

Remediation of mould damaged building materials—efficiency of a broad spectrum of treatments

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We compared the efficiency of some commercially available products and methods used for remediation of mould-contaminated building materials. Samples of gypsum board and pinewood were artificially contaminated with toxin-producing isolates of *Stachybotrys chartarum* and *Aspergillus versicolor*, respectively, then, ten different remediation treatments were applied according to the manufacturers' instructions. Microbial and chemical analyses of the infested materials were carried out both immediately before and after treatment, after six weeks of drying at room temperature, and after another six weeks of remoistening. The aim of the study was to determine whether the investigated methods could inhibit the mould growth and destroy some selected mycotoxins produced by the moulds. None of the decontamination methods tested could completely eliminate viable moulds. Some methods, especially boron based chemicals, ammonium based chemicals, and oxidation reduced the contents of mycotoxins produced by *S. chartarum* (satratoxin G and H, verrucarol), whereas the one which uses an ammonium based chemical reduced the amount of sterigmatocystin produced by *A. versicolor* with statistical significance. No remediation treatment eliminated all the toxins from the damaged materials. These results emphasize the importance to work preventively with moisture safety throughout the construction processes and management to prevent mould growth on building materials.

Introduction

Microorganisms are ubiquitous in the environment. Some species of moulds have found an ecological niche in damp buildings. Such moulds are frequently able to produce mycotoxins. Mycotoxins are secondary mould metabolites which exhibit high toxicity, for example cyto-, neuro-, and genotoxicity,

and are immunosuppressive.¹⁻⁴ Mycotoxins derived from mouldy spots at surfaces indoors may become airborne^{5,6} and thus inhalable by the inhabitants. Exposure to airborne mycotoxins is associated with symptoms in the lower respiratory tract, skin and eye irritation, chronic fatigue syndrome, immunosuppression⁷ and compromising of lung function.⁸

There may be hundreds of different mycotoxins in indoor environments of buildings affected by water-damage. For example, *Stachybotrys chartarum*, a mould that can use paper-lined gypsum board (containing cellulose) as a carbon source^{5,9} can produce over 90 different mycotoxins and other bioactive secondary metabolites. Other mycotoxin-producing moulds frequently encountered in damp indoor environments include *e.g.* *Penicillium*, *Aspergillus*, and *Alternaria*. Recently, in a study on moisture-damaged indoor environments, Täubel and colleagues¹⁰ utilized a liquid-chromatography tandem mass spectrometry (LC-MS/MS) method by which it is possible to

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Environmental impact

Our study shows that none of 10 different mould remediation agents and methods tested was able to totally remove mould from infested building materials. They were also ineffective in destroying the mycotoxins. Since it is known that moulds which are present on surfaces indoors may become airborne the results indicate that there is a risk of inhaling mycotoxins in buildings even after mould remediation.

detect up to 186 different fungal and bacterial secondary metabolites.

A variety of decontamination methods have been used to deactivate moulds including UV light (while almost 90% of *Aspergillus versicolor* died after UV exposure, *S. chartarum* was not much affected),¹¹ chlorine and sodium hypochlorite,^{12,13} ethanol,¹² gamma and UV radiation,^{14,15} ozone,¹⁶ chlorine dioxide,^{17–19} and various paints and cleaners.^{20–22} Bleach that contains sodium hypochlorite with antimicrobial activity is recommended by the US Environmental Protection Agency (EPA).¹² However, information is scarce on the different methods' ability to destroy moulds growing on building materials, and, in particular, whether mycotoxins are deactivated. It has been shown²³ that the efficiency of a combined sodium hypochlorite and detergent-containing solution in eliminating mould spores and deactivating mycotoxins differed between different mould species and substrates; remediation was successful in clothes and indoor paper materials but not in carpet and wood materials. The study utilized an indirect way of measuring the mycotoxins, viz. a yeast toxicity assay.

The aim of the present study was to compare the efficiency of ten different mould remediation methods including the use of heat, oxidation, boron based chemicals, chloride-based chemicals, and ammonium chloride based chemicals. Two common building materials contaminated with two common indoor water-indicating moulds, viz. *S. chartarum*-infected gypsum board and *A. versicolor*-infected pinewood, were used. Remediation efficiency was determined with regard to mould viability, by using microscopy and cultivation, and the presence of some mycotoxins known to be produced by these molds, by using liquid chromatography- (HPLC) and gas chromatography- (GC) tandem mass spectrometry (MSMS).

