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First results of plant processing on ground stone tools: phytolith evidence and GC-MS from archaic Messapian settlements - San Vito dei Normanni and Cavallino (Puglia, Italy)

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Abstract **– A large number of ground stone tools have been unearthed in the archaic Messapian settlements of San Vito dei Normanni - Castello d'Alceste (Brindisi) and Cavallino (Lecce), in southern Puglia. The questions raised by these tools concern exploitation of food resources and distribution of productive activities within the settlements. First results of phytolith analyses conducted on ten grinding tools are presented. Quantitative and morphological analysis aimed at estimating the amount of phytoliths in the sediment, but also at identifying the types of plants used in the sites. Phytoliths indicated the nature of the vegetal processed matter, including cereals plants used for human and animal nutrition. Together with those from the analysis of organic residues already conducted at the sites these results contribute to the understanding of the food processing and dietary practices of the archaic Messapic populations.**

I. INTRODUCTION

Investigations conducted by the University of Salento at the Messapic villages of San Vito dei Normanni - Castello d'Alceste (Brindisi) and Cavallino (Lecce) have brought to light the remains of two indigenous settlements, spanning from the Iron Age to the Archaic period, the time of their sudden abandonment (fig. 1).

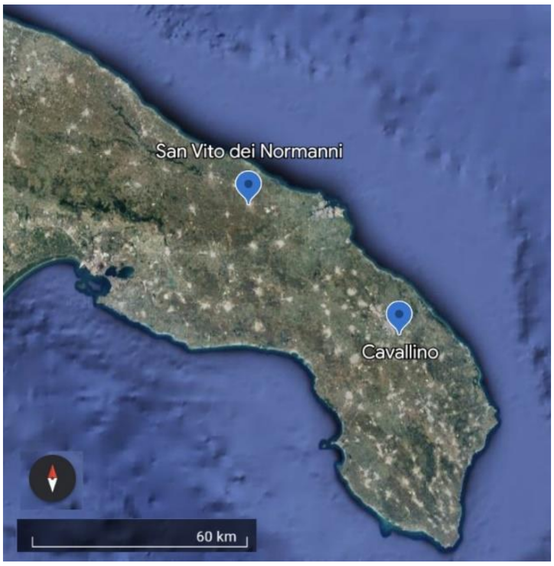

Figure 1: Site localization via Google Earth

Both sites underwent a moment of intense development in this phase, as evidenced by the construction of masonry houses with tiles roofing [1] and buildings for ritual activities [2] and the discovery of large quantities of fine pottery of Greek import, mainly related to wine consumption practices [3].
Following the ongoing archaeometry research conducted to understand the dietary habits of the inhabitants of these settlements [4], we decided to use phytoliths analysis to further investigate plant processing and diet.
The first applications of phytoliths to archaeology date back to the early 20th century and concern palaeoecological studies. [5], but it is since the late 1970s that attention has focused primarily on the origin and spread of agriculture [6]. Current lines [7] of research include (but are not limited to) standardisation of the lexicon related to morphometric studies and the creation of online databases; radiocarbon dating and other radioactive isotope investigations; broadening horizons on yet unstudied *taxa* or cultural practices related to food and beverage production and consumption.
This is the first time that such studies have been applied to archaic Apulian contexts.
Moreover, one quern from Castello d'Alceste was selected for organic residue analyses by Gas Chromatography - Mass Spectrometry (GC-MS), with the aim of clarifying

its original use through the chemical characterization of the organic compounds absorbed by the porous stone.

II. MATERIALS AND METHODS

For phytoliths extraction sediment samples have been taken from ten grinding stone tools from both archaeological sites. Six ground stone tools were selected from Cavallino, including one pestles, one grinder and four saddle querns, whereas four ground stone tools were selected from the site of S. Vito dei Normanni - Castello d'Alceste, including one grinder and three grinding stones of various types. Three sediment samples were taken for each selected artefact. The number of total samples, which may seem small, is in fact consistent with data commonly processed in similar situations [8]. Analyses were performed during a training internship held at the Archaeology of Social Dynamics research group, Spanish National Research Council (CSIC) in Barcelona, supervised by Dr. Marta Portillo.

