Profile of Bioaerosol Contaminants in Primary School Classrooms: Systematic Literature Review 2016 - 2020

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Abstract

People spend plenty of time in indoor environments, thus the exposure to indoor air pollutants contributes greatly to one's health, well-being and productivity. Bioaerosol is one of the main contaminants, especially in primary schools, as children are less likely to wash their hands, perform protected coughing and sneezing, and they are more likely to make indirect physical contact with each other; such as exchanging belongings, which may promote the spread of infectious diseases and can ultimately affect their academics. This study aims to evaluate the level of bioaerosol contamination in primary schools and to investigate risk factors associated with its exposure.

The methodology is a detailed systematic literature review that evaluates available data regarding bioaerosol contaminants in primary school classrooms between the year 2016-2020. Google Scholar and Portal Garuda were used as the main databases. 16 literatures were selected; 11 measured bacteria, 10 measured fungi and 1 measured virus, active or passive were employed for the bioaerosol sampling strategy. It was found that in subtropical countries, the average colony count of bacteria, fungi and viruses are higher compared to tropical countries. Factors such as those related to the weather, the building itself as well as its occupants, are associated with bioaerosol contaminations at school. More studies are needed to describe bioaerosol levels at classrooms.

Keywords
Bioaerosol, Primary School Classrooms, Types of Contaminant, Contaminant Concentration, Contaminant Factors

1. Introduction
Air quality where individuals often spend most of their time contributes to someone's health (WHO, 2010). IAQ parameters are divided into physical, chemical, and biological parameters. Bioaerosol (biological aerosol) contributes about 5% to 34% of air pollution in the indoor environment of public facilities (Mandal and Brandl, 2011). Bioaerosol contaminants include bacteria, bacterial endotoxins (cell membranes of gram-negative bacterial) fungi, toxins, mycotoxins, glucans, pollens, viruses, allergens, and antigens (molecules that induces an immune response (Rogoff, 2014).

Exposure to bioaerosol contaminants can cause health problems such as allergies and infections; infectious diseases such as pneumonia and measles; respiratory disorders such as asthma, tuberculosis, COVID-19; to lung cancer (McLean *et al*., 2004) or pancreatic/liver cancer (Felini *et al*., 2011). Therefore, an assessment of the microbiological quality of indoor air from a public health point of view in various regions around the world is required, especially to protect vulnerable groups such as children.

Children are more vulnerable to environmental exposure than adults as they breathe more air, eat more food and drink more fluids than adults proportionate to their body weight (WHO, 2006). In general, children spend a lot of time at their school, especially in classrooms. The school environment itself differs massively to an adult's work environment because children tend to have distinct habits such as unprotected coughing and sneezing. Furthermore, children are less likely to wash their hands and are more likely to exchange items between each other (e.g., stationery), which promotes the spread of infectious diseases (Chithra and Nagendra, 2018).

Several studies have indicated that bacterial and fungal aerosol exposure in indoor environments are associated with non-communicable diseases, such as allergies, respiratory and immunotoxin diseases (Cavaleiro-Rufo *et al*., 2017). Simons *et al*. (2010) found that students' performance and absenteeism are impacted by their exposure to poor air quality in schools.

Prevention and control measures on poor IAQ, especially on bioaerosol contaminants, is required to create a good primary school environment with an IAQ that can make individuals comfortable and healthy. However, before prevention and control is carried out, it is necessary to identify present bioaerosol contaminants in primary school classrooms. Hence, his systematic literature review study will discuss the types, concentrations and factors of bioaerosol contaminants in primary school classrooms according to literature published between 2016 to 2020.

2. Material and Methods

This study is a Systematic Literature Review (SLR), which identifies, assesses, and interprets research findings about bioaerosol at schools, that was published between 2016-2020. The systematic literature review in this study follows the PERSPERO research protocol.

Each literature goes through five stages which include the screening based on the year of publication and language, screening by the title and abstract, screening by the result, selection based on the accessibility and completeness of articles, and selection based on the eligibility.

