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Original article

The mysterious mould outbreak - A comprehensive fungal colonisation in a climate-controlled museum repository challenges the environmental guidelines for heritage collections

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ABSTRACT

Within the last decade, fungal colonisations have increased in Danish museum repositories. The growth is unexpected, as many Danish museums strive to comply with the environmental guidelines for heritage collections. When fungal growth develops in heritage collections, it threatens the heritage preservation and occupational health of museum staff. Therefore, it is crucial to prevent. This study characterised a fungal colonisation in a 1450m^2 museum repository, striving to meet the guidelines for heritage collections with relative humidity below 60%. After fungal discovery, the repository was examined twice by an environmental laboratory with morphological identification of fungi and quantification of fungal biomass based on fungal enzyme activity. However, the reports were not sufficient to qualify a recovery process. A research study with a broader approach was conducted to further elucidate the problem. The study included 1) building examination, 2) fungal surface sampling and morphological ID, 3) ID of fungal isolates with DNA sequencing, and 4) activated fungal air sampling and morphological ID. Although the relative humidity was measured to meet the guidelines for heritage collections with no evidence of moisture or microclimate, hyaline and white fungal colonies were distributed on heritage artefacts throughout the repository. There was no growth on interior and building structures. Cultivation of air samples on DG18-agar and V8®agar showed the presence of common indoor fungi, while artefact samples cultivated on the same media showed no growth. In contrast, cultivation of air samples and artefacts samples on the low water activity agar MY50G followed by DNA-sequencing showed high concentrations of the xerophilic fungi *A. halophilicus, A. domesticus, A. magnivesiculatus* and *A. vitricola*, belonging to *Aspergillus* section *Restricti*. These fungi are characterised by growing at low water activity corresponding to low relative humidity. The museum repository seemed to provide this environment with relative humidity below 60%. The study emphasised that examining the same fungi using different approaches may obtain very different results. Furthermore, the study questioned if the environmental guidelines for heritage collections adequately prevent the risk of xerophilic fungal growth. Xerophilic fungi are not adequately included in the risk assessment underlying the preventive conservation framework. Consequently, the risk is not included in the revised environmental guidelines accepting RH between 40-60% to support more sustainable heritage storage. It has not been studied if the revision would increase the risk of xerophilic fungal growth before it was accepted and implemented. This study indicates that it could be the case. Close collaboration between mycologists and museum professionals may develop more standardised and targeted detection and prevention practices for heritage repositories. The risks of xerophilic fungal growth should be included in the preventive conservation framework ensuring heritage preservation and occupational health of museum staff.

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1. Introduction

Museums have a leading role in preserving the tangible and intangible heritage of extinct nature and human cultures for posterity, making collection care and preventive conservation central in any museum. Long term conservation of heritage collections can only be achieved if the repositories support this aim by meeting the environmental guidelines [1–3]. Even if museums strive to obtain good environmental conditions, unforeseen incidents may occur, causing physical, chemical, and biological degradation of the heritage collection.

A direct consequence of excess moisture may be the development of fungal growth. Common causes of fungal growth in heritage collections are sudden water damages with insufficient recovery response [4–8] and infiltration where humid outdoor air propagates leaky buildings with inadequate climate control [9–12]. In addition, fungal growth in climate-controlled museum repositories has been reported [13–15]. Fungal growth in heritage collections is devastating, as fungi have strong degradative properties threatening heritage reservation [16–19]. In addition, fungi secrete harmful substances, adversely affecting human health [20–22]. When a museum collection poses a health hazard, the value for research and dissemination is significantly reduced, which challenges the very purpose of museums. Therefore, it is crucial to act.

A standard security framework responding to hazards damaging cultural heritage includes the acts: *avoid, block, detect, respond, and recover,* which are also central tools in the recommendations for the preventive conservation preserving heritage collections [23–25], the integrated pest management (IPM) [12], and the disaster management reducing the impact of natural and human disasters on cultural heritage [26].

A strategy to *avoid* fungal colonisations is to keep the growth conditions unfavourable by controlling relative humidity (RH) and temperature (T) within the guidelines. The 2019 ASHRAE Handbook recommend RH 35-65% with no limits of T [1]. The European Standard does not define RH and T more precisely than high and low levels [2], while the Bizot Guidelines, the AICCM Guidelines and AIC Guidelines recommend RH 40-60% and T 15-25°C [3]. Although the guidelines differ slightly, the acknowledged preventive conservation literature agrees that RH must exceed 70-75% before fungal growth develops [23–25]. Ongoing IPM also prevents fungal growth [8,9], and one prevention strategy could be monitoring airborne fungi [27–29].

If the preventive strategies avoiding fungal growth fail, *blocking* further growth may reduce the damage. A thorough building inspection could determine the cause of excess moisture to eliminate the source [30]. It is also important to isolate the contaminated artefacts from non-contaminated areas [8,9] and ensure that the ventilation system does not facilitate the dispersal of fungal particles [31,32].

A prerequisite to *respond* and *recover* is to *detect* the fungal colonisation. Sanmartín et al., 2018 and Ding et al., 2020 reviewed methods for sampling, analysing, and identifying microorganisms in cultural heritage and addressed three approaches: morphological, chemical, and molecular techniques [33,34]. These approaches are also the most frequently used in examining public institutions, domestic environments, and workplaces [30,35]. In general, there is broad agreement that no methods provide a complete overview, why none of the methods should stand alone.

