"THE DATE OF THE LINENS FOR THE METHOD OF THE C14.
PARTICULAR CASE OF THE SHROUD OF OVIEDO"

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1. INTRODUCTION

As we have said in multitude of occasions, the research realized by the Investigation Team of Sindonología's Spanish Center (E.D.I.C.E.S.) on the Shroud of Oviedo, it is based on the interpellation to the proper object considering it to be an object of archaeological value. And in Archaeology one of its fundamental variables is the time, to be able to place the above mentioned object in the historical context where it was used.

The chronological determination of the objects in Archaeology can be carried out of two forms: relative and absolute

In the first form the object in question is placed with regard to others, establishing relations of the type: "more modern than ", " more ancient than " or " contemporary to ".

Whereas in the chronological absolute methods one tries to know the real time; between these we can mention: dendrochronology, compared or crossed chronology, carbon 14 (14C), termoluminiscence (TL), potassium - argon, series of the uranium (uranio/torio), fission tread, racemization of amino acids, arqueomagnetisme and paleomagnetism, and resonance of spin electronic[1].

Of these absolute methods the only one that today is used for the date of fabrics is 14C, since it is the best method of date for ancient textiles[2].

2. ANTECEDENTS

Since the first moment the E.D.I.C.E.S. there questioned the need to carry out a date of the Shroud of Oviedo with the maximum of the technical current guarantees, and for it as the first measure we put in contact with the Archaeological National Museum (M.A.N.) to obtain information on as the textile dates were realized at present, in the above mentioned Museum they sent us to the National Museum of Decorative Arts (M.N.A.D.) that in the last years they realized dates for the method of the radiocabon in Coptic fabrics with good results

The laboratory where the M.N.A.D. was sending their samples was Beta Analytic, Inc. of Miami, USA, the major world laboratory of date for radiocarbon with more than 120.000 samples reported of all kinds.

. In the Minutes of the I International Congress on the Shroud of Oviedo, in the presentation " Results of the evaluation of the observations and of the examinations on some captures carried out

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[1] V.M. FERNÁNDEZ MARTÍNEZ, *Teoría y método de la arqueología*, SINTESIS, Madrid, 2000.

[2] A. DE MOOR, *3500 years of textile art*, Ed. Lannoo, Tielt, 2008.

on the Shroud of Oviedo on May 24, 1985 and 7/8 of May, 1994 " of Pier Luigi Baima Bollone, Nello Balossino, Mario Moroni, Stefano Zaca[3] is exposed:

"In order to do the first attempt of check of the age of the Shroud with the method of the radiocarbon, one has consulted the Laboratory AMS of the University of Tucson's Arizona and the IsoTrace Radicarbon Laboratory of Toronto, Mario Moroni sent to both a fragment of the Shroud thought by Frei in 1979. To Tucson's Laboratoryit was sent a sample of 20,79 mg of weight, indicated with the abbreviation AA 6049. And to the Laboratory of Toronto, a sample of 14 mg of weight, classified with the number TO 2442.

Tucson communicated the result on October 31, 1990, and Toronto on September 11, 1991. The ages determined with the method of the Carbon 14 have turned out to be identical as it is possible to see in the following table:

DATE OF THE SAMPLE	*TUCSON*	*TORONTO*
Radiocarbon Date B.P.	*1292 + 53*	*1300 + 40*
Calendar Date 68% c.i.	*666-771 a.C.*	*666-724 a.C.*
Calendar Date 95% c.i.	*642-869 a.C.*	*653-786 a.C.*
Probability 100% cal. date *s 13 C*	*710 a.C.* *-25%*	*679 a.C.* *-25%*

The data are not of easy interpretation, due to the known difficulties of date of textile structures and of the concrete conditions of conservation of the sample, from the moment in which it was taken (1979), till when it come to us, some years after Frei's death, happened in 1983.

It is necessary to bear in mind in addition that the Shroud suffered the explosion of the Holy Chamber, which took place on October 11, 1934, and of they remain identifiable remains in the spectrum RX

The radiodate performed by us wants to be simply the stimulus to do more precise investigations that allow to confront the problem in the conditions more opportune and correct.

It has been done radiodate, but the obtained results do not offer sufficient guarantees"

In addition in the same presentation with reference to the captures realized by Max Frei Sulzer they indicate us: **" … but there are not known the conditions of conservation of the sample, part of the fact from which they came to us in one commercial envelope opened "**

According to certificate of Jodi Barnhill The University of Arizona[4], of October 31, 1990 sent to Valsecchi Giuseppina on the analysis of 14C in the sample of linen V6009, the radiocarbon age is 1292 +53 BP, with 1 σ = 666-771 A.D. and 2 σ = 642-869 A.D

The abbreviation AA6049 of the radiodate for Tucson indexed by Baima Bollone does not correspond with the numeration of the sample indicated in the certificate that I have, that is V6009, though it should be the same sample to the being identical the results given in it.

In the meeting supported between the Archbishop of Oviedo D. Carlos Osoro Sierra with the Director of the E.D.I.C.E.S. D. Guillermo Heras y D ª Maria Soledad Carretero on June 28, 2004, between the topics treated with regard to the date of the linen, which was a hanging question of

[3] Minutes of the I International Congress on the Shroud of Oviedo, Oviedo, 1994, pag. 415, 428-430
[4] Copy of the existing Certificate in my files.

resolving, the E.D.I.C.E.S. stay presenting in six months its offer and in one year it shoud take the corresponding decision

Proving Mr. Archbishop his acceptance and interest for our approaches. In addition he would organize for the following Easter a cycle of Conferences in Oviedo in which there would intervene the major possible number of members of the E.D.I.C.E.S. to announce the people of Oviedo and Asturias in general the results obtained up to the date on the Shroud of Oviedo.

At the end of November of 2.004 D. Angel Pandavenes Alonso takes possession as a new Dean of the Chapter of the Cathedral of Oviedo, declaring himself from this moment the major impeller of everything relating to the Holy Shroud of Oviedo

On March 31 of 2.005 in meeting supported in the Chapterhouse of the Offices of the Archbishopric of Oviedo between canons of the Chapter and members of the E.D.I.C.E.S., it is indicated in the point 7 of the Minutes raised on Date of the Shroud of Oviedo: " The Team of Research of Sindonology's Spanish Center (E.D.I.C.E.S.) esteem suitable to declare on the Date of the Shroud of Oviedo. For it it is going to study seriously the possibility of carrying out an analysis for the procedure of 14C, beside studying other aspects that they allow to identify the epoch to which the Linen belongs ".

Between September 11 and 14 of 2.006 we go to Turin: D. Ángel Pandavenes Alonso, D. Jose M. Rodriguez Almenar, D. Guillermo Heras Moreno, D ª M ª Sol Carretero, D. Philip Montero Ortego, D ª Pilar Marin Sepúlveda and D ª Esther Roa Cilla to put in knowledge of Sindonology's International Center of Turin (C.I.S.T.) our researches and the possibility of collaboration between both Centers.

3. CAPTURE OF THE SAMPLE

On November 17 of 2.006 at 11:00 one came to unstitch the Shroud of its fabric of support on the part of Pilar Marín. Taking a sample of the fabric of the Shroud in presence of the Dean of the Chapter. For it I firstly carried out a microscopic meticulous observation of the linen to find the place where less pollution existed on the fabric and in addition it supposed the minor degradation on the same one

The chosen place was the continuity of the existing step in the top part, next the tear, where previously it had taken some sample (Fig. 1 and 2)

Fig. 1.- Wrong said of the Shroud showing the zone where the sample took for the datación for 14C.

Fig. 2.- Extension of the zone of cut before carrying out it

The cut sample tape-worm an approximate dimensions of 35 x 5 mm, as it can be observed in the Fig. 3 and 4, being in use for the execution new clean and sterile material.

The whole process was recorded on video as silent witness, as well as also photographic digital captures of the zone were done before and after the cut. That were attached to the Minutes raised by the presents for it witness in the File of the Cathedral

Fig. 3.- Extension of the cut sample.

The cut sample got in an Eppendorf´s pipe with hermetic closing, and moved personally to the Engineering department and Science of the Materials of the Technical Top School of Industrial Engineers (U.P.M.), where I carried out its weighed one with a result of 0,0357 g, proceeding again to a meticulous microscopic observation of the sample to 400 increases before doing its division in two parts (Fig. 5). Although in the edge of one of its ends it was possessing remains of oxide of iron of the nails used in the subjection of the fabrics support. This observation revealed not appraisal of biofilms, incorporations, strange threads, etc. on the fibers of the threads.

I have to indicate that at all time the sample of the Shroud was guarded by my person up to its sending to BETA ANALYTIC for its date, so that the chain of custody was not breaking

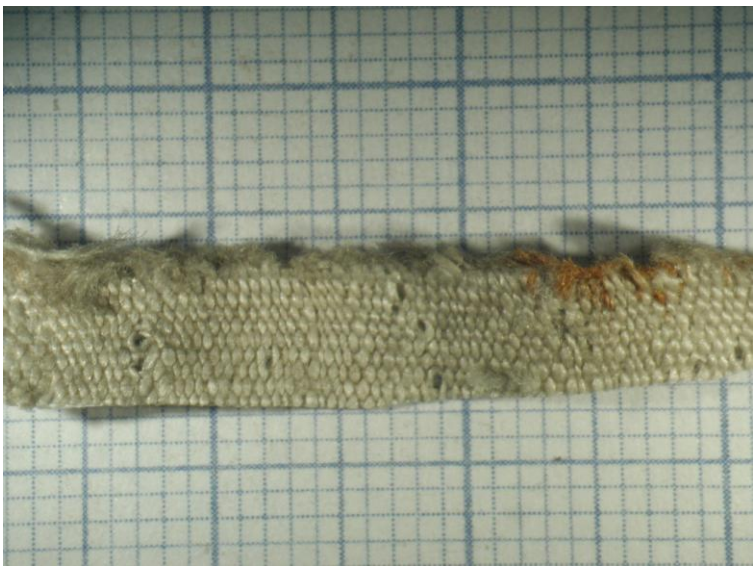

Fig. 4.- Cut sample of the Shroud 35 x 5 mm.