Materials and methods

Chemicals

Solvents and reagents were of analytical or HPLC grade and used without any further purification. The buffers were degassed and filtered through 0.45 µm filters (Millipore, Bedford, MA) before use. Water was distilled and deionised. Methanol, dichloromethane, and sodium hydroxide were purchased from Fischer Scientific (Loughborough, United Kingdom) and acetonitrile and toluene from Merck (Darmstadt, Germany). *N*-Heptafluoro-butyylimidazole (HFBI), and verrucarol (VER) were purchased from Sigma-Aldrich (Schnelldorf, Germany), 1,12-dodecanediol, ammonium acetate, and sodium acetate from Fluka (Schnelldorf, Germany), sterigmatocystin (STRG) from Biopure (Tulln, Austria), and reserpine from Varian, Inc. (Walnut Creek, CA). Trichodermin was a kind gift from Poul Rasmussen (Leo-Pharma, Denmark), and trichodermol (TRID) was derived from trichodermin by hydrolysis. Crude satratoxin G and satratoxin H (SATG and SATH) mycotoxin standards were kindly provided by Bruce B. Jarvis (Dept. of Chemistry and Biotechnology, University of Maryland).

Sample preparation

Materials. Gypsum board (Gyproc) and pine block were obtained from a local building supplies store. The materials,

visually clean and dry, were sawed into pieces with an area of 120 cm² and 30 cm², respectively, and sterilized by autoclaving. Negative control samples were collected from these materials. The materials were wetted with sterile water for 12 h to simulate water damage.

Inoculation of fungi. *S. chartarum* chemotype S (IBT 9460, a macrocyclic trichothecene- and trichodermin-producing strain) and *A. versicolor* (IBT 16000, a STRG-producing strain) were used to study the efficiency of the remediation of the mould-contaminated gypsum board and pinewood respectively. Both strains were kindly provided by Ulf Trane (Technical University of Denmark). Spore suspensions were collected from fungal cultures grown in Petri dishes on malt extract agar. 3 ml sterile water was added to each Petri dish which was gently agitated and a sterile plastic inoculation loop was carefully used to loosen the fungal spores from the plate. The spore suspensions were then collected with a sterile plastic pipette. To obtain confluent growth the suspension (containing spores and some hyphae) was sprayed over the materials with the assistance of a compressed air-system (*air-brush*). Following spraying *S. chartarum* onto gypsum boards the surface was divided into 4 parts, one for each sampling occasion (Fig. 1). The *A. versicolor* suspension was applied simultaneously on 4 pieces of pinewood. All samples were produced in duplicate and a positive control was included.

Experimental setup. The test samples were hung up on steel rods, with a distance of about 5 cm, in moisture chambers. The chambers were kept at room temperature with a relative humidity (RH) of 95 ± 5%. The temperature and RH were monitored by using an electronic logger (*TinyTag*). After 6 weeks of incubation confluent mould growth was achieved and the first samples (batch 1) were taken for analysis. The rest of the samples were subjected to the different remediation procedures. The second batch of samples was analysed immediately after treatment (24 h). The remaining test pieces (inoculated and remediated) were taken out from the moisture chambers and stored at room temperature and 40% RH for six weeks (third batch) to simulate drying of the materials which most often accompanies a remediation process. After the drying period the samples were again placed in the moisture chamber for another 6 weeks (fourth batch) to simulate re-moistening of the building materials in a re-occurring water-damage. The positive controls were held in the moisture chambers over the whole time period of the experiment to obtain the best conditions for mould growth and metabolic activity, i.e. to illustrate "the worst case scenario" of a water-damage without remediation.

Remediation procedures. The remediation methods used are listed in Table 1. The respective manufacturer's protocols, when available, were thoroughly followed. One of the material samples was only dried but otherwise treated according to the same schedule as the others. In short, the procedures were as follows. *Ozone*: an ozone producing unit was connected to an airtight box containing the building materials to be tested. The ozone level was determined using Draeger test tubes (ozon10/A, ca. 15 ppm) and the exposure period was 30 min. *Peroxide*: a commercially available product containing butane peroxide and hydrogen peroxide (1 : 1, 7%) plus propylene glycol was used. The

