# OCCURANCE OF AIRBORNE FUNGAL SPORES WITH POTENTIAL ALLERGOGEN EFFECT IN URBAN AND RURAL EDUCATIONAL INSTITUTIONS FROM IAŞI COUNTY, ROMANIA

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**Abstract**

Air contamination by airborne fungal spores in five educational institutions placed in urban and rural locations of Iasi county, Romania was investigated in 2014 over a period of 3 months (April-June) using the Petri plate gravitational settling (passive) method. Petri plates contained nutrient media (PDA) in three different compositions (classic, with rose-bengal and with streptomycin) were exposed to room air for a 15-min period face upwards to collect particles settling by gravity. The location differed in habitat characteristics, such as urbanisation level, vegetation and microclimate and these characteristics could affect spore occurrence in indoor air.
The identification of the fungi was made according to their microscopic properties and through references. The fungal genera most commonly isolated in all five locations were *Penicillium*, *Cladosporium* and *Aspergillus* (40.5, 26.3 and 23.5% of the total, respectively).
Our results showed that fungal spores density in the educational institutions air was within the sanitary level accepted for public buildings, with exception of one classroom from the rural area (Mogoşeşti-Siret), which has potential to develop adverse health effects to the occupants (1196 UFC/m$^3$ air).

**Key words**: indoor air - airborne fungal spores density - educational institution - urban and rural areas

Fungal spores and other airborne structures are ubiquitous in the indoor environments and the prolonged exposure to these polluted environments may cause various symptoms such as respiratory illnesses, allergic reactions, headaches, dizziness, nausea, fatigue, and dry skin. Many researchers have associated the indoor air pollution with sick building syndrome (Bassam Aboul-Nasr et al., 2014; Daisey et al., 2003; Jurado et al., 2004; Salonen et al., 2014; Ulea et al., 2009, 2013). A building can be diagnosed as sick (SBS) if > 20% of its occupants reveal one or more of the above mentioned symptoms for two weeks and such symptoms disappear when leaving the building (Kim et al, 2005).

Generally, the majority of the indoor airborne fungal microbiota is derived from outdoor sources and is transferred inside through windows and doors (Burge et al., 2000; Shelton et al., 2002). The physicall condition from different building structures, such as humidity level, temperature and the presence of organic and anorganic substrates influences significantly the fungal populations (Bartlett et al., 2004; Frankel et al., 2012b; Wu et al., 2007).

*Penicillium*, *Aspergillus*, *Cladosporium*, *Alternaria*, *Fusarium* and *Mucor* were found to be the most common fungal genera that contaminate the indoor environments and can cause extreme allergic reaction or respiratory and other related diseases in humans. Collection of airborne spores can provide valuable information about the indoor air quality in many types of buildings (Hu et al., 2002).

This study characterized the air pollution level with indoor airborne fungal spores that can cause an allergic response in five educational institutions placed in urban and rural locations of Iasi County, Romania. The investigation followed the Petri plate gravitational settling method of sampling to help establish standards for future references and in order to recommend ways to reduce pollution levels in classrooms.

## MATERIAL AND METHOD

Air contamination by airborne fungal spores in five educational institutions placed in urban and rural locations of Iasi county, Romania was investigated in 2014 over a period of 3 months (April-June) using the Petri plate gravitational settling (passive) method.

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The used method suppose that Petri dishes which contained potato-dextrose-agar (PDA) media in three different compositions (classic, with rose-bengal and with streptomycin) are exposed to room air for a 15-min period face upwards to collect particles settling by gravity. Petri plates were put 80–100 cm above the floor and at 80-100 cm from the wall during sampling.

The experiment was conducted with a threefold repetition for each microbiological determination and the counts obtained were averaged. Microbiological media plates were prepared using Masterclave 09 plate maker and an aliquot portion of 15mL of media was poured using APS 320 automated Petri plate filler (AES Laboratoire, France).

Petri plates used for fungal sampling were incubated aerobically at 28°C for 5-7 days. After incubation, the fungal concentration per cubic meters of air (CFU/$m^3$) was calculated according to Omelyansky (1940).

Light microscopy (1000x magnification) was used to determine the colonial features and the morphological structures of the fungi. The determination of the morphological structures of fungi was carried out on fungal material mounted in lactophenol by slide culture technique. Fungi were identified to genus level based on morphological and physiological characteristics following the works provided by Ellis (1971,1997), De Hoog et al. (2000), Barnett and Hunter (1999).

