INTERNATIONAL JOURNAL OF CONSERVATION SCIENCE

ISSN: 2067-533X Volume 10, Issue 2, April-June 2019: 291-306 www.ijcs.uaic.ro

FUNGAL MONITORING IN AN EXHIBITION ROOM WITH EGYPTIAN MUMMIES IN THE MUSEUM OF NATURAL SCIENCES OF LA PLATA, ARGENTINA

Daniela S. NITIU[1,6*], Andrea C. MALLO[1,7], Lorena A. ELÍADES[2,6], Mauro GARCÏA SANTA CRUZ[3,6], Mario C.N. SAPARRAT [2,4,5,6]

[1] Cátedra de Palinología, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata. Calle 64 N°3. CP: 1900. La Plata. Buenos Aires. Argentina.
[2] Instituto de Botánica Carlos Spegazzini, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata. Calle 51 N° 477. CP. 1900. La Plata, Buenos Aires, Argentina
[3]Facultad de Bellas Artes, Universidad Nacional de La Plata. Calle 8 N°1326. CP. 1900.La Plata, Buenos Aires, Argentina
[4] Instituto de Fisiología Vegetal, Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata. Diag. 113 esp.61.CP. 1900. La Plata, Buenos Aires, Argentina
[5] Cátedra de Microbiología Agrícola, Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata. Av. 60 CP. 1900. La Plata, Buenos Aires, Argentina
[6] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.
[7] Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC, PBA), Argentina.

Abstract

The cultural properties kept in museums are exposed to different factors that may affect the integrity. The aims of the present study were i) to determine the environmental fungal load present both inside and outside the Egyptian Room of the Museum of Natural Sciences of La Plata, Argentina, as well as inside two showcases where Egyptian sarcophagi are preserved, along a one-year monitoring, and ii) to evaluate the possible impact of temperature and relative humidity on the preservation of these mummies. The fungal load was determined by means of a volumetric air sampling methodology and the environmental variables by means of specific instruments. The fungal load found in the four sites studied was of 32843,23 CFU/m^3, belonging to 21 fungal taxa, mainly to the phylum Ascomycota. The most representative were Beauveria bassiana, Fusarium oxysporum, Penicillium sp. and Rhodotorula sp. (as a yeast representative), were common to the four sites, showing a significant similarity between the outdoor and indoor environments. The temperature of the exhibition room and that of the two showcases were either similar or lower than the values recommended by the UNI 10829:1999 standards. In contrast, throughout the year, the relative humidity recorded was higher than the recommended values.

Keywords: *Environmental monitoring; Fungal load; Cultural heritage; Egyptian mummies; Museum of La Plata; Argentina*

Introduction

Museums are reference sites for the preservation and exhibition of the natural and cultural heritage of a country, as well as a source of invaluable information for specialists of various scientific disciplines. Thus, governmental requirements at national and global levels

* Corresponding author: danielanitiu@yahoo.com.ar

establish the need to monitor and improve the conditions for the control and conservation of materials kept in these enclosures [1].

The environmental conditions of the building, including the temperature, relative humidity and specific humidity, along with the degree of ventilation, cleanliness and activities that are developed in a certain exhibition room, play a key role in the proliferation of biodegradation and biodeterioration agents such as fungi, which are the main biological agents responsible for causing serious damage to both pieces of a collection and complete collections [2-4]. The availability of spores and other fungal propagules in the indoor air of museums and the presence of fungi colonizing goods with patrimonial value are conditioned by several factors such as the state of preservation of the goods, their components, and their physical structure.

The fungi associated with the biodeterioration of patrimonial objects can be grouped into two main categories: i) cellulolytic and keratinolytic fungi, which are fungi with specific enzymatic abilities able to degrade different polymers and ii) opportunistic fungi (such as those belonging to the order Mucorales), which, if there is sufficient humidity, can grow in practically all types of materials but are unable to depolymerize their structural components [5-7].

At the Museum of Natural Sciences of the city of La Plata, Argentina, various improvement interventions have been carried out since 2013 in various sectors of the building, including the exhibition halls, the collection storage warehouse, and the working areas, in order to both install new security systems and minimize the problems of humidity caused by the deterioration of walls and the appearance of spots with the consequent proliferation of fungal biofilms. This has led to an important restoration and remodeling of the interior space and improvements in the museum accessibility and circulation. This remodeling has included the relocation of the Egyptian Room, which contains a collection of mummies acquired from the Boulaq Museum (current Museum of El Cairo, Egypt) at the beginning of the last century by Dardo Rocha, the founder of the city of La Plata. This collection includes an adult female mummy called *Tadimentet*, an adult male mummy called *Herwodj*, and a funerary package, all from Saqqara, one of the necropolises of the city of Memphis, capital of Egypt during the Late Period, a historical period that took place 2300 years ago and coincided with the last pharaonic dynasties in the valley of the Nile River. Its antiquity dates from 664-323 BC. In 2010, these mummies were studied by means of non-invasive computed tomography performed in a specialized Hospital of the province of Buenos Aires, which allowed deepening the knowledge about mummification techniques and probable causes of death [8, 9]. As a result of the aforementioned remodeling of the Museum, in 2015, tasks were begun to record the environmental conditions and microbiological load in the Egyptian Room so as to monitor the state of preservation of the mummies.

The aim of the present work is to present the results of the study of the environmental fungal load determined inside the Egyptian Room, in the indoor air of two of the showcases where the Egyptian collection is preserved, and in the air outside the Egyptian Room, along a one-year monitoring. The study included the evaluation of the temperature and relative humidity and the possible impact of these environmental variables on the preservation of the goods.

Material and methods

Characterization of the sampling sites

After an intense work of restoration and improvement of the Egyptian Collection, in 2013, the Museum of Natural Sciences of La Plata opened the Room currently called "Fragments of history on the banks of the Nile", which holds a collection composed of more than 300 pieces, including two mummies, 40 blocks of sandstone belonging to lintels, friezes, door jambs with hieroglyphic inscriptions, ceramics of various burials, and four figurines called

ushebtis, all of which are part of the current permanent exhibition of the Museum (www.museo.fcnym.unlp.edu.ar). This Egyptian Room has an area of 300m^2 and is located on the top floor of the Museum. In the present study, three representative sites of the Room were analyzed: Site 1 - the visiting area, which is the most exposed site due to the movement of visitors and personnel and the direct connection with the outdoor air (Fig. 1a), Site 2 - a showcase containing the female mummy *Tadimentet*, and Site 3 - a showcase containing the male mummy *Herwodj*. The air outside the Room was also studied (Site 4).

