instead, reflects the presence of riparian vegetation developed at the flanks of the Tiber River, which is located at around 2 km from the site.

The significant frequencies of *Pinus* (10%) suggest either the presence of ornamental pine trees that Romans were used to plant in both urban contexts and rural villas (Allevato et al., 2010, 2016), or the presence of pine populations not far from the site. Considering that the closest natural pine communities are currently located in the calcareous slopes of the Martani Mountains (Schiller and Brunori, 1992), only 30 km far from Spoletino, it is likely that also in the past most of the highly produced and easily dispersed pollen of *Pinus* found in our record came from this area.

As to the record, of *Fagus* (>2%), its provenance is to be related to the pure beech stands located in the highest sectors of Cimini mountains, located at less than 20 km from Spoletino, that in Roman times were quite well developed (Magri and Sadori, 1999).

Among the arboreal pollen, the high amount of *Olea* (17%) stands out, documenting a local olive exploitation during Roman times. In this period, similar frequencies are hardly reported in other pollen records of Italy. For example, in Apulia a real conversion of the territory in the extensive olive orchard as it currently appears started only from the 6<sup>th</sup> century with the Byzantine domination, while during Roman times *Olea* cultivation shows a decline to be related to adverse climate conditions (Di Rita and Magri, 2009). The high frequencies of *Olea* at Spoletino contrasts with the main cultivation practice of the area at present, which is addressed to the production of white grapes varieties and high-quality wines.

The absence of *Vitis* pollen in the Spoletino pollen record induce to exclude any local vineyard when the archaeological site was active.

*Juglans* pollen with frequencies exceeding 1% suggests the presence of local walnut trees, which are commonly cultivated in gardens and orchards during Roman times known as Jovis Glans trees (Jupiter's acorn or nut of the Gods) (Mercuri et al., 2013).

Direct and indirect evidence of farming activity can be found also in the record of non-arboreal pollen. The admixture of Amaranthaceae, Brassicaceae, *Asphodelus*, *Plantago*,

Cichorioideae and Fabaceae strongly suggests the presence of herbaceous communities typical of meadows disturbed by livestock and other agropastoral activities. However, Brassicaceae and Amaranthaceae were also possibly cultivated as human food.

In Roman times, the cultivation of *Brassica* species, mostly cabbage varieties, was suggested in Campanian sites (Russo Ermolli et al., 2014; Di Rita et al., 2018). Pollen of Chenopodiaceae, which in coastal sites mostly indicates the development of halophilic communities on salty soils; in inland sites, it mostly reflects either the cultivation of *Beta vulgaris* or the development of nitrophilous pioneer weeds, such as *Chenopodium album*, competing with field vegetables (Grundy et al., 2004).

Although cereal type pollen is recorded only with one grain, its find is consistent with the presence of cereal crop in the area.

Apart from these anthropogenic pollen indicators the record is dominated by pollen of grasses (Poaceae: 5%), which could come from different environments such as open meadows, riparian reeds, as well as from pioneer plant communities in cultivated fields.

The pollen of sedges (Cyperaceae/Juncaceae 0.5%) may have been produced by freshwater marsh communities developed in the Tiber river margins, as usually occur in the floodplains of rivers within environments characterized by stagnant water (Di Rita et al., 2015).

