AJAB
Asian J Agri & Biol. 2018;Special Issue:97-102.
Original Research Article

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

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Received:
November 09, 2017
Accepted:
February 28, 2018
Published:
May 30, 2018

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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 problem. 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. 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 equipment~~s~~, 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 equipment~~s~~, 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 28ºC 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/m^3 and 262.0(125.0) CFU/m^3), 285.0(236.0) CFU/m^3, 248.5(74.0) CFU/m^3 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/m^3). 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 1. Indoor mold isolated from preschools in urban and sub urban area.

Names of mold	Morphology	Preschools	Sources
Aspergilus niger	*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 curley lines on the surface.	A, B, C, D, E, F, G, H, I, J, K, L	The spores that are produced by *Aspergillus* species can be found both indoor and outdoor.
Microsporum canis	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	A	*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 which occurs in rare cases. The main sources are cats and dogs
Candida albican	*Candida albican* 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.	A, B, C, H	*Candida albican* 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.
Penicillium notatum	The colonies of *P. notatum* is usually green in colour and it consist of a conidiophore that is dense forming a shape that is like a broom. When	A, C, D, F, G, H, L	*Penicillium* spores are commonly found indoors on water damaged building materials, walls, wallpaper, floor, carpet

	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 2: Comparison of indoor mold between preschools in the studied areas

Variable	Urban (n=135)	Comparative (n=135)	Z value	P value
	Median (IQR)	Median (IQR		
Mold (CFU/m^3)	403.0(250.0)	262.0 (125.0)	-5.783	<0.001**

* =Significant at p<0.05, ** = Significant at p<0.001

Table 3: Comparison of indoor mold between houses in the studied areas

Variable	Urban (n=135)	Comparative (n=135)	Z value	P value
	Median (IQR)	Median (IQR		
Mold (CFU/m^3)	285.0 (236.0)	248.5 (74.0)	-2.189	0.028*

* = Significant at p<0.05, ** = Significant at p<0.001

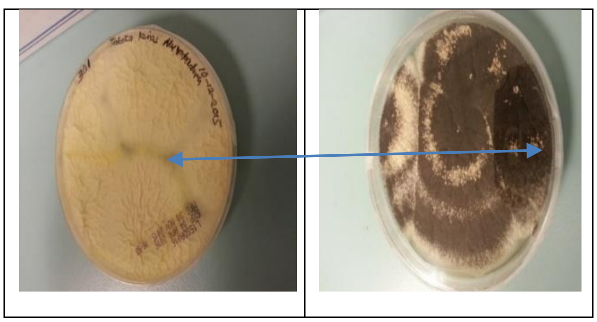

Figure 1. *A. niger* showing reverse and surface appearance.

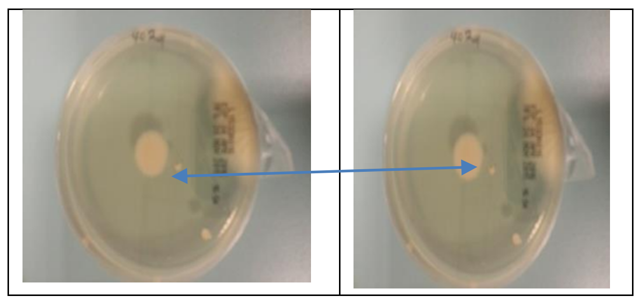

Figure 3. *Candida Albican* in a reverse and surface appearance

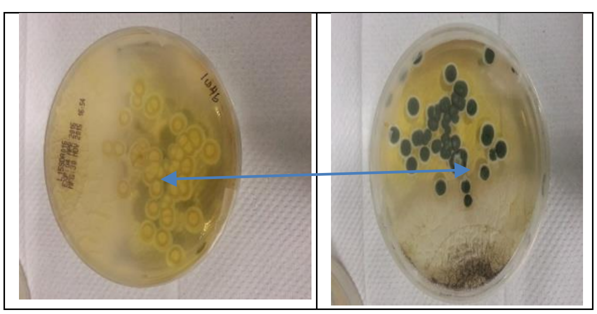

Figure 2. *Penicillium notatum* in a reverse and surface appearance

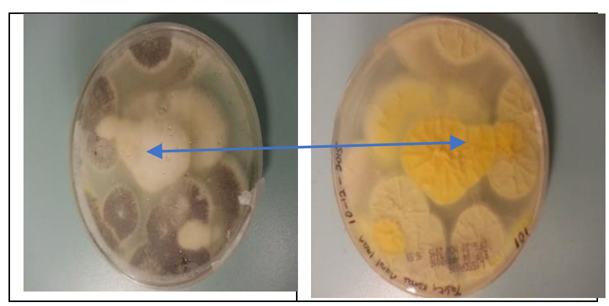

Figure 4. *Microsporum canis* in a surface and reverse appearance

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

A very big thank you to all the teachers, parents and staffs of the schools for their support and assistance during the period of data collection.

References

Arbes SJ, Gergen PJ, Elliot L and Zeldin DC, 2005. Exposure to indoor allergens in day-care facilities: results from 2 North Carolina counties. J. Allergy Clin. Immunol. 16(1): 133-139.

Ayuni NA, Juliana J and Ibrahim MH, 2014. Exposure to PM_{10} and NO_2 and association with respiratory health among primary school children living near petrochemical industry area at Kertih, Terengganu. JOMB. 3(4): 282-287.

