Concentration and size distribution of biological particles in school classrooms

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ABSTRACT
Fungal and bacterial aerosol particles concentrations are measured in a school classrooms at two different floors using the 6-stage Andersen impactor as a bioaerosol sampler. The average bacterial concentration is higher than the average fungal concentration. The concentrations were 957 and 955 cfu/m³ for bacterial particles at first and second floors, respectively while the fungal particles concentrations were 146 and 235 cfu/m³ at first and second floors, respectively. Most of the biological particles were concentrated at the size range of respirable particles (< 5 µm ) that can penetrate into the alveoli and may cause lung diseases. The human activity is a main factor for the production of microbiological particles. Environmental factors play also a role on the fungal growth. Bacterial concentration is almost twice the guide value of WHO while the fungal concentration is underestimation.

Keywords
bacterial particles; fungal particles; human activity; respirable particles; environmental factors; size distribution

INTRODUCTION
Biological particles, including all airborne microorganism, are emitted from almost any natural or anthropogenic surfaces. Humans significantly impact the composition and concentration of microbial aerosols in indoor air [1]. Human activities is an important source of indoor bioaerosols [1]. Animals, flowerpots and wastebaskets are also other sources for the emission of bioaerosols [2]. Poor indoor air quality has been shown to cause several health hazard. Recently, indoor air quality in workplace has received great attention [3]. Humans spend most of their time indoors where biological particles can impact on public health [4]. Exposure to biological particles containing airborne microorganism and their by-products can result in different lung diseases [5]. Studies have been done in schools and children environments reveal a negative health effects on children [6,7]. In many indoor environments bacterial and fungal aerosol particles may lie in the respiratory size range (<10 µm) [8]. From my previous study there is a positive correlation between the deposition of microorganisms in the human lung and some respiratory diseases [9]. There are several sampling techniques for collecting biological particles. One of the most commonly used is the Andersen impactor. The collected biological particles are sized aerodynamically and can be directly related to human lung deposition. The concentration and size distribution of the particles are vital parameters in the deposition lung models. Therefore the objective of this study is to investigate the concentration and size distribution of bacteria and fungi in a school classrooms using Andersen impactor. The occupants are students with different ages.

MATERIALS AND METHODS
The study was performed in class rooms of primary school at first and second floors during the teaching time where the occupants are adult female students and children with age from 5 to 7 years old. The class rooms with an area of 17.5 m². The temperature ranged from 30 to 34 °C with an average value of 34 °C. The average relative humidity recorded 30% in first floor and 33% in the second floor. The conditions of the study sites are summarized in table 1. Sampling was performed under normal ventilation where all windows and door are open and there was a mechanical ventilation as well. Air sampling was taken by 6- stage Andersen impactor. The impactor operates at flow rate of 28.3 L/min. The collected particles are size fractionating according the aerodynamic cut-size diameters of the impactor (7.0, 4.7, 3.3, 2.1, 1.1 and 0.65 µm). The impactor was located in the center of the room. For collecting fungal particles, Sabourauds Dextros agar (SDA) was used. Nutrient Agar was used as a collecting media for bacterial particles. A volume not less than 27 ml of culture medium was placed in a removable glass Petri dish where plastic ones should not be used because the static charge generated reduces the collection efficiency. The Petri dish was inserted in the impactor. The sampling time ranged from 10 to 15 min for each run to avoid overestimation of the particle colonies. The samples were incubated at 37 °C for 24 to 48 hours. Colonies on each plate were counted. The concentration of biological particles was estimated as colony forming unit per cubic meter of air (cfu/m³) by:

\[ C = \frac{N}{V_x} \text{ cfu/m}^3 \]

Where:
C is the particles concentration
N is the number of colonies on each stage of the impactor

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$V$ is the impactor flow rate ($\text{m}^3/\text{h}$).

t is the sampling time (hour).

The parameters of the size distribution, Median Aerodynamic Diameter (MAD) and Geometric Standard Deviation (GSD) were given by the following equations [10].

$$\ln \text{MAD} = \frac{\sum n_i \ln d_i}{\sum n_i}$$

$$\ln(GSD) = \left(\frac{\sum n_i (\ln d_i - \ln \text{MAD})^2}{\sum n_i}\right)^{1/2}$$

Where MAD is the Median Diameter, $n_i$ is the fraction in stage $i$, $d_i$ is the cutoff diameter of the stage $i$ and GSD is the Geometric Standard Deviation. MAD is defined as the diameter at 50% cumulative fractions. GSD of the size distribution is defined as the diameter at 84% cumulative number divided by the diameter obtained at 50%.

During the sampling, temperature and relative humidity were recorded. The condition of the measurement is summarized in Table 1.

### Table 1. The condition of the measurement

| Study location | Area ($\text{m}^2$) | ventilation | Average Temp. ($^\circ\text{C}$) | Average relative humidity (%) | occupants          |
|----------------|---------------------|-------------|---------------------------------|-------------------------------|-------------------|
| First floor    | 17.5                | normal      | 34                              | 30                            | Adult females     |
| Second floor   | 17.5                | normal      | 34                              | 33                            | Children          |

### RESULTS AND DISCUSSION

Table 2 summarize the concentrations of measured biological particles. Concentration of bacterial particles (957 and 955 cfu/m$^3$) in first and second floors, respectively is higher than the concentration of fungal particles (146 and 235 cfu/m$^3$) in first and second floors, respectively. The human activities and environmental parameters (temperature and relative humidity) affect the airborne particles [11]. It was reported that the main source of bacteria is the humans while the main source of fungi is the outdoor environment [12].

