Xerophilic fungi in museum repositories challenge our perception of healthy buildings and the preservation of cultural heritage

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ABSTRACT

Within the last decade, fungal infestations have emerged in Danish museum repositories challenging museum staff's health and heritage preservation. The growth is unexpected, as most repositories are climate-controlled, according to the international guidelines for heritage collections. This pilot study aims to enlighten unexpected fungal growth in three climate-controlled repositories. The environmental conditions were assessed with measurements of relative humidity (RH), temperature, and material moisture content (MC), showing no evidence of elevated moisture. Morphological and molecular identification showed the growth of *A. halophilicus*, *A. domesticus*, *A. magnivesiculatus* and *A. vitricola*; four xerophilic fungi able to grow at low water activity. Except for these species, none of the detected airborne species gave rise to growth. The growth of xerophilic fungi is inexplicable but may be associated with a revision of the international environmental guidelines for heritage collections expanding the RH range. The study questions if the revision adequately prevents the risk of fungal growth to ensure heritage preservation and the occupational health of the museum staff.

INTRODUCTION

Museums have a leading role in preserving the tangible and intangible heritage of extinct nature and human cultures for posterity and communicating history to people and society. To meet this obligation, most heritage collections are stored in museum repositories serving as documentation of history from ancient to present and as sources for future research and dissemination (ASHRAE, 2019; Elkin and Norris 2019).

Fungal infestations have become an increasing problem in heritage collections in museums, galleries, and archives (Ranalli et al. 2009, Sterflinger, 2010; Sterflinger & Piñar, 2013). In Denmark, the growth is unexpected since the relative humidity (RH) in most repositories is controlled according to international environmental guidelines for preserving heritage collections (ASHRAE, 2019; Kerschner, 2013).

The environmental guidelines for heritage collections have been an ongoing discussion among conservators and conservation scientists for more than 30 years. Based on Garry Thomson's work "*The Museum Environment*" (Thomson et al., 1978, 1986), and interpretations of research on the ageing of hygroscopic materials, the consensus has been to maintain RH strictly controlled in the range of $50 \pm 5\%$ RH and 20-21 $\pm$ 2°C (reviewed by Atkinson, 2014). In the last decade, the heritage society has argued that these strict setpoint values should be expanded to values less energy-intensive (Ashley-Smith et al., 2013; ASHRAE, 2019; Atkinson, 2014; Bickersteth, 2014; Staniforth, 2014). The discussions have led to an agreement on keeping the temperature (T) in the range of 15-25°C and RH in the range of 40-60% (Bickersteth, 2014; Kerschner, 2013). The 2019 ASHRAE Handbook advise RH 35-65% with no limits of T supported by the British Standard (ASHRAE, 2019; British Standard, 2012), while The European Standards do not define RH and T more precisely than "high" and "low" levels (European Standard, 2010; European Standard, 2013; European Standard, 2018). Revising the guidelines provides more sustainable storage of heritage collections by reducing the energy consumption, the carbon footprint and the cost. However, it is not well researched whether the revision increases the risk of fungal growth in the heritage collections.

When fungal growth develops in heritage collections, it challenges our perception of healthy buildings and the preservation of cultural heritage. Fungal growth deteriorates materials and, thereby, heritage artefacts (Caneva et al., 2009; Ranalli et al., 2009; Sterflinger, 2010; Sterflinger & Piñar, 2013). In addition, fungal growth poses a human health hazard (Afshari et al., 2009, Borchers et al., 2017; Nevalainen et al., 2015; Rudert & Portnoy, 2017) and may affect the occupational health among the museum staff. Therefore, preventive conservation by controlling and monitoring the environmental conditions to avoid fungal growth is of the highest importance.

In this pilot study, we examined the environmental conditions, the fungi present in dust and the fungi causing growth on heritage artefacts in three Danish museum repositories climate-controlled according to the environmental guidelines for heritage collections. The aim was to enlighten inexplicable fungal growth in the three repositories to assess if the preventive conservation strategies sufficiently ensured healthy buildings supporting heritage preservation and the health of museum staff.