The inclusion criteria on finding the literature are that the literature must be available in the database of Google Scholar and Portal Garuda (*Garba Rujukan Digital*), be in either English or Bahasa Indonesia, was published between 2016 – 2020, and must answer study questions regarding the description of bioaerosol contaminants in the form of types, concentration on sampling results and factors that could affect the concentration of bioaerosol contaminants in primary school classrooms. After the literatures are selected, data from the literature will be processed by identification, extraction, synthesis and interpretation using the software Microsoft Word and Microsoft Excel in which the results will be presented in narrative form, figures and tables.

3. Data Collection

From the searching that have been carried out using keywords and databases, 62,135 literatures were found which were then filtered based on inclusion criteria until 16 literatures were selected with the results of a systematic literature review of the description of bioaerosol contaminants in primary school classrooms in the form of: types of bioaerosol contaminants, concentrations of bioaerosol contaminants and factors that influence the concentration of bioaerosol contaminants (**Table 1**). The steps are `as follows (**Figure 1**):

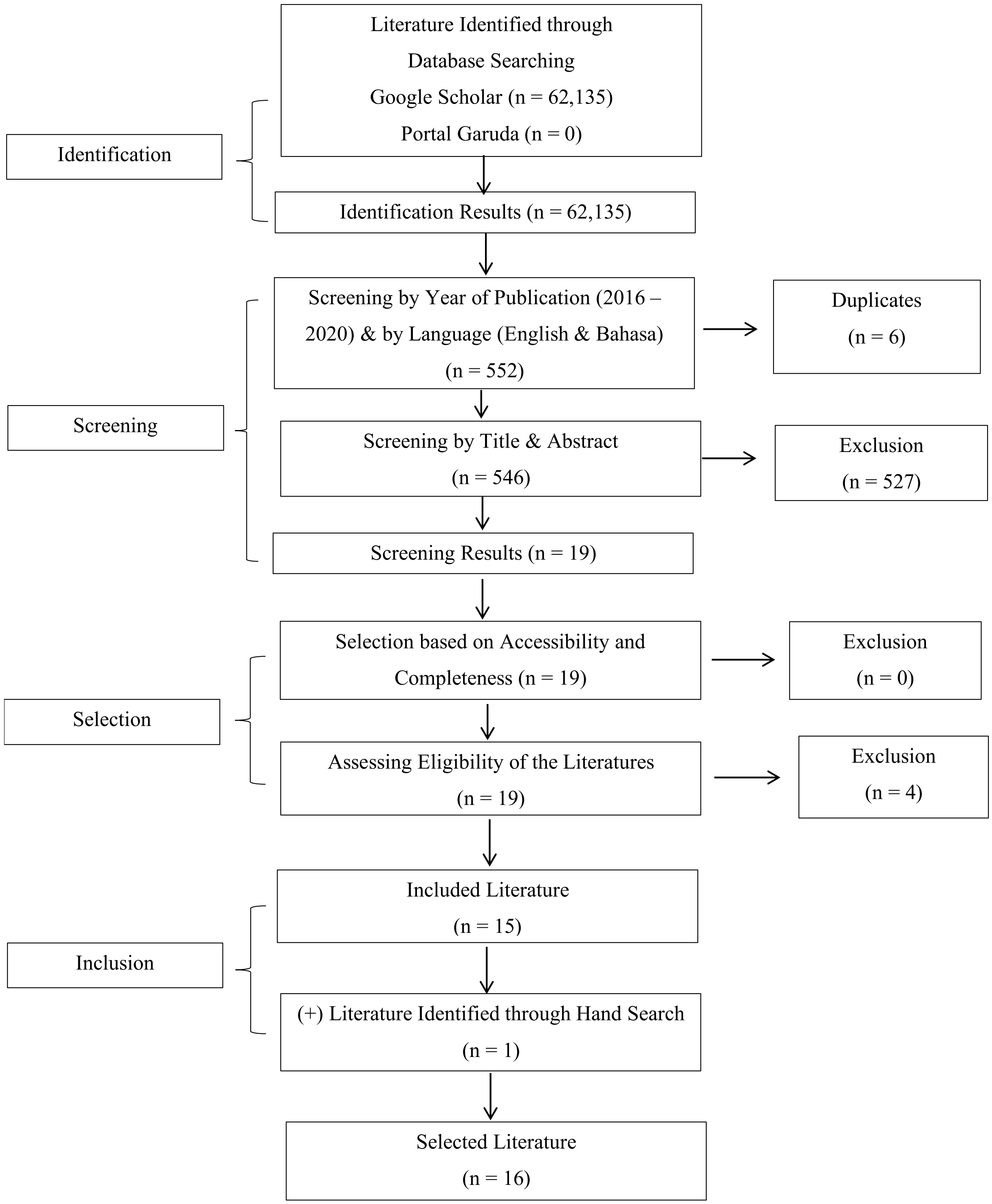

Figure 1. PRISMA Flow Chart

Table 1. List of Codes for the 16 Reviewed Literatures

Code	Reviewed Literatures
A1	*Air microbial quality in certain public buildings, Egypt: A comparative study* by Abdel Hameed Awad, Yuosra Saeed, Yasser Hassan, Yousef Fawzy and Mohamed Osman (2017)
A2	*Airborne Influenza A Virus Exposure in an Elementary School* by Kristen K. Coleman and William V. Sigler (2020)
A3	*Assessment of Airborne Particles in Indoor Environments: Applicability of Particle Counting for Prediction of Bioaerosol Concentrations* by Seyed Hamed Mirhoseini, Mahnaz Nikaeen, Kazuo Satoh and Koichi Makimura (2016)
A4	*Assessment of Bacterial Aerosol in a Preschool, Primary School and High School in Poland* by Ewa Bragoszewska, Anna Mainka, Józef S. Pastuszka, Katarzyna Lizończyk and Yitages Getachew Desta (2018)
A5	*Association between fungal spore exposure in inner-city schools and asthma morbidity* by Sachin N. Baxi, William J. Sheehan, Joanne E. Sordillo, Jonatahan M. Gaffin, Perdita Permaul, Peggy S. Lai, Margee Louisias, Carter R. Petty, CHunxia Fu, Diane R. Gold and Wanda Phipatanakul (2019)
