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Artificial vs natural *Stachybotrys* infestation—Comparison of mycotoxin production on various building materials

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Abstract

The genus *Stachybotrys* belongs to filamentous fungi found in indoor environment, mostly on cellulose-rich substrates after water-damage. The major purpose of this study was to investigate the influence of different building materials in case of mold infestation on the mycotoxin production of *Stachybotrys* species. Fifteen *Stachybotrys* mycotoxins including satratoxins, phenylspirodrimanes, and recently discovered stachybotrychromenes were in the focus of the investigations. Artificial and natural infestations were compared to determine whether environmental factors, for example, time of growth, temperature, humidity, and material additives have an influence on the observed mycotoxin profiles. It turned out that mycotoxin profiles from *Stachybotrys* spp. on building materials can be influenced by cellulose, paints, and paste of the materials. The total toxin levels of artificially and naturally contaminated gypsum board samples ranged up to 30 µg/cm^2, whereas wallpaper samples showed total toxin levels in the range of 20-66 µg/cm^2. A naturally infested sample disclosed the conversion of the dialdehyde components to the corresponding lactone isomers under the influence of light.

KEYWORDS

building material, LC-MS/MS, phenylspirodrimanes, satratoxins, stachybotrychromenes, *Stachybotrys*

1 | INTRODUCTION

Mold growth in the indoor environment can generally cause allergic, infectious, or toxic symptoms in humans.[1,2] It is conceivable, that spores or mycelium might potentially serve as fungal fragments for indoor air contamination in the course of the aerosolization process[3] and that inhalation exposure along this route can lead to adverse health effects. In this context, various respiratory diseases were frequently reported and proved to be associated with mold in damp buildings.[4-7] In terms of potential toxic health effects, mycotoxins are regarded as health hazards.[8] Mycotoxins are defined as secondary metabolites produced by fungi and exhibiting toxic activities, based on in vitro toxicity toward human and vertebrate cell lines with corresponding IC_{50} values <1000 µmol/L.[9] The genus of the filamentous fungus *Stachybotrys* features a diversity of mycotoxins, primarily belonging to classes of macrocyclic trichothecenes (MCTs), atranones, and phenylspirodrimanes (PSDs).[10] *Stachybotrys* is one of the world's ten most feared fungi,[11] especially due to the cases

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of stachybotryotoxicosis in agriculture[12,13] and pulmonary hemorrhage in infants exposed to infested buildings.[14] Commonly, water-damaged buildings containing cellulose-rich materials are preferable environments and substrates.[15,16] Although the class of the PSDs is the most abundant among the mycotoxins produced by *Stachybotrys* spp.,[17] most investigations regarding *Stachybotrys* contamination in the indoor environment focused on the highly toxic MCTs, such as satratoxins, verrucarins, and roridins.[18-23] Aleksic et al recently investigated building materials artificially contaminated with *Stachybotrys*; however, only the MCT production was studied.[24] *Stachybotrys chartarum* is classified into two chemotypes, namely A and S; chemotype S (CT S) strains produce the class of the MCTs in addition to the PSDs, whereas strains of the chemotype A (CT A) are capable producers of atranones and PSDs. Besides, the same applies for *S. chlorohalonata*, which was segregated from *S. chartarum*.[25] Furthermore, it should be noticed, that approximately only one-third of the *Stachybotrys* species found in the indoor environment belong to *S. chartarum* CT S.[16] Other studies addressed the volatile organic compounds emitted by *Stachybotrys* during growth[26] or hemolysins like stachylysin.[27] But the fact that the PSDs significantly occur in higher levels in both CTs and include a versatile variety of bioactive compounds,[28] is evidence for the potential of these compounds in regard to further research and investigations. Thus, Došen et al demonstrated that PSDs are relevant in dust samples and can putatively serve as exposure biomarkers for *Stachybotrys* growth in the indoor environment.[29] Likewise, the pre-contamination of new gypsum plasterboard (GB) with fungal species such as *Stachybotrys* is of relevance, which can significantly increase fungal growth after water damage.[30] *Stachybotrys* growth is particularly observed on wallpaper and gypsum[31] and mycotoxin production on artificially and naturally infested building materials was rather studied for other genera.[32,33] Therefore, the objective of this study was to elucidate the toxigenic potential of *Stachybotrys* growth on different building materials. Based on a developed high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) method,[34] several *Stachybotrys* toxins with focus on the PSDs (Table 1) were investigated. Distinct *Stachybotrys* species were grown on various building materials such as woodchip-fleece wallpaper (wfWP), textured vinyl wallpaper (tvWP), gypsum plasterboard (GB), and gypsum plasterboard moisture resistant (GBmr) to compare corresponding toxin production. Ultimately, artificial and natural infestation was compared in order to estimate whether the unacquainted environmental factors potentially influence the overall profiles of the produced mycotoxins.

Practical Implications

- Growth of the filamentous black mold *Stachybotrys* in indoor environments can be of health concern.
- The production of several *Stachybotrys* toxins on building materials such as woodchip-fleece-wallpaper, textured vinyl wallpaper, gypsum plasterboard was investigated to evaluate particularly the relevance of phenylspirodrimanes.
- The immunosuppressive phenylspirodrimanes can reach levels in the μg/cm^2 range on building materials infested with *Stachybotrys*.
- The obtained data are of relevance for future risk assessment.

2 | MATERIALS AND METHODS

2.1 | Solvents, reagents, and mycotoxin standards

All solvents used in this study were of LC-MS grade quality. Acetonitrile (MeCN) and methanol (MeOH) were purchased from Fisher Scientific. The used ASTM type 1 water was produced with a Purelab Flex 2 system (Veolia Water Technologies). Potato dextrose broth (ready-to-use) was purchased from Sigma-Aldrich. Formic acid (FA), sodium acetate, and sodium dodecyl sulfate (SDS) were purchased from Merck KGaA and glycerol (anhydrous) from Applichem. Tris-HCl, NaCl, Na_2EDTA, isopropanol, and agar (Kobe I) were purchased from Roth GmbH.

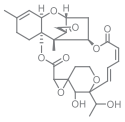
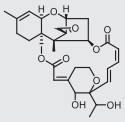
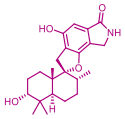
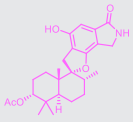
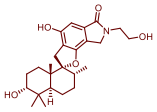
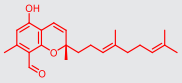
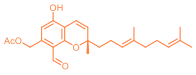
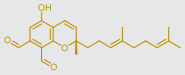
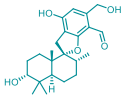
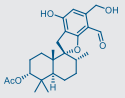
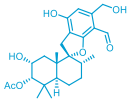
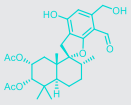
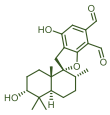
Stachybotrys toxins were isolated and characterized as previously described (Jagels et al[34,35]) and stored in MeCN and MeOH stock solutions at −20°C.

2.2 | Origin of fungal strains for artificial contamination and of the naturally contaminated samples

For artificial contamination of the materials, *S. chartarum* CT S IBT 40293 and *S. chartarum* CT A IBT 40288 were kindly provided by M. Gareis (Ludwig-Maximilians-Universität München, Germany). *S. chartarum* CT S DSM 12880 was purchased from Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). *S. chlorohalonata* CBS 109283 was obtained from the Westerdijk Fungal Biodiversity Centre (CBS, Utrecht, the Netherlands).

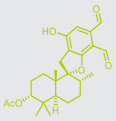
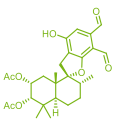
Seven naturally contaminated samples (samples A-G) were collected in Germany from water-damaged buildings. Sample A was a painted woodchip WP (wWP, unknown fleece content) from an infested bedroom and sample B a painted textured fiberglass WP derived from a living room. Sample C was a synthetic WP sampled out of a showroom. The samples D-G were all GBs, whereas sample D was originally from a storage room in a school and samples E, F, and G from contaminated office rooms. The materials visibly infested with mold were sampled in situ, and pieces were cut out from the contaminated places and taken to the laboratory for further treatment. Microbial co-contamination of other genera or bacteria was not pursued, and therefore, no statement can be made regarding the influence of other microorganisms on the metabolite profile and was not taken into consideration in the following.