However, detection with methods complementing each other is not always sufficient to qualify recovery. During a collection review in a Danish museum repository, striving to meet the environmental guidelines for heritage collections with RH below 60%, unexpected fungal colonisation was discovered. Two employees suffered from acute adverse health effects, the work was stopped, and the process *block, detect, respond, and recover* was initiated. Surprisingly the process did not clarify the fungal colonisation, which appeared inexplicable. However, within the last decade, similar fungal colonisations have emerged in Danish museums, indicating that the phenomenon could be more frequent than expected [15].

2. Research aim

This study characterises a comprehensive fungal colonisation in a climate-controlled museum repository striving to meet the environmental guidelines for heritage collections with RH below 60%. The study enlightens why the preventive conservation strategies failed and why two external reports examining the colonisation did not detect the fungal species causing the growth. The study discusses if the preventive conservation framework and the international environmental guidelines prevent the risk of fungal growth adequately. The aim is to call for a more targeted approach improving detection methods and preventive conservation practices in museum repositories, ensuring heritage preservation and occupational health.

3. Materials and methods

3.1. Study site and previous interventions

ROMU is a historical museum located in Zealand, Denmark. The repository was established in 2010 in a 1450 m^2 rebuilt and dehumidified warehouse facility (Fig. 1) on the advice of a conservation company, ensuring best practice when establishing museum repositories in existing buildings. The Danish building legislation and the guidelines for preserving heritage collections from the Danish Cultural Board were also followed. The heritage collection included approx. 30.000 historical artefacts and 280.000 archaeological artefacts documenting Danish history and prehistory. The repository replaced several small storage facilities, and before the heritage artefacts were moved, they were cleaned and freeze disinfected for pests.

Based on the environmental guidelines for heritage collections, RH was prophylactic controlled (Fig. 1) with a desiccant dehumidifier, Munters MLT 1400E, 1400m^3/h, and setpoint value 50% RH aiming to keep RH below 55%. The dehumidifier was oversized with a utilisation percentage of 61% and had a built-in hygrostat close to the process air inlet (Fig. 1: IV), alarming if RH exceeded 60% (supplementary document S1). A Tinytag Ultra 2 TGU-4500 datalogger conducted annual RH and T monitoring.

After the fungal discovery in 2012, the first step was to block further growth. The building envelope was inspected for leaks, and nothing unusual was noted. Data loggings showed RH 50-57% annually (Fig. 3), technical service of the dehumidifier showed no operational disturbances, and no alarm indicated elevated RH. In June and Juli 2012, an environmental laboratory examined the museum repository. Report 1 included morphological identification (ID) of fungal species from three museum artefacts cultivated on contact plates with Vegetable Juice Agar (V8®). Report 2 expanded the results with 1) morphological ID of fungal species from three surfaces cultivated on V8® contacts plates and quantification of colony-forming units (CFU), and 2) Fluorometric quantification of

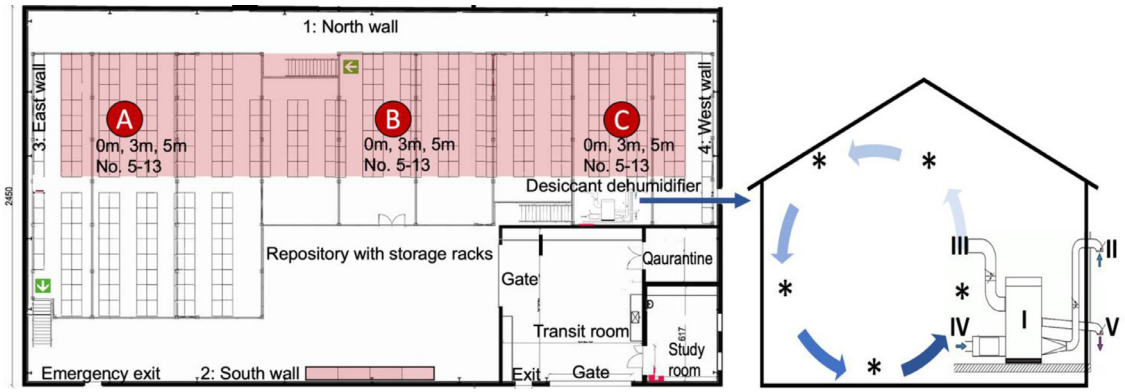

Fig. 1. The repository was constructed on a steel frame with trapezoidal steel walls, eternit roof covering 900m^2 ground floor and a 550m^2 mezzanine with floor to ceiling height 7m. The rebuilding included repairing leaks and removing water pipes, floor drains, doors, and windows to prevent water damage. Walls/ceiling were insulated with 350mm mineral wool, 0.2mm PE vapour barrier and 26mm gypsum board. The floor was 150 mm painted concrete on a moisture barrier. Racks/mezzanines were made of steel. A desiccant dehumidifier (I) controlled RH by drying outdoor air (II), distributing it through ducts in the repository (III), circulating the process air (IV), and returning the wet air outside (V) (non-measurable principal-drawing). T was not mechanically controlled. The dehumidifier, air inlet, air outlet, and ducts were installed as the manual specified. The red marking: fungal growth. No. 1-13: Measuring points, and A-C: Fungal sampling points.

the activity of β-n-acetylhexosaminidase (NAHA) [36,37] estimating the fungal biomass on three surfaces with Mycometer®Surface. Surprisingly the two reports did not provide adequate knowledge to qualify recovery, and dehumidification was continued blocking growth.

