# Bioaerosol

2

Paola De Nuntiis and Franco Palla

**Abstract**

Cultural heritage constitutive materials can provide excellent substrates for microbial colonisation, highly influenced by thermo-hygrometric parameters. In cultural heritage-related environments, a detrimental microbial load may be present both on artworks surface and in the aerosol. Confined environments (museums, archives, deposits, caves, hypogea) are characterised by peculiar structures and different thermo-hygrometric conditions, influencing the development of a wide range of microbial species, able to induce artefact biodeterioration and to release biological particles in the aerosol (spores, cellular debrides, toxins, allergens) potentially dangerous for the human health (visitors/users). In order to identify the real composition of the biological consortia, highlighting also the symbiotic relationships between microorganisms (cyanobacteria, bacteria, fungi) and macro-organisms (plants, bryophyte, insects), an interdisciplinary approach is needed.

The results from in vitro culture, microscopy and molecular biology analysis are essential for a complete understanding of both microbial colonisation of the cultural objects and the potential relationship with illness to human. Concerning the bioaerosol, of crucial importance are the time and techniques for sampling.

P. D. Nuntiis (✉)
Italian National Research Council – Institute of Atmospheric Sciences and Climate (CNR-ISAC), Bologna, Italy
e-mail: p.denuntiis@isac.cnr.it

F. Palla
Department of Biological, Chemical, Pharmaceutical Sciences and Technologies, Laboratory of Biology and Biotechnologies for Cultural Heritage, University of Palermo, Palermo, Italy
e-mail: franco.palla@unipa.it

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2022

F. Palla, G. Barresi (eds.), *Biotechnology and Conservation of Cultural Heritage*,
https://doi.org/10.1007/978-3-030-97585-2_2

**Keywords**

Biodeterioration · Microbial colonisation · Preventive conservation

## 2.1 Indoor Environments (Libraries, Museums, Storerooms, Hypogea, Churches)

Several aspects of indoor environments need to be defined. Their main function is to preserve the objects, made of different materials that make up a country's historical and artistic cultural heritage, in addition to serving educational purposes and representing cultural and social identity. Indoor environments may either be constructed in situ to preserve artworks in their place of origin (hypogea) or custom-built using innovative techniques and with air-conditioned premises.

Alternatively, they may be realised in existing monumental buildings which constitute artworks themselves, but also house works of art. The type of building structure will determine the risk and potentially the type of deterioration and problems associated with managing the indoor environment and collections.

In turn, the location of indoor environments, in urban or rural centres, industrialised or green areas and coastal or mountain regions (Thomson 1986; Camuffo 2019) will determine their different external and internal microclimatic conditions, the aerosol composition and its biological impact. As indoor air is linked to the atmosphere surrounding the building (Brimblecombe 1990), also a wide number of visitors will have a negative effect on the indoor environment. Moreover, the shape of indoor spaces may have a further influence, as they can range from large, very high premises to small interconnecting areas arranged either on several floors or on a single level, attics or basements, with or without windows, varying exposure, adjoined or separate, with showcases, display cabinets or clima boxes representing micro-environments enclosed within the main exhibition macro-environments (Michalski 1994; De Guichen and Kabaoglu 1985; De Guichen 1980; Cassar 1995; IBC 2007; Lazaridis et al. 2015).

Indoor microclimatic conditions deeply depend on whether active heating/air-conditioning indoor and lighting systems are present or not in a given environment. Most indoor environments, especially those constructed in loco (hypogeum), or in historical buildings or premises mainly destined for other uses (churches), lack active microclimate control systems. Even when conditioning systems are present, they are often adjusted to the needs of human comfort on the part of museum staff or church worshippers rather than those of artefact conservation. In addition, unless these systems are regularly serviced, they can be a further source of potential chemical and, above all, biological pollutants (Sedlbauer 2002; Varas-Muriel et al. 2014).

The study and control of the microclimate (Cavallini et al. 1991; IBC 2007; Bernardi 2009; Camuffo 2019) is essential to curb "biological risk", as microclimatic conditions have a major impact on spore germination. The main indoor microclimatic parameter is relative humidity. Very high relative humidity levels in the winter months, or in conditions of moist warm wind, may result in condensation followed

**Fig. 2.1** Hypogeum archaeological site, in addition to the glass ceiling (*dark area at the top*), allowing natural light to penetrate, two older-generation lighting systems are visible: fluorescent tubes lacking UV guards and halogen spotlights generating considerable heat and relative air movement. This lighting system combined with high relative humidity levels increases the risk of biodegradation. The site is currently under monitoring and closed to the public

by microbial colonisation on the internal or external surfaces of a building and even worse on the frescoes of the masonry walls (Camuffo 2007; Ranalli et al. 2018).

Under these conditions, churches are more at risk of biodeterioration than hypogea due to the different materials they contain ranging from stone architectural and decorative features to organic materials like wood used for floorings, ceilings and furnishings and objects linked to worship (Nugari 2003). The thermo-hygrometric excursions during daily liturgical celebrations typical of the mountain churches, have not to be overlooked (Camuffo et al. 1999). With the possible exception of storerooms, another problematic parameter in all these indoor environments is natural or artificial light. Light heats the illuminated surface generating hygrometric and mechanical stress and warms the circulating air mass triggering convection that not only enhances the inertial deposition of suspended particulate matter but also pigment discoloration and biodegradation (Fig. 2.1). Light is needed to display objects, but existing lighting systems are often outdated and obsolete. Nonetheless, a variety of lighting systems are currently available that ensure optimum object preservation, saving both energy and money, including through the use of sensors that trigger light only when visitors enter an environment (IBC 2007).

Biological contamination can also occur in objects newly acquired from uncontrolled indoor environments (donations from private collections or excavated materials in contact with the soil), but is also common after natural or man-made disastrous events like flooding. Visitors also act as carriers of outdoor spores transported inside the building through clothes, skin and hair, they contribute to

**Table 2.1** Average contribution ascribed to individual museum visitors

| Contribution | Cause | Quantity |
|---|---|---|
| RH—water vapour | Breathing, sweating | Approx. 40–100 g/h |
| T—heat | Movement | Approx. 100 W/h |
| $CO_2$—carbon dioxide | Breathing | Approx. 20 l/h |
| Dust | | |
| Fibres | Clothing | Approx. 0.2 g/m$^3$ h |
| Microorganisms | Sweating, talking, sneezing | |
| Organic fragments | Skin, hair | |

Cited by Mandrioli (2015)

increasing $CO_2$ linked to breathing, temperature, water vapour and microorganisms through sweating, talking and sneezing (Table 2.1). Indoor exhibition/conservation environments such as museums and churches often contain different materials and multi-material artworks, which give rise to a number of conservation problems linked to the intrinsic features of the materials (Hueck 2001) and their microclimatic requirements for conservation and management. Related to this aspect are storerooms located in attics or basements, which are often poorly maintained, full of dust and insects, and have suboptimal ventilation and unstable microclimatic conditions due to their location and frequent lack of air-conditioning systems. These environments are often neglected in terms of conservation measures as they are deemed less important, despite housing large quantities of precious artefacts made of different materials for long periods of time. In addition, storerooms often house restored works of art that emit further pollutant gases generated by restoration treatments (Cappitelli et al. 2004).

Artworks dust protection has been recently achieved using the Compactus shelves, but these microenvironments can cause fungal colonisation, especially in historical libraries on leather or fabric books binding (Micheluz et al. 2015). A regular and adequate cleaning, is a highly recommended preventive conservation strategy in every environment (Getty Conservation Institute 1994); in fact, humidity associated with mild-moderate temperatures and accumulated dust may create a favourable habitat for a biodeterioration of many materials (Sterflinger and Pinzari 2012). In the already contaminated material, moisture is well preserved in the biofilm, so even conditions of RH $>$ 50% are sufficient for the survival of some microorganisms (Camuffo 2019).