METHODS

Selection of study sites

The three museum repositories (R1-R3) were selected based on three main criteria: 1) the storage facilities should be climate controlled according to the international environmental guidelines for heritage collection, 2) the collections should include heritage artefacts with fungal growth, and 3) the museum staff should have reported work-related health nuisance.

Risk assessment of occupational health

Examination of the repositories was conducted after a risk assessment of occupational health. Personal safety equipment was chosen based on the risk assessment according to the Danish work environment legislation, and the equipment was used during the fieldwork.

Examination

The examination included 1) outdoor and indoor building inspection of the storage facilities and photo documentation, 2) acquiring data loggings of RH and T from the museums, 3) measurement of RH, T, and material moisture content (MC), 4) inspection of the heritage collection for fungal growth and photo documentation, and 5) fungal examination conducted with air sampling, and surface sampling on heritage artefacts followed by morphological identification and DNA sequencing of selected fungal isolates.

Measurement of RH, T and material moisture

Three measurements were carried out at each measuring point. Measurements of RH and T were conducted with an Elsec 765 Environmental Monitor according to the manufacturer's instructions. Measurement of MC in building structures: the floor, the ceiling, and the walls, were conducted with Gann Moisture Measuring Hydromette compact B according to the manufacturer's instructions specifying MC in selected building materials as 1) dry, 2) risk and 3) wet. Measurements of surface temperature on selected heritage artefacts, the floor, the ceiling, and the walls were conducted with Testo 835-H1 IR thermometer with an inbuild moisture meter.

Fungal surface sampling and morphological ID

Nine heritage artefacts with visible fungal colonies were selected at the museums. The artefacts were made in three different materials: pinewood, leather, and wool. Fungal particles were sampled from colonies on the heritage artefacts with sterile rayon swabs (Sarstedt tube applicator). The nine swabs were inoculated on agar with different a_w: V8® Vegetable Juice Agar (V8), Dichloran 18% Glycerol Agar (DG18), and Malt Yeast 50% Glucose Agar (MY50G) (Samson et al., 2019). The V8 and DG18 plates were incubated for seven days at 25°C in darkness. The MY50G plates were incubated for 21 days at 25°C in darkness. The appearing colonies were transferred to fresh agar plates with streak inoculation and incubated for 7 and 21 days at 25°C in darkness. Further isolation was conducted with three-point inoculation on the agars suggested for the morphological ID of the species in question (Sklenář et al., 2017; Samson et al., 2019). The isolated fungal species were identified visually at 40 and 400x magnification, according to Sklenář et al., 2017; Samson et al., 2019, Samson et al., 2014.

Fungal air sampling and morphological ID

Air sampling was conducted outdoor and indoor in three areas in the repositories by MAS 100 ECO for one minute on V8-agar, DG18-agar and MY50G-agar. The indoor air sampling was conducted after air circulation making the deposited dust on surfaces airborne to simulate activity (Schrock et al., 2011). The agar plates were incubated, isolated and identified similar to the surface samples.

Fungal identification with DNA sequencing

The morphological ID of surface samples was confirmed by DNA sequencing. DNA was purified from single colonies growing on agar plates using a Fast DNA Spin Kit for Soil (MP Biomedicals, USA). PCR amplification of fungal DNA regions was conducted by use of a Taq DNA Polymerase Kit (Ampliqon, Denmark) according to the manufacturer's manual. To obtain a good separation of the xerophilic species, calmodulin primers (cdm5/cdm6) were used in PCR amplification (Sklenář et al., 2017). A single isolate gave no PCR product with cdm5 / cdm6, and ITS primers were used. DNA fragments were sequenced by use of a BigDye Terminator v.1.1 Cycle Sequencing Kit (Thermo Fisher, USA) and by the use of a SeqStudio Genetic Analyser from Applied Biosystems (Thermo Fisher, USA). The sequences obtained were analysed using the EMBL-EBI homepage BLAST service (ebi.ac.uk). The ClustalX2 program was used for sequence alignments.

