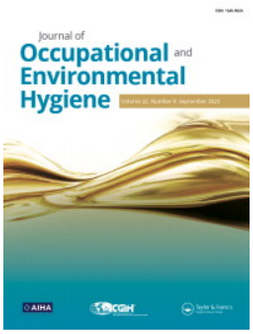

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Exposure to fungi, bacteria, and endotoxin in a museum, with staff reporting work-related symptoms

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ABSTRACT

Occupational exposure to airborne fungi in indoor environments may pose respiratory health risks. This study aimed to characterize exposure to fungi, bacteria, and endotoxin in a historical museum where the staff reported airway symptoms. Air samples were collected during three separate workdays using personal and stationary samplers. Fungi and bacteria were quantified and identified using MALDI-TOF MS, and the total inflammatory potential was measured through Reactive Oxygen Species (ROS) production in a human cell line. The geometric mean of staff exposure was 5.9×10^3 CFU/m^3 fungi, 1.8×10^3 CFU/m^3 bacteria, and 7.93 EU/m^3 endotoxin. Staff reported symptoms of the airways, with immediate symptoms upon entering two departments, which exhibited elevated fungal concentrations compared to other departments. The most prevalent fungal species were *Aspergillus conicus, A. domesticus, A. pseudoglaucus*, *A. pseudogracilis*, and *Cladosporium* spp. Concentrations of bacteria were highest when staff were present and without dominance by any particular species. Staff exposure and stationary samples induced ROS production in a cell line, which correlated with concentrations of fungi, bacteria, and endotoxin. Fungi were detected on the museum artifacts, and concentrations of fungi and bacteria increased during handling of the artifacts. In conclusion, staff in the two departments where airway symptoms were reported were exposed to 2×10^4 to 7×10^4 CFU/m^3 of fungi, primarily *Aspergillus* spp. and *Cladosporium*. spp. This exposure constituted both xerophilic species and species commonly found in water-damaged buildings, and they seemed to have developed on artifacts. These findings underscore the importance of developing preventive strategies to protect the health of museum staff. Moreover, it highlights the challenge of managing fungi adapted to varying humidity conditions.

KEYWORDS

Maldi-TOF MS; nasal symptoms; ODTS; occupational health; personal exposure; xerophilic fungi

Introduction

Exposure to fungi in indoor air can constitute a potential risk factor for respiratory problems. In an arts and crafts museum where books were handled, an employee exposed to high concentrations of fungi repeatedly experienced symptoms similar to those of Organic Dust Toxic Syndrome (ODTS) (Kolmodin-Hedman et al. 1986). Other cases include Hypersensitivity Pneumonitis (HP) associated with exposure to the yeast *Aureobasidium pullulans* from an indoor hydroponic system (Engelhart et al. 2009) and with *Fusarium vasinfectum* from walls in a mobile home, which the affected individual was self-renovating (Dickson and Tankersley 2015). In an epidemiological study, 30% of 103 museum staff members were sensitized to at least one fungal allergen, with prolonged museum employment of more than 5 years identified as a risk factor (Wiszniewska et al. 2009).

Fungi have been documented to colonize various materials within museum artifacts (Kavkler et al. 2015; Nigam et al. 1994; Okpalanozie et al. 2018; Micheluz et al. 2016; Abe 2010; Bastholm et al. 2021; de Carvalho et al. 2018), with airborne fungi detected in museum rooms, repositories, archival storage facilities, and cultural heritage buildings (Rojas and Aira 2012; Bastholm et al. 2022; Ilies et al. 2023; Zielińska-Jankiewicz et al. 2008; Awad et al. 2020; de Carvalho et al. 2018). Cases of fungal presence on museum artifacts have been associated with water damage (Pinheiro et al. 2019;

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Sterflinger 2010), but also with xerotolerant and xerophilic fungi that can grow with low water activity have been repeatedly found on museum artifacts (Bastholm et al. 2022; Abe 2010; Zalar et al. 2023; Hagiuda et al. 2022) and library books (Micheluz et al. 2015). However, the potential health effects of exposure to these xerophilic fungi have not been investigated.

Few studies have measured airborne bacteria in museum-related environments (Saridaki et al. 2023; Gorny et al. 2016). In occupational settings where large amounts of organic materials are handled, bacterial endotoxin exposure has been associated with symptoms of the airways and ODTS (Basinas et al. 2012). Moreover, endotoxin may have synergistic effects with fungal β-glucan (Engstad et al. 2002). No studies have been found on endotoxin exposure in museum settings; however, air concentrations have been documented previously in residential (Frankel et al. 2012) and educational (Lai et al. 2015) settings.

Exposure to fungi, endotoxin, and bacteria can induce inflammation, with reactive oxygen species (ROS) production playing a pivotal role in the ensuing immune response (Natarajan et al. 2019). ROS serve as key signaling molecules in inflammatory disorders (Mittal et al. 2014), contributing significantly to the pathogenesis of conditions such as asthma, ODTS, and HP by promoting inflammation, tissue damage, and disease progression in the lungs. ROS production upon exposure to airborne microorganisms and dust from occupational settings can be studied *in vitro* in a human promyelocytic leukemia cell line (HL60) (Frankel et al. 2014). This method offers the advantage of evaluating ROS production in response to the complete mixture of airborne particles encountered during a workday. During a workday, employees are typically exposed to several different airborne fungi and bacteria, endotoxin, and other particles (Vitte et al. 2022; Rasmussen et al. 2021).

