

Mold contamination in schools with either high or low prevalence of asthma

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Abstract

Background: Mold exposures have been linked to the development and exacerbation of asthma. The purpose of this study was to determine whether the Environmental Relative Moldiness Index (ERMI) metric, developed to quantify mold exposures in homes, might be applied to evaluating the mold contamination in schools.

Methods: Settled dust samples (n = 10) were collected on each level of a water-damaged school in Springfield, Massachusetts and two samples per level in five Idaho schools. Each dust sample was analyzed for the 36 molds that make up the ERMI. The concentration of 2.5- μm particulate matter (PM_{2.5}) was measured in each school at two locations during the spring of 2013.

Results: The average ERMI value in the Springfield school, 15.51, was significantly greater (p < 0.001) than the average ERMI value, -2.87, in the Idaho schools. Ten of the twenty-six Group 1 molds, which are associated with water-damaged environments, were in significantly greater concentrations in the Springfield school. The populations of Group 2 molds, which are common indoors even without water damage, were essentially the same in Springfield and Idaho schools. The average PM_{2.5} concentration in the Springfield and Idaho schools was 11.6 and 3.4 $\mu\text{g}/\text{m}^3$, respectively.

Conclusions: The ERMI scale might be useful in comparing the relative mold contamination in schools.

Asthma is the most common chronic disease among children in the United States (U.S.) (1) and is likely caused or exacerbated by environmental exposures, modified by the host genetic and epigenetic backgrounds (2). Although children spend 6–8 h a day at school, relatively little attention has been given to children's exposures in schools compared to homes.

Banda et al. (3) recently reported that asthma-related hospitalizations of children, ages 5–18, were more likely associated with classroom triggers, whereas for younger children, it was home exposures that were associated with asthma-related hospitalizations. They suggested that the relationship between hospitalization and asthma triggers 'in different indoor environments warrants further study'. Baxi et al. (4) found that in 12 different schools, 100% of the classrooms tested positive for mold in the air. As a result, they suggested that studies linking mold exposure in schools and asthma should be pursued. As a result of the Health Effects of

School Environment study (HESE) of 21 European schools, it was recommended that: 'Schools should be routinely tested for global indoor molds' evaluation' (5).

To standardize the quantification of mold contamination in homes, the Environmental Relative Moldiness Index (ERMI) metric was created (6). The ERMI scale was based on a random national sampling of homes conducted by the U.S. Department of Housing and Urban Development (HUD) and a DNA-based analysis of 36 indicator mold species in each sample. The 26 Group 1 species are associated with water damage, and the ten Group 2 molds are common in homes, independent of water damage. Although the ERMI scale was developed to describe relative mold contamination in homes, it might also be applied in other indoor environments.

The purpose of this study was to determine whether the ERMI metric might be applied to evaluating the mold contamination in a water-damaged school compared to schools

without a history of water damage. This evaluation also included a comparison of 2.5- μm particulate matter ($\text{PM}_{2.5}$) concentrations in these schools.

Methods

Schools Studied

A water-damaged school, located in Springfield, Massachusetts (MA), was the subject of a Health Impact Assessment conducted by the EPA because the school had a long history of water problems. A comparison school in Springfield was sought for this study, but none volunteered. The only schools accessible for comparison mold sampling were in northwest Idaho because five schools were already taking part in an EPA Region 10 study of indoor air in schools. The Idaho schools had no history of water damage. Student demographics and asthma prevalence values were obtained from school or state datasets for Massachusetts and the northwest region of Idaho (7).

Dust sample collection

Swiffer SweepertTM cloths (Proctor and Gamble, Cincinnati, OH, USA) were used to collect settled dust from random locations (e.g., tops of light fixtures, bookshelves, doors) in each school. The technician, using disposable gloves, wiped each sample site with a single Swiffer SweeperTM cloth until the cloth became gray to black. The cloth was then placed in sealable bag. The used gloves were discarded and new gloves applied for each succeeding sample. For the Springfield school, ten Swiffer SweepertTM cloth dust samples were collected from each of the three building levels. Two Swiffer SweeperTM cloth dust samples were collected per level in each of the Idaho schools. (At the Idaho school that had two levels, one of the samples contained insufficient dust for analysis). The samples were sent refrigerated, overnight to the EPA laboratory in Cincinnati for analysis.