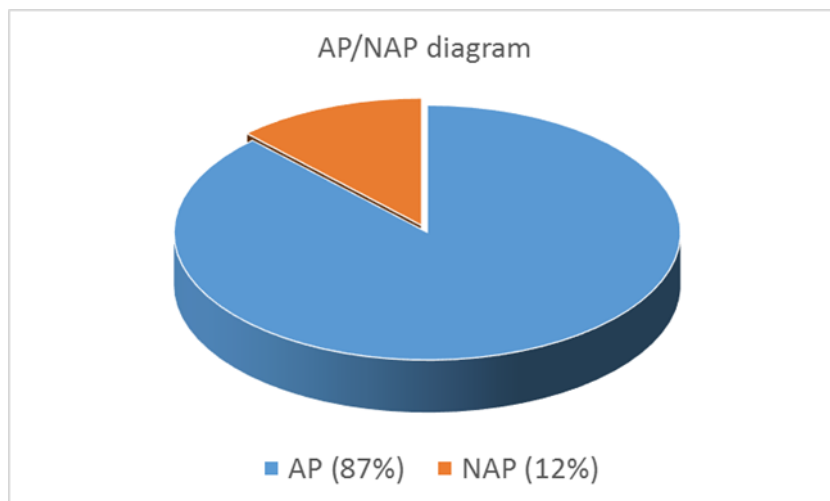


Figure 45: Chart of the Arboreal vs Non-Arboreal Pollen

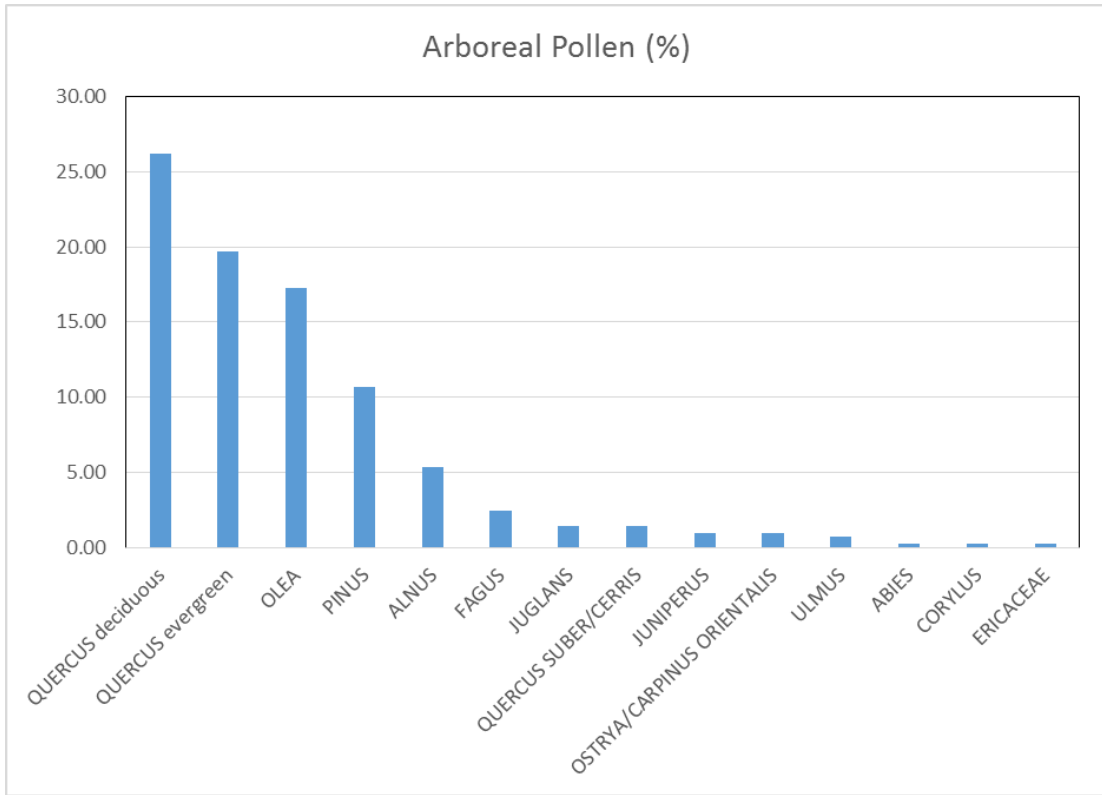


Figure 46: Chart of the percentage of the Arboreal Pollen

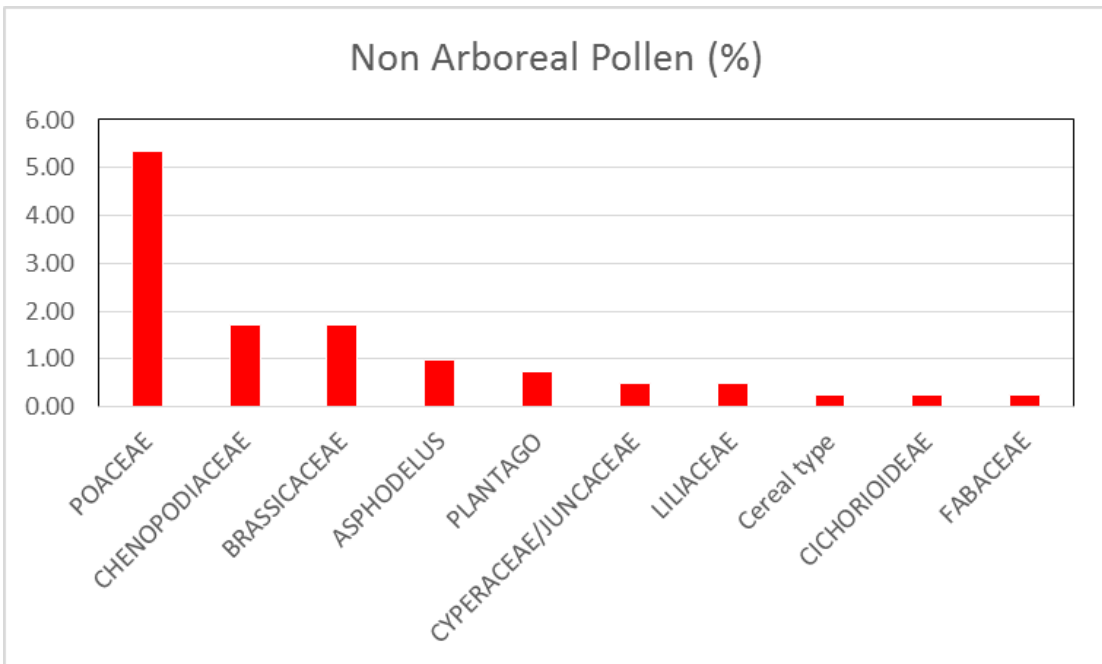


Figure 47: Chart of the percentage of the Non Arboreal Pollen

### 6 - 3 - Diatoms

The diatoms sampled from the cistern of the Roman villa of Spoletino provide valuable information on the water environment. In particular, most of the diatoms are typical of freshwater or slightly brackish environments (See figure 48-49-50). All the diatom species identified are benthonic forms, except *Cyclotella ocellata*, which is however rare in the Spoletino samples. Some species are littoral (e.g. *Epithemia*) or epiphytic, growing on submerged plants, as in the case of the dominant species *Achnanthes hungarica*. They suggest that the water level in the cistern was always rather low, so that at times the cistern could be almost dried out, as indicated by several diatom species typical of wet soils and aerobic sites, such as *Hantzschia amphioxys*, *Aulacoseira italica*, and *Pinnularia borealis*, the latter being rather common on wet walls.

Several species found in the Spoletino cistern live in waters with high electrolyte content. The diatoms identified are never acidophilous and generally indicate a rather oligotrophic environment. The waters were not polluted, and relatively low in nutrients. Species of running waters were absent, as it is normally expected in a cistern.