Fig. 1. Museum of Natural Sciences of La Plata, Argentina. (a) diagram of Egyptian Room with Sampling sites: Site 1: indoor environment (SO2), Site 2: showcase with famale mummy (VO1); and Site 3: showcase with the male mummy (VO2) and outdooor environment (SO4). Location of dataloggers in the Room indicated with (H). (b) exhibition Room with Egyptian mummies

Air fungal load

The fungal load of the indoor air of the Egyptian Room, the air of each of the two showcases, and the outdoor air was studied between July 2015 and June 2016, with a volumetric sampler type Hirst Zefon Z-Lite IAQ Air Sampling Pump® based on the principle of inertial impact of the particles, and adapted for the subsequent culture of the samples. The samples were collected by means of Millipore filter holders equipped with Whatman® - ICT grade 1 qualitative sterile filter paper, with a diameter of 25mm. The resulting system was applied to the suction pump for 5 minutes at a rate of 0.015m^3 per minute. In the laboratory, each filter was suspended in 20mL of sterile water under aseptic conditions (fragmented and mixed). The resulting suspension was spread on each plate (1.0mL per plate), and three replicates per sample were made on solid medium (Rose Bengal Agar, Biopack, C.I. 45440 article 947901) [10]. The plates were incubated at 25°C and 63% relative humidity (RH) in the dark until the appearance of fungal colonies, which were counted as Colony-Forming Units (CFU). The material of each colony was observed under an optical microscope and the fungi were identified taxonomically on the basis of morphological and cultural characteristics [11]. The CFU were converted into volumetric units, calculated as: number of colonies x volume of dilution/volume of air sampled. The stock cultures of these samples were kept at 4°C, 2% ($w \cdot v^{-1}$) in malt-agar extract, and then deposited in the culture collection of the Spegazzini Institute, Universidad Nacional de La Plata, La Plata, Argentina (LPSC). The data obtained were used to estimate the total concentration and specific concentration for each site and to analyze the

seasonal variation. The indoor/outdoor index [12] between the air of the Egyptian Room and the air inside the showcases was calculated considering the quality of the outdoor air as one of the determining factors that influence the indoor pollution levels [13, 14], in addition to the air removal and exchange processes typical of an exhibition room [15].

Environmental parameters

To evaluate the building environment, we used the methodology developed from the adaptation of the procedure of the Getty Conservation Institute [16]. This evaluation seeks to describe the sensitivity of the collections, the behavior of the building, and the risks represented by the environment and humans [17]. The building environment was monitored using the guidelines proposed in the [18, 19] . This monitoring consisted in recording and analyzing the environmental conditions of the different spaces through the continuous measurement of the temperature and RH during the study period. These two variables were considered because it is known that they have a great impact on the conservation of historical pieces kept in museums. Eight measurement campaigns, corresponding to 2 days for each season of the year, were carried out. To this end, dataloggers were placed in the sampling sites to determine the temperature and RH with a frequency of 10 minutes. The results for the indoor air showed an average annual temperature of 15°C and an average annual RH of 64%.

Considering the characteristics of the objects held in the Egyptian Room, the reference values recommended for their conservation, corresponding to the categories "mummies" and "painted wood", were determined according to the UNI10829: 1999 standard [19].

Statistical analysis

The statistical analysis was carried out using Spearman's Correlation test (XL STAT, version 2011). The test was used to examine the relationship between the concentration of the fungal taxa most represented in the indoor sites monitored and the environmental parameters temperature and RH. A level of significance of $P < 0.05$ was considered. The mean and standard deviation for each taxon were calculated.

Results

Air fungal load

A total of 21 fungal taxa were recorded after the eight seasonal aeromycological monitoring campaigns carried out in the four sites studied (inside the Egyptian Room, inside the showcase holding the female mummy *Tadimentet*, inside the showcase holding the male mummy *Herwodj*, and outside the Egyptian Room). Most of the genera corresponded to taxa belonging to the phylum Ascomycota (Table 1), with *Rhodotorula* being the only representative of the phylum Basidiomycota. A total of 328436,23 CFU/m3 of air were estimated during the whole sampling period and the most representative taxa in all the sites were *Beauveria bassiana*, *Penicillium* sp., *Fusarium oxysporum* and *Rhodotorula* sp., each exceeding 10% of the total sampled.

The indoor air of the Egyptian Room (Site 1) showed 66220,57 CFU/m3, corresponding to 16 fungal taxa (Table 1), among which the most abundant were *Penicillium sp.* (14.90%), *Fusarium oxysporum* (14.36%), *Aspergillus niger* (14.23%), *Beauveria bassiana* (8.32%), *Talaromyces sp.* (7.79%), *Cladosporium cladosporioides* (7.79%), *Penicillium sp*.1 (6.98%), *Mycelia sterilia* (5.64%), *Cladosporium sp.* (5.37%), *Aspergillus sp*. (4.43%), *Rhodotorula sp.* (3.36%), *Aspergillus terreus* (2.68%), *Paecilomyces sp*. (2.01%) and *Fusarium solani* (1.48%). niger, with 4173,6 CFU/m3. In the autumn season, the maximum contribution was provided by thirteen taxa (Table 2), whereas during the spring, the maximum contribution was that of *Aspergillus*

Table 1. Fungal types identified and values of total CFU/m^3 in the four sampling sites. Percentage of taxa distribution

Sporal type	Site 1	Site 2	Site 3	Site 4	Total	Distribution of taxa (%)
Acremonium sp.	177.77	0.00	266.66	0.00	444.43	0.14
Alternaria sp	0.00	355.55	533.32	444.43	1333.30	0.41
Aspergillus sp.	2933.26	977.75	0.00	0.00	3911.01	1.19
Aspergillus niger	9421.99	4622.11	5333.20	11555.27	30932.56	9.42
Aspergillus terreus	1777.73	355.55	177.77	2044.39	4355.45	1.33
Beauveria bassiana	5510.97	47554.37	266.66	19910.61	73242.61	22.30
Cladosporium sp.	3555.47	1333.30	0.00	3199.92	8088.69	2.46
Cladosporium herbarum	0.00	0.00	0.00	266.66	266.66	0.08
Cladosporium cladosporioides	5155.43	3199.92	2133.28	266.66	10755.29	3.27
Fusarium oxisporum	9510.87	16444.03	14132.98	2311.05	42398.94	12.91
Fusarium solani	977.75	0.00	0.00	0.00	977.75	0.30
Micelia sterilia	3733.24	977.75	7999.80	3022.15	15732.94	4.79
Paecilomyces lilacinus	1333.30	977.75	355.55	88.89	2755.49	0.84
Penicillium sp.	9866.42	17599.56	12621.91	14844.07	54931.96	16.73
Penicillium sp1.	4622.11	0.00	1599.96	88.89	6310.95	1.92
Penicillium restrictum	0.00	0.00	0.00	88.89	88.89	0.03
Penicillium rubrum	266.66	888.87	355.55	2399.94	3911.01	1.19
Phoma sp.	0.00	6933.16	0.00	0.00	6933.16	2.11
Rhodotorula sp.	2222.17	19377.29	7910.91	16266.26	45776.63	13.94
Talaromyces sp.	5155.43	6310.95	1422.19	2311.05	15199.62	4.63
Tricoderma	0.00	0.00	0.00	88.89	88.89	0.03
Total count	**66220.57**	**127907.91**	**55109.73**	**79198.02**	**328436.23**	**100.00**

