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Submicron fungal fragments as another indoor biocontaminant in elementary schools

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There has been great concern about mold in school environments, but few comprehensive assessments of mold have been performed in such settings. Even spore counts or microscopic enumeration only may not be sufficient for evaluating fungal exposure. We explored the levels of submicron fungal fragments with potential heath impact due to their small size in elementary schools and investigated the variation in the concentrations of such particles before and after the rainy season. The concentrations of (1,3)-β-D-glucan in submicron fungal fragments, airborne mold and bacteria, and PM_{10} were measured both indoors and outdoors in 70 classrooms at 8 elementary schools from May (before the rainy season) to July (after the rainy season) in 2012. Temperature and relative humidity were also monitored. We compared the levels of submicron fungal fragments among schools before and after the rainy season. The associations of the levels of submicron fungal fragments with other variables were analyzed. Overall, the concentrations of (1,3)-β-D-glucan ranged from 10 to 347 pg m^{-3}, and the indoor/outdoor ratios were greater than 1 in every school. After the rainy season, the (1,3)-β-D-glucan concentrations decreased by about 35%, and similar significant decreases in the concentrations of airborne mold and bacteria and PM_{10} were observed. This difference was prominent for PM_{10} ($P < 0.001$). Only relative humidity was negatively associated with the concentration of submicron fungal fragments ($P = 0.007$). Our findings confirmed the comparable amounts of submicron fungal fragments in school environments. More comprehensive exposure assessments for smaller-sized fungal particles should be performed for better understanding of their health impact, particularly with regard to seasonal changes.

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Environmental impact

Mold could play an important role in triggering and exacerbating allergic symptoms among sensitized individuals. For better understanding of the causal role of mold exposure in health outcomes, a comprehensive exposure assessment for mold should be performed in various environments using different evaluation methods simultaneously. For this reason, the present study is the first to identify and measure, in school environments, smaller-sized fungal particles with potential adverse health impact due to their smaller size and composition. Moreover, the observation of comparable amounts of submicron fungal fragments in classrooms could be used as background information for establishing a comprehensive profile of fungal exposure and exploring the causal association of fungal exposure with health outcomes of students, as well as of teaching and non-teaching staff.

Introduction

A high prevalence of allergic diseases in children has been shown in many studies.[1–3] Children are more vulnerable to exposure to many allergens owing to their immature immune system.[4] Because they spend considerable time indoors, they are at great risk to such exposure if their homes, daycare centers, and schools are not well maintained. Thus, preventing the development or increase in severity of symptoms of allergic diseases in buildings or spaces occupied by children requires careful management.

The home environment in many epidemiological studies has been a target for evaluation of allergen exposure since houses are often considered the primary sites of exposure. However, schools as examples of nonresidential areas can be regarded as major sources of allergen exposure. Exposure to allergens can affect children's health, as elementary school children spend 6 h or more a day in school, and most of that time is often spent in one classroom. Moreover, the school environment would be important for children with atopic dermatitis and asthma in the concept of "allergic march" which refers to natural or typical progression of allergic diseases that often begin as atopic dermatitis and end as allergic rhinitis.[5] That is, many children with asthma may have allergic rhinitis if allergen exposure in

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the school environment is well controlled although parents avoid allergens in their dwellings. However, few environmental surveys in school classrooms have been performed. In particular, sufficient assessment of the level of mold has not been performed although mold has been found to be an allergen of particular concern in the school environment.

Exposure to mold has been associated with adverse health effects.[6] In particular, the World Health Organization (WHO) reported that exposure to mold is highly linked to the development and exacerbation of symptoms of allergic or respiratory diseases such as asthma.[7] However, the causal effect of mold exposure on allergic diseases is still unclear since spore counts on culture media or microscopic enumeration only followed by air sampling has often been used for assessing fungal exposure in many epidemiological studies.[8–12] Spore counts by culturing from the sample collected may make it difficult to evaluate the exposure to mold due to the different growth rates of types of mold, and the dependence of culturability on media.[13,14] In addition, microscopic enumeration requires considerable technical expertise, and results could even vary depending on the observer. For these reasons, assessment of spore concentration only may not be enough for exploring fungal exposure even though these methods could be relatively easy for sampling and analysis.

Recently, several studies showed that measurement of (1,3)-β-D-glucan—which exists in fungal cell walls—in submicron fungal fragments by biomass analysis could be applied for exposure assessment of mold, even suggesting it as a new tool to determine associations with respiratory diseases such as asthma.[15] These fragments reach deep into the lung tissue and persist for long periods in the air due to their small size. Moreover, these particles are more common than airborne spores, and may have high impact on health outcomes as sources of allergens.[16] Therefore, investigating the level of submicron fungal fragments in school environments and residences can lead to a better understanding of exposure to mold and health outcomes of elementary school children. Moreover, the extreme weather conditions in South Korea, such as summer heatwaves followed by hurricanes with heavy rain from June to July every year, might favor mold growth. Additional evaluation of exposure to mold in schools is therefore necessary after the rainy season.

