

Research Article

Assessment of mold contamination in hurricane-damaged homes in Houston, Texas after sanitization by volunteers

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Abstract

The purpose of this pilot study was to evaluate the effectiveness of mold sanitation in homes that suffered hurricane-related water damage. After a home is flooded, sanitation of the structure for mold is necessary before the interior of the home can be rebuilt. In this study, homes ($n = 6$) in Houston, Texas that had been flooded by Hurricane Harvey were sanitized by volunteers. At either 6, 8, 15, 25, 34, or 56 days after the sanitation was completed, a Button™ sampler was used to collect a 48-hour air sample, so that the mold cells in the air could be quantified. Each air sample was then analyzed by quantitative PCR (qPCR) assays for the 36 molds in the Environmental Relative Moldiness Index (ERMI) panel of indicator molds. Quantifying the 36-ERMI molds in air samples results in “ERMI-like” values. The ERMI-like values in the sanitized homes were inversely correlated (Pearson p -value 0.04) with the log of the number of days after the sanitation was completed, an indication that it takes time after sanitation for the mold levels to stabilize. This pilot study demonstrated that the ERMI-like metric was useful in assessing post-sanitization mold levels in previously flooded homes.

Introduction

Hurricanes often result in water damage to homes which can promote mold growth [1,2]. Mold exposure can lead to adverse health effects, including asthma, allergies, and other respiratory problems [3]. Currently, there is no threshold for mold exposure in the US [4]. Other countries, however, have suggested various limits to indoor mold levels.

In Japan, the indoor airborne fungal levels are suggested to be maintained under 1,000 colony forming units (CFU)/m³ air, or the indoor/outdoor (I/O) ratio under 2, in cases where the fungal levels exceed 1,000 CFU/m³ air [5]. The European Collaboration Action categorized airborne fungal levels exceeding 1,000 CFU/m³ air as “high” and those exceeding 10,000 CFU/m³ air as “very high” [6]. Unfortunately, culturing air samples to estimate fungal/mold contamination has many limitations, including short sampling times, differences

in culture-medium requirements, difficulty in spore identification, etc. [7].

As an alternative to culturing fungi/molds from air samples, quantitative PCR (qPCR) assays for many common molds were developed [8]. To quantify mold levels in homes, the Environmental Relative Moldiness Index (ERMI) was created [9]. The ERMI metric was based on the analysis of dust samples that were collected in the 2006 HUD Healthy Homes Survey from 1,096 representative homes across the US [9]. Each dust sample was analyzed for 36 indicator molds categorized into two groups; 26 Group 1 molds associated with water damage and 10 Group 2 molds that primarily originate from the outside air. Studies have shown that the ERMI metric is useful in assessing the relationship between mold exposures and asthma [10]. However, the ERMI metric is based on the analysis of a dust sample that is not available in a recently sanitized home. Therefore, a 48-hour air sample was

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used to create an ERMI-like metric for use in analyzing mold levels when a dust sample is not available.

The ERMI-like metric was described in an earlier study of the effectiveness of high-efficiency particulate air (HEPA) filtration treatment in reducing indoor air contamination in the homes of children with asthma [11]. Like the ERMI metric, the sum of the logs of the Group 2 molds (hereafter, sum Group 1) was subtracted from the sum of the logs of the Group 1 molds (hereafter, sum Group 2). However, since the results are from air samples, not dust, the term ERMI-like was used to clarify the difference from the ERMI [11]. The ERMI-like metric was found to be effective in quantifying the reduction in mold contamination in HEPA-treated homes of children with asthma [11]. In this study, the effectiveness of the mold sanitation of flooded homes was evaluated using the ERMI-like metric.

Materials and methods

After Hurricane Harvey had flooded homes in Houston, volunteers helped six families by performing mold sanitation in their homes. The homes were sanitized between October 19 and November 30, 2017.

The first step in the sanitation process was the removal of all non-structural materials from the home like clothes, curtains, furniture, or appliances. Any visibly, water-damaged material, like drywall and flooring, were removed. Then all nails, screws, staples, and residual scraps of material were removed. A digital moisture meter (MMD4E Moisture Meter, General Tools Company, Cincinnati, OH, USA) was then used to measure the moisture level in the wooden framing. After the moisture level was determined to be below 17%, the team then scrubbed the full area of all exposed surfaces in the home with hard bristle brushes. If the home gutting process included the ceiling or the ceiling joists, these surfaces were scrubbed also. Walls were scrubbed by starting at the top (ceiling) and then down to the floor. Every square inch of the gutted area was scrubbed seven times. One scrub includes an up and down motion on the same area (scrub up/down, left/right, clockwise/counter-clockwise).

After the walls and/or ceiling in a room were scrubbed, the floor was scrubbed. A hard bristle push broom was used to cover the entire surface area of the floor. Then the team vacuumed all exposed and scrubbed surfaces in the home. (Brush attachments were used to brush down all surfaces while vacuuming.) After the vacuum work was complete, a commercial cleaning agent called ShockWave™ (Fiberlock Technologies, Inc., Andover, MA, USA) was prepared by mixing the concentrate and water, following the manufacturer's instructions. The ShockWave™ mix was then sprayed lightly/ evenly over the surface area of the scrubbed and vacuumed areas. The home was then allowed to dry.

