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Deterioration, decay and identification of fungi isolated from wooden structures at the Humberstone and Santa Laura saltpeter works: A world heritage site in Chile

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ABSTRACT

The use of wood in construction has been part of mankind's history but wood placed into the environment is affected by biotic and abiotic agents and is degraded over time. Even in extreme environments, such as dry desert sites, deterioration of wood can take place. One site located in the Atacama Desert in northern Chile is the Humberstone and Santa Laura saltpeter works where offices and other structures were built of wood. Founded in 1872, the Humberstone and Santa Laura Saltpeter Works was designated a UNESCO World Heritage Site in 2005 for its historic significance. Since significant deterioration in the wooden buildings has taken place, investigations were initiated to better understand the degradation underway so conservation efforts to protect the historic buildings can be developed. The objectives of this study were to identify the type of deterioration and decay taking place and to isolate and identify fungi from wood samples of structural elements at both sites. Samples of deteriorated wood showed extensive degradation that resulted in a defibration of the wood. The middle lamella between cells was degraded and remaining secondary walls separated due to high concentrations of salts. This resulted in a serious corrosion of the exterior layers of wood cells. Although high salts inhibit fungi, many different fungi were isolated. Sequencing of the ITS region of the rDNA was used and fungi were identified as *Penicillium chrysogenum*, *Engyodontium album*, *Eupenicillium tropicum*, *Penicillium digitatum*, *Pseudotaeniolina globosa*, *Cladosporium phaenocomae*, *Aureobasidium pullulans*, *Penicillium virgatum*, *Coprinopsis sp.* and *Phanerochaete sordida*. Several of these fungi appear to be halophilic.

1. Introduction

Nitrate or saltpeter exploitation, which is still carried out today, began long ago in Chile during the pre-Colombian era (Minvu, 2003). The 'República' was a period of time for very active mining (Vial, 1987), and these mining activities were located mainly in Arica and Parinacota, Tarapacá, Antofagasta, and Atacama regions of Chile, which are desert areas with limited farming and forestry activities. This mineral, used as fertilizer and raw material for making explosives, made Chile the only manufacturer and exporter in the world at the end of War of the Pacific (1879–1883) because it had vast areas with high concentrations in its deposits (Vial, 1987). Saltpeter became Chile's major mining export product until the first decades of the 20th century. Mining remained a strong industry until the great depression of 1930's and the development of synthetic products of similar characteristics that eventually decreased the demand for Chilean nitrates (Culverwell, 2000).

The Humberstone and Santa Laura saltpeter works are a remarkable example of an industry that left a deep mark in Chilean history (Fig. 1). The sites (20°S 12′ 20.9″, 69°W 47′ 38.6″, and 1050 masl), are located in the so-called 'Pampa del Tamarugal', 47 km

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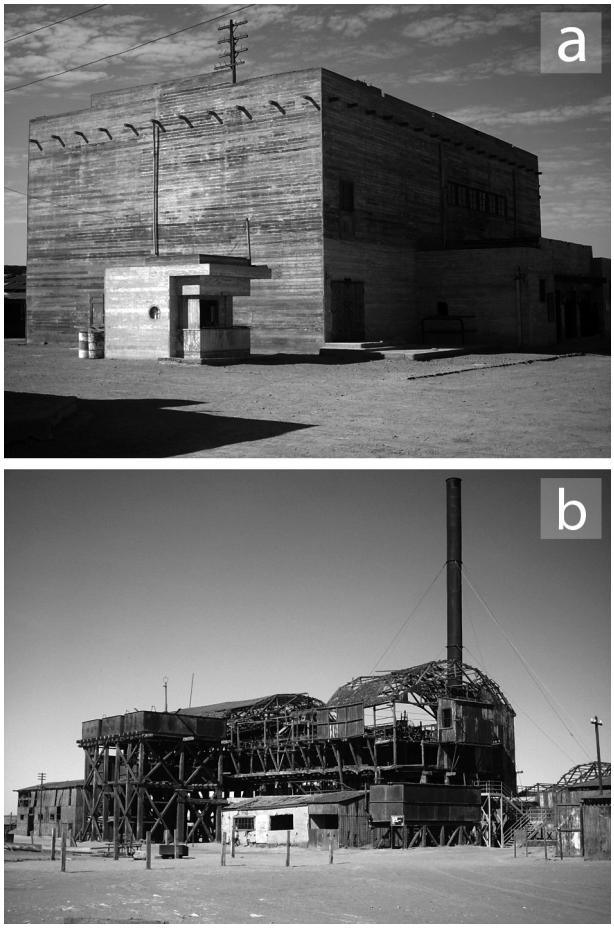

Fig. 1. Examples of the wooden historic structures at Humberstone (a) and Santa Laura (b) saltpeter offices in the Atacama Desert, Chile.

