

Análisis sobre el crecimiento de hongos en diferentes revestimientos aplicados a sistemas ligeros

Analysis of fungal growth on different coatings applied to lightweight systems

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Abstract

*Walls are essential for buildings because they delimitate surroundings while influencing durability. Regarding the development of buildings, lightweight systems have emerged, which usually have internal closures of gypsum boards with several coating options. The south region of Brazil has subtropical climate, which promotes the development of fungi, which harm human health in buildings. Therefore, a test was performed according to ASTM D3273-16, for the periods of 4 and 10 weeks, to assess the resistance to growth of fungi on gypsum boards with coatings of acrylic paint, epoxy paint, smooth speckle, textured speckle and waterproof polymer. Results showed more severe deterioration of the samples coated with acrylic paint, and at 10 weeks the worst case reached a grade below 4 according to the classification of the standard and provided the development of the *Aspergillus* and *Aureobasidium* fungi, whereas the epoxy paint sample favored the growth of genera *Penicillium* and *Aspergillus*. The tests also showed significant differences in mold damage for the periods of 4 weeks (recommended by the standard) and 10 weeks (timespan of this study).*

Keywords: Fungi, biological evaluation, durability coating, lightweight walls

Resumen

Las paredes son esenciales, porque delimitan el entorno e influyen en la durabilidad de los edificios. Actualmente, han surgido sistemas ligeros que tienen cierres internos de planchas de yeso con diferentes revestimientos. La región sur de Brasil tiene un clima subtropical, que promueve el desarrollo de hongos que dañan la salud humana. Por lo cual, se realizó una prueba según la norma ASTM D3273-16, durante los periodos de 4 y 10 semanas, evaluando la resistencia al crecimiento de hongos en planchas de yeso con pintura acrílica, pintura epóxica, masilla lisa, masilla texturizada y polímeros impermeabilizantes. Los resultados mostraron un deterioro más severo en las muestras recubiertas con pintura acrílica y, a las 10 semanas, el peor caso alcanzó una calificación inferior a 4 (clasificación de la norma) y mostró el desarrollo de los hongos *Aspergillus* y *Aureobasidium*, mientras que la muestra de pintura epóxica favoreció el crecimiento de los géneros *Penicillium* y *Aspergillus*. Las pruebas mostraron diferencias significativas en el daño por moho a las 4 semanas (recomendado por la norma) y a las 10 semanas (período de este estudio).

Palabras clave: Hongos, evaluación biológica, revestimiento de durabilidad, paredes ligeras

1. Introduction

Biodeterioration is caused by the presence of microorganisms such as bacteria and fungi and becomes apparent as materials undergo modifications. It is divided in four classes (Sterfliger; Piñar, 2013; Alsopp; Seal; Gaylard, 2004):

- I. Physical or mechanical biodeterioration: the organism does not feed on the material, although the pressure caused by its movement or growth causes the substrate to break.*
- II. Aesthetic biodeterioration: only metabolic organisms or products are present, which do not damage the material but limit its acceptability.*
- III. Chemical assimilatory biodeterioration: the microorganism uses the material as a source of energy or food.*

- IV. Chemical dissimilatory biodeterioration: there can be excretion of waste products that disfigure or damage materials and result in chemical damages.*

Microorganisms are essential for biodeterioration and on the production of food and medicine, although many fungi species are pathogenic, impairing health and the economy while contributing to pollution of indoor air and diseases (Ghosal; Macher; Ahmed, 2012). Tham et al. (2017) mention that fungi that grow on trees, plants and grass bring about outdoor fungal spores, whereas indoor fungal spores relate to the moisture that lies on furniture. Simon-Nobbe et al. (2008) state that several diseases result from inhalation, ingestion or contact with fungal spores, such as allergies, allergic bronchopulmonary mycoses, sinusitis and allergic asthma. Due to their ability to colonize the human body, they can do even more damage to the immunologic system than pollen or other sources. According to Sharpe et al. (2016), the increasing exposure to indoor moisture arising out of fungal contamination is a worldwide public health problem that

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increases the risk of allergic diseases, afflicting next to a third of the European population.

