Viable and not viable spore concentrations in National Gallery of Umbria (Italy)

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Received: 20 March 2020 / Accepted: 19 January 2021 / Published online: 15 March 2021
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Abstract The conservation actions towards artworks holding a common patrimony for the community are of primary importance, but also those related to their “container” as museums, libraries or archives are to consider. Fungal spores and bacteria carried by air flows to the artwork surface can colonize it causing biodeterioration through physical and/or chemical alterations of the materials with the irreversible loss of their value. The quality control of the indoor air surrounding the historic building is essential, as well as for the protection and conservation of the artwork, also for the protection of the health of operators and visitors. The aim of this study was to monitor airborne fungal particles, through volumetric spore traps, for improving the knowledge about the conservation and protection of artworks in the museum environment analysing the principal relationships between indoor environmental conditions and potentially biodeteriogenic fungal spore growth. The evidence of no significant relationships between spore concentrations and environmental conditions recorded inside the different expositive rooms testified the regular and correct maintenance of the air conditioning system inside the considered building (National Gallery of Umbria, central Italy). Moreover, in a specific museum room a

significant spore concentration decreasing trend was recorded mainly due to a structural modification in the same building.

Keywords Artworks · Biodeterioration risk · Airborne fungal spore · Museum

1 Introduction

The preservation and maintenance of artworks collections are fundamental for the existence of a museum and, at the same time, the assumption of its social function, holding a common patrimony for the community. Preserving the assets present in museums means preventing and limiting both the damage caused by the natural degradation of the artworks constituent materials, and those attributable to its utilization (Caneva et al., 2008). Conservation actions are directed not only to the artwork, but also to the “container” that houses it, and to the environment that interacts with it (Cacace, 2007). To identify promptly and effectively the risk conditions for the artworks collections, it is important to evaluate the interrelationships between the environment and the quality of indoor air, by a chemical, physical and biological point of view. In confined spaces as museums, libraries or archives, in addition to atmospheric agents that mainly have a chemical-physical action, the biological

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component of air constitutes a potential element of degradation of the artworks. Organic art pieces are the most sensitive to light, humidity, temperature and air pollution being susceptible to the microorganisms development (Ruga et al., 2008). Fungal spores and bacteria moved by air flows can colonize artwork surfaces and with favourable environmental conditions may cause biodeterioration (Caneva et al., 2007). Physical and/or chemical alterations of the materials (substrate degeneration) may be caused by biological pollution determining aesthetic alteration of the artworks with the irreversible loss of their value.

The indoor air quality control is essential, as well as for the protection and conservation of the artwork, also for the protection of the health of operators and visitors. In particular, the determination of biological pollutants allows to define the levels of biodeterioration risk and of the hygienic-sanitary level for both workers and visitors. Moreover, it is useful to optimize the environmental protection procedures for helping the preventive conservation, and for a more careful analysis of the causes of alteration and degradation (Sorlini, 1993; Pinna, 2003; Caple, 2012; Lucchi, 2018; Caneva et al., 2019).

The aim of this study was to contribute to the knowledge improvement about the conservation and protection of artworks in the museum environment through the airborne fungal particles monitoring. Moreover, the principal relationships between indoor environmental conditions and potentially biodeteriogen fungal spore growth were investigated utilizing two different spore traps.

2 Materials and methods

2.1 Studied area

This study was carried out in the conservation and exhibition areas of the “National Gallery of Umbria”, the principle Museum of Umbria located in Perugia (Italy). Since 1878 it has been housed on the upper floors of the historic “Palazzo dei Priori”, splendid example of civil gothic architecture, whose first section was constructed in 1292. It extends on 4000 m² with 40 expositive rooms.

The collection, spread out in several spaces from room 1 to room 40, comprises the greatest representation of the Umbrian School of painting, ranging from

the XIII to the XIX century. The exposition begins from greater room at third floor of the building where artefacts datable to XIII century are conserved to the new rooms at second floor with more recent artefacts datable between XVI–XIX centuries.