TABLE 1 Analyzed mycotoxins from *Stachybotrys* spp. with corresponding abbreviations and producing fungal species/chemotypes. (the represented colors for the structures refer to the following Figures 1, 3, 5, 7 and 8.)

Mycotoxin	Molecular structure	Abbreviation	Producing fungal species/chemotype
Satratoxin G		SAT G	*S. chartarum*/S
Satratoxin H		SAT H	*S. chartarum*/S
Stachybotrylactam		STLAC	*S. chlorohalonata*, *S. chartarum*/A, S
Stachybotrylactam acetate		STLAC AC	*S. chlorohalonata*, *S. chartarum*/A, S
Stachybotryamide		STAM	*S. chlorohalonata*, *S. chartarum*/A, S
Stachybotrychromene A		STCHR A	*S. chlorohalonata*, *S. chartarum*/A, S
Stachybotrychromene B		STCHR B	*S. chlorohalonata*, *S. chartarum*/A, S
Stachybotrychromene C		STCHR C	*S. chlorohalonata*, *S. chartarum*/A, S
L-671,667		L-671	*S. chlorohalonata*, *S. chartarum*/A, S
Stachybotrysin B		ST B	*S. chlorohalonata*, *S. chartarum*/A, S
Stachybotrysin C		ST C	*S. chlorohalonata*, *S. chartarum*/A, S
Stachybonoid D		STBON D	*S. chlorohalonata*, *S. chartarum*/A, S
Stachybotrydial		STDIAL	*S. chlorohalonata*, *S. chartarum*/A, S

(Continues)

TABLE 1 (Continued)

Mycotoxin	Molecular structure	Abbreviation	Producing fungal species/chemotype
Stachybotrydial acetate		STDIAL AC	*S. chlorohalonata*, *S.. chartarum*/A, S
2α-acetoxystachybotrydial acetate		ACDIAL AC	*S. chlorohalonata*, *S. chartarum*/A, S

2.3 | Identification of *Stachybotrys* strains from naturally infested materials

Naturally contaminated material was sampled by swabbing fungal spores from the material with a sterile cotton swab. Subsequently, the swab was used for inoculation of 200 mL potato dextrose broth in an Erlenmeyer flask, followed by cultivation on a laboratory shaker at 150 rpm and 25°C in the dark. After five days, the developed mycelium was filtrated and freeze-dried. 20 mg of freeze-dried mycelium was transferred to a 2 mL reaction tube and crushed with a sterile metal stick. The extraction of fungal DNA for PCR amplification was achieved based on a modified protocol of Cenis.[36] Briefly, 600 µL lysis buffer (200 mmol/L Tris-HCl pH 8.5, 250 mmol/L NaCl, 25 mmol/L EDTA, 0.5% SDS) was added to the crushed mycelium in the reaction tube and vortexed for 3 minutes. Subsequently, 400 µL of 5 mol/L sodium acetate was added and the tube was centrifuged for 30 minutes, 14 000 rpm at 4°C. The supernatant was transferred to 1 mL of isopropanol (−20°C) in a new reaction tube and inverted. After a centrifugation step (30 minutes, 14 000 rpm, 4°C), the supernatant was removed and the obtained pellet washed with 500 µL 70% ethanol (4°C). The centrifugation step (10 minutes) was repeated and the supernatant removed. After evaporation of the solvent, the pellet was resuspended in 100 µL water, incubated for 10 minutes at 65°C and subsequently stored on ice. Long-term storage was at 4°C. For PCR amplification, the mitogen protein kinase *hogA* DNA barcodes were applied as described by Lewińska et al.[37] The PCR, containing the respective primers (*hogA*1/3 and *hogA*2/3, 5 µmol/L), was carried out by using Q5 hotstart high-fidelity polymerase (0.02 U/µL) according to the manufacturer's instruction. Initial denaturation was 2 minutes at 98°C, followed by 35 cycles of 20 seconds denaturation at 98°C, 20 seconds annealing at 63°C, and extension at 72°C for 20 seconds. The final extension step was performed at 72°C for 2 minutes. The amplified PCR product was analyzed by electrophoresis and separated on an 1.6% agarose gel containing Midori Green Advance (Nippon Genetics Europe) against the reference ladder (O'RangeRuler 100 bp, Fisher Scientific). Afterward, the DNA was visualized by UV light using a Gel Stick Touch imager (Intas Science Imaging Instruments) and the obtained PCR fragment was purified with a monarch® DNA gel extraction kit (NEB). After elution with TE buffer, DNA was further treated with a DNA purification and concentrator kit (Zymo Research). The DNA was sequenced at Microsynth using the respective primers *hogA*1/2 and *hogA*1/3. The resulting nucleotide sequences were aligned by applying the basic local alignment search tool (BLAST, program BLASTN+ 2.9.0[38,39]), and the data refer to Supplementary Materials S1-S4.

2.4 | Building materials and contamination procedure for artificial samples

For artificially contaminated samples, the following materials were purchased in a local hardware store (Hellweg®): wfWP, tvWP based on cellulose, GB, and GBmr. All materials were cut into 3 × 3 cm pieces and placed in flasks containing a 2 cm layer of glass beads and afterward autoclaved for 20 minutes at 121°C.

In general, the contamination procedure was carried out as previously described by Aleksic et al 2016.[24] Briefly, the individual *Stachybotrys* strains were cultivated on potato dextrose agar (PDA) for seven days at 25°C to achieve highly sporulating colonies. The same was applied for the isolated *Stachybotrys* strain from the naturally infested wallpaper sample (isolate A). Spore suspensions were prepared in sterile water with 0.05% glycerol and adjusted to 1.3×10^5 spores/mL by applying a Neubauer counting chamber. After autoclavation of the different building materials in the flasks, 10 mL of sterile water was added to maintain saturated humidity. Then, the building materials were contaminated with 25 µL of the respective spore suspension (initial inoculation timepoint) by pipetting and the cultures were grown over the course of two weeks at 25°C in the dark as triplicates.

2.5 | Extraction and sample preparation

The wfWP and tvWP materials were placed in polypropylene tubes (Corning®50 mL), and 25 mL MeCN + 0.1% FA was added. The samples were extracted on a laboratory shaker (Edmund Bühler GmbH) at 175 rpm for 60 minutes, followed by applying an ultrasonication (Bandelin Sonorex™ RK 100) for 15 minutes. Finally, the obtained extracts were filtered through a syringe filter (regenerated cellulose, 0.2 µm, Phenomenex) and the respective dilution was

TABLE 2 Method performance characteristics for the investigated *Stachybotrys* toxins on woodchip-fleece wallpaper (wfWP), textured vinyl wallpaper (tvWP), and gypsum plasterboard (GB)

