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Original Research Article

Indoor air quality (IAQ) characteristics and its microbial community identifications at two selected schools in Pahang, Malaysia: a preliminary study

Hizrri A[1], Zati Nabilah MG[1], Nurul Amni Z[1], Shahida N[1], Maryam Z[1], Hazrin AH[1], Mohd Faez S[1], Mohd Shukri MA[1]*

[1]Department of Biotechnology, Kulliyyah of Science, International Islamic University Malaysia, Kuantan Campus, Pahang, Malaysia

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**Corresponding author email:*
mamshukri@iium.edu.my

Abstract

It is important to assess IAQ characteristics and to identify possible microbial contaminants in schools' indoor environment because children are more vulnerable to air pollutants as they inhale more air pollutants per kilograms of body weight. Hence, this study aims to assess and to compare the level of selected IAQ parameters and microbiological contaminants inside the classroom of schools in urban area and rural area during occupied and non-occupied period. This study also aims to identify airborne bacteria species and fungi genera within classroom of schools in those area. For methodology of the study, schools were selected based on their location. School X (SX) was located in Kuantan, Pahang, while school Y (SY) was located in Pekan, Pahang. The physical IAQ parameters (Temperature, Relative Humidity (RH), Carbon Dioxide (CO_2) were measured using VelociCalc® Multi-Function Ventilation Meter 9565 (TSI®, Minnesota, USA), and airborne particulate matter (PM) were measured using DustMate (Turnkey Instruments, UK). Surface Air System Indoor Air Quality (SAS IAQ), (PBI International, Italy) was used to collect the microbial contaminants and subsequently CFU were counted. The data were recorded for 30 minutes for each time-slot for 8 hours during occupied and non-occupied period within selected classrooms. Bacteria identification was done using 16S rRNA gene sequence analysis and fungi were identified macroscopically through direct identification technique up to genus level. The results were compared to standard reference limit based on Industrial Code of Practice on Indoor Air Quality (ICOP, 2010) regulated by the Department of Occupational Safety and Health (DOSH, 2005). This study found that temperature (SX, Occupied; 34.9±3.9, Non-Occupied; 32.8±0.7), (SY, Occupied; 30.7±0.2, Non-Occupied; 30.6±0.5), RH (SY, Occupied; 74.4±2.9, Non-Ocupied; 70.05±1.0) and bacterial CFU counts (SX, Occupied; 558±308), (SY; Occupied; 903±415, Non-Occupied; 1176±303) exceeded the standard limit regulated by DOSH. Number of gram-negative bacteria dominated over gram-positive bacteria in both settings. *Bacillus sp.* (*B. atrophaeus*, *B. subtilis*, *B. pumilus*, *B. altitudinis*. *B. tequilensis*, and *B. aerophilus*) were the most dominant species, followed by *Staphylococcus sp*. (*S. warneri*, *S. sciuri*, *S. haemolyticus*, and *S. gallinarum*). The common fungal species isolated in both schools during occupied and non-occupied period were *Aspergillus, Alternaria, Cladosporium, Penicillium, and Mucor*.

Keywords: Indoor Air Quality, Schools, Bacteria, Fungi

Introduction

Schools had become second most important indoor environments for children to spend their day time (Alves *et al.,* 2015). According to USEPA, (2005) level of indoor pollutants were higher than outdoors up to 5 times, thus it could affect children's health. Children were more susceptible to air pollutants because of greater air respirations, greater metabolism and growth rates, and immature immune systems (USEPA, 1995; Nur Azwani 2015). Overcrowded classrooms, insufficient air ventilations, poor maintenance, increased usage of cleaners, and chemically formulated products could worsen IAQ level in the classrooms (Pegas *et al.,* 2011; Alves *et al.,* 2013). The deterioration of buildings' materials and equipment in school environment would cause more IAQ problems. Consequently, failure to overcome IAQ problems would affect health, comfort, and performances of students and teachers in school environment (Mohai *et al.*, 2011).

