ORIGINAL ARTICLES AAEM

Ann Agric Environ Med 2006, 13, 55–64

A YEAR-ROUND AEROMYCOLOGICAL STUDY IN ZAGREB AREA, CROATIA

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Šegvić Klarić M, Pepeljnjak S: A year-round aeromycological study in Zagreb area, Croatia. *Ann Agric Environ Med* 2006, **13**, 55–64.

Abstract: A 1-year aeromycological study was conducted in the area of Zagreb, the capital of Croatia, in order to establish seasonal variations in the composition and concentration of aeromycota. Sampling was carried out at 3 locations: the city centre, the Pharmaceutical botanical garden and the mountain of Medvednica, at regular intervals using the Mas 100 Eco Air-sampler with Sabouraud-dextrose agar. Airborne fungi peaked during spring and summer (110–284 cfu/m^3), while lower levels were detected in autumn and winter at each of the 3 sampling sites (6–128 cfu/m^3). Significantly lower concentrations were found in Medvednica region ($p<0.01$) during most sampling periods. Yeasts were present in higher concentrations in autumn and winter (11–46 cfu/m^3) than during spring and summer (9–11 cfu/m^3) in the city centre and botanical garden. In the Medvednica region, yeasts were found at significantly lower concentrations than at other locations only during the autumn and winter (1–16 cfu/m^3). The dominant fungi contributing to these differences were species of *Cladosporium*, *Penicillium* and *Alternaria*. These genera comprised between 30.4–79.5% of the samples. Other stable components of aeromycota were *Fusarium*, *Aspergillus* and sterile mycelia (11.1–44.0%). Total counts of airborne fungi as well as individual counts of *Cladosporium* and *Alternaria* showed significant positive correlations with temperature and solar radiation ($p<0.05$). *Alternaria* also showed a significant correlation with wind speeds while *Cladosporium* was negatively correlated with atmospheric pressure ($p<0.05$). Yeasts showed a significant positive correlation with relative humidity, yet were negatively correlated with temperature and solar radiation in the city centre and the botanical garden. In contrast, a significant positive correlation in the case of yeasts was observed in the Medvednica region with respect to temperature and solar radiation ($p<0.05$).

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Key words: airborne fungi, airspores, *Alternaria*, *Cladosporium*, *Penicillium*, seasonal patterns.

INTRODUCTION

Fungi are ubiquitous, occurring as saprophytes or parasites on a variety of substrata. From there they become airborne. Concentrations of airborne fungi depend on several factors: seasonal and daily variations in meteorological conditions (temperature, humidity, wind speed, rainfall, solar radiation), vegetation, air pollution, agricultural, industrial and other human activities [9]. Seasonal variations in fungal concentration are related to geographical climatic factors of a particular region. Most reports showed that higher concentrations of airborne fungi were recorded during the summer (July, August, September) and early autumn (October), while lower concentrations were measured during the winter months of January and February [1, 12, 17, 18, 29].

Among airborne fungi, members of *Cladosporium*, *Alternaria*, *Penicillium* and *Aspergillus* were permanently present in many aeromycological calendars. The genus *Cladosporium* is one of the most abundant airborne fungi

Received: 7 July 2005
Accepted: 27 April 2006

in warm areas with peaks in summer and early of autumn [12, 16, 17, 20, 28]. Also, *Alternaria* shows a similar seasonal pattern in response to temperature while *Penicillium* and *Aspergillus* were noted at higher concentrations during the winter months (December to February) [12, 17, 18]. Meteorological factors influence the composition and concentration of airborne fungi: high temperature increases *Alternaria* spore concentration; frequent rainfall correlates with *Fusarium* levels; relatively low wind speed (0.5 m/s) releases a high number of conidia of *Penicillium* sp. and *Aspergillus fumigatus* [1, 2, 24].

Various plants can be infected with plant pathogens *Fusarium* and *Alternaria* species, which can be dispersed into the air. On the other hand, the presence of plants, known to produce essential oils and aerosol, which showed sporistatic, fungistatic, and fungicidal activities [25, 26], possibly reduces airspora viability. Increase of CO_2 concentration stimulates fungal sporulation suggesting that levels of the airspora correlate with air pollution [13]. In addition, increase of SO_2 (product of fossil coal combustion) in cold months can reduce airborne fungi concentration [1]. Airborne fungi are sometimes associated with respiratory diseases, such as chronic bronchitis, asthma, allergies, hypersensitive pneumonitis and infectious diseases such as aspergillosis [9, 23, 30]. Resano and co-workers [27] found fungal sensitisation in 20% of patients diagnosed with rhinosinusitis and/or bronchial asthma and the most frequently involved fungi were *Alternaria* and *Cladosporium*. The examination of common airborne fungi distribution in a particular region can be helpful in: identifying association between domestic fungal sensitisation and clinical diagnosis; and clinical prevention of the seasonal allergic diseases [33]. Therefore, the purpose of our study was to determine seasonal variations in the composition and concentration of the aeromycota at different locations in Zagreb, the capital of Croatia.

MATERIALS AND METHODS

The study area. The study was conducted at 3 locations in Zagreb area: the city centre (C); the Pharmaceutical botanical garden "Fran Kušan" (BG); and the mountain of Medvednica (M).

The first sampling site C is characterised by heavy traffic and streetcars as public transport. Near the sampling station there is a city-park with trees *Platanus orientalis*.

The second location BG is a residential area with less traffic. The pharmaceutical botanical garden (2.4 ha) contains continental and Mediterranean plant species, including a great number of medical plants such as *Picea abies*, *Mentha piperita*, *Melissa officinalis*, *Pelargonium radula*, *Rosmarinus officinalis*, *Artemisia absinthium*, *Salvia officinalis*, *Micromeria thymifolia*, *Tanacetum parthenium*, *Lavandula officinalis*, and many others. These particular species contain essential oils, which showed antifungal activities [25].

The third station M is a picnic area with the mountain house "Puntijarka" at the height of 957 m, represented with plant association such as *Lamio orvale-Fagetum sylvaticae*, *Abieti-Fagetum*, *Blechno-Fagetum* and *Aceri Fraxinetum*.

