Isolation and identification of mold from naturally ventilated preschools in urban and suburban area in Selangor

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Abstract
Mold includes airborne particles of biological origin. Exposure to mold in damp buildings has been associated with the risk of experiencing health problems. Children may be exposed to mold spores through the respiratory tract, when they inhale or through skin contact. This study is a comparative cross-sectional study which was carried out in Malay preschools. A total of 270 respondents and 12 preschools from both locations (Puchong and Hulu Langat) were involved in this study. This study aimed at identifying different types of fungi present in selected preschools in Selangor. Indoor isolation of fungi was done using an instrument known as PBI Duos SAS Super 360™ bioaerosol sampler and Sabouraud dextrous agar (SDA) was used for the cultivation and identification of mold in selected preschools. Mold colonies were represented as colony forming units (CFU m⁻³). Further identification of fungi was done based on their macroscopic and microscopic characteristics. A total of 12 preschools and 36 houses were selected to participate in this study. Mold isolated from preschools in study and comparative area include; Candida albican, Aspergillus niger, Microsporium canis and Penicillium notatum. Analysis has shown a significant difference between mold level which were isolated in industrial and suburban preschools (p<0.05) for preschools and homes sampled. In conclusion, this study found that indoor exposure to mold were higher among preschools in the urban area than those in suburban area. Water damaged materials provide a good environment for the growth and multiplication of mold.

Keywords: Mold, Indoor Air Quality, Preschools.

Introduction
Indoor air comprises of mixture of bioaerosol and non-biological components which includes mold, bacteria and allergens. It is also made up of dust particles, smoke from tobacco sources and cooking equipments, exhaust particles from vehicles and particles from thermal power plants (Kalogerakis, et al., 2005). Human activities indoors such as talking, sneezing, coughing, body movement like walking, running, cleaning and use of convenience, use of VOC emitting materials, smoke from tobacco and cooking equipments, cleaning products, furniture and finishes used in homes and so on. The choice of school locations should be done in places with lower exposure to outdoor pollutants. (Maeir et al., 2002). Mold related symptoms are eye and throat irritation, stuffy nose, sore throat, headaches and dizziness. All mold can cause health effects, allergic reactions and asthma attacks in children. (Meyer et al., 2004).
Indoor pollutants can be generated from outdoor air and some indoor activities carried out in schools and homes. Several studies have been proven that human activities are an important source for indoor pollution. (Kamaruddin, et al., 2015; Yusoff, et al., 2016; Suhaimi et al., 2015; Wesley and Jalaludin, 2015; Nur Azwani et al., 2015; Chen and Hildemann, 2009). Sources of bioaerosol include fungi, pollen, bacteria, protozoa, animal dander (Douwes et al., 2003), human bodies can generate indoor pollutants directly through activities like talking, sneezing, and coughing, while other activities like washing, flushing toilet, sweeping floor can generate bioaerosol indirectly (Kalogerakis et al., 2005; Martin et al., 2015).

Materials and Method

Study location
This study is a cross-sectional comparative study carried out in preschools in Puchong, (industrial area) and Hulu Langat (suburban area). Preschools in the industrial area were chosen based on different characteristics. Those that were within 5 km close to industry, factories, close to major roads and sources of indoor air pollutants, while for comparative were those that were less exposed to indoor air pollutant.

Mold sampling
Isolation and identification of mold was done using the PBI Duos SAS Super 360 microbiological air sampler and Sabouraud Dextrous agar (SDA). 500 liters of air sample for 2 minutes was used during sample collection. The incubation of cultures was done for 5 days at the temperature of 28°C. Culture purification was done by sub-culturing from the primary cultures into a new agar media and incubated again for another 5 days at 28°C to get a pure culture. Macroscopic identification was carried out by observing the appearance of the colonies. Further identification was done by staining using Lactophenol cotton blue. Microscopy was carried out which involves checking for the presence or the absence of hyphae. The morphology which is the shape, method of arrangement of the spores and also the size and appearance of the hyphae produced by the colonies. (Hoog and Guarro, 1995).

Results and Discussion

The result of this study in Table 1 shows the different types of mold that were isolated from preschools in studied and comparative area. The schools were represented using alphabets. The schools in the studied area were represented as A-F while those in the comparative area were represented as G-L. In this study, A. niger was isolated in all the 12 preschools used in this study followed by P. notatum, C. albican and M. canis which was isolated also from a school in the industrial area.

M. canis is known to be a more frequent cause of ringworm in humans. It is known as a causal agent of skin diseases in animals and humans especially in children. It causes infection in the hair, skin and nails in rare cases. The main sources are cats and dogs. Candida albican is known to be a normal flora in humans. Indoor sources of this organism can be from human and animal droppings. They can also be isolated from polluted water, soil, air and plants. Penicillium spores are commonly found indoors on building materials that have being damaged by water, walls, wallpaper, floor, carpet mattress and wooden materials. Indoor exposure to mold spores and pet allergens can lead to increase the rate of asthma attack, increase in incidence of wheezing, daytime breathlessness, symptoms like headache, sore throat, tiredness, and cough. The results of this study in Table 2 and Table 3 show the median (IQR) concentration for mold in schools and in homes of respondents as 403(250.0) CFU/m³ and 262.0(125.0) CFU/m³, 285.0(236.0) CFU/m³, 248.5(74.0) CFU/m³ for studied and comparative area respectively. The result obtained in this study did not exceed the WHO guideline for 8 hour exposure to mold colonies which is (500 CFU/m³). Mold isolated in this study were significantly different in homes and schools for both locations studied. (Z= -5.783, p= <0.001), (Z= -2.189, p= 0.028) which indicates that the level of mold in preschools can be influenced by the indoor level of dampness and settled dust.

