International Journal of Molecular and Clinical Microbiology 12(1) (2022) 1596-1604

International Journal of
Molecular and Clinical Microbiology

# Investigation of Indoor and Outdoor Fungal Bioaerosols and Environmental Factors in Indoor Air Quality of Nursery Schools

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ARTICLE INFO

*Article history:*
Received 3 November 2021
Accepted 22 January 2022
Available online 1 February 2022

*Keywords:*
*Bioaerosol,*
*Mycoflora,*
*Indoor air,*
*Outdoor air,*
*Particulate Matter*

ABSTRACT

Bioaerosols are airborne particles that contain bacteria, viruses, and fungi. Human reactions to bioaerosols are very different. This study attempted to determine indoor and outdoor fungal density in nursery schools in Birjand city and compares the relationship between air fungus density and particulate matter (PM) with mean temperature and relative humidity (RH) in three seasons: Fall, Winter, and Spring. This cross-sectional study was conducted in six nursery schools within a period of 2017-2018. Bioaerosols sampling from the indoor and outdoor air was performed by air-trapping method (28.3 L/min) for 35 min, collected in Sabouraud dextrose agar medium. The air temperature and relative humidity (RH) were measured. The mean temperature of indoor and outdoor was 23.49±4.259 and 20.87±5.57, and the relative humidity of 36.04±12.86 and 22.4±7, respectively. The most isolated fungal species was *Penicillium* spp. There is a difference between the dispersal of fungi and suspended particles and the humidity of the two environments ($p=0.001$). Moreover, there is only a difference in the distribution of fungi in fall ($p=0.035$). Generally, the contamination of indoor is linked to a load of outdoor airborne fungal spores**.** It can be safely concluded that bioaerosol has outdoor sources and ventilation plays an important role in improving the quality of the indoor air**.**

## 1. Introduction

Bioaerosols particles are one of the pollutants that can cause a decrease in indoor air quality (IQA)(Hewitt et al., 2012). The bioaerosols are a wide range of airborne particles, comprising bacteria, fungi spores, viruses, pollen, and endotoxins (part of the outer cell wall of Gram-negative bacteria), and they can contribute to around 5-34% of indoor air pollution (Wang et al., 2012). According to preview studies, aerosols contain particulate matter (PM) which includes coarse particles (pm10), fine particles (pm2.5), ultra-particles (pm1)(Jedrychowski et al., 2006; Srikanth et al., 2008). These particles can act as carriers for fungal spores (e.g. *Penicillium* spp., *Aspergillus* spp., *Mucor* spp., *Rhisopus* spp) are commonly related to allergy, infection, irritation, and toxicity for human and animal and have a significant impact on public

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health and environmental protection (Chegini et al., 2020; Faridi et al., 2017; Schwab and Straus, 2004).

Many studies have shown that the outdoor bioaerosol concentration affects the indoor air (Branco et al., 2014a; El-Morsy, 2006). In general, infants and toddlers, spend approximately 90% of their time in indoor environments, thus, indoor air is important for their health. Children are considered a risk group since they are more vulnerable to microorganisms than adults. On the other hand, environmental factors such as temperature, humidity, and ventilation have a profound effect on the presence of aerosols indoors(Branco et al., 2015; Kabir et al., 2012).

Unfortunately, there are no guidelines or sufficient reference data in Iran for the fungal count in indoor and outdoor air in childcare centers. An important indoor air pollutant is mold as they can also release volatile organic compounds (VOCs) and particulate matter (PM) leading to negative health effects (El-Morsy, 2006; Jedrychowski et al., 2006). Therefore, periodic monitoring of indoor airborne particles is very important to control the quality of ventilation.

This study attempts to investigate the levels and characteristics of indoor and outdoor fungal density in nursery schools in Birjand city and compares the relationship between air fungus density and particulate matter (PM) with mean temperature and relative humidity (RH) in three seasons: Fall, winter, and spring.

## 2. Materials and Methods

### *3.1. Design and sampling sites*

This study is a cross-sectional descriptive-analytic study. The study population was the indoor and outdoor air ambient of six nursery schools located at urban sites of Birjand province, Iran 2017-18. In this study, due to the closure of most kindergartens in the summer, therefore the distribution of fungal agents in indoor and outdoor environments was investigated in three seasons: Fall, Winter, and Spring. The sampling location in this study was nursery schools in Birjand in 2017-2018. The city of Birjand was geographically divided into five regions: north, south, east, west and center, and 6 nursery schools were randomly selected. Due to the higher commuting time in the morning than the evening shift, sampling was performed about 8am to 13 pm. A summary of the sampling locations is shown in table 1.