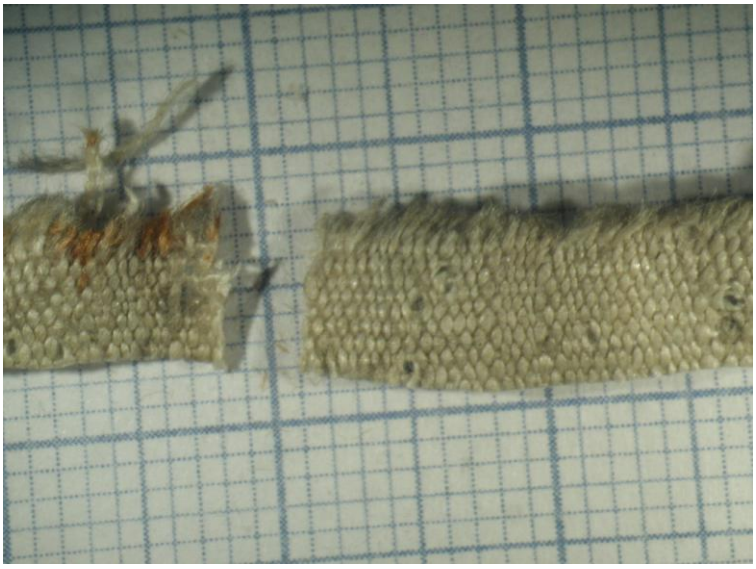

Fig. 5.- The cut sample once divided to eliminate the zone contaminated by oxides of iron.

After the cut, both resultant chunks had a few approximate dimensions of 21 x 5 mm and 13 x 5 mm, and weight of 0,0228 g and 0,0125 g respectively (Fig. 6 and 7).

I introduced her again the sample to dating in a new Eppendorf´s pipe with hermetic closing and I record externally with the name SO-1711, to remember the day and month in which it was taken.

The rest of the sample stayed in my power, guarding it with the remains of threads of the Shroud that I preserve, up to the moment that is necessary its restitution to the Cathedral of Oviedo.

Fig. 6.- Sample sent to BETA for the date.

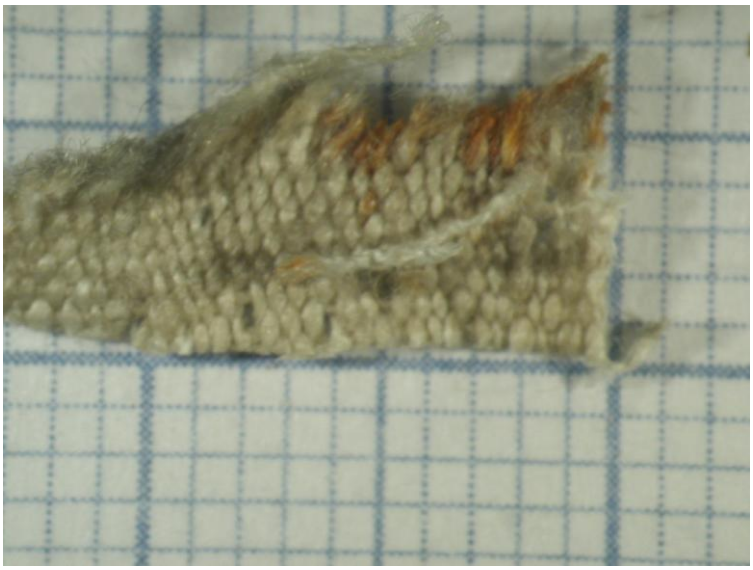

Fig. 7.- Zone eliminated by the contamination of oxides of iron..

Close to the sample of the Shroud (SO-1711) four more samples of fabrics were sent for its date by 14C. One proceeding from my file and three facilitated ones for the National Museum of Decorative Arts. They all were sent in the same conditions from the Technical Top School of Industrial Engineers (UPM) in order that it was not possible to know the origin of the same ones.

4. RESULTS

The samples were sent to BETA ANALYTIC 03-01-2007 by valise UPS, and on February 13 of 2.007 there is received the report of BETA ANALYTIC with the results obtained of the date of the Shroud (Fig. 8).

Sample Data	Radiocarbon Age messured	Rate 13C/12C	Conventional Radiocarbon Age (*)
Beta-225639	1240 ± 50 BP	-25,2 0/00	1240 ± 50 BP
SAMPLE: SO-1711			
ANALYSIS: AMS-Standard Delivery			
MATERIAL/PRETREATMENT: (textile): acid / alkali / acid / cellulose extraction			
2 SIGMA CALIBRATION: Cal AD 660 a 890 (Cal BP 1280 a 1060)			

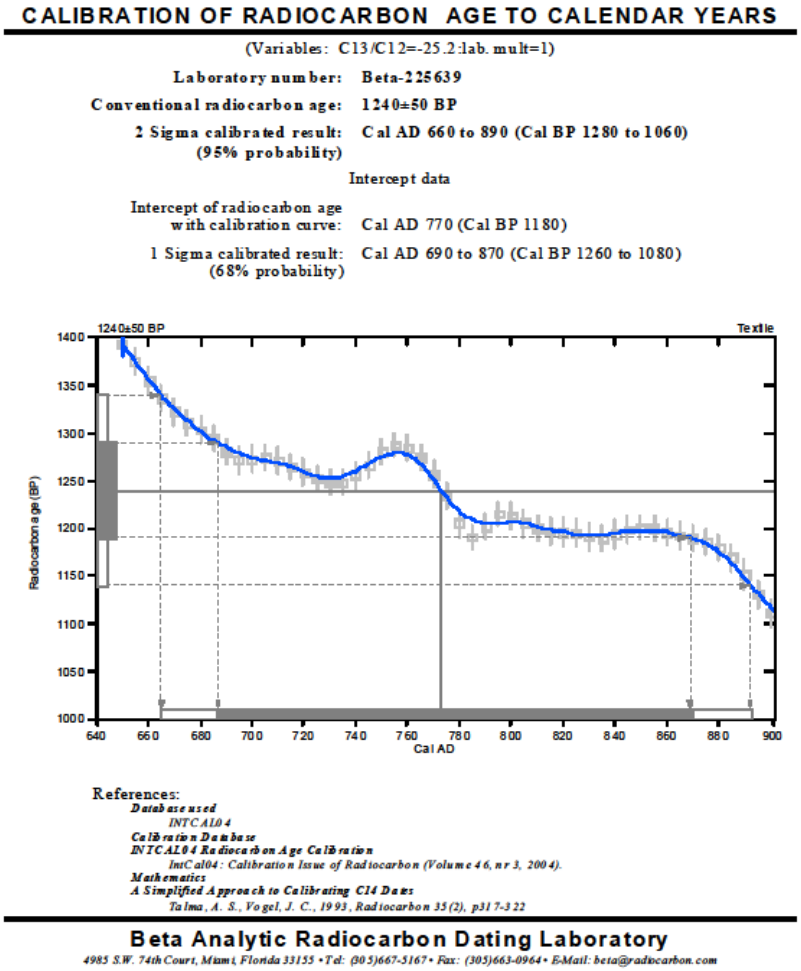

Fig. 8.- Copy of the Report of BETA ANALYTIC with the results obtained in the date by 14C of the Shroud of Oviedo

On 13-04-2007 I presented in my second communication to the International Congress II on the Shroud of Oviedo (" *Other studies of chemical and biological character: Date of the Linen* ") these results obtained in the radiodate. The E.D.I.C.E.S. agreed with the presentation of this result, in spite of that we knew that not all the attendees were going to understand and accept the obtained result. Since already the experience was had by the results of the date of the Sindone of Turin given

in 1988, since it is difficult to accept a few analytical results that do not adjust to the personal desires of the people, but a scientist always has to exhibit and to communicate the information that he obtains in a essay and later to find justification to the same ones if these do not agree with waited before the beginning.

From the same moment I propose and promise to myself to try to determine the reasons that could have influenced the date increasing the concentration of 14C present, and that therefore must have been a contamination of organic origin. That is to say, with a compound of Carbon more modern that it had made increase the original concentration of 14C, and this carbon must be been combined chemically by the cellulose of the linen not being eliminated in the pretreatment carried out before the analysis of 14C and that therefore would have buckled the date. Lookiing for it organic radical causers of this contamination (oil, aloe, storaque, etc.). Due to the nearness of the dates of the Congress II (April 2.007) an analysis was done by Infrared Microscopy by aim of Total Attenuated Reflectance (ATR) by crystal of Germanium connected to a spectrophotometer, seeing that in the current clean linen existed a band of tension to 1750-1690 cm 1 of (=C=O and one shoulder to 1650 cm 1. But the Shroud did not have this first peak and there was more marked the second one (Fig. 9). Dr. Gary Elis that realized the analysis commented to me that both peaks in the current linen can be due to a contamination with fats of the hands.

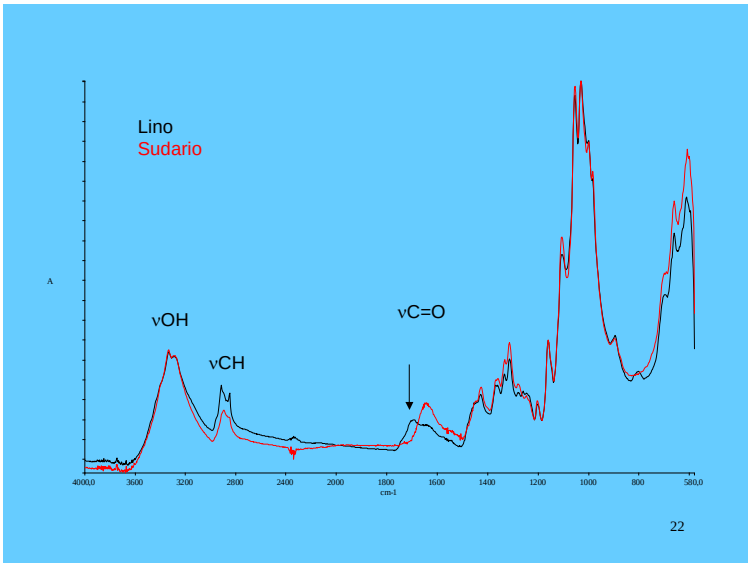

Fig. 9.- IR Spectra of fabric of current linen and Shroud by means of Total Attenuated Reflectance.

