Case study of fungal growth on newly cast concrete floors

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Abstract. In several cases, the Danish Technological Institute has experienced widespread fungal growth on newly cast concrete floors, with a moisture barrier and floating wooden flooring. The reason for fungal growth is usually due to an inadequate drying period. Existing recommendations require that the relative humidity (RH) of air in equilibrium with the concrete, measured in the middle of the concrete, should not exceed 85-90% RH. In this study, six randomly picked apartments in a newly built apartment complex, were chosen for a case study of fungal growth and moisture on newly cast concrete. The study demonstrates that at least some species of fungi can grow very well on newly cast concrete if the surface is dusty and moist. The study also demonstrates that a few samples on the surface will often be representative for the whole floor. The study finds that there is a need to revise the existing guidelines for acceptable moisture content in the concrete before mounting the floor. This might have an impact on the entire building process and/or the design of the floor construction. The study also finds that there is a need for a guideline for measuring moisture and fungal growth on newly cast concrete floors.

1. Introduction
Many studies have found that fungal growth and moisture damage in buildings will lead to an unhealthy indoor environment for the building occupants [1–5]. Fungal growth in buildings should therefore be avoided and, when discovered, eliminated.

In several cases, the Danish Technological Institute has experienced widespread fungal growth on newly cast concrete floors. This is often seen in a typical floor construction where a moisture barrier and floating flooring is laid. The purpose of the moisture barrier is to protect the wooden floor against moisture from the concrete. However, what happens on the surface of the concrete has largely been ignored. It is generally assumed that fungal growth is unable to establish on newly cast concrete, due to its high pH of 12-14. However, although most fungi grow at a pH range of 3-8, some Aspergillus species, e.g., Aspergillus versicolor, can grow just as well at a pH above 10 [6].

Fungal growth on the concrete floor is usually due to an inadequate drying period of the newly cast concrete [7]. Before laying of the wooden floor, moisture should be measured within the concrete slab at a depth of 0.4 times its thickness, and existing national Danish recommendations require that the relative humidity (RH) of air in equilibrium with the concrete, should not exceed 85-90% RH [8,9]. The moisture content in the middle of the concrete floor will be considerably higher than at the surface, due to evaporation. When a moisture barrier is laid on the surface of the concrete, the moisture content in the concrete floor will equalize. However, a study finds that fungal growth can establish on any surface, if the RH at the surface, e.g., a concrete floor, exceeds 75% and the surface is dusty [10]. It is therefore relevant to investigate whether a reassessment of the existing guidelines for measuring moisture content of the concrete before laying the flooring, is necessary to better accommodate the risk of fungal growth on the surface of concrete floors, and not only the risk of damaging the flooring.

It is often not possible to see the extent of fungal growth on the surface with the naked eye – especially on concrete – so, sampling on the surface of which fungal growth is suspected is necessary. Choosing the most optimal sampling method is key to getting an accurate picture of the extent of fungal growth on the surface. For commercial use in Denmark, the three most used sampling methods are contact agar plate sampling, sticky tape sampling and NAHA-enzyme based biomass sampling [7].
The assessment of the extent of fungal growth and the need for remediation, is often based on only one or two samples per 50-100 m² of surface/construction, which are assumed to be representative of the entire surface. This has led to discussions on whether these few samples are adequate to determine the need for remediation of the entire construction. Extended focus, and increased knowledge, on how to avoid fungal growth in new constructions, and how to efficiently assess the need for remediation would help prevent or reduce the waste of resources and ultimately lower the cost of building.

In this study, we examine fungal growth on newly cast concrete floors and compare two different sampling methods: sticky tape sampling for direct microscopy and contact agar plate sampling for cultivation, identification and counting of fungi.

We test 1) whether sticky tape and agar plate sampling methods lead to similar conclusions regarding the extent of fungal growth, 2) whether a few samples will be representative of the extent of fungal growth in the entire construction and 3) whether the current recommended limit of 85-90% RH in the middle of the concrete floor is sufficient to avoid fungal growth. The results are evaluated in relation to the existing guidelines for mounting wooden floors on newly cast concrete.

2. Methods
Six randomly picked apartments (A-F) in a newly built apartment complex, were chosen for a case study of fungal growth on newly cast concrete floors. In all six apartments, the floor construction was made with hollow core decks, 100 mm of expanded polystyrene (EPS) concrete, a noise reducing membrane, approximately 60 mm of wear layer with floor heating, a moisture membrane, and a wooden flooring on top (figure 1). We were informed that the surface was vacuumed but not thoroughly cleaned before laying of the flooring. Two apartment sizes were included in the study: 44 m² and 86 m². Fungal growth samples and moisture measurements were taken a year and a half after the concrete floor was cast.