### *3.2. Sampling procedure from indoor and outdoor*

A total of 96 samples were prepared using air-trapping according to a previous study conducted by Sepahvand et al, where active sampling method and single stage Anderson method were applied (Sepahvand et al., 2020) . The sampling procedure included collision of bioaerosols with single stage Andersen's impactor (Quick take-30, USA). The volume of air required based on the NIOSH0800 standard was 28.3 l/min. Each sampling lasted 35 min, plates containing a culture medium of Sabouraud Dextrose Agar (SDA) medium containing chloramphenicol (Merck, Germany) were placed in Andersen's impactor according to the instructions provided by the manufacturer company. The divice was placed at the altitude of 1.5 m above the ground, about the height of the breathing zone of the children at the center of each class. After that, the plates were transferred to the laboratory, and incubated at 25-27°C for 3-7 days. After the growth and appearance of fungi on the surface of the culture medium, fungi were identified based on macroscopic and microscopic characteristics. Before sampling, to prevent the secondary contamination and error, all sampling equipment was disinfected with alcohol (70%) and autoclaved. During the sampling of meteorological parameters such as temperature and relative humidity (RH), the effect of environmental factors was measured and recorded.

### *3.3. Particulate matter (PM) sampling*

Particulate matter sampling performed for assessment of particulate air from indoor and outdoor using Tess 5200 (HAZ-DUSTE, Mass Counter-Taiwan) for 10 min and 1.5 m above the ground. This device is ideal for particulate air monitoring applications and was calibrated by a suitable calibrator (zirofilter, USA) prior to air sampling. Aerosol PM Concentration reported in $mg/m^3$.

*3.4. Macroscopic and microscopic identification of fungi*

To determine the fungal genera and species, methods applied in the related literature were used. The characteristics of the colonies grown on the SDA were identified according to the colony state /shape and their microscopic morphological criteria. The microscopic checkup of the fungal species was carried out using fungus culture on lam, using Lactophenol Coton Blu (LPCB) solution. Furthermore, slide cultures and needle mounts (tease mounts) were used for microscopic determination according to Aneja K et al (Aneja KR, 2007).

*3.5. Statistical analysis*

All statistical analyses were performed using the SPSS16.0 software. We used t-test or one-way analysis (ANOVA) for comparing parametric continuous variables and for nonparametric variables using the Kruskal-Wallis test between groups. Statistically significant differences were determined when the probability p-value was lower than 0.05.

**Table 1.** Summary of the main characteristics of the studied nursery school

| kindergarten | Classroom localization | Area (m2) | Number of children | Number of Staff | location |
|---|---|---|---|---|---|
| Taha | first floor | 32 | 31 | 7 | East of town |
| Golhaiezendegy | first floor | 18 | 15 | 4 | North of town |
| Nikan | first floor | 12 | 10 | 8 | Central of town |
| Mehrafarin | third floor | 30 | 21 | 14 | West of town |
| Golpira | first floor | 15 | 15 | 5 | Central of town |
| Atefeha | first floor | 20 | 22 | 8 | South of the city |

## 3. Results

We collected 96 samples (48 outdoor and 48 indoor samples) from 6 nursery schools during three seasons (Fall, Winter and Spring) as indicated in figure 1.

The mean temperatures of indoor and outdoor were 23.49±4.25 and 20.87±5.57 and the mean RH were 36.04±12.86 and 22.4±7 respectively. The average of fungal density, aerosol, temperature, and RH from six sites was calculated and shown in table 2. There were significant differences between fungal density, aerosol, and RH of indoor and outdoor environment (p=0.001). However, there was no significant difference in terms of temperature (p=0.380). The particle sizes which include $PM_{10}$, $PM_{2.5}$, and $PM_{1.0}$ were also evaluated. Our result showed a significant difference amongst the three types of particles isolated from the indoor and outdoor environment. The mean± SD of fungal density from the indoor and outdoor was determined in the three seasons (Fall, Winter and Spring) showed a significant difference between fungal density of indoor and outdoor in Fall (p=0.036) (Table 2).

Relationships between fungal density, RH, temperature, and suspended particles were shown in table 3. The results of Spearman's correlation analysis demonstrate that was no significant relationship between fungal density and temperature (r=0.091; p=0.380), but the relationship between fungal density and RH was also significant (r=0.0404; P=0.001). There was no significant relationship between fungal density and suspended particles (r=0.089; P=0.389) (Table 3).

