

ANNEX: PALYNOLOGICAL STUDY

Sebastián Pérez Díaz, Sara Núñez de la Fuente

Palynological report on 15 sediment samples
from the archaeological site of Los Azogues,
Santander (Cantabria).

A.1

INTRODUCTION

The reconstruction of past societies, through their material remains, requires collaboration between different disciplines, both in the field of social sciences and the so-called earth sciences, in order to explain the mechanisms of change and evolution of these societies. In this multidisciplinary context, the study of botanical remains recovered from archaeological sites helps to characterise, from a social and economic point of view, the human groups that inhabited them.

Palaeobotany is essential for explaining the relationship between humans and the environment, attempting to provide an explanatory framework for questions such as forest dynamics, climate evolution, the possibilities for plant life, anthropisation of the environment (deforestation), the existence of detectable economic activities that leave their mark on the landscape, land use, etc.

This report presents the results of the analysis of 15 sediment samples from the archaeological site of Los Azogues (Santander, Cantabria). This analysis is particularly interesting given the location and use of the deposit. The site is located on one of the outer walls of the Cathedral of Santander, and corresponds to a medieval necropolis with a chronology that extends from the 12th to the 15th century.

This report has been prepared by Dr. Sara Núñez-de-la-Fuente, a professional archaeologist specialising in archaeoaplinology, and Dr. Sebastián Pérez-Díaz, from the Department of Geography, Urban and Regional Planning at the University of Cantabria (Santander).

MATERIAL AND METHODS

Sampling

In February 2023, a total of 15 sediment samples were collected for palynological study (Table A1). All of them were taken following the standardised method in archaeological palynology, minimising the risk of contamination of the sample with pollens from the current pollen rain.

More in detail, in the case of Los Azogues archaeological site, there was an open and continuous stratigraphic profile more than 4 metres deep, so it was decided to take a vertical sample on the stratigraphic profile itself (figures A1 and A2). Sampling was always carried out by levels or stratigraphic units (SUs), previously defined by the archaeologist responsible for the excavation, in order to cover the widest possible temporal and cultural interval.

Once the sampling area was decided, the first step was to clean the stratigraphic profile chosen to remove current pollen contamination; this should always be carried out from top to bottom. After this, approximately 20 grams of sediment were placed in individual zip-lock bags, suitably labelled, and the material used was cleaned with distilled water between samples.

Sampling was carried out starting from the oldest sample (the deepest) to the most recent (the shallowest), i.e. always from the bottom upwards, in order to avoid the risk of contamination by falling sediment. In general, we have opted for a sampling resolution of approximately 10 cm intervals between each sample, depending, of course, on the strength of each stratigraphic unit considered. In fact, in some of them, precisely to avoid the risk of contamination due to the presence of landslides, stone blocks, roots, burrows, etc., a different sampling interval has been used. In some cases, several samples were taken from the SU unit to study the potential variability along the same SU.

Chemical treatment of palynological samples

The chemical treatment used basically follows the so-called classical method (Girard and Renault-Miskovsky, 1969; Goeury and Beaulieu, 1979; Faegry and Iversen, 1989; Burjachs, 1990; Moore et al., 1991; Burjachs et al., 2003; López-Sáez et al., 2003). This method consists in that, after washing the sediment, it is subjected to different chemical attacks with acids and bases (hydrochloric acid, sodium hydroxide, potassium hydroxide), whose objective is to successively eliminate carbonates, organic matter and silicates, in such a way that at the end of the process only the spore-pollinic content remains. The latter is concentrated

by means of a dense liquor, in this case Thoulet's liquor (Goeury and Beaulieu, 1979), which makes it possible to separate the pollen and non-pollen microfossils from the rest by densimetric differences.

