

Fourier-transform Raman spectroscopic study of a Neolithic waterlogged wood assemblage

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Abstract The use of Fourier-transform Raman spectroscopy for characterising lignocellulosics has increased significantly over the last twenty years. Here, an FT-Raman spectroscopic study of changes in the chemistry of waterlogged archaeological wood of *Pinus* sp. and *Quercus* sp. from a prehistoric assemblage recovered from northern Greece is presented. FT-Raman spectral features of biodeteriorated wood were associated with the depletion of lignin and/or carbohydrate polymers at various stages of deterioration. Spectra from the archaeological wood are presented alongside spectra of sound wood of the same taxa. A comparison of the relative changes in intensities of spectral bands associated with lignin and carbohydrates resulting from decay clearly indicated extensive deterioration of both the softwood and hardwood samples and the carbohydrates appear to be more deteriorated than the lignin. The biodeterioration of the archaeological timbers

followed a pattern of initial preferential loss of carbohydrates causing significant loss of cellulose and hemicellulose, followed by the degradation of lignin.

Keywords FT-Raman · Waterlogged wood · Archaeological oak · Archaeological pine · Greece

Introduction

Fourier-transform Raman spectroscopy has increasingly been applied to the characterisation of lignocellulosics [1]. The advent of FT-Raman spectroscopy and the introduction of the near-infrared laser source (excitation wavelength, 1064 nm) have helped to overcome difficulties presented by conventional Raman spectroscopy, particularly fluorescence [2]. Several applications of FT-Raman spectroscopy to complex biological matrices, such as wood, have been reported [1, 3, 4]. FT-Raman spectroscopy has been used to examine and assess organic plant material, identifying characteristic bands of wood and its chemical components such as carbohydrates, sugars, extractives and resins [5, 6].

An FT instrument is built around an interferometer, and several advantages associated with the FT approach have been reported [2]. The nondestructive analysis and the minute sample sizes are important benefits, especially when dealing with archaeological material, as well as the ease of sample handling, since most solid samples can be measured directly without extensive sample preparation [7], and can afterwards be reused for further analysis or returned to an archive.

The use of FT-Raman spectroscopy to distinguish between hardwoods and softwoods has been demonstrated

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in fresh wood [8, 9]. There have been numerous applications of the technique on fresh/sound lignocelluloses, but there has only been moderate use of FT-Raman spectroscopy to examine waterlogged archaeological material [9]. For example, FT-Raman spectroscopy has been used to study the changes to sixteenth-century wood exposed to accelerated ageing, and the effects of the exposure of historical wood (sixteenth-century wooden beam) to sodium hydroxide solution and high temperatures at various time intervals have also been investigated [11, 12]. The chemical characterisation of waterlogged archaeological wood is of great importance, as it can not only reveal the degradation processes that have occurred but it can also direct conservation practices. For example, the identification may guide the appropriate conservation methods or dictate the molecular weight of polyethylene glycol used in the treatment, and an understanding of the preservation of excavated material may assist in the proper handling of archaeological timbers [10].

General vibration band assignments for fresh wood have been reported as follows. The wood components as cellulose, hemicelluloses and extracts appear in the region 2500–3500 cm^{-1} . The region 1500–1800 cm^{-1} is the most informative for lignin. While they often overlap, peaks in the region 1000–1500 cm^{-1} are informative for cellulose and hemicellulose. Finally, the low-frequency Raman spectral region between 250–1000 cm^{-1} has the holocelluloses and extractives as its main contributors [13].

Here, an FT-Raman spectroscopic study is presented that explores the changes in the chemistry of waterlogged archaeological pine wood (*Pinus* sp.) and oak (*Quercus* sp.) from the Neolithic site of Anargyroi, on the margins of Cheimatidis Lake in northern Greece (Fig. 1). The archaeological samples were identified at genus level, based on their anatomy. The pine was most likely to be either *Pinus sylvestris*, *Pinus nigra*, *Pinus peuce*, *Pinus leucodermis* or *Pinus pinea*, and the oak was most likely to be either *Quercus cerris*, *Quercus aegilops* or *Quercus trojana*. The material could not be identified at the species level due to the poor preservation state of the material. All of the abovementioned species are represented in both the Neolithic and modern pollen profiles of the area [10]. Extended analysis of the assemblage has been carried out and the preservation state of the material was determined through electron microscopy and selected physical parameters (such as maximum moisture content and density). Biodeterioration was diagnosed in all samples [10, 14]. FT-Raman spectral features of biodeteriorated wood were associated with the depletion of lignin and/or carbohydrate polymers at various stages of degradation. In addition to the data for the archaeological wood, spectra of sound wood from the same genus were obtained (Fig. 1). Fresh samples of *Pinus peuce* Griseb. and *Quercus trojana* were selected.

