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The application of FTIR microspectroscopy in a non-invasive and non-destructive way to the study and conservation of mineralised excavated textiles

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Abstract

The potential of Fourier Transform Infrared (FTIR) microspectroscopy, to inform the study and conservation of mineralised excavated textiles is discussed, highlighted by two case studies of 5th c. BCE finds. In both cases the textiles were funerary from pyre burials, used to wrap the remains of the incinerated bones of the deceased, and placed inside copper alloy urns, to be buried. FTIR spectra of the textile fibres were acquired by an FTIR microscope in reflectance mode. In regard to the two case studies, this was a non-invasive and non-destructive method, since it did not involve removal of material for sampling, as both finds were in a fragmentary condition, it did not apply any pressure, nor did it require pressing of the minute fragments used as samples. Past fibre analyses in case study A reported cultivated and wild silk, which had been used as evidence of the presence of silk in Classical Greece, while more recent studies, controversially pointed to either cellulosic or wool fibres. The unlimited application of FTIR microspectroscopy in reflectance mode due to its non-invasive/destructive nature, enhanced fibre identification but most importantly led to further analyses of specific fragments that revealed the find was suffering from active biodeterioration, subsequently treated. Case study B, where the textiles had been preserved folded, was a particularly unique find from its period, since one of the textiles present was bearing evidence of embroidery. Similarly, the extended application of FTIR microspectroscopy indicated good preservation of the organic matter within the fibres, informing thus the conservation decision-making process, which involved partial unfolding of the textiles, an action that revealed an additional decoration pattern, and minute fragments of the embroidery thread preserved.

Keywords: FTIR microspectroscopy, Reflectance, Non-invasive/destructive, Excavated textiles, Mineralisation, Textile conservation

Introduction

Fourier Transform Infrared (FTIR) is a vibrational spectroscopic technique, which has found numerous applications to the study of excavated textiles. For organic compounds (like textile fibres), molecular vibrations lead to specific absorption bands in the spectra produced, which can facilitate identification [1, 2]. Comparison of the spectra of mineralised excavated cellulosic fibres with spectra of modern fibres, has enabled fibre identification

[3], and observation of certain peak differences attributed to mineralisation [4]. When applied to the study of artificially mineralised samples, FTIR was reported to be able to detect the organic matrix left, even after long-term burial [5], and differences between partially mineralised samples [6]. As a matter of fact, FTIR revealed that textile fibres originally characterised as pseudomorphs were rarely totally mineralised [7], and even identification in terms of proteinaceous or cellulosic fibres was possible in samples that were previously described as completely mineralised [8, 9].

FTIR was actually reported useful in studying and documenting the effects of degradation and ageing on

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textile fibres at a molecular level, when applied to historic textiles successfully showing different stages of degradation of cellulosic fibres [10], and to biodegraded historic textiles, where it was able to detect structural changes in the cellulosic fibres caused by fungi [11, 12] and qualitative analysis of materials present in cross-sections of the fibres when combined with a synchrotron radiation source (SR) [13]. In fact, the ability of FTIR in detecting changes induced by degradation due to ageing in regions of the produced spectra of cellulosic fibres proposed it as an alternative technique to dating cellulosic textiles [14, 15], with promising results, although quite sensitive to the environmental factors and the effects of chemical processing (like bleaching), the fibres might have undergone [16].

FTIR can operate in transmission, reflectance or attenuated total reflectance (ATR) modes. Transmission requires the removal of a sample. In addition, only very thin samples (around 20 microns) are suitable for transmission, so preparation of the sample by pressing or grinding may be necessary. This also helps to circumvent problems arising from the lensing effect of fibres, due to their tubular shape. Nevertheless, this kind of preparation is destructive to the sample, especially in the case of excavated textiles similar to the ones discussed here, which are too brittle to withstand such processes without complete destruction. ATR might obviate the need for the removal of a sample, but pressing against a crystal window (e.g. diamond) is necessary, which again can prove to be destructive for extremely sensitive excavated fabrics. Reflectance may not require sample removal or preparation, making thus the method non-invasive and non-destructive in the context of excavated textiles. However, the drawbacks when using the FTIR microscope in reflectance mode are that the infrared beam might be scattered due to the cylindrical shape of the fibres, and that it does not penetrate the specimen in depth to perform bulk rather than surface analysis [e.g. 3]. The former might diminish the quality of the spectra, while the latter might reduce the reproducibility of the results, especially in specimens partially coated by deposits, such the mineralised textile fibres. In addition, the poor quality of spectra obtained for severely degraded excavated textiles was reported to even preclude fibre characterisation [17].

This paper investigates the potential of the application of FTIR microspectroscopy, in a non-invasive and non-destructive way, that is without permanent removal and alteration or destruction of material from the find [18], to inform the study and conservation of two excavated textile finds. The textiles discussed here, were funerary from pyre burials, used to wrap the remains of the incinerated bones of the deceased, along with fruit and/or other offerings, and placed inside copper alloy vessels for burial. More than one textiles were identified in each case study, which were considered quite exceptional finds, since textile preservation in a burial context in Southern Europe is very rare, due to the unfavourable environmental conditions prevailing. The temperate climate occurring in this region excludes both very hot and extremely cold temperatures, and is characterised by great temperature and moisture levels fluctuations between summer and winter. These conditions are destructive to the preservation of organic material, such as textile fibres, as they induce physical (i.e. shrinkage and swelling) and chemical (i.e. hydrolysis) degradation processes and encourage micro-organism growth that leads to enzymatic hydrolysis of cellulose and proteins [7, 19]. Special conditions need to develop for preservation to occur, and these are usually achieved by the proximity of textiles to metals (mineralisation) or their exposure to fire (carbonisation) [e.g. 20–23].

In both cases discussed here, the textile finds had been preserved by mineralisation as a result of having been buried inside copper alloy vessels. Mineralisation is known as the process of textile preservation achieved when the organic fibres of a textile, which are in close contact with a metal object, are gradually replaced by the metal corrosion products. In addition, the metal ions acting as microorganism inhibitors, are protecting the fibres from attack by micro-organisms. Copper ions form complexes or bonds with the end groups or hydroxyl groups in the cellulose, thus stabilising it and protecting it from further degradation [4]. The process of mineralisation may terminate at any time (partly mineralised fibres/textiles with some that retain their original organic composition) or continue until replacement of the organic matter of the fibres is complete (completely mineralised fibres/textiles, known as pseudomorphs), providing thus, textile finds at varying degrees of mineralisation [4, 24]. Empirically speaking, the higher the degree of mineralisation, the more the original physical and chemical properties of the fibres have changed and are most probably not known. However, the newly acquired properties include a significant loss in weight, extreme hydrophobia, brittleness and zero mechanical strength and elasticity [24] (Fig. 1).