Sampling, isolation and identification methods. Samples were collected over a period of 12 months between October 2002–September 2003. Each month, samples were obtained 4 times in 6 replicates at 1 location. Thus, 864 samples were collected in total.

The samples were usually taken on Sundays between 12:00–15:00, except when it was raining or snowing. In that case, sampling was carried out on another day of the week during clement weather, and always between 12:00–15:00. Fungi were sampled using the Mas 100 Eco Air-sampler (Merck) (hole-to-agar impactor), with 400 holes, and Sabouraud agar plates (9 cm diameter) supplemented

Table 1. Averages of meteorological measurements: temperature (T), relative humidity (RH), atmospheric pressure (P), wind speed (WS), rainfall (R), and solar radiation (SR) in Zagreb and Medvednica from October 2002–September 2003.

		X	XI	XII	I	II	III	IV	V	VI	VII	VIII	IX
Zagreb	T (ºC)	11.5	12.0	1.5	1.5	1.3	9.5	13.6	23.0	25.5	26.0	28.2	19.3
	RH (%)	71.8	76.3	87.0	68.3	76.5	49.8	37.3	54.5	51.5	49.0	51.5	56.6
	P (hPa)	1020.8	1013.4	1023.2	1023.5	1025.9	1026.9	1017.0	1014.9	1014.0	1016.5	1015.7	1023.1
	WS (m/sec)	0.05	0.1	0.1	0.2	0.2	0.4	0.2	0.3	0.2	0.1	0.2	0.2
	R (mm)	107.9	75.8	71.3	73.1	30.5	8.1	28.9	19.8	75.2	78.6	17.4	78.6
	SR (h)	121.7	76.1	21.5	87.3	117.8	183.8	180.8	272.5	287.3	297.0	303.3	214.1
Medvednica	T (ºC)	810	8.0	-4.7	-2	-5.6	3.8	6.0	17.5	17.0	20.0	24.0	18.3
	RH (%)	84.0	85.0	97.0	81.5	90.0	58.5	52.3	74.5	55.0	58.5	59.0	71.6
	P (hPa)	902.8	899.0	905.13	904.7	915.8	909.6	902.0	904.2	904.7	906.1	906.8	910.6
	WS (m/sec)	0.2	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.1	0.2
	R (mm)	133.5	87.8	86.1	115.1	71.3	16.1	43.9	26.4	37.3	117.1	49.7	120.5
	SR (h)	126.3	74.1	16.9	88.1	108.7	180.5	184.9	263.1	270.5	260.3	275.9	204.8

Table 2. Airborne fungi detected in central Zagreb (C) with annual mean concentrations (cfu/m^3), frequencies (%) and concentration range during the 12 months.

Airborne fungi (C)	%	cfu/m^3	range (cfu/m^3) (minimum–maximum)	detected in months
Cladosporium	79.5	81.55	1.04–192.97	all
Penicillium	70.4	13.12	1.86–31.46	all
Alternaria	59.4	15.84	0.42–66.46	all
Fusarium	44.0	3.87	0.83–12.91	all
Aspergillus	43.0	3.63	1.46–8.54	all
Paecilomyces	6.9	0.42	0.20–1.66	XI, XII, IV, V, VII
Chrysosporium	10.4	1.24	0.63–5.83	XI, I, II, III, IV, VII, IX
Phoma	5.9	0.32	0.20–1.25	X, XI, XII, I, II, III, IV, V, VI
Sterile mycelia	11.1	1.66	0.20–7.92	I, IV, V, VI, VII, VIII, IX
Botrytis	3.8	0.19	0.42–0.83	X, XI, XII, V
Mucor	2.7	0.21	0.20–1.67	XI, IV, VII, VIII
Rhizopus	7.1	0.64	0.20–4.79	X, XI, II, VI, VII, VIII, IX
Trichoderma	2.4	0.12	0.20–0.42	X, XII, III, IV, V
Ulocladium	2.4	0.12	0.20–0.42	XI, I, II, VI, VII
Curvularia	1.4	0.10	0.20–0.63	II, III, VII
Absidia	1.4	0.08	0.20–0.42	XII, III, VII, VIII
Geotrichum	3.1	0.17	0.20–1.04	II, III, IV, V
Arnium	0.3	0.03	0.2–0.63	VII, IX
Botryomyces	0.3	0.02	0.20	XI
Trichothecium	0.3	0.02	0.20	III
Sclerotium	1.0	0.07	0.20–0.42	I, IV, IX
Dreschlera	1.0	0.05	0.20–0.42	IV, V
Pithomyces	1.0	0.05	0.20	II, IV, V
Scopulariopsis	1.0	0.05	0.20–0.42	III, IV
Unidentified fungi	22.5	2.37	0.42–6.46	all

with 50 mg/l of streptomycin and 20.000 I.U. of penicillin. The impaction velocity is approximately 10.8 m per second [19]. Airflow rate is 100 l/min. The sampling height 0.7–1.2 m and the sampling time was 2 min.

After field sampling, the plates were incubated at 25ºC ± 2 for 5 days, and colony forming units of moulds and yeasts in cubic meter (cfu/m^3) were counted. Fellers correction was not applied [7].

The airborne fungi were identified on the basis of their macro- and microscopic characteristics after subculturing on Czapek and Malt, Potato dextrose and Synthetic nutrient agar according to keys [6, 28].

Meteorological measurements. Meteorological data: temperature, relative humidity, atmospheric pressure and wind speed in sampling time, and average monthly rainfall and solar radiation (Tab. 1) were obtained from the Meteorological and Hydrological Service in Zagreb (www.meteo.hr/index.html).

Statistics. The data obtained as cfu/m^3, were statistically analysed by the 1-way analysis of variance (ANOVA), followed by the multiple comparison procedure (Bonferroni test). The Pearson correlation coefficients between the cfu/m^3 and meteorological data (temperature, relative humidity, solar radiation, atmospheric pressure, wind speed) were also analysed. The level of $p<0.05$ was considered statistically significant for all tests performed.

RESULTS

The mean and standard deviation of the concentration of airborne viable moulds and yeasts over a period of 12 months are given in Figures 1-2. Higher levels of airborne moulds were counted during the late spring and summer. The peaks of concentration were recorded from May to September at all the sampling sites and were in the range from 110 cfu/m^3 in June (M) to 284 cfu/m^3 in August (C). In June and August significantly higher concentrations of

Table 3. Airborne fungi detected in the Pharmaceutical botanical garden (BG) with annual mean concentrations (cfu/m^3), frequencies (%) and concentration range during the 12 months.