A recent review describes a lack of quantitative studies linking indoor exposure to fungi directly to harmful health effects (Perez et al. 2024). In this study, the staff exposure in a historical museum setting was characterized. Measurements were conducted as personal exposure samples during work tasks and stationary area samples in various work areas. In addition, surface samples from artifacts were analyzed as potential sources of exposure. An advanced method for identification of fungal and bacterial species, Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF-MS), was used in this study. Additionally, endotoxin levels were measured, and the Total Inflammatory Potential (TIP) of staff exposures was assessed using a human cell line. The aim is to obtain knowledge about occupational bioaerosol exposures in a museum where staff members experienced symptoms in the airways.

Methods

The museum

The studied museum is a local historical museum dedicated to preserving and communicating cultural heritage. The museum was temporarily located in containers, some of which were accessible to the public while others served as storage repositories. The departments of the museum included in this study were Office 1, where museum artifacts were also present, Office 2, where the staff was often present, and four museum departments with artifacts (Departments 1-4). Each department was located in a separate container. Visible fungi were observed on artifacts in Departments 1 and 2. During the third day of measurement, an air purification system was active with a HEPA filter mounted to reduce particle concentrations in Department 2.

Staff and questionnaire

Contact with the museum was obtained due to a staff member experiencing airway symptoms, suspected to be caused by fungal exposure. The museum staff consisted of a mixture of employees and volunteers (part-time staff). The staff was preparing to move all of the museum's activities to a renovated facility at the time of measurement, thus, the staff mainly worked on cleaning the current facility and artifacts and packing them for moving. Measurements were made during three separate days in late summer 2023. During the first two days of measurements, one person was at work, and during the third day, five people were at work.

Seven staff members participated in the study and were given a questionnaire on skin and airway-related symptoms occurring within the last week and/or 12 months and on the use of protective equipment. The questionnaire was based on previously used questionnaires (Rasmussen et al. 2023).

Exposure assessment

To determine personal exposure, participants were fitted with backpacks containing pumps attached to two personal air samplers (Gesamtstaubprobenahme sampler (GSP), BIG Inc., USA) attached on the front of the backpack, i.e., in the inhalation zone of the participants (14031 E 2021). A 37 mm polycarbonate filter

(pore size 0.8 μm, SKC) was mounted in one sampler, and a 37 mm Teflon filter (pore size 1.0 μm, Merck) was mounted in the other. The sample flow was adjusted twice before sampling and kept at a flow rate of 3.5 L/min. The average sampling time was 169 min.

Stationary samples, outdoor references, temperature, and humidity

Stationary samples were collected using GSP samplers in four areas during each of the three days: Office 2, Departments 2, 3, and 4. The average sampling time was 333 min.

To determine potential exposures in Department 1 before and during packing of artifacts on day 3 and to get information about the sizes of particles (aerodynamic diameter) with airborne microorganisms, stationary samples were collected using an Andersen six-stage cascade impactor (ACI; Thermo Fisher Scientific Inc. Waltham, MA, USA) with a flow rate of 28.3 L/min. The ACI sampled bioaerosols into six size fractions. Stage 1 (particle size range for nasal/oral cavity exposure): 7.0 to 12 µm, stage 2 (Pharynx): 4.7 to 7.0 µm, stage 3 (Trachea and primary bronchi): 3.3 to 4.7 µm, stage 4 (secondary bronchi): 2.1 to 3.3 µm, stage 5 (terminal bronchi): 1.1 to 2.2 µm, and stage 6 (Alveoli): 0.65 to 1.1 µm. The ACI was placed on a 1 m stand and sampled for 10 and 20 min on Dichloran glycerol agar (DG18) twice, 15 and 20 min on Malt yeast 50% agar (MY50) twice, and 10 and 15 min on Nutrient agar (NA; Thermo Fisher Scientific Oxoid, Basingstoke, UK) plates with actidione (cycloheximide; 50 mg/L; Serva, Germany) twice. The agar plates from the ACI were incubated at 25 °C upon return to the laboratory.

Outdoor reference samples were taken outside the museum using a GSP sampler mounted with a polycarbonate filter; the sampling time was 330 min. Temperature and relative humidity (RH) were measured inside different departments and outdoors using Tinytag Plus Range (TGP-1500) every 5th minute throughout the sampling periods.

Surface samples

Surface samples were taken from 10 artifacts from different departments using a moistened swab (eSwabs pre-moistened in modified Amies medium; Copan). The artifacts included: paper, a glass cabinet, wooden shelf, Southwest rain hat, typewriter, book, and navigation system.

Extraction, quantification, and identification of bacteria and fungi

Polycarbonate filters from both personal and stationary GSP samplers were extracted the morning following the sampling day due to the long transport time from the sampling location to the laboratory. Filters were extracted at room temperature in 5.0 mL sterile extraction solution (MilliQ water with 0.05% Tween 80 and 0.85% NaCl) by orbital shaking at 500 rpm for 15 min. Suspensions from the filters were plated in serial dilutions for quantification and identification of bacteria and fungi. For enumeration of bacteria, samples were plated on NA and incubated at 25 °C for seven days. For enumeration of fungi, samples were plated on Dichloran Glycerol agar (DG18; water activity a_w = 0.95; Thermo Fisher Scientific Oxoid, Basingstoke, UK) in duplicate and incubated at 25 and 37 °C for seven days. Fungi were also plated on Malt Yeast Extract 50% Glucose Agar (MY50 agar, a_w = 0.86; Oxoid, Denmark) in duplicate and incubated at 15 and 25 °C for 14 days.

E-swabs (surface samples) were vortexed for 5 min and plated in 5 serial dilutions on MY50 agar in duplicate, followed by incubation at 15 and 25 °C for 14 days.