The findings in this study are similar to the findings in previous studies by Savilahti et al., 2001; Jo and Seo 2005; Godwin and Batterman 2007; Viegas et al., 2010 and Mi et al. 2006 who found that the frequently isolated fungi species in indoor school environment were Penicillium spp, Cladosporium, Alternaria and Aspergillus spp.
These studies found that symptoms such as headaches, sore throat, wheezing and cough were experienced after exposure to mold spores. Previous studies by Wanner et al (1993), McGrath et al (1999) and Cooley et al (1998) in indoor environment also isolated *Penicillium, Aspergillus, Eurotium, Wallemia, Cladosporium*. Some studies had found that school environment could be a source of exposure to allergen for children who do not have pets at home (Salo et al., 2009; Fromme et al., 2008; Kim et al., 2005; Zhao et al., 2006).

A local study “Indoor air pollutant exposure and eosinophil cationic protein as an upper airway inflammatory biomarker among preschool children” conducted by Wesley & Jalaludin in industrial and suburban area among preschool children isolated a median (IQR) mold concentration level as 361.00 (0.00) CFU/m$^3$ in exposed area and 344.00 (95.00) CFU/m$^3$ in comparative area which is in line with this study. Several studies have also found mean level of indoor mold concentrations as 92-505 CFU/m$^3$ and an average of 305 CFU/m$^3$ in classrooms (Jo and Seo, 2005; Godwin and Batterman, 2007; Viegas et al., 2010).

<table>
<thead>
<tr>
<th>Names of mold</th>
<th>Morphology</th>
<th>Preschools</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus niger</em></td>
<td>A. niger is one of the most common and easily identifiable species of the genus Aspergillus. It is identified by its white to yellow colour which is made up of black conidial that are dark brown to black in colour. Their walls are not smooth. It has a wooly appearance on the surface. When placed in a reverse position, it is pale yellow in colour and has curly lines on the surface.</td>
<td>A, B, C, D, E, F, G, H, I, J, K, L</td>
<td>The spores that are produced by <em>Aspergillus</em> species can be found both indoor and outdoor.</td>
</tr>
<tr>
<td><em>Microsporum canis</em></td>
<td>When cultured on SDA, the colonies spread out flat. It is known with a white to cream-colour. A dense cottony surface can be clearly seen on the surface of the colonies. It produces a bright yellow colour when placed in a reverse position</td>
<td>A</td>
<td><em>M. canis</em> is known to be a more frequent cause of ringworm in humans. It is known as a causal agent of skin diseases in animals and humans especially in children. It causes infection in the hair, skin and nails which occurs in rare cases. The main sources are cats and dogs</td>
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<td><em>Candida albican</em></td>
<td><em>Candida albican</em> when cultured on SDA appears smooth. It is made up of blastoconidia. When viewed with the aid of a microscope, their budding are spherical to subspherical. The colonies on Sabouraud’s Dextrose Agar (SDA) shows white to cream-colour. It is yeast-like.</td>
<td>A, B, C, H</td>
<td><em>Candida albican</em> is known to be a normal inhabitant in 40 to 80% of normal human beings. It is known as commensal in the gastrointestinal tract of humans. Environmental sources of this organism can be from human and animal excreta. The can also be isolated from polluted water, soil, air and plants.</td>
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<tr>
<td><em>Penicillium notatum</em></td>
<td>The colonies of <em>P. notatum</em> is usually green in colour and it consist of a conidiophore that is dense forming a shape that is like a broom.</td>
<td>A, C, D, F, G, H, L</td>
<td><em>Penicillium</em> spores are commonly found indoors on water damaged building materials, walls, wallpaper, floor, carpet</td>
</tr>
</tbody>
</table>
viewed under a microscope. It has a white apron growth which can be seen at the edge of the colonies. The colonies appear white and can be seen clearly on reverse side. Mattress. The spores are spread in the air and can easily be inhaled into the body.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Urban (n=135)</th>
<th>Comparative (n=135)</th>
<th>Z value</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>Mold (CFU/m³)</td>
<td>403.0(250.0)</td>
<td>262.0 (125.0)</td>
<td>-5.783</td>
<td>&lt;0.001**</td>
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* = Significant at p<0.05, ** = Significant at p<0.001

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<td>Mold (CFU/m³)</td>
<td>285.0 (236.0)</td>
<td>248.5 (74.0)</td>
<td>-2.189</td>
<td>0.028*</td>
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* = Significant at p<0.05, ** = Significant at p<0.001

**Conclusion**

Most of the schools in industrial area had moisture damaged ceilings which is a favorable place for the growth of mold. The presence of mold in preschools was attributed to moisture damaged ceilings and wood used in preschools. Indoor mold is often found in public places like schools and daycare centers. Furthermore, majority of the schools used for this study had low ventilation rates. This is because during school hours the doors were closed which reduces the rate of ventilation inside the classrooms. Low ventilation rate in schools can be linked to adverse health effects in children and increase in asthmatic
symptoms among children. Reduction in the rate of ventilation in schools can lead to the reduction in the academic performances of children. Preschools should provide a good ventilated environment for children. Moisture damaged facilities such as ceiling and wood should not be used in preschools. Mold remediation should be done in building with moisture damaged walls. Remediation should be done when the children are not around to avoid exposing them to mold spores.

Acknowledgement

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References


Zhao ZH, Dong T and Jiang TA, 2006. Comparative study of asthma, pollen, cat and dog allergy among pupils and allergen levels in schools in Taiyuan city, China and Uppsala, Sweden. Indoor Air. 16(6): 404-413.