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The assessment of fungal bioaerosols in the crypt of St. Peter in Perugia (Italy)

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ABSTRACT

The inspection of the quality of the indoor air in which a work of art or a historical artefact is kept becomes essential for its conservation. The determination of organic pollutants represents an important tool in pre-emptive conservation. The study investigated the quality of the air in the crypt of the Basilica of St. Peter in Perugia (Italy) through different methodologies. The objectives included the analysis of the levels of biological particulates of fungal origin, and the determination of the degree of variability of the airborne spore concentrations, as indicative of the level of contamination of the environment. The quantitative analysis of the airborne fungal component demonstrates that across the whole period considered there were wide variations in the bioaerosols, heterogeneous spore distributions and different peak concentrations in the areas studied. The qualitative analysis of the airborne fungal component allowed the determination of the different fungal genera present, both in the interior of the crypt and in the outside environment. The analysis of the data shows an increasing trend over the period considered, with the highest values during the months of June and July.

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Introduction

The conservation of our cultural heritage has for many years favoured protection against the risk of degradation, to limit any damage before it gets worse ('pre-emptive conservation'). In this way, the focus of the conservation moves from the work of art itself to the physical environment in which it is kept (Getty Conservation Institute, 1994). The regulation of the quality of the indoor air in which a work of art or a historical artefact is kept becomes essential for its conservation. In particular, determination of organic pollutants is useful, so that the procedure to optimise the environmental protection represents an important tool in pre-emptive conservation, and above all allows the definition of the levels of biodeterioration risk (which arises from the life processes of living organisms that produce changes in the materials of their surrounding environment) and of sanitary hygiene for the associated operators and visitors (Sorlini, 1993; Mandrioli and Caneva, 1998; Urzì et al., 2001; Pinna, 2003; Aira et al., 2007; Angelosante et al., 2007; Ruga et al., 2007, 2008; Chen et al., 2010). The methodologies and the operational techniques for the prevention and recovery of damages caused by the biodeterioration improve the information about the biological risk factors of the workers. These diseases may be particularly dangerous and related to the conservation and restoration of ancient artefacts often degraded by the action of living organisms. The numerous examples of diseases that affect the restorers may be caused by the manipulation of artefacts from infected areas or environments, or by artefacts that act as vectors of pathogenic agents not particularly dangerous in the broad sense, but which have been isolated for a long period, and against which the human body struggles to develop specific immunity. In the confined environments where particularly valuable historical, artistic or cultural objects are kept, such as museums, libraries, archives, churches and hypogea, there are a wide range of microorganisms and macroorganisms that represent potential risks for the degradation of these objects. These can also act in synergy with other factors, such as the atmospheric conditions, the nature of the artefact, its location within the environment, the materials that can be subject to attack, and any physicochemical degradation processes that are already present (Ruga et al., 2008).

Moreover, an artefact that has been previously restored, it may sometimes be subject to an increased susceptibility to biological degradation due to the type of substances used in recovery operations (Gu, 2003; Cappitelli et al., 2004).

In confined environments, there is a lack of the natural circulatory mechanisms that are normally part of the external environment, which results in greater amounts and settling of dust and

