Molds and mycotoxins in dust from water-damaged homes in New Orleans after hurricane Katrina

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Molds and mycotoxins in dust from water-damaged homes in New Orleans after hurricane Katrina

Abstract Dust collected in New Orleans homes mold-contaminated because of the flooding after hurricane Katrina was analyzed for molds and mycotoxins. The mycoflora was studied by cultivation and quantitative PCR for selected molds. The most commonly found mold taxa were *Aspergillus*, *Cladosporium*, and *Penicillium*. Verrucarol, a hydrolysis product of macrocyclic trichothecenes predominately produced by *Stachybotrys* spp. was identified in three dust samples by gas chromatography-tandem mass spectrometry, and sterigmatocystin (produced by various *Aspergillus* spp.) was found in two samples by high pressure liquid chromatography-tandem mass spectrometry. This is the first demonstration of mycotoxins in Katrina-associated dust samples. The analytical methods used represent valuable tools in further studies on bioaerosol exposure and health risks.

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Practical Implications
In the aftermath of natural disasters like hurricane Katrina water-damages on infrastructure and public and private property are often associated with health risks for remediation workers and returning residents. In the case of New Orleans evaluations of health hazards, health studies, and assessments of bioaerosol have been conducted previously. However, until now mycotoxins have not been addressed. Our study shows, for the first time, the presence of mycotoxins in dust collected in houses in New Orleans mold-contaminated because of the hurricane Katrina. The results may highlight the potential health threats posed by mold aerosols in post-disaster inhabited areas.

Introduction

In August 2005, hurricane Katrina, one of the costliest and deadliest natural disasters in the history of United States, struck Louisiana, Mississippi, and parts of Florida, Georgia, and Alabama. In New Orleans, the city levee system catastrophically failed; about 80% of the city flooded to varying depths of up to about 6 m, causing a large loss of lives and property damage (Knabb et al., 2005). Because of the floods, approximately 80% of all houses and their contents were heavily contaminated by mold growth. In this study the objective was to analyze house dust samples collected in water-damaged buildings in New Orleans for the presence of molds and some selected mycotoxins (toxic secondary metabolites of molds) potentially produced in damp indoor environments (Bloom et al., 2007b). Verrucarol (VER) and trichodermol (TRID), hydrolysis products of macrocyclic trichothecenes (including satratoxins) and trichodermin respectively (all predominately produced by *Stachybotrys* spp.), were analyzed by gas chromatography-tandem mass spectrometry (GC-MSMS). Sterigmatocystin (STRG, mainly produced by *Aspergillus* spp.), satratoxin G (SATG), and satratoxin H (SATH) were analyzed by high pressure liquid chromatography-tandem mass spectrometry (HPLC-MSMS). The dust mycoflora was studied by cultivation and by a mold-specific quantitative PCR assay (MSQPCR) (Haugland et al., 2002).

Materials and methods

Chemicals and standards

Solvents and reagents were of analytical or HPLC grade and used without any further purification. Buffers were degassed and filtered through 0.45 μm filters (Millipore, Bedford, MA, USA) before use, and water was distilled and deionized. Cultivation media were prepared at the Department of Laboratory Medicine in Lund according to protocols provided by the Centraalbureau voor Schimmelcultures, the Netherlands (http://www.cbs.knaw.nl/). All cultivation media were supplemented with 100 ppm chloramphenicol (Sigma, St Louis, MO, USA). Methanol, dichloromethane, sodium chloride and sodium hydroxide were purchased from Fischer Chemicals (Leicester, UK) and acetonitrile, toluene, and acetone from Lab Scan (Dublin, Ireland). N-heptafluorobutyrylimidazole (HFBI), STRG, and VER were purchased from Sigma (Schnelldorf, Germany). 1,12-Dodecanediol, ammonium acetate, and sodium acetate were purchased from Fluka (Schnelldorf, Germany and Bachu, France). Reserpine (5 ng/μl) was purchased from Varian Inc. (Walnut Creek, CA, USA). Trichodermin was a kind gift from Poul Rasmussen (Leo-pharma, Ballerup, Denmark), and TRID was derived from trichodermin by hydrolysis. Crude SATG and SATH mycotoxin standards were kindly provided by Professor Bruce B. Jarvis (Department of Chemistry & Biotechnology, University of Maryland, College Park, MD, USA).

