STUDY ON THE DECAY EXTENT OF WOODEN COMPONENTS OF DANXIA TEMPLE ANCIENT BUILDING BY POLARIZED LIGHT, FLUORESCENCE AND X-RAY DIFFRACTION METHODS

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In the present study, the decay extent of wooden components from the Halls of Pilu and Tianran ancestor of Danxia Temple ancient buildings was observed by polarized light, fluorescence, and XRD methods. The findings of the investigation can be summarized as follows. Sample No. 1 was identified as red birch wood (*Betula albo-sinensis*) and sample No. 2 was identified as maple wood (*Pterocarya stenoptera*). The brightness of crystalline cellulose birefringence in the cell walls of vessels, wood rays and wood fibres, both in decayed red birch wooden components (DRBWC) and in decayed maple wooden components (DMWC), was not obvious, indicating that the cellulose was seriously consumed by fungi. However, the brightness of green fluorescence in the cell walls of both DRBWC and DMWC was very evident, indicating that the lignin was mainly retained or was not consumed by fungi. XRD analysis indicated that wood decay fungi did not change the structure and crystal layer distance of the crystallization zone, but the diffraction intensity decreased to a certain extent. The crystallinity of cellulose was reduced by 11.16%, from 43.29% to 38.46%, in DRBWC and by 52.16%, from 40.68% to 19.46%, in DMWC, demonstrating a reduction in crystalline cellulose. The degradation of cellulose in wooden components will eventually lead to a reduction in their load-bearing capacity. According to the tendency of brown rot fungi of consuming mostly cellulose and hemicelluloses while avoiding lignin, we concluded that both DRBWC and DMWC were seriously degraded by brown rot fungi, in addition to the attack of termites. The low resistance of the two wooden components to fungal decay and termites is the main cause of their deterioration. The results on the extent of decay will provide scientific data for the future conservation and restoration of the Danxia Temple relics.

Keywords: Danxia Temple ancient buildings, wooden components, wood identification, decay extent, brown rot fungi, bright-field light, polarized light, fluorescence

INTRODUCTION

The Danxia Temple, with its wooden structures, was originally built in about 824 A.D. It is located in Liushan Town, Nanzhao County, Nanyang City, Henan Province, China. It is a national cultural relics protection unit, and is one of the eight famous temples in Henan Province. It has very high historical, scientific and artistic value. However, some wooden components, especially the lower part of wooden pillars, have been degraded to different degrees under the influence of factors, such as ambient temperature and humidity changes, microorganisms, insects, light and so on, in the environment due to long-term exposure to air.[1-2] The degradation behaviors inevitably lead to changes of anatomical structures[2-8] and to the degradation of chemical composition.[2,8,9-12] The changes of anatomical structures eventually decreased the mechanical strength of wooden components and affected the safety and the life span of ancient buildings.[13]

Methods of polarized light, fluorescence[2,7-8] and X-ray diffraction (XRD)[14-15] are used to evaluate the decay extent of wooden components

in ancient buildings, due to the advantages of requiring a minimal number of samples and resulting in less damage to ancient buildings compared with conventional gravimetric techniques.

The goals of this study have been to gain an insight into the internal cause of decay, by identifying the tree species and determining the distribution and content of cellulose and lignin, observed by polarized light, fluorescence and XRD methods. Such observations would provide reliable data support and theoretical basis for scientific assessment of the extent of decay, and for reasonable protection and repair of wooden components of cultural relics in the future.

EXPERIMENTAL

Materials

Samples were collected from wooden components in the ancient building of Danxia Temple in Nanzhao County, Nanyang City, Henan Province, China. Birch wood (*Betula* sp.) was collected from the lower part of the wooden pillar in Pilu Hall, and maple wood (*Pterocarya* sp.) was collected from the lower part of the wooden pillar in Tianran ancestor's Hall with an increment borer (10-100-1027, Haglöf AB, Mora, Sweden).[1] The two pillars were gnawed seriously by termites, as could be identified by the naked eye. Control samples were obtained from about 10 years old living trees (30 cm diameter at breast height), at the height of 1 m from the roots using an increment borer.