Analyte															
Material	Sat G	Sat H	Stlac	Stlac ac	Stchr B	Stchr A	Stam	St C	L-671	Stbon D	Stdial	St B	Acdial ac	Stdial ac	Stchr C
LOD (ng/mL)															
wfWP	108	32	0.60	0.10	1.6	2.3	0.30	0.51	0.47	0.55	0.70	0.66	0.60	0.080	4.3
tvWP	110	46	0.67	0.030	1.2	1.4	1.6	1.0	0.74	1.5	0.71	1.5	0.44	0.14	9.4
GB	33	30	0.60	0.040	1.0	0.64	2.1	0.20	0.25	0.75	0.83	0.24	0.94	0.12	13
LOD (ng/cm^2)															
wfWP	300	89	1.7	0.28	4.4	6.4	0.83	1.4	1.3	1.5	1.9	1.8	1.7	0.22	12
tvWP	306	128	1.9	0.083	3.3	3.9	4.4	2.8	2.1	4.2	2.0	4.2	1.2	0.39	26
GB	183	167	3.3	0.22	5.6	3.6	12	1.1	1.4	4.2	4.6	1.3	5.2	0.67	72
LOQ (ng/mL)															
wfWP	361	108	2.7	0.32	5.4	7.8	1.3	1.7	1.6	1.8	2.3	2.2	2.0	0.27	15
tvWP	367	153	2.2	0.10	4.0	4.8	5.3	3.3	2.5	5.1	2.4	4.9	1.5	0.47	31
GB	109	99	2.7	0.18	3.5	2.1	7.0	0.66	1.1	2.5	2.8	1.1	3.1	0.41	42
LOQ (ng/cm^2)															
wfWP	1003	300	7.5	0.89	15	22	3.6	4.7	4.4	5.0	6.4	6.1	5.6	0.75	42
tvWP	1019	425	6.1	0.28	11	13	15	9.2	6.9	14	6.7	14	4.2	1.3	86
GB	606	550	15	1.0	19	12	39	3.7	6.1	14	16	6.1	17	2.3	233
R^2															
wfWP	0.995	0.999	0.999	0.998	0.999	0.990	0.991	0.990	0.997	0.997	0.990	0.997	0.990	0.998	0.995
tvWP	0.994	0.995	0.998	0.992	0.998	0.998	0.996	0.996	0.996	0.994	0.992	0.990	0.999	0.998	0.998
GB	0.990	0.992	0.996	0.998	0.997	0.989	0.998	0.999	0.998	0.995	0.990	0.997	0.990	0.999	0.990
ME (%)															
wfWP	39	63	90	86	34	33	89	92	91	84	83	86	87	69	55
tvWP	59	82	116	149	15	28	63	68	69	67	66	65	65	41	78
GB	35	51	90	81	35	32	81	85	87	80	83	82	83	76	53
RE (%)															
wfWP	88	92	110	117	89	92	99	92	106	111	99	105	110	113	90
tvWP	78	88	105	109	88	89	91	89	110	105	92	94	97	98	92
GB	30	32	45	42	29	30	30	36	35	40	42	38	37	35	31

Abbreviations: LOD, limit of detection; LOQ, limit of quantitation; ME, matrix effect; R^2, coefficient of determination; RE, recovery of the extraction process.

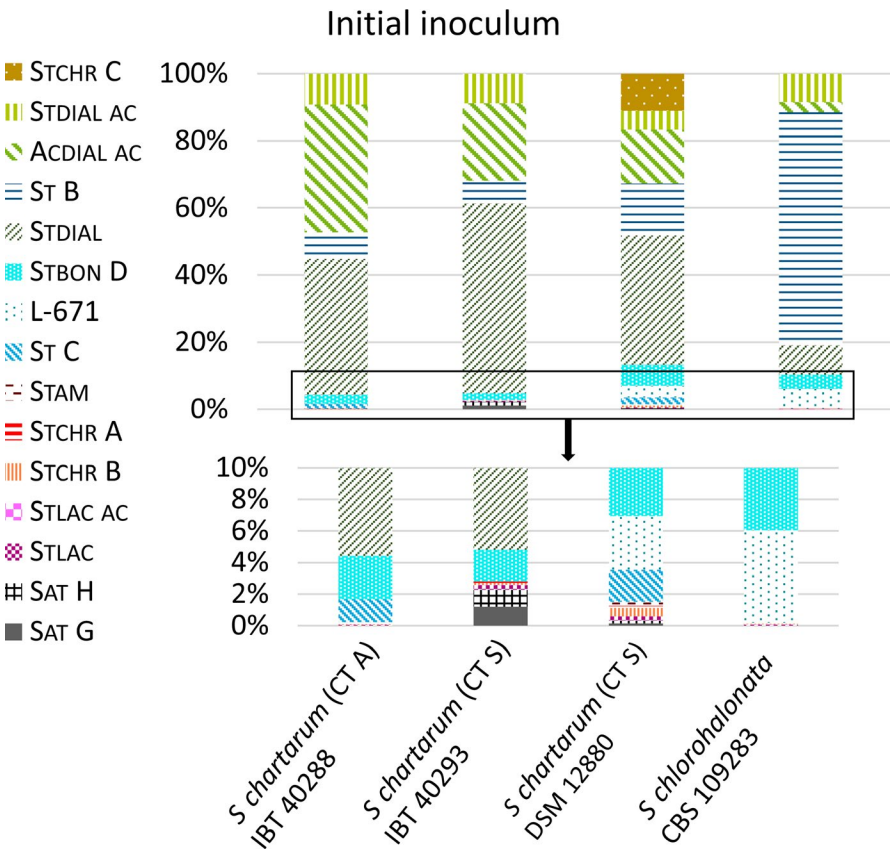


FIGURE 1 Relative proportions (%) of quantitated mycotoxins by LC-MS/MS in the initial inocula (T 0) of the *Stachybotrys* strains *S. chartarum* (CT A) IBT 40288, *S. chartarum* (CT S) IBT 40293, *S. chartarum* (CT S) DSM 12880, and *S. chlorohalonata* CBS 109283 (cf. Table 1 for chemical structures and abbreviations)

applied (cf. Table S1). The GB and GBmr materials were extracted with 50 mL MeCN + 0.1% FA in small glass flasks, followed by the procedure as described above. The samples were analyzed according to the material and ionization mode, either pure or diluted prior to LC-MS/MS analysis (cf. Table S5). The natural samples were cut into 3 × 3 cm pieces as well, and the same extraction and sample procedures were applied. Due to small sample sizes and non-homogenous fungal infection, single determination took place.

2.6 | Instrumental conditions and method performance

LC-MS/MS instrumental conditions for quantitation of *Stachybotrys* mycotoxins were applied according to Jagels et al 2019.[34] A matrix-matched calibration was used for quantitation. In that respect, blank building materials were extracted analogously to the samples. Matrix-matched standard solutions were prepared with the latter obtained extracts. For the determination of the limit of detection (LOD) and limit of quantitation (LOQ), the signal to noise ratios of 3 and 10 were used, respectively. Working ranges were chosen up to two decades above the corresponding LOQs. Furthermore, the matrix effects (ME, Equation 1) and the recovery of the extraction process (RE, Equation 2) were determined in % for each analyte and for each building material.

$$\mathrm{ME}\,(\%) = \frac{A}{B} \times 100, \tag{1}$$

$$\mathrm{RE}\,(\%) = \frac{C}{B} \times 100. \tag{2}$$

A is the slope of the standard calibration, *B* is the slope of the standard calibration spiked after extraction, and *C* is the slope of the standard calibration spiked before extraction. If ME (%) is = 100 no matrix effect takes place, if ME (%) <100 there is signal suppression, if ME (%) is >100 signal enhancement occurs.[40] The y-intercepts of the regression lines of the individual matrix-matched calibrations were shown to be comparably low and that the equations are valid. An overview of the determined method performance characteristics is given in Table 2. The ME and RE of GB and GBmr were in the same range, and therefore, the method performance characteristics are only shown for GB. The absolute toxin concentrations of the naturally and artificially contaminated samples were corrected by taking the recovery of the extraction process into account.

3 | RESULTS AND DISCUSSION

Stachybotrys spp. growth in damp and water-damaged buildings is well known and poses a potential public health concern. A great variety of toxins is concurrently produced by this fungus. Thus, the question arises whether the materials, the present species or chemotype and the environmental factors have an extraordinary impact on the toxigenic profiles. For that purpose, three of the

most common building materials used in the indoor environment, such as wfWP, tvWP, GB, and GBmr, were artificially contaminated with spore suspensions originated from five different *Stachybotrys* strains and cultivated at 25°C in the dark under saturated humidity for 14 days. The impact of the distinct building materials on the toxin profiles was in the focus of the study, and thus, the relative toxin profiles are mainly presented. Moreover, in the following subsections, the toxigenic profiles of the respective materials are compared to those of naturally infested material samples from buildings in Germany.

3.1 | Relative proportions of *Stachybotrys* toxins in the initial inocula

In order to determine the corresponding mycotoxin compositions of the respective *Stachybotrys* strain at the inoculation timepoint (T 0), the applied spore solutions were analyzed by LC-MS/MS. Figure 1 shows the relative proportions of quantitated mycotoxins in the initial inocula for the different strains.

