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The impact of high-density polyethylene materials on microbiological purity in the process of storing and preserving textiles

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Abstract

Textiles made of natural fibers, both contemporary and historical, are at constant risk of degradation caused by, among others, microbial enzymatic activity. Fungi, bacteria, or actinomycetes are capable of producing proteolytic and cellulolytic enzymes, enabling microbial growth on textiles and leading to their decay. Ensuring proper storage conditions, including the usage of protective materials, allows long-term preservation of historical textiles in a good condition. These studies involved a broad microbiological analysis in order to verify whether high-density polyethylene (HDPE) materials can protect historical textiles during their storage. The results demonstrate interesting differences in microbial counts between washed textiles stored without HDPE covers and those stored in such covers; the latter presented considerably higher counts of microorganisms. However, when textiles had previously been sterilized, HDPE covers helped maintain appropriate microbiological purity. A considerable portion of historical textile collections, particularly exceptional silk liturgical vestments or burial garments, are kept by church institutions and stored in sacristies, treasuries, or other church rooms. The specificity of these places, both cultural and related to the age of the buildings themselves, makes them exceptional environments for storing textiles. To date, these places have rarely been researched, particularly in microbiological aspects. The analysis recounted below encompasses qualitative and quantitative assessment of fungi and bacteria present in the air of the treasury of the Wawel Royal Cathedral in Krakow, Poland, as well as the analysis of their destructive potential.

Keywords

HDPE, textiles storage, microbiology hazard, preserving textiles, biodeterioration

Historical textiles, which are a special group of cultural heritage items, have accompanied people for hundreds and even thousands of years. As today, textiles served various purposes in the past: they were used in daily life as utility textiles or clothes, they had diplomatic and representative functions in political life, as well as broad religious and decorative value.[1] At present, they let us learn more about the history of mankind – not only of its cultural and behavioral aspects, but also of technological development. That is why their appropriate preservation for future generations is such an important issue. Unfortunately, since these objects were made of natural fibers, such as silk, wool, cotton, or linen, they are constantly undergoing irreversible degradation processes. Because of their brittleness, their storage is problematic in various technical aspects both under different environmental conditions and techniques of storage.[2–4] Ensuring proper storage conditions for all cultural heritage collections is crucial for their preservation in a good physical state so that they can be a testimony of the past cultures to future generations. Proper care of historical collections, which encompasses the creation of properly prepared space/room for storage, their appropriate inventory, documentation, and provision of protective covers, as well as preventive conservation, is an integral part of the system aiming at the protection of museum collections.[5] The basic assumptions of this system include protection

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against external environmental conditions, fire, flooding, or vandalism. More detailed guidelines concerning protection of museum collections include: maintaining stable indoor climate conditions (relative humidity and temperature), no UV radiation, ventilation pollutants (such as ozone, O_3, and volatile organic compounds, VOCs), or particulate matter (PM2.5 particles with a diameter of 2.5 μm or less and PM10 particles with a diameter between 2.5 and 10 μm), as well as minimizing organic contamination.[6–12] Maintaining stable temperature and relative humidity is particularly important in storing brittle materials such as textiles, since these parameters considerably affect their preservation. As for relative humidity, the values recommended by most authors should range from 50–55%.[1,7,13,14] When too low, humidity can result in excessive fiber drying, thereby leading to an increase in their brittleness and raising the risk of mechanical damage. When too high, it can be conductive to hydrolytic reactions and cause degradation of cellulose and collagen fibers. Moreover, it can favor microbial growth. Therefore, maintaining humidity at a stable level and, above all, avoiding uncontrollable fluctuations over short periods of time become crucial aspects. Gradual humidity changes, in turn, which are associated with annual seasonal changes, are less harmful.[1] Maintaining stable levels of relative humidity in order to avoid stress to museum items has become the main recommendation of the novel European Standard EN 15757:2010 "Conservation of cultural property – specifications for temperature and relative humidity to limit climate-induced mechanical damage in organic hygroscopic materials."[15] Furthermore, optimal temperature in storerooms for historical cloths should range from 16–18°C, which is not always possible due to economic reasons. Nevertheless, temperature should be properly correlated with relative humidity.[1,3,13]

The manner of storage is a separate issue in preservation and protection of historical cloths. Due to their brittleness and frequently considerable degradation, the manner of storage should be adjusted to the size, type or shape of an item. The most common ways of storing textiles include: lying flat, hanging in a vertical position,