Entrapment treatment of the sample

The entrapment treatment of the samples included the following steps.[2,7-8] First, the small samples were placed into a vacuum dryer to remove the air from the wood. Then, the samples were successively immersed into 20%, 40%, 60%, 80% and 100% polyethylene glycol (PEG molecular weight = 2000) aqueous solution. The process was conducted in an oven at 60 °C for 48 h for every gradient, while the 100% PEG aqueous solution was used twice. Next, the samples were embedded in PEG: they were placed at the bottom of an embedding mold, a 100% PEG aqueous solution was added, and the set-up was covered with a plastic embedding box. The embedding box was placed into a freezer for about 10 min.

Sample sectioning

The sectioning procedure included the following steps.[2,7-8,16] The embedded samples were sectioned with a microtome (HistoCore AUTOCUT, Leica). Transverse, radial and tangential sections were made of about 10-15 μm thickness. The slices were dehydrated with 50%, 75%, 95%, and 100% ethanol solution; each concentration treatment lasted for 10 min. Then, the slices were defatted with dimethylbenzene solution for 3 min, and sealed with neutral gum. To avoid the effect of dye on polarized light and fluorescence, the slices used for observation under polarized light and fluorescence were not dyed with safflower O dye.

Observations under polarized light and fluorescence

The deformation of cell walls was analyzed under bright-field light; the distribution and content of cellulose were analyzed under polarized light; while the distribution and content of lignin were analyzed under fluorescence (the blue light excitation) with a fluorescence microscope (ECLIPSE Ni-U, Nikon) for the analysis of the decay extent in the wooden components.[2,7-8,17]

X-ray diffraction (XRD) measurement

X-ray diffraction analysis (XRD) was used as a supplementary technique to polarized light microscopy to evaluate the degradation of cellulose in the samples. A diffractometer (Ultima IV, Rigaku, Japan) with Cu-Kα radiation (λ = 0.1542 nm) was used for the measurements at 40 kV and 40 mA. Scattered radiation was detected in the 2θ range of 10–60° at a rate of 1.8°/min. Generally, the crystalline bands at about 14.6–15.5°, 16.5–17.0°, 20.0–21.0°, 22–22.5° (2θ) are assigned to 101, $10\bar{1}$, 102, 002 crystallographic planes of cellulose I; those at about 18.5–19.2° being assigned to the amorphous part of cellulose with contributions from lignin and hemicelluloses.[14-15,18] The crystallinity index (*CrI*), which represents the relative content of crystalline cellulose, was calculated by the formula:[19]

$$CrI = \frac{I_{002} - I_{am}}{I_{002}} \times 100\% \quad (1)$$

where *CrI* is the crystallinity of cellulose, I_{002} is the peak intensity (002) at 2θ = 22.5°, and I_{am} is the peak intensity at 2θ = 18.0°.

RESULTS AND DISCUSSION

Anatomical structural characteristics and identification of tree species of samples

The properties of woods in the same genera vary to different degrees. Therefore, accurate identification of tree species can provide more effective support for material deterioration analysis.

From the microstructures in Figure 1, we found that porosity belongs to diffuse-porous wood; the vessels are oval to circular in outline; the vessel groupings are predominantly radial multiples of two to four cells and a few exclusively solitary; in addition, the vessel arrangement is in a radial pattern; no tyloses and deposits were observed in vessels from the transverse section (Fig. 1a). No helical

thickenings were displayed in vessel elements (Fig. 1b). Perforation plates belong to the scalariform type with ≤10 bars (Fig. 1b). Intervessel pits are mostly alternate and a few are alternate-opposite (Fig. 1c). Axial parenchymas are apotracheal in diffuse, diffuse-in-aggregates and boundary shape (Fig. 1a). Wall thickness of non-septate fibres ranges from thin to thick (Fig. 1a). Ray width is 2 to 3 cells, body ray cells are procumbent, with mostly 2 to 4 rows of upright and/or square marginal cells (Fig. 1b, c); no special cells were discovered in the rays (Fig. 1b, c); no axial and radial intercellular canals were found in the wood (Fig. 1a, c).