Before any rebuilding, the post-sanitation mold levels

were evaluated either at 6, 8, 15, 25, 34, or 56 days after the sanitation protocol was completed. The number of days varied for each home because of the complex schedules of the volunteers and homeowners. After the six homes had been sanitized, air samples were collected by the volunteers, as directed by the lead EPA researcher, onto 25-mm diameter, 1- μ m pore-size PTFE filters (Merck Millipore, Billerica, MA) using a Button™ sampler (SKC, Inc., Eighty-Four, PA, USA) [11]. Air samples were taken at a flow rate of 4 l/min for 48-hours using SKC potable pumps (SKC, Inc.). The Button™ samplers were returned to the US EPA laboratory for removal of the filter from the Button™ sampler, under controlled conditions, followed by the mold analysis.

Each filter was placed into a 2-ml extraction tube containing 0.3 g of glass beads and the DNA extracted and purified, as previously described [12]. Then, each of the 36 molds that make up the ERMI panel was quantified using qPCR assays [8]. The resulting data was described as cell equivalents (CE) per filter for each of the 36 molds. Then the data was divided by the volume of air per sample yielding a concentration of CE per cubic meter of air sampled (CE/m³ air). The sum Group 2 value was then subtracted from the sum Group 1 value to generate the ERMI-like value for each home, as previously described [11].

Pearson correlations analysis was used to determine the correlation between ERMI-like values, the sum Group 1, or sum Group 2 values, and the log of the days after sanitation was completed. The Student T - test was used to evaluate the significance of the differences in average ERMI-like values, the sum Group 1, or sum Group 2 values in the set of three homes sampled less than 25 days after sanitation compared to the set of three homes sampled 25 days or longer after sanitation.

Results

Table 1 shows the results of the qPCR analysis of the air-sample filters for each of the 36 ERMI-panel molds in each of the six homes in Houston, which were sampled at either 6, 8, 15, 25, 34, or 56 after the mold sanitation was completed. All homes received the same sanitation treatment and therefore each home appeared to the volunteers to be fully sanitized when the sanitation was completed, i.e., free of water damage and visible mold. The ERMI-like values and the values of the sum Group 1 mold were significantly (p - values for both 0.04) inversely correlated with the log of the days after sanitation (Table 2). By contrast, the sum of Group 2 molds was not significantly ($p = 0.13$) correlated with the log of the days after sanitation (Table 2).

The average ERMI-like value for the set of three homes sampled at less than 25 days after sanitation was 16.6 compared to 4.1 for the set of three homes sampled after 25 or more days. The latter set of three homes had significantly ($p = 0.009$) lower average ERMI-like values (Table 3). The average



Table 1: Results for the mold analysis of the six homes (H) in Houston using quantitative PCR assays and expressing the resulting concentrations as cell equivalents per m³ of air (CE/m³ air). The homes were sampled at either 6, 8, 15, 25, 34, or 56 after the mold sanitization was completed.

Home (H) number Days after sanitation Concentration (CE/m ³ air)	H-1 6 CE/m ³	H-2 8 CE/m ³	H-3 15 CE/m ³	H-4 25 CE/m ³	H-5 34 CE/m ³	H-6 56 CE/m ³
Group 1						
<i>Aspergillus flavus</i>	570	16	0	0	0	0
<i>Aspergillus fumigatus</i>	15	7	0	0	2	0
<i>Aspergillus niger</i>	5900	110	8	15	5	3
<i>Aspergillus ochraceus</i>	5	7	0	0	1	0
<i>Aspergillus penicillioides</i>	140	65	41	460	7	2
<i>Aspergillus restrictus</i>	7	10	5	3	0	0
<i>Aspergillus sclerotiorum</i>	5	6	0	0	0	0
<i>Aspergillus sydowii</i>	0	0	29	55	0	8
<i>Aspergillus unguis</i>	25	160	0	13	0	1
<i>Aspergillus versicolor</i>	0	45	0	59	0	0
<i>Aureobasidium pullulans</i>	0	4	0	0	2	2
<i>Chaetomium globosum</i>	2	72	8	18	0	0
<i>Cladosporium sphaerospermum</i>	30	64	3	36	5	3
<i>Eurotium amstelodami</i>	240	140	13	51	71	1
<i>Paecilomyces variotii</i>	1	45	22	9	0	2
<i>Penicillium brevicompactum</i>	0	1	0	0	0	0
<i>Penicillium corylophilum</i>	1	530	15	21	0	0
<i>Penicillium crustosum</i>	0	0	0	0	0	0
<i>Penicillium purpurogenum</i>	0	2	2	1	0	0
<i>Penicillium spinulosum</i>	0	0	0	0	0	0
<i>Penicillium variable</i>	61	250	150	30	3	0
<i>Scopulariopsis brevicaulis</i>	6	0	0	21	0	0
<i>Scopulariopsis chartarum</i>	9	10	0	1	2	13
<i>Stachybotrys chartarum</i>	0	8	0	16	0	0
<i>Trichoderma viride</i>	0	25	5	18	0	0
<i>Wallemia sebi</i>	1100	120	11	110	18	2
Sum of the Logs Group 1	24.23	31.25	13.90	23.04	6.74	4.17
Group 2						
<i>Acremonium strictum</i>	0	11	0	0	0	0
<i>Alternaria alternata</i>	0	11	0	0	14	0
<i>Aspergillus ustus</i>	50	42	6	110	0	1
<i>Cladosporium cladosporioides 1</i>	200	870	42	59	250	4
<i>Cladosporium cladosporioides 2</i>	2	3	0	21	0	0
<i>Cladosporium herbarum</i>	2	49	12	0	110	4
<i>Epicoccum nigrum</i>	0	51	0	0	1	0
<i>Mucor group</i>	3	2	0	3	1	0
<i>Penicillium chrysogenum 2</i>	140	130	64	750	3	1
<i>Rhizopus stolonifer</i>	0	0	0	0	0	0
Sum of the Logs Group 2	7.23	12.93	5.29	8.49	6.07	1.20
ERMI-Like	17.00	18.32	8.61	14.55	0.67	2.97

