



Occurrence of fungi in air and on plants in vegetation of different climatic regions in Croatia

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Abstract

Research was carried out on the presence and variation in occurrence of fungal genera in air and on plant samples collected from three different climatic regions in Croatia in relation to temperature and relative humidity throughout one year (1998). The sampling method employed in this study was exposure of 150 Sabouraud agar plates with antibiotics to the air for 10 min. and by placing 10 pieces of each plant sample (N = 150) on same agar. The spores belonging to 15 fungal genera and sterile mycelium were identified. *Cladosporium* (65.3–74%), *Penicillium* (4.43–13.9%), *Alternaria* (2–4.7%), and sterile mycelium (7.4–15%) were the most prevalent fungi in the air of all three climatic regions, while *Fusarium* (20.3–36.2%) was the most prevalent fungal genera on plant samples especially in modest continental climate. Significant differences were obtained for comparative fungal contamination of air and plant samples, and by comparing monthly occurrence in all three parts included. The climate factors and presence of vegetation that produce essential oils with fungicide activity may be the reason for reduced fungal contamination of outdoor environments in the Mediterranean climate.

1. Introduction

Fungi from a wide variety of genera have a great capacity to colonise many kinds of substrata and develop in extreme environmental conditions, on soils, plants and animal remains (Comtois, 1990). Spores from these fungi are usually airborne.

Airspora of several species of moulds are known to induce numerous human diseases such as chronic bronchitis, asthma, fungal allergies, hypersensitivity pneumonitis and aspergillosis (Ferguson et al., 1988; Jonathan et al., 1989; Repp and Muler-Wening, 1989). Tilak (1991) estimated that between 2 and 30% of respiratory allergies are due to fungal spores, and cited *Alternaria* and *Cladosporium* as producing the highest number of allergies. Fungal allergens are found primarily in the spores, but they are also occurring in other structures, e.g., mycelia and material

secreted into the environment (Bush, 1989; Verhoeff and Burge, 1997).

The implications for human health make it advisable to determine local changes in airborne mycoflora, especially since some researches that have been carried out, showed that fungal allergies have been increased during the past decade (Allergy service of the University Hospital of Cordoba, 1990).

The airspora concentrations of outdoor environments depend on numerous factors including; time of day, meteorological factors, seasonal climatic factors, and type of vegetation (Street and Hamburger 1976; D' Amato et al., 1984).

Therefore, the aim of our study was to determine the common airborne fungi of the outdoor environment in correlation to fungal contamination of plants in different climatic conditions in Croatia.

2. Materials and methods

2.1 The study area

Plant and air samples were collected from three geographical parts in Croatia; modest continental climate, including Zagreb-capital city Croatia, and vicinity (lat 45°48' N, long 15°18' E), Mediterranean climate northern Adriatic coast (Primorje), including city Rijeka and vicinity (lat 45°19' N, long 14°26' E), and South Croatia, including city Split (lat 43°30' N, long 16°26' E), and island Hvar (lat 43°10' N, long 16°26' E).

Total count of air (N = 150) and plant (N = 150) samples were collected during 5 months (January, February, May, June, November) in 1998 (3 years after the war in Croatia). from all three parts in Croatia. Monthly meteorological data (relative humidity and temperature) were collected from Hydrometeorological state department in Zagreb (Croatia).

Sampling area in the continental part is represented with following plant associations; *Quercus carpinetum orientalis* and *Quercus castanetum*.

Sampling area in Primorje is represented with few plant associations, including; *Quercus-Carpinetum orientalis*, *Paliuretum adriaticum*, *Stipo-Salvietum officinalis*, and cultivars including; plough fields, vineyards and olive-grows.

Sampling area in Dalmacija is represented with different plant associations on the seaside, including *Erico-Cistetum cretici*, *Stipo-Salvietum brachypodietosum*, *Stipo-Salvietum officinalis*, *Erico-Rosmarinetum*, *Vitici-Tamaricetum*, halofile *Juncetum maritimo acuti*, and cultivates including; vineyards and olive-groves of *Lavandula sp.* -cultivated on island Hvar.