3.2. Broader research study

In 2019-2020, a broader research study was conducted. Before the fieldwork was initiated, a risk assessment of occupational health determined the personal safety equipment eliminating fungal exposure. The research study included 1) thorough building examination, 2) fungal surface sampling and morphological ID, 3) ID of fungal isolates with DNA sequencing, and 4) activated fungal air sampling and morphological ID.

3.3. Thorough building examination assessing the indoor climate

Measuring RH and T was conducted to determine the indoor environment. A Profort Multiguard®Technic system was installed, enabling closer remote-controlled monitoring of RH and T through the GSM network alarming if RH exceeded 60%. Nine Tinytag Ultra 2 TGU-4500 data loggers were placed sectionally for one week in levels 0m, 3m and 5m in area A-C (Fig. 1), measuring if RH and T varied in areas with a suspected different climate. The building envelope was inspected for potential leaks. Moisture content (MC) was measured in selected building structures with Gann Moisture Measuring Hydromette Compact-B according to the manual specifying MC as dry, risk, and wet (Fig. 1: 1-6). Surface temperature (ST) was measured on building structures and artefacts with Testo 835-H1 IR thermometer with inbuild moisture-meter according to the manual (Fig. 1: 1-13). RH and T were measured with Elsec 765 Environmental Monitor according to the manual (Fig. 1: 1-13). Three RH, MC, T and ST measurements were conducted at each measuring point.

3.4. Surface sampling and morphological ID

The repository was inspected for fungal growth, and eighteen contaminated artefacts in six materials; silk, wool, wood, leather, ceramics, and cast iron were selected; three in each material. The growth was photo-documented, fungal colonies were sampled with 3M no. 480 polyethene tape and transferred to slides for microscopy according to the ASTM standard [38]. Fungal biomass was sampled on the 18 heritage artefacts with sterile rayon swabs. The swabs were streak inoculated on V8®, dichloran-18%-glycerol-agar (DG18), and malt-yeast-50%-glucose agar (MY50G). V8® and DG18 were incubated for seven days at 25°C in darkness, and MY50G were incubated for 21 days at 25°C in darkness. The fungal 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 media suggested for the morphological ID of the species in question [35,39]. The fungal species were morphologically identified in the microscope by comparing with reference works [35,39].

3.5. Identification of fungal isolates with DNA sequencing

The morphological ID of isolates from surface samples was confirmed by DNA sequencing from an extern laboratory. DNA from isolates was purified using a Fast DNA Spin Kit for Soil (MP Biomedicals, USA). PCR amplification of fungal DNA regions was conducted using a Taq DNA Polymerase Kit (Amplicon, Denmark) according to the manufacturer's manual. To obtain a good separation of the xerophilic fungal species, calmodulin primers (cdm5/cdm6) were used for PCR amplification [39]. A single isolate gave no PCR product with cdm5/cdm6, and ITS primers were used. DNA fragments were sequenced using a BigDye Terminator v.1.1 Cycle Sequencing Kit (Thermo Fisher, USA) and a SeqStudio Genetic Analyser from Applied Biosystems (Thermo Fisher, USA) according to the manufacturer's manual. The sequences obtained were analysed using the EMBL-EBI homepage BLAST service (ebi.ac.uk).

3.6. Activated air sampling, morphological ID and data treatment

Indoor air sampling was conducted for one minute by MAS 100 ECO on V8®agar, DG18, and MY50G in areas A-C (Fig. 1) with activated sampling [40]. One air sample was taken outdoor. The samples were processed and identified as stated in 3.4. Concentrations of airborne fungi were presented as CFU/m^3. Concentrations and species richness in area A-C using three different agar media were compared in SAS version 9.4 as mixed model with random effect area of sampling.

4. Results

4.1. Two previous reports from an external laboratory

Report 1 identified *Acremonium* sp. *Aspergillus niger, Alternaria* sp., *Aureobasidium pullulans, Aspergillus* sp., *Cladosporium herbarum,*

Table 1
Contact plate samples: Morphological ID and colour of the appearing fungal species when cultivated on V8*.

Sample	Identified fungi	Colour on V8
Blackboard	*Alternaria* sp.	Brown
	Aspergillus niger	Black
	Aureobasidium pullulans	Yellow/pink
	Cladosporium herbarum	Brown
	Penicillium sp.	Green/blue
	Stachybotrus chartarum	Black
Piggybank	*Aspergillus niger*	Black
	Aspergillus sp.	Green
Book	*Acremonium* sp.	Yellow/white
	Penicillium sp.	Green/blue

* Colour according to Samson et al. 2019

Table 2
Contact plate samples: Morphological ID, CFU, and colour of the appearing fungal species when cultivated on V8*.

Sample	Identified fungi	CFU	Colour on V8
Interior	*Aspergillus fumigatus*	1	Green
	Cladosporium herbarum	8	Brown
	Mucor spinosus	1	White
	Penicillium sp.	1	Green/blue
Interior	*Penicillium* sp.	50	Green/blue
	Acremonium sp.	1	Yellow/white
	Aspergillus fumigatus	1	Green
	Aspergillus versicolor	4	Grey/blue
	Aureobasidium pullulans	1	Yellow/white
	Cladosporium herbarum	13	Brown
Dust	*Cladosporium herbarum*	30	Brown
	Mucor spinosus	2	White
	Penicillium sp.	10	Green/blue

* Colour according to Samson et al. 2019

Fig. 2. Fungal contaminated blackboard and archaeological ceramic urn.

