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ORIGINAL PAPER

Biological and microclimatic monitoring for conservation of cultural heritage: a case study at the De Rossi room of the Palatina library in Parma

Cesira Pasquarella · Carla Balocco · Elisa Saccani · Emanuela Capobianco · Isabella Viani · Licia Veronesi · Filippo Pavani · Giovanna Pasquariello · Valentina Rotolo · Franco Palla · Roberto Albertini

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Abstract Biological particles in heritage-related indoor environments (museums, libraries, archives) represent a hazard to artifacts (biodeteriogenic action), operators and visitors' health. The aim of the study was to evaluate environmental biological contamination and microclimate conditions in different periods of the year in De Rossi room of the historical Palatina Library in Parma. Microclimatic measurements were recorded continuously for a period ranging from 11 to 17 days in: January–February, May and September 2017. Monitoring of bacterial and fungal contamination was performed for air by active and passive sampling and by nitrocellulose membranes on shelves and manuscripts. Microorganisms were isolated by in vitro culture (Tryptic Soy Agar and Sabouraud Dextrose Agar) and characterized by molecular investigation. Viable and non-viable fungal spores were collected by Hirst spore trap. Concerning air, the highest bacterial mean values were 76.67 cfu/m^3 (in May) and 3.33 IMA (in February, May and September), while for fungi 60.67 cfu/m^3 and 4.33 IMA in September. The highest fungal values, both on shelves and books, were recorded in September with *Alternaria*, *Arthrinium*, *Aspergillus*, *Cladosporium* and *Penicillium* spp. being the mainly isolated fungi. Air temperature, relative humidity and air velocity reached mean values ranging, respectively, from 14.71 to 26.60 °C, from 41.62 to 44.83% and from 0 to 0.04 m/s. This case study provides an assessment of the environmental quality over a long period, representing a reference model to better understanding microbiological contamination of cultural heritage environment toward the improvement of artwork conservation strategies and the safeguard of human health.

Keywords Cultural heritage · Library · Biological monitoring · Microclimatic monitoring

C. Pasquarella (✉) · E. Saccani · E. Capobianco · I. Viani · L. Veronesi · F. Pavani
Department of Medicine and Surgery, University of Parma, Via Volturno, 39, 43125 Parma, Italy
e-mail: ira.pasquarella@unipr.it

C. Balocco
Department of Industrial Engineering, University of Florence, Via Santa Marta, 3, 50139 Florence, Italy

G. Pasquariello
Central Institute for Restoration and Conservation of Archival and Book Heritage, Ministry of Cultural Heritage and Activities, Via della Stamperia, 6, 00187 Rome, Italy

V. Rotolo · F. Palla
Department of Biological, Chemical and Pharmacological Sciences and Technologies, University of Palermo, Via Archirafi, 38, 90123 Palermo, Italy

R. Albertini
Department of Medicine and Surgery, University of Parma, Via Gramsci, 14, 43126 Parma, Italy

1 Introduction

In cultural heritage indoor environments, biological particles represent a hazard for artifacts (biodeteriogenic action) and for humans (infectious, allergenic and toxic effects) (Mandrioli et al. 2003; Sterflinger 2010; Di Carlo et al. 2016). Fundamental steps for prevention are the knowledge of biological agents (quantitative and qualitative) and of factors affecting their circulation, survival and growth (Balocco et al. 2013). Recommended range for microclimatic parameters in archives and libraries (ASHRAE 2003; UNI 10829 1999; MIBAC 2001) is achievable only if a heating, ventilation and air conditioning (HVAC) system is installed. Regarding environmental microbial contamination, there are a reduced number of studies and there are still no reference methods for air and surface sampling; other than that threshold values have not been defined yet (Mandrioli et al. 2003; Di Carlo et al. 2016; Pasquarella et al. 2015, INAIL 2017). A working model for a global assessment of environmental microclimate and contamination has been proposed (Pasquarella et al. 2015) and applied in this study for monitoring different seasons at the Palatina Library in Parma.

2 Materials and methods

2.1 Setting

The De Rossi room dates back to the late nineteenth century and hosts incunabula and manuscripts from the eleventh to the nineteenth centuries, among them the largest collection of Jewish manuscripts outside Israel. The room is 6.90 m wide, 12 m long (volume of 496.8 m^3). Only staff taking books to be given for consultation is allowed to enter. No HVAC system is present. During winter, a radiator in the middle of the room is switched on at the lowest temperature; during summer, the window is opened daily in the early morning for 1 h. This window overlooks a courtyard in a no traffic urban area of the historic city center.