Figure 2 illustrates the microstructures of samples No. 2. The porosity of samples No. 2 belongs to diffuse-porous wood; the numbers of vessels with oval to circular outline are less than 5 vessels per square millimetre; the vessel groupings are predominantly exclusively solitary (90% or more); moreover, the vessel arrangement is in a diagonal pattern; no tyloses and deposits were discovered in vessels from the transverse section (Fig. 2a). No helical thickenings were found in vessel elements (Fig. 2c). Perforation plates belong to the type of simple perforation plates. Intervessel pits were alternate (Fig. 2c). Axial parenchymas are apotracheal in diffuse, diffuse-in-aggregates and a few bands less than three cells wide (Fig. 2a). Wall thickness of non-septate fibres ranged from thin to thick. Ray width is 1 to 2 cells, body ray cells are mainly procumbent, with mostly 2-4 rows of upright and/or square marginal cells; no special cells and crystals were found in these rays (Fig. 2b, c); no axial and radial intercellular canals were found in the wood samples (Fig. 2a, c).

Based on the observation of anatomical structural characteristics, the authors of this paper assumed that sample No. 1 belongs to birch wood (*Betula* sp.) and sample No. 2 belongs to maple wood (*Pterocarya* sp.).[1] According to the principle of "local selection" in the construction of ancient buildings, we consulted the "Names of Chinese Main Woods"[20] and "Chinese Timber Records",[21] in order to establish the main origin of the wood by the distribution of tree species, and it was concluded that red birch wood (*B. albo-sinensis*) and maple wood (*P. stenoptera*) are most widely distributed in Henan Province. Based on the analysis, we believed that sample No. 1 should be identified as *B. albo-sinensis* (Betulaceae), and sample No. 2 – as *P. stenoptera* (Juglandaceae).

Red birch wood has many attractive characteristics, such as straight grain, heavy basic density (about 0.58 g/cm^3), high hardness, high strength and high impact toughness. Nevertheless, it also has low resistance to wood decay fungi and termites.[21] Maple wood also has many outstanding characteristics, such as smooth structure, poor shrinkage, but low basic density (about 0.39 g/cm^3), low strength and low resistance to wood decay fungi and insects.[21] The low resistance of the two woods to decay and termites is one of the main reasons for materials' deterioration in the process of long-term use. The accurate identification of tree species provides a basis for the analysis of material deterioration and the subsequent protective treatment against decay and insects.

Bright-field light, polarized light and fluorescence analyses of decayed wooden components

In general, the distribution and content of the cellulose crystalline regions of cell walls in wood can be qualitatively determined by using a polarizing microscope. The higher the brightness of the crystalline cellulose birefringence is, the higher the relative content of cellulose is.[2,7-8,22] Meanwhile, the brightness of green fluorescence observed by the fluorescence microscope can provide the distribution and content of lignin.[2,7,17] Accordingly, the greater the brightness of green fluorescence intensity is, the higher the relative content of lignin is.[2,7-8,17,23]

Figure 3 shows a comparison of the microstructures under bright-field light, polarized light and fluorescence in DRBWC (Fig. 3a-c) and the birch model specimen – NDRBWC (Fig. 3d-f). Under the bright-field light (Fig. 3a, d), almost no deformations in the cell walls of vessels, wood rays and wood fibres in DRBWC (Fig. 3a) can be noted, compared to NDRBWC (Fig. 3d). The thickness of these cell walls did not become thinner than that in NDRBWC either. It was probable that the PEG treatment reinforced the cell walls of vessels, wood rays and wood fibres in DRBWC.[2,7] Under polarized light, the brightness of crystalline cellulose birefringence in the cell walls of vessels, wood rays and wood fibres in DRBWC (Fig. 3b) was not obvious, compared to the model specimen (Fig. 3e), demonstrating that cellulose in DRBWC was seriously consumed by the decay fungi.

