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Concentration and Size Distribution of Bacterial and Fungal Bioaerosols and the Related Exposure Dos

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IN this study, concentration and size distribution of bioaerosols (bacteria and fungi) were obtained in indoor at minimum ventilation and during the operation of air condition system (ACS). Indoo/Outdoor concentration ratio (I/O) of bacteria and fungi were also determined. In addition, the indoor exposure dose (IED) of bacteria and fungi were calculated. A six-stage Andersen cascade impactor was used for sampling the microorganisms during summer season at Minia University, El-Minia (Egypt). Mean bacterial and fungal concentrations were higher (727±160 and 252±60 CFU/m^3, respectively) at minimum ventilation than the concentrations during ACS (506±120 and 176±44 CFU/m^3, respectively). Indoor bacterial concentration level is three times higher than the fungal level. On the other hand, outdoor bacterial concentration (220±55 CFU/m^3) is lower than the outdoor fungal level (300±77 CFU/m^3). I/O concentration ratio of bacteria was greater than unity indicating that the main source of bacteria is the human occupancy while the corresponding I/O concentration ratio of fungi was less than unity indicating that the fungi are released from outdoor sources. Mean IED of bacteria (37.4 CFU/kg) was about three times higher than the IED of fungi (12.5 CFU/kg) .

Keywords : Human lung; Bioaerosols; Concentration; Exposure dose; Six-stage Andersen impactor.

Introduction

Bioaerosols are released from indoor and outdoor air including natural and anthropogenic sources [1, 2]. Indoor air is a predominant source of different microbes [3]. There are a complex mixture of microbes, fragments, byproducts, bacteria, fungi, endotoxin and volatile microbial organic compounds in indoor environment [4]. Human activities significantly effect on the composition and the concentration level of microbes in indoor air [5].

The composition of microbes of indoor air is influenced by both indoor and outdoor sources [6,7]. The outdoor air contribution depends on the ventilation of the building [8]. Microbial particles can occur in air as single cells or aggregates of cells and in fragments. They are often transported attached to other carrier particles (particulate matter, PM) such as skin flakes, soil, dust, saliva or water droplets forming a biological particles or bioaerosols [9,10].

The impact of bioaerosols on the human health depends on their biological properties as well as on their size and size distribution that determine the site of particle deposition in the respiratory tract [10]. Bacteria and fungi cells have a size range from 0.3 to 100 µm [11]. In many indoor environments, bacterial, fungal particles and their fragments fall in the inhalable size range (<5 µm) which can enter the lower parts of the human lung [2,12] and can cause different infectious diseases, allergic reactions and asthma [13-18]. People spend most of their time in different indoor environments [2,5,19-21]. The adverse health effects depends on the absorbed dose of air pollutants [22].

Therefore, the objectives of this study are i) to investigate the concentration level of bacteria and fungi in indoor air at two different ventilation conditions; at minimum and during the operation of air conditioning system. In addition in outdoor air, ii) to evaluate indoor/outdoor concentration ratio of bacteria and fungi (I/O ratio) and iii) to calculate the exposure dose due to the inhalation of these particles.

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Materials and Methods

Sampling site

Samples were taken from indoor air at Physics laboratory of Minia University (El-Minia/ Egypt) during a summer season. The lab is about 10 m above the ground level with a surface area of 50 m^2. Outdoor sampling were performed outside the building. Minia University_is located in the middle of El-Minia city at about 300 km from the Capital, Cairo (upper Egypt).

Bioaerosol sampling

Airborne bacteria and fungi were collected using Six-stage Andersen impactor (ACI) with a flow rate of 28.3 L/min. Andersen impactor fractionates the collected microorganisms according to their aerodynamic diameters. The cut-off diameters of the impactor are 0.65, 1.1, 2.1, 3.3, 4.7 and 7 µm. The stages of the impactor simulate the human respiratory tract. The collection efficiency of Andersen impactor was previously validated [23, 24].