2.2 Monitoring

2.2.1 Timing

Microclimate data were recorded in 2017: January 27–February 7, May 19–29 and September 5–19, performing the microbial monitoring on the last day of each period and on December 18th.

2.2.2 Biological environmental sampling

Air sampling was performed at five points at 1 m from the floor: n.1, Incunabula sector, n.2 and n.5, Jewish sector, n.3, near the window and Palatino book shelf, n.4, middle of the room; and at one point at four meters from the floor: n.6, near the Bible book shelf. Active sampling using DUO SAS 360 equipped with RODAC plates (55 mm diameter), with a suction volume of 250 L, and passive sampling (Petri dishes Ø of 9 cm) were carried out. The results were expressed as colony-forming units per cubic meter (CFU/m^3) and as Index of Microbial Air Contamination (IMA) (Pasquarella et al. 2000), respectively. Tryptic Soy Agar and Sabouraud Dextrose Agar + chloramphenicol were used for bacteria (36 ± 1 °C for 48 h) and fungi sampling and isolation (22 ± 1 °C for 120 h).

Surface microbial contamination was evaluated by Microbial Build-up (MB = total number of microorganisms accumulated on a surface in an unknown period of time prior to the sampling) and Hourly Microbial Fallout (HMF = number of microorganisms that settle on a surface during 1 h) (Pitzurra et al. 1997), using nitrocellulose membrane (Sartorius AG, Germany Ø of 47 mm), to be subsequently transferred to culture media. Shelves surfaces, points n.1 (1a, 1b), n.3, n.5 (5a, 5b), n.6,—page and cover of manuscripts (XIVin century Hebrew Bible, Ms. Parm. 2810, and XI century Tetraevangelo, Ms. Pal. 5), were sampled.

The morphological analysis of isolated colonies was performed by optical microscopy, while molecular investigation was performed using the genomic DNA (extracted by the GeneJET Genomic DNA Purification kit) as template for in vitro amplification (18-26S ITS-rRNA) of target sequences specific for fungi (Palla and Barresi 2017).

A Hirst spore trap sampler (Burkard), positioned in the middle of the room for 7 days in January–February, September and December, was used for

microscope identification of airborne fungal spores according to UNI CEN/TS 16868 2015.

2.2.3 Microclimatic monitoring

The microclimate monitoring (performed at 1 m from the floor) included stratigraphic and altimetric measurements of air temperature and radiant temperature, relative air humidity and air velocity. The instruments technical characteristics complied with the specific requirements (ISO 7726: 1998). Microclimatic values were acquired every 10 min using two hot wire anemometers, two air temperature and air humidity radio sensors and one globe thermometer radio connected with a multiple data acquisition device. Temperature and relative humidity were measured at points n.1, n.2 and n.3; mean radiant temperature at point n.4; internal air velocity at point n.4 and at the point n.3.

2.2.4 Meteorological data

Concurrently with microclimatic monitoring, outdoor temperatures and relative humidity values were acquired.

2.3 Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences version 20.0 (SPSS Inc. Chicago, IL, USA). Continuous variables were described as mean, standard deviation, maximum and minimum. Correlation between variables was evaluated using Rho-Spearman correlation coefficient; p value ≤ 0.05 was considered as statistically significant. Data error analysis on the microclimatic data was carried out by standard deviation and Chi-squared test (Bevington and Robinson 2003).

3 Results and discussion

Table 1 shows bacterial and fungal contamination in the different periods of the year. A wide seasonal variability was observed. Focusing on fungi, the highest mean values were measured in September (60.67 CFU/m^3, 4.33 IMA), with values up to 100 CFU/m^3 and 9 IMA; the lowest mean value was measured in December by active sampling (6.67 CFU/m^3) and in February by passive sampling (0.50 IMA). An increase of air fungal contamination in May and in September was observed in all the sampling points, with the highest values close to the window and in the middle of the room. This is consistent with the increase of fungi outdoor (Albertini et al. 2014) which may enter the room through the window. A significant variation for fungal contamination among the different periods, both for active ($p = 0.028$) and passive ($p = 0.05$) sampling, was observed, significantly correlated to temperature values ($p = 0.03$ active and $p = 0.004$ passive sampling). A significant correlation for fungi by active and passive sampling ($p = 0.008$) was observed. Consistently with the air results, in September the highest values of surface microbial contamination both on the shelves and on books were observed. Fungi detected by Hirst reached the highest values in February.