Table 2: Homes are listed by the number of days between completion of the sanitation (Days) and the air-sampling event. The Environmental Relative Moldiness Index-like (ERMI-like) values and the sum Group 1 and Group 2 mold values are shown for each home. The inverse Pearson correlation *p* - values between Log Days and each metric category are shown.

Home	Days	Log Days	ERMI-like	Group 1	Group 2
1	6	0.778	17.00	24.23	7.23
2	8	0.903	18.32	31.25	12.93
3	15	1.176	8.61	13.90	5.29
4	25	1.398	14.55	23.04	8.49
5	34	1.531	0.67	6.74	6.07
6	56	1.748	2.97	4.17	1.20
Pearson <i>p</i> - value			0.04	0.04	0.13

Table 3: Statistical evaluation (Student T - test) of the comparison of the average ERMI-like values, sum Group 1 and sum Group 2 mold values for the set of three homes sampled less than 25 days after sanitation was completed versus the set of three homes sampled 25 days or longer after sanitation was completed. (SD = Standard Deviations).

Days after sanitation	ERMI-like	Sum logs Group 1	Sum logs Group 2
	Mean (SD)	Mean (SD)	Mean (SD)
< 25 days	16.6 (1.9)	26.2 (4.4)	9.6 (3.0)
≥ 25 days	4.1 (4.1)	8.3 (5.0)	4.2 (2.6)
T - test; <i>p</i> - value	0.009	0.001	0.08



sum of Group 1 molds for the set of three homes sampled at less than 25 days after sanitation was 26.2 compared to 8.3 for the set of three homes sampled after 25 or more days. The latter set of homes had significantly ($p = 0.001$) lower average sum Group 1 mold values. However, the average sum Group 2 values were not significantly different between the two sets of homes ($p = 0.08$).

Discussion

The goal of home sanitation/remediation is to eliminate all active mold growth, all surface spores, and the possibility of continued mold growth in flood-impacted homes. In earlier studies after Hurricane Katrina, increased mold levels were reported in flooded areas in New Orleans [13-15], followed by increases in lung and respiratory diseases [16]. Although some studies reported that remediation reduced microbial contamination [2,17], other studies found that remediation was less than completely effective [13,18,19]. The problem has been the lack of standardization in evaluating the sanitation/remediation efforts to reduce mold.

The results from this small, pilot-study indicate that a 48-hour air sample may provide a useful metric to evaluate the effectiveness of mold sanitation efforts. Ideally, the ERMI-like values in these homes would have been measured immediately after the sanitation was completed and then periodically afterward to determine when the ERMI-like values had stabilized. This goal became impractical because of the demands on the volunteers' time.

Nevertheless, after sanitation, it appears to take several weeks (> 25 days) for the ERMI-like values to be reduced compared to the lesser time frame of < 25 days. The water-damage mold population, sum Group 1, also seems to be reduced more slowly, perhaps because it takes longer for the home to completely dry and the residual Group 1 mold spores to dissipate. On the other hand, the outside mold population, sum Group 2, seemed to equilibrate quickly, probably because these gutted homes were open to the outside air. However, a more rigorous post-sanitation, the mold-monitoring schedule will be needed to confirm the success of the sanitation protocol.

This study has the many limitations that one might expect in the middle of an ongoing disaster, specifically the small number of homes evaluated. Logistically, it was very difficult to conduct a larger study when the volunteers were focused on helping as many families as possible. Nevertheless, the results of this study show the potential value of using the ERMI-like metric to evaluate home-sanitation efforts. In preparation for future hurricanes, it would be appropriate to have trained teams available for rapid sanitation responses and mold testing.

Conclusion

This pilot study shows the potential value of the ERMI-

like metric for the assessment of the effectiveness of home sanitation, but larger studies with additional post-sanitation sampling are needed to confirm this finding.

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Data availability statement: All data will be available at the NIH-PMC website.

Disclaimer

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