A6	*Concentration and size distribution of biological particles in school classrooms* by Mona Moustafa (2017)
A7	*Faktor-faktor yang berhubungan dengan Keberadaan Bakteri Udara di Ruang Kelas* (Factors that are related with the existence of bacteria in classrooms) by Vita Wiana Budi Cahya (2016)
A8	*Identification and characterization of air bacteria from some school of Baghdad city* by Rana M. Badri, Rana R. Alani and Sura Sami Hassan (2016)
A9	*Impact of a green roof system on indoor fungal aerosol in a primary school in Greece* by Ioanna Pyrri, Artemis Zoma, Nikolaos Barmparesos, Margarita Niki Assimakopoulos, Vasikili D. Assimakopoulos, and Evangelia Kapsanaki-Gotsi (2020)
A10	*Indoor bacterial load and its correlation to physical indoor air quality parameters in public primary schools* by Zewudu Andualem, Zemichael Gizaw, Laekemariam Bogale and Henok Dagne (2019)
A11	*Indoor Culturable Fungal Load and Associated Factors among Public Primary School Classrooms in Gondar City, Northwest Ethiopia, 2018: A Cross-sectional Study* by Zewudu Andualem, Zemichael Gizaw and Henok Dagne (2019)
A12	*Indoor exposure to bioaerosol particles: levels and implications for inhalation dose rates in schoolchildren* by Joana Madureira, Lívia Aguiar, Cristiana Pereira, Ana Mendes, Micaela Machado Querido, Palua Neves, and João Paulo Teixeira (2018)
A13	*Influence of indoor conditions on microbial diversity and quantity in schools* by Dahae Seong, R. Sean Norman and Shamia Hoque (2019)
A14	*Microbiological assessment of indoor air quality of some selected private primary schools in Ilishan-Remo, Ogun State, Nigeria* by Enitan SS, Ihongbe JC, Ochei JO, Effedua HI, Adeyemi O and Phillips T (2017)
A15	*Monitoring of fungal aerosols in some educational buildings from Iaşi, Romania* by Florin Daniel Lişpa, Eugen Ulea and Irina Paraschiva Chiriac (2016)
A16	*Occupancy implications on indoor air quality (IAQ) in selected primary school classrooms around Kuantan*, Pahang by Hazrin, Maryam, Hizrri, Norhidayah, Samsudin and Mohd Shukri (2017)

4. Results and Discussion

4.1 Types of Bioaerosol Contaminants

A. Bacteria

Bacteria are unicellular organisms that are very small, nucleoid, do not have a nuclear membrane and chlorophyll, are saprophytes/parasites, reproduce by binary fission and are included in prokaryotic protists (Harti, 2005). In general, bacteria often come from gram-positive stale spores or non-spores, gram-negative bacilli and gram-positive cocci (Lowy, 2008).

