MICROBIAL ENVIRONMENTAL MONITORING IN MU-SEUMS: PREVENTIVE CONSERVATION OF GRAPHIC COLLECTIONS

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1. Introduction

The air in indoor and outdoor environments contains a large number of suspended particles of various origin, size and shape which make up the atmospheric aerosol. A proportion of these particles is of biological origin and constitutes the *bioaerosol* or *biological aerosol*. Most of them are fungal and bacterial spores, *Bryophyta*, *Pteridophyta*, lichen propagules, algal cells, pollen grains, protozoan cysts and viruses. The *bioaerosol* is a potential biodeteriogen for graphic collections which include prints, drawings, watercolors, books, photographs, etc. preserved in museums, libraries and archives. Paper items of historical and artistic value are subject to aggression by specific bacterial and fungal microflora. If certain conditions are met, biological particles deposited on the surface of artworks can form colonies, resulting in damage to the artwork itself.

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It is, however, necessary for these microorganisms to find favorable nutritional and environmental conditions for their development.

Paper is a hygroscopic material, its biodegradability being conditioned both by organic substances and percentage of water content; biological attacks also depend on microclimatic conditions.

Environmental relative humidity (RH) determines different levels of water absorption for various types of artworks. For this reason, biological environmental monitoring combined with microclimatic monitoring is essential to assess contamination levels and is the first step in assessing biological risk for graphic collections. This article presents the partial application of a methodological model involving an *integrated system* of biological and microclimatic monitoring based on an interdisciplinary research project for storage and exhibition of artworks in confined spaces [1-2].

This model was tested in the National Institute of Graphic Arts (Istituto Nazionale per la Grafica - ING) in Rome, on a collection of ancient drawings belonging to the Fondo Corsini. The integrated system relies on: 1) biological aerosol monitoring with active and passive methods; 2) sampling fungal spores with a spore trap (Hirst type); 3) monitoring allergens with dust sampling; 4) monitoring airborne particles with a laser particle counter; 5) microbial sampling of surfaces (cultural objects and furnishing materials) with non-destructive and non-invasive techniques using nitrocellulose membrane filters; 6) microclimatic monitoring with multi data-logger for continuous measurements of air temperature, relative humidity, air velocity, radiant temperature; 7) Computational Fluid Dynamics (CFD) application for transient simulations for the study of air and surface temperature distribution, air velocity field, local perturbations, dust and particle re-suspension processes and pollutant diffusion that are closely connected to indoor air quality for heritage paper conservation. This research is a pilot study which focuses on the biological contamination of the graphic collections and is a contribution to standardizing a system of diagnosis-intervention for the preventive conservation of cultural heritage artworks of organic nature kept in indoor environments (i.e. museums, libraries and archives).

2. Museum environments

A multitude of artworks in museums are made of a complexity of materials that present different conservation problems related to microclimatic conditions. Inside these confined spaces, the microclimate is influenced by architectural characteristics, materials and construction techniques, and by the outdoor climate; at the same time, the level of biological contamination is influenced by various factors such as the air conditioning system, the type of artworks and furnishing materials, and also by the presence of people (staff and visitors).

Inside museums, air is the principal means of transportation for biological agents (*bioaerosol*) present in the environment [3] and people are one of the major sources of biological contamination. For organic cultural property preserved in indoor environments, microfungi and their spores play a crucial role. The transport and dispersal of fungal spores is a complex phenomenon: they remain suspended in the air until their falling speed, which is proportional to the square of their radius, becomes smaller

than the speed of the air movements that keep them suspended [4]. Dust is one of the principal carriers of microorganisms; if present on surfaces, it can absorb moisture creating a favorable environment for biodeteriogen growth. Furthermore, 82% of dust is made up of organic material and for this reason is a source of nutrition for a variety of microbial species. Microbial contamination of surfaces is closely connected to the concentration of aerobiological microorganisms; in fact, as is now well known, dust and aerobiological particles that settle on the surface of artworks are a hazard with a potential risk of causing damage to them [1].