All visible bacterial and fungal colonies were counted after the specified incubation period. For GSP and ACI samples, concentrations were calculated as time-weighted averages of colony-forming units (CFU)/m^3, taking into account the sampling time and the flow rate. Sample concentrations were then calculated as geometric mean (GM) values of appropriate sample dilutions (plates with a CFU count between 1 and 100).

A representative dilution, i.e., with optimal coverage and separation of individual colonies, was used for species identifications using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). A Microflex LT mass spectrometer (Bruker Daltonics) was used using the Bruker Biotyper 3.1 software, with the BDAL library for bacteria (V11) and the Filamentous Fungi library (V4) for fungi. The fungal library was extended with xerophilic *Aspergillus* species. Bacteria were identified using the extended direct transfer method, and fungi were identified using a modified ethanol extraction protocol. The instrument was calibrated weekly using a bacterial test standard (Bruker Daltonics). Isolates were analyzed in duplicates on the MALDI-TOF MS, and the following cutoffs were used for species identifications: isolates with scores of 1.80 or higher were identified to species level, isolates with scores between

1.70 and 1.79 were identified to genus level, and isolates with scores lower than 1.70 were unidentified.

Total inflammatory potential (TIP) and endotoxin

Suspensions from GSP polycarbonate filters were used in duplicate to determine the total inflammatory potential (TIP) of the samples using a granulocyte-like cell assay (HL-60 cells; ATCC, CCL-240). As negative reference, the extraction solution for polycarbonate filters was used, a positive references, pure endotoxin (0.5 EU/ml of Escherichia coli O55:B5 endotoxin, Lonza Pharma & Biotech), an air sample from a home with more fungi than bacteria, and a sample from a farm with more bacteria than fungi were used. The three positive references were used as internal positive references in every assay.

The HL-60 cells were exposed to the dust suspensions and references, after which the production of reactive oxygen species (ROS) was measured by a luminol-dependent chemiluminometric assay using a thermostatic (37 °C) ORION II Microplate Luminometer (Berthold Detection Systems) (Madsen et al. 2020, Timm et al. 2006). Relative-light units per second (RLU/sec) were measured for 1 sec every 120 sec for 180 min. Data are presented as ROS production over time, together with concentrations of fungi, bacteria, and endotoxin (units/mL) in the same samples. In addition, for each sample, RLU over the full period of 180 min was summed, thereby expressing the TIP of the sample as the area under the curve (AUC). The AUC was expressed as time-weighted averages (AUC/m^3).

The dust on the Teflon filters was extracted in 5.0 mL pyrogen-free water with 0.05% Tween 20 by orbital shaking (500 rpm) at room temperature for 60 min and centrifuging (1000 × g) for 15 min. The supernatant was stored at −80 °C until they were diluted 1.5 times and analyzed (in duplicate) for endotoxin using the kinetic Limulus Amebocyte Lysate test (Kinetic-QCL endotoxin kit, Lonza Pharma & Biotech, Walkersville, Maryland, USA). A standard curve obtained from an Escherichia coli O55:B5 reference endotoxin was used to determine the concentrations in terms of endotoxin units (EU; 11.0 EU ≈ 1.0 ng). The data are presented as EU/m^3 air, and the limit of detection was 0.05 EU/ml, corresponding to a detection limit of 0.085 EU/m^3.

Data analyses

Data were $\log_{10}$-transformed before analysis to approximate normality and stabilize variance, as raw concentration data were right-skewed. After transformation, the data were visually assessed for normality using Q-Q plots, and residuals from the General Linear Model (GLM) were checked to confirm that model assumptions (normality and homogeneity of variance) were met. To compare exposures and concentrations measured using a stationary sampler in different areas of the museum, the GLM procedure in SAS was used. The LSD (Least Significant Difference) option was used to conduct pairwise comparisons of means. Concentrations before and during activity with packing of artifacts, and the geometric mean aerodynamic diameter of particles with bacteria on NA and fungi on DG18 and My50 were compared using the paired t-test. Results were considered statistically significant at $p < 0.05$. Pearson correlations were calculated to assess the relationship between exposure and TIP. Statistical analyses were done using SAS 9.4 (Cary, North Carolina, USA).

Results

Self-reported symptoms

The staff were nonsmokers, and more than 50% reported nasal symptoms that resolved after leaving work. Some reported sneezing at work and the sudden onset and resolution of influenza-like symptoms (Table S1). The staff reported immediate airway symptoms when entering Departments 1 and 2. While staff often used gloves at work, facial masks were used only after symptoms appeared.

TIP, exposure, and concentrations of microorganisms

The average RH was higher than 60% in all departments, and indoor temperatures were affected by outdoor temperatures (Table S2).

Profiles of ROS production of HL60 cells during exposure to personal and stationary samplers are presented in Figures 1 and 2, respectively, without normalization to sampling time. Negative (extraction solution) and positive reference samples—endotoxin, a sample from a home, and a sample from a farm are shown in Figure 1. All museum samples induced ROS production. No peak response was observed for the negative reference, while peak responses were observed after 75 min for pure endotoxin, 13 min. for the home sample, and 23 min for the farm sample. Peak responses were observed 10-15 min. after exposure to the personal (Figure 1b–d) and stationary (Figure 2a–d) museum samples. In samples with the highest