Table 2. Seasonal data for each sampling site in terms of total CFU/m^3, maximum and minimum concentration recorded, geometric mean, standard deviation, number of fungal types by period and total CFU/m^3 for each site

Site 1 (indoor)						
	Winter	**Spring**	**Summer**	**Autumn**	**Total count**	
Total	20424	15628.8	12165.6	17937.6	66156	
Max	621.6	4173.6	2664	2486.4		
Min	0	0	0	0		
Arit.mean	10212	7814.4	6082.9	8968.8		3195.90
Standard deviation (n = 8)						
N° of sporal types	9	5	4	13		
Site 2 (Fem)						
	Winter	**Spring**	**Summer**	**Autumn**	**Total count**	
Total	66688.8	40404	9412.8	11277.6	127783.2	
Max	12165.6	14474.4	2308.8	3996		
Min	0	0	0	0		
Arit.mean	33344.4	20202	4706.4	5638.8		
Standard deviation (n = 8)						21824.50
N° of sporal types	6	8	12	10		
Site 3 (Male)						
	Winter	**Spring**	**Summer**	**Autumn**	**Total count**	
Total	12533.02	97064.24	18132.88	37599.06	233450.24	
Max	12533.02	33865.82	9599.76	14399.64		
Min	0	0	0	0		
Arit.mean	6266.51	48532.12	9066.44	18799.53		6265.80
Standard deviation (n= 8)						
N° of sporal types	1	7	7	13		
Site 4 (outdoor)						
	Winter	**Spring**	**Summer**	**Autumn**	**Total count**	
Total	41736	21489.6	6571.2	9412.8	79209.6	
Max	19891.2	6926.4	1864.8	1509.6		
Min	0	0	0	0		
Arit.mean	20868	10744.8	3285.6	4706.4		
Standard deviation (n= 8)						11580.90
N° of sporal types	10	3	9	12		

The indoor air of the showcase of the female mummy (Site 2) showed a total of 127907,91 CFU/m3, corresponding to 15 fungal taxa (Table 1), among which the most representative were *Beauveria bassiana* (37.18%), *Rhodotorula sp.* (15.15%), *Penicillium sp.* (13.76%), *Fusarium oxysporum* (12.86%), *Phoma sp*. (5.42%), *Talaromyces sp.* (4.93%), *C. cladosporioides* (2.50%) and *Cladosporium sp*. (1.04%). In the summer season, the maximum contributions were provided by twelve taxa (Table 2), and *Beauveria bassiana* was the maximum exponent, with 46531,2 CFU/m3 during winter.

Regarding the indoor air of the showcase of the male mummy (Site 3), a total of 55109,73 CFU/m^3 corresponding to 14 fungal taxa were quantified (Table 1). Among them, the most abundant were *Fusarium oxysporum* (25.65%), *Penicillium* sp. (22.90%), *Mycelia sterilia* (14.52%), *Rhodotorula* sp. (14.35%), *Aspergillus niger* (9.68%), *Cladosporium cladosporioides* (3.87%), *Penicillium* sp.1 (2.90%) and *Talaromyces* sp. (2.58%). In the spring campaign, the maximum contributions were provided by thirteen taxa (Table 2), being *Fusarium oxysporum* the maximum exponent, with 7814,4 CFU/m^3.

Finally, the air of the external environment (Site 4) showed CFU/m^3, corresponding to 17 fungal taxa (Table 1), among which the most abundant were *Beauveria bassiana* (25.11%), *Rhodotorula* sp. (20.52%), *Penicillium* sp.1 (18.72%), *Aspergillus niger* (14.57%), *Cladosporium* sp. (4.04%), *Mycelia sterilia* (3.81%), *Penicillium rubrum* (3.03%), *Fusarium oxysporum* (2.91%), *Talaromyces* sp. (2.91%) and *Aspergillus terreus* (2.58%). In the summer season, the maximum contributions were provided by 12 taxa (Table 2), among which *Beauveria bassiana* was the maximum exponent, with a value of 19891,2 CFU/m^3 during winter campaign.

A large number of the spores suspended in the Room's environment are potential sources of agents able to colonize and degrade the constituent materials of the mummies, and thus able to cause their biodeterioration. Although the showcases were found to share a high number of taxa with the indoor environment, *Alternaria alternata* was present only inside both showcases and *Phoma* sp. was present only inside the showcase of the female mummy, which indicates that they could differentiate *in situ* based on the material of each enclosure.

The total value of the indoor/outdoor index between the indoor air of the Egyptian Room and the outdoor air was 0.79, indicating a concentration of fungi outside the room. The taxa involved were *Aspergillus niger*, *A. terreus*, *Beauveria bassiana*, *Penicillium* sp., *P. rubrum* and *Rhodotorula* sp., corresponding to values of the indoor/outdoor index lower than 0.87. On the other hand, *Cladosporium* sp., *C. cladosporioides*, *Fusarium oxysporum*, *Mycelia sterilia*, *Paecilomyces* sp., *Penicillium* sp.1 and *Talaromyces* sp. showed a value of the indoor/outdoor index higher than 1.1, thus indicating a greater representation inside the Room (Table 3).The total value of the indoor/outdoor index between the Room and the showcase of the female mummy (Site 2) was 2.0, whereas that for coincident taxa was lower than 0.73, indicating a notable concentration in Site 2 with respect to the Room. The total value of the indoor/outdoor index between the Room and the indoor of the male mummy showcase (Site 3) was 0.93, whereas that for coincident taxa was lower than 0.57 (Table 3) and a similar concentration of spores was found in both sites.

The total number of spores inside and outside the Egyptian Room was quite similar. In contrast, the number of spores in the showcase where the female mummy is preserved was markedly higher, given mainly by the presence of *Beauveria bassiana* in the winter season, which could be indicating the presence of an internal source that favors the development of such spore (Table 2).

These results would explain two particular situations: on the one hand, the showcase conserving the female mummy is in a sector of the exhibition room where there is a direct circulation of people, so the air flow is more dynamic, partly exerting the effect of "resuspension", moving a greater number of particles; on the other hand, the showcase where

the male mummy is kept is confined to an area against the wall, below the lower section of a staircase to the upper floor, showing greater stability of the surrounding atmosphere and therefore of its interior. The ventilation and the movement of people act primarily as a means of transport for fungi and their diaspores, not being a source of nutrients or conditions that promote their biodegradation action.

