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EVALUATING BIODETERIORATION RISKS IN A COLLECTION OF CONTEMPORARY ART PAINTINGS: ENVIRONMENTAL ASSESSMENT AND CHARACTERIZATION OF FUNGAL COMMUNITIES

Erika TARILONTE[1,*], Saioa CENDÓN-SÁNCHEZ[2], Ane ZARATE[2], Aitor REMENTERÍA[2], Andoni RAMÍREZ-GARCÍA[2], Oskar GONZÁLEZ-MENDIA[1], Miren Itxaso MAGUREGUI[1]

[1] Department of Painting, Faculty of Fine Arts, University of the Basque Country (UPV/EHU), Barrio Sarriena s/n, 48940, Leioa, Spain
[2] Department of Immunology, Microbiology and Parasitology, Faculty of Science and Technology, University of the Basque Country (UPV/EHU), Barrio Sarriena s/n, 48940 Leioa, Spain

Abstract

This study focused on the fungal community present on a collection of contemporary art paintings and assessed the potential risks they pose. The fungal load within the storage facilities was analyzed before and after implementing a dedicated room designed for improved conservation. Likewise, environmental variables were measured. Monthly air sampling revealed inadequate microbial air quality in the pavilion where the paintings were kept, with excessively high relative humidity levels and frequent oscillations. However, post-renovation, the microbial air quality improved significantly due to enhanced isolation and better control of environmental parameters. In both outdoor and indoor environments, the dominant identified fungi were Cladosporium, Penicillium, Aspergillus, and Alternaria, with three of these genera also detected on the paintings. This suggests that the majority of fungi present originated from external spore infiltration. Surface sampling of the paintings showed fewer fungal colonies compared to other inert storage surfaces, although some paintings did exhibit visible colonies. Microscopic examination of surfaces and cross-sections revealed no immediate serious damage, but the potential for long-term fungal growth was identified as a threat to the paintings. Effective isolation and environmental regulation were deemed crucial for mitigating fungal risks and preserving the collection.

Keywords: *Canvas painting; Contemporary art; Microbial air quality; Environmental monitoring; Fungi*

Introduction

Canvas paintings are among the most prevalent and significant artworks in many contemporary art collections. Therefore, their appropriate storage is a key issue for a successful conservation of our cultural heritage. Unfortunately, due to a wide range of reasons, canvas paintings are not always stored under optimal conditions and are subjected to uncontrolled environmental parameters such as fluctuations in relative humidity (RH) and temperature or to atmospheres with poor air quality and lack of ventilation [1, 2]. These factors can lead to the appearance of different types of alterations in the artworks, including biodeterioration [3]. In fact, paintings are an excellent substrate for microorganisms to grow since the constitutive organic materials can be used as a carbon source [4]. These materials are found in the support (e.g., fibers),

* Corresponding author: erika.tarilonte@ehu.eus

ground layer (e.g., animal glue), paint layers (e.g., drying oil), protection layer (e.g., natural or synthetic resins), or in substances applied during conservation–restoration interventions [5, 6].

Among the microorganisms that can colonize canvas paintings, fungi play a major role considering that they are extremely resistant and ubiquitous in the air and dust. Under certain conditions, fungi may coexist with artworks without causing significant damage, but when conditions are altered, the growth and vegetative development of fungi can be accelerated, with the increase in temperature or RH being two crucial factors for that [7, 8]. During colonization, fungal microorganisms produce aggressive metabolic products and extracellular enzymes, which can cause biodegradation and alterations in the properties of the materials present in paintings [9]. At first, it can be limited to aesthetic damage, but when the fungal hyphae penetrate into the paintings, they can cause color changes, punctures, and loss of paint, among other things [10].

Considering the risk for the cultural heritage, lately, much research has focused on the analysis of the fungal communities in canvas paintings [11-14]. These studies usually rely on cultivation strategies and molecular techniques for the quantification of fungal concentration and the characterization of fungi [15-17]. Indeed, the latter is a crucial step to determine the deterioration that the microorganism may cause [18]. Furthermore, in order to investigate the damage affecting the painting, these studies may apply microscopic analysis to the surface and cross sections of the paintings [14].

Besides the examination of the paintings, aerobiological studies are increasingly important in this field since fungi spread via spores in the air. For that reason, the microbiological quality of the air of sites such as museums, historical buildings, or libraries is monitored in order to assess the risk of biodeterioration [19-24]. In this way, microbiological monitoring together with temperature and RH control are fundamental to guarantee a correct conservation of canvas paintings. While there are indeed well-established international agreements for the storage of cultural properties regarding temperature and RH developed by ICOM (International Council of Museums) together with UNESCO (United Nations Educational, Scientific and Cultural Organization) [25], there is no specific criteria for assessing air quality in quantitative terms regarding microorganisms. In this context, national and international standards [26, 27] related to public health may be used as a reference.