Sampling

The field sampling was conducted during January 2007 in five severely mold-contaminated single-family houses (Table 1) in New Orleans, LA, USA, which had been flooded during hurricane Katrina. Sampling was made approximately 5 months after flood water receded; at that time the houses had not been remediated or re-inhabited. Bulk composite samples were collected in small plastic bags from the middle of the floor surface area of selected rooms using a small plastic brush and dust pan (washed with soap and water, sprayed with 70% ethanol, and dried before using in each home), and kept at −20°C before analysis.

Cultivation and identification

Approximately 100 mg of each dust sample was suspended and serially diluted (10^1, 10^2, 10^3, 10^4, 10^5 times) in 0.85% NaCl solution. An aliquot from each dilution was plated onto malt extract agar, czapek yeast extract agar, and dichloran 18% glycerol agar, and incubated at 22°C for 7 days. Colony forming units were counted and fungal taxa identified using phase-contrast microscopy according to Samson et al. (1995).

Quantitative PCR

Parts of the dust samples (10 mg) were sent to Anozona (Uppsala, Sweden), contract partner of Fugenex (Yorkshire, UK), who are licensed to use DNA extraction and MSQPCR analysis according to Haugland and Vesper (2002) (US patent 6,387,652) as described elsewhere (Haugland et al., 2002; Vesper et al., 2004). Information on the primer and probe sequences is provided at http://www.epa.gov/microbes/moldtech.htm.

Sample preparation, extraction and purification

Dust (~1 g) was prepared for chemical analysis (Bloom et al., 2007a,b). In brief, samples were extracted with methanol (2–3 ml) in 10-ml glass test tubes with Teflon-lined screw caps, stored in the dark for 48 h at room temperature, and centrifuged. The supernatants were decanted into new tubes. Extraction was repeated with dichloromethane after which the methanolic and dichloromethane phases were pooled, evaporated under a gentle stream of nitrogen, dissolved in dichloromethane, and applied onto polyethyleneimine

Table 1 Description of five flooded homes in New Orleans from which bulk dust samples were collected

	House 1	House 2	House 3	House 4	House 5
Sample Date	1/8/2007	1/8/2007	1/8/2007	1/29/07	1/25/2007
Amount of flood water	~3 m	~3 m	~3 m	~3 m	~1.5 m
Type of building	Single	Single	Single	Single	(1/2) Attached Double
	Bricked, ranch style	Bricked, ranch style	Bricked, ranch style	Bricked, ranch style	Raised wood frame, shotgun style
	Concrete floor	hardwood, tile, terrazzo, and area rug flooring	Tile, concrete floors	Carpet and concrete flooring	Hardwood, linoleum, and carpet flooring
	Living, kitchen, bath, (3) bedrooms	Living, kitchen, bath, (3) bedrooms	Living, kitchen, bath, (3) bedrooms	Living, kitchen, bath, (3) bedrooms	Living, kitchen, bath, (2) bedrooms
Samples collected	Combined bulk sample	Combined bulk from hardwood, tile and walls; bulk from tile and hardwood	Combined bulk from tile and concrete	Bulk from concrete	Bulk from linoleum and hardwood

bonded silica gel columns (PEI, 1 ml, JT Baker, Phillipsburg, NJ, USA). Samples were eluted with 5 ml dichloromethane, evaporated, re-dissolved in 0.5 ml methanol, and filtered through 0.45 μm Millex syringe filters (PTFE, Millipore, Bedford, MA, USA) into new Teflon-capped analysis vials. After adding 1 ng of reserpine as an internal standard, 50 μl was taken for direct HPLC-MSMS analysis. The remaining 450 μl were mixed with 500 pg 1,12-dodecanediol (internal standard in GC-MSMS), evaporated, hydrolyzed in 0.2 M methanolic NaOH, and extracted with water and dichloromethane. The organic phase extracts were taken to new tubes, evaporated to dryness and placed in a desiccator overnight. The dried extracts were then subjected to derivatization by adding 80 μl of acetonitrile-toluene (1:6, v:v) and 20 μl of HFBI, followed by heating at 70°C for 60 min. Samples were then left standing in excess of derivatizing agent at room temperature at minimum of 4 h before analysis by GC-MSMS.