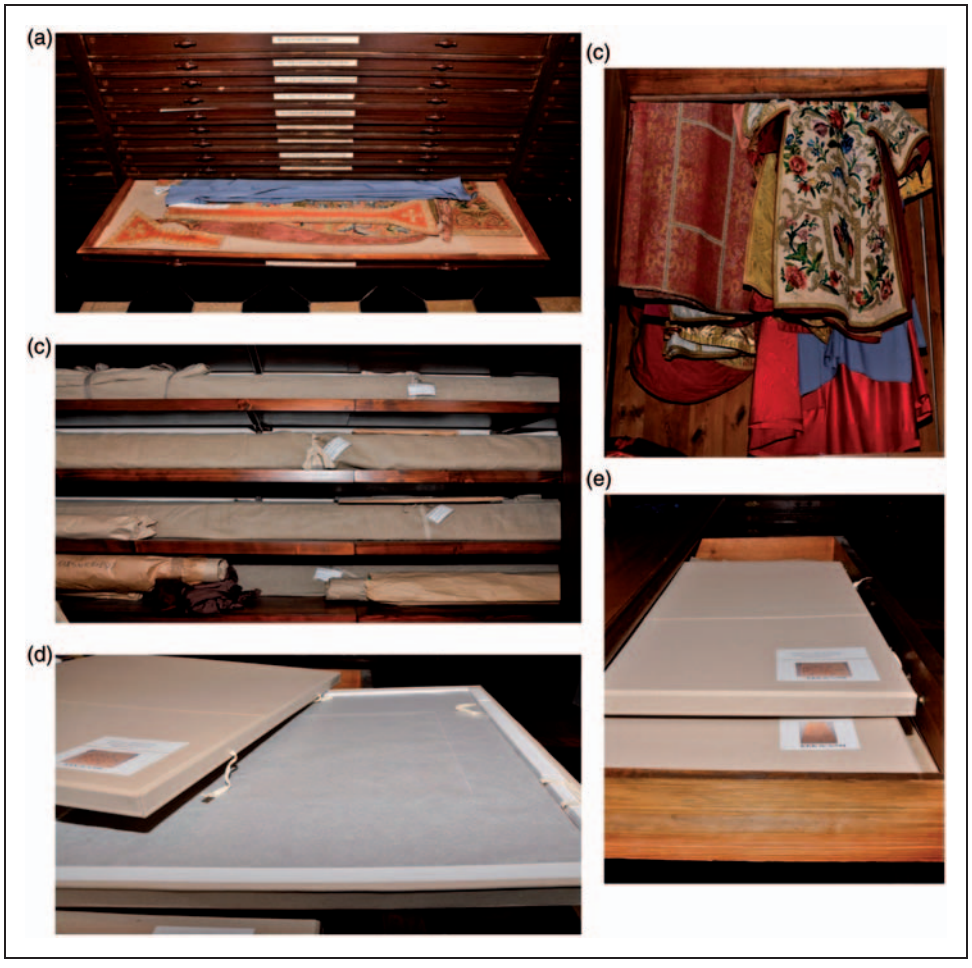

Figure 1. Storing historical textiles: (a) laid flat; (b) rolled up; (c) hung; (d, e) placed in acid-free boxes.

or rolling up (Figure 1). The first form of storage, i.e. laying textiles flat usually unfolded in drawers, is used for relatively small items whose shape and size enable such storage. Also, it can be a good solution for heavily destroyed items since it provides a calm storage with no stretching or stress to their fibers. Costumes or garments that could crumple or fold when laid flat are hung in wardrobes using specially prepared hangers. Large textiles in a suitable condition are usually rolled up and packed in tubes made of acid-free paper or covers of unbleached muslin (or sometimes cotton) with adequately protected ends. Rolled items are stored on stands preventing tensions. Smaller items can also be stored in boxes of acid-free paper, or muslin and paper separators can be used (Figure 1).[13,16]

The aforementioned increase in temperature and relative humidity can cause microbial growth, of both bacteria and fungi, on stored items. If conditions are favorable, microorganisms are capable of developing and initiating biodeterioration, i.e. enzymatic decomposition of organic matter of which natural textiles are made.[4,17–19] Other factors enabling microbial growth are organic contaminants found in dust carried through ventilation ducts and by visitor and employee presence/movement. Hence additional preventive measures are undertaken during storage of historical textiles. The aim of preventive measures is to isolate items from the external environment, thereby protecting them from contaminants or insects. An example of such a practice is the usage of a special high-density polyethylene (HDPE) material for packing and storing historical textiles. This packaging material is commercially available and its main properties include: low moisture absorption, vapor permeability, and high durability. This material is used in conservation practice for packing items in order to isolate them from the outer environment. These materials are supposed to enable textiles to breathe and simultaneously protect them from the outer environment, physical and organic impurities as well as excessive humidity. This study attempted to verify whether or not covers made of this polyethylene material have negative effects on the microbiological status of protected textiles. Especially in case of usage it for storing textiles after conservation treatments that must return to their primary place of storage which is often contaminated by microorganisms. This HDPE material was used in study concerning microbiological aspect for the first time in this research.

Experimental details

Materials and methods

The material used in the first part of the experiment included raw and non-colored samples of contemporary textiles (silk, wool, cotton and linen) of 25 cm^2. As has been mentioned above, the aim of this stage was to verify whether storing textiles in HDPE covers affects microbial growth on their surfaces.

The study involving the storage of selected samples in polyethylene covers was conducted in two time periods (3 and 6 months) in a specific environment of the treasury of the Wawel Royal Cathedral in Krakow, Poland. The treasury was selected for the experiment mainly because it is located in the historical building of the Wawel Royal Complex and stores over 2000 historical textiles. Thanks to this, it has a specific microclimate typical of places used for storing historical, mainly church, textiles. All textile samples were initially sterilized and then placed in the cathedral treasury for spontaneous contamination for the period of 3 months. Subsequently, they underwent standard conservation washing. Four groups were distinguished for each textile type. The first group included dry samples after conservation washing stored in the treasury without covers. The second group consisted of dry washed textiles packed in HDPE covers. The third group contained washed and dried textiles sterilized with ethylene oxide in a fumigation chamber which were stored in the Treasury without covers. Finally, the fourth group included washed and dried textiles sterilized with ethylene oxide in a fumigation chamber which were wrapped in HDPE (Figure 2). Covers made of this material were prepared in accordance with the standard procedure; the size of the covers was adjusted to the size of the samples and then sealed with tape made of the same material. In order to recreate the most credible practical conditions, the cover material was not sterilized since such a practice is not used in protecting museum items. After the test periods, textiles underwent a quantitative microbiological analysis. The samples were rinsed with 10 mL of saline solution and then the suspension was cultured on culture media for fungi (malt extract agar, MEA, and Sabouraud glucose agar, SGA) and bacteria (trypticase soy agar, TSA). The inoculated media were incubated at a temperature of $28 \pm 2^{\circ}C$ for 72 h for bacteria and 5–7 days for fungi. Subsequently, the colonies obtained were counted. The results were analyzed statistically.

Conservation washing. Textile samples were washed in 1% solution of Pretepon G (Tomchem, Poland) in demineralized water at a temperature of 35°C, pH 6.5–7.5 for 30 min. Subsequently, textiles were rinsed three times in demineralized water at a temperature of 30–35°C. Finally, after ordering the warps and wefts, all fragments of materials were left to dry.

Sterilization procedure. The sterilization was conducted in a fumigation chamber and included three steps.