DNA-based mold analysis

The dust from each Swiffer SweepertTM cloth was recovered, sieved through 300- μm mesh, and 5.0 ± 0.1 mg of dust was added to an extraction tube and then spiked with 1×10^6 cells of *Geotrichum candidum* as an external reference, as previously described (8). Each extraction tube was shaken in the bead beater (Biospec Products, Bartlesville, OK, USA) for 1 min and the DNA purified using the DNA-EZ extraction kit (GeneRite, South Brunswick, NJ, USA).

Methods and assays have been reported previously for performing the analyses (8). Briefly, the standard reaction assays contained 12.5 μl of 'Universal Master Mix' (Applied Biosystems Inc., Foster City, CA, USA), 1 μl of a mixture of forward and reverse primers at 25 μM each, 2.5 μl of a 400 nm TaqMan probe (Applied Biosystems Inc.), 2.5 μl of 2 mg/ml fraction V bovine serum albumin (Sigma Chemical, St. Louis, MO, USA), and 2.5 μl of DNA free water (Cepheid, Sunnyvale, CA, USA). To this mix was added 5 μl of the DNA extract from the sample. All primer and probe sequences used

in the assays as well as known species comprising the assay groups are at the website: <http://www.epa.gov/nerlcwww/moldtech.htm>. Primers and probes were synthesized commercially (Applied Biosystems, Inc.).

Calculation of relative mold contamination

After the concentrations of each of the 26 Group 1 (G1) molds and ten Group 2 (G2) molds were determined, the ERMI values were calculated, as shown in eqn (1). The sum of the logs (SL) of the concentrations of the G2 molds (s_2) is subtracted from the sum of the logs (SL) of the concentrations of G1 molds (s_1) (9).

$$\text{ERMI} = \sum_{i=1}^{26} \log_{10}(s_{1i}) - \sum_{j=1}^{10} \log_{10}(s_{2j}) \quad (1)$$

Statistical analysis

The statistical differences between the average sum of the logs of the Group 1 molds (SLG1), the average sum of the logs of the Group 2 molds (SLG2), and the average ERMI values in the Springfield and Idaho schools were compared using the Student's *t*-test. The statistical differences in concentrations of individual mold species between the Springfield school and the Idaho schools were evaluated by the Wilcoxon rank-sum test. The Holms procedure was used to adjust for the multiple comparisons. Analyses were performed in SAS version 9.3 (SAS Institute, Cary, NC, USA) and R version 2.14 (R Foundation for Statistical Computing, Vienna, Austria).

Estimation of $\text{PM}_{2.5}$ concentrations

The $\text{PM}_{2.5}$ was measured in two locations in each school. The locations selected were based on the expert opinion of the investigator and their knowledge of each school's air handling system. In the Springfield school, the $\text{PM}_{2.5}$ sampling occurred in June 2013 and utilized the RTI MicroPEM, Model M-300 (RTI International, RTP, NC, USA). Particulate matter in the Idaho schools was sampled in April 2013 using a Fluke 983 Particle Counter (Fluke, Inc., Everett, WA, USA), and the particle counts were converted to mass, as previously described (10).

Results

Some of the characteristics of the six study schools are found in Table 1. Based on state records, the Springfield school had a high proportion, about 85%, of students from low-income families of Puerto Rican heritage. The students in the Idaho schools were primarily from middle class, Caucasian families. The prevalence of asthma in the Springfield school was about 21% and, for the Idaho schools, about 7%. The average concentration of $\text{PM}_{2.5}$ in the Springfield school and the Idaho schools was 11.6 and 3.4 $\mu\text{g}/\text{m}^3$, respectively (Table 1).

Visible mold growth was observed only on the first level of the Springfield school, but wet carpeting was detected on the second and third levels. No visible mold was observed or wet carpeting detected in the Idaho schools. The overall average

Table 1 Some characteristics of the study schools and the average 2.5- μm particulate matter concentrations

Location (state)	Levels (n)	Year	Population (n)*	PM _{2.5} ($\mu\text{g}/\text{m}^3$)
Idaho 1	2	Multiple†	185	4.4
Idaho 2	1	Multiple	300	3.5
Idaho 3	1	2004	300	3.5
Idaho 4	1	1998	600	2.3
Idaho 5	1	Multiple	400	3.4
Massachusetts	3‡	1972	800	11.6

*Student population is an approximation based on the average number of students per school year.

†The school has undergone many additions and renovations and therefore cannot be assigned a specific year.

‡The lowest level of the school is located underground. All other levels are above ground.