In this study, we evaluated the levels of submicron fungal fragments, as well as airborne molds, bacteria, and particulate matter, in both indoor and outdoor areas of elementary school classrooms. To our knowledge, the present study is the first to explore the level of submicron fungal fragments in school environments. The concentrations of submicron fungal fragments before and after rainy seasons and their correlation with physical factors (*i.e.*, temperature and relative humidity) were also compared and analyzed.

Methods

Selection of elementary schools

We aimed to select schools with buildings over 30 years old and schools with old buildings that were renovated in the last 10 years. We finally selected 8 schools from 15 in the provinces of Chungcheong and Gyeonggi in South Korea.

Sampling and analysis

The period with monthly average precipitation of less than 150 mm for 30 years (1981–2010) was categorized as "before the rainy season" and the period with monthly average precipitation of more than 300 mm was defined as "after the rainy season". We collected samples from 70 classrooms of 8 schools, and the first and second samplings were performed from May 15th to June 1st and from July 12th to 20th in 2012, respectively. Measurements were conducted in 8–9 classrooms per school, with a total of 70 classrooms. Samplings of submicron fungal fragments, particulate matter smaller than 10 μm (PM_{10}), and airborne bacteria and molds, as well as measurements of temperature and relative humidity, were conducted simultaneously. We also collected data on the number of floors, potted plants, and students using a check-list.

Sampling and analysis of submicron fungal fragments. Samplings of submicron fungal fragments in indoor and outdoor areas of classrooms were performed as described by Seo *et al.*[15] Briefly, two-stage cyclone samplers developed by the National Institute for Occupational Safety and Health, each loaded with a 37 mm gamma-irradiated polycarbonate filter (pore size, 0.8 μm; SKC Inc., Eighty Four, PA, USA) were used for airborne sampling. Two cyclone samplers (one for measuring and the other for blank sampling) were placed in each classroom with students (for indoor sampling) or on the rooftop of the school building (for outdoor sampling). Each sampling was performed for about 7–8 h at an average flow rate of 3.5 L min^{-1}, and during the whole class period. After the sampling, the filter cassette was covered with aluminum foil, placed on ice in a cooler box, and then stored in a refrigerator until analysis. We also measured the relative humidity and temperature in elementary school classrooms 60 min after the start of the sampling of submicron fungal fragments and used the arithmetic mean of the sampling as the representative value. We measured the outdoor temperature of elementary school buildings, relative humidity, and other weather parameters using thermometers and hygrometers.

Analysis of (1,3)-β-D-glucan was performed as described in detail in previous studies.[15] Briefly, the filter from the cassette was transferred to a 15 mL conical tube (Fisher Scientific, Pittsburgh, PA, USA) with 5 mL phosphate-buffered saline solution (Fisher Scientific), and submicron fungal fragments were extracted by sonication (Model 231; Fisher Scientific) for 1 h followed by 2 min of vortexing (Model FS20; Fisher Scientific).[17] A kinetic chromogenic *Limulus* amebocyte lysate analysis (Glucatel; Associates of Cape Cod, East Falmouth, MA, USA) was used for measuring (1,3)-β-D-glucan. An aliquot of 25 μL from a mixture of 0.5 mL extracted solution and 0.5 mL of 0.6 M NaOH in each sample was placed in a microplate reader (ELx808 TM; Bio-Tek Instruments Inc., Winooski, VT, USA), and the concentration of (1,3)-β-D-glucan was measured for about 150 min using absorbance at 405 nm every 30 s. The final result of the analysis was expressed in pg m^{-3}, followed by adjustment for the total air volume of each sampling.

Concentration of PM_{10}. We measured the concentrations of PM_{10}, along with sampling of submicron fungal fragments. The measurement was carried out for 8 h using an optical particle meter (GT-331; Met One Instrument, Grants Pass, OR, USA) placed 1.2 m from the floor surface at a position at least 1 m from the wall at the center of each classroom. The 8 h average result of each classroom was calculated in units of μg m^{-3}.