RESULTS

Description of study sites

The museum repositories belonged to three Danish cultural history museums in the region of Zealand. Repository 1 and 2 were established in two rebuilt warehouse facilities thermally highly insulated to keep a stable indoor climate (fig. 1). Repository 3 was built as a museum repository based on a sustainable museum storage concept (Christensen et al., 2016).

Fig 1. Repository 1 established in a rebuilt warehouse facility highly thermally insulated to keep a stable indoor climate

The collections included several hundred thousand historical and archaeological artefacts documenting Danish history and prehistory. The heritage artefacts consisted of a wide range of organic and inorganic materials in different states of preservation. Small museum artefacts were packed in museum boxes and paper-based packing materials with low acidity, as recommended for storage of heritage collections, while large artefacts were freely shelved (fig. 2). Interior, such as shelving, was made of painted steel.

Outdoor and indoor building inspection

Outdoor and indoor building inspections showed no evidence of damages on the building envelope, causing propagating water. There was also no evidence of structural faults in the building constructions, causing elevated moisture in building structures.

The three museums were striving to comply with the international environmental guidelines for heritage collections, with RH fluctuating between 40–60% annually. Desiccant dehumidifiers controlled RH, with setpoint values at 50-55% RH. The temperature was passively controlled through a highly insulated building envelope.

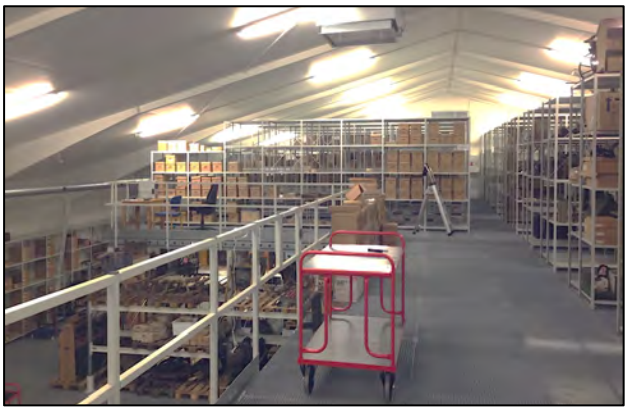

Fig 2. Repository 1 appeared in a good order

The data loggings of RH and T conducted as a part of the preventive conservation strategies at the museums showed that the dehumidifiers managed to keep RH slowly fluctuating between 52-63% RH during six months, while T fluctuated between 10°C and 23°C in the same period (fig. 3-5).

