Chapter

Technical - Experimental Methods Used in Artworks' Expertise

Verginica Schröder, Daniela Turcanu-Carutiu, Adina Honcea, Rodica-Mariana Ion, Sorin Grigore and Loreley-Dana Jianu

Abstract

The works of art analyzed in this research study are part of the Ovidius University Gallery collection that ensures the permanent visibility of the research results consisting in the experimental synergistic method as well as the innovative materials intended for restoration and conservation. We are sure that after the scientific investigation, the authentication and restoration of these works of art will increase their value. The synergistic methodology, to which we refer, can be defined as a sum of methods and procedures in the trans- and interdisciplinary field, which introduces the notion of "health" in the field of restoration artworks, changing the paradigm of approach as a whole, analyzing pigments, supports and all the specific painting materials. Nondestructive analytical procedures will be implemented to develop and optimize the conditions for identifying the individual types of biological impact present in works of art and of case studies on real samples. In the research activity, we used the different techniques to investigate and characterize traditional organic binders used in works of art, to see the effect on the consolidation and durability of materials, to test their functionality and usefulness while validating a viable laboratory model in relation to the natural system.

Keywords: works of art, synergistic method, biological impact, restoration, integrity

1. Introduction

The paintings submitted for scientific research in order to find the best conservation solutions are at an alarming stage of deterioration, some even in advanced stages. This study focuses on four works of art that each have specific problems different from one another, therefore requiring a differentiated treatment, personalized after an accurate diagnosis is given.

The works in the collection that are the object of the scientific investigation are the following: *Girl with the child*, painted by Vladimir Yegorovich Makovsky, belongs to the Russian School of painting from the nineteenth century; *A miracle of Jesus* attributed to author, Laurent de la Hyre, French School from the seventeenth century; *Adoration of the Shepherds* unknown author, French School from the eighteenth century; and *Shepherd boy with sheep in the forest of the castle*, attributed to author, Wendel Dietterlin, German School from the sixteenth century.

Masterpieces are currently of the Etta Ionescu Art Gallery of the Ovidius University of Constanta and belonged to Mr. Catalin Lazureanu, the last descendant of a large family of art collectors. The son lacking adequate space to keep in good

condition the works of art inherited from his mother Etta, who lost much of the property during the communist period, stored them in small improvised spaces, in boxes, in packages dosed in different spaces of an apartment, in Constanta city.

This study was intended, also, to find new hypotheses regarding authenticity of the paintings, attribute the artworks to the author or one to the school of painting.

For this purpose, were used studies of art history and museology, technical methods, such as: analytical documentation, photographing ultraviolet radiation in the dark room by auto exposure and auto-focus, analyses with Vilber Lourmat Quantum program, Image Master™ technology.

Fluorescence of natural products is an interesting characteristic of many components with application in most fields of biology [1] and the methods of art diagnosis such as the analysis and studies in expertise and conservation [2–4].

Through this UV photography analysis technique, the conservators can carry out identifications and evaluations related to the particularity of pigments [4], the integrity of pigment at surface layers.

You can also assess the age differences of the painted surfaces and identify the restoration areas. This analysis shows that based on the contrast intensity, it is possible to implement an automatic segmentation of the UV image of a retouched painting [5].

The UV radiation interacts with the apparent layer, and the technique has proven to be a nondestructive technique for first evaluation and diagnostic expertise of artworks. UV fluorescence can indeed be used for a preliminary identification of pigments. Some of them are easily distinguished, for example the titanium and zinc white pigments can be recognized with UVR because they display a strong UV absorbance band [6]. In addition, the method is recognized as a useful tool in *in situ* analysis of recently restored wall paintings [7].

Also, this fluorescent property of natural pigments starts a new trend in contemporary art such as the fluorescent painting or the conservation problems of these works [8].

The optical and fluorescence microscopy techniques were used for evaluation of microstructural aspects of painting canvas and the state of degradation and biological activity on the painted surfaces with implications for choosing the conservation method [9].

Also, a combined strategy including the air biological contamination and the isolation of microorganisms from the works of art surfaces were evaluated to determine their biodeterioration potential on canvas and painting pigments.

The biodegradation of oil paintings is a complex process involving multiple phenomena and dynamic interactions between microorganisms and the substrate. Visible phenomena include: the pigmentation of surfaces, the degradation of colors, the penetration into the material, and the modification of its resistance.

Microorganisms induce successive colonization of painted surfaces. These phenomena are accompanied by the presence of specific metabolism compounds (organic or inorganic acids), or enzymes from the extracellular matrix [9], synthesized according to the type of substrate (lipases, esterases, proteases, cellulases, etc.). The isolation and identification of microorganisms, especially of the fungi type, that have cellulolytic action responsible for biodegradation of works of art with natural substrate are of great importance in understanding their degradation [10].

As a result, the details regarding the communities of microorganisms associated with a certain type of painted substrate are of major importance before beginning the process of manipulation (transport, relocation) or restoration.

Another necessary aspect for a good evaluation and intervention in stopping the degradation process and counteracting its effects is the identification of the abiotic-microclimate environment conditions that favor the installation and development of the microorganisms responsible for biodegradation.

2. The evaluation of works of art in order to identify their biodegradation state

2.1 Description of the studied artworks

Vladimir Yegorovich Makovsky was an important Russian painter. His paintings depict a lyrical, idyllic image of the characters in the compositional scenes, *Girl with the child* (**Figure 1**) being an example in this sense, painted by the artist at the age of 45.

Figure 1.
Title of artwork, Girl with the child*, author Vladimir Yegorovich Makovsky (1846-1920), Signed B. Маковский, dated 1891, oil/canvas, size 70 x 115 cm (cod of painting P17).*

V. E. Makovsky was born in Moscow to the family of a famous artist, one of the founders of the Moscow School of Music, and Eduard I. Makovsky. Ever since childhood, the boy (and his brother, later also a famous painter Konstantin E. Makovsky) had been surrounded by an artistic atmosphere, constantly seeing famous masters visiting his father's house, hearing their arguments, and talking about art, he was imbued with thoughts about his high purpose and, therefore, very early on he felt his calling [11].

