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Laboratory test to assess sensitivity of bio-based earth materials to fungal growth

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ABSTRACT

The effect of molds present in buildings on the health of the occupants is a major issue hence, when a building material is developed, its sensitivity to microbial growth should be assessed. However, few studies have investigated fungal growth on bio-based building materials with the resources available in a laboratory specializing in materials. The objective of this paper is thus to propose a simple and efficient experimental method useful for construction materials laboratories, adapted from methods proposed in the literature. For this purpose, fungal growth was investigated under different environmental conditions on earth-based material with or without the addition of straw or hemp shiv. Samples were inoculated with a strain of *Aspergillus brasiliensis* and were incubated for 12 weeks at 76, 84 or 93% RH, and 30 °C or 20 °C. Reproducible results showed that earth-based materials were more sensitive to fungi when they were enriched in plant aggregates. Fungal development was observed on earth material containing plant aggregates after 4 weeks of exposure at 93% RH and 30 °C, whereas it was observed after 8 weeks on raw earth material under the same conditions. Additionally, the possibility of quantifying fungal development with increased sensitivity by using image analysis is proposed. Due to the growth of fungal species other than *A. brasiliensis*, a natural inoculation approach is recommended. One of the conclusions is that liquid water is more favorable to mold growth than relative humidity alone. The addition of liquid water is thus recommended to accelerate the test.

1. Introduction

Development of construction materials is often planned to meet objectives and requirements concerning mechanical and/or thermal properties. However, the objective is not necessarily twofold, and other constraints can be defined, which may be as various as fire safety, visual aspect, durability and occupants' health. On this last point, mold risk has been the focus of increasing interest in recent years, for two main reasons. First, buildings are being designed to be increasingly airtight, which limits unwanted air infiltrations but also leads to increased levels of indoor relative humidity. Second, numerous research projects and recent constructions are considering bio-based materials, mainly for the low embodied energy and for the renewability of the raw material. These are claimed to be healthy and to increase the indoor comfort of the occupants [1] but it is commonly accepted that the use of plant matter would lead to an increased risk of mold growth. Furthermore, it is estimated that 20%–40% of the housing in Northern Europe and North America is affected by indoor molds [2]. Microbial Volatile Organic Compounds (MVOC), responsible for the unpleasant odor, and spores and mycotoxins, which are responsible for various health issues [3,4], are by-products of this active fungal growth. The incidence of the spores on human health depends on the concentration, exposure time and host factors. An exposure to these fungal by-products can generate allergic conditions and impact asthmatic well-being particularly among immunocompromised persons [2]. The set of such health problems is part of the Sick Building Syndrome (SBS) or Building Related Illness (BRI). As people spend more than 80% of their time inside buildings [5], the impact is large.

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For these reasons, more and more attention is being paid to mold growth on building materials [6–14], with applications of bio-based material becoming a topical issue. Some studies investigating such growth deal with wood-based or paper-based materials and also inorganic materials such as cement or gypsum plaster board [3,6,12]. Fungal growth has also been studied *in situ* on straw bales with lime-based render for building envelopes [15]. Hoang et al. [7] have shown that bio-based materials are more sensitive to fungal growth because of the nutrients they contain and their high hygric capacity. However, the methodologies followed in these studies were varied and mostly adapted from practices in biology laboratories [16,17]. Although such access to relevant devices has its importance, there are some significant differences in terms of purpose between the field of construction materials and that of biology. To develop new materials, it is important to elaborate a method to evaluate their sensitivity to fungal growth that would be easy to implement in a materials laboratory, without necessarily identifying the fungal species developed. Moreover, a screening method able to test various samples in a reproducible manner may help to select relevant materials, in the very early stages of the development of new materials in laboratories.

Some standard protocols exist to evaluate mold growth on materials, e.g., the standard ASTM D3273 [18] concerning the growth of mold on coated surfaces, in which the material is tested for only four weeks in one set of environmental conditions. However, no standard is specific to bio-based materials.

