

Article

Museal Indoor Air Quality and Public Health: An Integrated Approach for Exhibits Preservation and Ensuring Human Health

Dorina Camelia Ilieș¹, Bahodirhon Safarov², Tudor Caciora^{1,*}, Alexandru Ilieș¹, Vasile Grama¹, Gabriela Ilieș³, Anca Huniadi⁴, Berdenov Zharas⁵, Nicolaie Hodor⁶, Mircea Sandor⁴, Martin Balázs Zsarnóczy⁷, Emilia Pantea⁸, Grigore Vasile Herman¹, Paula Dejeu⁹, Mariana Szabo-Alexi¹⁰ and Lorant Denes David¹¹

- ¹ Department of Geography, Tourism and Territorial Planning, Faculty of Geography, Tourism and Sport, University of Oradea, 410087 Oradea, Romania; dilies@uoradea.ro (D.C.I.); ailies@uoradea.ro (A.I.); vgrama@uoradea.ro (V.G.); grigoreherman@yahoo.com (G.V.H.)
 - ² Department of Digital Economy, Samarkand State University, Samarkand 140105, Uzbekistan; safa-rovb@rambler.ru
 - ³ Faculty of Geography, “Babes-Bolyai” University, Sighetul Marmăției Extension, 435500 Sighetul Marmăției, Romania; gabriela.ilies@ubbcluj.ro
 - ⁴ Faculty of Medicine and Pharmacy, University of Oradea, 410073 Oradea, Romania; ahuniadi@uoradea.ro (A.H.); msandor@uoradea.ro (M.S.)
 - ⁵ Department of Physical and Economic Geography, Faculty of Science, L. N. Gumilyov Eurasian National University, Nur-Sultan 010008, Kazakhstan; berdenov-z@mail.ru
 - ⁶ Faculty of Geography, “Babes-Bolyai” University, 400090 Cluj Napoca, Romania; nicolaie.hodor@ubbcluj.ro
 - ⁷ Institute of Sustainable Economy, Kodolanyi Janos University, H-1139 Budapest, Hungary; martin@kodolanyi.hu
 - ⁸ Faculty of Environmental Protection, University of Oradea, 410087 Oradea, Romania; epantea@uoradea.ro
 - ⁹ Medical Laboratory service M.D., Bethany Medical Clinic Oradea, 410004 Oradea, Romania; office@betania-centrumedical.ro
 - ¹⁰ Department of Physical Education, Sport and Physical Therapy, University of Oradea, 410087 Oradea, Romania; mariszabo@yahoo.com
 - ¹¹ Institute of Rural Development and Sustainable Economy, The Hungarian University of Agriculture and Life Sciences (MATE), H-2100 Godollo, Hungary; dr.david.lorant@gmail.com
- * Correspondence: caciora.tudoriulian@student.uoradea.ro; Tel.: +40-740941144



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Abstract: The quality of the indoor microclimate in museums is a problem of great interest to the contemporary society, given that it is in close connection with the health and comfort of visitors and employees, as well as with the integrity of the exhibits. Taking into account the fact that museums are places that have a special role in the community’s life and therefore attract a very large number of visitors of all ages, a very important issue is to determine the degree of safety that the indoor microclimate presents. Thus, the quality of the indoor microclimate was investigated inside an iconic museum in Romania, dating back to the 19th century, because pollutants from external or internal sources of the building, generated secondary, often anthropogenic, as a tendency to defend/adapt to climate change (CC), contribute to both local and regional pollution, but also lead to challenges in identifying links between air quality (AQ) and climate change (CC). The methodology used was based on monitoring the main parameters of the microclimate (temperature, relative humidity and CO₂) over a period of between October 2020 and March 2021, 21 weeks, as well as on determining the microbiological contamination of the air and some indoor exhibits located in three different areas of the museum. At the same time, the study aims to identify cheap, easy to implement and non-invasive solutions for removing fungi identified on exhibits for long-term preservation and reducing the risk of various pathologies in humans following prolonged exposure. The results obtained show that the indoor microclimate in the old heritage building favours the development of fungi, which have a high degree of contamination of the air (over 800 CFU/m³) and of the exhibits, representing a potential risk for the health of the visitors and museum workers. Thus, six species of yeast and five different fungi genera were identified in the air, while on the exhibits were individualised six fungi genera, a species of yeast and a bacterium. The most viable solution for cleaning materials, prolonging their lifespan and reducing the risk of disease in humans was represented by the use of essential oils (EO). Three essential oils (lavender, mint and lemon) were applied on an exhibit with

five different microorganism genera, and it was observed that they have the ability to inhibit the spores from moulds and bacteria, being a very good alternative to the usual chemical treatments that are used in the cultural heritage field.

Keywords: cultural heritage; museum environment; microaeroflora; fungi; essential oils; antifungal; cleaning; public health; human pathology

1. Introduction

Museums, as art and culture promoters, play a fundamental part in the daily life of any community [1,2]. Considering the fact that they deal with the preservation, analysis, cataloguing and exhibition of various old items of scientific, artistic or historical interest, the determination of favourable or unfavourable conditions in which these are kept and the interaction with visitors and employees has a particular significance [3]. In museums, storage and exhibition halls, the microclimate control, microbiological monitoring and cleaning process are very important in the prevention of fungal contamination of the indoor items and air [4–6].

Monitoring the indoor microclimate and the microbiological load has a double role. On the one hand, an inadequate microclimate can give rise to and maintain microorganisms that in turn can colonize and biodeteriorate (physical and chemical degradation) valuable tangible cultural heritage. Recent studies in the field reveal that microclimatic conditions (temperature, humidity, dust, light, etc.) have a critical role in the proliferation of colonies of germs and fungi [7–9]. Deterioration of the exhibits due to biological factors and agents is a serious problem, causing damage to the constitutive material [10,11]. The effects of microbiological attack upon the materials should be stains, discoloration, loss of strength [12,13]. The microbial attack can also cause spots, white films [14]. Continuous monitoring and control of environmental conditions should be a high priority in overall prevention and conservation activities [15–18]. For the evaluation of the attack and risk to which the museum heritage items are exposed to and not only, it is necessary to perform the assessment of the microbial load both on the surface of the item as well as in the air of the room where it is stored or exhibited within the museum etc. [19].

On the other hand, an inadequate indoor microclimate can affect the health of visitors, restorers and staff working in the museum [19,20]. This is extremely important considering that museums are usually crowded places that attract visitors belonging to all age categories, some of them implying a high degree of vulnerability (especially children and elderly). The main threat in this regard is given by the microbiological contamination and especially by the fungi [21]. The vast majority of fungi identified inside museums are of medical interest and with pathogenic potential for humans, especially in the case of subjects with weak immunity system or with allergies [9,22–24].

The monitor and control of indoor environments, in museums or other heritage buildings, were done by various researchers. In this direction we mention both older and recent studies in the field [25–32]. Similar studies dealing with this topic were done also in Romania, among which we mention those of Dăneasa [33] and Herbel [34], who have analysed Romanian wooden churches; Grøntoft and Marincas [35] who analysed indoor air pollution impact on cultural heritage in the National Military Museum in Bucharest and the Tismana Monastery in Gorj County. Other research projects [36–43] present the technology for restoration and protection against biodegradation of various materials from heritage buildings (made of wood, canvas etc.).

In recent years, as presented also by Sciarpi et al. [44], in this field, the emphasis is set on preventive preservation and not on reactive conservation. Preventive conservation involves monitoring the internal microclimate and identifying the safest methods to reduce the risk to which exhibits and visitors are subjected [45]. Thus, cleaning and preventing the colonization with fungi of valuable materials and therefore stopping their deterioration

and safeguarding the health of visitors and employees, is a task of great importance in the public museums. Prevention of fungal growth on cultural heritage objects is a challenge for restorers and conservators [4,46]; in this context the non-toxic and non-destructive treatments should be found and tested [47]. In recent years, there has been a growing interest in the use of various natural substances with antimicrobial action. These substances are most often represented by essential oils (EO), which are extracted from natural sources (aromatic plants) and have a low toxicity [48]. They are a powerful resource in green conservation strategies, as an alternative to chemical ones, the compatibility with artwork constitutive materials, being environmentally friendly and having no effects on human health. They are considered the future generation of biocontrol agents and methods in the context of sustainable development [49]. Given that one of the basic characteristics of cultural heritage is authenticity, essential oils seem to be the most viable solution [50] for cleaning materials in a non-invasive way and preserving them in good condition [51].

