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SEASONAL VARIABILITY OF AMBIENT BIOAEROSOL CONCENTRATIONS OVER DENSELY POPULATED CENTRAL AREA IN SOFIA, BULGARIA: FOCUS ON SPRING AND SUMMER

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Abstract

In terms of air quality, Sofia, Bulgaria, is one of Europe's most polluted capitals. The city represents a unique pollution challenge due to the combination of specific environmental and orographic features. Until now, no studies have been conducted to monitor outdoor bioaerosol concentrations. This pioneering research presents a systematic study of the seasonal cycle of bacterial and fungal aerosol concentrations in Sofia. Although the overall study extends over a full year, this paper focuses specifically on results from the spring and summer seasons. Airborne microbial samples were collected from the 1st of March 2024 to the 31st of August 2024 at an open-air location situated in a central urban area. The findings reveal a clear seasonal distribution pattern for bacterial and fungal aerosols. The mean bioaerosol concentrations during the spring-summer period were ranked in descending order as follows: a) bacterial aerosols: summer (258.0 CFU/m^3) > spring (197.0 CFU/m^3); (b) fungal aerosols: summer (987.0 CFU/m^3) > spring (228.0 CFU/m^3). This data on bioaerosol levels can be used to support future aeromicrobiology studies, policymaking, and urban planning in Sofia.

Key words: bioaerosols, airborne bacteria, meteorological parameters

Introduction. Airborne biological particles, or bioaerosols (e.g., bacteria, fungi, pollen), significantly impact urban air quality. Monitoring these aerosols

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is vital for environmental management and assessing health impacts. Bioaerosols have been associated with respiratory diseases, allergies, and adverse effects on human health [1]. They also contribute to the overall burden of particulate matter in urban environments [2]. Despite advances in aeromicrobiology, long-term studies in urban areas remain limited. Sofia's specific environmental factors, including its location in a valley and proximity to Vitosha Mountain, exacerbate pollution retention and require focused research. The city's rapidly increasing population and urbanization further amplify the need for bioaerosol monitoring. Studies conducted in cities such as Beijing, Marseille, and Warsaw have highlighted significant seasonal and regional variability in bioaerosol concentrations, driven by local climate, geography, and anthropogenic activities [3,4]. The diversity in atmospheric biological particle concentrations is influenced by meteorological and geographical conditions, as well as the urbanization characteristics of a given area. Geographic location and climate play a crucial role in determining outdoor airborne bacteria levels, as bioaerosol transport is primarily driven by hydrodynamic and kinetic processes, while their distribution and persistence are shaped by local human activities and prevailing meteorological conditions [5]. However, no comprehensive studies have been undertaken in Sofia, leaving a critical gap in understanding bioaerosol dynamics in this unique setting. This study is the first of its kind in Bulgaria, monitoring ambient bioaerosol concentrations in central Sofia to evaluate seasonal dynamics. The findings aim to inform future policies on air quality management and public health.

Materials and methods. ***Sampling site.*** Samples were collected on the roof of the Faculty of Biology, Sofia University. The choice of the site was justified by its location in a highly urbanized central area of the city of Sofia, as well as by its proximity in the north direction to two of the main transport vectors in the capital, separated by an extensive green area. South side of the building borders to an area with compact building development. There are no specific industrial pollution sources surrounding the sampling site. The sampling location was 20 meters above ground to minimize ground-level turbulence effects.

Sampling protocol. Bioaerosols were collected using a Six-Stage Viable Cascade Impactor (FSC-A6), operating at a flow rate of 28.3 L/min. Nutrient agar and Yeast Extract Glucose Chloramphenicol agar were used for bacterial and fungal sampling, respectively. Samples were collected three times per week throughout the study period (March-August). The six-stage impactor, widely used for quantifying bioaerosol contamination, separates particles based on their aerodynamic diameter, ranging from >7 µm in stage 1 to 0.65–1.1 µm in stage 6. The sampler was sterilized with 70% ethanol before each use, ensuring the integrity of the samples. The sampling duration for each run was set to 10 minutes, following established protocols [6]. Petri dishes with appropriate nutrient media were loaded in the sampler to target viable bacterial and fungal bioaerosols. The collected samples were incubated under specific conditions to determine colonyforming units (CFU/m^3), enabling an accurate representation of bioaerosol concentrations. The sampling period for this study focuses on the spring and summer months of 2024 (March to August) as part of a larger one-year research project.