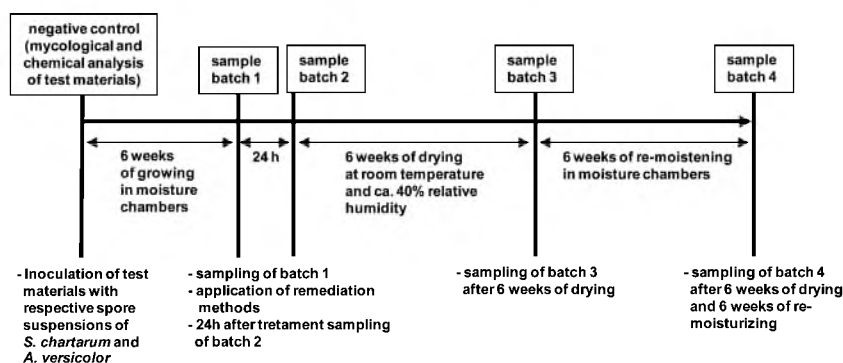


Fig. 1 Schematic of the project design.

manufacturer recommends spraying the solution onto the material. However, as spraying should be performed by specially trained professionals the solution was instead applied using a paint brush (thus also applying mechanical force). The dosage, just as recommended by the manufacturer, was 0.13 l undiluted solution (9 g of butane peroxide and hydrogen peroxide respectively) per m² of the surface of the material. The solution is corrosive but is consumed in the sanitation process. *Boron-based chemical 1*: a commercially available boron-based solution used for remediation of mould and bacteria was used. This solution contains *N*-didecyl dimethyl ammonium chloride (2%), disodium octaborate tetrahydrate (10%), and monopropylene glycol. The undiluted solution (ca. 0.25 l m⁻², corresponding to 5 g m⁻² of *N*-didecyl dimethyl ammonium chloride and 25 g m⁻² of disodium octaborate tetrahydrate) was applied on the building materials by brushing. The solution is highly flammable, corrosive, and toxic to the environment; according to the manufacturer it remains in the material after sanitation. *Boron-based chemical 2*: another commercially available boron-based solution was applied by spraying, in the form of steam, onto the material. The application was performed by the Finnish supplier as they hold a license to the application process. This solution is, according to the manufacturer, mainly comprised of boric acid and borax, which after a chemical reaction form a neutral borate with pH ca. 7. The solution is harmful for water-living organisms according to its registration protocol. However, according to the Finnish supplier it is not classified as harmful (with Finland's

environmental administration) and thus does not require labelling. According to the manufacturer the solution remains in the material after sanitation. *Hot air*: heat (300 °C) was applied with a hot air gun which was swept over the surface of the test materials for 2 min at a distance of 12 cm. *Flaming*: an open flame from a propane burner was swept over the surface of the test material similar to the hot air gun. The flame was swept at a distance of 15 cm and slightly faster over the surface to avoid burning of the material. *Steam*: the test materials were washed with steam from a steam cleaner device by sweeping the mouthpiece of the machine over the material surface at a distance of ca. 10–15 cm for 2 min. *Ammonium chloride-based chemical*: a commercially available algae and mould wash solution used for sanitation of algae, moss, mould, etc. was used. According to the manufacturer the solution contains *N*-alkylbenzyl dimethyl ammonium chloride (200 g l⁻¹), sodium nitrilotriacetate, and C10-alcoholtoxyolate. This corrosive chemical was brushed onto the materials in diluted solution (1 : 5, v/v) at a dose of 0.2 l (corresponding to 6.7 g *N*-alkylbenzyl dimethyl ammonium chloride) per m² and left on the material surface for 48 h (according to the instruction of the supplier). According to the manufacturer the solution remains in the material after sanitation. *Sodium hypochlorite-based chemical*: a common sodium hypochlorite-based solution (1–5%), labelled as irritant and corrosive, was used. The solution was brushed onto the test material in a diluted form (50% in water), corresponding to ca. 6 g sodium hypochlorite per m², and was left on the material surface for 30 min. According to the manufacturer the solution does not remain in the material after sanitation. *Drying*: the test material was dried at room temperature.