2.2 Sampling method

Air samples (10 samples in each region during each month sampled) were collected daily (early morning) by exposure of Sabouraud agar plates with antibiotics penicillin and streptomycin (60 : 40) (diameter 10 cm) to air for 10 minutes. Samplings were done during good weather to avoid rain, snow, etc. The plates were held at one and a half meter. Plates were incubated on 25 °C for 4–7 days and colony forming units (CFU) were counted. Fungal species were re-isolated on different agar plates (Czapek, Malt, Potato-sucrose agar) for characterisation and determination of fructification organs in accordance with keys; Raper and Thom (1949), Booth (1971), Samson et al. (1981).

Plant samples (stems and/or mature leaves) were collected in sterile paper bags, and 10 pieces from each sample were placed on Sabouraud agar plates for analyse of contamination intensity (CI).

Plant species in the continental part: *Quercus robur*, *Corylus avellana*, *Carpinus sp.*, *Aesculus hippocastanum*, *Castanea sativa*, *Platanus orientalis*.

Plant species in Primorje: *Quercus ilex*, *Carpinus sp.*, *Salvia officinalis*, *Paliurus spina cristi*, *Aesculus hippocastanum*, *Platanus orientalis*.

Plant species in Dalmacija: *Erica verticillata*, *Cistus sp.*, *Salvia officinalis*, *Rosmarinus officinalis*, *Tamarix sp.*, *Laurus nobilis*, *Lavandula sp.*

Samples were incubated on 25 °C for 4–7 days and than the moulds were re-isolated on Czapek, Malt, Potato-Sucrose agar for identification.

2.3 Statistical analysis

The data obtained as the total number of colonies per agar plates (cfu) of each fungal genera were statistically analysed by nonparametric Kruskal-Wallis ANOVA test and Spearman rank correlation, which was performed by using the Statistica for Windows Version 5 software program. The distribution was tested by Kolmogorov-Smirnov "Goodness of fit" test. Probability values less than 0.05 ($p < 0.05$) were considered significant.

3. Results and discussion

The total percent of occurrence, mean, minimum, maximum, standard deviation and standard error of air and plant mycoflora from the continental part, Primorje and Dalmacija are listed in Tables 1 and 2.

The air mycoflora from all three sampling areas in Croatia, was dominated by 3 fungal genera; *Cladosporium* 73.3% in continental part, 74% in Primorje, 65.3% in Dalmacija; *Penicillium* 13.9% in the continental part, 4.43% in Primorje, 10.4% in Dalmacija; *Alternaria* 2% in continental part, 4% in Primorje, 4.7% in Dalmacija, and sterile mycelium 7.4% in continental part, 15% in Primorje, 14.6% in Dalmacija. Other isolated fungal genera including *Aspergillus*, *Fusarium*, *Botrytis*, *Absidia*, *Rhizopus*, *Mucor*, *Trichothecium*, *Trichoderma* and other moulds (*Bipolaris*, *Bispora*, *Culvularia*, *Epicoccum*) had a very low percentage of occurrence, or were totally missing in samples. The significant differences ($p < 0.05$) were established for *Penicillium*, *Cladosporium*

Table 1. Number of cfu of fungi in air samples collected in Croatia

	mean	sum	Min	Max	Std. Dev	Standard Error	Percent of occurrence (%)
<i>Absidia sp.</i>							
C	0.04	2	0	1	0.198	0.028	0.4
P	0.08	4	0	1	0.274	0.038	1.1
D	0	8	0	0	0	0	0
<i>Alternaria sp.</i>							
C	0.22	11	0	3	0.582	0.082	2.0
P	0.28	14	0	4	0.783	0.110	4.0
D	0.4	20	0	5	0.990	0.134	4.7
<i>Aspergillus sp.</i>							
C	0.02	1	0	1	0.141	0.020	0.2
P	0.08	4	0	3	0.444	0.063	1.1
D	0.1	5	0	3	0.505	0.071	1.2
<i>Botrytis sp.</i>							
C	0.04	2	0	2	0.283	0.04	0.4
P	0.02	1	0	1	0.141	0.02	0.5
D	0.02	1	0	1	0.141	0.02	0.3
<i>Cladosporium sp.</i>							
C	8.2	410	0	100	19.673	2.782	73.3
P	5.34	267	0	100	14.131	1.998	74.0
D	5.2	260	0	100	14.042	1.985	65.3
<i>Fusarium sp.</i>							
C	0.2	10	0	2	0.495	0.070	1.20
P	0.04	2	0	1	0.198	0.028	0.55
D	0.1	5	0	2	0.364	0.051	1.70
Other moulds: <i>Bipolaris</i> , <i>Bispora</i> , <i>Culvularia</i> , <i>Epicoccum</i>							
C	0.14	7	0	5	0.730	0.103	1.0
P	0	0	0	0	0	0	0
D	0.02	1	0	1	0.141	0.02	0.5
<i>Penicillium sp.</i>							
C	1.44	72	0	11	2.508	0.355	13.9
P	0.32	26	0	5	0.999	0.141	4.43
D	0.84	42	0	11	2.235	0.316	10.4
<i>Rhizopus sp.</i>							
C	0.04	2	0	1	0.283	0.04	0.37
P	0	0	0	0	0	0	0
D	0	0	0	0	0	0	0
sterile mycelium							
C	0.8	40	0	4	1.125	0.159	7.4
P	1.08	54	0	7	1.536	0.217	15.0
D	1.18	59	0	7	1.699	0.240	14.6