The level of air quality in Malaysia has degraded over past two decades prior to rapid urbanization. Preschool children in urban area had higher risk to be affected by indoor air particles as compared to preschool children in rural area (Nur Aida *et al.*, 2014). The situation has been supported by Chua *et al.,* (2015), who reported that level of PM_{10}, $PM_{2.5}$ and carbon monoxide were higher at school in urban area of Selangor as compared to other school located in rural area. Gram-positive *cocci* were the most predominant culturable bacteria isolated from indoor air. Study in school in Mexico had revealed that Gram positive bacteria were predominant than Gram negative bacteria. *Staphylococcus* and *Streptococcus* were among isolated bacteria that could cause infections in respiratory airways (D' Arcy *et al.,* 2012; Hurtado *et al.,* 2014).

To highlight, studies in primary schools' environment on indoor and outdoor pollutants, chemical compositions, and bacterial components were still insufficient (Almeida *et al.,* 2011; Liu *et al.,* 2000; Pegas, 2012). The characteristics, growth behavior, and adverse effects on human's health of bacteria species must be investigated. Furthermore, mode of transmission of some bacteria species, its cellular mechanisms during infections, and severity of the infections on human's health, were poorly understood (Meklin *et al.,* 2005).

Poor IAQ in classrooms would lead to uncomfortable learning environment and increase absenteeism of student to school. In longer term, high indoor pollutants level inside classrooms would induced adverse health effects on students and teachers. Therefore, this study aimed to determine characteristics of IAQ parameters and to identify potential airborne bacteria and fungi species at selected schools in urban and rural area in Pahang. Overall, the data established from this study would provide additional input on current status of indoor airborne contaminants especially in schools' environment in Malaysia.

Materials and Method

Sampling site description

Two different government schools in Pahang were selected by different background characteristics of the schools which represent rural and urban area according to Lee and Chang, (2000). The selected two schools were; School X (SX) located in Kuantan, Pahang, which started its activities in 2004. Residential area, office buildings, bus terminal, and constructions area were present near to the school location. On the other hand, School Y (SY) located in Pekan, Pahang. The school opened in 1952 and located in rural area of Pekan, Pahang. The school buildings were enclosed by natural environments and were closed to a cowshed. Sampling point at both of the schools were done in standard 4 classrooms.

Sampling Method

The sampling time were divided into four slots for 8h started from the beginning of the school period. All the instruments were set up 1 meter from the wall at the back of classroom, the height was set according to children breathing height (0.75 - 1.5 meter). Temperature, RH, and CO_2 were measured by VelociCalc® Multi-Function Ventilation Meter 9565 (TSI®, Minnesota, USA), while concentration of $PM_{2.5}$ and PM_1 were measured using DustMate (Turnkey Instruments, UK). The data were measured for 30 minutes for each time slots. Surface Air System Indoor Air Quality (SAS IAQ), (PBI International, Italy), was used to sample out airborne microbial contaminants. Petri dishes filled with nutrient agar (NA) and potato dextrose agar (PDA) was placed into sampler housing. Airborne particles were captured by impaction when 100 liters of air was aspirated over the agar surface. The sampling of airborne microbial

contaminants was triplicated at every session of sampling.

Microbial count

Samples were directly incubated in the laboratory for NA within 24 hours at 37 ºC while PDA within 3 days at 30ºC. Bacteria and fungi colony were counted after the incubation period and colony forming unit for each of bacteria and fungi were calculated according to the formula (1):

$$TBC/TFC = (Pr \times 1000) / V \quad (1)$$

Where:

V = Volume of sampled air

r = Colony Forming Units counted

Pr = Probable count obtained by positive hole correction

TBC/TFC = Colony Forming Units per 1000 litres

Fungi Identification

In purpose of fungi identification, fungi structures such as mycelium, sporulation structures, and fruit-bodies were observed. The cell morphology of fungi and bacteria colonies were observed using light microscope and Dino Eye. The images later were referred to Samson *et al.*, (2010) for genus identification.