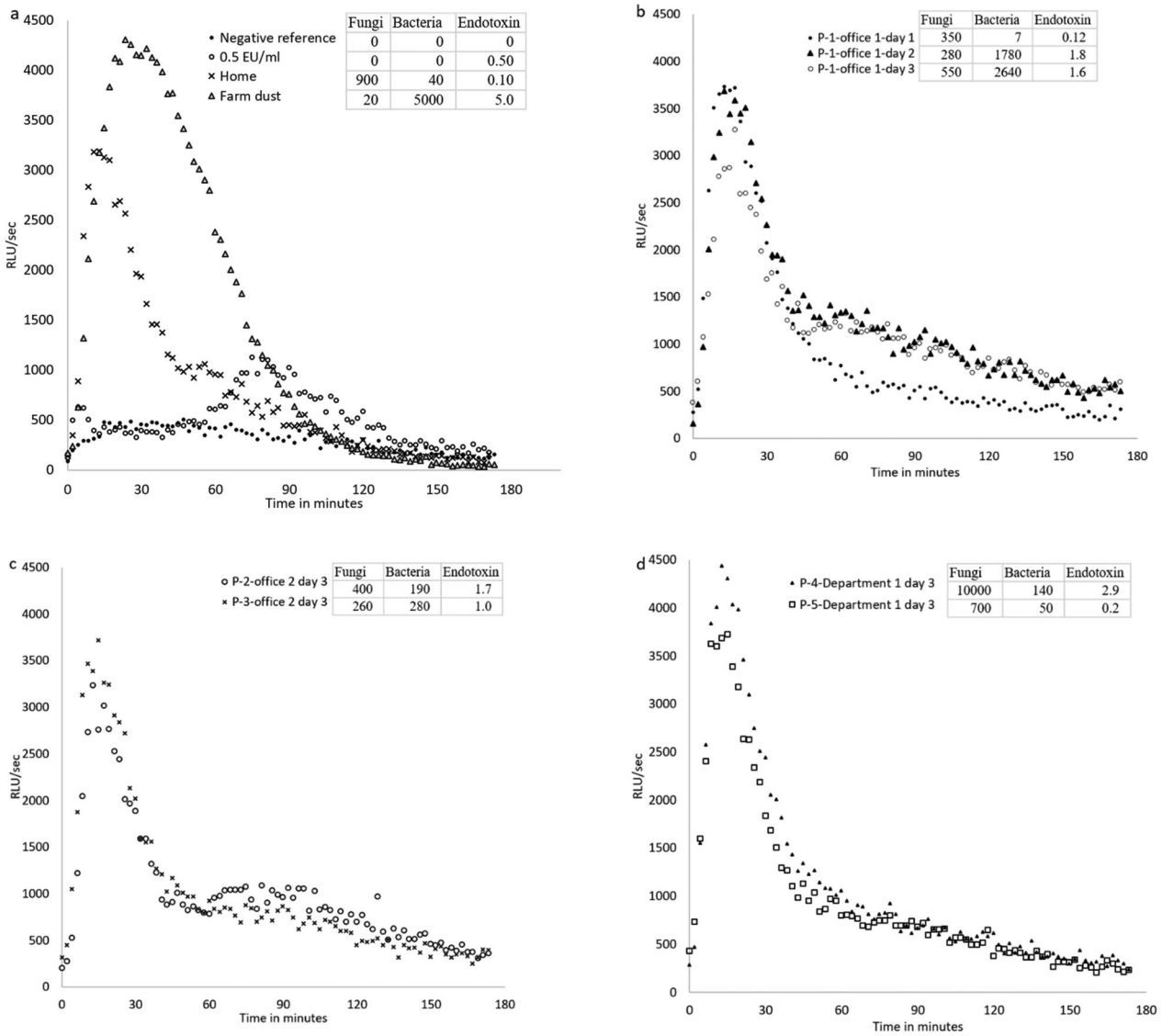

Figure 1. Profiles of reactive oxygen species (ROS) production in HL60 cells during exposure of cells to reference and personal air samples. The response is measured in relative luminescence units (RLU) per second over time. Each panel includes a table displaying concentrations of fungi (cfu/ml), bacteria (cfu/ml), and endotoxin (EU/ml) in the corresponding sample. Shown are results for: one person working in office 1 over three days (b); two persons in office 2 (c); and two persons in department 1 (d).

concentrations of bacteria or endotoxin, ROS production persisted beyond 60 min (Figure 1b). The highest peak response was found for P-4, who worked in Department 3 for 178 min. (Figure 1d). For the stationary samples, the highest peak exposure was observed in Department 2 on days 1 and 2 (Figure 2b).

The GM of TIP, fungi, bacteria, and endotoxin in personal exposures were 8.20×10^5 AUC/m^3, 5.9×10^3 CFU/m^3, 1.8×10^3 CFU/m^3, and 7.93 EU/m^3, respectively. The TIP ($p < 0.01$), bacteria ($p < 0.01$), and endotoxin ($p < 0.01$) of personal exposures were higher than in stationary samples.

The staff were exposed to different levels of fungi, bacteria, and endotoxin when working in different departments, with the highest TIP, fungal, and endotoxin exposure when working in Department 1, and the highest bacterial exposure in Office 1. High concentrations of fungi were found in Department 2 (Table 1).

TIP correlated with endotoxin ($r = 0.72$, $p < 0.01$), fungi ($r = 0.44$, $p = 0.05$), and bacteria ($r = 0.87$, $p < 0.01$). Endotoxin correlated with concentrations of bacteria ($r = 0.84$, $p < 0.01$).

