



Original article

Fungal monitoring of the indoor air of the Museo de La Plata Herbarium, Argentina



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ABSTRACT

Background: Biological agents, such as fungal spores in the air in places where scientific collections are stored, can attack and deteriorate them.

Aims: The aim of this study was to gather information on the indoor air quality of the Herbarium of Vascular Plants of the *Museo de Ciencias Naturales de La Plata*, Argentina, in relation to fungal propagules and inert particles.

Methods: This study was made using a volumetric system and two complementary sampling methods: (1) a non-viable method for direct evaluation, and (2) a viable method by culture for viable fungal propagules.

Results: The non-viable method led to ten spore morphotypes being found from related fungal sources. A total of 4401.88 spores/m³ and 32135.18 inert suspended particles/m³ were recorded. The viable method led to the finding of nine fungal taxa as viable spores that mostly belonged to anamorphic forms of Ascomycota, although the pigmented yeast *Rhodotorula* F.C. Harrison (Basidiomycota) was also found. A total count of 40,500 fungal CFU/m³ air was estimated for all the sites sampled.

Conclusions: Both the non-viable and viable sampling methods were necessary to monitor the bio-aerosol load in the La Plata Herbarium. The indoor air of this institution seems to be reasonably adequate for the conservation of vascular plants due to the low indoor/outdoor index, low concentrations of air spores, and/or lack of indicators of moisture problems.

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Muestreo de los hongos del aire en el interior del Herbario del Museo de La Plata, Argentina

RESUMEN

Antecedentes: Los agentes biológicos, tales como las esporas fúngicas suspendidas en el aire, en sitios donde se conservan colecciones científicas, pueden dar lugar al ataque y deterioro de las mismas por los hongos.

Objetivos: El objetivo de este trabajo fue proporcionar información acerca de la calidad del aire interior en el Herbario de Plantas Vasculares del Museo de Ciencias Naturales de La Plata, Argentina, en relación con el contenido de propágulos fúngicos y partículas inertes, mediante el uso de dos técnicas complementarias.

Métodos: El estudio se llevó a cabo con un sistema volumétrico y dos metodologías de muestreo: 1) método no viable de evaluación directa; y 2) método viable para el cultivo de propágulos fúngicos viables.

Resultados: A partir del sistema de recuperación directa se cuantificó un total de 4401,88 esporas/m³ con 10 morfotipos pertenecientes en su mayoría a anamorfos de Ascomycota. Asimismo se cuantificaron 32135,18 partículas inertes suspendidas por m³. Con el uso del sistema viable se estimó un total de 40.500 UFC/m³ aire para todos los sitios muestreados y se identificaron nueve taxa fúngicos

Palabras clave:

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que pertenecen también a formas anamórficas de Ascomycota, aunque se halló la levadura pigmentada *Rhodotorula* F.C. Harrison (Basidiomycota).

Conclusiones: Ambos métodos, viable y no viable de muestreo, son necesarios para el control de la carga de aerosoles en el Herbario de La Plata. El aire interior de esta institución parece razonablemente adecuado para la conservación de plantas vasculares, dado el bajo índice interior/exterior, bajas concentraciones de esporas o la ausencia de indicadores de problemas de humedad.

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Biodeteriogens like fungi, bacteria and Actinomycetes pose severe threat to biological collections, represent risk for human health, and are a cause of deterioration of different stored materials.^{5,14,38} Climatic and indoor environmental conditions such as temperature, humidity and air circulation influence the microbial prevalence.⁴¹ Microbes enter the indoor atmosphere through wind currents, and staff and visitors carry dust particles deposited by impaction. One of the main factors which influence indoor pollution levels is the outdoor air quality.^{25,29} Also, the indoor concentrations of fungal spores depend on both indoor and outdoor sources as well as on removal processes, such as air exchange and chemical reactions.^{33,36}

