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A microbial survey of the museal airborne fungal biodeteriogens

Hortensia Clara Radulescu, Georgeta Roșu, Cristina Popescu, Adriana Ispas, Pyerina Carmen Ghițuleasa, Veronica Lazar

Abstract: Tangible cultural heritage is exposed to multiple environmental risk factors able to affect its integrity and cultural function. Such factors are physical, chemical and also microbiological. Fungal biodeterioration is known to cause aesthetical and structural damage to materials, the effect increasing in the case of improper depositing or accidents like floods or water leakage. At the same time, air contamination with different fungal spores can add more a risking factor for heritage goods safety. Tracing of air biocontamination has a double significance: protection of museum` workers health from hazardous bio-aerosols and the control of the presence of biodeteriogens able to decompose museum pieces. The present paper will present the methodology used for the detection of the fungal species in the air of an ethnographical museum in Romania, in storage rooms, as well as in exposition rooms. Preliminary results show the predominance of fungal genera with known cellulolytic activity, such as *Aspergillus, Penicillium* and *Cladosporium* and direct correlation between the values of temperature and humidity and the concentration and types of air cultivable fungal species. Further more, the culture media used for the isolation of the air fungal species proved to be an important factor in the isolation methodology.

Key words: tangible heritage, heritage textiles, ethnographical textiles, ethnographical museum, fungal biodeterioration, air microbial load, aerobiology, cellulolytic fungi.

Un estudio microbiano de los biodeteriógenos fungosos aerotransportado en el museo

Resumen: El Patrimonio cultural material está expuesto a múltiples factores ambientales de riesgo que pueden afectar a su integridad y función cultural. Estos factores pueden ser físicos, químicos y también microbiológicos. El biodeterioro producido por hongos es un conocido causante de daño estético y estructural de los materiales, con un efecto mayor en el caso de deposición inadecuada o accidentes, como inundaciones o fugas de agua. Al mismo tiempo, la contaminación del aire con diferentes esporas de hongos puede constituir un factor de riesgo para la seguridad de los bienes patrimoniales. El rastreo de la biocontaminación aérea en museos tiene una doble utilidad: protección de la salud de los trabajadores en el museo ante bio-aerosoles peligrosos y el control de la presencia de agentes biodeteriorantes capaces de descomponer las piezas del museo. El presente documento presenta la metodología utilizada para la detección de especies de hongos en el aire de un museo etnográfico en Rumania, tanto en salas de almacenamiento, como de exposición. Los resultados preliminares muestran un predominio de géneros de hongos con actividad celulolítica reconocida, tales como *Aspergillus, Penicillium* y *Cladosporium* y una correlación directa entre los valores de temperatura y humedad con la concentración y los tipos de hongos presentes en el aire. Además, los medios de cultivo utilizados para el aislamiento de las especies fúngicas del aire se mostraron como un factor importante en la metodología de aislamiento.

Palabras clave: patrimonio material, textiles patrimoniales, textiles etnográficos, museo etnográfico, biodeterioro fúngico, carga microbiana del aire, aerobiología, hongos celulolíticos.

Introduction

Heritage Textile collections usually combine different types of natural fibres, either vegetal such as cotton, hemp, flax or animal, such as wool and silk. These natural macro-polymers are known to have physical-mechanical and physical-chemical properties that are modified over time by the action of different environmental parameters: temperature, humidity, light, ozone and microbial colonization (Bresee 1986; Luxford 2009; Duffus 2013). The combined action of all these factors leads to the ageing of the more fragile natural fibres, a process which today may be exacerbated by intensive cultural tourism, air pollution and climate change. The present debate regarding environmental conditions necessary for the conservation of collections is mainly focusing on the control of temperature and humidity being accepted that the more fragile a structure or a heritage object is, the tighter environmental controls are required (Atkinson 2014).

Most research considering environmental factors that affect the conservation status of heritage textiles is focused on the effect of temperature, humidity or light on these structures (Crighton 1992; Koussoulou, 1999; Luxford and Thickett 2011; Ren Yong-hua et al 2012), though other factors like the air microbial load or the air pollution can have an important impact to the safety of the Cultural goods, even bigger in certain cases, as water leakage accidents, or in a changing climate conditions.

