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A year-round investigation of indoor airborne fungi in Croatia

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This study assessed the composition of aeromycota at a grain mill and four dwellings (two apartments and two basements) as well as in outdoor air during one year in Zagreb, Croatia. The incidence of Aspergilli from sections *Flavi*, *Nigri*, and *Versicolores* was also assessed. Airborne fungi were collected using an air-sampler and DG-18 agar plates. The average concentrations of airborne fungi in the grain mill ranged from 14,310 to 40,000 cfu m⁻³, which was above the hazardous level (10⁴ cfu m⁻³), whereas the values statistically estimated using Feller's correction were up to six times higher. Concentrations in the apartment (163-1244 cfu m⁻³) were lower than in outdoor air (286-2090 cfu m⁻³) and lower than in the basement (697-1203 cfu m⁻³), except in the warmer period of the year when they were similar. The most abundant species throughout the year were *Cladosporium* spp. (90-100 %), *Penicillium* spp. (40-100 %), and *Alternaria* spp. (10-100 %), which are common for temperate climates. Aspergilli from the *Flavi* (50-100 %) and *Nigri* (15-40 %) sections as well as *A. ochraceus* (15-60 %) and *Eurotium* spp. (85-100 %) were the most abundant at the grain mill and were rarely found in outdoor air. In the basement, Aspergilli (*Versicolores*) were more abundant than in the apartment. The excess of aeromycoparticles in the grain mill throughout the year may have represented a serious health risk to mill workers. This is the first Croatian one-year study of indoor airborne fungi in a grain mill and dwellings; however monitoring should continue over a longer period.

KEY WORDS: *Flavi*; *grain mill*; *Nigri*; *residential areas*; *Versicolores*

Many studies have recently investigated the levels of airborne fungi in indoor environments and their role in the initiation and/or progression of chronic respiratory diseases like asthma and chronic rhinosinusitis (1-6). In addition, the European Environment Agency (EEA) has published a report on air quality in Europe in 2012 (7), where the most severe health effects were attributed to air pollution by particulate matter (PM). The highest mortality caused by PM clearly stems from particle fractions 2.5 µm in diameter, which represents 40-80 % of PM mass concentration in ambient air in Europe. Fungal spores are regularly found in outdoor and indoor air

as part of PM. Their size ranges usually from 1 to 10 µm with variations even among the same species. Besides fungal spores, fragments of mycelium may also be released in the air with sizes even smaller than fungal spores (8-10). Inhalation of these particles represents a threat to human health due to possible deposition in the lower respiratory system and interactions with lung tissue. No uniformness in the suggested guidelines for acceptable levels of fungi in indoor environments has yet been achieved and there is no dose-response relationship between the concentration of airborne fungi and adverse health effects. Moreover, the immunocompetence of an

exposed individual plays a crucial role in both the type and intensity of possible effects.

Airborne fungi are of special concern in industrial environments such as grain mills or agricultural warehouses, where their concentration may be up to a hundred thousand times higher than in outdoor air. It has been suggested that occupational concentrations of airborne fungi above 10^4 cfu m^{-3} should be considered a health hazard in non-sensitized subjects, although this does not mean that such a working environment will necessarily lead to any respiratory symptoms (9-13). Considering the limited number of studies addressed at the fungal burden in occupational environments and urban homes in our country, the aim of the present study was to investigate the composition and concentration of aeromycota in industrial and residential environments in Zagreb, Croatia, for which purpose measurements were performed in a grain mill, an apartment and a basement.

Special attention was paid to the occurrence of Aspergilli from sections *Flavi*, *Nigri*, and *Versicolores* for several reasons. In immunocompromised patients, most invasive respiratory infections are caused by *Aspergillus fumigatus*, *A. flavus*, *A. terreus*, and *A. niger sensu lato* (14, 15). *Aspergillus flavus* and *A. parasiticus* from the *Flavi* section are important producers of the highly toxic and carcinogenic aflatoxin B1 and these species grow on almost all crops (16). *Aspergillus versicolor* is the most frequently reported *Versicolores* fungus from damp indoor environments and its presence might be related to Sick Building Syndrome (17-20). Moreover, it represents a special threat for human health due to its very small spores and release of mycellium fragments (10) as well as ability to produce sterigmatocystin (21, 22). Finally, *Aspergillus* section *Nigri*, or black aspergilli, which can easily be isolated from occupational and residential environments, have been reported to produce ochratoxins and fumonisins (23).

This is the first quantitative and qualitative assessment of indoor airborne fungi in a grain mill and dwellings over a one-year period in Croatia.