Finally, a complete analysis should not neglect the assessment of building vulnerability, considering the physical and managerial criticalities related to poor maintenance of roofs, windows and masonry (Bonazza et al. 2021; Cacciotti et al. 2021, Sesana et al. 2021), especially caused by the extreme hydrometeorological phenomena, due to climate change and closely connected to the microclimate and indoor microbial exposure. The use of water-sensitive building materials in areas with hot and humid climates can also lead to indoor mould growth.

Environmental monitoring continues to be the main tool not only for recording but above all for actively keeping under control the causes of material degradation. Based on a series of data recorded over a sufficiently long period of time, continuous monitoring will permit to correctly analyse indoor and outdoor environmental measurements based on specific targets. The importance of on-site monitoring is also reiterated in the Sendai Framework for Disaster Risk Reduction 2015–2030 (Sendai Framework - United Nations 2015)[1] Priority 4 (Enhancing disaster preparedness for effective response and to "Build Back Better" in recovery, rehabilitation and reconstruction) in the sectors of risk due to climate change which also includes Cultural Heritage (Bonazza et al. 2018). The knowledge of climate change impact on cultural heritage in Italy is principality based on the identification of the main climatic parameters for degradation of indoor and outdoor artworks. A specific threat to cultural heritage concerns changes in biodegradation processes due to projections of seasonal rainfall (National Plan for Adaptation to Climate Change—PNACC[2] which makes effective the National Strategy for Adaptation to Climate Change—SNACC[3]).

## 2.2 Airborne Particles: Organic and Vegetable Dust and Biohazards

Bioaerosol is the scientific term used to define a suspension of aerosols or particulate matter of microbial, plant or animal origin and may consist of pathogenic or non-pathogenic, live or dead bacteria and fungi, algae, viruses, pollen, plant fibres, high molecular weight allergens, bacterial endotoxins, mycotoxins, peptidoglycans or glucans (Douwes et al. 2003) passively carried by air (Cox and Wathes 1995). For this reason, the term bioaerosol does not include insects (Mandrioli and Ariatti 2001).

Airborne biological material is composed of particles generated from natural sources by active or passive mechanisms and resuspended in the atmosphere as individual organisms or often aggregated with each other or with non-biological solid or liquid particles in suspension (Lighthart and Stetzenbach 1994). The biological aerosol includes many types of airborne particles varying widely in morphology and size that can be seen with a magnifying glass or microscope. Their aerodynamic diameter may range from molecular size to large and giant particle size (Jaenicke 2005; Hinds 1999; Pöschl 2005), e.g. viruses (1 nm–1 μm), bacteria (0.1–1 μm), fungal spores (0.5–50 μm), lichen propagules (10 μm–1 mm), bryophyte spores (1–100 μm), algal cells (1 μm–1 mm) and pollen grains (10–100 μm) (Després et al. 2012; Fuzzi et al. 2015). Airborne particles are

[1] https://www.undrr.org/implementing-sendai-framework/what-sendai-framework

[2] https://www.minambiente.it/sites/default/files/archivio/allegati/clima/pnacc.pdf

[3] https://www.minambiente.it/notizie/strategia-nazionale-di-adattamento-ai-cambiamenti-climatici-0

associated with other biological materials such as protozoan cysts found in aggregates or incorporated in solid or liquid particles, pteridophyte spores, plant fragments and products from metabolic activities. Another term commonly used to describe airborne particles derived from biological organisms is *primary biological aerosol particles* (PBAPs) that differentiates biogenic particles from secondary organic aerosols formed by further physical processes and chemical reactions in the atmosphere such as photo-oxidation (Després et al. 2012). When microorganisms are incorporated, for instance, into fog droplets for many hours, the environmental conditions are favourable for rapid growth, giving rise to *secondary biological aerosol particles*, commonly called SBAPs (Fuzzi et al. 1997, 2015; Després et al. 2012).

Aerobiology is a relatively new scientific discipline specifically dealing with airborne particles, how they behave in the air once generated, how the environment influences their dispersion and deposition and the impact these particles have on other organisms or materials such as artworks. The presence of bioaerosol in the atmosphere is strictly correlated to an active source able to produce material through physiological processes generating microorganisms or physical processes resulting in disaggregation and fragmentation of organisms. Sources of bioaerosol emission can be natural, for example, bacteria found in the air often belong to groups commonly present in the ground, and in fresh and sea water, while bacteria, algae and fungi are released into the air by a bubble-bursting mechanism influenced by the wind. Forests and vegetation are sources of pollen, spores and fragments, while anthropogenic sources include farming and agricultural processing and industrial activities.

The particles produced are emitted into the atmosphere by both physiological (e.g. the catapult expulsion mechanism for the dispersal of *Parietaria* (*officinalis, judaica, lusitanica, creatica*) allergens and physical processes (e.g. fungal spores released by the action of wind and rain on vegetation) (Mandrioli 1985). The process of fungal spore release mainly depends on atmospheric agents, relative humidity, air temperature, dew point temperature and wind turbulence (Jones and Harrison 2004). Peak fungal spore concentration in temperate and Mediterranean climates coincides with summer and autumn when relative humidity is higher but is strongly influenced by the frequency of precipitations that attenuate relative humidity values. Seasonal variations in pollen production have a major impact on the ratio between indoor and outdoor spore concentrations. In spring and summer, the peak concentration of most fungal spores detected in indoor environments is similar to that found outside, whereas in winter the indoor concentration is higher (Mandrioli et al. 1998; Sabbioni et al. 2008), thereby confirming the trapping effect of buildings. Post-injection conditions in the atmosphere are due to the survival of microorganisms and are controlled by physical and chemical parameters. The dynamic nature of the atmosphere makes difficult to assess the individual importance of each parameter. The major limiting factors are temperature, relative humidity, ultraviolet radiation, oxygen, carbon monoxide, nitrogen dioxide and formaldehyde (Mandrioli 1998). Although bioaerosol is ubiquitous its concentration in the atmosphere varies with season and location, and it has been estimated to constitute up to 25% of total aerosol

mass and is sometimes numerically close to 50% of all aerosol particles on a global basis (Jones and Harrison 2004; Jaenicke 2005; Walser et al. 2015).

Recent studies implementing molecular techniques demonstrated that the fungal spectrum suspended in the air is much richer than previously known (Fröhlich-Nowoisky et al. 2009; Després et al. 2012; Pashley et al. 2012).

The transport or dispersal of bioaerosol in the atmosphere is a physical process based on kinetic energy exchanged when gas particles in the atmosphere clash with motionless air-dispersed particles. Particles do not behave consistently so that each stage is random in both duration and direction. Due to unfavourable environmental conditions such as dehydration and UV radiation (Griffith and De Cosemo 1994), the atmosphere contains not only vegetative forms but many forms of resistance like bacterial and fungal spores. Small particles, ranging in size from 1.0 to 5.0 μm, remain suspended in the air for a longer time, whereas larger particles tend to settle more quickly on surfaces due to their larger mass. Bioaerosol can be transported in the atmosphere for long distances (Gregory 1973; Schlesinger et al. 2006) and a longer time due to its vicinity to particle sources and resuspension of deposited particles (Tampieri et al. 1977; Mandrioli et al. 1980, 1984; Rantio-Lehtimäiki 1994). Kellogg and Griffin (2006) identified the global transport of desert dust as the main mechanism responsible for the transport of aerosol microbiota: pollens, fungi and bacteria. The average residence time of biological particles in the atmosphere can range from less than a day to a few weeks, depending on their size and aerodynamic properties (De Nuntiis et al. 2003; Després et al. 2012).

