



Indoor air fungal pollution of a historical museum, Egypt: a case study

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Abstract Fungi can cause serious damages to historical works of art. This study aims to evaluate fungal pollution in the indoor air environment, deposited dust and surfaces of works of art in a historic house museum “Prince’s Mohamed Ali museum”, Giza, Egypt. Fungal pollution was evaluated at six museum’s buildings differed in size, design, location and content, for 2 years of campaign. The deposited dust was collected once every 3 months, while surface swabs were collected twice, after 1 year and 2 years of exposure. Airborne fungal concentrations were evaluated using volumetric (Andersen 2 stage impactor) and gravimetric (index microbial air contamination) air samplers. Airborne fungal concentrations ranged within 47–784 CFU/m³ and 175–40,250 CFU/m²/h using volumetric and gravimetric samplers, respectively. Indoor/outdoor ratio confirmed that outdoor environment was the main source of indoor fungal pollution. The rate of deposited dust was ~ 90-folds lower indoor than outdoor. Fungal concentrations associated deposited dust ranged between 10² and 10⁴ CFU/g, representing

a cause of concern. In respect to medium type, fungal diversity profile was in order of deposited dust $\geq$ airborne $\geq$ surfaces. *Alternaria*, *Aspergillus* and *Cladosporium* were the common fungal taxa. Secondary and tertiary fungal colonizers were detected after 2 years of exposure, indicating the presence of adequate moisture content inside the museum. No clear relationship patterns were found between microclimatic conditions and fungal culturability. The measured indoor fungal pollution and microclimatic conditions represented a problem for works of art and early indicator for fungal accumulation and biodegradation.

Keywords Historical museum · Fungal pollution · Air · Deposited dust · Surface · Climate · Diversity index

1 Introduction

Air pollution detrimentally affects ecosystem, human health, building materials (Sablier and Garrigues 2014) and cultural heritage collections (Sterflinger and Piñar 2013). Deposited dust and its composition, biological particles and environmental conditions may trigger damage of cultural heritage collections (Lazaridis et al. 2015), by promoting the growth of microorganisms, soiling and chemical reactions (Grau-Bové and Strlic 2013).

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The outdoor environment, air-conditioning systems, vegetation and anthropogenic activities are the main contributors of microorganisms in indoor environments. Microorganisms may be freely found in the airborne state or associated particulate matters that they are settled on surfaces causing biodeterioration of materials (Górny et al. 2016). A detrimental levels of microorganisms may be found in aerosols/or on works of art surfaces in cultural heritage-related buildings (De Nuntiis and Palla 2017). However, the ecology and composition of substrate, surrounding environmental conditions, porosity and microbial metabolic activity are the major determinants of the type and extent of microbial colonization (Dakal and Cameotra 2012). Moreover, dust helps form a microenvironment on surfaces for microorganisms. Particle properties, environmental conditions and bio-receptivity determine the association between microorganisms with surfaces and dust (Urzi and Realini 1998).

Microclimatic conditions play a critical role in microbial proliferation (Pasanen et al. 2000, Karbowska-Berent et al. 2011; Camuffo 2013). Thermohygrometric parameters highly influence colonization of microorganisms on works of art substrates (De Nuntiis and Palla 2017). Relative humidity directly determines moisture content of materials (Ritschkoff et al. 2000), which promotes proliferation/growth of mold on surfaces (Flannigan and Morey 1996). The interactions between microorganisms with microclimatic conditions and air pollutants create alterations in cultural heritage structures (Anaf et al. 2013).

Fungi, primary biological aerosol particle, are common biological particles in the atmosphere worldwide (Zhai et al. 2018). Fungi are considered one of the major biodeteriogenic agents of surfaces (May et al. 1993). *Aspergillus*, *Penicillium*, *Mucor*, *Alternaria*, *Stachybotrys*, *Cladosporium*, *Monilia* and *Phoma*—fungal genera—display biodegradative activities (Dalal et al. 2011; Krakoval et al. 2012). Fungi excrete pigments, metabolic products and organic acids that may damage building materials (Gutarowska 2010). Urzi et al. (2001) concluded that once fungi settled on surfaces they contributed a great variety of alterations like black paints, intergranular growth, sugaring and biopitting. Moreover, fungal spores/debris can trigger asthma in individuals with atopic asthma (WHO 2009). Exposure to fungal debris/or spores is linked to allergenic sensitization,

allergy and asthma (Żukiewicz-Sobczak 2013, Sharpe et al. 2015; Yilmazkaya et al. 2018).