2.1 National Institute of Graphic Arts

The National Institute of Graphic Arts (*Istituto Nazionale per la Grafica* - ING) is a museum of international importance designed to preserve, protect and promote a legacy of works that document all the different sectors of the graphic arts. Its recent foundation, in 1975, is the result of merging the *Calcografia Nazionale* and the *Gabinetto Nazionale delle Stampe*. It is located in Palazzo Poli, which was bought in 1978 to unify this rich heritage, previously split between the historical repositories of the *Palazzo della Calcografia* by the Trevi Fountain and the *Villa Farnesina alla Lungara*. The work of restoration and refurbishment, which did not get underway until 1986, reached completion only recently, resulting in the effective unification of the collections in the summer of 2008 [5]. The ING collections are very heterogeneous and include drawings, prints, matrices, art videos and photographs; these works cover a long period going from the 16th to the 19th centuries. The two separate sections, *Fondo Corsini* and *Fondo Nazionale*, today kept in two storage rooms (repository no.1 and no. 2) make up the *Gabinetto Disegni e Stampe*.

The most important one, known as the *Fondo Corsini* and owned by the *Accademia Nazionale dei Lincei*, comes from the Corsini family collection and was the initial nucleus of the *Gabinetto Nazionale delle Stampe*. It consists of 6,400 drawings in 52 volumes (Figure 1 a-b)



Figure 1. a) View of cabinet n. 6 with shelves and volumes; b) Example of volumes with drawings.

The earliest nucleus of drawings comes from the library of Cardinal Neri Maria Corsini, who also inherited the collections of his uncle, Pope Clement XII, on the latter's election to the papacy in 1730. The most interesting drawings are *Study of drapery in silverpoint on a red ground* by Leonardo Da Vinci, *Profilo di giovane* by Filippo Lippi and *Nudo femminile* by Parmigianino. After 1895 scholars, such as Adolfo Venturi, Paul Kristeller and Federico Hermanin, decided to remove the drawings from the volumes and re-organize the collection. They were restored, arranged in *passe-partout* and placed in boxes using a classification based on authors or schools. This operation was criticized and today there is a project for the reconstruction of the bound volumes of the *Fondo Corsini* to produce a prototype accessible on the internet [13].

2.2 Drawings

Drawings on paper had various aims: originally they were designs for artistic projects used in architecture, painting or sculpture. Over the centuries, different techniques were used, together with various instruments (pencil, pen, crayon, graphite) on a wide variety of supports (paper, metal, wood, etc.) [6]. Drawing later came to be considered an art in itself and today there are a great many drawings recognized as graphic artworks.

The drawings examined in this article are on paper. Paper consists of cellulose (45-55%), lignin (20-30%) and hemicellulose (15-25%). Cellulose is the main component consisting of carbon with hydrogen and oxygen; its molecule is very large and is made up of simpler molecules which are repeated (interconnecting monomers). Lignin has a complex structure and is made up of aromatic polymers of phenylpropanoid units. Hemicelluloses are polysaccharides present in the cell walls of plants associated with cellulose and lignin [7].

Paper is a highly hygroscopic material, i.e. it absorbs and releases water and is strongly affected by changes in humidity and temperature; it is thus vulnerable to attacks by various biological agents. Cellulose, the principal constituent of this material, is an important source of carbon, essential for the nourishment of potential biodeteriogens, as well as other organic substances like adhesives, glues and inks that can all be a source of biodeterioration [8].

3. Materials and methods

3.1 Description of site: repository no.1 of the ING

Biological and microclimate monitoring was performed in repository no.1 (Figure 2), situated on the second floor of the ING. The repository has two windows and two doors which are always closed because it is used as a storage room and only staff are allowed entry; there is also an air conditioning system (during monitoring, in August 2011, it was switched off) and a thermohygrometric monitoring system that uses wireless technology transmitting environmental parameters to an operations center via radio. Repository no.1 contains graphic collections (drawings and prints) belonging to two different collections: *Fondo Corsini* and *Fondo Nazionale*; the artworks are kept in closed metal cabinets, made up of sliding shelves with holes for air circulation.