Meteorological and pollution data. During each sampling session, meteorological conditions and concentrations of key air pollutants were monitored and recorded. Meteorological data, including temperature, relative humidity, wind speed, and UV index, were sourced from the metropolitan municipality's weather station [7]. Information on gas pollutants (nitrogen dioxide, sulphur dioxide, ozone, carbon monoxide), $PM_{2.5}$, PM_{10}, and the air quality index (AQI) was obtained from the Metropolitan Municipality's pilot platform "AIRTHING" [8].

Data analysis. Spearman's rank correlation was used to analyze relationships between bioaerosol concentrations and environmental factors – temperature (T), relative humidity (RH), wind velocity (WV), UV radiation (UV), and PM_{10} and $PM_{2.5}$ concentrations. Statistical significance was established for results with a probability value of less than 0.05 ($p < 0.05$).

Results and discussion. ***Spring-summer dynamics of ambient bioaerosol concentrations.*** *Spring.* Meteorological spring in the Northern Hemisphere includes March, April, and May [9]. The period of the current survey (March 2024 – August 2024) included spring months – March (2024), April (2024), and May (2024). The analysis in this study specifically focuses on the spring and summer months within this period, to explore the seasonal dynamics during these warmer months. According to Annual Hydrometeorological Bulletin in Bulgaria, Spring 2024 was with temperatures close to normal, with average deviation from seasonal values up to $+0.9\,^{\circ}$C. Figure 1 shows the monthly variation of bioaerosols concentration during the spring sampling period.

The spring season in the inspected urban location is distinguished by pronounced fluctuations in detected bioaerosol levels. The number of fungi in aerosols varied from 12.0 CFU/m^3 (2nd week, March) to 650.0 CFU/m^3 (2nd week, May), while concentration of airborne bacteria was minimal in the 2nd week of April (55.0 CFU/m^3) and reached maximum of 772.0 CFU/m^3 in the 3rd week of May. The recorded values of ambient bacterial aerosol concentrations in Sofia during spring are lower, compared to the concentrations of bacterial aerosols from the same season in Gliwice, Poland, (80 to 1124 CFU/m^3) [4]. On the other hand, they are higher than the reported average concentrations in Sacley, France (March 5 ± 1 CFU/m^3; April–May, 14 ± 3 CFU/m^3) [10]. We supposed that the observed bioaerosol concentration maximum in May was due to the suitable environmental conditions and increased microbe sources, such as the blooming of plants in neighbouring green area. Some research [11,12] also observed that the concentrations of total airborne microbes gradually increase in spring. Figure 2 illustrates the size distribution of ambient bioaerosol particles during the spring sampling period.

The distinguishing feature of the observed pattern was that during March and April, the highest proportions of airborne viable bacteria were detected on