The sampling activity was defined in relation to the environments that would be more critical. Particular attention was devoted to rooms where organic nature artefacts were conserved, or where the anthropic impact is particularly high (the presence of users and visitors causes changes in environmental conditions, particularly in small rooms with higher risk of biodeterioration). The airborne spores sampling was realized in four rooms (8, 11, 23 and 40) characterized by the permanent presence of particular artistic and cultural relevance artefacts as they are painted on wooden boards made by “Fra Angelico”, by “Piero della Francesca”, by “Perugino” or paintings on canvas from the XVIII and XIX centuries made for example by “Sebastiano del Conca” (Garibaldi and Salari, 2006). The expositive areas are equipped by an air-forced system managed by a dedicated software that consists of two central heating and cooling systems. Thanks to the special detection probes present in the individual rooms, connected with the respective air conditioners, the air coming from outside is put into circulation after passing through special water and antibacterial liquid filters, essential for the reduction of microbial and polluting substances suspended in the air. In Fig. 1 the plan of the “National Gallery of Umbria” is reported with the indications of the 2 floors where monitoring activities were realized in the 4 rooms.

2.2 Aerobiological sampling

Aerobiological monitoring was done with the volumetric method, using two types of instruments (Ogden et al., 1974). The sampling was conducted weekly, from May 2011 to June 2017. The intent was to accurately represent the aerospore in the studied environments, also considering the fluctuations related to seasonal climatic conditions. Through the utilized methodology we studied both the non-cultivable aerodynamic fungal component (spore concentration/m³ of air) and the cultivable vital component (total fungal load) (Mandrioli et al., 2003).

Fig. 1 Gallery exposition building. Main entrance is at third floor, while the exit at second floor

2.3 Fungal particles counting

For the sampling of the airborne fungal particles, a Personal Volumetric Air Sampler spore trap 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 aspiration of nominal air volumes, and provides data on the fungal spore concentration (number of spores/m³). The simple counting of particles gives a first “quick and dirty” approximation of a microbial contamination of the air. The sampler was positioned, one time in a week for 10 min (for a total of 0.1 m³ of sampled air), both in the interior of the Gallery and in the external environment.

This study also examined the indoor environment of the Gallery, as a comparison with the outdoor

environment (referred to as ‘outside’). As some of any biological polluting agents present might come from the outside environment, the outdoor pollution also needs to be taken into consideration during the evaluation of the risk factors (Ruga et al., 2015).

2.4 Cultural analyses

For the analysis of the airborne viable fungal particles, the Andersen three-stage sampler was used (first stage > 7.0 µm; second stage between 3.3 and 4.7 µm; third stage between 1.1 and 2.1 µm) (Sorlini, 1993; Nugari and Roccardi, 2001; Nugari, 2003). During the sampling phase, the sampler was placed in the individual areas selected for the monitoring. The time for the air aspiration was again set to 10 min (at 28.3 l/min) determining a total of 0.283 m³ of sampling air

per week. The analysis of the airborne viable culturable fungal component has been carried out only in the indoor spaces. The comparison of indoor and outdoor fungal component vitality was not realized because the real objective of work was to evaluate the vitality of spores in the Gallery, considering that the scientific literature (Mandrioli et al., 2003; Caneva et al., 2008) suggests as in outdoor spaces the atmospheric pollutants, depending by their nature and concentration, should stimulate or inhibit the fungi metabolism influencing the spore vitality. After three and six days of incubation at 28 ± 2 °C, the developed microbic colonies were counted. The values obtained were expressed as colony forming units per cubic meter of air analysed (CFU/m³).

2.5 Identification of fungal groups

The Fungi identification was realized on the base of colonies macro-morphological and spore micro-morphological description. Spore reference images, Atlas and spore collection were used for the spore identification (Saenz Lain et al., 2006). In all the considered methods the “fungal strains” were classified to genus level.