The influence of fungal pollution on works of art has gained a great attention in the last decades. Climate change and diverse of human activities may increase/or modify fungal pollution. The climate change may alter abundance and distribution of fungal spores (Barnes et al. 2013). A change in temperature may influence colonization and growth of fungi directly through physiology of individual microorganisms or indirectly through physiological effects on their hosts (Sindt et al. 2016). The present study aims to evaluate the current status of fungal pollution in the air environment, deposited dust and on surfaces of some of works of art in a historic house museum in Egypt. Information on fungal pollution in a cultural heritage-related building is important to minimize/prevent biodeterioration and understands variability of fungal spores in relation to microclimatic conditions, ventilation and type of work of art.

2 Materials and methods

2.1 Description of the museum

Fungal pollution was evaluated in Prince's Mohamed Ali palace/Al Manial museum. It is considered one of the highly attractive museums in Cairo, Egypt, and it is preserved as a historic house museum. The museum was closed in the period between “2005 and 2015” for restoration works. The museum is characterized by its architectural Maghreb style, and it contains many of amazing and wondering works of art, Islamic arts, furniture and decorations.

The museum is located in the western bank of the Nile River, in the Al Manial district (El Rawda El Nilia island), south of Cairo (Fig. 1). The area of the museum is 61,711 m² of which 5000 m² is the area of buildings and ~ 34,000 m² is the area of the garden, which includes many of the rare plants. Generally, Al Manial district is characterized by high traffic density, diverse commercial/residential activities, parking, and it is located in the vicinity of the Cairo University that worsening its air quality.

The museum is mainly composed of five separate and distinctive buildings, namely reception hall, clock tower, Thorne hall, residence hall and golden hall. In



Fig. 1 Map of the Prince Mohamed Ali museum showing sampling locations, Map data ©Google, 2013

the present study, sampling campaign was collected from the museum's buildings, differing in size, design and location. The selected museum's buildings had no history of water damage, leaks or floods. The sampling was taken at the depository, reception hall, residence hall, Thorne hall, hunting museum and restoration laboratory. Table 1 shows the description of each of the sampling location/building.

2.2 Airborne fungal sampling

The sampling was carried out for 2 years of campaign from December 2016 to November 2018. The air samples were taken during the working hours and normal activities, between 9.00 a.m. to 14.00 p.m., two times per month. The air sampling was collected from a fixed position ~ 1 m height above the floor level at the center of building and outside the main door of the building (control samples).

Table 1 Description of sampling locations

Sampling Site	Description	Ventilation type	Location
Depository	It is used as a storage room and it contains a number of artifacts and objects	Mechanical ventilation (independent air-conditioning systems)	It locates at the west edge of the garden in the vicinity of a light traffic road and commercial area
Reception hall	It consists of two floors; the upper floor is comprised of two halls; one is designed in Shami style and the other hall in Moroccan style. Its purpose was to receive official guests. It contains many of rare antiques like carpets and Arabian furniture	Natural ventilation	It locates near to the main gate and close to heavy traffic road
Residence hall	It was dedicated to residence of the Prince Mohamed Ali. It consists of two floors; the ground floor was allocated to the reception, dining rooms and prince's library The top floor was allocated to bed rooms	Natural ventilation	It locates in the southeast edge inside the botanic garden away from traffic and near to west river Nile bank
Thorne hall	It consists of 2 floors the ground floor is known as trusteeship, and the 2nd floor contains many of amazing pictures, Aubusson and chairs coated with velvet	Mechanical ventilation (independent air-conditioning systems) a natural ventilation	It locates at the southeast edge of the garden on the west bank of the river Nile behind to residence hall
Hunting Museum	The museum was included to the hall in 1963; it displays ~ 1180 pieces of animals, birds, stuffed butterflies and skeletons	Mechanical ventilation (HVAC, heating, ventilation and air conditioning)	It locates along the north wall in the vicinity of the reception hall, next to heavy traffic road
Restoration Laboratory	It is used for conservation and restoration works of collections and work of arts of the museum	Mechanical ventilation (independent air-conditioning systems) and natural ventilation	It locates at south wall away from light traffic road, behind the deposit

Fungi were evaluated by using volumetric (Andersen 2 stage impactor) and gravimetric (index microbial air contamination, IMA) air samplers (ISO-14698-1 2003). For volumetric air sampling, Andersen two-stage viable cascade impactor sampler (TE-10-160, Tisch Environmental Cleves, OH, USA) was used. It separates particles into fine ($< 2.5 \mu\text{m}$) and coarse ($> 7 \mu\text{m}$) size ranges, operating at flow rate of 28.3 L/min for 5 min to prevent overloading the collection plates. Two consecutive samples were taken during each sampling event (4 plates/location) and ~ 1 h between each sample. The short time of sampling constitutes a problem because the representativeness of sampling decreases and variability between side by side increases (Godish and Godish 2007). However, for gravimetric air sampling, Petri dishes, 90 mm in diameter (3 plate/location), were opened to the air according to 1/1/1 scheme (1 h/1 m from the floor/1 m away from wall/or obstacle), (Pasquarella et al. 2000). Malt extract agar (MEA) medium (Hi-media

laboratories, Mumbai, India) was used to collect and count fungi (Sarica et al. 2005).