Airborne mold and bacteria. Airborne mold and bacteria were sampled in classrooms using a one-stage Andersen sampler (Andersen Instruments, Atlanta, GA, USA) as described in an earlier study.[15] Briefly, 5 min air samplings were performed twice each for airborne mold and bacteria at a flow rate of 28.98 L min^{-1}. For mold- and bacteria-specific samplings, we used malt extract agars with an antibacterial agent (streptomycin, 40 mg L^{-1}; Difco Laboratories, Detroit, MI, USA) and tryptic soy agars with an antifungal agent (cycloheximide, 0.5 g L^{-1}; Sigma-Aldrich, St Louis, MO, USA), respectively. Colonies were counted after 4 day incubation, and the final concentrations were expressed as the corrected colony numbers followed by adjusting the results by the field blank correction and positive-hole correction for air volume (CFU m^{-3}).

Statistical analysis

The concentration of (1,3)-β-D-glucan in submicron fungal fragments showed a right-skewed distribution in normality tests. Geometric mean (GM) values were used for reporting the results in this study. Student's *t*-test and the paired *t*-test were performed to compare the concentrations of (1,3)-β-D-glucan in submicron fungal fragments, as well as concentrations of airborne mold and bacteria, before and after the rainy season. Pearson's correlation analysis was also carried out to determine associations among indoor temperature, relative humidity, (1,3)-β-D-glucan concentration in submicron fungal fragments, and airborne mold and airborne bacteria concentrations. All data were analyzed using the SAS® program (version 9.1; SAS Inc., Cary, NC, USA), and a significance level (α) of 5% was applied unless otherwise indicated.

Results

General characteristics of elementary schools

The general characteristics of the 8 selected elementary schools are presented in Table 1. Among the buildings of the selected schools, 6 were constructed more than 50 years ago, and 2 (schools D and E) were recently renovated. Student numbers per school ranged from 22 to 1650, and the number of students in schools located in urban areas was over 600. More than 70% of the classrooms were built before 1990, and approximately 65% of the classrooms were located on the second floor (Table 2). We found that most of the classrooms (about 63%) were more than 10 m away from the toilets.

Level of (1,3)-β-D-glucan in submicron fungal fragments

Overall, the concentrations of (1,3)-β-D-glucan in submicron fungal fragments ranged from 10 to 347 pg m^{-3}, and lower levels of (1,3)-β-D-glucan were observed in the more recently built schools C and F (Fig. 1). Meanwhile, the GM concentration of (1,3)-β-D-glucan in the recently renovated schools (40.9 pg m^{-3}) was about 38% higher than that of non-renovated schools (29.6 pg m^{-3}), but this was not significantly different ($P = 0.089$). All the indoor/outdoor (I/O) ratios were found to be more than 1 in every school (range, 1.05–3.56) (Fig. 2(a)). Interestingly, the I/O ratio value was over 1 in every classroom before the rainy season (range, 1.5–4.6), but it dropped below 1 in some schools after the rainy season (range, 0.6–2.5) (Fig. 2(b)).

Fig. 3 shows the GM concentrations of (1,3)-β-D-glucan in submicron fungal fragments before and after the rainy season. Overall, compared to the concentrations before the rainy season (36.3 pg m^{-3}), the concentrations significantly decreased by about 35% after the rainy season (23.9 pg m^{-3}) ($P = 0.011$). In the school-wise results, the concentration was found to decline by 21–68% after the rainy season compared to the concentrations before the rainy season.

No statistical differences for the GM concentrations of (1,3)-β-D-glucan in submicron fungal fragments were observed between classrooms built before and after 1990 (Fig. 4(a)). In addition, between schools located in urban and rural areas, the concentrations in urban schools were higher by about 40%, but this was not statistically significant ($P = 0.897$) (Fig. 4(b)). The GM concentration of (1,3)-β-D-glucan in the recently renovated schools (68.1 pg m^{-3}) was around twice that of other schools (34.9 pg m^{-3}), albeit with no statistical relevance ($P = 0.083$) (Fig. 4(c)).

Levels of PM_{10}, airborne mold, and bacteria before and after rainy season

The GM concentrations of airborne molds and bacteria before the rainy season decreased to 54% and 44% after the rainy season, respectively ($P < 0.001$ for mold, $P = 0.003$ for bacteria) (Table 3). In particular, a greater difference was observed for the concentrations of airborne mold. Similarly, the GM concentration of PM_{10} was found to be lowered by more than 60% after the rainy season compared to before it ($P < 0.001$). The temperature and relative humidity after the rainy season significantly increased by about 2.5 °C and 17.4%, respectively, compared to the period before the rainy season ($P < 0.001$).