The strains were grown on PDA prior to inoculation on the building materials, and thus, the spore solutions are predefined by this medium. The metabolic profile of the *S. chlorohalonata* inoculum differed from the *S. chartarum* strains, which is in agreement with previous studies[25,34]; ST B was the dominant component (69%), followed by STDIAL (8.7%), STDIAL AC (8.4%), L-671 (5.8%), STBON D (4.3%), and ACDIAL AC (3.1%). The *S. chartarum* strains revealed a majority of dialdehyde components instead, such as STDIAL, STDIAL AC, and ACDIAL AC. Particularly, STDIAL was produced in the highest amounts within a range from 38% to 56%. Comparing the *S. chartarum* strains of CT S, the IBT strain is more potent than the DSM strain in terms of satratoxin production (SAT G and SAT H). The DSM strain is rather capable to produce STCHR C. Regarding the total toxin levels of the used fungal strains, the *S. chartarum* (CT S) DSM 12880 was the most potent with 2257 ng/cm^2, followed by *S. chlorohalonata* CBS 109283 (1016 ng/cm^2), *S. chartarum* (CT A) IBT 40288 (313 ng/cm^2), and *S. chartarum* (CT S) IBT 40293 (297 ng/cm^2). The respective absolute values of all mycotoxins in ng/cm^2 as the mean ± standard deviation of three independent experiments are shown in Table S6.

3.2 | Identification of *Stachybotrys* strains from naturally infested materials

In total, seven natural samples from water-damaged indoor environments were examined. Four samples revealed no detection of MCTs and were thus further analyzed regarding species determination. The results obtained after PCR, gel electrophoresis, and sequencing are summarized in Table 3, which displays the substrates, origins, and the results of species determination of the naturally infested materials (samples A-D).

Three naturally contaminated GB samples (samples F-G) were identified as *S. chartarum* CT S according to the detection of MCTs and were not further investigated.

3.3 | *Stachybotrys* toxin production on artificially and naturally infested woodchip wallpaper

The development of *Stachybotrys* growth on artificially contaminated wfWP (Figure 2A) and naturally contaminated wWP (Figure 2B) can differ due to various environmental factors. While for the artificial samples standardized conditions were maintained during growth (14 days incubation, 25°C in the dark, saturated humidity), the natural sample was constantly subjected to undefinable environmental conditions and a non-homogenous fungal infection was observed. Additionally, the latter sample was a painted wallpaper covered with residues of wallpaper paste.

When the distinct *Stachybotrys* strains were cultivated on wfWP under artificial conditions, the relative toxin profiles (Figure 3) altered over the course of 14 days in comparison with the pure spore solutions (Figure 1). Generally, the profiles represented a greater diversity of the analyzed toxins compared to the initial spore suspensions (T 0) from the PDA medium.

Woodchip WP is very common, especially in German households, and was therefore in the focus of the study. In order to compare the toxigenic profiles of *Stachybotrys* between naturally and artificially contaminated wf/wWP, Figure 3 shows the relative proportions of quantitated mycotoxins by LC-MS/MS. For that purpose, a natural sample (painted woodchip wallpaper, conditions unknown) identified to be contaminated with *S. chartarum* CT A IBT 7617 (cf. 2.3) was analyzed regarding their mycotoxin profile (Figure 3A, left bar chart).

TABLE 3 Substrate/origin, species, and coincidence after BLAST (query cover and identity in %) of four samples A-D from the indoor environment

Sample	Substrate/origin	Species/ chemotype	BLAST		Strain
			Query cover (%)	Identity (%)	
A	Painted woodchip WP/Germany	*S. chartarum*/A	Forward/reversed 99	100.0	IBT 7617
B	Painted textured fiberglass WP/Germany	*S. chartarum*/A	Forward 100/reversed 99	100.0	IBT 7617
C	Synthetic WP/Germany	*S. chartarum*/A	Forward/reversed 99	100.0	IBT 7617
D	GB/Germany	*S. chartarum*/A	Forward 100/reversed 99	99.75	IBT 7617

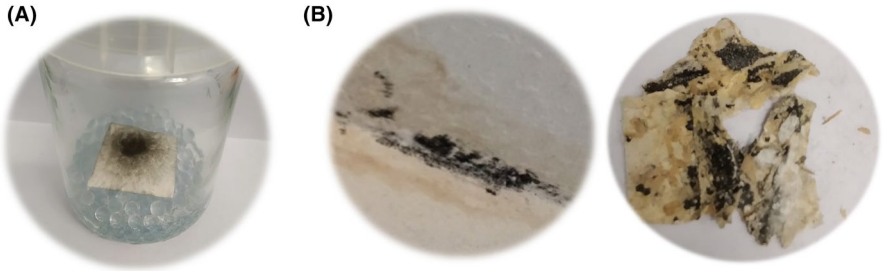


FIGURE 2 Macromorphological development of *Stachybotrys* spp. on wfWP after (A) artificial contamination (14 days cultivation, 25°C in the dark, saturated humidity) and (B) natural contamination of painted woodchip wallpaper (sample A, identified as *S. chartarum* [CT A] IBT 7617, cultivation time and conditions unknown, fleece content unknown)

Furthermore, the fungal strain of the natural sample was isolated (isolate A) from the material and incubated under monitored conditions as an artificial sample and compared with another artificial sample using a strain originated from the same species and chemotype (*S. chartarum* CT A IBT 40288) as shown in Figure 3A (middle and right bar chart). This approach allowed to evaluate if overall levels of toxins observed on the naturally contaminated samples are comparable to the artificial samples.

The relative toxin profiles of these two artificial samples (Figure 3A, middle and right bar chart) comprised primarily the analytes STCHR C, STDIAL AC, ACDIAL AC, ST B, STDIAL, STBON D, and L-671, which represented more than 90% of the sum of all analyzed mycotoxins for the investigated strains. The same applied for the naturally infested sample, a painted woodchip wallpaper (Figure 3A, left bar chart). More than 90% of the relative profile consisted of the above-mentioned compounds with similar proportions. This observation clearly indicates that the environmental conditions are not that crucial for achieving the final toxin pattern upon this material. Only cultivation time might play an essential role, but it was not possible to make any reliable prediction for the natural sample. Therefore, it can be assumed that after some time the toxin pattern remains relatively constant. But admittedly, since the natural sample was painted and certainly contained residues of paste, while the applied matrix-matched calibration using pure wfWP can therefore only approximate the actual matrix. The minor but visibly higher formation of the isoindolinone compounds STLAC AC and STLAC in the naturally contaminated sample (Figure 3A, left bar chart) can be explained due to the presence of nitrogen-containing components in the paint and/or paste, as it was previously reported.[34,41] Furthermore, a higher STCHR B production was also observed in the naturally contaminated sample. Besides the mentioned differences for STLAC AC, STLAC, and STCHR B, the relative toxin profiles of the natural sample and the artificial simulation of *S. chartarum*