In the context of the abovementioned, this study aims to assess the fungal load of the air and the exhibits inside the heritage building of a museum and the influence that the main parameters of the microclimate (temperature, relative humidity and CO₂ concentration) have on the proliferation of microorganisms. At the same time, the study aims to identify viable and non-invasive solutions for cleaning materials, so that the exhibits are treated and preserved for as long as possible, and the indoor environment is safe for human health. Moreover, the entire article takes into account the determination of the microclimatic conditions and the fungal load inside the museum and their impact both on the exhibited collections and the health of visitors and employees.

The Municipal Museum of History and Ethnography in Beius is located in the city of Beius, Bihor County, Romania (Figure 1). It is set up inside a house built in the 19th century in in Baroque eclectic style. Since 1985, the museum has two exhibitions (history and ethnography), the number of exhibits amounting to over 3000. The history exhibition includes numismatic pieces belonging to the Palaeolithic, Middle Ages and the Roman occupation period, while the ethnography exhibition is made up of the items which depict the traditions and crafts practiced throughout history in the land of Beius [52].

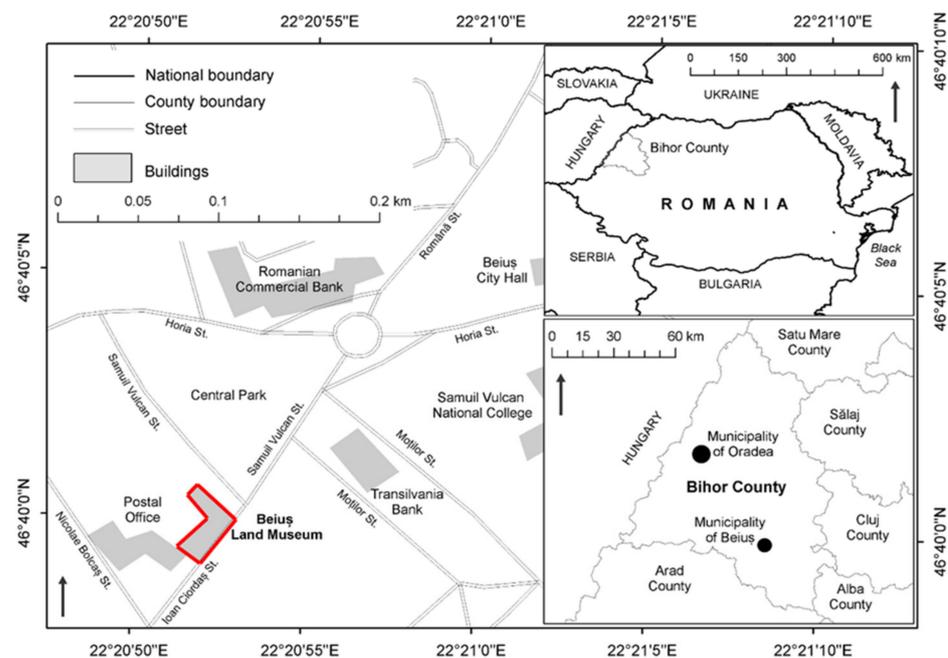


Figure 1. Location of the museum of Beius at municipality, county and national level.

The interior microclimate and the museum exhibits were previously the subject of study of two scientific studies [19,53]. In Ilies et al. [19], the emphasis is on determining the main parameters of the indoor microclimate in the period June–October 2020 (15 weeks).

Thus, the fluctuations in temperature, relative humidity (RH), CO₂ concentration, concentration of formaldehyde (HCHO) and volatile organic compounds (TVOC), natural and artificial brightness, as well as concentration of particulate matter were monitored, all of them being related to standards in terms of exhibit conservation and human health. The results obtained in this study show that the analysed indicators are mostly outside the international standards in force, which has certain effects on the integrity of materials and human health [54]. At the same time, they fluctuate a lot and very often, which can induce stress to materials by alternating their physical and chemical properties, accelerating the degradation process.

This study is meant to complete the series of data on the main microclimatic indicators (temperature, RH and CO₂) (it completes the previous summer-autumn monitoring with the winter-spring monitoring) as well as to complete the study by determining the microbiological contamination and its influence on human health and the preservation of the exhibits. The development of measurements during the winter-spring period (October 2020—March 2021) was established due to the fact that during this period, the museum benefits from a peak in the number of visitors. Many of them are children and pupils, given that most of the extracurricular activities take place indoors during this period and the museum is a favourite place. Thus, the determination of the microbiological load is all the more important as children are most prone to infection with different fungi and the winter season is characterised by weakened organisms [55], that favour the onset of infections [56].

2. Materials and Methods

2.1. Determination of Indoor Microclimate (Temperature, Relative Humidity and Carbon Dioxide)

Following the summer monitoring of the main parameters for indoor microclimate (temperature, RH and CO₂), comprehensively presented in Ilieş et al. [19], for this study it was decided to monitor the fluctuations of the three parameters throughout winter. Thus, the monitoring period lasted for 21 weeks, between 22nd October 2020 and 18th March 2021. The rooms selected for monitoring are, according to Figure 2: two exhibition halls located at the basement of the museum (total air capacity 127.4 m³), two exhibition halls at the first floor (total air capacity 424.8 m³) and main warehouse (139 m³ of air).

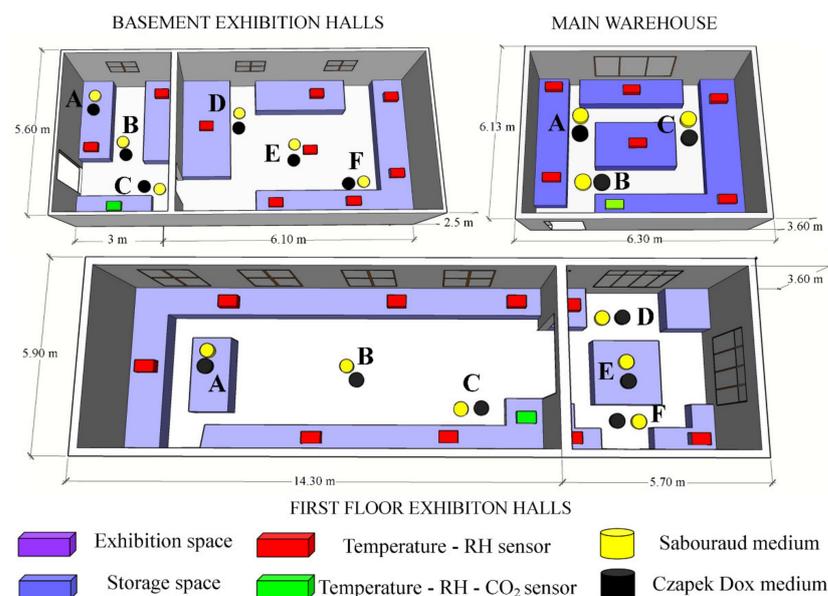


Figure 2. Space distribution of sensors for temperature—RH—CO₂ measurement inside the three halls of the museum, as well as in the sampling areas from air and from the exhibits for determining the microbiological load.

Three sets of Klimalogg Pro data-logger sensors were used for temperature and RH monitoring, scheduled to record hourly quantitative data with an accuracy of ± 0.1 °C (temperature) and $\pm 3\%$ (RH). Depending on the size of the halls, were used between 6 and 9 such sensors. The distribution of the sensors was made in a way that it covered the surfaces as evenly as possible (Figure 2), so that by performing the arithmetic mean on each sensor and for the entire set of sensors positioned in a certain hall, the fluctuations of the two parameters (temperature and RH) could be determined with as much accuracy as possible.