The aim of this research was to study the fungal community present in canvas paintings belonging to a representative Contemporary Basque Art Collection and to evaluate the risk of microbiological colonization before and after the storage facilities in which the collection is stored were conditioned. The collection we studied belongs to the Faustino Orbegozo Eizaguirre Foundation and gathers over 200 paintings by some of the most representative Basque painters of the 70s and 80s, which makes this collection a very significant part of the cultural heritage of the Basque Country [28]. Interestingly enough, the environmental conditions of the collection were not controlled until, as an outcome of this study, a refurbishment of the storage facilities took place, so the paintings were subjected to temperature and RH fluctuations characteristic of the oceanic climate in northern Spain for years. Therefore, this collection represents an excellent case study to evaluate the risk of biodegradation in canvas paintings and to carry out aerobiological studies in a site where cultural heritage objects are stored as a tool for monitoring microbial air quality. Indeed, due to inadequate storage conditions, the paintings showed different alterations, which could be attributed to microorganisms, such as surface-whitening areas and spots with macroscopic appearances similar to filamentous fungi. In order to study the biodegradative process, painting samples were analyzed by microscopic techniques, and surface sampling was carried out to quantify and identify the isolated fungal genera by using molecular techniques. Similarly, monthly air sampling was carried out inside and outside of the pavilion used to store the artworks. In this way, the effectiveness of the implemented treatments and measures was progressively evaluated.

Materials and Methods

Characteristics of the storage facilities

The research was carried out in the storage facilities of the private Contemporary Art Collection belonging to the Orbegozo Foundation located in Basque Country in northern Spain, from September 2022 to March 2024. The depot is an industrial pavilion made of a concrete structure but without any additional insulation. It consists of two floors; the lower floor is located at ground level and has one spacious room separated from the entrance area (Fig. 1A), while the upper floor is L-shaped and completely open (Fig. 1B). Paintings were mainly stored on floor 1 (Fig. 1D), although several of them were also in the separated room of floor 0 (Fig. 1C). During the study, from April to November 2023, the decision to remodel the room on floor 0 was taken, carrying out insulation and refurbishment works. The paintings were meticulously cleaned, indexed, and stored in the remodeled room. The room was equipped with a dehumidifier and a fixed datalogger to measure temperature and RH. Furthermore, a cleaning and maintenance plan was established (Figs. 1E and 1F).

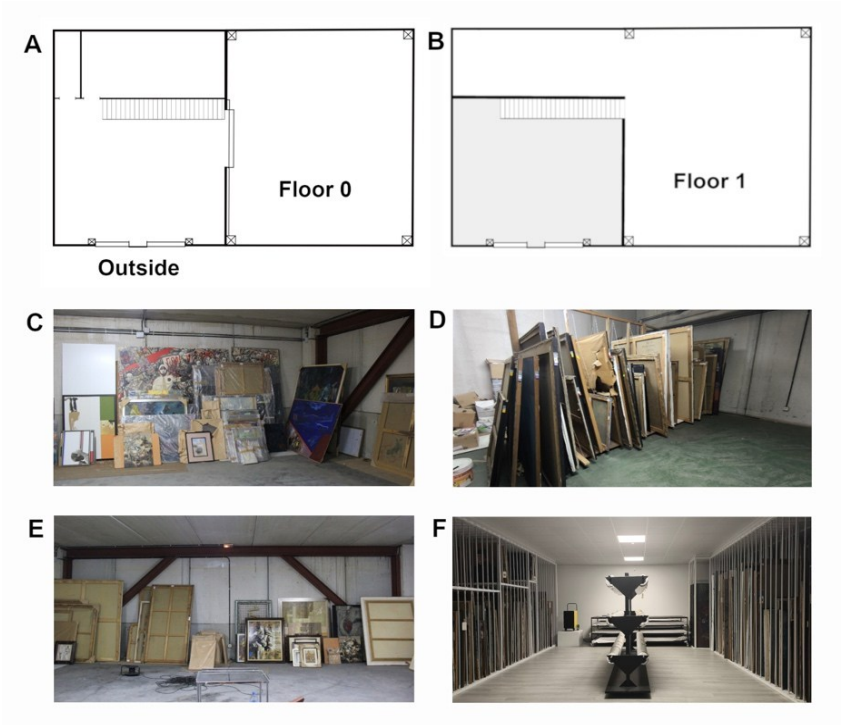

Fig. 1. Pavilion where the art collection is stored. (A-B) Plan of the pavilion; (C-D) The arrangement of the paintings in the pavilion: (C) floor 0, (D) floor 1; (E-F) the room of floor 0, (E) before and (F) after the refurbishment works

Air sampling and measurement of environmental factors

Microbiological contamination was determined by counting the number of fungal Colony-Forming Units per cubic meter (CFU/m^3) present in the air, and sampling was performed monthly between September 2022 and March 2024 (except for August) at different spaces of the pavilion, including the open air, so that three sampling points were selected: outdoor, floor 0, and floor 1 (Fig. 1A and 1B). At each sampling point, 100L samples of air were filtered in two Petri dishes (50L each) with Sabouraud agar (Condalab, Spain, Torrejón de Ardoz) at a 100L/min flow rate using portable equipment MAS-100 Eco® microbial air sampler. Sabouraud plates were incubated at 26°C for 72h.

Temperature and RH monitoring was carried out at the same points as those specified for the air sampling: outdoor, floor 0, and floor 1 (Figs. 1A and 1B). In each one of the two selected points inside the pavilion (floor 0 and floor 1), a continuous data logger was installed, an IAQ (Indoor Air Quality) Testo 174 H, which is an electronic thermohygrometer that records 1 data point/hour. Data was downloaded using ComSoft Professional software. For the outdoor environmental parameters, information available from the Regional Meteorology Agency, Euskalmet (https://opendata.euskadi.eus), was used.