The final portion of the sediment is preserved in glycerine gelatine for subsequent mounting and reading under an optical microscope. In a more detailed way, the chemical treatment followed can be summarised in the following stages:

1. After separating the sediment in beakers (approximately 30 grams per sample), distilled water was poured in and sieved if necessary. As a control element, a Lycopodium tablet was added, an exogenous element usually used to estimate pollen concentration (Stockmarr, 1971). Hydrochloric acid (HCl) is then added to remove carbonates and break up the Lycopodium tablets. When this has occurred, the HCl is neutralised by successive washes with distilled water and centrifugation (5 minutes at 2500 revolutions per minute or rpm).
2. Once the HCl has been neutralised, sodium hydroxide (NaOH) diluted to 20 % is added to act on the silicates. To achieve this, each sample is placed in a container with hot water for 20 minutes. The NaOH is then neutralised by successive washes with distilled water and centrifugation (5 minutes at 2500 rpm).
3. Finally comes the most delicate phase of the chemical treatment of the samples, since the aim is to definitively separate the pollen content from the rest of the sediment by densimetric difference (Girard and Renault-Miskovsky, 1969; Goeury and Beaulieu, 1979). To do this, after pouring a drop of HCl on each sample, a dense pollen concentration liquor called Thoulet's liquor (made from potassium iodide, cadmium iodide and distilled water) is added at density 2. After shaking for a variable time, usually about 50 minutes in a mechanical shaker or just 8 seconds in an ultrasonic cell disintegrator (Sonifer 450 CE ultrasonic disintegrator with threaded micro-tip), this liquor is filtered through glass fibre filters, in which the pollen content of the sample remains, according to the density mentioned above. After a further attack of HCl to remove the carbonates from the filter or hydrofluoric acid in the case of using glass fibre filters and its corresponding neutralisation, the sample is ready to be observed under the microscope.
4. All samples analysed in this work have been preserved in eppendorf tubes in gelatinised glycerine. In no case were they stained because of the obvious possibility that staining could mask the ornamentation of certain pollen types (Franco-Múgica et al., 1997).

Microscopic identification

The preparation of the samples for observation under the optical microscope was carried out using 76 x 26 mm slides, 1 mm thick, on which 24 x 60 mm coverslips were placed, finally

sealing them with hystolaque to avoid losing the sample and to fix it and make it easier to read. Identification was carried out by optical microscopy (Nikon microscope - 40x and 100x objectives).

Pollen microfossils

Pollen identification is possible thanks to the fact that one of the grain walls (sporodermis) is made up of a very resistant substance called sporopollenin, which allows it to be preserved over time, as well as resisting the chemical process to which the samples are subjected. The structure and chemical composition of the sporodermis of the spores of ferns and other pteridophytes is similar to that of pollens, which is why they can also be preserved and studied. The main diagnostic characters of pollens are the following:

- Number of grains. Pollen within the anther are bound together 4 by 4, in the form of tetrads, but when released from the anther, most are dispersed individually (monads). More rarely, they may occur in groups of 2 (dyads), 4 (tetrads) or >4 grains (polyads).
- Exine ornamentation and structure. The exine is the outermost layer of the sporodermis, itself made up of different layers, the outermost of which (ectexine) may be smooth (without ornamentation), baculate (presence of elements more than one micron and taller than wide), echinate (pointed elements more than 3 microns), etc.
- Distribution and shape of the apertures. The apertures are areas where the exine becomes thinner and may even disappear, favouring the exit of the pollen tube through which fertilisation of the ovocell occurs. They characterise the pollen grain according to their number, shape and distribution. There is a specific denomination for these types of characters according to the number of apertures, with some species lacking them and others with more than half a hundred (0: inarpetuate; 1: mono-, 2: di-, 3: tri-, 4: tetra-, 5: penta-, 6: hexa-, >6: poly-). Depending on the shape of these openings, pollen grains are classified as colpate (opening twice as long as wide), porate (opening as long as wide) and colporate, a mixture of both. The distribution of the apertures is also a defining element, whether they are located in the equatorial zone, distributed over the entire surface or restricted to the poles (in the equatorial zone: zonate; over the entire surface: panto-).
- Shape and size of the pollen or spore. Since the shape and size of the pollen grain may vary after the pollen has left the flower as a result of exposure to environmental conditions and sedimentation, these characteristics are taken into account as a guide rather than a determining factor.

Reference collections were used to identify pollen morphotypes. In addition, a variety of literature on the morphometric characteristics of palynomorphs has been used (Moore and Webb, 1978; Bonnefille and Rioulet 1980; Moore et al., 1991; Blackmore et al., 1992; Reille, 1992, 1995).

