GROWTH OF MOULDS ON BUILDING MATERIALS UNDER DIFFERENT HUMIDITIES

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ABSTRACT

The growth of *Aspergillus versicolor, A. ustus, Chaetomium* spp., *Cladosporium sphaerospermum, Penicillium chrysogenum, Stachybotrys chartarum, Trichoderma harzianum*, and *Ulocladium* spp. was evaluated on 27 different building materials at 25 °C and 70, 80 and 90 % relative humidity (RH) over a 7-month period.
Growth was evaluated by photographing the materials monthly, and after 7 months moulds growing on the materials were identified; biomass estimated by the MycoMeter test® and by GC-MS/MS analysis of ergosterol; and mycotoxins determined by HPLC-DAD-FLD.
Moulds grew more slowly than expected and no growth was visible on any material at 70 % RH, but at 80 % RH visible growth was seen on rough pinewood and wallpapered materials. At 90 % RH visible growth by *Aspergillus* and/or *Penicillium* on 25-75 % of the surface of all wallpapered materials, MDF, all plywood plates and pine wood. No growth was visible on pure or painted gypsum boards or concrete materials.

KEYWORDS: material, microbial growth, damp buildings, GC-MSD, relative humidity, Mycotoxin

INTRODUCTION

The association between mould growth and adverse health reactions as: extreme fatigue, headache, lack of concentration and memory, blocked nose, itching eyes, burning sensation of the skin, hoarseness, cough, asthmatic bronchitis, asthma and recurrent airway infections, especially sinusitis, has been demonstrated during the last years [1-3][1; 2].
The causal compounds, and thereby the mechanisms behind the health problems are still unknown, but moulds are capable of producing an array of biologically active compounds, especially mycotoxins which are probably involved in the health effects [3-7], volatile organic compounds (MVOC's) [8-10].
Available water is the most important factor for selecting of which moulds will actually grow on a given material [5], these moulds are called the *associated funga* [11]. The "extend" of humidity or water damage can be divided into two cases which each can be divided up in to two sub cases:

- Water damages due to leaks
  - Clean water from pipes etc.
  - Incoming water from roofs or flooding, water containing organic material will help the moulds grow
- Condensation damages
  - Massive condensation with liquid water on structure
  - High relative humidity on the material but not condensation.

Typically, only materials containing a good carbon source can support mould growth under high humidities, whereas the water ingress damages especially if the water contains organic material, will give rise to mould and bacterial growth on inorganic materials as mineral wool and concrete [12].
When looking at the high humidity damages, it is economically very important to know the minimal water activity (a_w-min) which can support fungal growth on different materials. These data are the basis of computer models, which can predict if constructions can support fungal growth. Several pa-

pers have suggested a_w-min values around 0.67-0.80, but growth also depends on temperature, time and composition of the material [13-19].
When testing materials for mould growth, the materials have usually been inoculated using a spore suspension, but spores will already start to germinate in the suspension and the water from the suspension will for some time give a much higher a_w in the top layer of the material [17; 20].
Several methods for assessing mould growth on building materials have been used, including visual inspection, electron microscopy [15; 17; 21], bio-markers as ergosterol [22] and chitinase determined by the Mycometer Test® [23].
This paper describes how typical moulds found in damp Danish buildings [12; 24] grew on different building materials at 25 °C and 70, 80 and 90 % relative humidity (RH) over a 7-month period.

METHODS

Conditioning of materials

The materials (typically 9 – 13 mm thick) were cut in plates of 20×14 cm, preconditioned at 65% RH for 3 months and placed in polyethylen boxes (21×15×5 cm). Water was applied, corresponding to what the materials would have to absorb to reach equilibrium at 70, 80 and 90 % RH respectively and the materials immediately packed in water tight polyethylene bags and X-ray sterilised (30 kGy γ-radiation).

Inoculation

Materials were inoculated using a dry cotton swap by a mixture of the 15 mould isolates shown in Table 1.

Table 1. Moulds used in this study.

IBT no.	Mould	IBT no.	Mould
16000, 18238	*Aspergillus versicolor*	9153, 9150	*Trichoderma harzianum*
8824, 8828	*Chaetomium* spp.	7168, 7719	*Ulocladium* spp
14920, 15904	*Penicillium chrysogenum*	7710, 7928	*Cladosporium sphaerospermum*
9460, 9466	*Stachybotrys chartarum*	14925	*Aspergillus ustus*

All strains used in this study have been isolated in mouldy buildings, have been identified and cultivated as described by Samson *et al.* [25], and are held at the IBT Culture Collection, Department of Biotechnology, Technical University of Denmark. *Aspergillus* and *Penicillium* cultures were identified to species level using secondary metabolite profiling and traditional identification methods [26; 27; 27; 28].