The settling velocity of fungal particle, a function of size, shape and density of particle and turbulence of the surrounding air, determines the accumulation/colonization of particles onto surfaces. In the present study, the settling velocity (m/h) was calculated as the ratio of the mean value of particle flux to surface ($\text{CFU}/\text{m}^2/\text{h}$) divided by the mean value of airborne particle concentration (CFU/m^3).

2.3 Deposited dust and its fungal fraction

The dry deposited dust was collected using a dust collector, 170 mm height and 80 mm diameter, positioned 2-m height above the floor indoors and ~ 6-m outdoor. The dust collectors were changed every 3 months, because low deposited dust rate was suspected inside the museum buildings. The deposited dust was carefully transferred to a dry sterilized pre-

weighted beaker, and the deposition rate was calculated as $\text{mg}/\text{m}^2/\text{day}$. A portion of the deposited dust (50–100 mg) was dissolved in 10 ml sterilized distilled water and shaken well for 30 min. Aliquots (0.5 ml) of the original samples were spread plated onto the surface of the MEA to determine the concentrations of fungi-associated deposited dust.

2.4 Fungal taxa-associated work of art surfaces

The fungal taxa accumulated on some selected works of art (wood, stone, metal, textile and leather) were collected by using a swab and template of 25 cm^2 surface area. The swabs were collected two times from the same collection, one after 1 year and the other after 2 years of exposure. The selected works of art were not subjected to any cleaning during the period of study. The swabs were eluted in 5 ml sterilized distilled water and shaken well for 20–30 min. Aliquots (0.5 ml) of the original samples were spread plated onto the surface of the MEA to identify fungal taxa-associated surfaces.

2.5 Fungal analysis

Fungal Petri plates were incubated at 28°C for 5–7 days and checked daily. For volumetric air sampling, positive-hole correction (Andersen 1958) was applied to the raw colony forming unit (CFU) recorded on each plate, and by using the CFU with sampling time and flow rate, the concentration was calculated and expressed as CFU per cubic meter of the air (CFU/m^3). For gravimetric air sampling, the resulting colonies were counted and expressed as $\text{CFU}/\text{square meter}/\text{hour}$ ($\text{CFU}/\text{m}^2/\text{h}$). Fungi-associated deposited dust was expressed as CFU/gram of dust (CFU/g), whereas fungi-associated surfaces were only identified.

2.6 Identification of fungi

Fungal isolates were identified by the direct observation of micro- and macro-morphological features on Sabouraud dextrose agar, Czapek Dox agar and malt extract agar, reverse and surface coloration of colonies compared with various literatures (Ellis 1971; Raper and Fennell 1977; Singh et al. 1991; Barnett and Hunter 1999; Pitt and Hocking 2009). Biodiversity of fungal taxa was measured by using Simpson's

diversity index (SDI). It is an index used to measure community diversity which takes into account the number of species present and the relative abundance of each species (using the software Statistics).

2.7 Fungal enzymatic activity

Cellulose, lipid and protein-decomposing fungi were screened using selective media. Mycelium disks (5 mm diameter) of the common culturable fungal taxa were cut up and placed onto the center of agar plates containing the selective medium.

Cellulose-containing agar medium [urea, 0.3; KH_2PO_4 , 2; $(\text{NH}_4)_2\text{SO}_4$, 1.4; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.3; cellulose, 5; peptone, 1; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 1; $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$, 1.6; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 1.7; COCl_2 , 2; g/l] was used to test cellulolytic activity (Montenecourt and Eveleigh 1977). The plates were incubated at $28 \pm 2^\circ\text{C}$ for 72 h and further incubated at 50°C for 8 h (Osman et al. 2008). The plates were flooded with Rose bengal (0.005%), and the appearance of a clear zone surrounding the isolate indicates cellulolytic activity.

Chromogenic substrate—phenol red agar medium (phenol red, 0.01%; olive oil, 1%; CaCl_2 , 10 mM, agar 2%), (Singh et al. 2006)—was used to screen lipase activity. The plates were incubated at $37\text{--}45^\circ\text{C}$ for 4 days, and the appearance of a yellow band over a pink background color indicates a lipolytic activity.

