COMPARATIVE STUDIES OF THE FUNGAL AEROSOLS IN SOME EDUCATIONAL BUILDINGS FROM URBAN AND RURAL ENVIRONMENTS IN NEAMT COUNTY, ROMANIA

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Abstract

The levels of airborne microbe in different educational buildings from urban and rural environments are unknown previously in Neamt County, Romania. Air contamination by airborne fungal spores was investigated in 2017 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 urbanization 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 most common culturable airborne fungal genera were *Penicillium*, *Aspergillus* and *Cladosporium* (89.4, 7.3 and 1.4% of the total, respectively).

Our results showed that fungal spores concentrations in the educational institutions air was situated at low level, with exception of one kindergarten classroom from the rural area, which has potential to develop adverse health effects to the occupants (957 CFU/m^3 air).

Key words: indoor air quality, airborne microbe, educational buildings, urban vs. rural area

Indoor air quality resulting from high levels of fungal spores and other airborne structures become a critical issue in the world because are ubiquitous. Humans spend approximately 80% of their time indoors 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 (Col B.G., Aksu H., 2007; Lipsa *et al*., 2016). According to Li and Kendrick (1995), while the relationship between respiratory illness and fungal spore exposure is clear, its mechanism is unknown. Moreover, Jo and Seo (2005) indicated that allergic reactions increased in school children when they were exposed to fungal spores and other airborne structures. 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). Additionally, there is a link between indoor fungal concentrations and seasonal variation, along with other geographical climatological and meteorological factors (Das S., Gupta-Bhattacharya S., 2008). According to Dassonville et al. (2008) buildings physical condition (age, structure, heating and ventilation systems) and number of inhabitants also affect indoor air quality and airborne fungal concentration.

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).

The objective of this study is to calculate the number of colony as CFU/m^3 on different cultivated media, and to identify the fungal genera that can cause an allergic response in educational institutions placed in urban and rural locations of Neamt 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.

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MATERIAL AND METHOD

Air contamination by airborne fungal spores in five educational institutions placed in urban and rural locations of Neamt County, Romania was investigated in 2017 over a period of 3 months (April-June) using the Petri plate gravitational settling (passive) method. This method is valued for precision in identifying captured organisms and for selectivity when sampling is aimed at a group of organisms.

The used method supposes 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 4860 fungal colonies in 480 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.

Seven fungal genera were isolated and identified in all three locations. In addition, a total of 79 nonsporulating colonies were registered.

Penicillium spp. was predominant (89.4%), followed by *Aspergillus* and *Cladosporium* genera (7.3 and 1.4%, respectively). *Penicillium* spp. was the dominant genera at all sampling stations.

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

Fungi isolated from sampled air are presented in *Figure* 1.

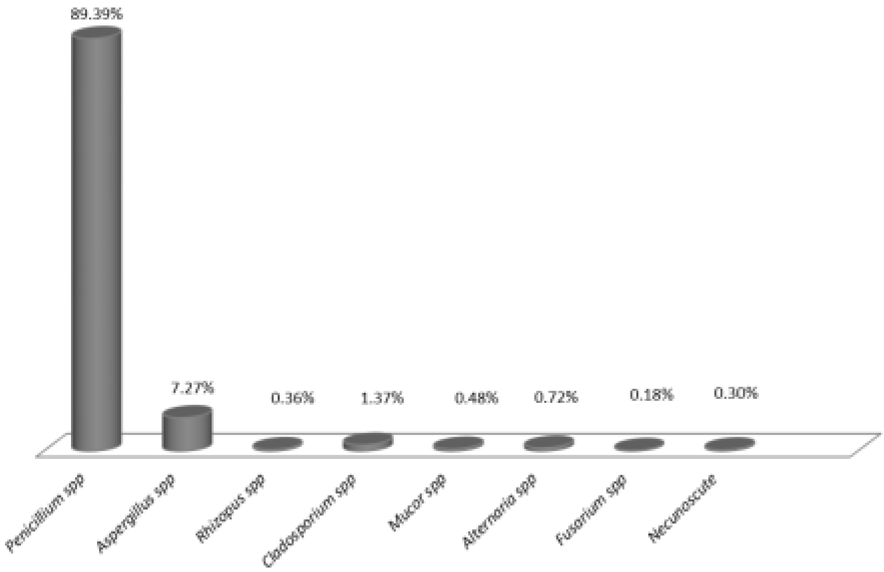

Figure 1 **Frequency of isolated fungi**

The concentration and occurrence of indoor air mycoflora in the present study was congruent with earlier studies conducted in different climate regions (Lipsa *et al*, 2014, 2016; Cheong and Neumeister-Kemp, 2005): 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 (98.7% – 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 196 to 987 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 220 to 987 CFU/m^3 in April, from 210 to 980 CFU/m^3 in May and from 196 to 904 CFU/m^3 in June, respectively. The peak of total fungal prevalence was recorded in April (40.3%), followed by May and June (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] $X \pm s$ (CFU/m^3)	Level of contamination
Primary school classroom (urban location)	April	232 ± 10	Low
	May	210 ± 11	Low
	June	196 ± 13	Low
Kindergarten classroom (rural location)	April	987 ± 10	High
	May	980 ± 17	High
	June	904 ± 15	High
Kindergarten classroom (urban location)	April	463 ± 18	Low
	May	493± 10	Low
	June	350 ± 19	Low
High school classroom (rural location)	April	290 ± 15	Low
	May	298 ± 12	Low
	June	379 ± 11	Low
High school classroom (urban location)	April	220 ± 13	Low
	May	295 ± 12	Low
	June	351 ± 17	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 kindergarten 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 kindergarten classroom from the rural location had higher values over the 3-months period (987, 980 and 904 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 (χ^2) test was applied to determine if there were any differences between the sampling period (April-June 2017) 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 Neamt 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 high school in both locations (not statistically significant): in rural area ranged from 290 to 379 CFU/m^3, while in the urban area the airborne mycota varied from 220 to 351 CFU/m^3.

The difference was statistically significant at 0.05 level in case of the kindergarten classrooms: in rural location the values ranged from 904 to 987 CFU/m^3 with possibilities to affect human health and in urban location the airborne mycota varied from 350 to 563 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, Aspergillus*a and *Cladosporium*, and several other fungal genera were recorded in the air of different educational institutions placed in urban and rural locations of Neamt County, Romania. These fungi are harmless for healthy people, but they may be dangerous for people from risk groups (with respiratory diseases). *Penicillium* spp. was predominant (89.4%), followed by *Aspergillus* and *Cladosporium* genera (7.3 and 1.4%, respectively). *Penicillium* spp. was the dominant genera at all sampling stations.

Indoor concentrations of fungal spores in the kindergarten 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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