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Aeromonitoring of Alternaria spores in the air of Zaporizhzhia city

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Key words: aeromonitoring, fungi, fungal spores, Alternaria.

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The aim of the study was to determine the patterns of changes in the concentration of mold spores of the genus *Alternaria* in the air of Zaporizhzhia city.

Materials and methods. The object of the study was the dynamics of *Alternaria* spore concentration in the atmospheric air of Zaporizhzhia city. The study was carried out at the Department of Medical Biology, Parasitology and Genetics of Zaporizhzhia State Medical University. Samples were taken annually from March 1 to October 31 by volumetric method using a Hirst-type trap. The device is yearly certified by the state metrological service with certificates. The samples were analyzed using a light microscope at ×400 magnification using the method of vertical transects. The results of the research were processed by applying the Statistica® for Windows 13.0 licensed software package.

Results. The seasonal dynamics of fungal spores of the genus *Alternaria* in the atmospheric air of Zaporizhzhia was analyzed over several years: from 2015 to 2020. The beginning, end and duration of sporulation, maximum values were determined.

Conclusions. Analyzing the average daily (for six years) indicators of the spore number in the air of Zaporizhzhia city, it can be concluded that their distribution is normal throughout the year, which corresponds to the intensity of spore formation, depending on changes in meteorological conditions, that is, an almost equally symmetric range from the optimum, which falls on the beginning of June and is associated with an increase in precipitation in this region. Thus, an average of 17 256 spores/m^3 were determined over six years. The maximum number of spores was 680 spores/m^3 (6.07). The average day fell on July 25, σ = 37.1. In general, sporulation lasted 119 days. Peak concentrations were recorded in summer and autumn.

Ключові слова: аеромоніторинг, плісняві гриби, спори грибів, Alternaria.

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Аеромоніторинг спор грибів роду Alternaria в повітрі міста Запоріжжя

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Мета роботи – визначення закономірностей змін концентрації спор грибів *Alternaria* в атмосферному повітрі м. Запоріжжя.

Матеріали та методи. Об'єкт дослідження – динаміка концентрації спор грибів роду *Alternaria* в атмосферному повітрі м. Запоріжжя. Робота виконана на кафедрі медичної біології, паразитології та генетики Запорізького державного медичного університету. Відбір зразків здійснювали з 1 березня до 31 жовтня волюметричним методом, використовуючи об'ємний пробовідбірник за типом Хірста. Аналізували зразки за допомогою світлового мікроскопа при збільшенні ×400, застосовуючи метод зчитування вертикальних трансект.

Результати. Проаналізували сезонну динаміку спор грибів роду *Alternaria* в атмосферному повітрі м. Запоріжжя у період із 2015 до 2020 року. Визначали початок, кінець і тривалість споруляції, максимальні значення.

Висновки. Проаналізувавши середньодобові (за 6 років) показники розподілу кількості спор у повітрі міста Запоріжжя, зробили висновок про нормальний розподіл протягом року. Це відповідає інтенсивності спороутворення залежно від змін метеорологічних умов, тобто майже рівносиметричного діапазону від оптимуму, що припадає на початок червня та пов'язаний зі збільшенням опадів у цьому регіоні. За 6 років моніторингу визначили в середньому 17 256 спор/м^3. Максимальна кількість спор становила 680 спор/м^3 (6.07). Середній день припав на 25 липня, σ = 37,1. Загалом споруляція тривала 119 днів. Пікові концентрації реєстрували влітку та восени.

The absolute biological risk factors for the emergence of allergic diseases are fungal spores, which form an important part of bioaerosols in the atmospheric air [1]. Fungal allergens in 21.2 % of cases are etiological factors of inhalation allergies, asthma and bronchial diseases. The occurrence and course of allergies depend on the degree of fungal spore concentration, the prediction of which is possible thanks to aerobiological monitoring [2,3].

Monitoring of non-biological components of atmospheric air is carried out on the legislative level around the world and in Ukraine in particular. Due to state funding, citizens of a country have public access to this data and can judge air quality through public Internet access. Unfortunately, the situation with biological pollutants, such as pollen and mold spores, is different. Only Switzerland and France have state monitoring networks, while in other countries, research is conducted privately [4,5]. Japan (143), the United States (85) and a number of European countries: Italy (88), France (85), Spain (77) and Germany (44) hold world leaders in terms of the number of aerobiological stations. To calculate the number of spores and pollen in most countries of the world, a Hirst-type volumetric trap is used, with the exception of the United States, where the Rotorod sampler is mainly used. About 50 % of these stations also control mold spores, which almost always include *Alternaria spp.* and *Cladosporium spp.* [4].