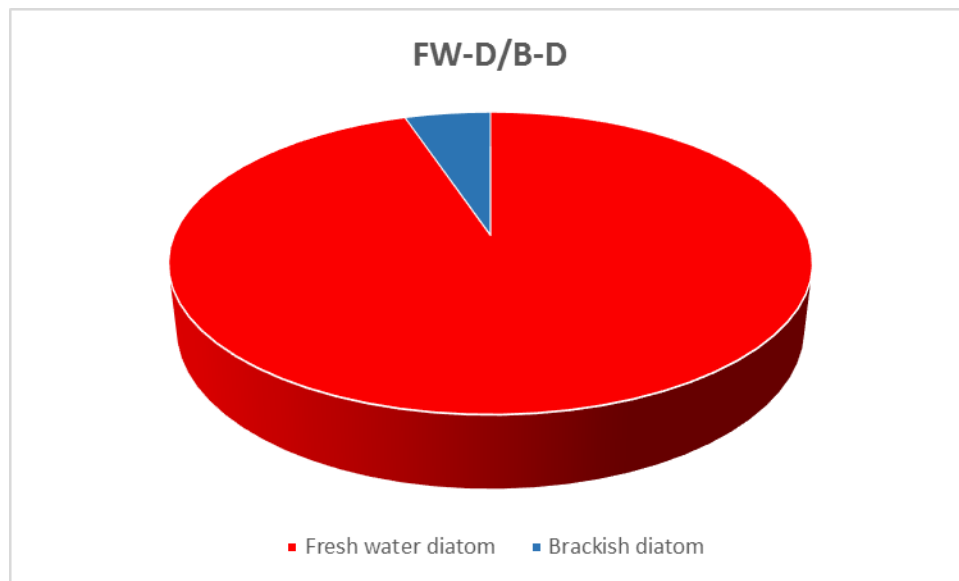


Figure 48: Chart of fresh water Diatoms/ brackish Diatoms

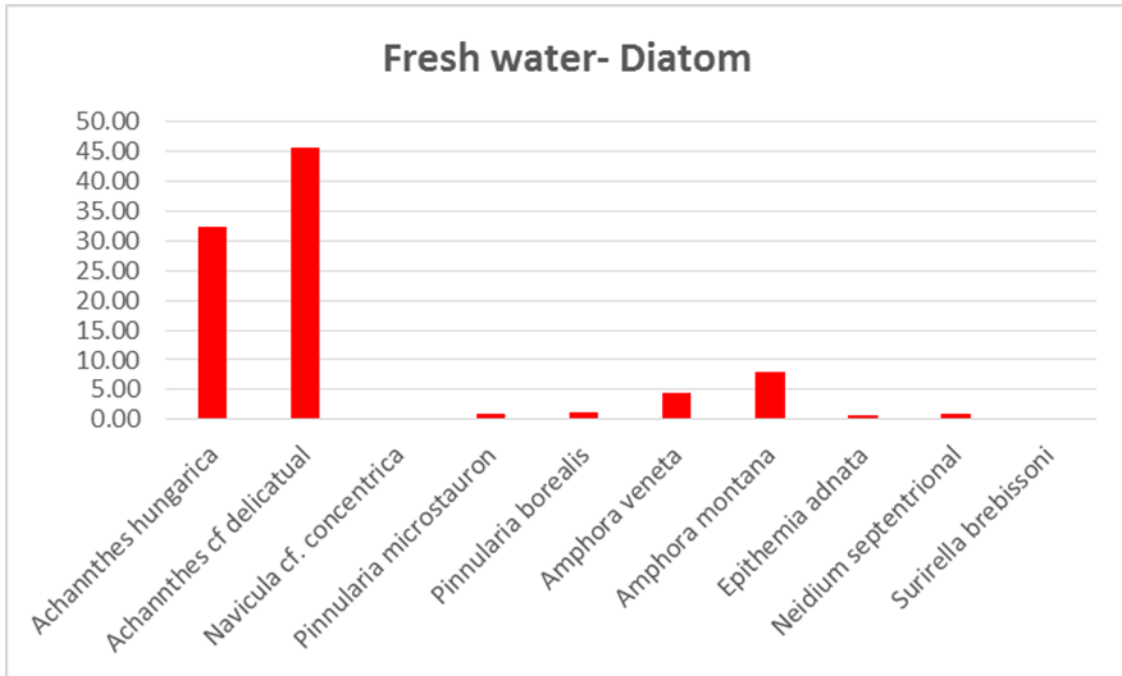


Figure 49: Freshwater diatoms

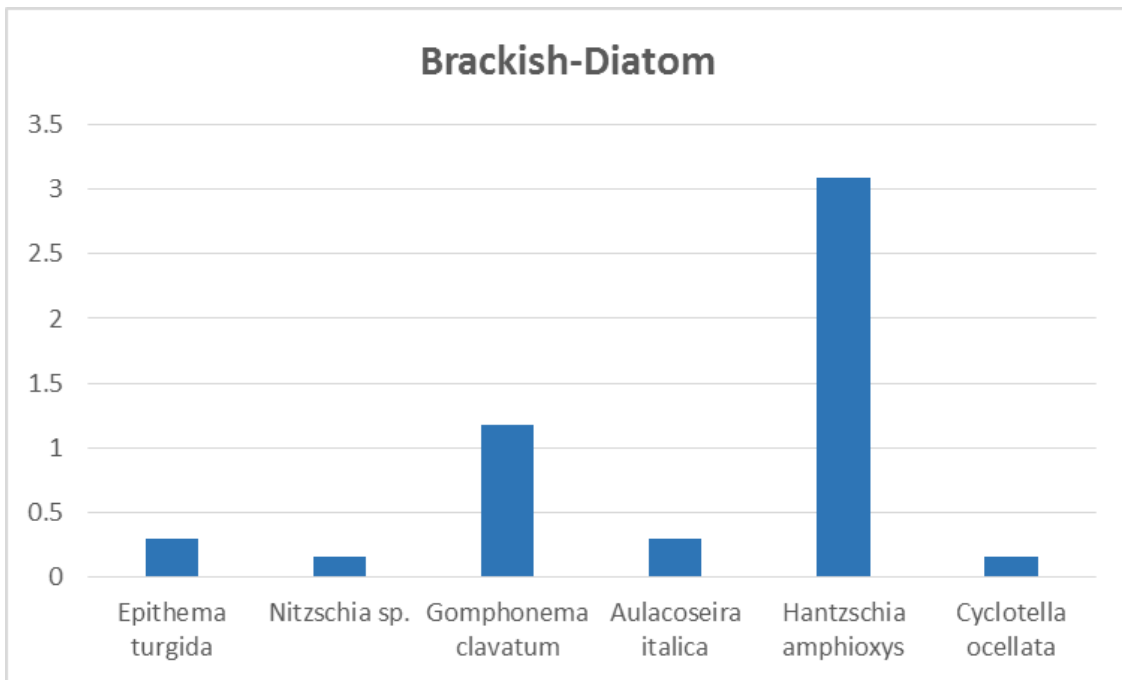


Figure 50: Brackish diatoms

## 7- CONCLUSION

My work of separation and identification of the materials from the cistern of the Roman Spoletino villa has made it possible to reconstruct different aspects of the environmental conditions around and within the cistern at the time of the sediment deposition. This was possible because the fine sediment filling the cistern allowed the preservation of wood. Besides, it was possible to retrieve, identify and count microfossils like pollen and diatoms. The chance to study such different materials, providing various aspects of environmental reconstruction is an important added value to the archaeological research. Unfortunately, no seeds species were found, which could have provided direct information on the local subsistence economy. The lack of carpological remains may be due to the fact that the sedimentary environment was not especially favorable to waterlogging, as the water level was low and the cistern was often dry, as indicated by the high number of diatoms typical of aerial environments and damp soils. By contrast, the wood fragments, which were not well preserved, could persist in sediments because they were already charred. On the whole, considering the limited amount of sediment that was treated for this thesis, a more in-depth study on different levels from the cistern may be expected to provide more detailed results.