2.6 Measurement of temperature and relative humidity

The study of the microclimatic conditions in conservation environments is a prerequisite for an understanding of the causes of any degradation, and to define the microbial diffusion processes to identify the most appropriate conservative mode (Monte and Ferrari, 1993; Mandrioli et al., 2003; Ascione and Minichiello, 2010; Sileo et al., 2013; Zorpas and Skouroupatis, 2016).

To obtain a more complete picture of the environmental conditions some variables (temperature and relative humidity) were considered inside the Gallery contemporary to the aerobiological samples and in outdoors areas.

The considered environmental variables were measured using the digital thermo-hygrometer (RTGR328N Thermo/Hygro Sensor, Oregon Scientific), cable free, RF frequency 433 MHz. The range of temperature measurement is from -20 to $+60$ °C, the range of measurement of relative humidity is between 20 and 99%.

2.7 Statistical analyses

The weekly records of spore concentrations and environmental data were summarized with the calculation of monthly averages to permit a better interpretation of the biological phenomena in relation to the indoor environmental Gallery conditions. Descriptive statistics were realized to explain the principal distribution of the spore concentrations and environmental variables inside the considered rooms of the National Gallery.

Correlation analyses were realized to show the principal relationships between spore concentrations monitored in the different monitoring rooms and outdoor with both samplers. Also environmental variables were correlated each other.

Moreover, a trend analysis was realized using nonparametric Mann–Kendall tests for monotonic positive or negative trends, considering the significant number of fungal spore monitoring and their distribution not really adjustable to normal distribution for which this test is particularly adaptable (Drapela and Drapelova, 2011; Pohlert, 2016). To estimate the true slope of an existing trend, Sen’s nonparametric method was used (Salmi et al., 2002), for the significance levels, the following symbols are used: +, trend at $p < 0.05$; *, trend at $p < 0.01$; and **, trend at $p < 0.001$.

3 Results

In Fig. 2 the Boxplot charts realized with the total spore concentrations captured by Burkard sampler and viable spore concentrations monitored by Andersen sampler have been reported. The viable (Andersen) and total (Burkard captures viable and not viable spores) spore monitoring shows different levels of concentrations considering the first 2 rooms (8–11) and the other ones (23–40). In the last 2 rooms the presence of not viable spore was highest considering that Andersen spore concentrations appear comparable to that recorded in the first 2 rooms. In terms of spore distribution around the median value, only the viable spore concentrations manifested a particular behaviour considering room 23 where the values were recorded in a very small range around the same median value.

Fig. 2 Boxplot with all monthly spore concentrations averages sampled with both instruments in the monitored rooms

Qualitative analysis of airborne particulate matter recorded with both the spore samplers has identified the presence of several fungal groups, as shown in Table 1. The most frequently monitored genera were *Cladosporium*, *Penicillium* and *Aspergillus*. Moreover, the analysis of the different genera recorded with the Andersen sampler, allowed to confirm the presence and vitality of those genera already detected with the Burkard and to identify the presence of some other fungal groups not recognized by the Burkard.

Figure 3 presents values of 2 environmental variables as temperature and RH recorded in the 4 monitoring rooms. In consideration of the controlled art gallery indoor conditions, the variables, as imaginable, evidenced very homogeneous values, during the year, with temperature average data around 20–21 degrees and RH values around 50%. Only RH values showed a slight decrease passing from the first to the last room but always included in a narrow range.

In Table 2 the correlation analyses between the spore concentrations in the different monitoring rooms

Table 1 Fungi genera identified with the Andersen (A) and the Burkard (B) Air Samplers in all the monitored rooms of the National Gallery of Umbria during the whole study period