Due to the indications received from the M.N.A.D. of the results obtained in the date by 14C of some fabrics on having been cleaned by petroleum oil and the difficulty of these hydrocarbons being eliminated, since also it happens with oily and lipid products that are combined by the cellulose of the linen, I proceeded to carry out a series of chemical essays to try to find a reason that was justifying the diversion obtained in the date of the Shroud.

Firstly one carried out infrared analysis with comparisons of the Shroud and fabrics of linen with several contaminant treatments (clean, stained with olive oil, with this one more aloe - mirra-incienso), the band typical of the oil not being detected fundamentally to 1744 cm 1 (Fig. 10), not the presence of none of these elements in the same one

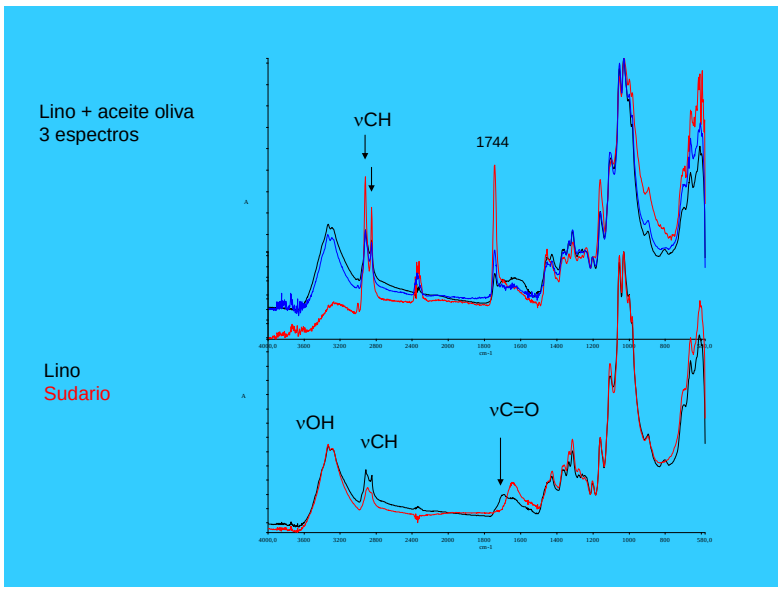

Fig. 10.- IR Spectra comparatives of linens spotted with olive oil.

Since it was had in mention initially like the most feasible a possible contamination by oils (the first sacred unction fulfilled in Toledo to the king Wamba 20-10-672) I asked again to determine the possible presence of hydrocarbons, terpens and oil acids in the linen of the Shroud (19-12-2007) by means of chromatography in liquid phase (HPLC) with toluene as non polar adsorbent.

Fig. 11.- Royal unction, Antiphonal León's Cathedral.

For it I sent two small chunks of thread of 5 and 3 mm (Fig. 12), detached with the chunk cut initially for the date (Fig. 7), to realize in them this chromatography. The obtained results were that it exist traces of oil acids in practically null quantities. It is necessary to return to notice that from the first moment in the analytical studies realized on the Shroud of Oviedo we have had to bear in mind the characteristics of the linen and its nature. For what the size of all the analyzed samples they have been microscopic, implying always a few analytical very careful and precise methodologies, since a mistake could lead us to not being able to repeat the tests. But at the same time these sizes have limited the technologies and results, not being able to use others that might offer us better others.

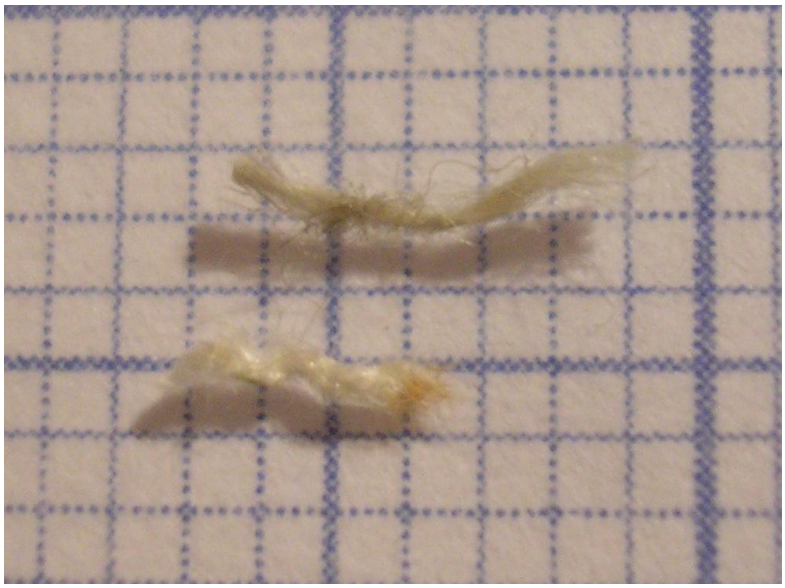

Fig. 12.- Chunks of thread of the Shroud sent for chromatographic analysis.

Before these results one advised us to carry out an analysis with pyrolysis / gas chromatography / mass spectrometry (Py-GC-MS), precise and advisable technology to determine organic compounds, macromolecules and polymers. After multitude of consultations I managed to find the one who might realize the above mentioned essays to low costs and it was the Institute Natural Resources and Agrobiology (I.R.N.A.S.) of Seville. Since always all the works realized in the Shroud of Oviedo on the part of the E.D.I.C.E.S. have been altruistically, and these essays would suppose a cost of 1.500 € + VAT according to the budget given by Analytical Engineering.

Put personally in contact with the I.R.N.A.S., they advised me that we should do a series of not destructive analyses to see the possible pollution of the linen before burning the sample and to lose it. Totally in agreement with this exposition 22-10-2008 I went to the I.R.N.A.S. of Seville delivered them in hand a sample of the Shroud of 7 x 5 mm and other one of linen boss L-54 facilitated by the Institute of Textile Research and Industrial Cooperation (U.P.C.) proceeding from Testfabriscs, Inc. (USA), which was the fabric of the received ones that more is alike the linen of the Shroud, in order that they were doing the preliminary essays that they believed opportune before realizing that of Py/GC/MS (Fig. 13).

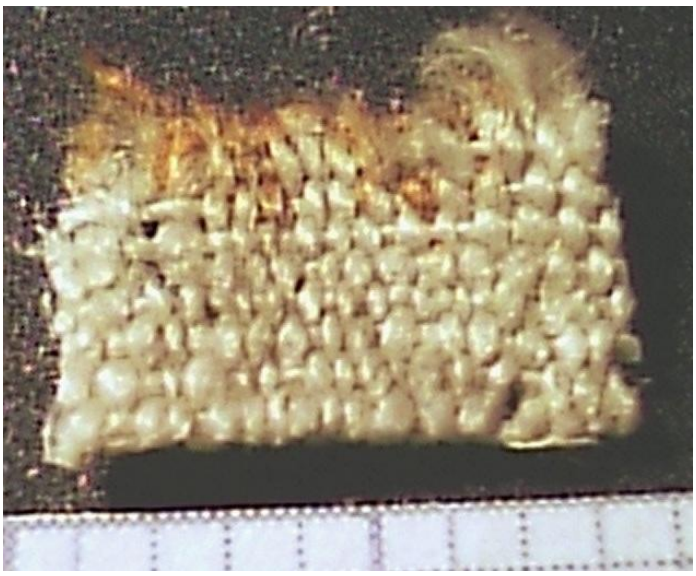

Fig. 13.- Initial State of the sample of the Shroud delivered to the IRNAS

The sample was brought to Madrid to the Institute of Structure of the Matter (CSIC) where a FT Raman and a FT IR is carried out with a surprising result. They do not find any rest of oily acids and the only pollution is due to **amorphous carbon** as can observe in the graphs of the Fig. 14 and 15 with typical peaks to 1360 cm^{-1} and 1590 cm^{-1}.

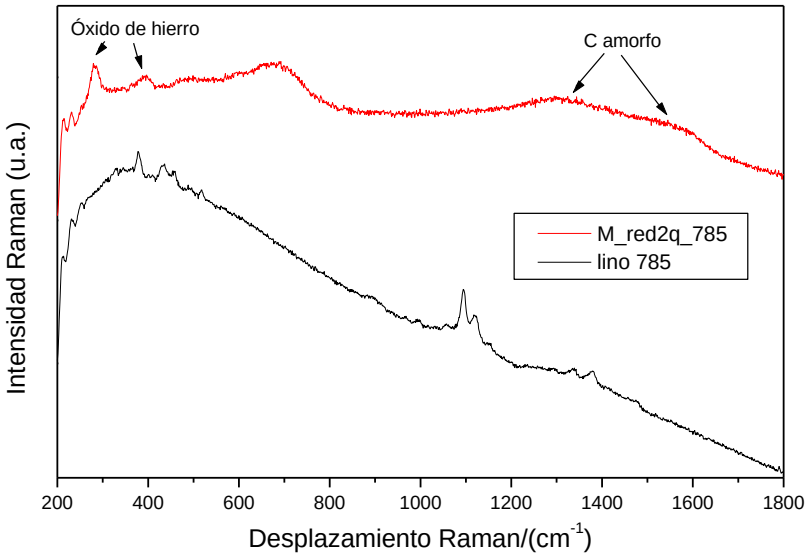

Fig. 14.- Excited micro-Raman Spectrum to 785 nm

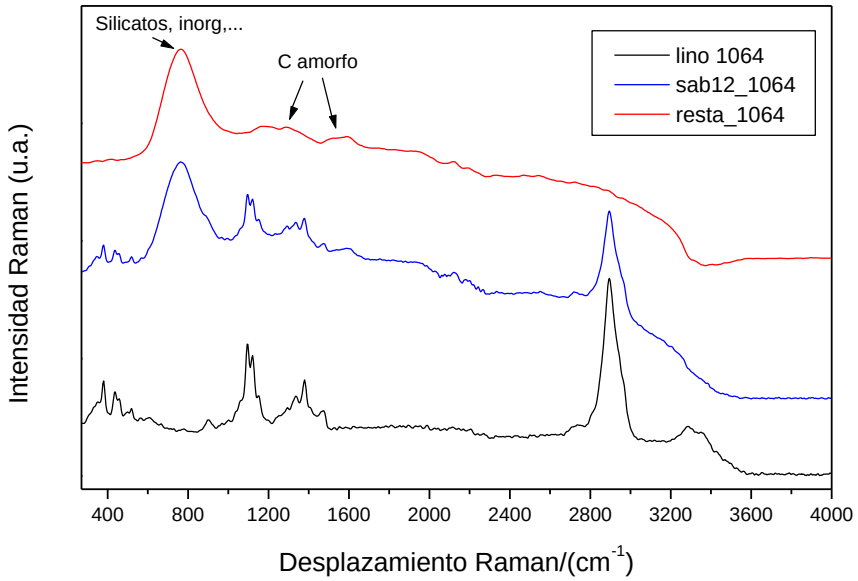

Fig. 15.- Excited micro-Raman Spectrum to 1064 nm (sab = Shroud).

