

Analysis of micro-particles vacuumed from the Turin Shroud

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Abstract

Aim of the present analysis of particles, in the range of 3-30 μm , vacuumed from the Turin Shroud, is to perform a classification and possibly to refer them to the environments in which, from historical information, it results that the Shroud was exposed.

Grains of pollen, linen and cotton fibers, mites, spores and fungi, mineral particles and particles possibly coming from body fluids have been studied and the classification of some of these particles has been possible.

The attention has been focalized to the pollen grains, to see if M. Frei's results can be confirmed or not, without forgetting a more general view of the analysis with the aim to detect which kind of new information can be useful for the Shroud studies.

The main results are: pollen analysis seems in agreement with M. Frei's results; there are few cotton fiber mixed with linen ones; many mineral particles are compatible with the Jerusalem soil.

1. Introduction

In 2009 the University of Padua financed the first author for the Research Project #CPDA099244 entitled: "*Multidisciplinary analysis applied to the Turin Shroud: study of the body image, of possible ambient pollution and of micro-particles capable to characterize the linen fabric*".

The research project is divided into 5 points, each of which aims at obtaining information regarding still not resolved important issues on the TS (Turin Shroud).

-1) Analysis of the formation mechanism of the body image with formulation of the most reliable hypothesis.

As it results from scientific publications^{1,2,3,4}, various proposals of the body image formation have been formulated, but none of these is able to satisfy the very special features of the image present on the ST. Image formation hypothesis based on radiation, thermal and ultraviolet rays have been considered and they have been opportunely verified with experimental tests in Ref. 4.

Basing on quantitative results of image analysis, a directional radiation proportional to the body-cloth distance has been considered as the most probable cause of image formation. Experimental results of image formation have been studied both from a macroscopic point of view, comparing them with the ones typical of the ST image, and under a microscopic point of view. It resulted that the best hypothesis of body image formation is that based on Corona Discharge⁵.

-2) Analysis of the possible causes of pollution of the TS samples taken in 1988, for radiocarbon dating purposes.

In 1988 a sample of fabric of the TS was taken from a corner and it was radiocarbon dated using the AMS technique. The published result⁶ provided a dating included among the 1260 and the 1390 within a confidence level of 95%, but also because of some statistical mistakes published in the same article, the result appears questionable⁷ due to the presence of a trend, possibly caused by environmental factors. This result has been reached with the help of experts of robust statistic that underline the presence of a contamination in the sample capable to vary the resulting date also of some millennia.

The analysis is also addressed to determine which are the possible causes of contamination. Fire, water, carbon monoxide, dirty and other sources of pollution, also caused by some kind of radiation, have been experimentally tested making AMS analyses on samples exposed to this kind of pollution. For the moment, it seems that only a neutron radiation could be the responsible of the detected systematic effect if we exclude the hypothesis of a Medieval mending, that seems very hard to be sustained.

-3) Analysis of the morphological features of the linen fibers contained in the TS.

The morphological features of the linen fibers coming from the TS have been compared with other linen fibers coming both from recent fabrics and from ancient fabrics coming Egypt and Israel showing some difference also related to the retting process. Such morphological features can be better highlighted if linen fibers are observed through a petrographic microscope in crossed polarized light at magnifications of 100x-600x. The analysis in polarized light highlights features of different types such as changes of thickness which cause chromatic changes and structural defects.

The analysis in cross-polarized light of the so-called "kink bands" (circumferential defects of linen fibers, consisting in folding of the primary fiber layer), typical of the linen fibers, showed interesting results in reference to the TS-image-fibers. It resulted that the chemical process that produced color also caused a remarkable increase of "kink bands" thus showing a structural change of the colored linen fiber.

From a mechanical point of view an opportune equipment able to measure the tensile strength of single flax fibers has been built for the purpose and at present interesting results also regarding TS fibers are under analysis: it seems possible an alternative mechanical dating. This dating, coupled with other two new dating methods under evaluation, based on Raman analysis and FT-IR spectra (focalized on the COC glicosidic group at 1093 cm^{-1} and on the Hydrocarbon group at 1380 cm^{-1}) seem to be a valid base to demonstrate that the TS is about 2000 years old.

-4) Analyses of microparticles present in the TS.

The various micro-particles of vegetable and mineral origin, having a diameter of about 3-30 μm micrometers, among which particles of pollen, are the object of the analysis that is widely discussed in the present work.

The initial aim was to perform a classification of these particles and, if possible, to refer it to the environments in which it results, from historical information, that the ST was exposed.

The analysis has been focalized to the verification of the presence of pollen typical of flora which lives in special zones described by the probable historical journey of the ST. This study has also been performed to verify some statements reported in previous papers^{8,9,10} that suppose a particular historical journey of the TS in the first centuries, on the basis of the pollen identification.

In addition some DNA analyses have been performed on vegetal and biological particles; the interesting results thus obtained are now under evaluation and will be hopefully soon published.

-5) Analysis of the body fluids particles.

Among the various micro-particles, there probably are mixed with some derived from the discharge of body fluids such as blood serum. Some scientists^{11,12} have already identified various particles coming from the TS as particles coming from body fluids of a man.

The present study is also addressed to try to classify the biological material of human origin, also trying to determine their chemical composition.

Starting from a previous study¹³, the present work is aimed to analyze the characteristics of dusts vacuumed from the TS, not only focalizing the attention on the pollen grains, but in general to evaluate which kind of useful information could be obtained.

2. Method and samples

In 1978 and in 1988 some dusts were vacuumed¹⁴ from the back of the TS and sampled in various filters. Some statistical analyses have been done on these dusts¹³, and other analyses were performed principally for conservation problems^{15,16}. The sampled dusts were vacuumed from space between the TS and the Holland Cloth, in filters named from “e” to “i” in reference to the vacuuming area shown in Ref. 14. The dusts, object of the present study, come from very small parts taken from filters “e”, “f”, “g”, “h” and “i” and sampled in stubs suited for SEM (Scanning Electron Microscope) analysis.

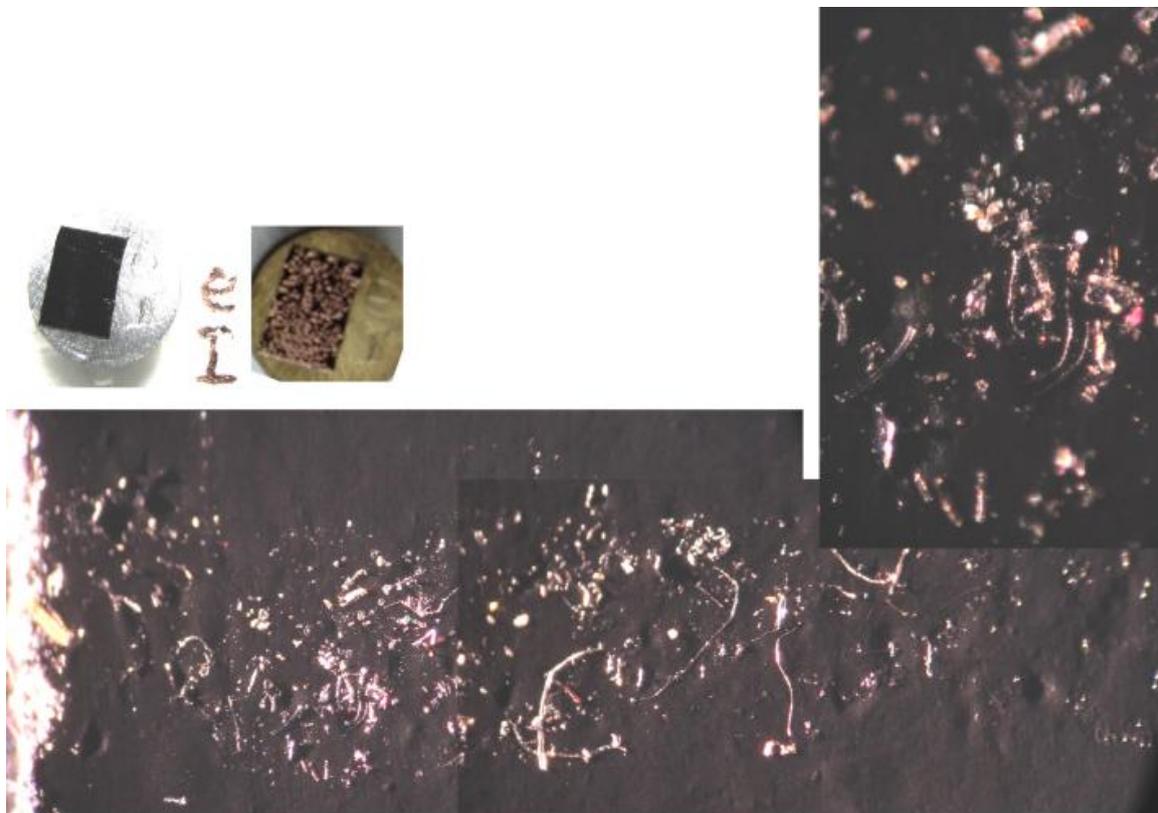


Fig 1. Example of map obtained using an optical microscope on Stub EI in order to save the color information relative to the dust samples.

A dozen of stubs were used and each stub has been classified with the first letter corresponding to the name of the filters “e” to “i”. A proper adhesive was put on the aluminum stubs and some micrograms of dust were placed on the adhesive. Before the analysis, the samples have been metallized using gold. It is therefore to evidence that the EDS (Energy Dispersive x-ray Spectroscopy) results connected with SEM analysis show a peak corresponding to gold, not typical of the sample under analysis, but due to the metallization.

To avoid the invasive procedure of metallization, some samples have been analyzed by ESEM (Environmental Scanning Electron Microscope) that in general has a lower resolution than SEM, but it does not request an invasive procedure. Also in this case an EDS analysis was performed, but in this case it is not obviously evident the Au peak.

Before to metallize the samples, a series of photomicrographs were obtained using an optical microscope in incident light in order to maintain the color information that is successively destroyed with metallization; Fig. 1 shows an example while other photos are reported in the Appendix.

To register the position of the single particles in the stub under analysis, an area of interest was previously defined and this area has been mapped by acquisition of a number of images needed to generate a photo-mosaic. It was observed a reasonable step between successive shots in such a way to ensure the overlap of a part of them, which is necessary for the Mosaic.