Regarding the permissible limits for exposure to fungal spores to assess health impact are some recommended concentrations for indoor environments (Mănescu, 1989):

- For clean area, level of air contamination should be lower than 500 CFU/$m^3$.
- For area with intermediate level of air contamination should be between 500 and 700 CFU/$m^3$.
- For area with high level of air contamination (not acceptable) should be upper 700 CFU/$m^3$.

Statistical analysis was conducted with SPSS 16.0 for Windows. Quantitative data are presented as mean ± standard deviation. Results with $p < 0.05$ were considered statistically significant.

## RESULTS AND DISCUSSION

In the indoor air of three different educational institutions a total of 5470 fungal colonies in 490 Petri plates were isolated and quantified to determine the frequency of occurrence and then identified. Koch sedimentation (passive) method was used because of its inherent practicalities, low cost and ease of use to obtain preliminary or qualitative information regarding the air fungal spores.

Ten fungal genera were isolated and identified in all three locations. In addition, a total of 104 nonsporulating colonies were registered.

*Penicillium* spp. was predominant (45.9%), followed by *Cladosporium*, *Aspergillus* and *Alternaria* genera (22.3, 17.4 and 7.3%, respectively). *Penicillium* spp. was the dominant genera at all sampling stations.

In contrast, *Geotrichum* and *Verticillium* genera were found only in the primary school classroom from the rural location and were also registered with less than one percent. Other fungi with less than one percent registered were the genera *Mucor, Rhizopus* and *Fusarium*. Nonsporulating fungi were a constant presence in all location during this study.

Fungi isolated from sampled air are presented in Figure 1.

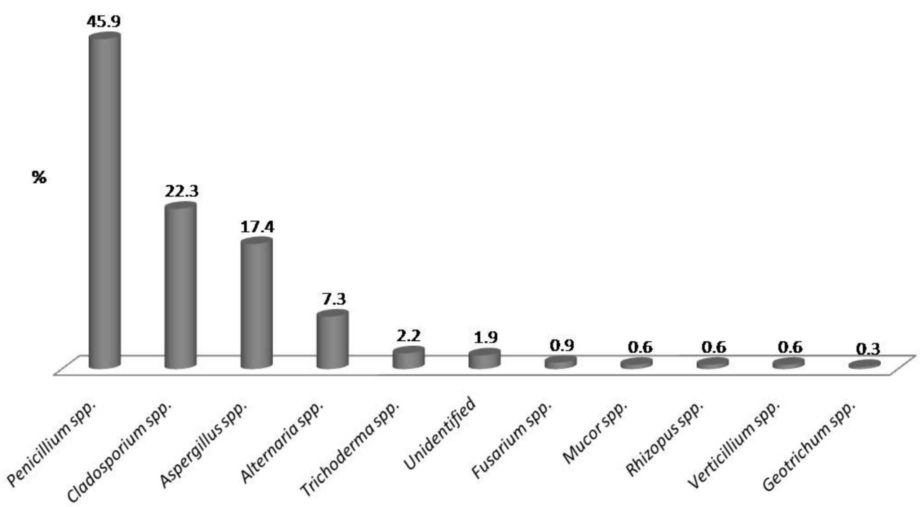


Figure 1 **Frequency of isolated fungi**

The concentration and occurrence of indoor air mycoflora in the present study was congruent with earlier studies conducted in different climate regions (Cheong and Neumeister-Kemp, 2005; Li and Kendrich, 1996; Shelton et al., 2002): The most common indoor fungus are *Penicillium*, *Cladosporium*, *Aspergillus* and *Alternaria* species and their spores represent the most frequent and predominant aeroallergens in the educational buildings (Sarica et al., 2002; Ulea et al., 2013). These results are compatible with our findings; the above mentioned genera were found in high frequency in our work (92.9% – Figure 1).

The quality of indoor air from the educational institutions depends on internal sources, such cleaning procedures, air ventilation, temperature and relative humidity. The air contamination in educational rooms was measured and the results varied from 145 to 796 CFU/m$^3$, and with one exception, remains below above-mentioned recommendations.

The results reported for fungal flora in every sampling site are the average (arithmetic mean concentration) and standard deviation of the counts obtained during the sampling period. Concentration of airborne mycota varied at different locations from 172 to 765 CFU/m$^3$ in April, from 145 to 796 CFU/m$^3$ in May and from 209 to 779 CFU/m$^3$ in June, respectively. The peak of total fungal prevalence was recorded in June (35.7%), followed by May and April (Table 1).

