

Aerobiological monitoring of the “Sistine Chapel”: airborne bacteria and microfungi trends

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Abstract

The present paper reports the results of a bacteriological and mycological monitoring carried out on the airborne microflora of the Sistine Chapel. The general aim of the study was to evaluate the impact of the flow of visitors, as well as seasonal effects, on the qualitative and quantitative variations of microorganisms. Two sampling campaigns were carried out in May and November 1997. A Surface Air System (SAS) sampler (active system) and a sedimentation based sampler (passive system), supported by an original plinth, were used. Temperature, humidity and carbon dioxide were detected. VITEK SYSTEMS jr. for *Staphylococcus* spp. and microscopic observation for microfungi were the identification methods. In spite of the conditioning and filtration air system, initial results with both samplers, show a positive correlation between the airborne microorganisms and presence and number of visitors. The SAS samples showed higher microbial load, for both bacteria and fungi, than the passive ones, but the epidemiological meaning of the differently collected data varies. The increase during visiting hours of human *Staphylococcus* spp. is stronger than the airborne bacterial load increase. The microfungi most frequently isolated were *Cladosporium* spp. and *Penicillium* spp. These preliminary data underline the significance of the survey for the protection of such a precious environment, and encourage the Authors to continue the ongoing monitoring.

Introduction

The Sistine Chapel is undoubtedly one of the world's most famous halls on account of the frescoes that adorn it, especially the timeless masterpieces of Michelangelo. The Chapel measures 40.5 × 13.2 m at the base and rises to a height of 20.7 m. The passing centuries have seen numerous restoration interventions, many of them deleterious, with consequent stratification of materials, some of an organic nature, that have often ruined the frescoes and rendered them unintelligible. For this reason a restoration intervention of the Vaults, the Lunettes and the Universal Judgment was commenced in 1980. The work was finally completed in 1994.

The restoration work was accompanied by microbiological and mycological studies of the frescoed walls that aimed at the definition of non-destructive sampling procedures and techniques, verification of the fluctuations of microbial and fungal concentrations during the course of the restoration work and the drawing up of restoration methodologies that would reduce the risk of pollution of exogenous origin to more or less minimal proportions. In the two-year period 1988–1989 microbiological samples were carried out on the frescoed surfaces of the vaults both before and after the restoration (Montacutelli et al., 1990), and during the following five years – 1990 to 1994 – the sampling was extended to the surface of Michelangelo's “Universal Judgment”, again both before and after the restoration work (Montacutelli

et al., 1997). On completion of the restoration work the Chapel was equipped with an air conditioning system that makes it possible to maintain the relative humidity at values between 55 and 60 percent and to limit the fluctuations of air temperature to values ranging between 22 and 24°. The equipment included an air change and filtration system that ensures daily air renewal inside the Chapel.

With a view to checking the efficacy of this system, microbiological air sampling campaigns were undertaken in the Sistine Chapel in various seasons of the year. The general aim of the study was to evaluate the qualitative and quantitative variation of the microorganisms before, during and after the presence of visitors; a second aim was to evaluate the seasonal influence on these parameters.

Materials and methods

Three sampling campaigns were carried out in the Chapel on the following dates: 21 May 1997, 13 November 1997 and 27 February 1998.

During each sampling operation the following parameters were recorded: temperature, relative humidity (%) and dew point; all measured with the IGRIST psychrometer (Model IGN/B). The air speed was recorded with the Terman anemometer without flow direction connector. The carbon dioxide concentration in the air was measured with the BABUK/M multisampler and the BSO 103 probe. The actual air samplings were carried out with Surface Air System (SAS) samplers using "surface plate", Model MTM-3 Plus (Multitime Monitoring System-3 Plus), which have a capacity of 180 litres of air per minute (active system) and make it possible to take three samples at once; samples were taken at intervals of 15 minutes over a period of one hour, i.e. a total of five samples for each culture media. The results were expressed as CFU/m³ of air intake. Gravimetric samplers based on an original support were also used (Figure 1). The idea to develop this new support was linked to this particular type of investigation which seeks to understand not only how many and which type of microorganisms are deposited by gravity before, during and after the arrival of visitors, but also how many actually deposit on the walls and ceiling vaults of the Sistine Chapel. While passive methods most commonly used today allow collection only of microorganisms deposited by gravity, this sampler makes it possible to collect even those particles moving about according