C = continental part; P = Primorje; D = Dalmacija.

Table 2. Number of cfu of fungi on plant samples collected in Croatia

	mean	sum	Min	Max	Std. Dev	Standard Error	Percent of occurrence (%)
<i>Absidia sp.</i>							
C	0.24	12	0	2	0.517	0.073	5.6
P	0.16	8	0	3	0.510	0.074	4.0
D	0.06	3	0	1	0.240	0.034	1.4
<i>Alternaria sp.</i>							
C	0.72	36	0	4	0.948	0.134	16.9
P	0.58	29	0	5	1.144	0.162	15.2
D	0.72	36	0	5	1.280	0.181	16.6
<i>Aspergillus sp.</i>							
C	0.14	7	0	2	0.405	0.057	3.3
P	0.26	13	0	3	0.633	0.089	6.6
D	0.18	9	0	5	0.748	0.106	4.1
<i>Botrytis sp.</i>							
C	0	0	0	0	0	0	0
P	0.02	1	0	1	0.141	0.020	0.5
D	0.04	2	0	2	0.283	0.040	0.9
<i>Cladosporium sp.</i>							
C	0.4	20	0	4	0.782	0.111	9.4
P	0.78	39	0	4	1.055	0.149	19.8
D	0.38	19	0	4	0.945	0.134	8.7
<i>Fusarium sp.</i>							
C	1.64	82	0	7	1.735	0.245	36.2
P	0.8	40	0	3	0.930	0.131	20.3
D	1.06	53	0	5	1.544	0.218	24.4
<i>Mucor sp.</i>							
C	0.04	2	0	2	0.283	0.040	0.94
P	0	0	0	0	0	0	0
D	0	0	0	0	0	0	0
Other moulds: <i>Bipolaris</i> , <i>Bispora</i> , <i>Culvularia</i> , <i>Epicoccum</i>							
C	0.8	40	0	5	1.161	0.164	18.4
P	0.6	15	0	5	1.054	0.149	4.5
D	0.06	3	0	2	0.313	0.044	4.5
<i>Penicillium sp.</i>							
C	0.04	2	0	2	0.283	0.040	0.94
P	0.34	17	0	4	0.917	0.130	8.6
D	0.24	12	0	3	0.656	0.093	5.5
<i>Rhizopus sp.</i>							
C	0.26	13	0	2	0.564	0.080	6.1
P	0.28	14	0	5	1.011	0.143	7.1
D	0.16	8	0	5	0.738	0.104	3.7
sterile mycelium							
C	0.36	18	0	3	0.776	0.109	8.4
P	0.4	20	0	3	0.756	0.106	10.7
D	0.38	19	0	5	1.086	0.156	8.7
<i>Trichoderma sp.</i>							
C	0.24	12	0	5	0.771	0.109	5.6
P	0.1	5	0	5	0.707	0.100	2.5
D	0.12	6	0	2	0.385	0.054	2.8
<i>Trichothecium sp.</i>							
C	0.1	5	0	5	0.771	0.109	2.3
P	0	0	0	5	0.707	0.100	0
D	0.2	10	0	2	0.385	0.054	4.6

C = Continental part; P = Primorje; D = Dalmacija.

and *Fusarium*, which were significantly higher in air samples from the continental part (Figures 1–3). The dominant fungal genera of plant mycoflora in examined sampling areas, was *Fusarium* 36.2% in the continental part, 20.3% in Primorje, 24.4% in Dalmacija. Significant difference between occurrences of plant mycoflora was established for *Fusarium* and other moulds (*Bipolaris*, *Bispora*, *Culvularia*, *Epicoccum*), which were significantly higher ($p < 0.05$) in the continental part, while *Rhizopus* was present in higher number in the continental part and Primorje (Figures 4–6).