Microorganisms can compromise the structure and function of the materials involved in biological collections in different ways. Fungi are an important cause of chromatic and structural alterations due to mycelial growth and pigment production, degrading cellulose and producing acids like oxalic, fumaric, succinic and acetic acid, which alter the normal pH of the substrate.^{20,21}

Biological collections are of vital importance for cultural heritage worldwide since they constitute an invaluable source of scientific information. In this sense, numerous standardized protocols are applied at present to protect and maintain biological materials over time.⁴⁷ Biodeterioration and biodegradation processes are problems that curators have to deal with nowadays. The monitoring and control of these agents that may colonize and degrade materials are critical measures for the safe preservation of this cultural heritage. Several studies about the prevalence and viability of microbial propagules in the air have been carried out.^{12,16,51} These studies are vital to have a detailed overview of the environmental air quality inside biological collections¹⁶ due to their usefulness as indicators of the conditions of conservation. The handling of biodeteriorated specimens is another serious problem for people's health since these microorganisms might be also pathogenic, allergenic and/or toxinogenic.^{27,35,45,46}

The biological material stored in herbaria are mostly collections of dry botanic specimens, organized by a given system, classified by a phylogenetic and alphabetic order⁶ and preferably stored under controlled conditions for conservation. The specimens are mounted on cardboard and every material must be stored in cold (-20°C) quarantine before incorporation. Then, they are transferred to special cabinets for preservation. One of the problems of conservation of these collections is related to the substrate, comprised by both the plant material (each specimen) and the support (cardboard), because both are rich in organic components for the development of insects, fungi and bacteria, which are important biodeterioration agents.^{34,51} Another problem of these collections is the phytosanitary conditions of the plant material itself. So, safety practices as the control of environmental conditions, people movement, and monitoring of the collections are standard measures to be taken.

The Museo de Ciencias Naturales de La Plata, Argentina ($34^{\circ}55' \text{S}$, $57^{\circ}57' \text{W}$) (Fig. 1a and b), houses the Herbarium of Vascular Plants (LP), which is an important institution containing significant collections of Asteraceae, Poaceae, Fabaceae and Pteridophyta (ca. 500,000 specimens). This institution is one of the largest in Latin America, and includes type material (5000 specimens) collected

by famous botanists such as A. L. Cabrera and C. L. Spegazzini.^{19,26} Although the plant collection is properly preserved in special cabinets and with controlled handling of environmental conditions, there is no available information about the indoor air quality of this Herbarium.

Fungi have proved to be the most important biodeterioration agents, especially on supports made of cellulose,⁴⁴ which is the main component of these plant collections. On the other hand, dust serves as a source of nutrients to some insects and fungi, and creates a microenvironment on collection surfaces, facilitating the absorption of moisture and promoting the proliferation of pests.¹³ For this reason, the aim of this work was to characterize both the indoor air mycobiota and the content of inert particles in the LP Herbarium, by using viable and non-viable volumetric methodologies.

Material and methods

Study site

The LP Herbarium, with a total surface of 350m^2 and ca. 1400m^3 , has several workplaces (Fig. 1c), including areas for research and administration, and a main room where plant specimens are adequately preserved under standard protocols and controlled temperature and humidity conditions (Laura Iharlegui, personal communication). Taking into account the age of the building, the place where the collections are stored (sites I and II) is in good condition due to its regular maintenance. The working and circulation areas (sites IV and V) do not present visible deterioration, whereas the secondary entrance (III) is the most exposed site due to the staff movement and the direct connection with the exterior and the exterior corridor (VI). Six representative areas of the Herbarium were analyzed in this study: I – the main access to the Herbarium (180m^3), II – the central corridor (43m^3), III – the secondary entrance (27m^3), IV – the visiting room (41m^3), V – the lobby (60m^3), and VI – the exterior corridor (open space) (Fig. 1c).