Table 2 shows the fungal genera isolated. *Alternaria, Aspergillus, Cladosporium* and P*enicillium* spp. were isolated in all the periods either from air or from surfaces. In some cases, the genera were isolated only in one period. From the air, *Botrytis* and *Eurotium* spp. were isolated in May and *Torula, Polythrincium, Epicoccum* and *Helminthosporium* spp. in February by Hirst spore trap. In certain samples, fungal species were isolated from both air and from surfaces, meaning that they had settled on that specific surface or re-aerosolizated after sedimentation. In one case, in September at point n.3, *Cladosporium cladosporioides* was isolated from the air, the shelf, the cover and internal page of the Ms. Pal. 5 book.

Figure 1 shows microclimate data recorded and related descriptive statistics. A wider range of temperature was observed, from a minimum of 14.09 °C in January–February to 28.41 °C in September. Temperature was quite stable in winter even though with some oscillations. When the heating system was switched off, the effects of external climatic parameters were evident, especially in the area close to the window (point n.3). In May, the temperature increased in all the monitored points; at the point n.3 a constant day–night oscillation was observed. A clear decrease in temperature was observed in September; point n.3 showed some oscillations, higher than the ones in May, but not constant in the whole period, as it was in May. The trend of the mean radiant temperature was similar to that of dry bulb temperature, proving the absence of strong radiant asymmetries. Relative

Table 1 Bacterial and fungal contamination of air and surfaces in different periods of the year: mean (standard deviation), minimum and maximum values

				Periods of the year			
				February	May	September	December
AIR	Settle plates	IMA	B	3.33 (2.42)	3.33 (1.97)	3.33 (1.86)	1.33 (1.03)
				0–7	1–6	0–5	0–3
			F	0.50 (0.84)	3.67 (3.20)	4.33 (2.73)	0.67 (0.82)
				0–2	0–9	1–9	0–2
	DUOSAS	CFU/m^3	B	45.33 (17.47)	76.67 (42.06)	43.33 (30.40)	27.33 (13.49)
				32–76	36–128	12–88	12–52
			F	7.33 (5.89)	36.00 (6.20)	60.67 (34.54)	6.67 (4.13)
				0–16	28–44	20–100	0–12
	HIRST	Fungal spores/m^3	F	5.86	n.d.	1.86	2.41
SURFACE (shelves)	Nitrocellulose membrane	HMF	B	n.d.	2.00 (0.82)	4.25 (2.06)	2.25 (0.96)
					1–3	2–6	1–3
			F	0.25 (0.50)	2.25 (1.26)	4.00 (2.94)	0.50 (0.58)
				0–1	1–4	1–8	0–1
		MB	B	9.67 (4.93)	9.50 (3.51)	16.67 (7.61)	6.50 (5.13)
				2–16	5–14	4–24	2–16
			F	1.33 (1.03)	6.00 (9.63)	20.83 (17.99)	9.83 (11.30)
				0–3	0–25	1–44	2–32
SURFACE (books)	Nitrocellulose membrane	MB	B	17.20 (33.48)	3.00 (2.65)	1.80 (1.10)	4.20 (4.92)
				0–77	0–5	1–3	0–10
			F	0.40 (0.89)	0.33 (0.58)	2.00 (2.00)	0 (0)
				0–2	0–1	0–4	0

B bacteria, *F* fungi, *CFU*/m^3 colony-forming units/cubic meter, *IMA* Index of microbial air contamination, *HMF* Hourly Microbial Fallout, *MB* Microbial Build-up, *n.d.* not determined

humidity ranged from a minimum of 39.25% in September, near the window, to a maximum of 47.28%, at Incunabula sector, in September. At point n.3, at the window, it appeared the most variable, compared with the points n.1 and n.2, where the levels were almost constant despite changes in outdoor conditions. The air temperature was in compliance with UNI 10829 (13–18 °C) only in January–February period, while MIBAC (18–22 °C ± 1.5) and ASHRAE (21–22 °C) standards were never respected. However, the mean relative air humidity values were in compliance with the MIBAC (40–55% ± 6) and ASHRAE (40–50% ± 6), but not with UNI 10829 (50–60%) standards. The air velocity ranged from 0 m/s (May and September) to 0.04 m/s (January–February). During the winter season, the radiator was switched on, producing a greater thermal stratification and air movements on the microclimate conditions, while in May and September the air was stagnant.