Surface sampling of paintings and inert surfaces in the building

Five paintings that showed surface-whitening phenomena, initially associated with fungal activity, were selected for surface analysis (Fig. 2). From those paintings, three areas affected by the surface-whitening phenomena and three non-affected areas (controls) were selected to analyze CFU/cm^2. To carry out the sampling, a swab impregnated in PBS medium was rubbed on a 3×3cm^2 surface marked using a plastic frame. Then, the sample was seeded on a Sabouraud agar Petri dish and grown at 26ºC for 7 days. Furthermore, affected areas of the paintings were sampled again after being cleaned with a swab impregnated in pH-adjusted aqueous solution to evaluate the effectiveness of the treatment. Additionally, fifteen inert surfaces of the pavilion (extinguisher, entrance door, upper railing) were sampled to compare the amount of microorganisms on those surfaces with the ones obtained in the painting samples.

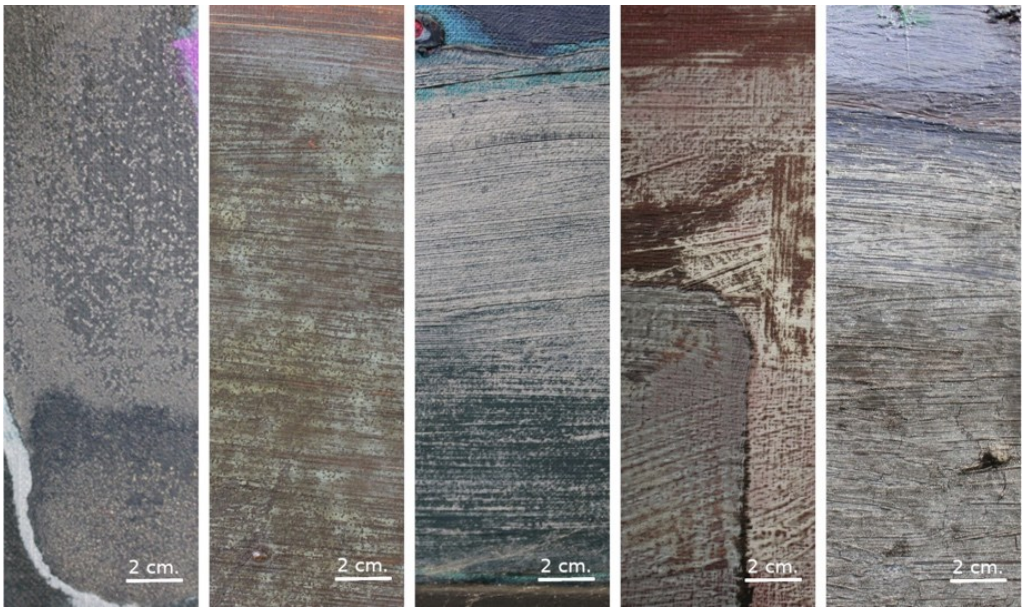

Fig. 2. Surface-whitening phenomena on several paintings.

On the other hand, three paintings with filamentous spots on the varnish layer that could be associated with colony-like structures (Fig. 3) were also selected for sampling and identification by molecular techniques. Thus, the filamentous spots were sampled precisely with the swab, which was entered in 5ml Sabouraud broth and incubated at 26ºC for 72h. After this period, 0.1ml of media was seeded onto a Sabouraud agar Petri dish and extended across the entire plate using a Digralski loop. Finally, Sabouraud plates were incubated at 26°C for 72h.

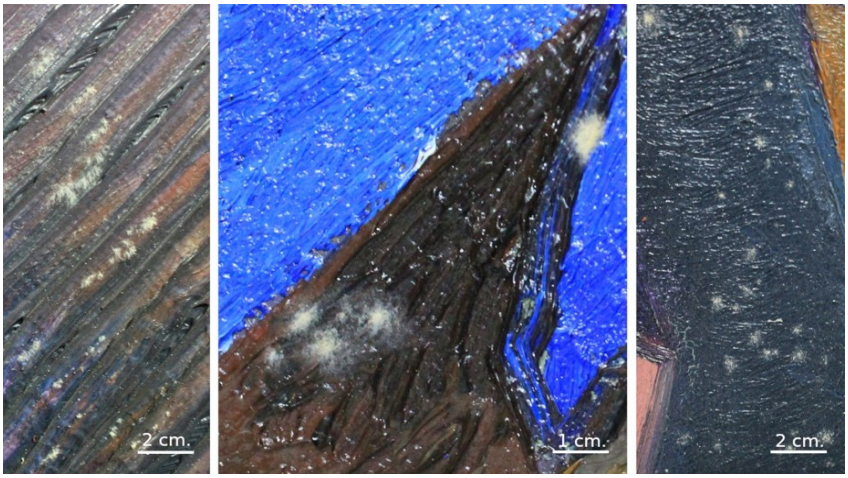

Fig. 3. Filamentous spots visible to the naked eye on different paintings

Fungal concentration and colony selection

After the incubation of Petri dishes, the number of CFU grown was counted, and its concentration in the air (m^3) and on the surfaces (m^2) was calculated. In addition, the diversity of colonies, that is, the number of different types of colonies, was also calculated in the Petri dishes corresponding to the air sampling from the outside of the pavilion and floor 1, where most of the paintings were stored. Thus, the Petri dishes corresponding to the sampling period immediately prior to the insulation works carried out on the pavilion were selected for this purpose. The different types of colonies of filamentous fungi were grouped based not only on their macroscopical characteristics (size, shape, pigmentation, relief, type of conidiophore or sporangiophore, etc.) but also on the microscopical characteristics (type of hyphae, spores, etc.) of the fungi observed with a magnifying glass and microscope. Following this classification, at least one colony of each group was selected, isolated on a new Sabouraud agar plate, and incubated at 26ºC for 7-10 days for molecular identification.