Penicillium sp., and *Stachybotrys chartarum* from the examined museum artefacts (Table 1). The colours of the species on V8® were brown, green/blue, black, and yellow, while the colour of the growth on the heritage artefacts was white (Fig. 2). The report concluded minor to moderate growth.

Table 3
Fungal quantification on surfaces: Fluorometric quantification of fungal biomass with Mycometer®Surface.

Sample	Mycometer®Surface value	Level
Artefact	1595	C
Interior	242	B
Interior	538	C

Category A: The Mycometer surface value ≤ 25. The level of fungi is not above the normal background level.
Category B: 25 < Mycometer surface value ≤ 450. The level is above normal background level due to high concentrations of fungal particles in dust or the presence of old growth.
Category C: Mycometer surface value > 450. The level is high, above normal background levels, due to mould growth.

Report 2 assumed that the fungal colonisation was due to RH 75-100%. The report identified *Acremonium* sp. *Aspergillus fumigatus, Aspergillus niger, Aspergillus versicolor, Aureobasidium pullulans, Cladosporium herbarum, Mucor spinosus*, and *Penicillium* sp. on the interior samples, of which the *Cladosporium herbarum* and *Penicillium* sp. had the highest CFU (Table 2). The colours of the dominating species were brown and green/blue. The NAHA activity was above and high above Mycometer®Surface background level, showing high concentrations of fungal particles and high levels of fungal growth (Table 3).

4.2. A thorough building examination assessing the indoor climate

The outdoor and indoor building inspection confirmed a well-maintained building envelope with no evidence of leaks causing water damage, no discolouration on walls/ceiling and no evidence of rising soil moisture (Fig. 4). Annual data loggings from 2010-2012 showed RH fluctuating between RH 50-57% and T 4-21°C (Fig. 3).

The Profort RH loggings from 2019-2021 supported the previous loggings, showing RH between 46-57% (Fig. 3) with an average RH of 54% and relative standard deviations of 2-3% (Table 4). RH logging in 2019 with nine loggers in potential different climatic zones also showed low relative standard deviations of 1% (Table 4). The raw data loggings and related annual average RH, the annual distributions, the standard deviations, and the relative standard deviations are shown in supplementary document S2.

Manual measurements of RH and T on the day of building examination showed RH 52-58% and T 12-22°C (Table 5). Measurements of MC in area A-C at the floor, the ceiling, and the walls showed no elevated moisture (Table 5). The surface temperature was slightly lower on the floor and the west- and north-facing walls than on the east- and south-facing walls and the ceiling. The surface temperature on the heritage artefacts corresponded to the air. When comparing RH and T to the water-vapour chart, there

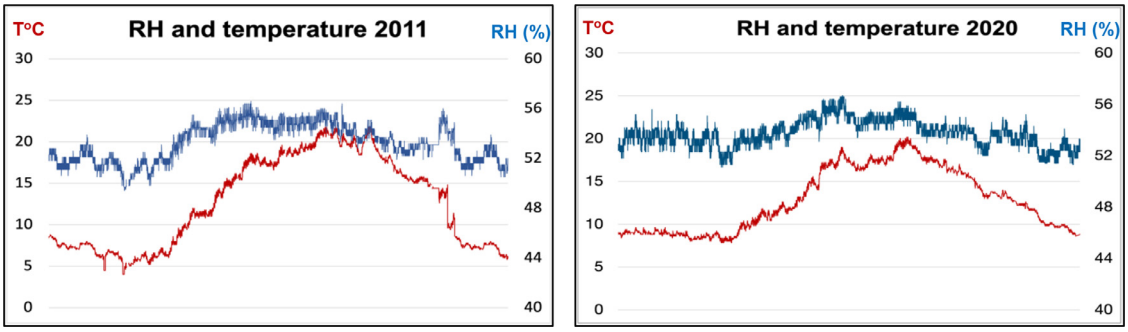

Fig 3. Annual variations of T(°C) and RH (%) were monitored with Tinytag Ultra 2 TGU-4500 datalogger in 2011 and Profort Multiguard®System sensor in 2020 showing stabile indoor RH below 60%.

Table 4
Datalogging: Min T, min-max RH, average RH, standard deviation and relative standard deviation calculated from loggings in area A-C[a)].

Measuring	Year	Periode	Datalogger	Area[a)]	Min T	Min-max RH (%)	Average RH	STDE	REL STDE (%)
Suspected fungal outbreak	2010	6 month	Tinytag	A	7.5	48 - 57	-	-	-
	2011	12 month	Tinytag	A	4.5	50 - 57	-	-	-
	2012	12 month	Tinytag	A	6.5	50 - 57	-	-	-
Research study	2019	9 month	Profort	A	8.4	46 - 57	54	1.4	3
	2020	12 month	Profort	A	7.8	51 - 57	54	1.0	2
	2021	9 month	Profort	A	4.2	49 - 57	54	1.6	3
Assessment of potential different climatic zones (April 2020)	2019	1 week	Tinytag 1	A: 0m	7.0	54 - 55	54	0.3	1
	2019	1 week	Tinytag 2	A: 3m	8.4	52 - 54	54	0.5	1
	2019	1 week	Tinytag 3	A: 5m	9.6	52 - 52	52	0.3	1
	2019	1 week	Tinytag 4	B: 0m	7.2	53 - 56	54	0.5	1
	2019	1 week	Tinytag 5	B: 3m	8.4	52 - 54	53	0.6	1
	2019	1 week	Tinytag 6	B: 5m	10.8	52 - 53	52	0.3	1
	2019	1 week	Tinytag 7	C: 0m	7.0	53 - 56	54	0.4	1
	2019	1 week	Tinytag 8	C: 3m	8.2	52 - 54	53	0.4	1
	2019	1 week	Tinytag 9	C: 5m	10.8	52 - 53	52	0.4	1

