Article

Microbial Air Quality in the Built Environment—Case Study of Darvas-La Roche Heritage Museum House, Oradea, Romania

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Abstract: Problems in the degradation and biodegradation of cultural heritage objects exposed or stored in public buildings and museums and of construction materials are caused (between others) by the activity of microorganisms. Biodeterioration can be observed not only at the level of the building materials of museum buildings, but also at the level of materials from which art objects are made (natural or artificial) and is determined by factors such as the chemical composition and nature of the composition material, the microclimate characteristics and exposure objects, but also through the manner and frequency of surface cleaning and housekeeping in museums. Based on this, the present study offers, through classical methods, a qualitative and quantitative identification of microorganisms inside a heritage museum building located in a temperate climate country. The purpose of the work was to determine to what extent the bacteriological microflora inside can directly and indirectly contribute to the health quality of the building's occupants as well as the degradation of its materials and structures. The results emphasize the presence of some fungi and bacteria, among them *Alternaria* spp., *Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp., and *Botrytis* spp. All of the analyzed rooms have a high and very high degree of fungal contamination (between 524 and 3674 UFC/m^3), which can represent a danger to both human health and the integrity of the exhibitions. This is more pronounced considering that some of species of fungi identified are associated with sick building syndrome, problems in humans due to harmful exposure to viruses, bacteria, and pathogens, which generate possible symptoms such as rhinorrhea, nasal congestion, hoarseness, coughing, sneezing, and irritability for the personnel and visitors.

Keywords: mycology; heritage building; biodeterioration; Darvas-La Roche Museum House; sick building syndrome; public health

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1. Introduction

Cultural heritage may bring an array of benefits to society by playing a crucial role in achieving sustainable development through poverty reduction and tourism benefits [1], but the health of cultural heritage buildings is of prime importance while providing recreational

services to tourists. Here, the term sick building syndrome (SBS) is used, the concept of which was first introduced by the World Health Organization (WHO) in 1983, and refers to nonspecific symptoms that cause illness to human health and damage to materials, caused by exposure to harmful agents associated with the occupancy of certain workplaces [2,3]. The issue has become a global concern as the impact of the deteriorating conditions of cultural heritage buildings on human health is prominent, as evidenced by the research on heritage buildings around the world [4,5].

SBS can also have an irreparable impact on historical exhibits, arranged in museums, due to their age and the materials that they are made of. The exhibits are prone to degradation by the combined action of bacteriological microflora and indoor air pollutants [6,7]. Thus, it is necessary to carefully determine the internal microclimate conditions and mycology of the building in such a way as to limit their impact on the valuable artifacts and the health of the employees and visitors [8,9]. The importance of the indoor microclimate lies in the fact that a specific indoor microclimate can be ensured in buildings to maintain the optimal conditions for the conservation of the building itself and for the comfort of the occupants [10,11].

The health of heritage buildings is interlinked with the microclimate and building mycology. In the case of heritage buildings, some important factors such as the age of the building, whether it is a wooden structure, relative humidity, and ineffective monitoring are conducive to microbial growth. Microbial contamination of indoor environment of a building can endanger not only the sustainability of the building but also impact the health of visitors and staffs. Globally, there have been numbers of studies on the indoor environment or microclimates of heritage buildings and its impact on human health [12–15]. Additionally, the microclimatic variables and pollutants from the surrounding environment may sometimes cause irreversible damage to the heritage art objects stored in the museum or heritage building if proper environmental control measures are not taken [16–18].

Although the specialized literature contains numerous studies on the microclimatic conditions and mycology of heritage buildings [19,20], the present study is required because of the uniqueness of the studied location and the stability of the causal relationships between microclimate, mycology, and population health. The effects of microclimate, building mycology, and SBS on human health has been the least researched in the context of the country. These kinds of studies can help to better understand the deterioration of heritage objects and improve conservation strategies by slowing down their natural and anthropogenic degradation, which is often irreversible, and in ensuring the safety of human health [21].

In the age of the postmodern period, studies on the indoor microclimate of heritage buildings have come to fore. Brimblecombe et al. [9] released a study using a multidisciplinary approach regarding the indoor environment of a modern museum building in the UK, namely, the Sainsbury Center for Visual Arts, in Norwich. The indoor microclimate was monitored and analyzed as were the bacterial groups and its concentrations (Gram-negative Flavobacteria and Gram-positive Actinobacteria/Bacillus and Staphylococcus). They also asserted that the indoor environment of a heritage building is connected to seasonal variation. Another recent study explored the indoor microclimate of museum buildings in different countries. It should be noted that the indoor environment of heritage buildings varies from one context to another, and also relies on several factors, for instance, the heating system, air conditioners, dust concentration, presence of fungi and bacteria, ventilation, lighting, the number of visitors, the physical parameters, and the outdoor environment [13,14,22–27].

The study of building mycology has gained in popularity because it has an impact not only on the health of the building, its materials, environment, and structures, but also on its occupants [28]. Thus, microorganisms are considered as major biodeteriogens of materials of artistic value due to their high metabolic versatility, and so are able to easily colonize organic materials and inorganic materials that make up the composition of heritage objects, causing aesthetic and structurally irreversible changes to their surface and interior either

of a physical nature (hyphal growth and penetration into the artifact substrates can cause discoloration, bio pitting, cracking, exfoliation, patina formation, etc.) and/or of a chemical nature (acid formation, pigments, extracellular enzymes, oxidation/reduction reactions, formation of secondary minerals, etc.) [29,30].

The presence of fungi in heritage buildings is a common feature around the world. Research findings have confirmed that among other factors, the presence fungus is one of the most potent causes of indoor pollution in cultural heritage [31]. Fungi, with their enzymatic activity and a proper environment, can live on and destroy museum objects. Recently, the study of fungal contamination has been quite popular because of its impacts not only in buildings, but also in work places [21,32–35]. Cabral [33] mentioned that in deteriorated houses and buildings, a high indoor humidity favors fungal growth (e.g., *Penicillium* and *Aspergillus*), and a prolonged exposure to volatile organic compounds and mycotoxins produced by *Penicillium*, *Aspergillus*, and *Stachybotrys* can negatively influence the health and well-being of the occupants, personnel, and visitors. Table 1 presents some of the studies in the field, together with the materials and methods used and the results obtained, especially regarding the types of microorganisms identified in the case studies.

Table 1. Summary of important research in the specialized literature aimed at determining microbiological contamination in buildings.