Fungal and bacterial species

Several *Aspergillus* and *Penicillium* species were found in the air samples, and some are described as

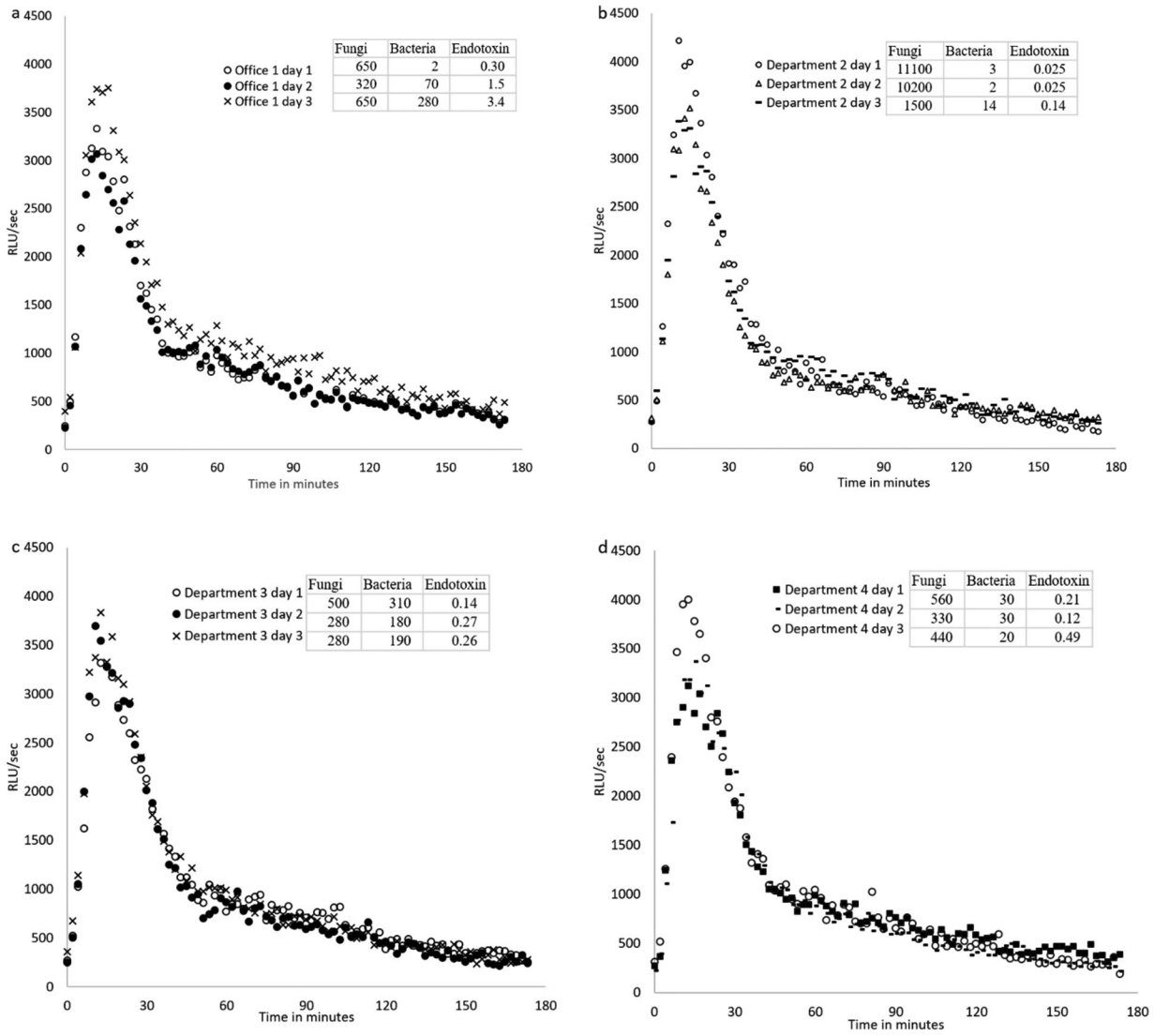

Figure 2. Profiles of reactive oxygen species (ROS) production in HL60 cells during exposure of cells to air samples taken in office 1 (a), departments 2, 3, and 4 (b, c, and d) using stationary samplers. The response is measured in relative luminescence units (RLU) per second over time. Each panel includes a table displaying concentrations of fungi (cfu/ml), bacteria (cfu/ml), and endotoxin (EU/ml) in the corresponding sample.

xerophilic (Table 2). Exposure during work in Department 1 was associated with exposures to *Aspergillus conicus*, *A. pseudoglaucus,* and *A. domesticus*. In all areas, airborne *Cladosporium* was found (Table 2).

The fungal species: *A. conicus, A. niger, Cladosporium* sp., *P. camemberti, P. chrysogenum, P. commune, P. corylophilum, Penicillium* sp., and *Saccharomyces cerevisiae* (baker's yeast) were found on the artifacts.

The staff was exposed to different species of bacteria, including human skin-related species such as *Micrococcus luteus*, *Moraxella osloensis*, *Staphylococcus epidermidis*, and *S. hominis*, which were also found using stationary samplers in Office 1 (Table 3).

Packing of artifacts

Using the ACI in Department 1, airborne fungi and bacteria were sampled in six size fractions before and during work with packing artifacts (Tables S3 and S4; Figure 3). Concentrations of fungi and bacteria were higher during the activity than before (ps < 0.05), and the fungal concentration increased by a factor of 6.6 while bacteria increased by a factor of 2.8. Several of the fungal species found during the packing activity were also found before, but in lower concentrations. The geometric mean aerodynamic diameter of fungal spores before the packing activity was 2.6 µm and during the activity, 2.8 µm ($p > 0.05$). Before the work activity, the geometric mean aerodynamic diameter of

particles with bacteria was 2.8 µm, and during the activity, 3.3 µm ($p < 0.05$).

