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A multidisciplinary approach to the study of cultural heritage environments: Experience at the Palatina Library in Parma

C. Pasquarella [a,*], C. Balocco [b], G. Pasquariello [c], G. Petrone [d], E. Saccani [a], P. Manotti [a], M. Ugolotti [e], F. Palla [f], O. Maggi [g], R. Albertini [h]

[a] Department of Biomedical, Biotechnological and Translational Sciences, University of Parma, Italy
[b] Department of Industrial Engineering, University of Florence, Italy
[c] Central Institute of Graphic Arts, Ministry of Cultural Heritage and Activities and Tourism, Rome, Italy
[d] Department of Industrial Engineering, University of Catania, Italy
[e] Hygiene Unit, University Hospital of Parma, Italy
[f] STEBICEF Department, Laboratory of Biology and Biotechnology for Cultural Heritage, University of Palermo, Italy
[g] Department of Environmental Biology, "Sapienza" University of Rome, Italy
[h] Department of Clinical and Experimental Medicine, University of Parma, Italy

HIGHLIGHTS

- An integrated system including biological, particles, microclimate and CFD analysis was applied.
- The tracing and diffusion of particles inside the room were studied.
- A wide variability in biological and particle values was observed.
- Cultural and molecular methods were combined to evaluate microbial contamination.
- Simulation results were consistent with experimental data.

GRAPHICAL ABSTRACT

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ABSTRACT

The aim of this paper is to describe a multidisciplinary approach including biological and particle monitoring, and microclimate analysis associated with the application of the Computational Fluid Dynamic (CFD). This approach was applied at the Palatina historical library in Parma. Monitoring was performed both in July and in December, in the absence of visitors and operators. Air microbial monitoring was performed with active and passive methods. Airborne particles with a diameter of ≥0.3, ≥0.5, ≥1 and ≥5 μm/m^3, were counted by a laser particle counter. The surface contamination of shelves and manuscripts was assessed with nitrocellulose membranes. A spore trap sampler was used to identify both viable and non-viable fungal spores by optical microscope. Microbiological contaminants were analyzed through cultural and molecular biology techniques. Microclimatic parameters were also recorded. An infrared thermal camera provided information on the surface temperature of the different building materials, objects and components. Transient simulation models, for coupled heat and mass-moisture transfer, taking into account archivist and general public movements, combined with the related

* Corresponding author.

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Microclimate
Computational fluid dynamics
Particle tracing

sensible and latent heat released into the environment, were carried out applying the CFD-FE (Finite Elements) method. Simulations of particle tracing were carried out.
A wide variability in environmental microbial contamination, both for air and surfaces, was observed. *Cladosporium* spp., *Alternaria* spp., *Aspergillus* spp., and *Penicillium* spp. were the most frequently found microfungi. Bacteria such as *Streptomyces* spp., *Bacillus* spp., *Sphingomonas* spp., and *Pseudoclavibacter* as well as unculturable colonies were characterized by molecular investigation. CFD simulation results obtained were consistent with the experimental data on microclimatic conditions. The tracing and distribution of particles showed the different slice planes of diffusion mostly influenced by the convective airflow.
This interdisciplinary research represents a contribution towards the definition of standardized methods for assessing the biological and microclimatic quality of indoor cultural heritage environments.

1. Introduction

Biological particles in indoor environments, such as museums, libraries and archives, can represent a hazard both for artifacts, due to their biodeteriogenic action, and the health of operators and visitors due to their potential infectious, allergenic and toxic effects (Mandrioli et al., 2003). Every building has its own microbial ecology equilibrium, which depends on its structure, the materials used for its construction and furnishings, and the people working and visiting the building itself. People represent one of the most important sources of microbial air contamination. Skin is a natural source of microorganisms, which are released into the environment through the continuous process of desquamation. Hair is also a significant potential source of microbial contamination. Microorganisms are also introduced into the environment when people talk, cough and sneeze. However, microorganisms can also come from animals and a variety of indoor and outdoor environmental sources, contaminated materials and objects, malfunctioning ventilation systems, any activity involving the modification or renovation of buildings, which inevitably generates dust and debris, increasing microbial contamination and that of fungal spores in particular. Depending on their size, particles settle on the ground and surfaces at different rates, contaminating any surface, for example graphic collections (prints, drawings, watercolors, books, codices, photographs, paper, etc.). Surfaces can also become contaminated through contact with other contaminated surfaces. The survival and development of microorganisms in the air and on surfaces will depend on microbial structural and metabolic characteristics and the presence of favorable conditions, such as nutritional and microclimatic conditions.

Biological risk, which is the probability that damage will occur, depends on the presence of biological hazards, and on the exposure and vulnerability of the materials and people (operators and visitors) involved. The first step in preventing such damage is a thorough knowledge of biological particles and all the factors that may affect their circulation, survival and growth in the environment, as a basis for any further preventive strategy. The monitoring of microbial contamination on the surface of heritage objects and in the air surrounding them, both from a quantitative and qualitative perspective, along with an evaluation of the microclimatic conditions, is essential for the study of environmental quality. Nowadays, the application of Computational Fluid Dynamics (CFD) allows a map of the global microclimatic conditions to be drawn, which is fundamental to the conservation of cultural heritage (Bakker, 2003; Tennekes and Lumley, 1972). Numerical models allow the prediction of damage-related processes in materials, and also knowledge of indoor air movement, air temperature and humidity distribution over time (Balocco et al., 2013).

As for the biological monitoring of air and surfaces, different methodologies and measuring techniques for biological monitoring have been adopted (Pasquarella et al., 2008), but a standardized and universally accepted methodology that can guarantee reliability, reproducibility and comparability of results is yet to be found. On the basis of experiences carried out in environments at high risk of contamination/infection (e.g. healthcare environments, food industries, spacecraft) (Guarnieri et al., 1997; Castiglia et al., 2008; Pasquarella et al., 2010, 2012a,b,c; Pitzurra et al., 2007); a working model for the evaluation of microbial air and surface contamination in cultural heritage environments has been defined (Pasquarella et al., 2011, 2012a,b,c). This model, completed with the evaluation of microclimatic parameters and CFD simulation, has been applied as a pilot study to the De Rossi Room at the Palatina Library in Parma.

To our knowledge, no study has yet adopted a multidisciplinary approach to investigate biological environmental pollution and related factors.

2. Materials and methods

2.1. Setting

The historical Palatina Library is located on the second floor of the Pilotta Building, which dates back to the late 16th century. The library was opened in 1761 and contains over 700,000 volumes. In particular, the De Rossi Room is one of the most important rooms in the Library, hosting the largest collection of Jewish manuscripts outside Israel and a number of incunabula, manuscripts from the 15th century.

The room is 6.90 m wide, 12 m long with a total volume of 496.8 m^3. It has two internal partition walls and two external walls. The smaller of these external walls, with an area of 48.28 m^2, has a central window with an area of 3.5 m^2 and is south-west oriented, and the wider external wall is 42.20 m^2 and south-east oriented. It has a cross-vaulted ceiling.