Gelatin agar medium (gelatin, 10; agar 20, phosphate buffer 0.2 M, g/l, pH 7.5) (Ammar et al. 1991) was used to test protease activity. The plates were incubated at 25°C for 4 days. The plates were flooded with 10 ml acidic mercuric chloride solution (Cowan 1974), and the appearance of a clear zone around the colony indicates a proteolytic activity.

2.8 Microclimatic conditions

Temperature and relative humidity were measured indoor and outdoor of the museum buildings using a portable digital thermohygrometer (PC-500 TRH-II, Sato Keiryoki Co, China) at the time of each sampling event a long over the sampling campaign.

2.9 Statistical analysis

Shapiro–Wilk test was used to verify the normality of the data distribution. In the present study, the data showed normal and non-normal distributions using

both air sampling methods. The data were analyzed using descriptive/non-descriptive statistics such as: mean, and 25th, median, 75th percentiles. Mann–Whitney *U* test was used to ascertain the significance of differences ($P \leq 0.05$) between microclimatic conditions and between fungal concentrations inside and outside sampling locations. Spearman's rank correlation test was used to determine the relationships between fungal airborne concentrations and microclimatic conditions at $P \leq 0.05$.

3 Results and discussion

3.1 Fungal air quality and quantity

Table 2 shows the 2-year range, mean and median fungal concentrations using both air sampling methods. Fungal concentrations ranged within 47–784 CFU/m³ and 175–40,250 CFU/m²/h using volumetric and gravimetric air samplers, respectively. The highest indoor median concentrations, 4765.5 CFU/m²/h and 236 CFU/m³ were found in the reception hall using gravimetric and volumetric air samplers, respectively. In regard to microbial risks on buildings, the Italian Ministry of Cultural Heritage proposed threshold limit values of ≤ 150 CFU/m³ for fungi (MIBAC 1998) and ≤ 100 –120 CFU/m³ or the collections should be subjected to disinfection (Parchas 2008). In the present study, the obtained results exceeded the proposed limit values in all the sampling locations, suggesting that works of art should be

subjected to disinfection. However, biological risk threshold is reached with different timing and degree, depending not only on fungal counts but also on other factors such as climatic and environmental conditions, and type and composition of the substrate; therefore, more further studies and evaluations are necessary before recommendation of using disinfectants.

Gravimetric air sampling determines fall-out of fungal particles (Whyte 1995). It is important to investigate soiling and degrading of the exposed materials. Gravimetric method variability depends on particle size and shape and motion of the surrounding air (Pelczar et al. 1996); however, it is an economical and available method (Humphreys 1992). On the contrary, volumetric air sampling determines airborne fungal exposure, but shows high variability due to short sampling time and heterogeneous contamination (the nearer the source, the higher the contamination).

In the present study, mean fungal concentration profiles were in the order of outdoor > depository > residence hall > reception > restoration lab > Thorne hall > hunting museum, using gravimetric sampler and residence hall > outdoor > restoration lab > reception > depository > Thorne hall > hunting museum, using volumetric sampler. Obviously, the degree of fungal contamination was not related to ventilation type due to its normal and non-normal distributions in respect to sampling location. However, hunting museum, the building was equipped with HVAC system, had the lowest fungal loads.

Table 2 Range, mean and median of airborne fungal concentrations using volumetric and gravimetric air samplers and their settling velocity

Location	Volumetric sampler (CFU/m ³)				Gravimetric sampler (CFU/m ² /h)				Settling velocity (m/h)
	M $\pm$ SD	Min	Median	Max	M $\pm$ SD	Min	Median	Max	
Outdoor	364 $\pm$ 117	212	297	635	8281 $\pm$ 4457	2812	8125	17,031	22.7
Depository	224 $\pm$ 188	68	168.5	784	4890 $\pm$ 11,196	469	1250	40,250	21.8
Reception hall	258 $\pm$ 89	153	236	393	4372 $\pm$ 2354	175	4765.5	7500	16.9
Residence hall	383 $\pm$ 138	125	187.5	656	4661 $\pm$ 5841	937.5	3281	22,656	19.9
Thorne hall	221 $\pm$ 80	98	218	349	2278 $\pm$ 1469	781	1953	5156	10.3
Hunting museum	170 $\pm$ 99	47	154	438	2267 $\pm$ 1510	212	2500	4218	13.3
Restoration lab.	260 $\pm$ 147	47	225	623	3838 $\pm$ 1413	467	3984.5	5625	14.7

M mean, *SD* standard deviation, *Min* minimal value, *Max* maximal value

In the present study, the settling velocity of fungal particles varied between 10.3 to 22.7 m/h (Table 2), indicating that the microenvironmental conditions differently affected behavior of fungal particles. In the reference to aerodynamic diameter and settling velocity (Mammarella 1971), the settled fungal spores had sizes $\geq 10 \mu\text{m}$ because they may be carried by the inert dust particles. Generally, microbes carried by particles fall-out with an average deposition rate of 16.5 m/h (Whyte 1995), and fungal spores have size ranges of $\sim 2\text{--}15 \mu\text{m}$ (Yu et al. 2013; Skóra et al. 2015). Fine size particles may be related to outdoor sources due to their ability to suspend in the air for longer time and transmit ventilation system; however, coarse size particles are usually local origin/sources.