On having returned the sample used in the accomplishment of this analysis to me, I verify that it is stained in its edges by remains of a black substance, that according to the information of the Dr. Domingo it owed to the adhesive used for to hold the sample in the equipment (Fig. 16)

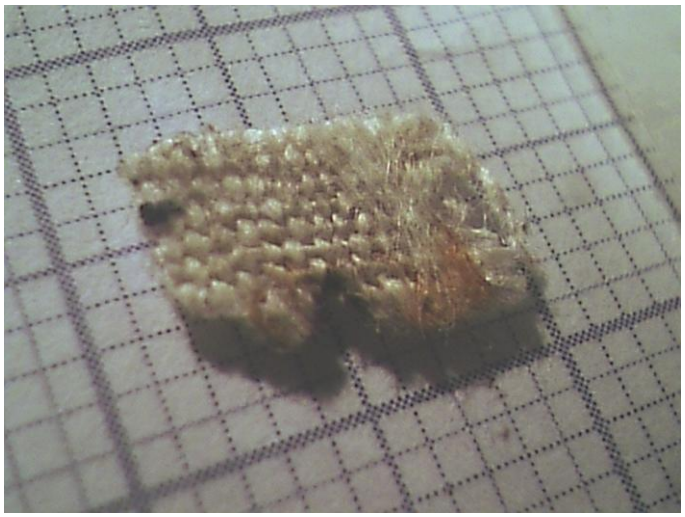

Fig. 16.- State of the sample after realizing FT Raman assays.

Trying to recover the sample, since it was that of major size of the Shroud that I had in my power, I observed microscopically that besides this one pollution was existed by some threads with a number of fibers that were spotted with a film of black color (Fig. 17). The aspect of these spots they were totally different, so much from the fibers that had been burned by the beam laser, as the spotted ones with oxide of iron (Fig. 18), as that they were possessing remains of the adhesive (Fig. 19).

Fig. 17.- Fibers spotted with black color of the FT Raman assay.

Fig. 18.- Fibers carbonized by the beam laser close to stained with oxides of iron.

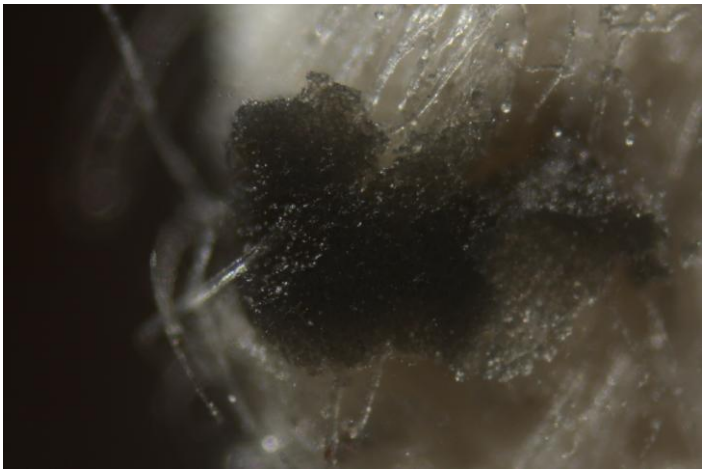

Fig. 19.- Adhesive used in the subjection of the sample to do the FT Raman assay.

In view of the results of these observations, I put to examine some of the samples of small chunks of threads of the Shroud that I had guarded. In all of them also one were appreciating black fibers of similar morphology and color as the existing ones of the Raman assay.

Fig. 20.- Black fibers in a chunk of thread of the Shroud taken in 1.990

Repeated this observation in the rest of guarded samples, all they revealed the existence of the above mentioned black fibers. Some detached of the threads (Fig. 20) and others forming spots in areas isolated of the same ones (Fig. 21).

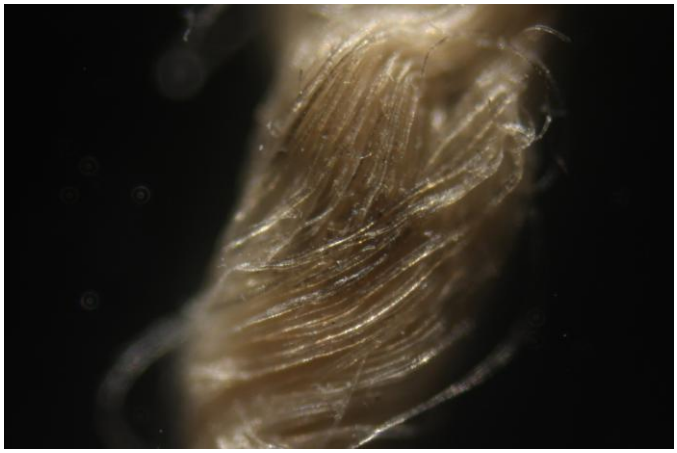

Fig. 21.- Spot isolated of black fibers in a chunk of thread of the Shroud.

To try to see the morphology of the external surface of these contaminated fibers, there were realized a series of microscopic observations in an Electronic Microscope of Environmental Scan to low vacum. So with retrodispersed electrons (BSED) produced by elastic collisions between the electrons of the beam and the nucleus of the atoms of the sample, as with secondary electrons (LFD) produced by inelastic collisions between the electrons of the bundle with the electrons of the sample (ideal for the formation of images.

Under optical stereoscopic microscope I prepared a support with three sets of fibers: Clean, black and with oxides of iron (Fig. 22). The clean fibers show a smooth surface alone where are observed the dislocations or nodules of growth of the fiber (Fig. 23, 24 and 25). The stained with oxides of iron fibers present a surface covered homogeneously with a film, which in FLD principally, is alike a dent (Fig. 26, 27); whereas the black fibers have a not so marked surface, with a few discontinuous coverings (Fig. 28, 29).

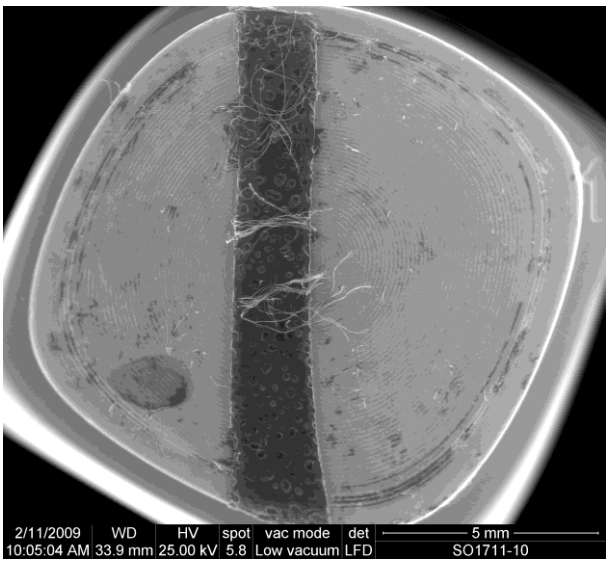

Fig. 22.- Sample holder of aluminium with the three sets of fibers

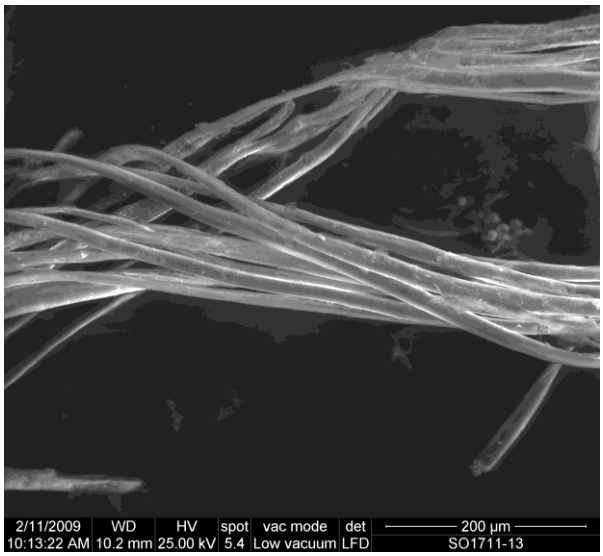

Fig. 23.- Set of cleaned fibers with secondary electrons

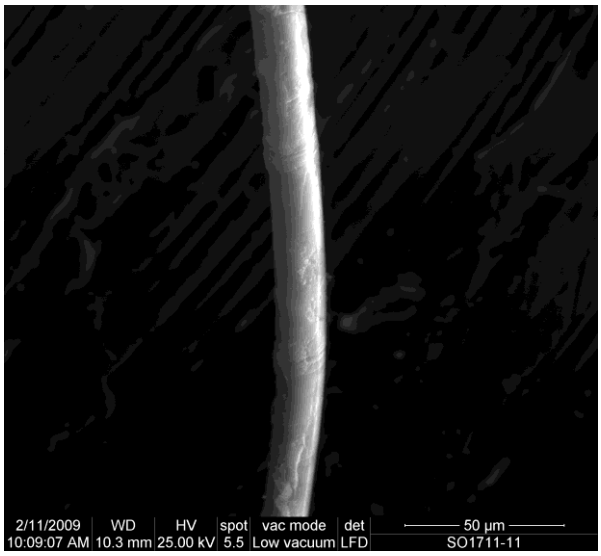

Fig. 24.- Image of cleaned fibers with secondary electrons (LFD)

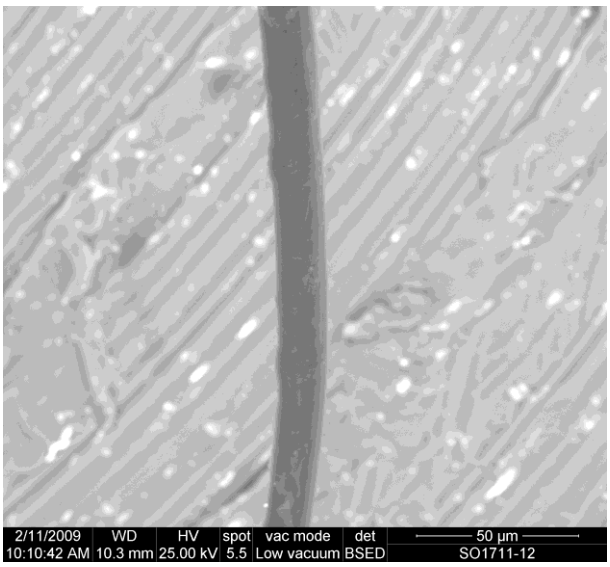

Fig. 25.- Image of cleaned fibers with retrodispersed electrons (BSED).