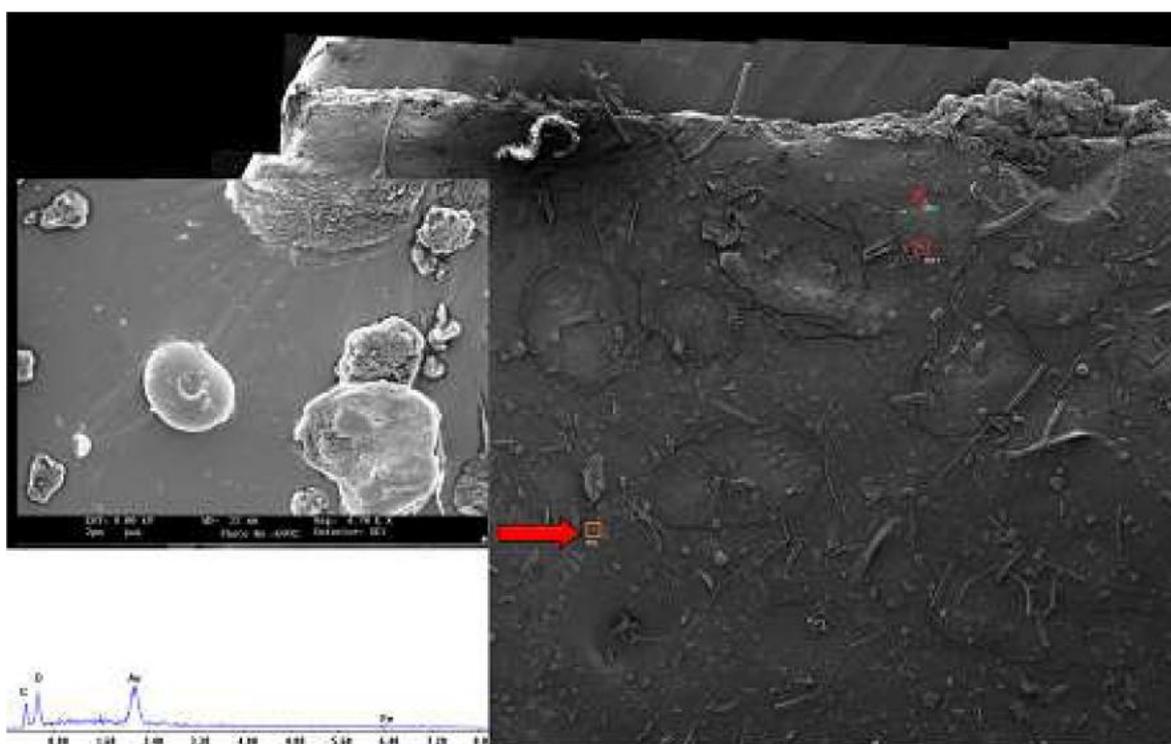


Fig 2. Example of map obtained from Stub EC (on the right) and a detail of an organic particle (on the left) with the corresponding position on the map evidenced by the arrow; the EDS spectra indicates that there are atoms of carbon (C) and oxygen (O) in addition to the gold (Au) due to the metallization thus showing that the particle under analysis is of organic type.

The subsequent analysis of spores, pollen and mineral particles detectable on the map has been obtained on dust particles with sizes in the range of 3-30 μm both by morphological investigation at high magnifications (5000-7000 X), and through the acquisition of the relative spectra (EDS) in order to have additional information of the chemical composition of the particle under analysis. It has been possible therefore to assess if a particle is of organic origin and its elemental composition.

M. Frei was accused of fraud because he reported the SEM photos of pollen grains similar to those detected in his glasses and not just the pollen grains coming from the TS. This accusation can be right for scientists of our days, but other scientists in the 1970-1990 used to publish photos corresponding but not equal to the pollen grain under examination.

The finding of this pollen grain in the TS dusts confirms the seriousness of M. Frei and partially explains why the palynologists preferred to show photo reproducing a better conserved grain; the pollen grain shown in Fig. 3 and probably also the others observed by M. Frei were not perfect because very old, and therefore a perfect grain was preferred to show the corresponding features.

The *Phillyrea Angustifolia* is a woody plant blooming in March-May with a bushy or small tree evergreen that can reach a height of 2-4 meters; it is a species typical of the Mediterranean Scrub and is not very demanding as it is a plant, that is particularly suited to cultivation in difficult terrain, drought and grows in areas especially on the coasts.

Fig. 4 shows a pollen grain classifiable to the family of Liliacées, perhaps of *Fraxinus angustifolia*. Fig. 5 instead shows a pollen grain not easy to classify. Fig. 6 reports some other pollen grains or particles still under examination.

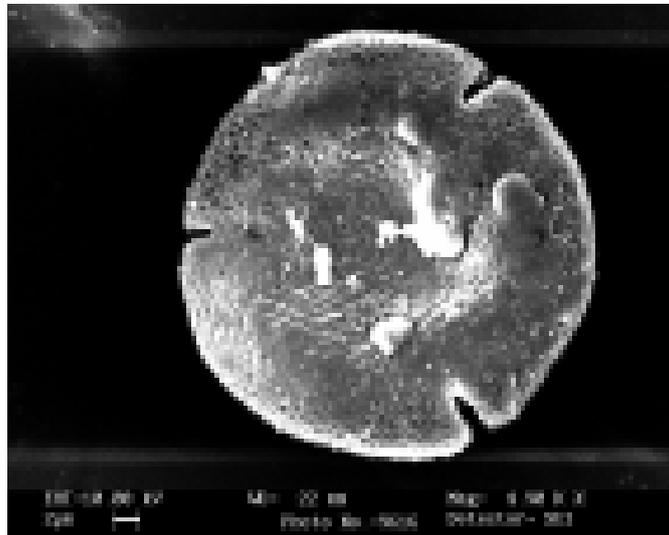


Fig 4. Pollen grain classifiable to the family of Liliacées, perhaps of *Fraxinus angustifolia*.



Fig 5. Pollen grain not easy to classify.

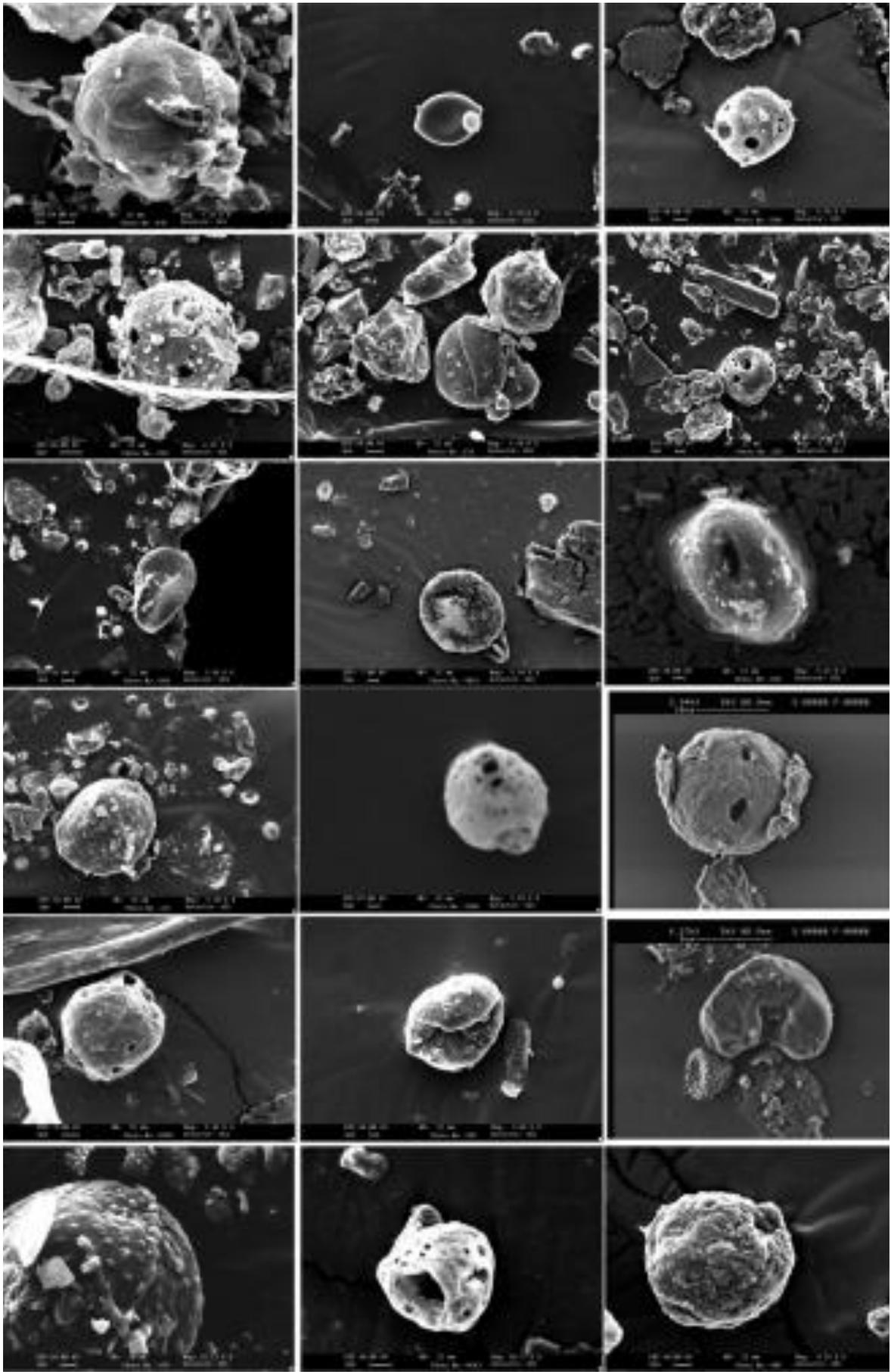


Fig 6. Other pollen grains and particles still under examination.

3.2. Linen and cotton fibers

The linen fibers are produced by the beams contained in the fibrous layer of the stem of some Librarian herbaceous plants belonging to the family of *Linacee*. The fibers are obtained by a process of maceration which solubilizes peptic and woody substances that hold them welded together.

The chemical composition of linen fiber differs from that of cotton for the lowest cellulose content and a greater presence of hemicellulose and lignin.

The fibers of linen have length of 6-50 mm and a diameter of 6-40 micrometers, pseudo-cylindrical shape, transverse striations, a narrow central channel and nodal thickenings of the walls called kink bands or dislocations, which confer to the fibers in a typical form of bamboo, see Fig. 7.

The observation at high magnification allows to identify some characteristics of the linen fibers, while the EDS has provided the chemical analysis of organic and inorganic impurities surface, in the form of individual particles or crystalline aggregates.

The detailed analysis shows that the deposits present on the fibers are composed in most of the cases of Calcium (Ca), therefore we can suggest the presence of Calcium Carbonate (CaCO_3) encrustations, see Figg. 8 and 9.

