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Pollen content in a II century BC remedy from the Pozzino shipwreck (Tuscany, Italy): its sources and association with the ingredients

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ABSTRACT

Six tablets, probably an ancient *collyrium*, were discovered in a tin pyxis recovered during the archaeological excavation of the so-called Pozzino shipwreck found in the Baratti gulf, near Piombino (Tuscany, Italy). The tablets were previously studied from a historical point of view; micro-morphological and chemical analyses were then performed to establish their composition. In addition, the tablets were subjected to pollen investigation, which revealed the occurrence of a significant amount of pollen grains. The list of pollen morphotypes is long and suggests multiple sources for the grains. Many of the morphotypes belong to *Olea*; many others belong to plants which display showy flowers and are commonly visited by bees, confirming the presence of a bee product already detected by the chemical analysis. The present paper focuses on the analysis of the pollen content of the medicine and offers conclusions resulting from its study. We hypothesize that the olive pollen grains were added to the tablets together with one or more ingredients, such as pollen bread and/or *oleum acerbum* (an oil obtained by pressing unripened drupes and which was used in antiquity for therapeutic applications).

KEYWORDS
Ancient medical remedy; bee product; *collyrium*; *Olea*; *oleum acerbum*; pine resin

1. Introduction

Ancient preparations for body care, such as ointments, creams, or even medicines, have been rarely discovered among archaeological findings. The assessment of the composition of the scarce remains has not always allowed to firmly establish whether their use was therapeutic or cosmetic (Boyer et al. 1990; Gourevitch 1998; Evershed et al. 2004; Ribechini et al. 2008; Colombini et al. 2009; Pérez-Arantegui et al. 2009; Pérez-Arantegui et al. 2011; Ribechini, Modugno, et al. 2011; Ribechini, Pérez-Arantegui, et al. 2011). Therefore, our knowledge of ancient medical preparations mainly derives from the ancient writers, e.g. Theophrastus "*Histora Plantarum*" and "*De Odoribus*"; Aulus Cornelius Celsus "*De Medicina*"; Caius Plinius Secundus "*Naturalis Historia*"; Lucius Iunius Moderatus Columella "*De re rustica*"; Pedanius Dioscorides "*De Materia Medica*". In this context, the discovery of several well-preserved medical tablets in the Pozzino shipwreck (II century BC) is of surprising importance, as it is a direct testimony of an ancient medicinal preparation.

The shipwreck was found on the seabed of the Baratti gulf, Piombino (Tuscany - Italy), at a depth of about 18 m, near the ancient Etruscan city of Populonia (Ciabatti 1990; Romualdi 1990; Romualdi and Firmati 1998) (Figure 1a). The excavation was carried out in different stages from 1982 to 1990 and revealed the remains of a small hull (15 m long, 3 m wide) with part of its cargo. Based on the analysis of the cargo, it is believed that the vessel, dating around 140 BC, was coming from the Greek coasts or Greek islands (Romualdi 1990; Romualdi and Firmati 1998). In that period, Populonia was a town of primary importance along the sea-trade routes between the eastern and western Mediterranean.

The ship cargo also included a medical kit. It consisted of a small stone *mortarium*, a bronze cupping vessel, an iron probe, numerous tin *pyxides*, and 136 wooden vials (Giachi 2013). All these objects were found next to an iron lock that possibly belonged to a destroyed wooden box.

At the time of the discovery, one of the tin *pyxides* (signed as A/6) was still closed. X-ray analysis of the interior revealed a regularly stratified content. The opening of this *pyxis*, carried out with an ultrasonic scaler, revealed the presence of six grey tablets: five of them were nearly intact, while the top one was irregularly broken. The tablets (Figure 1b) have a discoid shape and measure about 4 cm in diameter with a maximum central thickness of 1 cm.