Fig. 26.- Image of fibers spotted with oxide of iron with LFD

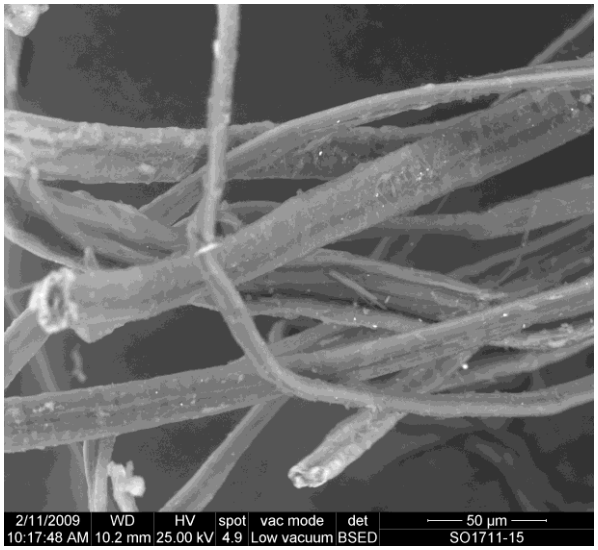

Fig. 27.- Image of fibers spotted with oxide of iron with BSED.

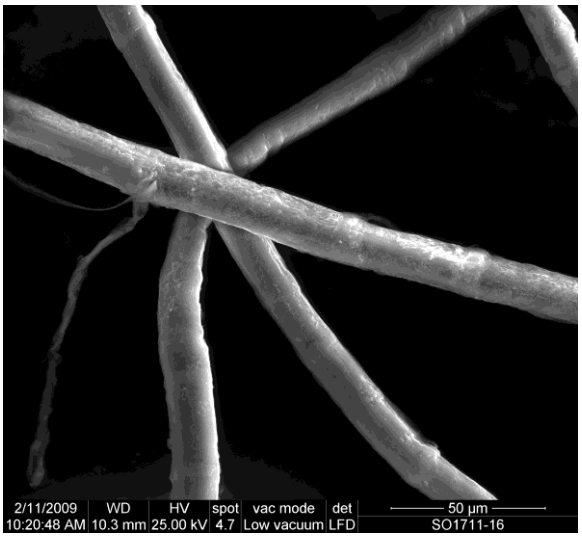

Fig. 28.- Image of black fibers with LFD

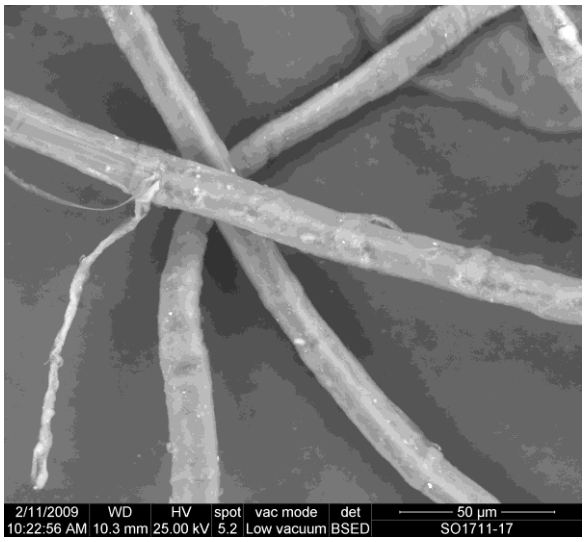

Fig. 29.- Image of black fibers with BSED.

At the same time as the microscopic observations, microanalyses were carried out by sonde of Dispersive Energy of X-rays (EDS), finding similar spectra in both types of fibers: clean and black, with only peaks of Carbon, Oxygen and Aluminium (Fig. 30, 31). Whereas in the fibers spotted with oxides of iron they are detected: Carbon, Oxygen, Aluminium, Sulphur, Chlorine, Calcium and Iron (Fig. 32). In three cases, the presence of Aluminium is due to the support of the samples.

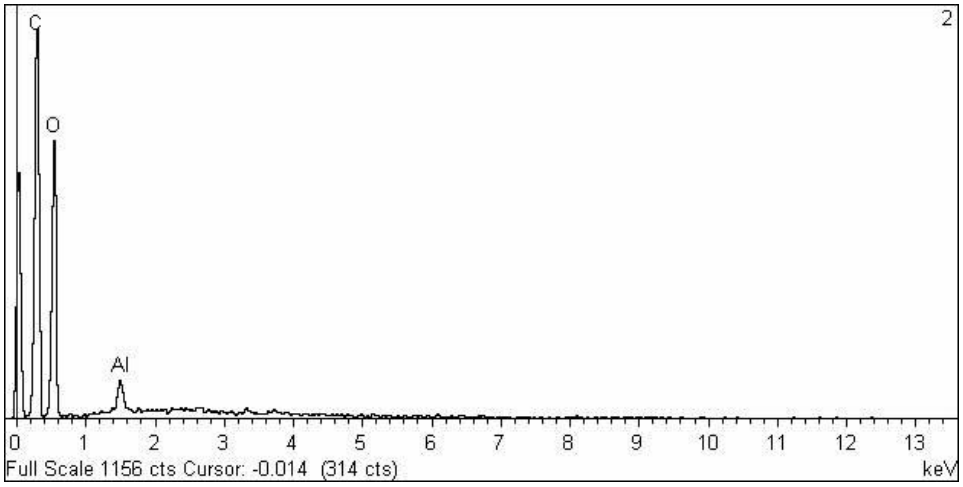

Fig. 31.- EDS Spectrum of cleaned fibers.

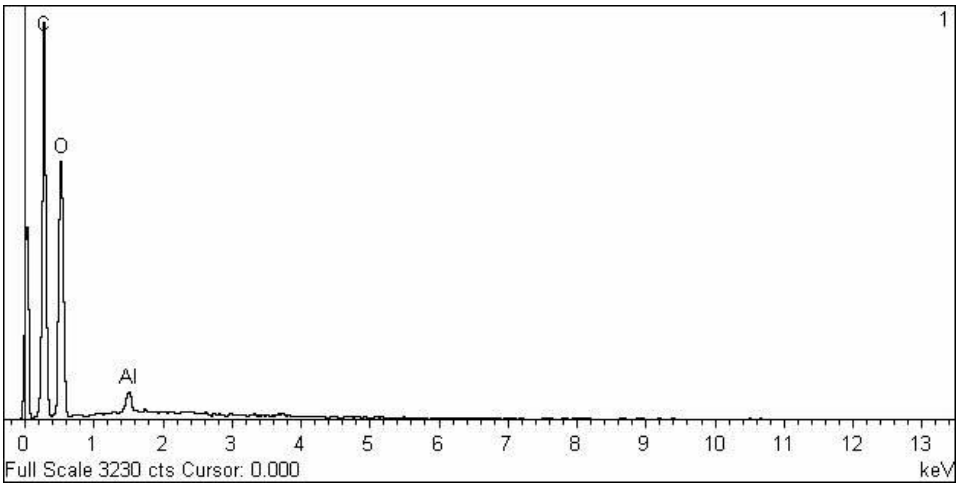

Fig. 32.- EDS Spectrum of black fibers.

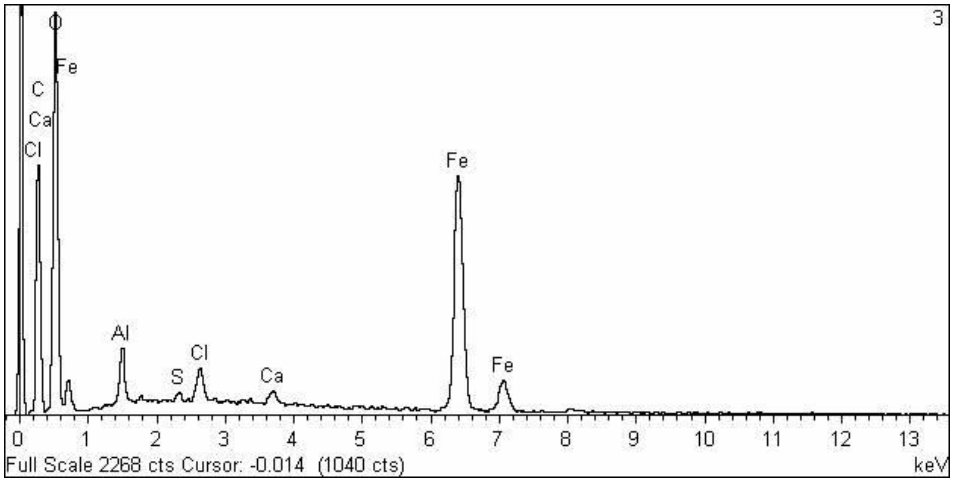

Fig. 33.- EDS Spectrum of fibers with oxides of iron.