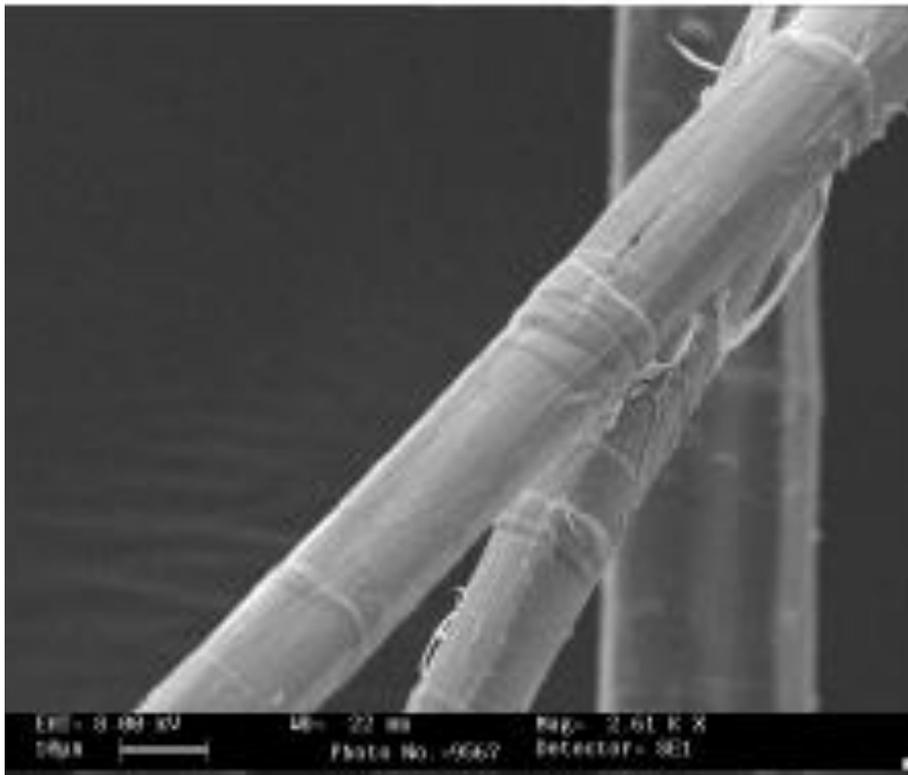


Fig 7. Modern linen fibers without encrustations showing the typical bamboo-structure.

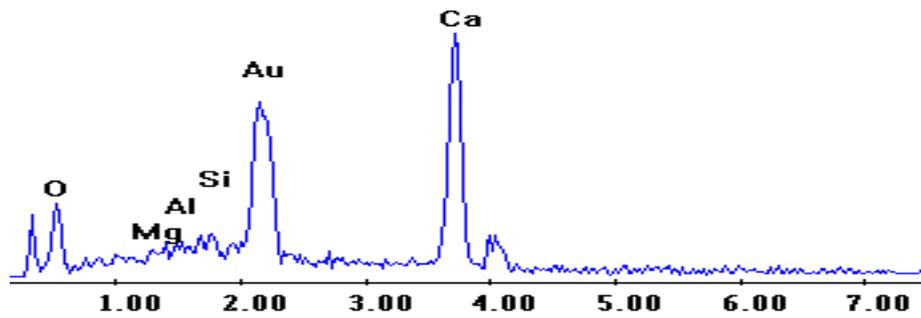
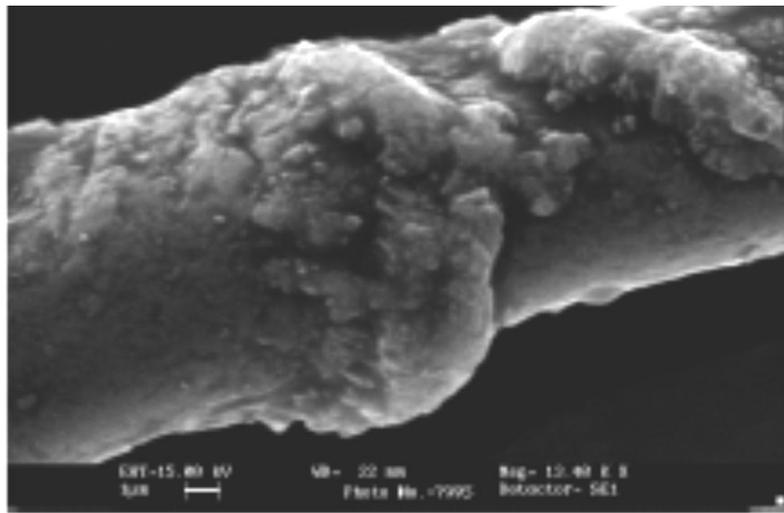


Fig 8. Encrusted linen fiber coming from the TS, with relative spectrum ,showing the presence of Ca (the absence of the peak of Au is due to the fact that this fiber was not metalized).

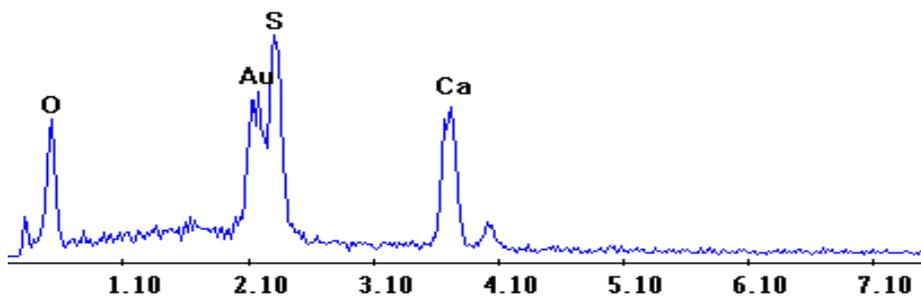
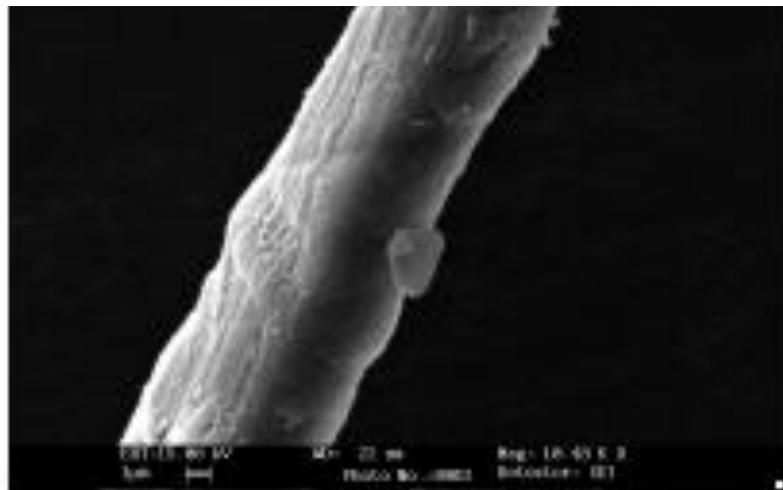


Fig 9. Linen fiber coming from the TS, with relative spectrum, showing the presence of Ca (the absence of the peak of Au is due to the fact that this fiber was not metalized).

Cotton is a vegetable fiber obtained in plants belonging to the genus *Gossypium*, family *Malvaceae*; the cotton fibers coming from the TS are from plants of the species *Herbaceum*.

A single fiber of cotton, whose length is approximately 2500 times its diameter, is composed of a primary wall, by a thicker secondary wall and a central canal, said lumen, which contains the protoplasmic juices. The primary wall, consisting of cellulose (80%), hemicellulose and pectins (3%), waxes, fats and proteins (2%) is covered by a thin cuticle characterized by overlapping layers of laminated nature. Typical morphology of the fibers of cotton, tape twisted, is clearly visible at magnification greater than 100x.

Fig. 10 shows some TS fibers; the ones indicated by the arrow seems of cotton; Fig. 11 shows a typical cotton fiber coming from the TS.

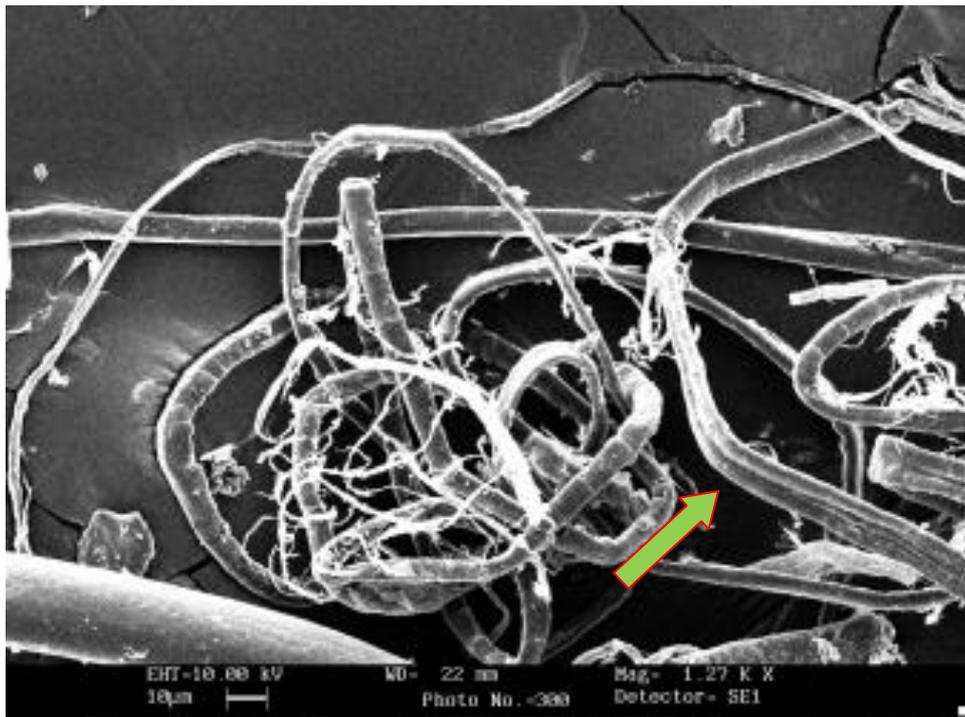


Fig 10. Fibers coming from the TS with a probable cotton fiber indicated by the arrow.



Fig 11. Typical tape structure of a cotton fiber coming from the TS.

3.3. Mites, spores and fungi

In some stubs, especially GI and HI, have been found remains of mites; the *Acarina*, vulgarly known as mites, are an order of *arachnids*, see Fig 12.

Other biological particles have been identified as spores of filamentous fungi belonging to the genus *Aspergillus Glaucus*, see Fig. 13, and *Cladosporium sp.* These types of organisms are present in large number in the stubs.