In this present study, the efficiency and reliability of FTIR microspectroscopy in reflectance mode, initially tested and subsequently applied to mineralised excavated textiles, is discussed. Several successful applications of FTIR to the identification and condition assessment of textiles involve the use of ATR [e.g. 1, 15, 16, 25] that applies enough pressure to the sample to make the technique destructive for very degraded mineralised textiles. Moreover, the applications of ATR reviewed took place mainly on historic rather than excavated textiles,

Fig. 1 Unmanipulated image showing how the hydrophobic textile fragment repels a water droplet, which forms a perfect sphere on its surface

the condition of the former being markedly better [e.g. 10, 12, 13], and when excavated textiles were reported, these were from Egypt [e.g. 14], a place that has thankfully provided us with an abundance of important finds in remarkably good condition. Unfortunately, this is not the case with mineralised textiles from Southern Europe, where the rarity and poor condition of the finds make the application of non-invasive and non-destructive methods of analysis a priority, both from a conservator's ethical viewpoint and in certain cases by law [e.g. 26, 27]. The aim of this study was to test whether FTIR applied in a truly non-invasive and non-destructive way, would be able to detect whether any 'signature' band assignments of the organic matter of the fibres, were still evident in the spectra despite mineralisation, as this would inform both fibre identification and the conservation strategy to be followed.

Materials and methods
Preliminary experiments
Two sets of preliminary experiments were carried out to compare FTIR microspectroscopy in reflectance and transmission modes with ATR, and to test the reproducibility of reflectance microspectroscopy. Flax (*Linum usitatissimum*), cotton (*Gossypium hirsutum*), hemp (*Cannabis sativa*), sparto (*Spartium junceum*), along with silk (*Bombyx mori*) and sheep wool (*Ovis* aries) reference samples were studied using a Perkin-Elmer FTIR Spectrum One instrument with a Universal ATR attachment (diamond window, single bounce at 45°), or an Autoimage microscope. Spectra were acquired over the range of the instrument 4000 to 500 cm^{-1}. For ATR spectroscopy, 16 scans were accumulated with a spectral resolution of 4 cm^{-1}. For microspectroscopy 32 scans were

accumulated with a resolution of 4 cm^{-1}. For transmission, individual fibres were removed from the samples and first flattened at 10 ton pressure between steel plates using a Specac manual hydraulic press. For reflectance, the whole specimen was placed on a small gold mirror inserted into the central hole of the microscope slide. In each case the microscope aperture was adjusted so that an area of 70×100 μm was analysed. Grams AI v8© software was used to process the spectra. No baseline correction of the spectra produced by the reference or excavated textiles spectra took place.

A second preliminary experiment was carried out with an objective to test the consistency of the spectra produced by the FTIR microscope, especially along fibres treated with a copper oxohydroxide solution to simulate the effects of mineralisation, which would have been partially coated by minerals (see Supplementary Information). Five reflectance spectra were recorded for fibres from the copper oxohydroxide treated reference samples. In each case the area probed was moved across the length of the same fibre and spectra were collected from areas that had been affected in different ways by the method of treatment (e.g. completely, partially, minimally or not covered by deposits).

The application of FTIR microspectroscopy to the case studies
Based on the preliminary experiments presented previously, FTIR microspectroscopy in reflectance mode was applied to mineralised fibre samples from two case studies of excavated textiles. Spectra were acquired over the range of the instrument 4000 to 500 cm^{-1}. 32 scans were accumulated with a resolution of 4 cm^{-1}. The fragmentary condition of the textiles ensured that the technique was also non-invasive, since loose fragments were temporarily removed from the finds, analysed, and subsequently returned [18] (Fig. 2). The whole fragment/sample was placed on a small gold mirror inserted into the central hole of the microscope slide, with the microscope aperture adjusted at 70×100 μm. Taking advantage of the fragmentary condition of the textiles and the non-destructive quality of FTIR microscopy in reflectance mode, numerous samples were analysed in a size range from 2×2 mm to 5×5 mm approximately. Ten different areas of each sample were analysed (five for the warps and five for the wefts). Since these spectra were consistent they were synthesised to give an average one. The spectra of the samples were compared to those of modern fibres used as references, to check whether the characteristic peaks for the major organic polymers were present in the former [e.g. 7]. Grams AI v8© software was used to process the spectra.

Fig. 2 A view of case study A, the Kerameikos textiles during conservation, showing the numerous, loose fragments present, some of which were used for FTIR analysis

Fig. 3 Case study A, the Kerameikos textiles before conservation

Case study A textile find was excavated in 1936 by the German Archaeological Institute at Athens [28] (Fig. 3). The textiles were inside a copper alloy lebes buried in the grave of the Alcmaeonidae family (the family of the Athenian general Alcibiades) at the Kerameikos cemetery. The shape and decoration of the copper lebes and the stratigraphy of the grave indicated that the findings were dated to the last third of the 5th c BCE (approximately 430–400 BCE). In the late 1960s the find was first analysed by microscopic examination and staining tests, and the textiles were reported to having been made of silk of the cultivated *Bombyx mori* species [29]. The results of this study have been mentioned as evidence for the use of silk in Classical Greece by pioneering researchers in the field of archaeological textiles [e.g. 22, 30]. In later years the find was sampled and analysed again, at first identified as wild silk by the application of microscopy and High Performance Liquid Chromatography (HPLC)—aminoacid sequence [31, 32] and later as wool by the application of Scanning Electron Microscopy (SEM) [33]. Although samples had been removed several times from this very important and unique find, and analysed with destructive techniques solely for the purpose of fibre identification, this was still debatable. FTIR was initially applied

aiming at a fibre identification aid. Research through the Museum's archives did not reveal any evidence of the find having been treated in the past, therefore the possibility of a foreign material introduced that could possibly contaminate the analyses results was excluded. Upon receipt, the find was kept in a transparent acrylic box with a lid, stored in a drawer. However, the lid was broken which meant dust had probably accumulated to the fibres.

Case study B textile find was retrieved in 1983 during a rescue excavation conducted by the Hellenic Ministry of Culture, which brought to light part of a Classical (5th c BCE) cemetery, at Piraeus (Fig. 4). Inside a sealed copper vessel, a substantial amount of textiles had been preserved folded in a bundle, one of them bearing evidence of a decoration, indicative of embroidery. Only the holes created by the needle and not the decorative thread seemed to have been preserved. To the author's knowledge, only two other 5th c BCE textile finds from the area of Koropi in Attica, with evidence of embroidery have been preserved [34], indicating that embroidered textiles from ancient Greece are rare finds. Consequently, the need to unfold the textile bundle in order to allow for a more comprehensive study of the decoration technique, was high. FTIR microspectroscopy was applied to several fragments/samples from this find, with the aim of detecting any 'signature' band assignments of the organic matter of the fibres. Preservation of the organic matter would mean the textiles retained at least some of their original physicochemical properties, one of which is hygroscopicity [e.g. 7] that decreases as the oxidisation of cellulose increases [35]. This would in turn indicate that the introduction of moisture to the fibres, by the method of humidification, could be safely and successfully applied to proceed with unfolding. Safety was a prerequisite since
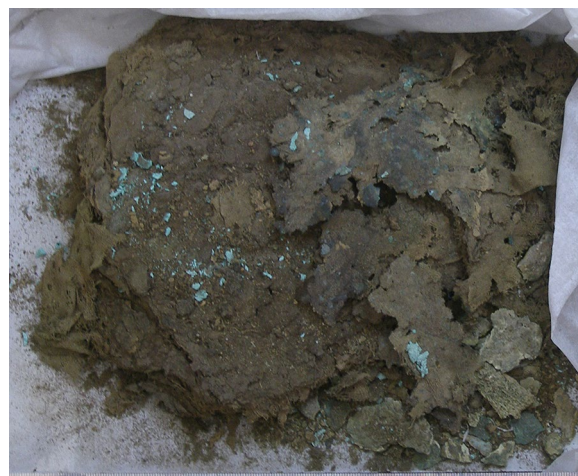

Fig. 4 Case study B, the Nikaia textiles before conservation

parts of a similar find had been reported to turn into pulp when attempting to unfold them by the introduction of moisture [36]. For FTIR analysis, the procedure described above for the Kerameikos samples, was also followed for this find. In this case, research through the Museum's archives did reveal that the find had never received any conservation, cleaning or consolidation treatment in the past, again excluding the probability of any contaminants present. Upon receipt, the find was kept in a cardboard box lined with tissue paper (the samples collected were not in direct contact with the storage material, to avoid any possible contamination) with no lid resulting thus in accumulation of dust to the fibres.