Sampling and data analysis

The indoor air was sampled on December 21st, 2011, at 9:30 am. The collection room (sites I–II) has a central air conditioning system and a dehumidifier device to control temperature and humidity. These parameters were recorded using a HOBO U14 LCD data logger. At the moment of the sampling, the mean temperature and mean relative humidity (RH) inside the collection site were 21.6°C and 53.5% respectively, while those outside were 27.5°C and 55% respectively. The study was performed during a period of recess of activities and absence of staff and visitors to reduce the effect of turbulence in the air.

The indoor air sampling was done on a volumetric Hirst type system with a Zefon Z-Lite IAQ Air Sampling Pump[®] by using two procedures: a non-viable and a viable method. In the non-viable method the pump was connected to the Air-O-Cell[®] cassette for direct microscopic evaluation of bioaerosols, whereas in the viable method a holder with a cellulose filter was connected to the same device to obtain a sample to culture viable fungal propagules. Both

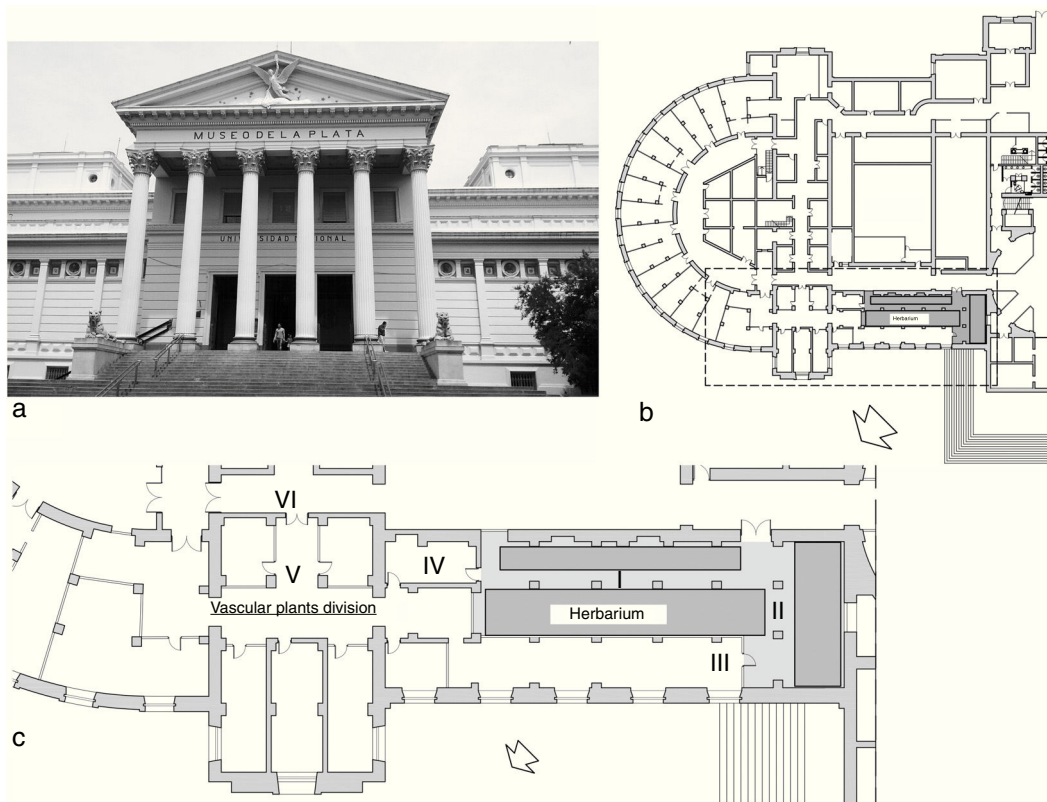


Fig. 1. Museo de La Plata and its Herbarium: Panoramic view (a), map of the left side of the Museum (b), diagrammatic scheme of the facilities of the Herbarium (c): Sampling sites: I – main access to the Herbarium, II – main corridor, III – secondary entrance, IV – visiting room, V – lobby, VI – exterior corridor. Maps: Arch. García Santa Cruz et al. Laboratorio de Arquitectura y Hábitat Sustentable, Universidad Nacional de La Plata.

sampling procedures were standardized at the same air flow and time of sampling for comparison of results. The sampling was carried out for 5 min at 1.5 m high in the center of the rooms.