Chen Q and Hildemann LM, 2009. The effects of human activities on exposure to particulate matter and bioaerosols in residential homes. Environ. Sci. Tech. 43(13): 4641-4646.

Cooley JD, Wong WC, Jumper CA and Straus DC, 1998. Correlation between the prevalence of certain fungi and sick building syndrome. Occup. Environ. Med. 55(9): 579-584.

Douwes J, Thorne P, Pearce N and Heederik D, 2003. Bioaerosol health effects and exposure assessment: prospects and progress. Ann. Occup. Hyg. 47(3): 187-200.

Godwin C and Batterman S, 2007. Indoor air quality in Michigan schools. Indoor Air, 17(2): 109-121.

Hoog GS and Guarro J, 1995. Atlas of clinical fungi. Centraal bureau voor schimmelcultures.

Jo WK and Seo YJ, 2005. Indoor and outdoor bioaerosol levels at recreation facilities, elementary schools, and homes. Chemosphere. 61(11): 1570-1579.

Kalogerakis ND, Paschali D, Lekaditis V, Pantidou A, Eleftheriadis K and Lazaridis M, 2005. Bioaerosol measurements in domestic and office premises. J. Aero. Sci. 36(5-6): 751-761.

Kim JL, Elfman L, Mi Y, Johansson M, Smedje G, Norbäck D, 2005. Current asthma and respiratory symptoms among pupils in relation to dietary factors and allergens in the school environment. Indoor Air. 15(3): 170-182.

Kamaruddin AS and Jalaludin J, 2015. Ondoor air quality and its association with respiratory health among Malay preschool children in Shah Alam and Hulu Langat, Selangor. Adv. Environ. Biol. (9)9: 17-26.

Kamaruddin AS, Jalaludin J and Hamedon TR, 2016. Exposure to industrial air pollutants and respiratory health school and home exposure among primary school children in Kemaman, Terengganu. Int. J. Appl. Chem. 12(1): 45-50.

Maier WC, Arrighi HM and Morray B, 2006. Indoor risk factors for asthma and wheezing among Seattle school children. Environ Health Perspect. 105 (2): 208-214.

Martin LJ, Adams RI, Bateman A, Bik HM and Hawks J, 2015. Evolution of the indoor biome. Trends Ecol. Evol. 30(4): 223-232.

McGrath JJ, Wong WC, Cooley JD and Straus DC, 1999. Continually measured fungal profiles in sick building syndrome. Curr. Microbiol. 38(1): 33-36.

Meyer HW, Suadicani P, Valbjorn O, Sigsqaard T and Gyntelberg F, 2004. Mold in floor dust and building-related symptoms in adolescent school children: A problem for boys only? Indoor Air. 14 (10): 65-72.

Mi YH, Norback D and Tao J, 2006. Current asthma and respiratory symptoms among pupils in Shanghai, China: influence of building ventilation, nitrogen dioxide, ozone, and formaldehyde in classrooms. Indoor Air. 16 (6): 454-464.

Nur Azwani MNR., Juliana J and Chua PC. 2015. Indoor air quality and respiratory health among Malay preschool children in Selangor. Biomed Res. Int. 248178.

Noor Hisyam N and Juliana J. 2014. Association between Indoor PM_{10}, $PM_{2.5}$ and NO_2 with airway inflammation among preschool children at industrial and sub-urban areas. Adv. Environ. Biol. 8(15): 149-159.

Ross M.A, Curtis L, Scheff, PA, Hryhorezuk DO, Ramakrishnan V, Wadden RA and Persky VW, 2000. Association of asthma symptoms and severity with indoor bioaerosols. Allergy. 55(8): 705-711.

Salo PM., Sever ML and Zeldin DC, 2009. Indoor allergens in school and day care environments. J. Allergy Clin. Immunol. 124 (2): 185-192.

Savilahti R., Utti J, Roto P and Husman T, 2001 Increased prevalence of atopy among children exposed to mold in a school building. Allergy. 56 (2): 175-179.

Seppa¨nen O, Fisk WJ and Lei QH, 2006. Ventilation and performance in office work. Indoor Air. 16 (1): 28-36.

Suhaimi NS, Jalaludin J and Abu Bakar S, 2015. Association between school and residential air pollutants with respiratory symptoms among school children at an industrial area. Adv. Environ. Biol. 9(26): 77-87.

Viegas C, Brebbia CA and Longhurst JWS, 2010. Air fungal contamination in two elementary schools in Lisbon, Portugal. Air Pollution. 136: 305-311.

Wanner HU, Verhoeff AP, Colombi A, Flannigan B, Gravesen S. Mouilleseaux A, Papadakis J and Seidel K, 1993. Indoor air quality and its impact on man. Biological Particles in Indoor Environments. Report 12. Commission of the European Communities, Brussels, Luxembourg.

Wesley AD and Jalaludin J, 2015. Indoor air pollutant exposure and eosinophil cationic protein as an upper airway inflammatory biomarker among preschool children. Procedia Environ. Sci. 30(2015): 297-302.

WHO, 2009. WHO guidelines for indoor air quality: dampness and mold. World Health Organization Copenhagen. 248.

Yusoff AF, Jalaludin J and Suhaimi NF, 2016. Association between air pollutants with feno among primary school children at petrochemical industries. Int. J. Appl. Chem. 12(1): 34-38.

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.