Further analyses applied to the case studies

Guided by the FTIR analysis results, further analyses were applied to the finds. For case study A, samples that gave the lowest quality spectra, were further examined with high magnification microscopy. A PHILIPS XL30 Environmental Scanning Electron Microscope (magnification up to 50,000) was used for the analysis at 15 keV, coupled with an X-ray microanalyser (EDAX CDU LEAP Detector, using the eDX© software). In turn, based on the SEM results, standard automated forensic Polymerase Chain Reaction (PCR) analysis was applied directly to the samples. Regarding the non-destructive concerns, it is important to note that some of the same samples that gave poor quality FTIR spectra were analysed by SEM and subsequently PCR. Samples were studied at the SEM without coating but still placed on a double sided carbon coated tape to secure them on the stub. Their poor mechanical strength did not allow for removal from the tape without destruction (crumbling into powder), making thus SEM partially (if removed) destructive. Nevertheless, selected mounted samples were successfully used for PCR analysis which is anyway destructive.

Similarly, for case study B, FTIR analyses results directed to certain conservation treatments that necessitated further analyses. These were Computed Tomography (CT) and Three-Dimensional (3D) scanning. A PHILIPS Brilliance 64 CT scanner at 8 kV, 0.98 mm resolution [37], and an Artec Studio 10 Professional 3D scanner were used respectively to document and record the profile, shape and position of the textiles in the bundle before conservation.

Results and discussion
Preliminary experiments

As stated above, the aim of these experiments was to compare the ability of the three techniques and the consistency of FTIR in reflectance mode, when detecting the absorbance bands assigned to specific components, characteristic of the organic matter of the fibres [e.g. 7,

38]. The ATR spectra showed the band assignments, characteristic of the organic polymers within the fibres. In more detail, for flax (i.e. cellulosic) fibres at 2900 cm^{-1} ν(C–H) stretching vibration, indicative of polysaccharides, 1635 cm^{-1} corresponding to vibration of water molecules absorbed in cellulose, 1365 cm^{-1} δ(C–H), 1335 cm^{-1} δ(CH$_2$), 1155 cm^{-1} ν(C–C) ring breathing band, asymmetric, 1105 cm^{-1} ν(C–O–C) glycosidic ether band, 1025 cm^{-1} ν(C–OH), alcohol and 895 cm^{-1} ν(C–O–C) arising from the polysaccharide components (i.e. cellulose); for silk and wool (i.e. proteinaceous) fibres at 3275 cm^{-1} ν(N–H) bending free and H-bonded, assigned to amide bonds, 1615 cm^{-1} ν(C=O) from carbonyl stretching assigned to amide I, 1515 cm^{-1} δ(N–H) bending assigned to amide II, and 1235 cm^{-1} ν(C–N) stretching to amide III, in peptide bonds (–CONH–) that link the aminoacids of proteins together [e.g. 1, 2, 7, 25, 39–42] (Tables 1, 2).

In the case of the FTIR microscope transmission mode some of the bands shifted towards the higher end of the spectrum (e.g. 1105 to 1124 cm^{-1}, 1025 to 1041 cm^{-1} for flax; 3275 to 3325 cm^{-1}, 1615 to 1690 cm^{-1}, and 1515 to 1528 cm^{-1} for silk and wool). More bands shifted in the same direction in the spectra of the uncompressed fibres examined in reflectance mode (e.g. 1155 to 1173 cm^{-1} for flax; 1690 to 1720 cm^{-1} and 1235 to 1248 cm^{-1} for silk; 1235 to 1260 cm^{-1} for wool). For the cellulosic fibres, the band assignment at 895 cm^{-1} suggestive of the ν(C–O–C) bond in cellulose slightly shifted towards the lower end of the spectrum at 892 cm^{-1} for transmission and 889 cm^{-1} for reflectance (Figs. 5, 6, 7). The 1735 cm^{-1} ester band assignment shifted towards the higher end of the spectrum at 1752 cm^{-1} but only appeared in the hemp and sparto spectra (Fig. 8). Similarly for the reflectance mode acquired spectra, the 1595 cm^{-1} band assignment (shifted to 1586 cm^{-1} in reflectance) indicative of lignin did not appear in the cotton fibres, since they do not contain lignin, neither did it appear in flax (with a lignin content ~ 2%), and only appeared in the hemp spectrum as a shoulder rather than a band (lignin content ~ 3.3%) [e.g. 25, 43] and in the sparto spectrum as a clear band (lignin content ~ 3.3%) [e.g. 44, 45] (Fig. 8). These results suggest the lignin content of flax fibres was too low for FTIR in reflectance mode to detect it. Flax and hemp fibre samples were taken from textiles, they were thus processed fibres. Fibre processing has been reported to reduce the lignin content of fibres [25]. Sparto fibre samples were directly extracted from the bast of the plant, they were therefore unprocessed fibres, suggesting the lignin content in them remained at a higher percentage than in the hemp fibres, although originally hemp and sparto have very similar lignin content percentages (Fig. 8 and Tables 1, 2). Generally, the reflectance spectra

Table 1 Shifts in band assignments for the cellulosic reference samples as detected by the application of FTIR at different modes

Band assignment	Wavenumber (cm^{-1}) FTIR-ATR, literature	Wavenumber (cm^{-1}) FTIR transmission	Wavenumber (cm^{-1}) FTIR reflectance
ν(C–H) bond, indicative of polysaccharides	2900	2900	2900
ν(C=O) ester (only in hemp and sparto)	1735	1735	1752
Indicative of adsorbed water within the fibre	1635	1635	1635
ν(C=C) aromatic in-plane, indicative of lignin (only in hemp and sparto)	1595	1595	1586
δ(C–H) bond in cellulose	1365	1365	1365
δ(CH$_2$) bond in cellulose	1335	1335	1335
ν(C–C) ring breathing, indicative of polysaccharides in cellulose	1155	1155	1173
ν(C–O–C) glycosidic ether band, indicative of polysaccharides in cellulose	1105	1124	1124
ν(C–OH) bond in cellulose	1025	1041	1041
ν(C–O–C) bond in cellulose	895	892	889

Table 2 Shifts in band assignments for the proteinaceous reference samples as detected by the application of FTIR at different modes

Band assignment	Wavenumber (cm^{-1}) FTIR-ATR, literature	Wavenumber (cm^{-1}) FTIR transmission	Wavenumber (cm^{-1}) FTIR reflectance
ν(N–H) amide bond	3275	3325	3325
ν(C=O) amide I bond	1615	1690	1720
δ(N–H) amide II bond	1515	1528	1541
ν(C–N) amide III bond	1235	1235	1248 (silk), 1260 (wool)

produced broader peaks with different intensities, but they appeared nonetheless characteristic. The band shifts were not considered to be a major drawback since 'finger-printing' was still possible, and positive identification of the fibres viable.