Molecular identification

Fungal DNA extraction was carried out using the method described by Hervás-Aguilar and collaborators [29]. Briefly, a sample of the fungus grown on a Sabouraud plate was obtained using an inoculation loop, and it was introduced into a 1.5mL tube containing 0.1mL lysis buffer (2% Triton X-100, 1% SDS, 100mM NaCl, 1.0mM EDTA, and 10mM Tris-HCl, pH 8). Then, 150mg of sterile 0.45mm glass beads were added to the tube, and it was vortexed for 30 seconds before incubating at 65ºC for 30 minutes. Every 10 minutes of incubation, the tube was vortexed for 30 seconds. After that, 0.1mL of a phenol/chloroform/isoamyl alcohol (25:24:1) solution was added, and the tube was vortexed for 5 minutes. Finally, the tube was centrifuged at 14,000rpm for 5 minutes, and the liquid phase was recovered and stored at -20ºC until use.

The concentration and the quality of the DNA extracted were verified by 1% agarose gel electrophoresis, using Gel Red Nucleic Acid Stain 10000X (Biotium, San Francisco, United States), at 90V for 60 minutes. The result was visualized using the gel documentation system Syngene U: Genius (Syngene, India, Bangalore).

To identify the colonies, a PCR was first developed to amplify the sequence between the Internal Transcribed Spacer 1 (ITS 1) and the ITS4 sequences using the primers ITS1: TCCGTAGGTGAACCTGCGG and ITS4: TCCTCCGCTTATTGATATGC [30, 31], obtaining a DNA product with a size between 350 and 880 base pairs.

Then, the PCR fragment obtained was verified by agarose gel as mentioned above and purified using the NZY Gelpure kit (Nzytech, UK, Cardiff). The amount of DNA and the quality

of the sample were checked using a NanoDrop Lite spectrophotometer (Thermo Scientific, Waltham, United States). Afterwards, the samples were sequenced by Eurofins Genomics (Ebersberg, Germany) using the Sanger method. Finally, the BLAST (Basic Local Alignment Search Tool) tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to compare sequences and to identify the genera or species.

Analysis of the penetration of the fungal colonies into the paintings

Three different techniques were used to visualize and analyze the fungal growth on the paintings. First, a Dino-Lite AM4113MT digital microscope (Dino-Lite Europe, Almere, The Netherlands) was used to directly observe the affected surfaces with potential fungal colonization. Secondly, a microsample from the painting containing the filamentous spots was extracted with a scalpel and deposited onto a glass slide with a drop of Calcofluor White Stain (Sigma Aldrich) to visualize the microscopic morphology of the fungus under the fluorescence microscope Nikon Eclipse Ni microscope (Nikon Corporation, Tokyo, Japan), and cross sections of those areas were analyzed by OM using a Nikon Eclipse Ci POL microscope in order to see whether the hyphae had entered into the painting.

Results

Microbiological air quality and environmental variables

The results extracted from the microbiological air sampling showed high levels of CFU/m^3 in the immediate surroundings of the pavilion (Fig. 4A), with an annual average around 800-900CFU/m^3 and without extreme seasonal variations. Spring was the season exhibiting a tendency to present lower values, and February and March gathered the lowest values (390 CFU/m^3 in both). From March onwards, the values rose progressively, remaining at values exceeding 1000CFU/m^3 until November, with autumn being the season with the highest values (1100-1200CFU/m^3), and not being until December when they fell below this peak and then decreased until they remained below 400CFU/m^3 during March.

The trend indoors was similar but showed slightly lower values. Before the refurbishment, both floor 1 and floor 0 presented high levels of CFU, with no significant differences between them and with an annual average between 600-800CFU/m^3. As occurred in the outdoor area, spring and winter were the seasons with a tendency to present lower values (340-600CFU/m^3), increasing in summer and autumn to values ranging between 800-1000 CFU/m^3. March was also the month that presented the lowest values (160-210CFU/m^3) which rose progressively with values that remained high (around 1000CFU/m^3) until November-December, months in which they fell again, remaining relatively stable and without large increases during all the cold months (Fig. 4A).

While the refurbishment works were being carried out, the fungal load in the air initially increased (April 2023), probably due to the movement of dirt and dust. Nevertheless, after the works were finished and the paintings cleaned and stored (November 2023), the fungal load decreased dramatically in floor 0, without following the trend of previous years. In fact, from December 2023 to March 2024, the number of colonies was around 200 CFU/m^3 on floor 0, while floor 1 continued with values similar to those recorded prior to the refurbishment works, close to 500CFU/m^3 (Fig. 4A).