Briefly, in the non-viable system the air stream is accelerated by the suction pump at a flow of 15 l/min and impacts on a glass slide that contains a sticky and optically clear sampling medium which collects and holds bioaerosols and inert particles. The glass cover slip containing the sample traces was removed from the Air-O-Cell[®] cassette and mounted on a slide, then stained with lactophenol cotton blue and covered with a slip. These samples were observed using an Olympus BH2 microscope at a magnification of 400 \times along seven transverse lines covering 22% of the total area of the preparation. Likewise, a magnification of 1000 \times was used in some cases to achieve an accurate identification. Different bioaerosols, including sexual and asexual fungal spores, were detected and identified with reference atlases of the Kingdom Fungi and related ones,^{3,18,24,28,39} as well as with a specialized electronic database.⁷ Aerosol counts were then converted into elements per m³ air following Baxter.⁴

Since environmental dust may be a source of nutrients and support for the development of fungal microorganisms, the inert particle content in the air was analyzed counting the particles by means of the non-viable system. The criteria of the American Conference of Governmental Industrial Hygienists on Total Suspended Particulates (TSP < 100 μ m) was applied for particles of inorganic origin like opaque particles and hyaline fibers and of human origin like skin cell fragments.²⁸ Particle counting and estimation of concentration were made by the same methodology as that used for non-viable bioaerosols.

In the case of culturable-air sampling, the cassette was replaced by a sterile holder with a GE Osmonics mixed-cellulose ester filter of 0.43 μ m pore and 25 mm diameter and the resulting system was applied to the pump for 5 min for each sample at a rate of

15 l/min. After sampling, in the laboratory, each filter was suspended in 50 ml of sterile water under aseptic conditions and the resulting suspension was vigorously shaken (15 min at 2000 rpm). To recover the greatest amount of fungal propagules adsorbed on the capture surface, the filter and aliquots (1 ml) of the suspension as well as its decimal dilutions (1:10; 1:100) were spread onto plates containing a 2% (w/v) Corn Meal Agar (CMA) medium amended with glucose (2 g l⁻¹), chloramphenicol (50 mg l⁻¹) and streptomycin (100 mg l⁻¹). This medium is broadly used for recovery of fungi from several types of samples such as soil, litter and air.^{8,40,42}

The plates were incubated at 25 °C and 63% HR in the dark until colony development. After one week, colonies which had developed on each plate were counted (Colony Forming Units, CFU), analyzed microscopically, and identified taxonomically, based on cultural and morphological features.¹¹ The CFU recovered from each sample were then converted into volumetric units calculated as: number of colonies \times volume dilution/volume of air sampled. Furthermore, some representative fungi were isolated in axenic culture. Stock cultures of these isolates were kept at 4 °C on 2% (w v⁻¹) malt extract-agar slants, and then deposited in the culture collection of the Instituto Spegazzini, Universidad Nacional de La Plata, La Plata, Argentina (LPSC).

On the data obtained from both sampling procedures, the specific prevalence⁴⁸ and species richness³² of each taxon were estimated. The indoor/outdoor index was also estimated.³¹

Results

Sample analysis using the non-viable method

Ten spore morphotypes from fungal and related sources, one fern spore type and one pollen grain type were found as biological

Table 1
Fungal bioaerosols identified and total concentrations estimated for the non-viable sampling in bioaerosols/m³ air, and for the viable sampling in CFU/m³ in the indoor air of the LP Herbarium.