Two standards have been issued in Europe, the first, EN 15757:2010 by Technical Committee 346 of CEN (European Committee for Standardization) and the second, PAS 198:2012, by the British Standards Institute that are addressing limits and ranges of values of temperature and humidity for safeguarding of collections (Atkinson 2014). In Romania, at national level there is a Governmental decision no. 1546 from 2003 which specify rules for maintain a microclimatic stability for temperature and humidity, the intensity of illumination and the control of air pollution with toxic gases (Gov. decision no. 1546/ 2003).

The biodeterioration which is seen as a process that induces undesired changes to the properties of a material determined by the presence and activity of organisms, is considered as an important risk factor for the conservation of cultural heritage objects (Pinzari et al 2011). Recent research underlines the effect of the bacterial and fungal communities on heritage objects and different molecular techniques are being currently developed for the non-invasive investigation of the microbial biodeterioration of the organic substrates (Capodicasa 2010; Sterflinger 2010; Montanari et al 2012; López-Miras et al 2012; Piñar et al 2015).

Air microbiological load is highly monitored in the health care sector but it could also be a risk factor for professional illness of workers in archives, libraries and heritage storage rooms. Through a rich enzymatic equipment microorganisms are able to degrade a broad range of materials (Allsopp et al 2004). They can also weaken the organic heritage materials and pave the road to an increased physical and mechanical degradation. Air can contain microbial species with a highly degradative potential for the heritage materials (Dyda et al 2016) and external atmospheric conditions can influence the quality and quantity of bacterial and fungal species inside museums and heritage buildings (Bulski 2016; Garcia 2016).

The aim of the research was the study of fungal species present in the air in the storage rooms and permanent exposition rooms of the Romanian Peasant Museum, an ethnographic museum in Bucharest, Romania. In order to achieve this aim, a monitoring methodology was developed and applied on the course of several months, starting with cold to warm weather conditions. The present paper presents the preliminary results of applying the chosen methodology.

Materials and methods

The museum characteristics

The National Romanian Peasant Museum, (MNTR) is an ethnographic and anthropological museum with extremely valuable collections, which has nearly 90 000 pieces representing costumes, ceramics, woods objects, icons and interior tapestry. The Museum is placed in Bucharest city center, at Victoria square, in a touristic area. Ethnographical textiles collections have a great significance, both because there are a great number of items, more than 30.000 pieces, and because they reflect all Romanian provinces starting with the first half of the 19th century. The heritage ethnographic textiles are fragmented in three collections deposited in modern storage rooms: The interior textiles collection, The rug collection and the Costumes collection [Figure 1]. A particular concept of the museum permanent exhibitions expose the ethnographic textiles together with objects from the traditional culture, wooden made, such as a church, a traditional house in open air, to increase visitor contact and understanding of the Romanian traditional culture. The structural materials of the ethnographic textiles are mainly natural fibers: hemp, flax, cotton wool and silk.

Figure 1.- The rug collection in a storage room (National Romanian Peasant Museum, Bucharest).

Measurement of the temperature and relative humidity

Simultaneously with the air microbial load sampling, the temperature (0C) and relative humidity (%) were measured in the same sampling points using a laboratory digital termohygrometer (Mannix, model SAM 990DW, Cole-Parmer).

Measurements of air microbial load

The air microbial load was determined in 7 storage rooms: interior textiles, rugs, light clothes, warm clothes, popular costumes and diverse textiles. In every room, the sampling was done in three points: in the front of the room, near

the ventilation system opening, between shelves and in the back of the room. The exposition rooms are situated on two levels, and on each level, they are interconnected. The air microbial sampling was done in a specific point kept the same for all the four rounds of samplings. Usually, the air sampling in the exposition rooms was done in the places where a bigger air load was supposed to be, i.e., under the roof of the "troița", a traditional wooden house for praying and resting. The sampling was done in seventeen exposition rooms for the first 2 rounds of samplings and in 10 rooms, for the last 2 round of samplings. The microbial air load sampling was achieved with SAMPL´AIR (Biomérieux) with 90 mm Petri dishes attached to the device and flowrates of 100 L/min and 200 L/min. The air sampling for measuring the microbial load was done in October 2015, January 2016, May 2016 and June 2016.

Determination of the cultivable fungal concentration in indoor air samples

The air fungal Petri dishes samples were incubated and the number of colony forming units was established for the determination of the fungal concentration expressed in CFU per cubic meter ($CFU.m^{-3}$).