In order to determine CO₂ concentration in the air, Extech SD800 datalogger was used. This is a device which determines the CO₂ concentration with an accuracy of ± 40 ppm, temperature with an accuracy of ± 0.8 °C and relative humidity up to $\pm 4\%$. Such a device was positioned in each of the three halls, in the most popular areas for tourists and museum staff, at a height equal to the height of an average man (between 1.6 and 1.8 m) (Figure 2).

Monitoring throughout the 21 weeks has generated over 194,000 individual data, subsequently processed and graphically rendered using software IBM SPSS 1.0.0.1406 and RStudio Desktop 1.4.1717. At the same time, the results were compared with the reference standards in the field [57,58], in order to identify their suitability for human health and the preservation of exhibits.

2.2. Determination of Fungal Load in the Air and on the Exhibits

In order to determine the microbiological contamination inside the museum in Beius, both the collection of samples from the air and from the exhibits were taken into consideration. This was carried out in order to determine, as complexly and completely as possible, the indoor situation and the possible effects on human health and the integrity of the exhibits.

The fungal loading assessment of the air in the rooms was made with the help of Koch sedimentation method [59]. This is a selective method to detect the particles which passively sediment as a result of gravity. It has the advantage of being very easy to implement and cheap; the analyses can be repeated by anyone at any time [60]. Thus, three areas were chosen in each room for positioning the two Petri dishes (9 cm in diameter) containing both Sabouraud sterile growth medium with Chloramphenicol addition and Czapek Dox sterile growth medium (Figure 2). The dishes were placed at the height of 1 m from ground level for about 30 min. During this time, the access to the halls of the visitors and the staff was forbidden, so that the measurements would not be influenced on their activity.

As for the assessment of fungal contamination degree of exhibits, this was done with the help of sterile pads. Samples were taken from seven different traditional clothing exhibits in the basement (3 exhibits), first floor (2 exhibits), respectively in the museum's storage area (2 exhibits) (Figure 3). The age of these items of clothing is mostly between 50 and 80 years, only the traditional coat located in the warehouse being about 100 years old.

Following the sampling performed with sterile pads, the Petri dishes containing Sabouraud and Czapek Dox culture media were seeded.

Both samples taken from the air and those from the exhibits were incubated and monitored for seven days at a temperature of approximately 28 °C. The developed yeast species were processed and incubated at 37 °C to obtain pure colonies. The final identification of fungi and yeasts was made after assessing the macroscopic, microscopic characters and biochemical properties, with the help of the API[®] 20 C AUX identification system. The API[®] 20 C AUX system represents a gallery of 20 microwells which contain dehydrated substrata for the testing of 19 assimilation biochemical reactions. The wells are inoculated with the yeast suspension prepared beforehand at a 2 McFarland turbidity. The reactions reading was made at 48 and 72 h, while the yeast process identification is obtained by informatics decoding of the 7 digits numeric profile, using the APIWEBTM software program.

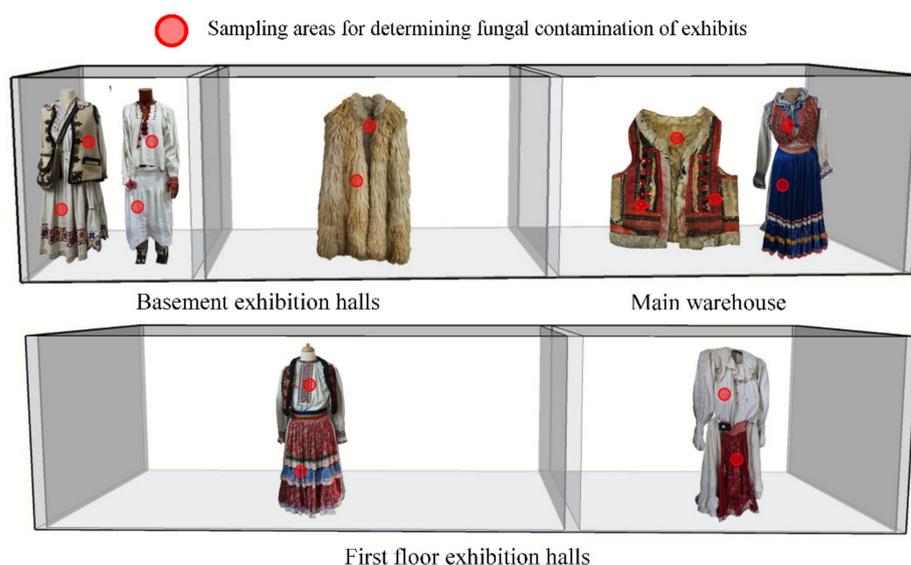


Figure 3. The places from the aged clothing exhibited in the museum from where the samples were taken.

The fungal colonies became macroscopically visible at 72 h. Afterwards, they entered a maturation process, changing their diameter, shape, colour and texture, some species completely invading the growth medium.

The quantitative analysis of fungal contamination degree was made by counting the colonies developed on the growth medium (expressed in CFU/mL) and by equating the result reported to the air volume with the help of two classical calculus formulas, as follows:

Formula from the Polish standard PN 89/Z-04008/08, according to Puchianu et al. [60].

$$\text{CFU/m}^3 \text{ air} = (n \times 10.000)/(S \times t \times 0.2) \quad (1)$$

where n represent the number of used Petri plates, S = surface of Petri dish in cm^2 (for a diameter of 9 cm, $S = 3.14 \times R^2 = 63.5 \text{ cm}^2$) and t = dish exposure time.

Omelianski calculus formula [61] which relies on the observation according to which on a surface of 100 cm^2 exposed to air over a period of time is sedimented a number of microorganisms equal to the one contained in 10 dm^3 of air.

$$\text{CFU/m}^3 \text{ air} = (n \times 10.000)/(S \times k) \quad (2)$$

where n = number of colonies developed on the dish surface, S = surface of Petri dish (for a diameter of 9 cm, $S = 3.14 \times R^2 = 63.5 \text{ cm}^2$), k = air exposure time coefficient (expressed in minutes, $k = 1$ for 5 min; $k = 2$ for 10 min; $k = 3$ for 15 min, etc.).

2.3. Non-Invasive Solution for Exhibits Cleaning

Given the degree of fungal contamination of the exhibits inside the museum, an overwhelming importance is represented by identifying non-invasive methods of removing fungi from them, both for conservation over a long period of time and for the health of those who come into contact with them. In this respect, essential oils (EO) are necessary as a very good alternative to the classic chemicals used to clean the exhibits.

In order to test the inhibitory effects of the essential oils, an exhibit was chosen from which samples were taken to determine the fungal contamination in the previous stage; respectively, a traditional women's waistcoat, aged approximately 100 years, located in the museum's warehouse. The choice was based on the fact that the item has a real value, being made up of several types of materials (cotton, wool and leather) and no less than five different types of microorganisms were identified on it, which makes it perfect for testing natural extracts. The essential oils selected for testing were as follows: *Mentha*

piperita—mint, *Lavandula angustifolia*—lavender and *Citrus limon*—lemon, due to their known antifungal effects identified in the literature [48,62–65].

Three examination areas of the traditional waistcoat were delimited (25 cm²), so that the determination to be performed on all the materials of which the coat is made. This was done so as to have an overview of the effects that essential oils have on the microflora of several types of materials composing the same object. Therefore, the examinations were performed on cotton threads (EA1), natural wool (EA2) and leather of animal origin (EA3), a single essential oil being applied inside them (Figure 4).



Figure 4. The areas examined within the traditional sheepskin waistcoat and the type of essential oil applied on each of them.

As presented in the previous subchapter, the sampling stage was carried out using sterile pads, in a non-invasive manner, and the samples were immediately sown on the Sabouraud culture media. In each examined area, 5 drops (150 µL) of essential oil were subsequently applied using glass containers with sprayer. In order for the application of the oils to be done evenly, 50 drops of sterile distilled water were added to each container (7.5 mL). The working methodology involved spraying the entire prepared content of 150 µL of essential oil and 7.5 mL of distilled water uniformly over the entire surface of 25 cm² delimited within the traditional sheepskin waistcoat. The mixture was allowed to act at room temperature for 15 min, after which new samples were taken which were also seeded on the Sabouraud media.