Makovsky took the first painting lessons from V. A. Tropinin and at 15, under his leadership, he painted the painting *A Boy Who Sells Kvass* (1861). In 1861–1866, Makovsky studied at Moscow School of painting, sculpture and architecture, where he received good professional training under the guidance of artists E. S. Sorokin and S. K. Zaryanko.

The painter is very famous for his portraits of beautiful young girls and scenes from the rural area, which he masterfully painted, in an almost impressionistic style, with a rather high-speed brush, in visible coughs.

The painting is that of a very young girl dressed in simple clothes from the country and with a diaper on her head, with smooth skin, with red cheeks, and with bare feet on dry and dusty bread. The little girl holds a child in her arms, who is supposed to be her brother. The tones are warm, the scene is very beautiful representing a moment of a hot summer day. To suggest this, the painter used the colors red, white, and yellow ocher in contrast to the very dark green, almost black, of the tree in the background.

Nevertheless, the work of art has been kept in better condition than the other works of the collection, considering that it is 129 years old.

The painting surface shows cuts, cracks, erasures, and losses of pigments in some places. The work is painted in oil on canvas, large dimensions 70 × 115 cm, and is signed В. Маковский with brown on the lower left.

Instrumental methods used here and in all the cases that will follow were: photographing ultraviolet radiation (254 nm, 365 nm) in the dark room by auto exposure and auto-focus, analyzed with Vilber Lourmat Quantum program, Image Master™ technology.

Results from the photograph in the ultraviolet light analysis not only showed that the signature and the paint layer are original (**Figure 2**), but also showed clearly the places of some restoration interventions. The darker areas show the places where made interventions of restoration (**Figure 3**).

During this study, it was found that the work belongs to В. Маковский and not to К. Е. Маковский as initially believed.

Figure 2.
The ultraviolet light analysis for the signature В. Маковский.

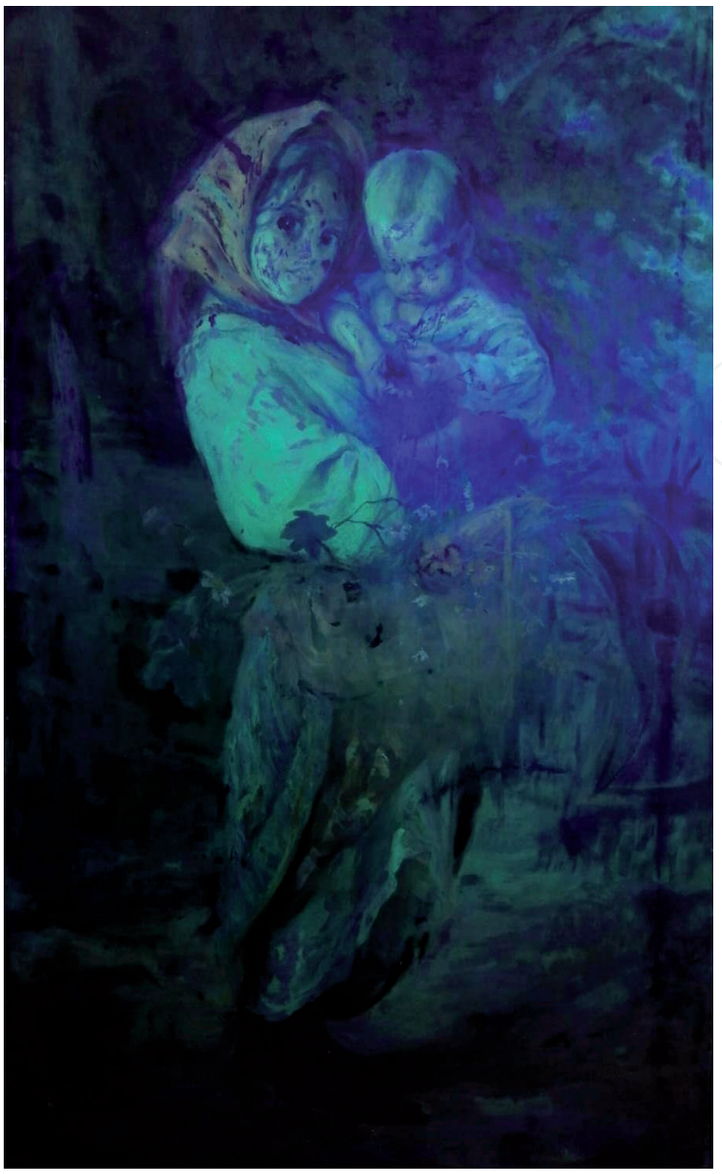

Figure 3.
The ultraviolet light analysis to the painting Girl with the child.

The subject in the painting entitled A *miracle of Jesus* represents the healing of Peter's mother-in-law recorded in Holy Scripture (**Figure 4**).

The painting surface shows cracks, erasures, and losses of pigments in some places. The work is painted in oil on canvas, large dimensions 86.5 × 72 cm, and is signed L. de Hyre with dark brown in the center down.

The author is the French painter Laurent de Hyre (27.02.1606–28.12.1656), born in Paris, belonging to the early French Baroque. The painter was influenced by Italian painting through the artworks of Italian painters who came to Paris, especially Primaticcio's works. For this reason, his painting was initially attributed to the Italian style, erroneously, when the signature was not known. During the in-depth study with the optical equipment and analysis with ultraviolet light, the signature was discovered, which with the naked eye is not visible, due to the age of the painting surface. It is very interesting that although he loved Italian painting, there are no witnesses to prove that the artist was in Italy, ever [12].

The composition presents the moment when Jesus after speaking in the Synagogue in Capernaum goes to the house of Peter where on a bed the woman was lying with a high fever. Jesus touches her hand reducing her to a new life, she rises, blesses Him, and begins to serve those around her. The figures are well outlined

Figure 4.
Title of artwork, A miracle of Jesus*, attributed to Laurent de la Hyre, French School, around 1635, oil/canvas, size 86.5 x 72 cm (cod of painting P10).*

in a clear-dark compositional space that induces dramatic mystery with a strong emotional impact and an energetic contrast between the brightly lit areas and others shaded in shades with predominantly brown chromatic tones of a pathetic religious interest. The influence of the Italian painting can be seen in the lively color and the delicacy with which the characters build, being unique in the French painting from the Baroque period culminating with Simon Vuet. He formed his own style from an early age, emphasizing the beauty of the colors but also their symbolism within the chromatic composition.