While different microbiological studies on construction materials have already been documented, this field of investigation is still emerging due to the diversity of environmental conditions and materials, and the multiplicity of questions addressed. Indoor microbial growth on building materials was recently reviewed by Verdier et al. [19], who compared several methods for sampling and analyzing the proliferation of micro-organisms, and described the most common microbial communities and the building parameters. Mold growth is dependent on various environmental factors, which have to be taken into account when developing a testing protocol. Some of them have been identified as having a particularly strong influence:

1. Water availability. In steady state conditions, fungal growth begins at around 80% of relative humidity according to Nielsen [4]. A minimum relative humidity of 77% was reviewed by Krijgsheld et al. [20] for fungal species, but the optimal value was 97%.
2. Substrate (or medium, or material). The proliferation depends on the quantity of nutrients available and the porosity and roughness of the material [21].
3. Temperature. The optimal temperature for many fungal species is between 20 °C and 30 °C [22–24]. However, some microbial growth has been recorded between 0 and 50 °C depending on the species [25].
4. Time of exposure. The longer the material is exposed to humidity, the higher is the risk of microbial growth [4].

There is a clear need to move forward on this topic, one of the most obvious reasons being the absence of a suitable standardized protocol. This is one of the issues identified in the framework of the Bioterra project, a national collaborative project involving members from both materials and microbiology laboratories. This project is focused on earth-based materials including plant aggregates, as they are assumed to sometimes present mold growth and as limited research has been published on this topic up to now. Mold formation was observed on earth panels containing hemp shiv ten days after manufacturing [26]. It was also observed after removal of the formwork of earth-straw walls [27], particularly inside the building, where ventilation was less effective.

One of the main objectives of this paper is to propose a simple and efficient method, adapted from methods already proposed in the literature, for assessing the sensitivity of materials to fungal growth, which will be useful for laboratories interested in construction materials. To achieve this objective, the second section of this paper provides a short literature review of existing tests and procedures. These considerations led us to design an in vitro protocol for the study of fungal growth, as described in the third section, dedicated to the presentation of the materials and procedures. The protocol was applied to samples made of raw earth as the mineral matrix, with the addition of two types of plant aggregates: barley straw or hemp shiv. In the fourth section, the results are presented regarding the rate of mold proliferation on the material, and the experimental procedures applied are discussed.

2. Literature review of mold growth evaluation on building materials

As underlined in the introduction, there is no consensus on the methodology that should be applied to study mold growth on building materials, although research has already been done on this topic. In this section, the main techniques found in the literature are presented. Some laboratory tests are based on standards intended for plastics (ISO 846 [28]) or insulation materials and their facings (ASTM C1338 [29]), for example. Recently, Johansson et al. [30] summarized these standards and proposed an innovative method intended for building materials. This constitutes the main basis for the present work.

2.1. Decontamination

Decontamination has to be performed just before starting the study of mold growth in order to remove the fungi already present in the material. A simple method is to expose the samples to high temperature for a given time. It is mentioned by Simons et al. [31] that, even if not all the bacteria were removed with a heat treatment at 100 °C, almost all molds were eliminated. Some authors have sterilized materials with gamma rays [7,12], which proved much more effective. However, this technique is costly and the device is rather unusual in material development laboratories. Some authors chose not to sterilize their samples to avoid unknown changes in the substrate [15,32].

2.2. Fungal selection and inoculation

Regarding inoculation, some authors suggest that natural inoculation would be more representative of real conditions [7,32], while artificial inoculation is preferred by others. The latter technique consists of inoculating the specimens with an inoculum preparation, which accelerates the test and improves repeatability [30]. Moreover, it is easier to quantify and compare the fungal growth when the initial state (spore quantity) is known.

Various species identified on indoor building materials have been listed by Verdier et al. [19]. The most frequent genera of species isolated are *Cladosporium*, *Penicillium*, *Aspergillus* and *Stachybotrys*. *Aspergillus niger* is used in many references [7,17] because it is often observed on building materials and has been involved in health issues [33,34]. This is a filamentous fungus, which has been observed all over the world in various environments (forests, dunes, indoors, etc.). It can be pathogenic for humans and its presence is not accepted in a hospital environment. The optimal water activity (a_w) for its growth is around 0.95 [35]. According to different authors, the optimal temperature is around 30 °C. Krijgsheld et al. [20] observed the greatest growth between 35 °C and 37 °C, it occurred between 27 °C and 37 °C according to Passamani et al. [35], and the proliferation was greater at 28 °C than at 20 °C in the study done by Lasram et al. [36]. *A. niger* may thus be considered as a representative species for a global evaluation in vitro.

Hoang et al. [7] inoculated a single strain (*A. niger*), but various fungal species have been used by others [6,9,12,17,37]. The inoculation was performed by means of a spray [6], micropipette [7] or dry cotton swab [12]. The latter was used in order not to modify the water activity.