Size distributions of airborne bacterial particles are shown in Fig. 1 and Fig. 2 for the measurements in the first and second floors, respectively. The concentrations of bacterial particles in the first floor are high at the size (1.1 µm) and also increased in the size range (3.3 – 7.0 µm). In the second floor, the concentration of bacterial particles reaches the maximum value in the size range (1.1 – 2.1 µm) and (4.7 – 7.0 µm).

Size distributions of airborne fungal particles are shown in Fig. 3 and Fig. 4 for the measurements in in first and second floors, respectively. The high concentrations of fungal particles are observed at the size (2.1 µm) in both floors.

### Table 2. Average number concentrations of bacterial and fungal particles

| Floor          | Number concentration (cfu/m$^3$) |
|----------------|----------------------------------|
|                | Bacteria | Fungi   |
| First floor    | 957       | 146     |
| Second floor   | 955       | 235     |

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Fig 1: Size distribution of bacterial particles in classroom on first floor

Fig 2: Size distribution of bacterial particles in classroom on second floor
Parameters of size distribution: median aerodynamic diameter ($d_{ae}$) and geometric standard deviation ($\sigma_g$) are listed in table 3. Median aerodynamic diameters of both microorganisms, bacteria and fungi are higher in second floor (2.69 µm for bacteria and 2.95 µm for fungi) than measured in the first floor (2.47 µm for bacteria and 2.77 for fungi). This may be attributed to the human activities. Hospodsky et al. ([12] found that the geometric mean diameter of microorganisms and particulate matter increases in occupancy environment as compared with vacant environment. These results also emphasized by the observation of Fox et al. [13] where a large size of bacteria was recorded with shed skin. Chunxiao et al. [14] found that the concentration of biological particles is much higher in the occupied period than unoccupied one in the classrooms.

It is observed that the dispersion of bacterial particles is higher than the dispersion of fungal particles where the geometric standard deviation of bacteria are 2.24 in first floor and 2.09 in second floor while the value of geometric standard deviation of fungi are 1.4 and 1.67 in first and second floor, respectively.
Table 3. Parameters of size distribution: median aerodynamic diameter ($d_{ae}$) and geometric standard deviation ($\sigma_g$)

| Floor          | Bacteria |   | Fungi |   |
|---------------|----------|---|-------|---|
|               | $d_{ae}$ | $\sigma_g$ | $d_{ae}$ | $\sigma_g$ |
| First floor   | 2.47     | 2.24 | 2.77  | 1.4 |
| Second floor  | 2.69     | 2.09 | 2.95  | 1.67|

The deposition of the aerosol particles in the human lung depends mainly on their size. In this result the high concentration of microbiological fungi and bacteria found were found in the respirable size (< 5 µm). This type of particles have the ability to pass into the alveoli and can cause lung diseases [15].

Table 4 summarize the comparison of the present average bacterial aerosols concentration with other studies. The average concentration of bacterial aerosols (956 cfu/m$^3$) exceeds the value reported by WHO [16] (500 cfu/m$^3$) and other studies by Lee et al. [17] and Mirhoseini et al. [18]. While the present results are lower than studies of Mentes et al. [19] and Pegas et al. [20].

Table 5 summarize the comparison of the present average fungal concentration with other studies. The average concentration of fungal aerosols (190 cfu/m$^3$) is lower than the value reported by WHO [16] (500 cfu/m$^3$) and other studies by studies by Mirhoseini et al. [18]. The present results lie in the range 103-1116 cfu/m$^3$ reported by Chao et al.; [21], Mentes et al. [19] and Bonetta et al. [22] while it is lower than the range (463-3125 cfu/m$^3$) reported by Hargreaves et al. [23] and Haas et al. [24].

Table 4. Comparison of the present average bacterial aerosols concentration with other studies

| Reference           | Bacterial concentration (cfu/m$^3$) | Sites               |
|---------------------|--------------------------------------|---------------------|
| Present work        | 956                                  | School classrooms   |
| WHO, 2002           | 500                                  | Guideline           |
| Mirhoseini et al., 2016 | 430                          | School classrooms   |
| Mirhoseini et al., 2016 | 395                          | University classrooms|
| Pegas et al., 2010  | 1284                                 | Schools             |
| Mentes et al., 2009 | 1251                                 | Kindergarten        |
| Mentes et al., 2009 | 1131                                 | Primary school      |
| Lee et al., 2006    | 505                                  | Homes               |
Table 5. Comparison of present average fungal aerosols concentration with other studies

| Reference | fungal concentration (cfu/m$^3$) | Sites               |
|-----------|----------------------------------|---------------------|
| Present work | 190                               | School classrooms |
| WHO, 2002  | 500                               | Guideline          |
| Mirhoseini et al., 2016 | 187                               | School classrooms |
| Mirhoseini et al., 2016  | 126                               | University classrooms |
| Chao et al., 2002; Mentes et al., 2009; Bonetta et al., 2010 | 103-1116              | Offices           |
| Hargreaves et al., 2003; Haas et al., 2007 | 463-3125              | Residence places  |

CONCLUSION

The average bacterial concentration is higher than the average fungal concentration. The concentrations were 957 and 955 cfu/m$^3$ for bacterial particles in the first and second floors, respectively while the fungal particles concentrations were 146 and 235 cfu/m$^3$ in the first and second floors, respectively. Most of the biological particles were concentrated at the size range of respirable particles (< 5 µm) that can penetrate into the alveoli and may cause lung diseases. The human activity and the environmental factors play an important role on the bioaerosol production. This study is a baseline for estimation the air quality in indoor air.

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