In the photos in the ultraviolet light are observed dark traces of restoration, and clear signature (**Figure 5**).

Hyre's paintings are in the Louvre Museum in Paris, Strasbourg, Ruen, and Le Mars. In 1635 he painted the painting of Saint Peter healing the sick, which is

Figure 5.
The ultraviolet light analysis for the signature L de la Hyre.

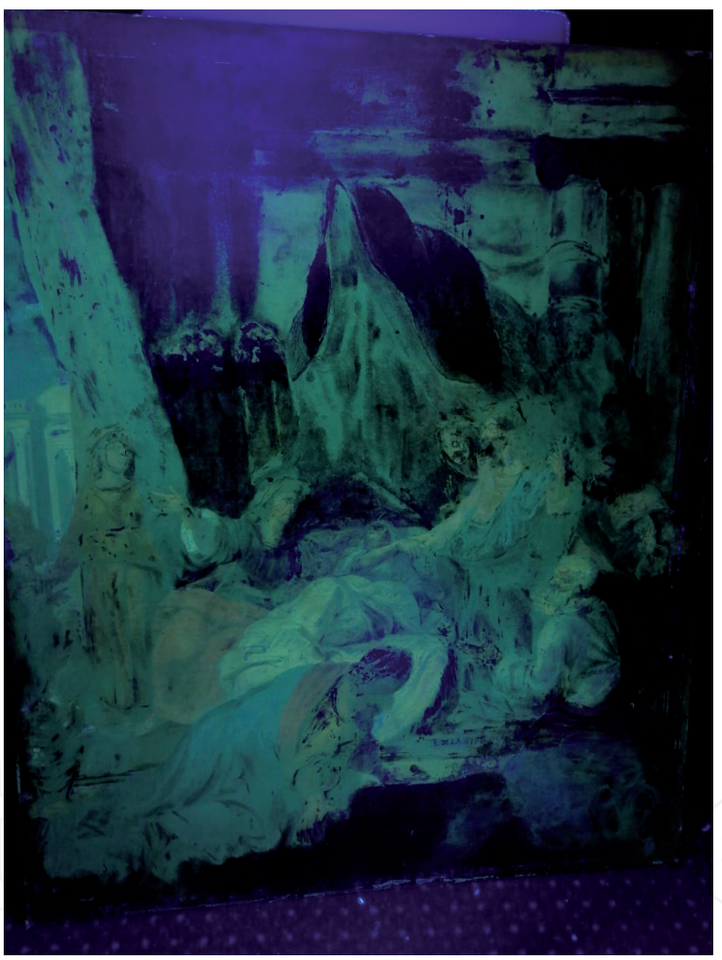

Figure 6.
The ultraviolet light analysis to the painting A miracle of Jesus.

displayed at the Louvre, Hyre having a preference for the religious theme, in the center of his attention is Saints Peter and Paul, the story being important in his compositions. In 1648, La Hyre became a founding member of the French Royal Academy of Painting and Sculpture, being elected one of the 12 elders who had management positions in the Academy [13].

The religious composition *Adoration of the Shepherds* (**Figure 7**) has as characters figures inspired by daily life: Holy Virgin, pastors and other characters, which makes us consider that the painting was painted at the eighteenth century, compared to the artwork *A miracle of Jesus* (**Figure 6**) whose Baroque characters are inspired by Rome and Ancient Greece, attributed to the seventeenth century, the early period.

Figure 7.
Title of artwork, Adoration of the Shepherds*, unknown author, French School, around 1770, oil/canvas, size 98.5 x 76 cm (cod of painting P13).*

The painting belongs stylistically to the French Academy in Rome, where most of the painters who painted between 1750 and 1800 Jean Baptiste Marie Pierre and his disciples Louis Durameau or Etienne de Vallee Poussin although the French borrowed the Italian way given a common feature that distinguishes them from Italian painters, in the mannerisms with which they approach the compositions representing the "Adoration of the shepherds," the angels hold a drapery that writes Vervum Caro Factum Est, disposed in the same way above the Virgin Mary, keeping reminiscent of the Rococo French manner. The French Academy in Rome offered to young French artists, by a scholarship, the opportunity to see and copy the masterpieces of antiquity or the Renaissance with which they returned to Paris [14].

Approaching the Italian style meant that the initial expertise was mistaken as belonging to the Italian school, especially since the work has no signature. The optical analyses with ultraviolet light showed the restoration areas (**Figure 8**).

The painting titled *Shepherd boy with sheep in the forest of the castle,* attributed to Wendel Dietterlin*,* presents an idyllic scene of a young shepherd with a bag at his neck, who sits on a stone, at the bank of a river, with a stick in his hand (**Figure 9**). The river divided into two parts the composition of the landscape at sunset. At the distance are the sheep on the other side of the river, over a dense forest and at the top of a hill is a castle. This poetic configuration shows us an idyllic scene with a shepherd resting near the sheep on the riverbank, under the castle on the coast.

Wendel Dietterlin (1550–1599) worked in Strasbourg although he was born in Pfullendorf in Wüttenberg into a family of artists, called Grapp, whose children were still painters. To be distinguished from one of the children bearing the same name he is known as Wendel Dietterlin the Elder. Most of his paintings have been lost, randomly found in different private houses or at auction houses. Few of his painting are clearly signed with both names, a clear example would be "Lazar's Resurrection," dated 1587. He is known more as a designer, engraver, and author of the Treaty of Ornament in Architecture. In Pfullendorf is the castle of Sigmaringen dating from the eleventh century and which served as an inspiration in his artistic works [15].

In some of his engravings, Wendel Dietterlin signs with Wendelinus, who is found in the work *Shepherd with sheep* in the castle forest. The WENDELINUS

Figure 8.
The ultraviolet light analysis to the painting Adoration of the Shepherds.