Airborne fungi (BG)	%	cfu/m^3	range (cfu/m^3) (minimum–maximum)	detected in months
Cladosporium	79.5	61.94	2.92–150.00	all
Penicillium	66.3	9.34	2.92–16.88	all
Alternaria	59.0	18.07	0.20–60.83	all
Aspergillus	34.7	4.10	0.83–4.58	all
Fusarium	36.1	3.49	1.46–11.25	X, XI, I, III, IV, V, VI, VII, VII, IX
Sterile mycelia	17.3	1.62	0.20–6.46	XI, XII, I, III, IV, V, VI, VII, VIII, IX
Chrysosporium	5.9	0.95	0.20–3.12	XII, I, II, III, IV, V, VI, IX
Geotrichum	5.9	0.93	0.20–5.83	I, III, IV, V, VII, VIII
Rhizopus	6.6	1.01	0.42–7.10	XI, I, V, VI, VII, VIII, IX
Botrytis	3.8	0.27	0.63–1.46	XI, XII, IX
Basidiomycete type[a]	2.7	0.24	0.20–2.70	II, VIII
Phoma	2.7	0.21	0.20–0.83	XI, XII, II, V, VI
Paecilomyces	2.7	0.19	0.20–1.25	X, I, II, VI, VII
Ulocladium	2.7	0.19	0.20–1.67	I, III, IV, VI
Sclerotium	2.7	0.19	0.20–0.63	X, XII, IV, VI, VII
Mucor	2.7	0.14	0.20–0.63	XI, III, V, VII
Absidia	1.7	0.08	0.20–0.42	III, IV, VI, IX
Trichoderma	4.1	0.39	0.20–1.25	X, XI, II, III, V, VI, VIII
Curvularia	0.3	0.03	0.20	XI, VII
Phialophora	0.3	0.03	0.20	IV, V
Dreschlera	0.3	0.02	0.20	V
Gliocladium	0.3	0.02	0.20	XI
Nigrospora	0.3	0.02	0.20	III
Arnium	1.0	0.07	0.20–0.63	VIII, IX
Unidentified fungi	18.7	1.94	0.20–5.00	all

[a]characterised with white mycelium, clamp-connections and arthroconidia

the airborne moulds were found at C (182-284 cfu/m^3) than at BG (128-201 cfu/m^3) and M (110-223 cfu/m^3) ($p<0.05$), while in May these concentrations were significantly higher at both C (225 cfu/m^3) and BG (204 cfu/m^3) than at M (112 cfu/m^3) ($p<0.01$). During July (170-197 cfu/m^3) and September (190-255 cfu/m^3) concentrations did not differ significantly ($p>0.05$). From early autumn (October) to early spring (April) concentrations of airborne moulds were lower than in other periods of the sampling (6-128 cfu/m^3). In October, December, January and March these concentrations were significantly higher at C (25-128 cfu/m^3) and BG (30-108 cfu/m^3) comparing to M (6-30 cfu/m^3) ($p<0.01$). In November and April the levels of airborne fungi were significantly lower at M (30-48 cfu/m^3) than at C (77 cfu/m^3) ($p<0.01$), while at BG and M concentrations did not differ significantly ($p>0.05$). Significant correlation was found between monthly means of airborne fungi concentrations and both, temperature and solar radiation ($p<0.001$) (Tab. 5).

Yeasts were found in higher concentration during the autumn and winter periods at C (15-46 cfu/m^3) and BG (11-36 cfu/m^3), compared to their levels in spring and summer (10-16 cfu/m^3 at C, 9-11 cfu/m^3 at BG). Measured concentrations did not differ significantly ($p>0.05$). The levels of the yeasts showed a significant positive correlation with relative humidity and significant negative correlation with temperature and solar radiation (Tab. 5).

At site M, yeasts showed a different pattern; they were found at significantly lower concentrations during autumn and winter (0.62-16.0 cfu/m^3) than at C and BG ($p<0.01$). In spring and summer months these levels were higher (7.2-15.4 cfu/m^3) and similar to those recorded at C and BG ($p>0.05$). The levels of the yeasts at site M showed significant positive correlation with the temperature and solar radiation. The airborne fungal genera are listed in Tables 2-4 in descending order based on their frequencies, which were calculated as positive samples in the total number of samples for each location.

Table 4. Airborne fungi detected on Medvednica (M) mountain with annual mean concentrations (cfu/m^3), frequencies (%) and concentration range during the 12 months.

Airborne fungi (M)	%	cfu/m^3	range (cfu/m^3) (minimum–maximum)	detected in months
Cladosporium	73.3	53.44	0.63—196.7	all
Alternaria	46.2	10.99	0.42–56.67	only not detected in II
Penicillium	30.4	4.42	0.20–23.3	all
Sterile mycelia	21.5	2.62	0.20–12.92	XI, XII, I, III, IV, V, VI, VII, VIII, IX
Fusarium	18.4	1.55	0.20–2.29	only not detected in XII
Aspergillus	11.8	0.68	0.42–1.46	X, XI, XII, III, IV, VI, VII, VIII, IX
Sclerotium	9.7	0.69	0.20–2.50	XI, XII, II, V, VI, VII
Geotrichum	8.7	0.97	0.20–7.30	I, II, III, IV, VII, VIII
Basidiomycte type[a]	10.4	5.81	0.63–35.83	XI, XII, III, IV, V, VII, VIII
Absidia	10.4	0.76	0.20–4.37	I, III, IV, V, VI, VII, IX
Chrysosporium	5.5	0.64	0.20–2.71	XI, I, II, III, IV, V, IX
Mucor	2.7	0.23	0.42–1.25	V, VI, VII
Rhizopus	3.5	0.22	0.42–0.83	V, VI, VII, VIII, IX
Botrytis	1.7	0.08	0.20–0.63	X, XI, XII
Chaetomium	0.7	0.06	0.42	XI
Paecilomyces	0.7	0.03	0.20	X, VI
Ulocladium	1.4	0.07	0.20–0.63	IV, V
Trichoderma	3.1	0.33	0.20–1.66	X, I, VI, VII, VIII
Arnium	0.3	0.02	0.20	VII
Nigrospora	0.3	0.02	0.20	IV
Curvularia	1.0	0.10	0.20–1.04	VII, IX
Pithomyces	1.0	0.06	0.20–0.63	V, VIII
Phoma	1.0	0.05	0.20–0.42	XII, V
Phialophora	1.0	0.05	0.63	V
Unidentified fungi	14.9	2.35	0.20–8.12	all

[a]characterised with white mycelium, clamp-connections and arthroconidia

Cladosporium was the most abundant genus at all locations (up to 79.5%), followed by *Penicillium* (up to 70.4%), *Alternaria* (up to 59.4%), *Fusarium* (44.0%) and *Aspergillus* (up to 43%). These fungi were detected in all 12 months at C and BG in presented order with similar AMC and frequencies (Tab. 2-3).