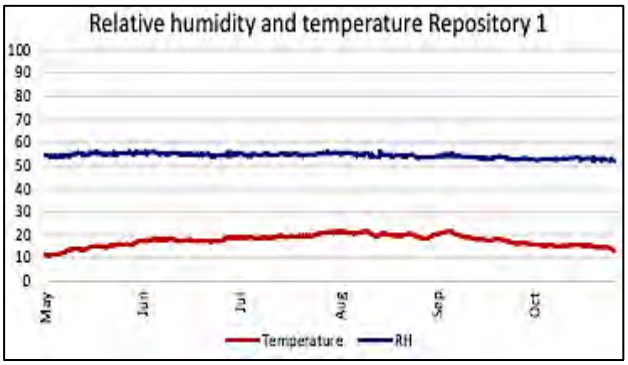

Fig 3. Six months variations of RH and T in repository 1

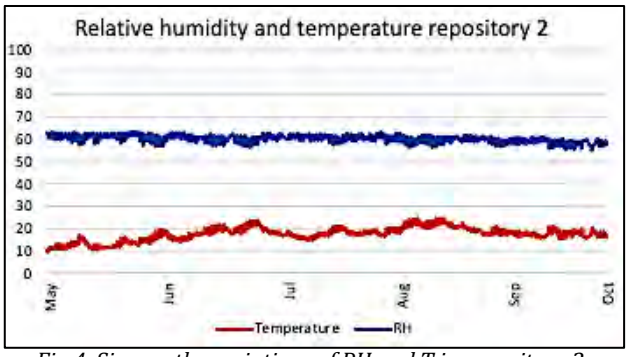

Fig 4. Six months variations of RH and T in repository 2

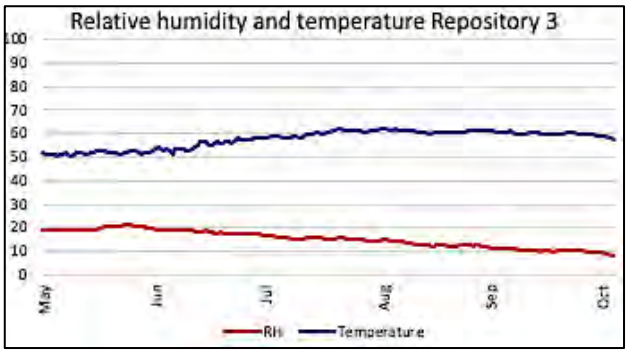

Fig 5. Six months variations of RH and T in repository 3

In addition, RH and T were measured on the day of examination (table 1). The measurements supported the data-loggings of RH and T.

Table 1. RH and temperature on the day of examination

	Repository 1	Repository 2	Repository 3
$\overline{RH}$ (%)	56	58	52
$\overline{T}$ (°C)	18	16	19

Measurement of RH, T and material moisture

Measurements MC in the floor, the ceiling, and the walls showed no elevated moisture levels indicating intrusive moisture (table 2).

Table 2. Material moisture on the day of examination in museum repository R1-R3

	R1 $\overline{MC}$	R2 $\overline{MC}$	R3 $\overline{MC}$
Floor (concrete)	Dry	Dry	Dry
Ceiling (gypsum board)	Dry	Dry	Dry
Wall north (gypsum board)	Dry	Dry	Dry
Wall south (gypsum board)	Dry	Dry	Dry
Wall east (gypsum board)	Dry	Dry	Dry
Wall west (gypsum board)	Dry	Dry	Dry

Measurements of surface temperature on the heritage artefacts, the floor, the ceiling, and the walls (table 2) showed no evidence of microclimate when RH and T were compared to the water vapour chart. The surface temperature was slightly lower on the floor and the north-facing and west-facing walls than on the ceiling, east-facing and south-facing walls, while the heritage artefacts had the same temperature as the air (table 3).

Table 3. Measurements of surface temperature on the day of examination in museum repository R1-R3

	R1 $\overline{T}$ (°C)	R2 $\overline{T}$ (°C)	R3 $\overline{T}$ (°C)
Floor (concrete)	13	11	13
Ceiling (gypsum board)	22	19	23
Wall north (gypsum board)	16	14	16
Wall south (gypsum board)	21	18	21
Wall east (gypsum board)	19	17	19
Wall west (gypsum board)	17	15	17
Heritage artefact pine wood	18	16	19
Heritage artefact leather	18	16	19
Heritage artefact wool	18	16	19

Morphological ID of fungal isolates from artefacts

Inspection of the three museum repositories and their stored heritage collections showed no fungal growth concerning building structures and interiors. The fungal growth was solely associated with the stored heritage artefacts and appeared as distinct hyaline and white colonies on artefacts (fig. 6-8). It was not just artefacts made of organic materials such as wood, leather and textile that showed growth, but also artefacts made in inorganic material such as cast iron and ceramics (fig. 6). The growth was not widespread throughout the museum repositories; the growth was most sparse in repository 3, concentrated to few artefacts, while repository 1 and 2 showed heavier but still sporadic growth.

Fig 6. A prehistoric urn with fungal growth (repository 1)

Fig 7. A bicycle leather case with fungal growth (repository 2)

Fig 8. A wooden tool with fungal growth (repository 3)

Cultivation and morphological identification of surface samples from fungal colonies on the museum artefacts showed no growth on V8 and DG18 agar (table 4). Growth solely developed on MY50G characterised by low a_w. The fungal colonies were morphologically identified to the xerophilic fungal species *A. halophilicus* and unidentified *Aspergillus sp.* (table 4).