4 Conclusions

This case study provides an assessment of the environmental quality over a long period. The results highlighted a wide quantitative and qualitative variability in microbial contamination over time, as well as variability in microclimate conditions. Further

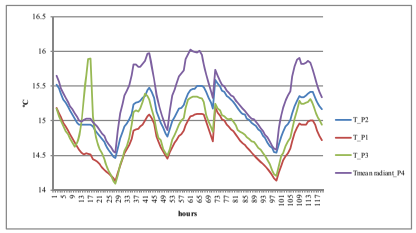
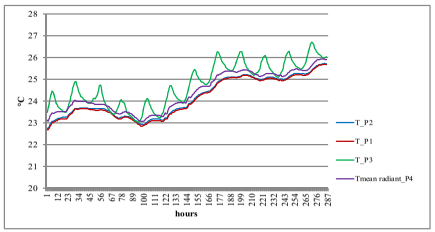
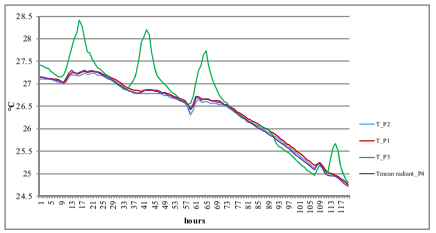
Table 2 Fungal genera isolated from air and surfaces in the different periods of the year on monitored sampling points indicated by specific code

Fungal genera	7th February			29th May			19th September			18th December		
	Air	Surface		Air	Surface		Air	Surface		Air	Surface	
		Shelf	Book		Shelf	Book		Shelf	Book		Shelf	Book
Alternaria spp.	4*	1b*m*, 3 *m*		1,2,3,4,5,6	5b*m*,6 *m*		1,3,4,4*	1a*h*,1a*m*,3 *m*,6 *m*	6p,6c	4*	5b*m*,3 *m*, 6 *m*	
Arthrinium spp.	2,3,4,6	5a*m*		2,5	3 *m*,6 *m*		1,2,3,4,5	1a*h*,1a*m*,1b*h*,5a*h*				
Aspergillus spp.		1a*m*, 3 *m*	6c	6	1b*m*,5a*m*, 5b*m*,3 *m*	3p,3c	1,2,3,4, 5,6	1a*m*,5b*h*,3 *m*, 6 *m*		1, 4*,6	5a*h*,5b*m*, 3 *m*	
Botrytis spp.				2,3								
Cladosporium spp.	3,4*	1b*h*	6c	1,2,3,4,5,6	1a*h*,5a*h*, 5a*m*, 5b*h*,5b*m*		2,3,4,4*	1a*h*,1a*m*,1b*h*,5a*h*,5b*h*,3 *m*, 6 *m*	3p,3c,6c	3,4, 4*,5	1b*h*,3 *m*, 6 *m*	
Epicoccum spp.	4*											
Eurotium spp.				6								
Helminthosporium spp.	4*											
Leptospheria spp.							4*					
Penicillium spp.	1,2,5	5a*m*,5b*m*		1,3,4,6	1b*h*,1b*m*		1,4,5,6	6 *m*		2	5a*m*,5b*m*, 3 *m*,6 *m*	
Pythomices spp.	4*						4*					
Polythrincium spp.	4*											
Tetraploa spp.							4*			4*		
Torula spp.	4*											

Air: 1,2,3,4,5,6 air sampling points (IMA, CFU/m^3); 4* Hirst sampling

Surface—shelves (Microbial Build-up, *m*; Hourly Microbial Fallout, *h*): 1a*m*, 1b*m*, 1a*h*, 1b*h*: Incunabula sector; 5a*m*, 5b*m*, 5a*h*, 5b*h*: Jewish sector; 3 *m*: Ms. Pal. 5 shelf; 6 *m*: Ms. Parm. 2810 shelf

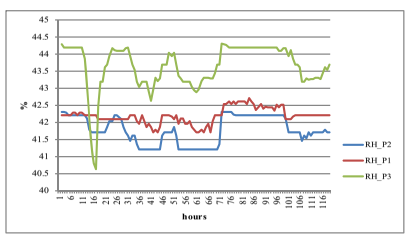
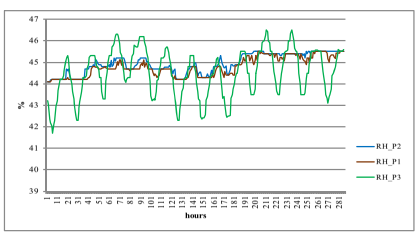
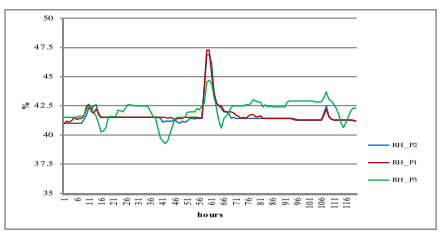
Surface—books (Microbial Build-up): 3c: Ms. Pal. 5 (cover, c); 3p: Ms. Pal. 5 (page, p); 6c: Ms. Parm. 2810; 6p: Ms. Parm. 2810