At site M, *Penicillium* was in third place in descending order (30.4%), behind *Cladosporium* (73.3%) and *Alternaria* (46.2%), followed by sterile mycelia (21.5%) and *Fusarium* (18.4%) (Tab. 4). At this sampling site, *Cladosporium* and *Penicillium* were constantly present, while *Alternaria* and *Fusarium* were not found during 1 month in the winter period. Also, *Penicillium*, *Alternaria*, *Fusarium* and *Aspergillus* (11.8%) were present in lower AMC and frequency at M than at C and BG.

Some white and dark sterile mycelia were detected predominantly in the summer period at all locations, with higher frequencies at sites M and BG (up to 21.5%) and lower at C (11.1%). *Chrysosporium* and *Rhizopus* were found sporadically during all seasons at each location, but with higher AMC and frequency at C and BG (5.9-10.4%) than at M (up to 5.5%). On the other hand, basidiomycete type (characterised with white mycelium, clamp-connections and arthroconidia), *Absidia* and *Geotrichum* were more frequently found at site M (8.7-10.4%) particularly in August and September, compared to BG and C.

Concentrations of basidiomycete type at M did not correlate with meteorological parameters. *Trichoderma*, *Botrytis*, *Phoma*, *Paecilomyces*, *Mucor*, *Curvularia* and *Sclerotium* were found sporadically over the sampling period at all sites with lower frequency (1-5%) and AMC (lower than 1 cfu/m^3). *Arnium*, *Nigrospora*, *Gliocladium*, *Dreschlera*, *Phialophora*, *Scopulariopsis*, *Chaetomium*, *Botryomyces* and *Trichothecium* were rare in samples, found only one or three times during sampling. Since *Cladosporium*, *Penicillium*, *Alternaria*, *Fusarium* and *Aspergillus* were constant airborne fungi, dominating over

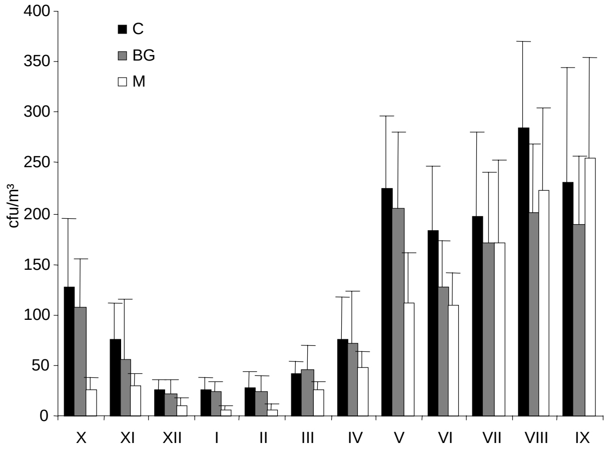

Figure 1. Monthly variations in concentration of total airborne moulds in Zagreb area. Monthly concentrations (cfu/m^3) of airborne moulds in the city centre (C), Pharmaceutical botanical garden (BG) and Medvednica (M) mountain presented as monthly mean and standard deviation.

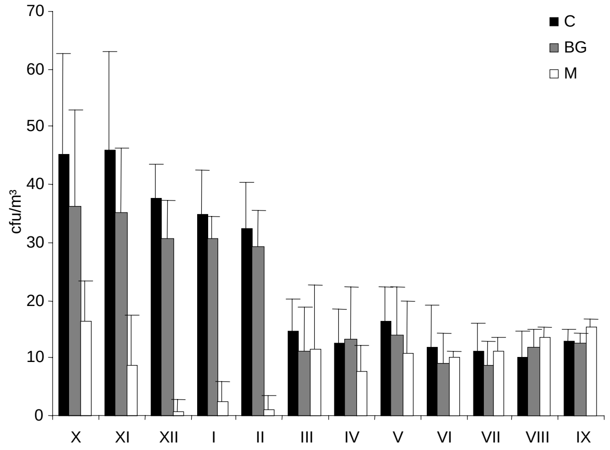

Figure 2. Monthly variations in concentration of airborne yeasts in Zagreb area. Monthly concentrations (cfu/m^3) of airborne yeasts in the city centre (C), the Pharmaceutical botanical garden (BG) and Medvednica (M) mountain presented as monthly mean and standard deviation.

a sampling period, variations in their monthly mean concentrations were statistically analysed.

Cladosporium and *Alternaria* (Fig. 2-3) showed similar seasonal patterns at all locations, with some statistical difference in airspora counts from month to month. Generally, they were present at higher concentrations in late spring and summer (70-200 cfu/m^3 for *Cladosporium*, 6-70 cfu/m^3 for *Alternaria*), than in autumn and winter (1-93 cfu/m^3 for *Cladosporium*, 0-9.4 cfu/m^3 for *Alternaria*). Concentrations of the airborne *Cladosporium* peaked in May (150 cfu/m^3) at BG, and in August and September at C and M (200 cfu/m^3). From May to September, these concentrations were similar at C and BG, except in June and August when the significantly higher *Cladosporium* levels were counted at C than at BG ($p<0.05$). During October, January, May, June and August, *Cladosporium* was found at significantly higher concentrations at C than at M ($p<0.01$). In October and March, higher levels were detected at BG than at M ($p<0.05$). In September, significantly higher level of *Cladosporium* was found at M than at BG ($p<0.05$) while the levels recorded at C and M did not differ significantly ($p>0.05$). Spores of *Alternaria* peaked in August at all locations, with similar concentrations (56-70 cfu/m^3). Significantly higher concentrations were counted during October, May, July and September at C and BG, then at M ($p<0.01$), while

Table 5. Correlation coefficients (r) among cfu/m^3 of airborne moulds (total), yeasts, *Cladosporium, Alternaria, Penicillium, Fusarium, Aspergillus* and Basidiomycete type and meteorological data: temperature (T), relative humidity (RH), rainfall (R), solar radiation (RS) atmospheric pressure (P) and wind speed (Ws) in Zagreb (r_1) and Medvednica (r_2).