Particle deposition is the aspect of most interest to cultural heritage and, in particular, the biodeterioration of artworks. However, bioaerosol in the atmosphere is only one of the potential risks arising when deposited material encounters favourable environmental conditions for the colonisation of artefacts. Particles are usually removed from the air by sedimentation and deposition on all surfaces, not only horizontal planes. Deposition occurs by gravitational settling, molecular diffusion and impact. A highly effective but discontinuous means of bioaerosol removal in outdoor environments is rainout and washout, which happens during precipitations when damp deposition captures the particulate in precipitations and deposits it on the ground. Precipitation is the most efficient removal mechanism for particles 0.1–10 μm in diameter. Computation of bioaerosol deposition velocity is a complex problem as particles are irregular in shape and their structural features hamper calculation of particle density, e.g. despite its size, the two air bladders of *Pinus* pollen grains make them particularly light, thereby increasing dispersal distance (Schwendemann et al. 2007). In addition, particles vary in relation to atmospheric humidity changes: small particles are dispersed among air molecules increasing in velocity, whereas large particles shift the surrounding air creating vortices and falling more slowly. Particle deposition is slowed down if the descending particle trajectory is close to a vertical surface, whereas the velocity changes when particles are clustered together. Particles are also affected by thermophoresis and diffusiophoresis, temperature and concentration gradients and electrostatic forces that not only induce particle accumulation with blackening of the surfaces involved but also biodeterioration when conditions are favourable. Particles

settled by dry or wet deposition can be involved in resuspension mechanisms and hence return once again into the atmosphere. Once deposited, biological particles can interact with the substrate, be it the nasal mucosa, a leaf surface or a fifteenth-century fresco, giving rise to an allergic reaction in sensitive patients, a plant disease following fungal or bacterial colonisation or mechanical and aesthetic deterioration of a painted surface. Fungal attack of a fresco surface, due to its porosity, can lead to hypha penetration of the painted layer resulting in flaking and detachment of the fresco surface, coloured stains obscuring the painting and the production of acid metabolites or enzymes able to transform complex molecules into simple water-soluble molecules. Over time, this process will weaken the painted layer damaging the material and value of the artwork. Studies on biodeterioration are a constantly evolving field for scientific and technological research. They currently focus not only on bioaerosol sampling and identification methods but also on transport and deposition mechanisms and above all on the ecology of the species involved.

## 2.3 Impact of Colonised Aerosol on Artwork Surfaces and Potential Enemies of Human Health

Studies on the biodeterioration of cultural heritage are not confined to the microbiology of biodeteriogens and material degradation processes; they require a multidisciplinary approach to understand the chemical and biological relations between the air and the materials it surrounds. Research in this sector not only serves to identify potential risk factors for artwork preservation, devise specific preventive conservation protocols or to identify the most suitable treatments, but it also serves to safeguard the health of operators (conservationists, restorers) and visitors from the risk of exposure through inhalation and contact with contaminated surfaces or objects. The risk for human health stems not only from harmful microbial species or the products of their metabolic activity, e.g. allergens, present on artefacts or in the air, but also from the hazardous residues of biocide treatments used in the cultural heritage sector. Another aspect, neglected in the past, is that some components of the bioaerosol as well as some environmental factors may have synergistic effects; for instance, you may think of the hypothesis in the health sector that hypersensitivity pneumonitis can develop after infection with the influenza virus (Gudmundsson et al. 1999). Indeed research on SARS-CoV-2 virus and COVID-19 disease has been strongly oriented, as never before, to study environmental and biological synergies, having impacts that will also affect the field of cultural heritage protection. Bioaerosol research started thanks to the interest of the health sector (allergology) and agriculture (phytopathology). The cultural heritage sector is just one of many areas in which bioaerosol can cause damage to persons and/or objects, with major economic and other related consequences. The biodegradation of cultural heritage, therefore, has a primary cultural, scientific and economic impact. As previously mentioned, bioaerosol on artworks is only harmful for preservation in concomitance with other factors: microclimatic conditions, the nature of the object, its state of preservation and chemical and physical degradation processes already in place.

Biological degradation is seldom caused by a single microorganism but is produced by complex communities, real ecosystems that develop on the artefact. Under favourable microclimatic conditions (RH, T and light), the bioaerosol deposited on the surface can grow and reproduce itself using the substrate as a nutrient (heterotrophic) or support (autotrophic), causing damage to the material component (Hueck 2001) of the cultural heritage whether it consists of traditional materials and/or modern materials like polymers. The substrate may be a statue, painting, old parchment, cave painting, glass window, liturgical vestment or fresco, made of a single organic or inorganic material or several different materials combined. The inorganic substrate can provide microorganisms with an exclusive supply of mineral salts and a limited amount of water depending on the material's porosity. The microorganisms colonising these artworks are therefore photo- or chemoautotrophic, i.e. able to self-synthesise the molecules required for their development by photosynthetic reaction (photosynthesis) or chemical reactions (chemosynthesis). Heterotrophic species can only penetrate the same artwork after autotrophic organisms, whereas organic materials are colonised by heterotrophic bacteria able to utilise the nutrients available in the material itself. The organisms causing damage to works of art are called biodeteriogens, but do not correspond to all the bioaerosol deposits generally found on them. Some of these particles may be viable but not culturable as they form colonies on solid media under certain growth conditions (time, temperature and nutrients). Many bioaerosol particles cannot be cultured on conventional media, but their existence can be proved using other methods (Năşcuțiu 2010; Blais-Lecours et al. 2015). An indoor environment (museums, galleries, archives, churches and hypogea) can be particularly suitable for microbial growth as it protects the microorganisms themselves from extreme variations in outside temperature and UV rays that can damage the bioaerosol. Desiccation, radiation, oxygen, ozone and its reaction products together with various pollutants can operate cumulatively affecting the viability of microorganisms (Griffith and De Cosemo 1994). For this reason, biological spores survive better in air than vegetative cells, as the humidity in the air is an important potential source of microorganism stress. Fungi are among the most harmful organisms associated with the biodeterioration of organic and inorganic materials (Sterflinger 2010). Many organisms excrete waste metabolic products, including pigmented or acid compounds that may disfigure materials, altering their colours or causing mechanical damage. The presence of heavy metals in some pigments used in paintings on the other hand can increase the resistance to microbial deterioration (Ciferri 1999).

Aesthetic biodeterioration should not be underestimated as it severely alters the perception of beauty and the legibility of artworks although generally it is less aggressive towards the materials. For example, the fungi present on different types of materials can determine the formation of visible films, spots, exfoliation, disruption and pitting. Chemical processes lead to the transformation, alteration and decomposition of the substrate and are much more common than in the past. In addition, the pores and fractures caused by chemical and mechanical interactions can host further biodeteriogens (Urzì et al. 2000). A chemical action may be attributed both to assimilation processes (when the organisms use the material as nourishment

by means of extracellular enzyme activity or ion exchange) and to the excretion of metabolic intermediates or substances having an inhibitory or waste function (such as acid, alkaline and complexing substances and pigments). In physical biodeterioration, the organism breaks or simply deforms the material with growth or movement. Although there are many studies in the literature on microbial contamination relating to works of art preserved in different structures such as museums, crypts, churches, hypogea, libraries and archives (Valentin 2003; Nugari et al. 2003; Gaüzère et al. 2014; Tarsitani et al. 2014; Kavklera et al. 2015; Ruga et al. 2015; Ranalli et al. 2018), there is still much to do on the definition of danger thresholds for biodeterioration processes.