	27 January - 7 February	19 - 29 May	5 - 19 September
Temperature (°C)			

	T_P2 (°C)	T_P1 (°C)	T_P3 (°C)	$T_{mean\ radiant}$_P4 (°C)
Mean	15.10	14.71	14.89	15.34
Max	15.59	15.19	15.89	16.03
Min	14.46	14.13	14.09	14.54
Stand dev	0.28	0.28	0.35	0.4

	T_P2 (°C)	T_P1 (°C)	T_P3 (°C)	$T_{mean\ radiant}$_P4 (°C)
Mean	24.13	24.08	24.70	24.36
Max	25.73	25.69	26.71	25.95
Min	22.74	22.69	23.05	23.07
Stand dev	1.28	1.28	1.36	1.27

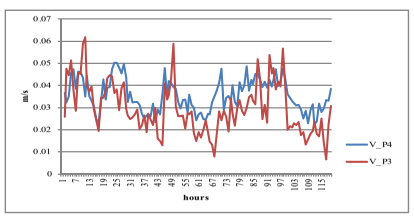
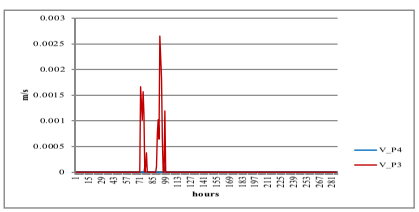
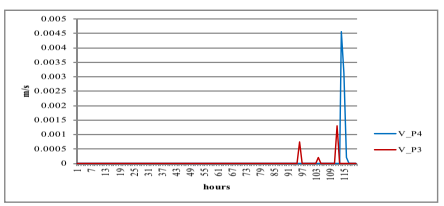
	T_P2 (°C)	T_P1 (°C)	T_P3 (°C)	$T_{mean\ radiant}$_P4 (°C)
Mean	26.37	26.42	26.60	26.38
Max	27.24	27.27	28.41	27.30
Min	24.77	24.76	24.80	24.71
Stand dev	0.73	0.74	0.81	0.76

Relative humidity (%)			

	RH_P2 (%)	RH_P1 (%)	RH_P3 (%)
Mean	41.77	42.19	43.64
Max	42.3	42.7	44.3
Min	41.2	41.7	40.63
Stand dev	0.41	0.23	0.66

	RH_P2 (%)	RH_P1 (%)	RH_P3 (%)
Mean	44.83	44.69	44.41
Max	45.50	45.50	46.50
Min	44.10	44.10	41.70
Stand dev	1.81	1.80	1.44

	RH_P2 (%)	RH_P1 (%)	RH_P3 (%)
Mean	41.62	41.69	42.10
Max	46.90	47.28	44.68
Min	41.00	41.00	39.25
Stand dev	0.91	0.89	0.83

Air velocity (m/s)			

	V_P4 (m/s)	V_P3 (m/s)
Mean	0.04	0.03
Max	0.05	0.06
Min	0.02	0.01
Stand dev	0.01	0.012

	V_P4 (m/s)	V_P3 (m/s)
Mean	0.00000072	0.00005142
Max	0.000103333	0.002651667
Min	0	0
Stand dev	0.013	0.003

	V_P4 (m/s)	V_P3 (m/s)
Mean	0.00006572	0.00001876
Max	0.00455	0.001293333
Min	0	0
Stand dev	0.005	0.0014

◀**Fig. 1** Microclimatic parameter values recorded at sampling points (P1, P2, P3, P4) (the numbers are the same as the ones reported in 2.2.2 Sampling points paragraph)

biological and microclimatic monitoring, including the physical and microbiological evaluation of the artwork, should be implemented, evaluating also the usefulness of a HVAC system, without, however, striving for the compliance with a theoretical microclimate, but always keeping in mind the integrity of the books. Considering that reference methods for air and surface microbial sampling are not available and threshold values have not been defined, this case study represents a contribution for defining standardized methods and better understanding microbiological contamination of cultural heritage environment, toward the improvement of artwork conservation strategy and the safeguard of human health.

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