International Biodeterioration & Biodegradation 63 (2009) 371–378

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Fungal colonization on excavated prehistoric wood: Implications for in-situ display

A. Pournou [a,*], E. Bogomolova [b]

[a] Department of Conservation of Antiquities and Works of Art, Technological Educational Institute of Athens, Ag. Spyridonos, Aegaleo, 12210 Athens, Greece
[b] Laboratory of Systematics and Geography of Fungi, V.L. Komarov Botanical Institute of Russian Academy of Sciences, Prof. Popov Str. 2, 197376, St. Petersburg, Russia

ARTICLE INFO

Article history:
Received 20 July 2008
Received in revised form
2 November 2008
Accepted 3 November 2008
Available online 17 March 2009

Keywords:
Fungi
Prehistoric
Archaeological wood
In-situ display

ABSTRACT

Excavations at the Neolithic settlement of Dispilio, Greece, have revealed significant amounts of waterlogged wood, some of which is being considered for in-situ display. This study investigated the presence of fungi and their biodeterioration patterns on the excavated material. Data will be used to assess the current threat from degradation and to aid in the design of appropriate control measures for any future display strategy. Fungi were isolated using different culture media and species were identified from slide cultures using light and fluorescence microscopy. Wood micro-morphology was examined using scanning electron and light microscopy. Seventeen fungal species were identified, all of which are typical terrestrial species, suggesting that they have developed in the exposed, post-excavation environment, rather than under waterlogged burial conditions. According to the literature many of the isolated species are potential wood deteriogens. In addition to fungi, abundant bacterial growth was observed in most samples. Microscopic examination of wood cells showed patterns of decay associated with soft-rot fungi, and tunnelling and erosion bacteria, suggesting past attack related to the waterlogged burial environment. The results obtained indicated fungal colonization of exposed timbers, presenting a potential threat to their long-term survival. Any in-situ display strategy, such as continuous or periodic water-spraying of the excavated wood, should include measures to monitor and control fungal growth.

1. Introduction

The prehistoric settlement of Dispilio was discovered in the early 1930s beside the lake of Kastoria, in northwestern Greece, following a fall in water level (Hourmouziadis, 2002; Whitley, 2004). Evidence showed it was in use from the middle–late Neolithic to the early Iron Age and was constructed on wooden platforms, supported by long piles, which were driven into the lake sediment.

To date, Dispilio is the only lakeside settlement systematically excavated in Greece, offering a unique opportunity to study wood unearthed from this period (Chatzitoulousis, 2006). Recent excavations have revealed further horizontal and vertical wooden structural elements consisting of wooden beams, piles, and branches (Fig. 1).

The excavation site is characterised by high annual fluctuations in pH and Eh, resulting in an environment that ranges from highly reducing to moderately oxidizing (Eh: −270 to +20) and from acidic to alkaline (pH: 6.5–8.7). Furthermore, as the water table fluctuates strongly, the unearthed wood inside the excavation trenches may be completely submerged during winter, and exposed to drying during summer months (Fig. 2).

Within the current post-excavation oxygenated environment, the biodeterioration processes taking place are different from those that prevailed during burial. Fungi appear to be the most aggressive deteriogens present throughout the site.

Lifting and active conservation of the timbers is not currently feasible due to cost restrictions. Plans are therefore in place to display the majority of the timbers in situ, within an archaeological park, which is currently under construction. Periodic or continuous water-spraying of the exposed wood is considered the most likely course of action.

This study's goal was to confirm and document the presence of fungi on the excavated waterlogged wood and examine the biodeterioration patterns that may have been caused by them. Knowledge of the fungal species growing on the excavated wood, and their deterioration potential, will be crucial in the design of appropriate measures for their control during future in-situ display.

* Corresponding author. Tel.: +30 210 5385469; fax: +30 210 5385406.
E-mail address: pournoua@teiath.gr (A. Pournou).

0964-8305/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ibiod.2008.11.001

Fig. 1. Wooden piles, branches and beams found as vertical and horizontal structural elements.

2. Materials and methods

2.1. Sampling

Wood samples were retrieved in winter from the top portion of six piles, after they had been fully submerged in the trench water for at least two months. Each sample was cut longitudinally in two parts. The first part was sprayed with 70% v/v ethanol in water, placed in a sterile polyethylene bag, and stored at 5 °C; this part was used for morphological examination. The second part was simply put in sterile polyethylene bags and kept at 5 °C; this part was used for isolation of fungi.

2.2. Identification of wood species

Thin sections of material in transverse (TS), tangential (TLS), and radial (RLS) directions were cut by hand using a double-edged razor blade. Sections were then mounted in 50% v/v glycerol in water. The keys of Philips (1979), Schweingruber (1990), and Paraskevopoulou (2003) were used for the identification of the species.

Fig. 2. Wooden pile, exposed to drying.

2.3. Cultivation, isolation, and identification of fungi

In order to allow the growth of water-and soil-inhabiting fungi, as well as wood-degrading fungi, a range of media (corn meal agar, Czapek agar, oat-meal agar, and 2% malt extract agar; Centraalbureau voor Schimmelcultures, 1990) were used for the isolation of micromycetes from wood samples. Small portions of wood were aseptically detached by scalpel and transferred to the culture media using a sterile needle or forceps. Eight wood samples were taken for analysis; five inoculations were made from each sample. After 10 days of cultivation, fungal colonies were isolated into pure culture. Fungi were grown on Czapek agar media under room temperature for 10–30 days, until sporulation appeared. Then, micromycetes were identified from slide cultures based on their cultural features with traditional methods and on their morphological ones using light and fluorescence microscopy.

2.4. Light microscopy

Hand-cut sections were stained with safranin O and counterstained with picro-anilin blue, (Peacock and Bradbury, 1973). They were then examined using an Olympus CX 41 microscope equipped with an Olympus C-5050 zoom digital camera system.

2.5. Scanning electron microscopy

Material was cut using a double-edged razor blade, dehydrated through an ethanol series and then left to air-dry in a desiccator for 48 h. It was then mounted on aluminium stubs using carbon glue, coated with palladium/gold in a POLARON SC7640, QUORUM sputter coater, and examined using a JEOL MP-35031 scanning electron microscope at 15 kV.

3. Results

3.1. Identification of wood species

Three of the six wood samples were identified as *Pinus sylvestris* L., *Pinus nigra* Arnold, or *Pinus mugo* Turra, as the wood of these species cannot be differentiated based on their anatomical microscopic characteristics. Two samples were identified as *Juniperus drupacea* Labill and one sample, a knot, was also a conifer but was not possible to identify.

3.2. Fungi isolated from wood samples

Seventeen fungal species were identified in cultures isolated from wood samples (Table 1). Even though the samples were fully waterlogged, the fungal species isolated were representative of those typically found in terrestrial habitats. No obligate water inhabitants or specific wood pathogens were found. Furthermore, abundant bacterial growth was observed in most samples.

3.3. Micro-morphology of decay

Light and electron microscopy revealed severe deterioration in all samples (Fig. 3), caused by both fungi and bacteria (Fig. 4). Conical cavities (Fig. 5a), aligned with the S_2 microfibrils (Fig. 5b) and "active penetration" of cell walls by hyphae were frequently observed (Figs. 6 and 7).