Discussion

In this study, bioaerosol exposure of part-time staff in a historical museum was characterized. Staff members reported nasal symptoms and symptoms resembling those of ODTS. Elevated exposure to fungi was linked to immediate airway symptoms upon entering specific departments. Exposure levels to fungi (maximum in Department 1: 7×10^4 CFU/m^3) were lower than those reported in a case of museum-related ODTS (10^6 CFU/m^3) (Kolmodin-Hedman et al. 1986). In this study, the fungal species *Aspergillus conicus, A. domesticus, A. pseudoglaucus*, *A. pseudogracilis*, and *Cladosporium* spp. dominated the exposures and may be the primary causative agents of the reported symptoms, and exposure to endotoxin and bacteria may also contribute. Thus, ROS, which are key signaling molecules in inflammatory disorders, correlated with airborne concentrations of fungi, bacteria, and endotoxin. High concentrations of fungal spores were associated with the highest peak ROS production, as seen for the exposure of P4 working in Department 1, while the highest bacterial and endotoxin concentrations seemed to be associated with a prolonged ROS production, as seen for P1 during working in office 1. Previous studies have shown that exposure to endotoxin may act synergistically with fungal exposure to exacerbate respiratory inflammation (Park et al. 2006).

Table 1. TIP, exposure and concentrations (GM) inside and outside the museum; number in the same column followed by the same letter are not statistically different.

		TIP AUC/m^3	Fungi CFU/m^3	Bacteria CFU/m^3	Endotoxin EU/m^3
P-value		<0.01	<0.01	0.07	<0.01
Office 1	P ($n = 3$)	3.54×10^{5b}	2665^{b}	2217^{a}	4.8^{a}
Office 2	P ($n = 2$)	3.46×10^{5b}	2804^{b}	1980^{a}	11.6^{a}
Department 1[1)]	P ($n = 2$)	6.18×10^{5a}	4.13×10^{4a}	1289^{a}	11.4^{a}
Office 1	S ($n = 3$)	1.91×10^{5c}	2098^{b}	129^{ab}	5.4^{a}
Department 2[2)]	S ($n = 3$)	1.03×10^{5c}	2.30×10^{4a}	18^{b}	0.18^{bc}
Department 3[3)]	S ($n = 3$)	1.15×10^{5c}	1416^{b}	31^{b}	0.86^{b}
Department 4[4)]	S ($n = 3$)	1.28×10^{5c}	1774^{b}	113^{b}	0.95^{b}
Outdoor	S ($n = 3$)	5.02×10^{4d}	316^{c}	208^{b}	0.19^{c}

P = personal samples, S = stationary samples, n = numbers of samples.[1)] Visible fungi on the artifacts; human activity.[2)] Most artifacts in glass display cases; visible fungi on artifacts; an air purification system was active during day 3, no human activity in the department.[3)] Mainly wooden bookcases with mugs; no visible fungi; no human activity.[4)] Styled like a formal historical living room, furnished with vintage wood and fabric furniture; no visible fungi; no human activity.

Table 2. Occupational exposure[1)] to fungal species during work in offices 1 and 2 and department 1. Stationary measurements in office 2 and other departments of the museum as well as outdoors. Data presented as averages of positive samples in CFU/m^3.

Fungal species	Office 1 P ($n = 3$)	Office 2 P ($n = 3$)	Depart 1 P ($n = 2$)	Office 1 S ($n = 3$)	Depart. 2 S ($n = 3$)	Depart 3 S ($n = 3$)	Depart 4 S ($n = 3$)	Positive Samples	Out-door References
Alternaria chartarum [X]						45	44	3	3
Al. infectoria [X]				23				1	
Aspergillus conicus [H,X]	51		13500	44	4700	45		5	
A. creber					400			1	
A. domesticus [X]			5058		5600			3	
A. fumigatus [rc2, H]				28				1	
A. glaucus [X]	94							1	
A. montevidensis [X]	25				76			1	
A. niger [rc2, H]	51							1	
A. pseudoglaucus [X]	25		4289	44	2022	44		7	
A. pseudogracilis[X]			4421		2400			3	
A. versicolor [rc2, H]		91			103	66		4	
A. villosus [X]					103			1	
Botrytis cinerea		91				79		3	
Cladosporium cladosporioides[X]		90	499		26	39	32	8	26
C. cucumerinum						39		1	
C. herbarum [X]	61	151	209	186	390	29	299	11	26
Cladosporium sp.[X]	900	349	810	617	789	309	469	17	
C. sphaerospermum [X]		300						1	
Penicillium brevicompactum, [H,X]/ *olsonii*	128	91		210	76	46	85	9	3
P. camemberti [H]			970	23				2	
P. chrysogenum[X]	158		4221	5			87	6	
P. citreonigrum[X]									3
P. citrinum						15		1	
P. commune [H]	5		190	23	152	92		8	
P. corylophilum [H,X]	180	78		65				6	26
P. digitatum					227			1	
Penicillium sp.			4221		5320			4	
Wallemia sebi [H,X]					170			1	

[1)]P = personal samples, S = stationary samples, n = numbers of samples. [X]Fungi considered as xerophilic or xerotolerant, [H]Described to be associated with moisture damage buildings (Vesper et al. 2007), [rc2]Risk class 2 pathogen. See also footnotes in Table 1. Positive samples are numbers of positive samples in the museum. Concentrations are presented as a hetamap with darker red presenting highest concentrations.

Table 3. Occupational exposure[1)] to bacterial species (CFU/m^3; average of positive samples) during work in offices 1 and 2 and department 2. Stationary measurements in other departments of the museum as well as outdoors.