Study Authors	The Case Study	The Methods Used	Microorganisms Identified in Aerosols, Surfaces, Heritage Objects, Museum Workers (Oropharygeal and Skin)
Borrego et al. [36]	Archives buildings—Historical Archive of Museum of La Plata; Archive of Historical and Cartographic Research Department from the Geodesy Direction; Archive of Notaries of Buenos Aires Province; National Archive of the Republic of Cuba	Classical methods	*Penicillium* spp. *Cladosporium* spp.
Singh [28]	Historic Buildings		*Serpula lacrymans, Coniphora puteana, Antrodia vaillantii, A. Xantha, Asterostroma spp, Donkioporia expansa, Paxillus panuoides, Phellinus contignuus, Tyromyces placentus.*
Jurado et al. [37]	Salón de Reinos, Museo Nacional del Prado, Madrid, Spain		*Bacillus Cytobacillus, Streptomyces, Penicillium, Cladosporium, Mucor, Alternaria, Botryotrichum* and *Stagonosporopsis*
Lazaridis et al. [38]	Criminology Museum of the University of Athens, Historical Museum of Crete, Neophytos Doukas Library, Greece		Air concentrations of cultivable airborne microorganisms (heterotrophic bacteria, cellulose metabolizing bacteria, acid producing bacteria and mesophilic fungi) were determined.
Boniek et al. [35]	Nossa Senhora da Conceição Church, Brasil	Classic and Molecular identification based on rDNA sequencing	**Indoor air:** Sixty filamentous fungal isolates; **On artworks:** *Cladosporium cladosporioides* and *Aspergillus versicolor*
Pangallo et al. [39]	Slovak National Gallery, Bratislava, Slovakia	The sequencing of 16S or 18S rDNA PCR products	***Indoor air:*** *Burkholderia cepacia, Pasteurella pneumotropica, Staphylococcus epidermidis, Mycetocola* sp. *Pseudomonas migulae Microbacterium* sp., *Arthrobacter* sp. *Bacillus* sp., *Pseudomonas stutzeri, Acinetobacter lwoffii, Staphylococcus* sp., *Arthrobacter agilis, Staphylococcus haemolyticus, Aerococcus viridans Staphylococcus* sp.; ***Objects:*** *Psychrobacter psychrophilus, Streptomyces alboniger, Micrococcus luteus, Streptomyces griseoflavus, Bacillus* sp.; *Agromyces italicus Leifsonia poae, Bacillus megaterium; Bacillus megaterium, Streptomyces almquistii, Paenibacillus* sp.
Abdel-Kareem [40]	Museum of Jordanian heritage	The classical method (plates with different culture media) and electronic microscopy (SEM)	*Aspergillus, Penicillium, Chaetomium Alternaria*

Table 1. *Cont.*

Study Authors	The Case Study	The Methods Used	Microorganisms Identified in Aerosols, Surfaces, Heritage Objects, Museum Workers (Oropharygeal and Skin)
McNamara et al. [41]	Artefacts from Heritage churches in Germany	Clasic methods and molecular biology techniques	*Aspergillus fumigatus, Aureobasidium pullulans, Capnobotryella renispora, Coniosporum perforans, Engyodontium album, Geomycetes asperulatus, G. Pannorum, Leptospaeria maculans, Stanjemonium ochoroseum, Verticillium lecanii, V. psalliotae, Rhodotorula minuta, Ustilago* spp.
Harkawy et al. [42]	Monastery library and incunables, Poland	Impactor measurements, stationary and personal bioaerosol samples took using GSP (Ströhlein GmbH, Kaarst, Germany) and Button Aerosol (SKC Ltd., Eighty Four, PA, USA) samplers equipped with pumps (model 224-PCTX8, SKC Ltd.).	**Filamentous fungi:** *Acremonium striatum Acremonium* spp. *Alternaria* spp. *Aspergillus flavus Aspergillus niger Aspergillus versicolor Chaetomium elongatum Chaetomium* spp. *Oidiodendron rhodogenum Oidiodendron truncatum Penicillium aurantiogriseum Penicillium verrucosum Penicillium spp. Ulocladium* spp. *Wallemia sebi;* ***Yeasts:*** *Candida famata Geotrichum candidum Rhodotorula glutinis*
Khan and Karuppayil [43]	Fungal pollution of indoor environments and its management	Specialistic methods	The review concerning the biotic indoor air pollution, role of fungi as biological contaminants and the impact upon human health.
Hasnain et al. [44]	International Center for Chemical and Biological Sciences (ICCBS) building, Karachi, Pakistan	Meteorological data and Statistical methods	*Cladosporium* spp. *Alternaria* spp. *Periconia* spp. *Curvularia* spp. *Stemophylium* spp. *Aspergillus* spp. *Penicillium* spp.
Pavic et al. [45]	Museums from Serbia	Classic methods and modern methods	*Firmicutes, Proteobacteria, and Actinobacteria phyla of Bacillus* and *Staphylococcus (Staphylococcus, Acinetobacter, Agrococcus, Janibacter, Rhodococcus, and Stenotrophomonas)*
Di Carlo et al. [46]	Diocesan Historic Archive, Sibilla Antrum and the Saints Cave, Italy	Clasic methods and molecular biology techniques	**Fungi:** *Aspergillus* spp. *A. Niger Alternaria* spp. *Cladosporium* spp. *Penicillium* spp. *P. Chrysogenum Scopulariopsis* spp. *Chaetonium* spp.; **Bacteria:** *Arthrobacter* spp. *Bacillus* spp. *B. Flexus B. Simplex B. Thuringiensis B. Weihenstephanensis Micrococcus* spp. *M. Luteus Terribacillus* spp.

Table 1. *Cont.*

Study Authors	The Case Study	The Methods Used	Microorganisms Identified in Aerosols, Surfaces, Heritage Objects, Museum Workers (Oropharygeal and Skin)
Dziurzynski et al. [47]	Museum of King John III's Palace at Wilanow, Warsaw, Poland	Clasic methods and sequencing methods	*Acinetobacter, Bacillus, Enhydrobacter, Micrococcus, and Staphylococcus Acinetobacter, Enhydrobacter, Staphylococcus Bacillus* and *Micrococcus*
Scarlat et al. [48]	Museums form Romania	API and the cefoxitin susceptibility test	*Dysbiosis, β-hemolytic microorganisms, methicillin* and *penicillin resistant staphylococci.*

In the present study, the experiments were conducted at Casa Darvas-La Roche, which is located in the Municipality of Oradea, Romania (Figure 1). These aimed to determine the bacteriological microflora inside the museum to protect the health of the employees and visitors as well as the integrity of the artifacts. The study considered not only the establishment of fungi and bacteria at the genera level in the indoor air, but also the calculation of the degree of fungal contamination in each of the studied rooms and the potential impact that they may have. The building is classified as historical, and was built in the Art Nouveau style, between 1911 and 1912. It went through extensive renovation and reconstruction processes until 2020, when it was reopened to the public in the form of a museum that houses numerous exhibitions depicting the life of a wealthy Jewish family from the end of the 19th century and the beginning of the 20th century [49,50]. It is one of the most spectacular Art Nouveau museums in southeastern Europe, both in terms of architecture from the outside as well as the priceless objects that it houses.