Genus	A	B	Genus	A	B
<i>Acremonium</i>	+	–	<i>Humicola</i>	+	–
<i>Agaricus</i>	–	+	<i>Laccaria</i>	+	+
<i>Agrocybe</i>	–	+	<i>Lepiota</i>	–	+
<i>Alternaria</i>	+	+	<i>Leptosphaeria</i>	–	+
<i>Amphisphaeria</i>	–	+	<i>Memmoniella</i>	+	–
<i>Arthrinium</i>	–	+	<i>Mucor</i>	+	–
<i>Ascobolus</i>	–	+	<i>Paraphaeosphaeria</i>	–	+
<i>Aspergillus</i>	+	–	<i>Paecilomyces</i>	+	–
<i>Bipolaris</i>	+	+	<i>Penicillium</i>	+	–
<i>Botrytis</i>	–	+	<i>Periconia</i>	–	+
<i>Cephalosporium</i>	–	+	<i>Peronospora</i>	–	+
<i>Cerebella</i>	–	+	<i>Phaeosphaeria</i>	–	+
<i>Chaetomium</i>	+	+	<i>Pithomyces</i>	+	+
<i>Cladosporium</i>	+	+	<i>Pleospora</i>	–	+
<i>Coprinus</i>	–	+	<i>Puccinia</i>	–	+
<i>Curvularia</i>	–	+	<i>Rhizopus</i>	+	–
<i>Didymella</i>	–	+	<i>Scopulariopsis</i>	+	+
<i>Drechslera</i>	–	+	<i>Sporidesmium</i>	+	+
<i>Epicoccum</i>	–	+	<i>Stemphylium</i>	+	+
<i>Exosporium</i>	–	+	<i>Stereum</i>	+	–
<i>Fusarium</i>	+	–	<i>Torula</i>	+	+
<i>Ganoderma</i>	–	+	<i>Trichophyton</i>	+	–
<i>Geotrichum</i>	+	–	<i>Xylaria</i>	–	+

A = Andersen sampler; B = Burkard sampler; + sampled genus; – not detected genus

are evidenced. The unique significant correlation values are reported considering rooms 8 and 11 for both the monitoring instruments ($r = 0.63$ for Burkard and $r = 0.88$ for Andersen). No significant values have been reported considering the indoor and outdoor (external monitoring) spore concentrations captured with Burkard trap.

The correlation analysis between temperature and RH recorded both inside studied rooms that externally the Gallery building showed various significant values (Table 3). The main relationships were shown by the rooms situated at the same floor, between rooms 8 and 11 with environmental conditions in accordance with each other, and between rooms 23 and 40. Moreover, temperature values recorded at second floor (rooms 23 and 40) were in accordance with temperatures

Fig. 3 Boxplot with all monthly Temperature and Humidity averages in the monitored rooms

Table 2 Correlation analysis between spore concentrations monitored in the different monitoring rooms and outdoor (external) with both samplers (* $p < 0.05$; ** $p < 0.01$)

	Room 8	Room 11	Room 23	Room 40
<i>Burkard</i>				
Room 8	1			
Room 11	0.63*	1		
Room 23	0.50	0.43	1	
Room 40	0.27	0.22	0.29	1
External	0.35	0.32	0.47	0.33
<i>Andersen</i>				
Room 8	1			
Room 11	0.88**	1		
Room 23	0.35	0.25	1	
Room 40	0.31	0.28	0.40	1

recorded outside (room 40 showed also RH related to the external RH).

Moreover, some correlation analyses were carried out considering temperature and RH, recorded during sampling activity in the different rooms, in relation to the spore concentrations (Table 4). These analyses not evidenced significant relationships and in particular during the investigations no temperature or humidity thresholds were exceeded ($T^{\circ} > 25^{\circ}$; $RH > 60\%$) above which bio-deterioration phenomenon may be intensified.