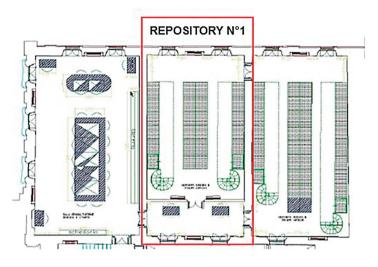


Figure 2. Map of repository no.1.

3.2 Microclimatic monitoring

The microclimatic monitoring system allows continuous monitoring of temperature (T) and relative humidity (RH) values using 8 sensors positioned inside repository no.1, inside the cabinets, the volumes, the boxes and on the surfaces of the works and one sensor placed outdoors (Figure 3 a-c).



Figure 3. Position of sensors a) inside cabinet b) inside repository c) outdoors

Thermohygrometric sensors record data hourly; all recorded measurements are then stored in a computer.

3.3 Microbial air sampling

Microbial air sampling was carried out by both active sampling, in order to measure the concentration of microorganisms in the air, and passive sampling, in order to measure the rate at which viable particles settle on surfaces [9]. The sampling was performed on 8^{th} and 9^{th} August 2011.

Active sampling was carried out using a Duo-SAS 360 impactor with a flow rate of

180 liters per minute (L/min). The sampler was placed in the monitored room at 1 meter above the floor and at about 1 meter away from any physical obstacle; a volume of 200 L of air was aspirated. RODAC plates containing SDA (*Sabouraud Dextrose Agar*) with chloramphenicol were incubated at 25°C for 5 days to isolate the microfungi. The results were expressed as colony forming units per cubic meter of air (CFU/m3) [10]. The samples were taken at 5 different points inside the repository (Figure 4) and at 1 point outside the window; each sampling was performed 3 times.



Figure 4. Air sampling points

Active air sampling was performed also using a spore trap (Hirst type), with a flow rate of 10 liters per minute (L/min) to assess the concentration of fungal spores and pollens in a time span of 24 hours following standards given by the Italian Association of Aerobiology [11]. The sampler was located in the middle of the storage room.

Passive sampling was carried out using Petri dishes with a diameter of 9 cm containing SDA with chloramphenicol, incubated at 25°C for 5 days, to determine the Index of Microbial Air contamination (IMA). Petri dishes were left open for 1 hour, at 1 meter above the floor and at a distance of about 1 meter from walls and obstacles. The results were expressed as colony forming units per square decimeter per hour (CFU/dm^{2/h}). The sampling points were the same as those used for the Duo-SAS 360 (Figure 4).

3.4 Microbial sampling of surfaces

Microbial sampling was performed on the surface of the artworks (drawings), volumes and on the furnishing materials (shelves) and was carried out in a non-destructive way using nitrocellulose membrane filters with a diameter of 47 mm and an area of 17.34 cm2 [10]. To measure the Microbial Buildup (MB), that represents the total number of microorganisms accumulated on a surface in an unknown period of time prior to the sampling, the membrane was pressed onto the surface for 30 seconds by the operator's fingertips protected by sterile gloves (Figure 5a). To measure the Hourly Microbial Fallout (HMF), which represents the number of microorganisms that settle on a surface during this time, the membrane was left on the surface for 1 hour (Figure 5b). Using sterile tweezers the membrane was then transferred onto the Petri dishes containing SDA with chloramphenicol.



Figure 5. Surface sampling a) Microbial Buildup (MB) b) Hourly Microbial Fallout (HMF)

4. Results and discussion

The presence of the thermohygrometric monitoring system inside the ING allowed continuous measurement of the temperature and relative humidity values during the year. The results recorded in August 2011 are shown in Figure 6.

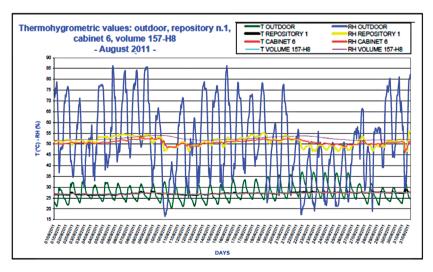


Figure 6. Thermohygrometric values for repository no.1, cabinet no.6, volume 157-H8, outdoors, in August 2011

Temperature and relative humidity values inside repository no.1 were lower than outdoors. This is because the ING is a historic monumental building with thick masonry walls which act as insulators.