Table 1 Remediation methods

Index	Method	Manufacturer
A	Ozone	Ozone-generator device Airmaster
B	Peroxide	ALRON Chemical Ltd.
C	Hot air	Black & Decker
D	Flaming	Commercially available burner
E	Steam	Kärcher Ltd.
F	Boron-based chemical 1	Svenska reimpregnering Ltd.
G	Boron-based chemical 2	WSJ Sanitation Oy Ltd.
H	Ammonium chloride-based chemical	BIOkleen Ltd.
I	Sodium hypochlorite-based chemical	Colgate-Palmolive Ltd.
K	Drying	

Analytical procedures

Mycology. The materials were tested for moulds at each sampling occasion (Fig. 1). Phase-contrast microscopy was used to study mould cultures and tape-lift samples. Tape-lifts were collected by pressing an adhesive tape onto a mouldy surface. A drop of lactic phenol was then put on a microscope slide, the tape was pressed onto the slide, and another drop of lactic phenol was added. Finally a cover slip was overlaid and the slide was put under the microscope. The mould spores were examined and identified to genus level.²⁴ Separately, 1 mm broad stripes were cut from the gypsum board paper and placed on Petri dishes containing MEA. In the case of pinewood the surfaces were

gently pressed onto MEA. Agar plates were kept at room temperature and inspected for growth after 5, 7, and 10 days of incubation.

Mycotoxin analysis. Pieces (30 cm²) of the paper layer of the gypsum board contaminated with *S. chartarum* (1/4 at each sampling time) were cut off with a sterile scalpel and transferred into glass test tubes for methanol extraction following analysis of SATG, SATH, VER, and TRID. The surface top layer (30 cm²) of the pinewood samples contaminated with *A. versicolor* was cut off in a systematic fashion, collected in test tubes, weighed, and extracted following analysis of STRG.

A ProStar HPLC/1200L triple quadrupole MSMS instrument (Varian Inc., Walnut Creek, CA, USA) was used for measuring SATG, SATH, and STRG, and a CP-3800 GC triple quadrupole MSMS instrument (Varian Inc.) was used for measuring VER and TRID. VER is a hydrolysis product of all macrocyclic trichothecenes including satratoxins⁶ and can thus be used for screening *Stachybotrys* trichothecenes.⁹ TRID is formed by hydrolysis of its acetate ester, trichodermin, a mycotoxin produced by *Trichoderma* sp. and some strains of *S. chartarum*.²⁵ As in our previous study, VER, TRID, and STRG were quantified using calibration curves and internal standards and expressed as ng cm⁻² (sample area) or ng g⁻¹ (sample weight). SATG and SATH could not be quantified since we did not have access to pure standards; instead, the ratio to the amount of internal standard was used for establishing rank orders.^{5,6}

Statistical analysis. SPSS package version 17.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical computations. Data are expressed as median (batch 1) or mean (batch 2–batch 4) and standard deviation. The effects of treatment on deviation in mycotoxin levels were evaluated by repeated measures analysis of variance (rANOVA). Therefore, differences between the median of batch 1 and the specific level of every subsequent batch (2, 3 and 4) were calculated and analysed. Pairwise differences were assessed by paired *t*-tests with Bonferroni correction. All comparisons were two-tailed and a *p* value of <0.05 was taken as being statistically significant.

Results and discussion

Mycology

Before remediation *S. chartarum* and *A. versicolor* showed confluent growth on the infected building materials. After the drying period (3rd batch) no noticeable differences with the 2nd batch samples were observed but after re-moisturising the materials (4th batch) fungal growth increased rapidly.

All samples of gypsum board paper and pinewood were culture-positive for *S. chartarum* and *A. versicolor*, respectively, except those which had been treated with the algae and mould wash solution; the latter were culture-positive for other (unidentified) moulds. Prints of pinewood samples gave such strong growth that species identification was difficult to perform. Cultivation is, however, not a quantitative measurement of the amount of mould present in the materials as it does not take into account the dead biomass (which also may contain mycotoxins and allergens). Furthermore, the cultivation on MEA, a rather

rich culture medium, does not illustrate the growth conditions on building materials *in situ*. It rather gives the opportunity to study the survival potential of the mould.

Mycotoxins

An overview of the toxin contents in the different materials before and after the treatments is presented including mean values of duplicate samples and positive controls (Fig. 2 and 3). Also shown in Fig. 3 are levels of significance for the differences between the toxin levels of the treated samples and the untreated positive controls over the experimental time span.

For further assessment of treatment efficiency coefficients of variation (CV) using the results from batch 1, where all samples are untreated, were calculated to determine a so-called biological variation between samples. This helped to distinguish between treatment affected changes in toxin levels and changes due to biological and methodological variations. For *Stachybotrys chartarum* infected gypsum board CVs of 24%, 25%, 27% and 20% were found for SATG, SATH, TRID and VER, respectively. For the *Aspergillus versicolor* infected pinewood material and the related toxin STRG a CV of 44% was found.