Nutrient agar (NA) was used as a culture media for collecting bacteria and Sabourauds dextrose agar (SDA) supplemented with chloramphenicol was used as a culture media for collecting fungi. A volume not less than 27 ml of culture medium was placed in each plate then they were inserted into each impactor stages. The sampling time of each run was 15 min to avoid overestimated number of particles. Sampling of bacteria and fungi were performed indoor and outdoor air. After microorganism sampling, the plates were incubated at 37 ˚C for 2-5 days. Total number of bacterial and fungal colonies were counted. The airborne bioburden calculated in terms of colony-forming units (CFU) per cubic meter air is presented by Equation (1):

$$C = \frac{n}{v} \ldots\ldots\ldots CFU/m^3 \quad , (1)$$

where n is number of collected colonies on each stage of the impactor, v is the flow rate of the impactor in m^3/h, t is the sampling time in hr.

Measurements were performed at two different ventilation conditions; minimum ventilation (i.e. all windows and doors are kept closed) and ventilation at the effect of air condition (AC).

During the measurement the impactor is located in the center of the lab at a level of 1.5 m above the ground to simulate the breathing zone of the human. Temperature and relative humidity were recorded during the measurements (Table 1)

Results and Discussions

Bioaerosol concentration

Mean concentrations of bacteria and fungi measured in indoor and outdoor are summarized in Table 2. Indoor bacterial concentration ranged from 506 ± 120 CFU/m^3, during the operation of air conditioning system (ACS), to 727 ±160 CFU/m^3, during minimum ventilation where all windows and doors are kept closed, with a mean value of 600 ± 140 CFU/m^3. The indoor bacterial concentration is relatively higher than the guide line (500 CFU/m^3) of World Health Organization [25]. On the other hand, the present indoor bacterial concentration level is lower than the microbial contamination limit, 1000 CFU/m^3, defined by Occupational Health and Safety Research Institute Robert Sauve, IRSST [26]. The number of occupants and human activities are the main sources of indoor bacteria [27, 28]. The average outdoor concentration of bacteria was 220 ± 43 CFU/m^3 which means that the indoor bacterial level is about 3 times higher than the outdoor level.

Indoor fungal concentration ranged from 176 ± 44 CFU/m^3, during the operation of air conditioning ventilation (ACS), to 252 ± 60 CFU/m^3, during minimum ventilation, with an average value of 200 ± 45 CFU/m^3. The indoor fungal concentration is lower than the guide line (500 CFU/m^3) of World Health Organization [25]. In all indoor conditions, the concentration of fungi is lower than the bacterial concentration indicating that the outdoor environment is the main source of fungi [27]. Temperature and relative humidity have impact to the level of microbe [29,30]. The concentration of microbes in indoor at minimum ventilation is higher than during air conditioning ventilation. This may be attributed to the high temperature, 28 ˚C and relative humidity, 48% during minimum ventilation while the corresponding temperature was 25 ˚C and the corresponding relative humidity was 33% during air conditioning system. It was confirmed that high temperature and relative humidity initiate the growth of bacteria and fungi [1,22, 31].

The average outdoor concentration of fungi was 300 ± 32 CFU/m^3 which is relatively higher than the indoor fungal concentration as well as higher than the outdoor bacterial concentration.

TABLE 1. Temperature and relative humidity at indoor ventilation conditions and outdoor.

Ventilation condition	Temperature (°C) ±SD	Relative humidity (%)±SD
Min.ventilation	28.6±7.5	48±22
ACS	25±5.5	33±12
Outdoor	34±8.5	39± 17

TABLE 2 . Mean concentrations of bioaerosols in indoor (at minimum ventilation and air condition system, ACS) and outdoor air.

Bioaerosols	Concentration (CFU/m^3) ±SD			
	Indoor			Outdoor
	Min. Ventilation	ACS	Mean	
Bacteria	727±160	506±120	600±140	22055±
Fungi	252±60	176±44	200±45	300±77

Deposition rates of bacterial and fungal particles increased with increasing ventilation rates in the indoor environments [32]. The type of ventilation system affects the concentration of indoor microbes and removing processes [3]. Mechanical ventilation is more efficient than natural ventilation in filtering particles from air [3]. The increased deposition rates leads to reduction in concentration levels of microbes [32]. This could explain the low concentration of bacteria and fungi in the ventilated indoor conditions.