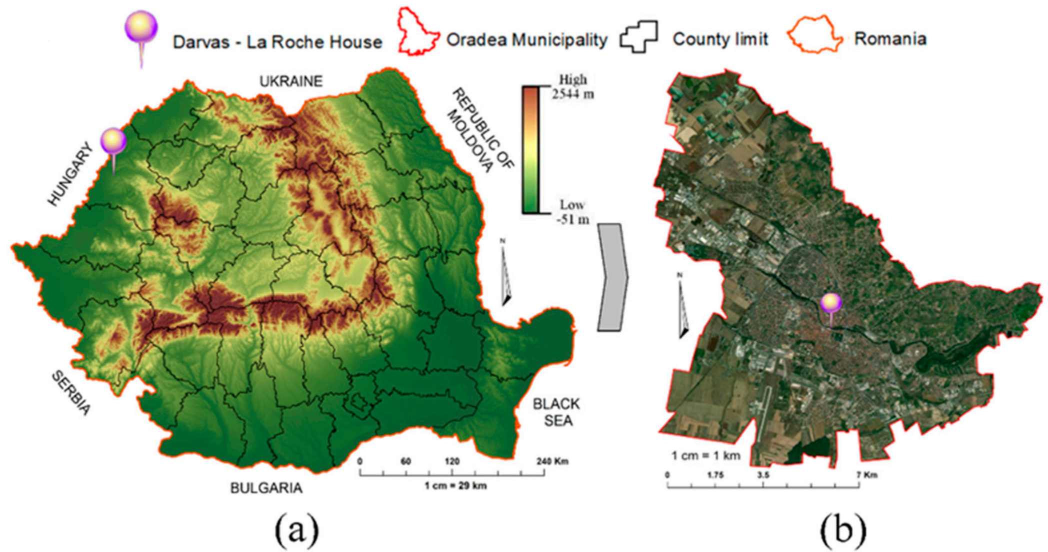

Figure 1. Location of the Darvas-La Roche House at the level of country (Romania) (**a**) and the Municipality of Oradea (**b**).

The current study is part of a much larger research to determine the indoor microclimatic conditions and how they affect the health of employees and visitors and the physical integrity of the objects. In the study by Ilies et al. [26], the determination of the physical parameters of the internal microclimate (temperature, humidity, particulate matter, natural and artificial light) as well as the pollutants present inside (carbon dioxide, formaldehyde, volatile organic compounds, oxygen concentration, sulfur dioxide, ozone, nitrogen dioxide, nitric oxide, hydrogen sulfide, carbon monoxide, and methane) was carried out. The results showed that high temperatures combined with low relative humidity induced a heightened stress on the exhibits and people because they favor the lifting of suspended particles and the release by new construction materials of amounts above the permissible limit of formaldehyde and volatile organic compounds. Based on their study, the current research aimed to identify the micro-biological contamination, with an emphasis on the load and species of fungi, that can develop in these indoor environmental conditions. These aspects have the potential to influence the growth and development of the bacteriological microflora, which can then endanger the artifacts and human health.

The novelty of the study resides in the diversity of the methods used to determine the internal microclimate and the microbiological contamination inside, and the fact that no such studies have been carried out thus far in Art Nouveau heritage buildings in Oradea, despite the fact that they represent the identity of the city. In this sense, the present study represents a first. At the same time, most studies in the field only determined the internal microclimate and did not consider the microbiological load; this being a very important

aspect for the presentation of a complete and complex picture regarding the influence of indoor conditions on the exhibitions and human health. Among the limitations of the study, it is worth mentioning the limited accessibility considering the historical monument status of the case study, which is of national interest and needs to be protected as best as possible.

2. Materials and Methods

In order to determine the degree of microbiological contamination inside the Darvas-La Roche Museum House, air samples were taken in three different rooms: the exhibition hall—ground floor (137.7 m^3 of air); the bedroom—1st floor (114 m^3 of air); and the great hall—1st floor (277.3 m^3 of air). Within these rooms, 23 data collection points were individualized (Figure 2), and spatially distributed in such a way as to cover the entire space as evenly as possible. Data were collected on Mondays in December 2022; these days were chosen because the museum is closed to the public, which favors the taking of samples for the determination of aerofungiflora, as a reduced activity in the human component does not risk inducing erroneous values in the obtained results.

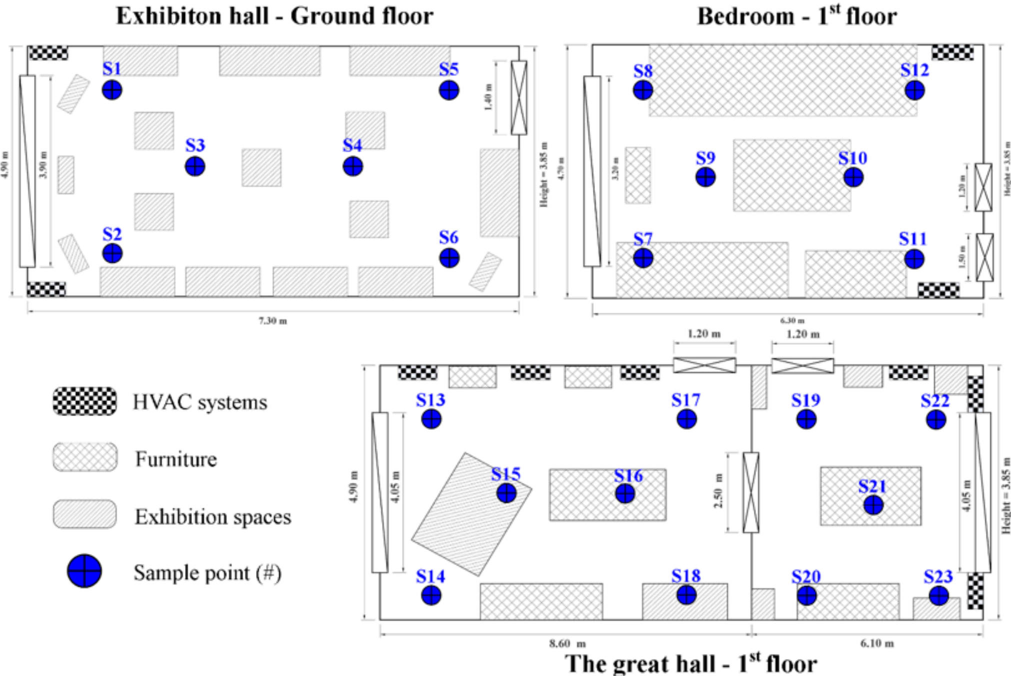

Figure 2. Spatial distribution of the sampling points for the assessment of the fungal load within the three analyzed rooms in Darvas-La Roche House.