Regarding the environmental conditions, the registered average annual outdoor temperature was about 15-16ºC. Inside the pavilion, temperatures were generally 4-5ºC higher, with annual average parameters of 18-19ºC (Fig. 4B). In terms of RH, the annual outdoor average was around 74-77%, with maximum peaks close to 100% practically every month of the year and minimum values between 16 and 37%. In the indoor area, the HR was generally 5% lower, with annual averages between 60% and 70% (Fig. 4C). In addition, the oscillations within a day were sharp and frequent, with RH fluctuations between 10% and 20% in less than 24 hours and even 40% in longer periods. The measurements taken after the refurbishment indicated a slight increase in the temperature of the conditioned room (floor 0), with parameters between 16 and 18ºC, and

a noticeable decrease and stabilization of RH, with an average of 55% and maximum fluctuations of 5% (Figs. 4B and 4C).

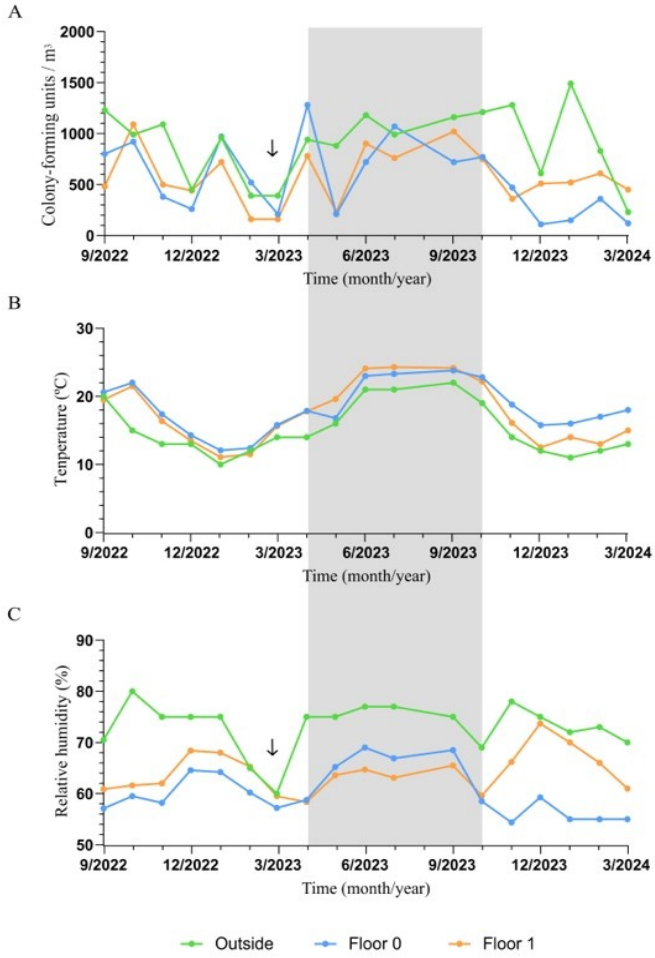

Fig. 4. Microbiological air sampling and environmental variables outside and inside the pavilion (the region in grey corresponds to the refurbishment period). (A) Number of CFU/m^3 in air per month in each sampling point; (B) Monthly average temperature in each sampling point; (C) Monthly average RH in each sampling point

Fungal genera species isolated in the air samples

The most representative genera were identified in the air samples from outside and from the floor 1. For this purpose, a macroscopic and microscopic analysis of the fungal colonies on the plates was carried out to group them according to the different types of colonies. One or two colonies of each group, with at least two colonies counted in a plate, were selected for molecular identification. Following these criteria, nine genera were identified outside the pavilion (Fig. 5A), while seven genera were identified on floor 1 (Fig. 5B), where the paintings were kept before the remodeling of the ground floor. Most of the colonies belonged to the genera *Cladosporium*, *Penicillium*, and *Aspergillus* in both sampling points. Other fungi identified included the genera *Mucor*, *Alternaria*, and *Epicoccum*, among others (Fig. 5).

Using the data from microbiological identification, the abundance of each fungal genus in the air was determined monthly. In general, the results in both sampling points were very similar,

with the most abundant genera, both outdoor (Fig. 6A) and on floor 1 (Fig. 6B), being *Cladosporium* and *Penicillium*.

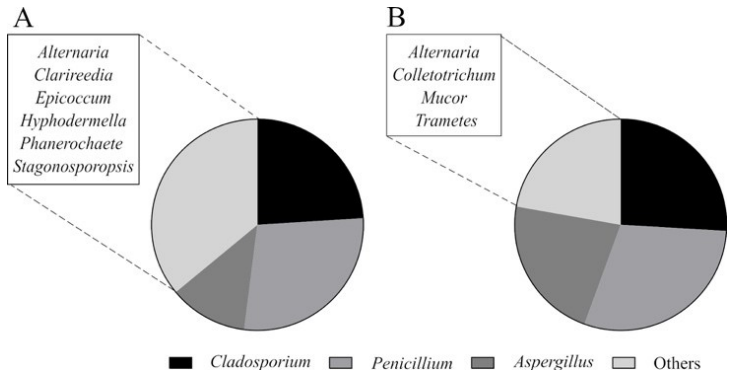

Fig. 5. Identification of fungal species isolated in air sampling:
(A) Classification and abundance of identified genera outdoors;
(B) Classification and abundance of identified genera on floor 1

Among them, the genus *Cladosporium* was the most prevalent genera, except in January and February outdoors and in December and April on floor 1. Regarding the genus *Aspergillus*, which is a very common opportunistic fungal pathogen, it was isolated in both sampling points but only during September, December, and April (Fig. 6).