Bacterial identification: 16S ribosomal RNA gene analysis

The genomic DNA extraction was conducted following the protocols mentioned in the Vivantis GF-1 Bacterial DNA Extraction Kit. Later, Polymerase chain reaction (PCR) was conducted to amplify the genomic DNA. Universal primers (F16S-8/R16S-E939) were used at the beginning of this step. The PCR components consisted of several reagents such as 5 or 10 µL purified genomic DNA (depending on the DNA concentration), 17 or 22 µL nuclease free water, 25 µL PCR Master mix, 1.5 µL forward primer and 1.5 µL reverse primer. Next, Eppendorf® PCR Mastercycler for 30 cycles was used to conduct DNA amplification. The thermal cycle steps were pre-denaturation, denaturation, annealing, extension and final extension. Amplified 16S rRNA gene was purified before sequencing, using protocols specified in Vivantis GF-1 PCR Clean Up Kit. Sequencing and oligodeoxyribonucleotides synthesis were completed by the 1st Base Laboratory, Malaysia. Initial sequencing of both strands was carried out by using ABI PRISM ® 377 DNA sequencer by using forward and reverse PCR primers. Sequences were extended by designing downstream primers based on the available determined sequence. The 16S rRNA sequence obtained was aligned and compared with the sequences stored in GenBank using Blastn analysis tool. Sequences were selected from Blast-Webpage and added directly into MEGA6 software using integrated web-browser.

Data Analysis

The IAQ parameters data obtained from the study were compared to Industrial Code of Practice on Indoor Air Quality (ICOP, 2010), regulated by the Department of Occupational Safety and Health (DOSH, 2005) as shown in Table 1. SPSS version 20.0 was used to run statistical analysis. Mann-Whitney U test was used to determine the differences between IAQ parameters measured in urban and rural area schools.

Results

All the temperatures measured in both schools exceeded the acceptable limits set by ICOP 2010. The mean indoor temperatures throughout the occupied and non-occupied period were higher at SX as compared to SY.

This finding was parallel to study by Fischer, *et al.*, (2012), that revealed air temperature in urban environment were higher than temperature in rural environment. urban air temperatures are noticeably higher than corresponding temperature in the rural environment. High temperature in the classrooms will affected the thermal comfort levels of the students (Ismail *et al.*, 2010). Clothing, activities in the classroom, students' physiology and age, were the individual factors that influenced individual's thermal comfort requirement (Alves *et al.*, 2013). Even though, thermal comfort requirement varies according to individuals, it has been reported that, with only slight changes of temperature inside the classroom, even within the comfort zone, the changes could affect student's concentration towards lesson in class (Alves *et al.*, 2015).

Results of this study also revealed that RH in SY was higher than SX, parallel to the study by Oke, (1987), that reported, RH in urban area had a tendency to be lower than in rural area due to more concrete buildings in urban area that did not have ability to contain moistures as compared to soil environment in rural

area. Besides that, rural area had higher amount of water that cooled the air by absorbing heat during evaporation process. In both schools, the percentage of RH were decreased when temperature raised. This situation was parallel with study done in Terengganu, Malaysia. The study revealed that as the temperature arises from morning to afternoon, the percentage of RH were started to decrease (Ismail *et al.*, 2010).

This study also found that, $PM_{2.5}$ level in SX were higher than SY. The findings were similar to study by Chua *et al.*, (2015), who reported the average concentrations of $PM_{2.5}$ were greater in urban schools as compared to rural schools. $PM_{2.5}$ concentrations also could be affected by students' activities. Students at SX were entering the classroom after their physical education class during the sampling period. Dust particles from outdoors could be trapped on clothes and shoes of the students. Resulted in increased of indoor PM concentrations (Alves *et al.*, 2013). Mann-Whitney U Test revealed that there was a significant difference in $PM_{2.5}$ concentrations during non-occupied period (p<0.05) Even though students were absent, $PM_{2.5}$ could be originated from traffic emissions and industrial activities, as well as constructions sites that present in surrounding area of the schools in urban area (Nur Azwani *et al.*, 2015). PM_1 also could have been originated from vehicles' combustions (Wang *et al.*, 2003). In addition, Emilia *et al.*, (2014), suggested that PM generations were not solely related to traffic emissions, but also could came from agricultural activities.

CO_2 concentrations in SX and SY were below 1000 ppm limit set by DOSH, 2010. This was mainly due to general ventilated classrooms that allowed air exchange through opening of doors and windows. Air movement generated by ceiling fan allowed high air exchange rate with outdoor air, resulted CO_2 level were lower during occupied period as compared to non-occupied period in both school. CO_2 concentrations were increased as students started to occupy the classroom in the morning. The CO_2 level were declined after students left the classroom after school period (Fischer *et al.*, 2012). CO_2 also accumulated in the classroom when the windows and doors were shut during the school hours (Lee and Chang, 2000).