On the other hand, no statistical differences were found between outdoor GM concentrations of airborne molds and bacteria (airborne mold, 176.5 CFU m^{-3} before the rainy season and 153.0 CFU m^{-3} after the rainy season ($P = 0.530$); airborne bacteria, 325.5 CFU m^{-3} before the rainy season and 335.0 CFU m^{-3} after the rainy season ($P = 0.6230$)). However, the outdoor GM concentration of PM_{10}, and average temperature and relative humidity after the rainy season decreased significantly ($P = 0.004$; 52.8 μg m^{-3} to 19.5 μg m^{-3}) and increased significantly ($P = 0.004$; 23.4 °C to 26.7 °C, and 52.4% to 66.7%), respectively. In addition, the average I/O ratio of airborne molds and bacteria appeared to be overall higher than 1 before and after the rainy season (mold, 2.28 before rainy season and 1.21 after rainy season; bacteria, 1.63 before rainy season and 0.90 after rainy season).

Table 1 Characteristics of elementary schools included in the present study

School	Location	Number of pupils	Number of classrooms	Number of classrooms for air sampling	Year of construction	Renovation
A	Rural	43	9	9	1942	No
B	Urban	712	26	9	1923	No
C	Urban	1649	25	9	2000	No
D	Urban	697	24	9	1947	Yes
E	Rural	102	9	9	1915	Yes
F	Rural	80	9	9	1990	No
G	Rural	22	8	8	1944	No
H	Rural	43	8	8	1931	No
Sum			118	70		

Pearson's correlation coefficient

The concentration of (1,3)-β-D-glucan in submicron fungal fragments was negatively correlated only with relative humidity (Pearson's correlation coefficient = −0.346; $P = 0.007$) (Table 4). Similarly, negative correlations were also observed between relative humidity and the levels of airborne mold and bacteria, as well as PM_{10}, and this association was more prominent for PM_{10} (Pearson's correlation coefficient = −0.525; $P = 0.02$).

Discussion

In this study, the concentrations of airborne mold/bacteria, as well as submicron fungal fragments, unexpectedly decreased by about 35–55% after the rainy season. In general, molds can grow well after precipitation or floods due to abundant moisture.[18] However, the opposite can be expected in terms of airborne microbial concentrations. High relative humidity is an important facilitator for the growth of microorganisms on walls, ceilings, and floors, but it could decrease the release of fungal spores and their hyphae from their origins. These results are consistent with those reported by Almaguer *et al.*; that is, fungal spores were more abundant during the dry season than in the rainy season.[19] Burge and Rogers also reported that precipitation and humidity could act as inducers or repressors of aerosolization of spores, hyphae, and their debris.[20] These explanations may also be supported by the negative correlations of relative humidity and airborne concentrations of particulate matter with the airborne concentrations of molds in this study. In particular, a sharp decline in the concentration of particulate matter could contribute to a decrease in the concentrations of other variables. The growth of microorganisms would be retarded by lower relative humidity, but paradoxically, these conditions also facilitate the easy release of airborne submicron fungal fragments,[15,21] as well as airborne molds from walls and/or ceilings.

Although the concentrations of (1,3)-β-D-glucan in submicron fungal fragments in schools have never been studied, a few studies have demonstrated their presence in some other environments. According to the results reported by Seo *et al.*,[15] the GM concentration of submicron fungal fragments in homes with and without asthmatic children was 50.9 pg m^{-3} (range, 17.2–247.1 pg m^{-3}) and 26.7 pg m^{-3} (9.9–81.0 pg m^{-3}), respectively. A field study involving collection of submicron fungal fragments was performed in houses severely damaged by mold due to hurricanes.[18] This study showed that the GM levels of (1,3)-β-D-glucan in submicron fungal fragments were 59.6–192.7 pg m^{-3} in summer and 338–520.5 pg m^{-3} in winter. Other results reported by Singh *et al.* showed that GM concentration of (1,3)-β-D-glucan in PM_1 samples was 300 pg m^{-3}.[22] Similar studies were carried out in agricultural environments such as grain, swine, and horse farms.[22,23] The GM concentration of (1,3)-β-D-glucan in PM_1 samples was 24.4 ng m^{-3}, and median levels of (1,3)-β-D-glucan in submicron fungal fragments shown in a study of Lee *et al.* were 4.0×10^3 pg m^{-3}

Table 2 Characteristics of 70 classrooms selected for air sampling from 8 elementary schools

		Number of classroom
Year of construction	Before 1990	52 (74.3%)
	After 1990	18 (25.7%)
Distance of classroom from the restroom	Within 10 m	26 (37.1%)
	More than 10 m	44 (62.9%)
Potted plants in the classroom	Presence	33 (47.1%)
	Absence	37 (52.9%)
Number of pupils per classroom	Less than 15	36 (51.4%)
	Greater than 15	34 (48.6%)
Floor of classrooms	1st Floor	25 (35.7%)
	Above 2nd floor	45 (64.3%)