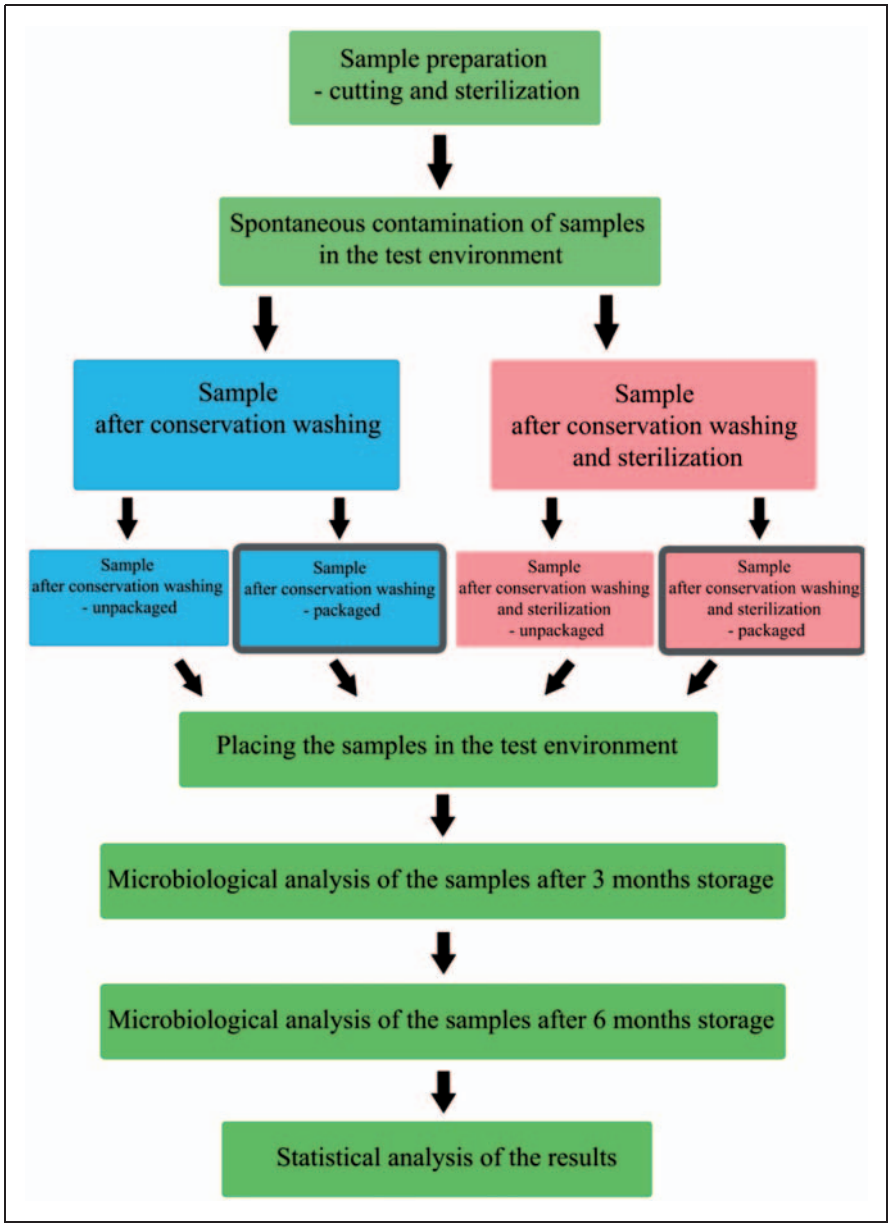

Figure 2. Plan of the experiment.

The first preparation stage takes 5 h under conditions of temperature 24°C, air pressure 800 mbar, and humidity 50%. At the end of this stage the pressure is decreased to 150 mbar for better water removal. Subsequently, the sterilization stage occurs with temperature 22°C, humidity 45%, and gas pressure 800 mbar (CO_2/EO = 91/9%) for 60 h. Finally, in a ventilation step, the air is changed 15 times with usage of a high-efficiency particulate air (HEPA) filter.

Statistical analysis. The statistical analysis was conducted with the use of a parametric test for the analysis of variance (factorial ANOVA) and a post-hoc Tukey's test. The level of statistical significance was $p < 0.01$.

Microbial analysis

The second stage of the study involved a microbiological analysis of indoor air in the Cathedral Treasury in order to learn about and characterize the site at which the tests were conducted. Because of the unique character of this place and few literature reports on such storage places for historical textiles, a complete microbiological analysis was carried out. The study encompassed both quantitative and qualitative microbiological evaluation of indoor air, biodiversity analysis of fungi present in the air and its changes over time using PCR-DGGE (polymerase chain reaction–denaturing gradient gel electrophoresis), as well as the verification of the

destructive potential of fungal and bacterial isolates obtained from cultures.

Analysis of selected physical parameters of the indoor climate. In the treasury of the Wawel Royal Cathedral, temperature and humidity are constantly monitored (Thermohygrometer MAX-MIN, Viking AB, Sweden). For an overall assessment of the indoor microclimate, these parameters were also analyzed during the experiment.

Microbiological analysis of the indoor environment. Air samples for the microbiological analysis were collected for 1 min onto selected culture media with a MAS-100 Eco sampler (Merck, Darmstadt, Germany) with air flow rate of 100 L/min. For bacteria, TSA and R2A media were used, whereas for fungi MEA, SGA, and agar with chloramphenicol DG18 were applied. The samples were incubated at a temperature of $28 \pm 2^{\circ}C$ for 72 h for bacteria and 14 days for fungi, with periodical inspection and isolation of dominant strains. Moreover, the material was also obtained via spontaneous contamination of sterile textile samples (silk, wool, cotton, linen) placed in the treasury for a period of 6 months. Following contamination, the samples were rinsed with saline and cultured on MEA and SGA media for fungi and TSA medium for bacteria. For isolation of proteolytic microorganisms, a sucrose-free Weary & Canby medium was used whilst microbes with cellulolytic properties were isolated with the use of a Czapek Dox agar without sucrose, where the only source of carbon was respectively a protein and cellulose from spontaneous contaminated fabrics. The media were incubated at a temperature of $28 \pm 2^{\circ}C$ for a maximum period of 4 weeks and inspected periodically. Microorganisms were then subcultured until pure cultures were obtained and identified. All microbiological media used during the study were provided by BTL (Lodz, Poland).

