ABSTRACT: Fungal spores can cause a range of health problems in humans such as respiratory diseases and mycotoxicoses. Since children are the most vulnerable, the presence of fungal spores in the facilities of preschool and school institutions should be investigated readily. In order to estimate air contamination by fungal spores, air sampling was conducted in eight facilities of the preschool institution in Novi Sad during February and March, 2007. Sedimentation plate method was used for the detection of viable fungal spores, mostly being members of subdv. Deuteromycota (Fungi imperfecti).

In 32 samples a total of 148 colonies were developed, among which five genera were identified: *Penicillium*, *Cladosporium*, *Aspergillus*, *Alternaria* and *Acremonium* while non-sporulating fungal colonies were labeled as sterile mycelia. Most frequently recorded genera were *Penicillium* with 46 colonies and *Cladosporium* with 44 colonies. The genera *Aspergillus* and *Alternaria* were represented with 3 colonies each and *Acremonium* with only 1 colony. The greatest number of colonies emerged in the samples from the day care facilities “Vendi” (58 colonies) and “Panda” (49 colonies). Most diverse samples were obtained from the day care center “Zvončica”, with presence of all identified genera.

These results showed notable presence of fungal spores in the indoor air of Preschool institution facilities and indicated the need for further, more complete seasonal research. Obtained information is considered useful for the evaluation of potential mycofactors that endanger health of children.

KEY WORDS: children, day care, fungi, indoor air, Preschool institution, spores

INTRODUCTION

Fungi are ubiquitous microorganisms that are known as important bio-contaminants of the indoor environment (Aydogdu et al., 2005; Aydogdu and Asan, 2008). Their spores are easily liberated in the air and may cause diverse health problems in humans such as: irritations, infections, allergies and toxicoses (Mentrez and Foard, 2004; Stetzenbach et al., 2004). The most common sources of airborne fungal particles indoors are outdoor air and fungal growth encouraged by favorable environmental factors...
(dampness, high temperature and the presence of organic and inorganic substrates) (Pessi et al., 2000; Liao et al., 2004).

Children spend most of their time inside the rooms of educational institutions which are common sites of air quality problems (Daisey et al., 2003; Aydogdu et al., 2005). Therefore, a good indoor air quality in buildings of day care centers and schools is essential for children’s safety. Various studies (Ferng and Lee, 2002; Koskinen et al., 2004; Zuraiim et al., 2009) showed that poor environmental conditions in day care centers can threaten the health of preschool children. Over the last decade, potential health consequences of fungal contaminants in indoor environment resulted in an increased interest for determination of potential exposure levels of these contaminants (Dotterud et al., 1996; Haverinen et al., 1999; Smedje and Norback, 1999; Su et al., 2001; Daisey et al., 2003).

Aerobiology studies the release of biological particulate matter into the atmosphere, its atmospheric transport, deposition and re-suspension (Schefinger et al., 2013). In Serbia, aerobiological research is focused on the studying of outdoor environment, in particular airborne pollen (Sikoparja et al., 2011). Furthermore, no aeromycological research of the indoor environment in school and preschool facilities has been conducted so far. The aim of this study was to investigate the presence of viable fungal spores in the indoor air of the day care facilities of preschool institutions in Novi Sad (Serbia).

**MATERIAL AND METHODS**

Airborne fungal spores were collected in February and March 2007, in eight facilities (child day care centers) of the preschool institution "Radosno detinjstvo" in Novi Sad: “Zvončica”, “Plavi zec”, “Vila”, “Petar Pan”, “Dunavski cvet”, “Maslačak”, “Panda” and “Vendi”. Each of these facilities was visited once. The presence of airborne fungal spores was investigated in the room where children spent most of the time during their stay in the day care center. The visited child day care centers were situated in different parts of the city. They also varied according to building age and size, number of children and staff, as well as the sizes of the investigated rooms. All of the facilities were equipped with a central heating system and ventilation was provided naturally through windows. Floors were predominantly covered with carpet.

Sampling was performed before usual daily activities, between 6.30 and 8.30 am. Sedimentation plate method was used, with malt extract agar (“Torlak”, Belgrade, Serbia) as the growth media. Four Petri plates were placed 1 m above the ground level in different parts of each investigated room and were exposed to air for 10 minutes by removing the cover lid. Sampled plates were incubated at 25°C for 5 to 7 days. After the incubation period, grown colonies were identified and counted. Their number corresponded to the number of viable airborne spores and it was expressed as the number of colony forming units (CFU) per plate.