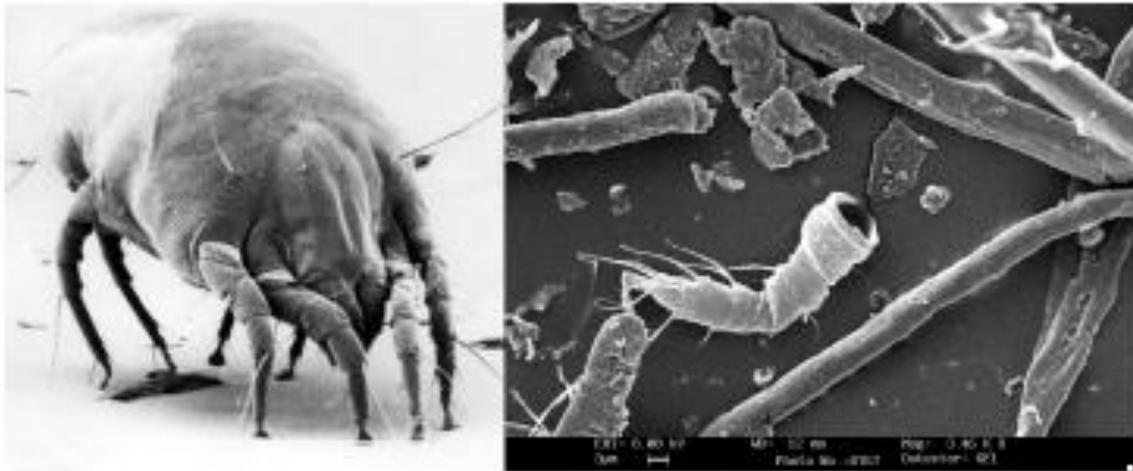


Fig 12. Mite on the left and remnants of mites in the TS dust on the right

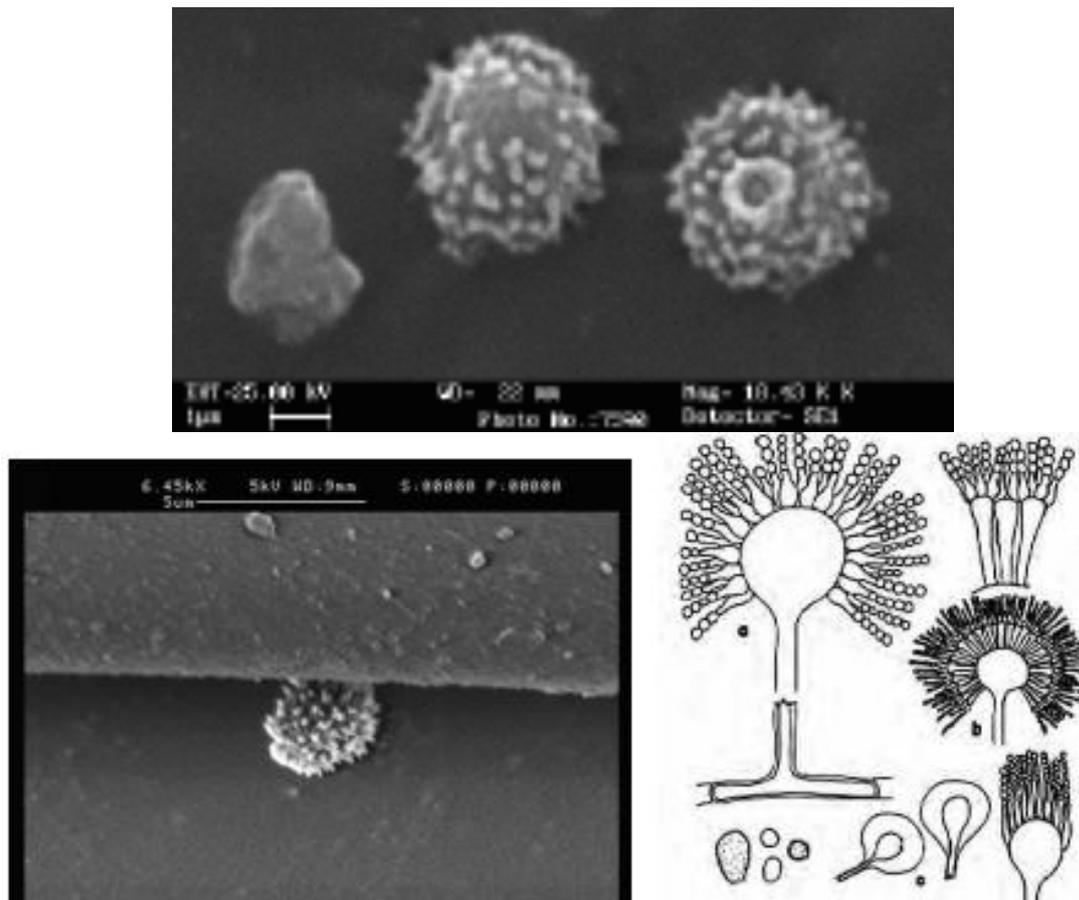


Fig 13. *Aspergillus Glaucus* of the TS dusts and corresponding morphology.

3.4. Mineral particles

Many mineral particles were found in the TS dusts, with sizes in the range of about 3-30 μm . They have different colors but prevalently are white or reddish. The attention has been focalized on the reddish particles, also because they are not so common in the soil of north Italy where the TS is now conserved for centuries.

It was instead found that the Jerusalem soil contains many red particles. Therefore a comparison between the reddish particles coming from the Jerusalem soil and the reddish mineral particles coming from the TS is here reported.

Fig. 14 shows a set of white or grey particles coming from a layer of soil just under the S. Sepulcher in Jerusalem and (on the bottom) reddish particles coming from the soil of Mt. Zion in Jerusalem. Two EDS spectra of these reddish particles are reported in Fig. 15.

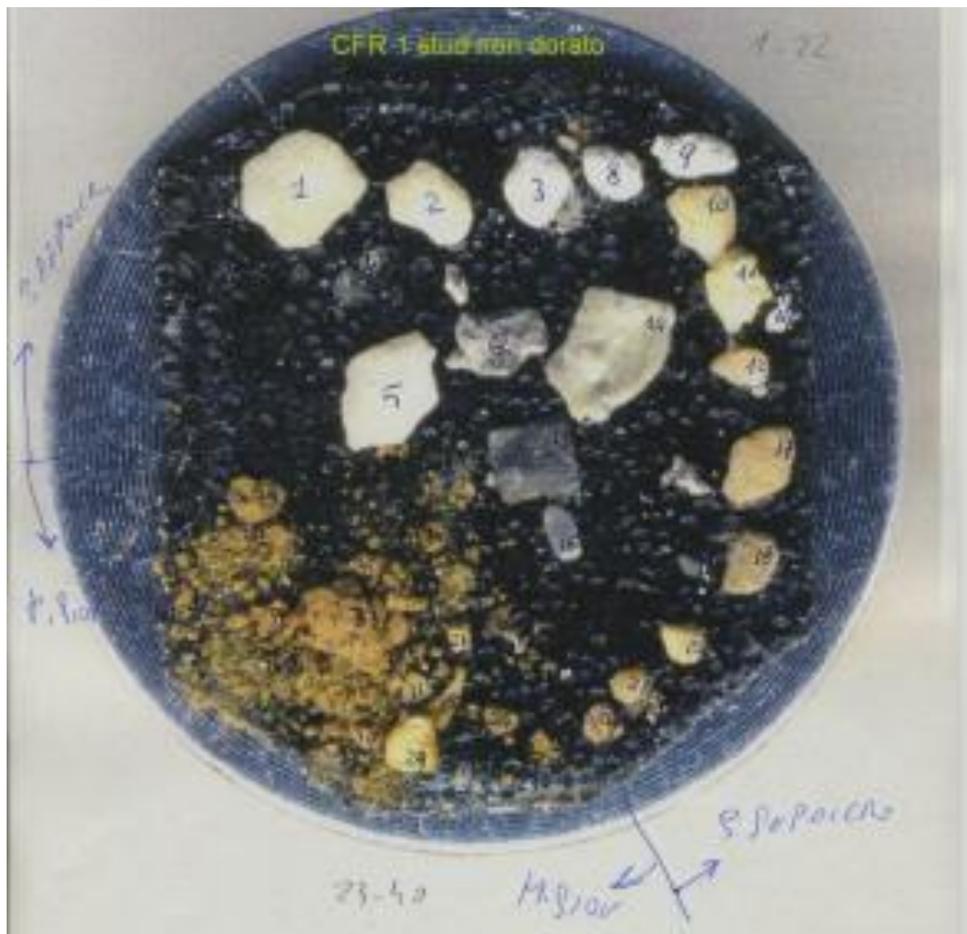


Fig 14. Mineral particles sampled in Jerusalem; the reddish ones (on the bottom) coming from Mt. Zion are those of interest for the present analysis.

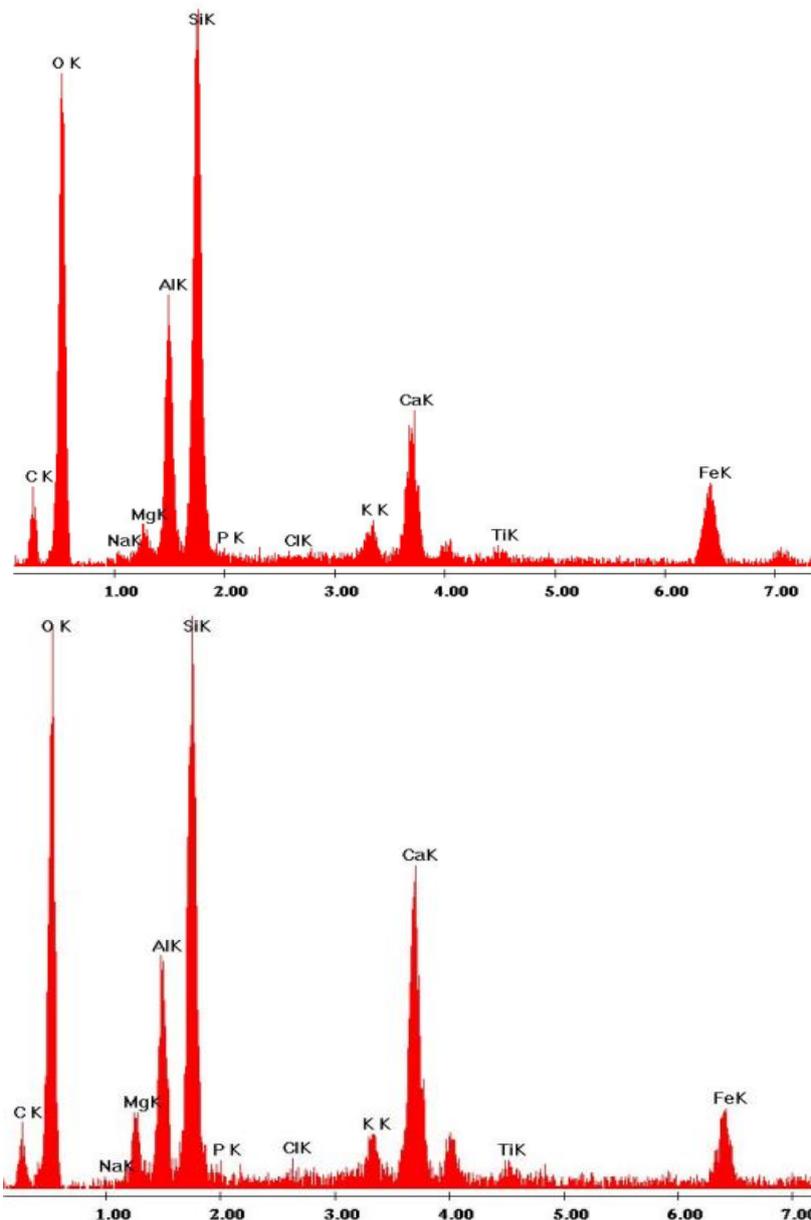


Fig 15. EDS spectra of the reddish mineral particles coming from Mt- Zion of Fig. 13.