Methods

Fourier-transform Raman spectra were obtained using a Bruker IFS66 instrument with an FRA 106 Raman module attachment and Nd/YAG laser excitation at 1064 nm. 180° scattering was used in the sample illumination geometry. Spectra were recorded over the wavenumber range 50–3500 cm^{-1} at 4 cm^{-1} spectral resolution with 1000 scans accumulated, and the laser powers used were between 0 mW and 180 mW. This range of powers is designed to accommodate the recording mode, which commonly starts at the bottom and gradually increases in power until satisfactory spectra are achieved without damaging the specimen. The wavenumber positions of sharp bands are accurate to $\pm 1 \text{ cm}^{-1}$. The Opus software, provided by Bruker, was used to collect spectral data and determine the peak positions. To investigate the spectral differences associated with the deterioration in the samples, compared with equivalent modern samples, a peak component analysis procedure using Galactic GRAMS/386 software was employed. Sample preparation was minimal and involved only preparing a flat surface for the wood which could then be introduced into the instrument.

A selection of three oak (Fig. 2) and three pine (Fig. 3) waterlogged archaeological samples were analysed. According to the analysis of the maximum moisture contents and densities of the samples, they can be considered to be highly degraded—classified as group I or II, suggesting extended deterioration of the wooden mass with little solid core remaining (Table 1).

Sample preparation was kept to a minimum and included only air drying. Provided that the surface of the wood was flat, the sample was inserted into the analyser; otherwise a flat surface was produced using a razor blade. Spectra of fresh wood samples from the same genera were also collected for comparison. The fresh wood samples were processed in the same way as the archaeological samples. The modern wood samples used were selected from heartwood. Heartwood is normally considered superior in hardness, strength, toughness and degradation resistance to equally sound sapwood from the same log [15], so it is preserved in all archaeological samples.

Results

FT-Raman spectroscopy was used to identify the depletion of carbohydrates from different archaeological samples at various stages of deterioration. All compounds of the wood structure exhibit bands in the FT-Raman spectrum that can be assigned. It has been demonstrated in relevant applications of Raman spectroscopy to archaeological material that as cellulose, hemicellulose and lignin