Regarding the consistency of FTIR spectra acquired in reflectance mode when applied to copper oxohydroxide treated samples, results showed that spectra acquired from different areas on the fibres were consistent, showing the same bands although with occasional small differences in relative intensity. For the flax oxohydroxide treated samples the bands at 2900 cm^{-1} ν(C–H) stretching vibration, indicative of polysaccharides, the 1635 cm^{-1} corresponding to vibration of water molecules absorbed in cellulose, 1124 cm^{-1} ν(C–O–C) glycosidic ether band, 1041 cm^{-1} ν(C–OH) alcohol, and 889 cm^{-1} ν(C–O–C) arising from the polysaccharide components, appeared in the spectrum. Additional bands appeared in the region of the spectrum from ~ 1350 to 1500 cm^{-1} and these could be indicative of the interaction between the copper ions and cellulose. The penetration of copper

ions to the amorphous regions of cellulose could rearrange and separate them, thus producing these absorption bands [4] (Fig. 9). For the reference samples, at least, a single spectral analysis seemed representative. This was encouraging for the analysis of the excavated samples, although variations across such 'non-ideal' specimens would be the more likely.

The Kerameikos textiles

The spectra were consistent, both along the warps and the wefts, of all the different textiles analysed. As far as fibre identification was concerned, none of the spectra showed any bands assigned to characteristic components of proteins, not giving thus any evidence for the presence of either silk or wool fibres in the find [46]. In addition, there were samples that showed all the bands assigned to the characteristic components of cellulose, as mentioned above and in Table 1 (reflectance mode column), indicating organic matter had been preserved in the fibres analysed. However, several samples produced spectra showing only two of the band assignments characteristic of components within cellulose (namely at 1635 cm^{-1} and 1124 cm^{-1}). Similarly to the copper oxohydroxide treated flax samples, bands showed at ~ 1447 cm^{-1} (i.e. within the region ~ 1350 to 1500 cm^{-1}) that could be assigned to the interaction of copper ions with cellulose as explained above. An additional band appeared at 1740 cm^{-1}, possibly assigned to the C-O bond, that could be attributed to the hydrolysis and oxidation of cellulose (reported at 1720 cm^{-1} at spectra acquired by FTIR-ATR and described as a shoulder and not a neat band, which is similar to the Kerameikos spectra) [35] (Fig. 10). Selected samples that produced such spectra were further analysed by SEM–EDS. SEM analysis showed that the fibre morphology in these samples had changed to
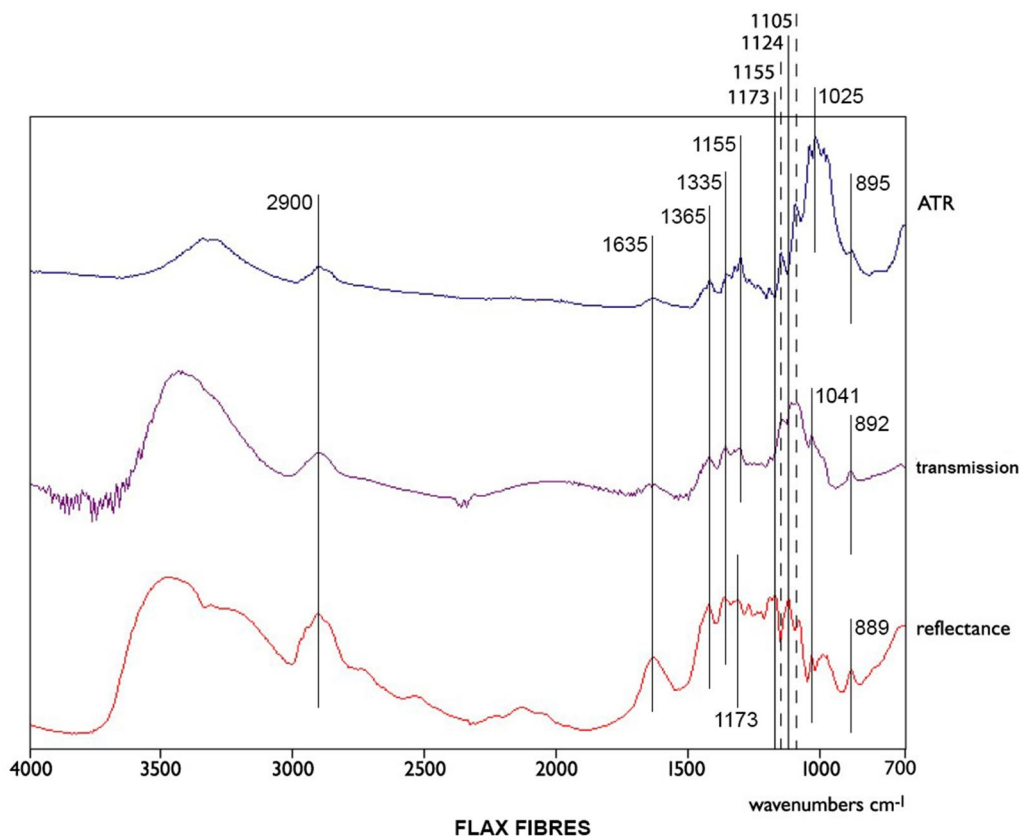
Fig. 5 The FTIR spectra of flax samples: ATR (top), transmission (middle) and reflectance (bottom)

an unrecognisable degree, and they had been attacked by micro-organisms sometime in the past. In certain areas the fibres were masked by profuse 1.5–2 micrometers sub-globose dehydrated fungal conidia-like structures (Fig. 11) or hyphae, also indicative of the presence of fungi. EDS analysis showed copper (Cu) corresponding to the mineralised state of the samples, and other elements like calcium (Ca), silicon (Si) and aluminium (Al) that could be attributed to dust deposits [47] (Fig. 12).

Identification of a micro-organism detected on a textile find, is considered to be very important not only for its treatment and preservation but also for fibre identification, since different micro-organisms seem to attack cellulosic and proteinaceous fibres. In addition, the identification of the specific microorganism would afford information on the way it attacks fibres and on its metabolism. As a result, the deterioration mechanism to which the fibres were subjected could be better defined and the most efficient method for de-activating the micro-organism could be selected [48]. Standard automated forensic PCR analysis applied directly to samples from the find, identified sequences of the cellulolytic fungus *Aspergillus terreus,* potentially active [49]. This fungus is cellulolytic,

it has therefore the capacity to hydrolyse cellulose (which is the main structural element of e.g. flax fibres) and not proteins (the main substance of silk fibres) [50]. The presence of a cellulolytic fungus in the find, was considered to further support the FTIR fibre identification results. Flax and silk reference samples were inoculated with the fungus *Aspergillus terreus* with the aim of studying the effects of fungal growth on the morphology of the fibres. SEM analysis showed similar effects between the cellulosic reference samples and the excavated ones.