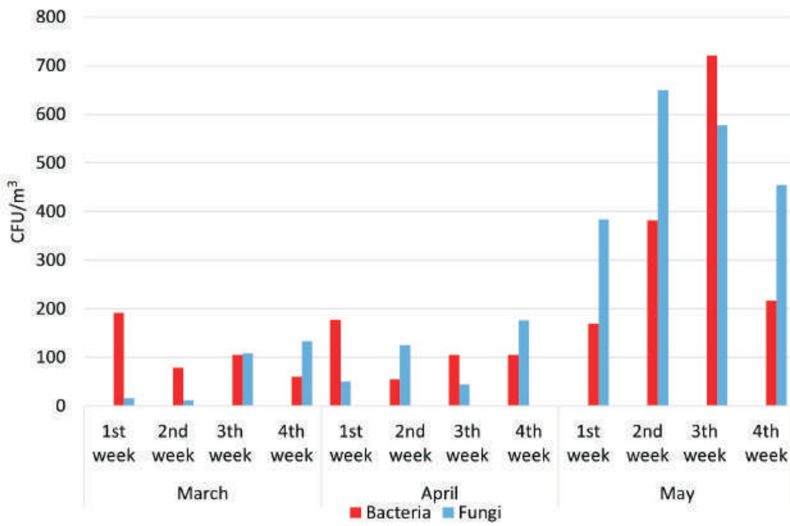

Fig. 1. Spring dynamics of ambient bioaerosol concentration

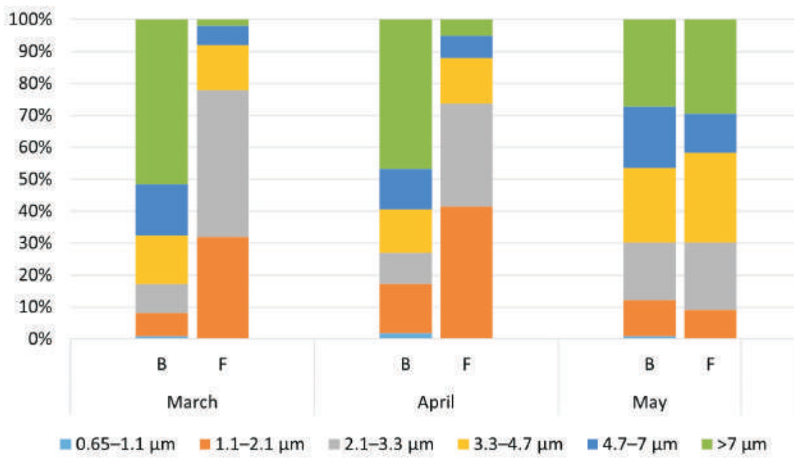

Fig. 2. Size distribution of ambient bioaerosols in spring

stage 1 ($\geq$ 7.0 µm) with a fraction of 51% for both months, while fungal aerosol particles of the same size were significantly less abundant (2% March, 5% April). In the last spring month (May) we observed reduction in course mode bacterial bioaerosols. For the same period, the fungal aerosol particles remained distributed multimodally.

Summer. Meteorological summer in the Northern Hemisphere includes June, July, and August [9]. The summer of 2024 in Sofia was relatively warm. During the summer sampling period, the capital city Sofia remained slightly cooler than the rest of the country due to its higher altitude. However, on the hottest summer days, temperatures exceeded 35 °C, mostly in July and August [13].

A significant pattern of increase in bacterial and fungal counts was noted from June to August in the air of the sampling location (Fig. 3). For that period, fungal aerosol concentration reached its maximum for the entire season – 2666.0 CFU/m^3 (2nd week, July). In the first week of July the highest concentration of bacterial aerosol (1260.0 CFU/m^3) was measured. A significant decline in both bioaerosol counts was detected in August.

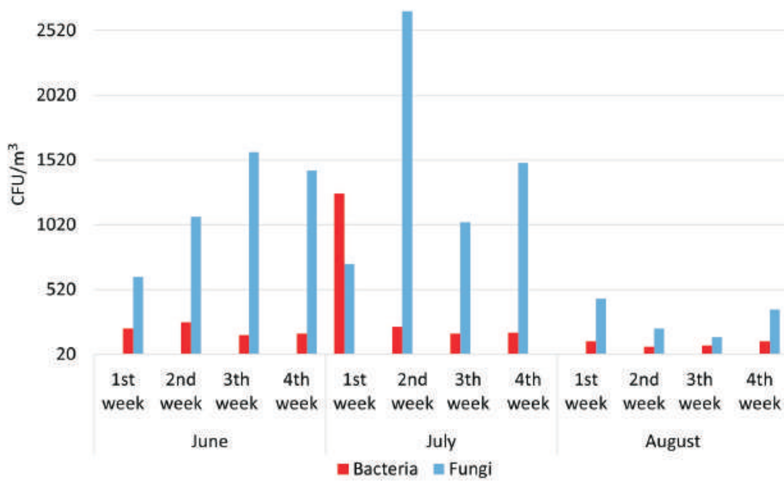

Fig. 3. Summer dynamics of ambient bioaerosol concentration

Previous studies have shown that the abundance of microbial aerosols during the summer is significantly higher than the rest of seasons [3, 12]. In our study the latter is prominent only for the fungal ambient aerosols in the investigated location. The decline of bioaerosol concentrations, observed in August, corresponds well with decreases of airborne microflora occurred during hot periods in some other reports [14, 15]. We believed that the combination of elevated temperature, relatively low RH, and strong solar radiation during the second half of the season created unfavourable environment in the area, which was not suitable for survival of airborne microorganisms. The distributions of particle size of microbial aerosols during summer are shown in Fig. 4. No clear distribution pattern was observed for both bacterial and fungal bioaerosol particles.