Indoor/Outdoor bioaerosols concentration ratio (I/O Ratio)

For comparing the strength of indoor and outdoor sources of microorganisms, the indoor/ outdoor ratio (I/O ratio) of bacterial and fungal concentrations are calculated. The indoor -to- outdoor (I/O) ratio indicates the source of microbes [22]. If I/O ratio is greater than unity, it suggests that the microorganisms are derived from indoor sources and if the ratio is lower than unity, it indicates that the major sources for emitting microorganisms are from outdoor.

The I/O concentration ratio of bacteria and fungi are summarized in Table (3). I/O concentration ratio of bacteria is greater than unity

TABLE 3. Indoor/Outdoor concentration ratio of bioaerosols (I/O ratio).

Bioaerosols	I/O Concentration Ratio		
	Min. Ventilation	ACS	Mean
Bacteria	3.3	2.3	2.7
Fungi	0.84	0.89	0.7

at both conditions of measurements where the I/O ratio was 3.3 and 2.3 at minimum ventilation and during ACS, respectively with mean value of 2.7. This result indicates that the main source for bacteria is the human in indoor environment as well as the building materials that host the growth of microorganisms [22]. The I/O of fungi was 0.84 and 0.89 at minimum ventilation and during ACS, respectively with mean value of 0.7 suggesting that the indoor fungi mostly emitted from external sources. These results are in agreement with other studies [22,33-35].

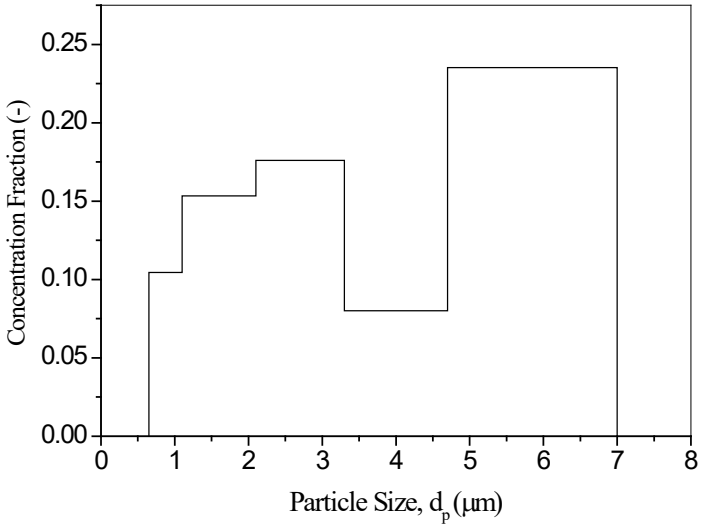

Fig. 1. Size distribution of bacteria at indoor minimum ventilation.

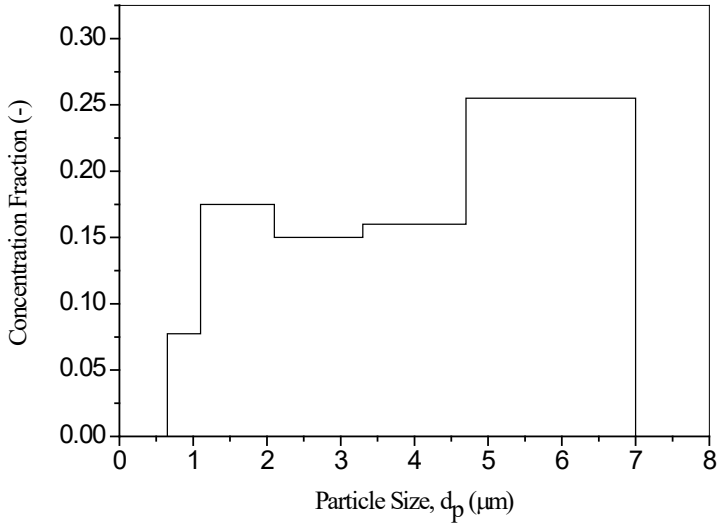

Fig. 2. Size distribution of bacteria at indoor ACS ventilation .

Size distributions of bioaerosols

Size distributions of indoor bacteria are presented in Fig. 1 and 2 at minimum ventilation and during ACS, respectively. The distributions are bimodal, at both conditions, according to the accumulation mode, with a high concentration at the size range 2.1-3.3 μm, and the coarse mode with a high concentration at the size range 4.7-7 μm. The parameters of the size distribution, Median Aerodynamic Diameter (MAD) and Geometric Standard Deviation (GSD), defined by Hinds [36], was 2.9 μm for bacterial aerosols with GSD of 2.2 at both conditions of measurements.