The protocol used for phytolith extraction is the one tested on the excavation of Tell es-Safi/Gath (Israel) by Ofir Katz and his colleagues [9]. This procedure allows a rapid extraction of phytoliths from the sediment, as it reduces the number of washing/drying cycles the samples undergo.

Each sample was weighed with a precision balance (0.1 mg resolution) to obtain an ideal amount of sediment between 20 and 50 mg, then placed in a 0.5 ml centrifuge tube. To this, 50 µl hydrochloric acid [6NHCl] ware added to dissolve the carbonates in the sediment. At the end of the reaction with the acid, 450 µl of Sodium polytungstate at 2.40 g/ml density [SPT, Na6(H2W12O40)-H2O] was added to the solution. The tube was then immersed in an ultrasonic bath for 5 min, then vortexed and centrifuged for 5 min at 5000 rpm. After the centrifuge, the tube was vortexed again; at this point, the remaining mineral portion (consisting mostly of quartz and clay) have settled to the bottom of the tube, and phytoliths and light particles remains in suspension in the SPT solution. The supernatant is then withdrawn with an automated pipette and placed into a new 0.5 µl tube, which is vortexed for the last time. Finally, 50 µl supernatant is taken and deposited on a rectangular microscope slide and covered with a 24x24 mm coverslip. The amount of 50 µl represents 10% of the initial volume of 500 µl derived from the initial weight, and therefore it will be possible to calculate the concentration of phytoliths in the sample.

The resulting slides were then observed with a transmitted light microscope (Olympus BX51) at 200x and 400x magnifications.

The terminology used in the results description follows the International Code for Phytoliths Nomenclature (ICPN) 2.0. [10]

To perform GC-MS, a sample (1-2 g) was taken with a pincer from the active surface of the stone tool together with surrounding soil. The sample was crushed into a fine powder using a mortar and pestle, solvent extracted and derivatized using established protocols [11] after the addition of 1 ml of a standard solution (nonadecane 1 mg/ml) for the quantification of lipid extract.

The total lipid extract (TLE) was obtained by adding a chloroform/methanol mixture (2:1 v/v, 5 mL). Extraction was performed twice by ultrasonication (30 min at 40° C). After centrifugation, half of the TLE was evaporated under a gentle stream of nitrogen and hydrolysed by adding 5 ml of NaOH/MeOH+H2O (9:1) and heating at 70°C for 1 hour in ultrasonic bath. The liquid fraction was acidified with HCl (1 M) and extracted twice with chloroform (5 ml). The solvent was then evaporated under a gentle stream of nitrogen. The extracts were derivatized with 50 µl of N, O-bis(trimethylsilyl)tetrafluroacetamide (BSTFA, Sigma) at 70 °C for 30 min and analysed by gas chromatography-mass spectrometry using an Agilent Technologies 6850 II series gas chromatograph coupled to an Agilent 5973 Network mass spectrometer operated in electronic ionisation (EI) mode (70 eV). The split/splitless injection system operating in the splitless mode was maintained at 300 °C and helium was used as the carrier gas at a constant flow rate of 1 ml/min. The temperature programme of the GC was kept at 50° C for 1 min, and then increased to 300° C at 10°C/min with a 15 min isothermal hold. The GC was fitted with a fused silica capillary column (5% phenylpolymethylsiloxane, 30m, internal diameter 0.25mm x 0.25µm film thickness). The mass range was scanned in the range of m/z 50 - 600 in a total cycle time of 1 s. Compounds were identified partially by their retention time, based on comparisons with analysed reference compounds, and by their mass spectra, interpreted manually with the aid of the NIST Mass Spectral Library.

III. RESULTS AND OBSERVATIONS

A. Cavallino

A total of fifteen samples was analysed from Cavallino. As shown in the table below (tab. 1), the concentration of phytoliths in the samples ranges from a minimum of 8.800 to a maximum of 299.000 phytoliths/1g of sediment, with average values around 138.000 phytoliths/1g of sediment. These are low values, but not dissimilar to those found at other archaeological sites across the Mediterranean [12]. A rather high percentage, almost a quarter of the sample, is represented by phytoliths with an unrecognizable morphotype, due both to silica dissolution and mechanical disaggregation.