Fig. 16 reports some dusts coming from the Stub H5 of the TS dusts and the arrow shows the reddish particle tested, whose spectrum is reported in Fig. 17. Fig. 18 reports some dusts coming from the Stub H6 and the arrow shows the reddish particle tested, whose spectrum is reported in Fig. 19.

It appears evident that the spectra of particles taken from Mt. Zion (Fig. 15) are quite similar to those of Stubs H5 and H6 (Fig. 17 and 19). Even if more accurate analyses should be necessary for a complete identification, those mineral particles are very similar to the typical local clay of Jerusalem (and of other Mediterranean areas influenced by the winds of the Sahara desert). They seem to belong of the Illite-Smectite family also containing gypsum.

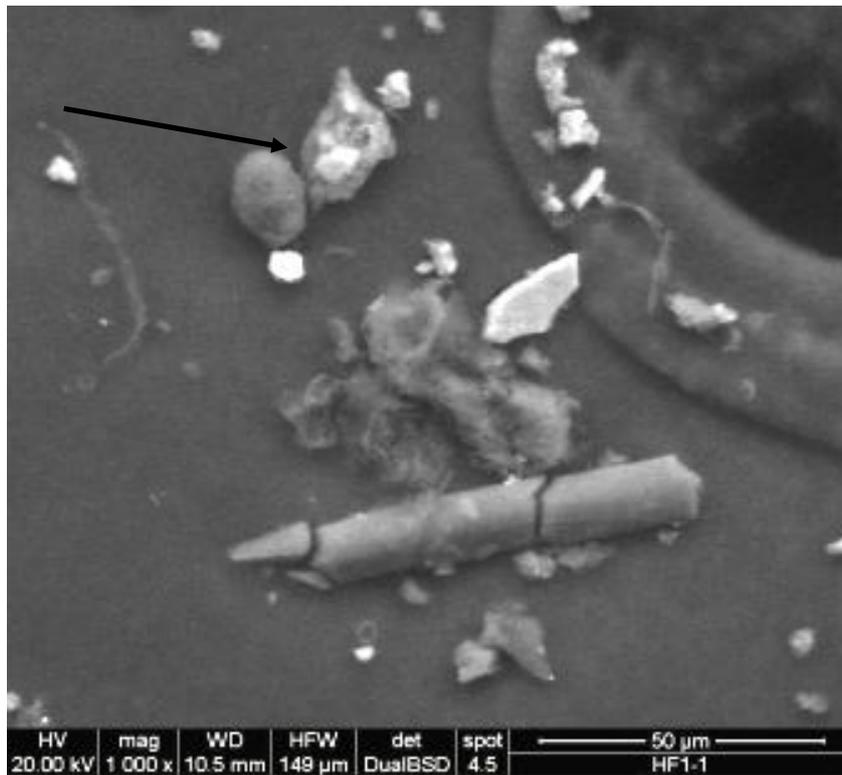


Fig 16. Dust particles of Stub H5: the arrow shows the reddish particle whose spectrum is reported in Fig. 17.

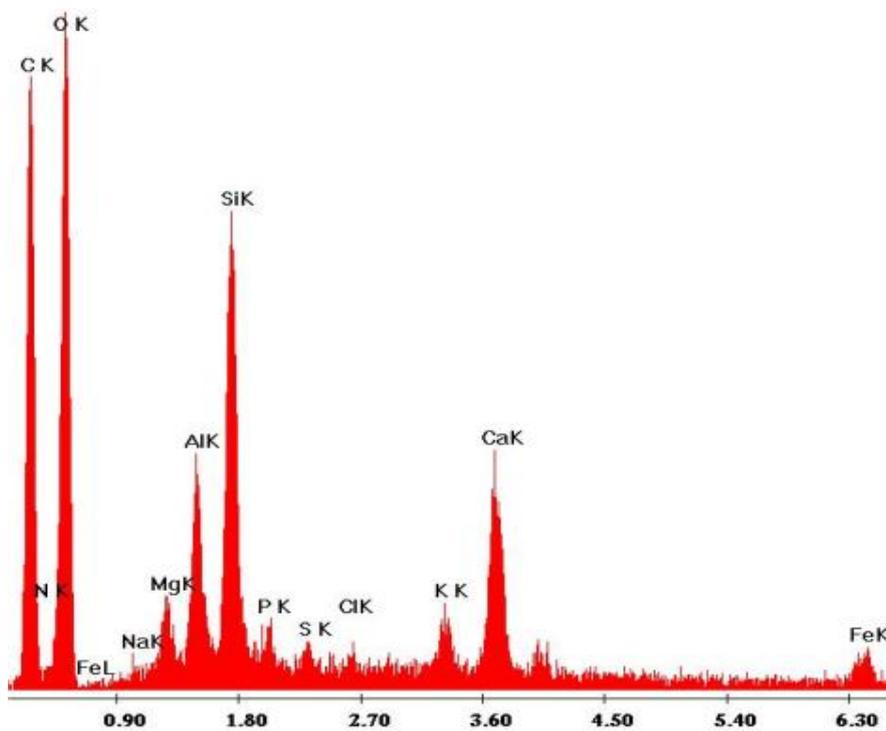


Fig 17. EDS spectrum of the particle shown in Fig. 16.

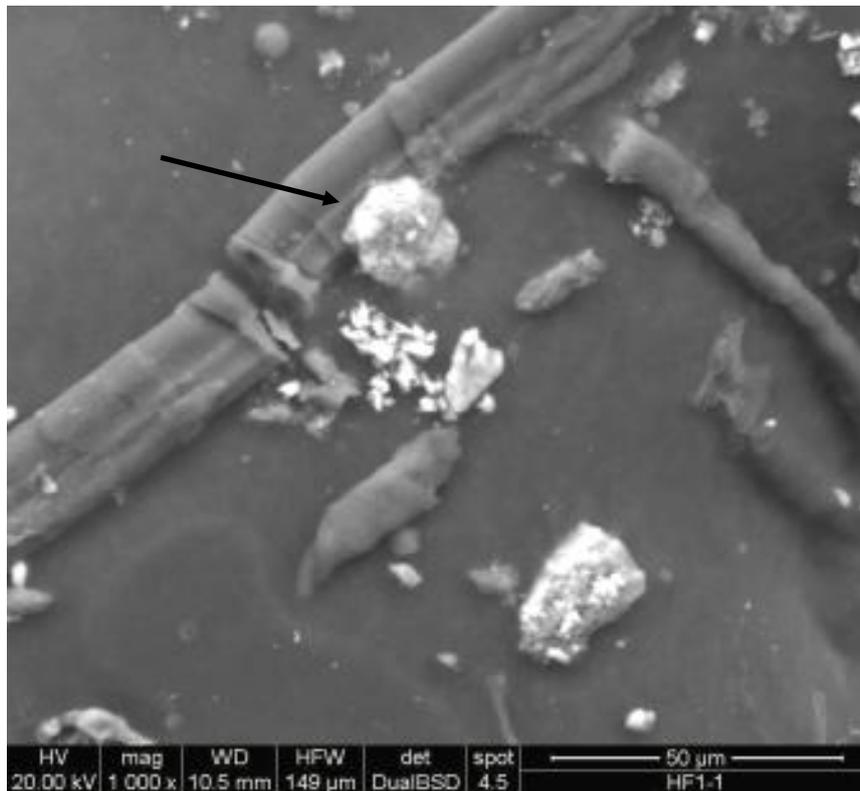


Fig 18. Dust particles of Stub H6: the arrow shows the reddish particle whose spectrum is reported in Fig. 18

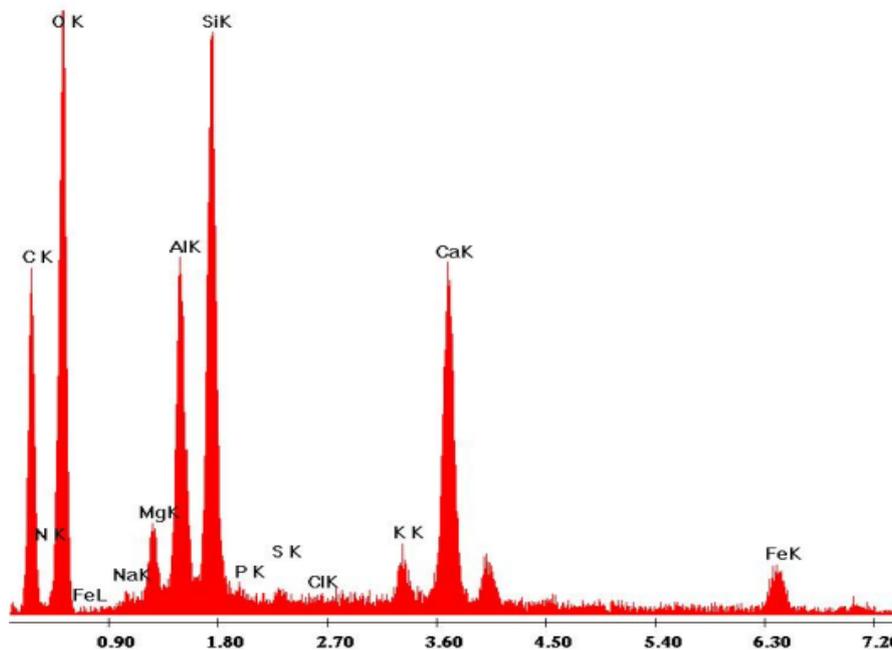


Fig 19. EDS spectrum of the particle shown in Fig. 18.

3.5. Particles possibly coming from body fluids

Among the vacuumed dusts, there are some biological particles not easy to be identified. Many red particles of the dusts are of mineral type, see Section 3.4, but there are also few of them looking like biological. It is well known^{11,12} that there is human blood on the TS and therefore these few red particles of biological type were tested from a spectral point of view to see if they can be compatible with blood.