Non Pollen Palynomorphs

A very important advance related to palaeoenvironmental studies, which has been developed since the mid 1970s, is the study of what have been called non-pollen palynomorphs (NPPs). This is a set of elements found in the palynological residue, consisting of both organic and mineral matter, including algal spores, cyanobacteria, fungal spores and thallus remains, fungal fruiting bodies, fragments of bryophytes or pteridophytes, animal microfossils, microfossils of unknown biological nature, etc. (López-Sáez et al., 1998, 2000; van Geel, 2001; Galop and López-Sáez, 2002).

The study of non-pollen palynomorphs does not involve additional chemical preparations and treatments, but the same as those used in traditional palynological analyses (van Geel, 2001). It is therefore an important and indispensable source of additional information on palaeoecological and palaeoenvironmental aspects that are difficult to detect with traditional pollen analyses based exclusively on the study of pollen.

In the palynological protocol, non-pollen palynomorphs can effectively contribute to understanding aspects such as the degree of water pollution, the temporal evolution of trophism, the selective use of fire, the natural or anthropic origin of fires, the relationship between periods of dryness and humidity, the level of water circulation, the variation in the water table, the degree of erosion, and even the level of anthropisation of a site in the sense of being able to quantify the degree of occupation, etc.

For the identification of these NPPs, abundant literature references have been used (van Geel, 1978; Pals et al., 1980; van Geel et al., 1981, 1983, 1983, 1989, 2003; Bakker and van Smeerdijk, 1982; Pantaleón et al., 1996; López-Sáez et al., 1998, 2000). The MNPs identified have been named according to the typology established for each of them by the school of Dr. B. van Geel of the University of Amsterdam (The Netherlands), although in most cases it is possible to identify them at a generic or specific level.

Processing and representation of palynological data

When considering whether a pollen sample is representative for the interpretation of a palaeopalynological analysis, two concepts must be taken into account: pollen sum and taxonomic diversity. In this work, it is accepted that a sample is representative of the surrounding vegetation when (López-Sáez et al., 2003):

- The pollen base sum counts 200 pollen grains, discounting hydrohydrophilic taxa, non-pollen microfossils, Aster type, Cardueae and Cichorioideae.
- At least 20 different taxa are present in the pollen base sum.
- The percentage of indeterminable pollens does not exceed 50% of the pollen base sum.

The last step followed in the pollen analysis was the elaboration of a graph showing the development of the different pollen and non-pollen types along the sequence. The data processing and graphical representation was carried out with the help of the TILIA and TGview programs (Grimm, 1992, 2004), together with the image processing program Inkscape (free software) for the improvement of the figures. For the elaboration of the pollen diagram, as already mentioned, hydro-hydrophilic taxa, non-pollen microfossils and Aster, Cardueae and Cichorioideae have been excluded from the base sum, since they are usually over-represented due to their zoophilic character (Bottema, 1975; López Sáez et al., 1998, 2000, 2003). The relative percentage of these excluded palynomorphs has been calculated with respect to the total sum (figure A3).

A.3

RESULTS AND DISCUSSION

The first comment concerns the state of preservation of the sporopollen remains. In general, the plant microfragments were in a fairly poor state of preservation, which made it very difficult to assign them to pollen types. As far as the representativeness of the samples is concerned, 9 of them were either sterile or not statistically representative (as they did not contain the minimum number of pollens mentioned above). Only 6 samples out of the total have reached the minimum threshold mentioned above (more than 200 pollen grains, discounting hydro-hydrophilic taxa, non-pollen microfossils, type Aster, Cardueae and Cichorioideae, a minimum of 20 different taxa and with an adequate taxonomic variety, and indeterminate values lower than 3%).

With regard to the composition of the plant cover in the area around Los Azogues (figure A3), tree pollen values range from 40.8-46.6%. At the local scale, the most representative taxa are deciduous; Among them are deciduous *Quercus* (*Quercus robur*, *Quercus pirenaica* or *Quercus faginea*, 5.7-10.8%), hazelnut trees (*Corylus*, 1.9-9.4%), birch trees (*Betula*, 5.7%) and lime trees (*Tilia*), showing the existence of small patches of mixed deciduous forests in the area, well adapted to an Atlantic climate dominated by humidity. In relation to these same environmental conditions, some taxa typical of riparian environments are documented, such as willows (*Salix*, 5.7-11.3%), alders (*Alnus*, maximum values of 7.7%), ashes (*Fraxinus*, maximum values of 7.5%) and elms (*Ulmus*), although taking into account local topographic and environmental factors, they could well be related to stable running water courses, or to the aforementioned mixed deciduous forests. The 16th century historian Juan de Castañeda described the area around the city of Santander in 1592 as follows: "it has many mountains, mainly of oaks, holm oaks, chestnuts and beches". In the same way, the description of this landscape is repeated, in this case referring to the region of Las Asturias de Santillana, which is said to be "all full of large forests and populated with many forests and groves of large and infinite chestnut, walnut and oak trees" (Díez-Herrera, 1987).