Table 4. Fungal isolates from nine heritage artefacts in museum repository R1-R3 identified morphologically

Museum		V8	DG18	MY50G
R1	Wood	-	-	*A. halophilicus* *Aspergillus sp.*
	Leather	-	-	*A. halophilicus* *Aspergillus sp.*
	Wool	-	-	*A. halophilicus*
R2	Wood	-	-	*A. halophilicus* *Aspergillus sp.*
	Leather	-	-	*A. halophilicus* *Aspergillus sp*
	Wool	-	-	*A. halophilicus* *Aspergillus sp.*
R3	Wood	-	-	*A. halophilicus*
	Wood	-	-	*A. halophilicus*
	Leather	-	-	*A. halophilicus*

Morphological ID of fungal isolates from air

Cultivation and morphological identification of air samples from the three museum repositories showed the presence of 28 different fungal species (table 5). There was an overlap of species in the three museum repositories. Of the 28 species, two are classified as risk group 2 that can cause human disease and might be a hazard to workers, 15 species are classified as risk group 1 that are unlikely to cause human disease (IFA, 2021), and 11 species are unclassified (table 5).

ID of isolates from artefacts by DNA sequencing

DNA sequencing was conducted on pure fungal isolates from the nine examined heritage artefacts (table 6). The DNA sequencing confirmed the morphological identification of *A. halophilicus,* causing growth in all three museum repositories. Besides, *A. domesticus, A. magnivesiculatus* and *A. vitricola* were identified. The three species were not identified to species level morphologically.

Table 5. Fungal isolates from air samples in three museum repositories (R1-R3) identified morphologically and their risk class in relation to health hazard (IFA, 2021)

Fungal species	Risk class	R 1	R 2	R 3
***Aspergillus* sp**	-	✓	✓	✓
A. calidoustus	2	✓		
A. candidus	1			✓
A. domesticus	-	✓	✓	
A. halophilicus	-	✓	✓	✓
A. magnivesiculatus	-	✓		
A. montevidensis	-		✓	
A. nidulans	1	✓		
A. niger	2	✓		
A. ruber	-			✓
A. versicolor	1	✓	✓	✓
A. vitricola	-	✓	✓	
Penicilium sp.	-	✓	✓	✓
P. brevicompactum	1	✓	✓	✓
P. buchwaldii	-	✓		
P. citreonigrum	1			✓
P. chrysogenum	1	✓	✓	✓
P. corylophilum	1	✓		
P. crustosum	1		✓	
P. thomii	-			✓
Alternaria sp.	1	✓	✓	✓
Botrytis cinerea	1		✓	
Botrysporium sp.	-	✓		
Cladosporium sp.	1	✓	✓	✓
Chaetomium globosum	1	✓	✓	✓
Engyodontium album	1	✓		
Epicoccum nigrum	1	✓		
Mucor circinelloides	1		✓	

Table 6. Fungal isolates from nine heritage artefacts in three museum repositories identified with DNA sequencing

Museum	Material		Similarity
R1	Wood	*A. halophilicus*	100 %
		A. domesticus	100 %
		A. vitricola	100 %
	Leather	*A. halophilicus*	100 %
	Wool	*A. halophilicus*	100 %
		A. magnivesiculatus	100 %
R2	Wood	*A. halophilicus*	100 %
		A. domesticus	100 %
	Leather	*A. halophilicus*	100 %
		A. vitricola	100 %
	Wool	*A. halophilicus*	100 %
		A. domesticus	100 %
R3	Wood	*A. halophilicus*	100 %
	Wood	*A. halophilicus*	100 %
	Leather	*A. halophilicus*	100 %

DISCUSSION

In this study, we documented growth of *A. halophilicus, A. domesticus, A. magnivesiculatus, and A. vitricola* on heritage artefacts in three climate-controlled Danish museum repositories. We also showed that air samples detected more fungal species than surface samples from colonies on artefacts and that there was an overlap of species in the three museum repositories.