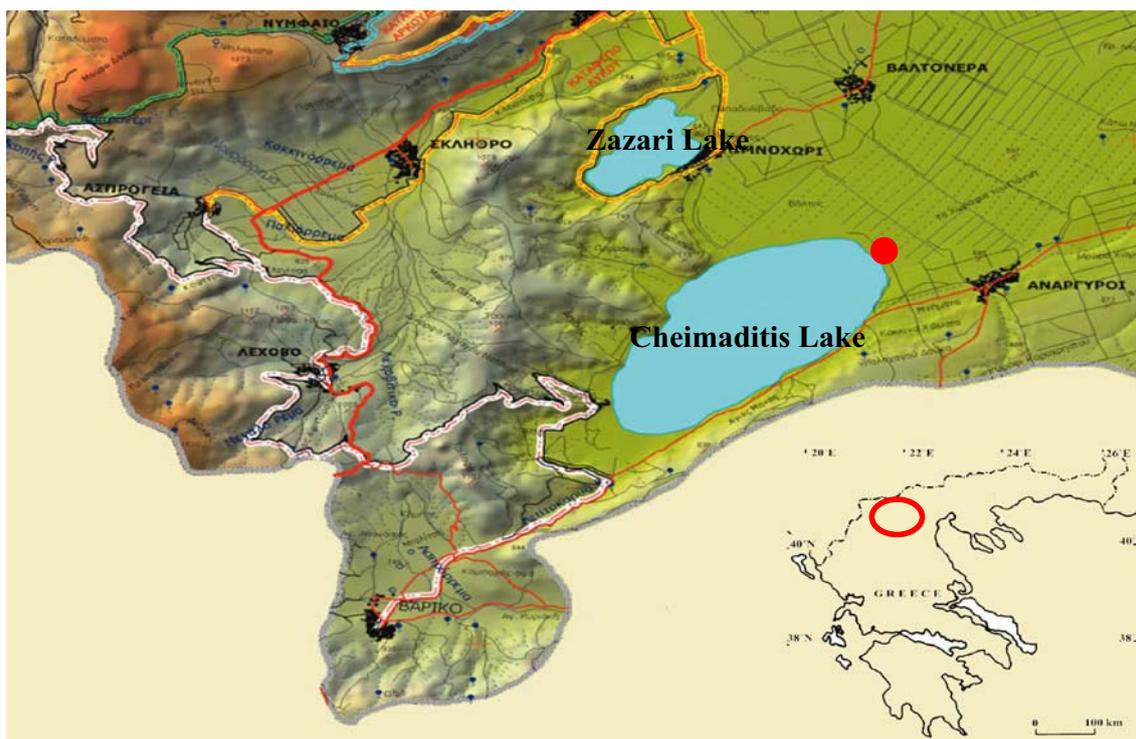


Fig. 1 The area of Cheimaditis Lake where the Anargyroi site is located (Source: [10])

deteriorate, their characteristic peaks in the spectra typically reduce in intensity or completely disappear [6], and this was observed in the archaeological wood specimens from Anargyroi. There are reports of variations in the peaks of cellulose, hemicellulose and lignin present in softwoods and hardwoods [14], as summarised in Table 2. The spectra presented here refer to the two genera of wood (oak and pine) identified in the archaeological assemblage, and are compared with spectra for sound wood from the same taxa.

Oak samples

According to reports of fresh modern wood samples [16–20], oak is expected to have an intense peak at 2893 cm^{-1} , coinciding with α -cellulose and hemicellulose, with a shoulder at 2936 cm^{-1} relating to the presence of extractives and hemicellulose (Fig. 4). In the archaeological samples (see Fig. 2), these two peaks were very weak, indicating that significant degradation of α -cellulose, hemicellulose and extractives had occurred. The expected

Fig. 2 Archaeological oak samples analysed: **b** A.07.04; **c** A.03.04; **d** A.01.04



Fig. 3 Archaeological pine samples analysed: **b** A.06.04; **c** A.02.05; **d** A.05.04



very intense peak with a shoulder at $1601/1657\text{ cm}^{-1}$ [21] attributed to Indulin AT lignin, was present with a poor low-wavenumber shoulder in the archaeological samples (Fig. 4). This indicated that lignin was not completely degraded. The overlapping cellulose and hemicellulose peaks at $1096/1125\text{ cm}^{-1}$ had completely disappeared, highlighting the degradation of cellulose and hemicellulose mentioned above. The phenol mode expected at 1190 cm^{-1} , the lignin-carbohydrate complex peak at 1271 cm^{-1} , the cellulose peak at 1341 cm^{-1} and the very weak lignin and extractives peak located at 1297 cm^{-1} had also all disappeared. In the low-frequency region of the Raman spectra of archaeological oak wood samples, the shoulders on the cellulose peaks located at $353/380$ and $436/459\text{ cm}^{-1}$ and the cellulose and hemicellulose peak at 897 cm^{-1} had disappeared. The hemicellulose peaks at $496/521\text{ cm}^{-1}$ had also shifted to 538 cm^{-1} and decreased in band intensity.

The spectra of the three archaeological oak specimens are therefore consistent with moderately extensive degradation, with some hemicellulose, α -cellulose and possibly some extractives remaining (2893 cm^{-1} and 2936 cm^{-1}). The lignin appears to be less degraded than the carbohydrates ($1601/1657\text{ cm}^{-1}$) and gives the strongest peak. In comparison with the reference material for

fresh hardwood samples, the archaeological samples A.01.04 and A.03.04 (see arrows in spectra d and c, respectively, in Fig. 4) are extensively degraded with little lignin and few carbohydrates remaining. Sample A.07.04 (spectrum b) appears to have lost less carbohydrate and lignin than the other specimens; however, it is also extensively deteriorated. This could be attributed to spatial variations in the depositional environment and the durability of the specific timber. FT-Raman spectra may vary from one part of the tree to another, between heartwood and sapwood [10].