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potentially contaminating material. These can arise from faulty air conditioning systems, from the presence of people, who represent one of the major carriers of microorganisms, and from sources that are in direct contact with the external environment, such as entrances and exits, or windows or doors (Orlandi et al., 2011; Quian et al., 2012; Gauzere et al., 2014). These airborne particles can be deposited on the works of art, and under favourable conditions of temperature, humidity, light and substrate, they can use the different surfaces not just as a physical support for their growth, but also as a nutrient source. This can cause irreversible structural changes or damage to such protected works of art, with a loss of strength and structure, and also changes from an aesthetic point of view (Lopez-Miras et al., 2013). To avoid irreversible deterioration of such artefacts, and to evaluate the indoor biological risk, it is important to periodically carry out aerobiological monitoring of the biological components of the air of their environment, which provides the characterisation of the air quality from a biological point of view, both quantitatively (e.g., degree of contamination) and qualitatively (e.g., presence of potentially biodeteriogens species or with potential allergenicity) (Gravesen, 1979; Kurup et al., 2000). This allows the identification of any contaminant sources and the carrying out of suitable interventions (Nugari and Roccardi, 2001; Ruga et al., 2008). The Aerobiology represents an important tool for the preventive conservation and the public health. Moreover, it allows to define important knowledge for operators involved in the artefacts conservation and restoration, providing also important information in terms of hygienic, safety and health protection. In environments such as crypts, with typical characteristics of the underground environment, alterations to the surfaces can arise from the development of either photoautotrophic microorganisms in the presence of natural or artificial light, or of heterotrophic microorganisms when there are organic substances, even in small quantities. In particular, wall paintings, such as murals or frescos, are usually poor in organic substances, and the development of heterotrophic microorganisms is conditioned not only by microclimatic factors, but also by the biological pollutants present in the air that arise from the propagation of biodeteriogens or organic substances that represent sources of food for many microorganisms (Nugari et al., 2007). In this way, such artefacts can suffer from degradation, and the problem of biodeterioration arises when live cells of biodeteriogens microorganisms are deposited on the surfaces. Under favourable conditions of temperature and humidity, heterotrophic microorganisms can be sufficiently able to develop and multiply, such as the bacteria and fungi that can be found in minimal dust deposits or organic residues that are generated by the primary and secondary autotrophic colonisers (Nugari et al., 1993; Saarela et al., 2004). Microscopic fungi, in particular, are the most important factors of all of the biological changes that frescos can undergo, because with their nutritional needs, their reactions toward external stimuli, and their ability to adapt to often adverse environmental conditions, microscopic fungi can show great diversity and great metabolic activity (Ciferri, 1999; Gorbushina et al., 2004; Zammit et al., 2009; Pangallo et al., 2012; Sterflinger and Piñar, 2013; De Leo and Urzì, 2014). Their metabolic products cause further chemical damage (Beech, 2004). The capacity of fungi to dissolve carbonates depends on available carbon sources, such as oxalic and citric acids which may mobilize cations with chelating activity (Hirsch et al., 1995; Wollenzien et al., 1995; Milanesi et al., 2006). Fungal organisms, for the large capacity of metabolic adaptation, can colonize and use as a nutritional substrate also synthetic polymers (Lugauskas et al., 2003). In presence of some polymers in which the organic fraction is absent the fungi development is linked to the organic additives present in the silicone resin used to protect and consolidate stone materials (Cappitelli et al., 2007).

Therefore, the aim of this study was to investigate, through different methodologies, the presence of airborne microrganisms, potentially biodeteriogens for the stone artefacts in a semi-underground environment.

In particular, the study objectives included: characterisation of the quality of the air in the crypt of the Basilica of St. Peter in Perugia (Italy) through analysis of the levels of biological particulates of fungal origin, and determination of the degree of variability over time of the airborne spore concentrations, as indicative of the level of contamination of the environment; analysis of the quality of the sampled particulates, and determination of airborne fungal genera; identification of possible relationships between the inside and outside concentrations of the particulates, and the microclimatic conditions, particularly for the temperature and relative humidity of the air.

Materials and methods

Studied area

This study was carried out in the conservation and exhibition areas of the crypt of the Basilica of St. Peter in Perugia (property of Foundation for Agricultural Education in Perugia, Italy), situated at a depth of 3 m below the floor of the church of St. Peter. In 2000 some measures were taken to consolidate the plasters and the fresco painting with acrylic resins. A special care was required by the disinfection of painted surfaces, affected with deteriogens organisms (algae), realized through biocide applications. The crypt is oriented northeast to southwest, following the same orientation of the church above (Fig. 1a), and it can be accessed down a ladder placed at the bottom of the left aisle, which ends in the first section. From here, there is a corridor, and running along its south side there are five brick niches, forming apses of equal size (Fig. 1b). On the north side, there is access to an exedra (Fig. 1c). The corridor continues along to the final part of the crypt, where there is a final area of equal size to that at the entrance to the crypt.

Aerobiological sampling

The objectives of this study were achieved mainly through the airborne fungal particles monitoring. The monitoring has been done with three different methods. The sampling was defined in relation to the environments that would be more critical and the areas in which there might be higher risk of biodeterioration (e.g., the parts located near to the entrances and exits, the areas characterised by the wall paintings). In particular, the sampling for airborne spores was carried out: in the first area (referred to as the 'entrance'), as this was closer to the entry and exit from the crypt; in the 'exedra', as this was the area closest to the frescos and connected with the church above through three little holes (15×10 cm) in the ceiling; and in the final area (referred to as the 'internal area'), as this was considered to be the innermost part of the crypt. Fig. 1a shows the plan of the crypt where the monitored areas are indicated. The sampling was conducted on a weekly basis from March to July, 2011.

The monitoring was realized during the same morning period utilizing contemporary the three sampling methods.

Fungal particles counting

The simple counting of particles gives a first "quick and dirty" approximation of a microbial contamination of the air. For the sampling of the airborne fungal particles, a Personal Volumetric Air Sampler spore trap for glass slides was used (Burkard Company Ltd.). This was based on the original Hirst model (1952), and it operates on the principle of impact for depression, through the

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