Proposal of methodology for testing resistance of building materials against mold infestation

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Abstract. Molds attack buildings on a broad scale, cause significant damage and carry a number of negative effects on human health. The design of the methodology is based on EN 60068-2-10 “Environmental Impact Testing – Parts 2-10: Tests – Test J and Instructions: Mold Growth” and ČSN 72 4310 “Testing the Resistance of Building Products and Materials against Mold”. However, the first of these standards is intended for electrical industry and the other was published in 1975 with no later revisions. The design of the new methodology aims at simplifying and updating and, last but not least, imitating the success of already implemented methodology for testing resistance to green algae growth. The proposed methodology was successfully verified on both standards and real samples.

1. Introduction

Due to biological infestation, properties of building materials can change. The process of reducing the qualitative properties of building materials as a result of life processes and activities of living organisms is called bio-corrosion and the organisms are called biodeteriogens. In building materials such as building stone, concrete, plaster, or plastics, a high degree of bio-corrosion occurs. Biodeteriogens need appropriate conditions for their life, such as substrate humidity, heat, nutrients, favorable pH of substrate (acidity or alkalinity), and oxygen (except anaerobic bacteria). Some species need light [1, 2].

The entire complex of biodeteriogens that interact with each other usually enters the bio-corrosion process of materials. It is a combination of physical and chemical actions, sometimes even other influences. Growth processes of microorganisms cause pressures that disturb the integrity of matter. Products of metabolic transformation affect stability of mineral components of the building material and dissolve them. This results in an ion exchange between the organism and the material. Practical consequences of these phenomena are destructive changes of the material that manifest themselves as functional (physicochemical, chemical, etc.) and morphological (color spots, pitting, etc.) [3].

A noteworthy group of organisms involved in bio-corrosion are microscopic fibrous fungi. They are multiplied by mycelium growth and by spores. Spores are mold reproductive units that germinate in wet places with nutrients, then grow and produce reproductive organs. From them, ripe spores are released into the environment and being very small and light, carried by the air over long distances [2, 4]. In suitable conditions for sporulation, they grow on walls or other surfaces in the indoor and outdoor environments of buildings. The limiting factor for mold growth on walls and various substrates in houses and apartments is moisture. However, high humidity is unnecessary as long as the water is bioavailable. Based on the species representation we can deduce the substrate moisture level and vice versa. In places
where moisture is evident at first glance, hydrophilic genera is found most often, such as *Alternaria*, *Epicoccum*, *Fusarium*, *Phoma*, *Stachybotrys*, *Trichoderma*, *Ulocladium*. In humid areas, genera *Cladosporium*, *Mucor*, *Rhizopus* often occur. *Aspergillus* and *Penicillium* are xerophilic and usually found in drier areas [5, 6].

Generally, microscopic fungi in the interior spaces, in addition to disrupting the substrate on which they grow, bring further undesirable consequences. They can have a very negative impact on human health, as they are significantly involved in a wide range of allergic and fungal diseases. They create specific proteins, glycoproteins, polysaccharides and many other substances in their hyphae and spores, which can cause allergic reactions in sensitive individuals after inhalation. The most common fungus in the air and on the walls, such as *Cladosporium* and *Alternaria*, are also the most important allergens [5]. Other products of these microscopic fungi may be mycotoxins, which are less likely to enter the atmosphere but may have carcinogenic and toxic effects. Most of these fungi can also have pathogenic effects on immune-impaired individuals. Some species also release volatile metabolites into the environment, such as secondary alcohol 1-octen-3-ol (characteristic fungal odor), various aldehydes, ketones, mono- and sesquiterpenes, often foetid. These substances often cause headaches and eye burning. Microscopic fungi may be one of the factors contributing to the syndrome of diseased buildings [4, 6].

Testing the resistance of building materials against mold growth is dealt with by ČSN 72 4310 Testing the resistance of building products and materials against mold [7], but this standard was issued in 1975 and the design of the tests is time consuming and technically demanding. The design of the new methodology is based on this standard and newer standards EN 60068-2-10 Environmental Impact Testing - Parts 2-10: Testing - Test J and Instruction: Mold Growth [8], which is intended for the electrical industry and EN 15458 Paints - Laboratory method for testing the efficacy of preservatives in the coating against water algae [9].

The design of the new methodology aims at simplifying and updating the test and, last but not least, imitating the success of already implemented methodology for testing resistance to green algae growth. The method mainly uses macroscopic observations of mold growth on building materials under defined conditions. Several variants were examined to find the least technical demanding and yet functional method.

2. Methodology proposal

Test specimens or filter paper with tested substance are placed on the surface of Sabouraud agar in individual Petri dishes. The test is performed in 2–3 parallel determinations. Two types of culture controls are prepared - a nutrient medium dish and a dish with nutrient medium and sample-sized filter paper on the surface of the agar. It is also possible to use a negative control - a filter paper impregnated with a biocidal substance. The samples are sterilized by a UV-C lamp prior to the testing.

Both the samples and the surface of the agar are covered with a thin layer of the mixed mold culture. A maximum of 0.5 ml of suspension is applied per 100 cm² of the surface of the specimen. The inoculation mold culture is prepared by washing the individual mature mold cultures prepared from the collection strains. Strains can be selected according to source standards, taking into account the strains typical of the material and environment. At least 5 species of strains are used. The suspension is prepared by washing the spores away from the culture with mineral solution (3 g of NaNO₃, 1 g of KH₂PO₄, 0.5 g of MgSO₄·7H₂O, 0.5 g of KCl, 0.01 g of FeSO₄·7H₂O in 1000 ml of distilled water, sterilization for 30 minutes at 0.1 MPa). Inoculation takes place under sterile conditions. The resulting spore concentration in the solution should be 1–2·10⁶ spores/ml.