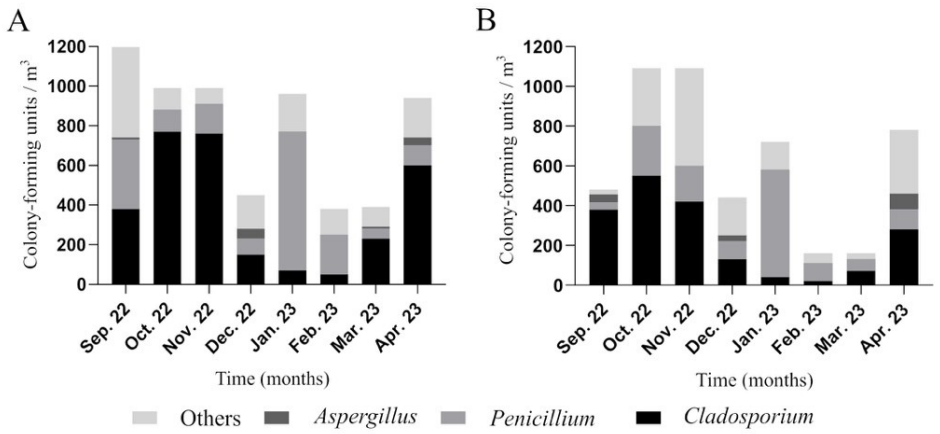

Fig. 6. (A) Outdoor monthly distribution of the genera; (B) Monthly distribution of the genera on floor 1

Study of fungal colonization on paintings

The results obtained from the CFU/m^2 count on the paintings surfaces, affected and non-affected by the surface-whitening phenomena, showed that there were no significant differences in the number of fungal colonies. Indeed, the number of colonies was relatively low in both cases. Furthermore, when analyzing the surfaces of other objects in the pavilion, it was observed that the number of CFU/cm^2 was significantly higher than on the surface of the paintings (Fig. 7A).

On the other hand, it was observed that after cleaning the affected areas of the paintings, the already low number of fungal colonies was further reduced and, in several cases, entirely eliminated (Fig. 7B).

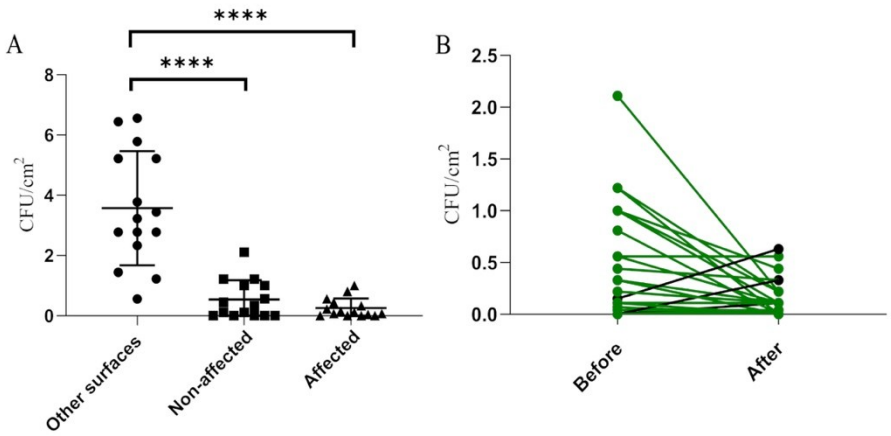

Fig. 7. Number of CFU/m^2 on different surfaces of objects and paintings. (A) Comparison of number of CFU/m^2 on other surfaces and on non-affected and affected areas of the paintings; (B) Number of CFU/m^2 in affected paintings before and after cleaning with a swab impregnated in pH-adjusted aqueous solution. Samples where the number of CFU/cm^2 was reduced or maintained after cleaning are marked in green

Regarding the filamentous spots, a total of five visible colonies were isolated from three different paintings, and molecular identification was carried out. The results showed that these fungi were *Penicillium*, *Alternaria*, *Aspergillus,* and *Peniophora*. Hence, these alterations can indeed be associated with fungal growth.

Analysis of the penetration of the fungal colonies into the paintings

The surface of the affected areas was studied with the digital microscope, and it was observed that the morphology of the filamentous spots (Fig. 8) looked clearly like fungi. This theory was confirmed when a microsample was studied with Calcofluor White Stain, and a fungal structure was noticed by fluorescence microscopy (Fig. 9A). In addition, as detailed above, four different genera were identified in the three paintings analyzed. However, when examining the cross section, no penetration of hyphae was seen, and, therefore, the fungal growth was merely superficial and did not seem to damage the painting (Fig. 9B).

