

## **Fungal Colonization on Fiber Cement Exposed to the Elements in a Tropical Climate**

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### **ABSTRACT**

This study describes a preliminary assessment of non-asbestos fiber cement samples exposed to the elements for forty months in a tropical climate. Fungal colonization was determined by culture, optical and scanning electron microscopy. Preliminary assessment of its correlation with quantitative colour change was carried out. A positive Pearson's coefficient ( $r = 0.9$ ) was observed only between total fungal structures and color change on the reverse surface, but not on the surface exposed to the north; on the latter particulate material from the burning of fossil fuels contributed to the dark colour. *Cladosporium*, *Pestalotia/Pestalopsis* and *Trichoderma* were three main fungal genera detected on fiber cement exposed for 40 months in the tropical climate of São Paulo. Mycelia sterilia also had high frequency.

### **KEYWORDS**

Fiber cement, Cellulose fibers, Fungal colonization, Natural weathering

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## **1 INTRODUCTION**

Fiber cement is a composite building material that can be used in a wide range of applications. Since asbestos is considered carcinogenic [Chiappino, 2006] there is a tendency to increase the use of asbestos free fiber cement composed of natural and synthetic fibers. These fibers increase strength and toughness of the composite hence they are extremely important to the mechanical performance of fiber cement products [Dias, 2005].

Cellulose fiber deterioration in the alkaline cement matrix has been considered the main problem of durability of fiber cements [Gram 1983, Sarja 1988, Bentur & Akers 1989]. Synthetic fibers like poly (vinyl alcohol) (PVA), on the other hand, have been considered durable in the cement matrix [Kalbskopf et al. 2002]. The main effects of fibers deterioration are embrittlement and decrease of mechanical strength.

Biodeterioration is not a frequent worry in fiber cement technology. Some researchers declare that the alkalinity of the matrix inhibits the deterioration of fiber cement products by this mechanism. De Souza et al. [1997] have studied composites of cement-bonded wood particleboard, which presents a high organic/inorganic ratio compared with conventional fiber cement formulations. These authors concluded that fungi and termites did not cause significant deterioration (weight loss) even after accelerated carbonation of composite. In this research the fungi studied were basidiomycetes (Phylum Basidiomycota).

In the current work the term fungi is designated to moulds (Fungi Imperfecti, also sometimes known as Deuteromycota); they are composed of filamentous structures called hyphae, which can differentiate and produce rounded forms called spores or conidia.

Fungi can grow on the surface of materials and cause dark discoloration. On roofs this can compromise the thermal comfort inside the buildings. However colour changes roof surfaces affect the thermal performance of the entire building, users comfort or energy consumptions, they also can affect urban microclimate due to spectral reflectance and infrared emittance [Synnefa et al, 2006].

Cellulose is easily transformed by cellulolytic microorganisms including bacteria and fungi. Due to high alkalinity of fresh cement materials usually microorganism can not grow before carbonation occurs. There have been some patents registered for the incorporation of biocides into cellulose to prevent microbial attack on fiber cement. Nevertheless there is little literature describing the colonization of fiber cement in natural weathering conditions and which microorganisms are more frequent.

This study describes mould colonization of non-asbestos fiber cement composed of PVA and cellulose fibers exposed to the elements for forty months in a tropical climate, in São Paulo, Brazil.

## **2 MATERIAL AND METHODS**

### **2.1 Materials**

Table 1 presents the materials and the formulation (labeled PVA 14) that were used for the preparation of specimens. Six-millimeter length poly (vinyl alcohol) (PVA) fibers, unbleached long cellulose fibers and newspaper waste were employed as reinforcement. Densified silica fume, ordinary Portland cement and limestone filler from the Brazilian market were also employed.

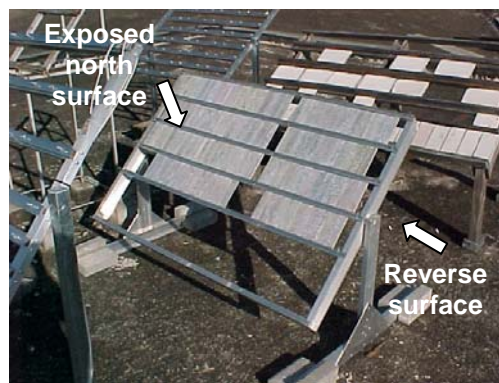
### **2.2 Natural Aging**

Fiber cement specimens were exposed in São Paulo, a large industrial metropolis with a mean annual temperature of 19.5 °C, an annual rainfall of ~1500 mm, a mean relative humidity of 78% and

presence of urban and industrial pollution. These specimens were exposed in metallic racks facing to the true north with a slope of approximately 45° (Fig. 1). After 40 months of exposure they were submitted to fungal analysis.