We compared the occurrence of isolated fungal genera in air and plant samples for each sampling area. The significant correlation ($p < 0.05$) was established for *Alternaria* in continental part, and sterile mycelium and *Fusarium* in Primorje. For the rest of isolated fungal genera from three sampling areas in Croatia, the significant correlation wasn't established between their occurrences in air and plant samples.

Among the fungal genera *Cladosporium* dominates the outdoor mycofungal flora all over the world. Earlier studies of the air mycofungal flora in Croatia also identified *Cladosporium* as the predominant genera (up to 50%) of examined samples (Pepeljnjak and Cvetnić, 1985; Cvetnić and Pepeljnjak, 1997). Our study of occurrence of fungal genera in the air showed that *Cladosporium* dominated especially in the continental part of Croatia. Similar results were obtained in surveys described in some geographic areas in Spain, *Cladosporium* (50%) dominated especially during the spring (Herrero, 1997), also in Turin (Italy) (Marchiso et al., 1997), and in Yokohama (Japan) (Takahashi, 1997) the results correspond to our findings. Studies that have been carried out in Nigeria and Kuwait, showed that *Alternaria*, *Aspergillus* and *Penicillium*, take precedence over *Cladosporium* (Lawande and Onyemehkwe, 1984; Moustafa and Kamel, 1976). However, comparisons of this kind are often difficult, since results greatly depend on sampling methodology, times of the year, time of day and climatic conditions.

In our study *Alternaria* was rarely present in air, which is in agreement with the results of some earlier studies (7–9%) of Larsen (1981) and Al-Doory and co-workers (1980). On the other hand, our results are not corresponding with recent findings in Spain and Italy where *Alternaria* was present in higher number especially during spring (15–17%). In plant samples *Alternaria* was present with much higher number of spora. It seems that the survey of fungal genera

depends on the type of substrata, and this was the case especially for *Alternaria* and *Fusarium*, which was also found with much higher numbers of spora on plants. In literature, the genus *Fusarium* has generally been reported with a low incidence in the air of many cities (0.15–3.1%) (Cavalo et al., 1980; Al-Doory, 1980; Takahashi, 1997) and that corresponds to our findings. On the other hand, *Fusarium* was present on plant samples in all sampling areas with a much higher number of spora than in air samples. Literature reported a high number of nonsporulating white (34–51%) mycelia (Sorenson et al., 1974). We found a higher incidence of sterile mycelium in the air, than in plant samples. Probably the type of substrata make possible the sporulation and development of identifiable characteristics.

Most of the studies on aeromycology show a wide range, finding low and high incidence of *Aspergillus* spores in the air from 2–95% (Abdel Hafez and Shoreit, 1985). In our study, the incidence of *Aspergillus* (0.2–1.2%) in the air was very low or totally missing, while the incidence on plant samples (3.3–6.6%) was higher. However, the recent studies reported very high incidences of *Aspergillus* in corn dust (Hill et al., 1984) and grain stores, with a wide range of *Aspergillus* species (*A. fumigatus*, *A. niger*, *A. ochraceus*, *A. ventii*, *A. oryzae*) (Molina et al., 1996), which depends on storage temperature, ventilation, storage period, the presence of vegetation from harvest, and infections by insects.

The genus *Absidia* has rarely been reported in aeromycological surveys. It is not abundant in the air of towns, but it is more common on vegetation.

Among isolates a lot of fungal genera were also found in low incidence in the air (0–1.7%) (*Bipolaris*, *Bispora*, *Culvularia*, *Epicoccum*) which corresponds with earlier findings in Croatia (Cvetnić and Pepeljnjak, 1997). Only in the continental part, in plant samples did these moulds have higher incidence (18.4%).