	Pearson (linear) correlation r	Average of T (ºC)	Average of RH (%)	Average of R (mm)	Average of SR (h)	Average of P (hPa)	Average of Ws (m/sec)
Airborne moulds (total)	r_1	0.92[a]	-0.53	0.05	0.84[a]	-0.54	0.05
	r_2	0.87[a]	-0.50	0.14	0.73[a]	0.16	-0.49
Airborney easts	r_1	-0.69[a]	0.87[a]	0.40	-0.85[a]	0.19	-0.54
	r_2	0.77[a]	0.49	0.18	0.60[a]	-0.18	-0.40
Cladosporium	r_1	0.91[a]	-0.51	0.06	0.84[a]	-0.61[a]	0.03
	r_2	0.82[a]	-0.42	0.23	0.70	0.19	-0.40
Alternaria	r_1	0.76[a]	-0.43	0.06	0.73[a]	-0.34	0.01
	r_2	0.76[a]	-0.50	-0.08	0.67[a]	0.07	-0.68[a]
Penicillium	r_1	-0.27	0.30	0.24	-0.30	0.45	0.33
	r_2	0.40	-0.09	0.20	0.25	0.33	-0.06
Fusarium	r_1	0.31	-0.29	0.33	0.06	-0.37	-0.31
	r_2	0.76[a]	-0.38	-0.23	0.56	-0.29	-0.48
Aspergillus	r_1	0.30	-0.20	0.08	0.22	-0.29	0.38
	r_2	0.04	-0.02	0.01	0.19	-0.24	0.33
Basidiomycete type	r_2	0.56	-0.40	0.09	0.49	0.05	-0.34

[a] marked values are statistically significant ($p<0.05$)

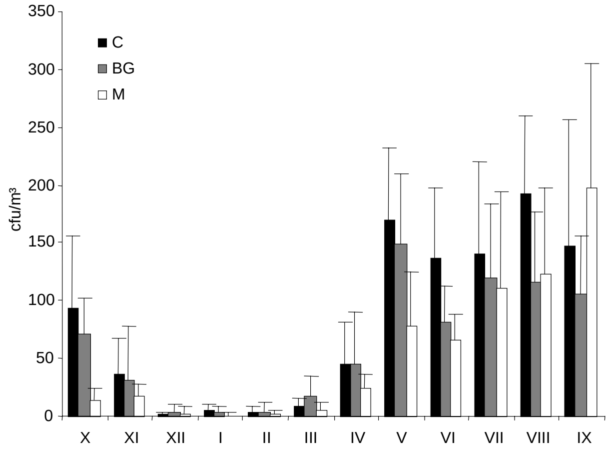

Figure 3. Monthly variations in concentration of *Cladosporium* airspores in Zagreb area. Monthly concentrations (cfu/m^3) of *Cladosporium* in the city centre (C), Pharmaceutical botanical garden (BG) and Medvednica (M) mountain presented as monthly mean and standard deviation.

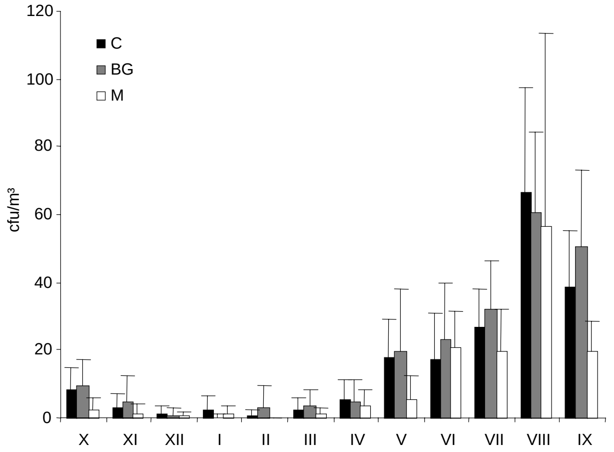

Figure 4. Monthly variations in concentration of *Alternaria* airspores in Zagreb area. Monthly concentrations (cfu/m^3) of *Alternaria* in city centre (C), Pharmaceutical botanical garden (BG) and Medvednica (M) mountain presented as monthly mean and standard deviation.

between C and BG significant difference was not found in that period ($p>0.05$). During November, December January, March, April, June and August, these concentrations did not differ significantly at all sites ($p>0.05$). Only in February Alternaria was not found in samples collected at M. Significant positive correlation was found between monthly means of *Cladosporium* and *Alternaria* concentrations and both, temperature and the solar radiation ($p<0.001$) (Tab. 5). Also, positive correlation was found between *Alternaria* concentrations and wind speed, and negative correlation between *Cladosporium* concentration and atmospheric pressure ($p<0.05$).

Penicillium (Fig. 4) was present at higher concentrations during autumn and winter, particularly in the areas of C and BG (9-17 cfu/m^3), while at M lower levels were detected over this period (0-5 cfu/m^3) (Fig. 4). Peaks were measured in September at all locations (from 17 cfu/m^3 at M to 32 cfu/m^3 at C). From October to March and in April significantly higher concentrations were found at C and BG than at M ($p<0.05$). In May and June, lower concentrations were measured in areas C and M compared to BG ($p<0.05$). In July and September, significantly higher levels of penicillia were found at site C than at M and BG, respectively ($p<0.05$). Significant correlation was not observed between monthly concentrations of airborne penicillia and meteorological measurements ($p>0.05$).