Molecular identification of molds and bacteria. Fungal DNA was extracted using a commercial Gene MATRIX Plant & Fungi DNA Purification kit (Eurx, Gdansk, Poland). A six-day fungal growth was collected from MEA with a sterile scalpel and then homogenized with glass beads using a Minilys homogenizer (BertinTechnologies, Montigny le Bretonneux, France). The subsequent stages of isolation were conducted in accordance with the manufacturer's protocol. Bacterial DNA was extracted from 48 h cultures on broth agar using a Bacterial & Yeast Genomic DNA Purification kit (Eurx, Gdansk, Poland) in accordance with the enclosed protocol. Microbial identification was conducted on the basis of an ITS (internal transcribed spacer) region sequence analysis for fungi and a partial sequence of 16S rDNA analysis for bacteria. Amplification of selected sequences was conducted in 25 μL of the reaction mix consisting of 1.3 μL of DNA template, 0.4 μmol of each primer (Genomed, Warsaw, Poland),[20] 200 μL/L of each dNTP (Sigma-Aldrich, Saint Louis, USA), 2 U of Taq DNA polymerase (Invitrogen, Thermo Fisher Scientific), 1 × polymerase buffer and 1.5 mmol of $MgCl_2$. The PCR with the use of U968 (5′-AACGCGAAGAACCTTAC-3′) and L1401 (5′-CGGTGTGTACAAGACCC-3′) primers followed the program of initial denaturation at 95°C for 5 min, 34 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 1 min and extension for 1 min at 72°C, as well as final extension at 72°C for 10 min. In the identification of fungal strains, ITS1 (5′-TCCGTAGGTGAACC TGCGG-3′) and ITS4 (5′- TCCTCCGCTTATTGATA TGC-3′) primers were used,[20] and amplification was conducted according to the following program: initial denaturation at 95°C for 5 min, 34 cycles of denaturation at 94°C for 50 s, annealing at 56°C for 50 s and extension for 50 s at 72°C, as well as final extension at 72°C for 10 min. The PCR products were isolated by electrophoresis from 1.3% agarose gel and visualized under UV light using Simply Safe dye (5 μL of dye was added to 100 mL agarose solution; Eurx, Gdansk, Poland).

The PCR products were purified and sequenced (Genomed, Warsaw, Poland). Based on the nucleotide sequences obtained, species identification was conducted using the NCBI (National Centre for Biotechnology Information) database and the Basic Local Alignment Search Tool (BLAST) for DNA sequence analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Fungal diversity analysis. The diversity analysis of fungi found in the air of the Wawel Royal Cathedral was conducted with the use of PCR-DGGE. This method enables us to learn about the genotypic structure of a tested environment based on the analysis of differences in selected genomic sequences. The nested-PCR technique, which consists of two PCRs, was applied for the amplification of the fungal ITS1 region. In the first PCR, ITS1F and ITS4 primers were applied, and the obtained amplicons were used as a template for the second PCR, which was conducted with the use of ITS1F-GC (with a GC clamp) and ITS4 primers.[20] The conditions for the reaction followed those reported by Lech and Ziembinska-Buczynska.[21] The PCR products obtained were isolated by electrophoresis in concentration gradient in a DCode Universal Mutation Detection System (BioRad, Hercules, USA). The amplicons with a GC clamp were separated from 8% polyacrylamide gel (acrylamide: bisacrylamide, 39.5:1) containing 30–60% of the denaturation factor (formamide–urea). Following electrophoresis, the gel was

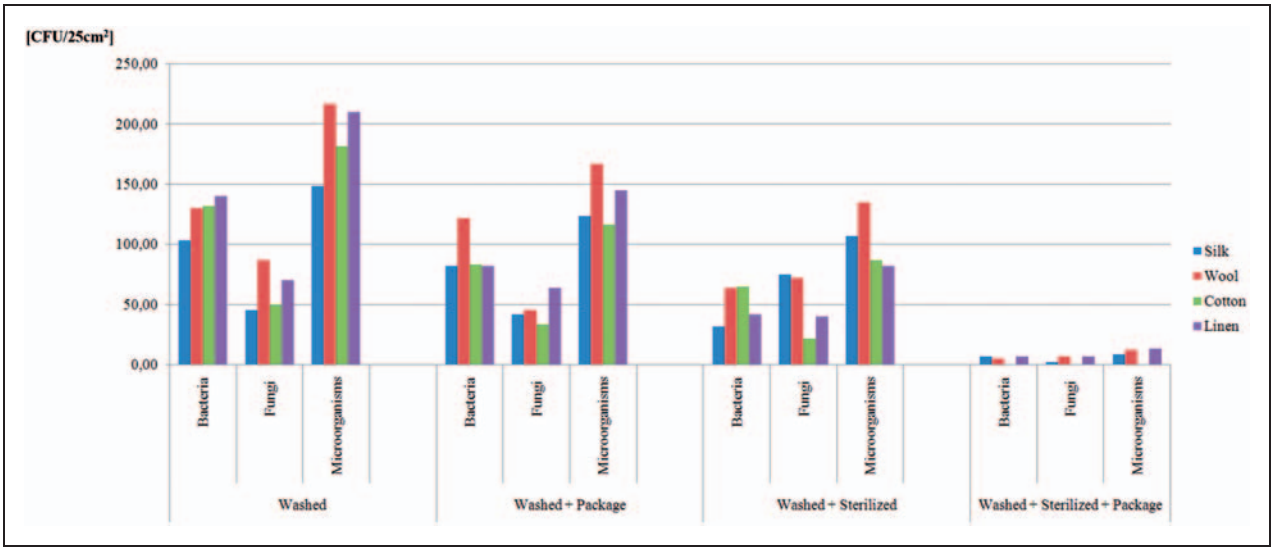

Figure 3. Mean numbers of bacteria, molds, and overall microbial counts, including textile types, conservation treatments, and the manner of storage – a 3-month analysis.

dyed in SYBR Green (1:10,000, Invitrogen) and washed in miliQ water for 20 min. The band pattern (fingerprint) obtained was visualized under the UV light and photographed. The densitometric analysis, attesting to the concentration of the products obtained, was conducted using Image J (National Institutes of Health, USA). Finally, the Shannon biodiversity index was calculated.[22]

Identification of prevailing genotypes based on the DGGE profile. The DGGE profile (band pattern) of the cathedral's indoor environment was used for the genetic identification of dominant genotypes in order to capture and identify non-culturable species. Selected bands were excised from the DGGE fingerprint with a sterile scalpel and then a repeated re-amplification of the excised products was conducted in the same conditions as the first amplification. The PCR products were isolated by electrophoresis, and the visualized bands were excised again for DNA extraction with the use of a commercial GenElute PCR Clean-up Kit (Sigma Aldrich) to purification the PCR products. The products obtained underwent sequencing.

Evaluation of the biodeterioration potential. The biodeterioration potential of isolated microbes was evaluated by analyzing proteolytic and cellulolytic properties. The former were tested with a gelatin liquefaction test (5-day incubation at 28°C; BTL, Lodz, Poland). The latter were tested with CMC (carboxymethylcellulose) media (BTL, Lodz, Poland),[23] using a triple-point inoculation method and incubation at 28°C for 48 h for bacteria and for 4 days for fungi. Afterwards, the plates with microbial growth were dipped in Lugol's solution and inspected for the presence of clear zones indicating cellulase production.