Monthly occurrences of fungal genera in air and plant samples are represented as the average of colony forming units (CFU) (Figures 7, 8) collected from each sampling area in relation to relative humidity and temperature. Results for all sampling areas show that peaks of airspora occurrences are higher during warmer month's (May, June) (except in Dalmacija in November), and the lowest peaks are recorded during the winter month's (January, February). By contrast, the occurrences of fungal spora in plant samples increased in the winter months with the highest peak

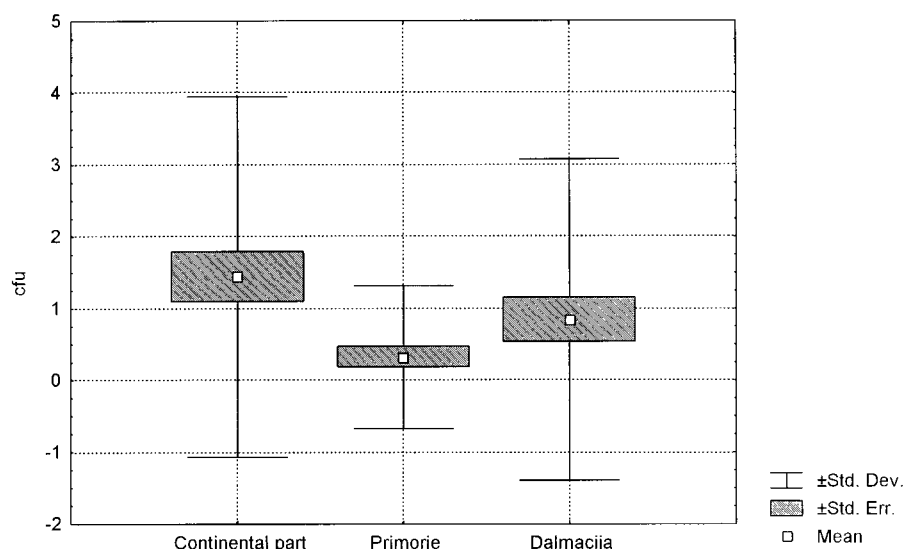


Figure 1. Occurrence of *Penicillium* sp. in the air samples.

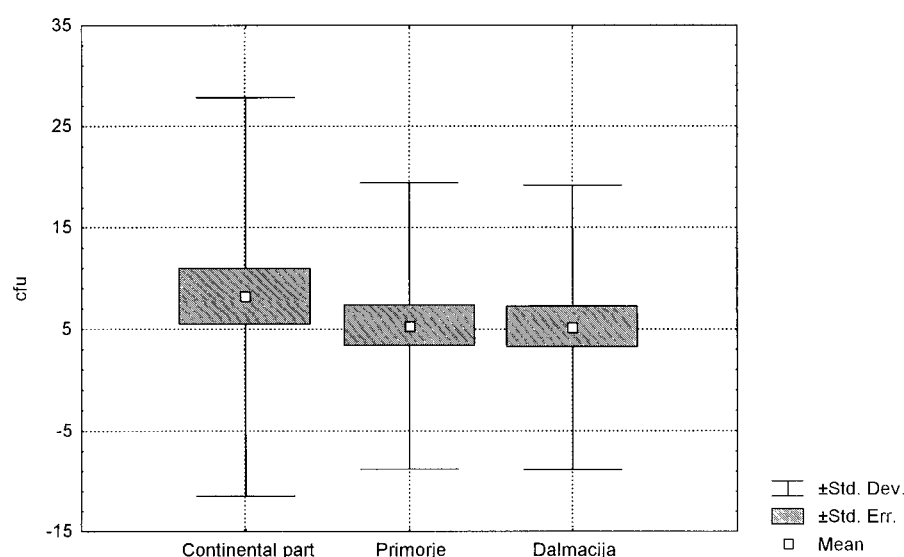


Figure 2. Occurrence of *Cladosporium* sp. in the air samples.

in January, and decreased in May and June. This was the case for all sampling areas, with lower occurrences established in Dalmacija, especially in May and June.

Airspora occurrence depends on climatic conditions; temperature and relative humidity. Many studies on aeromycology showed that temperature 15–25 °C and relative humidity 60–70% increase the airspora occurrence of the outdoor environment, which was also the case in our study. In the Mediterranean

climate, hot and dry summer months are responsible for reduced sporulation (Levetin and Horowitz, 1978). However, too much rain, low temperature or dryness in the winter in the continental climate also seems to reduce fungal spora in outdoor air. In the present study, the occurrences of the fungi detected in the outdoor air were significantly lower ($p < 0.05$) in Dalmacija during June when the temperature was higher (more than 20 °C) and relative humidity was lower (50–60%) than in other parts of sampling. On the other