Isolation of the fungal strains

The air sampling for measuring microbial load was done using Sabouraud Dextrose Agar (Biomérieux) and Sabouraud Dextrose Chloramphenicol (Biomériuex) culture media. After the incubation at 280C for 21 to 28 days, the colonies exhibiting different morphological characteristics, such as color of the colony on the face and reverse of the Petri dish, the texture of the colony surface, were isolated on Sabouraud Dextrose Agar with 3 concentrations in dextrose 2%, 4%, 6% and Potato Dextrose Agar. After the incubation of the fungal cultures, isolates exhibiting different morphological characteristics were selected for taxonomical identification.

Characterization of the fungal isolates

The fungal isolates were characterized by optical microscopy with lactophenol cotton blue staining technique at the Axio Imager A2 Optical Microscope (Carl Zeiss). The colonies surface morphology was observed at the Stereomicroscope Discovery V8 (Carl Zeiss). The growth characteristics were assessed by cultivation on four different media: Potato Dextrose Agar (Merck), Malt Extract Agar (Sanimed), Czapek-Dox Agar (Sanimed), and Yeast Extract Agar (Merck) and measurement of the growth rate at different intervals until the 14th day of incubation. The identification of the fungal isolates was done using dichotomous keys (Barnett and Hunter 1998).

Results and discussions

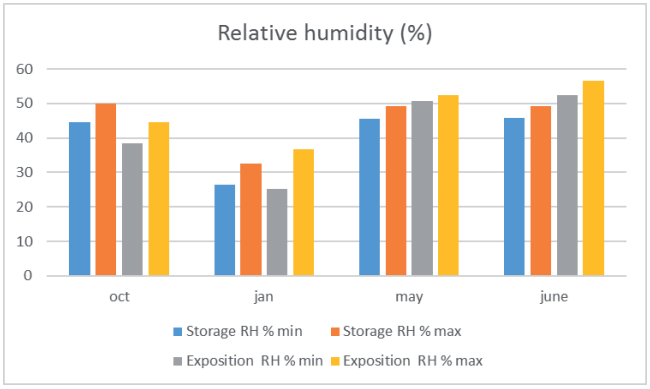
Air isolation of the cultivable fungal species and temperature and humidity measurements

When using Sabouraud Dextrose Chloramphenicol Agar (SABCh) the growth of fungal colonies after incubation of the plates was much more reduced then in the case of using Sabouraud Dextrose Agar (SAB) without any antibiotic. It can be suggested that at the initial sampling of an air sample, it is important to use culture media rich in nutrients for different fungal taxons, with different metabolic and physiologic behavior. A restricting media, such as it is SABCh, even though chloramphenicol it is an antibiotic used against bacteria, may also inhibit the formation of reproductive structures in the case of fungi. Another important aspect obtained during sampling and isolation of the fungal species from the air microbial load, was the type of culture media used in the 2nd round of cultivation. Our results showed that the use of another culture media, different from the culture media used in sampling increased the chance to obtain a fungal isolate. Thus, when using PDA (Potato Dextrose Agar), the colonies isolated from the SAB plates could further grow. When using the same type of culture media, i.e. SAB (Sabouraud Dextrose Agar), some of the colonies lost their ability to grow.

Differences in the number of colonies or species of obtained fungal isolates using air sampling flowrates of 100 L/min and 200 L/min were not found. However, the air sampling area was shown as an important factor determining cultivable fungal diversity. The fungal isolates increased in diversity in the case of the air samples taken at the opening of the air system ventilation in the storage rooms or under the roof of the" troița", the wooden praying construction from the permanent exposition room.

The measurement of the temperature and relative humidity during all 4 rounds of sampling comprising cold to warm weather showed a difference of 1 ^{0}C to 3 ^{0}C between rooms either storage or exposition rooms. Indoor air relative humidity had the highest levels in May, between 45% and 55 %. In January, this range was considerable low, with levels between 25 % and 35 % in both storage and exposition rooms [Graph 1]. In the months with increased levels of indoor relative humidity, the diversity of air cultivable fungal isolates was higher compared with the months with lower levels of indoor air relative humidity as for example in June, some yeasts were also isolated from the air samples.

The range of air fungal concentrations differed from one month to another, coinciding with differences in the temperature and relative humidity levels [Table 1]. Thus, the lowest fungal concentrations in the air samples were obtained in January, when both atmospheric parameters decreased drastically, especially the relative humidity. In storage rooms, without heating, the air fungal concentration was around 20.



Graph 1.- The measurement of relative humidity during the four rounds of air microbial sampling in storage and expositions rooms.