Figure 9.
Title of artwork, Shepherd boy with sheep in the forest of the castle*, attributed to Wendel Dietterlin, German School, around 1580, oil/canvas, 59 x 47 cm (cod of painting P7).*

signature is placed under the foot of one of the subjects and follows a low form, in the dark area of the painting, which makes it difficult to see (**Figure 10**). For this reason, at the initial expertise the signature was not observed, and the landscape was wrongly attributed to the Italian school, which was prolific in landscapes with castle as genre in the sixteenth century as the Flemish. The work, executed in a mannerist style, was studied with optical magnifying devices and subjected to the analysis of the whole surface in ultraviolet light where the signature and the restoration areas were noted. The work has been attributed to the author following the results of the investigations so far (**Figure 11**).

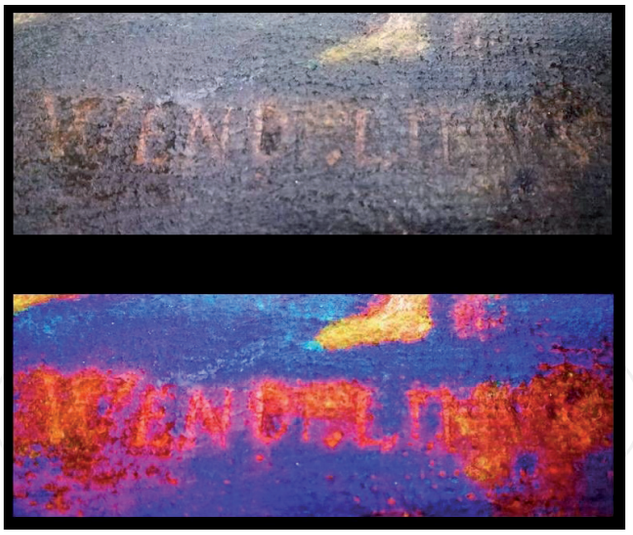

Figure 10.
The ultraviolet light analysis for the signature WENDELINUS.

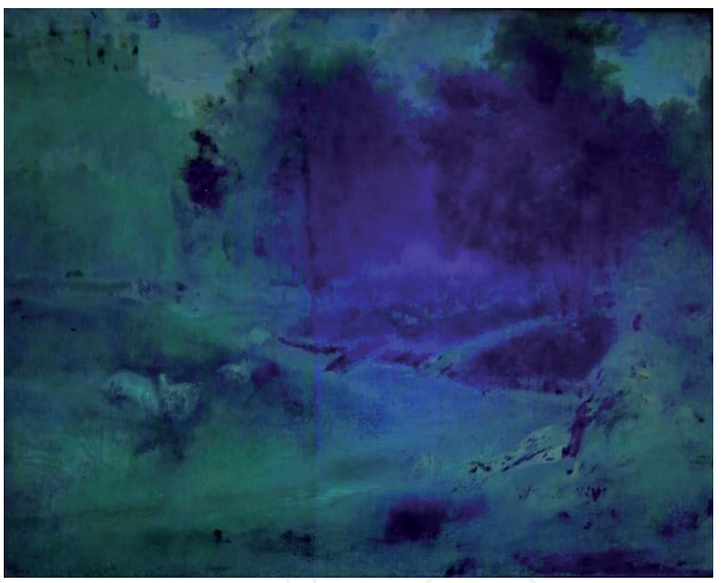

Figure 11.
The ultraviolet light analysis to the painting Shepherd boy with sheep in the forest of the castle.

All these artworks, which represent main topics for scientific analysis, present conservation interventions made by unauthorized persons. Due to those amateur actions, we can see deformations of the painting support, of the canvas, and traces of varnishes applied unevenly on the painting surface that represent the principal cause of the damage. These bad human interventions led to irreversible chemical phenomena that attacked the original pigments and to the loss of the initial color brightness. Being original paintings, they also keep untouched places that can be the subject of scientific study, without being affected by previous restorations. These virgin surfaces represent a special advantage for dating and authentication. Luckily, these works were painted with very good pigments on natural fiber, linen, or hemp. These superior materials used had the quality of helping the paintings to have a longer life. The processes that have started over time are of a chemical and biological nature. This study aims to find a modern solution to stop harmful actions and repair as much as possible what has been degraded.

2.2 The state of degradation and biological activity on the painted surfaces

For the maintenance and conservation of the collection of paintings from the Ovidius University Gallery, a scheme of periodic evaluation of the conditions regarding the microbial load was designed in order to establish the initial decontamination and maintenance measures by air conditioning.

The expertise consists in applying some analysis techniques that include the following stages:

1. Storage of artworks in the quarantine space
2. Evaluation of airborne microbial impact
3. Evaluation of the state of degradation/integrity of the support materials (canvas, wooden frames)
4. Examination of biological presence/activity on the painted surfaces

2.2.1 Analysis techniques and sampling

For the analysis, four old paintings have been selected, described in Section 2.1, with areas showing modifications of the varnish layer and the color layer.

For each painting, five points were identified for sampling, four of which were on the painted side and the fifth was on the material surface of the canvas (the back side of the painting), see **Table 1**. The sampling was performed using noninvasive techniques, by delicate abrasion using a sterile cotton swab, on a surface, then removing this to a Petri dish with the culture medium.

In order to identify if there is a source of contamination from the airborne microorganisms' culture media, plates were exposed in the areas of interest for 15 minutes (Ae 1, Ae 2, Ae 3, Ae 4 samplings).

As a medium of bacteria isolation, Glucose Nutrient Agar, Columbia Blood Agar were used, and Sabouraud Chloramphenicol Agar were used for microfungal colonies isolation.

The samples were kept at 24–25°C in Benckmark My Temp digital incubator, and colonies growth was evaluated after 3–5 days. For identifications, colonies morphological details and gram stains were used. Also, automat instrument ViteK 2 BioMerieux was used for automat identification and antibiotic susceptibility testing.