Under fluorescence, the brightness of green fluorescence in the cell walls of vessels, fibres

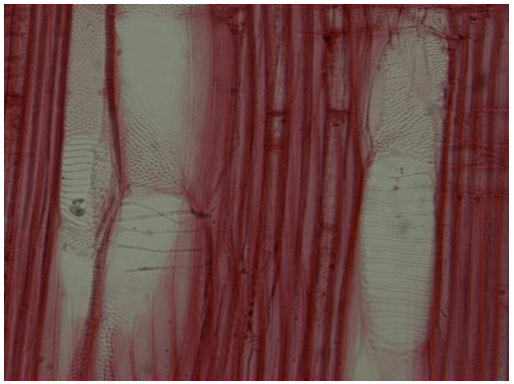
and rays in DRBWC (Fig. 3c) was very apparent as in the model specimen (Fig. 3f), implying that lignin in DRBWC was abundantly retained or was not consumed by decay fungi. Under the polarized light (Fig. 3b, e), the brightness of crystalline cellulose birefringence in the cell walls of vessels, wood rays and wood fibres in DRBWC (Fig. 3b) was not obvious, compared to NDRBWC (Fig. 3e), indicating that the cellulose in DRBWC was seriously consumed by decay fungi. Under the fluorescence (Fig. 3c, f), the brightness of green fluorescence in the cell walls of vessels, fibres and rays in DRBWC (Fig. 3c) was very evident, as in NDRBWC (Fig. 3f), indicating that the lignin in DRBWC was abundantly retained. That is, the lignin was not consumed by rot fungi. The result is consistent with those of FTIR analysis of DRBWC.[1]

As a rule, brown rot fungi can consume cellulose and hemicelluloses, and retain lignin; while white rot fungi can consume not only cellulose and hemicelluloses, but also lignin. According to the observation under polarized light and fluorescence (Fig. 3b, c), we concluded that the cellulose was seriously consumed by brown rot fungi and the lignin was retained in DRBWC. In addition, the micro-area distribution of lignin in the cell walls of wood fibres was analyzed. We found that the brightness of green fluorescence in the cell corner (CC) was stronger than those in the middle lamellar layer (CML layer) and secondary wall layer (S layer) both in DRBWC (Fig. 3c) and NDRBWC (Fig. 3f). The stronger brightness of green fluorescence observed in CC suggested that these regions contained a relatively higher content of lignin than the adjacent CML and S layers. This finding is consistent with the results reported on the micro-area distributions of lignin in other wood species.[24-34]

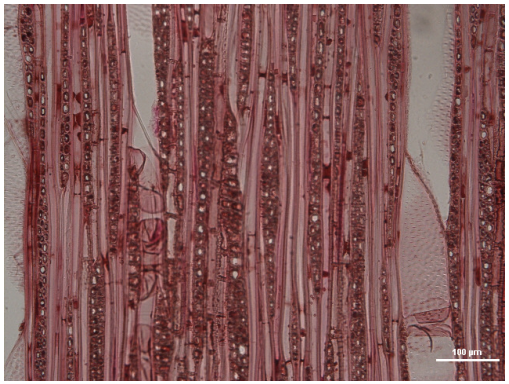
Figure 4 shows a comparison of the microstructures in DMWC (Fig. 4 a-c) under bright-field light, polarized light and fluorescence, compared to the maple model specimen (NDMWC) (Fig. 4 d-f). Under bright-field light (Fig. 4 a and d), part of deformations in the cell walls of vessels, wood rays and wood fibres in DMWC (Fig. 4 a) are revealed, compared to NDMWC (Fig. 4 d), showing that the samples were attacked by fungi. Under polarized light (Fig. 4 b and e), the brightness of crystalline cellulose birefringence in the cell walls of vessels, wood rays and wood fibres was not obvious in DMWC (Fig. 4 b), compared to NDMWC (Fig. 4 e), indicating that the cellulose in these cell walls was seriously consumed by rot fungi. However, the brightness of crystalline cellulose birefringence in vessels was higher than that in wood rays and wood fibres (Fig. 4b), indicating that the cellulose in wood rays and wood fibres was more seriously consumed by rot fungi than that in vessels. In general, the cell walls of vessels contain abundant guaiacyl lignin, whereas those of wood fibres contain abundant syringyl lignin contents, the resistance of guaiacyl lignin to decay is generally higher than that of syringyl lignin. Due to the property of the higher resistance to decay in guaiacyl lignin, the cell walls of vessels showed more evident brightness of crystalline cellulose birefringence than that of wood fibres in DMWC (Fig. 4 b). Under fluorescence (Fig. 4 c and f), the brightness of green fluorescence in the cell walls of vessels, fibres and rays was very evident in DMWC (Fig. 4 c), similarly to NDMWC (Fig. 4 f), indicating that abundant lignin in these cell walls was retained, that is, lignin was not consumed by decay fungi. The result was consistent with those of FTIR analysis of DMWC.[1]