Table 1

**Monthly distribution of fungi recovered from the indoor air of educational rooms**

| Sampling location | Sampling month | No. of fungal colonies counted[a] X ± s (CFU/m$^3$) | Level of contamination |
|---|---|---|---|
| High school classroom (urban location) | April | 225 ± 13 | Low |
| | May | 275 ± 18 | Low |
| | June | 295 ± 13 | Low |
| Kindergarten classroom (rural location) | April | 189 ± 10 | Low |
| | May | 236 ± 12 | Low |
| | June | 248 ± 15 | Low |
| Kindergarten classroom (urban location) | April | 172 ±10 | Low |
| | May | 145± 12 | Low |
| | June | 209 ± 24 | Low |
| Primary school classroom (rural location) | April | 765 ± 35 | High |
| | May | 796 ± 42 | High |
| | June | 779 ± 31 | High |
| Primary school classroom (urban location) | April | 320 ± 13 | Low |
| | May | 395 ± 14 | Low |
| | June | 421 ± 19 | Low |

[a]Average and standard deviation of airborne microfungi as determinate by Petri plate gravitational settling (passive) method.

Fungal concentrations show a lower level of contamination in educational institutions placed in urban and rural locations with exception of the primary school classroom from the rural location. In this case the fungal contamination was found to be higher as the recommended concentration (>700 CFU/m$^3$) and has potential to develop adverse health effects to the occupants. The presence of a good ventilation system to eliminate some indoor sources inside building and appropriate methods for maintaining and cleaning classrooms are required.

The level of fungal spores in the air of primary school classroom from the rural location had higher values over the 3-months period (765, 796 and 779 CFU/m$^3$), which could not be explained by the number of scholars, but could be explained by the presence of mould, room characteristics, cleaning procedures and lower ventilation.

Salonen (2009) mentioned that in location with mould growth it is to expect that the microbial levels are higher. In addition, the moisture damage may cause an increasing of fungal spores concentrations.

Pastuszka et al. (2000) reported that *Cladosporium*, *Alternaria* and *Aspergillus* are the main fungi to which children may be sensitised and to which allergic symptoms can be provoked. According to Belousova et al. (2001), asthma and SDS symptoms are common in highly contaminated schools with *Alternaria* spp. Also, *Alternaria*, *Cladosporium*, *Curvularia*, *Fusarium*, *Trichoderma* and *Verticillium* genera may produce mycotoxicosis in humans.

Chi-square ($\chi^2$) test was applied to determine if there were any differences between the sampling period (April-June 2014) and fungal densities. Statistical significantly difference was found only in case of the primary school classroom from the rural location ($p < 0.05$).

The comparative recorded data for the occurrence of airborne fungal spores with potential allergogen effect in urban and rural educational institutions from Iaşi County revealed that in the rural area the quality of indoor air was inferior in comparacy to the urban locations. The concentration level of appearance during the sampling period was not very different for kindergarten in both locations (not statistically significant): in rural area ranged from 189 to 248 CFU/m$^3$, while in the urban area the airborne mycota varied from 145 to 209 CFU/m$^3$.

The difference was statistically significant at 0.05 level in case of the primary school classrooms: in Mogoşeşti-Siret (rural location) the values ranged from 765 to 796 CFU/m$^3$ with possibilities to affect human health and in Iasi (urban location) the airborne mycota varied from 320 to 421 CFU/m$^3$.

According to Corden and Millington (2001), higher temperatures, relative humidity, geographical region and specific reservoirs of contamination (e.g. agricultural activities) can induce fungal spore concentrations and increasing of fungal contamination risk.

The fungal flora in indoor air may affect human health and as a consequence many clinical and epidemiological investigations must be undertaken.

## CONCLUSIONS

*Penicillium Cladosporium*, *Aspergillus*, *Alternaria* and several other fungal genera were recorded in the air of different educational institutions placed in urban and rural locations of Iasi County, Romania. These fungi are harmless for healthy people, but they may be dangerous for people from risk groups (with respiratory diseases). *Penicillium* was the most prevalent fungal genera (45.9%), followed by *Cladosporium*, *Aspergillus* and *Alternaria* genera (22.3, 17.4 and 7.3%, respectively).

Indoor concentrations of fungal spores in the primary school classroom from the rural location was found to be higher as the recommended concentration (>700 CFU/m$^3$) and has potential to develop adverse health effects to the occupants. To eliminate some indoor sources inside building the presence of a good ventilation system to is required and appropriate methods for maintaining and cleaning classrooms are required.

In all other location the air quality presented concentrations which were lower than 500 CFU/m$^3$, so that no negative health effects for occupants were expected.

According to the obtained results, we conclude that the studied location show parameter values that do not comply entirely with the Romanian legislation for air quality. These parameters are directly related to public and occupational health and are excellent indicators of sick building syndrome (SBS). Appropriate methods for cleaning classrooms and lowering occupancy could decrease fungal spores concentrations in these environments.

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