The airborne fungi were species primarily found in indoor environments such as domestic homes (Samson et al., 2019). The fungal species originated from dust layers on the interior and building structures which became airborne with air circulation before sampling. However, *A. halophilicus, A. domesticus, A. magnivesiculatus,* and *A. vitricola*, growing on the museum artefacts, also contributed to the airborne fungi. Except for the four xerophilic species, none of the detected airborne species gave rise to fungal growth in the repositories.

It was surprising that the fungi growing on the museum artefacts were all xerophilic fungal species from *Aspergillus* Section *Restricti*, which can grow on substrates with low a_w and other extreme environments (Sklenář et al., 2017). When fungi can grow on substrates with low a_w, it is equivalent to growth at low RH. Museum repositories may provide this environment, as they are usually climate-controlled within RH 40-60%.

An ever-present joker, when working with fungi, is that you find what you are looking for. When fungal infested museum repositories are examined, the dominating methods are surface sampling followed by cultivation and morphological / molecular identification, as in the study of domestic environments (Mazzoli, 2018). In housing, though, fungal growth is primarily caused by damp indoor environments or water damages, which is a different premise than climate-controlled museum repositories. In this study, an agar with a low a_w (MY50G) was included, making it possible to identify the xerophilic fungi growing on the museum artefacts. If this agar had not been included, the xerophilic fungi would have been non-detected.

When fungal growth develops in heritage collections, it affects not only heritage preservation; it may also affect the health of museum staff. Fungal growth acts as a pollutant, releasing physical and chemical substances suspected of adversely affecting human health (Afshari et al., 2009; Borchers et al., 2017; Nevalainen et al., 2015; Rudert & Portnoy, 2017).

In this study, two of 28 species, *A. calidoustus* and *A. niger,* were classified as risk group 2 according to the German classification of biological agents in relation to health hazard (IFA, 2021). Fungi in risk group 2 can cause human disease and might be a hazard to workers. *A. calidoustus* and *A. niger* were detected from the airborne dust in the repositories and did not give rise to growth in the repositories.

Of the remaining 26 species isolated from dust, 15 species were classified in risk group 1, including biological agents that were unlikely to cause human disease, and 11 species were unclassified, including the four xerophilic species growing on the museum artefacts.

When the hazard of specific fungi has not been classified and there, in general, are no limit values for fungi with regard to occupational health (Afshari et al., 2009), risk assessment of potential human exposure during work is complicated. In addition, there is no clear consensus on which risk-class specific fungal species are belonging to internationally, as the classification of biological agents depends on national regulations. However, studies on fungal contamination in museums show that museum workers may be at greater risk of developing health symptoms and disease when they are exposed to fungi during work (Gutarowska et al., 2015; Skora et al., 2017; Skora et al., 2015; Wiszniewska et al., 2009, 2010). Therefore, the detected fungal species pose a potential risk to the occupational health of museum staff at the three museums.

A more accurate risk assessment of the fungal exposure during work requires other and more analyses, including measurements of concentrations of the airborne fungi during the handling of fungal infested museum artefacts and more in-depth knowledge of the hazard of the present species.

In this study, air-circulation was conducted with a standardised method before sampling with a stationary microbial air sampler. The intention was to enlighten the fungal composition in three climate-controlled museum repositories, considered healthy buildings according to the environmental guidelines for heritage collections. The study completely changed the perception of the museum repositories as healthy buildings. The growth of xerophilic fungi poses a hazard to both the preservation of heritage artefacts and occupational health.

When a museum collection poses a health hazard, the accessibility to the heritage artefacts is significantly reduced, and the value for research and dissemination of knowledge through exhibition and education is diminished. These consequences threaten the very purpose of museums, where preservation, research and dissemination of knowledge are essential cornerstones. Therefore, preventive conservation strategies to avoid fungal growth is of the highest importance.

The study documented the growth of xerophilic fungi but did not clarify the cause. The three museums were striving to comply with the international environmental guidelines for heritage collections and have been storing museum artefacts for more than a decade without problems with fungal growth. The fungal growth was only associated with the heritage artefacts. There was no growth on surfaces with suspected elevated a_w such as close to the floor, the outer walls and other colder zones.