HPLC-MSMS and GC-MSMS

A ProStar HPLC/1200L triple quadrupole MSMS system (Varian Inc., Walnut Creek, CA, USA) was used. 20 μl of sample was injected (in duplicate) using an autosampler (Varian, model 410) into a Polaris 5 μM C18-A 150 × 2.0 mm RP-18 column. An initial methanol concentration of 20% was held for 1 min, after which it was raised linearly (9 min) to 70% before it was again raised linearly (10 min) to 100% and held for 9 min. At the end of the run, the concentration of methanol was linearly lowered again (1 min) to 20% and kept 8 min for stabilization. See (Bloom et al., 2007b) for further details.

Derivatives were analyzed by GC-MSMS using a CP-3800 GC triple quadrupole MSMS system (Varian Inc.). Analyses were made in negative chemical ionization mode, at 70 eV, an ion source temperature of 150°C, and with ammonia as ionization gas (0.4 kPa). Duplicate sample volumes of 1 μl were injected onto a FactorFOUR™ fused-silica capillary column (VF-5 ms, 30 m × 0.25 mm i.d) (Varian Inc.) in the splitless mode. A mix of HFBI and acetone (1:3, v:v) was injected in between samples to eliminate any trace of un- or semi-derivatized VER/TRID. Details on MSMS conditions are provided elsewhere (Bloom et al., 2007a,b).

Results

Cultivation

The mycoflora of the cultivated dust samples is described in Table 2. House 1 and House 3 contained the highest number of fungal propagules and House 4 the lowest. Indicator organisms of water damage, e.g. *Chaetomium* sp., *Trichoderma* sp., and yeasts, were identified in approximately half of the samples. Only one sample (House 4) did not show any growth of *Aspergillus* spp., and *Stachybotrys* spp. were not found in any sample.

PCR

The results are summarized in Table 3. As in the cultivation studies House 1 and House 3 contained the largest amounts of fungi and House 4 the least. *Stachybotrys chartarum* was identified in all samples

Table 3 Number of mold and bacterial DNA-sequences per mg dust

Sample	Total mycoflora	*A. versicolor*	*S. chartarum*	*Aspergillus* spp. *Penicillium* spp.	*Streptomyces* spp.
House 1	716,000	n.d.	7972	7160	448
House 2a	10,040	n.d.	14	237	n.d.
House 2b	67,760	n.d.	13	2087	n.d.
House 2c	21,168	n.d.	66	117	n.d.
House 3	1,353,200	n.d.	572	11,824	n.d.
House 4	385	n.d.	n.d.	n.d.	n.d.
House 5	159,120	27	36	253	n.d.

n.d., not detected.