The incubation was carried out at 24 ±2°C and relative humidity 96 ±2%. Petri dishes were placed in a sealed cultivation vessel, where high relative humidity was maintained by a saturated Na₂SO₄ solution. A desiccator was used as a suitable vessel, as its shape eliminates dripping of the condensing water onto the samples. For the same reason, Petri dishes were tested open. The vessels were placed in a cultivation box. The test lasted 28 days. During the incubation, the culture vessel was opened once a week in the biohazard box, exchanging air and documenting the condition.
During the test and at the end of the test, the growth of fungi on the test bodies and the nutrient medium in the vicinity was visually evaluated. Mold spores in both positive controls must be germinated within 6 days of incubation. Resistance is then assessed as follows:
• fungicidal material - mold does not develop, inhibitory zone is visible on substrate agar,
• fungistatic material - mold does not develop on specimen, colonies are not visible in microscope, the mold grows on agar.
• Resistant material - Quantification 1–5 by scale:
  1 - slight growth, growth visible only in microscope, scattered colonies
  2 - gradual growth - numerous small colonies, up to 25% of the surface
  3 - intense growth, up to 50% of the surface
  4 - very intense growth, up to 75% of the surface
  5 - full growth - 100% of the surface

3. Testing procedure
A number of samples were used to verify the design of the methodology - pulp, filter paper impregnated with Ajatin, fiber-cement board, standard concrete and a set of concretes with various additives. The testing bodies were sized 65 × 30 × 10 mm or 50 × 50 × 10 mm. The spore suspension was prepared from a collection of strains kept in the laboratory, using the following strains: *Aspergillus versicolor*, *Penicilium purpurogeum*, *Phoma sp.*, *Alternaria alternata*, *Cladosporium cladosporoides*.

The tests were carried out according to the described test design and were incubated and monitored for 6 months.

![Figure 1. Petri dishes in the cultivation vessel.](image)

4. Results and evaluation
Tests of all materials were conducted according to the described methodology and their results corresponded to the composition of the sample. Parallel determinations showed insignificant differences and were evaluated consistently. Spore germination was observed on day 3 after inoculation. The prolonged cultivation time confirmed no changes in molding and change in overall material evaluation(see figures 2 and 3). Therefore, it is unnecessary to maintain the standard testing period of 3–6 months.
Figure 2. Samples after 28 days of incubation.

Figure 3. Samples after 6 months of incubation.

The results of individual sample testing are given in table 1. Figures 4 and 5 are photographs of samples graded 1 and 5, respectively.

Table 1. Evaluation of the resistance against mold testing.

| Sample                                | Evaluation |
|---------------------------------------|------------|
| Ajatin 6%                             | fungicidal |
| Pulp                                  | 5          |
| Standard concrete                     | 1          |
| glass-fiber-cement board              | 1          |
| Concrete with admixture 1             | 2          |
| Concrete with admixture 2             | 4          |
| Concrete with admixture 3             | 3          |
| Concrete with admixture 4             | 1          |
| Concrete with admixture 5             | 2          |
| Concrete with admixture 6             | 4          |
| Concrete with admixture 7             | 5          |
| Concrete with admixture 8             | 3          |
| Concrete with admixture 9             | 4          |
All grades of non-resistant materials were observed in the evaluation, only the biocidal product was fungicidal. The materials evaluated as grade 1 showed fungistatic effect on smooth surfaces, but small colonies grew on the areas damaged by cutting during sample preparation (see figure 6). This should be taken into account during preparation of testing specimens and their evaluation.

**Figure 4.** Sample of glass-fiber-cement board grade 1.

**Figure 5.** Sample of concrete with admixture 7 grade 5.

**Figure 6.** Growth of small colonies on cut surfaces.

5. **Discussion**

For testing of mold resistance in laboratory conditions, a number of standardized methods are available, apart from those mentioned above [7, 8, 9], mainly the ASTM C1338 [10] and ASTM G21-96 [11] methods and their derivatives. Some are intended for a particular type of material, others for a whole
group of materials. The principles of these methods are generally the same: mold spores are introduced onto surface of test material samples, incubated in a suitable environment and after several weeks, typically four, the surfaces of the test specimens are analyzed for mold growth. The methods differ particularly in the mold species used, the density of spores and the method of inoculation, the conditions of cultivation (relative humidity and temperature), the use of nutrient media and the final evaluation of the test. Some factors and their influence on the final results are discussed by professional public. Typically, five species of microscopic fungi are used to inoculate, and the methods combine different species. However, each mold has different ability to grow on different building materials, even under the same conditions, and also has different moisture requirements [6]. The use of nutrient media is also discussed as the real building materials are less nutritive, so the representativeness of the test decreases in presence of the medium [6]. Standard test protocols rely on the high relative humidity of the environment as a source of moisture, but it can be assumed that building materials will occasionally be exposed to direct humidity, however briefly. Real moisture and temperature fluctuations also occur in real constructions [12]. Furthermore, the microscope evaluation method is questionable, as the observer can overlook the mold growth without pigmentation [12]. Given all these controversies described, literary sources recommend caution when interpreting the results because in real applications, conditions are uncontrollable [12].

6. Conclusions
While mold resistance tests provide an overview of short-term performance of the samples under controlled conditions, they offer little information on the durability of long-term product performance in real-world applications. This must be taken into account when extrapolating the test results into real conditions. Also, the test results represent no metric value of absolute mold resistance for comparison between tests or between products.

This work aimed to propose a simple, technically low-demanding methodology for testing the resistance of building materials against mold infestation. The proposed methodology was validated on real samples and is suitable for screening material resistance, especially during research and development of new materials.

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