The settling velocity of fungal particles was the highest in the mechanically ventilated and undisturbed building (depository) and the lowest in the hunting museum. Fungal particles showed different behavior in naturally and mechanically ventilated buildings (Table 2), depending on fungal concentration, conditions of the surrounding air (laminar flow, turbulent air and calm air) and size of particles. Air stability (calm air) situation, poor ventilation and relatively higher relative humidity accelerate fall-out of fine particles (Lacey 1991). However, traffic, human activity, ventilation rate and uncontrolled accesses through doors additionally influence the degree of the settling velocity of fungal particles.

Indoor-to-outdoor (I/O) ratio documents the presence/or absence of indoor biocontaminant sources (ACGIH 1999). The I/O ratio ≤ 1 indicates regular indoor environmental conditions, while $\text{I/O} > 1$ indicates irregular conditions (AIHA 1989). In the present study, the mean of I/O ratios did not exceed 1 except in the residence hall, indicating the presence of indoor fungal source (mass balance condition). The I/O ratios exceeded 1 in the depository, residence hall and reception hall in some months along-over the study period, indicating improper indoor environmental conditions. Generally, outdoor air environment was considered the main contributor of indoor airborne fungi.

3.2 Monthly fungal variation

The monthly fungal variations using volumetric and gravimetric air samplers are shown in Fig. 2a, b, respectively. Fungal concentrations varied monthly,

with the highest concentrations detected in May, in the depository and residence hall. The source type, abundant taxa, ventilation condition, human activity and microclimatic conditions temporally affect fungal concentrations (Dong et al. 2016; Zhai et al. 2018). Concentrations of fungal spores are the highest in the summer and fall seasons and lowest in the winter season (Shelton et al. 2002). In Egypt, indoor temperature and relative humidity are widely fluctuated in hot months, and the spring season is characterized by dusty storms, dry weather and high amount of dead plant debris, the primary source for fungi. In addition the accidental events (e.g., rain/or storms) increase fungal concentrations in the air (Barnes et al. 2001).

3.3 Dust deposition and its fungal fraction

The deposited dust rates ranged within 130–859 mg/m²/day outdoor and 0.1–8.4 mg/m²/day indoors, ~ 90 -folds higher outdoor. The seasonal deposited dust rate was the highest in the spring and lowest in the winter (Table 3). The maximum deposited dust rate was found in the reception hall (5.2–8.4 mg/m²/day) and the minimum in the depository (0.1–1.2 mg/m²/day).

The concentrations of fungal-associated deposited dust ranged between $\sim 10^2$ and 10^4 CFU/g (Fig. 3). The concentrations were significantly higher ($P \leq 0.05$) in the summer in the residence and reception halls ($P = 0.05$) compared to other seasons and locations. This variation may be attributed to high rate of ventilation exchange rate, deposited dust and its characteristics (composition and size) and degree of human activity. Generally, dust particles support microbial growth, exposure relevance and resuspension of deposited particles. Therefore, keeping museum clean/or minimize deposition dust rate is an important challenge in cultural heritage-related buildings. The museum materials may be subjected for dust accumulation and microbial colonization when available conditions met microclimatic conditions, moisture, porosity and substrate type and its composition (organic, inorganic or synthetic).

In the present study, a total of 31 fungal types were identified in the air and deposited dust, differing in respect to building. *Aspergillus*, *Penicillium* and *Cladosporium* were the dominant fungal taxa because they can grow in diverse habitats in diverse ways and resistant to various environmental stresses (Reponen