Table 2 Molds cultured from the dust samples using three different agar media

	CFU/mg dust (Fungal taxa)		
Sample	MEA	CYA	DG18
House 1	1280 (*Penicillium*, *Aspergillus*, *Trichoderma*, yeast, *Cladosporium*, *Mycelia sterila*)	9700 (*Aspergillus*, *Trichoderma*, *Cladosporium*)	922 (*Penicillium*, *Aspergillus*, yeast, *Mycelia sterila*)
House 2a	0	53 (*Cladosporium*, *Aspergillus*, *Mycelia sterila*)	490 (*Penicillium*, yeast, *Mycelia sterila*)
House 2b	55 (*Cladosporium*, *Aspergillus*, *Chaetomium*)	100 (*Cladosporium*, *Aspergillus*, *Mycelia sterila*)	18 (*Aspergillus*, *Paecilomyces*)
House 2c	85 (*Aspergillus*, *Cladosporium*, *Mycelia sterila*)	60 (*Cladosporium*, *Aspergillus*, bacteria)	10 (*Mycelia sterila*)
House 3	20580 (*Aspergillus*, *Trichoderma*, yeast, *Cladosporium*, *Chaetomium*, *Alternaria*, *Fusarium*, *Mycelia sterila*)	13880 (*Aspergillus*, yeast, *Trichoderma*, *Cladosporium*, *Chaetomium*, *Mycelia sterila*)	20870 (*Penicillium*, *Trichoderma*)
House 4	0	0	100 (Yeast, *Mycelia sterila*)
House 5	9 (*Aspergillus*)	61 (*Penicillium*, *Alternaria*, *Mycelia sterila*)	9 (*Aspergillus*)

CFU, colony forming units; *Mycelia sterila*, non-sporulating mycelium; MEA, malt extract agar; CYA, czapek yeast extract agar; DG18, dichloran 18% glycerol agar.

Table 4 Mycotoxins in the studied dust samples

Sample (~1 g dust)	Pg mycotoxin/mg dust				
	TRID	VER	STRG	SATG	SATH
House 1	n.d.	0.6	16	n.d.	n.d.
House 2a	n.d.	n.d.	n.d.	n.d.	n.d.
House 2b	n.d.	n.d.	n.d.	n.d.	n.d.
House 2c	n.d.	n.d.	28	n.d.	n.d.
House 3	n.d.	n.d.	n.d.	n.d.	n.d.
House 4	n.d.	0.6	n.d.	n.d.	n.d.
House 5	n.d.	18	n.d.	n.d.	n.d.

n.d., not detected.
TRID, trichodermol; VER, verrucarol; STRG, sterigmatocystin; SATG, satratoxin G; SATH, satratoxin H.

except House 4 where also the other specified fungal species/taxa and *Streptomyces* bacteria were absent. *Aspergillus versicolor* was only detected in House 5 and *Streptomyces* only in House 1.

Mycotoxin analysis

The mycotoxin findings are summarized in Table 4. VER was found in House 5 (18 pg/mg dust) and in trace amounts in House 1 and House 4 (0.6 pg/mg dust). In these analysis ions of m/z 638 (M − HF) were fragmented and quantification was performed based on product ions of m/z 213 as described earlier (Bloom et al., 2007a). STRG was found in House 1 (16 pg/mg dust) and in House 2c (28 pg/mg dust). Here, ions of m/z 325 were fragmented and product ions of m/z 310 were used for quantification (Bloom et al., 2007b). TRID, SATG, and SATH were not found in any of the dust samples. The limits of detection for VER, TRID, and STRG standards are 72, 37, and 19 pg added to 1 mg of dust respectively (Bloom et al., 2007a,b).

Discussion

There have been several reports concerning public health-related issues in post hurricane New Orleans including initial microbiological and chemical contaminant assessments, e.g. of semi-volatile compounds, arsenic, lead, and fecal indicator microorganisms (Pardue et al., 2005; Presley et al., 2006; Schwab et al., 2007; Sinigalliano et al., 2007). In microbial assessment studies in different locations high mean indoor/outdoor mold spore ratios were found; 4.11 (Schwab et al., 2007), 4.8 (Solomon et al., 2006), and 8.3 (Rao et al., 2007). The mold genera most commonly found indoors were, respectively, *Aspergillus* and *Penicillium*, *Cladosporium,* and *Curvaria* (Schwab et al., 2007), *Cladosporium*, *Aspergillus*/*Penicillium,* and *S. chartarum* (Solomon et al., 2006), *Aspergillus niger*, *Penicillium* spp., *Trichoderma,* and *Paecilomyces* (Rao et al., 2007), *Penicillium, Aspergillus* and *Paecilomyces* (Chew et al., 2006), and *Penicillium* and *Aspergillus* [Center for Disease Control (CDC)., 2006]. Molds and fungal glucans were detected at highly-elevated concentrations (Rao et al., 2007) and the mycoflora was not typical for non-water damaged buildings (Rao et al., 2007; Solomon et al., 2006). Interestingly, the area concentrations of molds on indoor surfaces were consistent with measured airborne mold levels (Rao et al., 2007; Schwab et al., 2007), and after remediation intervention mold and endotoxin levels in the indoor air tended to be lower (Chew et al., 2006). In a CDC report from October 2005, glucan and endotoxin levels were significantly correlated and highest indoors (CDC, 2006). Highly-elevated concentrations of endotoxin were also found by Rao et al. (2007). However, Solomon et al. (2006) found no association between flooding and endotoxin concentrations or between endotoxin concentrations in flooded and non-flooded areas in New Orleans.