In Ukraine, aerobiological monitoring with volumetric method is carried out in Kyiv at the State Institution "O. M. Marzieiev Institute for Public Health of the NAMSU" under the leadership of O. I. Turos, in Zaporizhzhia – on

Table 1. Summary table 2015–2020

Descriptive Statistics											
Year	Mean	Median	Mode	Max	Sum	%	Std. deviation	Skewness	Std. err. Skewness	Kurtosis	Std. err. Kurtosis
2015	29.07	20.07	6.07	1272	15096	87	33.1	0.16	0.019	0.015	0.038
2016	20.07	16.07	4.07	1356	23067	134	35.5	0.17	0.016	0.76	0.032
2017	1.08	30.07	6.07	1918	14798	86	37.6	-0.37	0.019	0.97	0.039
2018	7.08	31.07	27.07	415	13430	78	44.6	-0.21	0.021	-0.52	0.041
2019	28.07	27.07	5.07	964	22417	130	37.8	-0.03	0.016	-0.12	0.032
2020	2.08	30.07	14.06	359	14727	85	47.1	0.09	0.020	-0.54	0.040
Mean	25.07	20.07	6.07	680	17256	100	37.1	-0.08	0.019	0.39	0.037

the basis of Zaporizhzhia State Medical University under the leadership of O. B. Prykhodko and National Pirogov Memorial Medical University (Vinnytsia) under the guidance of V. V. Rodinkova [6]. But only Zaporizhzhia and Vinnytsia implement counting of mold spores in Ukraine [7].

The results of aerobiological observations are used to construct an allergy prediction, which has been carried out since 2011 by the Vinnytsia Scientific Aerobiological Group to prevent the population from a possible outbreak of pollen and fungal allergies [6]. Therefore, there is a great need to monitor spores of fungi that can cause such allergies, in particular *Alternaria spp*.

Aim

The aim of the study is to determine the patterns of changes in the concentration of mold spores of the genus *Alternaria* in the air of Zaporizhzhia city.

Materials and methods

The object of the study was the dynamics of mold spores of the *Alternaria spp*. The analysis of the spore concentration was carried out on the basis of data collected over several years: from 2015 to 2020. Sampling was carried out with volumetric method using a Hirst-type trap. The device is yearly certified by the state metrological service with certificates. The samples were analyzed with a light microscope at ×400 magnification using the method of vertical transects.

The results of the research were processed by applying the Statistica® for Windows 13.0 licensed software package.

Results

Seasonality was determined using the 95th percentile method, where the beginning of the season was defined as the date when 5 % of the spore total number per year was recorded, and the end – as the date when 95 % was observed. Descriptive statistics of the results obtained after monitoring fungal spores of the genus *Alternaria* in the period from 2015 to 2020 are shown in *Table 1*.

The graph (*Fig. 1*) shows the average daily number of *Alternaria* spores in 2015.

Fungal spores appeared on June 6 (5th percentile). The maximum number of spores was recorded on July 7 with a value of 1272 spores/m^3. Sporulation occurred for 84 days and ended on September 10. Thus, according to the results of five-year monitoring, 2015 was the shortest sporulation season. In total, 15 096 spores/m^3 were determined during the year, which was 87 % of the annual observations. The average day was July 29, σ = 33.1 days. The distribution of spores has a positive asymmetry on average at the level of 0.16 ± 0.019.

The graph (*Fig. 2*) shows the average daily number of spores in 2016.

The season started earlier than the previous year and fell on May 27. The maximum number of fungal spores was recorded on July 4 with a value of 1356 spores/m^3. The sporulation period was 109 days and ended on September 13. In total, 23 067 spores/m^3 were determined for the year, which was 134 % of the annual observations and was the maximum value for 5 years of observations. The average day was July 20, σ = 35.5 days. The distribution of spores has a positive asymmetry on average at the level of 0.170 ± 0.016.

The graph (*Fig. 3*) shows the average daily number of spores in 2017.