Table 3. Indoor/outdoor index between the indoor air (Site 1) and the outdoor air (Site 4), between the inside of the showcase with the female mummy (Site 2) and the indoor air (Site 1), and between the inside of the showcase with the male mummy (Site 3) and the indoor air (Site 1)

Sporal type	**I(S1)/(S4)**	***Sporal type***	**I(S2)/(S1)**	**Sporal type**	**I(S3)/(S1)**
Aspergillus niger	0.82	*Aspergillus sp.*	0.33	*Acremonium sp.*	1.50
Aspergillus terreus	0.87	*Aspergillus niger*	0.49	*Aspergillus niger*	0.57
Beauveria bassiana	0.28	*Aspergillus terreus*	0.20	*Aspergillus terreus*	0.10
Cladosporium sp.	1.11	*Beauveria bassiana*	8.63	*Beauveria bassiana*	0.05
Cladosporium cladosporioides	19.33	*Cladosporium sp.*	0.38	*Cladosporium cladosporioides*	0.41
Fusarium oxisporum	4.12	*Cladosporium cladosporioides*	0.62	*Fusarium oxisporum*	1.49
Micelia sterilia	1.24	*Fusarium oxisporum*	1.73	*Micelia sterilia*	2.14
Paecilomyces lilacinus	15.00	*Micelia sterilia*	0.26	*Paecilomyces lilacinus*	0.27
Penicillium sp.	0.66	*Paecilomyces lilacinus*	0.73	*Penicillium sp.*	1.28
Penicillium sp1.	52.00	*Penicillium sp.*	1.78	*Penicillium sp1.*	0.35
Penicillium rubrum	0.11	*Penicillium rubrum*	3.33	*Penicillium rubrum*	1.33
Rhodotorula sp.	0.14	*Rhodotorula sp.*	8.72	*Rhodotorula sp.*	3.56
Talaromyces sp.	2.23	*Talaromyces sp.*	1.22	*Talaromyces sp.*	0.28

Taxa characteristic of the outdoor environment (such as *Alternaria alternata*, *Cladosporium herbarum*, *Penicillium restrictum* and *Tricoderma* sp. as well as taxa exclusive of the indoor environment (such as *Acremonium* sp. *Aspergillus* sp. and *Fusarium solani*) were present throughout the sampling period. The microbiological data showed a high number of taxa in common between both environments, showing a connection between the sites, mainly by means of air currents, which could influence the diversity and concentration of spores in the indoor environment. This dynamics could also be due to the high number of people who circulate daily, since it is one of the main attractions of the Museum and is connected with other areas.

Environmental variables and fungal load

The values of temperature and RH recorded during the year were different for each site of the indoor environment. Both in the Room and inside the two showcases, maximum values of temperature (between 29.7°C and 28.8°C) were recorded in summer, whereas minimum values (between 12.0°C and 12.7°C) were recorded in winter (Fig. 2). Regarding RH, the Room was the site with the greatest difference throughout the seasons, with the highest value in spring (69%) and the lowest value in winter (47%). Regarding the showcases, Site 2 recorded the maximum value of RH (59.7%) in autumn and the minimum (56.6%) in summer, whereas Site 3 recorded the maximum RH (63%) in autumn and the minimum (54.2%) in winter (Fig. 2).

As mentioned above, considering the characteristics of the historical pieces preserved in the Egypcian Room and analyzing specific bibliography, the reference values recommended for their conservation, i.e. those corresponding to the categories “mummies” and “painted wood”, were determined according to the UNI 10829:1999 standard. Depending on the conservation and tolerance values were available. Taking into account the temperate humid climate of the city of La Plata and the building characteristics of the Museum, it is difficult to reach the optimal values at a reasonable energy and environmental cost.

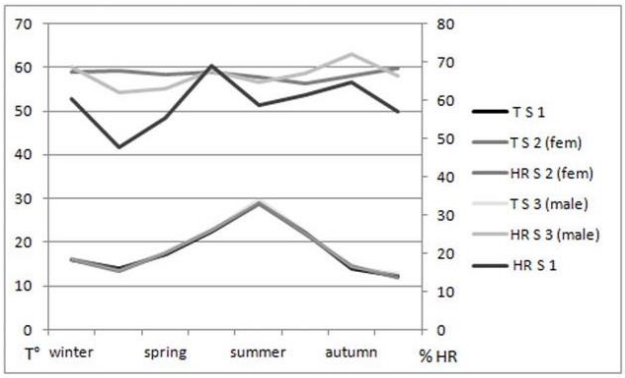

Fig. 2. Values of temperature and relative humidity of the indoor sampling sites (Site 1, Site 2 and Site 3) during the eight seasonal sampling campaigns

Since the development of a large part of the fungal taxa responds to sensitive changes in the temperature and RH in the short term, the daily variation in each of the sites studied was analyzed in a seasonal scale. This analysis showed that, in winter, the daily variation in temperature in Site 2 was higher than the recommended values, whereas in Site 3 it was optimal on most days. Regarding the daily variation in RH, it was optimal in the female showcase and higher than the recommended values in the other sites (Fig. 3). In spring, the daily variation in temperature in both showcases was optimal on most days, whereas the daily variation in RH in Site 2 was optimal and that in the other sites was higher than the recommended values (Fig. 4). In summer, the daily variation in temperature in the two showcases was optimal on most days, whereas the daily variation in RH was optimal only inside the female showcase and higher than the recommended values in the other sites (Fig. 5). In autumn, the daily variation in temperature was optimal on most days, whereas the daily variation in RH was optimal only inside the female showcase and higher than the recommended values in the other sites (Fig. 6).

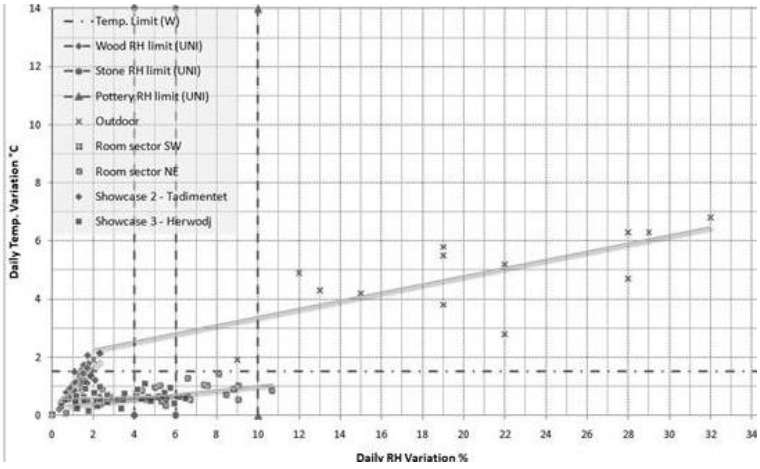

Fig. 3. Records of daily variation in temperature and relative humidity in the indoor samping sites (Site 1, Site 2 and Site 3) as well as in the outdoor environment (Site 4) during winter. Limit values of temperature and relative humidity taken from the UNI10829: 1999 standard and Williset al. (2014) for wood, leather and ceramics