[a)] Refers to the areas in Fig. 1.

Fig. 4. The repository was well-maintained with no evidence of discolouration on walls/ceiling due to leaks or water damage.

Table 5
Building examination: Average MC, RH, and T in sampling points 1-13[a)], June 2019.

No	Area[a)]	Sample	$\overline{MC}$	$\overline{RH}$ (%)	$\overline{T}$ (°C)
1	North	Wall north (gypsum board)	Dry	56	17
2	South	Wall south (gypsum board)	Dry	52	22
3	East	Wall east (gypsum board)	Dry	53	20
4	West	Wall west (gypsum board)	Dry	55	18
5	A-C	Floor (concrete)	Dry	56	12
6	A-C	Ceiling (gypsum board)	Dry	58	18
7	A-C	Air (3m)	-	52	22
8	A-C	Heritage artefacts silk	-	55	18
9	A-C	Heritage artefacts wool	-	56	18
10	A-C	Heritage artefacts wood	-	56	18
11	A-C	Heritage artefacts learther	-	54	18
12	A-C	Heritage artefacts ceramics	-	55	18
13	A-C	Heritage artefacts cast iron	-	55	18

[a)] Refers to areas in Fig. 1.

was no evidence of microclimate on the floor, the ceiling, the walls, and the artefacts (Table 5).

4.3. Surface sampling and morphological ID

The heritage artefacts were made in a wide range of materials in all states of preservation, such as wood, leather, textiles, bone, feather, paper, synthetic polymers, metal, glass, ceramic, and stone. Small artefacts were packed in boxes and paper-based materials with low acidity, as recommended for heritage collections, while large artefacts were freely shelved (Fig. 2). A close inspection showed hyaline and white fungal colonies on approx. half of the historical museum artefacts distributed throughout the repository (Fig. 1). The growth was solely on museum artefacts in both organic and inorganic materials (Fig. 5a-c) and primarily on artefacts packed in boxes. There was no fungal growth on interior and building structures. Tape-lift samples showed the presence of *Aspergillus* sp. producing both mycelia, conidiophores and ascomata (Fig. 5c).

Surface samples from the 18 contaminated museum artefacts cultivated on the three media showed no cultures on V8® and DG18. In contrast, cultures developed on MY50G (Table 6). The dominant fungal species from each artefact were isolated, in total, one species from each artefact. The species were identified as *A. halophilicus* and *Aspergillus* spp. (Table 6). The morphological identification was verified by DNA sequencing, which confirmed *A.*

a. b. c.

Fig. 5. Fungal contaminated heritage artefacts: 5a. A suitcase, 5b. A gas flare, and 5c. Mycelium from heritage artefact with ascomata and conidiophores.

Table 6
Identification of fungal species causing growth: Morphological ID(*) and molecular ID(**) of the fungal isolate from artefacts.

Material	Area	Museum artefact	V8®[1)]	DG18[2)]	MY50G*	Fungal ID**	Similarity
Silk	A	Bonnet	-	-	*A. halophilicus*	*A. halophilicus*	100 %
	B	Bonnet	-	-	*A. halophilicus*	*A. halophilicus*	100 %
	C	Bible cover	-	-	*Aspergillus* sp.	*A. halophilicus*	100 %
Wool	A	Lace pillow	-	-	*A. halophilicus*	*A. halophilicus*	100 %
	B	Hat	-	-	*A. halophilicus*	*A. halophilicus*	100 %
	C	Coat	-	-	*Aspergillus* sp.	*A. domesticus*	100 %
Wood	A	Carved box	-	-	*A. halophilicus*	*A. halophilicus*	100 %
	B	Shrine	-	-	*Aspergillus* sp.	*A. vitricola*	100 %
	C	Mousetrap	-	-	*Aspergillus* sp.	*A. domesticus*	100 %
Leather	A	Compass box	-	-	*Aspergillus* sp.	*A. vitricola*	100 %
	B	Glove	-	-	*A. halophilicus*	*A. halophilicus*	100 %
	C	Wallet	-	-	*A. halophilicus*	*A. halophilicus*	100 %
Ceramics	A	Urn	-	-	*Aspergillus* sp.	*A. magnivesiculatus*	100 %
	B	Lampshade	-	-	*Aspergillus* sp.	*A. domesticus*	100 %
	C	Jug	-	-	*Aspergillus* sp.	*A. halophilicus*	100 %
Cast iron	A	Lattice	-	-	*Aspergillus* sp.	*A. magnivesiculatus*	100 %
	B	Frying pan	-	-	*Aspergillus* sp.	*A. halophilicus*	100 %
	C	Waffle iron	-	-	*A. halophilicus*	*A. halophilicus*	100 %

1-2) No growth was shown on V8® and DG18.