Since *Aspergillus terreus* is an aerobic fungus, a low oxygen environment was created in order to control its growth and prevent further biological damage of the fibres. The find was enclosed in a heavy weight transparent barrier film (ESCAL™) with resistance to oxygen permeation. Oxygen absorbers RP System™ type A and K were placed inside the package, to absorb the remaining oxygen. The percentage of oxygen achieved within the package ranged between 0.2 and 0.4%. The minimum oxygen percentage for *A. terreus* to grow is around 2–5% [51] hence it was considered that further growth of the fungus on the find was prevented [52].
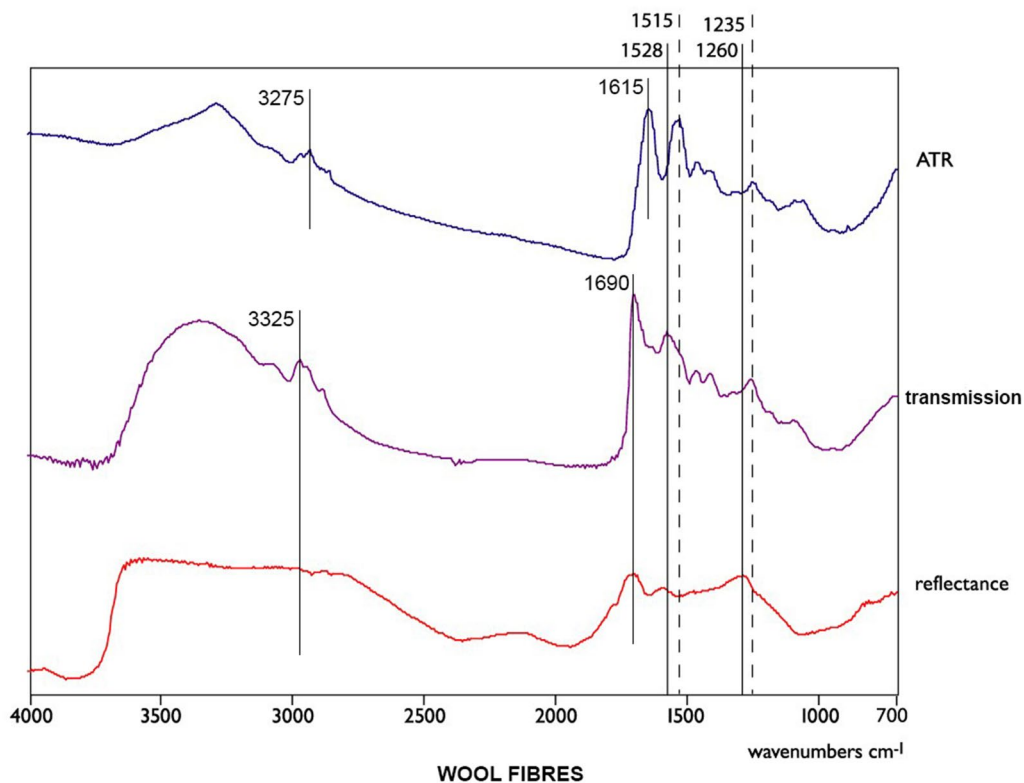
Fig. 6 The FTIR spectra of wool samples: ATR (top), transmission (middle) and reflectance (bottom)
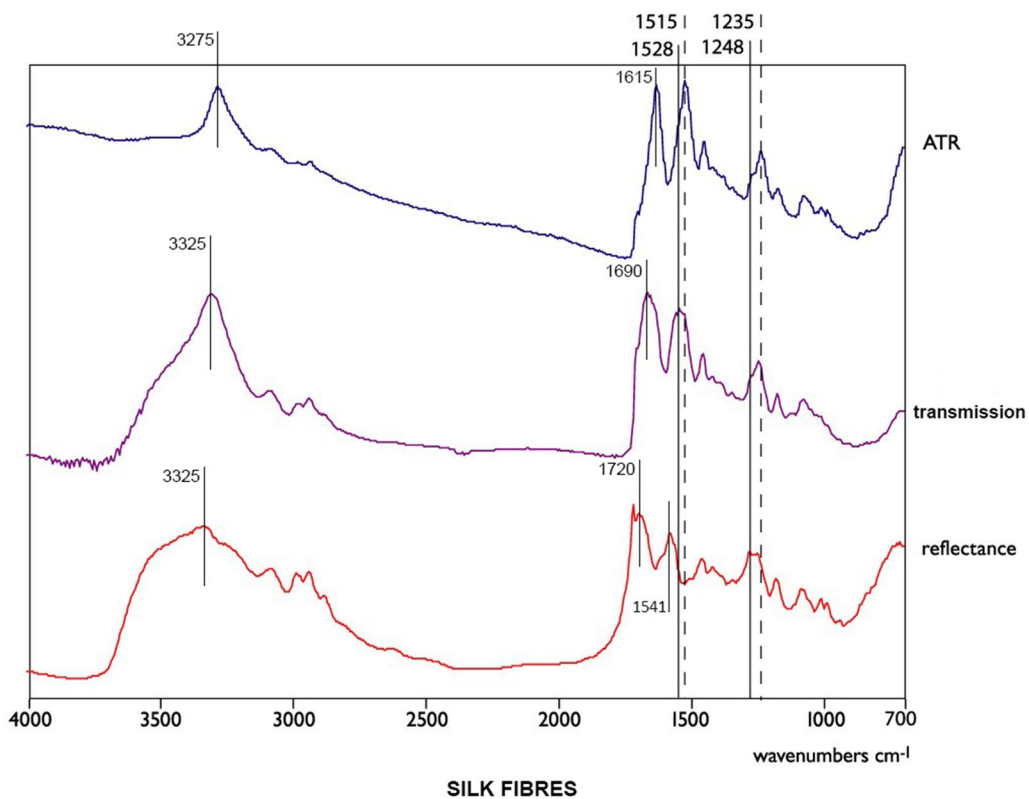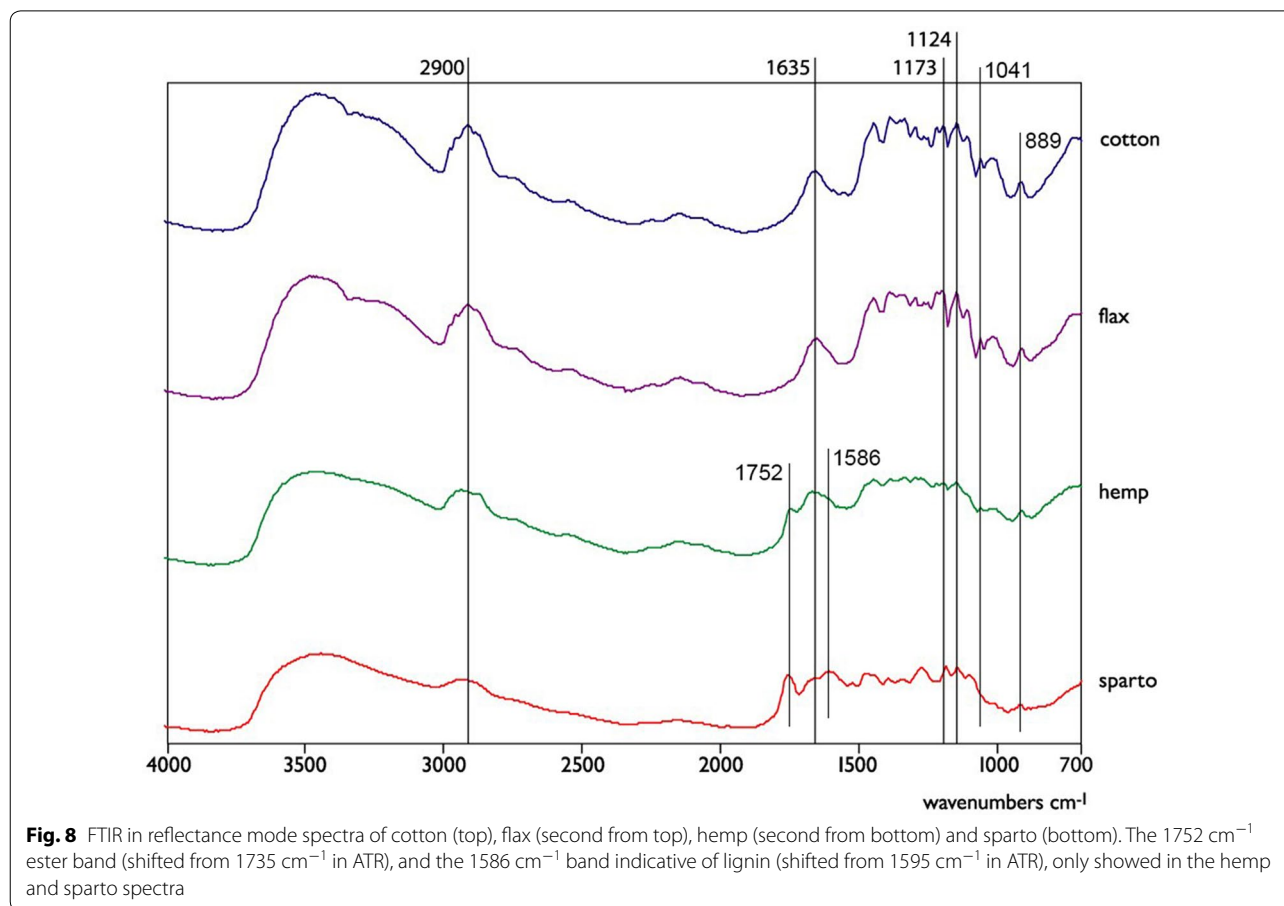