The aerofungiflora was sampled using the Koch sedimentation method, a technique chosen for use in the case of particles that passively sediment due to gravity [51]. The advantages of the method include the fact that it is low cost, there is no need for special identification equipment, it has an easy-to-implement working procedure, is a rapid method to assess the growth of fungal colonies on culture media, and presents useful results for monitoring the air quality in the rooms and in establishing preventive measures. The disadvantages reside in the fact that it is a non-volumetric identification method, it is valid for biologically viable particles that settle on the Petri plates in a certain time interval, and the culture media can also be influenced by the physical/chemical factors in the air in the case of long exposures and has the probability of overloading in the case of a high degree of air contamination [52].

The Koch sedimentation method was implemented through the use of Petri dishes with sterile Sabouraud culture medium. Petri dishes 9 cm in diameter were used, which were exposed indoors for 30 min, at a height of approximately 1.7 m, representing the height of a man of average stature. Each sample taken was inoculated into nutrient agar to

identify the bacteria as well as in Sabouraud agar containing chloramphenicol in order to isolate the fungal genera [36]. All air samples were incubated at 24–25 °C for a period of 10 days to allow the fungal colonies to grow. These started to enter a maturation process, being visible after 24 h, showing changes in terms of the shape, diameter, color, and texture. The final identifications were made through examining the macroscopic and microscopic characteristics of the grown colonies [53,54].

The evaluation of the degree of fungal contamination in the air in the rooms was expressed in CFU/m^3 (number of fungal colony forming units/m^3 of air) and the equivalence of the result relative to the air volume with the help of calculation formulas from the Polish standard PN 89/Z-04008/08 [55] and Omelianski formula, respectively [56].

The formula used in accordance with PN 89/Z-04008/08 is:

$$\boldsymbol{UFC/\mathrm{m}^3 = \frac{n \times 10.000}{S \times t \times 0.2}}$$

where n is the number of Petri plates used; S is the surface of the Petri plates expressed in cm^2 (in this case, for a 9 cm diameter Petri plate; $S = 3.14 \times R^2 = 63.5\ cm^2$); and t is the total exposure time of the plate (expressed in minutes).

Omelianski's calculation formula, as shown by Cernei et al. [56], prove that on a surface of 100 cm^2 exposed to air for a certain period of time sediments a number of microorganisms equal to that contained in the 10 dm^3 of air, through:

$$\boldsymbol{UFC/\mathrm{m}^3 = \frac{n \times 10.000}{S \times k}}$$

where n is the number of colonies grown on the surface of the plate; S is the surface of the Petri plate; and k is the coefficient of exposure time to air (expressed in minutes; each unit k is assigned 5 min).

In addition to the determination of the total number of fungal colony forming units per m^3 of air for each Petri plate, the determination of the average quantity of UFC/m^3 at the level of each room, the spatial distribution of these at the level of the analyzed rooms as well as the average number of fungal colonies for each sample collected and for each room were considered. Moreover, the determination of the genera of the fungal colonies aimed to determine the impact they could have on the health of the visitors and employees as well as on the integrity of the exhibits arranged within the museum (Figure 3).

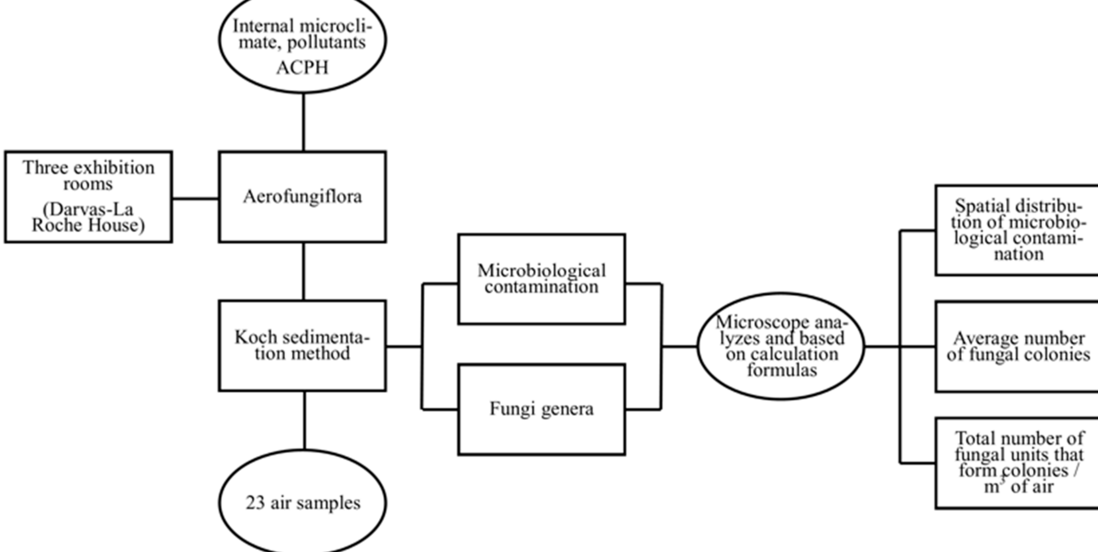

Figure 3. Flowchart representing the methodology of data acquisition and processing as well as the qualitative results obtained.

Considering that the indoor microclimate is controlled with the help of HVAC systems, which have a significant impact on the physical parameters, pollutants, and bacteriological microflora, the correlation of information on them with the obtained results was considered [57,58]. Thus, the HVAC systems include air filtration devices, but at the same time, the windows are left open for better ventilation of the rooms. To determine the air change per room per hour (*ACPH*), the application of a simple calculation method was considered, based on the measurements made in the rooms with a handheld telemeter. The formula is as follows:

$$\boldsymbol{ACPH = \frac{CFM \times 60}{A \times HC}}$$

where *CFM* represents the cubic feet per minute of air purification systems; 60 refers to the minutes within an hour; *A* represents the area in m^2 of the rooms; and *HC* is the ceiling height in m of the analyzed exhibition areas.