These results seeming to be coincidental with the obtained ones in the analyses FT Raman. Only it was appreciating the presence of Carbon and Oxygen, then this film of black color that covers some zones of the fibers had to have an organic origin that does not differ from those of the clean linen. To perform a checking of this information I carry out again a microanalysis FT Raman, but this time instead of using a surface of linen that we did not have, we carry out it on a hank of black fibers separated by me individually of chunks of threads stored (Fig. 33)

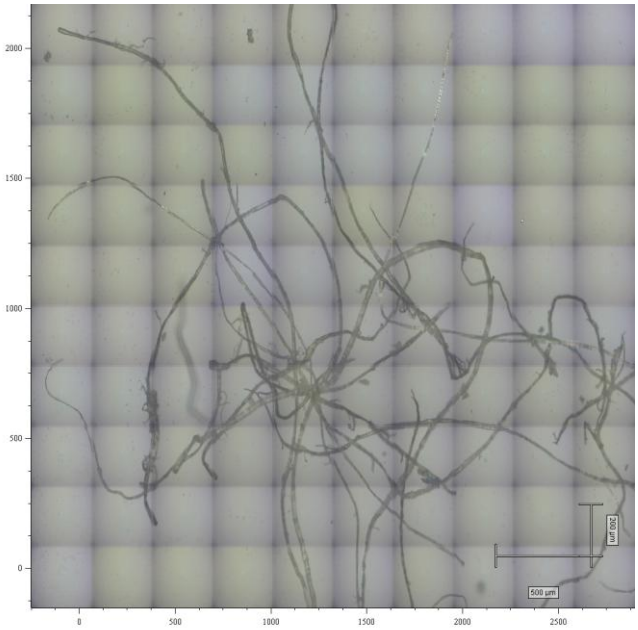

Fig. 33.- Hank of black fibers used in the Raman analysis.

I have to indicate that when these black fibers are extracted, the contamination only is partially in the length of the fiber, remaining clean in rest (Fig. 34 and 35). Being in addition the black zone much more fragile that the clean one, breaking with facility in its extraction of the thread with help of a few tweezers, due undoubtedly to the change in the structure of the cellulose of the linen.

Fig. 34.- Black fiber showing a black zone and the clean rest.

Fig. 35.- Detail of the black end of the previous fiber

The spectra FT Raman obtained show that the clean zones of the fibers only have the typical peaks of the cellulose of the linen (Fig. 36). Whereas the zones with the black contamination return to indicate that this one is due to an **amorphous carbon** (Fig. 37 and 38).

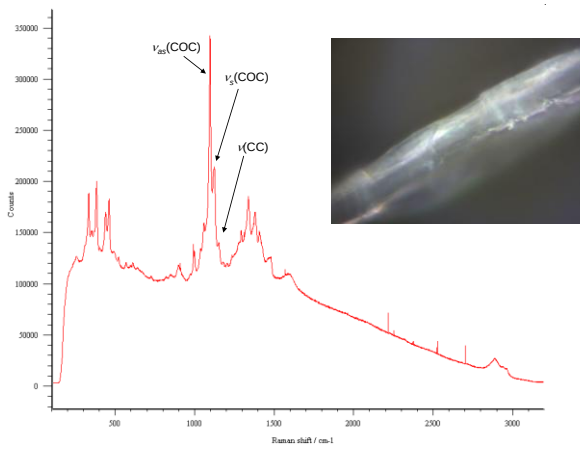

Fig. 36.- Spectrum FT Raman of the clean zone of a fiber

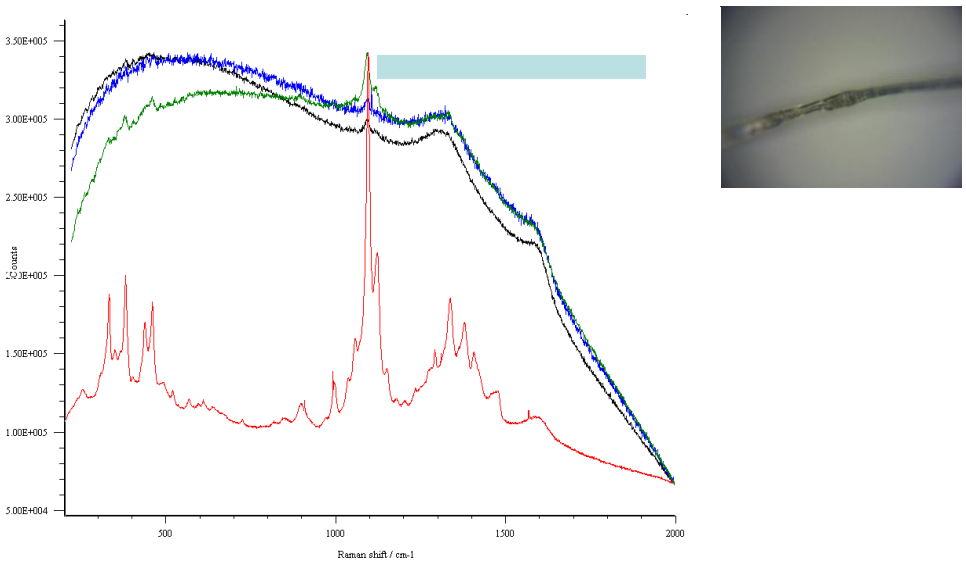

Fig. 37.- Spectra FT Raman of a black zone of a fiber on the clean one.

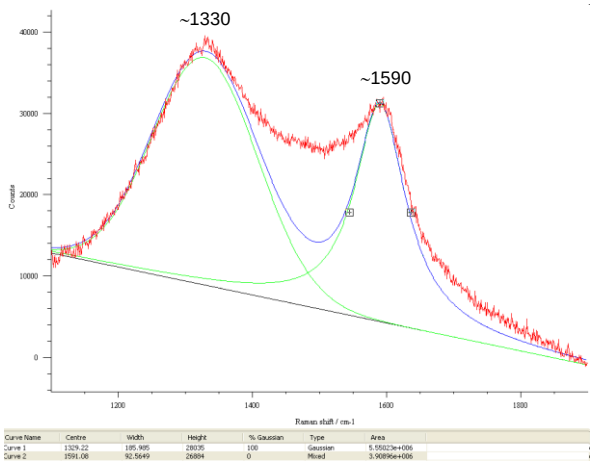

Fig. 38.- Mathematical treatment of the top spectrum of the Fig. 38 showing the typical peaks of the amorphous carbon to 1330 and 1590 cm-1

Come to this point, I wonder as such an energetic pretratamiento (acid / alkali / acid / cellulose extraction) carried out by BETA ANALYTIC, according to its Report, to the sample of the Shroud before the date, it has not eliminated this contamination. For it I request to BETA ANALYTIC that was sending the exact conditions in which there was treated the sample SO-1711 before the date. Answering that these were:

1. Dispersion in purified water .
2. Washing with HCl 0,1 N - 30 min - 80 ºC to eliminate carbonates.
3. Washing up to neutralization
4. Washing with NaOH 2 % - 2 h - 80 ºC to eliminate organic secondary acids
5. Washing for neutralization
6. Washing with HCl 0,1 N - 30 min - 80 ºC to neutralize.
7. Washing up to neutralization.
8. Wash with $NaClO_2$ (saturated sodium chlorite) to pH = 3 and 70 ºC. It eliminates all the components except the cellulose of the wood. Useful for very old or very contaminated wood
9. Washing up to neutralization.
10. Drying to 105 ºC during 12 h.

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I return to extract a few black fibers and using a MICROCON®, device for filtration by centrifugation, used to concentrate and to desalt macromolecular solutions like proteins, antibodies and nucleic acids up to 100.000 NMWL (Nominal Molecular Weight Limit), I submit to the black fibers to the same procedure used by BETA ANALYTIC (Fig. 39). On having finished the essay I verify that there are not even dissolved the fibers (Fig. 40), even have not lost its black contamination. Then if this black contamination is produced by an amorphous carbon, and the micro FT Raman spectroscopy has not detected molecular compositions, its origin must be an organic matter and on not having been eliminated in the previous treatment it can be the reason of increasing the original concentration in ^{14}C of the sample and therefore to have rejuvenated it.

To this we must add that according to the information facilitated by BETA ANALYTIC, after the previous treatment they have had 58,4 % of loss in the weight of the sample. If the black fibers do not dissolve in the same one, its contamination could have had a strong incident in the result of the date..

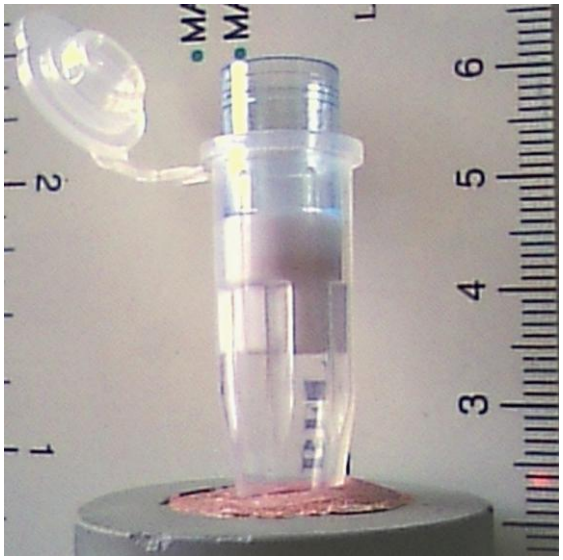


Fig. 40,- MICROCON device of filtration.

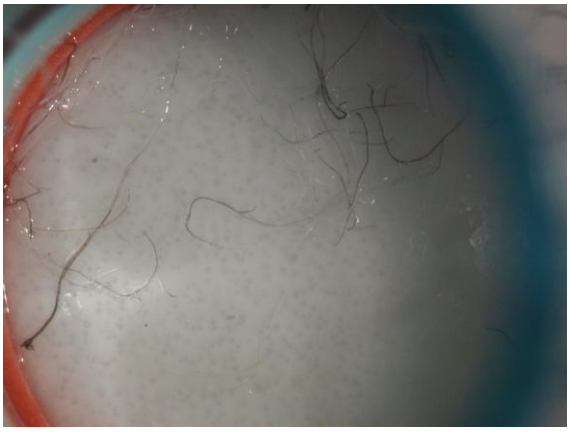


Fig. 41.- Black fibers after the previous treatment used by BETA ANAYTIC.