Concerning the positive control samples (Fig. 2) in the *S. chartarum* infected material the levels of the toxins observed initially (batch 1 and batch 2) increased dramatically after the drying period (batch 3); for TRID the increase was up to 4000% compared to batch 1. After re-moistening (batch 4) the levels of SATG and SATH were reduced but TRID and VER increased furthermore. In general, SATG was slightly more abundant than SATH. The amounts of STRG in the *A. versicolor*-infected pinewood samples remained quite constant with around 10% increase in batches 2–4 compared to batch 1, which may reflect more biological variation than further production of toxin.

The changes in toxin contents in samples following the different remediation methods were as follows.

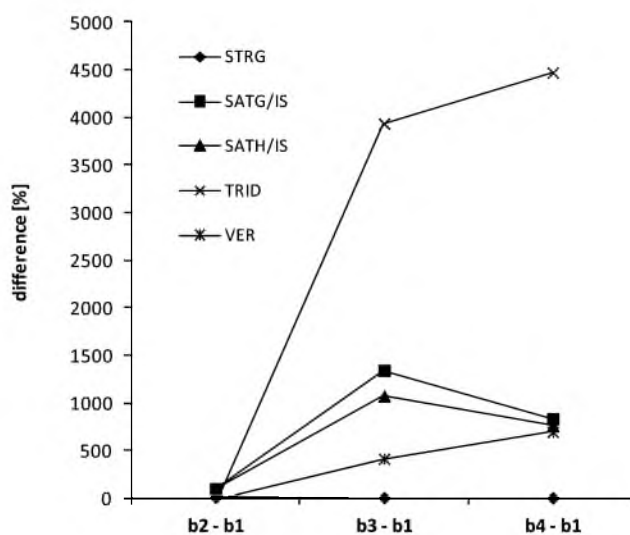


Fig. 2 Relative differences in the amounts of mycotoxins in the positive control samples over the experimental time span in relation to batch 1 (e.g. b2 – b1 denotes the difference between time point batch 2 and time point batch 1, etc.).