Results and discussion

Impact of HDPE material on microbiological purity during textile storage

The aim of the microbiological analysis was to demonstrate whether the usage of HDPE material for storing textiles, particularly historical ones, can affect microbial safety of stored items. Figure 3 presents the results of a quantitative microbiological analysis after three-month storage of textiles packed and non-packed in polyethylene materials, taking into account the type of textile and its initial microbiological purity as well as the process of washing and an additional sterilization treatment. The statistical analysis revealed that in textiles washed only, there are no significant differences in microbial counts (bacteria, molds, and overall microbial count) between packed and non-packed samples. As for textiles additionally sterilized (with ethylene oxide), the usage of HDPE material resulted in greater microbiological purity. The bacterial and fungal counts, irrespective of the type of textile, did not exceed 7 CFU/25 cm^2, i.e. the number of microbes present on the polyethylene material itself. However, due to the rigor of the chose statistical test, statistical significance was noted only for bacterial counts on wool and cotton, fungal counts on silk and wool as well as total microbial count on silk, wool and cotton. It must be noted that the lack of statistically significant results in the remaining cases is caused by a very high standard deviation with a very low level of microorganisms found on textiles after sterilization.

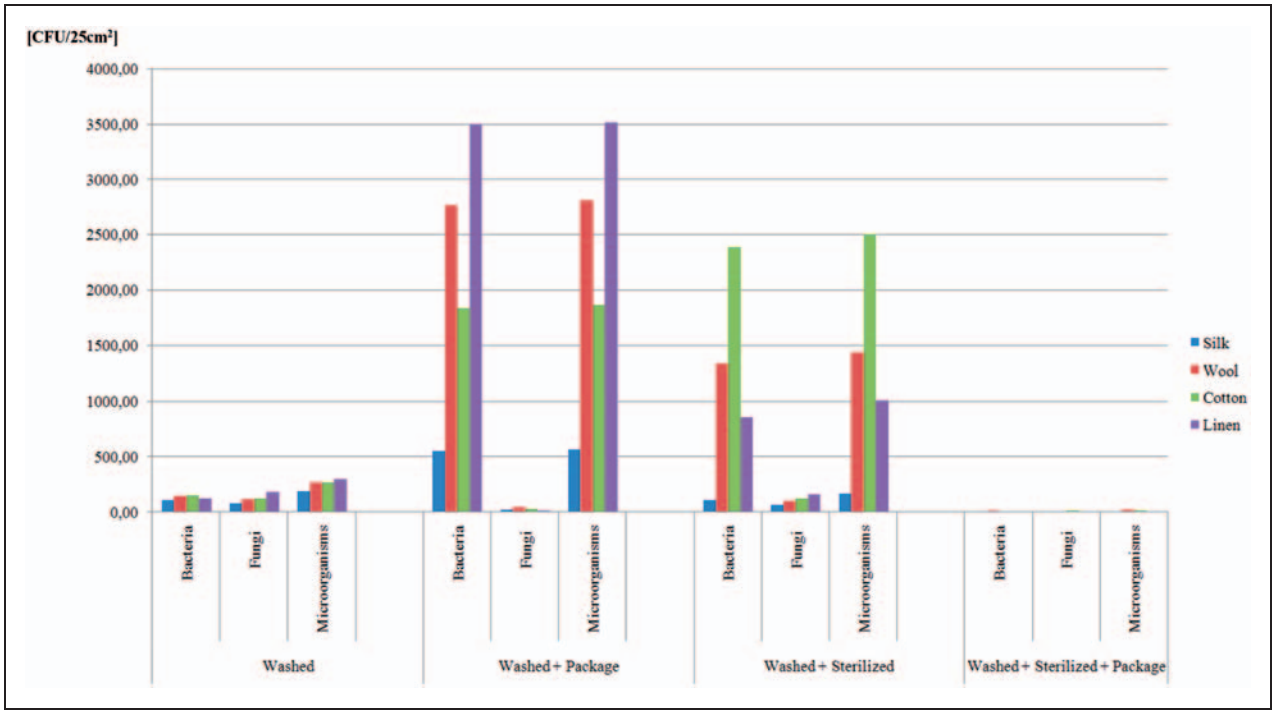

Figure 4. Mean numbers of bacteria, molds, and overall microbial counts, including textile types, conservation treatments, and the manner of storage – a 6-month analysis.

Very interesting results were obtained in the quantitative analysis of microbes present on non-packed and packed samples after six-month storage in the Cathedral Treasury (Figure 4). In this case, when comparing packed and non-packed textiles that had been only washed, a considerably higher number of microorganisms was observed on the former samples. The differences were statistically significant for bacteria and overall microbial counts for almost all types of textiles except for silk on which considerable microbial growth was observed as well but it was not statistically significant. The statistical analysis of the results obtained for washed and additionally sterilized samples also demonstrated significant differences between packed and non-packed samples. In this case, however, the bacterial and overall microbial counts were markedly lower on packed samples and the differences were statistically significant for wool, cotton and linen.

Based on these results, it can be concluded that storing textiles only after washing in tightly closed covers made of the tested polyethylene material for short periods of time has no influence on microbial safety of stored items. In long-term storage, however, keeping textiles in tightly closed covers causes considerable growth of microorganisms. This is probably associated with the development of a specific microclimate inside closed HDPE covers enabling survival and development of microbes. Such a situation can also be affected by humidity fluctuations. The situation changes completely when textiles previously sterilized are kept in HDPE covers. In this case, HDPE covers are good protective barriers, and textiles wrapped in them maintain high microbiological purity. The studies suggest that the tested material could be a good solution for storing historical textiles after conservation treatments but only for previously sterilized items. Nonetheless, the verification of these conclusions seems to require further tests with even longer storage periods.

The quantitative analysis that tested the possibility of using HDPE materials for storage revealed one more significant correlation. After three-month storage in the Treasury, the microbial count on non-packed sterilized textiles was lower than on textiles that had been only washed, which could have been expected. However, a reverse situation occurred after 6-month storage; non-packed sterilized samples revealed much more microorganisms (statistically significant results were obtained for bacterial and total microbial counts for wool, cotton and linen). A rapid settlement of free ecological niches by microbes is a known and common phenomenon. However, in the case of museum collections, it must be emphasized that sterilization of historical items and their subsequent placement in a storeroom environment without protection will lead to even faster inhabitation by microorganisms present in this environment which can have a destructive potential and cause rapid item deterioration.