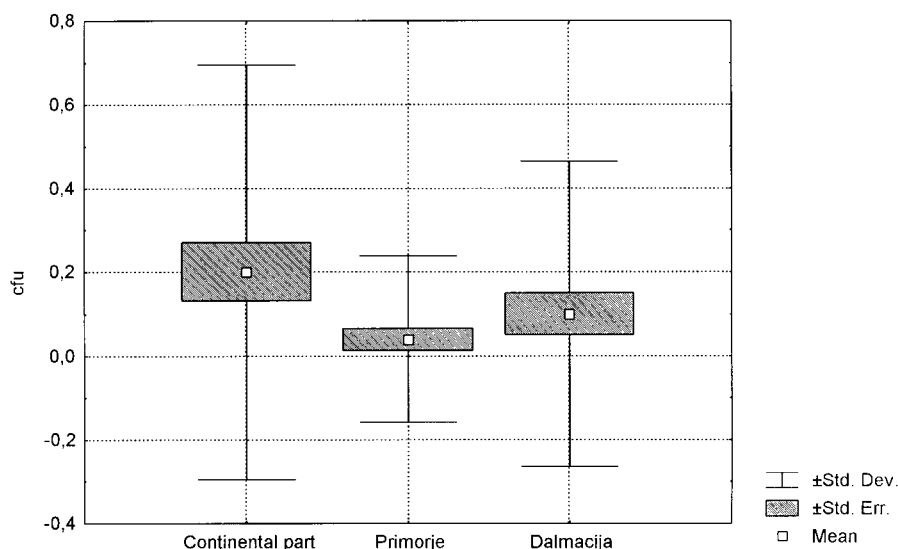


Figure 3. Occurrence of *Fusarium* sp. in the air samples.

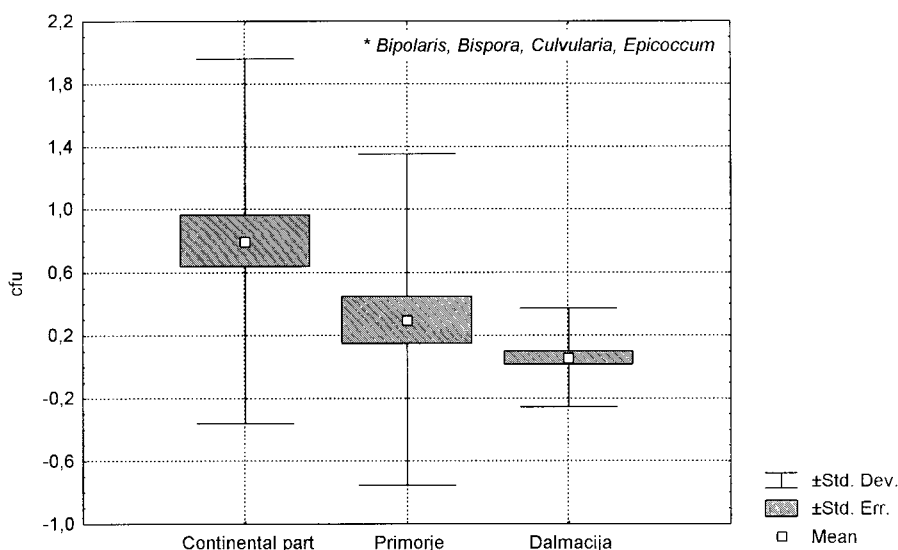


Figure 4. Occurrence of Other moulds* in the samples of vegetation.

hand, in Dalmacija a significantly higher occurrence ($p < 0.05$) of fungi was detected in November, when the relative humidity was (60–70%) and temperature was about 15 °C. Except for the climatic factors, it seems that the type of vegetation can reduce the spora occurrence as it was established in plant samples from Dalmacija. Among the genera identified, Nilsson (1990) mentioned the following genera as allergenic: *Alternaria*, *Aspergillus*, *Cladosporium*, *Culvularia*, *Fusarium*, *Penicillium* and *Epicoccum* (Tialk, 1991).