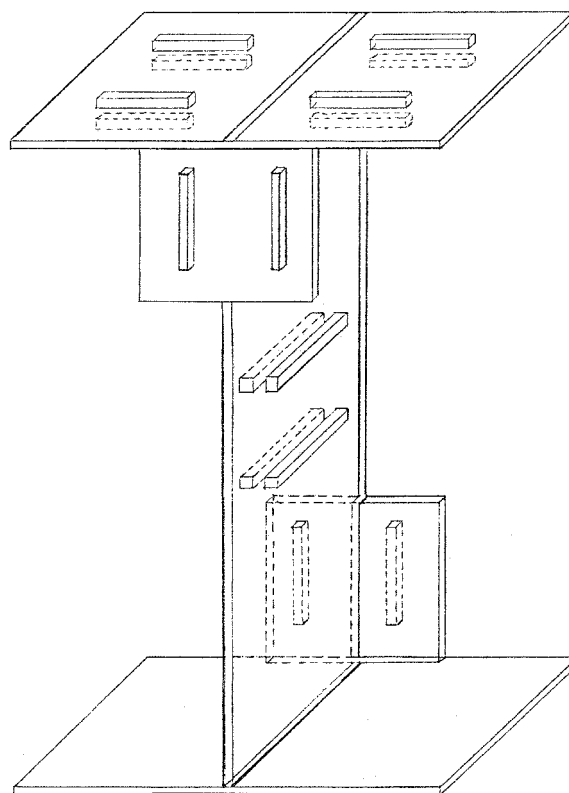


Figure 1. Gravimetric sampler based on original support

to air currents. The sampler is built to allow placement of "surfair plate" which contain the selected culture media, in a manner to cover all directions in the form of a sphere. Each sampler contains eight plates and the use of three samplers is necessary to allow each of the three selected cultura media to face all directions.

Two sampling locations were used for gravimetric sampling: one close to the active sampling apparatus, at a height of 3.5 m above ground, with three samplers, and the other above the altar, at a height of about 1.4 m, i.e. immediately below the wall of Universal Judgment, once again with three samplers. The data obtained were converted as numbers of microorganisms deposited on 1 m² of surface after one hour of exposure. Also this systems supported "Surfair plates" (International PBI) that have a surface area of 24 cm².

Tryptone Soy Agar (total bacterial count), Mannitol Salt Agar (*Staphylococcus* count) and Czapek Agar (total microfungi count) were the media used for microorganisms detection. The identification methods employed were VITEK SYSTEMS jr. (Bio



Figure 2. Gravimetric samplers close to the active sampling in Sistine Chapel

Mérieux) for *Staphylococcus* spp. and microscopic observation for microfungi.

Results

As far as the microclimatic parameters are concerned (Table 1), in each of the periods examined, even at the times when the flow of visitors was at its height, the values remained within acceptable limits for both the conservation of the artwork and the comfort and well being of the visiting public. The same applies to the recorded CO₂ values, since it is well known that this value must be kept below 1 ppm if the air is not to be perceived as vitiated (Bellante De Martiis et al., 1993). Table 2 shows the data (expressed as CFU) obtained by means of the two sampling methods in the three sampling periods relating to the total microbial load and, separately, to *Staphylococcus* and microfungi. The SAS samples showed a lower microbial load (expressed per m³) than the passive samples (expressed per m²), both for bacteria and for fungi. In the case of both samplers the results brought out a positive correlation between the airborne microorganisms and the number of visitors. These data demon-

strate that the capture of particles obtained with the new type of support, developed specifically for this work, was very effective. We therefore believe that an investigation of particles deposited on all surfaces, not only horizontal ones, and which seeks to identify which particles are present and moving about in the air at a particular time, can use both sampling methods together for better understanding of the phenomena.