Then, which is the origin of this contamination? I think that it can alone have two origins:

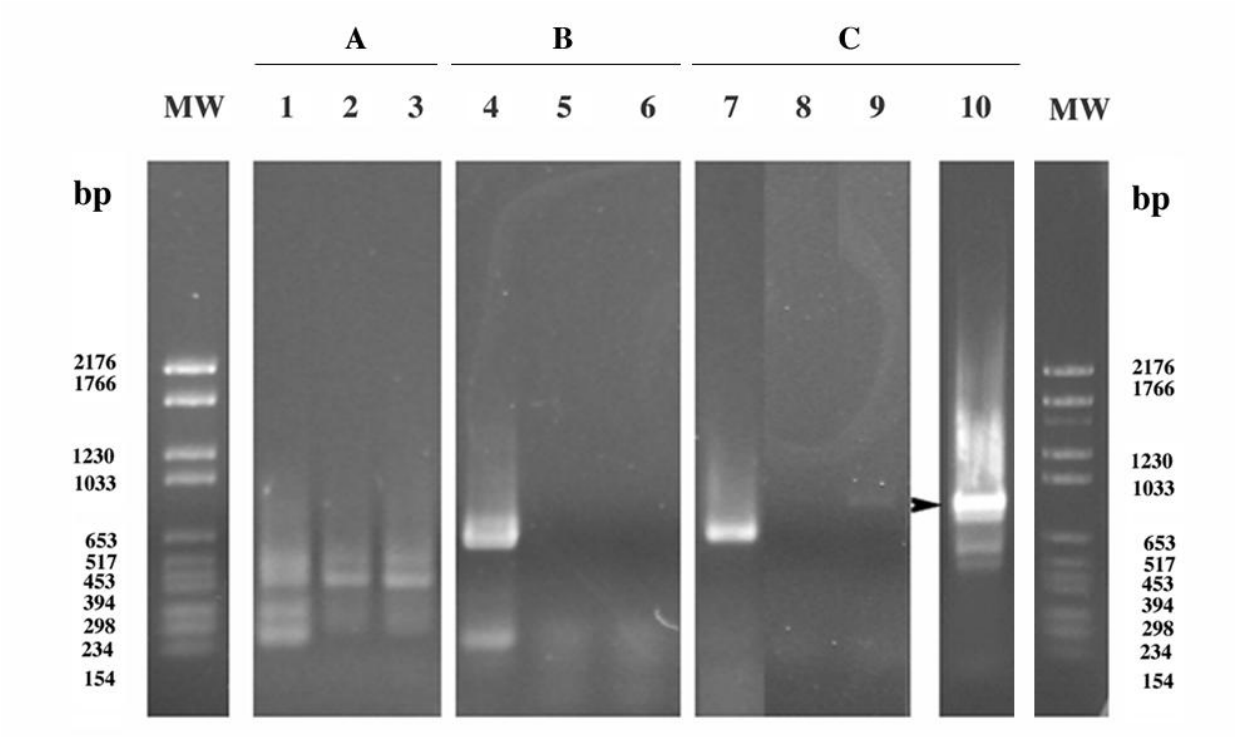
a) A biofilm of fungi and / or bacteria.

b) Environmental products of combustion

For the checking of the first point, microbiological analyses were realized with inoculates of black fibers, firstly in means of solid traditional standardized cultures for bacteria (TSA) to 30 ºC and for fungi (PDA) to 28 ºC. Its results were of negative growths after 60 days of incubation. This result only indicates us that microorganisms have not grown or because they are dead or because they are not viable in the means and / or conditions used. But we must not forget that normally there is major the quantity of microbiological genres that do not grow in the traditional means of culture that we use, that those who grow in them.

In spite of the difficulty that supposes trying to analyze a biological sample of possibly 2000 years of antiquity and that has been contaminated by the contact by many persons and environments, samples were sent to centers specialized in determinations of molecular biology of the DNA that have reported the following results:

1. There are no remains of bacterial DNA corresponding to ribosomic operons of sizes bigger to 800 pb.
2. The presence of bacterial DNA in the water prevents the detection of bacteria in samples in which there are no remains of DNA of bigger sizes to 800 pb.
3. One have not could amplified bands of DNA by one of the universal pairs of primers used to amplify 5.8S-ITS of fungi and yeasts.
4. A band of DNA has been amplified in minimal quantities by a pair of primers designed to amplify 5.8-ITS of plants.
5. This latter band have could be re-amplified to sequenced,
6. The sequence obtained (332 nt) corresponds with 100 % of identity to the mushroom dermatofite *Malassezia furfur* that is responsible of seborrheic dermatitis in the man.
7. The remains of DNA amplified come from contamination of human origin.



Results of DNA's amplification using different pairs of primers:
A: Using universal primers that amplify the intergeneic region placed between the ribosomic genes 16S and 23S in bacteria (ITS): 1: Negative control of MilliQ sterile water, 2: Dark fibers of the Shroud, 3: White fibers of the Shroud. Bands were not obtained using the primers that allow to amplify the ribosomic gene 16S..
B: Using universal primers that amplify the intergeneic fragment placed between the genes 18S and 23S in fungi (5.8S-ITS): 4: Positive control, 5: Dark fibers of the Shroud, 6: White fibers of the Shroud
C: Using universal primers that amplify the intergeneic fragment placed between the genes 18S y 23S in plants (5.8S-ITS): 7: Positive control, 8: Dark fibers of the Shroud, 9: White fibers of the Shroud, 10: Result of the reamplification of the band of present DNA in the rail 9.

In the presence of these negative results, we try to verify if the contamination that was provoking the black fibers was of environmental origin for combustions. For it I prepare again a support with black and clean fibers for its comparison for observation in SEM and microanalysis for EDS.

The placement of the fibers in the support, partially to the air, impeded the observation and analysis of the same ones (Fig. 42). Nevertheless, it is estimated as the black spots they do not have a homogeneous and constant film in the whole surface (Fig. 43).

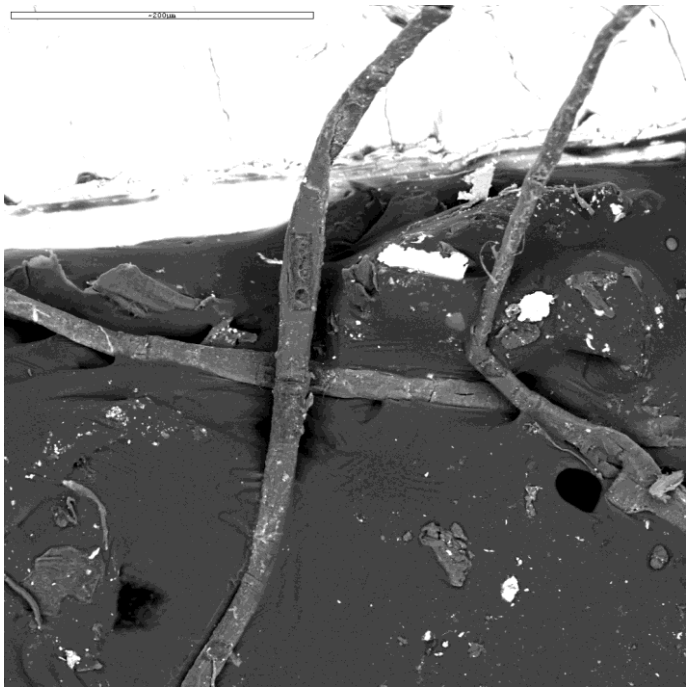


Fig. 42.- Placement of the black fibers in the support of the SEM.

Fig. 43.- Superficial aspect of the black fibers

Before the problems arisen in the observation and analysis of the previous sample, I return to prepare a new support with a different subjection with both types of fibers: clean and black. (Fig. 44 to 48).

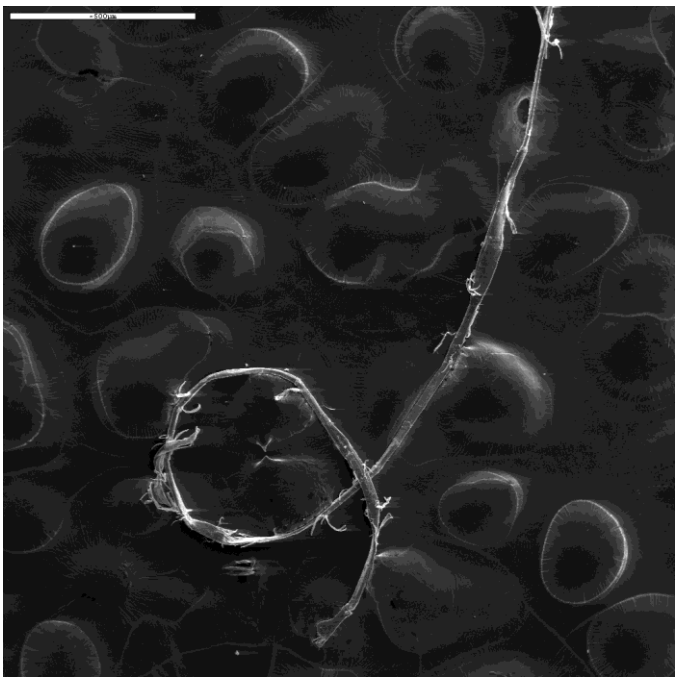


Fig. 44.- One of the black fibers showing its exterior degradation.