According to the tendency of brown rot fungi towards consuming cellulose and hemicelluloses, while avoiding lignin, and considering the findings of polarized light (Fig. 4 b) and fluorescence (Fig. 4 c) observation, we concluded that the cellulose was consumed by brown rot fungi and the lignin was retained in DMWC. The result was consistent with those of FTIR analysis.[1] In addition, the micro-area distribution of lignin in the cell walls of wood fibres was analyzed in this paper. We found that the brightness of green fluorescence in all of CC, CML layer and S layer was strong both in DMWC (Fig. 4c) and NDMWC (Fig. 4 f), showing that all of CC, CML layer and S layer retained a relative high content of lignin.

a) Transverse section

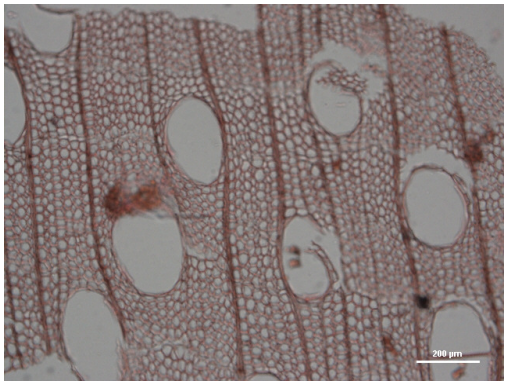


b) Radial section

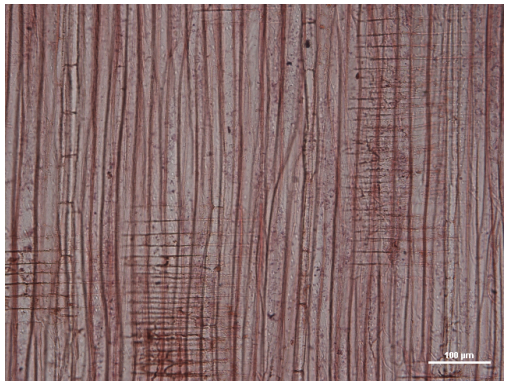


c) Tangential section

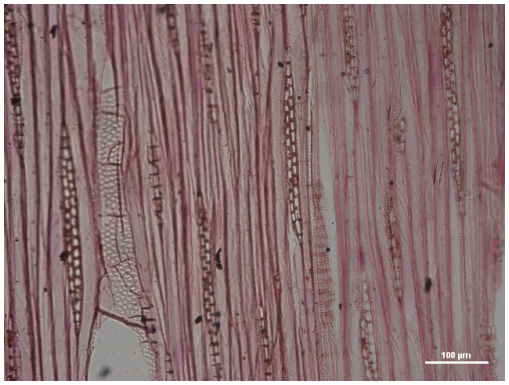
Figure 1: Microstructures of sample No. 1 under bright-field light