The tablets were carefully examined and the results of the first historical, chemical and micro-morphological investigations were published in Giachi et al. (2013). The chemical analysis revealed that the main components of the tablets (about the 83% of total) were hydrozincite and smithsonite (respectively lead hydroxycarbonate and carbonate); the tablets also contained small amounts of animal and vegetal lipids, beeswax, resin of Pinaceae, pollen grains and starch grains (Giachi et al. 2013). The presence of starch grains with traces of thermal treatment was probably due to a wheat paste added as a thickening or gluing agent (Giachi et al. 2013). Moreover, micro-morphological examination evidenced numerous thin threads of flax, very likely added to

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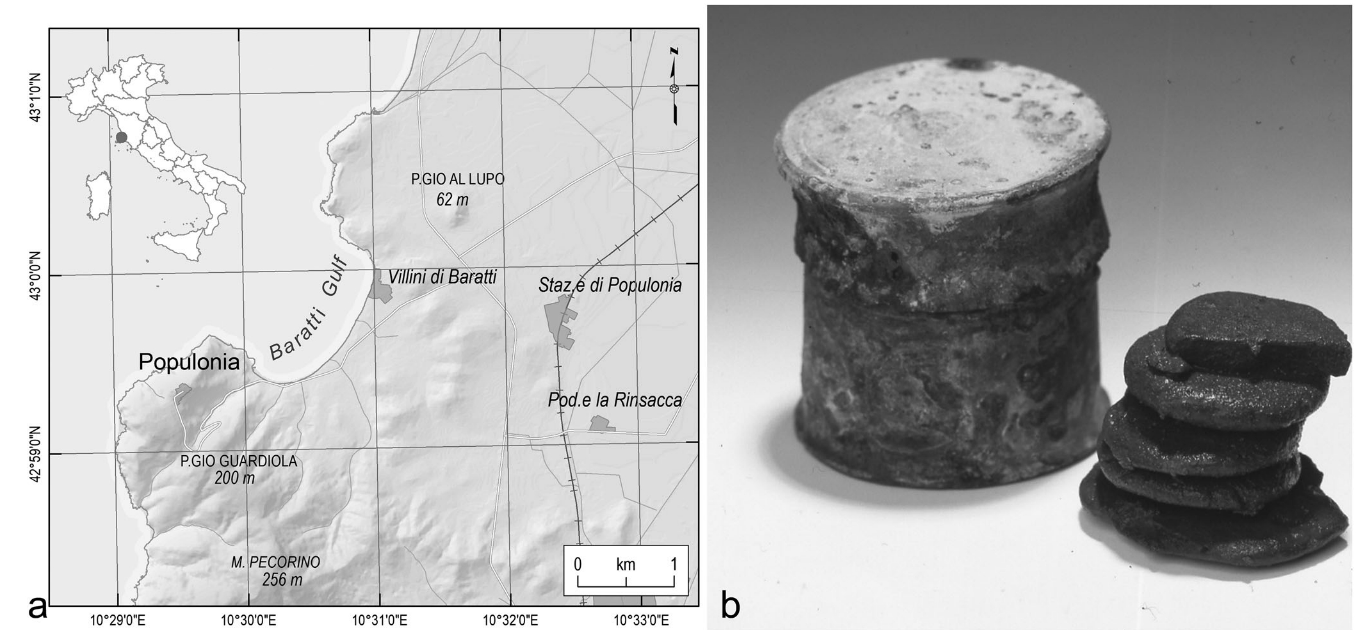

Figure 1. (a) The Gulf of Baratti where the Pozzino shipwreck was found (b) the tin pyxis A/6 and the discoid tablets.
Source: edited by Lorella Dell'Olmo, Laboratorio di Fitogeografia, Dipartimento di Biologia, Università degli Studi di Firenze, Italia

avoid any breaking or crumbling of the tablets. Charred fragments of plant tissue, trichomes, and appendages of Arthropods were also occasionally found.

The comparison of the collected data with historical and pharmacological literature and other archaeological evidence suggested that the tablets was very probably a *collyrium*, a poultice for ophthalmic use (Giachi et al. 2013).

A further investigation of the tablets was mostly devoted to collecting information about the pollen content and analyzing its significance, particularly with respect to the ingredients detected.

The present paper focuses on this last issue: it investigates the pollen content of the ancient medicine and the possible source of the pollen grains and asks whether these were introduced into the preparation with one or more ingredients or may represent an accidental contamination. Therefore, the identification of the pollen grains in the Pozzino remedy may improve our knowledge of its therapeutic ingredients and provide new information about ancient pharmacological technology.