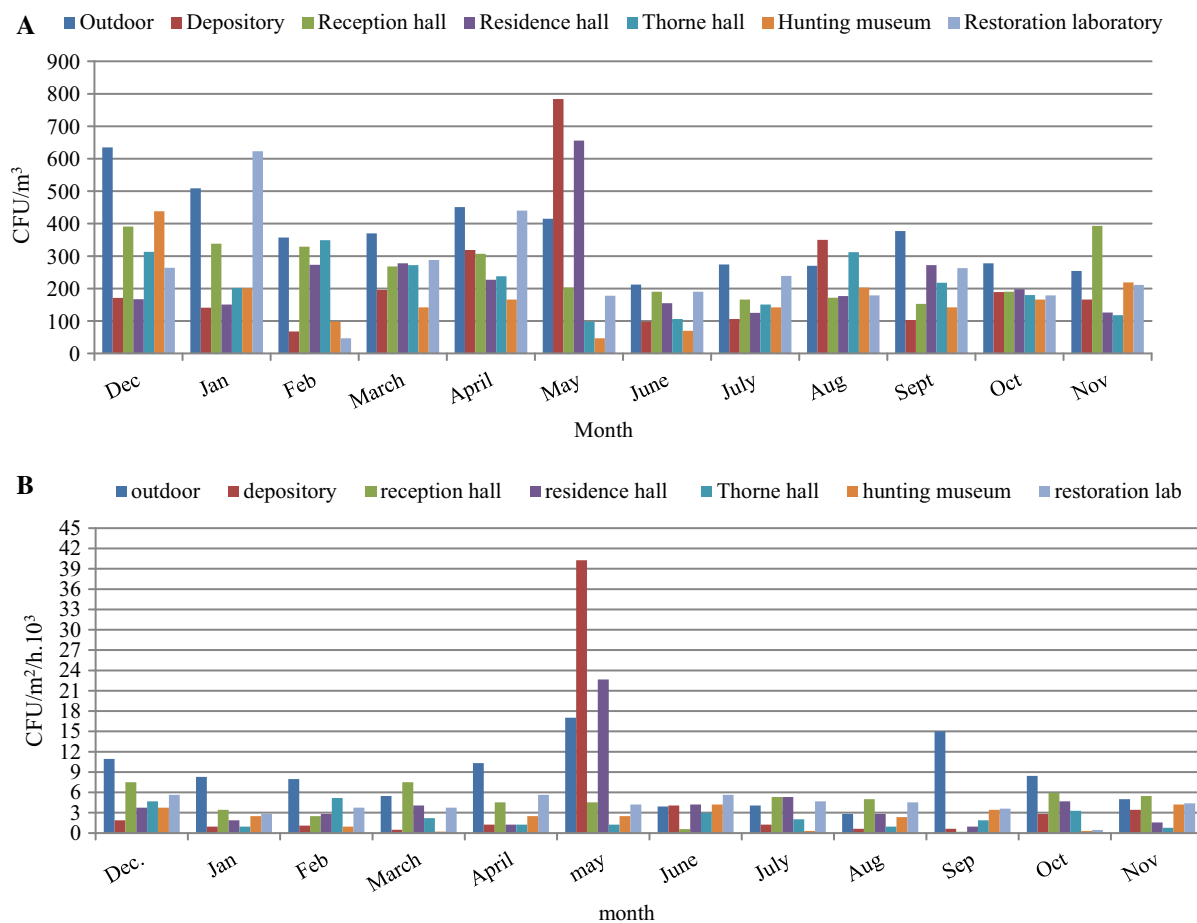


Fig. 2 Monthly mean airborne fungal concentrations at Al Manial museum: **a** using volumetric sampler and **b** using gravimetric sampler

Table 3 Seasonal mean deposition dust rate (mg/m²/day) indoor and outdoor of the museum's locations

Season	Building						
	Outdoor	Depository	Reception hall	Residence hall	Thorne hall	Hunting museum	Restoration lab
Winter	541	1.2	6.9	4.1	2.4	1.15	2.6
Spring	859	0.2	8.4	7	5.6	7.1	5.1
Summer	130	0.3	5.5	6.7	5.3	4.7	4.3
Autumn	274	0.1	5.2	5.7	3.2	4	3.5

et al. 2001). Some of the identified fungal types are included in the risk group-2: *Aspergillus fumigatus* and *Penicillium* (Directive 2000/54/EC), and most of fungal types can cause allergic reactions (Rylander and Jacobs 1994).

Simpson's diversity index (SDI) showed that the number of fungal diversity profiles were deposited dust > air state > swab in respect to medium and: residence hall > depository > deception hall > Thorne hall > hunting museum > restoration

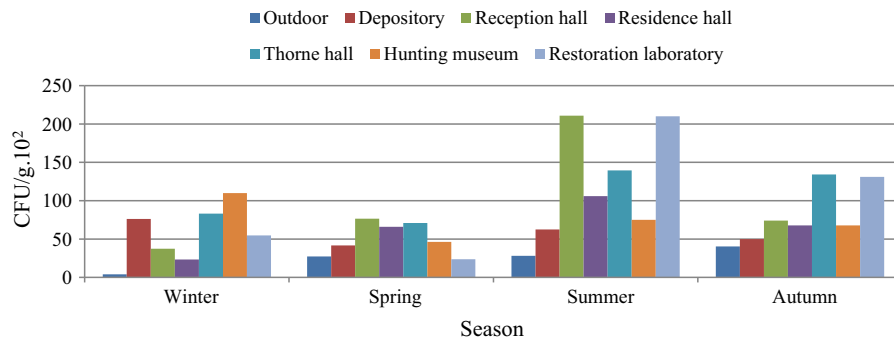


Fig. 3 Concentration of fungi-associated deposited dust at the museum's buildings

laboratory > outdoor in respect to location. This means that fungal diversity was the highest in the deposited dust (a good reservoir for fungi) and in the residence hall.