3. Results

3.1. Determination of Indoor Microclimate (Temperature, Relative Humidity and Carbon Dioxide)

According to ANSI/ASHRAE [57] and ASHRAE [58] standards on the quality of museum indoor microclimate for human health and preservation of exhibits, temperature must be kept throughout the year between 18 and 22 °C, relative humidity (RH) between 45 and 60%, while the amount of carbon dioxide must not exceed 1000 ppm. If the measurements performed during June–October 2020 have revealed the fact that only 13.8% of the days have met the ideal conditions (all three parameters to be within the accepted

range) [19], the period October 2020–March 2021 presents a worrying situation, given that in none of the days the ideal conditions for the integrity of human health and exhibits were met.

During the analysed period of time, the indoor of the museum in Beius has registered an average temperature value at 8.6 °C, with approximate variations of 16.7 °C, between maximum 16.3 °C recorded in the first week of monitoring and −0.4 °C in the thirteenth week. The first-floor exhibition halls have imposed as the area with the lowest temperatures, the average for the entire period reaching only 6.4 °C, followed by the basement exhibition halls with 8.7 °C, while the main warehouse was the warmest area, with an average of 10.8 °C. The largest fluctuations were recorded at the first-floor exhibition halls (14 °C), between 13.6 °C and −0.4 °C, followed by the basement exhibition halls with 11 °C (maximum temperature at 13.2 °C and minimum temperature at 2.2 °C) and the main warehouse which recorded a maximum fluctuation which differs by 9.5 °C (maximum temperature at 16.3 °C and minimum temperature at 6.8 °C). In accordance with the outside temperature, all the maximum temperature values belong to the first week of monitoring (22–28 October 2020), and particularly on 26 October, while all the minimum values belong to week thirteen (14–20 January 2021), and especially on 19 January. (Figure 5A).

The thermal instability inside the museum is also highlighted by the average weekly temperature values. This indicator was most prominent in the first-floor exhibition halls (11.8 °C), which has a maximum value of the weekly average at 12.8 °C and a minimum value at 1 °C. The basement presented a fluctuation of the average weekly values by 11.5 °C, reaching a maximum value at 15 °C and a minimum value at 3.5 °C. The smallest values were recorded in the warehouse, temperature fluctuations which differ only by 8.1 °C, with maximum temperature at 15.9 °C and minimum temperature only at 8.1 °C. Temperatures tend to closely follow the evolution of the weather outside, given that the museum does not have HVAC system, the trend recording a decrease until mid-February, after which the values register a slight upward trend towards the end of the monitoring period.

None of the 26 temperature measurement sensors have recorded any value ranging between 18–22 °C, which is considered to be ideal for human health and the integrity of the exhibits [58]. At the same time, large fluctuations, both in winter and summer, reveal an unstable environment from a termin point of view, which represents a potential danger.

As for the relative humidity (RH), during the analysed period, it was preserved for 84.2% of the time outside the 45–60% recommended range, in compliance with ASHRAE [58] standards. But meanwhile, the exceedances are not very high in terms of quantity, the RH average for the entire period being 63.9%, a value that exceeds by only 3.9% the upper limit of the ideal value. Broken down by monitoring areas, the warehouse imposes as the room with the best values, the average over the entire period (61.1%) approaching the standards in force. The rooms located on the first floor have recorded an average RH value of 64.9%, while the basement recorded 65.7%.

The highest absolute fluctuations were recorded in the main warehouse, where RH values have fluctuated by 32.9%, between maximum 78.9% in week 17 (11–17 February 2021) and minimum 46% in the last week (11–18 March 2021) of monitoring. In the other two areas, RH fluctuations were similar, between 54.3% and 75.2% differing by 20.9% in first floor exhibition halls, respectively between 55.4% and 76%, differing by 20.6% in the basement exhibition halls (Figure 5B).

The evolution of the weekly average values reveals two contradictory situations. In the case of the warehouse, the trend is linear, slightly downward, more pronounced towards the end of the monitoring period. In this area, weeks 13 and 20 have recorded the lowest average value (53.1%), while week 16 registered the highest average value (70.6%). The trend within the other two monitored areas of the museum presents a slight increase in the weekly average values of RH until the middle of the study period, after which they gradually decrease in the second half. In the case of both first-floor exhibition halls and basement exhibition halls, the highest weekly average values were recorded in the 16th week, 71.9% on the first floor and 72.3% in the basement. At the same time, the lowest

values were recorded during week 20, when 59.9% RH were registered on the first floor, and 55.9% in the basement (Figure 5B).

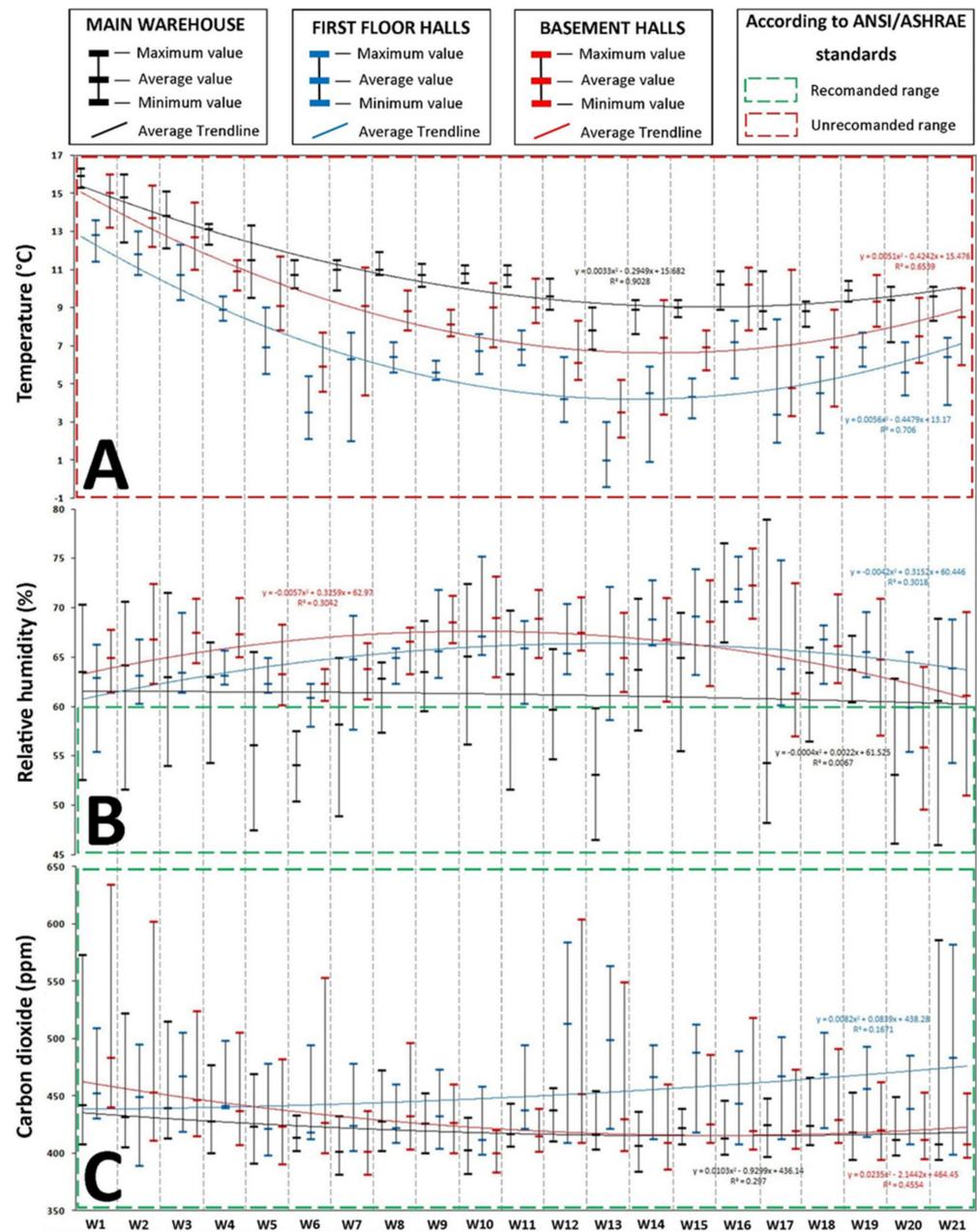


Figure 5. The fluctuations in terms of maximum, minimum and average value per week for temperature, relative humidity and amount of carbon dioxide in the three monitored areas of the Beius museum (A)—Temperature variations in the three monitored rooms; (B)—Variations of the relative humidity in the three monitored rooms; (C)—Variations of the carbon dioxide in the three monitored rooms).