Fusarium and *Aspergillus* (Fig. 5-6) were constantly present during the year, but measured in lower concentrations than *Penicillium*. Airborne fusaria peaked in October at C (15 cfu/m^3) and BG (11 cfu/m^3), when the rain was more frequent. During the winter period, *Fusarium* concentrations were low or not detected in the samples at all locations (0-3 cfu/m^3), and slightly increased in summer at C and BG (5-7 cfu/m^3). At these two locations significant difference in monthly concentrations was not found, while at M concentrations ranging from 0–3 cfu/m^3 were significantly lower than at C and BG. *Aspergillus* peaked in May only at C (9 cfu/m^3) ($p<0.05$), while during the year it was present in concentrations from 2–4 cfu/m^3 at C and from 1–4 cfu/m^3 at BG.

At site M, *Aspergillus* airspora levels were extremely low, approximately 1 cfu/m^3 or not detected during January, February and May. Monthly airspora counts of *Fusarium* at the site M correlated with temperature, while *Aspergillus* did not correlate with average of the monthly meteorological measurements.

DISCUSSION

Fungi are ubiquitous microorganisms with a great capacity to colonise many kinds of substrata and to develop in extreme environmental conditions, on soils, plants and animal remains, and from there they are airborne [3]. Peaks of concentration of the airborne moulds in spring and summer and lower levels detected in autumn and winter at each of the 3 sampling sites in Zagreb, are highly influenced by seasonal temperature changes and solar radiation.

During most of the sampling time lower levels of airborne fungi were found in Medvednica region and this might be determined by the sampling height (above 900 m), type of vegetation, lower temperatures, rare traffic and no pollution.

During the sampling year, monthly mean concentrations of the airborne fungi were lower in the Pharmaceutical botanical garden than in the city centre, but without significant difference. Significantly lower levels in the botanical garden were only detected in June and August. One of the reasons for decreased concentrations in this location is less traffic and consequently decreased air pollution. The other reason could be the presence of medical plants known to produce essential oils and aerosols with antifungal activities, which could reduce airspora viability [25, 26].

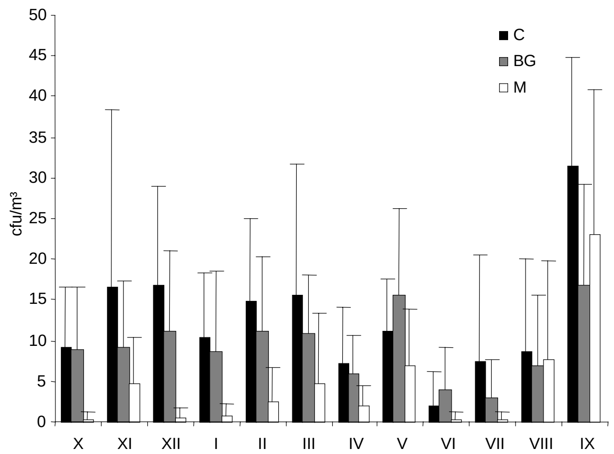

Figure 5. Monthly variations in concentration of *Penicillium* airspores in Zagreb area. Monthly concentrations (cfu/m^3) of *Penicillium* in the city centre (C), Pharmaceutical botanical garden (BG) and Medvednica (M) mountain presented as monthly mean and standard deviation.

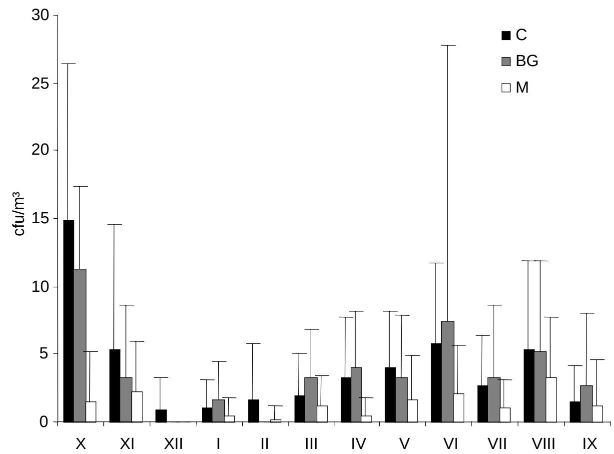

Figure 6. Monthly variations in concentration of *Fusarium* airspores in Zagreb area. Monthly concentrations (cfu/m^3) of *Fusarium* in the city centre (C), Pharmaceutical botanical garden (BG) and Medvednica (M) mountain presented as monthly mean and standard deviation.

Yeasts showed an opposite seasonal pattern. They were present with higher concentrations during autumn and winter when the relative humidity was higher, then in spring and summer at locations in the city centre and botanical garden. These findings are similar to those conducted in Italy [17]. In Medvedica, yeasts were found at lower levels in the autumn and winter months (except in October), as they were greatly influenced by low temperatures.

The dominant airborne fungi were *Cladosporium*, *Penicillium* and *Alternaria*. Species of *Cladosporium* are the most common saprophytic fungi living on organic materials worldwide. They produce dry spores 3-5 µm in size depending on species, which can be easily spread in the air by wind. Earlier studies on airborne mycoflora in Croatia also identified *Cladosporium* species as the dominant airborne fungi [5, 26].

Alternaria was present at lower concentrations than *Cladosporium* but the spores of *Alternaria* are larger in size (some species up to 150 µm in length) and they are comparable in biomass [1]. These 2 entities peaked in spring and summer, which is in agreement with studies conducted in Italy, Spain, Poland, Japan and Australia [8, 17, 18, 20, 29]. Asan *et al.* [1] found higher levels of *Alternaria* and *Penicillium* than *Cladosporium*. Also, some studies in Nigeria, Kuwait and Uganda showed that *Aspergillus*, *Alternaria* and *Penicillium* dominated over *Cladosporium* [10, 14, 22]. These differences may be due to climatic and geographical characteristics of the region and sampling methods.

Penicillium was frequently present in autumn and winter, and peaked in September, which can be the consequence of temperature decrease.