Fig. 7 The FTIR spectra of silk samples: ATR (top), transmission (middle) and reflectance (bottom)

Fig. 8 FTIR in reflectance mode spectra of cotton (top), flax (second from top), hemp (second from bottom) and sparto (bottom). The 1752 cm^{-1} ester band (shifted from 1735 cm^{-1} in ATR), and the 1586 cm^{-1} band indicative of lignin (shifted from 1595 cm^{-1} in ATR), only showed in the hemp and sparto spectra

The Nikaia textiles

Similarly, the non-destructive characteristic of FTIR microspectroscopy, meant that a large number of samples could be analysed. Spectra of the warps and wefts of all three textiles were similar, showing all bands assigned to components of cellulose (2900 cm^{-1}, 1635 cm^{-1}, 1365 cm^{-1}, 1335 cm^{-1}, 1173 cm^{-1}, and 1124 cm^{-1}) (Table 1). The band at 1635 cm^{-1} appeared quite broad possibly due to the ~ 1720 cm^{-1} band appearing more like a shoulder, assigned to the C-O bond, attributed to the hydrolysis and oxidation of cellulose [35]. Most importantly the bands at ~ 1447 cm^{-1} evident in the Kerameikos samples were not recorded in any of the spectra produced (Fig. 13). However, mineralisation was recorded by EDS analysis that detected copper (Cu) (and other elements like calcium (Ca), phosphorus (P), sodium (Na), silicon (Si) and aluminium (Al) that could be attributed to dust deposits [47]) (Fig. 14). Based on FTIR and EDS analysis, it would be safe to say that although the Nikaia fibres were mineralised, they had probably been affected by the process to a lesser degree than the Kerameikos textiles for example, indicating the structure of their molecular chains was similarly less affected [4], meaning that the

condition of the textiles was good enough to withstand unfolding without damage [53]. Unfolding would enable better study of the decoration (embroidery) evidence observed. However, there was great concern from an ethical viewpoint, whether the textile bundle should be unfolded. Unfolding would destroy any evidence of how the textiles had been placed inside the copper alloy urn, and it would terminate any potential of a different intervention and interpretation in the future. It was decided therefore, to only partially unfold loose parts of the textiles bearing evidence of embroidery.

CT and 3D scanning were applied that enabled detailed recording of the folded bundle before taking any interventive conservation action. CT scanning also showed that the bundle consisted only of folded textiles with no hard or sharp material present, like metal fragments or incinerated bones that could potentially harm the textiles during unfolding.