Not all bacteria which exist in the air present a bad impact on human health. Some bacteria are even important for the human body and the environment. Health risks arise when the concentration of some species become excessively high. For bacteria, health effects from bacteria exposure depend on the bacteria's species, human susceptibility, the produced metabolic products, as well as the concentration and duration of exposure. The main health effects associated with exposure to bacteria are hypersensitivity reactions (allergies), infection and irritation (Stetzenbatch *et al.,* 2004).

The types of bacteria found in this literature review based on their respective gram stains were gram-negative bacteria, gram-negative rods, gram-positive bacilli, gram-positive bacteria, gram-positive cocci, gram-positive rods forming endospores, and non-sporing gram-positive rods. While the types based on the genus or species found were *Alloiococcus otitis; Aeorcoccus; Arthrobacter citreus, globiformis, oxydans; Bacillus atrophaeus, cereus, ciculans, licheniformis, mycoides, subtilis, pseudomycoides, pumilus; Brevibacterium; Coagulation-Negative Staphylococcus* (CoNS); *Corynebacterium auris, propinquum, tuberculostearicum; Curtobacterium; Kocuria carniphila, rosea; Macrococcus caseolyticus; Micrococcus eruption; Mycobacterium; Rothia nasimurium; Staphylococcus arlettae, aurens, chromogens, equorum, epidermis, hominis, lentus, sciuri, species, warneri; Streptomyces; Paenibacillus amylolyticus, glucanolyticus;* and *Pseudomonas*.

Most bacteria that were found in 11 literatures include *Bacillus*, *Micrococcus*, and *Staphylococcus* species. The A4 literature cites that *Staphylococcus*, *Micrococcus*, *Bacillus*, and *Kocuria* are the most common types of bacteria found in the indoor environments (Górny and Dutkiewicz, 2002). Arthrobacter bacterial species have also been found in several indoor environments. The A1 literature found that the bacterial species *Bacillus* was commonly found in dust accumulation at almost all locations.

B. Fungi

Fungi is a kingdom of multicellular eukaryotic organisms that are unable to produce their own food and can potentially cause health problems by producing allergens and irritants. A mould is the general name for a group of visible fungi that grow as filaments or multicellular hyphae, which then combine into structures or mycelia.

Fungi can be found everywhere and represents a significant threat to public health in indoor environments (Samet and Spengler, 2003). They are capable to grow on natural and synthetic materials, especially absorb on wet and inorganic materials. Fungi absorbs impurities in the moist material and uses it as their growth substrate.

The types of fungi that were found in these reviewed literatures are *Acremonium; Alternaria; Aspergillus flavus, fumigatus, niger, and ochraceus; Aureobasidium; Basidiospores small hyaline; Botrytis / Botryotinia; Candida; Cladosporium; Curvularia; Fusarium; Geotrichum; Microsporum; Mucor; Penicillium; Rhizopus; Sphaeropsidales; Stemphylium; Sterile hyphae; Thielaviopsis; Trichoderma; Trichophyton; Verticillium*; and yeast.

According to a study by Zukiewicz-Sobczak (2013), around 2-6% of the total population in developed countries are allergic to fungi and are especially more susceptible to the genus *Alternaria, Cladosporium, Aspergillus, Penicillium* and *Fusarium* (Żukiewicz-Sobczak, 2013).

The species of fungi that are mostly found in the reviewed literatures are *Alternaria, Aspergillus, Cladosporium, Penicillium* and yeast. This finding is supported by the A3 literature which states that *Aspergillus, Penicillium, Cladosporium* and *Alternaria* can grow in different habitats by various ways, which makes these species more dominant than other fungi species (Sharma, 2011).