2.1. Are sticky tape samples and agar plate samples comparable?
Sampling for fungal growth was primarily conducted using contact agar plates containing V8-agar, which is commonly used when sampling for fungal growth in buildings [11]. Each plate has an area of 24 cm² and was cultivated at 26 °C for 6-7 days before the total number of colony-forming units (CFU) were counted and the cultures identified. The extent of fungal growth was divided into four categories based on number of CFU (table 1). Simultaneously, sticky tape samples with an area of 6.25 cm² were taken at 16 sampling points across all apartments. These were analysed by microscopy at 200 times magnification. At least 15 fields of view were analysed. Each field of view has a grid of squares, each of them 50 x 50 µm, and the percentage of squares covered by mycelium and number of spores were recorded. Averaging the recorded percentages and spore counts for each sample results in an overall evaluation of sticky-tape samples in four categories (table 1) similar to those for agar plate sampling.

To test whether results from contact agar plates and sticky tape samples (based on spore count or mycelium fragments) differed significantly, we converted categories to values from 0 to 3, see Table 1. We then ran a Friedman test, accounting for sample location, followed by a Wilcoxon signed-rank test, while correcting for multiple comparisons using the Bonferroni correction [12] in R version 4.0.4 [13].
Table 1. The extent of fungal growth from none to substantial based on sampling method. Contact agar plate samples are evaluated using the number of colony-forming units (CFU) and sticky tape samples are evaluated based on the number of spores or the percentage cover of mycelium fragments.

| Sample type            | None (0) | Low (1)         | Moderate (2)                     | Substantial (3)                   |
|------------------------|----------|----------------|----------------------------------|-----------------------------------|
| **Contact agar**       | 0 CFU    | <10 CFU         | 10-50 CFU                        | >50 CFU                           |
| **Sticky tape – Spores** | 0 spores per cm² | 500-2000 spores per cm² | 2000-5000 spores per cm² | >5000 spores per cm² |
| **Sticky tape – Mycelium fragments** | 0% of squares covered | 0-10% of squares covered | 10-70% of squares covered | >70% of squares covered |

2.2. Are a few samples enough to determine extent of fungal growth?

Initial sampling was carried out in all six apartments, by creating two openings in the flooring of each apartment, one in the kitchen and one in the bedroom next to the bathroom (figure 2).

![Figure 2. Layout of apartments A-F. Sampling points with substantial fungal growth = full circle and a cross in red. Sampling points with moderate fungal growth = dotted circle and a cross in yellow. Sampling points with no sign of fungal growth = full circle in green. Apartments B, C, D, F = 44 m² and Apartments A and E = 86 m².](image-url)
The accuracy of the sampling results from the initial sampling compared to the entire surface, was investigated by removing the wooden flooring in all six apartments and conducting control sampling widespread over the surface. In the first apartment that was investigated (A), 22 sampling points were selected (figure 2). It was later decided that between 6-10 sampling points were sufficient to investigate if the two initial sampling points were in fact sufficient. Thus, for the next five apartments (B-F) only between 6-10 sampling points were selected (figure 2). The sampling was conducted on the concrete surface by opening the moisture membrane immediately before sampling, to avoid contamination of the concrete surface prior to sampling. Through R version 4.0.4 [13], we used a Kruskal-Wallis test for each apartment to examine whether there was a significant difference in the results obtained from the two initial samplings compared to the extended control sampling.

2.3. Is fungal growth avoided if the relative humidity is below 85-90%?
Relative humidity and temperature were measured on the surface of the concrete floor by inserting a slim RH and temperature measuring probe (Testo) between the moisture membrane and the concrete floor. Initially the wooden flooring was gently lifted with a crowbar to insert the probe under the moisture membrane. The probe was left until it had reached equilibrium, or for at least 30 min, before the results were noted. After the wooden flooring was removed, a small hole was made in the moisture membrane before the sampling for fungal growth, and through the hole, the probe was inserted under the moisture membrane and left until it had reached equilibrium, or for at least 30 min. The instruments were calibrated both before and after the investigations. After measurements were recorded, a larger hole in the membrane was made to conduct sampling for fungal growth.

The results from the moisture measurements were binned into three groups based on RH (< 75%, 75-90%, > 90%). To test whether there was a significant difference in CFUs depending on the RH level we ran a Kruskal-Wallis test in R version 4.0.4 [13].