The number of colonies forming unit (CFU) from air, by sedimentary methods on Petri dishes was according to EN ISO 1469-1 [16], and Pasquarella methods [17, 18] was adapted using the formula to Dorohoi, 2000 [19].

$$\text{Microbial CFU/m}^3 \text{ in the air (IMA)} = n \times 10^4/S \cdot z$$

where n is the colonies number, S is the plate surface area (cm^2), and z is the time coefficient.

The light and epifluorescence microscopy techniques were used for morphological details identification.

Samples for biological analyses for the evaluation of canvas structures and painted surfaces characteristics were collected from a surface of 16 cm^2 using a sterile cotton swab, from each painting, and 5-mm^2 canvas samples were also taken and analyzed under a stereomicroscope and epifluorescence microscope.

The assessment of the microbiological contamination of the canvas was done by isolation on simple agar culture plates.

No.	Samples (biological analyses cods)	Features
1.	P17 Rd 1	Red pigment
2.	P17 Wh 2	White pigment
3.	P17 Bk 3	Black pigment near the wooden frame (bottom right)
4.	P17 Bk 4	Black pigment near the wooden frame (top right)
5.	P17 Tx 5	At the back of the canvas
6.	P10 Rd. 1	Scarlet pigment
7.	P10 Wh 2	White-gray pigment
8.	P10 Bk 3	Black pigment—near the wooden frame (bottom right)
9.	P10 Bk 4	Black pigment near the wooden frame (top left)
10.	P10 Tx 5	At the back of the canvas
11.	P13 Rd 1	Red pigment
12.	P13 Wh 2	White-gray pigment
13.	P13 Bk 3	Matte black pigment
14.	P13 Br 4	Dark brown pigment
15.	P13 Tx 5	The back of the canvas
16.	P7 Wh 1	White, light blue pigment
17.	P7 Bu 2	Blue pigment
18.	P7 Gr 3	Green pigment with traces of depigmentation
19.	P7 Bk 4	Black pigment
20.	P7 Tx 5	The back of the canvas
21.	Ae 1	Sample 1, centrally located in small room (1-m distance from painting pieces)
22.	Ae 2	Sample 2, exhibition, large room (0.5- to 1-m distance from painting pieces)
23.	Ae 3	Sample 3, exhibition hall, centrally located on the tourist route
24.	Ae 4	Sample 4, small room, near the wall area (1-m distance)

Table 1.
Codification of biological samples and the characteristics of the sampling surfaces.

2.2.2 Indoor airborne microbial exposure assessment

The air microbiota is influenced by a number of factors: altitude, season, human agglomerated, degree of ventilation, temperature, and relative humidity.

Indoor airborne microbial quantifications are a significant parameter to evaluated healthcare-associated infections. Also, the microbial monitoring accounted for microclimate quality and helped to identify critical situations that require corrective intervention [20]. The level of temperature and relative humidity are favorable conditions for interspecific changes and to the vector spread [19, 21].

Bacterial and fungal contamination was identified at all airborne microorganism's assessment points. The most diverse contamination was found in the small room where the paintings in the collection were stored for evaluation. The large number of bacterial colonies belonging to the same species of gram + bacilli was found in sample Ae3, (**Figure 12**), the colonies having invaded the entire surface of the culture plate.

2.2.3 The assessment of the support material (canvas) of the paintings

The study assesses the quality of the initial materials from which the fabrics were made, the arrangement and the quality of the working technique (fabric, treatment/canvas preparation).

The degree of degradation, the vulnerability of the material resistance depending on the thickness and the quality of the microfilaments, and the sensitization by the biological action were noted.

After the microscopic examination of the fibers, it was found that the fabrics contain fibers of different sizes in their structure (**Figures 13a–d**).

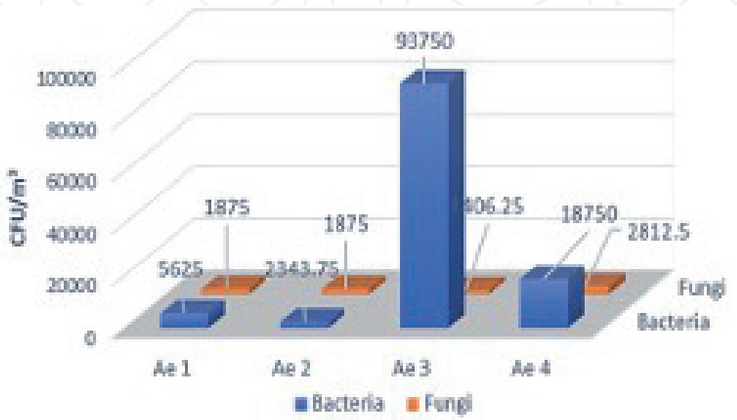

Figure 12.
Microbial isolated stains after 15 minutes exposure of cultures plates to airborne microorganisms in the exhibition area.