The interaction between airborne fungi and the human body had been ignored for a long time (CABRAL, 2010). Yet, the acknowledgment of the sick building syndrome (SBS) in the 1970s and its correlation with high concentrations of specific indoor fungi brought back up the study of indoor fungi. SBS occurs when the building users show symptoms related to exposure to chemicals, particles or biologic material (APA, 2009). According to Al-Hunaiti et al. (2017), household dust carries a wide range of biological contaminants, toxic for humans.

Johansson, Ekstrand-Tobin and Bok (2014) argue that the interaction between environmental factors, material properties and fungi characteristics are determinants for their growth on buildings, considering the amount of water in the material as the most important element. If the environmental conditions are favorable to mold, there is a risk it might develop on the materials in spite of the critical level of moisture and temperature of each specific material, aside from the fact that different treatments can affect the fungi growth (Johansson; Svensson and Ekstrand-Tobin, 2013).

Fungal growth can occur directly on concrete, paints, and some construction materials can even arrive pre-contaminated with fungi (Adams et al. 2016). Moisture, ventilation and temperature are among the main factors that relate to indoor air pollution and fungal development (Stanković; Nikolić; Arandjelović, 2011). The area that undergoes fungal damage on construction materials varies with respect to temperature and the construction material (Andersen et al. 2011, Ghosal; Macher, 2012). The intensity of impact on the microbiome depends on the building's occupation levels, the movement of air and on the occupants themselves (Adams et al. 2016). The biological contaminants come mainly from outdoor air, anthropogenic sources and construction materials (Awad et al. 2018). Even with the diversity of studies related to fungal growth and building materials, the results are still not easily answered due to the wide variety of materials and other interference factors (Giuseppe, 2013).

Regarding the damages to which buildings are susceptible, there may be repercussion on construction materials deriving out of climatic changes that occur during their use (Grynning et al., 2017), added to urban pollution, low quality of construction materials and problems of design and execution. Considering such damages, most buildings tend to present high levels of degradation and premature aging, which can negatively affect building attributes such as safety, aesthetics and durability (Possan; Demoliner, 2013).

Evaluating the exposure of buildings, the intensity of the source of outdoor air inside of ventilated buildings varies according to the type of ventilation. The ventilation rate affects the relative contribution of outdoor air, in a manner that rooms with natural ventilation or open windows present microbial profiles similar to outdoor air and less influence from other sources (Adams et al. 2016). These factors add up to architectonic design and can be lessened regarding to control of moisture and ventilation of buildings, air respiration and thermal treatment techniques (Singh; Yu; Kim, 2011).

Johansson, Svensson and Ekstrand-Tobin (2013) affirm that fungi can survive periods and conditions that are unfavorable to their growth. Yet, conditions are hardly constant along time in a building, as temperature and humidity vary and may favor the fungi growth.

In buildings, walls acts as compartmentalization, being related to safety and performance (Ibem et al, 2013; Thomsen, 2014). Predominantly, paints are applied over interior and exterior walls as surface finishing due to their significant influence on durability, aesthetically protecting and valuing buildings (Chai et al, 2011).

Bashir and Hafeez (2016) concluded that fungal grown on the superficial surface of painted area is a warning that there is sufficient organic material on the walls, that can harm human health. Also, the authors pointed out that the paint quality should be and moisture repellent.

Hoang et al. (2010) state that the susceptibility to fungal growth on materials like Drywall and Light Steel Frame has not been fully understood yet. Guerra (2017) points out that hardboards, plasterboards and cardboards tend to be prone to the proliferation of microorganisms.

Bach and Rangel (2005) affirm that, for containing several nutrients in their composition, paints undergo biodeterioration from growth of microbial colonies in both wet and dry states, which alters their functions. As per Parjo et al. (2015), the fungi *Aspergillus niger*, *Stachybotrys*, *Cladosporium* are often found on covering boards, besides *Aureobasidium*, *Alternaria* and *Penicillium*, which can be found on dry paint films. Shirakawa et al. (2002) noticed fungal growth on the interface between layers of paint and on the interface between paint and substrate.