Air temperature data registered inside repository no.1 during the period of the sampling ranged between 26°C and 28°C; the situation remained stable during the whole month without excessive daily fluctuations. Relative humidity in the same period was between 46% and 54%. Results registered by the sensors inside the cabinet and on volumes and drawings were also constant, showing thermohygrometric balance (Figure 7).

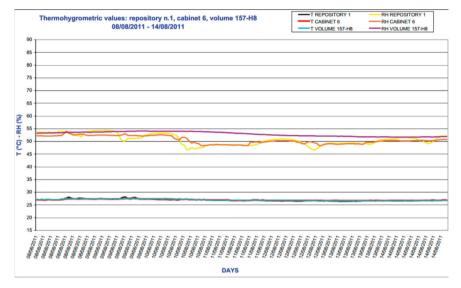


Figure 7. Thermohygrometric values in repository no.1, cabinet no.6, volume 157-H8 in the week of sampling

The values of relative humidity fell within the range recommended by the standards for the preservation of paper (*Atto di indirizzo sui criteri tecnico-scientifici e sugli standard di funzionamento e sviluppo dei musei* by MiBACT) [12]. The air temperature data were outside recommended standards, but given that their values, though high, remained constant, they cannot be considered dangerous.

Table 1 shows the microbial air contamination values in different sampling points on two monitored days and for three samplings.

Table 1. Microbial air contamination values in repository no.1 on two monitored days (8th and 9th
August 2011). The sampling points are indicated in Figure 4. Columns A-B show active method
(Duo-SAS 360); C-D passive method (Petri dishes, IMA)

	Fondo Corsini 08/08/2011 h 13	Fondo Corsini 09/08/2011 h 13	Fondo Corsini 08/08/2011 h 13	Fondo Corsini 09/08/2011 h 13			
	CFU\m ³	CFU\ m ³	CFU\dm²\h	CFU\dm ² \h			
	А	В	С	D			
Point 1							
1	380	350	2	11			
2	255	285	8	6			
3	480	520	4	9			
Average	371.7	385	6	8.7			
Point 2							
1	495	145	6	3			
2	460	185	9	13			
3	475	115	9	14			
Average	476.7	148.3	8	10			
Point 3							
1	440	170	6	7			
2	560	130	5	9			
3	480	190	6	7			
Average	493.3	163.3	5.7	7.7			
Point 4							
1	585	605	19	10			
2	1185	630	15	18			
3	١	440	14	15			
Average	885	558.3	16	14.3			
Point 5							
1	550	1	92	117			
2	640	2125	71	148			
3	835	1	73	patina			
Average	675	2125	78.67	132.5			

The number of fungal spores inside the repository was lower than that outside the room using both the active and passive methods. This result can be explained by the fact that the monitored ambient is a storage room closed to the public.

Table 2 shows the fungal species isolated by passive sampling. A prevalence of *Cla-dosporium cladosporioides* (Fresen.) G.A. de Vries, *Cladosporium cucumerinum* Ellis & Arthur, *Cladosporium herbarum* (Pers.) Link and *Penicillium* spp. was found. Qualitative analysis of the samples taken with the Duo-SAS 360 was carried out on only one sampling and revealed the presence of *Alternaria alternata* (Fr.) Keissl, *Cladosporium cladosporium cladosporium herbarum*.

FUNGAL SPECIES	1C1	1C2	1C3	1D1	1D3	2D2	2D3	3D1	3D2	3D3
Alternaria alternata						x				
Alternaria spp.						x	x			
Cladosporium cladosporioides	x	x			x	x			x	x
Cladosporium cucumerinum		x	х	x	x	x	x		x	x
Cladosporium herbarum	x	x		x		x	x		x	x
Epicoccum nigrum						x				
Penicillium spp.			x	x	x	x				

Table 2. Fungal species isolated by passive method (IMA). Sampling points refer to those in Table 1

An overview of data reported in the literature shows that the genera *Cladosporium* and *Alternaria* can be potential biodeteriogens for cultural heritage on paper but, at the same time, these genera are common and typical of atmospheric air and can be found in all indoor environments.