Incubation

Boxes with the materials were incubated for 7 months at 25 ± 1 °C, and 70 ± 2, 80 ± 2 and 90 ± 2 % RH, in 220 L stainless steel chambers placed in a room with a constant temperature. To each steel chamber, a hygrostate controlled the ratio of sterile filtered dry- and humid air. Two to three times a week the humidity in the chambers was checked with a Testo 610 (Testo, Lenzkirch, Germany) calibrated at a nationally accredited institution.

Growth evaluation

Growth was evaluated by photographing the materials monthly, and at the end of the incubation period the following analyses was performed:

- Colonies on the materials were identified after isolation on V8 agar. *Penicillium* and *Aspergillus* species were cultivated in 3 point cultures on Czapek yeast autolysate agar (CYA), MEA, yeast extract sucrose agar (YES), Creatine Sucrose Agar (CREA) at 25°C and *Aspergillus* also on CYA for identification [25].
- Materials were inspected visually and under a stereo microscope.
- Biomass estimated by GC-MS/MS analysis of ergosterol (ERG). Shortly, a 1-2 mm thick disk, 10 mm in diameter, was cut out of the material, 4-D_2-ergosterol (internal standard, IS) was added,

the sample was saponificed and extracted with pentane. ERG was derivatised to TMS-ether, and analysed on a Finnigan GCQ, operated in MS/MS mode [29].

- Biomass estimated by enzymatic activity of chitinase (Mycometer test®, MycoTec, Copenhagen)[23].
- Fungal colonies were scraped off the materials, extracted with methanol, and analysed by High Performance Liquid Chromatography (HPLC) coupled to a diode array detector (DAD) and a diode array fluorescence detector (FLD) [4; 5; 28], UV spectra of the peaks in the chromatograms were compared with reference standards of secondary metabolites and mycotoxins (approx. 400 compounds).

RESULTS

Growth on the materials was often very unequally distributed, meaning that certain areas would be totally without growth whereas some areas would be completely overgrown.

Chemical markers

No growth was recorded at 70 % RH on any material, but as seen in Table 2, at 80 and 90 % RH both the wooden materials and the wallpapered materials supported growth.

Table 2. Chemical markers measured after 7 months

		80 % relative humidity				90 % relative humidity			
		Mycometer		Ergosterol		Mycometer		Ergosterol	
No.	Material	(mLU)*		(ng/cm^2)		(mLU)*		(ng/cm^2)	
1	Calcium silicate plate	-8	-5	ND	ND	234	4	ND	ND
2	Painted[2] glass fibre wallpaper on wet zone gypsum board	242	-19	ND	ND	298	367	467	590
3	PVC floor on wet zone gypsum board	79	11	ND	ND	0	50	ND	ND
4	Wallpapered[3] chipboard (DDT)	1549	105	500	ND	0	632	780	977
5	Painted[1], wallpapered, gypsum board	64	69	370	147	1296	576	1112	5883
6	Painted[1] gypsum board (Fab. 2)	64	43	ND	ND	358	134	256	ND
7	Gypsum board (fibre gypsum)	58	53	ND	72	10	10	473	478
8	MDF plates	1846	810	615	190	1881	3147	1477	1760
9	Beech wood floor boards, previously mould contaminated[4]	460	1420	893	1593	1802	2375	1297	2869
10	Old plywood (25 years)	581	10	ND	224	908	739	792	1018
11	Mould contaminated plywood[5]	495	652	612	1132	603	375	1480	1657
12	Plywood	-3	-16	ND	ND	163	241	2008	633
13	Paper wool insulation	8	-20	ND	ND	-12	21	144	438
14	Mineral wool (MMMF)	49	-12	84	79	26	45	47	656
15	Pine wood on mineral wool (MMMF)	14	-6	616	43	3500	1086	1162	3974
16	Pine wood, contaminated with earth	165	67	2012	ND	530	1940	1601	912
17	Pine wood	1406	220	1413	2698	3500	3500	5817	3397
18	Planed pine wood	1440	18	525	ND	484	221	1735	6543
19	Wallpapered[3] levelling layer	1019	595	2025	127	1998	1243	1505	1015
20	Levelling layer	3	-18	ND	ND	32	2	ND	ND
21	Levelling layer (concrete)	156	6	ND	ND	133	39	ND	ND
22	High density concrete	58	-11	ND	ND	-2	60	ND	ND
23	Wallpapered[3] low density concrete	712	757	ND	ND	401	1235	2241	901
24	Low density concrete	-4	-22	ND	36	33	38	ND	ND
25	Wallpapered[3] gypsum board	697	220	106	99	3500	277	2601	961
26	Gypsum board	57	29	ND	ND	9	34	ND	496
27	Gypsum board	121	12	58	ND	506	130	600	549

*Mili-Luminans units, -20–25 mLU means no enzyme present, 25-150 mLU very little growth, 150 mLU – means active growth, max. Value 3500 mLU. ND: Not Detected. [1]Paint: White acrylic. [2]Wet zone paint. [3]Wallpaper glue, 100 % Cornstarch. [4]Ergosterol, ND prior to test. [5]Contaminated by *Ulocladium*, ergosterol prior to inoculation 247 and 165 ng/cm^2.