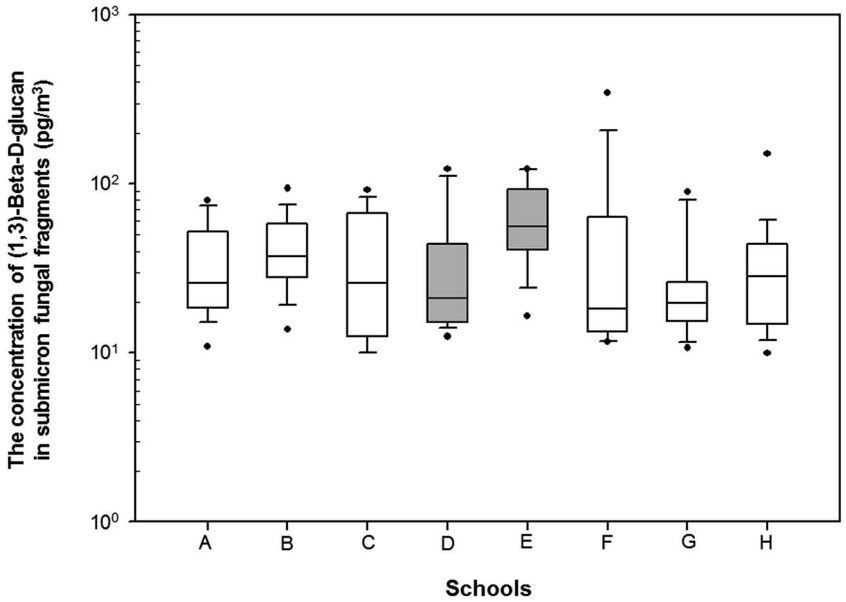

Fig. 1 A boxplot of the concentration of (1,3)-β-D-glucan in submicron fungal fragments for elementary schools. Each boxplot indicates an interquartile range with median, upper, and lower whiskers; upper and lower boundaries (3rd quartile/1st quartile). A gray-colored boxplot indicates an elementary school renovated recently.

for corn farms, 2.0×10^3 pg m^{-3} for swine farms, 8.4×10^3 pg m^{-3} for poultry farms, and $1.8–3.0 \times 10^3$ pg m^{-3} for mushroom farms. Madsen *et al.* also reported the presence of submicron fungal fragments (5.6 ng m^{-3} for average value and 3.5 ng m^{-3} for median) in biofuel plants.[24] In general, there were one order of magnitude differences for the level of (1,3)-β-D-glucan in submicron fungal fragments between agricultural and non-agricultural environments. For comparing the results between school and home environments, the average level of (1,3)-β-D-glucan (36.3 pg m^{-3}; range, 10.0–347.0 pg m^{-3}) for schools overall before the rainy season was lower than the GM concentration of submicron fungal fragments in homes with asthmatics, but higher than that of non-water-damaged homes. In addition, the range of (1,3)-β-D-glucan in this study was of same order of magnitude or similar to that of (1,3)-β-D-glucan in homes with asthmatics or mold-damaged houses as a result of hurricanes, respectively. Such differences might be caused by environmental conditions, different sampling and analytical methods used, diversity of microorganisms, and anthropogenic disturbance by students during sampling. In particular, anthropogenic disturbances in a classroom might markedly contribute to these differences since samplings were performed simultaneously in a class. Importantly, because the concentration of (1,3)-β-D-glucan in submicron fungal fragments in school environments was higher than that reported in non-water-damaged homes, even though the measurement was conducted in spring, when there is ample ventilation through windows, it is necessary to carefully consider the effect of the environments of obsolete school buildings on student health. Above all, the confirmation of the presence of submicron fungal fragments in school environments could contribute to a better understating of the impact of mold exposure on health outcomes.

A few studies showed the seasonal variations of airborne mold concentrations in school environments. Generally, there is a clear seasonal pattern with the levels of airborne mold in winter being lower than those in summer, fall, or spring. For example, Bartlett *et al.* reported that the GM levels of airborne mold in elementary schools of Canada were 172.3 CFU m^{-3} for winter, 422.8 CFU m^{-3} for spring, and 422.7 CFU m^{-3} for fall.[25] A similar manner was shown in the mold concentrations (median; 12 CFU m^{-3} in winter, 94 CFU m^{-3} in spring, 476 CFU m^{-3} in fall) collected in Minneapolis, MN, USA by Ramachandran *et al.*[26] Indoor mold levels in 44 classrooms from 11 elementary schools were investigated in South Korea during winter and summer periods.[10] The GM level of airborne mold in winter (3514–5105 CFU m^{-3}) was significantly higher than that in summer (202–316 CFU m^{-3}) as well. In addition, it was observed that the levels in fall are likely higher than those in spring.[9] These consistent results could be attributed to lower temperature and humidity in the winter period which would be unfavorable for mold growth. In this study, molds were collected from the late spring to early summer (May–July), and so it may be a little hard to explore the seasonal variation of mold levels and compare our results directly with those of previous studies. Rather, as mentioned above, our findings indicated that airborne mold concentration would display seasonal variation linked to the rainfall pattern, and moreover we believe that these results could be utilized as background information for investigating and establishing the behavior mechanism of mold spores in indoor areas and the profile of fungal exposure.