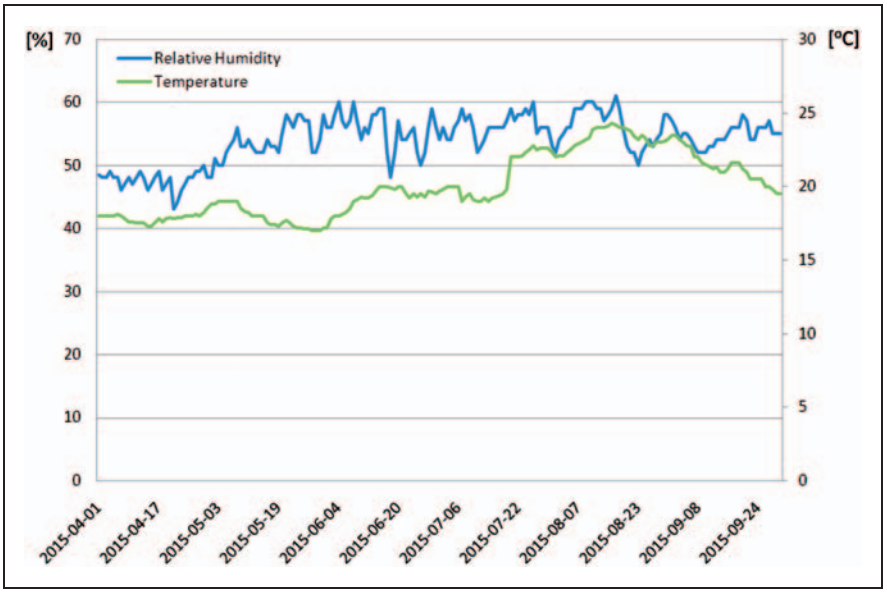

Figure 5. Changes of the indoor environment parameters (temperature and relative humidity) in the treasury of the Wawel Royal Cathedral.

Indoor microclimate analysis

In the treasury of the Wawel Royal Cathedral, physical parameters of the indoor climate (temperature and relative humidity) are constantly monitored. From the point of view of microbiological hazard, these parameters are very important since their uncontrolled, abrupt changes can lead to rapid microbial development. Possible abrupt and uncontrolled fluctuations of these parameters could affect the interpretation of the results obtained in this experiment. That is why it was decided to perform an analysis of the parameters in question from the period of six months (Figure 5). The analysis of temperature and relative humidity changes was conducted from 1 April 2015 to 30 September 2015. In this period, 185 measurements of each parameter were taken. Both parameters occurred to be relatively stable over the entire test period with temperature ranging from 17 to 24.3°C and humidity from 43 to 61%. Considering the recommendations regarding RH of 30–35% in the winter and 50–55% in the summer,[13] and not exceeding the humidity of 65% and temperature of 24°C,[1] it can be stated that the conditions in the treasury were good, particularly because the building in question is historical. Alarming humidity fluctuations were probably caused by cleaning and using the room for church services. The literature reports demonstrate that the age and structure of the building can also be associated with such changes.[15,24]

Microbiological analysis of the textile storing environment

The quantitative evaluation of microorganisms present in the indoor air of the treasury of the Wawel Royal Cathedral was conducted three times: at the beginning of the experiment, after 3 months and after 6 months. The results (Figure 6) suggest that the air in the treasury is characterized by relatively stable microbiological conditions. The greatest number of bacteria (445 CFU/m^3) and molds (140 CFU/m^3) were observed in the summer. This was probably caused by an increase in temperature and humidity observed during the monitoring of physical parameters of the indoor climate. However, it must be underlined that these results are similar to microbial counts found in the air of other Polish museums, the mean values of which range from 340–560 CFU/m^3 for bacteria and 75–14,000 CFU/m^3 for molds.[25] According to the Italian standards issued by the Italian Ministry of Cultural Heritage, microbial counts in museums should not exceed 750 CFU/m^3 for bacteria and 150 CFU/m^3 for fungi.[26]

As a result of triple air sampling and the microbiological analysis of textile samples placed at various sites in the treasury for spontaneous contamination, 15 bacterial (Table 1) and 17 fungal isolates (Table 2), most commonly occurring in the tested room, were obtained. The predominant bacteria belonged to the genus *Bacillus* (7 isolates: *B. barbaricus*, *B. cereus*, *B. fordii*, *B. infantis*, *B. megaterium*, *B. pseudomycoides*) and the genus *Micrococcus* (2 isolates: *M. luteus*, *M. yunnanensis*). Among molds, the prevailing group consisted of the following genera: *Aspergillus* (3 isolates: *A. flavus*, *A. fumigatus*, *A. niger*), *Penicillium* (3 isolates: *P. corylophilum*, *P. funiculosum*, *P. glabrum*), and *Alternaria* (2 isolates: *A. infectoria*, *A. tenuissima*) as well as two isolates from the genus *Cladosporium*

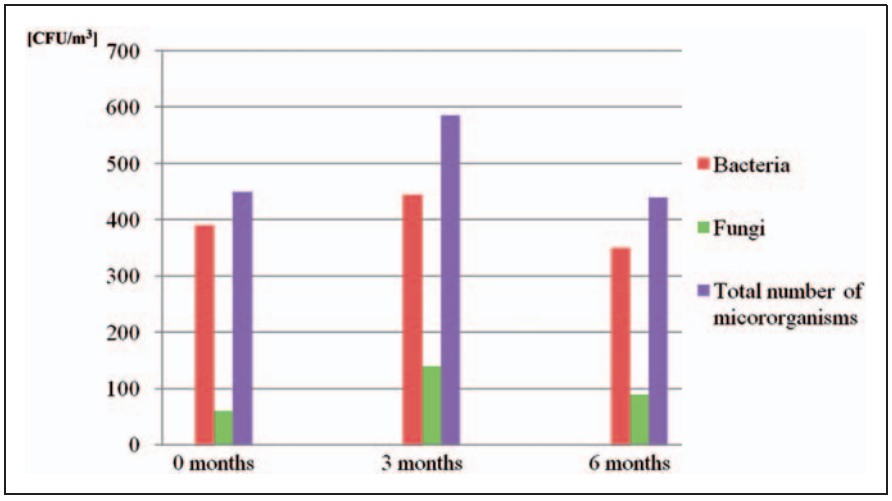

Figure 6. Quantitative microbiological evaluation of the indoor air in the treasury of the Wawel Royal Cathedral.