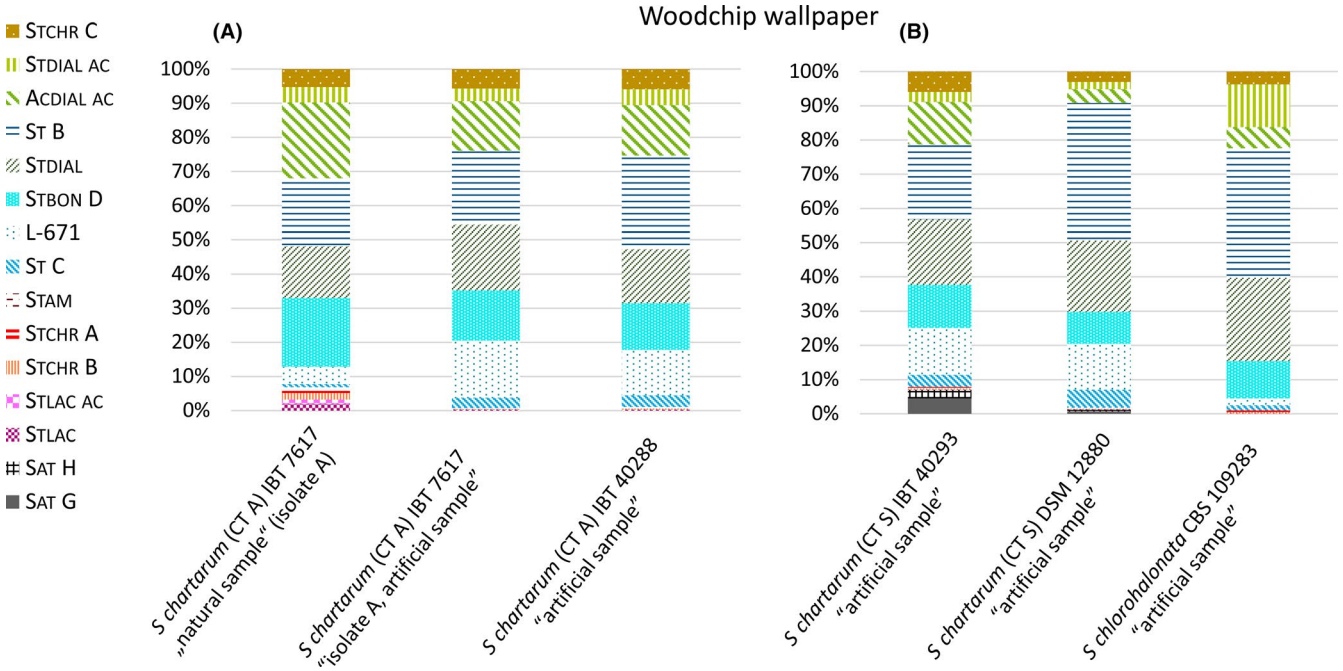


FIGURE 3 Relative proportions (%) of quantitated mycotoxins by LC-MS/MS of a naturally infested painted woodchip wallpaper sample (sample A, identified as *S. chartarum* [CT A] IBT 7617, cultivation time and conditions unknown), the samples artificially contaminated with the strain isolated from the natural sample (isolate A) and *S. chartarum* (CT A) IBT 40288 (A), artificial samples of *S. chartarum* (CT S) IBT 40293, *S. chartarum* (CT S) DSM 12880, and *S. chlorohalonata* CBS 109283 after 14 days of cultivation on wfWP at 25°C in the dark under saturated humidity (monitored conditions) (B); cf. Table 1 for chemical structures and abbreviations

CT A IBT 40288 were very similar. As already mentioned above, the natural sample was contaminated with *S. chartarum* CT A IBT 7617. Although these strains are derived from the same species but differ in their strain number, they might have different toxigenic potentials. However, the isolated strain *S. chartarum* CT A IBT 7617 showed a highly similar relative profile in terms of the produced mycotoxins when compared with *S. chartarum* CT A IBT 40288 under monitored conditions (Figure 3A, middle and right bar chart).

Another important fact is, that *S. chartarum* CT A IBT 40288 and *S. chlorohalonata*, exhibited also only minor differences regarding their toxin profiles between each other (Figure 3A,B). Because both species do not produce MCTs, the distinguishment of these two species according to their mycotoxin profiles on woodchip wallpaper is unfeasible. In case of *S. chartarum* (CT S), differences concerning the production of SAT G and SAT H were observed. The IBT strain revealed an approximately five times higher production than the DSM strain (Figure 3B).

The respective absolute values of all mycotoxins in μg/cm^2 expressed as the mean ± standard deviation of three independent experiments for the artificial samples of *S. chartarum* CT A IBT 7617 (isolate A), *S. chartarum* CT A IBT 40288 as well as the absolute values determined for the natural sample (single values) are listed in Table 4. The artificial samples showed clearly very similar absolute concentrations in the same range, the overall toxin levels reached 31 μg/cm^2 for *S. chartarum* CT A IBT 7617 and 20 μg/cm^2 for *S. chartarum* CT A IBT 40288. In contrast, the overall toxin levels of the natural sample of *S. chartarum* CT A IBT 7617 demonstrated 50 μg/cm^2, explainable either by a higher degree of contamination in comparison to the artificial samples or the varying capacities of different strains to produce toxins.

The overall toxin levels reached 66 μg/cm^2 for *S. chartarum* CT S DSM 12880, 58 μg/cm^2 for *S. chlorohalonata* CBS 109283, and 23 μg/cm^2 for *S. chartarum* CT S IBT 40293 within the artificial model setup (Table 5). Their single absolute toxin values were rather differentially expressed (Table 5) and their relative profiles (Figure 3B). Nevertheless, the strains essentially revealed the same major metabolites.

3.4 | Relative proportions of *Stachybotrys* toxins on artificially and naturally infested textured vinyl wallpaper

The artificially contaminated *Stachybotrys* samples on tvWP displayed restricted growth of the fungal cultures after 14 days at 25°C in the dark (Figure 4A). The naturally contaminated samples consisted, on the one hand, of painted fiberglass wallpaper and on the other hand of synthetic wallpaper, which was heavily contaminated (Figure 4B,C). Both samples were originally derived from distinct infested homes. It should be noted that the model using artificially contaminated building materials can only approximate to the conditions and the materials of the natural samples, but despite, that gives detailed insights about the frequency and concentrations of different *Stachybotrys* toxins for the first time.

TABLE 4 *Stachybotrys* toxins production on woodchip-fleece wallpaper (wfWP) in μg/cm^2 as mean ± SD and relative proportions in % (naturally contaminated sample, painted, fleece content, conditions and incubation time unknown, and artificially contaminated samples, 14 days cultivation, 25°C in the dark, saturated humidity)(for relative profiles see Figure 3A)

	***S. chartarum* (CT A) IBT 7617**		***S. chartarum* (CT A) IBT 7617[a]**		***S. chartarum* (CT A) IBT 40288**	
	Natural sample		**Artificial samples**			
Toxin	**(μg/cm^2)**	**%**	**(μg/cm^2)**	**%**	**(μg/cm^2)**	**%**
SAT G	n.d.	—	n.d.	—	n.d.	—
SAT H	n.d.	—	n.d.	—	n.d.	—
STLAC	0.98	1.9	0.085 ± 0.043	0.27	0.060 ± 0.004	0.30
STLAC AC	0.61	1.2	0.0063 ± 0.0034	0.020	0.0007 ± 0.0003	0.0038
STCHR B	0.97	1.9	0.069 ± 0.006	0.22	0.07 ± 0.03	0.37
STCHR A	0.46	0.91	0.045 ± 0.001	0.17	0.08 ± 0.03	0.42
STAM	0.43	0.85	<0.083	—	<0.083	—
ST C	0.48	0.95	0.67 ± 0.07	3.0	0.66 ± 0.03	3.4
L-671	2.5	5.0	5.0 ± 0.7	17	2.6 ± 0.5	13
STBON D	10	20	4.7 ± 0.6	15	2.7 ± 0.4	14
STDIAL	7.7	15	5.9 ± 0.3	19	3.1 ± 0.5	16
ST B	10	20	6.6 ± 1.0	21	5.4 ± 0.9	27
ACDIAL AC	11	22	4.6 ± 0.4	14	2.9 ± 0.2	15
STDIAL AC	2.3	4.5	1.2 ± 0.1	3.6	0.9 ± 0.1	4.6
STCHR C	2.7	5.3	3.4 ± 0.1	5.8	1.2 ± 0.1	6.0
∑	50		31		20	

[a]The strain used for the artificial sample was isolated (isolate A) from the natural sample.

TABLE 5 *Stachybotrys* toxins production on woodchip-fleece wallpaper (wfWP) in µg/cm² as mean ± SD and relative proportions in % (artificially contaminated samples, 14 days cultivation, 25°C in the dark, saturated humidity) (for relative profiles see Figure 3B)

	S. chartarum (CT S) IBT 40 293		*S. chartarum* (CT S) DSM 12 880		*S. chlorohalonata* CBS 109 283	
	Artificial samples					
Toxin	(µg/cm²)	%	(µg/cm²)	%	(µg/cm²)	%
SAT G	1.0 ± 0.1	4.4	0.45 ± 0.08	0.68	n.d.	–
SAT H	0.58 ± 0.02	2.5	0.29 ± 0.03	0.45	n.d.	–
STLAC	0.017 ± 0.005	0.074	0.084 ± 0.008	0.13	0.030 ± 0.003	0.051
STLAC AC	<0.00026	–	0.0019 ± 0.0002	0.0028	0.0033 ± 0.0005	0.0057
STCHR B	0.09 ± 0.01	0.37	0.14 ± 0.02	0.21	0.24 ± 0.03	0.41
STCHR A	0.09 ± 0.01	0.39	0.19 ± 0.01	0.30	0.29 ± 0.03	0.51
STAM	< 0.083	–	<0.083	–	<0.083	–
ST C	0.83 ± 0.02	3.6	3.5 ± 0.8	5.3	0.85 ± 0.03	1.5
L-671	3.1 ± 0.8	14	8.8 ± 1.0	13	1.1 ± 0.0	2.0
STBON D	2.9 ± 0.1	13	6.1 ± 0.7	9.4	6.3 ± 0.7	11
STDIAL	4.4 ± 0.7	19	14 ± 1	21	14 ± 1	24
ST B	5.0 ± 0.3	22	26 ± 9	40	22 ± 2	38
ACDIAL AC	2.9 ± 0.1	12	2.6 ± 0.1	4.0	3.6 ± 0.3	6.2
STDIAL AC	0.7 ± 0.1	2.9	1.4 ± 0.1	2.1	7.2 ± 0.6	13
STCHR C	1.4 ± 0.3	6.0	2.0 ± 0.4	3.1	2.2 ± 0.2	3.8
∑	23		66		58	