2. Materials and methods

Pollen analysis was performed on two samples from the broken top tablet: sample A, a 0.42 g fragment, and sample B, 0.5 g accurately isolated from the inner part of the same tablet. Each sample was first treated with a 10% HCl water solution to dissolve the main components of the tablet. The samples were then washed in water, water-sieved (meshes 0.5 mm), subjected to acetolysis (Erdtman 1960), and mounted in 50% glycerol/water solution. The resulting samples were finally observed under light microscope (LM), operating at 400-630 x. Identification was performed with the help of literature (Beug 1961; Punt et al. 1976–1996; Andersen 1979; Nilsson 1988; Moore et al. 1991; Reille 1992–1998; Osman 2009), reference pollen collection, and acetolysed fresh material. Absolute pollen frequency (APF) was reported as number of grains per gram. Pollen percentages were calculated on total pollen and spores.

After the first results showed the presence of *Olea* pollen, modern materials were observed to explore its possible sources. In order to verify the presence of olive pollen on the surface of olive drupes at different development stage, 30 drupes were indicatively collected every month, from summer to autumn. Their surface was washed with a 10% NaOH water solution, and the residual sediment observed at LM after they were washed with water.

3. Results

Numerous pollen grains were found in the samples (Table 1, Figure 2a-f), reaching a concentration of about 1400 grains per gram, which equals to a concentration of about 8000 grains per gram of the organic component. The state of preservation of the grains varied from good to very bad: in case of the latter, the grains were crushed, broken, or crumpled, some to the point of being unidentifiable. Intriguingly, many of the pollen grains that are commonly deemed very resistant (e.g. Amaranthaceae and Asteraceae) were the most affected by damage.

Groups formed by pollen grains belonging to different plants were also observed.

The majority of the pollen morphotypes occurred in both samples; of the remaining ones, fourteen were exclusively in sample A and eleven exclusively in sample B. In these last two groups, each of the morphotypes represents less than 1% of the total pollen content.

Considering the entire pollen content of the medical preparation, the quantities of AP (arboreal, shrubby, and climbing plant pollen) and NAP (non-arboreal plant pollen)

Table 1. Pollen percentages of sample A (a portion of the tablet), sample B (from the inner part of the tablet), and their sum (A + B) calculated on the total pollen and spores. Bee foraging according to: Ricciardelli D'Albore and Persano Oddo 1981; [(1)] Huang 2010. p = pollen; n = nectar; h = honeydew.

	sample A	sample B	A + B	Foraged by bees for:
AP	50.2	51.4	50.9	
Pinus cf. *halepensis*	1.5	0.5	0.9	p
Pinus cf. *pinaster*	0.4		0.2	p
Pinus sp.	1.1		0.5	p
Cupressaceae		0.3	0.2	p
Betula	0.8		0.3	p
Castanea	0.4	1.8	1.2	p, n, h
Quercus	0.4	0.3	0.3	p, h
Corylus	0.4		0.2	–
Platanus		0.3	0.2	–
Ulmus	0.4	0.3	0.3	p
Alnus	1.5	2.6	2.1	p
Populus	0.8		0.3	p
Salix	0.4		0.2	p, n
Buxus	0.4		0.2	p
Myrtus		0.3	0.2	p, n
Fraxinus	0.8	0.8	0.8	p
Olea	39.2	43.0	41.4	p
other Oleaceae	0.4	0.5	0.5	
cf. Prunus	0.4	0.3	0.3	p, n
Vitis	1.1	0.8	0.9	p
NAP	49.8	48.6	49.1	
Avena/Triticum group	11.8	12.8	12.4	p, h
Hordeum group	2.3	1.0	1.5	p, h
wild Gramineae	4.9	5.4	5.2	p
Urtica dioica type	2.3	3.3	2.9	–
Plantago cf. *lanceolata*	2.7	1.0	1.7	p
Plantago sp.		1.0	0.6	p
Amaranthaceae	5.3	4.1	4.6	p
Anthemis type	0.8		0.3	p
Aster type	0.4	0.8	0.6	p, n
Ambrosia type		0.3	0.2	p, n
Centaurea nigra type	1.1	4.9	3.4	p, n
cf. *Centaurea cyanus*		0.3	0.2	p, n
cf. *Carduncellus*	0.8	1.3	1.1	p, n
Artemisia	0.4	0.5	0.5	p
Cichorieae	0.4	1.3	0.9	p, n
Sinapis type	2.3	1.3	1.7	p, n
Peucedanum palustre type	0.4	0.3	0.3	p, n
Oenanthe fistulosa type		0.5	0.3	p, n
other Apiaceae	0.8		0.3	
Heliotropium europaeum type	0.4	0.3	0.3	–
cf. *Medicago*		0.3	0.2	n
cf. *Lotus*	1.1	0.5	0.8	p, n
Hypericum	0.8		0.3	p
Caryophyllaceae	1.1	0.8	0.9	p, n
cf *Filipendula*	0.8	1.3	1.1	p, n
cf. *Sanguisorba minor*	2.3	1.3	1.7	p
Helianthemum	0.8	0.3	0.5	p
Fumana		0.5	0.3	p
Succisa pratensis type	0.4		0.2	p, n
cf. *Nepeta*	0.4	0.3	0.3	p, n
cf. *Mentha*		0.3	0.2	p, n
Liliaceae s.l.	0.8		0.3	p, n
Cyperaceae	0.4	0.5	0.5	p
Typha/Sparganium	0.8		0.3	P [(1)]
Spore monolete		0.3	0.2	
cf. *Dryopteris*	0.4		0.2	
INDET	3.0	2.3	2.6	