Mensah-Attipoe et al. (2016) stress that the fungal grown visible on construction materials is caused by prolonged moisture on their surfaces and that the increase of fungal biomass brings about the difference of antifungal resistance. Johansson, Svensson and Ekstrand-Tobin (2013) concluded that fungal growth is induced when the combination of temperature and humidity exceeds the growth limit curves calculated. To guarantee fungi development, moisture content must be elevated, into 94-96%, and temperature could variate between 10 and 40°C (ASTM, 2016)

According to Andersen et al. (2011), is important to identify the fungus present in a moldy building. The antifungal resistance analysis is guided by ASTM D3273-16 (ASTM, 2016) through an accelerated test. The identification of fungi usually takes place by observing their morphology from spores captured or after cultivation. These methods contemplate approximately 90 species considered common and important indoors (Adams et al., 2013). Luo et al (2018) emphasize the needed to evaluate how coatings and surface treatment can affect the fungi growth and survivor.

Considering the presented scenario, this article assessed, comparatively, standard gypsum boards with 5 coating types through the accelerated test by ASTM D3273-16 (ASTM, 2016) and microscopic and morphological complementary analyses.

2. Method

2.1 Characterization of Systems

Samples of standard gypsum boards were arranged for testing, which measured 75 x 100mm and were tested in accordance with ASTM D3273-16 (ASTM, 2016), with five coating types:

- a) Water-based matte acrylic paint: three coats of water-based matte acrylic paint were applied over

the gypsum boards with a soft bristle brush, with intervals of 24 hours between each coat. This paint was white-colored with 80% water dilution.

- b) Smooth spackle: three coats of the product were applied with a plastic spatula, with intervals of 24 hours between coats.
- c) Textured spackle: three coats of the product were applied with a texture foam paint roller, with intervals of 24 hours between coats
- d) Water-based epoxy paint: two coats with 10% water dilution were applied with a short pile wool paint roller under a 24-hour interval between coats.
- e) Flexible waterproofing polymer: an asphaltic product was applied, which was diluted in water for priming. When its wet-dry state was reached (after approximate 30 minutes), five cross-coats of the waterproofing solution (product mixed with sieved CP-IV Portland cement in a 1:1 relation) were applied with a brush, with intervals of 90 minutes between coatings.

The application procedures and materials followed the specifications of its manufacturers.

2.2 Test for Resistance to Fungal Growth

The test for fungal growth resistance was based on ASTM D3273-16 (ASTM, 2016). The test cabinet comprises an isolated lid to minimize heat loss and the bars that support the apparatus are polymeric to avoid contamination. Under these bars there is a tray for storing the soil, which has a metallic mesh at the bottom. The operation counted with an interface of temperature control that granted isothermal conditions within the limits determined by the standard. Lastly, its base has a polypropylene tank. The components are described as follows:

- a) Soil: suitable for the propagation of plants, with 25% peat moss and pH range (5.5-7.0.)
- b) Fungi: presented in Table 1.

Table 1. Fungi used in the test

Fungus	Code
<i>Aureobasidium pullulans</i>	ATCC 9348
<i>Aspergillus niger</i>	ATCC 6275
<i>Penicillium sp.</i>	ATCC 9849

Fuente: ASTM D3273-16 (2016)

- c) Test panels: among the types the standard allows, type II was selected minding panels of gypsum boards of 75 x 100 mm, with thickness ranging from 13 to 25 mm.

The sequence adopted was to deposit the soil with water presence, incubate the fungi for 14 days and then proceed with the morphological analysis to assess fungal growth. The test procedure undertook the following sequence:

- a) The samples were arranged and conditioned at temperature of $23 \pm 2^\circ\text{C}$ and humidity of $50 \pm 5\%$ for four days before being deposited in the test cabinet.
- b) Exposure: the samples were suspended vertically 75 mm above the surface of the soil deposited to allow free circulation of air, as depicted in Figure 1.





