THE OCCURRENCE OF MICROSCOPIC FUNGI IN AIR SAMPLES FROM DIFFERENT EDUCATIONAL INSTITUTIONS FROM IAŞI, ROMANIA

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Abstract

Contamination of the air by fungi in three educational institutions placed in different location of Iasi City, Romania was investigated in 2013 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 identification of the fungi was made according to their microscopic properties and through references. The moulds most commonly isolated in all three locations were *Penicillium* spp, *Aspergillus* spp and *Alternaria* spp (58.7, 17.6 and 10.4% 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 laboratory for students, which has potential to develop adverse health effects to the occupants.

Key words: indoor air - fungal spores density - educational institution

Fungal spores and other airborne structures are ubiquitous in the indoor environments. Many researchers have reported that indoor air pollution with fungal flora can produces many undesirable health effects of different types, ranging from sensory annoyance, discomfort, respiratory disease (e.g. asthma), allergic reactions, and other adverse health effects which are associated with sick building syndrome (Daisey et al., 2003; Karwowska, 2003; Ulea et al., 2009).

Some species from *Penicillium*, *Aspergillus*, *Cladosporium* and *Alternaria* fungi genera can cause extreme allergic reaction or respiratory and other related diseases in humans. The physicall condition from different building structures, such as humidity level, temperature and the presence of organic and anorganic substrates influence the fungal concentration in their indoor air. Collection of airborne spores can provide valuable information about the indoor air quality in many types of buildings (Hu et al., 2002).

The aims of this investigation were to monitor and compare the densities and distribution of indoor airborne fungal spores that can cause an allergic response in three educational institutions placed in different location from Iaşi, Romania. The investigation followed the Petri plate gravitational settling (passive) method of sampling to help establish standards for future references.

MATERIAL AND METHOD

The fungal composition on the air from different educational institutions located in the city of Iaşi (NE Romania) was investigated. Areas monitored were two laboratories for students from the university campus, one classroom from a high school and one classroom from a nursery school.

Samples were collected from April to June 2013. Air samples from all location were taken using the Koch sedimentation method, which 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

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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 DISCUSSIONS

In the indoor air of three different educational institutions a total of 4671 fungal colonies in 728 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.

Eleven fungal genera were isolated and identified in all three locations. In addition, a total of 102 nonsporulating colonies were registered.

Penicillium spp. was predominant (58.7%), followed by *Aspergillus*, *Alternaria* and *Cladosporium* genera (17.6, 10.4 and 4.3%, respectively). *Penicillium* spp. was the dominant genera at all sampling stations.

In contrast, *Geotrichum* and *Stemphylium* genera were found only in the phytopathological laboratory and were also registered with less than one percent. Nonsporulating fungi were a constant presence in all location during this study.

Fungi isolated from sampled air are presented in Figure 1.

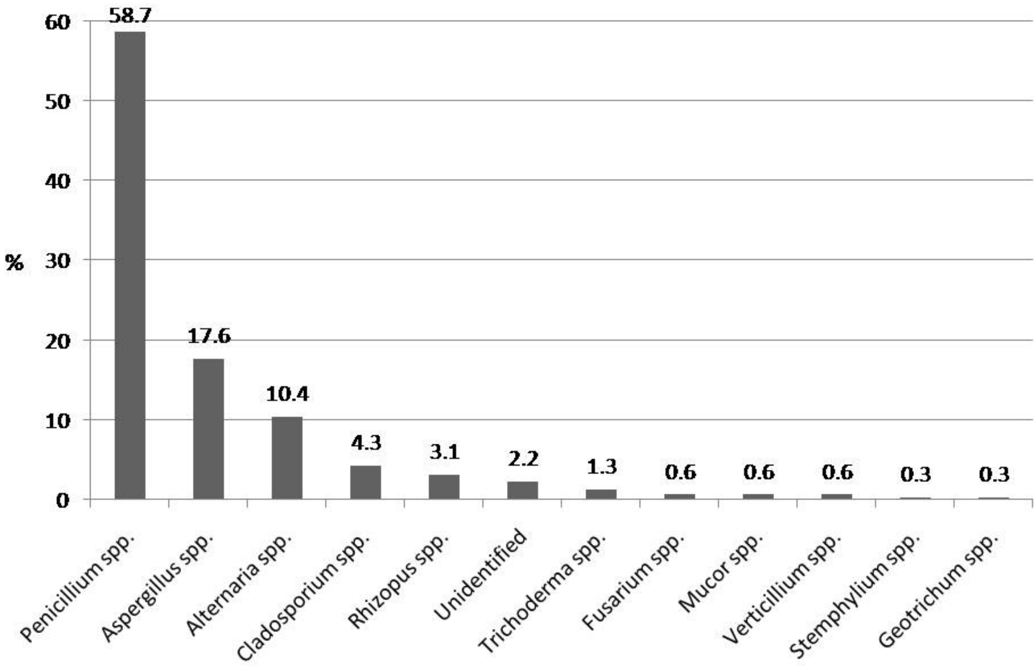

Figure 1 **Frequency of isolated fungi**

Hu et al. (2002) reported that *Penicillum*, *Cladosporium* and *Aspergillus* spores represent the most frequent and predominant aeroallergens in school buildings. The most common indoor fungus are *Cladosporium*, *Alternaria*, *Aspergillus* and *Penicillium* species (Sarica et al., 2002). These results are compatible with our findings; the above mentioned genera were found in high frequency in our work (91.0% – 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 120.0 to 831.0 CFU/m^3, and generally 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 120 to 748 CFU/m^3 in April, from 146 to 831 CFU/m^3 in May and from 138 to 803 CFU/m^3 in June, respectively. The peak of total fungal prevalence was recorded in May (36.5%), followed by June 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
Microbiology laboratory	April	169 ± 10	Low
	May	193 ± 13	Low
	June	226 ± 19	Low
Phytopathology laboratory	April	748 ± 23	High
	May	831 ± 29	High
	June	803 ± 30	High
High school classroom	April	386 ± 25	Low
	May	533 ± 10	Intermediate
	June	378 ± 21	Low
Nursery school classroom	April	120 ± 20	Low
	May	146 ± 15	Low
	June	138 ± 12	Low

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

Fungal concentrations were minimal in nursery school classroom and microbiological laboratory, and varied between minimal and intermediate level in the high school classroom. The fungal contamination in phytopathological laboratory 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 is required.

Only laboratory of phytopathology has higher values over the 3-months period (748, 831 and 803 CFU/m^3), which could be explained by the higher number of students, as well as room characteristics, cleaning procedures and lower ventilation. In May 2013, local fungal air density in case of high school classroom was higher than the sanitary level of 500 CFU/m^3, and is registered with intermediate level of contamination.

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 2013) and fungal densities. Statistical significantly difference was found only in case of one laboratory for students ($p < 0.05$).

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). According to Corden and Millington (2001), higher temperatures 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

Indoor concentrations of fungal spores in phytopathological laboratory 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.

In case of nursery school classroom and the high school classroom the airborne fungal spores concentrations was lower than 500 CFU/m^3, so that no negative health effects for occupants were expected.

The maximal number of fungal genera was found in the laboratory of phytopathology: *Penicillium*, *Alternaria*, *Aspergillus*, *Cladosporium*, *Rhizopus Trichoderma*, *Fusarium*, *Mucor*, *Verticillium*, *Stemphylium* and *Geotrichum*.

The fungal genera *Penicillium*, *Aspergillus* and *Alternaria* were the most prevalent with 58.7, 17.6 and 10.4% of the total, respectively.

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