2.2. Monitoring program

Monitoring was performed during two periods of the year: July and December 2012. Air microbial sampling, particle sampling and microclimate analysis were performed at a height of 1 m (12 sampling points), 2 m (12 sampling points) and 4 m (2 sampling points) (Fig. 1). Book surfaces (spine and edge) and shelves were sampled in the two areas where the most valuable books are stored (Jewish manuscripts and incunabula). The library was closed to visitors and operators during the sampling period. Researchers performed the sampling wearing sterile protective clothing (cap, mask, gown, gloves and overshoes) (Fig. 2).

2.2.1. Biological environmental monitoring

2.2.1.1. Microbial air sampling. Microbial air sampling was carried out by active samplings, to measure the concentration of microorganisms in the air, and by passive sampling to measure the rate at which the microorganisms settle on surfaces (ISO, 14698-1; Pasquarella et al., 2008). For active samplings, a DUO SAS 360 sampler (International PBI, Milan, Italy) equipped with RODAC plates (55 mm diameter) was used. The flow rate was 180 liters per minute (L/min) and the suction volume was 200 liters (L). The sampler was placed in the monitored room at different heights above the floor and about one meter away from any physical obstacle. Results were adjusted according to the table provided by the manufacturer and were expressed as colony forming units (cfu)/cubic meter (m^3). For passive samplings, Petri dishes with a diameter of

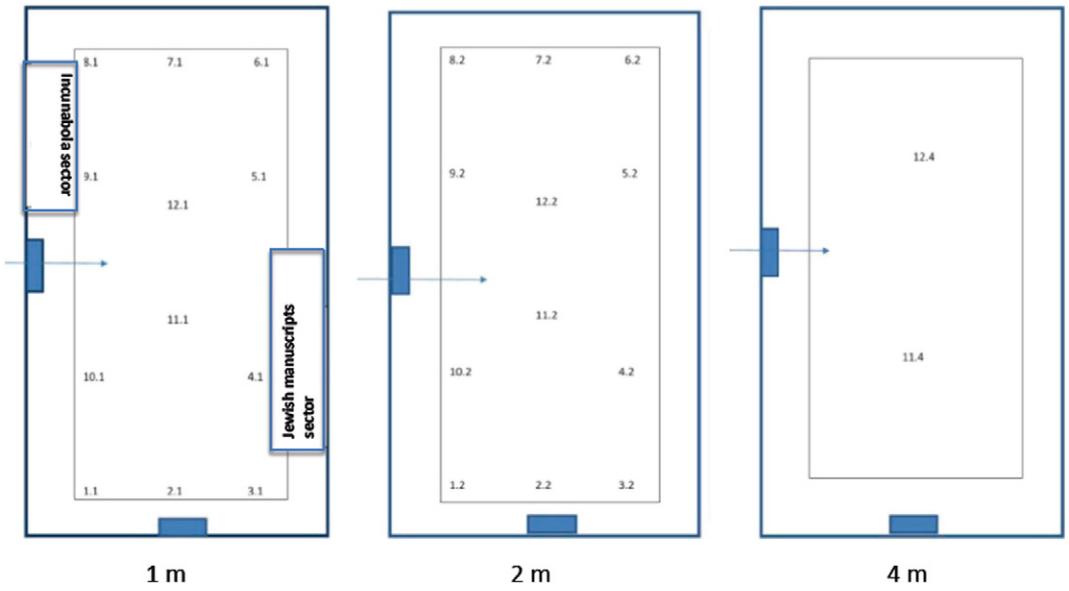

Fig. 1. De Rossi Room: sampling points at 1 m, 2 m and 4 m height.

9 cm were opened for 1 h, at different heights (1, 2 and 4 m) above the floor and about 1 m away from any obstacle, to determine the Index of Microbial Air contamination (IMA) (Pasquarella et al., 2000).

2.2.1.2. Surface microbial sampling. The surface contamination of shelves and ancient manuscripts was assessed using nitrocellulose membranes (Sartorius AG, Göttingen, Germany) with a diameter of 47 mm. Two parameters were measured: the Microbial Build-up (MB, the total number of microorganisms accumulated on a surface in an unknown period of time prior to the sampling) and the Hourly Microbial Fallout (HMF), the number of microorganisms that settle on a surface during 1 h, (Pitzurra et al., 1997; Poletti et al., 1999).

Fig. 2. Sampling in the De Rossi Room.

The MB measurements were performed by pressing the nitrocellulose membrane onto the surface of the sampled area for 20 s and then transferring this to a Petri dish containing the culture medium; to measure the HMF, the nitrocellulose membrane was left on the tested surface for one hour. The results for MB and HMF were expressed as cfu/square decimeter (dm^2), as suggested in (ISO, 14698-1).

2.2.1.3. Microbial isolation and identification by culturing techniques. Trypticase Soy Agar (incubated at 36 ± 1 °C for 48 h) was used for bacteria isolation and Sabouraud Dextrose Agar with chloramphenicol (incubated at 22 ± 1 °C for 120 h) was used for microfungi isolation. To identify fungal species in the surface and air samples collected in the Jewish and Incunabula sectors, the colonies isolated on the SDA were transferred to Malt Extract Agar, Czapek Yeast Agar and Potato Dextrose Agar. Microbial identification was performed by morphological identification of isolates by using optical microscope.

2.2.1.4. Microbial isolation and identification by molecular techniques. Molecular analysis was performed by in vitro amplification (PCR) of DNA target sequences. Total microbial DNA was directly extracted with the Sigma GenElute kit, from the microbial particles present on half of the surface of each nitrocellulose membrane.

DNA molecules were used as template in PCR (Polymerase Chain Reaction) reactions in order to amplify target sequences specific for bacteria or fungi. In particular, specific rDNA sequences (16S ribosomal gene or related ITS regions — Internal Transcribed Sequences, 16-23S rRNA genes) were amplified for the detection of bacterial species, while the ribosomal ITS regions (18-26S rRNA genes) were amplified for the detection of the fungal species (Palla, 2011, 2012). The amplification products were first analyzed by electrophoresis on 2% agarose gel and the nucleotide composition determined by Eurofins MWG Operon sequencing service. The sequences were analyzed (% of similarity) with all those stored in genomic databases (NHI—USA, EMBL—GERMANY), using the BLAST platform (Palla et al., 2013).

2.2.1.5. Microscope detection of fungal spores collected by a Hirst spore trap sampler. A Hirst spore trap sampler (VPPS 1000) with a flow rate of 10 L/min was used for microscope identification of both viable and non-

Table 1
Bacterial and fungal air contamination values obtained with active (cfu/m^3) and passive (IMA) sampling at 1, 2 and 4 m in July.

