MONITORING OF FUNGAL AEROSOLS IN URBAN AND RURAL SCHOOLS FROM IAŞI COUNTY, ROMANIA

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Abstract

The densities and distributions of airborne fungal spores known to cause respiratory tract disorders were monitored in three educational buildings located in urban and rural areas from Iaşi, Romania. Air samples from all locations were collected monthly over a period of 3 months (April-June 2016), using the Petri plate gravitational settling (passive) method. Petri plates containing nutrient media were exposed to room air for a 15-minute period, face upwards, to collect particles settling by gravity. A total of 4358 microfungal colonies were counted on 628 plates. During the sampling period 11 fungal genera were isolated and identified. Fungal spores density in the educational institution's air was within the sanitary level accepted for public buildings, with the exception of one high school classroom, which has the potential to put the occupants at risk of developing adverse health effects. Indoor results show differences for fungal genera distribution between the educational buildings. The dominant genera were represented by *Aspergillus*, *Penicillium* and *Fusarium* with 40.4, 26.3 and 9,8% of the total, respectively

Key words: air sampling, educational buildings, fungal aerosols, health effects

Microbial contamination inside educational institutions is of great interest because fungal spores and other airborne structures, which are ubiquitous in the indoor environments, represent a risk for human health and/or a cause for the biodeterioration of building constructions and different materials stored inside (Lipsa *et al*, 2016; Daisey *et al*, 2003; Ulea *et al*, 2009, 2013). Fungal spores are often reported to be hazardous for human health in indoor environment, because their possibilities to cause allergies, respiratory diseases (including asthma) and symptoms of sick building syndrome (SBS).

The purpose of this study was to evaluate and compare fungal biodiversity in the indoor air of urban and rural schools from Iaşi County, Romania. Also, we have estimated the levels of fungal contamination and discussed about the potential health risk incidences associated with this flora.

MATERIAL AND METHOD

The densities and distributions of airborne fungal spores known to cause respiratory tract disorders were monitored in educational buildings located in urban and rural areas from Iaşi, Romania. Air samples from all locations were collected monthly over a period of 3 months (April-June 2016), using the Petri plate gravitational settling (passive) method.

Potato dextrose agar (PDA) and Peptone glucose agar (PGA) media were used for the sampling and quantification of fungi. To these media streptomycin and rose-bengal stain was added in certain quantities. Czapek-Dox agar media was used for filamentous fungi identification.

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).

The experiment was conducted with a threefold repetition for each microbiological determination and the counts obtained were averaged. The fungal colony number was counted after 5 days of incubation at 28ºC.

After incubation, the fungal concentration per cubic meters of air (CFU/m^3) was calculated according to Omelyansky (1940) using the following formula:

$$N = a \cdot 10000/p \cdot t \cdot 0.2$$

where:
N = fungal CFU/m^3 of air;
a = number of colonies per Petri plate;
p = area of Petri plate in cm^2;
t = exposure time in min.

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Light microscopy (1000x magnification) was used to determine the colonial features and the morphological structures of the fungi.

Regarding the permissible limits for exposure to fungal spores to assess health impact are some recommended concentrations for indoor environments (Mănescu, 1989; World Health Organization, 1990):

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 DISCUSSIONS

Petri plates containing nutrient media were exposed to room air for a 15-minute period, face upwards, to collect particles settling by gravity. A total of 4358 microfungal colonies were counted on 628 plates. During the sampling period 11 fungal genera were isolated and identified.

The dominant genera were represented by *Aspergillus*, *Penicillium* and *Fusarium* with 40.4, 26.3 and 9,8% of the total, respectively (*figure 1*).