Bacterial species	Office 1 P ($n=3$)	Office 2 P ($n=3$)	Depart1 P ($n=2$)	Office 1 S ($n=3$)	Depart 2 S ($n=3$)	Depart 3 S ($n=3$)	Depart 4 S ($n=3$)	Posi-tive Samples	Out-door References
Acinetobacter lwoffii	879							1	
Agromyces cerinus		38						1	
Arthrobacter luteolus		38		15				2	
Bacillus cereus		38		15				2	
B. mycoides				8				1	
Brevibacterium casei		38						1	
Corynebacterium lipophiloflavum		79						1	
Co. mucifaciens	16	114	16					5	
Co. ureicelerivorans		114						1	
Curtobacterium flaccumfaciens	16	38		8			29	5	
Cytobacillus firmus				8				1	
Exiguobacterium mexicanum				8				1	
Frigoribacterium faeni	16		16	15				3	
Kocuria marina				40				1	
K. palustris				40			15	2	
K. rhizophila				60				1	
K. rosea				8				1	
Kytococcus sedentarius				8				1	
Microbacterium foliorum				8				1	
M. phyllosphaerae						11		1	
Micrococcus luteus	1758	197	128	450	5		171	10	
Moraxella osloensis	1758	310	401	15				6	
Oerskovia turbata				5				1	
Paenarthrobacter aurescens			16					1	
Planococcus okeanokoites				8				1	
Pseudomonas oryzihabitans		38		40				2	
Rhodococcus erythropolis			16					1	
R. fascians		189	64	40			15	6	77
Staphylococcus arlettae				8				1	
S. epidermidis	879	159	16	40				6	
S. equorum		76						1	
S. haemolyticus				8				1	
S. hominis	5275	250	1001	114			15	8	
S. lugdunensis	253							1	
S. petrasii				8				1	
S. saprophyticus				30				1	
S. warneri		79						1	
Streptomyces chartreusis			16					1	
S. xanthophaeus		38						1	

[1)]P = personal and S = stationary samples taken using GSP samplers. See also footnotes in Table 1. Positive samples are numbers of positive samples in the museum. Concentrations are presented as a hetamap with darker red presenting highest concentrations.

High concentrations of airborne *A. conicus* were found in different departments. Rare cases of eye disease in immunocompromised patients have been reported from exposure to this species (Smith et al. 2013; Jones 1978). The species *A. montevidensis*, which was found in low concentrations, has previously been found in clinical airway and clinical sputum samples, and it has been involved in cases of otitis, keratitis, and pulmonary infection (Siqueira et al. 2018; Bongomin et al. 2018). Exposure to *Aspergillus fumigatus* and *A. niger*, found in low concentrations, have previously been shown to cause different occupational related health effects in settings with exposures to greater concentrations than found in this study (Kofoed et al. 2024).

Aspergillus spp. was present as airborne particles of the size mainly depositing in the nose, throat, and bronchial region, while *Cladosporium* spp. was present as smaller particles able to deposit in both upper and lower airways. Hence, they may affect the airways differently.

No other publications were identified on personal exposures to fungi, bacteria, and endotoxins in museum environments. Consequently, the findings are compared with studies using stationary samplers. The fungal concentrations in all departments exceeded or were in the upper range of what has been measured in archive storage facilities and museums (Zielińska-Jankiewicz et al. 2008; Awad et al. 2020; Saridaki et al. 2023; Guiamet et al. 2011), and exposure to endotoxin was higher than measured in normal homes (Frankel et al. 2012), a laboratory (Niu et al. 2020), and outdoor air (Madsen 2006). Personal exposure to bacteria was higher than found in museums in Poland and Greece (Lenart-Boron et al. 2016; Saridaki et al. 2023). In contrast, stationary measurements were lower than reported in these studies. It should be noted that measured concentrations may be affected by the

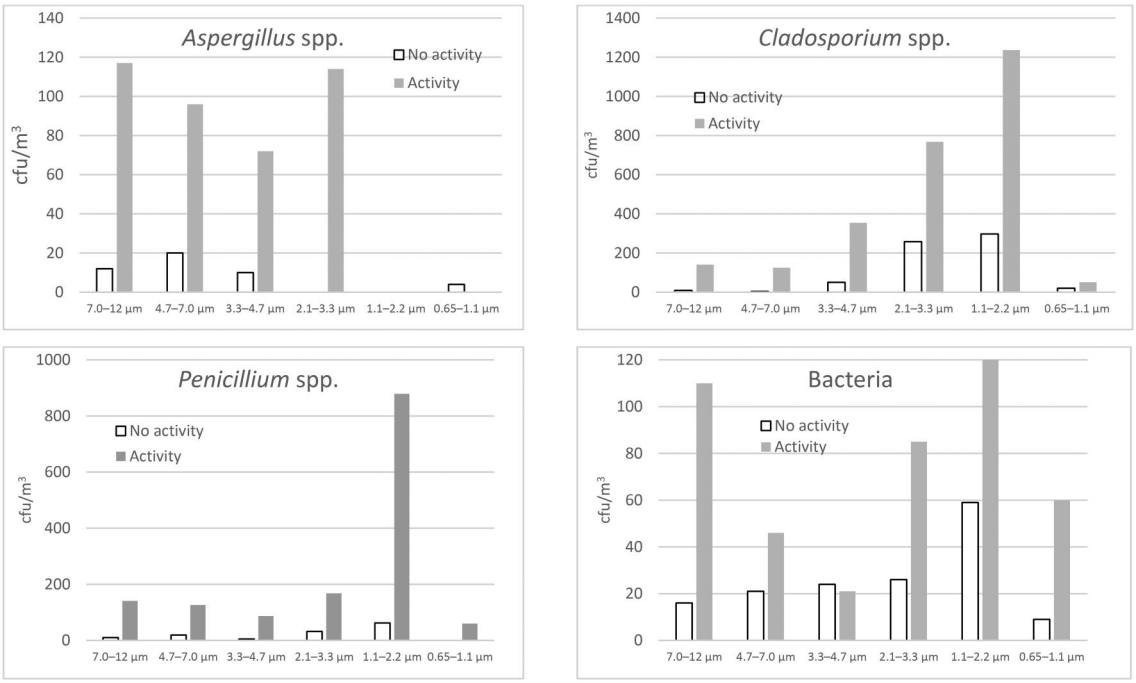

Figure 3. Concentrations of *Aspergillus* spp., *cladosporium* spp., and *penicillium* spp. and bacteria in department 1 as function of particle size fraction. Samples were taken using the ACI before and during work activities with packing of artifacts. Fungal and bacterial species can be found in Tables S3 and S4.