	Code	1 m				Code	2 m				Code	4 m			
		cfu/m^3		IMA			cfu/m^3		IMA			cfu/m^3		IMA	
		B	F	B	F		B	F	B	F		B	F	B	F
	1.1	30	10	18	2	1.2	310	30	n.d.	n.d.	1.4	n.d.	n.d.	n.d.	n.d.
	2.1	280	60	10	3	2.2	660	50	14	2	2.4	n.d.	n.d.	n.d.	n.d.
	3.1	340	10	10	1	3.2	490	0	n.d.	n.d.	3.4	n.d.	n.d.	n.d.	n.d.
	4.1	150	20	8	0	4.2	320	15	8	1	4.4	n.d.	n.d.	n.d.	n.d.
	5.1	50	20	9	2	5.2	190	20	n.d.	n.d.	5.4	n.d.	n.d.	n.d.	n.d.
	6.1	80	0	7	0	6.2	330	0	4	2	6.4	n.d.	n.d.	n.d.	n.d.
	7.1	100	50	3	0	7.2	110	10	n.d.	n.d.	7.4	80	10	n.d.	n.d.
	8.1	90	20	6	0	8.2	190	0	5	1	8.4	n.d.	n.d.	n.d.	n.d.
	9.1	330	15	35	1	9.2	90	15	4	0	9.4	n.d.	n.d.	n.d.	n.d.
	10.1	250	50	6	1	10.2	280	10	4	0	10.4	n.d.	n.d.	n.d.	n.d.
	11.1	290	30	8	2	11.2	330	15	8	1	11.4	250	15	5	0
	12.1	290	15	4	1	12.2	340	25	5	0	12.4	n.d.	n.d.	9	1
Median		200	20	8	1		315	15	5	1		165	12.5	7	0.5
Min.		30	0	3	0		90	0	4	0		80	10	5	0
Max		340	60	35	3		660	50	14	2		250	15	9	1

B: bacteria; F: fungi.
CFU/m^3: colony forming units/cubic meter.
IMA: Index of Microbial Air contamination.
n.d.: not determined.

viable fungal spores. The count was performed according to the method defined in UNI, 11108/04 (UNI, Italian Organization for Standardization).

2.2.2. Particle counting

Airborne particles with a diameter of ≥0.3, ≥0.5, ≥1 and ≥5 μm were counted with the laser particle counter Climet CI 754, certified and validated in accordance with the requirements in UNI EN 13205:2002 (UNI EN, 13205; ISO, 14644-1). The suction volume was 75 L/min. The measurements were carried out in triplicate at different heights, 1 m above the floor, with a start-up delay of 1 min and a delay time of 10 s between each of the three suctions of 25 L.

2.2.3. Microclimatic monitoring

The environment monitoring was based on stratigraphic and altimetric measurements of air temperature, relative humidity, air velocity and mean radiant air temperature. Differential pressure variations in specific zones were also evaluated.

The microclimate measurements were acquired every 2 min, using the following instruments connected to a radio master R-log data logger capture system: 2 hot wire anemometers, 2 air temperature and air humidity radio sensors, 2 differential pressure sensors, 1 globe thermometer.

Temperature and relative humidity monitoring were carried out for three different heights consistently spaced out from the internal wall. The globe thermometer was set at 2 m from the floor in the middle of the hall. Internal air velocity and differential air pressure, between monitored room and adjoining hall was evaluated in proximity to the internal door at 1 m and 2 m with respect to the floor.

An infra-red FLIR thermo-camera (FLIR T600) with a data matrix of 480 × 360 pixels, an accuracy of ±2% or ±2 °C reading; thermal sensitivity <0.04 °C at 30 °C; temperature range −40 °C to 650 °C, provided

Table 2
Bacterial and fungal air contamination values obtained with active (cfu/m^3) and passive (IMA) sampling at 1, 2 and 4 m in December.

	Code	1 m				Code	2 m				Code	4 m			
		cfu/m^3		IMA			cfu/m^3		IMA			cfu/m^3		IMA	
		B	F	B	F		B	F	B	F		B	F	B	F
	1.1	60	0	6	1	1.2	25	5	n.d.	n.d.	1.4	n.d.	n.d.	n.d.	n.d.
	2.1	25	5	4	1	2.2	30	10	n.d.	n.d.	2.4	n.d.	n.d.	n.d.	n.d.
	3.1	40	n.d.	10	2	3.2	50	5	n.d.	n.d.	3.4	n.d.	n.d.	n.d.	n.d.
	4.1	50	0	6	3	4.2	20	10	1	0	4.4	n.d.	n.d.	n.d.	n.d.
	5.1	20	5	6	2	5.2	70	5	n.d.	n.d.	5.4	n.d.	n.d.	n.d.	n.d.
	6.1	35		7	0	6.2	30	10	3	n.d.	6.4	n.d.	n.d.	n.d.	n.d.
	7.1	45	5	13	2	7.2	ND	0	n.d.	n.d.	7.4	30	n.d.	n.d.	n.d.
	8.1	40	5	9	2	8.2	35	5	8	n.d.	8.4	n.d.	n.d.	n.d.	n.d.
	9.1	70	20	15	3	9.2	50	0	2	1	9.4	1	n.d.	n.d.	n.d.
	10.1	75	20	7	3	10.2	30	5	n.d.	n.d.	10.4	n.d.	n.d.	n.d.	n.d.
	11.1	55	15	13	1	11.2	40	15	8	0	11.4	45	0	6	1
	12.1	60	15	9	2	12.2	70	5	6	1	12.4	n.d.	0	6	1
Median		47.5	5	8	2		35	5	4.5	0.5		37.5	0	6	1
Min.		20	0	4	0		20	0	1	0		30	0	1	1
Max		75	20	15	3		70	15	8	1		45	0	6	1

B: bacteria; F: fungi.
cfu/m^3: colony forming units/cubic meter.
IMA: Index of Microbial Air contamination.
n.d.: not determined.

Table 3
Surface bacterial and fungal contamination values in July and December: Microbial Buildup (MB).

Sector	July						December					
	Sampling point	1 m		Sampling point	4 m		Sampling point	1 m		Sampling point	4 m	
		cfu/dm^2			cfu/dm^2			cfu/dm^2			cfu/dm^2	
		B	F		B	F		B	F		B	F
Jewish manuscripts	Shelf (JS1)	161	40	Shelf (JS4)	c.g.	6	Shelf (JS1)	6	17	Shelf (JS4)	c.g.	167
	Book spine (JB1Sp)	6	6	Book spine (JB4Sp)	6	12	Book spine (JB1Sp)	29	58	Book spine (JB4Sp)	29	40
	Book edge (JB1E)	c. g.	6	Book edge (JB4E)	c.g.	6	Book edge (JB1E)	c.g.	185	Book edge (JB4E)	45	138
Incunabula manuscripts	Shelf (IS1)	c.g.	75	Shelf (IS4)	45	35	Shelf (IS1)	52	29	Shelf (IS4)	0	46
	Book spine (IB1Sp)	0	12	Book spine (IB4Sp)	12	6	Book spine (IB1Sp)	17	17	Book spine (IB4Sp)	c.g.	260
	Book edge (IB1E)	c.g.	6	Book edge (IB4E)	c.g.	6	Book edge (IB1E)	c.g.	277	Book edge (IB4E)	0	0

B: bacteria; F: fungi.
cfu/dm^2: colony forming units/square decimeter.
c.g.: confluent grown.
I: Incunabula sector; J: Jewish sector; B: book; E: edge; S: shelf; Sp: spine.
1: at 1 m; 4: at 4 m.

information on the surface temperature of the various building materials, objects and components.