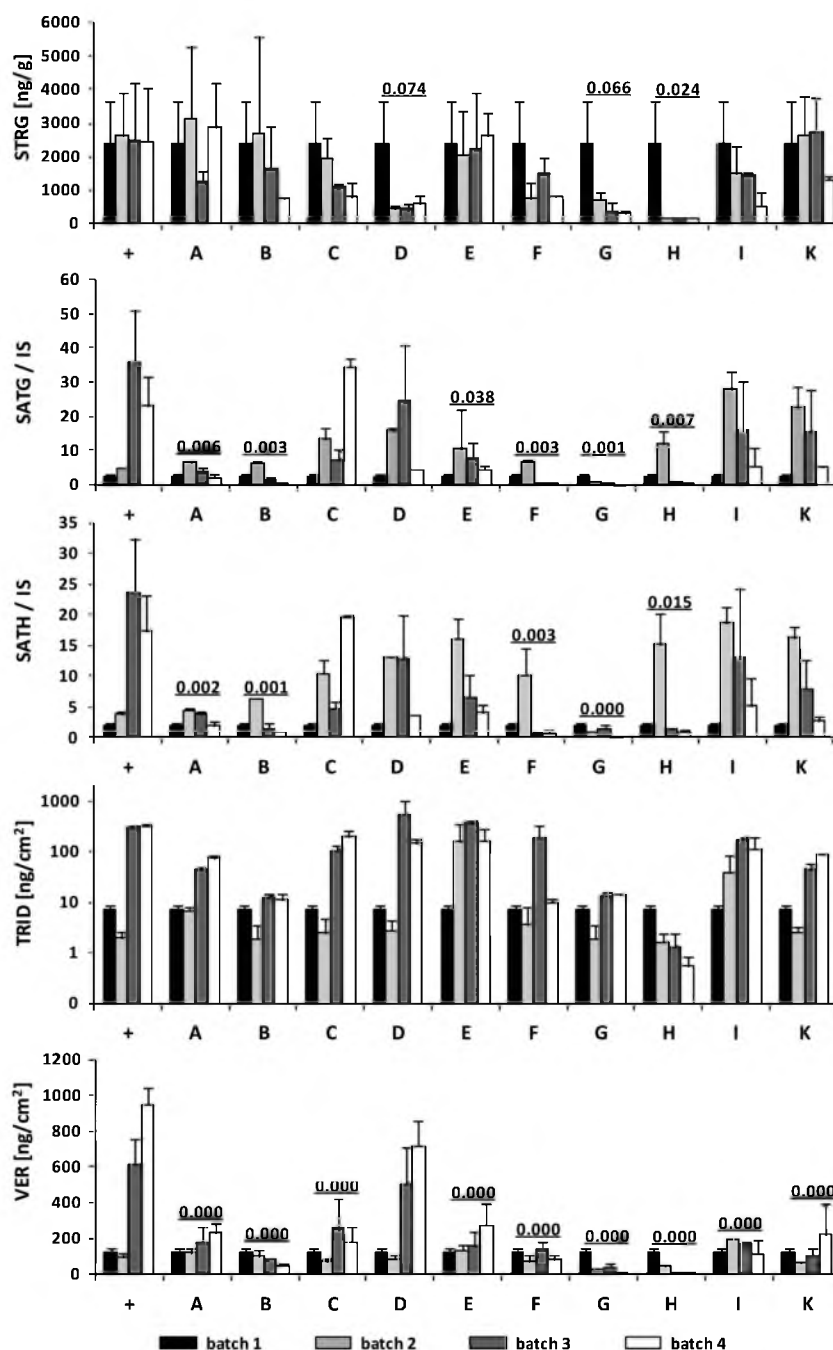


Fig. 3 Amounts of mycotoxins in the building material samples before (batch 1) and 24 h after (batch 2) treatment, and after drying (batch 3) and re-moistening (batch 4). Bars represent the median (batch 1) and mean values, and error bars are standard deviations. Underlined figures show the levels of significance in comparing positive control samples with the treated samples. Capital letters stand for different treatments: A—ozone, B—peroxide, C—hot air, D—flaming, E—steam, F—boron based chemical 1, G—boron based chemical 2, H—ammonium based chemical, I—sodium hypochlorite based chemical and K—drying, and + represents the positive control.

The effect of the *ozone* treatment was statistically significant for SATG ($p = 0.006$), SATH ($p = 0.002$) and VER ($p = 0.000$) whereas no effects were observed for TRID and the *Aspergillus* related toxin STRG. The amounts of SATG and SATH increased directly after treatment by about 100% (Fig. 4b and c). After the drying period and the re-moistening SATG levels decreased to a level of $\sim 20\%$ respectively batch 1. A similar trend was observed for SATH although levels in batch 1 and batch 4 were the same ($b_4 - b_1 = 0\%$). Although a statistically significant

difference between positive control and ozone treated samples was found for VER, the VER levels increased dramatically also in the treated sample materials during the drying period (50%, Fig. 4d) after treatment and further within re-moistening (100%, Fig. 4d). The results indicate that ozone does not inhibit the production of the studied mycotoxins (possibly except for the satratoxins). Ozone has been reported to be ineffective in treating building materials with mould growth,²⁶ especially in porous materials as gypsum board.¹⁶ This may seem surprising since

ozone contributes to primary and secondary chemical reactions²⁷ forming products which may be harmful both to microorganisms and materials.

The initial effect of the *peroxide* treatment was that the levels of SATG and SATH increased about 150% (Fig. 4b and c) whereas those of VER (Fig. 4d) and TRID decreased. After drying and re-moistening decreased amounts of SATG (–50% and –73%, respectively), SATH (–25% and –51%, respectively), and VER (–26% and –60%, respectively), as well as increased amounts of TRID (70%), were observed. By contrast, a 5-fold reduced STRG level was observed at the end of the experiment which was not significant compared to the positive control. As for the ozone treatment significant changes were found for SATG ($p = 0.003$), SATH ($p = 0.001$) and VER ($p = 0.000$).

This indicates that treatment with oxidizing agents as ozone and peroxide reduces the amounts of macrocyclic trichothecenes like SATG and SATH, also followed by lowering the amounts of VER, the hydrolysis product of satratoxins as well as a secondary metabolite in itself.

The treatment with *hot air* (gun) gave an initial increase of the amounts of the satratoxins, after which a slight decrease occurred (after drying), and again an up to 60-fold increase after re-moistening related to observed contents at the beginning of the experiment (Fig. 3). VER and TRID decreased immediately after treatment but increased after drying. STRG production (on wood) increased immediately after treatment but decreased during drying so that the amount at the last sampling time was around 1/3 of that of the positive control. VER showed a significant difference compared to the positive control ($p = 0.000$), which is actually related to a smaller increase of VER, but not to a serious reduction of VER over time (Fig. 4d).