**Table I.** Materials and fiber cement formulations [Dias 2005].

Materials	Dry weight fraction (%)
Portland cement	75.20
Limestone filler	12.72
Silica fume	6.68
PVA fibers	1.40
Unbleached cellulose fibers	1.20
Newspaper waste	2.80
	Volume fraction (%)
PVA fibers	3.00
Cellulose fibers	8.60



**Figure 1.** Rack with fiber cement specimens.

### 2.3 Fungal Sampling Method

Brush sampling was carried out by mean of a toothbrush previously packaged in aluminum foil and sterilized for 15 minutes at 120° C at 1 atmosphere of pressure and dried for 2 days at 80° C. The toothbrush was moistened in saline sterile solution and brush sampling was performed five times in a clockwise direction. Afterwards toothbrushes were kept in closed tubes containing 10 mL of sterile saline solution (0.85%). Fungi were recovered from the brush in an ultra-sonic bath Thornton C/7, T7 for 10 minutes and diluted 1:10 in sterile saline solution. This suspension was used to evaluate culture and total fungal structures.

### 2.4 Quantitative Culture

Culture was carried out with 100 µL of the fungal suspension as described in 2.3 and inoculated by spread plate method in two Petri dishes containing Sabouraud Dextrose Agar. The plates were incubated at 25° C for 48-72 hours and colony forming units (CFU) were recorded.

### 2.5 Quantification of Total Fungal Structures by Neubauer Chamber<sup>1</sup>

Fungal suspensions as produced in Section 2.3 were analyzed in Neubauer chamber<sup>1</sup> in optical microscope in order to quantify total fungal structures (hyphae and spores).

<sup>1</sup> Chamber used in to count biologic structures

## 2.6 Color Analysis by Spectrophotometry

BYK Gardner Color-Guide 45/ 0.6805 equipment was used. According to CIE Lab, L\* : Luminosity (black L\*=0 to white L\*=100); a\*: red to green (positive and negative values respectively) and b\*: yellow to blue (positive and negative values respectively) were analysed.

The total difference of colour was defined as  $\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$ , in this case the average values of L\*, a\* and b\* of unexposed samples were used as initial reference color.

## 2.7 Scanning Electron Microscopy

The samples were submitted to SEM. A LEO Leika S440 microscope with an acceleration voltage of 20 kV and current of 150 mA was used to examine the fractured section and the surface of specimens. All samples received carbon coating in a Sputtering Baltec SCD 050 equipment before SEM examination.

## 2.8 Statistical Analysis

Statistical analysis of fungal quantification was carried out according to the non parametric Mann-Whitney test ( $\alpha=0.05\%$ ). Pearson's correlation was used to analyze differences between colour and fungal quantification. BioEstat program was used for both analyses.

## 3 RESULTS

Results of fungal growth measurements are presented in Table 2 for both viable fungi and total fungal structures. According Mann-Whitney test viable fungi is significantly lower than total fungal structures in both exposed and reverse surfaces of fiber cement.

Viable fungal colonization assessment by culture method, according Mann-Whitney test, was higher in reverse surface than exposed surface. However, for total fungal structures evaluation there is no significant difference between exposed and reverse surface.

Table 3 presents L\*, a\* and b\* of unexposed samples which average is used as initial reference. Besides of this L\*, a\* and b\* of exposed to north and reverse ones. This Table also presents total difference of colour ( $\Delta E$ ) between each measure and the average of unexposed samples as describes in 2.6.

Fungal relative frequency is presented in Table 4. *M. sterilia*, a group of fungi that does not produce spores, was found in high frequency (54% and 67% on exposed and reverse surfaces, respectively).

**Table 2.** Fiber cement quantification per 5.4 cm<sup>2</sup> of sampled area of viable fungi in CFU and total fungal structures.

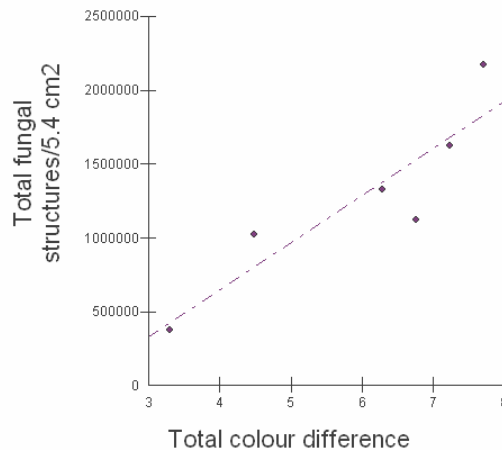
<i>Viable fungi CFU</i>		<i>Total fungal structures</i>	
<i>Exposed surface</i>	<i>Reverse surface</i>	<i>Exposed surface</i>	<i>Reverse surface</i>
470	1990	1.7 x 10 <sup>6</sup>	1.3 x 10 <sup>6</sup>
350	1230	1.9 x 10 <sup>6</sup>	1.6 x 10 <sup>6</sup>
335	513	2.4 x 10 <sup>6</sup>	2.2 x 10 <sup>6</sup>
285	650	3.4 x 10 <sup>6</sup>	3.7 x 10 <sup>5</sup>
216	570	1.3 x 10 <sup>6</sup>	1.1 x 10 <sup>6</sup>
265	1680	8.5 x 10 <sup>5</sup>	1.0 x 10 <sup>6</sup>