Figure 1. Samples hanged over the test chamber

c) *Evaluation: The fungal growth samples was performed every week along ten weeks through a scale of rating that estimates the surface's percentage of defacement ranging from 0 to 10, where 10 means no visual defacement and 0 total defacement. This scale is set by subtracting the percentage of tainted area from 100%. The procedures for measuring the damaged area were based on Pacheco (2016), with treatment by ArcMap 10.3 software program.*

d) *The evaluation of the damaged area relied on the use the ArcMap 10.3 software suite for analysis of images and georeferencing. The identification of various colored spots in the image allowed to distinguish the regions affected by fungi and was rendered by the aforementioned software suite, as depicted in Figure 2.*

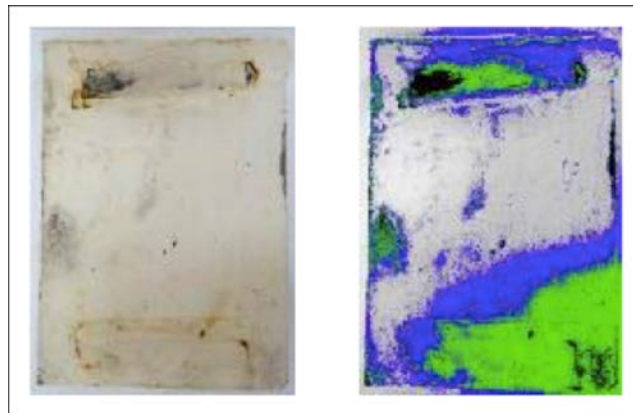


Figure 2. Damaged sample and image analyzed by the ArcMap 10.3 software to identify damaged areas

e) *Next, the area damaged by fungal growth was measured by the AutoCAD® software*

depth of field, high-resolution images, adjustable angles between -45° and $+45^\circ$, and resolution of $1 \mu\text{m}$. The samples analyzed were the most deteriorated to the naked eye.

2.3 Microscopic Analysis

The microscopic analysis was performed after nine weeks of testing. The assessment was performed with the automated digital microscope model Smart Zoom 5, ZEISS, with magnifications ranging from 10x to 1010x, extended

2.4 Identification of Fungi in Culture

Two samples from each coating group were chosen based on the probability of fungi presence by spots visible to the naked eye. After, the material with fungi was scraped and

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cultured in petri plates on potato dextrose agar (PDA) medium (Figure 3a, b) at 28 °C for 5 days.

The fungal strains were identified based on their reproductive structures already known and documented in specialized bibliography. Thus, a small fraction of these

structures was treated with KOH 3M, stained with phloxine and covered with glass cover slip (Figure 3c).

After, the material was visualized under the microscope ZEISS Primo Star with the AxioCam ERc 5S connected, and the ZEN imaging software was set to the objective lens of 40x, generating a magnification of 400x.

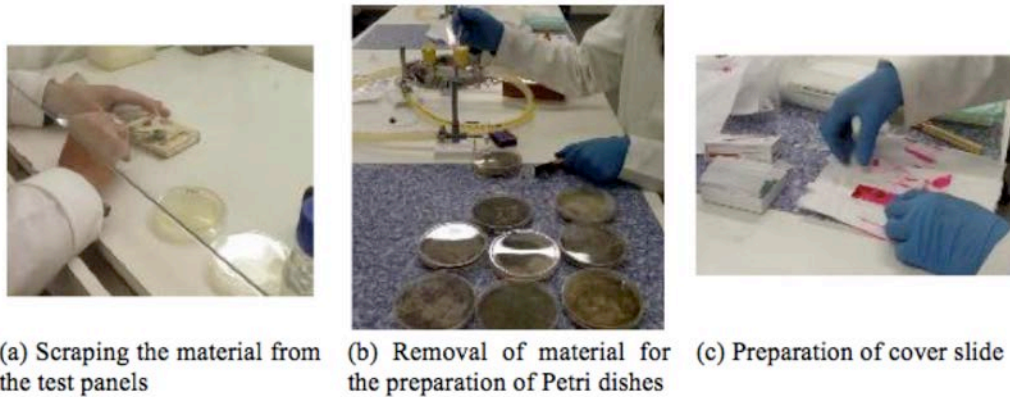


Figure 3. Morphological analysis procedures

The fungi developed reproductive structures after staying in the oven for 5 days, allowing the determination of the microorganism species. For that reason, plates were prepared with a small fraction of the material that was removed with a previously-flamed platinum inoculation loop and mixed to KOH 3M and phloxine coloring to assist the visualization.