An exception was registered for the entrance of the storage room with summer costumes, were the fungal concentration was 70. In the expositions rooms, in January, the fungal concentration was approximately double than in storage rooms, but still under the number of fungi obtained in the other 3 sampling months. The highest concentration of fungi in the air was detected in October, with a mean value of 153 in storage rooms and 223 in exposition rooms. October is a month with high outdoor air relative humidity, because of the seasonal rains, and warm temperature (sometimes up around 20 0C). The combination of a high humidity and warm atmosphere could create favorable conditions for increasing fungal content in the air samples.

Characterization of the fungal isolates

Observations made with the stereomicroscope and light microscopy showed that some fungal isolates presented a dense mycelium of melanised hyphae, without the development of reproductive structures, which made classical taxonomical identification difficult [Figure 2]. The morphological features, like color of the colonies, the intensity of growing was slightly different, depending of the type of culture media used. The identified fungal species in the air samples were Aspergillus niger, Aspergillus flavus, Cladopsorium sp., Paecilomyces sp., Stachybotrys sp. and Penicilium sp., and also some unidentified yeast species [Figure 3]. All these genera and species are usually found in the indoor and outdoor air and are known to decompose cellulosic substrates (Pinar et al 2016; Amore et al 2013; Foladi et al 2013; Hussain et al 2012; Aro et al 2005; De Vries and Visser 2001; Das et al 1997). When cultivating the fungal isolates on different types of culture media, in order to assess the growth characteristic, the best growth was observed on the PDA culture media, followed by Czapek-Dox media. The growth on MEA was poor and for most fungal isolates Yeast Extract Agar didn't allow a good growth and sporulation.

The preliminary results of this research suggest that the air microbial load, in terms of quantity and diversity can be correlated with atmospheric conditions, such as outdoor temperature and humidity and the sampling area. The culture media used in the air sampling and further fungal strains isolation play an important role for the accurate monitoring. In the case of the ethnographical museum, Sabouraud Dextrose Agar and Potato Dextrose Agar gave the best results in sampling and isolation of the fungal air contaminats. The developed methodology for monitoring the fungal air load in the ethnographical museum can be part of a preventive conservation strategy in this museum, in which daily measurements of the temperature and relative humidity are also performed. Further more, the implementation of such a strategy can also favor the workers' healthcare, especially in places such as storage rooms or archives.

Table 1.- Concentration of the fungi in the air samples from storage and exposition rooms (CFU.m^{-3}).

Sampling Round	Storage rooms			Permanent expositions rooms		
	Interior textiles f/m/b*	Rugs f/m/b	Summer costumes f/m/b	The "beauty" room	The "troiţa" room	"Triumph" room
October 2015	180/-/-**	190/-/-	90/-/-	210	210	250
January 2016	20/20/30	20/20/20	70/30/20	40	50	40
May 2016	45/75/80	85/130/115	50/80/80	110	35	90
June 2016	45/30/35	45/35/15	40/40/10	90	80	40

* front of the room (f)/ middle of the room (m)/back of the room (b); **in the first round from storage rooms air samples were taken from the front of the room only.