Correlation analyses between outside environmental data and internal spore concentrations in the different rooms not evidenced significant relationships, as it was easy to imagine, while significant values were recorded considering the influence of temperature and RH in relation to spore concentrations recorded outside the same gallery building through Burkard sampler. In this case, temperature and RH values showed a positive significant correlation with external spore concentrations ($T_{\max}$ 0.66*; $T_{\min}$ 0.62*; * $p < 0.05$), while RH a positive but not significant value (RH 0.56). The nonparametric Mann–Kendall test realized with the spore concentrations monthly averages monitored with Burkard showed no significant trends in the rooms apart those recorded in room 40 (Test $Z = -4.01$, Signific. ***) that evidenced a clear decrease during the considered period (Fig. 4). The spore concentrations recorded by Andersen trap showed an analogous behaviour with no trends in rooms 8, 11 and 23, while the last room (40) presented a higher significance level than the Burkard one (Test $Z = 3.40$; Signific. ***)

Finally, no significant relationships were evidenced considering spore concentrations captured with the 2 spore traps in each room. In particular a one-way ANOVA (Table 5) was realized with the spores sampled in room 40 (where monthly spore concentrations trends were shown) to exemplify the different outcomes due to the methodologies adopted. The f-ratio value was 16.07 (p value of 0.000097, significant at $p < 0.05$). A qualitative analysis concerning spore monitoring evidenced a certain seasonality in the *Cladosporium* species particularly in room 40 where the Burkard sampling showed gradually lower values over the years determining a decreasing trend, while Andersen sampling evidenced a very low significant trend with *Cladosporium* (Fig. 5). The *Cladosporium* spore concentrations sampled with both the spore traps during the summer months represented

Table 3 Correlation analysis between the environmental variables recorded inside the rooms (R) and outdoor (Ext) (* $p < 0.05$; ** $p < 0.01$)

	R_8 T°	R_8 RH	R_11 T°	R_11 RH	R_23 T°	R_23 RH	R_40 T°	R_40 RH
R_8 T°	1							
R_8 RH	− 0.49	1						
R_11 T°	0.68*	− 0.26	1					
R_11 RH	− 0.13	0.64*	− 0.16	1				
R_23 T°	0.26	0.01	0.23	0.20	1			
R_23 RH	− 0.22	0.57	− 0.03	0.61*	− 0.24	1		
R_40 T°	0.42	0.18	0.45	0.42	0.81**	0.12	1	
R_40 RH	0.09	0.34	0.11	0.41	0.30	0.60*	0.22	1
Ext_ T°	0.29	0.05	0.30	0.38	0.88**	0.05	0.91**	0.44
Ext_RH	0.16	0.13	0.19	0.23	− 0.23	0.45	0.21	0.61*

Table 4 Correlation analysis between the environmental variables (T°; RH) recorded inside the rooms and spore concentrations captured with Burkard and CFU presence by Andersen sampler (* $p < 0.05$; ** $p < 0.01$)

	Burkard	Andersen
<i>Room 8</i>		
T°	0.15	0.14
RH	− 0.19	0.17
<i>Room 11</i>		
T°	0.07	0.19
RH	− 0.15	0.52
<i>Room 23</i>		
T°	0.12	− 0.17
RH	− 0.05	0.00
<i>Room 40</i>		
T°	0.32	0.43
RH	0.20	− 0.03

more than 50% of the total spore recorded during the year.

4 Discussion

The main results about spore concentrations (viable and not viable) in the considered rooms evidenced only a relationship between the rooms 8 and 11 which are physically linked by a brief corridor, the other rooms showed independent data. By an indoor environmental point of view, also temperature and RH values appeared related between rooms 8 and 11, while another relationship was carried out considering rooms 23 and 40 situated at second floor of the Gallery building and subjected to the same characteristics of

Fig. 4 Trend analysis with all the monthly spore concentrations averages captured with Burkard and Andersen samplers in room 40

the conditioning system. The external environmental values showed correlations mainly with those recorded inside the last expositive room (40) probably

Table 5 ANOVA considering both monitoring samplers (Burkard and Andersen) utilized in room 40 where spore concentration trends were shown (* $p < 0.05$)

Treatments	Burkard	Andersen	Total
N	74	74	148
$\sum X$	3827.95	1786.67	5614.62
Mean	51.73	24.14	37.93
$\sum X^2$	440,724.4	56,197.86	496,922.3
SD	57.66	13.37	43.95
Result details			
Source	SS	df	MS
Between-treatments	28,154.22	1	28,154.21
Within-treatments	255,768.4	146	1751.83
Total	283,922.6	147

Fig. 5 Monthly *Cladosporium* spore concentrations captured with Burkard and CFU presence by Andersen sampler in room 40 during the study period

due to the stairs presence which connect ground zero of the building (exit) with the same last room.