Table 1. Main quantitative results from Cavallino samples

Sample	Initial weight (g)	Phytoliths/ 1g sediment
CV4	55,4	8.800
CV5	42,4	93.000
CV6	49,8	56.000
CV34	23,5	299.000
CV35	25,1	98.000

CV36	34,9	201.000
CV98	36,4	193.000
CV99	44,4	243.000
CV100	48,9	267.000
CV104	52,1	97.000
CV105	36,1	122.000
CV108	43,7	138.000
CV112	36,1	165.000
CV113	21,1	123.000
CV114	44,1	66.000

Morphological analysis shows that 60% of the total counted phytoliths refers to plants of the subfamily *Pooideae*, which includes annual herbaceous species used for food and fodder, such as wheat and barley. These outcomes are consistent with results from the archaeobotanical research conducted in the settlement: analysis of botanical macro-remains has, in fact, enlightened presence of spelt (*Triticum dicoccu*m), free-threshing wheat (*Triticum aestivum/compactum*) and barley (*hordeum vulgare*) [13]. Dicotyledonous plants were attested in much smaller percentages (8.6%), with a predominance of phytoliths associated with bark and leaves.

In terms of plant parts, a predominance of stems/leaves, can be seen in almost all samples. The exceptions are CV35, CV99-100, CV104-105 and CV112-113, where the percentage of inflorescence phytoliths is between 15 and 20% and the sum of short cells and inflorescences constitutes almost or more than half of the record (fig. 2). All these samples come from use surfaces of passive slab of saddle querns, except for CV104-105 which are from an handstone.

The percentage of multicellular phytoliths (anatomically connected) is low, due to mechanical degradation of the samples.

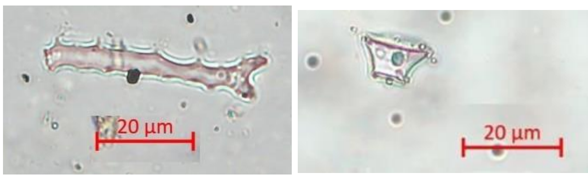

Figure 2: elongate echinate (left) and short cell rondel (right) from Cavallino (Photos: G. Sabetta).

B. *San Vito dei Normanni – Castello d'Alceste*

For this site a total of thirteen samples was examined and their main quantitative results are presented in the table below (tab. 2). The target of 200 phytoliths per slide was reached for all the samples, thus leading to a decisive increase in the density of micro residues, with a peak of 4,600,000 phytoliths/1g sediment and an average of 1,120,000 phytoliths/1g. These values are in line with those reported in the literature for other known settlements, especially in Levant [14].

It should be emphasised, however, that the preservation conditions of the phytoliths are not very different from those identified in Cavallino, and both silica dissolution and mechanical disaggregation can be observed.

Table 2. Main quantitative results from San Vito

Sample	Initial weight (g)	Phytoliths/ 1g sediment
SV28	35,2	1.800.000
SV29	25,4	3.500.000
SV30	57,5	1.100.000
SV71	22,9	1.200.000
SV72	27,1	370.000
SV73	37	310.000
SV94	45,6	1.230.000
SV95	40	1.130.000
SV96	50,9	1.400.000
SV97	48,3	970.000
SV101	37	890.000
SV102	37,7	1.700.000
SV103	47,9	4.560.000

Morphological analysis shows a clear predominance of grasses, which account for 84% of the sample. Morphologies that can be associated with dicotyledonous plants are less attested, with average percentages varying between 7.2% of leaves and 4.2% referring generically to wood/bark. Lastly, 4.2% is the average percentage of weathered morphotypes that cannot be recognised due to their poor state of preservation.

The phytolith record is dominated by the *Fam. Poaceae*, with occurrences mainly in the *Pooideae* subfamily. In one case, however, according to the short cell bilobate morphologies, it was possible to recognise the *Panicoideae* subfamily, in which millet (Panicum miliaceum) belongs. It is attested on only one artefact (samples SV101-102-103 in tab. 2) and comes – significantly - from the innermost sample collected on the surface of the tool (fig. 3, left). This artefact, a grinding slab, was selected for GC-MS analysis and results are discussed below.