Other morphotypes identified are *Pinus* sp, with maximum values of 9.3%. However, due to their high pollen production and wide geographical dispersion (due to their anemophilous pollination), their local landscape representation was residual. Studies of current pollen rainfall have shown that only when values of more than 60% are documented can we consider the existence of pine forests on a local scale (López-Sáez et al., 2013). In this case, we must assume the presence of pine forests on a regional scale, possibly in the nearby mountainous areas, where different palaeobotanical studies have documented the presence of these pine forests in the Asón area (Pérez-Díaz et al. 2016a), as the closest reference, but not in percentages (< 20%) that make us think of extensive pine forests, but rather in a landscape that would have a band of pine forests not too extensive above the deciduous forest. We also find in this location of Los Collados del Asón a landscape very similar to that of Los Azogues for the same chronology, with a composition of deciduous woodland ranging between 35.5% and 58%, formed mainly by hazel and oak trees, and accompanied by lime trees, elms and willows.

In the Cantabrian region, the existence of pine forests has been extensively documented in practically all the sites and peat bogs that have been studied over time. However, the presence of these pines, more extensive during the Pleistocene, was reduced with the arrival of the Holocene, which would have been a period of general climatic improvement. The warmer conditions of this climatic period would have led to the retreat of the pine forests and the development of deciduous formations (oak and beech forests), as has been described in regional pollen records from the eastern Cantabrian section, such as the Zalama, Los Tornos and Culazón peat bogs (Peñalba, 1989; López-Sáez et al, 2013; Pérez-Díaz et al., 2016b), in Lago de Ajo in the central part of the Cantabrian Mountains (Allen et al., 1996), or in the Alto de la Espina peat bog in Asturias (López-Merino et al., 2011).

Other interesting tree components are chestnut trees (*Castanea*), identified with maximum values of 7.5%. The presence of chestnut (*Castanea sativa* Mill) in SW Europe is still under debate, as many authors only consider the presence of this species in these territories as a consequence of its intensive cultivation since Roman times (Scarascia-Mugnozza et al. 2000, Conedera et al., 2004; Krebs et al., 2004). However, the palaeobotanical record documents it in SW Europe from the Pliocene and Lower Pleistocene (Huntley and Birks, 1983). In the Iberian Peninsula it has been identified in the fossil record at least since the Lower Pleistocene (Can Guardiola, Atapuerca), as well as in the Middle Pleistocene (Lezetxiki, Atapuerca, Torralba, Ambrona, Pinedo Formation, Bolomor), possibly in relation to glacial refuges. The autochthonous character of the chestnut tree for the Iberian Peninsula has been proposed from different scientific fields (García Antón et al., 1990; Morla Juaristi, 1996), and palaeobotanical data support this hypothesis (Carrión et al., 2003; Gómez-Orellana et al., 2007; Muñoz-Sobrino et al., 2004; Postigo-Mijarra et al., 2008, 2010, López-Sáez et al., 2017). In the context of the northern Iberian Peninsula, the presence of chestnut has been documented in the archaeological site of Laminak II from ca. 12500 cal BP (Uzquiano, 1994), in the Gesaleta peat bog from ca. 10000 cal BP (Ruiz-Alonso et al., 2019), in the Arbarrain peat bog since ca. 8000 cal BP (Pérez-Díaz et al., 2018), in Atxuri since ca. 4500 cal BP (Pérez-Díaz et al., 2015), to give just a few examples. Therefore, their presence in this context is

not strange. In any case, its scarce representation does not seem to derive from cultivation processes, but rather from wild species.