MATERIALS AND METHODS

Sampling and determination of airborne fungi

Airborne fungi were collected over a one-year period (2012) in two-month intervals at a grain mill

(GM) situated near Zagreb, Croatia, and in residential locations which included two apartments (AP) and two basements (BS). The differences between the chosen apartments and basements were considered irrelevant for this study. Samples of outdoor air (ODA) were also collected. The floor area of the APs was approximately 70 m^2 each, both inhabited and without visible mould growth. The BS's were situated in the same dwellings as the APs. These were unoccupied and served as a repository for the inhabitants. Visible mould growth as well as typical mouldy odour was observed in both basements. The GM was situated in an industrial area 40 km outside Zagreb and served for processing cereals, mainly corn and wheat, as well for short-term flour storage. It was organised as a building consisting of a big reception and depository at the ground floor, milling site located at the first floor, and sieving site located at the second floor. The air was quite saturated with grain particles, but no mouldy odour was observed. Samplings were done in the middle of the working week in the morning during the most intensive and constant milling operations at 20 locations, including site of grain/flour exchange, site of flour storage, site of sieving, and site of milling. Twenty samples were taken from the APs at locations: kitchen, dining room, living room, bedroom, and bathroom. Samples were also taken from 10 locations in the BS, two at each site. The total number of samples was 420. Air temperature ($^{\circ}C$) and relative humidity (%) were measured by thermo-hygrometer (Boneco, Widnau, Switzerland) at every location during each sampling. Fungi were sampled 1 m above ground using a Mas 100 Eco air sampler (Merck, Darmstadt, Germany) with 400 holes (hole to agar impactor) and dichlorane 18 % glycerol agar (DG-18) plates (25). The impaction velocity of the sampler was approximately $10.8 m s^{-1}$ and airflow rate $100 L min^{-1}$. Because of the high contamination level, a volume of 10 L (sampling time 6 s) was chosen for sampling in the GM, while $50 L min^{-1}$ (sampling time 30 s) was applied at the other sampling locations. After field sampling, the plates were incubated for 5 days at 25 ± 2 $^{\circ}C$, after which the developed fungal colonies were counted. The concentrations of airborne fungi were expressed as colony forming units (cfu) per volume of air sampled (expressed in cubic meter), i.e. cfu m^{-3} . Feller's correction (probable statistical total) was applied to all of the samples (24). The airborne fungi were identified on the basis of their macro- and microscopic characteristics after subculturing on Czapek, Malt Extract and Potato Dextrose agar (Fluka,

Sigma-Aldrich, Steinheim, Germany), according to the manuals (25, 26). In order to separate the aspergilli of interest, they were isolated on Czapek Yeast Agar [CYA, (26)] and Malt Extract Agar (MEA; Becton Dickinson, MD, USA) and incubated at 25 °C in the dark for seven days (*Aspergillus* section *Flavi* and section *Nigri*) and ten to fourteen days for *Aspergillus* section *Versicolores*. The identification of *Aspergillus* species was conducted according to Pitt and Hocking (25) and de Hoog et al. (26).

Statistics

Concentrations of airborne fungi at each location are represented as mean±SD of cfu m⁻³. For each plate, Feller's correction (probable statistical total) was applied - as the number of viable particles impinged on a plate increases, the probability of the next particle going into an "empty hole" decreases. The probable number of viable particles calculated from Feller's formula ($Pr=N \frac{1}{N} + \frac{1}{N-1} + \frac{1}{N-2} + \frac{1}{N-r+1}$), given by the manufacturer (Merck KgaA, Darmstadt, Germany; Pr=probable statistical total; r=Number of cfu counted; N=total number of holes in the sampling head).

The Kolmogorov-Smirnov test was used to verify whether the variables were normally distributed. Concentrations of airborne fungi were logarithmically transformed to normalise distribution. When normality was achieved, ANOVA and Tukey multiple comparison tests were used. Otherwise, Kruskal-Wallis test was applied, followed by Dunn's multiple comparison test. Spearman's correlation coefficients between average fungal concentrations at each location and ambient parameters (temperature and relative humidity) were also calculated.