Pine samples

The lignin band at 1599 cm^{-1} was the only remaining distinctive feature of the spectra for the archaeological specimens of pine. Pine timber would be expected to have an intense peak at 2893 cm^{-1} coinciding with α -cellulose and hemicellulose with a shoulder at 2936 cm^{-1} relating to the presence of extractives and hemicellulose [16–18, 20, 22]. These two peaks had disappeared in all of the archaeological samples, indicating the degradation of α -cellulose, hemicellulose and extractives. The expected very intense peak with a shoulder located at $1601/1657\text{ cm}^{-1}$

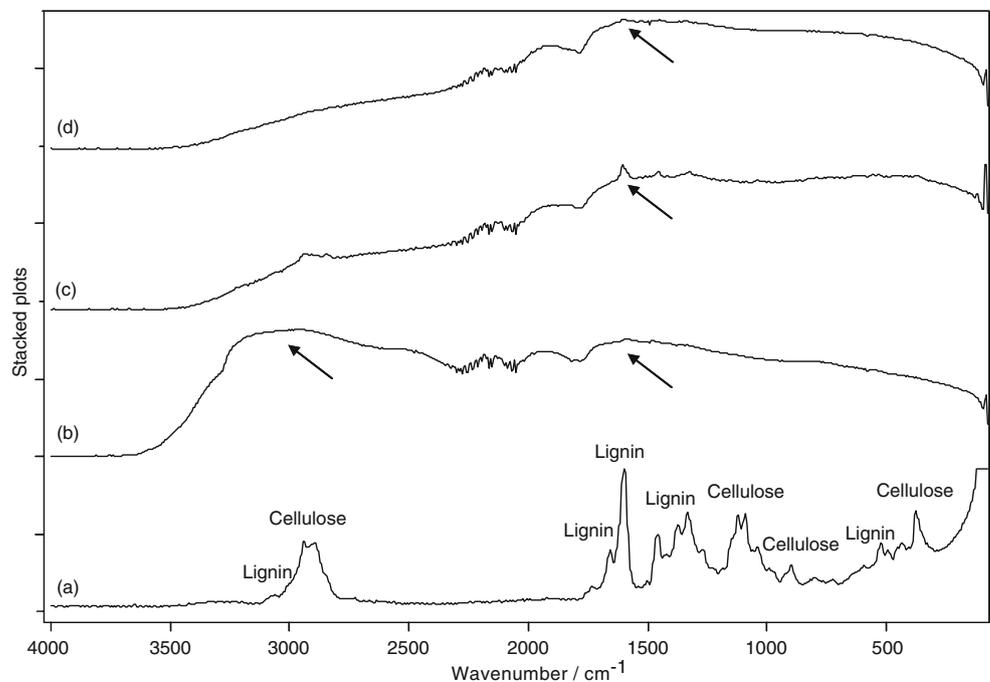
Table 1 Maximum moisture contents (%) and densities of the waterlogged samples, and subsequent classification of the preservation state of the wood

Samples	Maximum moisture content (%)	Density (g/cm^3)	Classification of the wood
A.07.04	592	0.148	Group I
A.03.04	397	0.154	Group II
A.01.04	745	0.154	Group I
Fresh oak <i>Quercus trojana</i> Webb	111	0.549	
A.06.04	445	0.146	Group I
A.02.04	606	0.130	Group I
A.05.04	351	0.250	Group II
Fresh pine <i>Pinus peuce</i> Griseb	144	0.460	

Table 2 FT-Raman band assignments for sound/fresh hardwood and softwood (sources: [26–32])

Band (cm ⁻¹)	Band assignment	Wood type (softwood/hardwood)	Band (cm ⁻¹)	Band assignment	Wood type (softwood/hardwood)
3068	Lignin	S	1270	Rosin	S
3020	Lignin	H	1269	Rosin	S
2939	Extractives / hemicellulose	H	1190	Phenol mode	S
2936	Extractives / hemicellulose	S	1185	Holocellulose	S+H
2907	Cellulose and hemicellulose	H	1180	Polystyrene butadiene	S+H
2900	Cellulose	S+H	1168	Cellulose	S+H
2895	Holocellulose	S	1125	Cellulose and hemicellulose	S
2893	Cellulose and hemicellulose	S	1119	Lignin and holocellulose	S+H
1736	Holocellulose	H	1118	Holocellulose	S+H
1657	Lignin	S	1096	Cellulose and hemicellulose	S
1650	α -Carbonyl groups coplanar with the adjacent aromatic ring	S	1093	Lignin	S+H
1601	Lignin (aromatic ring motion) or polystyrene butadiene	S	1050	Lignin	H
1596	Lignin aromatic ring	S	1009	Cellulose	S+H
1455	Lignin-carbohydrate complex	S	897	Holocellulose	H
1379	Hemicellulose	S+H	895	Crystalline and/or amorphous cellulose	S+H
1377	Holocellulose	S+H	524	Holocellulose	H
1341	Cellulose/hemicellulose/lignin-carbohydrate	S	521	Hemicellulose	S+H
1333	Holocellulose	H	496	Hemicellulose	S+H
1327	Lignin	S+H	459	Cellulose	S+H
1318	Xylan	S+H	436	Cellulose	S+H
1312	Hemicellulose	S+H	380	Cellulose	S+H
1297	Lignin	S+H	376	Holocellulose	S+H
1272	Rosin	S+H	372	Holocellulose	H
1271	Lignin-carbohydrate complex	S			