A total of 135 and 140 colonies collected from indoor and outdoor environments, thirteen different fungal genera (hyalinemycetes, dematiaceous, and yeast form) and 22 colonies of mycelia sterile were isolated. The frequency number of the fungi type is represented in figure 2. According to the results, *Penicillium* sp were the most prominent fungal genus isolated from outdoor and indoor followed by *Rhizopus* sp, *Cladosporium* sp, and *Aspergillus* sp.

The most prominent fungal genus isolated from two environments were classified into 4 classes including hyaline Hyphomycetes (44.28% and 44.44%), Zygomycetes (22.14% and 10.37%), Dematiaceous Hyphomycetes (17.14% and 30.37%), mycelia streilia (7.14% and 8.88%) and yeast form (5.71% and 9.62%) respectively (Figure 3).

**Table 2.** Comparison of fungal, bioaerosol density and particles with temperature and humidity in indoor and outdoor of nursery schools

| parameter | Indoor (m±SD) | Out door (m±SD) | p-value |
|---|---|---|---|
| **Fungal colony** | 2.90±1.1 | 3.30±1.576 | **0.001** |
| **Aerosol** | 121±68.118 | 104±201.60 | **0.001** |
| **Temperature** | 23.49±4.259 | 20.87±5.57 | 0.380 |
| **Relative humidity** | 36.04±12.86 | 22.4±7.7 | **0.001** |
| **Particles size** | | | |
| $PM_{1.0}$ | 0.53±0.38 | 1.81±3.04 | **0.006** |
| $PM_{2.5}$ | 3.75±4.59 | 9.11±10.58 | **0.006** |
| $PM_{10}$ | 109.9±67.54 | 76.82±44.68 | **0.002** |
| **Frequency of fungi isolated in different season** | | | |
| Fall | 24.8±7.89 | 13.6±1.51 | **0.036** |
| Winter | 16.38±0.931 | 19.7±1.24 | 0.176 |
| Spring | 20.1±1.38 | 16.7±1.23 | 0.363 |

**Table 3.**The results of Spearman's correlation analysis between fungal density, RH and environment factors

| Variants | RH[a] | TM[b] | PM[c] |
|---|---|---|---|
| fungal density | P=0.001 | P=0.380 | P=0.389 |
| | r=0.040 | r= 0.091 | r=0.089 |

a: Relative humidity; b: Temperature; c: Particulate matter

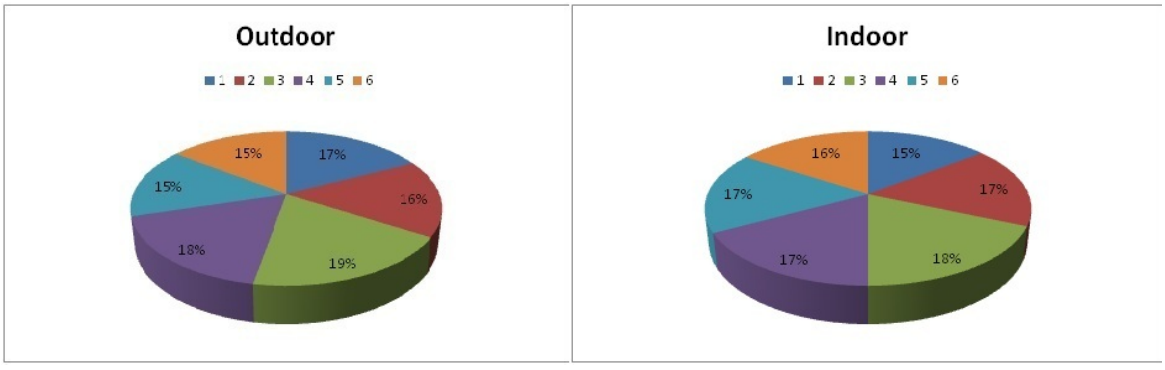


**Figure 1.** The percentage of fungal isolates from each studied nursery school.(1) Taha, (2) Golhaiezendegy, (3) Nikan, (4) Mehrafarin, (5) Golpira and (6) Atefeha.

