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Accelerated testing of mold growth on traditional and recycled book paper

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ABSTRACT

The growth of molds on paper containing cellulose is a frequent occurrence when the level of relative air humidity is high or when books become wet due to water leaks in libraries. The aim of this study is to differentiate the bioreceptivity of different types of book paper for different fungi. Laboratory tests were performed with strains of *Aspergillus niger*, *Cladosporium* sp., *Chaetomium globosum* and *Trichoderma harzianum* isolated from books. Four paper types were evaluated: couché, Pólen (offset), recycled and a reference paper containing only cellulose. The tests were carried out in chambers with relative air humidity of 95% and 100%. Mold growth was greatest in the tests at 100% relative humidity. Results of stereoscopic microscopy observation showed that *Cladosporium* sp. grew in 74% of these samples, *A. niger* in 75%, *T. harzianum* in 72% and *C. globosum* in 60%. In the chambers with 95% air humidity *Cladosporium* sp. grew in only 9% of the samples, *A. niger* in 1%, *T. harzianum* in 3% and *C. globosum* did not grow in any sample. The most bioreceptive paper was couché and the least receptive was recycled paper. The composition of the recycled paper, however, varies depending on the types of waste materials used to make it.

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1. Introduction

The purpose of libraries, archives and museums is to preserve the items collected, enabling access to the knowledge contained in books and documents and enjoyment of the artworks contained on paper. Long-term preservation of the collections of historic, artistic and cultural heritage of these repositories involves controlling the environment in which they are kept and exhibited.

According to King et al. (2001), most authors believe the main factor that must be controlled in a library is relative humidity of the air. In any given place, the ability of the air to hold moisture is directly proportional to its temperature. As the temperature rises, the air is able to hold more moisture, and when the temperature falls, mist or fog can form in the air and dew droplets can condense on the surface of materials (Craddock, 2001), providing conditions for fungal growth. The water activity (a_w) of a substrate is measured by the ratio between the vapor pressure of the water in the substrate and the pressure of pure water at the same temperature and relative humidity. Pure water thus has the maximum ratio of 1 (Florian, 1997). Pinzari et al. (2006) showed that degradation of cellulose by fungi can be initiated at water activity levels greater than 0.65.

Book collections are particularly propitious for growth of microorganisms, because they group in one place a large quantity of organic material, such as paper, starch, leather and cloth (Haines and Kohler, 1986; Parker, 1987; Ogden, 1992; Neve et al., 2009). Microorganisms, because of their rapid reproduction and physiological activity, generally are highly adaptive to environmental conditions. Thus, they can rapidly reproduce on various substrates, causing them to discolour or decompose (Kowalik, 1980). Fungi are not only the most important agents of biodeterioration, they also are a major cause of allergic reactions, particularly respiratory ones, in susceptible users (Florian, 1997).

There are many types of paper of varying composition and quality made for various uses, from simple wrapping paper to the most expensive papers used to print artworks. The Chinese are credited with the invention of paper, and the surviving examples show that this paper was of high quality, containing long and linear fibers, with only one compound, animal glue, as binder (Lima et al., 1988).

The invention of the printing press caused a huge increase in demand for paper, a trend that increased as mass printing techniques advanced. This caused a relative shortage of raw material, such as rags and linen and cotton fibers. New sources of cellulose were needed, and wood pulp proved to be an excellent raw material

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(Kuan et al., 1988). Paper produced from wood pulp has a dark color and contains a high amount of non-disintegrated material. Research into new techniques to produce paper from wood that is lighter, more resistant and with better printing quality culminated in different pulping and bleaching methods (Lima et al., 1988).

The aim of this work is to assess the bioreceptivity of different paper types for the growth of molds in tests without addition of nutrients. We tested three types of common commercial paper available in Brazil – dull couché matte, Polén (offset) and recycled paper – along with a reference paper containing only cellulose.

2. Material and methods

2.1. Material

2.1.1. Paper

Four paper types were tested. The first was a reference paper, produced by the São Paulo State Technological Research Institute from bleached eucalyptus cellulose pulp, without any products to enhance its physical and mechanical properties, such as binding agents, mineral loads, colorants, adhesives, additives, etc. This paper is highly adsorptive and has a pH around 6.8.

The couché matte paper is made from chemically extracted eucalyptus pulp. It is alkaline (pH = 9) and coated on both sides with kaolin and calcium carbonate (pigment), latex (adhesive) and bleach. The calcium carbonate provides the dull finish. The polén (offset) paper is also alkaline (pH = 9) and is produced from chemical pulp. During the manufacturing process, a colorant (pigment or aniline) is added to the pulp. The paper contains starch binder (from corn or manioc). The recycled paper also is alkaline (pH = 9). It can contain both post-consumer waste (used paper) and pre-consumer waste (rejects from paper mills) in varying levels.