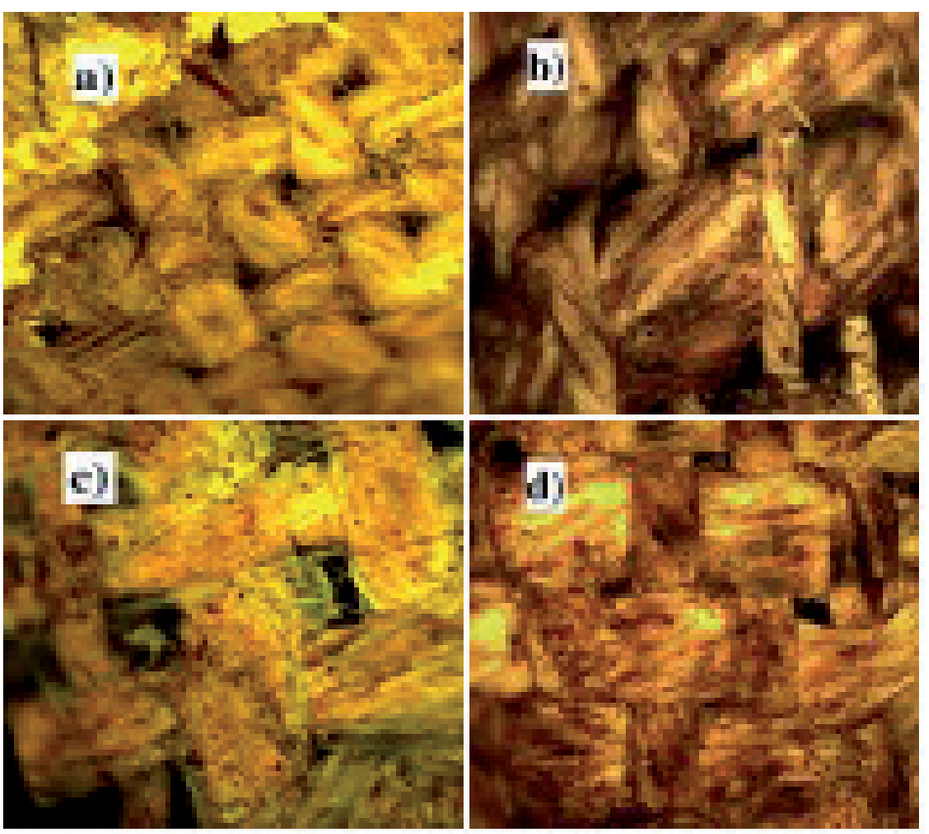

Figure 13.
Fabric images: a) P17, b) P10, c) P13, d) P7; epifluorescence microscopic examination (x 400 magnification).

Samples	General aspects of painting canvas	Count of isolated microorganisms
P 17 Tx 5	Compact fabric, without inter-nodular spaces; uniformly arranged, fine, equal thickness, rounded filaments	21 bacterial colonies (3 species)
P 10 Tx 5	Uneven fabric with inter-nodular spaces and irregularities; filaments of variable thicknesses and shapes, with lamellar appearance	14 bacterial colonies (4 species)
P 13 Tx 5	Large mesh fabric, uneven; coarse filaments with knots	6 bacterial colonies (4 species)
P 7 Tx 5	Fabric with filaments of different thickness, wrinkled, with degraded appearance	2 fungal colonies

Table 2.
Micro-structural details of fabrics correlated with the level of microbiological contamination.

Particular aspects such as knotted filaments, and the wrinkled or degraded appearance of the textile micro-structures/fibers, have been observed (**Table 2**).

Microbiological contamination is dissimilar as most bacterial colonies (21 and 14, respectively) were isolated from canvas surfaces P17 Tx 5 and P10 Tx 5, including hemolytic forms. Microbiological contamination with bacteria is more limited in P13 Tx 5. Also, in P7-Tx5, fungal strains with very fast growth capacity such as filamentous ascomycetes were isolated (**Figure 13** and **Table 2**).

The variances that appear regarding the identified colonies are due to the fact that, in such situations, the purity of the fibers, the primer level of animal origin, and the organic or inorganic pigments used influence the process of contamination.

Cellulose filaments with higher purity compared to those with lignin content are more vulnerable to bacteria, a fact confirmed by the larger number of colonies isolated from P17 Tx 5 compared to the other samples.

2.2.4 Painted surfaces

The assessment of the state of the painted areas in relation to biodegradation took into account the identification by means of the technique of isolation of the microorganisms by culturing techniques and the comparison of the manner in which the areas of the canvas colored with different pigments were contaminated. Thus, the analyzed surfaces were colored with red, white, light blue, green, and black brown pigment. The working hypothesis is the possibility of a difference of contamination correlated with the origin/structure/chemical component of the analyzed pigment. The presence of heavy metals in certain pigments could ensure a resistance of the painted layer [22].

Also, the microorganisms present on the painted surface were assessed by comparison with those isolated from the air microflora in order to determine if this microflora is the main source of contamination or if these paintings have acquired particularities regarding the biodegradation activity due to the substrate or the previous conservation environment.

The identification of the types of microorganisms would allow to establish the methods of decontamination taking into account the following aspects:

the chemical composition of the oils used;
species that produce biodegradation;
the response of species of microorganisms to biocides;
growth speed.

The results of the assessment of contamination of painted objects reveal the following aspects:

- In the large exhibition space (Ae 3), there are some differences between the isolated strains of the air and those isolated from the painted surfaces, a single species of the genus *Aspergillus* having been identified on the painted surfaces as well.

- The direct exchange between the environment microflora and the painting microflora was evident through the analysis of the air a few centimeters away from the painted surface (Ae 2) where in the environmental sample the same fungal species were found (*Aspergillus*, *Penicillium*, *Ascomycetes*) and one very common species of bacteria from the group *Aeromonas* sp. was present on the wall painting as well.

  The microflora samples collected from the small precinct (Ae 1, Ae 4), with a varied number of paintings with different types of substrates, highlight a large number of common species in the air samples with those found on the painted surfaces that were analyzed.

- The bacterial forms dominate the painted samples as regards the number of colonies as well as the number of species: *Aeromonas* sp., *Corynebacterium* sp., *Micrococcus* sp., and *Bacillus* sp. The species of the genus *Bacillus* present in our samples are indicated in the literature as predominant, competing species of other bacterial types, frequently isolated from oil painted surfaces. Some *Bacillus* strains show a high level of tolerance to the oils used in the paintings [22].

- The presence of bacteria on the painted surfaces indicates significant differences between the areas with pigments of different paintings as well as from one surface to another on the same painting or the same pigment (**Figures 14a–d**).