RESULTS AND DISCUSSION

Data related to average concentrations of airborne fungi (cfu m⁻³) and average temperature and relative humidity specific for each sampling site and each period of year are presented in Table 1. Levels of aeromycota in the GM (up to 40,000 cfu m⁻³) were significantly higher during the whole year than at the other sampling sites ($p<0.0001$). Statistically corrected concentrations at the GM were up to 6 times higher than the measured values. Compared to observations from studies on similar occupational environments such as another grain mill [max. concentration

1.7x10⁴ cfu m⁻³ (27)], rice mill [mean 13.71x10³-48.42x10³ cfu m⁻³; (28)], outdoor agricultural area [mean 72-1796 cfu m⁻³; (29)], agricultural non-point source during wheat harvesting season [mean 10⁵-10⁶ cfu m⁻³; (30)], or sawmills (mean 1700-7300 cfu m⁻³; (13)) our analysis showed an overload with airborne fungi in the GM throughout the entire year. Taking into account statistically estimated values (up to 2.6x10⁵ cfu m⁻³), levels of airborne fungi in the GM were 20 times higher than the occupational concentration of airborne fungi considered hazard to human health (>10,000 cfu m⁻³). Variations of airborne fungi levels in the GM showed no correlation to both temperature and relative humidity (Table 2). This was not surprising because the measured levels were the result of fungal contamination of grains that developed during storage, before delivery to the GM.

Seasonal variations of airborne fungi in the AP and BS did not exhibit a similar pattern. In the BS, airborne fungi exceeded 1000 cfu m⁻³ from May to November while the levels in January, March, September, and November were significantly higher as compared to the AP ($p<0.001$). Variations of airborne fungi levels in the BS also did not show a correlation to temperature and relative humidity (Table 2). Taken together, these observations suggest a more pronounced fungal contamination in the BS probably due to poor maintenance and ventilation.

Seasonal variations of airborne fungi levels in the AP and ODA showed a similar pattern and positively correlated to temperature changes ($R=0.6661$, $R=0.5409$) but not to relative humidity. However, the levels of aeromycota in the AP obtained in January, March, July, and September were significantly lower than in ODA ($p<0.001$).

The observed concentrations of fungi in ODA (240-2090 cfu m⁻³) were twice as high as the concentrations observed in similar investigation conducted in 2002/03 in Austria [100-1000 cfu m⁻³; (31)]. In that same investigation, apartments without visible mould growth had a maximum of fungal spore concentration of 300 cfu m⁻³. The highest observed concentration in the AP from our investigation was 13 times higher (4000 cfu m⁻³) and more than 16 times higher (5060 cfu m⁻³) in the BS. Our previous year-round investigation, which was also performed in 2002/03, at outdoor air locations in Zagreb (32) showed that airborne fungi peaked in August and September (up to 400 cfu m⁻³), which was five times lower than the levels measured in that same period in this study. Since Zagreb and the Austrian region

Table 1 Quantitative composition of airborne fungi throughout one year at locations as follows: grain mill (GM), apartments (AP), basements (BS), outdoor air (ODA)

Sampling period	Concentration of airborne fungi (cfu m ⁻³)		Min-max*	Temperature (°C)	Relative humidity (%)
	(mean±SD)	(mean±SD)*			
	Absolute values	Calculated values			
GM					
January	25630±16440*	161600±127186*	4000-40000	6.2	34
March	38250±7826*	249925±57579*	5000-40000	8.0	29
May	26745±16719*	160725±128276*	4100-40000	24	36
July	40000±0*	262800±0*	40000-40000	24.4	42
September	14310±11222*	48980±92190*	7400-40000	25	31
November	6270±2661*	6940±3241*	1700-12600	10.2	66
AP					
January	163±102.7	164±105.8	20-460	18.3	41
March	182±123.3	184±128.7	40-520	18.8	36
May	1244±1014	1462±1460	320-4000	23.9	42
July	997±448.5	1078±506.3	140-1660	26.9	37
September	462±176.9	477±192	200-960	28.5	30.5
November	223±107.1	224±109.1	40-420	18.6	49
BS					
January	697±931.4 ^{ab}	804±1199 ^{ab}	40-3600	9.9	29.5
March	797±917.6 ^{ac}	916±1218 ^{ac}	100-3800	11.2	35.5
May	1123±853.6	1275±1108	240-3320	18.3	47.5
July	1034±464.4	1123±557.3	480-2140	21	54.5
September	1041±751 ^{ac}	1172±1035 ^{ac}	340-3800	20.7	39
November	1203±1444 ^{ac}	1539±2205 ^{ac}	180-5060	13.9	40
ODA					
January	286±66.2 ^{ab}	288±71.2 ^{ab}	200-440	11.9	35
March	884±1711 ^{ac}	1586±3163 ^{ac}	180-5880	13.4	33
May	1350±550.6 ^{ab}	1506±696 ^{ab}	800-2600	22	32
July	2090±235.6 ^{ac}	2426±323.3 ^{ac}	1800-2500	27.3	35
September	860±250.5 ^{ac}	914±288.9 ^{ac}	640-1520	34.6	22
November	240±62.54	240±62.54	120-340	2.8	46