Eleven fungal taxa were isolated from the selected works of art's surfaces after 1 year and 2 years of exposure (Table 4). These taxa were identified on different surfaces and varied regarding the substrate type, representing a risk to museum's collections. Xerophilic fungi (*Alternaria*, *Aspergillus niger* and *Asp. fumigatus*) commonly associated and still attached to surfaces due to their ability to come and contact with the receptive surface. Organic made objects (textile and wood) were more able to be colonized by fungal types than inorganic ones. Porous materials (textile and carpet) can act as reservoir of particles and provide suitable microbial growth

conditions. Generally, fungi-associated surfaces reflect their ecology and taxonomy in relation to substrates (Portnoy et al. 2004).

The primary (e.g., *Penicillium*, *Aspergillus* and *Eurotium*), secondary (e.g., *Alternaria*, *Ulocladium*, *Cladosporium*, *Asp. flavus* and *Asp. versicolor*) and tertiary (e.g., *Stachybotrys*, *Aureobasidium* and *Chaetomium*) fungal colonizers are well-grown at water activity ranges of 0.6–0.8; ~ 0.8–0.9 and > 0.9, respectively. In the present study, the primary fungal colonizers commonly inhabited surfaces due to their ability of growing at low water activity. The detection of secondary (*Alternaria*, *Cladosporium* and *Asp. flavus*) and tertiary (*Aureobasidium*) fungal colonizers after 2 years of exposure may indicate the presence of an adequate moisture on surfaces; moisture is the main factor controlling mold growth (Flannigan and Morey

Table 4 Fungal taxa isolated from different objects after 1 and 2 years in the museum

Type	Wooden box		Wooden console		Carpet		Canava		Marble stone		Metal lantern		Leather		Textile cover	
	1 Y	2 Y	1Y	2Y	1Y	2Y	1Y	2Y	1Y	2Y	1Y	2Y	1 Y	2 Y	1 Y	2 Y
<i>Alternaria</i>	–	+	+	+	+	+	–	+	–	+	–	–	–	–	+	+
<i>A. niger</i>	+	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+
<i>A. fumigatus</i>	+	+	–	+	+	+	–	+	–	+	+	–	+	+	–	+
<i>A. flavus</i>	+	+	+	+	+	–	–	+	–	–	+	+	+	+	+	+
<i>A. terreus</i>	–	–	–	–	–	+	–	–	–	+	–	+	–	–	–	–
<i>Aureobasidium</i>	–	–	–	–	–	+	–	–	–	+	–	+	–	–	–	+
<i>Cladosporium</i>	–	–	–	–	–	+	–	–	–	+	–	–	–	+	–	+
<i>Eurotium</i>	–	+	–	+	+	–	–	+	–	–	–	–	+	–	+	+
<i>Penicillium</i>			+	–	+	+	+	–	–	–	–	+	+	–	+	–
<i>Rhizopus</i>	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+
Sterile hyphae	+	–	–	+	+	–	–	–	+	+	–	–	–	–	–	+

1996). Relative humidity differently determines level of water activity of various materials/substrates. Organic and inorganic materials should be stored at environment with relative humidity ranges between 40–70 and $\leq 40\%$, respectively (Erhardt and Mecklenberg 1994). This confirms that the measured microclimatic conditions in Prince's Mohamed Ali museum represent a risk for works of art.

3.4 Fungal enzymatic activity

Table 5 shows cellulolytic, lipolytic and proteolytic enzyme activities of the common fungal taxa. *Aspergillus*, *Cladosporium* and *Penicillium* showed cellulolytic activity. *Alternaria*, *Aspergillus*, *Aureobasidium* and *Rhizopus* exhibited lipolytic and proteolytic activities. Fungi producing extracellular enzymes play important critical role in degradation of organic and inorganic materials (Lopez-Miras et al. 2013; Lech 2015) and help fungal growth at low water activity substrates (Sterflinger 2010).