Table 1. Identification of bacterial isolates and their proteolytic and cellulolytic activity

Code of isolated strain	Bacterial isolates	Accession number of the closes sequence from NCBI (% similarity)	Gelatin test	Cellulolytic activity
B1	*Bacillus barbaricus*	JF727665.1 (99)	+	+
B2	*Bacillus cereus*	EU111736.1 (100)	+	+
B3	*Bacillus cereus*	DQ884352.1 (100)	+	+
B4	*Bacillus fordii*	KP282827.1 (99)	+	–
B5	*Bacillus infantis*	KJ126933.1 (99)	+	+
B6	*Bacillus megaterium*	FJ976617.1 (100)	+	+
B7	*Bacillus pseudomycoides*	KU258291.1 (99)	+	+
B8	*Kocuria polaris*	LT221178.1 (99)	–	+
B9	*Micrococcus luteus*	KR047779.1 (99)	–	–
B10	*Micrococcus luteus*	KT906675.1 (99)	+	+
B11	*Micrococcus yunnanensis*	KU684497.1 (99)	–	–
B12	*Micrococcus yunnanensis*	KT719588.1 (99)	–	–
B13	*Psychrobacillus psychrodurans*	KU285615.1 (99)	–	+
B14	*Psychrobacter pulmonis*	KR856350.1 (99)	+	–
B15	*Streptomyces albus*	KT581272.1 (99)	–	+

(*C. cladosporioides*, *C. ramotenellum*). The specificity of the tested place, i.e. the cathedral treasury (but also sacristies and other church rooms that store historical liturgical silk textiles), renders it difficult to compare the identified microflora with other studies reported in the literature. A vast majority of microbiological analyses conducted in storerooms for cultural collections concern museums or archives where the dominant microflora is made up by molds from the *Penicillium*, *Aspergillus*, *Cladosporium*, *Trichoderma*, *Alternaria*, and *Chaetomium* genera,[14,25,27,28] and bacteria belonging to the *Bacillus*, *Micrococcus*, *Pseudomonas*, or *Staphylococcus* genera.[25,28,29] Microbial species identified in these studies that have been isolated from historical textiles before or found responsible for their deterioration include the following molds: *Penicillium corylophilum*,[3] *Cladosporium cladosporioides*,[30] as well as *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Penicillium funiculosum*, and *Alternaria tenuissima*.[31] The microbial species identified in the air of the treasury have been found to occur on cultural heritage items other than textiles but also made of natural components, such as proteins or sugars. *Bacillus megaterium*, *B. cereus*, *Micrococcus luteus*, *Psychrobacter psychrophilus*, and

Table 2. Identification of fungal taxons and their proteolytic and cellulolytic activity

Code of isolated strain	Fungal isolates	Accession number of the closes sequence from NCBI (% similarity)	Gelatin test	Cellulolytic activity
F1	*Alternaria infectoria*	KR094444.1 (99)	–	+
F2	*Alternaria tenuissima*	KT310953.1 (99)	+	+
F3	*Aspergillus niger*	KT832690.1 (96)	+	+
F4	*Aspergillus flavus*	KR076749.1 (99)	+	–
F5	*Aspergillus fumigatus*	KF305755.1 (98)	+	+
F6	*Bjerkandera adusta*	AF455468.1 (99)	–	+
F7	*Cladosporium cladosporioides*	HM776418.1 (100)	+	+
F8	*Cladosporium ramotenellum*	KP701983.1 (100)	–	–
F9	*Coprinellus aff. radians*	FJ185160.1 (97)	–	+
F10	*Embellisia abundans*	KC311474.1 (99)	+	+
F11	*Leptosphaerulina chartarum*	DQ384571.1 (99)	–	+
F12	*Neosartorya hiratsukae*	KR909166.1 (99)	–	+
F13	*Ochroconis constricta*	KF437521.1 (97)	–	–
F14	*Penicillium corylophilum*	JN585947.1 (99)	+	+
F15	*Penicillium funiculosum*	GQ337426.1 (99)	+	+
F16	*Penicillium glabrum*	JF327812.1 (98)	+	+
F17	*Rhodotorula mucilaginosa*	KF646193.1 (99)	–	–

Kocuria sp. have been isolated from, among others, historical parchments,[32] paper documents,[33] archaeological textiles,[34] or paintings.[28] *Cladosporium cladosporioides*, *C. ramotenellum*, *Aspergillus flavus*, *A. niger*, *Penicillium corylophilum, P. glabrum*, or *Embellisia abundans* have been previously described as xerophilic fungi inhabiting niches such as libraries and archives.[35] Noteworthy is the isolation of a species of psychrotolerant bacterium *Kocuria polaris*, which is capable of producing an orange pigment that could cause stains on textiles,[36] and *Bacillus barbaricus* described as a new species responsible for the biodeterioration of wall paintings.[37] As for fungi, attention should be drawn to species that have not been isolated from historical textiles before, such as *Ochroconis constricta* – a keratinophilic fungus isolated from soil,[38] *Leptosphaerulina chartarum* with a high potential to produce xylanolytic enzymes,[39] and *Neosartorya hiratsukae* – a pathogenic fungus responsible for, among others, dermatitis.[40,41]