The paper samples were cut into uniform squares of 16 cm^2, with 144 specimens of each type, for a total of 576 samples.

2.1.2. Humid chambers

The humid chambers used in the tests consisted of glass jars hermetically sealed with metal lids and rubber seals. Each jar contained one paper sample, attached to the lid with a thread. The jars together with the paper samples were sterilized in an autoclave at 121 °C.

2.2. Microorganisms

Four fungal cultures previously isolated in libraries in Brazil (Reis-Menezes, 2009) were used in the bioreceptivity tests. The genera *Aspergillus*, *Chaetomium* and *Trichoderma* were chosen because they are used as test organisms by the Technical Association of the Pulp and Paper Industry (TAPPI, 1999). *Cladosporium* was selected because it is a fungus often isolated during sampling of air and books in Brazil (Gambale et al., 1977, 1983, 1993; Graudenz et al., 2002; Reis-Menezes, 2009). The species used in this experiment were *Aspergillus niger*, *Chaetomium globosum*, *Trichoderma harzianum* and *Cladosporium* sp.

2.3. Accelerated test

2.3.1. Temperature and humidity

Two relative humidity levels were tested, 100% and 95%. The former was attained by placing 30 mL of distilled water in the bottom of each sealed jar, and the latter by placing a saturated solution in the bottom of the jars, obtained by diluting 10 g of KH_2PO_4 in 30 mL of distilled water (Winston and Bates, 1960). The temperature of 25 °C was chosen since it is considered ideal for growth of the filamentous fungi isolated.

2.3.2. Inoculum

The inoculum was produced from seven day old growth of fungi on Sabouraud agar. The fungal propagules were suspended in a saline solution (0.85%) utilizing an ultrasound bath (Thornton – Inpec Eletrônica Ltda, type T7, model C/T). The cells were counted in a Neubauer chamber and the concentrations were adjusted to 10^2, 10^4 and 10^6 cells/mL using the dilution equation: $C_1V_1 = C_2V_2$. The sterilized paper samples, attached to the jar lids with thread, were inoculated with 50 μL of each concentration in a laminar flow cabinet. The inoculum (50 μL) was placed in the centre of the paper using a pipette. Then they were replaced in the humid jars without contacting the water or saturated solution at the bottom. Six replicates of each set of conditions were prepared. The sets were incubated in a heated chamber at 25 °C for 30 days. Control samples were also incubated under the same conditions but without inoculum. The inoculum was tested for viability using the Miles et al. (1938)'s method.

2.3.3. Observation of mold growth on the paper samples

After 30 days in the humid chamber, the samples (treated and controls) were removed from the jars and placed for seven days in Petri dishes together with a small piece of cotton moistened with 8% formaldehyde, to kill the fungi. The mold growth or absence thereof on the paper surfaces was evaluated under a stereoscopic microscope (Carl Zeiss Jena) and the color was analyzed as described below.

2.4. Color analysis

The purpose of the color analysis was to compare fungal growth for the same species on each paper sample after the period in the jars. The change in color between the control (without inoculum) and treated paper samples was determined with a BYK Gardner Color-Guide 45/0.6805 apparatus, a color-guide hand held spectrophotometer. The difference in color was measured by the formula:

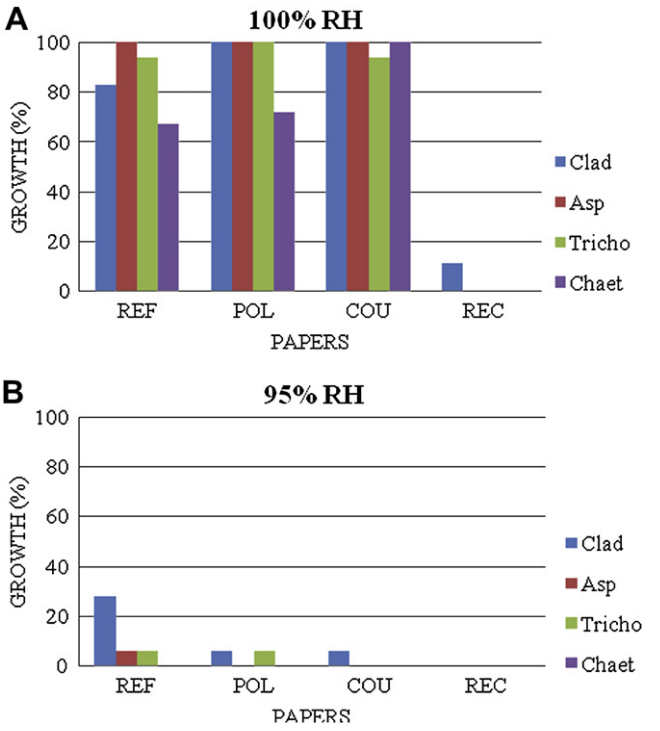

Fig. 1. Effect of relative humidity on the occurrence of mold growth on the four paper types.

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