The amount of CO₂ is the only measured indicator that is located throughout the monitoring period below the milestone of 1000 ppm, according to ANSI/ASHRAE Standard [57] as the maximum accepted for human health and integrity of the exhibits. Thus, the average of the three study areas for the entire period amounted to only 433.2 ppm; the highest average quantity being recorded in the first-floor exhibition halls (452 ppm), followed by basement exhibition halls (427.1 ppm) and main warehouse (420.6 ppm). The CO₂ value varied during the monitoring period in a range of only 253 ppm, between 634 ppm

recorded in the first week and 381 ppm for the seventh week. Regarding the evolution of the weekly averages, the analyses reveal a contradictory situation between the warehouse and basement halls, respectively the first-floor exhibition halls. The first two register a relatively linear trend, with a slight note of decrease in values. Inside the warehouse, the highest average value of the week belongs to the first week (441.8 ppm) and the lowest to the seventh week (401.2 ppm), while in basement halls these values are higher, 483 ppm the maximum and 401.5 the minimum. In the case of the first-floor exhibition halls the trend is an increasing one, with variations up to 100.8 ppm in terms of the average weekly value of CO₂ (512.6 ppm maxim in week 12, respectively 411.8 the minimum in week 10) (Figure 5C).

3.2. Determination of Fungal Load in the Air and on the Exhibits

The results of the examination of the samples taken from the air reveal that the museum indoor is characterised by an impure environment, with a real harmful potential for the human health and the integrity of the exhibits. The quantity and diversity of fungal species in the air being complemented by the large volume of suspended particles identified inside. The average amount of suspended particles for the three monitoring areas was 19.3 µg/m³, of which PM_{2.5} had values of 7 µg/m³, and PM₁₀ recorded 12.3 µg/m³ [19]. These values exceed the thresholds allowed by the international standards in force [66], which regulate that the amount of suspended particles must not exceed 12 µg/m³ in order to ensure human health.

After the seven days of incubation, on average were identified between 8 and over 28 fungal colonies on Petri dishes. The average of the fungal colonies depended mainly on the sampling site, so that rooms of the same area recorded a different number of fungal colonies. The smallest average number of fungal colonies was identified in the small basement hall (8 colonies), followed by the warehouse with 11 colonies, the large hall on the first floor with 24 colonies and the large basement hall with 28 colonies. The dishes containing the samples taken from the small hall of the first floor were totally invaded by fungal colonies, their number being thus impossible to be determined exactly (Figure 6A). As for the number of colonies with different appearances, they were four in the small halls of the basement and first floor, respectively five in the large halls; the warehouse individualizing as the area with the most fungal colonies with different appearance (9 colonies) (Figure 6B).

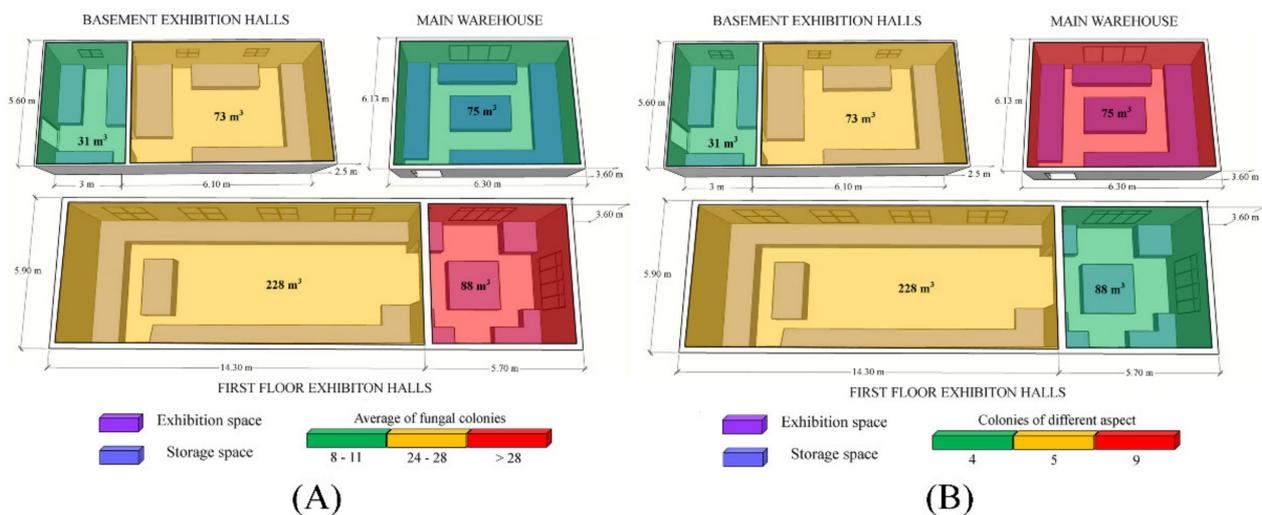


Figure 6. The degree of fungal contamination from the interior of the Beius museum, divided into monitoring areas in period October 2020–March 2021 ((A)—the average number of fungal colonies identified in each area; (B)—the number of fungal colonies with different appearance identified in each area).

Figure 7 presents samples collected from the three monitoring areas A–C (Figure 1) of the large hall located on the museum first floor, in the seventh day of incubation, when the colonies are visible and well developed. Based on these samples, it was very easy to determine the number of colonies grown on each dish (Area A—25 colonies; Area B—30 colonies; Area C—31 colonies) and the number of colonies with different appearance within them (Area A—5 colonies; Area B—4 colonies; Area C—3 colonies); so that after performing the arithmetic mean between these values, to obtain the average values related to this hall.

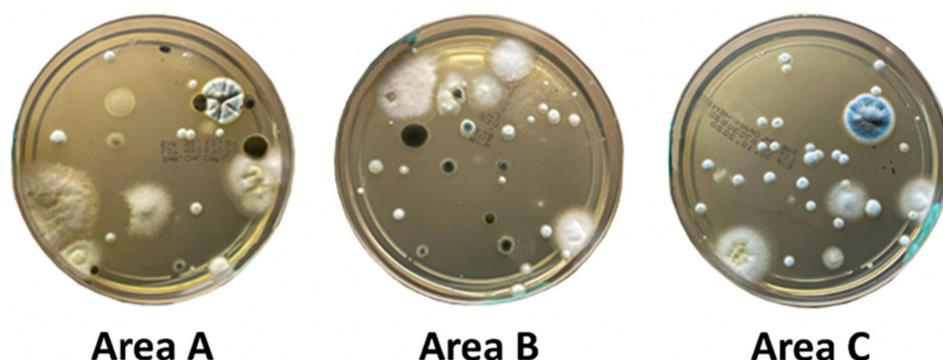


Figure 7. Fungal colonies developed on the plates taken from the large hall on the first floor of the Beius museum in period October 2020–March 2021 (Area A—left side; Area B—central side; Area C—right side).

At present, regarding the fungal loading of the museum indoor air, there are no regulations or standards, therefore; certain indicative norms were used, established based on studies in order to assess the air contamination degree in the rooms. According to European Commission's health standards [67,68], the degree of fungal contamination is determined depending on CFU/m³ air, as follows: Very low—<25 CFU/m³, Low—25–100 CFU/m³, Medium—100–500 CFU/m³, High—500–2000 CFU/m³, Very high—>2000 CFU/m³. In the case of the museum in Beius, the average number of CFU/m³ air falls, depending on the area, between 200 and over 2000 (Figure 8A). The most affected is the small hall located on the first floor which records > 2000 CFU/m³ air, related to an extremely high degree of contamination. The large halls located on the first floor and in the basement are represented by a number of 630 and 735 CFU/m³ air, respectively, and a high degree of fungal contamination. In the warehouse, also, a high degree of fungal contamination was determined (577 CFU/m³ air). The area with the lowest contamination rate is the small basement hall, where the average number of CFU/m³ air was only 210, the degree of contamination being an average one (Figure 8B).