Table 7
Concentrations of airborne fungal species: CFU/m^3 of fungal species cultivated on V8, DG18, MY50G. The CFU/m^3 is an average of the detected fungi from area A-C.

Fungal species	$\overline{\text{CFU/m}^3}$ V8®	$\overline{\text{CFU/m}^3}$ DG18	$\overline{\text{CFU/m}^3}$ MY50G
Aspergillus spp.	90	340	680
A. creber	0	10	0
A. fumigatus	0	20	0
A. glaucus	0	10	0
A. halophilicus	0	0	2600
A. montevidensis	0	20	0
A. nidulans	0	10	0
A. niger	40	0	0
A. pseudoglaucus	0	10	0
A. ruber	0	10	0
A. sydowii	0	10	0
A. versicolor	0	180	0
Penicilium spp.	20	60	0
P. brevicompactum	0	60	0
P. buchwaldii	0	40	0
P. citreonigrum	0	20	0
P. crustosum	0	20	0
P. chrysogenum	10	40	0
P. commune	0	30	0
P. coryphilum	0	20	0
P. palitans	0	30	0
P. thomii	0	20	0
Acremonium spp.	10	0	0
Alternaria spp.	0	60	0
Botrytis cinerea	10	0	0
Cladosporium spp.	50	40	0
Engyodontium album	10	0	0
Epicoccum nigrum	10	0	0
Fusarium spp.	10	10	0
Mucor circinelloides	30	10	0
Mucor spinosus	40	0	0
Paecilomyces spp.	0	10	0
Stachybotrys chartarum	10	0	0
Species richness	13^b	25^a	2^c
Concentration CFU/m^3	340^c	1090^b	3280^a

Numbers in the row followed by the same letter are not statistically significantly different.

halophilicus with 100% similarity. The unidentified *Aspergillus* spp. were identified to *A. domesticus, A. magnivesiculatus* and *A. vitricola,* with 100% similarity (Table 6).

4.4. Activated air sampling, morphological ID and data treatment

Cultivation, isolation, and morphological identification of fungi from activated air samples on V8®, DG18 and MY50G showed fungi from the genera *Acremonium, Aspergillus, Alternaria, Cladosporium* and *Penicillium* and 29 identified fungal species (Table 7). There was a variance between the species growing on the different media. The highest species richness was found on DG18 ($p<0.0001$). The species *A. halophilicus* dominated MY50G but was absent on V8® and DG18 (Fig. 6a-c). The total concentration of fungi (CFU/m^3) was significantly higher on MY50G than on V8® and DG18 ($p=0.002$), and it was higher on DG18 than on V8 agar.

5. Discussion

In this study, we documented extensive growth of *A. halophilicus, A. domesticus, A. magnivesiculatus* and *A. vitricola* on museum artefacts in a climate-controlled repository belonging to the Danish Museum ROMU. The fungal species were xerophilic belonging to the *Aspergillus* section *Restricti*, characterised by their ability to grow on substrates with low a_w corresponding to low RH [39]. The colonisation was unexpected as the museum was striving to meet the guidelines for heritage collections with RH 40-60%. It was also unexpected that two reports from an environmental laboratory did not identify the fungi causing the growth.

5.1. Preventive conservation and environmental conditions

Collection care and preventive conservation was a priority at Museum ROMU. The museum followed the preventive conservation recommendations for heritage collections, including building maintenance, climate control, climate monitoring, suitable storing materials, and IPM, among others, to avoid fungal growth in the heritage collection. An indoor environment with RH 40-60% should - in theory - not support fungal growth. Nevertheless, the fungal colonisation developed involving several thousand museum artefacts.

Sterflinger, 2010 points out that climate monitoring in a heritage building may be insufficient to reflect the actual climate in the building, including the different climatic zones. The impact of the stack effect, warming by sunlight, diurnal variations of temperature, air circulation, and isolation of the building envelope must be considered when assessing the indoor climate [18]. However, the museum repository was not a historical building; it was a rebuilt warehouse facility following the Danish building legislation and the guidelines for preserving heritage collections from the Danish Cultural Board. The building was sealed, dehumidified and highly insulated with good air circulation and minimal microclimate.

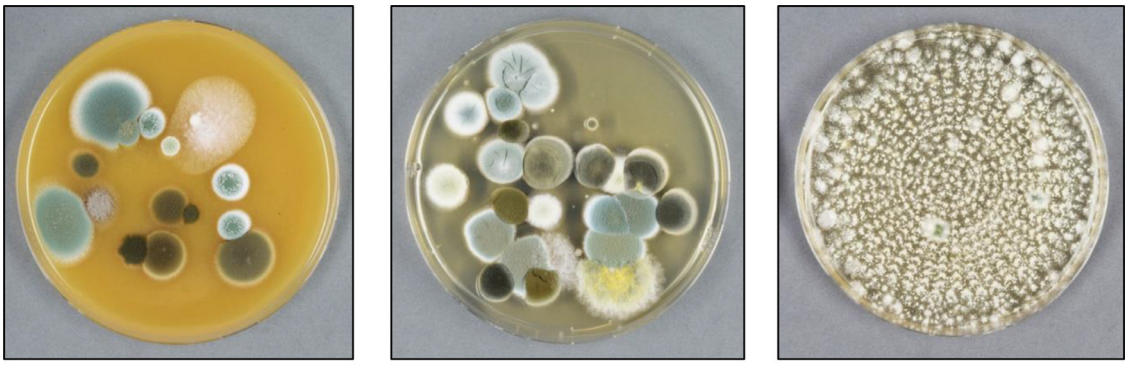

Fig. 6. Activated air-sampling from area A (fig. 1) on three medias with different a_w: 6a: V8®agar, 6b: DG18-agar, and 6c: MY50G-agar.