2.2.4. Computational fluid dynamics application

The CFD application for transient simulations, integrated with experimental data and applied to three-dimensional models of the studied environment, was used to assess the indoor microclimatic conditions and study the tracing and diffusion of particles inside the room (Balocco et al., 2013). The geometry of the analyzed environment was compared to a solid model, where all the different computational domains and the person standing were considered in the simulations. In order to solve the natural airflow convection inside the room, the Reynolds Averaged Navier–Stokes and energy equations were numerically solved under the assumption of Newtonian fluid and incompressible flow. Momentum equations are coupled with a standard k–ε closure scheme (Ignat et al., 2000; Launder and Spalding, 1974), applied in order to model turbulence caused by eddy viscosity. In order to firstly validate the applied numerical tool, results carried out by simulations were preliminary compared with available experimental data. Simulations were carried out in summer and winter climatic conditions. In order to evaluate the effect of the buoyancy driven airflow inside the room we assumed that any imposed pressure gradient acts on air filling the numerical domain. This condition reflects the current state of the room, which is not equipped with any ventilation or air conditioning system.

Comparison concerned time histories of temperature and relative humidity evaluated at three different points inside the room, corresponding to locations where probes were arranged during the experimental campaign. By imposing the same external environmental constraints occurring during acquisitions, different simulations were performed. In order to compute the path-lines of small particles in fluid flow inside the room, particle tracing post-processing was also carried-out, once the air velocity fields were solved. Simulations of particle tracing of different diameters (≥0.3, ≥0.5, ≥1 μm) were carried out using the formulation provided (Coulson and Richardson, 2009).

2.3. Statistical analysis

SPSS 19 (Statistical Package for Social Sciences) was used for statistical evaluations. Mann–Whitney tests were used to establish significant differences for IMA, cfu/m^3 and particles at the different height and different seasons. A p-value < 0.05 was considered statistically significant.

3. Results and discussion

3.1. Biological sampling

A wide variability in microbial contamination in both air and on surfaces for the different sampling points was observed in July and December.

3.1.1. Air sampling

Tables 1 and 2 show the bacterial and fungal contamination values obtained at the different sampling points, in July and December respectively, at 1 m, 2 m and 4 m. In the sampling performed in July the highest median levels of air bacterial contamination were found at 2 m height (315 cfu/m^3), for IMA at 1 m height (8 IMA), the lowest at 4 m (165 cfu/m^3),

Table 4
Surface bacterial and fungal contamination values in July and December: Hourly Microbial Fallout (HMF).

Sector	July			December					
	Sampling point	1 m		Sampling point	1 m		Sampling point	4 m	
		cfu/dm^2			cfu/dm^2			cfu/dm^2	
		B	F		B	F		B	F
Jewish manuscripts	Shelf (JS1)	6	6	Shelf (JS1)	0	0	Shelf (JS4)	0	0
	Book edge (JB1E)	35	6	Book edge (JB1E)	0	0	Book edge (JB4E)	c.g.	12
Incunabula manuscripts	Shelf (IS1)	0	0	Shelf (IS1)	0	17	Shelf (IS4)	29	0
	Book edge (IB1E)	6	0	Book edge (IB1E)	12	23	Book edge (IB4E)	6	0

B: bacteria; F: fungi.
cfu/dm^2: colony forming units/square decimeter.
c.g.: confluent grown.
I: Incunabula sector; J: Jewish sector; B: book; E: edge; S: shelf.
1: at 1 m; 4: at 4 m.

while for IMA at 2 m (5 IMA). As for micro-fungi, the highest median value was recorded at 1 m and 2 m (20 and 15 cfu/m^3 respectively), while the lowest at 4 m (12.5 cfu/m^3); as for IMA both at 1 m and at 2 m the median was 1 IMA, while at 4 m the median was 0.5 IMA. The highest value of bacterial air contamination was recorded at 2 m (660 cfu/m^3), and 1 m for the IMA (35); fungal contamination showed the highest value at 1 m for both cfu/m^3 (60) and for IMA (3).

In the sampling performed in December the higher median values of bacterial air contamination at 1 m in height were, both with active sampling and passive sampling,, 47.5 cfu/m^3 and 8 IMA, while bacterial median values decreased at higher levels: at 2 m (35 cfu/m^3 and 4.5 IMA) and 4 m (37.5 cfu/m^3 and 6 IMA). Fungal contamination, both at 1 m and 2 m, showed a value of 5 cfu/m^3, which decreased to 0 at 4 m. The highest value of IMA (3 IMA) was recorded at 1 m height.

3.1.2. Surface sampling

As for surface contamination, in the sampling performed in July the highest value of MB bacteria (161 cfu/dm^2) and fungi (75 cfu/dm^2) were recorded on two different shelves at 1 m height; in December a maximum value of fungal contamination of 277 cfu/m^3 was recorded on the edge of the book at 1 m height (Table 3).

Table 5
Fungal species isolated from air (cfu/m^3 and IMA) and surfaces (MB and HMF) in summer and winter, on the different sampling points indicated by specific code (the number of cfu/m^3 and IMA registered are reported in brackets).

	July				December			
	cfu/m^3	IMA	HMF	MB	cfu/m^3	IMA	HMF	MB
Alternaria alternata	4.1 (1)/11.2 (1)				4.2 (1)	9.1 (2)		JBSp1 (3)/JBE1 (3)/JS4 (5)/JBSp4 (1)/JBE4 (3)/IBSp1 (1)/IBE1 (1)
Alternaria sp.1	9.1 (1)							
Alternaria sp.2	9.1 (1)/11.2 (1)							
Alternaria sp.3						4.1 (1)	JBE4 (2)	
Alternaria sp.4						12.4 (1)		JS1 (1)/IS1 (2)/IS4 (3)/IBSp4 (2)
Alternaria sp.5						9.1 (1)		
Arthrinium phaeospermum	4.2 (1)				11.2 (2)			
Arthrinium sp.2				JS1 (3)				
Aspergillus niger				IS4 (1)				IBSp1 (1)
Aspergillus versicolor	11.4 (1)							
Aureobasidium pullulans	11.4 (1)							
Botrytis cinerea	4.2 (1)							IBE1 (1)
Cladosporium cladosporioides	4.1 (2)/12.2 (1)			JS1 (3)/ IS1 (4)	11.1 (1)			JBSp1 (2)/JBSp4 (5)
Cladosporium cucumerinum	9.1 (1)/11.1 (2)/11.2 (1)/12.4 (1)	4.2 (1)	JBE1 (1)			9.2 (1)	IS1 (2)/IBE1 (1)	
Cladosporium herbarum	12.1 (1)/12.2 (2)/12.4 (1)	12.4 (1)		IS4 (1)	11.1 (1)		IS1 (1)/IBE1 (1)	
Cladosporium macrocarpum							IBE1 (1)	
Cladosporium sphaerospermum	11.1 (1)				4.2 (1)		IBE1 (1)	JBSp1 (2)/JBE1 (21)/ IS1 (1)/IBE1 (44)/IS4 (4)
Cladosporium sp.5	11.1 (1)/11.4 (1)							
Cladosporium sp.6				IS1 (9)				
Epicoccum nigrum	4.1 (1)–11.1 (1)			JS1 (1)	12.1 (1)/11.2 (1)			JBSp1 (2)/JBE1 (4)/JBSp4 (1)
Eurotium spp.					9.1 (2)			
Fusarium spp.								JBE1 (2)
Yeast			ER1 (1)			4.1 (2)		
Penicillium chrysogenum								JBSp1 (1)/JBE1 (2)/JS4 (23)/JBE4 (20)/IBSp1 (1)/IBSp4 (40)
Penicillium expansum	4.2 (1)				12.1 (2)/ 4.2 (1)/12.2 (1)			JS1 (1)
Penicillium sp.2	12.2 (1)							
Penicillium viridicatum					11.1 (1)			
Penicillium sp.3				JBSp4 (1)		12.2 (1)		
Pithomyces chartarum				JBSp1 (1)/IBSp1 (1)				
Pleospora herbarum	11.1 (1)/ 12.2 (1)			IS4 (4)				
Rhizopus stolonifer				JBE1 (1)–JS4 (1)–JBE4 (1)–IBE1 (1)–IBE4 (1)				JS4 (1)/JBSp4 (1)/JBE4 (1)/IBE1 (1)/IBSp4 (1)
Shaeropsidales								JS1 (1)
Non–fruiting Mycelia						11.1 (1)/12.1 (2)		IS1 (2)/IBE1 (1)/IS4 (1)/IBSp4 (2)