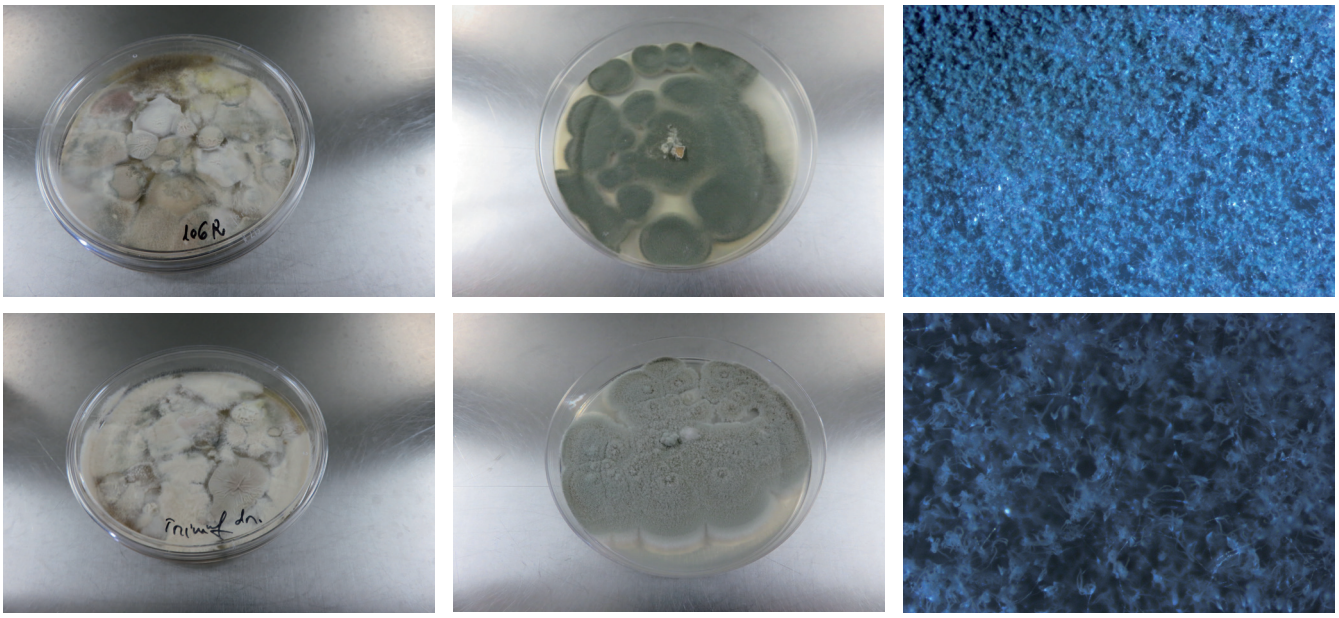


Figure 2.- Fungal growth after incubation of air samples plates (first image), isolation of a single colony (2nd image) and stereomicroscope image (3rd image) of fungal mycelium collected from storage room (up row) and exposition room (down row).

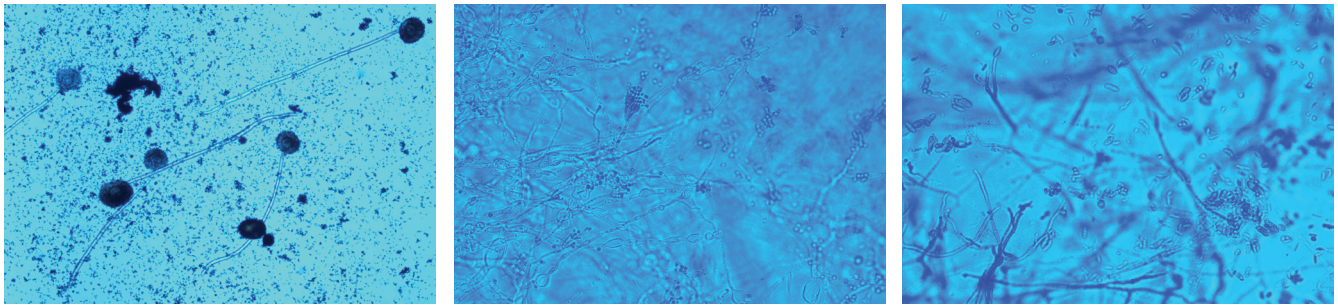


Figure 3.- Light microscope images of fungal isolates stained with lactophenol cotton blue: *Aspergillus* sp. (1st image), *Penicillium* sp. (2nd image) and *Cladopsorium* sp. (3rd image).

Conclusions

A methodology for monitoring the air fungal load was applied for the identification of the cultivable fungi in storage and permanent exposition rooms in an ethnographical museum. Fungal species belonging to different genera such as *Aspergillus, Cladosporium, Paecilomyces, Stachybotrys* and *Penicillium* were isolated and identified by classical methods. The comparison of results from air sampling done in cold and warm months, together with measurement of the temperature and relative humidity, have permitted to analyze the influence of these factors in fungal air content. The highest concentration of fungi in the air samples was obtained in the month with higher temperature and relative humidity values which could be favorable to sustain fungal growth.

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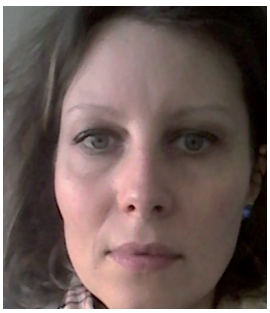

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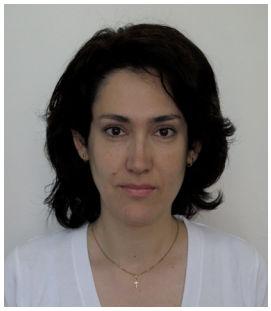

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