Loose and accessible textile fragments bearing evidence of embroidery were removed from the bundle and humidified (slow and controlled introduction of moisture into the fibres). When the fibres were pliable enough (after approximately 2 h), the textile was pinned
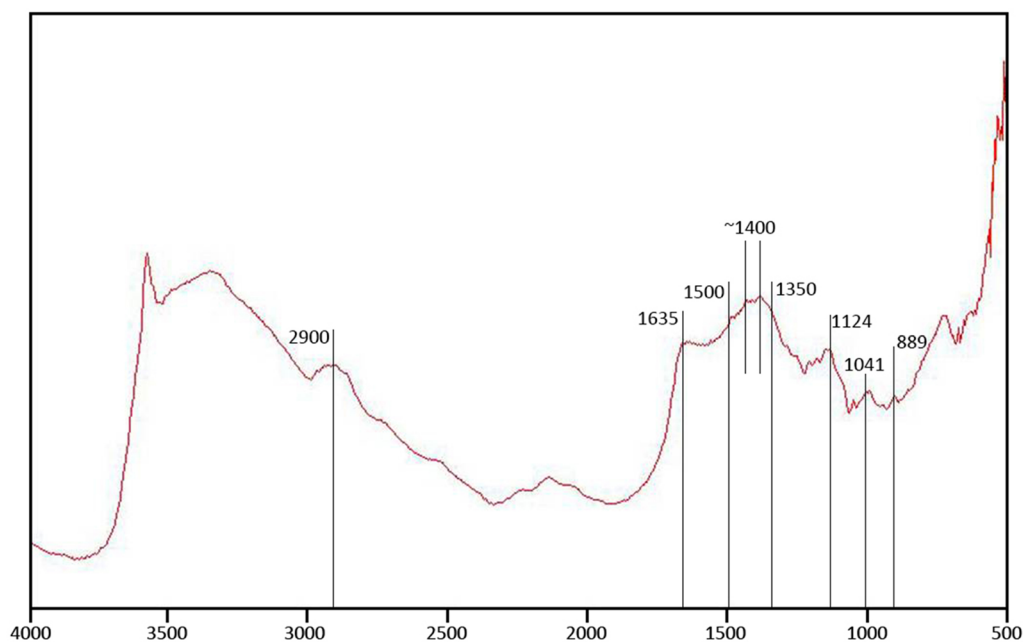
Fig. 9 Example of FTIR spectrum of oxohydroxide treated flax acquired in reflectance mode, showing certain bands assigned to components characteristic of cellulose (2900 cm^{-1} ν(C–H) stretching vibration, indicative of polysaccharides, the 1635 cm^{-1} corresponding to vibration of water molecules absorbed in cellulose, 1124 cm^{-1} ν(C–O–C) glycosidic ether band, 1041 cm^{-1} ν(C–OH) alcohol, and 889 cm^{-1} ν(C–O–C) arising from the polysaccharide components), and others indicating the effects of the treatment to the fibres (in the region of the spectrum from ~1350 to 1500 cm^{-1})
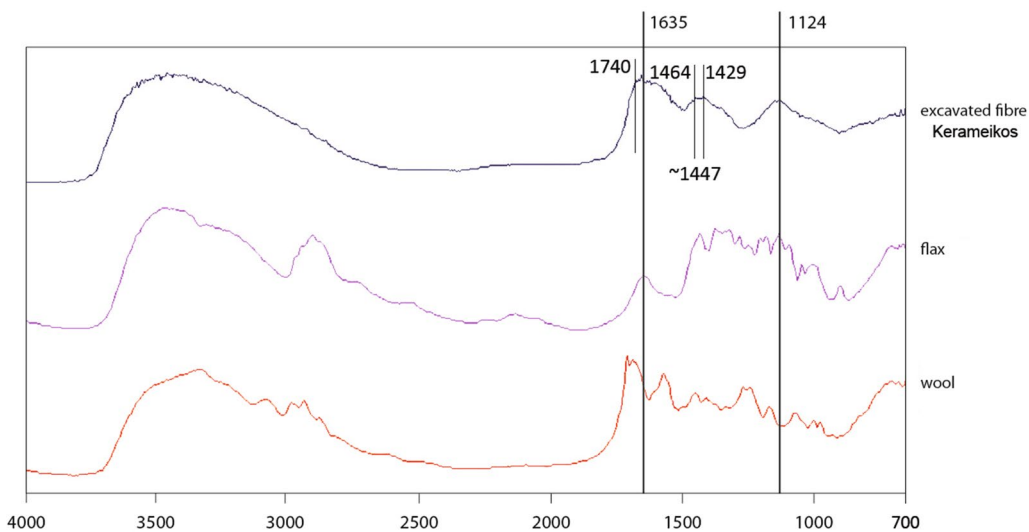
Fig. 10 The FTIR spectrum of excavated fibres (top) showed two of the band assignments characteristic of cellulose (middle), but none of proteins (bottom)
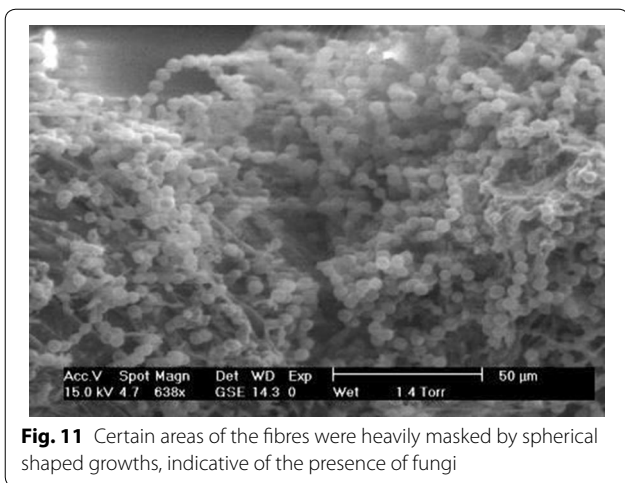
Fig. 11 Certain areas of the fibres were heavily masked by spherical shaped growths, indicative of the presence of fungi

into place with very fine entomological pins placed in the spaces between the crossing warps and wefts, and left to air dry, thus gradually unfolding it. Humidification and gradual unfolding were repeated in each fragment until any deep creases were removed or as long as it was safe for the fragile textile. Treatment was considered very beneficial as the fibres did not suffer from any mechanical stress and the majority of the creases and folds were removed. It proved also valuable to the study of the find since one flattened fragment revealed an additional decoration pattern that was not obvious before unfolding. Furthermore, better examination of the flattened pieces showed that minute fragments of the decoration thread have probably been preserved [54].

Conclusions

FTIR microspectroscopy applied in reflectance mode could be a truly non-invasive and non-destructive technique, especially when applied to excavated textiles that are in a fragmentary state that have been preserved in a particularly poor condition, as usually is the case with mineralised textiles. The technique was able to detect bands assigned to characteristic compounds of the organic matter present and preserved within the fibres, as well as bands attributed to the degradation of the organic matter and the effects of mineralisation, and even provided evidence of the latter occurring at different degrees.

In the case of the Kerameikos textiles, the application of FTIR microspectroscopy contributed to the unceasing controversy concerning fibre identification, even in samples where the morphology of the fibres had altered to an unrecognisable degree. Most importantly, it enabled characterisation of the condition of the samples analysed. Subsequently, this informed sample selection for further analyses, which diagnosed the find was suffering from bio-deterioration, still active. Cultivation and identification of the micro-organism detected further corroborated fibre identification, and informed the selection of the appropriate conservation treatment that deactivated it. A useful future analysis would be HPLC-aminoacid sequence of the micro-organism detected to compare it with similar analyses performed in the past with the aim of fibre identification.

For the Nikaia textiles, FTIR afforded condition assessment for the larger parts, of the folded textiles, showing
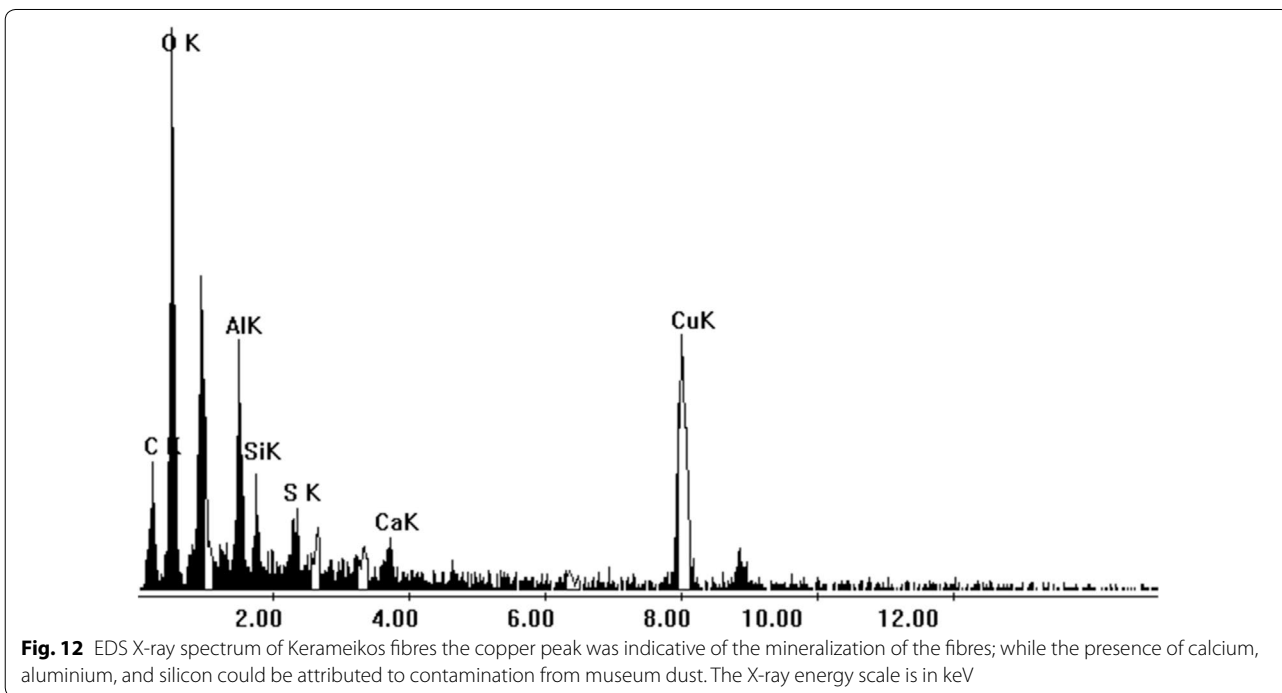