Size distributions of indoor fungi are presented in Fig. 3 and 4 at minimum ventilation and during ACS, respectively. The distributions are unimodal, at both conditions, according to the accumulation mode with a high concentration at the size range 2.1-3.3 μm.

Median Aerodynamic Diameter, MAD (1.8 μm) and Geometric Standard Deviation, GSD (2.5) were nearly similar at minimum ventilation and during ACS.

Size distributions of outdoor bacteria and fungi are presented in Fig. 5 and 6, respectively. The distribution of outdoor bacteria was bimodal with a high concentration at the size 1.1 μm and the second peak in the size range 3.3-4.7 μm. The corresponding outdoor fungal distribution was unimodal with a high concentration at the size range 2.1-3.3 μm.

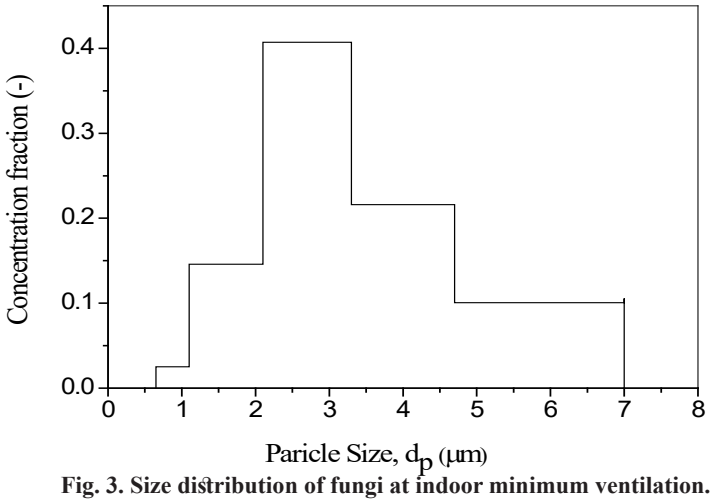

Fig. 3. Size distribution of fungi at indoor minimum ventilation.

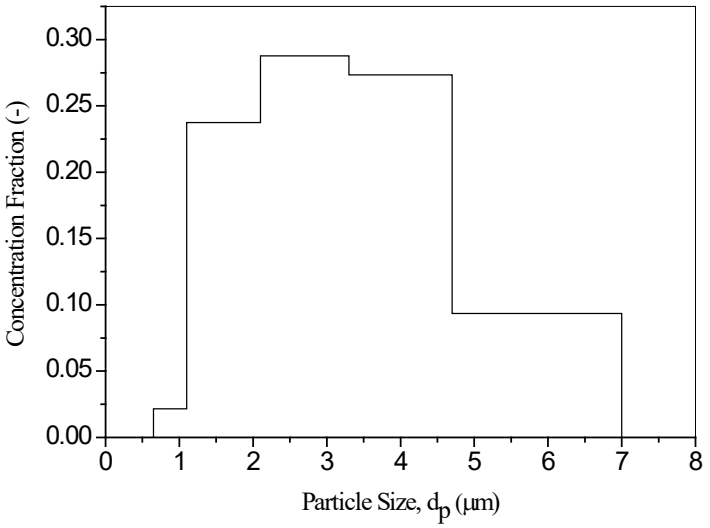

Fig. 4. Size distribution of fungi at indoor ACS ventilation.