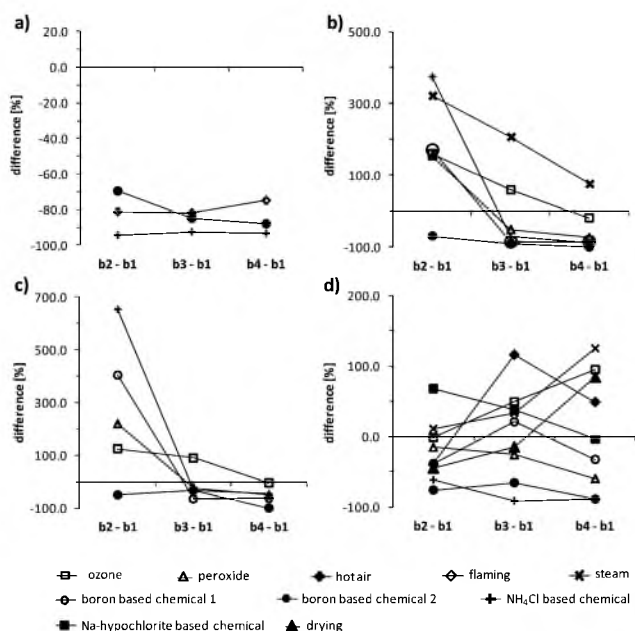


Fig. 4 Relative differences in the amounts of mycotoxins STRG (a), SATG (b), SATH (c) and VER (d) in treated samples with statistically significant differences to positive control samples in relation to batch 1 (e.g. b2 – b1 denotes the difference between time point batch 2 and time point batch 1, etc.).

Flaming the surface resulted in a 5-fold increase in the amounts of the satratoxins after treatment (batch 2) (Fig. 3). After drying the level of SATH was rather constant while SATG increased slightly; re-moistening resulted in a marginal decrease. VER and TRID decreased slightly immediately after treatment but increased sharply after the drying period; remarkably by a total of over 190 times for TRID and close to 50 times for VER. Re-moistening had only a little effect on VER but reduced the TRID level by a factor of 4. The results indicate, just as for hot air, that flaming may actually increase the amount of some *Stachybotrys* toxins. STRG (on pinewood) decreased directly after treatment and remained constant thereafter, which may suggest a stop in toxin production by *A. versicolor*. Only for the latter, a statistically significant trend ($p = 0.074$) for differences between the treated samples and positive controls was observed. Although excluded due to using a cut-off of $p = 0.05$, accounting the formerly mentioned CV for STRG in *A. versicolor* infected pinewood, a possible strong treatment related reduction of STRG, by around 90%, was found (Fig. 4a).

Treatment with *steam* increased the levels of the *Stachybotrys* toxins particularly TRID where the increase was 20-fold. Subsequent drying decreased the amounts of the satratoxins and increased the levels of VER and TRID whereas re-moistening decreased the levels of the *Stachybotrys* toxins except VER. Overall, the amounts of these toxins increased during the experiment's time span, but less than did the positive control. Statistically significant differences between the positive control samples and the treated samples were observed for SATG ($p = 0.038$) and VER ($p = 0.000$) (Fig. 3). SATG levels decreased over time relative to batch 1 (Fig. 4b), while VER levels increased (Fig. 4d). No effect on STRG was observed. The results indicate that the sanitation method with steam as used in this study did not reduce the studied toxins.

The use of *boron-containing chemical 1* resulted initially in increased amounts of the satratoxins and decreased amounts of VER and TRID. After the drying period the opposite was found, with a 50-fold increase in the amounts of TRID. Overall, the treatment resulted in significantly decreased levels of SATG ($p = 0.003$), SATH ($p = 0.003$), and VER ($p = 0.000$), and a moderate increase in the level of TRID (Fig. 3). The amount of STRG decreased by a factor of 5 directly after treatment; the levels after drying and re-moistening were about 1/3 of the amounts of the positive control but the difference was not significant. In general, this treatment seems to reduce the levels of the toxins possibly except for TRID, the amount of which varied widely between samples. Clearly, this treatment does not eliminate mycotoxins in the building material completely. In another study it was recently shown that another boron-based chemical, also containing tetrahydrate-disodium-octaborate (BORA-CARE), delayed and diminished the spread of mould growth²⁸ but its effect on mycotoxins was not assessed.