The air changes were nevertheless found to be efficacious: indeed, the microbial loads were found to return to the initial values in the samples taken two hours after the departure of the visitors, with the sole exception of the sample taken on May 21 (and then only as regards the total microbial load). It should also be noted that the number of isolates obtained by means of both methods was smaller in the winter sampling. This result confirms those obtained by other authors (De-Wei Li and Kendrick 1995; Ebner et al. 1989; Lacey 1981; Rosas et al. 1992; Simeray et al. 1993) indicating a consistently lower microbial loads in winter months, for both bacteria and fungi. Therefore airborne spores from the public visitors to this type of closed, air-conditioned and air-filtered environment are generally less present than in uncontrolled spaces. The epidemiological meaning

Table 1. Microclimatic condition and CO₂ measuring, collected at three sampling times

PARAMETERS	21/5/97			13/11/97			27/2/98		
	8	11	16	8	11	16	8	11	16
Air temperature	20.5	21.6	19.6	21.5	21.0	20.0	19.6	20.2	18.2
Relative humidity%	67.5	58.7	60.1	59.8	65.5	66.0	64.8	58.8	66.5
Dew point	15.0	13.5	11.9	13.8	15.1	13.8	13.3	12.5	12.0
Air speed	0.01–0.05	0.1–0.3	0.01–0.1	0–0.01	0.04–0.3	0.05–0.4	0.01	0.05–0.1	0.05
CO ₂ %	0.02	0.08	0.04	0.05	0.09	0.05	0.015	0.05	0.02

Table 2. Total CFU referred to total microbial, *Staphylococcus* and microfungi load, obtained with two methods (Passive and Surface Air System) the three sampling times (A: before, B: during, C: after visitors presence).

	21/05/97			13/11/97			27/02/98		
	A	B	C	A	B	C	A	B	C
Total Bacterial Load									
TOTAL CFU/m ² /h (Passive)	9688	4618	3681	1198	3542	1198	651	5156	807
TOTAL CFU/m ³ (SAS)	617	605	283	161	789	105	22	450	100
<i>Staphylococcus</i> spp									
TOTAL CFU/m ² /h (Passive)	1076	2778	417	130	2474	182	182	2292	260
TOTAL CFU/m ³ (SAS)	22	411	39	3	233	16	3	233	15
Microfungi									
TOTAL CFU/m ² /h (Passive)	486	1146	521	547	469	234	52	417	52
TOTAL CFU/m ³ (SAS)	50	178	84	39	144	24	11	56	11

of the differently collected data varies: in fact, the increase in the human *Staphylococcus* isolates during visiting hours is more marked than the increase in the total airborne bacterial load (Tables 2, 3). The most frequently detected genera of microfungi were *Cladosporium* and *Penicillium* (Table 4).

Conclusions

The results of the analyses highlight the efficiency of the air conditioning system, but bring out the need for exercising greater control over the flow of visitors, who are the principal vehicle for the introduction of microbial and fungal spores. The passive detection method devised for these analyses proved to be efficacious and enabled the isolation of microorganisms present not only due to the effects of gravity, but also those carried by turbulent air movements.

The data obtained suggest to develop a system like a passageway for entrance of the public with a counter air flow to “wash” visitors. Monitoring the effect of the special passageway would allow a full evaluation of the level of controls in this precious environment.

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Table 3. *Staphylococcus* species% detected with two methods [Passive and Surface Air System (SAS)] the three sampling times (A: before, B: during, C: after visitor presence).