Fig. 12 EDS X-ray spectrum of Kerameikos fibres the copper peak was indicative of the mineralization of the fibres; while the presence of calcium, aluminium, and silicon could be attributed to contamination from museum dust. The X-ray energy scale is in keV
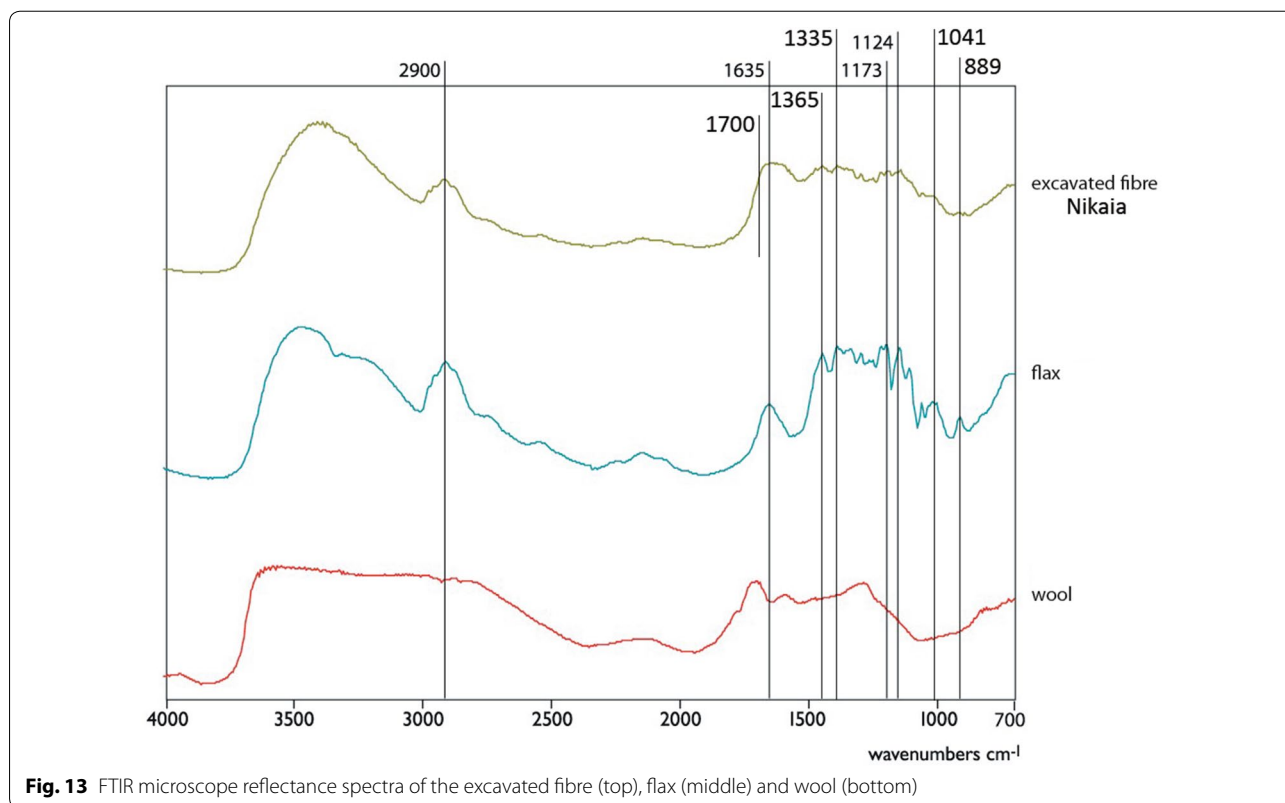
Fig. 13 FTIR microscope reflectance spectra of the excavated fibre (top), flax (middle) and wool (bottom)
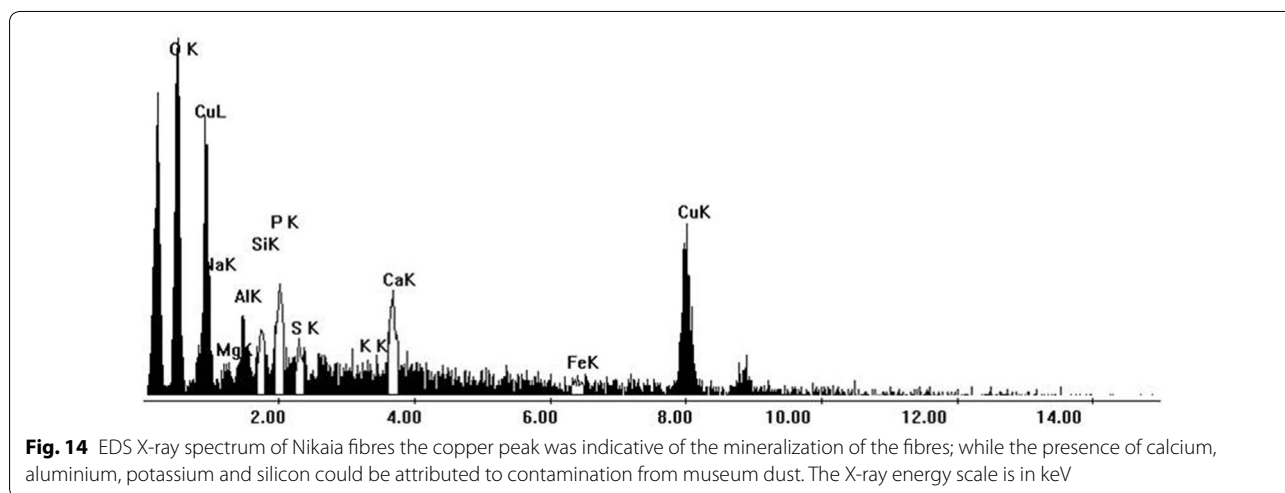

Fig. 14 EDS X-ray spectrum of Nikaia fibres the copper peak was indicative of the mineralization of the fibres; while the presence of calcium, aluminium, potassium and silicon could be attributed to contamination from museum dust. The X-ray energy scale is in keV

that the organic matter within the fibres had been preserved and remained unaffected at a molecular level to a degree that would allow for certain conservation treatments to be applied safely. This informed the conservation decision-making process, which involved partial unfolding, by the method of humidification, of certain loose fragments from the textile bundle. Likewise, in this case study, treatment enhanced the study of the find, since the treated parts revealed previously masked archaeological and technological information, such as an additional decoration pattern and remains of the decoration thread.

The non-invasive and non-destructive quality of the technique is an important prerequisite for the analysis of such rare, and yet very important finds, which are more

often than not in such a poor state of preservation that analyses will most probably have to repeated.

Additional file

Additional file 1. Additional figures.

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