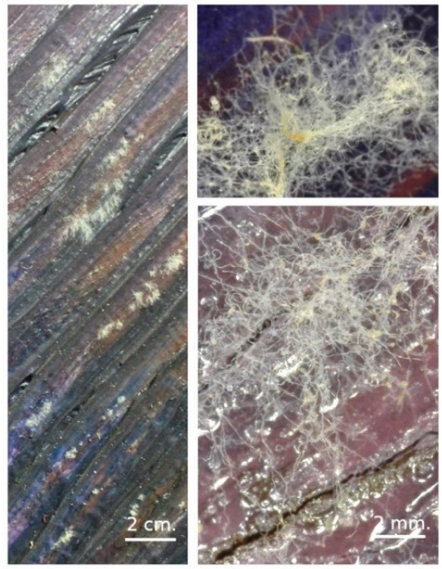

Fig. 8. Hyphal growth observed with the digital microscope: 200×

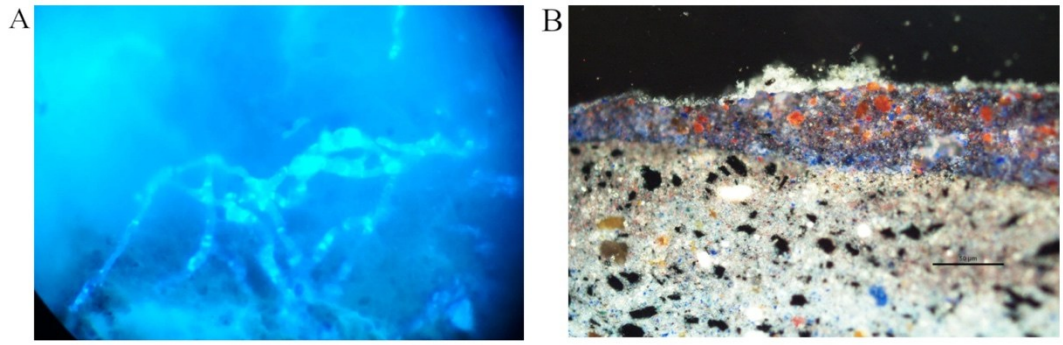

Fig. 9. Hyphal growth on the paintings. (A) A microsample extracted from the painting with filamentous spots observed after staining with Calcofluor White Stain under the fluorescence microscope: 400×. (B) Surface fungal growth observed under cross section with the optical microscope: 200×

In the surface-whitening phenomena areas we did not detect any significant amount of fungi, being comparable to the unaffected areas. In addition, the appearance observed under the digital microscope was totally different from the appearance of the spots and, in turn, very different from each other (Fig. 10). Moreover, no fungal structure with Calcofluor White Stain was found. In order to clarify the nature of these phenomena, we are carrying out a multi-analytical approach whose results indicate that the surface-whitening phenomena are related to other physico-chemical processes rather than to a biological origin.

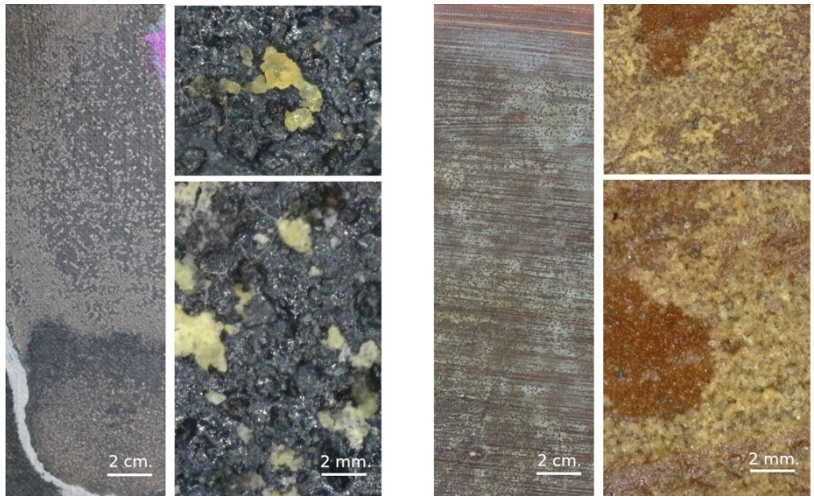

Fig. 10. Surface-whitening phenomena observed with the digital microscope: 200×

Discussion

In this study, the community of fungi present in the paintings belonging to the Orbegozo Foundation has been examined, and the potential risk they pose to the collection duly evaluated. In order to do that, the fungal load of the storage facilities has been analyzed before and after a specific room was set up for the better conservation of the paintings.

A monthly air sampling was carried out aimed at measuring the fungal load in the air, which indicated that the air quality inside the pavilion used to store the pictures was inadequate. In fact, the standard UNE 171330:2024 establishes that the number of fungal colonies must be

less than 200CFU/m^3 to consider air quality in microbiological terms to be acceptable and that 75% of the analyzed points must be below the limit values. Inside this building, however, more than 75% of the samples analyzed exceeded this value, and, in some months, the values reached 1000 and 1700CFU/m^3. Even if the standard used as reference is relative to public health and not specific to the storage of cultural heritage, it is clear that microbial air quality was inadequate. The abundance of fungi in the environment was a direct consequence of the conditions in which the pavilion was kept and the lack of isolation, maintenance, and proper cleaning.

After the remodeling of the floor 0 microbial air quality improved greatly thanks to the isolation and the control of environmental parameters, especially RH, which has a direct effect on fungal growth. This can be seen by the CFU/m^3 readings of the air falling below 200CFU/m^3, approaching the limit established by the UNE standard. In addition, the temperature and RH parameters were improved, remaining stable between 50-55% and with temperatures between 16-18ºC, within the recommended standards. Therefore, improving the isolation of the storage and regulating the climatic parameters can solve, or at least mitigate, the problems related to the protection of the stored collection and the maintenance of proper indoor air quality [32].