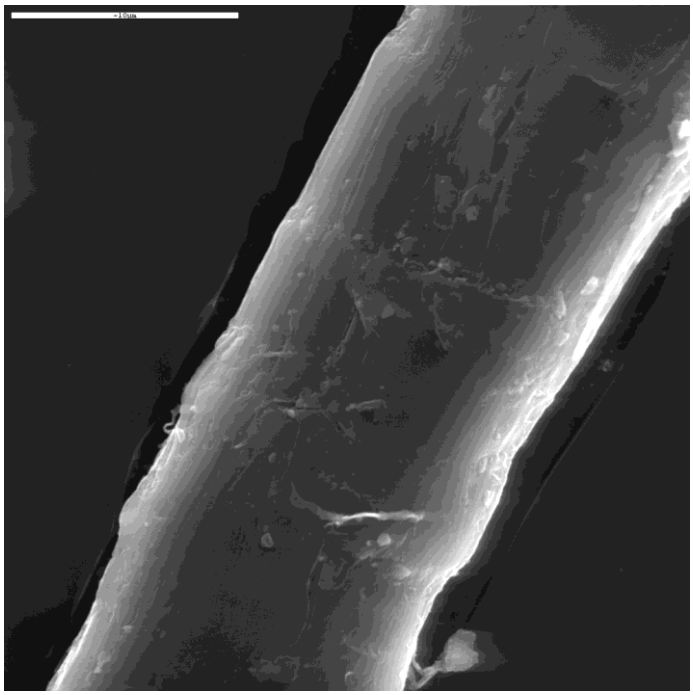


Fig. 45.- One of the clean fibers showing its smooth and uniform exterior

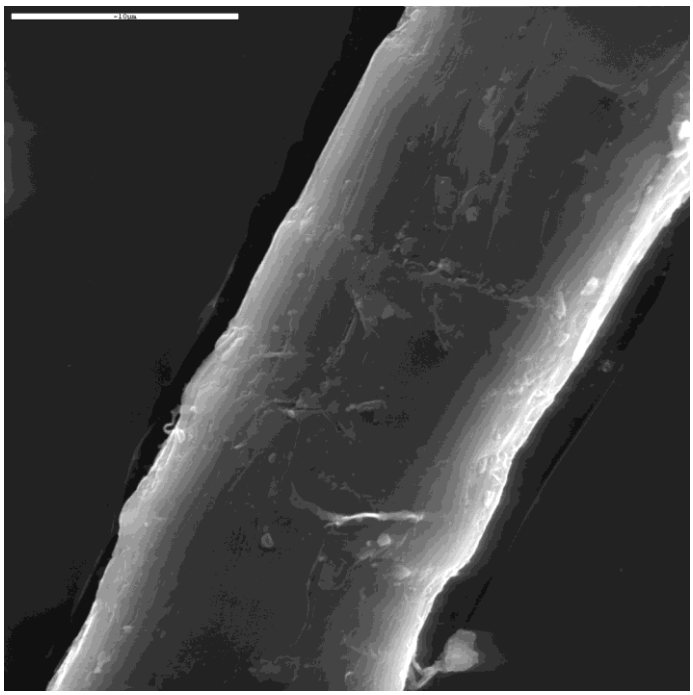


Fig. 46.- Other one of the clean fibers mounted in its carries, of aspect similar to that of the Fig. 45.

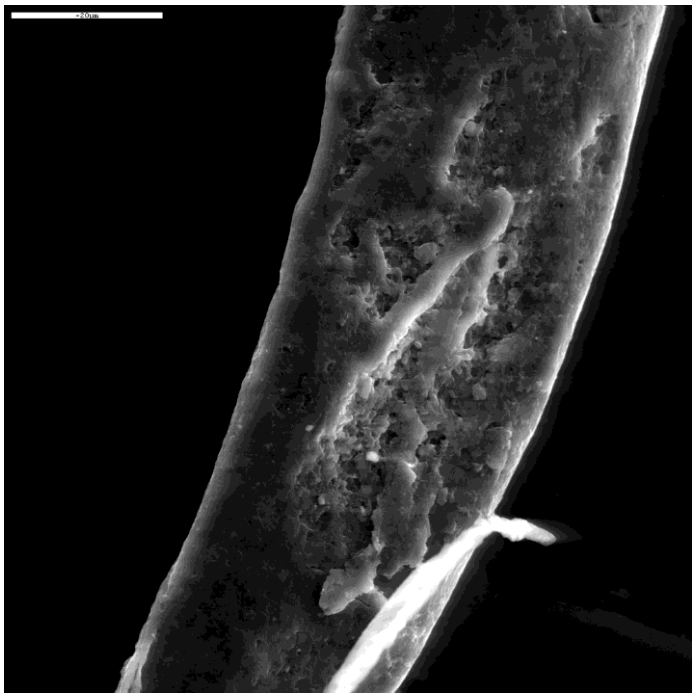


Fig. 47.- Black fiber showing as this is covered with a film that has destroyed the surface of the fiber.

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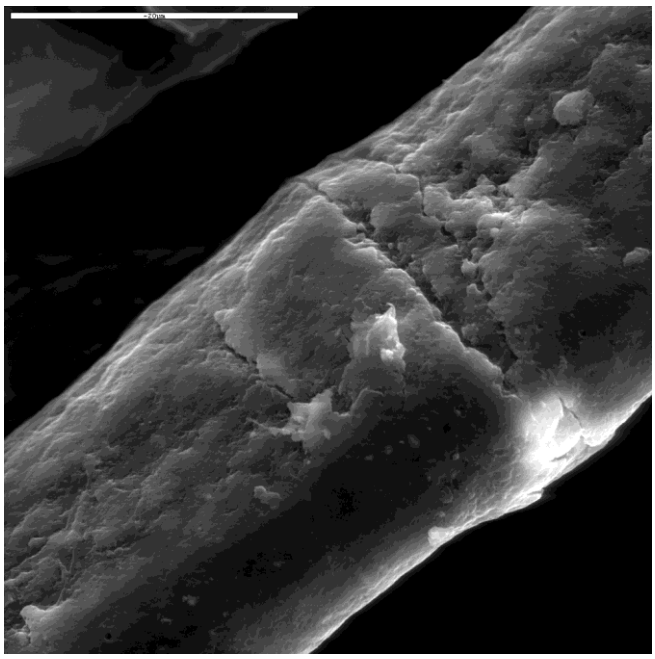

Fig. 48.- Black fiber showing its superficial covering with a divided nodule

The microanalyses realized by EDS indicate the presence of the majority elements like present in the following Table 1:

EDS SHROUD FIBERS														
	BLACK													CLEAN
	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Na	--	--	--	--	--	--	--	--	--	16,9	12,8	6,55	12	27
Al	9,46	14,33	9,00	39,32	12,88	9,01	16,03	10,92	8,37	6,24	7,81	8,55	5,83	2,34*
Si	11,24	4,63*	3,45*	8,17	13,64	3,66	32,54	24,94	16,15	12,75	13,20	17,30	13,97	15,48
P	14,21	9,30	18,87	14,14	8,86	24,43	6,71*	2,33*	1,55*	*	0,31*	2,33	1,43*	*
S	7,46	12,97	4,74*	4,86	13,17	4,32	8,32	10,43	8,21	7,59	5,20	5,26	6,36	2,39*
Cl	8,14	10,55	12,75	2,46	14,98	5,96	--	--	--	--	--	--	--	17,3
K	5,6	7,82	2,53*	2,23	5,78	3,08	--	--	--	--	--	--	--	--
Ca	40,69	27,29	38,4	22,83	22,6	44,34	36,41	19,68	19,63	15,78	21,51	21,90	23,57	20,28
Fe	--	--	--	3,62	--	--	--	16,7	12,00	7,57	9,27	15,20	16,7	1,30*
Cu	3,20*	13,13*	10,26*	2,38*	8,09	5,21*	--	14,98*	15,2	15,9	25,6	17,25	9,10*	10,55*
Ag	--	--	--	--	--	--	--	--	18,8	17,4	4,36*	5,67*	11,1	3,45*
	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0

Table 1: The first six columns were obtained of the first preparation and the rest of the second one.

In all spectra in addition the peaks of Carbon and Oxygen appear and that have not been included in the Table 1 by the nature of the cellulose of the linen and the samples to be metallized by graphite in order to do conductives. The marked elements (*) its concentrations are out of the analytical range, and the Aluminium initially I thought that could be due to the support of the sample, thought rejected by the results of the second one.

I want to indicate that these microanalyses are punctual on areas of approximately 10 x 10 µm in the superficial ones of the fibers

We can affirm that the elements marked in yellow in the black fibers (Na, To, If, S, Ca. Faith, Cu and Ag) are inherent in the covering of these. Whereas in the clean fibers is not observed Al, S, Fe, Cu and. It does not attend these metallic elements in the clean fibers and yes in the black ones incline us to reject initially the origin of this contamination as microorganisms that do not possess them in its structural composition.

And if the superficial contamination of the fibers of the linen have its origin in environmental incomplete combustions, these only can come from combustions of wood, oils, waxes, emission of vehicles or industrial.

The Sulphur can be the element that can aim at us on the origin of this contamination, so for example in the wood and waxes its concentration is minimal. It is not this in the liquid combustibles (diesel, kerosene, fuel oil) and mineral carbons. Then it seems to be that the origin of the contamination points us at these combustibles.

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5. CONCLUSIONS

This work has met enormously impeded in its accomplishment for the size and number of the analyzed fibers, and therefore the need of the employment of analytical technologies and top equipments not always available. Besides the work that supposes taking and separating individually the contaminated (black) fibers of the clean ones. Not forgetting the antiquity and nature of the linen

It is possible to assure that in the threads of the Shroud one find random a number of fibers colored superficially of black.

These fibers are a few times parted partially with the threads and others forming isolated spots in the surface of the same ones.

The surface of these black fibers takes the amorphous Carbon as a principal element, so its origin is organic.

That are not solved, not bleached with the previous acid traetment / alkali / acid / extraction cellulose used before the date by ^{14}C

That in this previous traetment gets lost 58,4 % of weight of the sample

If the black fibers do not dissolve with this previous traetment, it means that it increases its concentration in the sample before the date.

Then this superficial contamination, which has been to the manufacture of the linen, is the causer of the increase of the ^{14}C atom and therefore the rejuvenation of the same one.

The origin of the elements found in this black superficial film seems to be industrial combustibles. Not rejecting one possible symbiosis with microorganisms not detected in the realized analyses, which alone have indicated us not current viability.

The final and determinant serious checking could be the power of to fulfil two dates separately. One of black fibers and other one of clean alone. My desire and intention is this, but alone we will be able to try to fulfil the radiodate with the clean ones, so the quantity of sample necessary for the test (~10 mg) could be impossible to obtain of black fibers.

6. GRATEFULNESSES

I want to leave witness in this paper my gratefulness first to Pilar, my wife, for many hours that I have had to distract to our home to be able to realize these works.
To the members of the EDICES, especially to Guillermo for his support, confidence and spirit during all these years that we take together. I do not want to forget all those that left us
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Dr. FERNANDO CATALINA LAPUENTE – Institute of Science and Technology of Polymers. Superior Council of Scientific Researches (Madrid)
Dr. ENRIQUE PARRA CREGO – Dpt. of Chemical Analysis. University Alfonso X The Wise (Madrid).
Dr. SALVADOR ROVIRA – Dpt. of Conservation. Archaeological National Museum. Minister of Culture
Dra. ANA CABRERA LAFUENTE - National Museum of Decorative Arts**.** Minister of Culture
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Dra. M. CARMEN GUTIERREZ BOUZÁN - Institute of Textile Research and Industrial Cooperation. Technical University of Catalonia.
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Dr. RAFAEL GONZÁLEZ– Dpt. of Diffraction X-rays. Natural Sciences. Museum Madrid.
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