C. Viruses

Viruses are infectious agents that usually consist of nucleic acid molecules in a protein layer, are too small to be seen with a microscope, and can only multiply within the host's living cells (Oxford Dictionary-Lexico). From 16 literatures that were reviewed, there was 1 literature that examined the Influenza A Virus (IAV).

IAV is divided into several subtypes based on two proteins on the viral surface: *hemagglutinin* (H) and *neuraminidase* (N). There are 18 different hemagglutinin subtypes and 11 different neuraminidase subtypes (H1 to H18, N1 to N11).

Despite the potential for 198 different combinations of influenza A subtypes, there are only 131 detected subtypes in nature. The most recent influenza A virus subtypes that spread in humans are: A (H1N1) and A (H3N2). Influenza A subtype can be divided into various genetic, which are "clades" and "subclades" (CDC, 2019).

4.2 Bioaerosol Contaminants Concentrations

The results of bioaerosol contaminant concentration sampling from the reviewed literatures are presented based on the literatures' country of origin's respective climate (**Table 2**).

Table 2. Bioaerosol Contaminants Concentrations based on Research's Country

Bioaerosol Types		Country	Concentration Results*	Regulation Standard (ACGIH, 1995)
Bacteria	A1	Egypt	1687.5 CFU / m^3	High
	A3	Iran	430 CFU / m^3	Medium
	A4	Poland	2205 CFU / m^3	Medium
	A6	Egypt	955 CFU / m^3	Medium
	A7	Indonesia	67 CFU / m^3	Low
	A8	Iraq	147.3 CFU / m^3	Medium
	A10	Ethiopia	3186.5 CFU / m^3	High
	A12	Portugal	4775 CFU / m^3	High
	A13	USA	293 CFU / m^3	Medium
	A14	Nigeria	4265.3 CFU / m^3	High
	A16	Malaysia	507.5 CFU / m^3	Medium
Total	11	Total	Subtropic: 8 Studies	Tropic: 3 Studies
Fungi	A1	Egypt	950 CFU / m^3	Medium
	A3	Iran	187 CFU / m^3	Medium
	A5	USA	316.9 spora / m^3	Medium
	A6	Egypt	235 CFU / m^3	Medium
	A9	Greek	225 CFU / m^3	Medium
	A11	Ethiopia	620.8 CFU / m^3	Medium
	A12	Portugal	1102.2 CFU / m^3	High
	A14	Nigeria	178.8 CFU / m^3	Medium
	A15	Romania	149.6 CFU / m^3	Medium
	A16	Malaysia	491.6 CFU / m^3	Medium
Total	10	Total	Subtropic: 7 Studies	Tropic: 3 Studies
Virus	A3	USA	1900 (M gene copies / m^3 air)	-
Total	1	Total	Subtropic: 1 Studies	Tropic: -

*The total average of bioaerosol concentration levelled based on the quantitative standard by American Governmental Industrial Hygienists (ACGIH, 1995) are: low (<100 CFU / m^3), medium (100-1000 CFU / m^3) and high (>1000 CFU / m^3).

The result of bioaerosol contaminant concentration from each research's countries (**Table 2**) can be divided into two categories, subtropical countries and tropical countries (**Table 3**).

Table 3. Concentration of Bioaerosol Contaminants based on Subtropical and Tropical Countries

Bioaerosol Types	Concentration Results			
	Subtropical Countries	Level of Concentrations*	Tropical Countries	Level of Concentrations*
Bacteria (CFU / m^3)	1709.91	High	1613.27	High
Fungi (CFU / m^3)	519.55	Medium	273.33	Low
Virus (M gene copies / m^3 air)	1900	-	-	-

There are no data on significant correlation between subtropical or tropical climate zones and the bioaerosol concentration explained in the papers under study. obtained by the researchers. However, it was found that bioaerosol concentration in subtropical differs from tropical countries, and may be influenced by many factors, such as temperature, humidity, use of the MVAC system and primary school pupils' activities in certain seasons. This statement is supported by the A1 which states that the concentration of bioaerosol in the air varies without a clear distribution pattern in relation to the season.

In naturally ventilated buildings, particles generally move freely, and the number of microbes can increase during winter. Higher concentrations of fungi in spring and autumn are caused by the suitability of temperature and relative humidity that increases microbiological activity in the spring period. Deciduous plants and suspended particles are an important medium for bioaerosols to grow in the fall.