3. Results
In figure 2, an overview of sampling points (62 in total) in each apartment is given. Sampling points marked with red (41 samples), had substantial fungal growth on the contact agar plates, >50 CFU of the same species. Samples marked with yellow (14 samples) had moderate fungal growth, 10-50 CFU of the same fungal species. Samples marked with green (seven samples) had low fungal growth, <10 CFU of the same species. In all six apartments the concrete surface was slightly dusty from the building process, although the concrete floors were cleaned by the carpenters for visible dirt before mounting the top floor. At 27 of 62 sampling points RH was between 75% and 90%. At 22 sampling points RH was above 90% and at 13 sampling points RH was below 75%. An overview of the results of moisture measurements and fungal sampling on contact agar plates is shown in table 2.

Table 2. Results of moisture measurements and fungal sampling on contact agar plates. n = number of sampling points. Asp = Aspergillus versicolor, Pen = Penicillium sp.

| Apartment | n | Relative humidity (RH) | Temperature [°C] | Growth on contact agar plates |
|-----------|---|------------------------|------------------|-----------------------------|
|           |   | <75%  | 75-90% | >90% |                      |
| A         | 22 | 4     | 11     | 7    | 23-24               |
| B         | 6  | 1     | 1      | 4    | 23-24               |
| C         | 10 | 1     | 4      | 5    | 18-25               |
| D         | 10 | 5     | 3      | 2    | 23-25               |
| E         | 7  | 1     | 4      | 2    | 23-25               |
| F         | 7  | 1     | 4      | 2    | 23-25               |
| n         | 62 | 23    | 27     | 22   | 18-25               |

3.1. Are sticky tape samples and agar plate samples comparable?
Sticky tape sampling was conducted at 16 of the 62 sampling points. In 15 samples no sign of fungal growth was detected. In one sample moderate levels of mycelium fragments and spores were detected.
In eight of the 16 matching contact agar plate samples, substantial growth was found (>50 CFU of the same species). In five samples, moderate CFU levels (10-50 CFU) were found. In three samples, low CFU levels (<10 CFU) were found.

Average category values were 2.3 ± 0.79 SD for agar plate samples (moderate growth), 0.44 ± 0.63 SD for sticky tape samples based on mycelium cover (no growth) and 1.06 ± 0.25 for sticky tape samples based on spore count (low growth; figure 3). A Friedman test showed significant difference ($\chi^2(2) = 22.4$, $p < 0.001$) in the categorisation of fungal growth (none=0, low=1, moderate=2 or substantial=3) depending on the method used (CFU count on agar plates, spore count on sticky tape or mycelium fragment cover on sticky tape). A follow-up Wilcoxon signed-rank test using Bonferroni correction showed a significant difference in categorisation between all methods (CFU count and spore count: $p = 0.005$, CFU count and mycelium fragment coverage: $p = 0.002$ and spore count and mycelium fragment coverage: $p = 0.006$).

3.2. Are a few samples enough to determine extent of fungal growth?

Mean CFU count was lower for the initial samples compared to the control samples for apartment A (initial = 2 ± 1.41 SD, control = 44.15 ± 37.96 SD), B (initial = 33 ± 35.36 SD, control = 95.25 ± 28.25 SD), C (initial = 63.50 ± 51.62 SD, control = 86.88 ± 45.98 SD) and E (initial = 52.50 ± 3.54 SD, control = 88.60 ± 33.83 SD) and higher for apartment D (initial = 71.50 ± 54.48 SD, control = 68.25 ± 36.87 SD) and F (initial = 115 ± 21.21 SD, control = 74.20 ± 42.93 SD). However, a Kruskal-Wallis test showed no significant difference in mean CFU count of the two initial samples compared to the follow-up samples for any of the apartments (A: $\chi^2(1) = 3.01$, $p = 0.08$, B: $\chi^2(1) = 1.93$, $p = 0.16$, C: $\chi^2(1) = 0.85$, $p = 0.36$, D: $\chi^2(1) = 0.02$, $p = 0.9$, E: $\chi^2(1) = 1.35$, $p = 0.25$ and F: $\chi^2(1) = 2.24$, $p = 0.13$), cf. figure 4.
3.3. Is fungal growth avoided if relative humidity is below 85-90%?

Mean CFU count was 60.62 ± 46.76 SD for RH < 75%, 60.46 ± 44.05 for RH 75-90% and 72.05 ± 37.24 SD for RH > 90% (figure 5). A Kruskal-Wallis test showed no significant difference in fungal growth (measured as CFU) between the three intervals of RH ($\chi^2(6) = 8.47, p = 0.21$), cf. figure 5.

**Figure 5.** Comparison of CFU count based on agar plate sampling at the surface of the concrete floor at three intervals of relative humidity (RH) measured between the concrete floor and the moisture barrier.