Some *Cladosporium* species (*C. herbarum*) have been noted as the primary agents responsible for allergies and asthma in humans (Hasnain et al., 1994; Vijay et al., 1991). Furthermore, the studies that have been taken among asthmatic children in Sweden reported a prevalence of reactivity to *Cladosporium* (31–37%) and *Alternaria* (14%) (Croner and Kjellman, 1992). However, the mentioned fungal genera are predominant in the continental part in air and plant samples. On the other side, distribution of fungal spora showed

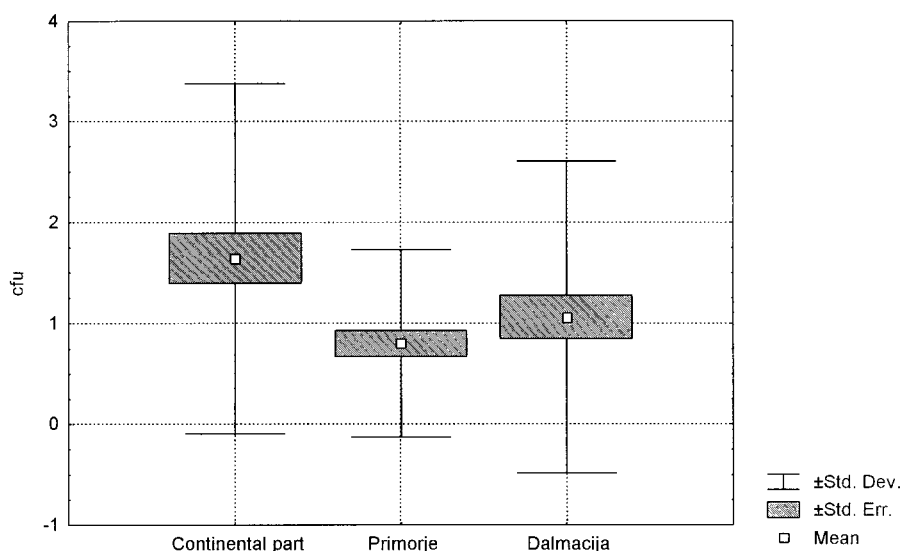


Figure 5. Occurrence of *Fusarium sp.* in the samples of vegetation.

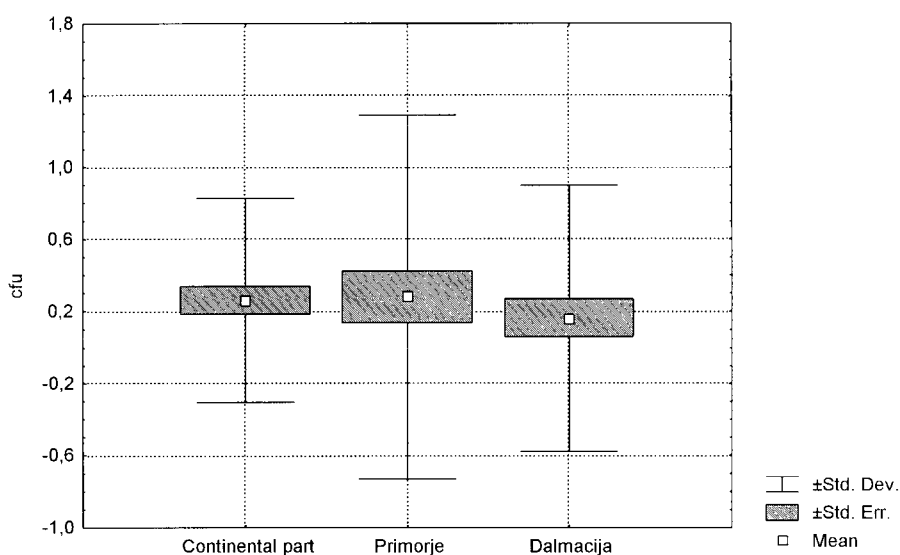


Figure 6. Occurrence of *Rhizopus sp.* in the samples of vegetation.

similarities in Primorje and Dalmacija. Earlier studies on mycological contamination of the ecosphere in Croatia during the past decade showed higher occurrences of fungal spora especially during the war in Croatia (1992–1994) in comparison with the 16-year period before the war. As a consequence of the war destruction (destruction of storage, organic material decay etc.), fungal contamination of air and storage houses of individual and industrial producers, was increased, which consequently increased mycological

and mycotoxycological risk (Pepeljnjak et al., 1999). Present findings corresponds with earlier findings taken before the war in Croatia, which means that mycological contamination of air and other substrata is decreasing, consequently decreasing mycotoxycological risk. Since the presence and concentrations of fungal spora in the outdoor environment depend on few factors; meteorological, seasonal climatic factors and the type of vegetation, reduced rainfall and dry Mediterranean climate can be one of the