**Table 3.** L\*,a\*,b\* of unexposed samples, exposed to north, reverse surface and ΔE, after 40 months aging.

<i>Unexposed</i>			<i>Exposed to north</i>				<i>Reverse surface</i>			
<i>L*</i>	<i>a*</i>	<i>b*</i>	<i>L*</i>	<i>a*</i>	<i>b*</i>	<i>ΔE</i>	<i>L*</i>	<i>a*</i>	<i>b*</i>	<i>ΔE</i>
63.15	-0.67	1.84	53.36	-0.27	4.56	7.30	54.15	0.10	3.24	6.28
59.64	-0.33	1.25	51.48	-0.05	5.58	9.42	53.18	0.04	3.30	7.24
54.80	-0.23	2.52	53.94	-0.10	4.56	6.76	53.73	-0.30	3.62	6.72
59.57	-0.76	2.11	54.15	-0.11	5.24	6.81	57.07	0.21	2.74	3.30
61.49	-0.62	3.09	53.29	0.24	5.45	7.71	53.64	0.10	2.96	6.75
63.40	-0.63	3.73	52.95	0.21	4.10	7.61	55.94	0.18	3.18	4.48
60.34	-0.54	2.42	53.19	-0.01	4.91	7.60	54.61	0.05	3.17	5.79

The last line in the Table 3 is the average of above values of the column.

Pearson's correlation was significant ( $r= 0.9$ ) only between total fungal structures and total color difference on the reverse surface (Figure 2) after 40 months aging. There was no correlation between viable fungi (CFU) and colour difference on both surfaces.

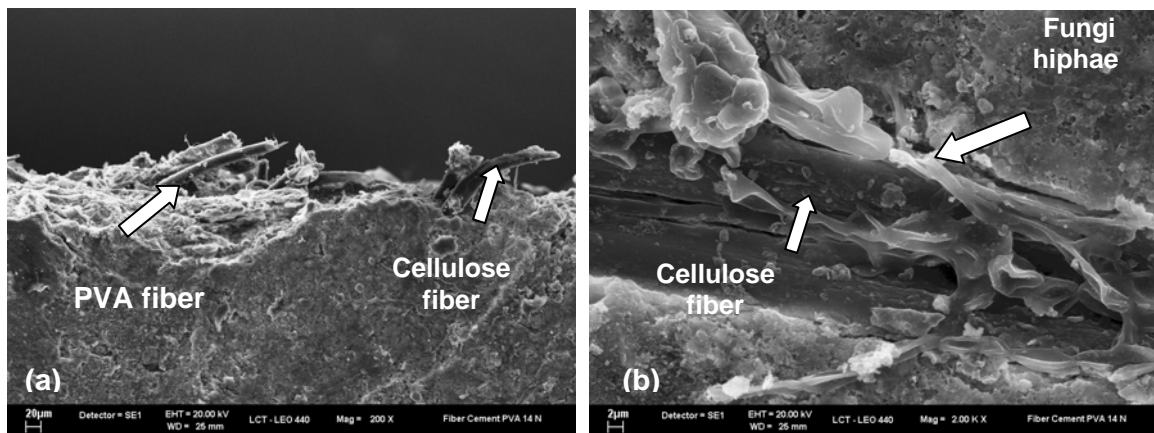


**Figure 2.** Diagram showing strong correlation between total fungal structures and color difference on the reverse surface (Pearson's correlation  $r=0.9$ ).