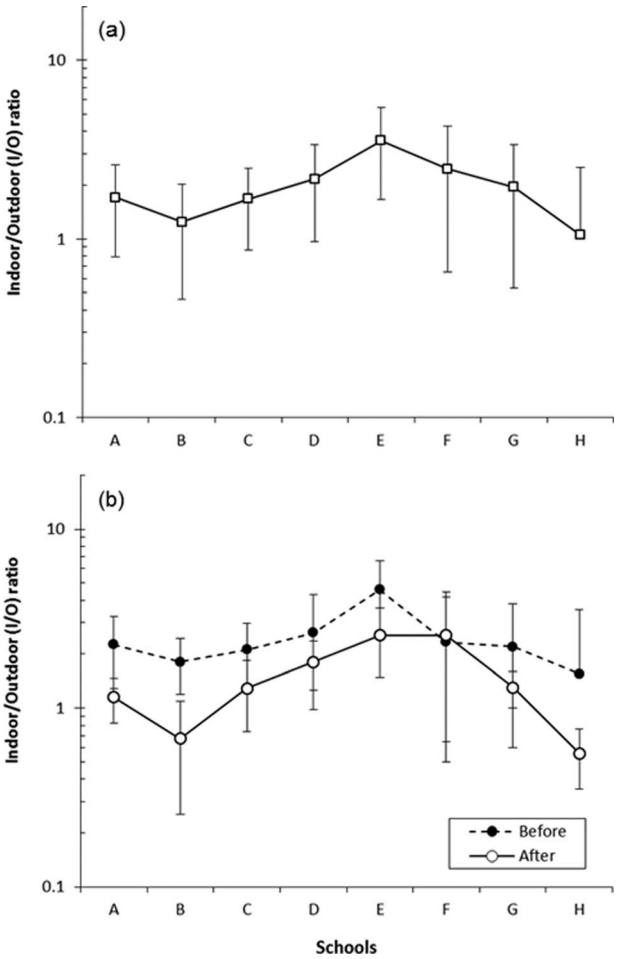

Fig. 2 Indoor/outdoor (I/O) ratios for elementary schools: (a) overall mean I/O ratio; (b) mean I/O ratio before and after rainy season.

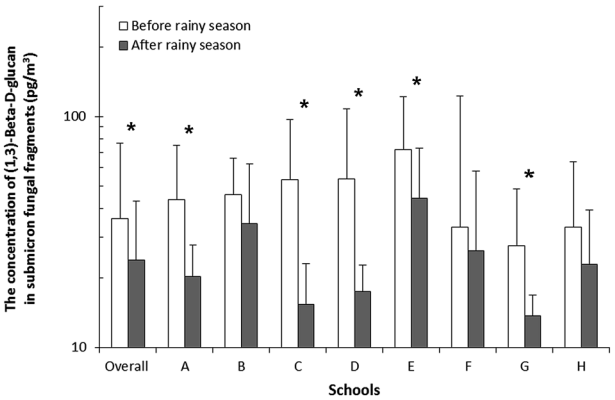

Fig. 3 The concentration of (1,3)-β-D-glucan in submicron fungal fragments for elementary schools before and after the rainy season ($^{*}P < 0.05$).

The concentrations of airborne mold in elementary schools vary on a large scale among countries, and so the concentrations of airborne mold collected in elementary schools for the spring period were compared. In Taiwan, the GM spore count sampled in 264 classrooms between April and May 2011 and analyzed by microscopic enumeration was about 2181 spores per m^3.[8] Kim *et al.* found that the mean concentration in Uppsala City, Sweden was 14 800 CFU m^{-3}.[27] Pegas *et al.* also reported that the corresponding value in Lisbon, Portugal was 1335 CFU m^{-3}.[11] In Boston and Minneapolis, USA, 121 spores per m^3 and 94 CFU m^{-3} were observed, respectively.[9,26] Bartlett *et al.* investigated the level of airborne mold in British Colombia, Canada.[25] They reported that a mean value of 422.7 CFU m^{-3} was observed. These results were similar to or higher than those in this study. Different sampling and analytical methods, sampling time, geographical and climatic differences, and different culture media could lead to the concentration variations. Thus these differences may hinder the comparison of our findings with others, but it is likely that high levels of airborne mold have been observed in tropical/subtropical areas or areas with oceanic climate. Reverse trend for mold concentration may occur in areas located in northern latitudes.