I: Incunabula sector; J: Jewish sector; B: book; E: edge; S: shelf; Sp: spine; 1 = at 1 m; 2 = at 2 m; .4 = at 4 m.

Table 6
Particle contamination values at 1 m, 2 m and 4 m in July.

July												
	1 m				2 m				4 m			
Points	≥0.3 μm	≥0.5 μm	≥1.0 μm	≥5.0 μm	≥0.3 μm	≥0.5 μm	≥1.0 μm	≥5.0 μm	≥0.3 μm	≥0.5 μm	≥1.0 μm	≥5.0 μm
1	17 835 966	1 770 200	372 933	8 900	16 911 066	2 118 633	738 800	29 133	n.d.	n.d.	n.d.	n.d.
2	17 728 100	1 921 066	515 900	21 933	16 779 833	2 168 300	782 766	29 466	n.d.	n.d.	n.d.	n.d.
3	17 646 900	2 087 833	655 733	42 433	16 695 000	2 199 166	820 400	30 633	n.d.	n.d.	n.d.	n.d.
4	17 591 800	2 061 433	633 100	32 266	16 718 633	2 209 933	834 000	35 533	n.d.	n.d.	n.d.	n.d.
5	17 574 966	1 981 633	586 000	25 566	16 662 533	2 151 200	781 666	34 866	n.d.	n.d.	n.d.	n.d.
6	17 374 333	1 966 766	585 600	23 266	16 731 966	1 900 900	569 566	12 633	n.d.	n.d.	n.d.	n.d.
7	17 385 433	1 937 700	570 533	23 833	16 642 233	2 011 700	660 800	23 200	n.d.	n.d.	n.d.	n.d.
8	17 274 466	2 035 333	644 466	33 433	16 546 400	2 104 100	747 133	30 533	n.d.	n.d.	n.d.	n.d.
9	17 477 900	2 029 833	641 533	27 600	16 861 800	1 951 500	607 966	16 700	n.d.	n.d.	n.d.	n.d.
10	17 499 400	2 124 866	714 266	28 966	17 005 366	2 185 333	791 566	30 366	n.d.	n.d.	n.d.	n.d.
11	17 416 833	2 139 033	730 033	33 566	16 772 000	2 270 566	852 000	36 566	17 056 266	1 666 066	356 566	7 333
12	17 773 000	1 735 433	347 433	8 600	17 018 766	2 032 100	654 700	28 666	17 248 066	1 972 733	589 866	16 733
Median	17 537 183	2 005 733	609 550	26 583	16 751 983	2 134 917	764 400	29 916	17 152 166	1 819 400	473 216	12 033
Min	17 274 466	1 735 433	347 433	8 600	16 546 400	1 900 900	569 566	12 633	17 056 266	1 666 066	356 566	7 333
Max	17 835 966	2 139 033	730 033	42 433	17 018 766	2 270 566	852 000	36 566	17 248 066	1 972 733	589 866	16 733

Table 7
Particle contamination values at 1 m, 2 m and 4 m, in December.

December												
Points	1 m				2 m				4 m			
	≥0.3 μm	≥0.5 μm	≥1.0 μm	≥5.0 μm	≥0.3 μm	≥0.5 μm	≥1.0 μm	≥5.0 μm	≥0.3 μm	≥0.5 μm	≥1.0 μm	≥5.0 μm
1	17 411 166	1 283 433	166 366	11 433	18 204 133	2 195 000	697 000	43 600	n.d.	n.d.	n.d.	n.d.
2	17 463 133	1 351 266	220 200	16 833	17 998 500	2 316 933	819 433	54 733	n.d.	n.d.	n.d.	n.d.
3	17 323 100	1 314 866	212 633	14 566	18 036 133	2 341 333	823 600	55 866	n.d.	n.d.	n.d.	n.d.
4	17 593 033	1 398 533	246 200	17 666	17 880 500	2 478 466	947 000	63 200	n.d.	n.d.	n.d.	n.d.
5	17 527 833	1 428 700	288 100	22 000	18 033 933	2 569 400	992 266	67 033	n.d.	n.d.	n.d.	n.d.
6	17 503 933	1 585 266	394 300	30 100	17 812 400	2 695 200	1 099 300	73 966	n.d.	n.d.	n.d.	n.d.
7	17 362 933	1 600 366	415 000	27 933	17 914 966	2 686 233	1 099 100	72 000	n.d.	n.d.	n.d.	n.d.
8	17 502 433	1 792 600	530 033	40 900	17 924 833	2 764 100	1 155 666	73 900	n.d.	n.d.	n.d.	n.d.
9	17 804 200	1 777 366	584 133	35 233	17 912 933	2 881 233	1 240 533	86 300	n.d.	n.d.	n.d.	n.d.
10	17 894 980	1 933 266	579 533	39 133	17 933 400	2 857 482	1 176 333	75 115	n.d.	n.d.	n.d.	n.d.
11	17 796 200	1 800 200	539 366	35 633	17 965 000	2 835 366	1 186 166	70 700	18 116 100	2 077 933	644 433	43 400
12	17 863 966	1 888 866	574 500	35 966	18 181 433	2 830 666	1 191 800	68 833	18 128 966	2 026 166	620 866	36 833
Median	17 515 883	1 592 816	404 650	29 017	17 949 200	2 690 717	1 099 200	69 767	18 122 533	2 052 050	632 650	40 117
Min	17 323 100	1 283 433	166 366	11 433	17 812 400	2 195 000	697 000	43 600	18 116 100	2 026 166	620 866	36 833
Max	17 894 980	1 933 266	584 133	40 900	18 204 133	2 881 233	1 240 533	86 300	18 128 966	2 077 933	644 433	43 400