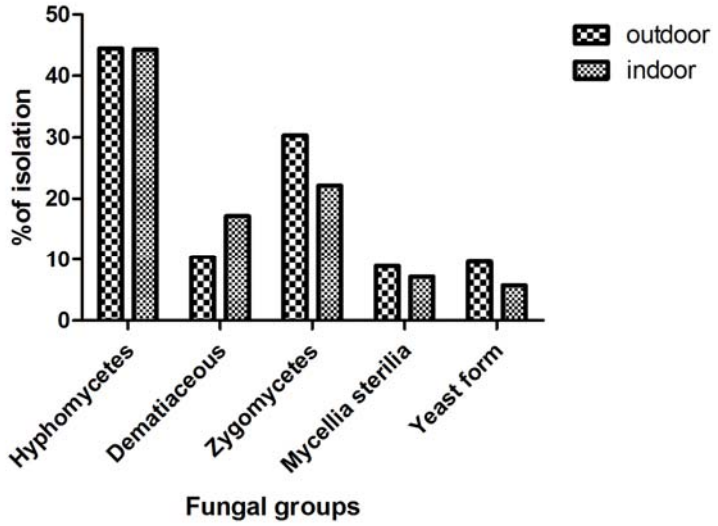


**Figure 2.** Frequency of fungi isolated from indoor and outdoor of nursery schools

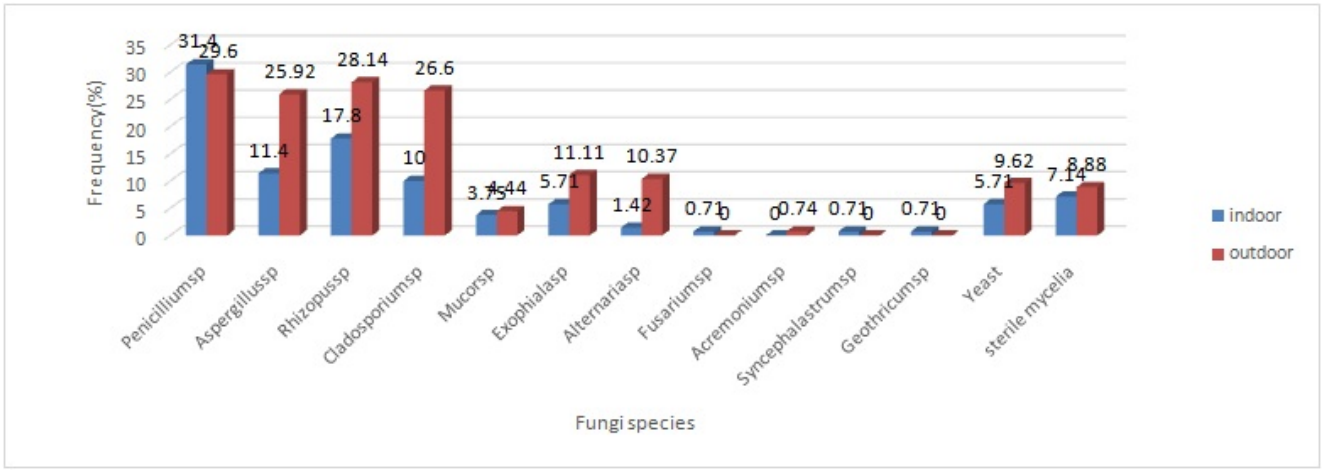


**Figure 3.** Total frequency of fungal distributed in different classes of fungi including Hyphomycetes (hyaline and dematiaceous), Zygomycetes, mycelia sterilia and yeast.

## 4. Discussion

In the present study, distribution and diversity of outdoor and indoor aerosol particle and airborne mycoflora have been reported from 6 nursery schools during Fall, Winter, and Spring. The results showed that the majority of the isolated fungi belonged to the hyaline hyphomycetes and zygomycetes, included *Penicillium* sp as the most frequent of fungal isolates (~30%) followed by *Rhizopus*, *Cladosporium* and *Aspergillus*. Although, *Rhizopus* was the common genera which isolated from the indoor environment, *Cladosporium* (26.6%) was isolated mostly from the outdoor.

In several surveys of airborne fungal spores, it has been demonstrated that the most prevalent fungi belonged to the genera *Aspergillus*, *Cladosporium*, *Penicillium*, and *Alternaria*. (Durugbo et al., 2013; Fernstrom and Goldblatt, 2013; Sepahvand et al., 2013; Shams-Ghahfarokhi et al., 2014). Aydogdu et al. studied fungi in the indoor air of primary schools in Edirne, Turkey. They reported that dominant fungal types included *Cladosporium*,

*Penicillium*, and *Alternaria* (Aydogdu et al., 2005). Yassin et al. evaluated the airborne fungi in an indoor and outdoor environment of an industrial zone in Egypt. Mainly members of the genus from the outside were *Aspergillus* and other genera isolated from the indoor were *Penicillium*, and *Aspergillus* (Yassin and Almouqatea, 2010).

Additionally, in a previous study of airborne mycoflora of outdoor air of Qeshm Island, southern Iran, a predominance of dematiaceous fungi versus hyaline hyphomycetes was reported by Barrati et al (Barrati B, Ghahri M, 2009). Shokri et al. reported the genera *Mucor*, *Cladosporium*, and *Rhizopus* as the dominant fungi in the air of forests and seashore areas of Babol, Iran (Shokri H, Khosravi AR, Naseri A, Ghiasi M, 2009).