In general, the GM concentrations of PM_{10}, airborne mold, and bacteria before the rainy season were lower in renovated schools (school D and E) than in non-renovated schools. The GM level of PM_{10} (23.4 μg m^{-3}) in renovated schools was about 3 times lower than that in the other schools (71.7 μg m^{-3}) ($P < 0.001$). Also, overall GM concentrations of airborne mold and bacteria were lower in renovated schools, but the corresponding concentrations of mold and bacteria in school E were similar to or even higher than those of non-renovated schools. Conversely, the GM concentration of (1,3)-β-D-glucan in submicron fungal fragments was about two-fold higher in renovated schools (63.3 pg m^{-3}; 35.7 pg m^{-3} for non-renovated schools), but the difference was not significant ($P = 0.237$). Similar results were found in a study by Lignell *et al.*,[28] where about 58.1–89.5% decreases in fungal concentrations were observed in schools after renovation. Similarly, bacterial concentrations decreased by up to 74% after renovation. Renovation of deteriorated facilities may decrease the concentrations of PM_{10}. Therefore, we believe that major reductions in the levels of PM_{10} and airborne mold with large spore size might occur in this study. The high microbial concentrations observed in school E can be explained by the fact that the renovation in this school was performed around 10 years ago, and 2 or 3 classrooms with water seepage or damage had been repaired. However, full-scale renovation was carried out in school D. The benefits of renovation may have been diminished, or perhaps partial renovation was not as effective in reducing the overall level of microbial concentrations in schools,[29] because the levels of submicron fungal fragments were high in renovated schools in this study. Small particles can persist longer in air than larger particles, and thus removal of submicron fungal fragments might be relatively difficult.[30] Poor or no association of the concentrations of particulate matter with submicron fungal fragments has been reported in the past.[31] As mentioned earlier, the level of small particles may not be reduced unless full-scale renovation or active management after renovation is performed. Our findings also indicate that small fungal particles would remain longer or not be easy to reduce even after

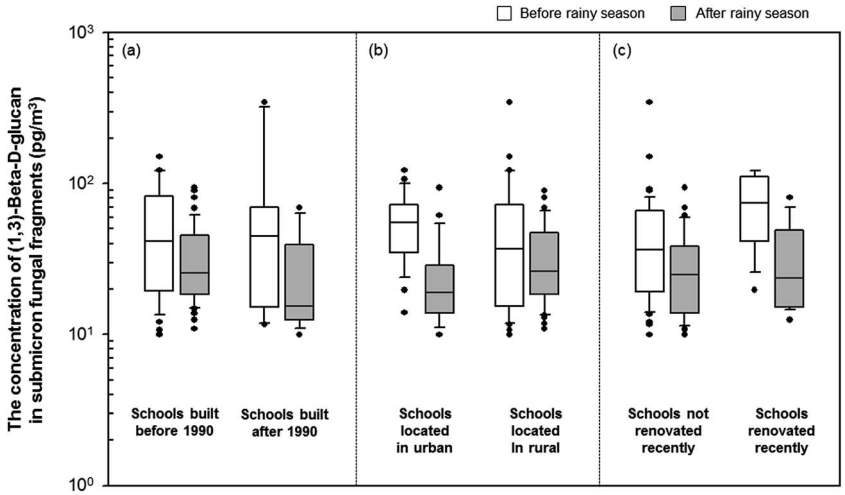

Fig. 4 A boxplot of the concentration of (1,3)-β-D-glucan in submicron fungal fragments for schools built before and after 1990. Each boxplot indicates an interquartile range with median, upper, and lower whiskers; upper and lower boundaries (3rd quartile/1st quartile). The white- and gray-colored boxplots represent the level of (1,3)-β-D-glucan in submicron fungal fragments before and after the rainy season, respectively.

renovation, and thus comprehensive assessment of exposure to mold, including fungal fragments, is necessary in the future.

The limitations of this study were that we did not compare levels of submicron fungal fragments in newly built buildings or investigate the temporal distribution of submicron fungal fragments, species of molds, or correlation between exposure to molds and health outcomes of students and teachers.