SPECIES	21/05/97						13/11/97						27/02/98					
	PASSIVE			SAS			PASSIVE			SAS			PASSIVE			SAS		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
<i>Micrococcus kristinae</i>																		
<i>Micrococcus</i> spp.			50.0		13.4	5.1			6.3	14.2					20.0		15.5	
<i>Staphylococcus aureus</i>	25.8	33.8			12.2	10.2		6.3						6.8			5.1	
<i>S. capitis</i>			18.2								6.9						9.9	
<i>S. caprae</i>						7.7											5.1	
<i>S. chromogenes</i>								6.3		33.3	6.4					33.3		
<i>S. cohnii</i>					11.6	10.2											5.2	
<i>S. epidermidis</i>	48.4	50.0		50.0	26.8	15.4	60.0	20.0	28.6	66.7	20.2		57.1	35.2	30.0		9.9	53.3
<i>S. haemolyticus</i>		16.2	50.0			18.0					19.3	43.8	42.9	14.8			9.9	
<i>S. hominis</i>				9.1	12.9	23.1					6.4	31.2		14.8	30.0		4.3	46.7
<i>S. lugdunensis</i>	25.8				10.5			6.3	14.3		6.9							
<i>S. saprophyticus</i>				22.7	12.6	10.2		13.7			20.2			6.8		66.7	5.1	
<i>S. sciuri</i>							40.0	20.0										
<i>S. simulans</i>														6.8			5.1	
<i>S. warnerii</i>								7.4	28.6		6.8				20.0		9.9	
<i>S. xylosus</i>								13.7	14.3		6.9	12.5		14.8			5.1	

Table 4. CFU of microfungi isolate with two methods (Passive and Surface Air System) at three sampling times (A: before, B: during, C: after visitor presence)

SPECIES	21/05/97						13/11/97						27/02/98					
	A		B		C		A		B		C		A		B		C	
	PASS.	SAS	PASS.	SAS	PASS.	SAS	PASS.	SAS	PASS.	SAS	PASS.	SAS	PASS.	SAS	PASS.	SAS	PASS.	SAS
<i>Acremonium strictum</i>						5												
<i>Acremonium</i> sp																		
<i>Alternaria alternata</i>	2	3																
<i>Alternaria</i> spp			2			4	2											3
<i>Ascomycetes</i>						2												
<i>Aspergillus versicolor</i>		1																
<i>Aspergillus</i> sp																		
<i>Botrytis cinerea</i>								3										
<i>Cladosporium cladosporioides</i>	2	21	10			53	1	23	2	6								
<i>Cladosporium cucumerinum</i>																		
<i>Cladosporium herbarum</i>	1	5	11			41	2	19	2	1	2							19
<i>Cladosporium macrocarpum</i>	1		3			37	1			1								
<i>Cladosporium sphaerospermum</i>	1	5			3	2	2	18		2								
<i>Epicoecum nigrum</i>						2												
<i>Epicoecum nigrum</i>			3															
<i>Hyphomycetes</i> spp						2												
<i>Hyphomycetes</i> spp							1			1								
<i>Mucorales</i>																		
<i>Paeclomyces fumosoroseus</i>																		
<i>Penicillium brevicompactum</i>						1												
<i>Penicillium canescens</i>	1							3										
<i>Penicillium citrinum</i>																		
<i>Penicillium expansum</i>		2																
<i>Penicillium glabrum</i>		3																
<i>Penicillium glabrum</i>		1																
<i>Penicillium griseofulvum</i>																		
<i>Penicillium italicum</i>						2												
<i>Penicillium janthinellum</i>						2		1										
<i>Penicillium mitzyskii</i>							1											
<i>Penicillium novae-zeelandiae</i>																		
<i>Penicillium olsonii</i>									2									
<i>Penicillium oxalicum</i>									1									
<i>Penicillium puberulum</i>										11								
<i>Penicillium purpogenum</i>										1								

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