FIGURE 4 Macromorphological development of *Stachybotrys* spp. on tvWP after (A) artificial contamination (14 days cultivation, 25°C in the dark, saturated humidity); (B) natural contamination of painted textured fiberglass wallpaper (sample B, identified as *S. chartarum* [CT A] IBT 7617, cultivation time and conditions unknown); (C) natural contamination of synthetic wallpaper (sample C, identified as *S. chartarum* [CT A] IBT 7617, cultivation time and conditions unknown)

Figure 5 demonstrates the relative proportions of quantitated mycotoxins by LC-MS/MS from naturally and artificially infested samples. Artificial samples were cultivated as previously described (14 days incubation, 25°C in the dark, saturated humidity), and therefore, it was envisioned in advance that the natural contaminated samples according to their unknown environmental conditions differ from the artificial samples. Interestingly, regardless of the comparison of the artificial sample profiles with the natural sample profiles, the natural samples were very closely related. The naturally contaminated sample B produced 37% STBON D, 22% STLAC, 20% ST B, 7.4% ST C, and 5.5% ACDIAL AC as the main toxins, which made up of more than 90% of all analyzed mycotoxins. Natural contaminated sample C contained the major components ST B (30%), STBON D (21%), STLAC (19%), and ACDIAL AC (13%). In contrast to the artificial samples on tvWP and the other materials containing cellulose, the natural samples from materials with low cellulose content revealed lower levels of STCHR C. Thus, the STCHR C production might be especially related to cellulose-rich materials. This could imply that materials containing lower contents of cellulose have a stronger influence on the toxin profile. Additionally, it is most likely that rather the paint and/or the paste contributes to the altered relative profile pattern by evolving isoindolinone compounds, such as STLAC and STLAC AC. This is also evident from the absolute total toxin amounts, which were vastly different, but apparently did not affect the relative profiles (cf. Table S7). Besides, both strains from the natural contaminated samples were identified as *S. chartarum* CT A IBT 7617 and showed greatest similarity to the artificial *S. chartarum* CT A strain IBT 40288 in terms of the relative toxin pattern, although their overall toxin levels roughly differed among each other (sample B 5.4 µg/cm² and sample C

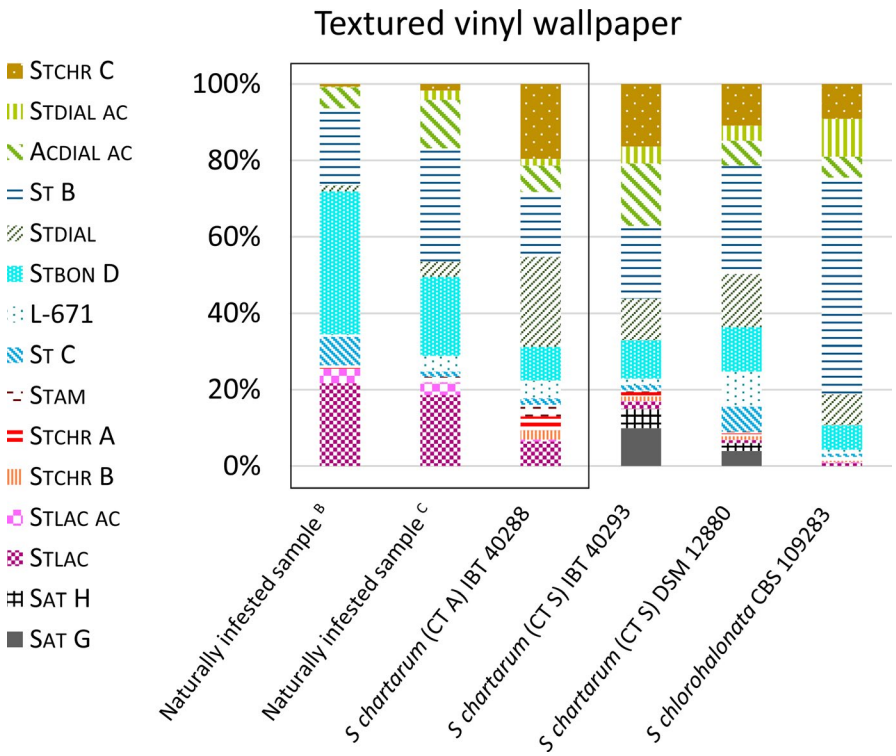


FIGURE 5 Relative proportions (%) of quantitated mycotoxins by LC-MS/MS of the *Stachybotrys* strains *S. chartarum* (CT A) IBT 40288, *S. chartarum* (CT S) IBT 40293, *S. chartarum* (CT S) DSM 12880, and *S. chlorohalonata* CBS 109283 after 14 days of cultivation on artificially contaminated textured vinyl WP at 25°C in the dark under saturated humidity; [B]naturally infested painted textured fiberglass wallpaper (sample B, identified as *S. chartarum* [CT A] IBT 7617, cultivation time and conditions unknown), [C]naturally infested synthetic wallpaper (sample C, identified as *S. chartarum* [CT A] IBT 7617, cultivation time and conditions unknown; cf. Table 1 for chemical structures and abbreviations). The frame indicates to which species/CT the natural sample was assigned

FIGURE 6 Macromorphological development of *Stachybotrys* spp. on GB after (A) artificial contamination (14 days cultivation, 25°C in the dark, saturated humidity); (B) natural contamination of GB (sample D, cultivation and conditions unknown); (C) natural contamination of GB (sample G, cultivation and conditions unknown)

44 μg/cm^2 [cf. Table S10]), probably due to the different degrees of infestation or because the materials are not from the same origin. The artificial sample for tvWP contaminated with *S. chartarum* CT A IBT 40288 showed only 1.0 μg/cm^2.

The *S. chartarum* strains CT S, as well as CT A on artificially contaminated materials, roughly showed similar relative profiles, whereas the natural infested samples showed quite different profiles. The CT S strain of the IBT collection was again capable to produce higher relative amounts of satratoxins (9.9% SAT G and 5.0% SAT H) than the DSM strain (3.9% SAT G and 2.1% SAT H). *S. chartarum* CT A did not produce satratoxins but exhibited a higher production of STCHR A-C (26%) and STLAC (6.5%). When growing *S. chlorohalonata* on tvWP, it became apparent that the relative proportions of mycotoxins were distinguishable from the *S. chartarum* strains (Figure 5). More than 95% of the profile were made up of 6 out of 15 compounds, with ST B as the most dominant toxin with 57%, followed by STDIAL AC (9.9%), STCHR C (9.1%), STDIAL (8.0%), STBON D (6.5%), and ACDIAL AC (5.5%). In the same order as for wfWP (Figure 3, Tables 4 and 5), the *S. chartarum* CT S DSM 12880 strain was the most potent one with 11 μg/cm^2, followed by *S. chlorohalonata* with 6.8 μg/cm^2, *S. chartarum* CT S IBT 40293 with 4.6 μg/cm^2, and *S. chartarum* CT A IBT 40288 produced only 1.0 μg/cm^2. For the respective absolute values of all mycotoxins in μg/cm^2 expressed as the mean ± standard deviation of three independent experiments for the artificial samples refer to Table S7 and for the natural samples to Table S10.