The lowest number of colonies was found coinciding with the months with the lowest humidity values and 2-3 months after recording the lowest temperatures of the year (Fig. 4). Furthermore, the highest density of fungal colonies in the air was reached between April and October. This result was expected for countries with temperate climates where the highest concentrations of fungal spores in the air are found from spring to autumn [33]. In fact, it is widely described that high temperatures and RH promote the growth of fungi [34-35]. Thus, the high temperatures, along with the inappropriate environmental conditions, could have further favored the growth of fungi.

In terms of the identified fungal microorganisms in the air, the three predominant genera observed in both external and internal environments were *Cladosporium*, *Penicillium,* and *Aspergillus*, which indicates that external fungal spores were surely entering the pavilion. Indeed, these genera are typically classified among the fungi most usually present in the air, alongside the *Alternaria* genus [36-38]. Those four genera are found on heritage objects and in the air worldwide [39-40]. However, in this study, *Alternaria* was only isolated in February and March, coinciding with the lowest temperatures of the year. Among the identified genera, the abundance of the *Cladosporium* genus throughout the year, particularly in the warmer months, can be attributed to its cosmopolitan nature, small size, and production of numerous conidia, facilitating its airborne dispersal. The presence of *Penicillium* was also abundant, owing also to its wide distribution and significant metabolic adaptability. The third genus, *Aspergillus*, was only isolated during three months. However, this scarcity may be due to the faster growth of other genera, which could have detrimental effects on its proliferation [41]. It is noteworthy that the four genera *Cladosporium*, *Penicillium*, *Aspergillus*, and *Alternaria* are associated with respiratory allergies as well as other diseases affecting the lungs and alveoli and can produce mycotoxins, which may cause fungal infections or chronic obstructive pulmonary diseases [42]. Therefore, it is important to control the quantity of spores in the air, not only to prevent biodegradation of the paint works but also because they could have health consequences for individuals.

In addition to the air sampling, the surfaces of several paintings and different inert surfaces in the building were examined before the remodeling of the pavilion. In general, the inert surfaces presented more CFU/cm^2 than the paintings. The lower counting of colonies on the paintings could probably be due to the fact that the surface of the paintings was not facing up, as they were stored vertically. Furthermore, the fact that the paintings are positioned adjacent to one another makes it more difficult for airborne fungus to settle.

Nevertheless, in some paintings, macroscopically visible colonies of filamentous fungi were observed and identified as *Alternaria*, *Aspergillus*, *Penicillium,* and *Peniophora*. In any case, the analyses performed on the paintings showed that the fungal colonization of the paintings was not causing serious damage at the moment, since the hyphae had not penetrated into the

paintings. However, in the long term the hyphae could have grown more and probably penetrated into the paintings, causing mechanical stress and physical damage (paint flaking and cracking), in addition to the action of the cellulolytic enzymes, which can dissolve fibers, stain the surface, or degrade the oil binders [43, 44].

Three of the genera identified in the paintings are the same as those identified in the air, *Alternaria*, *Aspergillus*, and *Penicillium*, confirming the hypothesis that most of the fungi present in the pavilion and on the paintings come from outside. However, a genus not present in the air, *Peniophora*, was also recognized, while the most common genus in the air, *Cladosporium*, was not. Consequently, in addition to airborne fungi coming from outside, it seems that a selection process could have happened in the paintings, likely caused by the materials used. This fact would be interesting to be further studied in the future because it is not a genre commonly found in works of art, and if detected, it is always in a minority presence [45, 46].

Regarding the correct conservation of the artworks, even if fungi present a real risk for the collection, the main cause of the surface-whitening phenomena seems to have a different source. In order to identify the nature of these phenomena, we are carrying out a parallel multi-analytical approach on cross sections, surface microsamples, and/or scrapings from both whitened and not whitened surfaces. The results obtained to date show that the whitening cannot be attributed to a single phenomenon but to different physicochemical processes, such as metal soaps, free fatty acids, and salts.

Conclusions

This research has managed to quantify, identify, and determine the origin of the microorganisms present in the storage room of an emblematic collection of contemporary Basque art belonging to the Orbegozo Foundation, highlighting the importance of controlling air quality in terms of levels of microbiological agents as a risk factor for the paintings, since these objects are sensitive to the attack of microorganisms. The measures implemented to correct the situation have also succeeded in stabilizing the temperature and RH and reducing the fungal load in the air.

Furthermore, the study demonstrates the critical role of environmental management — particularly in regulating relative humidity and ensuring proper insulation — in preventing fungal proliferation and preserving cultural heritage. The identification of dominant fungal genera such as *Cladosporium*, *Penicillium*, *Aspergillus*, and *Alternaria*, known for their allergenic and potentially pathogenic properties, underlines not only the biodeterioration risk but also the potential health implications for staff and visitors.

Additionally, the comparison of air samples and surface analyses suggests that, while airborne fungi constitute the primary source of contamination, the materials and orientation of artworks may influence the type and extent of fungal colonization. The detection of *Peniophora* exclusively on painted surfaces also opens new avenues for future research on material-specific microbial selection mechanisms in heritage environments.

Lastly, although the fungal colonization had not yet caused irreversible damage, this study reinforces the necessity for ongoing monitoring and the integration of preventive conservation protocols. It also stresses the value of a multi-analytical approach in distinguishing between microbiological deterioration and other surface phenomena, such as whitening, which may arise from distinct physicochemical processes.

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