Based on measurements of RH, T, and MC, it was not surprising that solely xerophilic fungi were causing the growth. The environmental conditions were not lucrative for the growth of the most common indoor fungal species. No one knows precisely when the fungal infestations in the three museum repositories occurred, as the data-loggings of RH and T have not been conducted continuously throughout the years due to technical challenges. However, periods without monitoring RH and T were very short. According to the museums, there had been no evidence of intruding moisture giving rise to elevated RH. Although there was no evidence of microclimate supporting fungal growth, a_w on surfaces has been adequate for xerophilic fungi germination. Data-loggings showed short-term periods with RH up to 63%. The question is, how much moisture is too much moisture regarding germination of xerophilic fungi?

The effect of RH and a_w on the germination of xerophilic fungal spores are not well researched. In general, germination of fungal spores can occur if RH and a_w are briefly raised. Subsequently, most fungi can grow at lower a_w (Deacon, 2009, Ponizovskaya et al., 2011). The growth of xerophilic fungi in the three museum repositories has occurred in parallel with the revision of the guidelines for environmental conditions for heritage collections and the global climate changes. If there is a causal relationship, it has not yet been studied.

Within the last decade, many Danish museums have implemented the revised environmental guidelines for heritage collections. Maintaining a strict museum environment concerning RH and temperature is energy-consuming and not adjusted to the fact that museums worldwide are located in very different climatic zones. Revising the guidelines supports a desire to reduce the carbon footprint and obtain more sustainable storage of heritage collections. In theory, an upper limit at 60 % RH should not increase the risk of fungal growth, as the guidelines for environmental conditions for heritage collections defines the limit of growth to 70% RH (ASHRAE, 2019; Caple, 2011; Elkin et al., 2019; EN:16893, 2018). However, it has not been studied if the revision could increase the risk of xerophilic fungal growth before it was accepted and implemented. This study indicates that this could be the case.

The growth of xerophilic fungi is not only observed in Danish museum repositories. Within the last decade growth of xerophilic fungi has been reported in several cultural heritage studies (Katja Sterflinger et al., 2018; Liu et al., 2018, Piñar et al., 2016). In particular, libraries and archives have been reporting xerophilic fungal growth (Micheluz et al., 2015, 2018; Polo et al., 2017), while studies in museum repositories are sparse.

The global climate changes are, in Denmark, manifested in more precipitation and cloudbursts. The climate changes may place greater demands on museum repositories in terms of density and dehumidification if a stable and preventive indoor climate is to be maintained. The examined storage buildings are dimensioned before awareness of climate changes and could be undersized to withstand the changing outdoor climate. When the limits of germination and growth of xerophilic fungi are not well defined, it is hard to recommend specific environmental conditions preventing these fungi.

CONCLUSION

This pilot study aimed to enlighten unexpected fungal growth in three Danish museum repositories climate-controlled according to the international environmental guidelines for heritage collections.

The study surprisingly showed the growth of *A. halophilicus, A. domesticus, A. magnivesiculatus* and *A. vitricola,* four xerophilic fungal species from the *Aspergillus* Section *Restricti*, characterised by the ability to grow on substrates with low a_w. Museum repositories may provide this environment, as they usually are climate-controlled regarding RH. The growth of xerophilic fungi may be associated with a revision of the environmental guidelines for heritage collections, museum repositories that cannot withstand the global climate changes, or both.

Xerophilic fungi in museum repositories challenge our perception of healthy buildings and the preventive conservation strategies used to ensure cultural heritage preservation. Larger studies of the prevalence of xerophilic fungi in museum repositories can provide a more comprehensive understanding of the causative factors and qualify the preventive conservation strategies museums must initiate to avoid the growth of xerophilic fungi in heritage collections. Global climate change is a reality. The risks it causes must be included in the preventive conservation strategies of museums to ensure healthy museum repositories supporting heritage preservation and occupational health of museum staff.

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