## 2.4 Revealing and Identifying Microbial Particles and Products

No automatic instruments are currently available for the direct measurement of viable and non-viable microorganisms in the air or on surfaces (Caneva et al. 2020). Nor is there a universal bioaerosol sampler: available devices must provide a representative sample trying to minimise stress (e.g. dehydration) and damage to the biological activity of microorganisms. The main aim of sampling is to identify the biological particles (qualitative sampling) presents in the sampled location and measure the variation of their concentration in the atmosphere (quantitative sampling).

Before embarking on sampling, it is important to establish what is being sought and where, which is the best sampling point in relation to the environmental characteristics and the presumed degree of contamination so as to set appropriate sampling times. Alongside biological sampling, it is particularly important to undertake parallel sampling of the main physical and chemical environmental parameters. Last but not least, the most suitable analysis techniques must be chosen to identify and quantify the bioaerosol isolated, especially in the case of viable material. It should be emphasised again that the exact bioaerosol fraction cannot be known using only culture-dependent methods since microbes may be viable but non-cultivable, underestimating both microbial diversity and concentration (Blais-Lecours et al. 2015). Nevertheless, the combination of culture-dependent and molecular analysis should be promoted to increase the observed microbial diversity (Palla 2015; Saiz-Jimenez and Gonzalez 2007) and should be adopted also in the field of cultural heritage diagnostics.

### 2.4.1 Sampling by Passive or Active Methods

The simplest technique, and hence the most commonly used by non-experts, is gravitational deposition, exposing a horizontal surface on which particles settle by gravity and remain trapped by an adhesive placed on the sampling surface or directly on a semisolid culture. Sampling efficacy will depend on air conditions, wind direction and speed, as well as particle concentration and dimensions. The passive

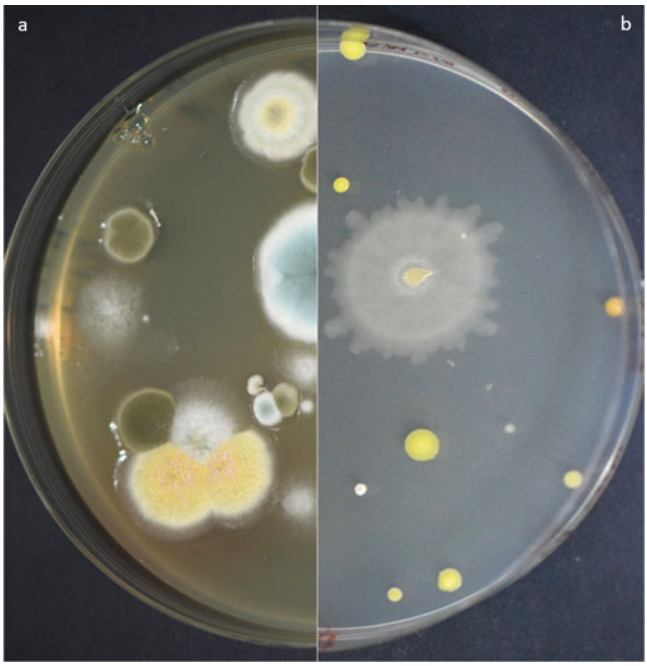


**Fig. 2.2** Passive sampling carried out in the same indoor environment using (**a**) Sabouraud medium (fungal colonies) and (**b**) Nutrient agar medium (bacterial colonies)

sampling allows a qualitative investigation, as the volume of sampled air and the efficiency of capture are not known. It is only suitable for undisturbed indoor environments and is also used for scientific tests in Italian heritage sites, such as the Sistine Chapel in Rome, the Palatina Library in Parma and in the crypt of St. Peter in Perugia (Montacutelli et al. 2000; Pasquarella et al. 2015; Ruga et al. 2015). Petri dishes measuring 90 cm in diameter, containing semisolid culture media (Sabouraud or Nutrient agar), are normally left open to the air for 1 h at 1 m from the floor and 1 m away from walls and then incubated at 30 °C for 16–72 h reaching bacterial or fungal colonies (Fig. 2.2). Culture plate sampling results first need to undergo culture analysis and are then expressed in colony-forming units per surface area (CFU/dm$^2$). To estimate microbial air contamination, an index of microbial air (IMA) is used, based on the count of microbial fallout on Petri dishes, expressed in CFU/dm$^2$/h or CFU/h (Pasquarella et al. 2000).

A sterile glass slide treated with adhesive can be used to sample non-viable bioaerosol with subsequent direct observation of the particulate under an optical microscope, the results are expressed in this case in particles per surface area (particles/cm$^2$). In addition to air sampling the affected surfaces of cultural objects can be sampled in a non-destructive and non-invasive way, using strips of adhesive tape (Fungi-Tape™) or sterile cotton swabs or nitrocellulose membrane filters (Sartorius AG, Göttingen, Germany), followed by observation under optical

microscope or scanning electron microscope (SEM) after adequate sample preparation. The nitrocellulose membrane filters consisted of a 47 mm square disc pressed onto the sample surface for 30 s and then transferred to Petri dishes containing agar for bacteria or fungi isolation (Pitzurra et al. 1997; Pasquarella et al. 2015, Micheluz et al. 2015). To avoid material loss in very small sampling points it is possible to perform a slight aspiration, using a vacuum pump for a short time, in order to guarantee the sufficient quantity of material for subsequent analyses (Paiva de Carvalho et al. 2019).

The Andersen microbial air sampler is a cascade impactor commonly used in diagnostics in the cultural heritage sector as it samples viable bioaerosol (Andersen 1958). The device is particularly suited to indoor sampling as the aspiration tube cannot be oriented according to the wind direction. The sampler has six or three stages in which the particles are separated by size and collected on Petri dishes containing culture medium. Each stage contains plates with 400 precision-drilled holes of decreasing diameter. The slower air speed in the first stages allows the larger particles to be captured, while the smaller particles are accelerated thanks to the narrower diameter of the holes through which they must pass before being captured by the agar-treated surface. The Petri dishes can then be removed and incubated to allow the captured microbes to grow for subsequent quantification and identification. Airflow is sampled at 28.3 l per minute, wall deposition is negligible and the particle capture rate is close to 100%. Sampling time depends on bioaerosol concentration but is generally in the order of a few minutes. The only limitation of this type of sampler is the high number of dishes generated during each sampling.

The Surface Air System (SAS Super ISO, PBI International, Milan, Italy) is a much more manageable and practical portable single-stage impactor specifically designed for indoor environments of hospitals and pharmaceutical and food companies. At the international level, it is currently considered the reference instrument for microbiological air sampling with an environmental bioaerosol capture rate of 100%. The device aspirates the air at a constant flow for periods varying from a few seconds up to an hour depending on the microbial contamination. The SAS SUPER ISO 100 (180 l of air per minute) is commonly adopted in the cultural heritage sector using 55 mm diameter contact plates, but if high fungal contamination is expected, maxi 84 mm plates or 9 cm Petri dishes can be used with a special adaptor because moulds tend to spread and consequently make counting difficult after incubation. The number of colonies counted on the surface must first be corrected for the statistical possibility of multiple particles passing through the same hole, and then the CFU per cubic metre of air sampled can be calculated.