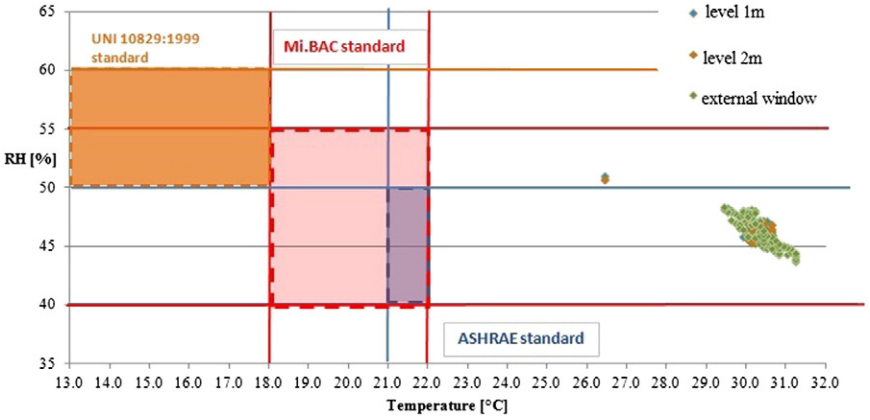

Fig. 3. Microclimatic parameter values recorded in July — comparison with standards.

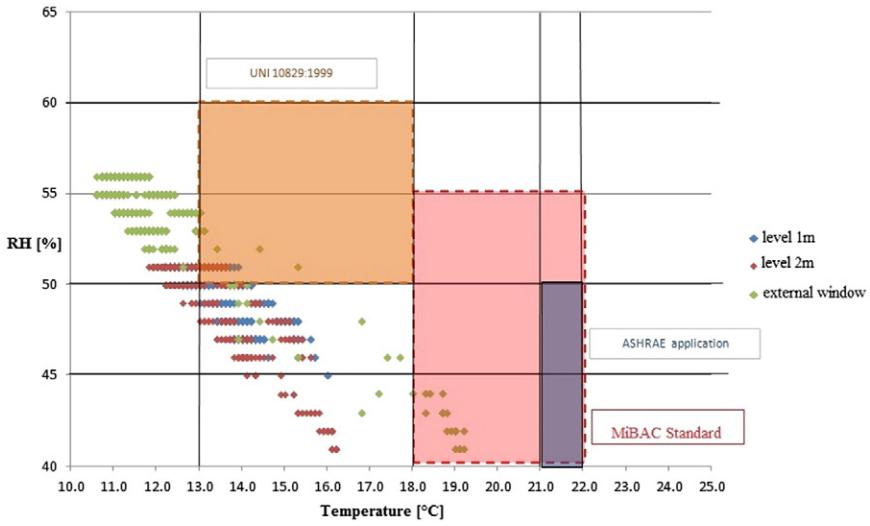

Fig. 4. Microclimatic parameter values recorded in December — comparison with standards.

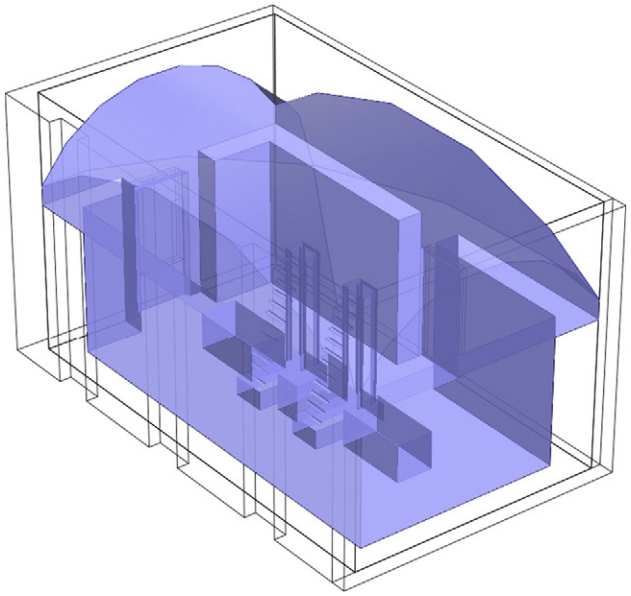

Fig. 5. The room geometry — the computational domain of the indoor air volume.

The highest HMF values both for bacterial (35 cfu/dm^2) and fungal contamination (23 cfu/dm^2) were observed on the edge of the book (Table 4).

3.1.3. Isolated microorganisms

Table 5 shows the bacterial and fungal genera and species, isolated from air and surfaces at the different sampling points, both in December and July, and the indication of the quantitative value. The most frequently isolated fungi were *Alternaria* spp., *Aspergillus* spp., *Cladosporium* spp. and *Penicillium* spp., which belong to genera of fungi biodeteriogens, which can potentially cause damage such as erosions, blemishes, pigmentation and changes in mechanical properties. Some fungi were found both in the air and on surfaces, while some fungi, for example *Aspergillus niger*, *Pithomyces chartarum* and *Rhizopus stolonifer*, were only found on surfaces. All the isolated fungi belong to the biodeteriogens genera. Some of them, for example *Alternaria* spp., *Aspergillus* spp. and *Cladosporium* spp., are also allergenic. In our opinion, the infectious potential of an *Aspergillus* spp. environmental contamination should be adequately considered for susceptible people. On the surfaces of the shelves and books examined, the genera *Aspergillus* spp. and *Cladosporium* spp. were the most abundant, to which must be added *Pleospora herbarum* and *R. stolonifer*; these fungi also belong to

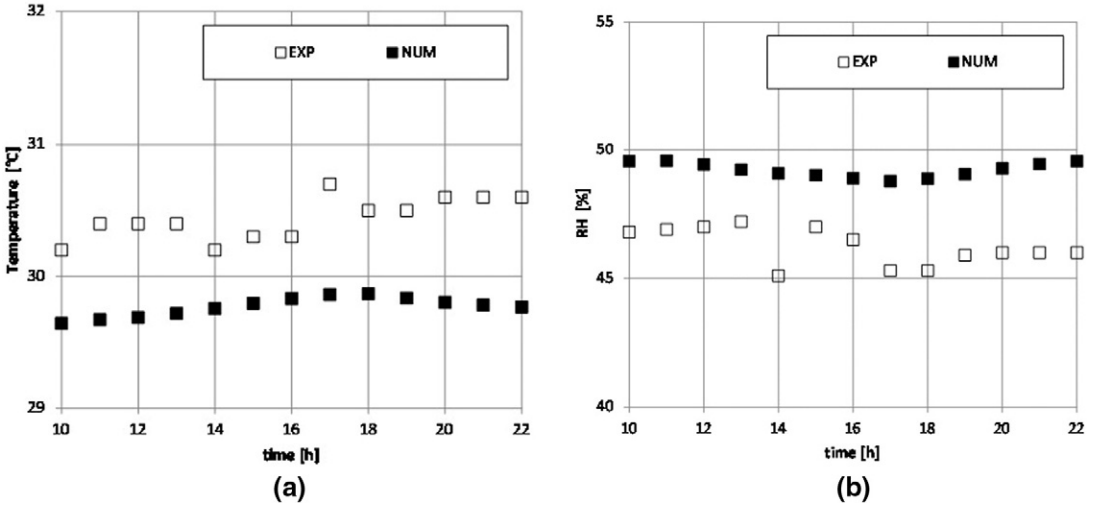

Fig. 6. Experimental (EXP) and numerical (NUM) values: temperature (a) and relative humidity (b) with relative difference, at the experimental point 11.1.