sampling devices and agar media used. For example, some of the cited studies used passive samplers with sedimentation on agar media, whereas this study used the GSP sampler, which actively samples the inhalable fraction of airborne particles (Kenny et al. 1999).

The temperature in the museum was at the lower end of the recommended range (16 to 25 °C) for exhibitions, while the average RH exceeded the recommended range of 40 to 60% (Museernes-Samråd 2021) with some fluctuations in both parameters. These conditions likely exposed artifacts to moisture, facilitating fungal growth. Consistent with this, several of the identified fungal species found in the air and on artifacts are known to be associated with moisture in buildings. However, the fungi found in high concentrations, *A. conicus*, *A. domesticus*, *A. pseudoglaucus*, *Cladosporium cladosporioides*, and *P. chrysogenum* are xerophilic fungi that can grow in environments with low water activity. Interestingly, of these xerophilic species, some are commonly (Vesper et al. 2007; Chakravarty 2022) or occasionally (Andersson et al. 2022) found in humid buildings. In the mentioned case study with ODTS, two species, *A. versicolor* and *P. verrucosum*, were identified (Kolmodin-Hedman et al. 1986). While *A. versicolor* is commonly found in humid buildings, *P. verrucosum* is xerophilic. Based on the presence of xerophilic species in both wet and dry buildings, strategies to avoid fungal growth are challenged. It has been suggested that xerophilic species may colonize dry material, and later, the dead hyphae of the xerophilic species form the basis for growth of non-xerophilic species (Micheluz et al. 2015). Recommendations to avoid fungal growth in museum artifacts have been suggested to include using fans to foster air movement and controlling temperature and humidity (Koul and Upadhyay 2018).

Some of the species found in the air in this study (*A. niger, P. funiculosum,* and *P. corylophilum*) have previously been found in museums and archive storage facilities (Zielińska-Jankiewicz et al. 2008; Bastholm et al. 2024), in indoor air in museums (*A. flavus, A. niger, C. cladosporioides, C. sphaerospermum*, *P. citrinum,* and *P. corylophilum*) (Rojas and Aira 2012), and on wood in a historical building (*A. conicus*) (Slimen et al. 2020). In this study, *Alternaria* spp. was found in low concentrations, but in a historical museum hall, it was found as the dominating genus (Saridaki et al. 2023). Although *Cladosporium* spp. were found both inside and outside the museum departments, higher concentrations were found inside, and they increased during packing activities. This suggests that the airborne *Cladosporium*

spp. concentrations derived from the artifacts, but whether it has colonized the artifacts or was present in sedimented dust is not known.

Several of the bacterial species found in this study are described as plant, soil, and human-related species. Findings of bacteria in the highest concentrations in personal samples, followed by office samples, indicate that bacteria derive from the staff and their activities. This was further supported by the presence of skin-related bacterial species such as *M. luteus* and *Staphylococcus hominis*. In Department 1, concentrations of airborne bacteria increased during activities. In a museum in Italy, concentrations of airborne bacteria increased during opening hours (Pasquarella et al. 2011), and in a study with stationary measurements in different rooms of a historical museum, the highest concentration of airborne bacteria was also found in an office (Grabek-Lejko et al. 2017).

Limitations

In this study, a combination of personal and stationary samplers was used to obtain knowledge about exposure and concentrations in museum departments with staff reporting work-related symptoms of the airway. A limitation of the study is the low number of participants, though all staff members at work participated in the study. Exposure levels may not reflect concentrations over time. This limitation was difficult to mitigate as the museum was undergoing deinstallation due to fungal contamination. Nevertheless, this study provides important information regarding indoor bioaerosol exposure in a museum facility.

In this study, a combination of three agar media and MALDI-TOF MS was used for the identification of fungi and bacteria. The use of additional agar media may result in the identification of more species.

Conclusion

The museum staff reporting airway-related symptoms were exposed to elevated levels of fungi and endotoxin. In the two departments where staff reported immediate symptoms, fungal concentrations ranged from 2 to 7×10^4 CFU/m^3. The predominant species were *Aspergillus conicus*, *A. domesticus*, *A. pseudoglaucus*, and *A. pseudogracilis*. Little is known about exposure to these species, and further research is needed to elucidate the health effects of exposure to elevated concentrations.

Recommendations

The exposure of affected staff consisted of both xerophilic species and those commonly found in water-damaged buildings, highlighting the challenge of managing fungi adapted to varying humidity conditions. This study underscores the importance of developing targeted interventions and preventive strategies to mitigate exposure and protect the health of museum staff. Fungi were found on museum artifacts, and handling these artifacts was associated with increased air concentration of fungi. This suggests that the artifacts serve as a source of exposure, highlighting the importance of limiting fungal growth to protect both staff and artifacts.

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Data availability statement

Exposure data can be found in the supplementary file.

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