This study also found that TBC was higher at SY as compared to SX during occupied and non-occupied period. TBC during occupied period had exceeded 500 CFU/m^3 set by DOSH. TBC of both schools had a significant different during non-occupied period. This could suggest that background area of a school could affect the TBC. In fact, SY was very closed to a cowshed. The classroom also was at the ground level, thus increasing the outdoor air pollutants intrusion into indoor environment. In addition, during occupied period, the presence of student increase could release the bacteria into the classroom environment through coughing, sneezing, talking, and shredding of skin epidermis. Therefore, presence of students inside the classroom had contributed to high TBC. The survival of microorganism in indoor environment also depended on RH, temperature, oxygen, availability of nutrient, air turbulence and also wind (Pegas *et al.*, 2011).

For TFC, the count at both schools were below the limit set by DOSH. TFC in SY was significantly higher than TFC in SX. This condition was probably due the building in SY was older than SX. Most probably, the airborne fungi originated from moisture damaged materials inside the classroom as previously suggested by Meklin *et al.* (2002). The buildings in SY also were lacking on scheduled maintenance, hence its' becoming more prone to mold problems. High RH and temperature as measured in SY had influence TFC in indoor environment as these parameters provide optimum conditions for fungi growth and sporulation (Bornehag *et al.*, 2001).

Identification of isolated bacteria in selected schools.

Airborne bacteria species identified at SX and SY during occupied and non-occupied period were isolated in Table 2 and Table 3, respectively. 80 *Bacilli* and 18 *Cocci* colonies were isolated in SX. While in SY, 91 *Bacilli* and 23 *Cocci* colonies were isolated. In SX, all 8 isolated bacteria sequences were Gram positive bacteria which consist of *Bacillus sp.*, *Staphylococcus* sp., *Bacillus cereus*, and *Bacillus licheniformis*. In SY, *Bacillus* sp., *Staphylococcus sp.*, and *Bacillus cereus* were also identified.

BLAST results of *Bacillus sp.* in SX revealed the closest species were *B. atrophaeus*, *B. subtilis*, *B. pumilus*, and *B. aerophilus*. Sources of *B. subtilis* were soil and water, but the bacteria also could reside in human gastrointestinal tract (Ralph and Ernest, 2006). *B. atrophaeus* was classified as non-pathogenic bacteria, therefore it could not harm children's health. However, *B. pumilus* which originated in soil environment could infect human even though the infections were rarely occurred. Most of the time, these bacteria were associated with plant growth, but

they also had caused 3 food poisoning cases in 2006 mainly involved rice. Infections with these bacteria resulted in dizziness, headache, diarrhea, and stomach cramps (Hormazabal and Granum, 2007).

Table 1. Interrelationship of IAQ Parameters among selected schools and its comparison to ICOP 2010 acceptable range/limit.

Parameters		ICOP 2010 acceptable limit/range	Occupancy					
			Occupied			Non-Occupied		
			SX (urban)	SY (rural)	p- value	SX (urban)	SY (rural)	*p*- value
Temperature (˚C)		23-26	**34.9 ±3.9**	**30.7 ±0.2**	0.507	**32.8 ±0.7**	**30.6 ±0.5**	0.275
Relative Humidity (%)		40-70	51.9 ±15.1	**77.1 ±2.3**	**0.001*	60.2 ±3.4	**70.1 ±0.1**	0.127
$PM_{2.5}$ (µg/m^3)		NA	22.89 ±12.16	18.57 ±2.79	0.827	20.56 ±0.82	12.75 ±2.73	**0.050*
PM_1 (µg/m^3)		NA	5.39 ±2.96	6.17 ±1.60	0.513	10.34 ±0.94	6.28 ± 1.27	0.127
CO_2 (ppm)		1000	429 ±222	370±62	0.701	518 ±28	457 ±12	0.127
Microbial Counts (CFU/m^3)	TBC	500	**558 ±308**	**903 ±415**	0.275	97.78 ±1.11	**1176 ±304**	**0.046*
	TFC	1000	443±44	874±131	*0.035	105±28.67	402±83	*0.028

Table 2: Identification of bacteria species at SX.

