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Biodegradation assessment of a 16th century fresco from Southern Portugal

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This work reports the study of the *frescoes* from the *Casa de Fresco dos Sanches Baena* in Vila Viçosa (Southeast Portugal) to allow their material characterisation, to identify the different microorganism populations and to assess their role in the deterioration of these paintings.

The *Casa de Fresco dos Sanches Baena* is a small semi-underground building constructed in a Palace garden over a well and used as a cool refreshing place by the owners. The frescoes that cover the ceilings (composed of 4 panels) present rich mythological scenes and have other decorative elements, which make them an unusual example of this art form. Unfortunately, due to partial abandonment the paintings are in an advanced state of degradation exhibiting partial detachment of paint layers and mortars, salt efflorescences and abundant biological colonisations (Fig.1).

Microsampling of paint layers was performed on representative areas of the paintings. The cross-sections were analysed by optical microscopy, microchemical analysis and scanning electron microscopy coupled with energy dispersive spectrometry allowing pigment identification and stratigraphy analysis. The results showed that azurite and malachite were used for the blues and greens, respectively. As for the reds and yellows, the results showed that ochres were used.

For the microbiologic sampling, sterile cotton buds and chisels were used and the biological materials collected in sterile recipients. Colonies morphologies were evaluated by microscope preparations. Gram's stains and some usually tests followed by *Bergey´s Manual of Systematic Bacteriology* [1] were used for bacteria identification. Fungal strains were identified following standard methods [2], based on its macro and micro-morphological characteristics, such as colony diameter, texture, colour, dimensions and morphology of hyphae and reproductive structures (for sporulating isolates). The microbiological study (fig. 2 and 3) by optical microscopy allowed the isolation of 34 fungi strains and 32 bacterial strains in the four painted panels that compose the frescoes. Panel 2 presented the highest microbial contamination with 19 fungi strains and 18 bacterial strains observed. The predominant bacterial strains were bacillus Gram+ and Gram-, from the genera *Bacillus* and *Pseudomonas*, respectively. Some strains of *Actinomycetes* were also isolated. The dominance of bacilli in the samples can be explained by their ability to survive for a long time as spores. As to the fungi populations, the dominant strains identified were from the genera *Cladosporium* and *Penicillium*, although others strains were also isolated, namely *Aspergillus* sp., *Acremonium* sp., *Sporotrix* sp., *Trichoderma* sp.. However, several isolated mycelia did not present reproductive structures and were designated as sterile mycelia.

The microbial activity in all panels was assessed by enzymatic assays, namely, dehydrogenase (DHA). The method was optimized using INT (2-(4-iodophenyl)-3-(4 nitrophenyl)-5-phenyl tetrazolium chloride) with quantification of INT-formazan and applied for the dehydrogenase