Fungal spores appeared on April 10 (5th percentile). The peak concentration of spores was recorded on July 6 with the maximum value for the entire observation period – 1356 spores/m^3. The sporulation period lasted 114 days and ended on September 17. In total, 14 798 spores/m^3 were identified for the year, which amounted to 86 % of the annual observations. The average day was August 1, σ = 37.6 days. The distribution of spores has a negative asymmetry on average at the level of -0.370 ± 0.019.

The graph (*Fig. 4*) shows the average daily number of spores in 2018.

Fungal spores appeared on May 24 (5th percentile). The maximum spores were recorded on July 27 with a value of 415 spores/m^3. The sporulation period was 132 days and ended on October 2. In total, 13 430 spores/m^3 were determined for the year, which amounted to 78 % of the annual observations. The average day was August 7, σ = 44.6 days. The distribution of spores, as in the previous year, had a negative asymmetry on average at -0.210 ± 0.021. There were two distinct waves of sporulation, in summer and autumn.

The graph (*Fig. 5*) shows the average daily number of spores in 2019.

Fungal spores appeared on May 22 (5th percentile). The maximum number of spores was recorded on July 5 with a value of 964 spores/m^3. The sporulation period was 112 days and ended on 10 September. In total, 22 417 spores/m^3 were identified during the year, which was 130 % of the annual observations. The average day was July 28, σ = 37.8 days. The distribution of spores had a negative asymmetry on average at -0.030 ± 0.016.

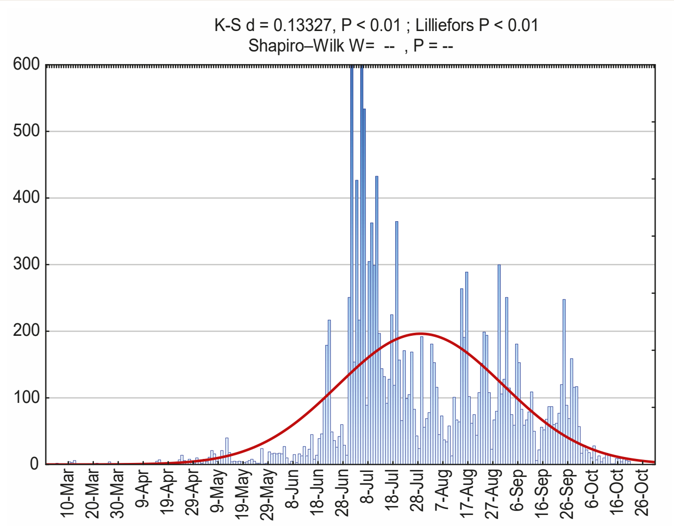

Fig. 1. Concentration of mold spores of the genus *Alternaria* in the air of Zaporizhzhia city in 2015.

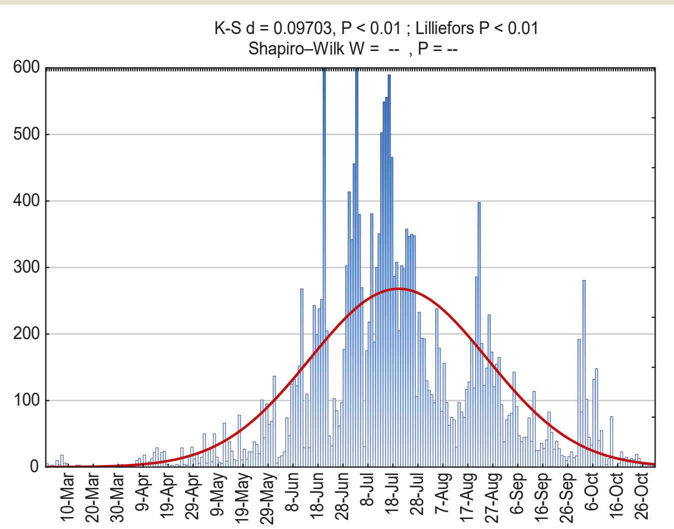

Fig. 2. Concentration of mold spores of the genus *Alternaria* in the air of Zaporizhzhia city in 2016.