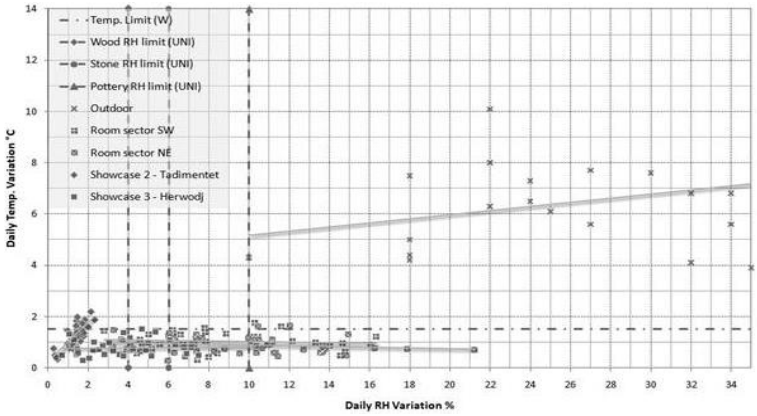

Fig. 4. Records of daily variation in temperature and relative humidity in the indoor samping sites (Site 1, Site 2 and Site 3) as well as in the outdoor environment (Site 4) during spring. Limit values of temperature and relative humidity taken from the UNI10829: 1999 standard and Williset al. (2014) for wood, leather and ceramics

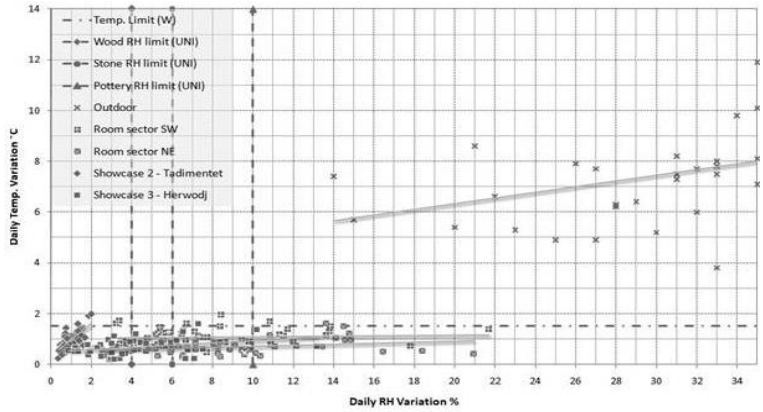

Fig. 5. Records of daily variation in temperature and relative humidity in the indoor samping sites (Site 1, Site 2 and Site 3) as well as in the outdoor environment (Site 4) during summer. Limit values of temperature and relative humidity taken from the UNI10829: 1999 standard and Williset al. (2014) for wood, leather and ceramics

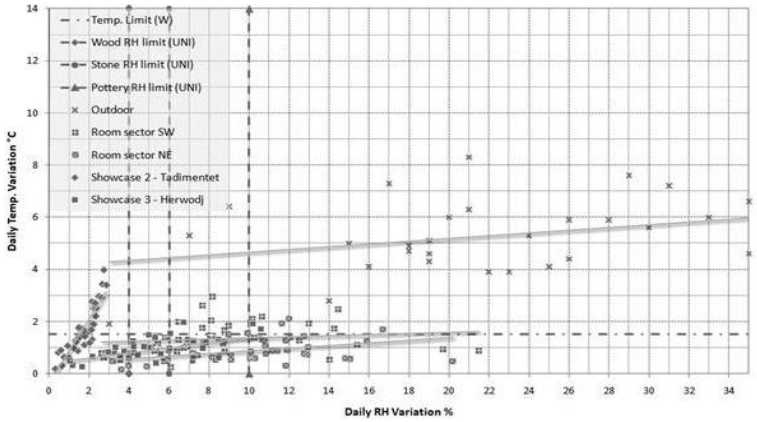

Fig. 6. Records of daily variation in temperature and relative humidity in the indoor samping sites (Site 1, Site 2 and Site 3) as well as in the outdoor environment (Site 4) during autumn. Limit values of temperature and relative humidity taken from the UNI10829: 1999 standard and Williset al. (2014) for wood, leather and ceramics

In general, in indoor environments, humidity is one of the critical factors for fungal development, since it determines the amount of water available for spore germination and microbial growth, which may be favored by the components of the different supports involved. In the present study, the maximum concentrations of fungal spores in winter were recorded inside the Room and inside the showcase of the female mummy, whereas those in spring were recorded inside the showcase of the male mummy (Table 2). The best represented taxa in those seasons and the most abundant taxa throughout the study were the genera *Beauveria bassiana*, *Penicillium* sp., *Fusarium oxysporum* and *Rhodotorula* sp. The correlation coefficient presented either positive or negative values between the concentration variables of each taxon and those of temperature and RH, but was not significant in any of the cases (Table 4).

Table 4. Spearman correlation analysis for the taxa best represented in the sampling with respect to the temperature and relative humidity. Significance level: $P < 0.05$. The mean and standard deviation were calculated for each taxa

Taxa	Variable	Correlation	p level	Min	Max	Mean	Standard Deviation
Beauveria	(CFU/m^3)			0.0000	524.0000	25.0000	106.7134
(n = 24)	temperature	-0.3704	0.0755	12.0280	29.7220	18.4718	5.4810
	humidity	0.1897	0.3718	47.7010	69.1120	58.6371	3.9364
Fusarium	(CFU/m^3)			0.0000	168.0000	18.7917	37.2395
(n = 24)	temperature	0.2422	0.2522	12.0280	29.7220	18.4718	5.4810
	humidity	0.0628	0.7688	47.7010	69.1120	58.6371	3.9364
Penicillium	(CFU/m^3)			0.0000	137.0000	18.7917	32.2477
(n = 24)	temperature	0.3150	0.1333	12.0280	29.7220	18.4718	5.4810
	humidity	0.2360	0.2647	47.7010	69.1120	58.6371	3.9364
Rhodotorula	(CFU/m^3)			0.0000	88.0000	11.3750	22.3311
(n = 24)	temperature	-0.1994	0.3486	12.0280	29.7220	18.4718	5.4810
	humidity	-0.2061	0.3323	47.7010	69.1120	58.6371	3.9364

Discussion

Several authors have evaluated the "Sick Building Syndrome", by estimating the microbiological quality of the air of different internal environments such as archives, libraries, museums, cathedrals, buildings, and laboratories, and found microorganisms that can grow on different organic and inorganic materials, causing aesthetic and/or structural biodeterioration [20-22]. Although there is no international standard that indicates whether an environment is sick or not, some references suggest that, for the environment to be approved for use, the microbial concentration must be below 1,000 CFU/m^3 [23, 24].