Spore type (non-viable system)	SI	SII	SIII	SIV	SV	SVI	Total count
<i>Alternaria</i>						238	238
<i>Arthrrium</i>						59	59
<i>Aspergillus/Penicillium</i>			953			59	1013
Ascospores				119	59		178
<i>Chaetomium</i>						59	59
<i>Cladosporium</i>			178			1609	1788
<i>Dreschlera/Bipolaris</i>					178		178
<i>Epicoccum</i>						59	59
<i>Leptosphaeria</i> type			59		59	119	238
Myxomycota				59		298	357
Pollen					59		59
Fern spores					178		178
Total part/m ³ air			1192	178	536	2504	4411
Indoor/outdoor index			0.48	0.07	0.21		
Fungal taxa (viable system)	SI	SII	SIII	SIV	SV	SVI	Total count
<i>Acremonium</i>	500						500
<i>Alternaria alternata</i>		500	2000			500	3000
<i>Cladosporium cladosporioides</i>	1500			500	1500	500	4000
<i>Cladosporium herbarum</i>						500	500
White yeast			500			500	1000
<i>Penicillium frequentans</i>						500	500
<i>Penicillium rubrum</i>						500	500
<i>Penicillium thomii</i>	500			1500		500	2500
<i>Rhodotorula</i> sp.			27,500			500	28,000
Total part/m ³ air	2500	500	30,000	2000	1500	4000	40,500
Indoor/outdoor index	0.63	0.13	7.50	0.50	0.38		

Table 2
Type and concentrations of inert particles (particles/m³ air) estimated in the indoor air of the LP Herbarium.

Inert particles	SI	SII	SIII	SIV	SV	SVI	Total count
Skin cell fragments	1013	8048	5842		5723	775	21,403
Hyaline fibers	1311		4709	417	1192		7631
Opaque particles	59	1669		715	536	59	3040
Total	2384	9777	10,552	1132	7452	834	32,135

dispersal units in the samples analyzed (Table 1). Other dusty particles such as skin cell fragments, hyaline fibers, and opaque particles, were also identified (Table 2). Total aerosol count converted into elements per m³ air corresponding to all the sites sampled showed a total of 4401.88 biological spores and 32135.18 total suspended particles (TSP).

Regarding the spore concentration, no spores were found in the main access (I) or in the central corridor (II) of the rooms containing the collections. In contrast, the secondary entrance (III) showed a concentration of around 1200 units/m³, where *Aspergillus/Penicillium* and *Cladosporium* spore types were prevalent. In the visiting room (IV), the total concentration was of about 180 units/m³ and the ascospore type was the most important. The total spore concentration in the lobby (V) was 350 units/m³ and *Dreschlera/Bipolaris* was the most important type. Also fern spores and pollen grains were found. Finally, the highest concentration of spore types was found in the exterior corridor (VI), with around 2500 units/m³ (Fig. 2) and *Cladosporium* being the prevalent type (Table 1).

Regarding the results obtained in the sampling of inert particles (Table 2), skin cell fragments represented 67% of the total concentration, hyaline fibers 24% and opaque particles 9%, being the central corridor (II) and the secondary entrance (III) the most affected by TSP.

Since the magnitude of the outdoor bioaerosol concentration might also be a determining factor affecting the indoor levels, an indoor/outdoor index was estimated for all interior sites and for individual sporal types, using the data obtained in the exterior corridor (VI) as an outdoor reference. The indoor/outdoor index in the

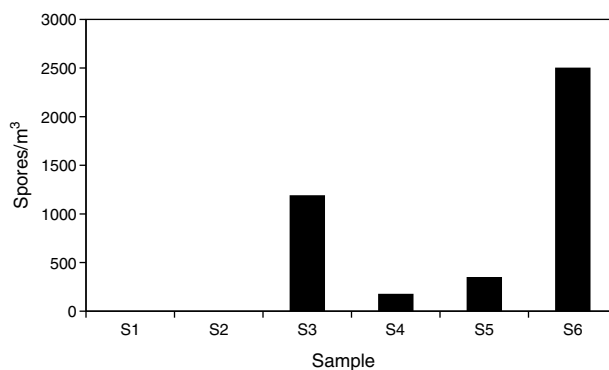


Fig. 2. Concentrations obtained by the non-viable method in the sampling sites (spores/m³ air).

collection room was 0% in the main access (I) and central corridor (II) and 46% in the secondary entrance (III). Values for the visiting room (IV) and the lobby (V) were of 7% and 21% respectively (Table 1).