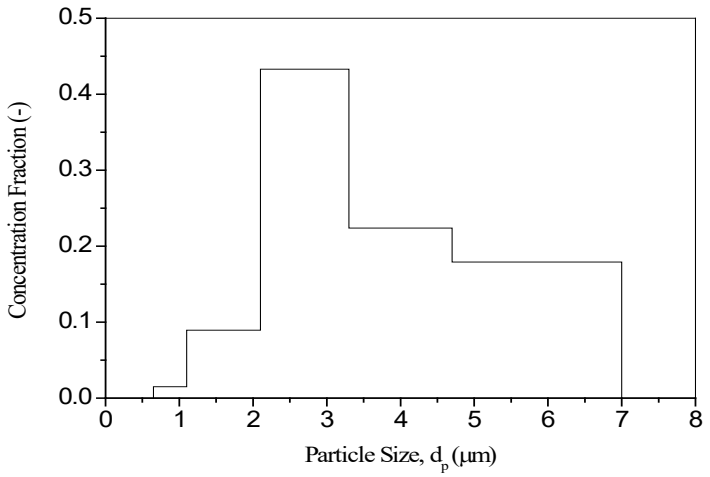

Fig. 5. Size distribution of bacteria at outdoor .

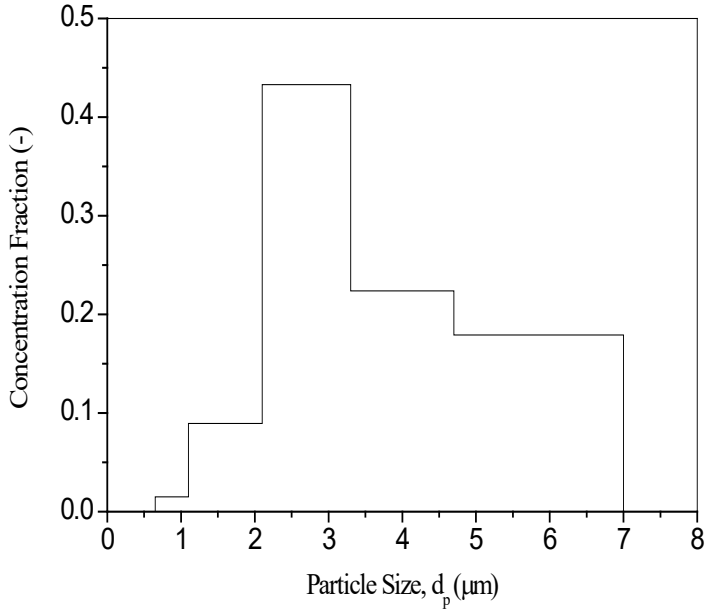

Fig. 6. Size distribution of fungi at outdoor.

Median aerodynamic diameter of bacterial distribution was 2 μm with a geometric standard deviation of 2.2 while median aerodynamic diameter of fungal distribution was 2.7 μm with a geometric standard deviation of 1.5. Similar size distributions of bacteria and fungi were confirmed by my previous studies [35,37] as well as other studies [22,27,38,39].

Indoor Exposure Dose (IED)

The assessment of infection risk depends not only on the number of inhaled bioaerosols but also on the individual's body mass [22]. Indoor exposure dose (IED) has been calculated on the basis of the US Environmental Protection Agency factors [40] and [41]. Based on the measured microbes concentrations and physical activity of the individuals and the exposure time, the IED was estimated based on Equation (2):

$$IED = \frac{C\ IR\ IEF}{BW} \quad , \qquad (2)$$

where, IED is the indoor exposure dose (CFU/kg);

C is the concentration of microbes (CFU/m^3);

IR is the inhalation rate coefficient, characteristic for the physical activity (m^3/h);

IEF is the indoor exposure fraction that represents the time spent over a day in the indoor environment (h) and BW is the mean body weight (kg).

TABLE 4 . Indoor Exposure Dose, IED (CFU/kg).

Bioaerosols	IED (CFU/kg)		
	Min. Ventilation	ACS	Mean
Bacteria	45.4	31.6	37.4
Fungi	15.7	11	12.5

In the present study, IR is 0.78 m^3/h taken for adult assuming light physical activity and the average of IEF which is 8 h. Mean body weight (BW) is 100 kg. Table (4) summarizes the mean indoor exposure dose (IED) of bacteria and fungi at minimum ventilation and during the operation of air condition system (ACS).

The indoor exposure dose of microbes (bacterial and fungi are higher at minimum ventilation than during the operation of air condition system; The IED of bacteria was 45.4 cfu/kg at minimum ventilation while the corresponding IED of bacteria was 31.6 CFU/kg during ACS. The mean value of bacterial indoor exposure dose measured at two ventilation conditions was 37.4 CFU/kg.