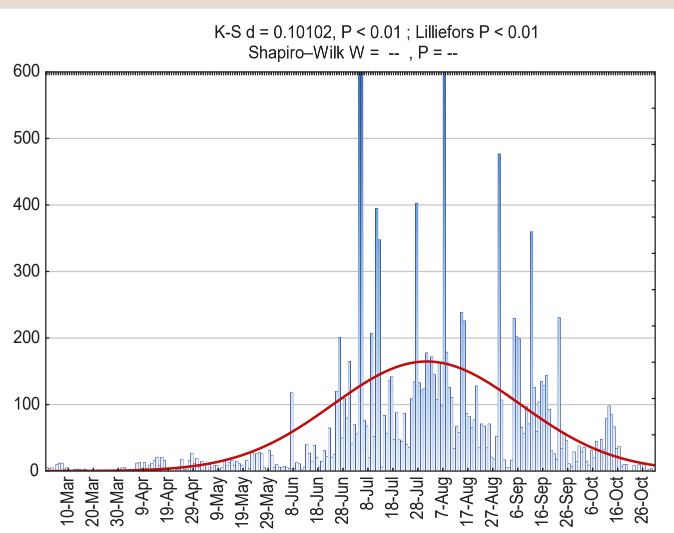

Fig. 3. Concentration of mold spores of the genus *Alternaria* in the air of Zaporizhzhia city in 2017.

The graph (*Fig. 6*) shows the average daily number of spores in 2020.

Fungal spores appeared on May 25 (5th percentile). The maximum of spores was recorded on June 14 with a value of 359 spores/m^3. The sporulation period was 142 days and ended on October 13. It was the longest sporulation season in the five-year observation period. In total, 147 270 spores/m^3 were determined for the year, which amounted to 85% of the annual observations. The average day was August 2, σ = 47.1 days. The distribution of spores was positive, with an average of 0.09 ± 0.02.

Discussion

Long-term monitoring allows to predict a high level of spores in the air for warning the population in good time. Consequently, people susceptible to fungal allergies have accurate information regarding the concentration of allergenic agents.

According to the study results, the highest concentration of mold spores *Alternaria spp.* in the air of Zaporizhzhia city were observed mainly in the summer period from July to August, as well as in autumn. Similar data can be found in the works of other authors. Studies carried out by P. Repiejko et al. in the southern part of Poland showed that peaks of spore concentration at five aeromonitoring stations also occurred in July [8]. But, in the air of northern Poland, the highest concentration of spores was observed in July–August (A. Chloupek et al.) [9]. However, Zaporizhzhia is located in the steppe zone and favorable weather conditions for the growing season of mushrooms, which is observed in early summer and early autumn. Our data are more consistent with the results obtained in the north of Italy. In air studies of cities in northern Italy, Marchesi noted the period with the highest rate of spores, with two main peaks in early and late summer [10]. When analyzing the correspondence of the spore number to weather conditions, no correlation was found. Further research for the development of forecasting methods is of interest.

According to research of G. Apangy et al., significantly higher concentrations of spores in the UK are observed in August, due to the large number of grains and oilseeds in these regions [11]. The same trend is observed in the studies of Danish scientists Y. Olsen et al., where high concentrations of spores are observed in August [12]. The authors also explain this in terms of the location close to agricultural sources and the harvest period. Mikaliunaite R. et al. also supports the fact that the concentration of spores is greater in areas with less urbanization than in urban air [13]. Reyes E. S. et al. in Valladolid (Spain) reported that more spores of *Alternaria* were observed in summer during the harvest season. Monthly peaks in different years were recorded in July and August. In addition to affecting the concentration of spores from agricultural sources, they also found a positive correlation with temperature and a negative correlation with relative humidity and rainfall [14]. Grinn-Gofron A. at al. studying the influence of thunderstorms on the level of fungal spores, including *Alternaria*, reported that the concentration of spores on stormy days was very high (more than 7000 spores/m^3) in Szczecin (Poland). Scientists noticed that before a thunderstorm, when the air temperature and ozone concentration were increased, the number of spores was increased, and vice versa, during a thunderstorm, these two indicators were decreased as well as the spore concentration [15].

Conclusions

1. Analyzing the average daily (for six years) indicators of the number of spore distribution in the air of Zaporizhzhia city, it can be concluded that the distribution is normal throughout the year, which corresponds to the intensity of spore formation, depending on changes in meteorological conditions, that is, an almost equally symmetric range from the optimum, which falls on the beginning of June and is associated with an increase in precipitation in this region. Thus, an average of 17 256 spores/m^3 was determined over six years. The maximum number was 680 spores/m^3 (6.07). The average day fell on July 25, σ =37.1. In general, sporulation lasted 119 days.