When the ratio was calculated for individual taxa (Table 1), four sporal types were present only in the exterior corridor (VI), two solely in the interior air of the Herbarium and four taxa both inside and outside (Fig. 3).

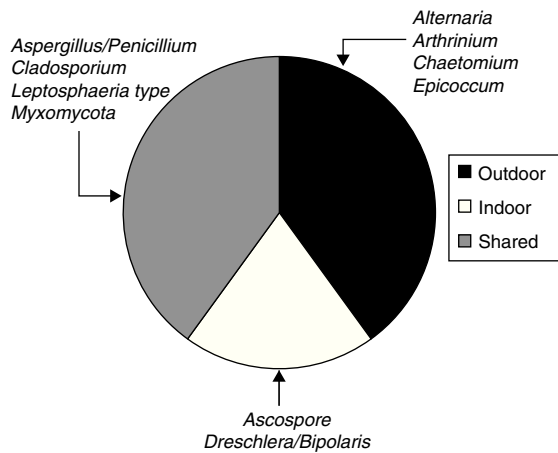


Fig. 3. Typical spores found by the non-viable method, inside the Herbarium, outside the Herbarium and in both sites.

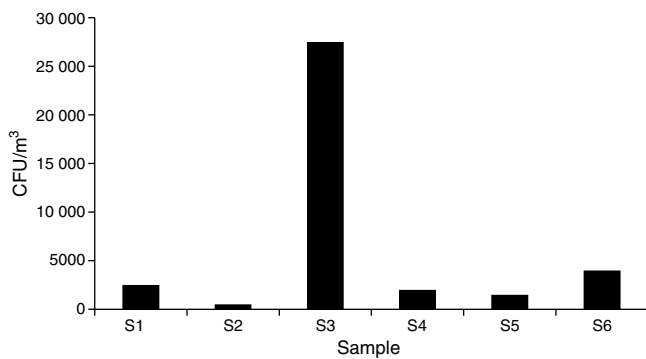


Fig. 4. Concentrations obtained by the viable method in the sampling sites (CFU/m³ air).

Sample analysis using the viable method

Nine fungal taxa were identified as viable spores when different air samples from the LP Herbarium were applied as inoculum source on an agar medium (Table 1). Although several decimal dilutions of up to 1:10³ were spread on agar plates, only informative data were obtained in the sampling of their own filter and the non-diluted suspension (≤ 40 CFU per plate).

The total estimated count of fungal CFU/m³ air showed 40500 elements for all the sites sampled.

Regarding the total concentration, 2500 CFU/m³ air were estimated for the main access (I), where *Cladosporium cladosporioides* (Fresen.) G.A de Vries, was the most important type. The central corridor (II) showed the lowest value, with 500 CFU/m³ and *Alternaria alternata* (Fr.) Keissl, being the main type. The secondary entrance (III) had the highest concentration, with 30,000 CFU/m³, mainly represented by *Rhodotorula* F.C. Harrison.

In the visiting room (IV) (2000 CFU/m³), *Penicillium thomii* Maire, was the most important taxon being close to *Penicillium frequentans* Westlingbut (Fig. 4).

When each spore type was considered, *Rhodotorula* sp. F.C. Harrison, *C. cladosporioides* (Fres.) de Vries and *Cladosporium herbarum* (Pers.) Link, were the most prevalent taxa. Similarly to the data obtained using the non-viable method, the exterior corridor (VI) showed the highest richness of airborne fungal propagules (Fig. 5).