FPA = 1400 grains/gram.

were nearly equivalent, but the AP list is far shorter than that of NAP (Table 1).

The most numerous pollen grains belonged to *Olea* (Figure 2a-c), which constitute about 41.5% of the total pollen content: they were from spherical to sub-spherical, 22-28 µm in diameter/polar axis, features that are consistent with *O. europaea* L. (olive) pollen (Nilsson 1988). Numerous *Olea* grains appeared lightly compressed (Figure 2c).

Poaceae belonging to the *Avena-Triticum* group, probably *Triticum*, are the second most abundant taxa. The grains, more or less crumpled, were often found in groups of 5 or 6 (Figure 2f). Amaranthaceae and *Carthamus* pollen-type grains (Asteraceae) were also present in a noticeable amount (Figure 2e). Numerous pollen grains belonging to plants used in ancient remedies were listed in the spectra, but they all were present in low amounts. Among them, *Sanguisorba minor* (Figure 2d), *Urtica*, and *Alnus*, probably *A. glutinosa,* and *Sinapis* pollen type. In addition to *Sinapis*, this pollen type includes many other Brassicaceae genera, such *Brassica*, *Raphanus* etc. (Moore et al. 1991).

The contemporary test carried out on 30 green olives showed that about 400 pollen grains were present on the olive surface at the end of June, when they provided a very small amount of oil. The pollen amount rapidly decreased to about 220 during July, down to 4 grains in late August and September.

4. Discussion

Pollen analysis of the ancient *collyrium* revealed the presence of a large amount of pollen grains which are attributable to a large number of plants. Previously, investigations carried out in ancient preparations had revealed the presence of pollen grains, but they mostly belonged to one or few plants, indicating the use of flowering branches as ingredients (Boyer et al. 1990; Bui-Thi-Mai and Girard 2003): the pollen grains of *Euphrasia officinalis* were the most frequent and numerous, followed by those of *Ribes nigrum*, *Artemisia* sp., *Xanthium strumarium* and Poaceae. The abundant and diverse pollen content of the Pozzino medical remedy and the different states of preservation of the grains suggest multiple origins for the grains; it does not include the pollen grains found in the other ancient preparations, with the exception of *Artemisia* and Poaceae.

It is hard to establish the ingredient with which each grain was introduced into the preparation. This is because the pollen grains may also be accidental environment contaminations or residues found on the tools used to make the remedy (Figure 3). However, it is reasonable to think that the grains that were found in remarkable quantities were introduced with some of the ingredients.

Most of the grains belong to *Olea* and are responsible for the noticeable AP percentage (about half of the total pollen). The employment of olive products had also been suggested by the chemical analysis, which detected the presence of oleic acid in the tablet thus hinting at the use of olive oil as an ingredient of the medicine (Giachi et al. 2013). In antiquity, the oil obtained by pressing unripen olives – called *omphacium* or *oleum acerbum* – was used for perfumes and medicinal preparations (Plinius *Naturalis Historia* 12, 130; Columella *De re rustica* 11, 2, 83; 12, 52, 1-ss; Dioscorides *De Materia Medica* 1, 29). This kind of oil could have contained olive pollen, since the use of warm water to wash the olive surface before pressing had likely not yet been introduced.