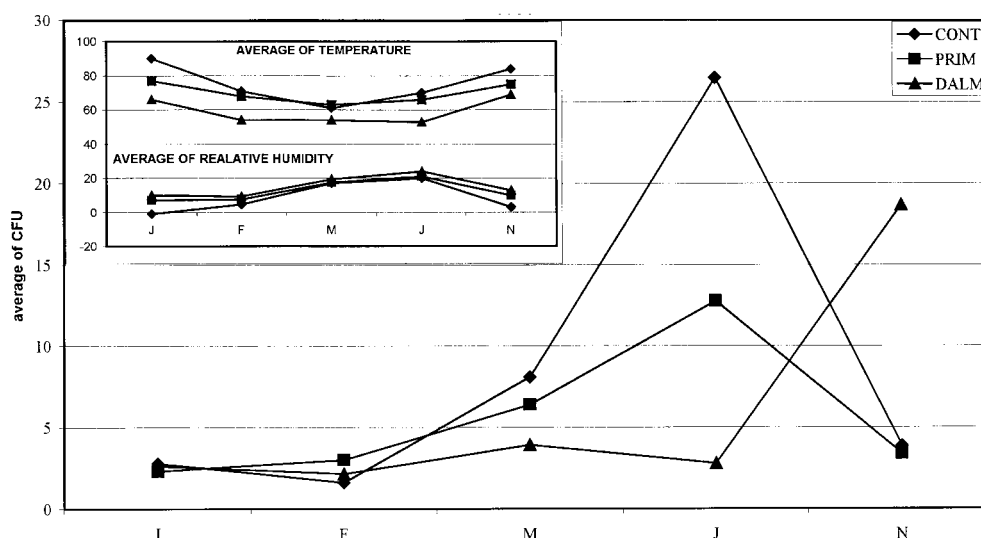


Figure 7. Average of monthly occurrence of airspora in different climatic regions in Croatia during 1998.

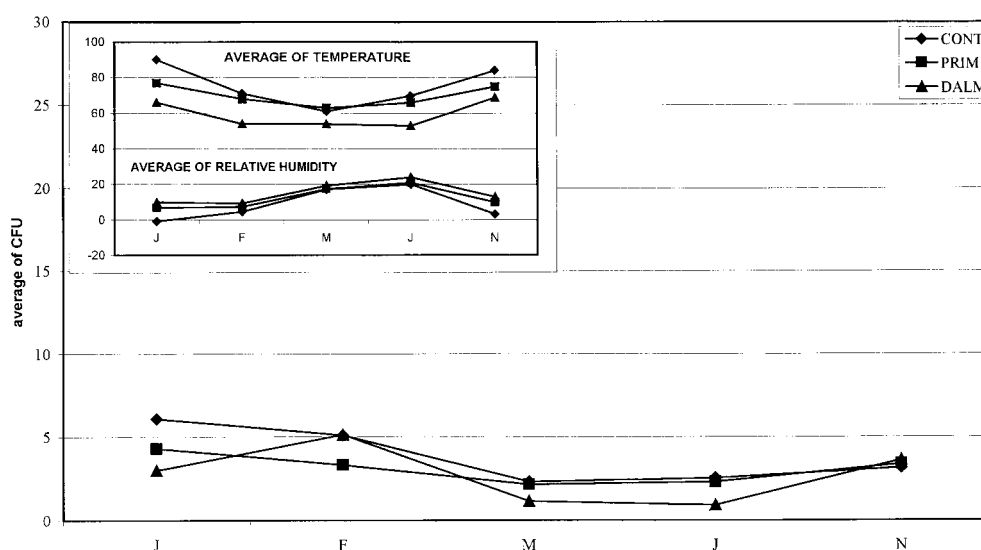


Figure 8. Average of monthly occurrence of fungal spora on plants in vegetation in different climatic regions in Croatia.

reasons for the reduction of fungal spora in Primorje and Dalmacija. On the other hand, the presence of some members of plant associations including; *Stipo-Salvietum officinalis* (Primorje and Dalmacija), *Erico-Cistetum cretici*, *Erico-Rosmarinetum*, and *Lavandula sp.* (Dalmacija, especially island Hvar), known to produce essential oils, and their aerosol which showed fungistatic and fungicide activity (Pepeljnjak et al., 1998; Kuštrak et al., 1998), can be one of reasons for reduced fungal spora in both, air and plant samples.

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