Generally the ergosterol contents correlated with the Mycometer Test®(Tabel 2), although the unequally distributed mould growth makes a thorough comparison of the two methods impossible based on this study.

Table 3. Results from visual and microscopic inspection of the materials after 7 months and isolation and identification of moulds growing on the materials.

No	80 % relative humidity	90 % relative humidity
1	-	Single colonies seen in MIC, PC
2	-	One colony visible, very hard also in MIC. (AV)
3	-	-
4	Growth of PC and AV on 20% of material	Growth on part of material, AV and PC
5	-	Yellow visible growth on most of the material, mixture of PC and AV.
6	-	Only growth a few places, PC and AV, in MIC Aspergilli heads were seen.
7	-	-
8	Two small A. flavus* colonies (approx 1 cm in diameter)	Massive growth over 60% of material, in MIC. 100% covered, 60% with olive Aspergillus growth and 40% with grey PC
9	Very hard to se anything visually, but in MIC conidiophores were seen on 40% of the material	Massive growth over whole material, MIC. mix of A. flavus, PC and AV.
10	Visually nothing, in microscope one could see PC conidiophores	Grey layer over most of material, mix. of A. flavus, AV and mostly PC
11	-	Grey layer over most of material, PC
12	-	Part of material was covered, but in MIC whole material was covered with fine mycelia of AV
13	-	White crystals, probably borates from material, no growth.
14	-	-
15	Visually nothing, in MIC fine mycelia and few conidiophores were seen on parts of the material.	Massive growth on material, mix of AV and mostly PC
16	Nothing visible, in MIC 5-6 pinpoint colonies with conidiophores was seen	Growth of 2-3 Eurotium sp. *, in MIC fine mycelia of PC covered rest material
17	70% covered by growth of AV and PC, easy to see.	Massive growth, mix of PC and AV
18	Grey over 30 % of material, growth of both PC and AV	Massive growth, mix of PC and AV
19	Massive growth, mix of PC, AV and a little A. flavus*	Massive growth, mix of PC and AV
20	-	-
21	-	-
22	-	-
23	Massive growth, mix. of PC and AV	Massive growth of PC and AV
24	-	-
25	Light growth of PC and AV	Massive growth of PC and AV
26	-	Nothing to see visually on in microscope
27	-	Few visible colonies, AV

MIC. : Microscope. – No growth. *Contaminant. *AV*: *Aspergillus versicolor. PC*: *P. chrysogenum*

As seen in Table 3, only two genera (*Eurotium,* anamorph *Aspergillus*) grew on the materials, which supports our general idea that the genera: *Ulocladium*, *Cladosporium*, *Stachybotrys*, and *Trichoderma* should be considered “water damage“ moulds that will grow only in constructions due to water ingress or massive condensation.

It was clear that the visual inspection was only good when the materials were heavily infested and should be supported by microscopy of "non mouldy" materials. The chemical markers could detect less growth than the visually based methods as well as assessing the mould growth are much easier.

Growth on the different materials
None of the materials was able to support mould growth at 70% RH, but the wooden materials supported massive growth down to 80% relative humidity, as it was clear that the rougher the surface, the faster the moulds grew. Wallpapered surfaces also supported mould growth at 80% RH, but growth was not as fierce as on the wooden materials.
No growth was visible at 80 or 90% RH on the 3 types of gypsum boards, not even using the stereo microscope. However, at 90% RH the ergosterol level at up to 600 ng/cm^2 and Mycometer levels up to 500 mLU showed that modest levels fungal mycelium present, probably the mycelium is extremely difficult to distinguish from the fibrous material surface.
The inorganic materials, as calcium silicate, mineral wool and concrete materials did not support fungal growth, as these materials do not contain an organic carbon source.

Analysis for secondary metabolites and mycotoxins
Although quite large quantities of biomass were scraped off the materials, no structurally known metabolites fungal except ergosterol were detected at any material. Aflatoxins were not detected (< approx. 50 pg on the FLD) on the materials (# 8, 9, 10 and 19) infested with *A. flavus*, or from CYA or YES agar cultures of the isolates. When comparing with mould infested water damaged materials previous analysed [4; 5], it seems like the secondary metabolism have been shifted off.

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