Pollen and anthracological finds complement each other in showing the main features of the local natural vegetation and of the tree crops in the territory of the villa. In particular, they indicate that the main floristic elements of the surrounding woodlands were oaks, both deciduous and evergreen, accompanied by other tree taxa, such as elms, in lower amounts. The presence of olive trees is testified by both pollen and wood remains, found in percentages higher than one would expect if olive trees would be wild. This indicates intentional olive plantation and management by the Roman farmers of the villa. This result is very interesting, as the area is currently mainly devoted to vineyards, which were missing during the Roman times, as suggested by the lack of *Vitis* pollen in the record. The finding of *Prunus cf. avium* suggests that cherry trees could be cultivated in the villa, which is possible considering that the species was imported by the Romans during the first century BC as reported by Pliny, who argued that this fruit crop was introduced to Rome from the Pontus area (south-eastern part of the Black Sea Basin) by Locullus in 73 BC (Zohary et al., 2012). Another evidence for local cultivations is the pollen find of *Juglans* with frequencies exceeding 1%, suggesting the presence of local walnut trees.

Indirect evidence of farming activity is also provided by other pollen taxa, including Amaranthaceae, Brassicaceae, *Asphodelus*, *Plantago*, Cichorioideae, and Fabaceae, which form herbaceous communities typical of meadows affected by livestock and other agropastoral activities.

The archaeobotanical questions that were asked by the archaeologists excavating in the Spoletino villa have found several answers, provided by the variety and complementarity of vegetal macro- and microremains (wood, pollen, and diatoms) preserved in the cistern. The integration of different archaeobotanical methodologies has offered a new perspective of the palaeoenvironmental research in this archaeological site, which is proving to be an exceptional settlement of Roman Imperial age.

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