The evidence of no significant relationships between spore concentrations and almost all indoor environmental conditions of the different expositive rooms may testify the regular and correct maintenance of the air conditioning system inside the Gallery building that avoided excessive peaks of temperature and humidity that could have caused the exceeding of thresholds above which fungal spores would have found growing incentives. Nevertheless, the detection of a certain seasonality of *Cladosporium* spore concentration within room 40 confirms the variability of the composition of fungal spores in the outdoor atmosphere during the year partially influencing indoor environment, above all considering one of the most widespread spore species (Grinn-Gofron and Bosiacka, 2015; Oliveira et al., 2009; Herrero and Zaldivar, 1997).

The conservation of typical artworks collections is directly related to the indoor environment of the museums, and it is especially sensitive to high levels of temperature and relative humidity (Camuffo et al., 1999; Sedlbauer, 2002; Brimblecombe et al., 2004; Bencs et al., 2007; Cappitelli et al., 2009; Kontozova-Deutsch et al., 2011; Lankester and Brimblecombe, 2012; Zorpas and Skouroupatis, 2016). High temperature and humidity also support biological activity; for instance, mould growth is more likely as T° rises for long period the threshold of 20 °C and RH rises above 65%, (Lefevre, 1974; Godoi et al., 2008). For artwork a climatically stable environment is recommended, where there is only slight variation in temperature and RH and where the air pollution concentration is sufficiently low. The synergism between pollutants, temperature and/or humidity can swell/shrink objects, and the results may be destructive above all considering sufficiently high fungal spore presence due to continuous visitors presence (Camuffo et al., 1999; Brimblecombe et al., 2004).

As regard as the room 40, for which a very significant decreasing trend was carried out about spore concentrations recorded with both the instruments, the graphical distribution of the data showed a progressive reduction of spore concentrations during the studied months. This phenomenon was due mainly to the improvement in the internal air conditioning system maintenance, done with more frequent air filter management, and above all with a structural modification in the same building of the National Gallery. Outside the room 40 along the stairs, through which

it's possible to reach the exit (ground zero), a large glass door was applied to better isolate the second floor of the exposition (characterized by highest not viable spore concentrations) consequently minimizing the air flow movement which potentially bring the air currents with different particulates (biological and not).

5 Conclusions

The spread of the surveys presented in this work could allow to deepen the interaction of environmental factors with indoor air spora or identify new ones, but above all to increase the availability of aerobiological data to analyse. This could provide the basis for the development of biohazard prediction models that could lead to the formulation of methodological standards and the definition of threshold reference values. Periodic environmental monitoring of microclimatic variables and the aerobiological component, can provide an important support to curators, directors of sites of historical, artistic and archaeological interest, in maintaining environments suitable for permanent and temporary exposures. Moreover, spore monitoring allows to implement procedures for the prevention of risk of fungal attacks on sensitive materials through environmentally friendly and easy to apply systems. The possibility of implementing preventive conservation measures with simplicity would allow further benefits to be achieved. First of all, the improvement in the management of financial resources, as the rationalisation of the various phases of the management of the works (storage, transport, maintenance, cleaning, security, exhibition, etc.) should be deeply considered. Moreover, other benefits could be: the possibility of the use of the most suitable and efficient systems for the prevention of fungal attacks on sensitive materials; the enhancement of conservation and exhibition sites; the collaboration with restorers, providing specific information suitable to maximize the restoration strategies to be adopted, reducing the incidence of extraordinary interventions optimizing the usability of Cultural Heritage.

Funding Open access funding provided by Università degli Studi di Perugia within the CRUI-CARE Agreement.

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