Other stable components of the airborne mycoflora were *Aspergillus* and *Fusarium*. Species of *Aspergillus* were constantly present in the samples from the city centre and botanical garden, and were not found during the winter months in Medvednica region when the temperature was below 0ºC. Higher frequency and concentrations of aspergilla were found in the city centre during sampling, which was probably influenced by heavy traffic, dust and pollution sources. Lugauskas *et al.* [16] found *Aspergillus niger* (57%) and *A. fumigatus* (84%) as the most frequent airborne moulds in polluted areas of some Lithuanian cities. *Fusarium* was detected with higher incidence in the city centre and botanical garden than at Medvednica site, with higher concentrations in autumn when the rainfall was more frequent, while sterile mycelia peaked in summer in all sampling locations. Some studies indicated positive correlation between frequent rainfall and *Fusarium* concentration [32].

Basidiomycete type (characterised with white mycelium, clamp-connections and arthroconidia) was detected in a few samples in February and August in the botanical garden, and more frequently at the Medvednica site with peak in September. Marchiso *et al.* [17, 18] found basidiomycete mycelia at higher concentrations between September and January.

Similar results were obtained for *Absidia* and *Geotrichum*. Species of *Geotrichum* are not common airborne fungi in the outdoor environment but prevailed in decaying organic material, and animal excrements [15, 16], and from there they become airborne. Decaying plant material and animal excrements may be the source of *Geotrichum* spores in the samples from the botanical garden and Medvednica. *Trichoderma*, *Botrytis*, *Phoma*, *Paecilomyces*, *Mucor*, *Curvularia* and *Sclerotium* were found sporadically at all the sampling locations with more or less lower frequencies and concentrations, apart from *Chrysosporium* and *Rhizopus*, which were detected in most of the sampling months.

Other airborne fungi identified, including *Arnium*, *Nigrospora*, *Gliocladium*, *Dreschlera*, *Phialophora*, *Scopulariopsis*, *Chaetomium*, *Botryomyces* and *Trichothecium*, were rarely present in the samples, which is in agreement with other literature reports [17, 18, 29].

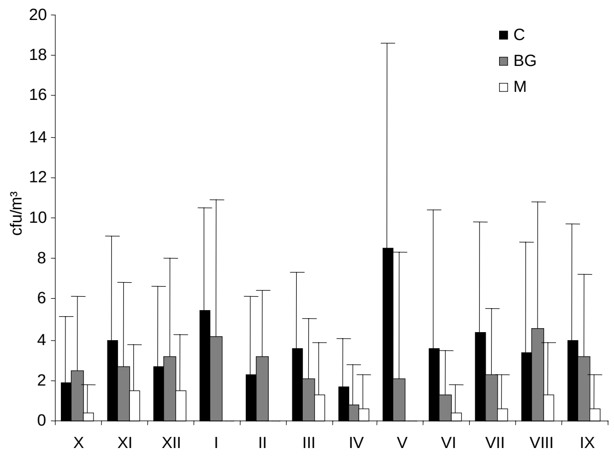

Figure 7. Monthly variations in concentration of *Aspergillus* airspores in Zagreb area. Monthly concentrations (cfu/m^3) of *Aspergillus* in the city centre (C), Pharmaceutical botanical garden (BG) and Medvednica (M) mountain presented as monthly mean and standard deviation.

The result of any aeromycological study is highly influenced by the method of sampling, type of sampler and collecting media, frequency of collecting, location and other factors. In this study, Sabouraud agar with antibiotics was employed as the medium for collecting moulds and yeasts. Therefore, some species, which are present at lower concentrations in the outdoor air, could be overgrown with the dominant ones and their presence and/or number may be underestimated. Subculturing on different media (Czapek and Synthetic nutrient agar) increases conidial germination of some fungi such as *Penicillium* and *Fusarium* species.

Various meteorological factors affect the type and concentration of airborne fungi. Many literature reports showed that higher temperature and solar radiation in spring and summer are responsible for the increase of airspora concentrations, while low temperature or dryness in the winter seems to reduce airborne fungi in the outdoor air.

Similarly, in the present study concentration of the airborne moulds peaked in August and September, positively correlating with temperature and solar radiation. Species of *Cladosporium* and *Alternaria* peaked in August and September, showing significant positive correlation with temperature and solar radiation, which is in agreement with studies conducted in other countries [4, 8, 17, 18, 20, 29]. Also *Fusarium* correlated with temperature changes in Medvednica.

Wind is an important factor for dispersion of airspora. *Alternaria* significantly correlated with wind speed. Studies in Italy and UK also found positive correlation between wind velocity and fungi producing larger spores [17, 18, 29]. According to Pasanen *et al.* [24], minimal wind speed for the release of *Cladosporium* sp. spores is 1 m/s, while *Aspergillus* and *Penicillium* release high number of spores at 0.5 m/s.

Cladosporium showed significant negative correlation with atmospheric pressure. Studies in Italy [17, 18] showed that the airspora release is insignificantly affected by the atmospheric pressure.

Von Vahl and Kersten [32] found significant positive correlation between frequent rainfall, high relative humidity and *Fusarium* spore concentration. In the present study, *Fusarium* peaked in October when the rainfall was more frequent (average 107 mm) than in other months (average 8-78 mm).

In the city centre and botanical garden, levels of the yeasts were positively correlated with relative humidity and negatively correlated with temperature and solar radiation, while in Medvednica they were positively correlated with both, temperature and solar radiation. These differences were probably caused by low temperature during winter months in Medvednica, (below 0ºC) when significantly lower levels of the airborne yeasts were measured.

CONCLUSIONS

Concentration of airborne fungi during the year shows variability mostly depending on seasonal, climatic and geographic conditions of the particular area. In this 1-year study, a higher incidence of airborne fungi was found in the city centre than in the botanical garden and Medvednica. Increased concentrations in the city centre can be a consequence of air pollution, traffic and dust. Lower levels found in the botanical garden and Medvednica can be due to decreased pollution, special environmental conditions and type of vegetation, which could reduce airspora viability.

At all the sampling sites, *Cladosporium*, *Penicillium* and *Alternaria*, as the most common aeroallergens, dominated over other detected airborne fungi, showing quantitative variations greatly influenced by temperature changes and solar radiation. Monitoring of airborne fungi can be helpful in prediction of their qualitative and quantitative variations depending on meteorological, geographical and seasonal climatic factors, which is of great importance for prevention of fungal allergic diseases.

Acknowledgements

This work was supported by the Croatian Ministry of Science, Education and Sports (No. 0006641).