It must be mentioned that the choice of a specific microbiological growth medium, the incubation temperature and the cultivation time result to differences on the selection of the viable, cultivable, airborne microbial community. Media can be specially prepared in the laboratory or purchased ready-to-use to allow a comparison of results. This is a key feature in the cultural heritage sector where national technical standards provide indications, but standardisation is still a long way off. Tryptic soy agar (TSA) is the medium most commonly used for a total microbial count (incubation for 24–48 h a 32 °C), while Sabouraud dextrose agar (SDA) is


**Fig. 2.3** Crypt environment. (**a**) Aerosol active sampling by portable sampler equipped with sterile disposable gelatine filter; (**b**) nutrient medium inoculated by Gelatine filter (completely water-soluble); (**c**) dissolving of gelatine filter during the contact with Nutrient Agar (the filter completely disappears in a few minutes)

used to count yeasts and moulds and usually includes an antibiotic (e.g. chloramphenicol) to avoid bacterial growth (incubation for 5 days at 25 °C).

Other SAS samplers are based on the capture of microorganisms by membrane filtration (SAS dust) or liquid filtration (SAS PCR) which allows identifying the material collected by means of a real-time PCR method. The air samplers that operate in impaction mode (AirPort MD8, Sartorius) collect airborne microorganisms by suctioning a defined air volume through a gelatine membrane filter (Fig. 2.3a) or a culture agar plate. Gelatine filters (Sartorius, AG Germany) allow the material collected to be transported to a culture medium for incubation as shown in Fig. 2.3b, c (Di Carlo et al. 2016).

Aspiration samplers are frequently used for the non-biological fraction of atmospheric particulate adopting membrane filters with pores of a few micron for non-viable bioaerosol (pollen grains and spores) where microorganisms remain trapped on the surface and can be examined under the light microscope either directly or after diaphanization, depending on the filter matrix.

Hirst impact sampler is used to measure the concentration trends of bioaerosol components like pollen, fungal spores, algae and other particles morphologically recognisable under optical microscope (Hirst 1995). The collection surface can be a microscope slide (for daily sampling) or a transparent plastic tape (for 7-day monitoring) treated with the application of a silicon layer to retain the impacted particles. Sampling efficiency is around 95% for particles with a diameter larger than 20 μm and 50% for particles with diameters 2–5 μm. The 2 × 14 mm slit allows the sampled air to move over a surface at 2 mm per hour so that the time trend of particulate concentration can be measured by subsequent light microscopy

Observation. The sampler's suction rate is 10 l per minute, equivalent to the average human breathing rate (Mandrioli et al. 1998), and for this reason is principally used in the field of health, in centres belonging to aeroallergen monitoring networks worldwide, performing the continuous monitoring of pollens and fungal spores (EAN, RIMA, REA, RNSA, NAB, etc.)[4] by a procedure already standardised in Italy (UNI 11108:2004)[5] and in Europe (CEN EN 16868:2019).[6]

The resulting data bank could be an interesting source of information on the daily concentrations of outdoor fungal spores in urban and rural environments for the cultural heritage sector. Three manufacturers (Lanzoni S.r.l., Bologna, Italy, Burkard Manufacturing Co. Ltd., Hertfordshire, England, Cavazza Sas, Bologna, Italy) currently adopt international recommendations to manufacture the commonly used outdoor samplers (VPPS 2000, volumetric spore trap and easy SPT100) and the portable version more often used indoors (VPPS 1000, indoor volumetric spore trap and easy SPT100 light).

### 2.4.2 Biochemical and Biomolecular Techniques

The analytical approach aimed at identifying the biological particles in the indoor air environment that can represent biodeterioration and health hazards includes a broad spectrum of methods. They are based on both conventional microbiological procedures and advanced techniques of molecular biology (Letch 2016; MacNeil et al. 1995). The identification of colonies isolated from air samples can be performed by observing the morphological features according to different manuals or identification keys. In many cases, however, it may be necessary to identify microbial consortia using specific staining methods (e.g. Lugol's staining, Gram staining), biochemical tests (e.g. enzymatic assay, metabolite profiling, ATP bioluminescence assay) and molecular analysis (Di Carlo et al. 2016; Lavin et al. 2014; Šimonovičová et al. 2015; Sanmartín et al. 2016; Castillo et al. 2016).

The application of molecular methods has allowed cultivation-independent investigations of microbial communities in diverse environments. Since not every microorganism in a microbial community can be isolated or cultivated, extraction and sequencing of total microbial DNA are useful to identify those microorganisms defined "not culturable" (Puškárová et al. 2016). Culture-independent methods (CIMs) are based on genetic identification (qualitative analysis) of bacteria and fungi as well as DGGE profiling and PCR that have been developed to study microbial communities from various environments (Šimonovičová et al. 2015;

[4] EAN, European Aeroallergen Network; RIMA, Rete Italiana di Monitoraggio in Aerobiologia; REA, Red Española de Aerobiología; RNSA, Réseau National de Surveillance Aérobiologique (France); National Allergy Bureau (USA).

[5] UNI 11108:2004—Method for sampling and counting airborne pollen grains and fungal spores (UNI, Italian standardisation body).

[6] CEN EN 16868:2019—Ambient air—Sampling and analysis of airborne pollen grains and fungal spores for networks related to allergy—Volumetric Hirst method.

Letch 2016). The strategy of PCR-mediated amplification of targeted sequences, followed by sequencing and comparative data analysis, has been used successfully on samples from air (Palla et al. 2014; Pasquarella et al. 2015). Similarly, non-PCR-based molecular techniques, such as microarray and *fluorescence* in situ *hybridisation*, have also been adopted (Su et al. 2012).

Molecular fingerprinting techniques (bacteria and fungi quantitative PCR, capillary electrophoresis single-strand conformation polymorphism fingerprinting) have been applied by many authors to analyse airborne bacteria and fungi in enclosed spaces, also in relation to bioaerosol in outdoor air, and the influence of microclimate parameters and total dust content on microbial contamination (Gaüzère et al. 2013; Skóra et al. 2015). Studies utilising culture-independent analyses of microbial communities in indoor environments give a complete overview, also based on their level of detail in documenting built environment data (Ramos and Stephens 2014).

Recent studies in DNA sequencing techniques have been carried out focusing on airborne and dust-borne microorganisms in selected museum, archive and library environments. An analytical approach using molecular fingerprinting has been applied to monitoring and characterising the airborne microbial diversity in the Louvre Museum over a long period of time (Gaüzère et al. 2014).

Microbiological contamination has been analysed in several Polish museums, libraries and archives by Skóra et al. (2015). The resulting nucleotide sequences of the identified microorganisms were analysed and compared to the sequences published in the National Center for Biotechnology Information (NCBI) database, using the BLASTN programme, confirming genetically the identified bacteria and yeasts that were previously macroscopically and microscopically characterised using Gram staining and catalase and oxidase tests. Combining cultivation-independent and cultivation-dependent studies, the fungal diversity in indoor environments was performed in order to shed light on the components of microbial consortia (Micheluz et al. 2015; Ortega-Morales et al. 2016). A widespread fungal infection was revealed in c*ompactus shelves* of Venetian library by Micheluz et al. (2015); particularly, xerophilic fungi were identified using a polyphasic approach based on morpho-physiological features and molecular studies. Molecular identification was performed by amplification and sequencing of internal transcribed spacers (ITS) of β-tubulin and actin genes. Moreover, airborne fungi possess great enzymatic potential to degrade materials, so their hydrolytic activity in attacking, proliferating and degrading these important artistic-historical items can be successfully detected using enzymatic assays and can be considered valuable data in completing the typical identification list of isolated strains. Recently, the biodegradative action of fungal microflora from mummified remains and fungal airborne communities was investigated using hydrolytic assays (Šimonovičová et al. 2015). Borrego et al. (2012) have determined indoor air quality in Argentine archives and the biodeterioration of documentary heritage using an analytical approach based on the qualitative determination of enzymatic fungal activity and acid production by fungi.