In the present study, the indoor air quality of three sites of the Exhibition Room "Fragments of history on the banks of the Nile" of the Museum of Natural Sciences of La Plata (Argentina) and that of the outdoor environment was analyzed by using a volumetric system to obtain fungal propagules, which were then cultured on a standard medium (Rose Bengal agar medium with chloramphenicol) for their recording and quantification. Although the medium used is highly recommended for the selective isolation and quantification of fungi from environmental and food sources, it has some limitations associated with the presence of the antibiotic, since it can have an additional effect on the inhibition of fungal spore germination and differentiation of diagnostic structures in the fungal cultures.

It is known that the activation of the germination of several ascospores and basidiospores as well as of other fungal propagules requires specific conditions, which make it difficult to obtain axenic cultures of characteristic taxa [25]. *Beauveria bassiana*, *Penicillium* sp., *Rhodotorula* sp. and *Fusarium oxysporum* were the taxa with the highest relative concentration (22.3%, 16.7%, 13.9% and 12.9%, respectively) in comparison with the other less frequent

fungi. Among the former, *B. bassiana* is a fungus with a remarkable proteolytic ability, partly associated with its role as an entomopathogen [26]. The availability of protein materials in mummies and historical scrolls, which are susceptible to colonization by microorganisms and invasion by insects, and the high load of *B. bassiana* spores in the indoor air of the showcases are in agreement with previous findings on the dominance of this fungal species in substrates rich in organic nitrogen [27]. On the other hand, many of the fungi identified have been mentioned by numerous authors as agents able to degrade different materials, due to their ability to synthesize a broad spectrum of enzymes with action on the aesthetic and/or structural alteration of historical pieces [28, 29].

Penicillium sp. was the most abundant taxon isolated in the four sampling sites, with an average higher than 14% in each site, whereas *Fusarium oxysporum* was the most abundant in the three indoor sites. *Penicillium* sp., *Cladosporium* sp. and *Aspergillus* sp. have been reported by [30-34] as the three main polluting genera of indoor environments worldwide and commonly isolated in houses, archives, libraries and museums [35-37].

The spectrum and activity of fungal propagules in the air are dependent on the preexisting colonization of the associated materials, their viability, and the transmission and flow of currents [38, 39]. The comparison of the inside of each showcase and that of the exhibition room showed differences, being the air of Site 2 (female mummy showcase) the one with the highest fungal load. This may be due to differences in the ventilation of each sector within the exhibition room (relative location) and/or the materials and their composition available inside each showcase, which can generate a particular microenvironment that favors the colonization of certain fungi as well as the differentiation of their propagules inside the showcase. Although [8, 9] have previously reported differences in the structure and chemical nature of each sarcophagus, the existence of mycofilms associated with specific materials of the mummies under study was not analyzed in this work.

Several authors have reported that temperature is the factor responsible for the existence of higher concentrations of bioaerosols in the air [40, 41]. However, this variable does not seem to be the conditioning factor of the spectrum of fungal spores found in our study area. Although the values recorded in Site 3 were slightly higher than those of the other sampling sites, the concentration of fungi in this site was lower than that found in Sites 1 and 2. Since this showcase is confined under the staircase, it is probable that the air flow that contributes to the spore load is low, and thus a stable atmosphere is maintained. In the other extreme, Site 2 showed the highest incidence of total fungal spores, which could be related to a greater flow of people circulation, given the location of the showcase, under which the fungi that grow associated with different supports can sporulate later, a fact that increases the load and dispersion of spores, as found for *Penicillium* spp. In addition, the possibility that the room's environment is a main source of "contamination" for the showcases of mummies through their holes cannot be ruled out. The Museum of La Plata is a 129-year-old neoclassical historic building, where the installation of modern climate controls is not possible. As the exhibition rooms do not have air conditioning to control the temperature and humidity or specialized air filters to eliminate different types of particles, including fungal propagules, the control of the indoor climate depends only on the natural ventilation. It is known that the preservation of mummified material is highly dependent on the successful elimination of moisture [42-45]. Therefore, it is a priority to avoid the rehydration of mummies because high RH values promote not only the gelatinization but also the hydrolysis of their protein components [46], a susceptible substrate for proteolytic fungi such as *B. bassiana* [47]. On the other hand, although the present monitoring identified several fungal taxa in the indoor air of the study area, it did not identify problematic representatives such as *Stachybotrys* sp. and *Trichoderma* sp., which are generally considered reliable indicators of sites with high humidity.

The ubiquitous spectrum of fungal taxa found is compatible with that previously reported in the indoor air of other exhibition areas and storage warehouses of the same Museum [48, 49]. Although the concentration and diversity of taxa in the external environment was higher than that in the exhibition room studied, the number of common taxa was high, probably due to the proximity to the external environment and/or to the movement of visitors and personnel inside the Museum. The indoor/outdoor index corroborated this phenomenon, and may allow inferring the characteristics of both atmospheres. In this sense, *Rhodotorula* sp., environmental yeast, was detected in all four sites. However, the presence of this fungus in association with other proteolytic fungi (as detected in Site 2) suggests that the main source comes from protein materials and derivatives available in the female mummy [50].

Conclusion

Although the results of the present study can be considered preliminary, they are key in the diagnosis of the state of conservation and prevention of the Egyptian mummies currently preserved in the exhibition room "Fragments of history on the banks of the Nile" of the Museum of Natural Sciences of La Plata, Argentina. The conservation of this heritage is a priority, because these mummies represent a biological and cultural source, which even offers the perspective of deciphering the past of Egypt at the genomic level [51]. Although the load of fungal spores found in the indoor air of this Egyptian Room and the two showcases where the mummies are preserved was high, the non-detection of fungi responsible for the “Sick Building Syndrome” or with ability to attack wood and its components (like *Stachybotrys* spp. and *Chaetomium globosum*) suggests that this Egyptian Room is able to hold archaeological collections and minimize the problems of humidity and its action on biodeterioration. In addition, prevention strategies such as intense cleaning and fungal environmental monitoring can ensure the adequate conservation of these materials of high heritage value.

Acknowledgments

The present study was financially supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) PIP 112-201101-00087; Agencia Foncyt PICT 2013-0418, Agencia Foncyt PICT 2015-1620 and Proyecto de Incentivos a la Investigación (N11/781) of the Facultad de Ciencias Naturales y Museo (FCNyM), Argentina.