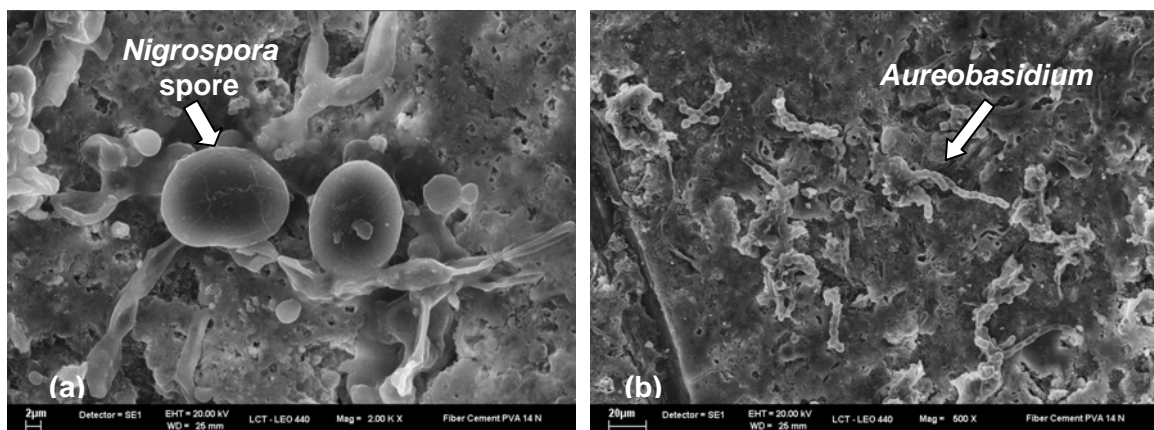
**Table 4.** Relative frequency of viable fungi detected by culture.

	Exposed surface (%)	Reverse surface (%)
<i>Cladosporium</i>	31	67
<i>Pestalotia/Pestalopsis</i>	54	40
<i>Trichoderma</i>	31	27
<i>Aspergillus</i>	8	20
<i>Nigrospora</i>	23	0
<i>Chaetophoma</i>	23	7
<i>Aureobasidium</i>	8	20
<i>Phoma</i>	8	13
<i>Alternaria</i>	0	7
<i>Monilia</i>	8	0
<i>Helminthosporium</i>	8	0
<i>Curvularia.</i>	8	0
<i>Fonsecaea</i>	0	7

Scanning electron microscopy images obtained after 40 months aging are presented in Figures 3 and 4. Figure 3 (a) shows a fractured region of fiber cement; cellulose fibers are bigger and more irregular than PVA fibers. Figure 3 (b) shows fungal hyphae growing on cellulose fiber.



**Figure 3.** (a) Fractured sample after 40 months exposure, (b) Fungi colonization on cellulose fiber.



**Figure 4.** Fungal colonization on fiber cement matrix after 40 months exposure. Round structure is similar to *Nigrospora* spore (a). Structures are similar to *Aureobasidium* genus (b).

#### 4 DISCUSSION

There is no correlation between total fungal structures quantified by Neubauer chamber in the optical microscope and viable fungi quantified by culture. Some portions of these fungal structures could be non viable and hence resistant to culture in the media due to environmental conditions like UV irradiation, temperature and desiccation. Another reason could be that the media used were not suitable for xerophilic fungal growth. In addition, some fungal structures detected by Neubauer chamber can be already dead.

As shown by scanning electron microscopy, in this preliminary assesment PVA fibers did not present fungal colonization. The cellulose fibers were colonized by fungi as well as the cement matrix.

In the surface exposed to the north irregular particles about 20% larger than fungal structures were found more often than on the reverse surface. The presence of particulate material from burning of fossil fuels is to be expected, since the natural aging station is located in São Paulo, the biggest city of Brasil with high pollution records.

Regarding L\* (black L\*=0 to white L\*=100) measurement both north-exposed and reverse samples showed significant lower values in relation to unexposed reference ones, confirming the darker surface in relation to unexposed samples after aging.

Fungi can contribute to the darkening of fiber cement as well as particulate material from the burning of fossil fuels. Some species of *Trichoderma* are quoted in the literature as cellulolytic fungi [Persson, 1991] and they are found in a relative frequency of about 30%. Others relevant fungal genera detected in this work, such as *Cladosporium* and *Pestalotia/Pestalopsis*, are also being isolated in different building materials in Brazil. *Cladosporium* has also been described as a cellulolytic fungus [Abrha, 1992]. *Mycelia sterilia*, a non sporulating group, was found in high frequency.

As described above the focus of this study was not to investigate the degradation of fiber cement by fungi, but the influence of fungal colonization on the surface darkening of this material. Future studies will focus on cellulose colonization inside the specimens submitted to natural aging.

## 5 CONCLUSION

Fungi detected in the current work contribute to darkening of fiber cement since most of them are usually melanin producing and their structures like hiphae or spores are brown to black. Particulate materials from burning of fossil fuels, typical from urban areas, contribute to dark discoloration, mainly on the specimens' surface exposed to the north.

## ACKNOWLEDGMENTS

The authors thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for sponsoring this research and for the grants to M.A. Shirakawa and C.M.R. Dias.

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