As for the number of fungal species/genera identified in the museum indoor air, eleven were determined. Six of these belong to yeast category and they were identified by API[®] C AUX system up to species level: *Candida guilliermondii*, *Candida sphaerica*, *Cryptococcus albidus*, *Cryptococcus laurentii*, *Cryptococcus neoformans* and *Sporobolomyces salmonicolor*.

The macroscopic and microscopic examination of viable fungal cultures have provided information regarding the mould genera and species. According to the macroscopic aspect of the colony (size, shape, outline, colour, consistency, dish invasion tendency) and to the microscopic one (hyphae, pseudohyphae, spores, blastospores, sporocystospores, sporocysts, conidiophores, metulae, phialides, conidiophores, etc.), the mould genera which had developed on the growth medium could be established [22]. Therefore, the fungal genera identified on the museum exhibits were: *Penicillium sp.*, *Aspergillus sp.*, *Stachybotris sp.*, *Trichoderma sp.*, *Cladosporium sp.*

On the two items of clothing located in the small basement hall (Figure 9A), at least five different species of fungi have been identified, the dishes being totally invaded by them. A similar situation was individualised in the case of the exhibit in the large hall, on the surface of which five genera of fungi were identified (*Penicillium sp.*, *Aspergillus sp.*,

Stachybotris sp., *Trichoderma sp.*, *Cladosporium sp.*), the invasion being a moderate-large one (Figure 9B).

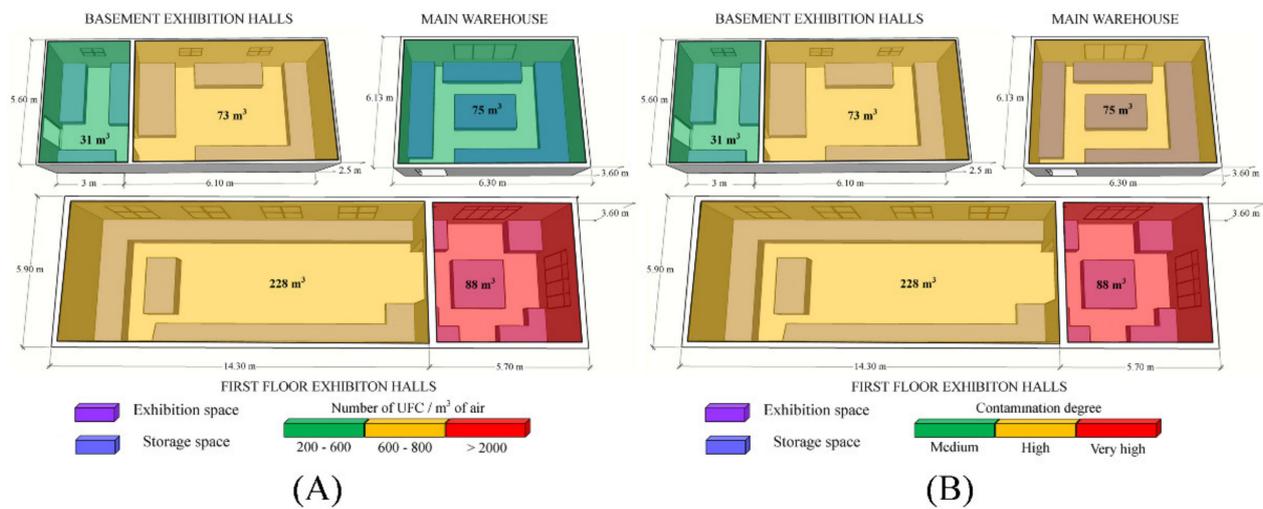


Figure 8. The degree of fungal contamination from the indoor of the Beius museum, divided into monitoring areas during the period October 2020—March 2021 ((A)—number of CFU/m³ air identified in each area; (B)—fungal degree of contamination identified in each area).

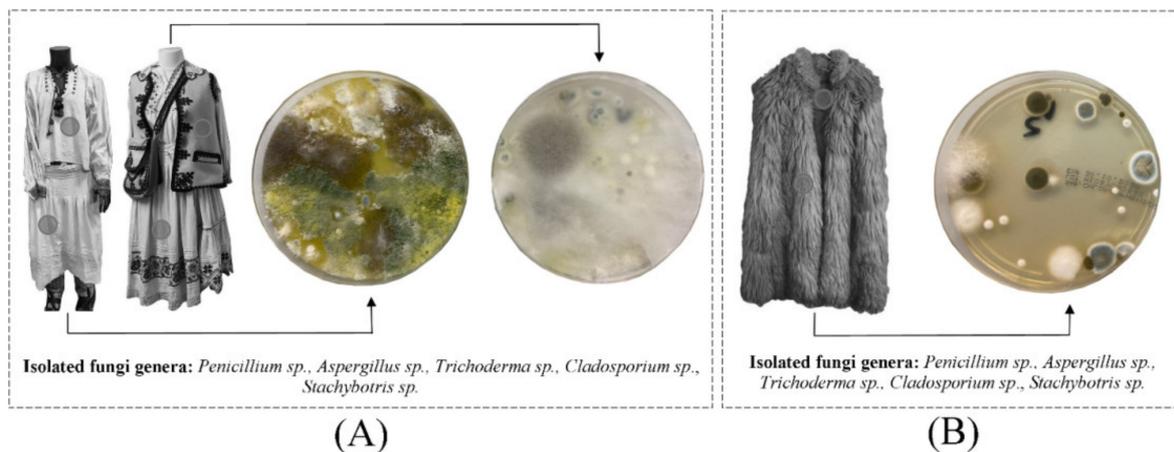


Figure 9. Isolated fungi genera and the appearance of the colonies on the exhibits placed in the basement exhibit halls at the museum in Beius throughout the period October 2020—March 2021 ((A)—samples collected from the exhibits found in the small basement hall; (B)—samples collected from the exhibits found in the large basement hall).

The samples collected from the exhibits found on the first floor of the museum (Figure 10A,B) have shown two fungal genera, *Penicillium sp.* and *Aspergillus sp.* In this case, the degree of invasion of the plates was moderate, denoting a safer microclimate than in the basement exhibition halls.

In the case of the warehouse, the samples collected from the two traditional clothing items have triggered two diametrically opposed situations. On the first clothing item (Figure 11A) was identified a single fungi genus (*Penicillium sp.*), while on the traditional waistcoat, in the three sampling sites were identified three mould genera (*Cladosporium sp.*, *Aspergillus sp.* and *Botrytis sp.*), one yeast (*Cryptococcus albidus*) and one bacterium (*Staphylococcus sp.*) (Figure 11B).

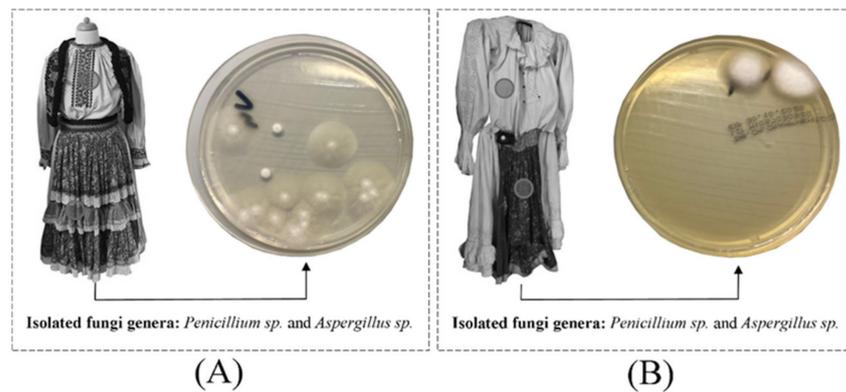


Figure 10. Isolated fungi genera and the appearance of the colonies on the exhibits placed on the first-floor exhibition halls of the museum in Beius throughout the period October 2020–March 2021 ((A)—samples collected from the exhibits found in the large first floor hall; (B)—samples collected from the exhibits found in the small first floor hall).