During the years, RH was measured with data loggers showing the dehumidifier managed to keep RH below 60%. The annual RH average was 54%, with low relative standard deviations (Table 4), indicating reliable data. In addition, measuring with nine loggers in potentially different climatic zones showed low standard deviations. The low RH was not surprising, as the desiccant dehumidifier was oversized with a utilisation percentage of 61% to ensure stable RH in the repository. Average RH and standard deviations in 2010-2012 could not be calculated, as there was no digital data from this period, but only transcripts. However, the built-in hygrostat in the dehumidifier, alarming if RH exceeded 60%, was not activated. In addition, there was no obvious evidence of microclimate supported by the absence of fungal growth along with the floor, the ceiling and the outer walls, which, in general, are areas suspected of microclimate [30].

A point of interest could be the passively controlled temperature, which is common in Danish repositories. In Denmark, an uninsulated concrete floor in a well-insulated building mitigates the outdoor climate impact with passive summer cooling and passive winter heating [41]. In 2011 and 2021, temperature monitoring showed T as low as 4°C during winter, which may be too low to prevent elevated a_w on surfaces. This assumption may be supported by an incident in 2018, where Museum ROMU moved two archaeological ceramic urns from an exhibition to the repository in January. Twelve months later, fungal colonies were developed on the urns (Fig. 2) despite the repository being dehumidified with RH below 60%. Future research may determine whether low indoor temperatures in museum repositories could cause locally elevated a_w adequate for fungal germination on artefacts even if RH is dehumidified below RH 60%.

5.2. Two previous reports from an environmental laboratory

The research study detected massive xerophilic fungal growth on the museum artefacts, which in contrast, was not reported by the external environmental laboratory. The inconsistent results illustrate the ever-present joker: When working with fungi, one may find what one is looking for. Fungal examination depends on the choices made: the sampling point, the sampling approach, and the analysis approach.

The external laboratory assumed RH 75-100% caused the colonisation; however, this suspicion was not confirmed, and data loggings showed RH below 60% - a very different premise. Nevertheless, the laboratory followed a protocol for examining water-damaged buildings. Cultivating fungi from a museum repository with RH below 60% on V8®agar is problematic. V8® has proven suitable for detecting fungal species associated with water-damaged buildings such as *Stachybotrys* and *Chaetomium* [42] and is not recommended for detecting xerophilic fungi [39].

When the laboratory chose V8® for cultivation, detecting the xerophilic fungi was not possible. The identified fungal species presumably originated from spores in dust, which explains why the colour did not correlate with the white growth on the heritage artefacts. A divergence in colour can be explained by the fact that the colour of cultivated fungal species depends on the substrate, pH, and aw [35]. However, *Alternaria, Aspergillus niger, Cladosporium, Penicillium* and *Stachybotrys chartarum* would not appear bright white, regardless of substrate. A closer dialogue on RH could have targeted the analysis approach detecting the xerophilic fungal species causing the fungal growth.

5.3. A research study addressing the environmental conditions with low RH

The research study addressed that RH was controlled below 60%. DG18 was chosen as a low a_w media applied in studies detecting fungi on dried and semi-dried foods [43]. It has also proven suitable for detecting indoor fungi in buildings [35,44]. MY50G was also chosen as a low a_w media detecting fungi in dried and semi-dried foods [45,46] and was recommended for detecting xerophilic fungi [39]. V8® was included as a reference, as it was used in the external reports.

Cultivation of artefacts samples showed only growth on MY50G agar with low a_w. The morphological ID of the appearing colonies was challenging, as xerophilic species are sparsely described in reference works, and only *A. halophilicus* was identified to species level. However, DNA-sequencing of the fungal isolates after PCR amplification with calmodulin primers identified the xerophilic fungal species *A. halophilicus, A. domesticus, A. magnivesiculatus,* and *A. vitricola* with 100% similarity. The ITS region is generally recommended as a universal fungal barcode for molecular identification of fungi [47]; however, calmodulin primers are more suitable for separating and identifying xerophilic fungal species [39].

When cultivating the activated air samples on V8®, DG18, and MY50G, the significant variance in the species and CFU/m^3 (Fig. 6a-c) reflected that different fungal species prefer different substrates regarding nutrients, pH, and a_w. The total CFU/m^3 was significantly higher on MY50G than on V8® and DG18, with *A. halophoilicus* strongly overrepresented. The fungi preferring DG18 and V8® were common indoor fungal species [35], presumably originating from dust becoming airborne with the activated air sampling. The CFU/m^3 for *Cladosporium* spp. and *Alternaria* spp. supported this assumption, as these species are common outdoor.