The IED of fungi was 15.7 CFU /kg at minimum ventilation while the corresponding IED of fungi was 11 CFU/kg during ACS. The mean value of fungal indoor exposure dose measured at two ventilation conditions was 12.5 CFU/kg. The higher IED of bacteria and fungi at minimum ventilation than during ACS is attributed to the higher concentration of bacteria and fungi at minimum ventilation than during ACS (shown in Table 1). These results were also confirmed by the Bragoszewska et al., [22] study where the mean IED of bacteria were 87.5 and 185.5 CFU/kg in winter at two different regions respectively. The corresponding IED of bacteria in spring were 193 and 249 CFU /kg, respectively.

Mean fungal IED were 9.9 and 21.4 CFU/kg in winter at two different regions respectively. The corresponding fungal IED in spring were 49.6 and 71 CFU/kg, respectively.

It is found that the average value of bacterial IED is more than two times of the fungal IED at all ventilation conditions which may be attributed to the higher concentration of bacteria than of fungi at both indoor ventilations. These results agree with Bragoszewska et al., [22] results where the bacterial IED was higher also than the fungal IED during both seasons, winter and spring.

In comparison the present results with others [21,22], it was found that the calculated IED is lower than the IED obtained by others; where 302.3 CFU/kg mean IED of bacteria was calculated by Bragoszewska et al., [21] and 175.4 CFU/kg by Bragoszewska [42]. One of the reasons of this discrepancy is due to the higher Egyptian body weight, in this study, compared to others.

Summary and conclusions

Analysis of the characteristics_of airborne bioaerosols gives a better understanding of the prevalence and ecology of indoor airborne particles, which may be useful in the management and overcome of the long- and short-term health issues for the individuals.

Measured indoor bacterial concentration was higher than indoor fungal concentration. The indoor concentration of bacteria was about twice higher than the outdoor level indicating that the people are the main source of bacterial emission. On the other hand, indoor concentration of fungi is lower than the outdoor level.

I/O ratio of bacteria was greater than unity while the corresponding I/O ratio of fungi was lower than unity indicating that the indoor source is the main source for bacteria while the outdoor sources represent the main source for fungi. Temperature, relative humidity and ventilation are significant factors initiating the microbes growth.

Size distribution of bacterial aerosols was bimodal in nature with a high concentration in the coarse mode while the corresponding size distribution of fungal aerosols was unimodal with a high concentration in the accumulation mode. The dispersion of bacteria is higher than the dispersion of fungi at both indoor and outdoor air. Indoor exposure dose of bacteria was three times higher than the dose of fungi.

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التركيز والتوزيع الحجمى للمعلقات الهوائية البيولوجية من البكتريا والفطريات و حساب جرعة التعرض

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في هذه الدراسة ، تم قياس تركيز البكتيريا والفطريات والتوزيع الحجمى لها خلال الحد الأدنى من التهوية (أثناء غلق جميع النوافذ والأبواب) وأثناء تشغيل نظام تكييف الهواء (SCA) داخل وخارج المبنى بجامعة المنيا, كما تم حساب نسبة التركيز الداخلى والخارجى لهذه الميكروبات(oitar O/I) حيث تحدد هذه النسبة مصدر هذه الميكروبات. هذا بالاضافة الى حساب جرعة التعرض لهذه الميكروبات.استخدم جهاز egaśt-xis nesrednA rotcapmi elbaivلتجميع البكتريا والفطريات وتقسيمها حسب الحجم. وقد وجد أن تركيز البكتريا والفطريات خلال التهوية الضعيفة أعلى من تركيزهما أثناء تشغيل نظام تكييف الهواء داخل المبنى. وكان تركيز البكتريا أعلى من تركيز الفطريات بالداخل. وجد أن نسبة التركيز الداخلى/الخارجى(O / I) من البكتيريا أكبر من الوحدة مما يدل على أن المصدر الرئيسي للبكتيريا هو الإنسان بينما كانت النسبة المقابلة للفطريات أقل من الوحدة مما يشير إلى أن الفطريات قد وجدت من مصادر خارجية. كانت جرعة التعرض للبكتيريا (gk/UFC4.73) أعلى بحوالى ثلاث مرات من جرعة التعرض للفطريات ((gk/UFC 5.21.