3.5 Microclimatic conditions related to fungal culturability

Microclimatic conditions are widely varied indoor because they are related with the presence/or absence

of windows/doors and controlling measures. Temperature measurements ranged between 11–29 °C indoors and 14–34 °C outdoor (Table 6). Relative humidity measurements ranged within 24–64% indoors and 32–52% outdoor. The mean values of relative humidity and temperature, respectively, were slightly higher in the depository (51%) and the reception hall (21 °C) compared to the other indoor sampling locations (Table 2). The 75th percentile showed that 25% of temperature measurements exceeded 23.3 °C in the depository and 27 °C in the reception hall. However, the 75th percentile showed that 25% of relative humidity measurements exceeded 58% in the depository and 55% in the reception hall, Thorne hall and hunting museum. Nonsignificant differences ($P \geq 0.05$) were found between temperature and relative humidity measurements inside the museum's buildings. Indoor and outdoor microclimatic conditions were closely similar, indicating no well-controlled measures for outdoor air masses and absence of systems for regulation of indoor conditions.

Spearman's rank correlations between airborne fungal concentrations and microclimatic conditions varied regarding location. Temperature negatively affected fungal culturability. Significant negative correlations ($P \leq 0.05$) were found between temperature and fungi at outdoor ($r_s = -0.6$), reception hall ($r_s = -0.71$) and Thorne hall ($r_s = -0.55$). On the contrary, relative humidity weakly supported fungal culturability. The correlations ranged between $r_s = 0.06$ at the depository to $r_s = 0.37$ at the Thorne hall. The relative humidity $\sim 50\%$ is reported as a border line for fungal growth, so climate control should be adjusted under this value.

Microclimatic conditions synergistically affect fungal culturability. The effect of ventilation condition, geographical factors and human activity may mask the influence of microclimatic conditions (Adams et al. 1986). The interactions of meteorological parameters, not only temperature and relative humidity (e.g., warming by sunlight, air stream and daily changes in temperature) but also building characters (e.g., area, design, type of works of art), affect airborne microbial concentrations (Camuffo 1998). These interactions may interpret the complexity correlations between airborne fungal concentrations and microclimatic conditions.

Table 5 Cellulolytic, lipolytic and proteolytic enzyme activities of the common fungal taxa

Taxa	Enzyme		
	Lipase	Protease	Cellulase
<i>Alternaria</i>	+	–	+
<i>Aspergillus niger</i>	+	+	–
<i>Aspergillus flavus</i>	+	+	+
<i>Aspergillus fumigatus</i>	+	–	–
<i>Aspergillus terreus</i>	–	+	–
<i>Aureobasidium</i>	–	+	–
<i>Cladosporium</i>	–	–	+
<i>Eurotium</i>	+	+	+
<i>Penicillium</i> spp.	–	+	+
<i>Rhizopus</i>	+	+	–
Sterile hyphae	+	+	+

Table 6 Mean, range and percentile records of microclimatic conditions

Relative humidity%	Temperature (°C)						Location					
	M ± SD	Min	Q1	Q2	Q3	Max	M ± SD	Min	Q1	Q2	Q3	Max
Outdoor	25 ± 6.5	14	18.3	26	29.8	34	42 ± 8.2	32	34	43	50	52
Depository	19.7 ± 5.5	11	16	19	23.3	29	51 ± 7.6	39	46	52	58	63
Reception hall	21 ± 5.7	13	16.3	18.8	27	28.5	48 ± 7.3	34	43	49	55	57
Residence hall	20 ± 5.4	13.5	15	18.5	26.8	27	48 ± 5	44	46	50	52	53
Thorne hall	18.8 ± 4.4	13	15.3	17.7	22.5	26	49 ± 7	36	45.5	49	55	64
Hunting museum	20 ± 4.5	13	16.5	19	23.7	27	49 ± 7.4	35	45	51	55	58
Restoration laboratory	20 ± 3.4	14	17.5	20.5	22.7	25	43 ± 8.2	24	39	46	49	54

M: mean; SD: standard deviation; Q1: 25th; Q2: median; Q3: 75th

4 Conclusion

Airborne fungal spores extremely varied regarding museum's building and sampling method. Fungal concentrations exceeded the threshold limit value of ≤ 150 CFU/m³ proposed by the Italian Ministry of Cultural Heritage. The settling velocity values indicated that fungal particles were mainly carried by local inert particles. The building with HVAC system (hunting museum) had the lowest fungal contamination. Organic made materials were colonized by more fungal taxa, and they were potentially at risk. The detection of primary, secondary and tertiary fungal colonizers reflected different microclimatic situations and substrate invaded. Deposited dust and naturally ventilated building had the highest fungal diversity. Microclimatic conditions had no clear effects on fungal culturability; however, fluctuations of microclimatic conditions represented a risk of the museum's collections. Multi-approaches should be used to characterize fungal pollution in a museum such as (1) consider contamination rate, density and settling velocity; (2) consider material type and fungal spore characteristics (type, size and shape); and (3) reduce deposited and resuspension particulate matter.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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