3. Results

The results indicate that the lowest air change per room per hour was the Exhibition Hall—Ground floor, which only has two air purification systems installed, the *ACPH* values being 5.17. With regard to the Bedroom—1st floor, it was individualized by a value of 6.27 in terms of *ACPH*, and the Great Hall—1st floor had the highest values of this indicator at 8.06, considering the fact that it has six air purifiers installed. At maximum capacity, the air purification devices in the three analyzed halls were able to recirculate the entire air from the rooms between 5.17 and 8.06 times per hour, depending on the characteristics of the rooms.

Examination and identification of the colonies revealed that the mold species developed exclusively, the colonies being visible from the third day. The identification of fungal genera was achieved following the evaluation of macroscopic and microscopic characteristics. The Petri plates were quickly invaded and most of them completely by the seventh day of incubation. All colonies developed on the culture media belonged to the category of molds.

Currently, there are no unanimously accepted standards or regulations regarding the fungal load of the air, therefore, in order to assess the degree of air contamination in the rooms, certain indicative norms established after some researches were used [59,60]. According to the sanitary norms applied to non-industrial institutions, assessment of the degree of air contamination in a room is based on the number of fungal units that form colonies (UFC/m^3 air). Thus, below 25 UFC/m^3 of air is considered to be a very low degree of contamination, between 25 and 100 UFC/m^3 of air represents a low degree of contamination, between 100 and 500 UFC/m^3 of air is attributed to a degree of medium contamination, while high contamination is between 500 and 2000 UFC/m^3 of air, and very high contamination exceeds 2000 UFC/m^3 of air (Table 2). All fungal colonies and the number of fungal units that form colonies were counted between the third and seventh day of incubation, starting from the eighth day and up to the tenth, when the colonies could no longer be counted due to their invading character.

Table 2. Assessment of the degree of air contamination in a room, according to the sanitary norms applied to non-industrial institutions [60].

Contamination Degree of the Indoor Air	Koch Sedimentation Method Based on Number of Fungal Units that Form Colonies Per m^3 of Air (UFC/m^3)
A—very low	<25
B—low	25–100
C—medium	100–500
D—high	500–2000
E—very high	>2000

After seven days of incubation, the analyses implemented on Petri plates resulted in the identification of a total number of 575 fungal colonies (for all 23 analyzed samples). The fungal colonies per sample were between a minimum number of 10 and a maximum number of 70, the average being 25 fungal colonies. In the case of the Exhibition Hall—Ground floor, 135 fungal colonies were identified, with an average of 22.5 colonies and maximum values between 20 and 25 colonies per sample (Figure 4a). Regarding the number of colony-forming fungal units, this exhibition hall stood out with a total value of 7083 CFU/m^3 and an average of 1180.5 CFU/m^3 of air, the absolute values varying within a relatively small gap between 1049 CFU/m^3 and 1312 CFU/m^3. These values of the number of fungal units that form colonies per m^3 indicate a room with a high degree of fungal contamination (indicator D) (Figure 4b).

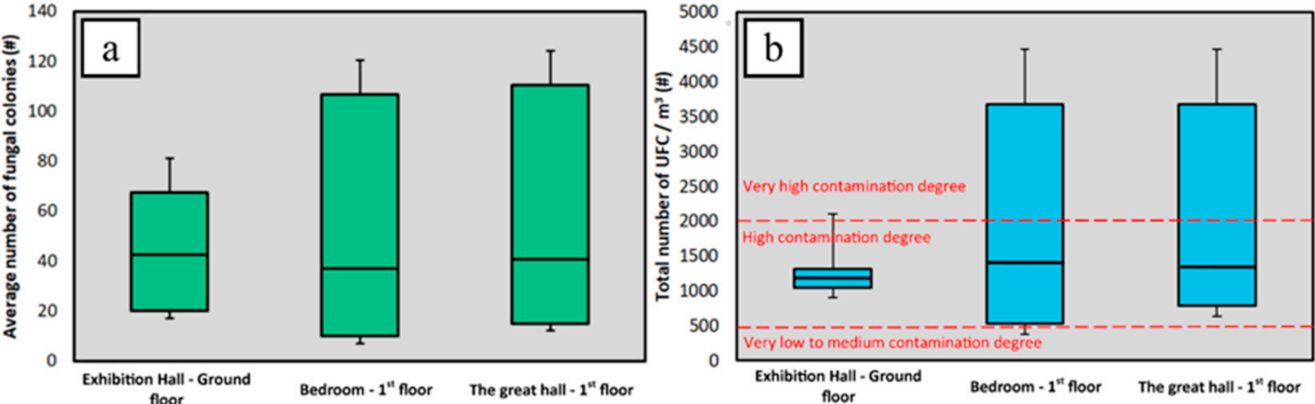

Figure 4. The degree of fungal contamination of the air inside the three analyzed rooms of the Darvas-La Roche House ((**a**)—the average number of fungal colonies; (**b**)—the total number of fungal units that form colonies, calculated per m^3 of indoor air).

For the Great Hall—1st floor, 18 data collection points were individualized, which totaled a number of 280 fungal colonies, with an average of 25.5 and intervals between 15 and 70 colonies (Figure 4a). The number of fungal unit forming colonies was established at a total of 14,692 CFU/m^3, the average being 1335.6 CFU/m^3, the results falling into the degree of contamination D (high), according to Table 1 (Figure 3b). Collection points number 14 and 19 were individualized for this room, which had minimum values of 15 fungal colonies and a number of 787 UFC/m^3. At the opposite end was sample 23, which had no less than 70 fungal colonies that totaled 3674 UFC/m^3, so fungal contamination was included in category E—very high.

The Bedroom—1st floor was identified as the room with the highest level of microbiological contamination among the analyzed rooms. A total number of 160 fungal colonies were determined in the six collection points, with an average of 26.7 colonies and absolute values between 10 and 70 fungal colonies/sample (Figure 4a). Regarding the number of colony-forming fungal units calculated per m^3 of air, this hall stood out with absolute values of 8395 UFC/m^3 and averages of 1399.2 UFC/m^3. The degree of fungal contamination of this room was high, category D, according to the sanitary norms applied to non-industrial institutions (Figure 4b). Analyzed point by point, sample 11 recorded the lowest values, both in terms of the number of fungal colonies (10 colonies) and the number of units that form fungal colonies (524 UFC/m^3) while sample 10 was identified as having 70 fungal colonies and 3674 UFC/m^3 (very high degree of fungal contamination).