Table 2 Mean and standard deviation of the values in the calculation of the Environmental Relative Moldiness Index for the Springfield school compared to Idaho schools

	ERMI *	SD†	SLG1‡	SD	SLG2§	SD
Springfield School	15.51	6.68	36.73	8.15	21.22	4.36
Idaho Schools	-2.87	3.91	17.20	4.02	20.07	3.08
p value	<0.001¶		<0.001¶		0.48	

*Mean Environmental Relative Moldiness Index value based on the analyses of 5.0 mg of dust for each sample.

†Standard deviation.

‡Mean sum of the logs of the Group 1 molds value.

§Mean sum of the logs of the Group 2 molds value.

¶statistically significantly different based on the Student's t-test.

ERMI for the Springfield school was 15.51 (Table 2). As the standard deviation of the ERMI values in the Springfield school, based on ten samples per level, was relatively narrow (± 6.7 , Table 2), we realized that many fewer samples per level were necessary to assess mold contamination. Therefore, in the Idaho schools, only two samples per level were obtained. Across all five schools, the average ERMI value was -2.87 and the standard deviation of the ERMI values was only ± 3.9 (Table 2).

The average ERMI value in the Springfield school was significantly greater ($p < 0.001$) than in the Idaho schools (Table 2). The average SLG1 value in the Springfield school (37.73) was also significantly greater ($p < 0.001$) than in the Idaho schools (17.20). However, the average SLG2 value for each school (21.22 in the Springfield school and 20.07 in the Idaho schools) was not significantly different ($p = 0.48$).

Ten Group 1 molds, including *Aspergillus ochraceus* and *A. unguis*, were found in greater concentrations in the Springfield school samples compared to the Idaho school samples (Table 3). Two Group 2 molds, *Aspergillus ustus* and *Cladosporium cladosporioides* 2, were significantly more abundant in the Springfield school, but *Cladosporium herbarum* was more abundant in the Idaho schools (Table 3).

Table 3 Average concentration of each of the 36 Environmental Relative Moldiness Index molds in the Springfield school compared to the Idaho schools

Mold and concentration (average no. cells/mg dust)	Springfield School	Idaho Schools	p-value	Z-value
Group 1 Molds				
<i>Aspergillus flavus</i>	9	1	0.004	2.78
<i>Aspergillus fumigatus</i>	13	22	0.073	1.79
<i>Aspergillus niger</i>	460	4	<0.001†	4.86
<i>Aspergillus ochraceus</i>	390	0	<0.001†	4.86
<i>Aspergillus penicillioides</i>	35	21	0.640	0.45
<i>Aspergillus restrictus</i>	115	1	0.027	2.21
<i>Aspergillus sclerotiorum</i>	4	0	<0.001†	3.72
<i>Aspergillus sydowii</i>	25	0	0.003	2.84
<i>Aspergillus unguis</i>	10	0	<0.001†	4.37
<i>Aspergillus versicolor</i>	12	17	0.164	1.4
<i>Aureobasidium pullulans</i>	6800	260	<0.001†	4.17
<i>Chaetomium globosum</i>	47	0	<0.001†	4.86
<i>Cladosporium sphaerospermum</i>	980	0	<0.001†	4.86
<i>Eurotium group</i>	145	290	0.058	1.9
<i>Paecilomyces variotii</i>	6	1	0.106	1.62
<i>Penicillium brevicompactum</i>	21	4	0.009	2.59
<i>Penicillium corylophilum</i>	35	7	0.012	2.46
<i>Penicillium crustosum</i>	16	27	0.194	1.32
<i>Penicillium purpurogenum</i>	0	1	0.495	0.71
<i>Penicillium spinulosum</i>	0	0	NM*	NM
<i>Penicillium variabile</i>	1	1	0.763	0.31
<i>Scopulariopsis brevicaulis</i>	7	4	0.848	0.19
<i>Scopulariopsis chartarum</i>	4	7	0.329	1
<i>Stachybotrys chartarum</i>	16	1	<0.001†	4.33
<i>Trichoderma viride</i>	11	1	<0.001†	4.21
<i>Wallemia sebi</i>	3450	67	<0.001†	4.81
Group 2 Molds				
<i>Acremonium strictum</i>	3	5	0.008	2.62
<i>Alternaria alternata</i>	240	130	0.074	1.79
<i>Aspergillus ustus</i>	150	2	<0.001†	4.84
<i>Cladosporium cladosporioides</i> 1	3550	5600	0.173	1.38
<i>Cladosporium cladosporioides</i> 2	29	9	<0.001†	3.78
<i>Cladosporium herbarum</i>	510	7000	<0.001†	4.59
<i>Epicoccum nigrum</i>	1450	3400	0.005	2.27
<i>Mucor group</i>	125	140	0.940	0.09
<i>Penicillium chrysogenum</i>	140	37	0.005	2.72
<i>Rhizopus stolonifer</i>	21	17	0.533	0.65

*Not Meaningful; this mold was not detected in any sample.