The use of *boron-based chemical 2* reduced statistically significant levels of SATG ($p = 0.001$), SATH ($p = 0.000$) and VER ($p = 0.000$) on the gypsum board, but after drying TRID increased 6-fold. After the re-moistening satratoxins were no longer detectable, VER was decreased, and TRID remained unchanged. The toxin levels were very low in comparison with the positive control. The STRG levels decreased after treatment and remained low. The results indicate significantly that this

treatment method is able to degrade the mycotoxins studied except TRID (Fig. 3 and 4).

The ammonium chloride-based solution led to an initial increase in the amounts of the satratoxins followed by a subsequent drastic decrease (batches 3 and 4). Levels of VER and TRID on the gypsum board, and STRG on wood samples decreased sharply after application and remained low; the difference was significant for SATG ($p = 0.007$), SATH ($p = 0.015$), VER ($p = 0.000$) and STRG ($p = 0.024$) (Fig. 3 and 4). The amounts of toxins were the lowest among all the tested treatments which indicates a degradation of the toxins and/or inhibition of toxin production of the studied moulds on the respective material. Another ammonium chloride-based chemical, containing *N*-alkylbenzyl dimethyl ammonium chloride (Allstar Corp., Valley Forge, PA), was previously used to kill *S. chartarum* on the gypsum board where, however, mould growth established itself afterward with additional fungal species,²⁹ like in this study. The effect of the product on mycotoxins was not studied.

Sodium hypochlorite treatment increased the amounts of all the toxins in the gypsum board (Fig. 3). After drying the amount of TRID continued to increase while the satratoxins and VER were kept rather constant. No clear effect of re-moistening was observed. Statistical testing showed statistically significant differences between VER in the positive control samples and in the treated samples. However a strong reduction of VER, shown as the relative difference between batch 1 and batch 4, could not be observed (Fig. 4d). The levels of STRG decreased slowly throughout the experiment but not significantly. The results indicate that sodium hypochlorite may stimulate the production of the toxins produced by *S. chartarum* but inhibit, or destroy, the production of the toxins produced by *A. versicolor*. According to the WHO¹³ VER and TRID as well as the satratoxins may be inactivated by sodium hypochlorite (3–5% concentration). Sodium hypochlorite is described in another study to kill *A. fumigatus* and reduce reactions in skin tests.³⁰

The remaining material sample was not subject to any other treatment than drying. Satratoxin contents increased between batches 1 and 2, and decreased again after additional drying and re-moistening. VER and TRID decreased marginally at sampling time 2 but increased after the second drying period and further on after re-moistening. Overall TRID increased as much as the positive control, VER also increased during the experimental schedule, whereby significant differences between the positive control and treated sample were observed ($p = 0.000$). Thus drying seems to have no effect on toxin stability or toxin production by *S. chartarum* on the gypsum board. The amounts of STRG (on pinewood) decreased at sampling time 2, remained constant after the second drying period, and decreased again after re-moistening. Accounting the calculated biological variation, this indicates that drying has no effect on toxin stability and/or toxin production of *A. versicolor* on pinewood. Overall, drying of the mould damaged material only did not destroy the mycotoxins.

Conclusions

None of the sanitation methods used could totally eliminate the moulds and mycotoxins from the building materials used. Interestingly, most methods resulted in an initial increase of the

Stachybotrys satratoxins, possibly due to stress. In general, the methods seemed to be more effective against *S. chartarum* growing on the gypsum board with special impact on macrocyclic trichothecenes SATG and SATH as well as VER. Amounts of STRG produced by *A. versicolor* growing on pinewood decreased following any of the used sanitation methods. However, considering the coefficient of variation of around 44%, calculated using the results from batch 1, it is not clear if the decrease in STRG is related to biological variation or an effect of treatment.

The different effects on different moulds and mycotoxins found in our study are in line with the results of Huttunen *et al.* where the immunotoxic potential of airborne particles and the number of microbes were used as indicators for a successful remediation of water damaged buildings.³¹ Studying different parameters for assessment of remediation, Haverinen-Shaughnessy *et al.* found no improvement in indoor air quality or in the health status of the residents, although the remediation was performed according to the manufacturer's manual, as in our study.³² The fact that the sanitation methods used in our study did not totally eliminate viable moulds and mycotoxins raises the question what obligation the suppliers have to ascertain the efficiency of their sanitation methods. More research is required for developing remediation methods which are both environmental friendly and efficient in eliminating mould growth and mycotoxins on building materials.

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