Another interesting morphotype is the yew (*Taxus*), which is infrequent in palynological records due to conservation problems and poor dispersal (Cortés et al., 2000) and which in this case has been identified in samples from stratigraphic units 15 and 16 (figure A3). In addition, its pollen grain has a low sporopollenin content, so that its susceptibility to oxidation is high (Havinga 1964, 1967), which means that its pollen representation is generally low. In this case, its low values (no more than 3%) are sufficient to indicate the existence of an isolated stand in the vicinity of the settlement. The presence of yew has been documented in the Cantabrian region in different sites and peat bogs since prehistoric times, with the presence of charred wood in the case of the caves of El Mirón (Zapata 2012), Mazaculos II (Uzquiano 1992, 1995) and the site of Peña Oviedo (Díez-Castillo, 1996, 2008), and through pollen analysis in the peat bog of Cueto de la Avellanosa (Núñez de-la-Fuente, 2018). Yew wood is known for its high value, as it is strong, dense and of very good quality. This has conditioned its use in the past for numerous activities, including the manufacture of weapons, documented at least since Palaeolithic times (Oakley et al., 1977; Thieme and Veil, 1985). Also made of yew wood were the bow and axe handle carried by Ötzi, "the Iceman", located in 1991 due to melting glaciers in the Alps (ca. 5300 cal BP), on the border between Italy and Austria (Spindler, 1994). Bows and spears made of yew wood were also known during the Middle Ages. In 1396 there is a reference in which Martin, crossbowman to the King of Navarre Charles III the Noble, was sent to the mountains of Burunda and Amescua to cut yew trees to make crossbow bows (Schwendtner, 2010). The excessive use of this tree for the manufacture of weapons led to the appearance of medieval legislation protecting the yew, even dating back to the early Middle Ages in several European countries. In Spain, the ancient Soria and Segovia charters protected the yew and holly, despite their use as fodder, allowing only those branches that could be cut by hand and not with an axe or knife to be used (Ruiz-Alonso, 2014). Yew has also been used historically as a construction element due to its robustness and durability, as fodder for animals and even, in sites related to animal housing, it seems that yew was used as an "insecticide", due to its known antibacterial and antimicrobial properties (Daniewski et al., 1998; Erdemoglu and Sener, 2001).

Something similar can be said about the presence in stratigraphic unit 7 (inhum. 81) of *Olea europaea*, although with very low values (0-1.9%). In this case, it could well be the wild species, associated with sclerophyllous formations of Cantabrian holm oak (together with strawberry tree, which has also been identified), or some occasional olive groves, although at the present stage of our knowledge it is not possible to provide more information in this respect.

Shrubs have a very low representation (maximum of 9.5%), with some taxa typical of Atlantic landscapes such as heaths (*Calluna vulgaris*, *Erica* type), gorse (*Genista/Ulex*) and others from drier and warmer environments, such as strawberry trees (*Arbutus*) or junipers (*Juniperus* type), the latter perhaps adapted to sclerophyllous formations of the Cantabrian holm oak forest type.

The herbaceous component is very important (values between 44.6 and 59.2%), indicating the dominance of open spaces composed of grass meadows (*Poaceae*, 15.4-26.4%), together with anthropic-nitrophilic and anthropozoogenic inspired communities (*Aster* type, *Cardueae*, *Cichorioideae*, *Chenopodiaceae*, *Urtica dioica* type, *Plantago lanceolata*) indicating a very important degree of anthropisation of the space (figure A4). The values of *Cichorioideae* (*Compositae*), usually over-represented in archaeological contexts due to their zoophilic pollination, stand out among them, reaching maximum values of 36.5%. No cultivated species, usually cereals, have been detected; however, the high values reached by the *Fabaceae* (9.2%) suggest some type of crop related to leguminous plants.

Other taxa identified are *Brassicaceae* and *Ranunculaceae*, with maximum values of 11 and 5.6%, respectively. As for the hydro-hydrophilic plants, both *Filicales monolet* and *trilete* are found with high values (42.8 and 11.2%, respectively) and the presence of *Polypodium vulgare* (1.9-24.9%) and *Cyperaceae* (0.6-11%), which are common species in humid contexts, is also noteworthy.

Finally, the non-pollen palynomorphs are varied. The presence of *Glomus cf. fasciculatum* and *Pseudoschizaea circula*, both indicators of the concurrence of anthropisation processes by sediment removal, should be highlighted, although in both cases their values are not too high (maximums of 7.7 and 4.4%, respectively).