2. The results of our data can be used to warn the public about possible outbreaks of fungal allergies or about the aggravation of the allergy symptom course in the future.

Prospects for future research are to study the influence of meteorological factors on the concentration of fungi *Alternaria*.

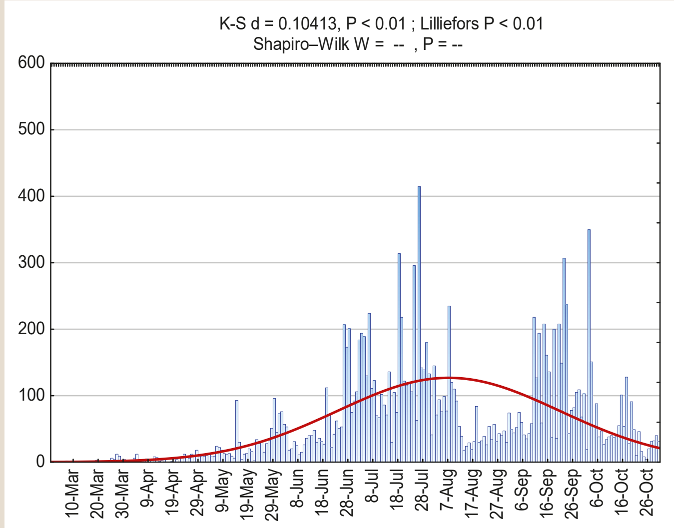

Fig. 4. Concentration of mold spores of the genus *Alternaria* in the air of Zaporizhzhia city in 2018.

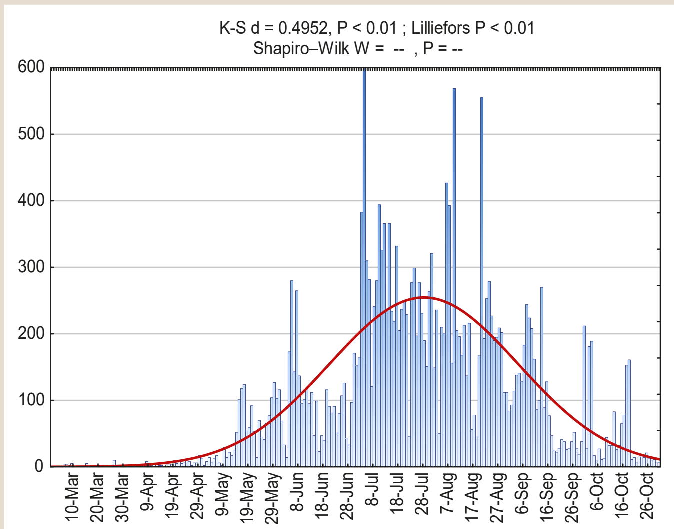

Fig. 5. Concentration of mold spores of the genus *Alternaria* in the air of Zaporizhzhia city in 2019.

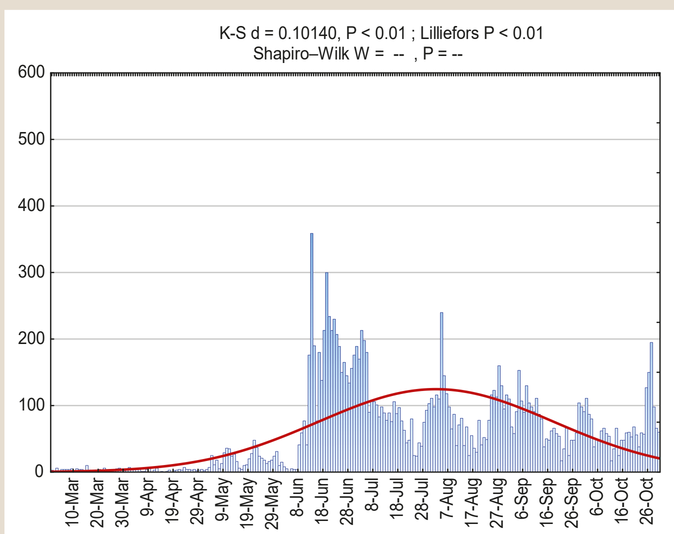

Fig. 6. Concentration of mold spores of the genus Alternaria in the air of Zaporizhzhia city in 2020.

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