Selected bacteria	Accession number	Closest BLAST match	Similarity (%)	Note
T1IG	JX010961.2	*Bacillus sp.*	100	Ex. Id
T1II	JQ653305.2	*Bacillus sp.*	100	Ex. Id
T1OC	KT720102.1	*Bacillus cereus*	100	Ex. Id
T1IF	KT720302.1	*Bacillus licheniformis*	100	Ex. Id
T2IA	HQ677210.1	*Bacillus sp.*	98	Ex. Id
T2IH	JQ795867.1	*Staphylococcus sp.*	99	Ex. Id
T2II	KT720199.1	*Bacillus sp.*	100	Ex. Id
T2OC	KT029134.1	*Staphylococcus sp.*	100	Ex. Id

Ex. Id = Excellent Identification

Table 3: Identification of bacteria species at SY

Selected bacteria	Accession number	Closest BLAST match	Similarity (%)	Note
U1IA	KU182829.1	*Bacillus sp.*	100	Ex. Id
U1II	KT720291.1	*Bacillus sp.*	100	Ex. Id
U1OF	KT719731.1	*Bacillus cereus*	100	Ex. Id
U2OB	LC099946.1	*Bacillus sp.*	100	Ex. Id
U3OI	KU168426.1	*Bacillus sp.*	100	Ex. Id
U2IK	KT803100.1	*Bacillus sp.*	100	Ex. Id
U2OF	KU167713.1	*Bacillus sp.*	100	Ex. Id
U3IC	KT026096.1	*Staphylococcus sp.*	100	Ex. Id

Ex. Id = Excellent Identification

In SY, there were four distinct species of *Bacillus sp*. were identified (*B. altitudinis*, *B. aerophilus*, *B. tequilensis*, and *B. subtilis)*. For both of *B. altitudinis* and *B. aerophilus*, their pathogenicity activities on human were still undiscovered (Shivaji *et al.*, 2006). Meanwhile, 16S rRNA gene analysis revealed that *B. tequilensis* was 99% similar to *B. subtilis* (Gatson *et al.*, 2006). *B. subtilis* could be isolated from

decomposing plant material, soil, water and air. These bacteria were capable to cause dermal allergic and eye irritation by producing enzyme subtilisin. Overall, infections of *B. subtilis* on human were rare and *B. tequilensis* infections on human were under reported (Khusro *et al.*, 2013).

B. cereus were isolated in both SX and SY. These bacteria were abundant in nature and could be found in soil and dried foods such as starches, grains, and legumes (Rusul and Yaacob, 1995). *B. cereus* were toxigenic bacteria and could cause food poisoning. Besides that, *B. cereus* could easily contaminate bread, dairy products, cakes, and also seafood. These bacteria also could contaminate *nasi lemak*, *nasi beriani*, and *nasi putih* (Sandra *et al.*, 2012). Improper preparation and cooking of food could encourage the presence of *B. cereus*.in the food. It has been reported that chicken soup and spices had contributed to contamination of *nasi ayam* with *B. cereus* (Rusul and Yaacob, 1995; te Giffel *et al.*, 1996). In Malaysia, food poisoning cases related to these bacteria were reported in Klang, Selangor, where 114 of hostels students experienced abdominal pain, nausea, and vomiting (Rampal *et al.*, 1984).

Bacillus licheniformis was identified only in SX. These bacteria could be isolated from soil and bird feathers. These species were known to cause contamination in cooked meats, dairy products, and vegetables (Lund, 1990; Rosenkvist and Hansen, 1995).

S. warneri and *S. sciuri* were two of *Staphylococcus sp*. isolated in SX. *S. sciuri* could be isolated from animal-originated food products, pets, farm animals, soil, and water. These bacteria were closely associated with animal, but its clinical relevance to people were increased (Hauschild and Schwarz, 2003; Stepanović *et al.*, 2005). *S. sciuri* could cause urinary tract infections, wound infections, pelvic inflammatory disease, endocarditis, and septic shock (Chen *et al.*, 2007). Meanwhile, *S. warneri* could infect oral regions, nasal cavities, as well as on human and animal skins. Infections of these bacteria could cause skin, eyes, and urinary tract infections. Severe infections could lead to immunosuppression and bacteraemia (Incani *et al.*, 2010). BLAST result revealed that *S. haemolyticus* and *S. gallinarum* were the closest *Staphylococcus sp*. in SY. *S. haemolyticus* were involved in some of human and animal diseases. These bacteria had high resistivity towards various antibiotics (Schaberg *et al.*, 1991; Kloos and Bannerman, 1994). On the other hand, *S. gallinarum* were closely associated with chicken as it could be isolated from poultry (Devriese *et al.*, 1983).