When the ratio was calculated for individual taxa in the viable method (Table 1), three spore types were present in the exterior corridor (VI), one solely in the interior air of the Herbarium and four taxa both inside and outside (Fig. 6).

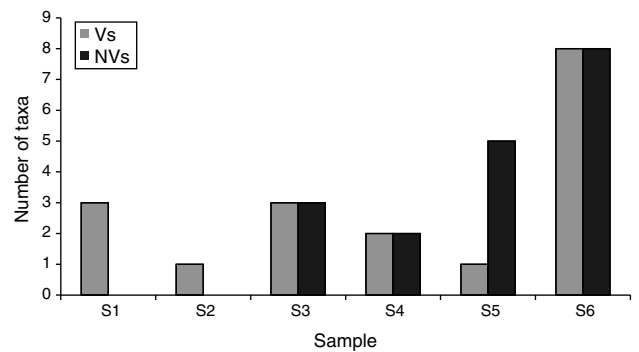


Fig. 5. Comparison of the richness of taxa obtained by the viable and non-viable methods in the sampling sites. V, viable method; NV, non-viable method.

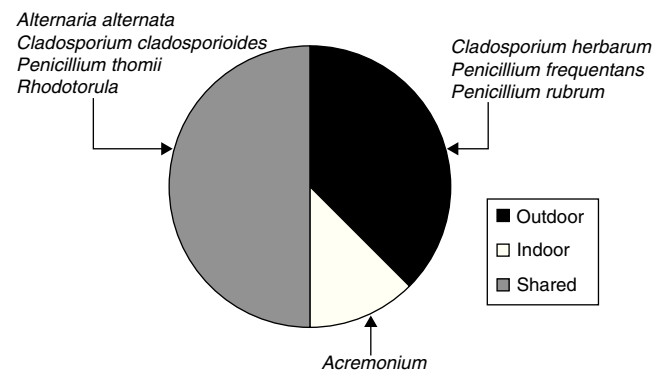


Fig. 6. Typical spores found with the viable method, inside the Herbarium, outside the Herbarium and in both sites.

Discussion

In this study, a preliminary analysis of the indoor air quality in six sectors of the LP Herbarium was done using a volumetric system for both viable and non-viable samples. Although a differential richness of spores and/or other airborne elements was found, it was dependent on each methodology applied. In this sense, the viable method was the most efficient in the identification of fungal taxa. While three fungal representatives were captured from sites I and II, the non-viable one did not record any fungal spore in those sectors. However, the apparent absence of propagules from one sampling type cannot discard their existence in the environment, when they are detected using another method. This fact is closely related to the existence of specific limitations for each type of sampling, being the morphological alteration and the viability and physiological requirements (including nutritional ones) the critical aspects for the non-viable and viable methods respectively.⁴³ Trunov et al.⁴⁹ suggested that disaggregated spherical spores, such as those belonging to *Aspergillus* and *Penicillium*, can be damaged by mechanical stress during their collection and be transformed to particulate elements that hinder their diagnosis and therefore their identification. On the other hand, the viable method also can fail when spore viability is affected and/or when the culture medium used for the growth of the air samples is not suitable. Although *Aspergillus*/*Penicillium*-type and *Chaetomium*-type spores were found in the secondary entrance and/or the exterior corridor, respectively, none of these taxa developed on an agar medium for fungi when samples from the viable method were inoculated.

While the two methods used in this work recovered a similar number of fungal spores, the taxa were different in each case (Figs. 3, 5 and 6). Only spores related to the genera *Alternaria* Nees and *Cladosporium* Link were detected by both methods. However, the non-viable method, which detected a higher number of

taxa than the viable method, is also useful for the identification and quantification of some ascospores, basidiospores and other propagules that show specific difficulties to germinate.³⁷ Both *Aspergillus-Penicillium*-type and *Cladosporium*-type spores were the ones that showed the highest relative concentration (23 and 40%, respectively) in comparison to the other types identified by the non-viable method. This method also allowed the capture and detection of several inert particles, which might be important in air pollution since they may be vehicle and/or substrate for microorganisms.^{15,22,41} Similarly, other authors have reported a higher number of airborne fungi using the non-viable method.^{1,2,7} In contrast, the viable method allows the accurate identification of most anamorphic fungal species such as those which require culture methods.