From a palaeoclimatic point of view, the chronological period in which the samples analysed (12th-15th centuries) fall into two different phases. The first lasts until ca. 1350 cal AD (Medieval Warm Period), characterised in south-western Europe by rising temperatures and precipitation. This phenomenon is evident in some deposits, although somewhat distant from the area around Santander, such as the case of the Prados de Randulanda peat bog (Álava), where the high resolution of its analysis allows us to identify this phase (Pérez-Díaz, 2012). In the case of the Azogues archaeological site, the presence of sclerophyllous vegetation may be evidence of this time.

After this last anomaly of an arid and warm medieval character, a new and rapid climatic change occurs, of a cold character, but in this case towards more humid conditions (Mayewski et al., 2004), which gives rise to what is known as the Little Ice Age. Its onset, according to authors, could be established between 1300 and 1400 cal AD (Desprat et al., 2003; Mayewski et al., 2004; Mann, 2007; Jalut et al., 2009), extending until the middle of the 19th century cal AD (ca. 1850 cal AD), with an initial drier phase until 1550 cal AD, and another wetter phase that lasts until the present day (Bradley and Jones, 1993).

Throughout the Little Ice Age, however, at least four moments are documented that represent temperature minima, related, among other factors, to the decrease in solar activity (Grove, 2001; González-Rouco et al., 2003; Steinhilber et al., 2009). These are the so-called Wolf (ca. 1280-1350 cal AD), Spörer (ca. 1460-1550 cal AD), Maunder (ca. 1645-1715 cal AD) and Dalton (ca. 1790-1820 cal AD) minima; of which the most pronounced would be the Maunder minimum. The coolest phase is between 1570-1730 cal

AD, and also another in the 19th century cal AD (Bradley and Jones, 1993), which will be mentioned later.

In the case of the Los Azogues archaeological site, due to the low resolution of the palynological study so far, this phase could not be identified. However, this phase is documented in other sites such as the aforementioned Prados de Randulanda peat bog, where from ca. 1320 cal AD, the palaeoclimatic reconstruction shows a prolonged thermal decline, accompanied by an irregular rainfall regime (Pérez-Díaz, 2012).

A.4

CONCLUSIONS

The palynological study of the medieval necropolis of Los Azogues shows relatively homogeneous characteristics throughout the sequence studied. From this analysis it can be deduced that the site would be composed at the landscape level by a deciduous forest in terms of the tree layer, where species such as oak, willow and hazel have a good presence in the landscape. These species are accompanied by other species typical of deciduous woodland, such as alder, birch and ash, thus demonstrating a relatively humid climate. Pine woods, although also represented in the landscape at this time, and given their relatively low percentage (maximum of 9.3%), would be found in more distant places, and possibly on a regional rather than local scale, or isolated pine trees in the surrounding area, without in any way constituting dominant formations in the vicinity of the Los Azogues site.

The above data regarding a humid climate are supported by the presence of hydro-hydrophilic vegetation, such as different types of ferns. However, the area surrounding the site would have been dominated more by open areas, in which herbaceous vegetation was the most important, with very few shrubs. Specifically, the landscape was dominated by grass pastures together with anthropic-nitrophilous communities, which could be evidence that this population would have been linked, among other things, to economic production activities, as these grass pastures would correspond to pastures for livestock use.

TABLE A.1. *Origin and representativeness of the samples studied at the archaeological site of Los Azogues (Santander, Cantabria).*

SAMPLES	STRATIGRAPHIC UNITS (SU)	PRESENCE OF POLLENS
15	UE-4-TOP	No
14	UE-4-BASE	No
13	UE-4	No
12	UE-13	No
11	UE-5	No
10	UE-6	No
9	UE-7-TOP	Yes
8	UE 7. INHUM 82	Yes
7	UE 7. INHUM 81	Yes
6	UE-7-BASE	No
5	UE-11	Yes
4	UE-15	Yes
3	UE-16	Yes
2	UE 19-TOP	No
1	UE 19-BASE	No



Figure A.1. *Location of samples from EU 4 at the archaeological site of Los Azogues (Santander, Cantabria)*



Figure A.2. *Location of samples in the stratigraphic profile of the Los Azogues archaeological site (Santander, Cantabria)*