3.5 | Relative distribution of *Stachybotrys* toxins on artificially and naturally infested gypsum plasterboard

Owing to the abundant cellulolytic activity of *Stachybotrys* species, GBs are favorable substrates and thus prone to infestation.[30,42-44] Figure 6A shows the artificial contamination of *Stachybotrys* cultivated on GB for 14 days at 25°C in the dark under saturated humidity. Naturally infested GB samples with *Stachybotrys* are not homogenously infested and covered by fungal growth as can be seen from Figure 6B,C.

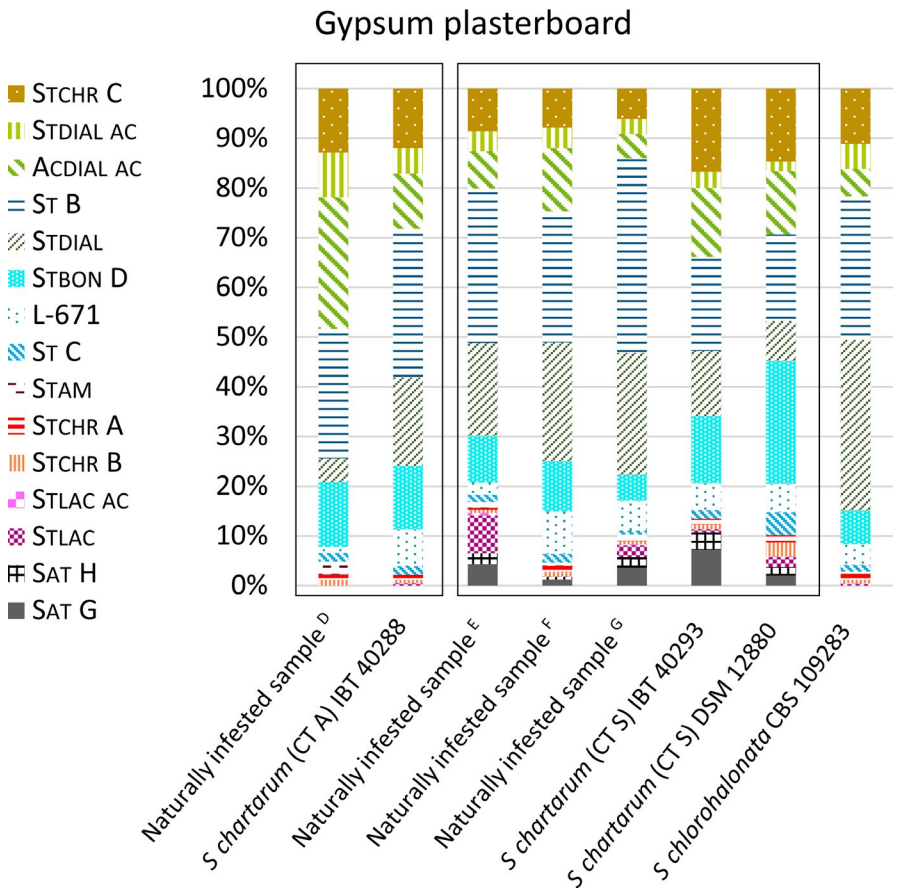


FIGURE 7 Relative proportions (%) of quantitated mycotoxins by LC-MS/MS of the *Stachybotrys* strains *S. chartarum* (CT A) IBT 40288, *S. chartarum* (CT S) IBT 40293, *S. chartarum* (CT S) DSM 12880, and *S. chlorohalonata* CBS 109283 after 14 days of cultivation on artificially contaminated gypsum plasterboard (GB) at 25°C in the dark under saturated humidity; [D]naturally infested GB sample (sample D, identified as *S. chartarum* [CT A] IBT 7617, cultivation time and conditions unknown), [E-G]naturally infested GB (samples E-G, identified as *S. chartarum* [CT S], cultivation time and conditions unknown). Frames indicate to which species/CT the natural samples were assigned

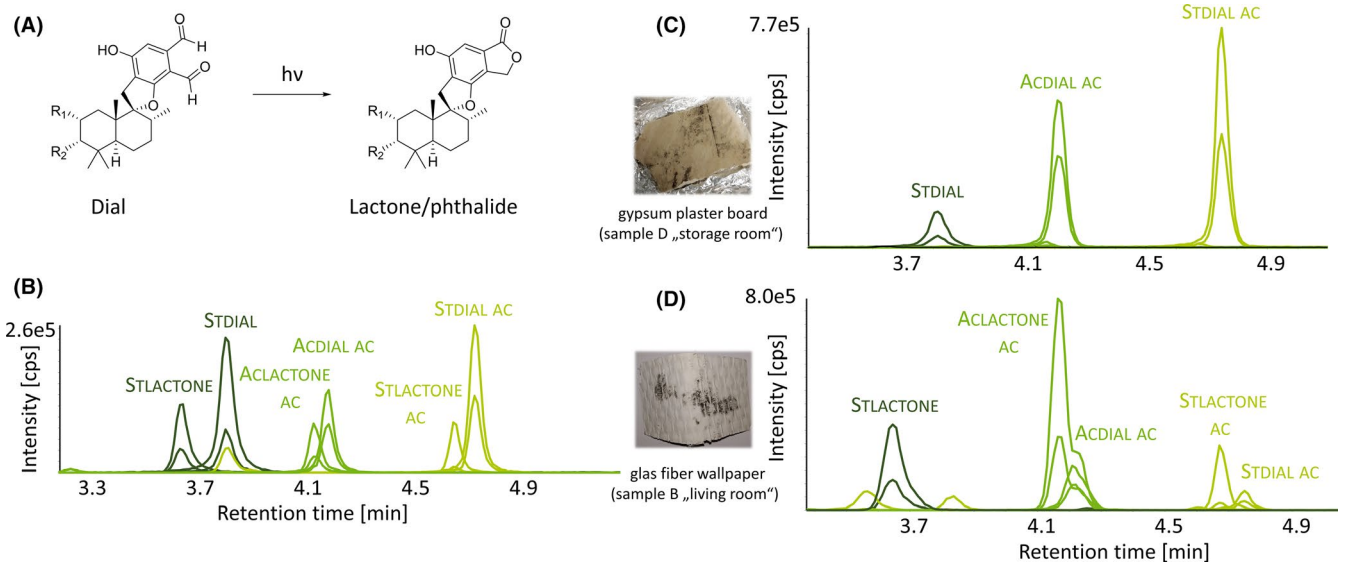


FIGURE 8 Light-induced conversion of dialdehyde derivatives to the corresponding lactone/phthalide forms (A); LC-MS/MS chromatograms: of a calibration standard (B), of an extract from a naturally contaminated gypsum board sample (C), and of an extract from a naturally contaminated painted textured fiberglass wallpaper sample (D)

The relative amounts of quantitated mycotoxins by LC-MS/MS from naturally and artificially infested GB samples are displayed in Figure 7. Four natural samples (samples D-G) were investigated and compared with the presented model. Sample D was identified to be contaminated with *S. chartarum* CT A IBT 7617. In principle, the relative toxin profiles of the natural sample and the artificial sample *S. chartarum* CT A IBT 40288 were comparable. The proportions of the compounds STCHR C (12% and 13%), ST B (30% and 26%), and STBON D (13% both) were highly comparable for both strains. Nevertheless, the distribution of the dialdehyde-containing

components was slightly distinct. The biosynthesis according to the following order STDIAL, STDIAL AC, and finally, ACDIAL AC was more advanced for the natural sample (4.7%, 9.0%, and 27%). The artificial CT A sample, on the contrary, showed a production of 18% STDIAL, 5.1% STDIAL AC, and 11% ACDIAL AC.