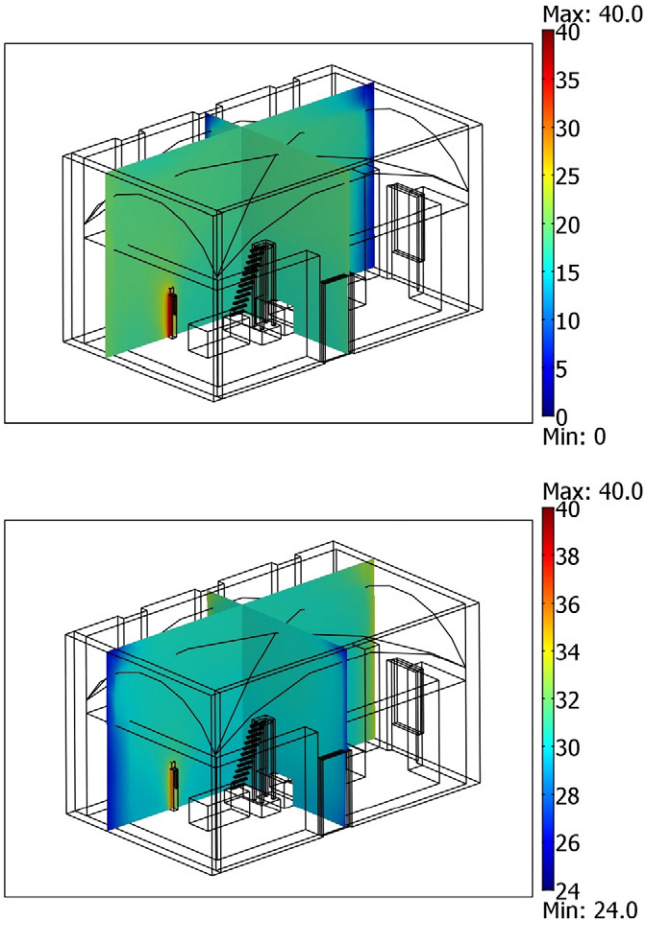

Fig. 7. Thermal distribution (°C) evaluated at 12:00 for summer (up) and winter (down) conditions.

the biodeteriogens species and are able to induce mechanical and esthetic damage. Fungal spores isolated with the HIRST spore trap confirmed the presence of *Cladosporium* spp., *Alternaria* spp., *Epicoccum nigrum*, *Fusarium* spp. and *Botritys* spp., but other genera were also found, for example *Polythrincium*, *Torula* and *Tetraploa*. The molecular identification of microorganisms confirmed the presence of some of the species that had already been isolated with culture techniques (*Aspergillus* spp., *Alternaria* spp., *Rhizopus* spp.), but also showed the presence of other fungi, such as *Chaetomium* spp. and *Scopulariopsis* spp., and some unculturable colonies. Bacteria such as *Streptomyces* spp., *Bacillus* spp., *Sphingomonas* spp. and *Pseudoclavibacter*, as well as unculturable colonies, were identified by molecular investigation, which represents a useful approach to reveal and identify microbial consortia colonizing both organic and inorganic substrates (Palla et al., 2010, 2013).

3.2. Particle counting

Tables 6 and 7 show the descriptive statistics of the number of particles ≥0.3, ≥0.5, ≥1 and ≥5.0 μm, at the different sampling points at a height of 1, 2 and 4 m, in July and in December.

A significantly higher concentration was observed in July for particles ≥0.5 μm ($p = 0.004$) and 1.0 μm ($p = 0.004$) in diameter, while a significantly higher concentration was observed in winter for particles ≥5.0 μm in diameter ($p < 0.001$). The comparison between 1 m and 2 m heights showed a significantly higher concentration at 1 m for particles measuring ≥0.5 μm ($p = 0.003$) and ≥1.0 μm ($p = 0.007$).

3.3. Microclimatic monitoring

Figs. 3 and 4 show experimental data related to temperature and relative humidity recorded respectively in July and December. Air temperature values ranged from a minimum of 29 °C to a maximum value of 31 °C in July, and from a minimum of 10.6 °C to a maximum value of 18 °C in December; for the relative humidity the measurements provided a range of a minimum of 25% and maximum of 86% for December, and a minimum of 30% to a maximum of 75%.

All the temperature values obtained in July and December are beyond the limits considered by the Standards for the paper conservation (ASHRAE, 2001, 2003, 2011; Ministry of Cultural Heritage, 2001); even the relative humidity values do not comply with the limits suggested.

3.4. CFD simulation

CFD was applied to the geometry of the studied room carried out by a solid modeling where all the different computational domains and the person standing considered in the simulations were highlighted in blue (Fig. 5). Fig. 6 shows experimental and numerical temperature and relative humidity values (left vertical axis), together with relative gap (right vertical axis).

Fig. 7 shows thermal distribution in longitudinal cross-sections of the room evaluated at 12:00 in summer and winter conditions. From thermal analysis the room appears to be well insulated with respect to external conditions, maintaining a constant temperature throughout the air domain. This finding agrees with experimental evidence.

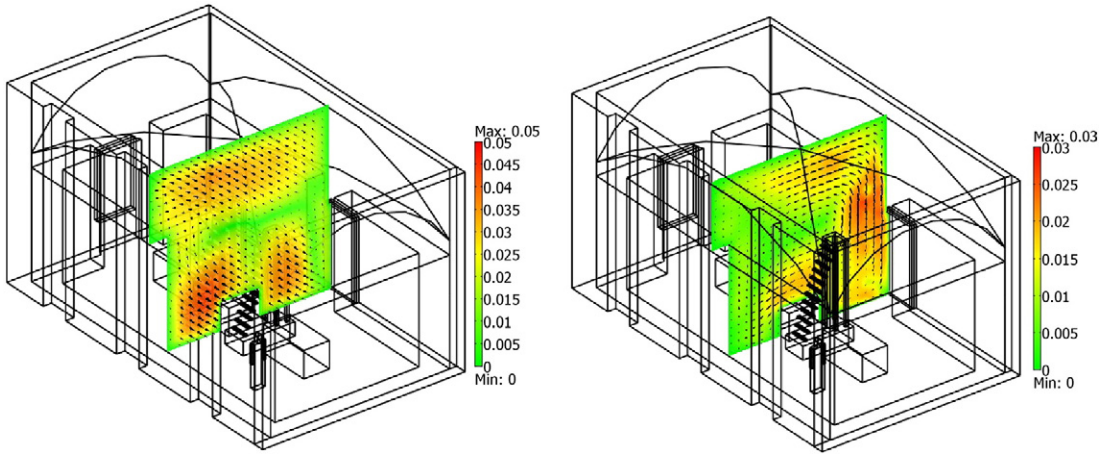

Fig. 8. Magnitude of air velocity (m/s) distribution evaluated at 12:00 on a vertical cross section of the room (half-room plan) for summer (left) and winter (right) conditions.