*p<0.0001 GM compared to all other sampling sites; ^{ac}p<0.0001 BS vs. AP; ^{ab}p=0.0008 BS vs. AP; ^{bc}p<0.0001 ODA vs. AP; ^{ab}p=0.0008 ODA vs. AP;
 ***p<0.0001 ODA vs. BS; ^{ab}p=0.0465 ODA vs. BS; ^{ac}p<0.0001 BS vs. ODA

Table 2 Correlation between concentration of airborne fungi (cfu m⁻³), temperature, and relative humidity

Sampling site Parameter	Grain mill		Apartments		Basements		Outdoor air	
	T (°)	rh (%)	T (°)	rh (%)	T (°)	rh (%)	T (°)	rh (%)
p-value (two-tailed)	0.8998	0.2336	0.0476	0.8479	0.2309	0.1730	0.0957	0.6215
R square	0.0045	0.3295	0.6661*	0.01035	0.3325	0.4068	0.5409	0.06659

T-temperature

rh-relative humidity

*p<0.05 significant positive correlation

investigated in the previously mentioned study are in the same climate zone, we can speculate that the higher levels of airborne fungi in ODA and in the AP and BS in this study could be related to current climate changes. However, the sample size and number of locations prevent us from reaching a firm conclusion.

The qualitative composition of airborne fungi at each site and in each sampling period is presented in Table 3. The qualitative composition of airborne fungi in the GM differed from the other sampling locations. The most dominant species in the GM belonged to *Aspergillus* spp. and *Eurotium* spp. (85-100 %). The most prevalent fungal genera in the residential areas were similar to those in ODA: *Cladosporium* spp. (45-100 % in AP and 55-95 % in BS), *Penicillium* spp. (85-100 % in AP and 70-100 % in BS), and *Aspergillus* spp. (20-80 % in AP and 50-95 % in BS).

High frequencies of *Aspergillus* spp., *Eurotium* spp., *Cladosporium* spp., *Penicillium* spp. and *Alternaria* spp. at places where people live and work raises concern because all of them have proven to be causative agents for fungal allergies and other respiratory disorders (27, 33). Aspergilli of interest (sections *Flavi*, *Nigri*, and *Versicolores*) were frequent only at certain locations. We did not specify any species from these sections because the applied methods were sufficient only for sectional classifications. Due to morphological similarities among species from each section, specific DNA sequence data is required (16, 34-38).

Species belonging to section *Flavi* were very frequent in the GM (50-100 %) and present throughout the year. The sampled plates after incubation sometimes revealed overgrowth by these species, indicating a very high concentration of their viable conidia in the air. *Eurotium* spp. was also dominantly present in the GM (90-100 % samples) throughout the year and their mycellia usually covered the entire plate. Black aspergilli were isolated from GM samples and the peak was observed in May (55 %). As for the other mould genera, *Cladosporium* spp. was frequent

in GM samples (60-95 %) followed by *Wallemia* spp. (40-90 %), *Ulocladium* spp. (5-80 %), and *Alternaria* spp. (5-10 %). Our failure to isolate the aforementioned species from samples taken in certain periods may have been due to overgrowth by section *Flavi* and/or *Eurotium* species.

We can also speculate on the observed frequencies of section *Versicolores* isolates. These species are known for their very small conidia (around 3 µm) and even if sampling is successful, their growth is too slow which causes them to become overwhelmed by other species. Despite this, we occasionally succeeded in isolating these species from GM samples (5-40 %). *Versicolores* species reached their peak in November, which was probably due to the fact that other airborne fungi were at their lowest concentrations. Considering the very high concentration of airborne fungi in the GM, exposed workers are under risk. Moreover, due to the enormous amount of fungi belonging to section *Flavi*, there is the possibility of toxigenic strains and consequently aflatoxin B1 (AFB1). Recently it has been proven that black aspergilli are able to produce fumonisins in high quantities (23). Consequently these mycotoxins might be inhaled through grain and flour dust or via conidia of black aspergilli. Well-known producers of fumonisins belong to the genus *Fusarium* and they are present in high amounts at places where maize is processed and stored (26). Because *Fusarium* spp. conidia are not likely to be airborne, we managed to isolate these species only in September (10 %). However, the presence of *Fusarium* spp. is expected throughout the year and so is the presence of fumonisins in maize.