†statistically significantly different; p-values are the result of a Wilcoxon rank-sum test and Holms adjustment procedure for multiple comparisons.

Discussion

The Springfield school has a long history of water damage, which was the reason it was the subject of the Health Impact Assessment. Group 1 molds were at twice the abundance in the Springfield school compared to the Idaho schools and led to an average ERMI value of 15.51, which is in the top 5% for 'relative moldiness' on the home ERMI scale (6). Examples of molds in significantly greater concentration in the Springfield school were *Aspergillus ochraceus* and *A. unguis*. In an earlier study, infant exposures in homes to higher concentrations of these two molds were linked to the development of asthma (11). By contrast, the five schools in Idaho, with no history of water damage, had low concentrations of Group 1 molds and an average ERMI value of -2.87 , well below average on the home ERMI scale (6).

On the other hand, the population of Group 2 indicator molds was about the same in both the Springfield school and Idaho schools. In the ERMI calculation, the sum of the logs of the Group 2 molds is used to adjust for external factors such as season, cleaning frequency, window use for ventilation (6). It is important to make this adjustment because of the geographic differences between the Springfield and the Idaho school locations. So although two Group 2 molds, *A. ustus* and *Cladosporium cladosporioides* 2, were in greater concentration in the Springfield school, this was balanced by the significantly greater concentration of *Cladosporium herbarum* in the Idaho schools. The result is that the average sum of the logs of the Group 2 molds is not significantly different in the Springfield and Idaho schools. Therefore, the differences in ERMI values between the Springfield school and the Idaho schools appear to be due to the differences in Group 1 mold growth.

The ERMI metric has only been used to quantify mold contamination in one other water-damaged school, in New Orleans (12). The average ERMI value in that school, 16.6, was about the same as the average ERMI value in the Springfield school (15.5). The National Institute of Occupational Safety and Health (NIOSH) investigated the New Orleans school for a 'Health Hazard Evaluation'. The NIOSH investigation found that twenty of the employees met the NIOSH physicians' case definition of work-related asthma. However, the mold contamination in schools cannot be causally linked to asthma in these studies, as many other environments, social factors, and exposures were not considered or quantified.

School is just one environment where children are exposed to contaminants, and we did not investigate other environments (e.g., home, daycare). Also, many social factors, including ethnicity and socioeconomic status, are important in the development of asthma (13), and the student populations in the Springfield and Idaho schools were not comparable. (Unfortunately, we could not obtain permission to sample in

other schools in the Springfield area for comparison). Also, many chemical and other biological exposures that have been linked to asthma were not measured in our study and they could be just as relevant as mold (14). Also, the $PM_{2.5}$ exposure measurements were limited to short sampling times and only a few locations in the school. However, the estimates of $PM_{2.5}$ concentrations were similar to those reported for other school-based studies in the U.S.A (15–17) and below WHO and EPA limits for outdoor air (18, 19).

In spite of the many limitations of the study, it appears that the ERMI metric that was developed for homes may be applied to schools with some modifications. For homes, a floor dust sample was used but this is not possible for school floors that are cleaned every day. Therefore, above-floor, settled dust was used in the mold sampling for schools. In homes, a single dust sample is the standard protocol for the ERMI analysis (6). In the investigation of the Springfield school, a dense sampling protocol was used (10 samples per level). However, we found that there was a great uniformity in the ERMI values in these samples. So the number of samples obtained in the Idaho schools was reduced to two per level, which might be more practical. More studies will be needed to determine the optimal sampling density for schools. In addition, the ERMI scale for schools would be improved with a random national survey of schools, as was done to create the ERMI for homes.

This study suggests that using a DNA-based approach to identifying and quantifying molds and the ERMI scale could be helpful in distinguishing the relative amounts of mold contamination in different schools. This might lead to improvements in school indoor air.

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Conflict of interest

The U.S. Environmental Protection Agency (EPA) through its Office of Research and Development collaborated in the research described here. Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official EPA policy. Mention of trade names or commercial products does not constitute endorsement or recommendation by the EPA for use. As MSQPCR technology is patented by the US EPA, the Agency has a financial interest in its commercial use.

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