Furthermore, the research study was based on methods complementing each other. The low-tech tape-lift sampling has proven suitable and effective as a rapid indicator qualifying further analysis. The method showed massive growth of *Aspergillus* spp. on heritage artefacts (Fig. 6c), in contrast to the external reports con-

cluding minor growth based on CFU. The research study also applied activated air sampling, where dust became airborne before sampling by a stationary sampler. This method enables sampling from a large area providing more detailed detecting than surface sampling and passive air sampling [48]. The external reports were based on a few point measurements, which may not represent 1450m^2 storing 300,000 artefacts.

5.4. Detecting fungal growth in museum repositories may require a targeted approach

The research study became more accurate and reliable by targeting the analysis approach to the environmental conditions with low RH. The xerophilic fungal growth was identified with the low a_w MY50G agar, followed by DNA sequencing of the fungal isolates using calmodulin primers. If this approach was not applied, the xerophilic fungi would have been non-detected.

Detecting fungal colonisations in heritage collections must be as accurate as possible to qualify recovery. Cleaning is a considerable task when several thousand museum artefacts are involved and requires substantial resources, as the methods must respect heritage preservation by avoiding accelerated ageing. It is also essential to ensure that the growth will not return after cleaning. These demands set the stage for a more targeted approach when detecting fungal growth in museum repositories.

In general, there is a lack of research assessing the pros and cons when applying detecting approaches in cultural heritage, and most studies are case studies based on the available methods. Sterflinger et al. 2018 suggest establishing an interdisciplinary network of researchers working with microorganisms in cultural heritage sharing knowledge and specialised laboratory facilities [49]. This study supports the need for future interdisciplinary collaboration on research contributing to a more targeted approach to detecting and preventing fungal growth in heritage collections.

5.5. Do the environmental guidelines for heritage collections prevent xerophilic fungi adequately?

Nobody knows when and why the fungal colonisation at Museum ROMU developed. Presumably, the preventive conservation strategies failed because the xerophilic fungi were not adequately included in the risk assessment underlying the preventive conservation framework. Preventive conservation is a fusion of research and best practices, and not all areas are well researched - including fungal growth in cultural heritage. The preventive conservation literature specifies an increased risk if RH exceeds 70-75% [23–25]. This study indicates that RH 70-75% is too high concerning xerophilic fungal growth risk assessment.

If the preventive conservation framework is to include the risk of xerophilic fungal growth, how much moisture is then too much? In general, germination of fungal spores may occur if a_w is briefly raised; subsequently, growth can develop at lower a_w [50,51]. Stevenson, 2017 showed that the limit growth of the xerophilic *A. peniciloides* was a_w 0.585, corresponding to RH 58.5% [52]. Similar studies have not been conducted for *A. halophilicus, A. domesticus, A. magnivesiculatus,* and *A. vitricola,* which caused the fungal colonisation at Museum ROMU. Specifying the growth limits for the xerophilic fungi is necessary if the risk is to be included in the preventive conservation framework for cultural heritage.

The fact that xerophilic *Aspergillus* growth occurred despite the low RH shows that we lack fundamental knowledge either on the true a_W that these fungi can grow at or knowledge on the dynamics between RH and a_W on the surface of the artefacts or both, particularly at low temperatures. We also lack knowledge about how the xerophilic fungi was introduced to the repository. *Aspergillus domesticus* and *A. vitricola* can be detected indoors but are not common outdoors [53]. Besides, there was no growth on interior and building structures. Further research could clarify if xerophilic fungal particles could be a natural part of heritage artefacts integrated into dirt from their historical use.

The xerophilic fungal colonisation at Museum ROMU is not exceptional. Xerophilic fungal growth is also shown in some cultural heritage studies [54–59] and studies from libraries and archives [60–64]. In the last decade, xerophilic fungal growth in climate-controlled museum repositories has also emerged [13,15]. This study indicates that the phenomenon could be more frequent than expected.

The fungal colonisation in Museum ROMU developed parallel with revising the guidelines for heritage collections and global climate change causing more precipitation. If there is a causal relationship – it has not been studied. The revised guidelines accepted RH 40-60% [3] instead of the previously suggested RH 50±5% [65]. In theory, the expansion of RH should not increase the risk of fungal growth. However, it has not been studied if the revision increases the risk of xerophilic fungal growth before it was accepted and implemented.

6. Conclusions and perspectives

This study characterised an unexpected, comprehensive fungal colonisation in a climate-controlled Danish museum repository with RH below 60%. The survey documented growth of *A. halophilicus, A. domesticus, A. magnivesiculatus* and *A. vitricola* on museum artefacts; xerophilic fungal species belonging to the *Aspergillus* section *Restricti*. These species are characterised by the ability to grow at low a_w corresponding to low RH, which explain why the preventive conservation strategies failed. The study emphasised that examining the same fungi using different approaches may obtain very different results. The results depend on the choices made concerning the sampling point, the sampling approach, and the analysis approach. The study also emphasised that knowledge sharing among the parties involved is crucial. Further research based on close collaboration between mycologists and museum professionals may develop more standardised, accurate and targeted detection practices allowing the comparison of studies. Furthermore, the study questions whether the guidelines for heritage collections prevent the risk of fungal growth adequately. The xerophilic fungi are not sufficiently included in the risk assessment underlying the preventive conservation framework. Consequently, the risk is not adequately included in the revised guidelines for heritage collections accepting RH 60% as an upper limit. 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 strategies. The risks of xerophilic fungal growth in heritage collections must be included in the preventive conservation framework to ensure heritage preservation and the occupational health of museum staff.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.culher.2022.02.009.

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