This recognition was achieved through the comparison of reproductive structures found in the samples with structures already known and documented in bibliography. The microscope used for this recognition was the ZEISS Primo Star as described.

3. Presentation and analysis of results

3.1 Visual analysis and software handling

The action of the fungi on the gypsum board for 4 and 10 weeks revealed not only visual defacement but also physical defacement from the dislocation of the cardboard within the gypsum board and cracking on some coverings. The use of metallic staples caused runoffs and rust stains on the edges of the samples, as well as on the lateral of one of the acrylic paint samples. Some of the images presented differences of reflections and shadows due to the local illumination, which were excluded from the measurements.

There were different ratings of degradation on the samples, as noted in Table 2, wherein 10 is the best and 0 is the worst situation, according to above mentioned standard. During the 4 week only the smooth spackle and textured spackle samples had shown signs of defacement, whereas during the 10 week only the waterproofing polymer and epoxy paint samples had not undergone apparent damages from the attack of these microorganisms.

Table 2. Rating of samples (higher degree of staining)

Sample	Rating for 4 weeks	Rating for 10 weeks
Acrylic paint	No modifications due to fungi	3.44
Smooth spackle	8.65	5.26
Textured spackle	9.34	4.50
Epoxy paint	No modifications due to fungi	
Waterproofing polymer	No modifications due to fungi	

To emphasize visual aspect modifications, Figure 4 presents the initial aspect measured during the 4 week and

the final aspect during the 10 week for the most visually damaged samples.


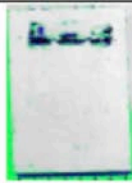

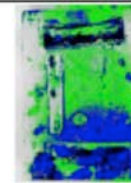



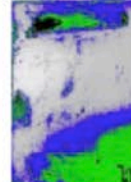



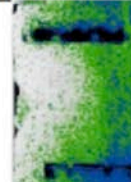








Analysis for 4 weeks - aspect	Image generated by the software	Analysis for 10 weeks - aspect	Image generated by the software
			
Acrylic paint – No significant modifications on account of fungal attack.		Acrylic paint – 65.57% of damaged area. Rating scale of 3.44.	
			
Smooth speckle – 13.35% of damaged area. Rating scale of 8.85.		Smooth speckle – 47.36% of damaged area. Rating scale of 5.26.	
			
Textured speckle – 6.63% of damaged area. Rating scale of 9.34.		Textured speckle – 55.02% of damaged area. Rating scale of 4.50.	
			
Epoxy paint – No significant modifications on account of fungal attack.		Epoxy paint – No significant modifications on account of fungal attack.	
			
Waterproofing polymer – No significant modifications on account of fungal attack.		Waterproofing polymer – No significant modifications on account of fungal attack.	

Figure 4. Analysis for 4 and 10 weeks of the samples with varied coverings

These results denote that the resistance to growth of fungi was higher for samples covered with waterproofing polymer and epoxy paint, since these coverings mitigate the superficial accumulation of water. The possibility of development of these microorganisms on these samples should not be discarded though, as it can still occur, albeit slowly. Regarding the analysis by the rating scale of standard, only smooth speckle sample 03 achieved rating below 9 during the 4 week, while only textured speckle sample 04

achieved rating above 8 during the 10-week, bearing in mind that two thirds of the samples did not reach rating 6.

Silva (2011) realizes that the boards that were exposed to a natural atmosphere had gotten contaminated by several fungi, among which appeared the ones used in this study. The author explains that the visual analysis is inaccurate, since many contaminations found in biologic tests had not been noticed in their analysis. Gajaca and Brazolin (2012) then complement by stating that physical

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modifications can occur without changing the color of the materials and their covering, which can be considered a false evidence of performance.