All three rooms analyzed recorded high and very high values of the degree of microbiological contamination of the indoor air, therefore, it is necessary to identify the areas where the colonies and fungal units are concentrated. The spatial distribution of the number of colony-forming fungal units (CFU/m^3) reveals a disproportionate distribution within the exhibition halls. In the Exhibition Hall—Ground floor, the highest values, up to 1312 UFC/m^3, were concentrated in the northeastern and central part, where the surround-

ing areas recorded values of 1049 UFC/m^3 (Figure 5). The Great Hall—1st floor followed the same pattern as in the previous case, but this time, the maximum values (3674 UFC/m^3) were assigned to a small area in the southeast part of the room, in the immediate vicinity of the window. In the rest of the hall, it varied between 787 UFC/m^3 (especially at the extremities of the hall) and 1312 UFC/m^3 (located in the central areas). A special situation was shown in the Bedroom—1st floor, where the maximum values (3674 UFC/m^3) are concentrated in the central areas of the room. Here the values increase with the approach to the center, from 524 and 787 UFC/m^3 at the extremities to 1312 UFC/m^3 in the immediate vicinity of the area of maximum microbiological contamination (Figure 5).

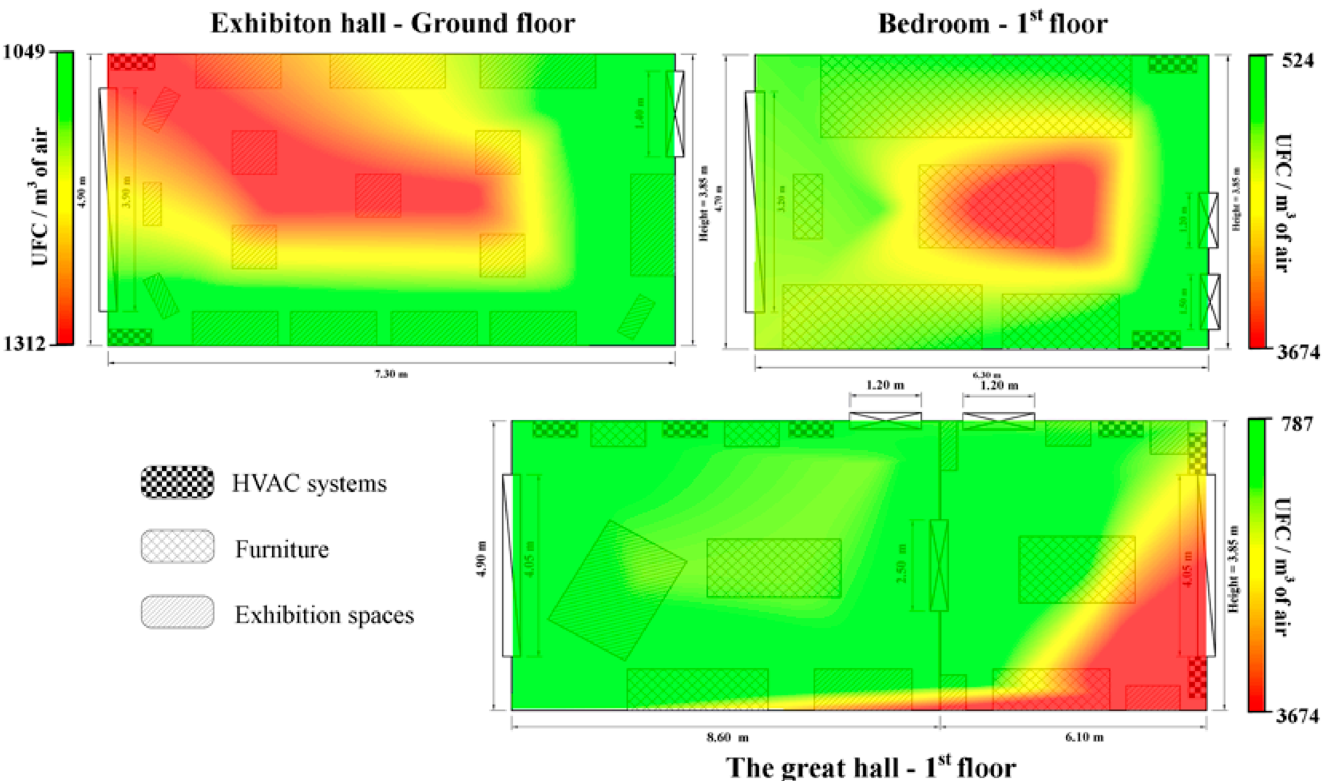

Figure 5. Spatial distribution of the number of units that form fungal colonies per m^3 of air in the three analyzed rooms in Darvas-La Roche House.

In addition to determining the degree of microbiological contamination in Darvas-La Roche House, a macroscopic and microscopic examination of the fungal colonies were used to identify the type of mold inside. These were sampled based on the physical properties of the colonies determined under the macroscope such as the size, shape, outline, consistency, and color as well as on the microscopic appearance (sporangiospores, conidiophores, methules, phialides, hyphae, pseudo-hyphae, conidia) [53,54]. The results obtained show that a number of five genera of mold were present on the 23 samples taken from the indoor air, namely, *Alternaria* spp., *Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp., and *Botrytis* spp. It was revealed that the same genera of fungi grew on the surface of the plates to a similar extent, their invasion being considerable. Figures 6 and 7 show the sampling model of the five types of molds based on microscopic methods as well as the appearance of the colonies that grew on the Petri plates on the seventh day after collection.

Among the types of fungi identified in the indoor air, some are recognized as having negative effects on human health, while others have a destructive potential on the integrity of the artifacts arranged within the museum.

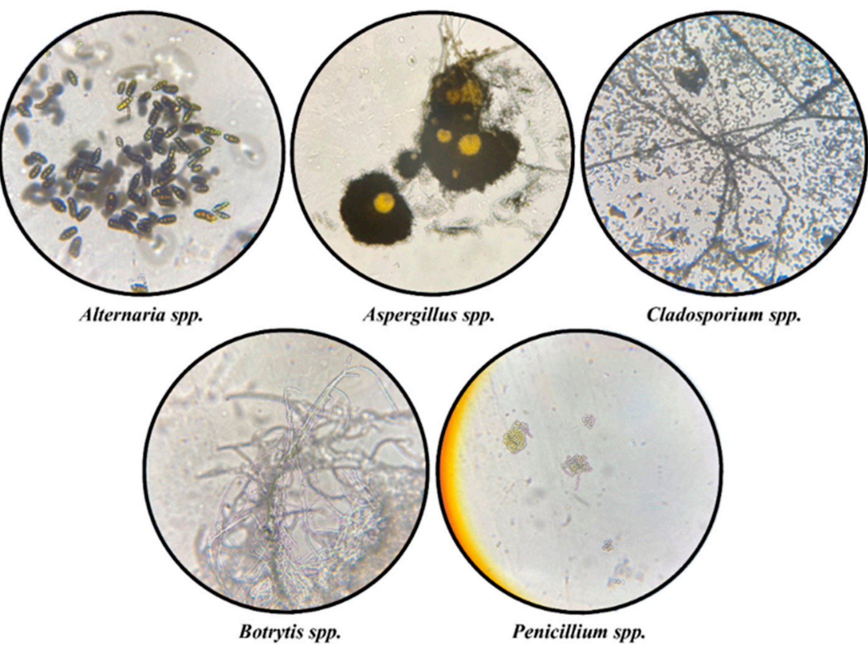

Figure 6. Appearance of the fungal colonies taken from the indoor air in Darvas-La Roche House on the seventh day of incubation.

