Indoor Air Quality: Focus on Fungi

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Aim

To perform an indoor air quality evaluation assessing the fungal communities present in the air and surfaces of four selected Portuguese Archives. The study also includes the surface sampling of ancient documents.

Introduction

Fungi can pose a threat to both human and cultural heritage. In documents, fungi are considered responsible for the formation of foxing spots and the general degradation of written heritage. In contact with the skin or when inhaled by workers and readers, fungi may cause health problems either by toxin's emission of by the presence of fungal debris or spores. Nevertheless, the complete study of these fungal communities is still giving its first steps since only now molecular techniques are being used [1]. This project aims to assess the indoor air quality in archives, with a particular emphasis on fungal development. One of its main goals is to develop a standard procedure for the identification of the individual components of fungal communities present on the cultural written heritage stored in archives. To identify them a culture independent technique - DHPLC - is being put to use. This method allows separation of PCR products using an ion-pair reversed-phase high performance liquid chromatography and has offered exceptional advantages when it comes to the identification and isolation of microorganisms from complex microbial communities [2] [3]. The study also includes a chemical and physical parameters analysis of the environment surrounding both documents and workers.

Methods

Chemical assessment

Equipment used in the chemical evaluation of the environment. Overall, 15 rooms were analysed

Handheld 3016 IAQ

Multi Rae and Babuc

Biological assessment

In the air...

Using Millipore Air Tester 20 rooms have been analysed so far

In the ceiling surface...

Sampling was done whenever fungal growth was visible (1 case)

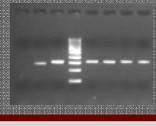
In the document´s surface...

A total of 15 documents have been analysed so far

PCR

Culture media: Agar, MEA and DG18

Amplification of the ITS2 and D2 genomic regions



Water activity determination (Rotronic,ITISE)

Results

Chemical results from one of the archives

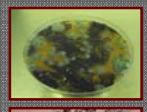
Parameter	Sampling site				
	Exterior (ref. point)	A	B	C	D
O_3 (ppm)	0,59	0,7	0,69	0,7	0,68
Formaldehyde (ppm)	0	0,003	0,002	0	0
CO (ppm)	0	1	0	0	0
VOCs (ppm)	0	0	2	2,6	0
CO2 (ppm)	449	604	504	471	451
Particulate matter (PM10) mg/m3	0,042	0,129	0,047	0,033	0,147
Temp.(ºC)	9,9	17	18,1	18,6	15,4
RH(%)	51,4	47,5	70,2	77,1	46,2

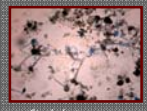
Parameters determined in the selected Archive. These were then analysed in terms of human health and document's conservation

Air sampling - Analysis performed after incubation (malt extract agar, 27ºC, 5-7 days)

Site	Fungi identified	CFU/m³	Total
Exterior	*Aspergillus terreus*	4	136
	Aureobasidium sp.	16	
	Fusarium incarnatum	12	
	Alternaria sp.	12	
	Aspergillus ochraceus	4	
	Ulocladium sp.	4	
	Cladosporium sp.	56	
	Penicillium sp.	12	
	Rhodotorula sp.	4	
	Yeasts	12	
Office	*Aspergillus* sp.	8	96
	Penicillium sp.	12	
	Alternaria sp.	12	
	Graphium sp.	12	
	Cladosporium sp.	24	
	Paecilomyces sp.	4	
	Yeasts	24	
Archive	*Aspergillus ochraceus*	8	36
	Paecilomyces sp.	4	
	Geotrichum sp.	4	
	Yeasts	20	

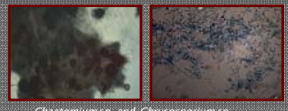
Colony forming units (CFU) determined in the air sample



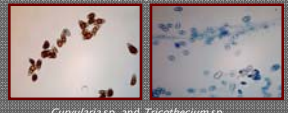


Stachybotrys sp. a potentially toxinogenic fungi producing *Satratoxin-H*

Identification of fungi in culture media



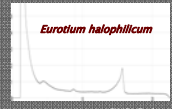
Chaetomium sp. and *Chrysosporium* sp. (400x)



Curvularia sp. and *Tricothecium* sp. (400x)

No growth in any culture media

DNA extraction
ITS2 and D2 amplification



DHPLC chromatograms:
DNASep Cartridge 0.5mL/min, Wave optimized buffers A and B, gradient 55%B at 61ºC

So far...

1. The chemical assessment performed alerted for values above the national norm (annex VII, decree-law n. 79/2006) regarding human health (O_3 and VOCs). For conservation purposes, the ozone level was also found to be higher than desirable.
2. The biological assessment of the environment yielded the identification of *Stachybotrys sp.* (potentially toxinogenic) which was followed by remedial action. This same assessment in documents surfaces made it possible to identify keratinophylic and cellulolytic fungi.
3. The dHPLC method is a non-labour intensive method for resolving complex mixtures of fungal DNA.

Bibliography

1) Michaelsen A, *et al* "Application of molecular techniques for identification of fungal communities colonizing paper material", International Biodeterioration & Biodegradation, Vol. 58 (2006) 133-141 **2)** Barlaan EA *et al* ,"Profiling and monitoring of microbial populations by denaturing high-performance liquid chromatography", in Journal of Microbiological Methods 61 (2005) 399-412 **3)** Goldenberg O *et al*, "Use of Denaturing High Performance Liquid Chromatography for Rapid Detection and Identification of Seven Candida Species, in Journal of Clinical Microbiology Vol.43, No.12, (2005) 5912-5915.

Acknowledgments

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