3.2 Microscopic analysis

The microscopic analysis revealed the presence of fungi on the samples (Figure 5), along with the change of material color. It also exposed the lack of visual evidences of the presence of microorganisms on epoxy paint sample 01, complying with the visual analysis, and evidences of contamination on the waterproofing polymer sample, contradicts the previous analysis, even though these conclusions still have to be confirmed with the morphological analysis.

For the rest of this analysis, the waterproofing polymer and epoxy paint samples was highly damaged when analyzed with the microscope, despite not having presented visual modifications. The layer of water maintained during the waterproofing polymer test may have affected its most external coat by generating intense physical damage even without the conditions for fungal development. This result indicates that the waterproofing material can reach higher critical values, as it did not reach moisture levels and temperature that would allow the fungi to grow (Johansson; Svensson and Ekstrand-Tobin, 2013). Silva (2011) also noted that maintaining a layer of water delayed and inhibited the attack by fungi in an outdoor area subjected to weather effects.

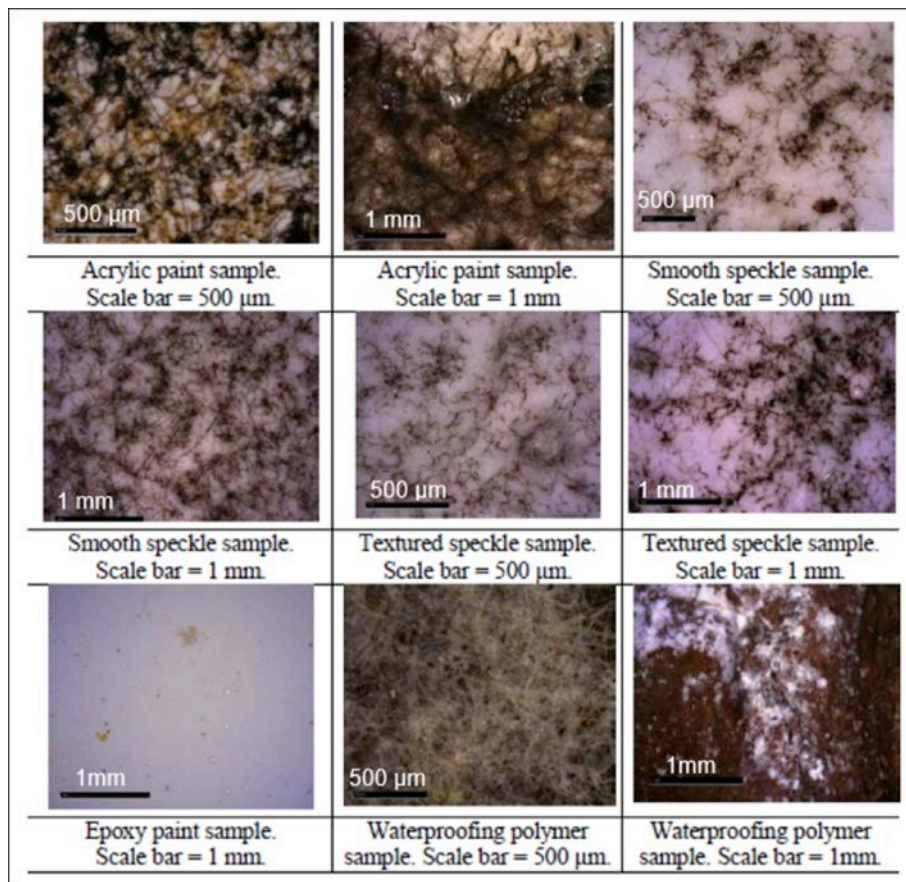


Figure 5. Images generated by microscopy

3.3 Morphological analysis

The morphological analysis by microscope identified the fungi based on Putzke and Putzke (2004) and

differentiated genus taxonomically in order to find the three types of fungi present in the test chamber, as specified in Figure 5.