Bioaerosol concentrations can also be classified based on its continents (**Figure 2**). Different geographical locations can affect the temperature and relative humidity of an area. Apart from temperature and humidity, other factors such as the behaviours or culture of each area can also be a factor that correlates with the concentration of bioaerosol contaminants in the classroom.

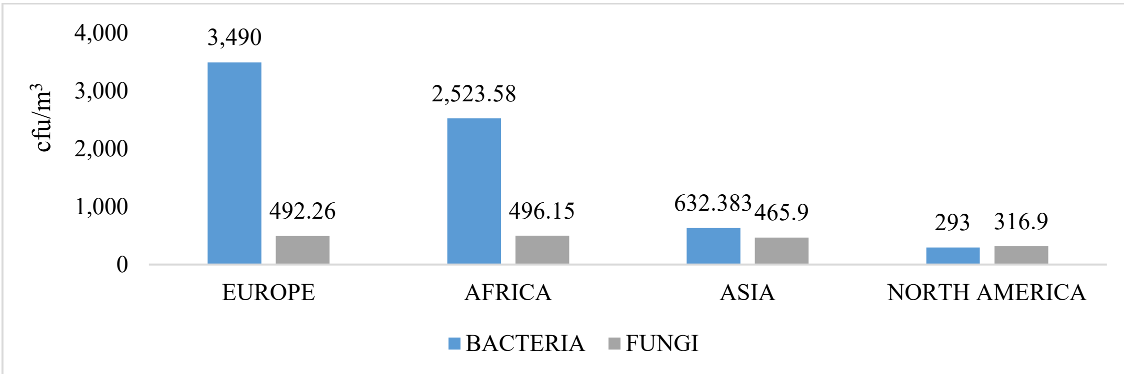

Figure 2. Bioaerosol Concentrations (bacteria and fungi) Classified by Continent

In this study, he highest concentrations of bacteria and fungi were found in Portugal (literature A12) with concentration reaching 4,775 CFU/m^3 for bacteria and 1,102.2 CFU/m^3 for fungi. While the lowest concentration of bacteria and fungi were in Indonesia (A7) for bacteria, that is 67 CFU/m^3 and the A3 literature for fungi in Iran, which was 187 CFU/m^3.

4.3 Factors or Elements affecting Bioaerosol Contaminants

Table 4 shows factors that are associated with bioaerosol contaminant concentration in the classroom that were obtained from 16 literatures. Those factors are temperature, humidity, classroom occupants, MVAC system, source of pollutants, classroom's structure, demographic state, seasons, class sanitation, building's age and the furniture inside the classrooms.

Table 4. Factors affecting Bioaerosol Contaminants Concentrations

No.	**Factors affecting Bioaerosols**										
	Temperature	Humidity	Classroom Occupants	MVAC System	Source of Pollutants**	Classroom's Structure	Demographic State	Seasons	Class Sanitation	Building's Age	Class' Furniture
A1	√	√	√	√	√	√	√				
A2	√	√	√								
A3	√	√	√	√	√						
A4	√	√	√	√		√	√			√	√
A5			√								
A6	√	√	√			√					
A7	√	√							√		
A8			√								
A9				√	√	√	√	√			
A10	√	√		√	√						
A11	√	√	√	√	√	√			√	√	
A12	√	√	√		√			√			
A13	√	√	√			√	√				
A14	√	√	√	√				√	√		
A15	√	√		√	√		√	√	√		
A16			√	√			√	√		√	√
Total	**12** 15.4%	**12** 15.4%	**12** 15.4%	**9** 11.5%	**7** 9%	**6** 7.7%	**6** 7.7%	**5** 6.4%	**4** 5.1%	**3** 3.8%	**2** 3.6%

*Primary school pupils refer to classroom occupants: class density and pupils' activity

** Sources of pollutant include CO_2, PM, emissions and other sources of pollutants

The following paragraphs explain three factors which are mostly found in the reviewed literatures:

A. Temperature

There are 12 literatures which states that temperature is a factor that influences the concentration of bioaerosols in primary school classrooms. Temperature is an important environmental factor for the continuity of bioaerosol contaminants as it affects enzyme activity. Temperature that is too low can cause enzyme activity to decrease, while temperature that is too high can denature the enzyme protein (Wiana and Cahya, 2016).