## References

Andersen AA (1958) New sampler for the collection, sizing, and enumeration of viable airborne particles. J Bact 76(5):471–484

Bernardi A (2009) Microclimate inside cultural heritage buildings. Il Prato, Padova, p 171. ISBN: 978-88-6336-032-5

Blais-Lecours P, Perrott P, Duchaine C (2015) Non-culturable bioaerosols in indoor settings: impact on health and molecular approaches for detection. Atmos Environ 110:45–53. https://doi.org/10.1016/j.atmosenv.2015.03.039

Bonazza A, Maxwell I, Drdácký M, Vintzileou E, Hanus C, Ciantelli C, De Nuntiis P et al (2018) Safeguarding cultural heritage from natural and man-made disasters. A comparative analysis of risk management in the EU. ISBN 978-92-79-73946-0. https://doi.org/10.2766/224310

Bonazza A, Sardella A, Cacciotti R, Kaiser A, Hanus C, De Nuntiis P, Hanus C, Maxwell I, Drdacký T, Drdacký M (2021) Safeguarding cultural heritage from climate change related hydrometeorological hazards in Central Europe. Int J Disaster Risk Reduct 63:102455, ISSN 2212-4209. https://doi.org/10.1016/j.ijdrr.2021.102455

Borrego S, Lavin P, Perdomo I, Gómez de Saravia S, Guiamet P (2012) Determination of indoor air quality in archives and biodeterioration of the documentary heritage. ISRN Microbiol 2012:1–10

Brimblecombe P (1990) The composition of museum atmosphere. Atmos Environ 24B:1–8. https://doi.org/10.1016/0957-1272(90)90003-D

Cacciotti R, Kaiser A, Sardella A, De Nuntiis P, Drdacky M (2021) Climate-change-induced disasters and cultural heritage: optimizing management strategies in Central Europe. Clim Risk Manag 32(15):100301. https://doi.org/10.1016/j.crm.2021.100301

Camuffo D (2007) Church heating and the preservation of the cultural heritage: guide to the analysis of the pros and cons of various heating systems. Electa Mondadori, Milan. ISBN: 88-370-5034-8

Camuffo D (2019) Microclimate for cultural heritage – measurement, risk assessment, conservation, restoration, and maintenance of indoor and outdoor monuments, 3rd edn, vol 23. Elsevier Science, New York, p 582. ISBN: 978–0–444-64106-9

Camuffo D, Sturaro G, Valentino A, Camuffo M (1999) The conservation of artworks and hot air heating systems in Churches: are they compatible? The case of Rocca Pietore. Italian Alps Stud Conserv 44(3):209–216. https://doi.org/10.2307/1506706

Caneva G, De Nuntiis P, Fornaciari M, Ruga L, Valenti P, Pasquariello G (2020) Aerobiology applied to the preventive conservation of cultural heritage. Aerobiologia 36:99–103. https://doi.org/10.1007/s10453-019-09589-9

Cappitelli F, Zanardini E, Sorlini C (2004) The biodeterioration of synthetic resins used in conservation. Macromol Biosci 4:399–406. https://doi.org/10.1002/mabi.200300055

Cassar M (1995) Environmental management: guidelines for museums and galleries. Routledge, London, p 165

Castillo NI, Ibáñez M, Beltrán E, Rivera-Monroy J, Ochoa JC, Páez-Castillo M, Posada-Buitrago ML, Sulyok M, Hernández F (2016) Identification of mycotoxins by UHPLC–QTOF MS in airborne fungi and fungi isolated from industrial paper and antique documents from the Archive of Bogotá. Environ Res 144(A):130–138. https://doi.org/10.1016/j.envres.2015.10.031

Cavallini T, Massa S, Russo A (1991) Optimal environmental conditions in museums. In: Science, technology and European cultural heritage: proceedings of the European symposium, Bologna, 13–16 June 1989. Butterworth-Heinemann Publishers, Oxford/Boston, pp 626–631. ISBN: 9780750602372

Ciferri O (1999) Microbial degradation of paintings. Appl Environ Microbiol 65(3):879–885. https://doi.org/10.1128/aem.65.3.879-885.1999

Cox CS, Wathes CM (1995) Bioaerosols handbook. CRC Press, Lewis Publishers, Boca Raton, p 656

De Guichen G (1980) Climate in museums: measurement. ICCROM, Rome

De Guichen G, Kabaoglu C (1985) How to make a rotten show-case. Museum (UNESCO) 146:64–67

De Nuntiis P, Maggi O, Mandrioli P, Ranalli G, Sorlini C (2003) Monitoring the biological aerosol. In: Mandrioli P, Caneva G, Sabbioni C (eds) Cultural heritage and aerobiology – methods and measurement techniques for biodeterioration monitoring. Kluwer Academic Publishers, Dordrecht, pp 107–144

Després VR, Huffman JA, Burrows SM, Hoose C, Safatov AS, Buryak G, Frohlich-Nowoisky J, Elbert W, Andreae MO, Pöschl U, Jaenicke R (2012) Primary biological aerosol particles in the atmosphere: a review. Tellus Ser B 64:15598. https://doi.org/10.3402/tellusb.v64i0.15598

Di Carlo E, Chisesi R, Barresi G, Barbaro S, Lombardo G, Rotolo V, Sebastianelli M, Travagliato G, Palla F (2016) Fungi and bacteria in indoor cultural heritage environments: microbial related risks for artworks and human health. Environ Ecol Res 4(5):257–264. https://doi.org/10.13189/eer.2016.040504

Douwes J, Thorne P, Pearce N, Heederik D (2003) Review bioaerosol health effects and exposure assessment: progress and prospects. Ann Occup Hyg 47:187–200. https://doi.org/10.1073/pnas.0811003106

Fröhlich-Nowoisky J, Pickersgill DA, Després VR, Poschl U (2009) High diversity of fungi in air particulate matter. Proc Natl Acad Sci 106:12814–12819. https://doi.org/10.1073/pnas.0811003106

Fuzzi S, Mandrioli P, Perfetto A (1997) Fog droplets – an atmospheric source of secondary biological aerosol particles. Atmos Environ 31:287–290. https://doi.org/10.1016/1352-2310(96)00160-4

Fuzzi S, Baltensperger U, Carslaw K, Decesari S, Denier van der Gon H, Facchini MC, Fowler D, Koren I, Langford B, Lohmann U, Nemitz E, Pandis S, Riipinen I, Rudich Y, Schaap M, Slowik JG, Spracklen DV, Vignati E, Wild M, Williams M, Gilardoni G (2015) Particulate matter, air quality and climate: lessons learned and future needs. Atmos Chem Phys 15:8217–8299. https://doi.org/10.5194/acp-15-8217-2015

Gaüzère C, Moletta-Denat M, Bousta F, Moularat S, Orial G, Ritoux S, Godon JJ, Robine E (2013) Reliable procedure for molecular analysis of airborne microflora in three indoor environments: an office and two different museum contexts. Clean–Soil Air Water 41(3):226–234. https://doi.org/10.1002/clen.201100699

Gaüzère C, Moletta-Denat M, Blanquart H, Ferreira S, Moularat S, Godon J-J, Robine E (2014) Stability of airborne microbes in the louvre museum over time. Indoor Air 24:29–40. https://doi.org/10.1111/ina.12053