REFERENCES

1. Asan A, Burhan S, Sarica S: Airborne fungi in urban air of Edirne city (Turkey). *Biologia* 2002, **57**, 59-68.
2. Bandyopadhyay R, Mughogho LK, Satyanarayana MV: Occurrence of airborne spores of fungi causing grain mould over a sorghum crop. *Mycol Res* 1991, **95**, 1315-1320.
3. Comtois P: Indoor mould aerosols. *Aerobiologia* 1990, **6**, 165-176.
4. Corden JM, Millington WM, Mullins J: Long-term trends and regional variation in the aeroallergen *Alternaria* in Cardiff and Derby UK-are differences in climate and cereal production having an effect? *Aerobiologia* 2003, **19**, 191-199.
5. Cvetnić Z, Pepeljnjak S: Distribution and mycotoxin-producing ability of some fungal isolates from the air. *Athm Environ* 1997, **31**, 491-495.

6. de Hoog GS, Guarro J, Gene J, Figueras MJ: *Atlas of clinical Fungi*. 2nd Ed. Centraalbureau voor Schimmelcultures/Universitat Rovira i Virgili, Baard-Delft 2000.

7. Feller W: *An Introduction to the Probability Theory and its Application*, 175. John Wiley and sons Inc., New York 1950.

8. Herrero B: Weekly variation of fungal colonies in the atmosphere of Palencia (Spain) throughout the year 1992. *J Allergy Clin Immunol* 1997, **7**, 611-618.

9. Horner WE, Helbling A, Salvaggio JE, Lehrer SB: Fungal allergens. *Clin Microbiol Rev* 1995, **8**, 161-178.

10. Ismail MA, Chebon SK, Nakamya R: Preliminary surveys of outdoor and indoor aeromycobiota in Uganda. *Mycopathologia* 1999, **148**, 41-51.

11. Kasprzyk I, Rzepowska B, Wasylów M: Fungal spores in the atmosphere of Rzeszów (South-East Poland). *Ann Agric Environ Med* 2004, **11**, 285-289.

12. Katial RK, Zhang Y, Jones RH, Dyer PD: Atmospheric mold spore counts in relation to meteorological parameters. *Int J Biometeorol* 1997, **41**, 17-22.

13. Klironomos JN, Rillig MC, Allen MF, Zak DR, Pregitzer KS, Kubiske ME: Increased levels of airborne fungal spores in response to *Populus tremuloides* grown under elevated atmospheric CO_2. *Can J Bot* 1997, **75**, 1670-1673.

14. Lawande RV, Onyemehkwe GC: Airborne fungi during Harmattan in Zaria, Nigeria. *Ann Allergy* 1984, **52**, 47-49.

15. Lugauskas A, Krikstaponis A, Sveistyte L: Airborne fungi in industrial environments - potential agents of respiratory diseases. *Ann Agric Environ Med* 2004, **11**, 19-25.

16. Lugauskas A, Šveistyte L, Ulevičius V: Concentration and species diversity of airborne fungi near busy streets in Lithuanian urban areas. *Ann Agric Environ Med* 2003, **10**, 233-239.

17. Marchiso VF, Airaudi D, Barchi C: One-year monitoring of the airborne fungal community in a suburb of Turin (Italy) and assessment of its functional relations with the environment. *Mycol Res* 1997, **101**, 821-828.

18. Marchiso VF, Airaudi D: Temporal trends of the airborne fungi and their functional relations with environment in a suburban site. *Mycologia* 2001, **93**, 831-840.

19. Meier R, Zingre H. Qualification of air sampler systems: The MAS 100. *Swiss Pharma* 2000, **22**, 15-21.

20. Mitakakis TZ, Guest DI. A fungal spore calendar for the atmosphere of Melbourne, Australia, for the year 1993. *Aerobiologia* 2001, **17**, 171-176.

21. Molina AM, Romero JA, García-Pantaleón FI, Comtois P Vilches ED: Preliminary statsistical modelling of the presence of two conidial types of *Cladosporium* in the atmosphere of Córdoba, Spain. *Aerobiologia* 1998, **14**, 229-234.

22. Moustafa AF, Kamel SM: A study of fungal spore population in the atmosphere of Kuwait. *Mycopathologia* 1976, **59**, 29-35.

23. Nilsson S: Regional and global distribution of aeroallergens. *Rev Paleobotany Palynol* 1990, **64**, 29-34.

24. Pasanen AL, Pasanen P, Jantunen MJ, Kalliokoski P: Significance of air humidity and air velocity for fungal spore release in the air. *Atmos Environ* 1991, **25A**, 459-462.

25. Pepeljnjak S, Kosalec I, Kalođera Z, Kuštrak D: Natural antimycotics from Croatian plants. **In:** Rai MK, Mares D (Eds): *Plant Derived Antimycotics*, 49-73. Binghamton, The Haworth Press, Inc. New York, London, Oxford 2003.

26. Pepeljnjak S, Šegvić M: Occurrence of fungi in air and on plants in vegetation of different climatic regions in Croatia. *Aerobiologia* 2003, **19**, 11-19.

27. Resano A, Sanz ML, Oehling A: Sensitization to *Alternaria* and *Cladosporium* in asthmatic patients and its in vitro diagnostic confirmation. *J Invest Allergol Clin Immunol* 1998, **8**, 353-358.

28. Samson RA, Hoekstra ES, van Oorschot CAN: *Introduction to Food-borne Fungi*. Centraalbureau voor Schimmelcultures, The Netherlands 1981.

29. Takahashi T: Airborne fungal colony-forming units in outdoor and indoor environments in Yokohama, Japan. *Mycopathologia* 1997, **139**, 23-33.

30. Tilak ST: Fungal spores and allergy. *J Palynol* 1991, **27**, 369-386.

31. Vijay HM, Burton M, Young NM, Copeland DF, Corlett M: Allergenic components of isolates of *Cladosporium herbarum*. *Grana* 1991, **30**, 161-165.

32. Von Vahl PG, Kersten W: *Fusarium* and *Didymella*-neglected spores in the air. *Aerobiologia* 1991, **7**, 111-117.

33. Wu P-C, Su H-J, Lin C-Y: Characteristics of indoor and outdoor airborne fungi at suburban and urban homes in two seasons. *Sci Total Environ* 2000, **253**, 111-118.