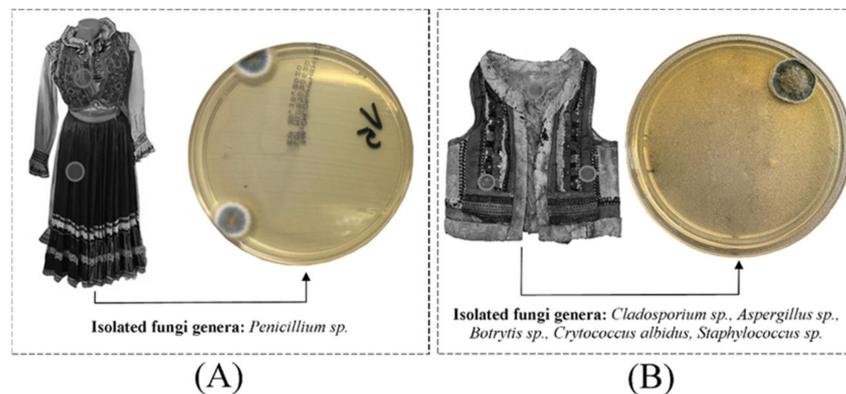


Figure 11. Isolated fungi genera and the appearance of the colonies on the exhibits placed in the main warehouse of the museum in Beius throughout the period October 2020–March 2021 ((A)—traditional women clothing; (B)—traditional waistcoat).

3.3. Non-Invasive Solution for Exhibits Cleaning

In Figure 12 are presented the examined areas of the traditional waistcoat along with the corresponding Sabouraud dishes. In the culture media, the upper half of each plate, denoted by the sign (–), was seeded with the sample taken before the application of the essential oil, and the lower half, marked with the (+) sign, was seeded with the samples taken 15 min after application of the essential oil on the tested area.

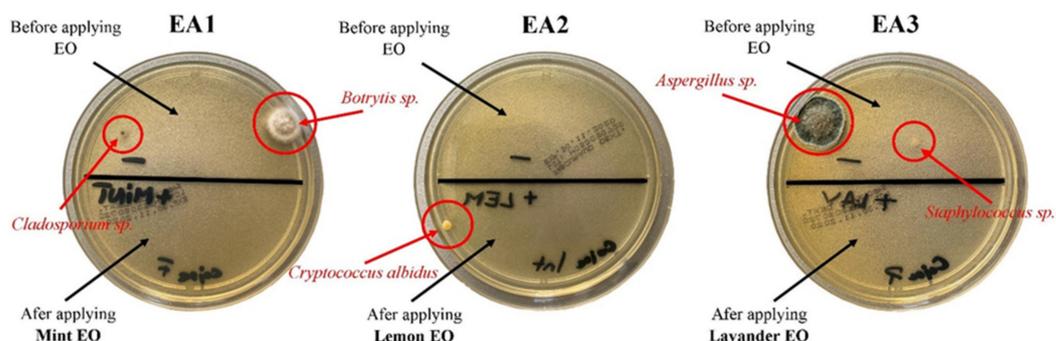


Figure 12. Sabouraud media corresponding each tested area from the textile in period October 2020–March 2021.

Three different types of moulds and a yeast species have developed on the surface of the Petri dishes [22]. The types of microorganisms identified in each of the three examined areas [6,61], as well as the effects of the application of essential oils are presented in Figure 13.

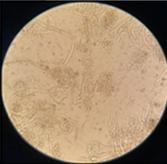
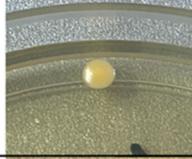
	Images - Microscopic images	Species	Material	EO applied	EOs effects		
Moulds			EA3 Leather	Lavander (<i>Lavandula angustifolia</i>)	Inhibitory effect		
						EA1 Cotton	Mint (<i>Mentha piperita</i>)
							
Yeast		<i>Cryptococcus albidus</i>	EA2 Wool	Lemon (<i>Citrus limon</i>)	Without inhibitory effect		
Bacteria		<i>Staphylococcus sp.</i>	EA3 Leather	Lavander (<i>Lavandula angustifolia</i>)	Inhibitory effect		

Figure 13. The microorganisms identified in each area examined and the effects of the oils applied on the textiles.

Round, flat, beige-colour colony developed on the culture medium together with the *Aspergillus sp.* was identified as a bacterial colony, namely *Staphylococcus sp.* (positive catalyst reaction, gram positive cocci arranged in bundles on light microscopy) [69]. This bacterial species was also inhibited by the lavender essential oil (*Lavandula angustifolia*) [70]. The yeast colony isolated from the inside of the traditional waistcoat (wool), respectively *Cryptococcus albidus*, have developed contrary to the expectation on the culture medium seeded with the sample taken after the application of the lemon essential oil. This result can be explained either as the absence of the inhibitory effect of the essence lemon oil over the isolated species, or as an insufficient duration of action of the essence oil over the tested area. Also, the particularity of the inner part of the traditional waistcoat must be taken into consideration, namely the considerable length of the wool threads that favour deep fungal and bacterial contamination, a layer that essential oil will not reach after a superficial spray.

4. Discussion

Microclimate, especially temperature and RH, plays an essential part in the deterioration of exhibits. The fluctuations of these indicators in time and space may induce a degree of stress to the constituent materials of the exhibits, which leads to irreversible changes in their physical and chemical properties, accelerating the deterioration [48,58]. This is also individualized inside the museum in Beius, where very low average temperatures are recorded (8.6 °C) and quite high values in terms of RH (63.9%) throughout the analysed

period of time. These values are outside the international standards in force, maximum fluctuations of 17.1 °C in the case of temperature and 32.9% as for RH. The registered values certainly present a risk for the conservation of the exhibits and can cause discomfort to the visitors and employees of the museum and can even generate serious health problems.

At the same time, the temperature, RH, CO₂ concentration values and also the suspended particles found in the air, can influence the colonisation degree of the exhibits with fungi [71]. The effect of air temperature (4–30 °C) and relative humidity (RH 11–96%) on the growth of several fungal species was studied in the laboratory by Pasanen et al. [72]. Results reveal that a short period of favourable conditions was enough to start fungal growth. Temperature was not a limiting factor for fungal growth on building materials, since fungi grew at even below 10 °C. Moreover, Sindt et al. [73] claim that “a change in temperature may influence colonization and growth of fungi directly through physiology of individual microorganisms or indirectly through physiological effects on their hosts”; this is all the more threatening as sudden and seasonal changes in temperature inside the museum are frequent. In terms of RH, it directly determines the amount of moisture in the materials, which favours the development of moulds and other microorganisms on surfaces [74]. Fungi causing systemic infections in humans are almost always acquired from an exogenous source and, although likely to experience wide ranges of humidity in their natural environments, few laboratory studies have been made on their survival at reduced RH. The interaction between microclimatic conditions and different microorganisms leads to alterations in the structure of elements belonging to cultural heritage and can induce pathogenicity in humans [75]. Relevant studies in this field [76–78] show that fungi are some of the main biodeteriogenic agents of surfaces, the genera with the most intense biodegradative activity being *Cladosporium*, *Aspergillus*, *Penicillium*, *Mucor*, *Alternaria* or *Stachybotrys*, and the vast majority of them being identified inside the museum in Beius. Thus, in order to maintain a clean environment, conducive to human activity, it is necessary both continuous and targeted monitoring of microbiological concentration on surfaces and in the air, and the adoption of non-invasive methods for air purification and surface decontamination.

Of the six yeast species, six species of moulds and one bacterium identified in the air and on indoor exhibits, four are considered pathogenic in humans, while the others are considered pathogenic only in rare cases (Table 1), especially in people with immunodeficiency caused by various diseases or in children. The increased pathogenicity of these microorganisms in the case of children makes the indoor microclimate of the museum in Beius to present an increased risk of infections. This is individualised due to the fact that during the cold period of the year, the museum is visited by numerous groups of students, who carry out their activity mostly indoors due to the cold weather. At the same time, the immune function of the human body often varies depending on the season, and usually in winter it is the lowest, which favours the onset of numerous diseases during this season [55,79]. Therefore, during the period of the year in which the measurements were carried out, the museum indoor may represent a risk of disease for the vast majority of visitors, restorers and museum employees.