Getty Conservation Institute (1994) Preventive conservation. In: Knell S (ed) Care of collections. Routledge, London, pp 83–87. https://www.taylorfrancis.com/chapters/edit/10.4324/9780203974711-18/preventive-conservation?context=ubx&refId=52f7f73c-7f59-4359-9bbd-9ee804d366aa

Gregory PH (1973) The microbiology of the atmosphere, 2nd edn. Aylesbury, Leonard Hall, p 377

Griffith WD, De Cosemo GAL (1994) The assessment of bioaerosols: a critical review. J Aerosol Sci 25(8):1425–1458. https://doi.org/10.1016/0021-8502(94)90218-6

Gudmundsson G, Monick MM, Hunninghake GW (1999) Viral infection modulates expression of hypersensitivity pneumonitis. J Immunol 162(12):7397–7401

Hinds WC (1999) Aerosol technology: properties, behavior, and measurement of airborne particles, 2nd edn. Wiley Interscience, New York, p 504

Hirst JM (1995) Bioaerosols: introduction, retrospect and prospect. In: Cox CS, Wathes CM (eds) Bioaerosols handbook. CRC Press, Lewis Publishers, Boca Raton, pp 5–14

Hueck HJ (2001) The biodeterioration of materials: an appraisal (reprinted). Int Biodeterior Biodegradation 48(1–4):5–11. https://doi.org/10.1016/S0964-8305(01)00061-0

IBC (ed) (2007) Oggetti nel tempo. Principi e tecniche di conservazione preventiva. Clueb, Bologna, pp 258. ISBN: 978-88-491-2866-6

Jaenicke R (2005) Abundance of cellular material and proteins in the atmosphere. Science 308(5718):73. https://doi.org/10.1126/science.1106335

Jones AM, Harrison RM (2004) The effects of meteorological factors on atmospheric bioaerosol concentrations, a review. Sci Total Environ 326(1–3):51–180. https://doi.org/10.1016/j.citotenv.2003.11.021

Kavklera K, Gunde-Cimermanb N, Zalarb P, Demšard A (2015) Fungal contamination of textile objects preserved in Slovene museums and religious institutions. Intern Biodeter Biodegr 97: 51–59. https://doi.org/10.1016/j.ibiod.2014.09.020

Kellogg CA, Griffin DW (2006) Aerobiology and the global transport of desert dust. Trends Ecol Evol 21(11):638–644. https://doi.org/10.1016/j.tree.2006.07.004

Lavin P, Gómez de Saravia SG, Guiamet PS (2014) An environmental assessment of biodeterioration in document repositories. Biofouling 30(5):561–569. https://doi.org/10.1080/08927014.2014.897334

Lazaridis M, Katsivela E, Kopanakis I, Raisi PG (2015) Indoor/outdoor particulate matter concentrations and microbial load in cultural heritage collections. Herit Sci 3:34. https://doi.org/10.1186/s40494-015-0063-0

Letch T (2016) Evaluation of a parchment document, the 13th century incorporation charter for the city of Krakow, Poland, for microbial hazards. Appl Environ Microbiol 82:2620–2631. https://doi.org/10.1128/AEM.03851-15

Lighthart B, Stetzenbach LD (1994) Distribution of microbial bioaerosol. In: Lighthart B, Mohr AJ (eds) Atmospheric microbial aerosols: theory and applications. Springer, Boston, MA, pp 68–98. https://doi.org/10.1007/978-1-4684-6438-2_4

MacNeil L, Kauri T, Robertson W (1995) Molecular techniques and their potential application in monitoring the microbiological quality of indoor air. Can J Microbiol 41(8):657–665. https://doi.org/10.1139/m95-091

Mandrioli P (1985) L'Aerobiologia e le sue moderne applicazioni. Aerobiologia 1:2–4. https://doi.org/10.1007/BF02447689

Mandrioli P (2015) The importance of environmental parameters for the conservation of mural paintings. Specificity of the Sistine environment. Conference proceedings: the Sistine Chapel, twenty years later. New breath new light, Auditorium Conciliazione, Roma 30-31/10/2014. Edizioni Musei Vaticani, Roma, pp 119–133. ISBN: 978-88-8271-366-9

Mandrioli P, Ariatti A (2001) Aerobiology: future course of action. Aerobiologia 17:1–10. https://doi.org/10.1023/A:1007602928928

Mandrioli P, Negrini MG, Scarani C, Tampieri F, Trombetti F (1980) Mesoscale transport of Corylus pollen grains in winter atmosphere. Grana 19(3):227–233. https://doi.org/10.1080/00173138009425007

Mandrioli P, Negrini MG, Cesari G, Morgan G (1984) Evidence for long range transport of biological and anthropogenic aerosol particles in the atmosphere. Grana 23(1):43–53. https://doi.org/10.1080/00173138409428876

Mandrioli P, Comtois P, Levizzani V (eds) (1998) Methods in aerobiology. Pitagora Editrice, Bologna, pp. 276. ISBN: 88-371-1043-X

Michalski S (1994) A systematic approach to preservation: description and integration with other museum activities. Stud Conserv 39(S-2):8–11

Micheluz A, Manente S, Tigini V, Prigione V, Pinzari F, Ravagnan G, Varese GC (2015) The extreme environment of a library: xerophilic fungi inhabiting indoor niches. Int Biodeterior Biodegradation 99:1–7. https://doi.org/10.1016/j.ibiod.2014.12.012

Montacutelli R, Maggi O, Tarsitani G, Gabrielli N (2000) Aerobiological monitoring of the "Sistine Chapel": airborne bacteria and microfungi trends. Aerobiologia 3:441–448. https://doi.org/10.1023/A:1026525432412

Năşcuțiu AM (2010) Viable non-culturable bacteria. Bacteriol Virusol Parazitol Epidemiol 55:11–18

Nugari MP (2003) The aerobiology applied to the conservation of works of art. In: Saiz-Jimenez C (ed) Coalition advanced course on biological problems in cultural heritage, Florence 8–-9 November 2002. Coalition Newsletter 6(SI): 8–10

Nugari MP, Ricci S, Roccardi A, Monte M (2003) Churches and hypogea. In: Mandrioli P, Caneva G, Sabbioni C (eds) Cultural heritage and aerobiology – methods and measurement techniques for biodeterioration monitoring. Kluwer Academic Publishers, Dordrecht, pp 207–224

Ortega-Morales BO, Narváez-Zapata J, Reyes-Estebanez M, Quintana P, De la Rosa SDC (2016) Bioweathering potential of cultivable fungi associated with semi-arid surface microhabitats of Mayan buildings. Front Microbiol 7:201. https://doi.org/10.3389/fmicb.2016.00201

Paiva de Carvalho HP, Sequeira SO, Pinho D, Trovao J, Fernandes da Costa RM, Egas C, Macedo MF, Portugal A (2019) Combining an innovative non-invasive sampling method and high-throughput sequencing to characterize fungal communities on a canvas painting. Int Biodeterior Biodegradation 145:104816. https://doi.org/10.1016/j.ibiod.2019.104816

Palla F (2015) Biological risk for the conservation and exhibition of historical–artistic artefacts in confined spaces. In: Lorusso S, Natali A, Palla F (eds) Risk management in the cultural heritage sector: museums, libraries and archives. Mimesis Edizioni, Milano-Udine, pp 95–108

Palla F, Di Carlo E, Barresi G, Billeci N, Chisesi R, Barbaro S, Sebastianelli S (2014) Bioaerosol in indoor environments for conservation of cultural heritage: potential risks for historical-artistic manifacts and human health. In: International Conference Indoor Air Quality in Heritage and Historic Environments, Prague, Kaiserštejn Palace, April 13–16 2014, p 47