Table 3. Fungi found in the samples

Sample	Fungus found
Acrylic paint	<i>Aspergillus</i>
Acrylic paint	<i>Aspergillus</i> and <i>Aureobasidium</i>
Smooth speckle	<i>Aspergillus</i>
Smooth speckle	<i>Aspergillus</i>
Textured speckle	<i>Aspergillus</i>
Textured speckle	<i>Aspergillus</i>
Epoxy paint	<i>Penicillium</i>
Epoxy paint	<i>Penicillium</i> and <i>Aspergillus</i>
Waterproofing polymer	<i>Aspergillus</i>
Waterproofing polymer	<i>Aspergillus</i>

Figure 6 shows the images generated by this analysis, which depict the reproductive structures of three fungi identified by what is described in the literature and their taxonomic groups.

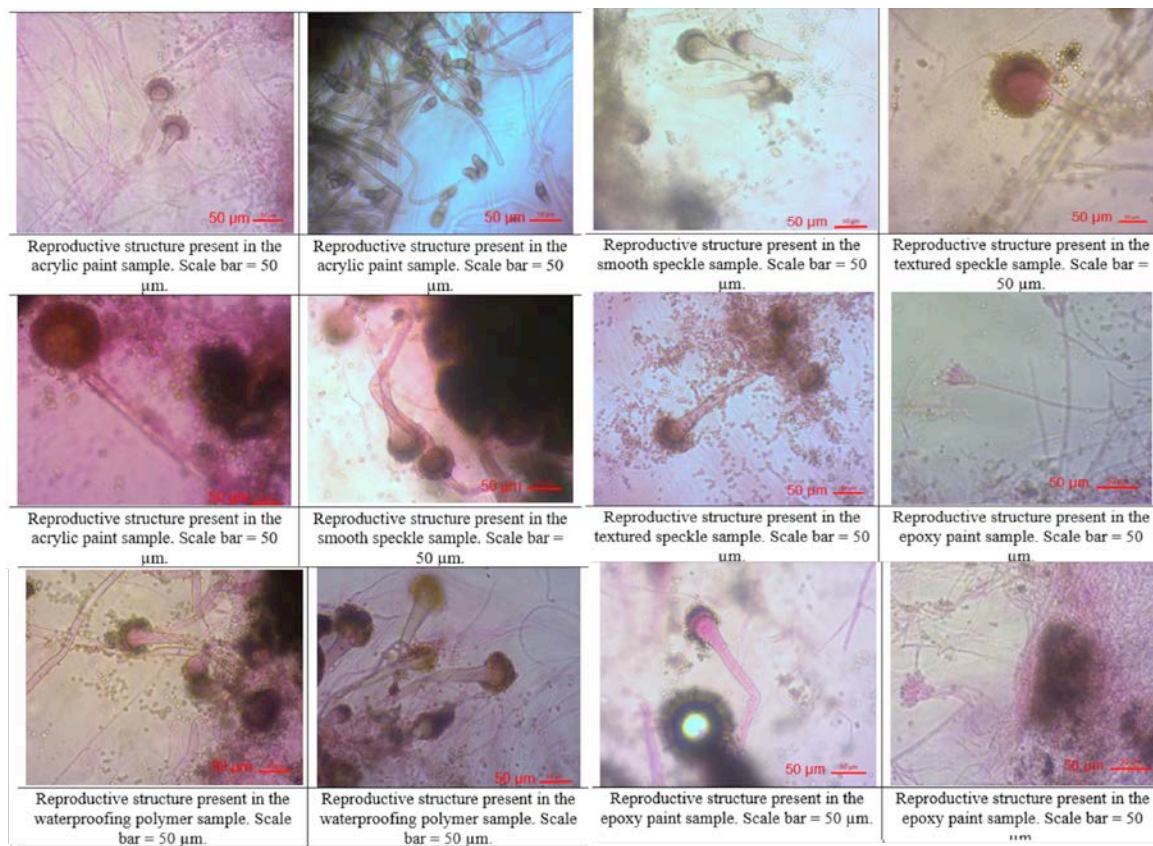


Figure 6. Images generated by the morphological analysis by microscope

As noted, all samples showed fungi development, even those which not presented visual signs of contamination, hence acting in accordance with the evidences from the microscopic analysis and confirming the reproductive structure of the three genera used in the study. Even without signs of defacement by microorganisms in the

mentioned analyses, the epoxy paint and the acrylic paint samples presented the formation of fungal colonies of *Aspergillus* and *Aureobasidium* and *Penicillium* and *Aspergillus* respectively. *Aureobasidium* and *Penicillium*, are among the fungi present in paint films listed by Allsopp et al. (2004). Furthermore, the fungus of type *Aureobasidium*