Furthermore, since molds play the major role in the process of biodeterioration of cultural heritage objects, a biodiversity analysis of fungi present in the air of the treasury was conducted. The test was carried out three times: at the beginning of the experiment, after three months and at the end of the experiment after six months. For this purpose, the author used the PCR-DGGE method, which enables the creation of a genetic profile (a fingerprint) of the investigated environment. The advantage of this method is the ability to obtain information about the genotypic diversity of microbes present in the tested environment without the necessity of conducting microbial cultures, which considerably improves the possibility of learning about species present in this environment. Apart from demonstrating the genotypic structure of an environment and the identification of strains that are non-culturable in laboratory settings, this method also enables us to trace biodiversity changes in the tested environment over time. DGGE fingerprints, which present the structure of microbial communities in the environment where cultural heritage objects are stored and on objects themselves, have been applied in numerous microbiological studies.[19,29,42–46] The level of fungal biodiversity (genotypic diversity) in the tested environment was determined based on the Shannon index (Figure 7(b)). The analysis demonstrated a change in fungal genotypic diversity over the study period with its highest level observed in the autumn. This trend is probably associated with increased humidity and stable high temperatures in this season, which was also reflected in the monitoring of the physical parameters of the indoor climate in the Treasury (Figure 4). Selected prevailing genotypes from the fingerprints underwent species identification, which resulted in the determination of five additional fungal species (Figure 7(a)). They included *Aspergillus fumigatus* (KP689196.1; 99%), *Alternaria altermata* (KJ173524.1; 99%), *Penicillium chrysogenum* (JF731255.1; 99%), as well as uncultured *Penicillium* sp. (LN833530.1; 99%), which constitute a typical microflora in storerooms for historical items,[14,45] and

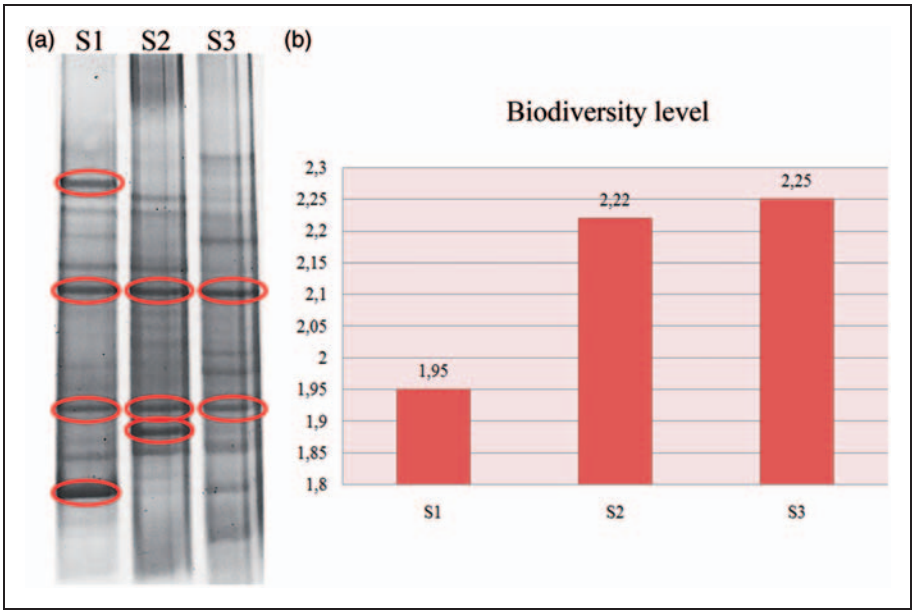

Figure 7. DGGE analysis of fungal community structure in the indoor air: (a) DGGE profile; (b) biodiversity level. Sample S1 – collected at the beginning of the experiment; S2 – collected after 3 months; S3 – collected after 6 months; taxons prevailing in the environment are encircled. Biodiversity level according to the Shannon index.

uncultured *Hypocreaceae* sp. (JF449870.1; 99%), which can be an environmental species. The identification of unculturable species and species that could not be obtained in cultures must be emphasized. This confirms the relevance of DGGE profiling in microbiological analyses of environments.

For complete characteristics of the tested environment in which textiles are stored and because other species were found, not isolated previously from historical textiles, an analysis of the destructive potential of obtained and identified microorganisms was conducted. Since the Wawel Royal Cathedral holds rich collections of not only silk textiles, but also items made of wool, cotton and linen, it was decided to test for both proteolytic and cellulolytic properties of microorganisms (Tables 1 and 2). Of among 15 bacterial strains, 60% were capable of protein breakdown and nearly 70% had cellulolytic properties. As for fungal strains, over 53% had proteolytic properties and almost 80% exhibited ability to produce cellulases. The analysis of microbial destructive potential indicates that cellulolytic strains prevail. They constitute a potential threat to cotton and linen that are often components of liturgical robes. A greater number of microbes capable of cellulase production can also be caused by a considerable share of wooden equipment and furniture inside the Treasury. Moreover, previously mentioned keratinolytic and xylanolytic properties of certain microorganisms, which can also affect their biodeterioration potential, must be taken into consideration as well. The results of enzymatic activity of the tested microorganisms are reflected in studies on fungi and bacteria isolated from various sites of cultural heritage storerooms.[3,20,28,32,34]

Conclusion

In this study, an attempt was made to verify whether polyethylene materials used in conservation for storing historical textiles affect microbiological purity of stored items. HDPE materials are supposed to protect textiles from non-organic and organic contamination and prevent excessive microbial inhabitation. The studies were conducted in two time periods: 3 and 6 months, using four types of fabric (silk, wool, cotton, and linen) after either conservation washing only or after washing with additional sterilization. The results and their statistical analysis indicate that in textiles washed only, HDPE could be used for transport or protection over short-term storage. Long-term storage in tightly sealed HDPE covers markedly increases microbial counts on packed textiles. When, however, textiles are additionally sterilized, the cover helps maintain very low microbial counts on their surfaces in both short- and long-term storage. This makes it a good barrier from the external environment. Nevertheless, these experiments are pilot studies only. To date, the usage of this material in historical textile storage has not been tested in terms of microbiological purity and requires further analyses with longer test periods. Additionally, the studies demonstrated a considerable increase in microbial counts on textiles which had undergone

sterilization and were stored without any cover compared with textiles washed only. This supports the opinions of numerous microbiologists stating that sterilized items that return to their primary place of storage become a ready niche to be inhabited by microorganisms. Sterilization can therefore weaken historical items by introducing physicochemical changes in their structures induced by a sterilizing agent, thereby making them more susceptible to biodeterioration.

When storing such brittle historical collections, it is crucial to ensure proper physical conditions of the indoor climate: relative humidity, temperature, no UV and proper ventilation. However, it is equally important to learn about the qualitative and quantitative status of microorganisms present on items themselves and in the air of storerooms. Knowledge of the diversity of fungi and bacteria present in storerooms, as well as their destructive potential, enables us to implement better preventive measures. As shown in the study presented above, the microflora of environments where cultural heritage items are stored can contain microbes that are pathogenic for humans. Therefore detailed knowledge about these environments can also influence safety of people who works with these valuable collections.

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