Fig. 4 FT-Raman spectra of oak: *a*) Modern *Quercus trojana* Webb (highlighting peaks of cellulose and lignin); *b*), archaeological sample A.07.04; *c*), archaeological sample A.03.04; *d*), archaeological sample A.01.04. Arrows indicate the characteristic peaks of lignin and carbohydrates remaining in the degraded archaeological specimens [16–22]



attributed to Indulin AT lignin [22] had shifted to 1598 cm^{-1} , indicating moderately extensive degradation of the lignin. The overlapping cellulose and hemicellulose peaks at $1096/1125\text{ cm}^{-1}$ have completely disappeared, highlighting the degradation of cellulose and hemicellulose mentioned above (Fig. 5).

The phenol mode expected at 1190 cm^{-1} , the lignin-carbohydrate complex peak at 1271 cm^{-1} , the cellulose peak at 1341 cm^{-1} and the very weak lignin and extractives peak located at 1297 cm^{-1} had also all disappeared. In the low-frequency region of the Raman spectra of the archaeological pine samples, the cellulose peaks located at $353/380$ and $436/459\text{ cm}^{-1}$, the hemicellulose peaks at $496/521\text{ cm}^{-1}$ and the cellulose and hemicellulose peak at 897 cm^{-1} had all disappeared. All samples showed moderately extensive degradation, with only some lignin remaining ($1601/1657\text{ cm}^{-1}$).

The FT-Raman spectra of the archaeological pine samples showed extensive degradation where the cellulose, hemicellulose, holocellulose, extractives and phenols had all completely deteriorated, leaving a highly degraded lignin (Indulin AT lignin) structure. For the archaeological pine samples, it is confirmed that all samples are extensively degraded with little lignin and few carbohydrate components remaining (Fig. 5, arrows). A small variation was observed in the extent of degradation between the samples. FT-Raman spectra were descriptive and highlighted this minor variation, where the sample A.06.04 (Fig. 5, spectrum b) appeared more robust than A.02.04 (spectrum c) and A.05.04 (spectrum d). As underlined by the oak samples, the FT-Raman spectra may vary from one part of

the timber to another, particularly between heartwood and sapwood, because heartwood is generally more resistant to decay than sapwood [10]. However, there is little literature and research work on these variations within archaeological waterlogged wood, and the assemblage used in this work could not support such a hypothesis. This could therefore be a future direction in the application of FT-Raman spectroscopy to archaeological samples.

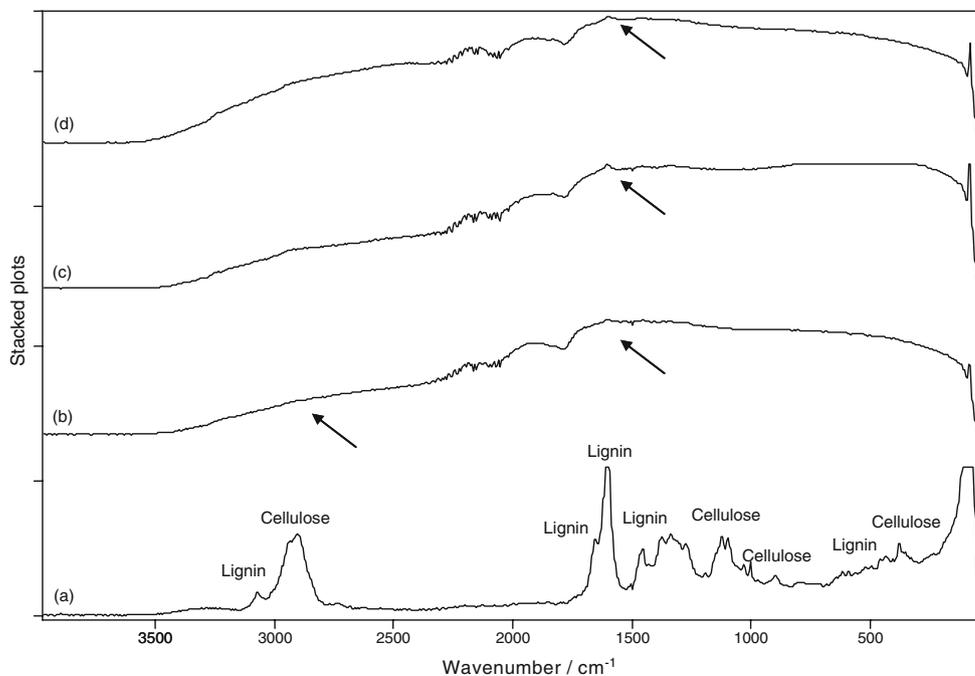
Comparing the data for the two species of archaeological wood, oak and pine, it was difficult suggest whether the hardwood was more durable than the softwood. However, statistical analysis of the results, or the application of analytical techniques that can be both supplementary and complementary to FT-Raman, for example FTIR, may facilitate such interpretations in the future.