The fungal taxa found in the present work (except for yeasts, and in one case, *Fusarium*) have been identified also in other studies on molds associated with hurricane Katrina (as mentioned before) (Chew et al., 2006; Rao et al., 2007; Schwab et al., 2007; Solomon et al., 2006). Chew et al. (2006) used PCR for detecting 23 mold species in air samples before, during, and after renovation of three New Orleans homes; *Aspergillus*, *Penicillium,* and *Cladosporium* were the most common molds, and the prelevance of *Stachybotrys* was 40%. In our study, *Stachybotrys* was identified in all samples but one when using PCR, but not in a single sample when using culture. However, spores of *Stachybotrys* spp. in indoor environmental samples are often non-viable (Miller et al., 2000). *Aspergillus*/*Penicillium* species were found in all studied samples, with both PCR and culture, except in one. This sample (House 4) was PCR-negative for the studied microflora, and culture revealed only little growth of yeast and non-sporulating mycelium, even so traces of VER were detected (Table 4). These seemingly conflicting results may be explained by (i) the sample had not been thoroughly homogenized (*viz,* sub-samples used for toxin analysis and PCR may not be representative for the sample as a whole), (ii) a producer of VER or macrocyclic trichothecenes other than *Stachybotrys* was present (for example *Myrothecium* sp.), (iii) the sample was too diluted to contain enough DNA for PCR or, (iv) PCR inhibitors were present. In addition, the homeowner of house 4 may have applied antimicrobial agents, a common practice in post-Katrina New Orleans. Several such agents contain oxidizing substances e.g. chlorine and ozone known to degrade DNA.

Neither SATG nor SATH were found, not even in the VER-positive samples, which is likely to be due to the limited analytical sensitivity for these compounds in HPLC-MSMS in comparison with VER detection using GC-MSMS (Bloom et al., 2007b). The trichodermin-negative results may indicate that the

Stachybotrys spp. found were of chemotype S type. *Aspergillus* spp. were identified in all samples positive for STRG (a mycotoxin produced by *Aspergillus* spp., e.g. *A. versicolor*, plus a limited number of other molds, Frisvad, 1989; Hajjar et al., 1989).

In general, the amounts of fungi found in the studied samples did not correlate with the amount of mycotoxins, *viz* the samples with the highest fungal concentrations did not contain the highest concentration of mycotoxins. Indeed, the interactions between different molds and between molds and other microorganisms, and the subsequent variation in the production of toxins and other secondary metabolites, are complex and currently only partly understood. Mold and mycotoxin exposure assessment methods need further improvements not only for achieving a better basic understanding of the indoor environmental microflora and its potential health impact but also in relation to consequences in juridical, political, and economical perspectives (Mudarri and Fisk, 2007).

This is the first report on the detection of mycotoxins in dust samples from New Orleans homes water-damaged because of the hurricane Katrina. The results emphasize the importance for returning residents and remediation workers to still today, 2 years after the Katrina disaster, use adequate personal protective equipment. Studies show that few reliably use the recommended protective appliances (CDC, 2006; Cummings et al., 2006). The demonstration of mycotoxins in flooded dwellings in New Orleans may highlight the potential health threats posed by mold aerosols in post-disaster inhabited areas and influence further guideline development for remediation of mold-affected buildings.

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