Significant differences have been observed in the composition of aeromycota in residences compared to ODA. Sometimes, plates taken in the BS were overwhelmed in growth by *Aspergillus* section *Versicolores*. This indicated drastic fungal contamination with these species at particular sites, since a frequency of *A. versicolor* above 20 % is considered an indicator of indoor fungal contamination

Table 3 Qualitative composition of airborne fungi at selected locations

Airborne fungi	January (%)				March (%)				May (%)				July (%)				September (%)				November (%)			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
<i>Absidia</i> spp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Alternaria</i> spp.	10	15	25	20	10	0	40	10	0	5	15	60	0	65	20	100	0	65	40	10	5	0	10	0
<i>Aspergillus ochraceus</i>	40	0	20	0	15	0	40	0	35	5	10	0	60	10	30	0	35	15	35	10	35	0	25	0
Section Flavi	80	0	0	0	50	0	5	0	70	0	5	0	100	10	0	10	90	5	10	0	55	0	15	30
Section Nigri	40	6.2	5.2	0	15	10	0	0	55	5	0	0	30	0	5	10	20	25	5	0	15	5	15	0
Section Versicolores	0	45	60	0	5	40	65	0	25	10	35	0	0	20	50	0	25	50	50	10	40	65	45	10
<i>Aspergillus</i> spp.	100	80	95	90	95	45	65	0	95	20	80	0	100	45	95	20	100	60	100	40	85	75	50	30
<i>Aspergillus terreus</i>	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
<i>Aureobasidium</i> spp.	0	0	0	5	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0
<i>Botrytis</i> spp.	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chrysonilia sitophila</i>	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cladosporium</i> spp.	60	45	55	100	55	65	95	100	95	100	95	100	0	95	80	100	70	90	75	100	65	65	80	90
<i>Culvularia</i> spp.	0	0	0	0	0	0	0	0	0	20	0	10	0	0	0	0	0	0	0	0	0	0	0	0
<i>Epicoccum</i> spp.	10	0	5	0	40	5	0	30	0	0	0	0	0	15	0	0	0	0	10	30	0	0	0	0
<i>Eurotium</i> spp.	90	60	40	50	100	20	65	0	100	45	85	20	100	50	85	60	100	15	80	0	100	25	70	30
<i>Fusarium</i> spp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0
<i>Mucor</i> spp.	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	10	0	0	0
<i>Penicillium</i> spp.	100	90	85	90	95	100	100	100	100	95	100	70	100	90	80	40	80	85	70	70	95	100	90	100
<i>Rhizopus stolonifer</i>	10	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	10	0	0	0	0	0	0
<i>Ulocladium</i> spp.	0	20	50	0	5	0	45	30	65	30	60	60	35	95	50	90	80	25	25	30	25	5	35	0
<i>Wallemia</i> spp.	0	30	30	10	60	5	20	0	90	30	15	0	40	10	15	10	50	10	0	0	85	15	55	50

1 - grain mill, 2 - apartment, 3 - basement, 4 - outdoor air

(21). One of the mould sources in the air of indoor environments is damp building material. *Aspergillus* species are often found on substrates such as walls of residences and working interiors, where their survival depends only on water activity (a_w). Aspergilli are primary colonizers and can be customized to a_w values below 0.8, which allows them to succeed in damp indoor environments more likely than other moulds (22, 39). *Aspergillus versicolor* is the most frequently reported fungal species in section *Versicolores* from damp indoor environments and its presence might be related to Sick Building Syndrome (17-20). Recent publications based on the molecular identification of *Aspergillus* species (*Versicolores*) indicated that *Aspergillus versicolor sensu stricto* were not common in buildings. However, it has also been reported that Aspergilli from this section isolated from indoor air are highly diverse in temperate regions (36), so we cannot exclude the presence of *A. versicolor sensu stricto* in our isolates. Considering the variety of recently identified *Aspergillus* species from this section (36), it is likely that more than one was able to produce sterigmatocystin. Therefore, their presence in high concentrations in indoor air could represent a serious threat to human health. Samples from the AP did not indicate overgrowth by these species, although their presence was persistent. Relative humidity measured at the AP in periods when indoor spaces are heated (January, November) ranged from 41 to 49 % and a high rate of condensation on windows was observed. In that same period, the ambient temperature was 18.3-18.6 °C and these conditions were considered supportive for sporulation and mycotoxin production by *A. versicolor* (40).

CONCLUSIONS

Considering the observed and analysed data, there is a threat of chronic exposure to secondary metabolites (sterigmatocystin, fumonisin, and aflatoxin). These metabolites can accumulate in spores and mycelium fragments in unknown concentrations. When people are exposed to high concentrations of mycotoxins, especially from contaminated food, one can reasonably easily establish a connection with a certain disease. However, when they are present in very low concentrations, it is very difficult to study and evaluate their effects. Moreover, there are many unknown facts about these effects when inhalation is the primary means of exposure. However, there is a remarkable

amount of studies proving toxic, mutagenic, and carcinogenic properties of mould secondary metabolites on respiratory surrogate tissues, cell lines, and animal respiratory system, as well as case-reports addressing adverse health effects in humans (4, 41-46). Therefore, monitoring of airborne fungi in occupational as well as living environments is of great importance in the prevention of unfavourable effects on human health.