According to J. Heller and A.Adler¹¹ the TS samples of human blood contain various elements such as Na, Mg, Al, Si, P, S, Cl, K, Ca, Fe. The elemental analysis performed by EDS spectroscopy on the red biological particles coming from the vacuumed dusts puts in evidence the presence of Si, Mg, Na, Cl, Ca and Fe.

These elements are present in blood and body fluids involved in various enzymatic processes and metabolism. The elements of interest in this specific case are:

- Sodium (Na): it is present in ionic form in blood and in intracellular fluids;
- Chlorine (Cl): it is mostly present in ionic form in extracellular fluids;
- Calcium (Ca): it may reach 1% in body fluids and it controls the coagulation process;
- Iron (Fe): an adult body contains about 4-5 g of iron, the 65% of which is contained in the hemoglobin. About 0.5 g of iron are contained in one liter of blood. Iron is an essential constituent of hemoglobin and myoglobin.

The red particle of Fig. 20 has been analyzed after metallization. The spectra of Fig. 21 reveal the presence of Si, Mg, Na, Cl, Ca and Fe. It is to note that these spectra are very similar to those obtained by P.L. Baima Bollone¹². Given this, it is possible to explain the presence of these elements assuming that the particles under analysis was in contact with blood, but at the same time, it is not true the opposite: the presence these elements does not allow to claim that it is unequivocally blood. Therefore, being the particle under analysis red, of biological type, and containing elements typical of blood, we can conclude that this particle was probably encrusted with blood, but the affirmation is obviously not sure.

As many touched the TS during many centuries, and remembering that various persons restored the TS (the Chambéry nouns spent many hours after the 1532 fire) it seems nevertheless hazardous to think that this possible blood particle came from the TS Man without thinking that the blood in question possibly derived for example from a puncture caused by a needle used during sewing.



Fig 20. Photomicrograph of the red particle under analysis made at optical microscope (on the left) and the same particle seen at SEM after gold metallization at the center and at the right, with the numbers of the corresponding spectra shown in Fig. 21.

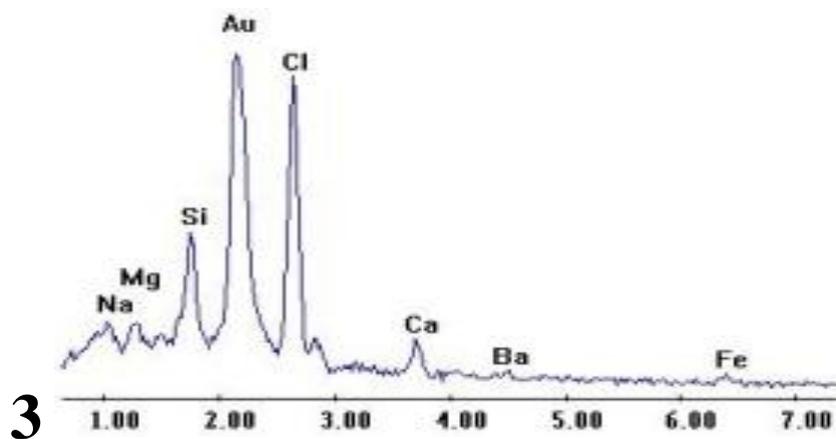
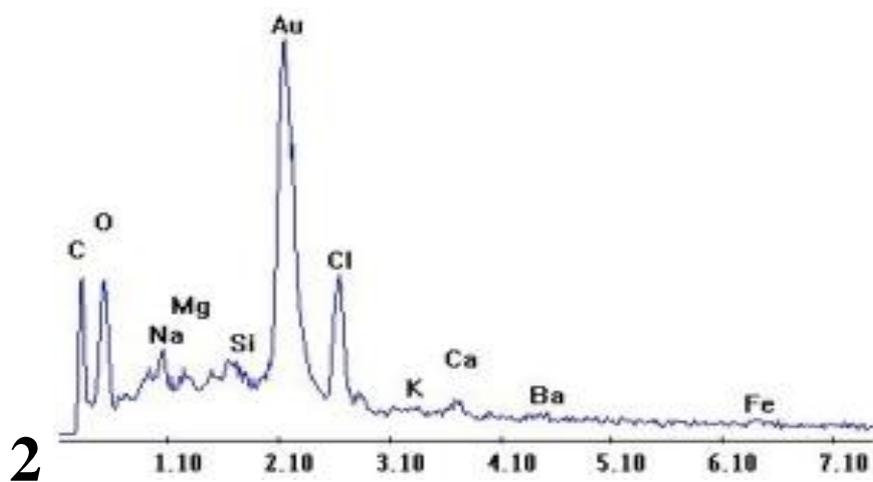
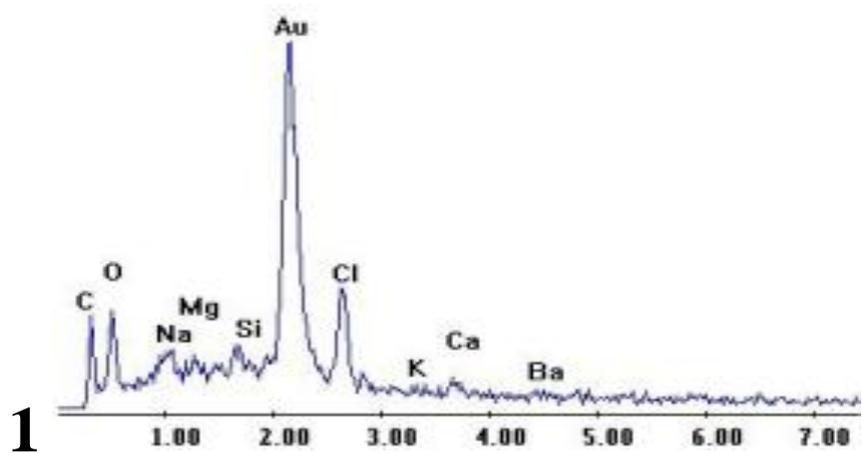


Fig 21 EDS spectra of the red particle of Fig. 20.

Conclusive remarks

The Padua University Research Project made possible the analysis of particles in the range from 3 to 30 μm , vacuumed from the Turin Shroud. They have been studied using optical microscopes, SEM, ESEM and EDS with the aim to classify them and possibly to refer them to the environments in which, from historical information, it results that the TS was exposed.

The following particles have been studied and some of them have been classified focalizing the attention to the pollen grains to detect if M. Frei's results can be confirmed or not. In addition it was not neglected the possible detection of new information perhaps useful for the TS studies in general.

- Pollen grains. The analyzed dusts do not contain the large number of pollen detected by M. Frei^{8,9} in his sticky tapes probably due to the different sampling method. About only one pollen grain was detected in each stub containing the vacuum dusts from the TS. A pollen grain of *Phillyrea Angustifolia* was detected, thus partially confirming the results of M. Frei and explaining why he preferred to publish in his papers, SEM photos of pollen corresponding to those seen by him without reporting just those present in his sticky tapes: in fact the pollen coming from the TS are clearly damaged. Also a pollen grain apparently of *Fraxinus* and one of *Cedar of Lebanon* have been detected.
- Linen and cotton fibers. Even if majority of the textile fibers composing the TS are of linen, there are also some cotton fibers of *Gossypium Herbaceum*. Some fibers show the presence of inorganic impurities on their surface; these encrustations seem of calcium carbonate.
- Mites, spores and fungi. Some remnants of mites have been detected in the vacuumed dusts, and this partially explains the Thymol treatment of the TS done in 1988; spores and fungi are very frequent in these dusts and among them the most frequent is *Aspergillus Glaucus*.
- Mineral particles. There are many mineral red mineral particles in the dusts (red because they contain iron) that are compatible with the Jerusalem soil. They seem to belong of the Illite-Smectite family also containing gypsum.
- Particles possibly coming from body fluids. A red biological particle was studied and also analyzed via EDS; it contains elements as Si, Mg, Na, Cl, Ca and Fe that are also present in human blood, but, due to the various kinds of pollution in the centuries, it is not possible to correlate it to the TS Man.

This analysis is obviously not conclusive because many open issues have to be clarified with future studies, also addressed to more specific tests. For example a larger number of dust particles should be used to confirm the results reported in the interesting studies on pollen^{8,9,10} and in the comparisons between the mineral particles coming from the TS and the Jerusalem soil. This last study could be the n-th clue in the TS research confirming its exposition in the Jerusalem environment.

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Análisis de micro-partículas de la Sábana Santa

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Observaciones concluyentes

El Proyecto de Investigación de la Universidad de Padua hizo posible el análisis de partículas en el rango de 3 a 30 μm , aspiradas de la Sábana Santa de Turín. Las cuales han sido estudiadas mediante microscopía óptica, SEM, ESEM y EDS, con el objetivo de clasificarlas y, posiblemente, referenciarlas a los ambientes en los que, a partir de información histórica, fue expuesta la SS (Sábana Santa).

Las siguientes partículas han sido estudiadas y algunas de ellas han sido clasificadas enfocando la atención a los granos de polen para detectar si los resultados de M. Frei pueden o no ser confirmados. Adicionalmente, no se descuidó la posible detección de nueva información la cual tal vez sea útil para los estudios de la SS en general.

- Granos de polen. Los polvos analizados no contienen el gran número de polen detectado por M. Frei^{8,9} en sus cintas adhesivas, debido probablemente al diferente método de muestreo. Fue detectado alrededor de un sólo grano de polen en cada contenedor de los polvos aspirados de la SS. Se detectó un grano de polen de *Phillyrea angustifolia* por lo que, parcialmente confirma los resultados de Frei M. y explica por qué prefería publicar en sus artículos micrografías de SEM del polen, correspondientes a aquellas observadas por solo por él, sin informar las presentes en sus cintas adhesivas: el polen proveniente de la SS está claramente dañado. También se ha detectado un grano de polen aparentemente de *Fraxinus* y uno de cedro del Líbano.

- Lino y fibras de algodón. Incluso si la mayoría de las fibras textiles que componen la SS son de lino, existen también algunas fibras de algodón de *Gossypium Herbaceum*. Algunas fibras muestran la presencia de impurezas inorgánicas en su superficie; estas incrustaciones parecen ser de carbonato de calcio.

- Los ácaros, esporas y hongos. Han sido detectados algunos restos de ácaros en el polvo recolectado, esto explica parcialmente el tratamiento de Thymol realizado a la SS en 1988, las esporas y los hongos son muy frecuentes en estos polvos, y entre ellos la más frecuente es la *Aspergillus glaucus*.

- Partículas minerales. Hay muchas partículas minerales rojas en los polvos (el color rojo es debido a que contienen hierro) que son compatibles con el suelo Jerusalén. Las cuales parecen pertenecer a la familia Illita-esmectita también contienen yeso.