Funding Body: 1) Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) PIP 112-201101-00087, 2) Agencia Foncyt PICT 2013-0418, 3) Agencia Foncyt PICT 2015-1620, 4)Proyecto de Incentivos a la Investigación (N11/781) of the Facultad de Ciencias Naturales y Museo (FCNyM), Argentina.

References

[1] L. Zendri, *La protección del patrimonio cultural de Argentina. De la legislación patrimonial a la consagración de un nuevo derecho. Derecho y Ciencias Sociales. Nº 16 (Temas relativos al desarrollo regional y local).* ISNN 1852-2971. **Instituto de Cultura Jurídica y Maestría en Sociología Jurídica. FCJ y S. UNLP.** 2017, pp. 40-55.

[2] A. Elnaggar, A. Sahab, G. Ismail, G. Mahgoub, M. Abdelhady, *Microbial study of Egyptian mummies: an assessment of enzyme activity, fungicides and some mummification materials for the inhibition of microbial deterioration*, **E-Conservation Magazine, 16,** 2010, pp. 39-49.

[3] C. Pasquarella, E. Saccani, G.E. Sansebastiano, M. Ugolotti, G. Pasquariello, R. Albertini, *Proposal for a biological environmental monitoring approach to be used in libraries and archives,* **Annals of Agricultural Environmental Medicine, 19**, 2012, pp. 209-212.

[4] B. Wills, C. Ward, V. Sáiz Gómez, *Conservation of Human Remains from Archeological Contexts*, **Regarding the Dead: Human Remains in the British Museum**. (Editors: A. Fletcher, D. Antoine and J.D. Hill). The British Museum. London, 2014, pp. 49-74.

[5] C. Meier, K. Petersen, **Schimmelpilze Auf Papier – Ein Handbuch Für Restauratoren**, Der Andere Verlag, Tönning, Germany 2006) pp.198.

[6] B. Blyskal, *Fungi utilizing keratinous substrates,* **International Biodeterioration and Biodegradation**, **63**, 2009, pp. 631-653.

[7] K.M. Naji, Q.Y.M. Abdullah, A:Q.M. Al-Zaqri, S.M. Alghalibi, *Evaluation the biodeterioration enzymatic activities of fungal contamination isolated from some ancient Yemeni mummies preserved in the National Museum*, **Biochemistry Research International**, **2014**, 2014. http://dx.doi.org/10.1155/2014/481508

[8] D.M. Santos, B.B. Daizo, S. Lesyk, F. Abramzon, H. Pucciarelli, *Prácticas Funerarias del antiguo Egipto*, **Revista Museo, 25**, 2011, pp. 56-65.

[9] S. Lesyk, F. Abamzon, *Evaluación tomográfica multicorte de momias egipcias en Buenos Aires*. **Revista Argentina de Radiología, 78**(3), 2014m pp. 171-180.

[10] D.J. Miller, P.D. Haisley, J.H. Reinhardt, *Air sampling result in relation to extent of fungal colonization of building materials in some wáter-damaged buildings*, **Indoor Air, 10**, 2000, 145-151.

[11] K.H. Domch, W. Gam, T.H. Anderson, **Compendium of soil Fungi**. Vol. 1, Academic Press, London, 1980, p. 860.

[12] E. Levetin, R. Shaughnessy, E. Fisher, B. Ligman, J. Harrison, T. Brennan, *Indoor Air Quality in schools: exposure to fungal allergens,* ***Aerobiologia,*** **11**, 1995, pp. 27-34.

[13] H.S. Lee, B.W. Kang, J.P. Cheongs, S.K. Lee. *Relationships between indoor and outdoor air quality during the summer season in Korea****. Atmospheric Environment,*** **31**(11), 1997, pp. 1689-1693.

[14] H. Kanaani, M. Hargreaves, Z. Ristovski, L. Morawska, *Deposition rates of fungal spores in indoor environments, factorseffecting them and comparison with non-biological aerosols*, **Atmospheric Environment, 42**(30), 2008, pp. 7141-7154.

[15] T.S. Nayar, P.S. Jothish, *An assessment of the air quality in indoor and outdoor air with reference to fungal spores and pollen grains in four working environments in Kerala, India*, Aerobiologia**, 29**, 2013, pp. 131-152.

[16] E. Avrami, K. Dardes, M. De la torre, S. Harris, M. Henry, W. Jessup, **Evaluación para la Conservación: Modelo Propuesto para evaluar las Necesidades de Control del Entorno Museístico**. Getty Conservation Institute, Los Angeles, 1999. http://www.getty.edu/conservation/

[17] M.G. García Santa Cruz, M.J. García Santa Cruz, H.R. Vázquez, L. Iharlegui, Evaluación para la conservación y monitoreo ambiental edilicio de los espacios de reserva del Museo de La Plata, Argentina. Libro de Ponencias del VII Encuentro de Museos Universitarios del Mercosur y IV Encuentro de Latinoamérica y el Caribe, UACh, Valvidia, 2016.

[18] * * *, Norma UNI10586:1997. *Documentazione: Condizioni climatiche per ambienti di conservazione di documenti grafici e caratteristiche degli alloggiamenti.* Milano: Ente Nazionale di Unificazione.

[19] * * *, Norma UNI10829:1999. *Beni di interesse storico e artistico: Condizioni ambientali di conservazione*.

[20] M.J. Aira, F.J. Rodríguez-Rajo, V. Jato, E. Piontelli, *Análisis cuantitativo y cualitativo de la aeromicota aislada de la Catedral de Santiago de Compostela (Galicia, España)*, **Boletín Micológico, 21**, 2006. pp. 27-34

[21] E. Bogomolova, I. Kirtsideli, *Airborne fungi in four stations of the St. Petersburg underground railway system*, **International Biodeterioration and Biodegradation, 63**, 2009, pp. 156-161.

[22] A. Arya, A.R. Shah, S. Sadasivan, *Indoor aeromycoflora of Baroda museum and deterioration of Egyptian mummy*, **Current Scence, 18**(7), 2001, pp. 793-799.

[23] * * *, Wonder Makers Environmental Inc. Post-remediation guideline. Information Series 147. (2001). http://www.wondermakers.com/public_html/Articles/Remediation%20Guideline s.pdf

[24] * * *, **Microbial Sampling and Analysis: Molds and Bacteria**, Eagle Industrial Hygiene Associates, 2004, http://www.eagleih.com/micro.html

[25] H.C. Redulescu, G. Rosu, C. Popescu, A. Ispas, P.C. Ghituleasa, V. Lazar, *A microbial survey of the museal airborne fungal biodeteriogens*, ***Ge Conservación*, 11**, 2017, pp. 86-94.

[26] P. Saranraj, A. Javaprakash, *Agrobeneficial entomopathogenic fungi – Beauveria bassiana: A review*, ***Indo-Asian Journal of Multidisciplinary Research*, 3**(2), (2017), pp. 1051-1087.