The grass data were further divided according to their anatomical origin to further assess which parts of the plants were attested at the site. The sample appears much varied and complex, with good representation not only of the green parts of the plant (stem/leaves), but also of the inflorescences and short cells, which are produced in both. There is a high concentration of phytoliths from inflorescences in samples SV28-29-30, taken from the saddle quern of US 4072 (excavations 2017-2018): amongst these, the presence of multicellular structures referable, perhaps, to emmer (*Triticum dicoccum*) is highlighted (fig. 3, right).

Samples SV94 to SV97 come from a counterweight press, discovered during the 2014 excavation campaign, in an area of the settlement used for large-scale food processing. The morphological analysis does not show any variations compared to the rest of the samples, but during the microscope observation it could be noted that the sediment taken from this object was very rich in silica fragments, homogeneously distributed throughout the slide. The high degree of silica degradation in these samples is most likely related to the better milling action that this type of millstone guaranteed compared to the more rudimentary saddle mills. In addition, some of the identified phytoliths show signs of degradation due to combustion: it remains to be clarified whether exposure to fire was accidental or intentional (fig. 4).

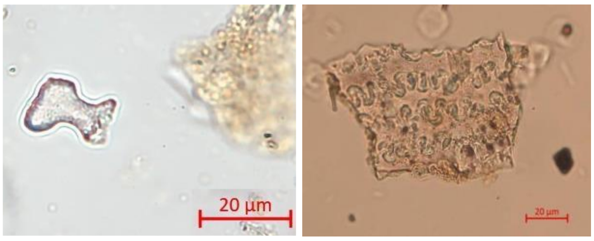

Figure 3: (left) microphotograph from sample SV102: short cells bilobate morphologically referable to the subfamily Panicoideae (400x magnification, 20 µm scale); (right) microphotograph of multicellular structure from sample SV29 (Photos: G. Sabetta).

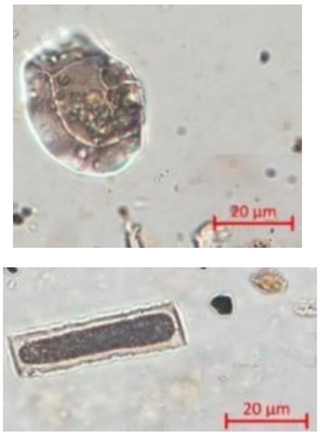

Figure 4: phytoliths with partial signs of melting due to increased temperature (Photos: G. Sabetta).

As mentioned above, GC-MS analysis was conducted on a sample from the active surface of a grinding quern from San Vito dei Normanni, recovered during the 2012 excavation campaign in what appears to be a productive area of the settlement (fig. 5, above). It shows a lipidic profile compatible with plant oils. The chromatogram (fig. 5, below) mainly shows the presence of palmitic ($C_{16:0}$), oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acids, with very low concentrations of stearic acid ($C_{18:0}$). The relative proportions of these fatty acids and the abundance of unsaturated acids $C_{18:1}$ and $C_{18:2}$ indicate a vegetable origin of the organic substances absorbed in the stone tools. Plant sterols (β-sitosterol) were also found, providing further evidence for a plant-derived content. The vegetable origin of the fatty acids contained in the sampled is also confirmed by the percentage of medium chain fatty acids ($C_{12:0}$, $C_{14:0}$ and $C_{15:0}$) greater than 10% [15].

The sample also displayed a series of α, ω -dicarboxylic acids, with a dominance of azelaic acid, a degradation product usually deriving from oleic acid, together with hydroxycarboxylic fatty acids, considered oxidation products of unsaturated fatty acids. The distribution of these components supports the presence of a plant oil [16]. The polyunsaturated linoleic acid ($C_{18:2}$) is compatible with the possible grinding of plants (particularly leaves and seeds).