- Partículas posiblemente procedentes de fluidos corporales. Una partícula biológica roja fue estudiada y analizada mediante EDS, la misma contiene elementos tales como Si, Mg, Na, Cl, Ca y Fe que también están presentes en la sangre humana, pero, debido a los diversos tipos de contaminación durante los siglos no es posible que se correlacionan con el Hombre de la SS.

Este análisis obviamente no es concluyente, debido a muchos temas pendientes por aclarar con estudios posteriores y dirigidos a pruebas más específicas. Por ejemplo, se debe utilizar un mayor número de partículas de polvo para confirmar los resultados reportados en los estudios sobre el

polen^{8,9,10} y en las comparaciones entre las partículas minerales procedentes de la SS y del suelo de Jerusalén. Este último estudio podría ser el enésimo elemento en la investigación de la SS confirmando su exposición en el ambiente de Jerusalén.

Appendix

Additional photographs of the stubs and of the micro-particles vacuumed from the TS are reported here below.

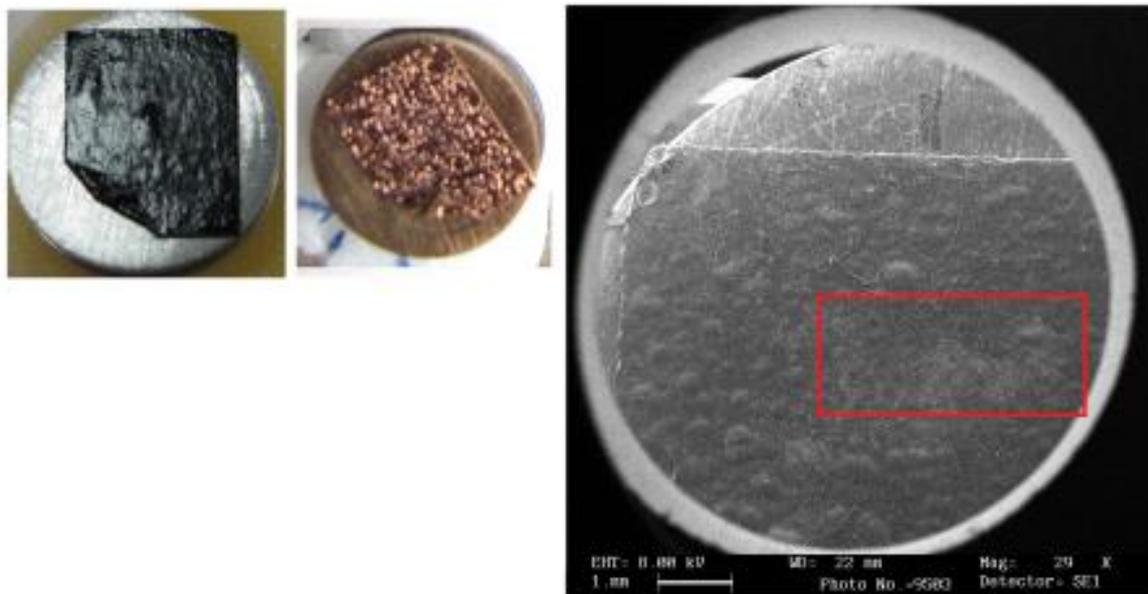


Fig. A1. From the left to the right, Stub F before and after gold metallization, and area of interest (in red) selected for the mapping.



Fig. A2. Stub HH after metallization.

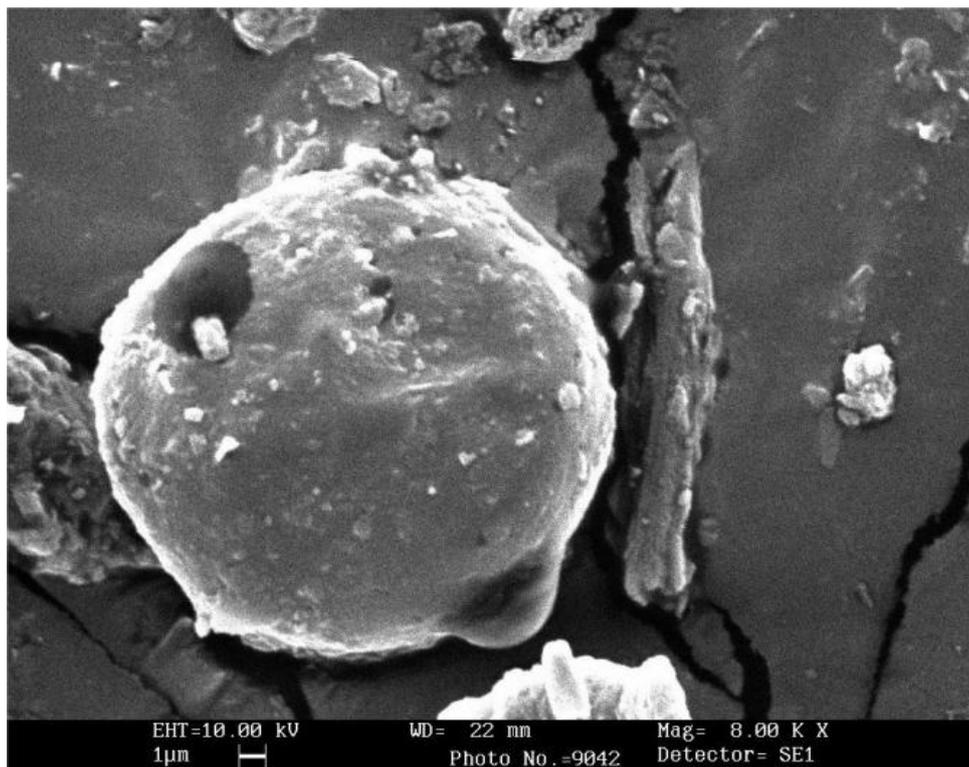


Fig. A3. Possible pollen grain of *Helianthemum versicarium* Boiss quite similar to that reported by M. Frei in Ref. 8 as pollen typical of Iranian steppe.

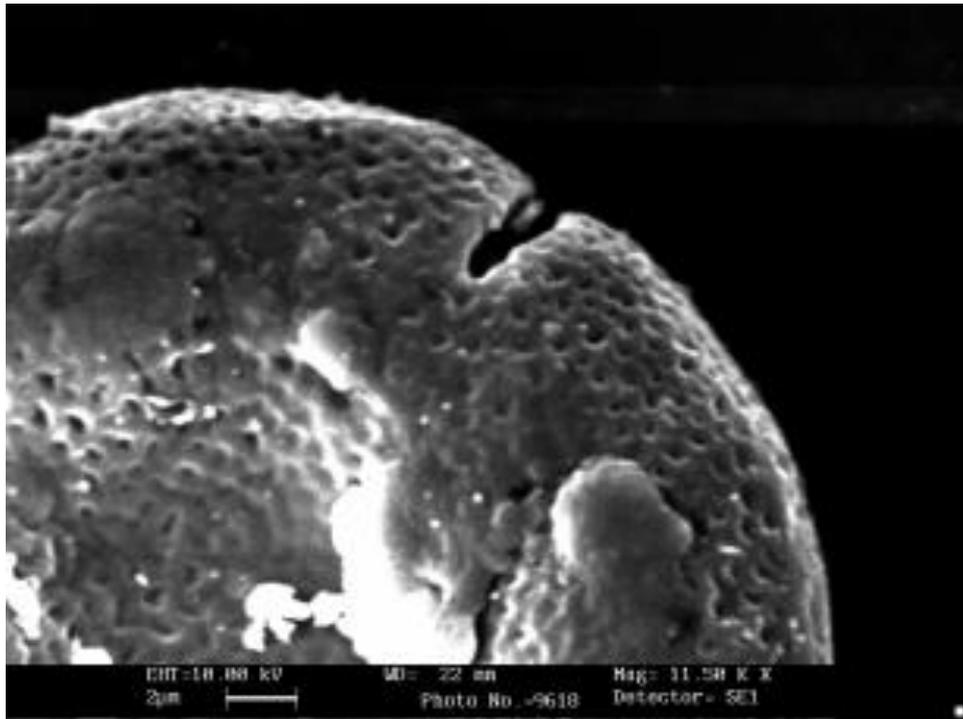


Fig A4. Detail of pollen grain of Fig. 4 classifiable to the family of Liliacées, perhaps of *Fraxinus angustifolia*.

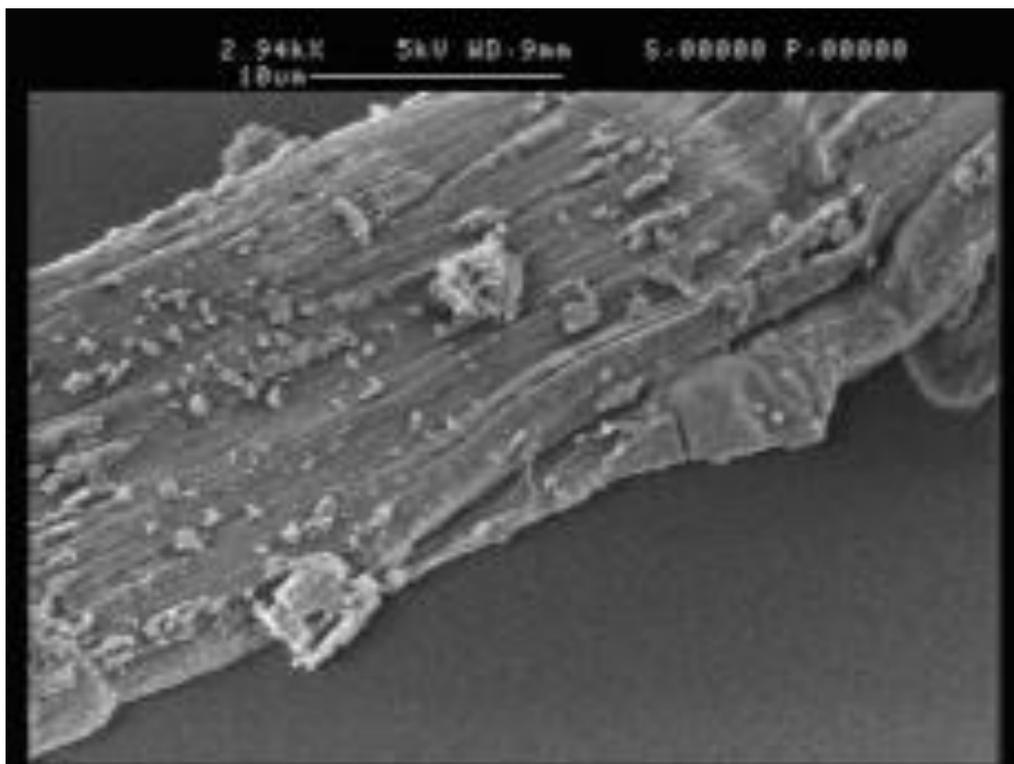


Fig A5. Encrusted linen fiber from Stub H1.