An indoor/outdoor ratio ≥ 0.63 and concentrations of bioaerosols ≥ 2500 part/m³ air were found in the samples from sites I and II. Although there are no international standards for indoor air quality regarding bioaerosols, our results suggest a safe air quality in the conservation areas of the LP Herbarium according to the findings of several authors.^{17,31,34} Additionally, although several taxa and/or morphological types were identified in the indoor air of the LP Herbarium by means of both methodologies, none of the samples analyzed included representatives of *Stachybotrys* Corda, *Fusarium* Link, *Trichoderma* Pers., or *Aspergillus versicolor* (Vuill.) Tirab, which are generally regarded as indicators of moisture problems.^{9,34} Therefore our results are compatible with a safe conservation for cultural heritage such as Herbarium material. However, most fungal types and taxa that we found in the Herbarium, such as *Penicillium* and *Cladosporium*, are ubiquitous fungal elements in indoor air.^{23,53} On the other hand, higher richness and concentration of airborne elements were found in site VI (exterior corridor), probably due to the proximity to the exterior and/or the movement of the staff within the Museum. Furthermore, the secondary entrance, which showed a higher indoor/outdoor index for the culturable sample, is also amenable to aerobiological contamination, possibly due to the fact that it is a crossing point between the Herbarium and the outside world, and it is frequently used to access to the collections. In this sense, *Rhodotorula* sp., which was detected only in this site (secondary entrance) using the viable method, was the element with the highest relative concentration, in comparison to others also identified (Table 1). Although *Rhodotorula* is a common environmental yeast that may be found in several contexts-including the air-, colonizing plants, humans, and other mammals, it has been recently recognized as an emerging pathogen in immunosuppressed patients, and the number of infections it causes has increased along the time.^{50,52} The presence of this fungus in association to a high level for an individual bioaerosol (not detected in the exterior corridor) suggests that the main source comes from within, for example from human origin.³¹ Everyday activities in the Herbarium such as handling organic materials, resuspension of spores as a result of cleaning activities, and the transportation of spores in the clothes,³⁰ plus the movement of the air and the relative humidity, could help to explain the results obtained.

In conclusion, both sampling methods were necessary to monitor the bioaerosol charge in the LP Herbarium. Under the environmental conditions in which this institution was analyzed, the status in this indoor air seems to be rationally adequate for the conservation of Vascular Plants due to the low indoor/outdoor index, low concentrations of airspora and lack of taxa indicator of moisture problems, at least in sites I and II, where the plants are conserved. Similar results were obtained during the same season of sampling in a neighboring repository of mummified legacy from the Museo de La Plata.³⁹ Therefore, although preliminary, our results may be applied for the diagnosis and prevention of

the potential effects of airborne fungi in biodeterioration since under certain climatic conditions such as high humidity and precipitation, their explosion and activity may be relevant.¹⁰

These findings are important since there are scarce available data or systematic studies on the quality of the indoor air in La Plata Museum and the incidence of bioaerosols, including fungi, which can potentially cause adverse health effects.³⁹ This information should have priority treatment to evaluate the need of measures for the proper handling of materials, and to take any additional action to control the conditions and safe preservation of the plant collections of the LP Herbarium and other similar institutions.

Sources: Maps of the Museo de Ciencias Naturales and Herbarium LP by Arch. Mauro García Santa Cruz, Lic. Jimena García Santa Cruz and Arch. Analía Gómez Laboratorio de Arquitectura y Hábitat Sustentable, Facultad de Arquitectura y Urbanismo Universidad Nacional de La Plata.

Conflict of interest

The authors declare no conflict of interest.

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