a) Transverse section

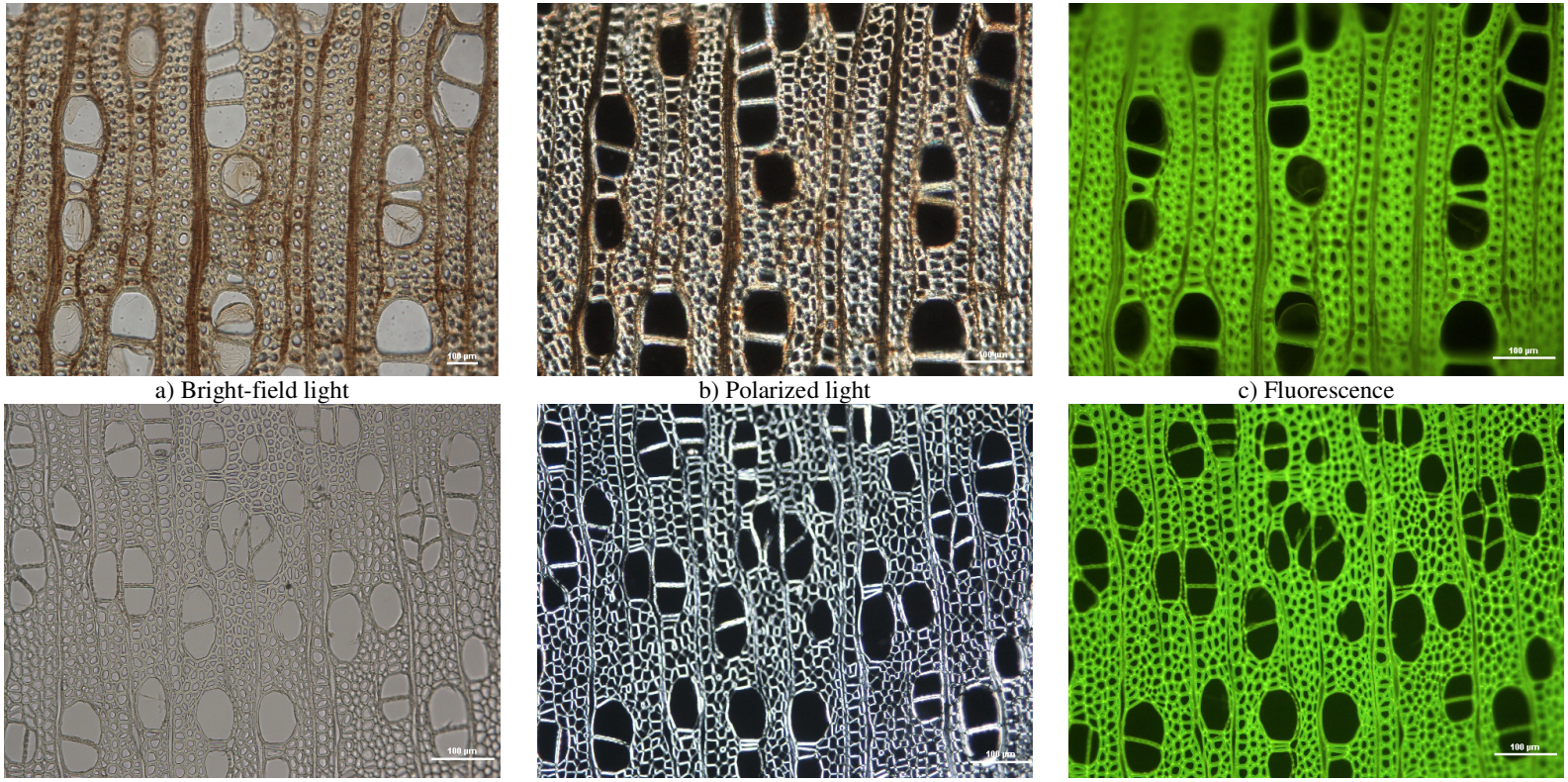


b) Radial section



c) Tangential section

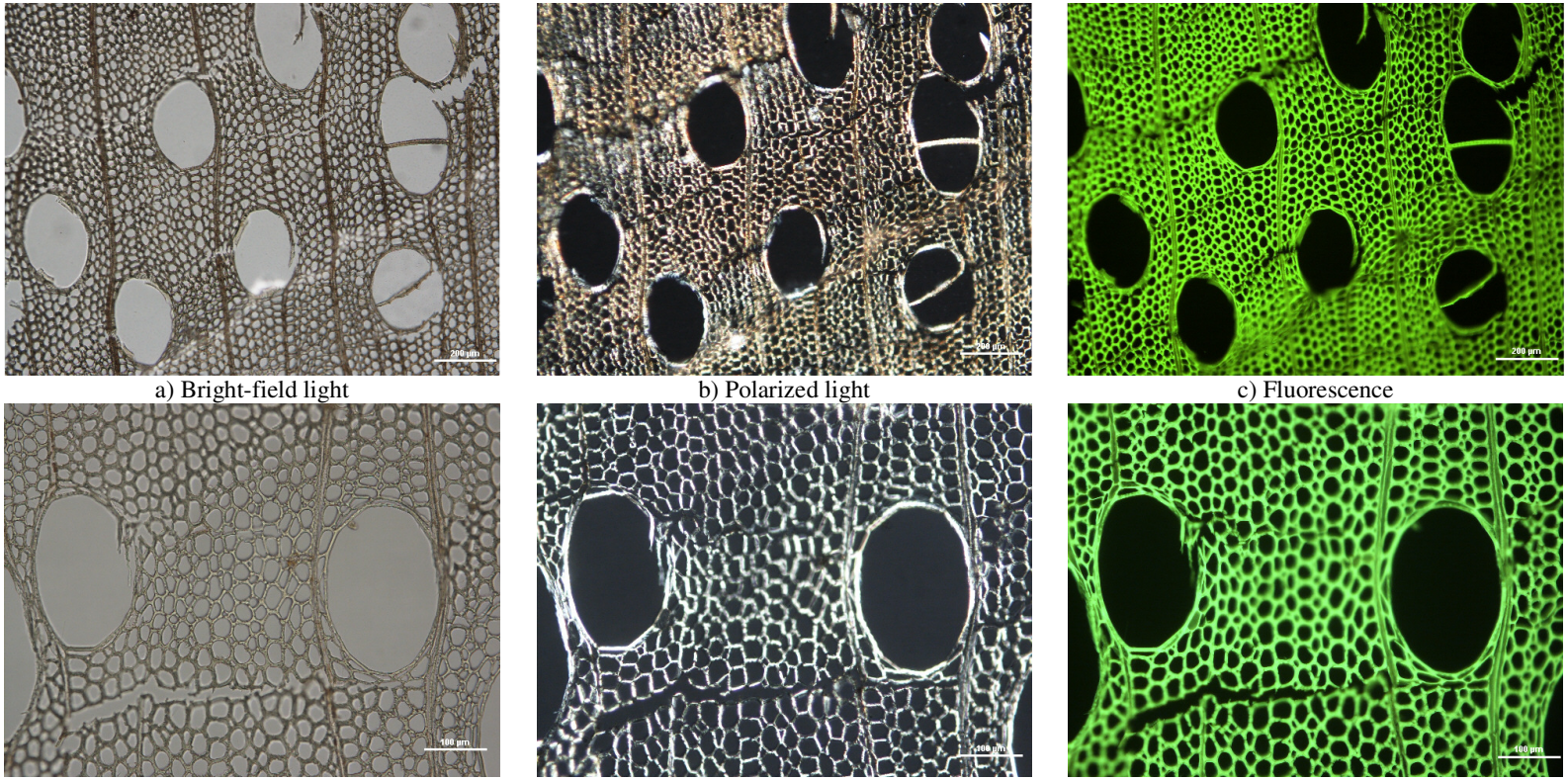
Figure 2: Microstructures of sample No.2 under bright-field light



a) Bright-field light b) Polarized light c) Fluorescence

d) Bright-field light e) Polarized light f) Fluorescence

Figure 3: Microstructures in transverse sections of birch wood under bright-field light, polarized light and fluorescence
(a), (b), (c): sample No. 1; (d), (e), (f): model specimen



a) Bright-field light b) Polarized light c) Fluorescence

d) Bright-field light e) Polarized light f) Fluorescence

Figure 4: Microstructures in transverse sections of maple wood under bright-field light, polarized light and fluorescence
(a), (b), (c): sample No. 2; (d), (e), (f): model specimen

XRD analysis of decayed wooden components

The crystallinity of wood refers to the percentage of the crystalline structure in the total comprising both crystalline and amorphous regions of cellulose, which reflects the degree of crystallinity of cellulose. There is a positive correlation between the crystallinity of cellulose and the mechanical strength of wood. The crystallinity of wooden components of ancient buildings decreased with the extent of wood decay, resulting in the reduction of mechanical properties. Therefore, the degree of crystallinity can be used to assess the material's deterioration in wooden components from ancient buildings.