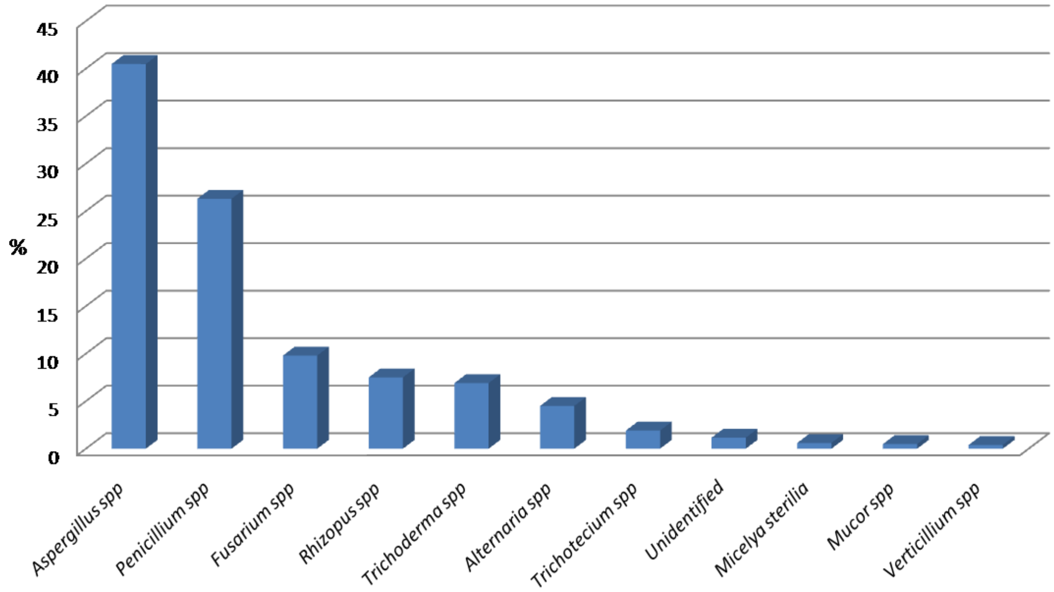

Figure 1 **Taxonomical composition of fungi isolated from the indoor air of urban and rural schools**

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 187 to 468 CFU/m^3 in April, from 256 to 758 CFU/m^3 in May and from 143 to 798 CFU/m^3 in June, respectively. The peak of total fungal prevalence was recorded in May (39%), followed by June and April (data not show).

Correlation between the fungal density and some parameters such as temperature, sampling month and the different locations were calculated. Significant correlation was found in case fungal colony numbers versus indoor temperature ($p < 0.001$). Chi-square (χ^2) test was applied to determine if there were any differences between the sampling period (April - June 2016) and fungal densities. Statistical significantly difference was found only in case of a school classroom from rural area ($p < 0.05$).

The general results showed that in all locations from rural and urban areas were differences in the distribution of fungal genera, but *Aspergillus* and *Penicillium* were the most prevalent. More exactly, the dominant genus in the rural schools was represented by *Aspergillus*, while in the urban schools the *Penicillium* spp. was predominant (*figure 2, 3 and 4*).

Aspergillus and *Penicillium* spores are the most frequent and predominant aeroallergens in the world. These two genera are significant indoor air allergens, while *Alternaria* and *Cladosporium* species are considered the most important fungal allergens in outdoor air (Aydogdu *et al*, 2005; Fischer G., Dott W., 2003; Sarica *et al*, 2002).

These results are compatible with our findings; the above mentioned genera were found in high frequency in our work (66.7%).

In April 2016, fungal concentrations were minimal in both areas, and varied in the range of minimal level. In this month the obtained data showed that the fungal contamination has no potential to develop adverse health effects to the occupants. Airborne fungal genera prevailed in both areas are presented qualitatively in *figure 2*. Numbers of isolated fungal genera are different: in rural area were isolated 6 genera (most prevalent *Aspergillus*, *Penicillium* and *Fusarium*), while in the urban area 11 genera were found (*Penicillium*, *Aspergillus* and *Rhizopus*).

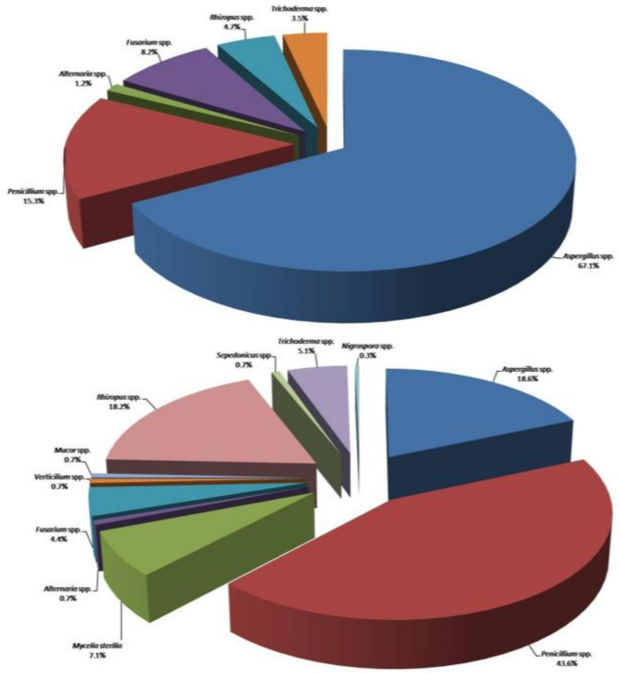

Figure 2 **Frequency of isolated fungi from rural (top) and urban (bottom) schools in April 2016**

In May 2016, fungal concentrations varied between minimal and intermediate level in case of the urban schools.

The fungal contamination in rural classrooms was found to be higher as the recommended concentration (>700 CFU/m^3) in Mogoșești-Siret, and at this location has potential to develop adverse health effects to the occupants. One explanation for this high concentration could be the fact, that the school is located close to the agricultural areas (tractors and other agricultural machines were present during the air sampling).

Airborne fungal genera prevailed in both areas are presented qualitatively in *figure 3*. Numbers of isolated fungal genera decrease qualitatively, but increase quantitatively: in rural area were isolated 4 genera (most prevalent *Aspergillus*, *Penicillium* and *Trichoderma*), while in the urban area 6 genera were found (most prevalent *Rhizopus*, *Penicillium* and *Aspergillus*).