The main sources of temperature are from the weather, building occupants, building equipment and the MVAC system. The impact of an unsuitable temperature affects the comfort and productivity of building occupants, for example, temperature is too high may cause heat stress.

A total of 14 literature states that temperature has a significant correlation with the concentration of bioaerosols. Three literatures, A1, A2, and A10 states that temperature has a negative effect on bacterial growth. This implies that, as the temperature decreases, the number of bacterial colonies will increase, and vice versa. Fungi has a negative correlation with temperature according to literature A11 and A12. Interestingly, the A3 literature stated that temperature had a positive effect on the growth of bacteria and fungi, but the correlation was weak.

B. Humidity

There are 12 literatures which states that humidity is one of the factors that influence the concentration of bioaerosols in primary school classrooms. Relative humidity is the ratio between the amount of water vapor in the air and the amount that can be accommodated at a certain temperature (Burroughs and Hansen, 2008). Higher humidity allows more microbial colonies to grow in a room. On the other hand, the lower the level of relative humidity is in a room, the smaller the number of microbial colonies which can be found (Rachmatantri, 2015).

Relative humidity can be affected by humidity generated by the weather, building occupants and water sources. Humidity affects the growth of mould, the comfort and productivity of occupants and can cause damage to building furniture. Low humidity causes dry and itchy eyes and skin. Meanwhile, high humidity causes a sticky sensation which can lead to discomfort.

Relative humidity has a different correlation in each study. The A1 literature had a positive correlation with bacteria and fungi, as did the A7 and A12 that focused on bacteria. On the other hand, literature A10 found that relative humidity had negative relation with the growth of bacteria. Meanwhile, study A11 stated that the correlation on relative humidity and fungal growth was negative.

C. Classroom pupils / Occupants

There are 12 literatures which states that classroom occupants are one of the factors that influence the concentration of bioaerosols in primary school classrooms. Literature A16 stated that building occupants such as number of occupant/class density, student activities and profiles play an important role in influencing IAQ in the classroom.

Class density can be presented as the number of occupants or pupils in the classroom divided by size of the room. The results of the A1 literature stated that the highest concentration of bioaerosols was found in the class which was most crowded. This is supported by literature A11 which stated that a class with a narrower room encourages the growth of fungi. Literature A14 stated that bioaerosol concentration is affected by the number of pupils in the class. For fungal species, literature A5 states that Alternaria is more often found in lower numbers and in fewer classrooms than Cladosporium/Penicillium/Aspergillus in unoccupied classrooms.

In addition to the presence of pupils in the class, student activity affects the concentration of bioaerosol in the classroom. The level of activity discussed in research A4 is sedentary/passive activity such as sitting, light intensity activity, medium intensity activity, and high intensity activity.

The A2 literature did not discuss activities in the classroom, but it was found that activities in the gymnasium had an impact on the virus concentration, because in the morning, the gymnasium was used for the subject of physical exercise by three alternating classes, while the same room was also used for lunch together during the day. Literature A6 states that bioaerosol growth is influenced by pupils' activity, this statement is supported by literature A12 and A8.

Pupils' profiles in the form of age and gender affects the concentration of bioaerosols in the classroom. In literature A13, the concentration of *Bacillus, Aerococcus, Corynebacterium* and *Penicillium* were influenced by the gender of the student. Lastly, the age of the building occupants has an impact on the concentration of bioaerosols as this research was conducted in the classroom of primary school children.

5. Conclusion

Sixteen literatures were selected in this study. All studies measured bioaerosol concentration by performing air sampling and then followed by laboratory analysis. Types of bioaerosol contaminants that were found in the reviewed literatures include: bacteria (11 studies), fungi (10 studies), and viruses (1 study). Although the number of literatures was limited, it was found that the average total concentration of contaminants in subtropical countries were higher than in tropical countries. The most dominant factors affecting the concentration of bioaerosol contaminants were temperature; humidity; 15.4% occupants or class pupils; and 11.5% MVAC system. The author suggests further research on bioaerosol in indoor air primary school classrooms. By understanding the exposure and risk factors associated with bioaerosol exposure, classroom air quality can be properly managed.

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