A school classroom can pose risks of exposure to certain environmental risk factors, including allergens, because children spend a major part of their time in one classroom. The concern regarding the adverse effect of indoor environments on children's health is valid because their immune systems are still developing and they breathe larger volumes of air compared to adults.[32] However, there have been few published studies on the evaluation of indoor risk factors in schools despite the differences in school and home environments in terms of size, activity, and population density. Moreover, mold is of particular concern in schools due to its link to the development and exacerbation of respiratory symptoms and allergic diseases such as asthma.[32] For these reasons, this study for identification and measurement of smaller-sized fungal particles capable of staying longer in the air and reaching deep into the lung could contribute to establish the comprehensive profile of mold exposure since spore count/enumeration only may not be sufficient for exploring the causal association of fungal exposure with health outcomes. In addition, this study is one of the first to confirm the presence of submicron fungal fragments in school environments, to our knowledge, and so could serve as useful background information to investigate the association between school environments and health outcomes of students, as well as of teaching and non-teaching staff.

Table 3 The geometric mean concentrations of airborne mold and bacteria, and arithmetic means of temperature and relative humidity in elementary schools before and after the rainy season[a]

	Airborne mold (CFU m^{-3}) [GM(GSD)]			Airborne bacteria (CFU m^{-3}) [GM(GSD)]			PM_{10} (μg m^{-3}) [GM(GSD)]			Temperature (°C) [mean ± SD]			Relative humidity (%) [mean ± SD]		
School	Before	After	*P*-value	Before	After	*P*-value	Before	After	*P*-value	Before	After	*P*-value	Before	After	*P*-value
A	449.6	177.1	0.006	544.8	311.7	0.017	69.0	17.6	<0.001	20.6	27.4	<0.001	60.0	68.1	<0.001
B	368.3	234.6	0.002	446.4	470.3	0.493	73.9	18.3	<0.001	27.1	26.8	0.411	46.6	71.9	<0.001
C	423.2	153.9	0.009	442.0	290.2	0.762	90.3	28.5	<0.001	25.6	26.9	<0.001	42.7	52.3	0.191
D	96.4	62.1	0.035	186.7	99.9	0.003	21.1	28.3	0.280	27.4	27.8	0.188	54.3	72.1	<0.001
E	642.1	148.0	0.002	1157.3	375.2	0.038	25.6	16.7	0.042	24.8	27.7	<0.001	35.0	60.3	<0.001
F	457.3	436.8	0.827	456.8	459.4	0.958	84.4	12.3	<0.001	23.7	24.5	0.052	48.7	71.2	<0.001
G	715.6	386.5	0.087	790.8	303.6	0.031	65.6	34.4	0.087	23.1	26.8	0.009	54.0	70.9	0.011
H	551.0	216.8	0.117	757.0	291.1	0.148	49.6	13.3	<0.001	21.8	27.7	<0.001	52.1	63.5	<0.001
Overall	402.9	184.7		531.8	299.2		50.8	19.8		24.4	26.9		48.8	66.2	

[a] Abbreviations: GM, geometric mean; GSD, geometric standard deviation; SD, standard deviation.

Table 4 Pearson's correlation coefficients for temperature; relative humidity; and concentrations of airborne mold and bacteria, PM_{10}, and submicron fungal fragments

	Temperature	Relative humidity	Airborne mold	Airborne bacteria	PM_{10}	Submicron fungal fragments
Temperature (°C)	1.000	0.243[b]	−0.319[b]	−0.228[b]	−0.412[b]	−0.132
Relative humidity (%)	—	1.000	**−0.208**[a]	**−0.244**[a]	−0.525[a]	−0.346[b]
Airborne mold (CFU m^{-3})	—	—	1.000	**0.778**[b]	**0.174**[a]	0.018
Airborne bacteria (CFU m^{-3})	—	—	—	1.000	0.073	0.108
PM_{10} (μg m^{-3})					1.000	0.212
Submicron fungal fragments (pg-glucan per m^3)	—	—	—	—		1.000

[a] *P*-value < 0.05. [b] *P*-value < 0.01; and significant correlations are marked bold.

Conclusions

It is evident that mold could play an important role in triggering and exacerbating allergic symptoms among sensitized individuals.[6,7,33] For better understanding of the causal role of mold exposure in health outcomes, comprehensive assessment of fungal exposure including smaller-sized fungal particles should be performed. For this reason, comprehensive data for classrooms should be collected and analyzed for appropriate evaluation of the association of school indoor environment with students' health. Moreover, the observation of comparable amounts of (1,3)-β-D-glucan in submicron fungal fragments in classrooms could serve as background information for establishing a comprehensive profile of fungal exposure. In particular, the decrease in submicron fungal fragments after the rainy season would be applicable for developing necessary strategies to prevent exposure to molds and to improve indoor air quality in school environments. A seasonal customized management in school environments is necessary, considering that exposure to small fungal particles would occur.

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