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Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Ahn BH, Park YH, Shin SH. Mouse model of *Aspergillus* and *Alternaria* induced rhinosinusitis. *Auris Nasus Larynx* 2009;36:422-6. doi: 10.1016/j.anl.2008.08.009
2. Bush RK, Portnoy JM. The role and abatement of fungal allergens in allergic diseases. *J Allergy Clin Immunol* 2001;107(Suppl 3):S430-40. doi: 10.1067/mai.2001.113669
3. Douwes J, Pearce N. Invited commentary: is indoor mould exposure a risk factor for asthma? *Am J Epidemiol* 2003;158:203-6. doi: 10.1093/aje/kwg149
4. Orlandi RR, Marple BF. Fungus and chronic rhinosinusitis: weighing the evidence. *Otolaryngol Head Neck Surg* 2010;143:611-3. doi: 10.1016/j.otohns.2010.07.002
5. Shin SH, Ponikau JU, Sherris DA, Congdon D, Frigas E, Homburger HA, Swanson MC, Gleich GJ, Kita H. Chronic rhinosinusitis: An enhanced immune response to ubiquitous airborne fungi. *J Allergy Clin Immunol* 2004;114:1369-75. doi: 10.1016/j.jaci.2004.08.012
6. Thompson GR 3rd, Patterson TF. Fungal disease of the nose and paranasal sinuses. *J Allergy Clin Immunol* 2012;129:321-6. doi: 10.1016/j.jaci.2011.11.039
7. European Environment Agency (EEA). Air Quality in Europe - Report No 4/2012. Copenhagen: EEA; 2012.
8. Fröhlich-Nowoisky J, Pickersgill DA, Després VR, Pöschla U. High diversity of fungi in air particulate matter. *Proc Natl Acad Sci USA* 2009;106:12814-9. doi: 10.1073/pnas.0811003106
9. Eduard W. Fungal spores: A critical review of the toxicological and epidemiological evidence as a basis for occupational exposure limit setting. *Crit Rev Toxicol* 2009;39:799-864. doi: 10.3109/10408440903307333
10. Cabral JP. Can we use indoor fungi as bioindicators of indoor air quality? Historical perspectives and open questions. *Sci*