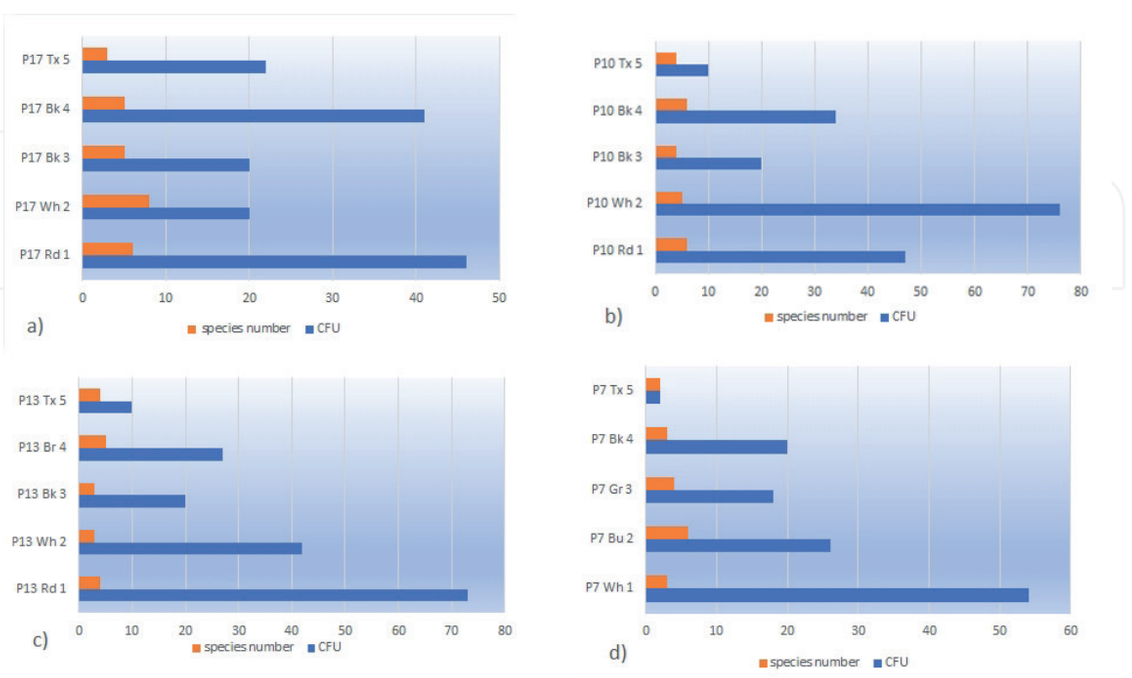

Figure 14.
Microbial diversity and the comparative number of colonies (CFU/cm²) and species isolated on the painting surfaces with different pigment substrates; Rd (red), Wh (white), Bk (black/brown), Gr (green), Bu (blue) and the convas (Tx 5) samples; a) P17 painting, b) P10 painting, c) P13painting, d) P7 painting.

The identification of bacteria on the surfaces of the analyzed paintings, as the dominant forms and the absence of these in the air samples, denotes the presence of organic materials on the surface of the paintings, which maintain bacterial activity. Most likely, this microbial load came from the previous storage of the art collection in noncompliant spaces and from a layer of dust that favored these deposits.

Our observations allow us to ascertain that the exchanges between the painted works and the aeroflora can be favored by the position of the painting in relation to the areas with high tourist traffic, in relation to the distance from the floor and to the variations of the conditions of temperature and humidity.

Depositing the paintings in a crowded manner in small enclosures with insufficient ventilation, without air conditioning, favors contamination between paintings; thus 8 bacterial and 3 fungal species were identified as being shared among the analyzed paintings, out of a total of 14 isolated morphotypes.

The identified fungal strains include *Penicillium* sp. and *Aspergillus* sp., which are known in the literature [23] as factors in the deterioration of the layers painted with oil or of the areas of the paintings treated with adhesives. The growth of these hyphae on the support material (canvas) on which the painting is applied favors the friability and the loss of the painted layers.

Bacteria associated with painted surfaces can form a characteristic biofilm depending on the age of the painting and the working technique (the nature and condition of the painted surface, the degree of maintenance, etc.).

The smaller number of species identified on the canvas (back side) compared to painted surfaces allows important valuations regarding the state of prior preservation of the analyzed paintings.

The presence of colored spores and the production of specific metabolites can produce pigmentation induced by organic acids, which generate major changes in the painted structure, which cannot be repaired.

3. Conclusions

The instrumental and experimental methods of analyzing the works of art belonging to the sixteenth, seventeenth, eighteenth, and nineteenth centuries, to different schools of painting namely Russian, German, and French, purchased from the art collector Catalin Lazureanu, through ARHEOCONS project and recently exhibited in the "Etta Ionescu" Art Gallery of the Ovidius University of Constanta, hosted by the National Military Museum "King Ferdinand I" in Constanta, have been used to establish the following aspects: attribution to the school or the author, the age of the paintings, the preservation mode, the quality and condition of the painted or support materials, the effects of biological contamination, and the severity of biodegradation.

The original provenance and the previous conservation mode have put their mark on the works of art. Identified species, *Aeromonas* sp., *Corynebacterium* sp., and *Micrococcus* sp., as well as the identification of hemolytic forms suggest contact with the dusty spaces of the deposits. The dust particles can not only act physically or chemically but also be sources of infection of the works of art with fungal spores, bacteria, and insect eggs. Microorganisms can reach the art objects, colonizing them, as they have as source of food the organic particles that enter the components of dust and, are favorable for development in an atmosphere with relatively high humidity.

The study allowed new hypotheses on the authenticity of the paintings, to attribute the works to the author or to the school of painting, to identify the

environmental factors, from the exhibition space that can create the risk conditions for the analyzed paintings. The results can be associated with the intervention methods for expertise as well as those related to conservation and/or restoration.

Following the study, it can be seen that all paintings require special conditions for conservation. The degradation process can be stopped by measures correlated with limiting the microbiological loading of the air and the painted or supporting components, repositioning the paintings in the exhibition areas without risk of contamination, cleaning the paintings, and restoring the damaged areas.

The study was approached according to clinical health methods analyses, results, personalized treatment, and principles that the ARHEOCONS project follows regarding the cultural heritage expertise.

Acknowledgements

This work was supported by a grant of the Romanian Ministry of Research and Innovation, CCCDI—UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0476/51-PCCDI/2018, within PNCDI III, ACRONIM: ARHEOCONS.

Conflict of interest

The authors declare no conflict of interest.

Thanks

Special thanks for consulting to Mr. Costel Coroban.