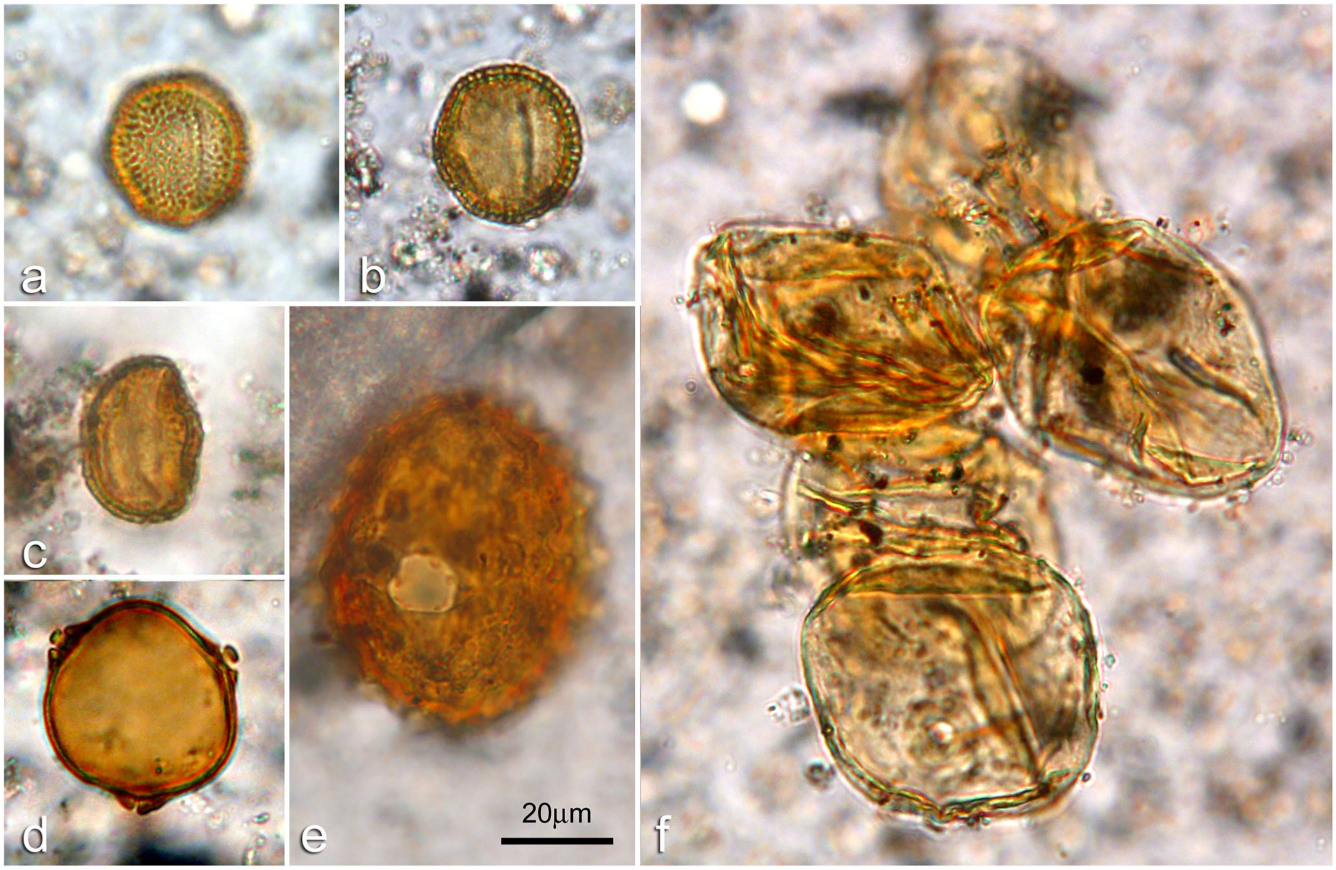

Figure 2. Pollen grains of the Pozzino medicine (LM): a-b) *Olea*; c) a compressed *Olea* pollen grain; d) *Sanguisorba minor*; e) *Carthamus* pollen-type; f) *Avena-Triticum* group. a-f are taken at the same magnification.