- Total Environ 2010;408:4285-95. doi: 10.1016/j.scitotenv.2010.07.005
11. Kuhn DM, Ghannoum MA. Indoor mold, toxigenic fungi, and *Stachybotrys chartarum*: infectious disease perspective. Clin Microbiol Rev 2003;16:144-72. doi: 10.1128/CMR.16.1.144-172.2003
 12. Opplinger A, Rusca S, Charrière N, Duc TV, Droz PO. Assessment of bioaerosols and inhalable dust exposure in Swiss sawmills. Ann Occup Hyg 2005;49:385-91. PMID: 15699057
 13. Šegvić Klarić M, Varnai VM, Ljubičić Čalušić A, Macan J. Occupational exposure to airborne fungi in two Croatian sawmills and atopy in exposed workers. Ann Agric Environ Med 2012;19:205-11. PMID: 22742790
 14. Klich MA. Health effects of *Aspergillus* in food and air. Toxicol Ind Health 2009;25:657-67. doi: 10.1177/0748233709348271
 15. Krishnan S, Manavathu EK, Chandrasekar H. *Aspergillus flavus*: an emerging non-fumigatus *Aspergillus* species of significance. Mycoses 2009;52:206-22. doi: 10.1111/j.1439-0507.2008.01642.x
 16. Varga J, Frisvad JC, Samson RA. Two new aflatoxin producing species, and an overview of *Aspergillus* section *Flavi*. Stud Mycol 2011;69:57-80. doi: 10.3114/sim.2011.69.05
 17. Nielsen KF, Thrane U, Larsen TO, Nielsen PA, Gravesen S. Production of mycotoxins on artificially inoculated building materials. Int Biodeter Biodegr 1998;42:9-16. doi: 10.1016/S0964-8305(98)00038-9
 18. Piecková E and Jesenská Z. Microscopic fungi in dwellings and their health implications in humans. Ann Agric Environ Med 1999;6:1-11. PMID: 10384209
 19. Tuomi T, Reijula K, Johnsson T, Hemminki K, Hintikka EL, Lindroos O, Kalso S, Koukila-Kähkölä P, Mussalo-Rauhamaa H, Haatela T. Mycotoxins in crude building materials from water-damaged buildings. Appl Environ Microbiol 2000;66:1899-904. PMID: 10788357
 20. Jussila J, Komulainen H, Kosma VM, Nevalainen A, Pelkonen J, Hirvonen MR. Spores of *Aspergillus versicolor* isolated from indoor air of a moisture-damaged building provoke acute inflammation in mouse lungs. Inhal Toxicol 2002;14:1261-77. doi: 10.1080/08958370290084908
 21. Engelhart S, Loock A, Skutlarek D, Sagunski H, Lommel A, Färber H, Exner M. Occurrence of toxigenic *Aspergillus versicolor* isolates and sterigmatocystin in carpet dust from damp indoor environments. Appl Environ Microbiol 2002;68:3886-90. doi:10.1128/AEM.68.8.3886-3890.2002.
 22. Nielsen KF. Mycotoxin production by indoor molds. Fungal Genet Biol 2003;39:103-17. PMID: 12781669
 23. Varga J, Kocsubé S, Suri K, Szigeti Gy, Szekeres A, Varga M, Tóth B, Bartók T. Fumonisin contamination and fumonisin producing black *Aspergilli* in dried vine fruits of different origin. Int J Food Microbiol 2010;143:143-9. doi: 10.1016/j.ijfoodmicro.2010.08.008
 24. Feller W. An Introduction to the Probability Theory and Its Application. New York: John Wiley and Sons Inc; 1950.
 25. Pitt JI, Hocking AD. Fungi and Food Spoilage. New York (NY): Springer Science+Business Media LLC; 2009.
 26. de Hoog GS, Guarro J, Gene J, Figueras MJ. Atlas of Clinical Fungi. 2nd ed. Utrecht: Centraalbureau voor Schimmelcultures/Reus: Universitat Rovira i Virgili; 2000.
 27. Lugauskas A, Krikštaponis A, Šveistyte L. Airborne fungi in industrial environments- potential agents of respiratory diseases. Ann Agric Environ Med 2004;11:19-25. PMID: 15236494
 28. Desai MR, Ghosh SK. Occupational exposure to airborne fungi among rice mill workers with special reference to aflatoxin producing *A. flavus* strains. Ann Agric Environ Med 2003;10:159-62. PMID: 14677906
 29. Adhikari A, Sen MM, Gupta-Bhattacharya S, Chanda S. Airborne viable, non-viable, and allergenic fungi in a rural agricultural area of India: a 2-year study at five outdoor sampling stations. Sci Total Environ 2004;326:123-41. doi: 10.1016/j.scitotenv.2003.12.007
 30. Hameed AAA, Khodr MI. Suspended particulates and bioaerosols emitted from an agricultural non-point source. J Environ Monit 2001;3:206-9. doi:10.1039/b007159p.
 31. Haas D, Habib J, Galler H, Buzina W, Schlacher R, Marth E, Reinthaler FF. Assessment of indoor air in Austrian apartments with and without visible mold growth. J Atmos Environ 2007;41:5192-201. doi:10.1016/j.atmosenv.2006.07.062
 32. Šegvić Klarić M, Pepeljnjak S. A year-round aeromycological study in Zagreb area, Croatia. Ann Agric Environ Med 2006;13:55-64. PMID: 16841873
 33. Ljubičić Čalušić A, Varnai VM, Čavlović AO, Šegvić Klarić M, Beljo R, Prester LJ, Macan J. Respiratory health and breath condensate acidity in sawmill workers. Int Arch Occup Environ Health 2013;86:815-25. doi:10.1007/s00420-012-0817-x
 34. Samson RA, Noonim P, Meijer M, Houbraken J, Frisvad JC, Varga J. Diagnostic tools to identify black *Aspergilli*. Stud Mycol 2007;59:129-45. doi: 10.3114/sim.2007.59.13.
 35. Godet M, Munaut F. Molecular strategy for identification in *Aspergillus* section *Flavi*. FEMS Microbiol Lett 2010;304:157-68. doi: 10.1111/j.1574-6968.2009.01890.x
 36. Jurjević Ž, Peterson SW, Horn BW. *Aspergillus* section *Versicolores*: nine new species and multilocus DNA sequence based phylogeny. IMA Fungus 2012;3:59-79. doi: 10.5598/imafungus.2012.03.01.07
 37. Jurjević Ž, Peterson SW, Stea G, Solfrizzo M, Varga J, Hubka V, Perrone G. Two novel species of *Aspergillus* section *Nigri* from indoor air. IMA Fungus 2012;3:159-73. doi: 10.5598/imafungus.2012.03.02.08.
 38. Nielsen KF, Mogensen JM, Johansen M, Larsen TO, Frisvad JC. Review of secondary metabolites and mycotoxins from the *Aspergillus niger* group. Anal Bioanal Chem 2009;395:1225-42. doi: 10.1007/s00216-009-3081-5
 39. Schulz T, Senkpiel K, Ohgke H. Comparison of the toxicity of reference mycotoxins and spore extracts of common indoor moulds. Int J Hyg Environ Health 2004;207:267-77. doi: 10.1078/1438-4639-00282
 40. Vujanović V, Smoragiewicz W, Krzysztyński K. Airborne fungal ecological niche determination as one of the possibilities for indirect mycotoxin risk assessment in indoor air. Environ Toxicol 2001;16:1-8. PMID: 11345539
 41. May JJ, Stallones L, Darrow D, Pratt DS. Organic dust toxicity (pulmonary mycotoxicosis) associated with silo unloading. Thorax 1986;41:919-23. PMID: 3590053
 42. Straus DC. Molds, mycotoxins, and sick building syndrome. Toxicol Ind Health 2009;25:617-35. doi: 10.1177/0748233709348287