Pashley CH, Fairs A, Free RC, Wardlaw AJ (2012) DNA analysis of outdoor air reveals a high degree of fungal diversity, temporal variability, and genera not seen by spore morphology. Fungal Biol 116(2):214–224. https://doi.org/10.1016/j.funbio.2011.11.004

Pasquarella C, Pitzurra O, Savino A (2000) The index of microbial air contamination. J Hosp Infect 46:241–256. https://doi.org/10.1053/jhin.2000.0820

Pasquarella C, Balocco C, Pasquariello G, Petrone G, Saccani E, Manotti P, Ugolotti M, Palla F, Maggi O, Albertini R (2015) A multidisciplinary approach to the study of cultural heritage environments: experience at the Palatina library in Parma. Sci Total Environ 536:557–567. https://doi.org/10.1016/j.scitotenv.2015.07.105

Pitzurra M, Savino A, Pasquarella C, Poletti L (1997) A new method to study the microbial contamination on surfaces. Hyg Med 22:77–92

Pöschl U (2005) Atmospheric aerosols: composition, transformation, climate and health effects. Angew Chem Int Ed Engl 44(46):7520–7540. https://doi.org/10.1002/anie.200501122

Puškárová A, Bučková M, Habalová B, Kraková L, Maková A, Pangallo D (2016) Microbial communities affecting albumen photography heritage: a methodological survey. Sci Rep 6(20810). https://doi.org/10.1038/srep20810

Ramos T, Stephens B (2014) Tools to improve built environment data collection for indoor microbial ecology investigations. Build Environ 81:243–257. https://doi.org/10.1016/j.buildenv.2014.07.004

Ranalli G, Zanardini E, Andreotti A, Colombini MP, Corti C, Bosch-Roig P, De Nuntiis P, Lustrato G, Mandrioli P, Rampazzi L, Giantomassi C, Zari D (2018) Hi-tech restoration by two steps biocleaning process of triumph of death fresco at the Camposanto monumental cemetery (Pisa, Italy). J Appl Microbiol 125(3):800–812. https://doi.org/10.1111/jam.13913

Rantio-Lehtimäiki A (1994) Short, medium and long range transported airborne particles in viability and antigenicity analyses. Aerobiologia 10(2):175–181. https://doi.org/10.1007/BF02459233

Ruga L, Orlandi F, Romano B, Fornaciari M (2015) The assessment of fungal bioaerosols in the crypt of St. Peter in Perugia (Italy). Int Biodeter Biodegradr 98:121–130. https://doi.org/10.1016/j.ibiod.2014.12.010

Sabbioni C, Mandrioli P, Bonazza A, De Nuntiis P, Guaraldi C (2008) Indagini diagnostiche finalizzate alla identificazione delle cause di degrado degli affreschi del camposanto monumentale di Pisa. Report tecnico contratto OPA Pisa – ISAC CNR, Bologna, p 154

Saiz-Jimenez C, Gonzalez JM (2007) Aerobiology and cultural heritage: some reflections and future challenges. Aerobiologia 23:89–90. https://doi.org/10.1007/s10453-007-9059-x

Sanmartín P, De Araujo A, Vasanthakumar A (2016) Melding the old with the new: trends in methods used to identify, monitor, and control microorganism on cultural heritage materials. Microb Ecol 71(4):1–17. https://doi.org/10.1007/s00248-016-0770-4

Schlesinger P, Mamane Y, Grishkan I (2006) Transport of microorganisms to Israel during Saharan dust events. Aerobiologia 22:259–273. https://doi.org/10.1007/s10453-006-9038-7

Schwendemann AB, Wang G, Osborn JM, Thatcher SL (2007) Aerodynamics of saccate pollen and its implications for wind pollination. Am J Bot 94(8):1371–1381. https://doi.org/10.3732/ajb.94.8.1371

Sedlbauer K (2002) Prediction of mould growth by hygrothermal calculation. J Build Phys 25:321–336. https://doi.org/10.1177/0075424202025004093

Sesana E, Gagnon AS, Ciantelli C, Cassar J, Hughes JJ (2021) Climate change impacts on cultural heritage: a literature review. Wiley Interdiscip Rev-Clim Chang 12(e710). https://doi.org/10.1002/wcc.710

Šimonovičová A, Kraková L, Pangallo D, Majorošová M, Piecková E, Bodoriková S, Dörnhoferová M (2015) Fungi on mummified human remains and in the indoor air in the Kuffner family crypt in Sládkovičovo (Slovakia). Int Biodeter Biodegrad 99:157–164. https://doi.org/10.1016/j.ibiod.2014.12.011

Skóra J, Gutarowska B, Pielech-Przybylska K, Stępień Ł, Pietrzak K, Piotrowska M, Pietrowski P (2015) Assessment of microbiological contamination in the work environments of museums, archives and libraries. Aerobiologia 31(3):389–401

Sterflinger K (2010) Fungi: their role in the deterioration of cultural heritage. Fung Biol Rev 24:47–55. https://doi.org/10.1016/j.fbr.2010.03.003

Sterflinger K, Pinzari F (2012) The revenge of time: fungal deterioration of cultural heritage with particular reference to books, paper and parchment. Environ Microbiol 14(3):559–566

Su C, Lei L, Duan Y, Zhang KQ, Yang J (2012) Culture-independent methods for studying environmental microorganisms: methods, application, and perspective. Appl Microbiol Biotechnol 93(3):993–1003. https://doi.org/10.1007/s00253-011-3800-7

Tampieri F, Mandrioli P, Puppi GL (1977) Medium range transport of airborne pollen. Agr Meteorol 18(1):9–20. https://doi.org/10.1016/0002-1571(77)90023-1

Tarsitani G, Moroni C, Cappitelli F, Pasquariello G, Maggi O (2014) Microbiological analysis of surfaces of Leonardo Da Vinci's Atlantic codex: biodeterioration risk. Int J Microbiol 2014: 214364–214371. https://doi.org/10.1155/2014/214364

Thomson G (1986) The museum environment, 2nd edn. Butterworths, Los Angeles, p 292

United Nations, Sendai Framework for Disaster Risk Reduction 2015–2030 (2015) United Nations Office for Disaster Risk Reduction (UNISRD)

Urzì C, De Leo F, De Hoog GS, Sterflinger K (2000) Recent advances in the molecular biology and ecophysiology of meristematic stone-inhabiting fungi. In: Ciferri O, Tiano P, Mastromei G (eds) Of microbes and art. The role of microbial communities in the degradation and protection of cultural heritage. Kluwer Academic Plenum, London, pp 3–19

Valentin N (2003) Microbial contamination in museums collections: organic materials. In: Saiz-Jimenez C (ed) Molecular biology and cultural heritage. Balkema Pub, Lisse, pp 85–91

Varas-Muriel MJ, Fort R, Martínez-Garrido MI, Zornoza-Indart A, López-Arce P (2014) Fluctuations in the indoor environment in Spanish rural churches and their effects on heritage conservation: hygro-thermal and $CO_2$ conditions monitoring. Build Environ 82:97–109. https://doi.org/10.1016/j.buildenv.2014.08.010

Walser SM, Gerstner DG, Brenner B, Bünger J, Eikmann T, Janssen B, Kolb S, Kolk A, Nowak D, Raulf M, Sagunski H, Sedlmaier N, Suchenwirth R, Wiesmüller G, Wollin K-M, Tesseraux I, Herr C (2015) Evaluation of exposure-response relationships for health effects of microbial bioaerosols—a systematic review. Int J Hyg Environ Health. https://doi.org/10.1016/j.ijheh.2015.07.004