In the present study, temperature, and RH of indoor and outdoor were measured simultaneously. Our results showed that significant differences between indoor and outdoor air fungal concentration and RH ($p=0.001$), while, the comparison the total mean of fungal with PM concentration and temperature was demonstrated no significant difference between indoor and outdoor air ($p=0.38$ and $p=0.38$ respectivelly). However, the amount of distribution and density of the fungi was significantly different between Fall and Winter ($p=0.036$).

Ehrampoosh et al. have demonstrated a significant relationship between indoor and outdoor suspended particle concentration (Ehrampoosh M et al., 2015). Hoseinzadeh et al. had reported that was a significant difference among fungus bioaerosol and RH (%) which is based on sampling points, while there was significant relation between RH (%) and temperature of daycare child center with total fungal count (Hoseinzadeh et al., 2017).

In this regard, there are several sources for fungi indoors such as building occupants and their activities and building materials. *Penicillium,* and *Rhizopus* spores are released into the air more easily than *Cladosporium* spores, explaining why *Penicillium* spores are common in the indoor and outdoor air (Hoseinzadeh et al., 2013). Compared to *Penicillium*, the size of *Cladosporium* spores are larger than other species. Since *Cladosporium* spore is wind-dispersed and may grow up on indoor surfaces when moisture is present (Klinmalee et al., 2009). These fungal spores are significant allergens and may cause asthma, especially in children with respiratory health conditions (Douwes et al., 2003). Unfortunately, there are no guidelines or sufficient reference data in Iran for the fungal count in the indoor air in childcare centers. The indoor mycoflora contamination is linked to a load of outdoor fungal spores and the indoor air fungal count variation may be affected by the outdoor environment.

Furthermore, exposure to bioaerosols tends to have corresponded various health effects (e.g. infectious diseases, allergies, and cancer). However, respiratory symptoms and the importance of lung function have been the most subject of studies that belong to the most important health problem by bioaerosols. Mycotoxin and glucans from molds, in addition to irritating airways, were also suggested to contribute to airway inflammation and asthma (Kim et al., 2018; Sousa et al., 2012). On the other hand, the possible associations between exposure to bioaerosols and some specific cancer have been reported (Bhatia, 2011).

It can be safely concluded that bioaerosol has outdoor sources and ventilation quality plays an important role in improving the quality of the indoor air. Since preschoolers constitute a large population proportion, creating a hygienic educational environment for this vulnerable age group can play a vital role in their well-being (Bragoszewska et al., 2018; Branco et al., 2014b). Certainly, poor ventilation in the classroom is a common problem in some countries during the cold season and its harmful effects not only include respiratory problems, but also lower students' academic performance and well-being.

This study has been some limitations. The present study did not investigate the distribution of aerosols in several months and seasons (Fall to Spring). However, there was a significant difference in aerosol size between the indoor and outdoor environments (table 2). It should be noted that the referred measurement was performed in the geographic regions which differed from Iran’s eastern regions in terms of climate characteristics and vegetation. Considerable variation in the fungal spore concentration in different months of the year was attributed to changes in seasons and conditions. Our findings were not sufficient for

interpreting the differences and explaining the dispersion of fungal spores. Rather, we are suggesting molecular methods for diagnosis of fungi species distribution pattern in our region (Birjand city) in future study.

## Conclusion

In this study performed to the identification and distribution levels of airborne fungal in nursery schools by the air-trapping method. The most commonly isolated species from indoor and outdoor was *Penicillium* spp. This result can be fundamental data for future research and may be useful in the development of preventive and educational strategies. Epidemiological investigations could be performed in multiple areas of the country and compared to data from clinical cases in order.

**Author Contributions:** Study concept and design: **MH.N** and **Z.S.**; analysis and interpretation of data: **T.SH**, and **F. S.;** drafting of the manuscript: **F. N.;** critical revision of the manuscript for important intellectual content: **A. A. R.**, **F. S.**, and **M. H. N.**; statistical analysis: **Z. S.**.

## Acknowledgements

This study was carried out in collaboration with staff of T Department of Environmental Health Engineering, Birjand University of Medical Sciences (BUMS) (Grant no.455705( ir.BUMS.REC. 1397.199)), Birjand, Iran. Thay are appreciated for their collaboration, specially thank MrsMarziehMoghani for help with sampling and Laboratory services.

## Declaration of interest

The author declares that has no conflict of interests

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