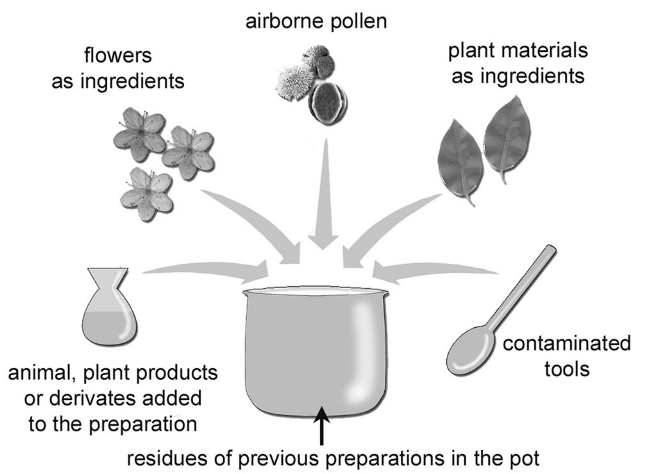

Figure 3. Possible sources of the pollen grains found in the preparations. High pollen concentrations are expected to be found when flowers or flowering branches were used as ingredients; high concentrations may also derive from the use of products or derivates such as resin or bees products. Lower pollen amounts may be due to: the contamination by airborne pollen; the dust deposited on the surface of the ingredients (for example, leaves) or the tools; the residue of previous preparations.

The procedure was still very new in Pliny's time, about two centuries after the Pozzino tablet preparation (Plinius *Naturalis Historia* 15, 23).

To verify this hypothesis, we conducted a test on the amount of olive pollen grains on a green olive surface. The result of the test showed that numerous pollen grains were present on the olive surface at the end of June, when the drupes are unripe and provide a very, very small amount of oil. The pollen amount rapidly decreased during the month of July and went down to a few grains in late August and September. Obviously, the amount of pollen grains can change significantly depending on the eventual amount of rain washing the olive surface.

Oleum acerbum could have been the source of the olive pollen, but, given the relevant pollen amount, it was more likely just one of several sources.

A general overview of the total pollen spectrum of the tablet shows that many of the listed plants have showy flowers and are commonly visited by bees (Asteraceae, Brassicaceae, Fabaceae; see Table 1). The use of a bee product as an ingredient of the tablet had been also clearly indicated by the chemical analysis, which showed the presence of beeswax (Giachi et al. 2013). This result is also in agreement with the crushed grains, the occurrence of clumps of pollen grains, and the appendages of Arthropods in the tablet we analyzed. Honey, beeswax, and propolis contain pollen grains and were known for their therapeutic properties in ancient time (i.e. Celsus *De Medicina*; Plinius *Naturalis Historia*). We can therefore presume that a considerable amount of pollen was introduced in the tablet together with the bee products.

Pollen analysis of beeswax is rare and usually shows a low of pollen content (Furnessa 1994; Chichinadze and Kvavadze 2013). Pollen grains penetrate the wax when the bees are moulding and working it, therefore most of the pollen grains detectable in beeswax derive from the pollen content of the combs.

Since a bee product seems to have been used as an ingredient of the Pozzino tablets and that olive pollen is by far the most abundant, it should be noted that olive is a nectarless species whose pollen is generally an accessory in the bee products of the Mediterranean regions. In South European, Middle Eastern, and North-West African honeys, the percentages of olive pollen are generally below 30% (Serra Bonvehi and Ventura Coll 1995; Ricciardelli D'Arbore 1998; Seijo et al. 2003; Terrab et al. 2003, 2004; Atrouse et al. 2004; Díez et al. 2004; Kaya et al. 2005; Dimou and Thrasyvoulou 2007; Silici and Gökceoglu 2007; Weinstein-Evron and Chaim 2016). In propolis from the northern and eastern Mediterranean, olive pollen does not usually exceed 20% (Ricciardelli D'Albore 1979; Gençay Çelemli and Sorkun 2006; Çelemli and Sorkun 2012). In a propolis sample collected near a Tuscan olive garden (Scandicci, near Florence) at the end of summer, the olive pollen reached only 3.3% of the total (Fiesoli 1992).

The pollen of non-nectariferous species is frequent in royal jelly in amounts that may differ from those observed in the honey from the same location (Ricciardelli D'Albore and Battaglini Bernardini 1978; Piana et al. 2006; Dimou et al. 2013), but it is known that it can reach very high values in pollen loads (Ricciardelli D'Albore and Persano Oddo 1981; Dimou 2012; Dimou et al. 2014). In particular, palynological studies in Greece have demonstrated that olive pollen is one of the dominant pollen types in the pollen loads of *Apis mellifera,* exceeding 45% of the total pollen (Dimou 2012; Dimou et al. 2014).