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The use of sedimentation plate sampling method enabled the recovering of cultivable fungi which easily produce reproductive structures necessary for proper determination (mostly belonging to the group Deuteromycota or *Fungi imperfecti*). Determination of fungal genera was based on micro- and macro-morphology, as well as reverse coloration and surface coloration of colonies, according to Watanabe (2002). Fungal colonies which did not sporulate after 10 days were recorded as sterile.

RESULTS

From the indoor air of eight analyzed sites, a total of 148 fungal colonies were isolated in 32 sampled Petri plates. The sterile colonies dominated with 51 CFU (34%). Sporulating fungi belonged to five genera of filamentous fungi (*Penicillium, Cladosporium, Aspergillus, Alternaria, Acremonium*). The most widespread genera, *Penicillium* and *Cladosporium*, were present in all of the investigated rooms (Tab. 1). They were also the most numerous with 46 CFU (31%) and 44 CFU (30%), respectively. *Aspergillus* and *Alternaria* were represented with 3 CFU (2%), while *Acremonium* was recorded with only one colony (1%) (Fig. 1).

Tab. 1. – Presence of isolated fungal genera in the investigated facilities

<table>
<thead>
<tr>
<th>Identified fungal genera</th>
<th>Number of colony forming units (CFU)</th>
<th>Total number</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vendi</td>
<td>Panda</td>
<td>Maslačak</td>
</tr>
<tr>
<td>Non-sporulating colonies</td>
<td>27</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td><em>Penicillium</em></td>
<td>26</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td><em>Cladosporium</em></td>
<td>5</td>
<td>28</td>
<td>2</td>
</tr>
<tr>
<td><em>Aspergillus</em></td>
<td>/</td>
<td>/</td>
<td>1</td>
</tr>
<tr>
<td><em>Alternaria</em></td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td><em>Acremonium</em></td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The total counts of CFU per each examined facility are shown in Fig. 2. Day care centers “Vendi” and “Panda” stand out with the highest number of detected viable airborne fungal spores – 58 CFU and 49 CFU, respectively. The number of fungal colonies developed in the samples from other facilities was notably lower (“Maslačak” – 11 CFU, “Zvončica” – 9 CFU, “Dunavski cvet” and “Plavi zec” – 6 CFU each, “Vila” – 5 CFU). The lowest number of CFU was obtained from “Petar Pan” (4). With five isolated genera, the diversity of recorded colonies was the highest in the day care center “Zvončica”, while in “Dunavski cvet”, “Panda” and “Vendi” only *Cladosporium* and *Penicillium* were identified (Tab. 1).
Fig. 1. – Distribution of detected fungal genera in the total number of developed colonies (CFU)

Fig. 2. – Total number of fungal CFU isolated in each examined children day care facility
DISCUSSION

The most common airborne fungal spores in the indoor environment belong to the following genera: *Cladosporium, Penicillium, Alternaria* and *Aspergillus* (E t z e l et al., 1998; R o l k a et al., 2005; K a l y o n c u, 2008) which is in accordance with our results. Moreover, *Penicillium* and *Cladosporium* which were present in all of the investigated sites and dominated among other fungal genera detected, were found to be predominant in many other indoor aeromycological studies (H u et al., 2002; S a r i c a et al., 2002; S h e l t o n et al., 2002; L e e et al., 2006; A y d o g d y and A s a n, 2008). Similar to our study, the research conducted by A y d o g d u et al. (2005) in the indoor air of 5 schools in Turkey recognized *Alternaria* and *Aspergillus* as the most frequent, followed by *Penicillium* and *Cladosporium*. *Acremonium* was the only genus in our research that was registered in a single sample with one colony. Other investigations of indoor air, in buildings with different purpose, have also recorded presence of *Acremonium* in small percentage (S h e l t o n et al. 2002; L u g a u s k a s and K r i k s t a p o n i s, 2004; R o l k a et al., 2005) but there was an indication of possible sample contamination. The occurrence and airborne spore concentrations in indoor environment show notable seasonal variability but also greatly depend on: geographical, climatic and meteorological parameters, surrounding vegetation, specific sources of indoor contamination, as well as the sampling time and procedure (A y d o g d u et al., 2008). Many of the airborne spores recorded in the indoor environments, particularly those of *Cladosporium* and *Alternaria*, originate from outdoor sources (H í n d y and A w a d, 2000). In contrast, species of *Aspergillus* and *Penicillium* are mainly derived from indoor sources (A y d o g d u et al., 2005).