Identification of isolated fungi in selected schools.

48 fungi colonies were isolated from SX, while 55 fungi colonies were isolated from SY. *Aspergillus* spp., *Cladosporium* spp., *Penicillium* spp., *Alternaria* spp., *Rhizopus* spp., and *Mucor* spp. were found in both schools. In SX, *Aspergillus* spp. were found predominant, followed by *Penicillium* spp., *Cladosporium* spp., with 27.08%, 20.83%, 18.75%, out of 48 isolated fungi colonies, respectively. In SY, the highest fungi genera iolated from the samples were *Mucor* spp. followed by *Aspergillus* spp. and *Penicillium* spp. with 27.27%, 21.81%, and 16.36% out 55 fungi colonies, respectively. The fungi genera found in this study were almost similar to another study in Malaysia. Hussin *et al, (*2011), revealed that *Aspergillus*, *Penicillium*, *Fusarium*, and *Rhizopus* were the most frequent isolated fungi genera isolated in primary schools. Another study also reported that, *Cladosporium, Penicillium,* and *Aspergillus* were the most common fungi genera isolated from indoor environment (Shelton *et al.*, 2002).

Penicillium could be found in various environment, namely soil, plants, air, indoors, and also food products. *Aspergillus* and *Penicillium* had the ability to grow on painted surfaces (Lugauskas *et al.*, 2003). Since classrooms' wall of SX and SY were painted, these fungi genera were found abundant in both schools. *Penicillium* was an opportunistic pathogenic fungus that infected people with human immunodeficiency virus (HIV), lead to fatal systemic mycosis (Hu *et al.*, 2013).

Meanwhile, *Mucor* also could be found in soil, plants, manure, air, vegetables, and decaying fruits (Samson *et al.*, 2000). SY was surrounded by many plants, that could contribute to high percentage of this fungi in rural classroom. *Mucor* caused mucormycosis, which a rare and fatal infections if not treated at an early stage. Sinuses and respiratory airways were common infected area. The infections also could spread throughout other body parts. Sinus infections were followed by headache, sinus pain, and fever. While infections on skin could resulted in excessive redness, pain, increase of body temperature, or swelling around a wound (Ribes *et al.*, 2000).

Cladosporium were among the most common fungi species that could be isolated from air. The fungi also could reside on wallpaper and carpet, especially, when water was present. Infections on human mainly

involved eye, skin, sinus, and brain. *Cladosporium* also could trigger allergic reactions and asthma on susceptible person (Samson *et al.*, 2000).

Alternaria were found abundant in outdoor environment during summer period. These fungi also could grow on ceiling tiles and fibre glass insulations. *Alternaria* could trigger asthma attacks, while could infect respiratory airways among HIV patients (Kobayashi *et al.*, 2009).

On the other hand, *Rhizopus* were more associated with cosmopolitan environment and were found originated from various sources such as rotten fruits and vegetables, bread, soil, and animal faeces. *Rhizopus* could cause zygomycosis, where the fungi infected subcutaneous tissue. People with deficiency or suppressed immune system were at high risk of getting infections that could be severe and fatal (Samson *et al.*, 2000; Ribes *et al.*, 2000).

Conclusion

To conclude, temperature, RH, and Bacterial CFU count in urban and rural school has topped ICOP 2010 limit. It has become a concern especially during occupied period where student tend to start losing focus and concentration due to thermal comfort effects. Besides, identified bacteria such as *B. subtilis, B. pumilus B. Cereus, S. warneri*, and *S. sciuri,* were known pathogen to human being. Besides that, *Aspergillus* spp., *Mucor* spp., and *Cladosporium* spp., were known to be associated with adverse health effects to human. Others fungi genera found in this study such as *Alternaria* spp., *Penicillium* spp., *and Rhizopus* spp., have high tendency to infect people with weakened immune system. Therefore, school compound must always be sanitized in order to prevent pathogenic microbial infections. Regular cleaning in the classroom also must be done to provide clean and conducive indoor environment for students to learn. The results of this study contains elementary data that crucial for the upcoming studies in the future. The results from this study can also assist in developing guidelines and policies for IAQ in school buildings.

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