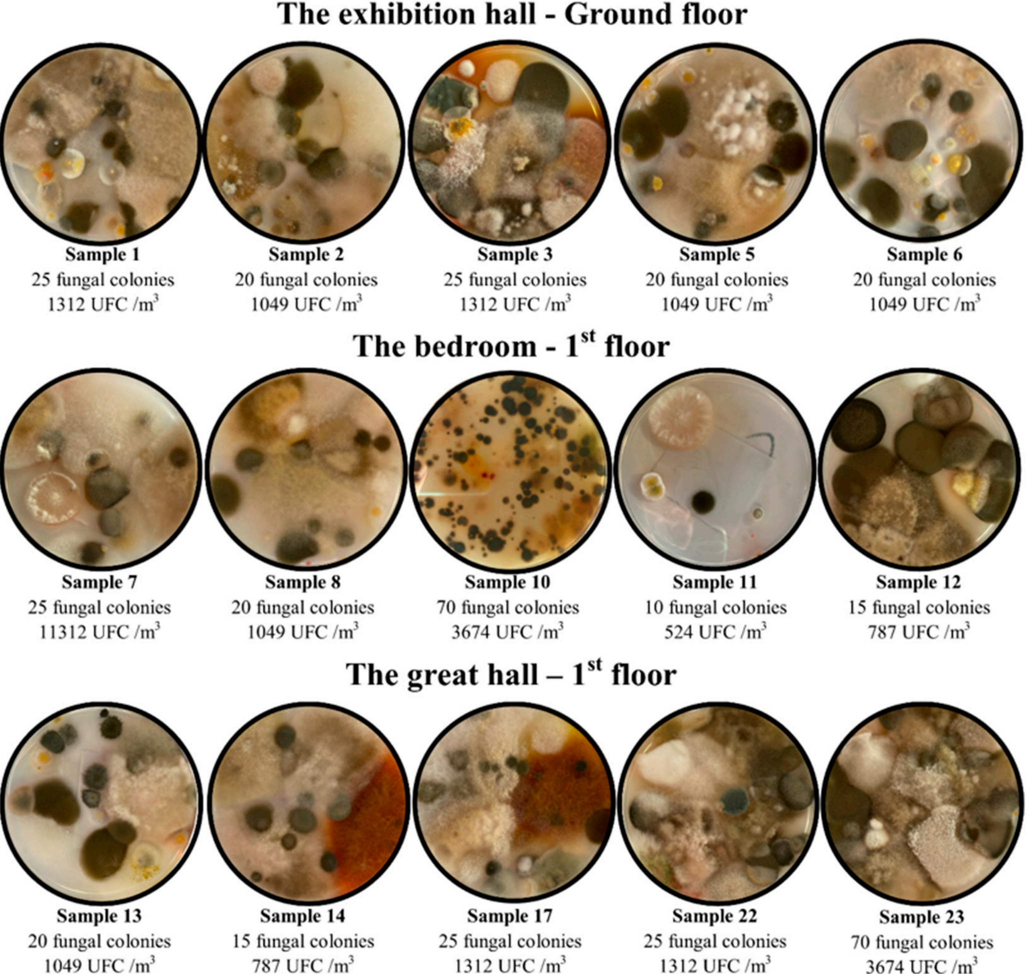

Figure 7. Appearance of fungal colonies at 7 days after sampling from the indoor air in the three rooms in Darvas-La Roche House.

4. Discussion

The inhalation of high concentrations of mixed organic dust including secondary metabolites and mycotoxins (e.g., *Aspergillus*), volatile organic compounds (VOCs), and allergens (glucans) is associated with sick building syndrome [60,61], which can affect, in the case of humans, the skin, respiratory, and neurological systems, potentially generating manifestations such as rhinorrhea, nasal congestion, hoarseness, coughing, sneezing, irritability, etc. Regarding the effects of the bacteriological microflora present inside on the exhibits, Elamin et al. [62] indicated that *Alternaria alternata*, *Aspergillus flavus*, and *Penicillium oxalicum* are fungal species that act on naturally aged fibers, causing hydrolysis, oxidation, depolymerization, and recrystallization. The most susceptible objects are those made of natural fibers and cellulose due to the predisposition of these materials to be damaged by the combination of indoor microclimatic conditions and bacteriological microflora, but old paintings and furniture are also at risk [63–65].

Most species of *Alternaria* (over 300 in total) are saprophytes, noting their presence in soils and organic matter. Some species of *Alternaria* that have clinical significance for humans are able to produce secondary toxic metabolites, some of which are mycotoxins with cytotoxic, carcinogenic, genotoxic, or mutagenic effects. In the case of individuals with high sensitivity or with low immunity, the spores of *Alternaria* spp. can trigger and maintain severe allergic reactions, upper respiratory tract infections, and bronchial asthma [66–69].

The *Cladosporium* genera consist of a large number of pigmented species (dematicaee) with wide distribution in the air as well as degraded organic matter. There are toxigenic and pathogenic species for humans (e.g., *C. cladosporioides*, *C. herbarum*, *C. oxysporum*, and *C. Sphaerospermum*) that have been associated with superficial infections of the skin, soft tissues, and appendages with onychomycosis up to disseminated infections with a high degree of mortality. At the same time, *Cladosporium* spp. are aero-allergenic and cause severe allergic reactions in the respiratory tract, sinusitis, lung infections as well as intrabronsyc lesions [70,71].

Botrytis spp. species are well-known as fungal pathogens of various plants and includes over 30 different species [72]. Botrytis species have not been reported as human pathogens, except for the fact that they can be associated with allergic reactions, allergic asthma, and pneumonia [73].