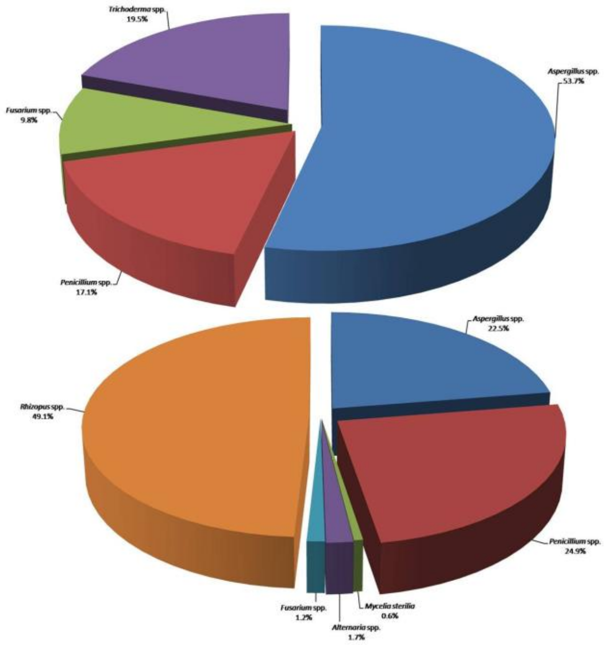

Figure 3 **Frequency of isolated fungi from rural (top) and urban (bottom) schools in May 2016**

In June 2016, fungal concentrations varied between minimal level in case of urban schools and high level of air contamination in case of the Mogoșești-Siret school.

Airborne fungal genera prevailed in both areas are presented qualitatively in *figure 4*. Numbers of isolated fungal genera remain as in May: in rural area were isolated 4 genera (most prevalent *Aspergillus*, *Rhizopus* and *Trichoderma*), while in the urban area 6 genera were found (most prevalent *Trichotecium*, *Penicillium*, *Aspergillus*).

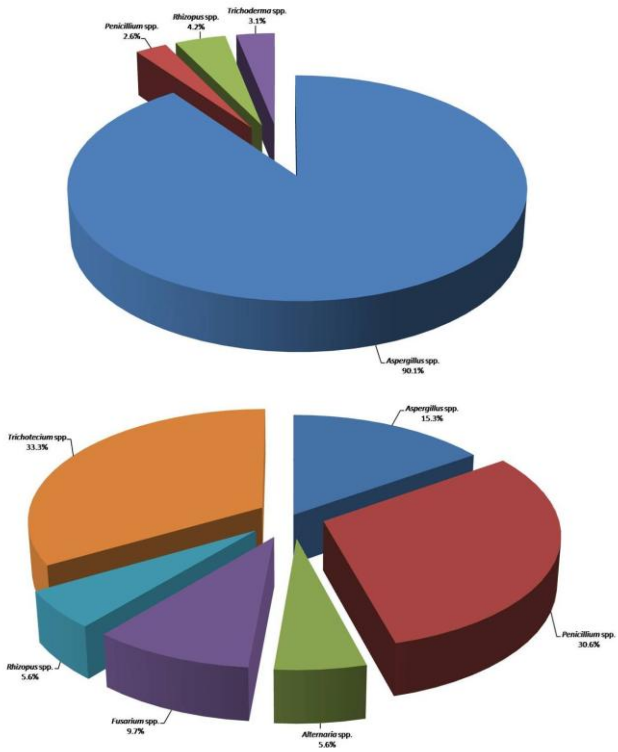

Figure 4 **Frequency of isolated fungi from rural (top) and urban (bottom) schools in June 2016**

Numbers of fungal genera decrease during the 3-month sampling period from eleven to six in urban location, and from six to four in rural location. *Aspergillus* spp *Penicillium* spp., *Rhizopus* spp, *Fusarium* spp., plus *Trichoderma* spp. were commonly isolated in the locations.

The quality of indoor air from the educational institutions depends on many factors, including cleaning procedures, air ventilation, temperature, relative humidity, geographical region and specific reservoirs of contamination (e.g. agricultural activities). Statistical significant correlation between temperature and fungal colony numbers was found. Corden J.M. and Millington W.M. (2001) reported that higher temperatures can induce fungal spore concentrations and increasing of fungal contamination risk.

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

CONCLUSIONS

It has been shown that total fungal spores exceeded the recommended upper limit for indoor concentrations (>700 CFU/m^3) only in case of one classroom environment from rural area, in May and June 2016. The fungal contamination has potential to develop negative health effects to the occupants. The presence of a good ventilation system to eliminate some indoor sources inside building is required. In case of one classroom from the primary school located in the urban area the airborne fungal spore concentrations was between 500 and 700 CFU/m^3, and is registered with intermediate level of contamination.

In all other location from urban and rural areas, the airborne fungal spore concentrations were under 500 CFU/m^3, so that no negative health effects for occupants were expected.

The dominant genus in the rural schools was represented by *Aspergillus*, while in the urban schools *Penicillium* was the predominant genus.

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