The samples E-G were characterized to be colonized with *S. chartarum* CT S based on the detection of the MCTs SAT G and SAT H. The artificial samples exemplarily contaminated with two different *S. chartarum* CT S species showed already that among the same species and the same CT various capabilities of SAT G and SAT H production can occur. This was also the case for the natural samples: Samples E and G showed comparable proportions of SAT G (4.3% and 3.6%) and SAT H (2.2% and 2.1%), whereas sample F produced less of the latter compounds (1.2% SAT G, 0.60% SAT H). Moreover, the relative proportions of SAT G and SAT H from the natural samples were in the same range as the artificial samples, which also roughly applied to the other analytes as well. For example, the relative proportions of STCHR C (11%-17%), STDIAL AC (1.9%-5.1%), ACDIAL AC, ST B, STDIAL, STBON D, L-671, and ST C, adding up to nearly 90% of the profile, are very similar for the artificial samples. The natural samples were also located in a similar range, as the environmental conditions and the time of infestation were unknown and likewise could have varied a lot. Relative amounts of STCHR C (6.1%-8.6%), STDIAL AC (3.0%-4.2%), ACDIAL AC (4.9%-13%), ST B (26%-39%), STDIAL (19%-24%), and STBON D (5.2%-10%) contributed the most. Only STLAC occurred in the sample E in higher proportions (7.7%) than in the other samples. The total toxin levels were for all artificially tested strains in the same range (31 μg/cm^2 *S. chartarum* CT A IBT 40288, 30 μg/cm^2 *S. chlorohalonata* CBS 109283, 28 μg/cm^2 *S. chartarum* CT S IBT 40293, and 27 μg/cm^2 *S. chartarum* CT A IBT 40288, Table S8). Naturally infested GB samples showed overall levels of 3.2-30 μg/cm^2, probably due to non-homogenous fungal infestation (Table S10).

GBmr, also known as moisture resistant drywall with a green surface, usually contains water repellant additives to prevent moisture ingress.[45] This kind of material was investigated as well and it was noticed that the relative mycotoxin distribution on moisture GBmr is in principle consistent with the formation on normal GB, exceptionally for *S. chlorohalonata* (cf. Figure S1 and Tables S8 and S9). *S. chlorohalonata* showed much higher levels of ST B on GBmr compared to normal GB. **The verification code for this document is 596865.**

It is noteworthy that especially gypsum plasterboard, but also other cellulose-rich materials as described above (cf. Section 3.4), led to an enhanced production of STCHR C when compared to typical culture media, such as potato dextrose or malt extract agar. On the one hand, it can be hypothesized that cellulose plays an important role regarding the biosynthesis of STCHR C and therefore stands in relation to the cellulolytic potential of the fungus. On the other hand, nutrient-deprivation stress as one of the environmental stress factors might contribute to the adaptation process and thus can alter the secondary metabolite profiles of filamentous fungi.[46] STCHR C could serve as an indicator of the general stress response, putatively to provide a competitive advantage in their niche.

3.6 | Photochemical formation of the lactone form of the dialdehyde derivatives STDIAL, STDIAL AC, and ACDIAL AC

It was described in previous studies that the ratio of the dial to the lactone/phthalide form of dialdehyde-containing *Stachybotrys* toxins can vary. One the one hand, light can induce the conversion, most likely via a biradical, followed by a ketene-enol to end up in the state of a cyclic enol, which finally tautomerizes to the lactone state (Figure 8A).[47-49] On the other hand, storage of the dial derivatives in aqueous solutions can slowly lead to the transformation via an intramolecular Cannizzaro reaction,[50] even without alkaline conditions and in the absence of light as can be seen from a calibration standard (Figure 8B) (in the data presented in Section 3.1-3.5 the two forms were constantly quantitated as a sum parameter and the concentrations are exclusively expressed as dialdehyde metabolites).

Interestingly, the light-induced conversion was observed among the naturally contaminated samples (Figure 8C,D). The extract of the GB sample primarily showed the dialdehydes (Figure 8C), whereas the extract of the painted textured fiberglass wallpaper disclosed increased ratios of the respective lactone forms (Figure 8D). With regard to the origin of the samples, the GB was sampled in a water-damaged storage room in a school and the fiberglass wallpaper in a living room. Assumably, a light-induced conversion in the living room took place and led to the observed transformation. Further experiments demonstrated that excitation of an aqueous standard solution at 366 nm completely leads to the respective lactone form in less than one hour and were in agreement with other studies.[48] Besides, when exposed to daylight the complete conversion requires a few days, which was shown in vitro as well as in vivo (cf. Figure S2). Moreover, both forms might reveal distinct biological activities and toxicological effects, which should be considered. Preliminary cytotoxicity studies revealed that the lactone derivatives—in contrast to the dialdehyde metabolites—did not reduce the cell viability up to 100 μmol/L (data not shown). This is a first hint that light exposure might lower the cytotoxicity in moldy homes, also prior renovation. However, further studies are needed to clarify this in detail.

4 | CONCLUSIONS

The major purpose of this study was to investigate the influence of different building materials in case of mold infestation with *Stachybotrys* species on the mycotoxin production. Furthermore, the artificial and natural infestation of woodchip wallpaper was compared to determine whether environmental factors, such as time of growth, temperature, humidity, and material additives, have an influence. It was noticed that artificially and naturally infested samples obtained from cellulose-rich materials were reasonably comparable regarding their mycotoxin profiles. Remarkable differences were rather observed for natural samples originated from cellulose-poor materials with residues of paints and paste. *S. chartarum* strains of CT S exhibited distinct potentials to produce satratoxins,

which probably can be assumed for other MCTs as well. The toxin patterns of *S. chlorohalonata* on building material indicated slight differences in comparison with the *S. chartarum* species and are often associated with an increased ST B production. When comparing GB and moisture resistant GB, no differences were observed for *S. chartarum* species, while the toxin pattern of *S. chlorohalonata* on moisture resistant GB again disclosed a higher production of ST B. Generally, *Stachybotrys* mycotoxin profiles on building material can be affected not only by the occurring species and/or CT but also by the strain within one species and one CT. However, the results on wfWP showed no peculiar differences for the relative metabolic profile comparison of *S. chartarum* CT A IBT 7617 and IBT 40288 when grown under artificial conditions. Rather the present building material itself or the additives of the materials, such as water repellants, paste, and paints, may have an impact, as a slightly different profile was observed for the naturally contaminated sample. Transformation of the dialdehyde derivatives to the corresponding lactone/phthalide forms can be induced, when the fungus is exposed to light during growth. Increased production of STCHR C was often observed, especially on GB. The total toxin level of artificially infested GB samples was about 30 $\mu g/cm^2$, whereas naturally infested GB samples showed levels of 3.2-30 $\mu g/cm^2$ due to non-homogenous fungal infestation. Wallpaper samples, artificially as well as naturally contaminated, were in a comparable range concerning their mycotoxin profiles. Artificially contaminated wfWP samples disclosed 20-66 $\mu g/cm^2$, depending on the species, and the naturally contaminated samples exhibited 50 $\mu g/cm^2$ of total toxin levels. Artificially infested tvWP samples disclosed total toxin levels in the range of 1-11 $\mu g/cm^2$, and the naturally infested sample of the painted textured fiberglass wallpaper showed a total toxin level in a comparable scale (5.4 $\mu g/cm^2$). It can be concluded that the presence of high cellulose-contents, paints, paste, and light is the most decisive factors to affect the toxigenic metabolite profiles of *Stachybotrys* spp. on building materials. Indeed, the activity of water (a_w-value) is an important parameter for *Stachybotrys* development and is related to the humidity, but conclusions about the humidity values for the naturally contaminated samples could not be drawn.

Ultimately, the question arises whether the results shown have a significance for human health. The satratoxins are clearly proven to be highly toxic and harmful, but they contribute in lower levels and are not formed by all species and chemotypes. Thus, it is still unclear whether and if so, to what extent the other presented *Stachybotrys*' toxins in their combination are relevant for detrimental diseases in humans. Reported different biological responses of spores from *Stachybotrys* strains grown on distinct wallboard type clarify the circumstances neither.[51] Nevertheless, the levels of toxins generated give cause for concern regarding human health when exposed to *Stachybotrys* spp. in a contaminated indoor environment.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Annika Jagels: Conceptualization (equal); Investigation (lead); Methodology (equal); Writing-original draft (equal). **Felix Stephan:** Investigation (supporting). **Simon Ernst:** Investigation (supporting); Methodology (supporting). **Viktoria Lindeman:** Investigation (supporting). **Benedikt Cramer:** Conceptualization (equal); Methodology (equal); Supervision (equal); Writing-review & editing (supporting). **Florian Hübner:** Conceptualization (equal); Methodology (equal); Project administration (equal); Supervision (equal); Writing-review & editing (equal). **Hans-Ulrich Humpf:** Conceptualization (equal); Funding acquisition (lead); Project administration (equal); Resources (lead); Supervision (lead); Writing-review & editing (equal).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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