Filamentous fungi that are widely able to contaminate the soil and air are those of *Penicillium* spp. They produce secondary metabolites, in other words, mycotoxins that have toxic effects on humans and animals at prolonged and continuous exposure (e.g., *Ochratoxina A* is a calcsilicate that is classed as a possible human cancerogenic by the International Agency of Cancer Research, targeting mainly the kidneys, being nephrotoxic, teratogenic, carcinogenic and immunosuppressive). Plant-pathogenic species, *P. citrinum*, *P. chrysogenum*, *P. digitatum*, *P. expansum*, and *P. marneffei* may cause infections in humans/animals through inhalation and sometimes by ingestion. Diseases that occur as a result of *Penicillium* infection are generally referred to as penicylosis (keratitis, endophthalmitis, otomycosis, pneumonia, endocarditis, and urinary tract infections). *Penicillium citrinum*, which produces the toxins ochratoxin and citrinin, has been associated with some conditions such as keratitis, asthma, and pneumonia. In the case of HIV-infected patients, those with hematological cancers, or those treated with immunosuppressants, *Penicillium marneffei* can cause various infections that lead to weight loss, skin lesions, lung damage, anemia, cough, and subfebriles [74,75]. *Penicillium digitatum*, *P. expansum*, and *P. chrysogenum*, although they are less associated with penicilolysis, can cause fatal infections in humans [76]. *Penicillium chrysogenum* and *P. expansum* have been reported to be causative agents of keratitis, necrotizing esophagitis, endophthalmitis, and asthma [75].

The genus *Aspergillus* consists of over 200 species that are ubiquitous in the indoor environment (especially in hospitals, housing, etc.), their development being favored with moisture, indoor plants, etc. Several species are well-known as important opportunistic pathogens in humans as a result of research. *Aspergillus fumigatus* is the most common and dangerous opportunistic fungal pathogen transported into the air, which is especially im-

portant among immunosuppressed hosts. The inhalation of spores (conidia) of *A. fumigatus* into the lungs can cause diseases such as invasive pulmonary aspergillosis, aspergilloma and various forms of hypersensitivity diseases such as allergic asthma, hypersensitivity, pneumonia, and allergic bronchopulmonary aspergillosis. Various species of *Aspergillus* produce mycotoxins: aflatoxins and sterigmatocystines produced by *A. flavus*; *ochratoxin A*, malformin, oxalic acid, and fumonisin B2 produced by *A. niger*; viriditoxin and gliotoxin produced by *A. fumigatus* and patulina, triptoquivalene, and cytolacine E produced by *A. clavatus*. Aflatoxins affect human health by being hepatotoxic and immunosuppressive. Fumonisins are considered carcinogenic, hepatotoxic, nephrotoxic, and immunosuppressive. Species of *Aspergillus* are also implicated in severe infections, for example, *Aspergillus flavus* can affect the respiratory tract with sinusitis, bones with oteomielitis, eyes with keratitis, nails with the production of onychomycosis; *Aspergillus fumigatus* is especially involved in respiratory tract infections with lung infections, *Aspergillus niger* can produce otomycosis and pulmonary aspergillosis [77–79]. *Aspergillus versicolor* is commonly found in indoor environments with high humidity and can produce a hepatoxic mycotoxin—sterigmatocistine, possibly another causative agent of invasive aspergillosis; it has been reported that this opportunistic pathogen contains more than 20 allergens and mainly irritates the nose, eyes, and throat [80–83].

Most of the pathologies that can be generated by fungi identified inside Darvas-La Roche House are those of the respiratory tract, which have an important socio-economic impact both through their frequency and because of their influence on the quality of life of patients. In particular, people with frequent and long-term exposure to an environment contaminated with the identified microbiological flora should follow a respiratory rehabilitation program aimed at reducing symptoms, optimizing exercise capacity and the reducing financial costs by stabilizing or limiting the systemic manifestations of these ailments [41,84–87].

5. Conclusions

High temperature values (often over 25 °C) combined with large amounts of suspended dust and a low relative humidity led to the formation of a rich and quite varied bacteriological microflora. The results obtained show the presence of five different genera of fungi: *Alternaria* spp., *Cladosporium* sp., *Botrytis* spp., *Penicillium* spp., and *Aspergillus* spp. These formed in the three analyzed rooms with no less than 575 fungal colonies (in average 25 colonies/collection point) and 30,170 colony-forming fungal units (average 1311 UFC/m^3 for each collection point). These values indicate a high degree of microbiological contamination (over 500 UFC/m^3 in all 23 situations), in accordance with the airborne microbial and fungal load. However, areas with very high values of the number of fungal units that form colonies (over 2000 UFC/m^3) were only found in limited areas within the three analyzed rooms, being completely isolated deviations from the calculated average. The Bedroom—1st floor is the room that recorded the highest microbiological pollution (26.7 fungal colonies and 1399.2 UFC/m^3 on average), followed by the Great Hall—1st floor (25.5 fungal colonies and 1335.6 UFC/m^3 on average), the lowest values were determined in the Exhibition Hall—Ground floor (22.5 fungal colonies and 1180.5 UFC/m^3 on average). All of these results were obtained despite the fact that the three analyzed rooms obtained an *ACPH* index between 5.17 and 8.06, the rooms, in theory, being well-ventilated. The problem could lie in the quality and functionality of the air purification systems, which may need repairs or adjustments, so that they match the properties of the rooms and the bacteriological microflora inside.

All of these characterizations work together to create the conclusion that the interior of the museum can present a risk to both human health and the biodeterioration of valuable artworks housed inside. These aspects are also supported by the types of fungi identified, some of which have the potential to induce allergic reactions, skin and tissue infections, or different symptoms (pneumonia, lung damage, anemia, etc.), while others can aggravate the pathogenicity that already exists. The most exposed are children, the elderly, people

with low immunity, or those who consume medicine that suppress or reduce the resistance of the body's immune system. In order for these potential risks to be reduced to a minimum, the permanent monitoring of the microclimate from the point of view of thermohygrometric parameters, pollutants, dust in the air as well as the microbiological load in the indoor air and the surfaces is recommended. At the same time, it is necessary to adopt environmentally friendly cleaning, reduce the sources of environmental contamination, include permanent ventilation, eliminate carpets that store dust, and recirculate the air.

The current study represents the first one for Darvas-La Roche Museum House, considering that no such measurements have been taken inside it, although it represents a landmark building for Oradea. We wish this research to be an example of good practice in terms of determining the internal microclimate in Art Nouveau heritage buildings in the municipality of Oradea, and hope that this methodology is applied to as many such case studies as possible, in order to determine the potential impact that the indoor microclimate has on the integrity of the exhibitions and the health of their employees/visitors. Among the limitations of the study, it should be mentioned that, being a heritage building, the interventions aimed at cleaning and regulating the interior microclimate must be undertaken with the utmost of care in order to not affect the degree of preservation of the artifacts. This can drastically limit the range of procedures available to stabilize the internal conditions.

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