The shapes of XRD diffraction patterns of DRBWC and DMWC were consistent with those of the model specimens, as can be seen in Figure 5 and Figure 6. The diffraction peak position of the 002 crystallographic plane is still concentrated around 22°, indicating that wood decay fungi did not modify the crystal layer distance in the crystalline region, but decreased the diffraction intensity to a certain extent. The crystallinity of cellulose was reduced by 11.16%, from 43.29% to 38.46%, in DRBWC and by 52.16%, from 40.68% to 19.46%, in DMWC (Table 1), demonstrating a clear reduction in crystalline cellulose and thus in mechanical strength. The results are consistent with the results of FTIR analysis[1] and the polarized light microscopy observations discussed earlier in this study (Fig. 3b and Fig. 4b).

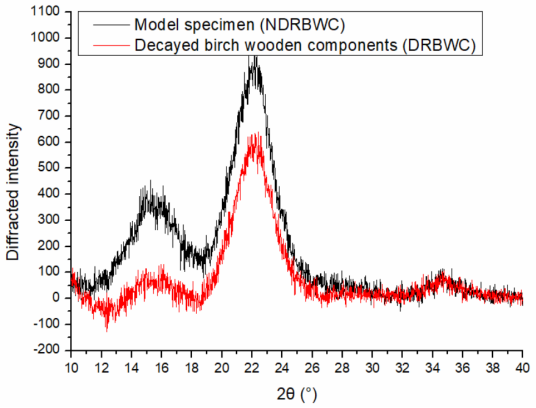


Figure 5: X-ray diffraction patterns of red birch wood

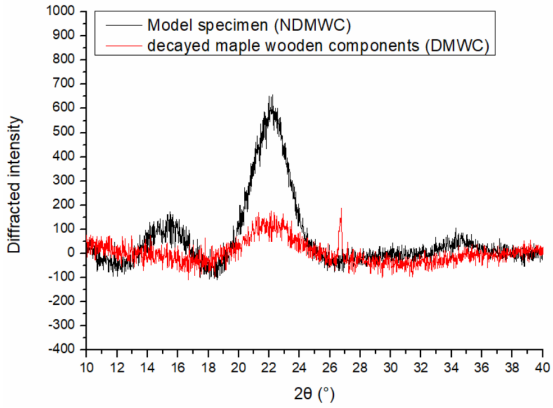


Figure 6: X-ray diffraction patterns of maple wood

Table 1
Average crystallinity values of samples

Samples		2θ	Crystallinity (%)
Red birch wood	Control	22.32	43.29
	RDRBWC	22.98	38.46
Maple wood	Control	22.24	40.68
	DMWC	22.35	19.46

CONCLUSION

The decay extent of wooden components in Pilu Hall and Tianran ancestor's Hall of Danxia Temple ancient buildings was determined by polarized light, fluorescence and XRD methods. The findings have led to the following conclusions.

(1) Based on the observation of anatomical structural characteristics and principle of "local selection" of construction materials in ancient buildings, it can be concluded that sample No. 1 should be red birch wood (*B. albo-sinensis*) and sample No. 2 should be maple wood (*P. stenoptera*). The low resistance of the two woods to fungal decay and termites is one of the main reasons for materials' deterioration in the process of long-term service.

(2) Though almost no deformations in the cell walls of vessels, wood rays and wood fibres in DRBWC and DMWC were noted, the brightness

of crystalline cellulose birefringence in these cell walls was not obvious, indicating that the cellulose in DRBWC and DMWC was seriously consumed by rot fungi. Meanwhile, the brightness of green fluorescence in these cell walls was very evident, indicating that the lignin in DRBWC and DMWC was not consumed by rot fungi.

(3) XRD analysis indicated that wood decay fungi did not change the structure and crystal layer distance of the crystalline regions of DRBWC and DMWC, but decreased the crystallinity of cellulose to different degrees.

Overall, both DRBWC and DMWC were not only seriously damaged by termites, but were largely affected by brown decay fungi. The degradation of wooden components in the ancient buildings of Danxia Temple will eventually lead to a reduction in their load-bearing capacity. Research into the extent of material deterioration will provide scientific data for future protection and repair.

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