Conclusions

The FT Raman results from this study cannot be considered quantitative since that would have involved the addition of a known concentration of an additive, noninterfering substance to the wood during analysis to compensate for fluctuations in laser power, geometric illumination conditions between different samples, and sample reflectivities over the range of specimens studied. At best the results are semi-quantitative, in that relative band intensities can be compared from sample to sample to evaluate the presence or otherwise of different components in systems subject to change.

FT-Raman spectroscopy, which has been applied mostly in the field of lignin chemistry for pulp and paper

Fig. 5 FT-Raman spectra of pine samples. *a*, Modern *Pinus peuce* Griseb.; *b*, archaeological sample A.06.04; *c*, archaeological sample A.02.05; *d*, archaeological sample A.05.04. Arrows indicate the remaining characteristic peaks of lignin and carbohydrates [16–22]



processes, could be a powerful tool to investigate structural transformations in the chemical constituents of archaeological wood. This spectroscopic study has identified extensive deterioration of both the pine and oak archaeological samples from the Neolithic site of Anargyroi. A comparison of the relative changes in the intensities of spectral bands associated with lignin and carbohydrates resulting from decay clearly indicated that extensive deterioration had occurred in both the softwood and hardwood samples. The biodeterioration of the archaeological samples followed a pattern of an initial preferential loss of carbohydrates, causing significant loss of cellulose and hemicelluloses, followed by degradation of the lignin. These observed degradation features in the archaeological samples could be attributed to both fungi and bacteria. There is no clear evidence in the FT-Raman results that suggests biotic or abiotic degradation processes. The reductions of the peaks from the components could also be attributed to the leaching of the organic components from the wood system during burial, reactions of the components in the buried environment, or hydrolysis in water. However, supportive electron microscopy has revealed extensive microbial activity. Microbial degradation is attributed to bacteria (like erosion and tunnelling bacteria) as well as Ascomycota and Deuteromycota (which cause soft-rot decay) that have been reported to initially attack the cellulose-rich secondary wood cell walls following minor degradation of the lignin-rich middle lamella region [23–25].

The decomposition, leaching and alteration of the wood cell wall compounds are directly connected with the weakness of the sample, which is likely to collapse with even basic handling. In order to select the appropriate conservation methods (including proper handling) and secure the preservation of this vulnerable excavated cultural property, the processes of deterioration must be well understood [10].

This study shows that FT-Raman spectroscopy can be regarded as an efficient tool for identifying the degradation processes in archaeological wood. However, the technique should be combined with other analytical techniques that can be both complementary and supplementary and may facilitate further insight into the chemistry of waterlogged archaeological wood. Minimum sample preparation, which may include simply drying, is an important task, given that such samples can then be reused in further analyses. However, extensive care, expertise and effort are required when processing and interpreting FT-Raman spectra. This study points to significant research potential in the application of FT-Raman spectroscopy to archaeological wood samples. A larger number of archaeological and reference samples can be examined in order to establish the potential of the Fourier-transform Raman technique in assessing the degradation of wood components. It should

also be highlighted that extensive research work is required in the analysis of archaeological wood at various specified stages of deterioration with the aim of creating a classification of groups and spectral features for comparison and reference purposes. Within such an integrated approach, chemometric techniques should be applied, and these may be useful for extracting systematic information and optimizing the extraction of information from FT-Raman analyses of archaeological lignocellulosic materials.

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