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developed only in the acrylic paint sample, and *Penicillium* was found only on the epoxy paint samples. *Aspergillus* was spotted on every sample except for one of epoxy paint, which coincides with the results of Bath and Rangel (2005) when they identified the fungus of genus *Aspergillus*. According to the authors, these results can be explained by two reasons: either the paints had extents of inefficient biocides or the fungi acquired resistance to these agents.

Mensah-Attipoe et al. (2016) noticed that *Aspergillus* fungus are more sensitive to variations of humidity than those species of *Cladosporium* and *Penicillium*. Still regarding genus *Aspergillus*, these authors noted that from day 0 to week 1 there was a significant reduction in colony forming units followed by a significant increase until week 4. Since testing conditions required constant humidity, there were no obstacles to growth and maintenance of *Aspergillus*. Still regarding to the genera *Aspergillus*, Shiriwaka et al (2002) reported its presence on painted surfaces, but not in deteriorated paints, thus, the test range of time may affect this genera identification.

Shirakawa et al. (2002) reported that *Aureobasidium* was present on the surfaces at the beginning of their tests, but the numbers fell during the fourth week and recovered in the next weeks, hence suggesting that the presence and concentration of fungi undergo modifications as time passes. Also, as the genera was present early in the surfaces, may have its initial fixation encouraged by the hydrophobic interaction of fresh paint surface. Analyzing real houses, Gi et al (2005) don't perceive the presence of *Aureobasidium*, them, the results agreed with this study, since in the real exposure and degradation of the materials, this genre can reduce its appearance and survivor.

These authors also state that this fungus seems to be left aside by modifications that affect the painting, which presumably boost other fungi in a preferential way, despite this microorganism being able to recover itself when exposed to more severe weather effects and being found mainly on old surface coverings. The results of this article are analogous, considering that this type of fungus developed only on samples covered with acrylic paint and was intensified as weeks passed.

Sharpe et al. (2016) perceived an increase of contamination risks of surfaces by genera *Aspergillus*, *Penicillium*, and *Cladosporium* due to the condensation, while the moisture within the building's tissue was associated only to the increased risk of *Aspergillus* and *Penicillium*.

4. Conclusion

The results point out a difference of classes based on the visual analysis established by ASTM D3273-16 (ASTM, 2016) and the test of resistance to growth of fungi for 4 weeks (standard test time), and 10 weeks (adopted in this study). During the 4 weeks of testing, only the smooth and textured speckle samples presented signs of mold on their surface, as they reached ratings next to 9 or higher, which correspond to milder degrees of rating. For 10 weeks, though, only the samples of epoxy paint and waterproofing polymer did not display apparent damages, whereas most of the other samples achieved ratings below 6.

All samples that underwent the morphological analysis turned out to have fungi on them, minding that the fungus of genus *Aspergillus* developed on 90% of these, going along with the results of Rahman et al. (2012), who had identified the predominance of this microorganism in their experiments. Genus *Aureobasidium* was spotted only on the acrylic paint sample, and the *Penicillium* fungus on the epoxy samples only. As per Rahman et al. (2012), fungi of genera *Aspergillus* and *Penicillium* are part of the group that is most frequently found indoors. The epoxy paint sample, even with no aesthetical evidences or physical damages, provided the development of two fungi simultaneously, being these *Penicillium* and *Aspergillus*. Similarly, the acrylic paint sample, on which genera *Aspergillus* and *Aureobasidium* developed, presented more severe aesthetical damages with rating below 4, in spite of less physical damage by the microscopic analysis. These results are consonant with Andersen et al. (2011), whom affirms that there exists an associated microbiota in each building material.

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