43. Piecková E, Wilkins K. Airway toxicity of house dust and its fungal composition. *Ann Agric Environ Med* 2004;11:67-73. PMID: 15236501
44. Piecková E. Adverse health effects of indoor moulds. *Arh Hig Rada Toksikol* 2012;63:545-59. doi: 10.2478/10004-1254-63-2012-2221.
45. Jakab GJ, Hmieleski RR, Zarba A, Hemenway DR, Groopman JD. Respiratory aflatoxicosis: suppression of pulmonary and systemic host defenses in rats and mice. *Toxicol Appl Pharmacol* 1994;125:198-205. PMID: 8171428
46. Kelman BJ, Robbins CA, Swenson LJ, Hardin BD. Risk from inhaled mycotoxins in indoor office and residential environments. *Int J Toxicol* 2004;23:3-10. doi: 10.1080/10915810490265423

Sažetak**Jednogodišnje istraživanje razine plijesni u zraku unutarnjih prostora**

Cilj rada bio je ispitati varijacije učestalosti (%) i koncentracija (cfu m^{-3}) plijesni u zraku mlina žitarica i četiriju stambenih prostora (dva stana i dva podruma) u odnosu na vanjski zrak tijekom jedne godine u Zagrebu (Hrvatska). Učestalost *Aspergillus* vrsta iz sekcija *Flavi*, *Nigri* i *Versicolores* također je ispitana. Plijesni iz zraka su uzorkovane pomoću uređaja Air-sampler MAS 100 Eco u kojem su postavljene DG-18-agarske ploče. Prosječna koncentracija aerogenih plijesni u mlinu bila je između 14.310 i 40.000 cfu m^{-3} , što je veće od koncentracije (10^4 cfu m^{-3}) koja se smatra opasnom za zdravlje. Procijenjene vrijednosti koncentracija plijesni u zraku mlina, dobivene Felerovom korekcijom, čak su šest puta veće od izmjerenih koncentracija. U stanovima ($163\text{-}1244 \text{ cfu m}^{-3}$) koncentracije aerogenih plijesni bile su manje nego u vanjskom zraku ($286\text{-}2090 \text{ cfu m}^{-3}$) i podrumima ($697\text{-}1203 \text{ cfu m}^{-3}$), izuzev u toplijim mjesecima kada su izmjerene vrijednosti bile slične. Tijekom godine dominirale su vrste iz rodova *Cladosporium* spp. (90-100 %), *Penicillium* spp. (40-100 %), i *Alternaria* spp. (10-100 %), koje su uobičajene u područjima s umjerenom klimom. *Aspergile* iz sekcija *Flavi* (50-100 %) i *Nigri* (15-40 %), *A. ochraceus* (15-60 %) i *Eurotium* spp. (85-100 %) dominirali su u zraku mlina, a u uzorcima vanjskog zraka rijetko su detektirane. Vrste *Aspergillus* iz sekcije *Versicolores* s većom učestalošću nađene u podrumima nego u stanovima. Veliko opterećenje mlina aerogenim plijesnima tijekom cijele godine može biti opasno za zdravlje radnika. Ujedno, ovo je prvo jednogodišnje ispitivanje sezonske varijabilnosti u sastavu plijesni u zraku mlina žitarica i stambenih prostora u Hrvatskoj. Međutim, monitoring treba nastaviti tijekom dužeg razdoblja.

KLJUČNE RIJEČI: *Flavi*; *mlin*; *Nigri*; *stambeni objekti*; *Versicolores*

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