Author details

Verginica Schröder[1*], Daniela Turcanu-Carutiu[2], Adina Honcea[3], Rodica-Mariana Ion[4,5], Sorin Grigore[6] and Loreley-Dana Jianu[1]

1 Department of Cellular and Molecular Biology, Ovidius University, Constanta, Romania

2 Faculty of Arts, Center of Artworks Expertise by Advanced Instrumental Methods (CEOAMIA), Institute of Science, Culture and Spirituality, Ovidius University, Constanta, Romania

3 Department of Microbiology and Immunology, Ovidius University, Constanta, Romania

4 ICECHIM, Group of Evaluation and Conservation of Cultural Heritage, Bucharest, Romania

5 Materials Engineering Department, Valahia University, Târgoviște, Romania

6 Synevo Laboratory, Microbiology Department, Constanta, Romania

*Address all correspondence to: verginica.schroder@univ-ovidius.ro; virgischroder@yahoo.com

References

[1] Duval R, Duplais C. Fluorescent natural products as probes and tracers in biology. Natural Product Reports. 2017;**34**:161-193. DOI: 10.1039/C6NP00111D

[2] Isacco E, Darrah J. The ultraviolet-infrared method of analysis, a scientific approach to the study of Indian miniatures. Artibus Asiae. 1993;**53**(3-4):470-491. DOI: 10.2307/3250528

[3] René de la Rie E. Fluorescence of paint and varnish layers (part I). Studies in Conservation. 1982;**27**(1):1-7. DOI: 10.1179/sic.1982.27.1.1

[4] Comelli D, Nevin A, Brambilla A, Osticioli I, Valentini G, Toniolo L, et al. On the discovery of an unusual luminescent pigment in van Gogh's painting, les Bretonnes et le pardon de Pont Aven. Applied Physics A. 2012;**106**(1):25-34. DOI: 10.1007/s00339-011-6665-9

[5] Cosentino A. Practical notes on ultraviolet technical photography for art examination. Conservar Património. 2015;**2**:53-62. DOI: 10.14568/cp2015006

[6] Cosentino A. FORS spectral database of historical pigments in different binders. e-Conservation Journal. 2014;**2**:57-68. Available from: http://e-conservation.org/issue-2/36-FORS-spectral-database

[7] Comelli D, Valentini G, Nevin A, Farina A, Toniolo L, Cubeddu R. A portable UV-fluorescence multispectral imaging system for the analysis of painted surfaces. The Review of Scientific Instruments. 2008;**79**(8):086112. DOI: 10.1063/1.2969257

[8] De Winter S. Conservation problems with paintings containing fluorescent layers of paint. In: CeROArt (online). EGG 1. Micheroux, Belgique; 2010. p. 1659. DOI: 10.4000/ceroart.1659

[9] López-Miras MM, Martín-Sánchez I, Yebra-Rodríguez Á, Romero-Noguera J, Bolívar-Galiano F, et al. Contribution of the microbial communities detected on an oil painting on canvas to its biodeterioration. PLoS One. 2013;**8**(11):e80198. DOI: 10.1371/journal.pone.0080198

[10] Coronado-Ruiz C, Avendaño R, Escudero-Leyva E, et al. Two new cellulolytic fungal species isolated from a 19th-century art collection. Scientific Reports. 2018;**8**(1):7492. DOI: 10.1038/s41598-018-24934-7

[11] Fiala V. Russian Painting of the 18th and 19th Centuries, Translated by Jean Layton. Prague: Artia; 1981. p. 18

[12] von Sandrart J. In: Dietterlin W, editor, StockauAcademia nobilissimæ artis pictoriæ. Albertina, Wien, Österreich; 1583. pp. 302-304

[13] Rosenberg P, Thuillier J. Laurent de La Hyre 1606-1656. Man, and Work (Exhibition Catalog 1989-1990 Museums in Grenoble, Rennes and Bordeaux). Geneva: Skira; 1988. p. 18

[14] Michel C. Charles - Nicolas Cochin et l'art des Lumières, École française de Rome; 1993. pp. 547-615. (Contains Pierre's letter on the causes of decadence in the art of France)

[15] Lesur N, Aaron O. Jean-Baptiste Marie Pierre 1714-1789. In: Premier peintre du roi. Paris: Arthena; 2009. pp. 10-15-450-500

[16] EN ISO 14698–1. Cleanrooms and Associated Controlled Environments—Biocontamination Control Part 1: General Principles and Methods. 2003

[17] Pasquarella C, Pitzurra O, Savino A. The index of microbial air

contamination. The Journal of Hospital Infection. 2000;**46**(4):241-256. DOI: 10.1053/jhin.2000.0820

[18] Pasquarella C, Balocco C, Pasquariello G, Petrone G, Saccani E, Manotti P, et al. A multidisciplinary approach to the study of cultural heritage environments: Experience at the Palatina Lybrary in Parma. Science of the Total Environment. 2015;**536**:557-567. DOI: 10.1016/j.scitotenv.2015.07.105-0048-9697

[19] Dorohoi D-O, Melniciuc PN, Nicolescu C. In: Vasiliana, editor. Tehnici de investigare a obiectelor de Patrimoniu. Iasi; 2000. p. 210

[20] Napoli C, Marcotrigiano V, Montagna MT. Air sampling procedures to evaluate microbial contamination: A comparison between active and passive methods in operating theatres. BMC Public Health. 2012;**12**:594. DOI: 10.1186/1471-2458-12-594

[21] Cambrea SC, Petcu LC, Iliescu DM. Relations of environmental factors and evolution of Boutonneuse fever in the county of Constanta – Romania. Journal of Environmental Protection and Ecology. 2018;**19**(2):914-922

[22] Phulpoto H, Qazi MA, Mangi S, Ahmed S, Kanhar NA. Biodegradation of oil-based paint by *Bacillus* species monocultures isolated from the paint warehouses. International Journal of Environmental Science and Technology. 2016;**13**:125-134. DOI: 10.1007/s13762-015-0851-9

[23] Tiano P. Biodegradation of cultural heritage: Decay mechanisms and control methods. In: Proceedings of the ARIADNE 9. Historic Materials and Their Diagnostics. Institute of Theoretical and Applied Mechanics of the Academy of Sciences of the Czech Republic Prague; 4-10 February 2002. p. 1-37. Available from: http://www.itam.cas.cz/ARCCHIP/w09/w09_tiano.pdf