Table 1. Fungal genera and species identified in the air and on the exhibits of museum in Beius, their pathogenicity and potential disorders caused to humans.

	Name of Genera/Species	Identification		Pathogenicity		Potential Disorders
		Air	Exhibites	Pathogenic	Rare Pathogenic	
Yeast	<i>Candida guilliermondii</i>	•		•		Onychomycosis; Rarely, invasive candidiasis [80,81].
	<i>Candida sphaerica</i>	•			•	Opportunist infections—especially in children [82].
	<i>Cryptococcus albidus</i>	•	•		•	Pulmonary, Cutaneous or Disseminated Cryptococcosis—especially in immunocompromised patients [83,84].
	<i>Cryptococcus laurentii</i>	•			•	Cutaneous Cryptococcosis, Keratitis, Endophthalmitis, Pulmonary abscess, Peritonitis, Meningitis, Fungemia [85,86].
	<i>Cryptococcus neoformans</i>	•			•	Pneumonia, Meningoencephalitis—in patients with immunocompromising disorders [87].
	<i>Sporobolomyces salmonicolor</i>	•			•	Dermatitis, Endophthalmitis—in patients with immunocompromising disorders [88].
Moulds	<i>Penicillium sp.</i>	•	•	•		Sindrom respirator allergic, Keratitis, Endophthalmitis, Otomycosis, Pneumonia, Endocarditis, Peritonitis, Urinary tract infections [89,90].
	<i>Aspergillus sp.</i>	•	•		•	Obstructive bronchial aspergillosis, Pulmonary aspergillosis and aspergilloma, Acute/chronic allergic sinusitis, Allergic bronchopulmonary aspergillosis, Endocarditis, Osteomyelitis, Endophthalmitis [91,92]
	<i>Trichoderma sp.</i>	•	•		•	Sneezing, asthma attacks, prolonged coughing and lung infections—in patients with immunocompromising disorders [93].
	<i>Cladosporium sp.</i>	•	•		•	Allergic respiratory syndrome, Allergic sinusitis, Dermatitis, Opportunist infections—in patients with immunocompromising disorders [94,95].
	<i>Stachybothris sp.</i>	•	•	•		Respiratory, cutaneous, ophthalmic allergic syndrome—especially in children [23].
	<i>Botrytis sp.</i>		•		•	Various allergies and respiratory problems—in patients with immunocompromising disorders [96].
Bacteria	<i>Staphylococcus sp.</i>		•	•		Bacteremia, Pneumonia, Endocarditis, Osteomyelitis [69].

Given that the museum is full of bacteriological microflora both in the air and on exhibits, this being a factor that contributes decisively to the deterioration of items over time and also having potential harmful effects on human health, non-invasive and non-destructive solutions must be found for cleaning and prevention [97–100].

After applying the essential oils of lemon (*Citrus limon*), mint (*Mentha piperita*) and lavender (*Lavandula angustifolia*) on the traditional Romanian waistcoat, we can conclude that they have the ability to inhibit the spores from moulds and bacteria, representing a very good alternative to the usual chemical treatments that are used for the conservation of elements belonging to the cultural heritage. Therefore, lavender oil has been shown to have inhibitory effects on *Aspergillus sp.* and *Staphylococcus* and the mint essential oil has an inhibitory effect over *Cladosporium sp.* and *Botrytis sp.*, for multiple materials having different properties.

Using these three essential oils was done only to test the inhibitory effects that natural biocides develop against fungi on a complex exhibit. Other natural extracts may have the same or perhaps even better inhibitory effects against the types of fungi identified. To this end, Spada et al. [101] and Bakkali et al. [48] show that some of the essential oils have specificities in terms of amplitude, but not in terms of mode of action or in terms of biological effects. Thereby, the essential oils can be considered as fungus decontamination agents for different types of fabrics belonging to cultural heritage [102], helping to the cleaning and preservation of the items exhibited in museums, creating at the same time purer environment for visitors and employees.

5. Conclusions

Establishing the favourable or unfavourable conditions inside museums, both in terms of exhibits, but especially the impact of these conditions on visitors and employees' health is a necessary step to reduce the risk that both categories are subjected to. Microclimate control, microbiological monitoring and the process of cleaning the exhibits and the rooms are very important in preventing fungal contamination of indoor items and air. An important role is played by preventive conservation, with basic cleaning and preventing the colonisation with fungi of valuable materials and therefore stopping their deterioration and safeguarding the health of visitors and employees. Thus, the study presents a dual approach, which on the one hand takes into account the microclimatic conditions that favor the appearance of fungi and on the other hand aims at the degree of microbial colonization of the air and surfaces inside the museum. These issues need to be taken into account more in the future by the scientific community, as old objects on display in museums, due to the degree of fungal load they contain, can pose a potential danger to museum staff and visitors. At the same time, in addition to monitoring the microclimate and the bacteriological load, it is necessary to adopt non-invasive reactive solutions for cleaning and/or protecting humans against their action.

The evaluation of the fungal load in air and on exhibits inside the Beius Land Museum and the influence of the main parameters of the microclimate (temperature, relative humidity, CO₂ concentration and suspended particles) on the profiling of microorganisms, together with viable and non-invasive solutions for cleaning, treating and preserving of exhibits were undertaken aiming to achieve a safe environment for human health. The data indicates that there is a high level of fungal contamination, inappropriate for exhibits and with direct impact over visitors' health. The six different types of yeast (*Candida guilliermondii*, *Candida sphaerica*, *Cryptococcus albidus*, *Cryptococcus laurentii*, *Cryptococcus neoformans*, *Sporobolomyces salmonicolor*), six different types of moulds (*Penicillium sp.*, *Aspergillus sp.*, *Trichoderma sp.*, *Cladosporium sp.*, *Stachybotrys sp.*, *Botrytis sp.*) and the bacteria (*Staphylococcus sp.*) identified in the air and on exhibits, have proven a damaging effect upon fabrics. Furthermore, the identified genera and species are involved in human pathology and the museum indoor air fits into a medium-high fungal contamination degree and represents a potential risk for human health, especially in the case of children and subjects with allergic status or allergic respiratory diseases. The development of these fungi is also supported by the non-compliant values of the main parameters of microclimate, the average temperature in the analysed period being only 8.6 °C, while the RH registered a concentration of 63.9% and the CO₂ did not exceed the average value of 433.2 ppm.

Given that fungi and bacteriological microflora easily contaminate tangible cultural heritage and this is a factor that contributes decisively to the deterioration of items over time, the essential oils of lemon (*Citrus limon*), mint (*Mentha piperita*) and lavender (*Lavandula angustifolia*) proved to be non-invasive and non-destructive solutions for cleaning and prevention since they have the ability to inhibit the spores from moulds and bacteria, representing a very good alternative to the usual chemical treatments that are used for the conservation of cultural heritage exhibits. The lavender oil has been shown to have inhibitory effects on *Aspergillus sp.* and *Staphylococcus* and the mint essential oil caused inhibitory effect over *Cladosporium sp.* and *Botrytis sp.*, from multiple materials having

different properties. Essential oils can be considered as fungus decontamination agents for fabrics helping to the preservation of the clothing exhibited in the museums. The control of the microclimate inside storage or exhibition halls together with the microbiological monitoring and green bioremediation and cleaning process are extremely important for preventing fungal contamination of the indoor items and indoor air, all directly linked to visitors and employees' health.

Museums protect and preserve our past and great works of art. But are they a real safe place for informal education and learning? Or they are a threat to the health of those who come to visit and those who work there? In these regards, our study offers a warning on the improper conditions encountered in museums, which can lead in time to the degradation of exhibits but can also cause many pathogens to people who come into contact with this environment. Careful monitoring of indoor air quality to protect human health and museum exhibits becomes extremely useful in analyzing the direct and indirect consequences of climate change as human responses to climate change.

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