East of Iquique, in the Tarapacá region of Chile (Fig. 2). Currently, both sites make up an historic industrial complex which represents the mining activity of saltpeter extraction (Minvu, 2003); Santa Laura possesses the essential features related to industrial material processing in the area, and Humberstone has the structures related to the community aspects of the mining village. The facilities of these old saltpeter sites, which have now been declared National Monuments and UNESCO Cultural Heritage Sites of Humanity in 2005, possess many buildings made from a variety of materials including corrugated zinc sheets, concrete 'pampino', and wood.

The structures at the sites have become important for their historical significance providing valuable information about the past mining heritage of Chile. However, as wood is exposed to the environment it can be vulnerable to deterioration and decay. Historic and archaeological woods, even in desert regions, are subjected to attack and their degradation raises serious conservation concerns for preserving the wood (Blanchette, 2000, 2010).

Butin and Peredo (1986) and Furci (2008) have indicated that Chile is a relatively rich country in terms of fungal flora. However, only about 3300 fungi species are known. According to Gamundi (2003), the most important collection of fungi was conducted by Mujica et al. (1980) in his work called 'Flora Fungosa Chilena'. Respect to investigations of decay in historical buildings and in archaeological wood the information is very limited (Ortiz et al., 2011, 2012).

The historic wooden buildings at the Humberstone and Santa Laura saltpeter works, have evidence of serious deterioration and decay but no information is known about the type of degradation taking place, the microorganisms involved or the deterioration processes that are occurring in this unusual environment. Because of their important World Heritage Site status, every weakness that might be affecting their structure needs to be understood in order to make decisions leading to their successful preservation over time and establishing plans to prevent loss. The purpose of this study was to investigate the types of deterioration and decay occurring in wood at Humberstone and Santa Laura saltpeter works and identify the fungi isolated from the wooden structures.

2. Materials and methods

2.1. Wood sample collection

Wood samples with different stages of deterioration and decay were collected from Humberstone and Santa Laura saltpeter works buildings located in Tarapacá region, Province of Iquique, commune of Pozo Almonte, Chile. In addition, wood samples showing no degradation were also collected to identify the types of wood used in the structures. The samples of wood were placed in sterile plastic bags and taken to the laboratory where they were stored at 4 °C.

Micromorphological evaluations were done to identify the type of deterioration and decay in the wood samples using previously published methods that characterize decay types and deterioration processes (Blanchette et al., 1994; Blanchette, 2000; Blanchette et al., 2004; Blanchette et al., 2010). Wood samples were prepared for scanning electron microscopy (SEM) using techniques described previously by Blanchette and Simpson (1992). Observations and photographs were made using a Carl Zeiss, model EVO – MA10 Scanning Electron Microscope. Wood species identification was made, through the use of keys, as reported by Diaz Vaz (1979). Elemental analyses of samples from wood timbers were done by multi-elemental inductively coupled plasma atomic emission spectroscopy (Blanchette et al., 2002).

2.2. Inoculation and culturing

Small wood segments were aseptically cut from the collected samples and placed in Petri plates containing culture medium for isolating fungi. The culture medium was malt extract agar (in g l^{-1}: Difco-agar 15, Bacto-malt extract 10), potato dextrose agar (in g l^{-1}: Difco-agar 15, glucose 20, and potato infusion 4), or a selective medium for basidiomycetes fungi were used (in g l^{-1}: Difco-agar 15, Bacto-malt extract 15, yeast extract 2, benlate (Methyl-1-(butylcarbamoyl)-2-benzimidazole-carbamate) 0.06, streptomycin sulfate 0.01 and lactic acid 2.5). The media was sterilized at 121 °C for 20 min. The culture media MEA and PDA were prepared with pH 5.6 and 6.5. The pH of these media was adjusted using NaOH or HCl. Plates were incubated at approximately 24 °C ± 2 °C.

2.3. Identification of fungi

The liquid medium for obtaining the dry mycelium for DNA extraction was prepared with (g l^{-1}): Bacto-malt extract 10. The obtained suspension was sterilized at 121 °C for 25 min. Erlenmeyer flasks (500 ml) containing 125 ml were inoculated with pure cultures of mycelium to be identified and were incubated at room temperature in a shaker at 150 rpm for 7 days. The mycelium was filtered and washed according to the protocol described by Montiel (2005). After washing, the mycelium was dried in an oven at 45 °C for 12 h.

DNA extraction was carried out using the previous published protocol of Cubero et al. (1999). Integrity of extracted DNA was