We can therefore assume that the large amount of *Olea* pollen in the Pozzino tablet may be due to the use of pollen loads or pollen bread, i.e. the pollen processed for storage (Bryant and Jones 2001), as an ingredient of the tablet. This hypothesis would also explain the compression exhibited by several grains (Figure 2c). The use of pollen bread is the most plausible, since the loads do not contain wax. The same source, i.e. pollen bread, is also probable for other non-nectariferous, anemophilous plants (Bryant and Jones 2001; Odoux et al. 2012; Dimou et al. 2014) including cereals such as *Triticum*, which is visited by the bees to collect honeydew (Ricciardelli D'Albore and Persano Oddo 1981).

Finally, it is possible that the olive flowers were added directly to the medicinal preparation, although no other evidence of the use of flowers was found in the tablet.

Another source of pollen for the tablet might have been plant resins, which can have a high pollen content that reflects the surrounding vegetation (Mariotti Lippi and Mercuri 1992). Indeed, Pinaceae resin was identified in the tablet (Giachi et al. 2013), in accordance with the occurrence of *Pinus* pollen, perhaps *Pinus halepensis* (Table 1). In ancient times, resin was used as an adhesive and sealing agent, perfuming agent, and preservative (Plinius *Naturalis Historia* 11, 6). Even in our case, pine resin might have been added as a preservative, owing to its antioxidant and antiseptic properties (Giachi et al. 2013).

A number of other plants are listed in the pollen spectra; many of them are known for their medicinal proprieties, but the amount of their pollen grains is not noteworthy and their pollen may have reached the tablet together with other ingredients or represent residues of previous preparations (Figure 3), e.g. *Urtica* (Plinius *Naturalis Historia* 22, 31-36), *Plantago* (Plinius *Naturalis Historia* 25, 80), *Mentha* (Plinius *Naturalis Historia* 22, 57-61), *Myrtus* (Plinius *Naturalis Historia* 23, 87). The recent recovery of pollen grains of *Mentha*, *Myrtus* and *Urtica* in a cess deposit at Megiddo, Israel, was supposed to represent a witness of their use for medical purpose well before the II century BC (Langgut et al. 2016). Apiaceae, a plant family which includes celery and carrots, deserve mention as they were previously detected in the Pozzino medicine by means of DNA analysis (Fleischer in Travis 2010).

As a whole, the plants listed in the spectra agree with the Mediterranean flora. The presence of *Corylus* and *Betula*, two plants which are indigenous of the northern and eastern Mediterranean, points to the flora of the Eurasia regions; however, these grains were only detected in sample A, a fragment of the tablet which included a part of the surface. Assuming that the differences in the plant lists of the two samples are not accidental, as it is possible, the occurrence of grains in sample A only might be the result of an airborne contamination after the tablet production.

5. Conclusion

The Pozzino tablets are a rare example of an ancient medical preparation that permit us to study their composition. The chemical, mineralogical and biological analyses allowed us to detect most of the ingredients (Giachi et al. 2013). The detailed pollen analysis presented in this paper offers the opportunity to confirm the previous results and deepen our understanding of the origin of its components. Indeed, the noticeable amount of pollen found in the Pozzino remedy suggests its direct relation to the preparation of the tablets, even though a few grains may have come from the tools or as an environmental contaminant or together with minor ingredients. Unlike other ancient remedies, which usually contain pollen grains from one or few plants, the pollen spectrum of the Pozzino tablet is very rich. This finding suggests a multisource origin of the grains, consistent with the presence of various ingredients, such as resin, bee products, and oleic acid.

A few morphotypes are present in a noticeable amount: most of all olive pollen. The olive pollen may have different sources: it likely came from the use of *omphacium* or *oleum acerbum*, an oil obtained from green olives that was notably used in ancient times for medicinal preparations. Another possibility is that the olive pollen entered the tablet together with a bee product, most probably pollen bread. These two possible sources are not mutually exclusive, since the chemical analysis evidenced the presence of oleic acid as well as beeswax. In addition to these most probable ingredients, the long list of pollen in the tablet hint to other possible minor sources or contamination from the surrounding environment or the tools used during the preparation.

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