The highest number of CFU, reported from day care centers “Vendi” and “Panda”, indicated notably higher amount of viable airborne fungal spores compared to the other investigated facilities. Main contributor to the high number of CFU in samples from the day care “Vendi” was the genus *Penicillium* (45%). This might indicate that the applied ventilation methods were not adequate for the given room, resulting in promotion of fungal proliferation inside the building. As opposed to this, *Cladosporium* was the main contributor to the high number of CFU recorded in the day care center “Panda” (57%). This result might be related to the immediate vicinity of a park area (Futoski park), which could be a source of *Cladosporium* – the mold usually developed in soil, on vegetation and decaying plant material (E l l i s, 1971). However, the applied sampling method could also have affected high concentrations of *Cladosporium* and *Penicillium* in facilities “Vendi” and “Panda”. With the application of culturing methods in aeromycological research, it is possible to overestimate the importance of these molds since their species grow easily in culture plates (A y d o g d u et al., 2005).

The Sedimentation Plate Method (also known as the gravity or settling plate method) was used for its practicality, low cost and the ease of use. It is a passive sampling technique that relies on particles settling out of air and is useful for the enumeration of bacteria, yeast and fungal spores (A t l a s and
Airborne fungi identified in the selected facilities of the preschool institution “Radosno detinjstvo” may affect the health of children and employees. According to Pastuszka et al. (2000), Cladosporium, Alternaria and Aspergillus represent the main group of airborne molds to which children may be sensitized and which may cause allergic symptoms. Alternaria and Cladosporium are considered to be the most important aeroallergens in the outdoor air, as well as the cause of mycotoxicosis in humans. Frequent exposure to airborne aeroallergens of the genus Alternaria can result in respiratory arrest in children and adolescents with asthma (O’Hollaren et al., 1991). Spores of Aspergillus and Penicillium represent the most widespread aeroallergens in the world (Aydogdu et al., 2005) and are recently recognized as the most important allergens of indoor air (Shen et al., 2000; Fisher and Dott, 2003; Kasznia–Kocot et al., 2007). Records on the Aspergillus and Penicillium viable fungal spores could indicate the presence of molds that are known to be responsible for mycotoxin production and emission of volatile organic compounds – VOC’s (Kasznia–Kocot et al., 2007).

CONCLUSION

Presence of airborne fungal spores in buildings of educational facilities can affect the health of children and staff. Results of our study indicated notable presence of viable fungal spores in the air of examined preschool facilities, hence the need for further, more detailed investigations. Seasonal air monitoring of fungal spores and application of more accurate, quantitative sampling method (the use of vacuum/culture samplers) would provide a more detailed insight about indoor air quality. Information obtained by such studies is required for the assessment of health-threatening factors, medical evaluation of children and staff health condition and for the implementation of remediation procedures.

REFERENCES


МОНИТОРИНГ СПОРА ГЉИВА У ОБЈЕКТИМА ПРЕДШКОЛСКЕ УСТАНОВЕ У НОВОМ САДУ

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Резиме

Споре гљива могу бити узрочници нiza различитих здравствених проблема код људи, као што су респираторна обољења и микотоксикозе. С обзиром на то да деца представљају једну од најугроженијих група, било би пожељно редовно преравати присуство спора гљива у просторијама предшколских и школских установа. У циљу процене квалитета ваздуха и евентуалног присуства спора гљива, током фебруара и марта 2007. године извршено је узорковање ваздуха у осам објеката Предшколске установе у Новом Саду. Употребљена је метода седиментационих плоча, а у центру истраживања биле су гљиве које су претежно припадни подраздела Deuteromycota (Fungi imperfecti).

У 32 узорковане петри плоче развило се укупно 148 колонија различитих родова гљива, од којих је укупно пет детерминисано: Penicillium, Cladosporium, Aspergillus, Alternaria и Acremonium. Гљиве чије колоније нису споруслисе, нису могле бити детерминисане и означене су као стерилне. Доминантни родови били су Penicillium са 46 колонија и Cladosporium са 44 колоније, док су родови Aspergillus и Alternaria били заступљени са по 3 колоније, а род Acremonium забележен је само са 1 колонијом. Највећи број колонија јавио се у узорцима из обданишта „Венди“ (58 колонија) и „Панда“ (49 колонија). Објекат „Звончица“ био је најразноврснији на основу присуства детерминисаних родова (забележено присуство свих 5 родова).

Добијени резултати указују на значајно присуство спора гљива у ваздуху испитиваних објеката и на потребу за даљим, потпунијим испитивањима. Информације добијене оваквим истраживањима изузетно су корисне за процену потенцијалних фактора који угрожавају здравље деце.

КЉУЧНЕ РЕЧИ: деца, гљиве, ваздух, предшколске установе, просторије, споре

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