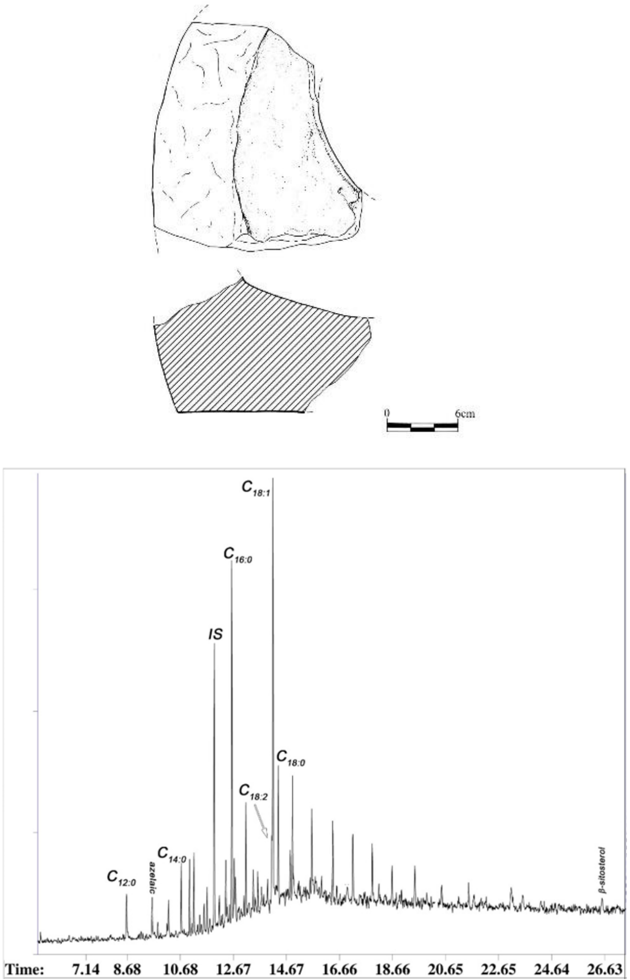

Figure 5: drawing of quern US 756 (above) and chromatogram of sample (below). IS: Internal Standard (F. Notarstefano)

The analysis therefore indicated that the stone tools were used in activities that produced the enrichment of the stone tool with fatty acids deriving from plants and/or vegetable oils.

Aside from the quantitative results, it is possible to draw some considerations about conservation practices of stone artefacts. The differences in phytoliths concentration detected in the samples from both sites can, in fact, be attributed largely to post-excavation treatment of the tools. While the findings from Cavallino were washed after the discovery, those from San Vito were not and returned better results.

Therefore, the study of micro-remains allows us to reflect on the need for correct planning of excavation and laboratory activities, to prevent the loss of informative potential. Having direct experience – in terms of phytolith concentrations in samples – of which practices ensure most satisfactory outcomes is certainly a starting point to guide future research.

IV. CONCLUSIONS

Sampling operations were conducted correctly and allowed the extraction of micro remains and their morphological analysis. Although being a first attempt for both sites, results are encouraging and consistent with the data available in scientific literature. In view of the positive results reported, phytoliths extraction from active surfaces of stone utensils from archaic archaeological contexts is possible and can be successfully combined to other archaeometry analyses for the reconstruction of ancient societies [17].

The hypotheses presented need to be corroborated by more comparison with further macro-botanical records at local and regional level. Nevertheless, it is desirable to extend the search for micro residues to storage and food preparation pottery as well as to other areas of the settlements, to get a more detailed view of food transformation chain, from production to consumption.

V. ACKNOWLEDGMENT AND AUTHOR CONTRIBUTIONS

Phytolith extraction and analysis was performed at Archaeology of Social Dynamics group (IMF - CSIC) by G. Sabetta during her PhD, under direction and constant supervision of Dr. M. Portillo.

F. Notarstefano conducted and interpreted the results of GC-MS analysis using the equipment of the Laboratory of Synthetic Organic Chemistry of the University of Salento, at the Department of Biological and Environmental Sciences and Technologies. All the work was carried out under the scientific direction of G. Semeraro, who conceived the study of the archaeological contexts.

All authors read and approved the manuscript.

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