[27] L.A. Elíades, M.N. Cabello, C. Voget, B. Galarza, C.M.N. Saparrat, *Screening for alkaline keratinolytic activity in fungi isolated from soils of the biosphere reserve "Parque Costero del Sur" (Argentina)*, **World Journal of Microbiology & Biotechnology, 26**, 2010, pp. 2105-2115.

[28] A.C. Ross, B. Halliss. *Microbiological deterioration of pulp wood, paper, and paint*. **Industrial Microbiology** (Editors: B. M. Miller and W. Litsky) Mc. Graw-Hill, New York, 1976, pp 309-354..

[29] M.S. Robledo, *Caracterización taxonómica, distribución y algunos aspectos fisiológicos de Aspergillus spp. que deterioran documentos en el Archivo General de la Nación*, **Tesis de Maestría** en Ciencias Biológicas, UNAM México, 1991.

[30] J. Singh, *European Heritage conservation and environmental monitoring-making informed decisions*, **European Heritage conservation environmentally sustainable Solutions**. Trinity College. Dublin, Ireland, 2003.

[31] M.L.E. Florian, **Fungal Facts. Solving Fungal Problem in Heritage Collections**. Archetype Publications Ltd., **London**, **UK**, 2004, p. 146.

[32] N. Valentin, *Microorganism in museum collections* **Coalition, 19**, 2010, pp. 2-5.

[33] E. Solís, *Estudio micológico del aire en áreas ocupacionales y exteriores del laboratorio de investigación en productos naturales ubicado en el edificio T-10 en la ciudad universitaria en zona 12 y el laboratorio ubicado en la zona 1 del centro de información y asesoría toxicológica del departamento de toxicología de la facultad de ciencias químicas farmacia de la universidad de San Carlos de Guatemala*, 2011, web: biblioteca.usac.edu.gt/tesis/06/06_3102.pdf. [Accessed: 06 jun. 2012].

[34] V.K. Özkan, *Microfungal contaminants on the surface of the books and the atmosphere of the library of health services vocational school in Marmaris, Turkey*, **International Biodeterioration and Biodegradation, 75**, 2014, pp. 83-88.

[35] A. Walter, **Basic Preservation Guidelines for Library and Archive Collections**, Natural Preservation Office, NPO Preservation Guidance. British Library, London, 2003.

[36] B. Gutarowska, Metabolic activity of moulds as a factor of building materials biodegradation, **Polish Journal of Microbiology, 59**, 2010, pp. 119-124.

[37] A.C. Mallo, D.S. Nitiu, L.A. Elíades, M.C.N. Saparrat, *Fungal degradation of cellulosic materials used as support for cultural heritage*, **International Journal of Conservation Science, 8**(4), 2017, pp. 619-632.

[38] B. Lighthart, L.D. Stetzenbach, *Distribution of microbial bioaerosol*, **Atmospheric Microbial Aerosols, Theory and Applications**, (Editors: B. Lighthart and A.J. Mohr) Chapman and Hall, New York, 1994, pp 68–98

[39] M.A. Stern, U. Alluitt, J. Corden, W. Millington, *The investigation of fungal spores in intramural air using a Burkard continuous recording air sampler*, **Indoor and Built Environment, 8,** 1999, pp. 40-48.

[40] D.W. Li, B. Kendrick**,** *A year-round study on functional relationships of airborne fungi with meteorological factors,* **International Journal of Biometeorology, 39**(2), 1995, pp. 74-80.

[41] A.M. Jones, R.M. Harrison, *The effects of meteorological factors on atmospheric bioaerosol concentrations-A review*, **Science Total Enviromental**, **326**, 2004, pp. 151-180.

[42] N. Valentin, *Microbial contamination and insect infestation in organic material*, **Coalition, 1**(6), 2003, pp. 2-3.

[43] I. Villalba, J. Mikan, J. Sanchez, *Actividades hidrolíticas y caracterización isoenzimática de poblaciones microbianas aisladas del patrimonio documental del Archivo General de Colombia*, **NOVA, 2,** 2004, pp. 50-58.

[44] S. Borrego, A.I. Perdomo, *Caracterización de la micobiota aérea en dos depósitos del Archivo Nacional de la República de Cuba*, **Revista Iberoamericana de Micología, 31**, 2014, pp. 182-187.

[45] M.E.B. Flores, P.G. Medina, S.P.D. Camacho, I. O. de Jesús Ramirez, M.E.T. Hernández, *Fungal spore concentrations in indoor and outdoor air in university libraries, and their variations in response to changes in meteorological variables*, **International Journal of Environmental Health Research, 24**(4), 2014, pp. 320-340.

[46] E.F. Hansen, *Protection of Objects from Environmental Deterioration by Reducing Their Exposure to Oxygen*, **Oxygen - Free. Museum Cases**, (Editor: Shin Maekahua.*)*, **Research in Conservation**, 1998, pp. 7-15.

[47] G.S. Westwood, S.W. Huang**,** N.O. Keyhani**,** *Allergens of the entomopathogenic fungus Beauveria Bassiana***, Clinical and Mollecular Allergy, 3**(1), 2005. doi:10.1186/1476-7961-3-1..

[48] D.S. Nitiu, A.C. Mallo, L.A. Eliades, M.C.N. Saparrat, H.R. Vazquez, *Monitoreo de la carga fúngica ambiental y de otros bioaerosoles de un depósito de restos humanos momificados del NOA del Museo de La Plata (Argentina): un estudio de caso*, **Boletín de la Sociedad Argentina de Botánica, 50**(4), 2015, pp. 427-436.

[49] D.S. Nitiu, A.C. Mallo, M.C.N. Saparrat, M.G. Garcia Santa Cruz, *Survey of the state of conservation of the Mylodon listai (Xenarthra-Mylodontidae) skin fragment from the Pleistocene of Argentina kept at the Museum of La Plata (Argentina),* **Ge-conservación, 10**, 2016, pp. 44-53.

[50] A.C. Mallo, L.A. Elíades, D.S. Nitiu, M.C.N. Saparrat, *Monitoring indoor air status in the Museo de La Plata Herbarium (LP) Argentina*, **Revista Iberoamericana de Micología, 34**(2), 2017, pp. 99-105.

[51] V. Schuenemann, A. Peltzer, B. Welte, W.P. van Pelt, M. Molak, C-C. Wang, A. Furtwängler, C. Urban, E. Reiter, K. Nieselt, B. Teβmann, M. Francken, K. Harvati, W. Haak, S. Schiffels, J. Krause, Ancient *Egyptian mummy genomes suggest an increase of Sub-Saharan African ancestry in post-Roman periods*, **Nature Communications, 2017**, 2017, DOI 10.1038/ncomms15694

Received: August 08, 2018
Accepted: May 25, 2019