Table 3 shows microbial surface contamination values of shelves, volumes and drawings.

Table 3. Microbial surface contamination values (MB and HMF). Samples 1-8 were taken o	n 8th
August 2011; samples 1A-6A on 9th August 2011.	

	SAMPLING POINTS	FUNGAL COLONIES
	1. HIGH SHELF	3
	2. LOW SHELF	25
МВ	3. VOLUME 157 H8: COVER	3
	4. DRAWING FC 127362: CLEAN AREA	1
	5. DRAWING FC 127362: FOXING AREA	1
	6. HIGH SHELF	6
HMF	7. LOW SHELF	2
	8. VOLUME 158: COVER	1
	1A. VOLUME 158 I1: COVER	11
мв	2A. DRAWING FC 129666: CLEAN AREA	0
	3A. DRAWING FC 129666: INSECT DAMAGE AREA	0
	4A. VOLUME 158 I3: COVER	4
HMF	5A. DRAWING FC 130069: FOXING AREA	0
	6A. DRAWING FC 130068 BIS: FOXING AREA	0

Fungal colonies are mainly present on shelves and volumes that are positioned at lower levels. This effect is due to the air flow that allows heavier particles to fall as a result of gravity. Moreover, CFUs, estimated by MB and HMF on the drawings, are lower than on the shelves. This result proves that the use of closed metal cabinets and boxes is optimal for the preservation of these artworks because the drawings are safe from microbial environmental pollution.

Qualitative analysis on the surfaces (Table 4) indicates the presence of *Cladosporium cladosporioides, Cladosporium cucumerinum* and *Cladosporium sphaerospermum* Penz. and also *Alternaria alternata, Aspergillus niger* Tiegh, *Humicola spp.*, and *Penicillium* spp. These fungal species are common in museum environments and could be biodeteriogens for paper, if optimal conditions for their growth exist.

FUNGAL SPECIES		МВ					HMF				
		2	3	5	1A	4	6	7	8	4A	
Acremonium terricola			x								
Alternaria alternata		x								х	
Arthrinium phaeospermum		x									
Aspergillus niger					x						
Cladosporium cladosporioides	x	x	x	x	x		x	x		x	
Cladosporium cucumerinum	x			x							
Cladosporium herbarum	x	x									
Cladosporium sphaerospermum	x					x	x		x		
Humicola spp.					x						
Penicillium spp.	x				x						
Phoma spp.											
Micelia sterilia hyalina		x				x				x	

Table 4. Fungal species isolated by surface sampling: **Microbial Buildup** (MB), **Hourly Microbial Fallout** (HMF). The sampling points refer to those reported in Table 3

Figure 8 shows results obtained by spore trap that confirm both the quantitative and qualitative data of the air and surface sampling.

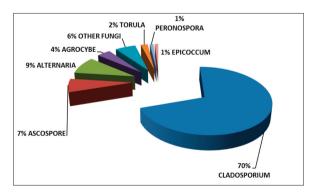


Figure 8. Fungal spores isolated by spore trap (Hirst type)

5. Conclusions

The study conducted at the ING, which allowed assessment of the quantity and quality of fungal spores in the air and on the surfaces of the drawings, showed that the monitored environment does not entail risks for the graphic collection. The risk of biodegradation, as is well known, depends on microclimatic conditions. In particular, the presence of water is crucial because it is essential for the metabolic processes of biological agents. This highlights the importance of an integrated approach to biological environmental monitoring for the cultural heritage and this research is a pilot study which has focused on the biological contamination of graphic collections. Further studies based on experimental campaigns will provide information on biological contamination and thermohygrometric values to create predictive risk models for both artworks and people (staff and visitors) which can help in standardizing a system of diagnosis-intervention for preventive conservation of the organic cultural heritage in indoor environments.

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