Otherwise, thermal transport is appreciably close to the simulated standing person inside the room, which represents the internal thermal source in the system. Isotherms appear locally shaped by the effect of the natural convection, determining a thermal plume above the simulated body. Magnitude of air velocity reaches its maximum value close to the thermal source, also determining a weak recirculation motion field in the remaining portions of the room (Fig. 8). On the other hand, the global flow pattern inside the air volume results from a combination of the aforementioned flow and a second one, determined by the different thermal conditions between external and internal walls. Magnitude of air velocity reaches its maximum value close to the thermal source, also determining a weak recirculation motion field in the remaining portions of the room. Because of summer or winter conditions, this second main fluid recirculation presents opposite rolling directions: one way the hottest walls are the external ones, so that air moves upward close to them, otherwise they become the coldest ones, so that air tends to move downward in their proximity. This assumption explains why the motion field computed behind the person appears much more intense in winter conditions than in summer conditions (Fig. 9). Transient simulation results that take into account the archivist movements show significant modification of flow patterns inside the room. This finding underlines the fact that, in the absence of imposed pressure gradient (forced indoor ventilation), the effect of a person moving in the buoyancy-driven flow may have a considerable impact, especially with respect to powders and any small particle transport (Fig. 10).

Obtained transient simulation results highlight the fact that in all situations similar to that being investigated and thus a thermal zone used for a rare books and manuscripts library, the mass and thermal flows combined with people movements define the internal air dynamic and air humidity concentration and distribution. This fact must be taken into account especially when particle diffusion and transport have to be foreseen for cultural heritage conservation and maintenance.

The tracing and distribution of particles based on diameter (≥0.3 and ≥0.5 μm) are provided in Fig. 10, which shows the different slice planes of particle tracing and diffusion in the air volume of the room during summer conditions. The particle tracing is mostly influenced by the convective airflow. Dispersion of particles of ≥1.0 μm in diameter is not shown, because they globally fall down due to the gravity force effect and sedimentation velocity.

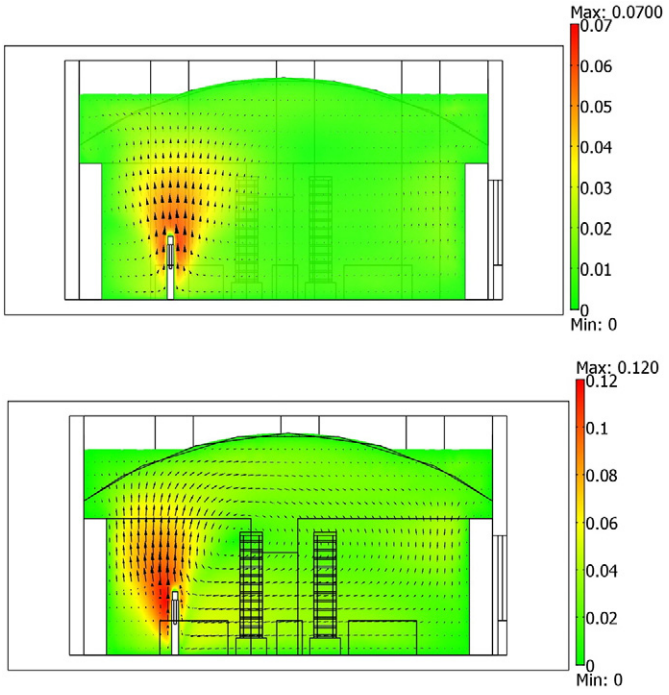

Fig. 9. Magnitude of air velocity (m/s) distribution evaluated at 12:00 for summer (up) and winter (down) conditions with people standing.

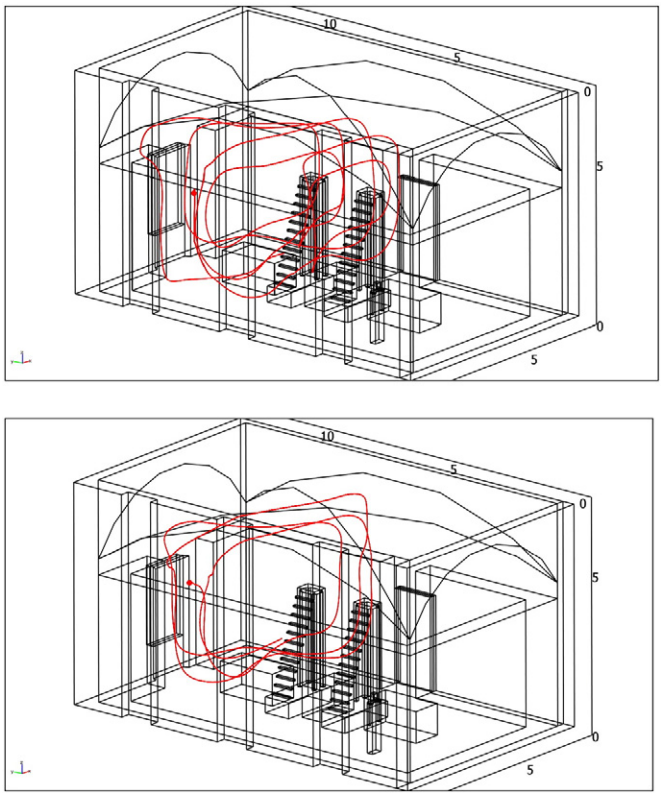

Fig. 10. Particle tracing: diffusion of particles with 0.3 μm diameter (up) and particles with 0.5 μm diameter (down).

4. Conclusions

The experience at the Palatina Library allowed us to apply the proposed multidisciplinary monitoring system. With this system, it was possible to assess air and surface biological contamination and to build a three-dimensional model of the environment. The CFD transient simulation results highlighted the fact that in all situations similar to that being investigated and thus a thermal zone used for a rare books and manuscripts library, the mass and thermal flows combined with people movements define the internal air dynamic and air humidity concentration and distribution. This fact must be taken into account especially when particle diffusion and transport must be foreseen for cultural heritage conservation and maintenance. A comprehensive overview on microbial colonization was obtained by combining culture analysis and molecular investigation. The results allow us to assess the negative impacts of microbial metabolic products on both cultural assets and on the health of workers and visitors. The question concerning an HVAC plant installation must be considered for the necessary ventilation and air change for the few occupants (archivists, librarians and some scholars) but also for contaminant removal (bacteria, spores, mildew and fungi species, furniture solvents and varnishes), which is crucial for the control of chemical instability of paper stressed by impulsive high thermal and hygrometric variations.

Our study represents a contribution towards the definition of standardized methods for assessing the biological and climate quality of indoor heritage environments. A large amount of comparable data on biological environmental contamination (air and surfaces) and microclimatic conditions, combined with CFD, is needed to understand the proper air quality for paper conservation, circulation and diffusion of microorganisms in the environment and to define the threshold values of

microbial environmental contamination for the preventive conservation of cultural heritage and the protection of the health of operators and visitors.

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