Fig A6. Linen and cotton fibers from Stub H3.

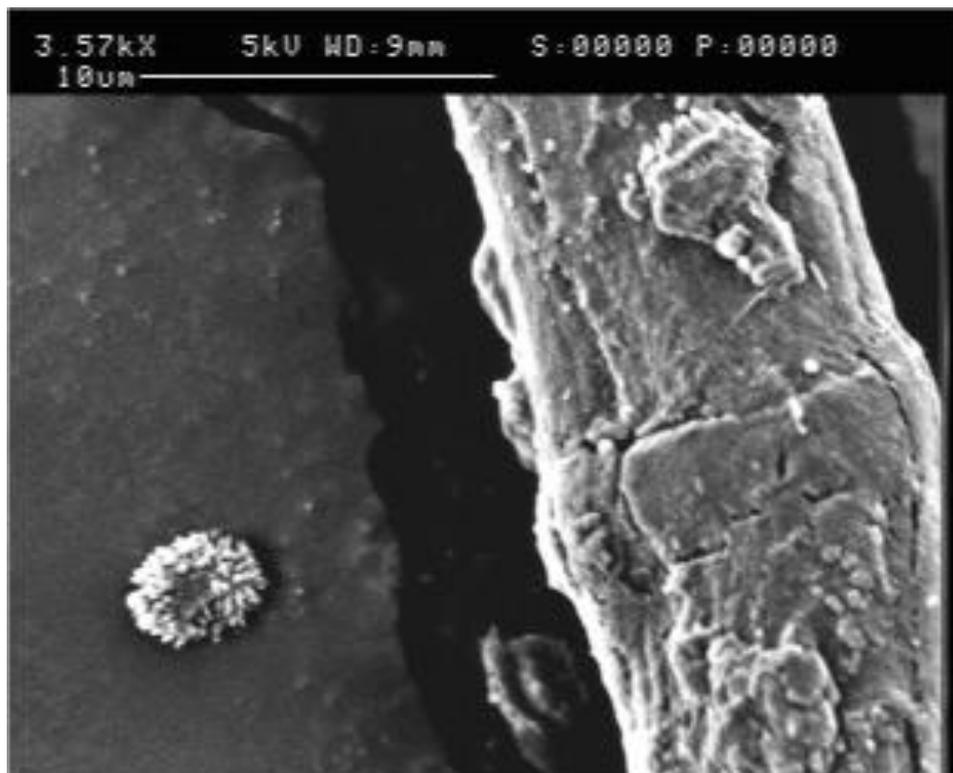


Fig A7. Dislocation of a TS linen fiber from Stub H3.

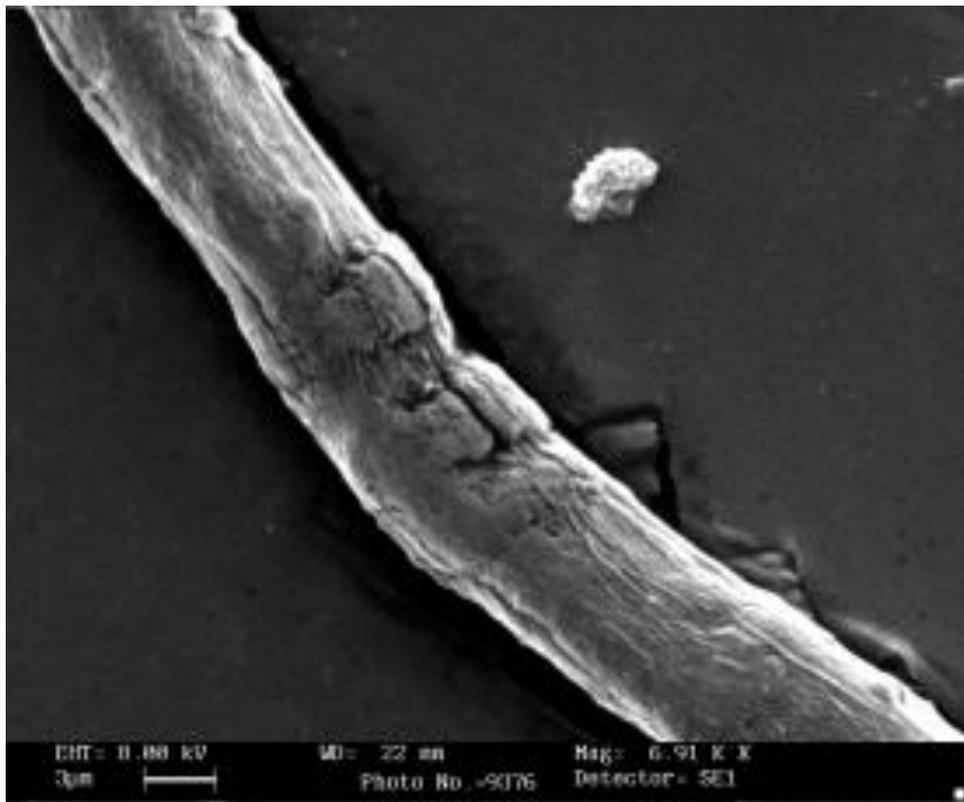


Fig A8. Dislocation of a TS linen fiber from Stub EI.

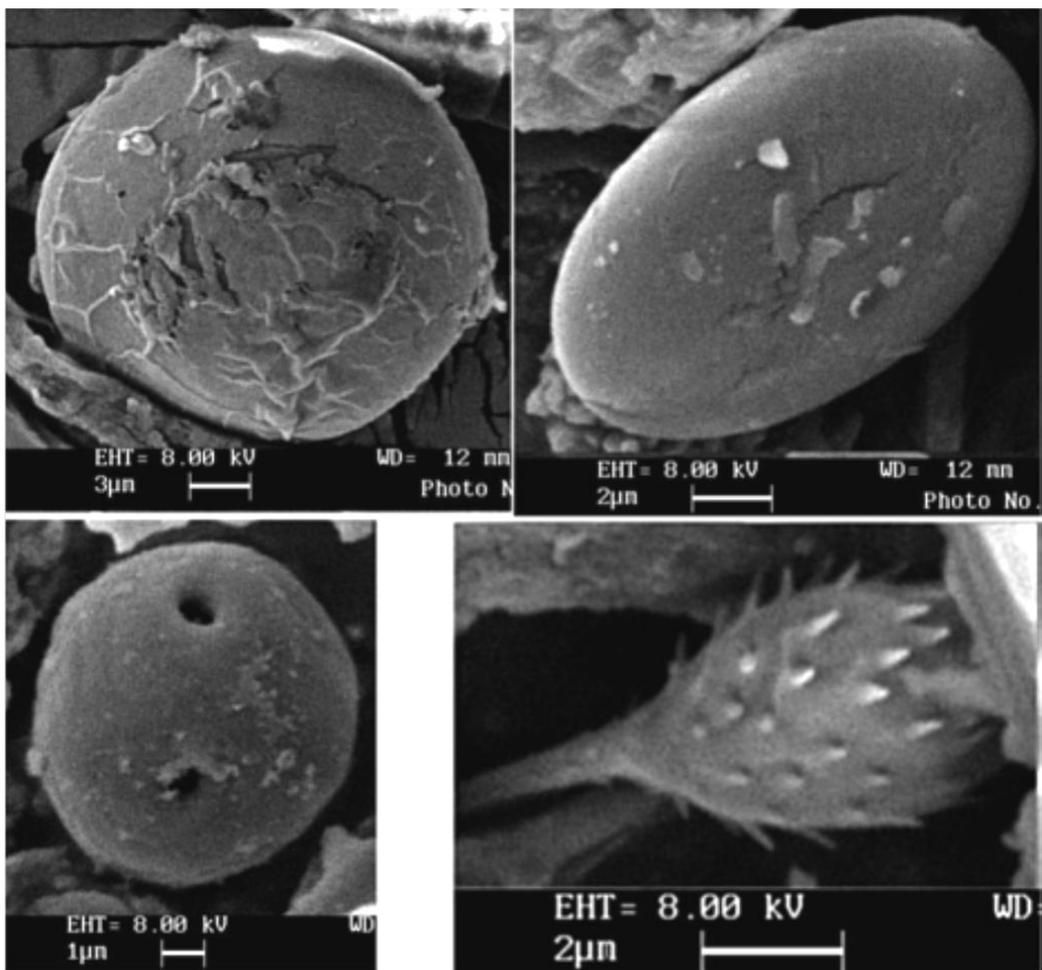


Fig. A9. Four biological particles.

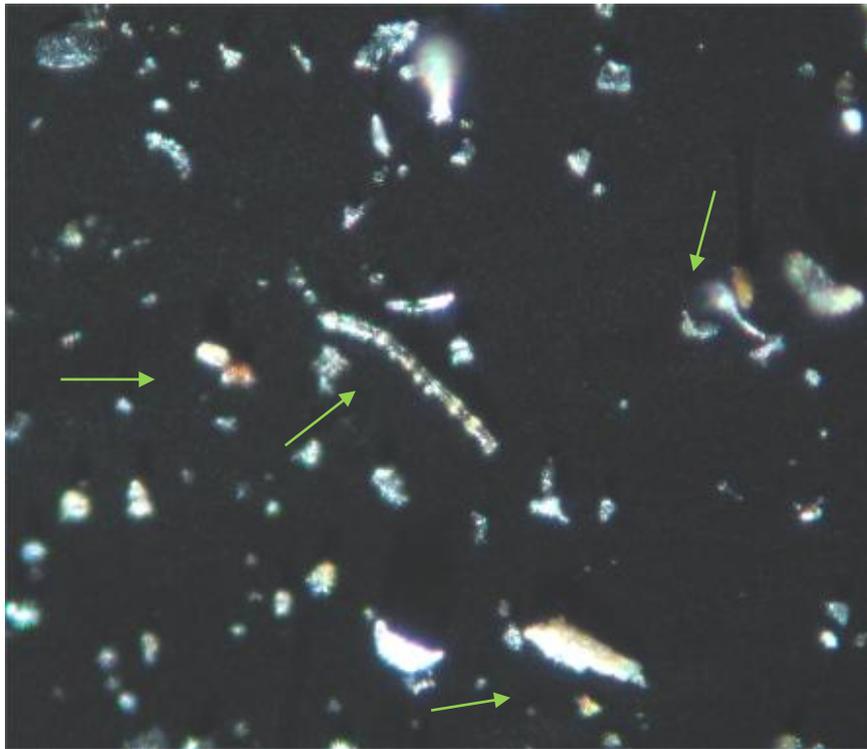


Fig. A10. Dust particles of Stub F seen in an optical microscope: a piece of linen fiber with red mineral particles are indicated by the arrows.