4. Discussion

The present study finds significant differences in fungal growth estimate depending on sampling method. Using contact agar plate sampling, widespread fungal growth over the surface was found, whereas sticky tape sampling only showed significant signs of fungal growth in one of 16 samples. Sticky tape sampling may severely underestimate the extent of fungal growth due to the uneven surface of the concrete [7], which results in reduced contact with the sticky tape. Contact agar plates will have better, if not 100% contact. Sticky tape sampling will also only find signs of fungal growth if sampling is done directly on the growth area, whereas contact agar plate sampling can find growth at a nearby area if viable fungal fragments are spread across the surface.

Because of the overall low estimate of fungal growth based on sticky tape sampling, we suggest that the two methods should be evaluated differently, at least when sampling newly cast concrete. Substantial growth is suspected if more than 50 CFU are found on a contact agar plate sample (as per usual). If sticky tape samples show more than 500 spores per cm$^2$ or if some of the squares are affected by mycelium (formerly classified as 1 – low growth) further sampling should be conducted. In short, sticky tape sampling can be a valuable first step, but on detection of spores or mycelium fragments, the sampling should be followed by other sampling methods, such as agar plate sampling.

The study shows no significant difference between the two initial samples taken in each apartment, and the control samples. Also, the figure showing the locations of the sampling (figure 2) demonstrate that the fungal growth was found to be widespread across the surface. This indicates that a few samples on the surface will often be representative of the whole floor and that the conditions are often similar across a coherent and comparable floor structure. However, it should be kept in mind that knowledge of the moisture levels and causes of the detected fungal growth plays a defining role in evaluating a potential problem with fungal growth. Furthermore, although no significant difference was found, average CFU count was lower for the initial samples than the control samples for four of the six apartments.

It is often stated that fungal growth on newly cast concrete will be limited, if possible, due to the relative high pH of the newly cast concrete. However, a few months after construction, when the building is ready for occupation, the pH value might have decreased due to decarbonatization of the concrete, as it is exposed to CO$_2$, during the drying process [14]. Further knowledge on this is necessary to better understand the potential risk of fungal growth on newly cast concrete floors.

The present study demonstrates that some species of fungi can grow very well on newly cast concrete, if the surface is dusty and moist. The most common species in this study were *Aspergillus versicolor* and *Penicillus* sp. A study by Andersen et al. (2010) has likewise found that *Aspergillus versicolor* and *Penicillus* sp. are amongst the most common fungal species found in water damaged buildings [15]. Furthermore, the study by Andersen et al. (2010) found that *Aspergillus versicolor* is often found
on concrete surfaces, and that concrete surfaces with a high moisture content in buildings are one of the most common contributors to fungal growth [15].

One caveat of this study could be, that the concrete surfaces were not cleaned properly, and that results therefore do not reflect live growth. However, the species composition found here is different from what is normally found in household dust samples in Denmark [16]. This supports our statement that the samples taken on the surface of the concrete floor, show substantial fungal growth on the surface.

Overall, we found high RH levels, well above 75%, at the surface of the concrete floor, and observation of fungal growth was widespread on the surface. This is supported by previous studies that claim that fungal growth can establish on any surfaces if the RH is above 75% and organic material is present e.g., dust or soil [10].

Initial RH between the concrete surface and the moisture membrane/flooring is unknown since measurements were taken a year and a half after the concrete floor was cast. Relative humidity may have been slightly higher at the time the flooring was laid out. Thus, at sampling points with low RH (<75%), there may have been a higher RH, just after the flooring was laid, and this may explain why no significant difference in fungal growth between the three moisture level intervals was found. To prevent fungal growth in new floor constructions, we find no evidence that allows for the flooring to be laid at a RH of 85-90% in the concrete. There is therefore a need to revise the existing guidelines for acceptable moisture content in concrete, before mounting the floor, or rethink what other measures can be taken to prevent fungal growth, e.g., a brush-on epoxy moisture barrier on top of the concrete floor. However, further research is needed. Revising the guidelines will have an impact on the entire building process and/or the design of the floor construction.

The study also finds that there is a need for a guideline for measuring moisture and fungal growth on newly cast concrete. Existing Danish guidelines state that the moisture content in the middle of the concrete floor should be measured to determine if the flooring can be laid out. However, this might be insufficient to determine RH critical for fungal growth on the surface of the concrete floor. The Danish Technological Institute has in several cases found that floor heating affects the RH on the surface of the concrete floor. This should be investigated further by a combination of simulations, field studies and controlled laboratory experiments. It would be relevant to research the connection between RH in the middle of the concrete floor and at the surface of the floor, at different scenarios, such as with or without floor heating, with or without moisture barrier and floating wooden floor vs. raised wooden floor. In addition, effects of cleaning the concrete floor or even preventive measures such as treatment with a disinfectant should be investigated.
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