An increase in air bioaerosol pollution during the summer months has been reported by several authors [16], but at the same time in some of the monitoring seasonal studies a decrease in the concentrations of bacterial and fungal bioaerosols was observed [14].

Influence of environmental factors on ambient bioaerosol concentrations. The factors governing the quantitative dynamics of ambient bioaerosol

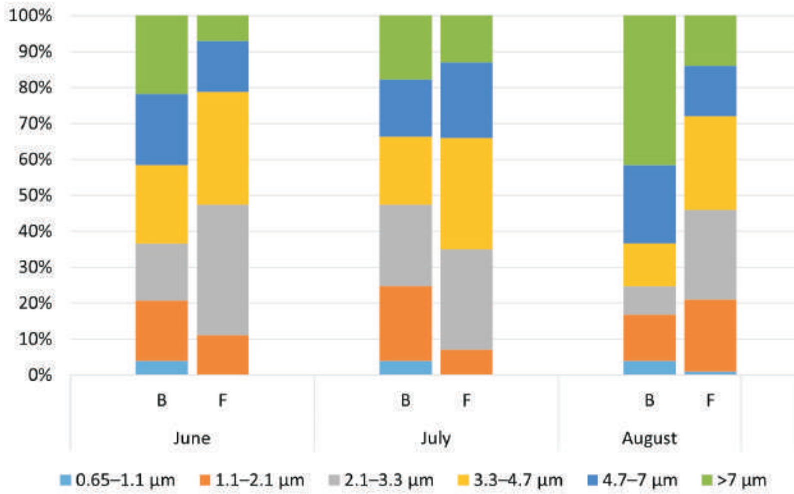

Fig. 4. Size distribution of ambient bioaerosols in summer

concentrations are overly complex and include meteorological parameters, climatic conditions, anthropogenic activities, and the geographical environment. The most important factors influencing bioaerosol concentrations for each site vary in different seasons, due to local fluctuations in weather conditions and meteorological parameters [17]. In this study, environmental parameters such as temperature, UV radiation, relative humidity, wind velocity and environmental indices (PM and conventional gases) were constantly monitored during the entire period in order to investigate their correlation with bioaerosol concentrations in investigated urban area. The Spearman correlation analysis was performed for the assessment. The bacterial and fungal aerosol concentrations were altered in a distinctive pattern by the environmental conditions: bacteria (spring): PM_{10} – 0.457; $PM_{2.5}$ – 0.338; T^a – 0.599; UV^b – (–0.645); WV^c (*Wind velocity*, m/s) – (–0.035); RH^d (*Relative humidity*, %) – (–0.384); CO^e (*Carbon monoxide* mg/m^3); –0.879; NO_2^f (*Nitrogen dioxide* $\mu g/m^3$); – (–0.450); SO_2^g (*Sulphur dioxide* $\mu g/m^3$) – 0.007; O_3^h (^{h} – *ozone* $\mu g/m^3$) – 0.239. In the same arrangement during the summer: 0.128; 0.161; 0.184; (–0.429); (–0.545); 0.491; 0.653; (–0.864); (–0.490); (–0.168). Fungi (spring): (–0.041); 0.013; 0.637; (–0.588); 0.214; 0.192; 0.540; (–0.888); 0.202; (–0.580). Fungi (summer): (–0.181); (–0.111); 0.282; (–0.201); (–0.256); 0.249; 0.726; (–0.518); (–0.374); 0.237.

During spring, levels of both bacterial and fungal aerosols had moderate positive correlation with the temperature (0.599/0.637, $p < 0.05$), which contrasts with the findings of other researchers [4]. Another common feature for both types of investigated bioaerosols during spring season in Sofia was moderate negative correlation with the ultraviolet radiation ($-0.645/-0.588$, $p < 0.05$). Negative relationship between bacterial aerosol concentrations and UV radiation during spring was also observed by other research groups [4]. The distinctive pattern in bacterial and fungal aerosols concentration levels during spring season included strong positive correlation between bacterial aerosols and CO (0.879, $p < 0.05$) and moderate positive correlation between fungal aerosols and the same factor (0.653, $p < 0.05$). In contrast, the negative correlation between bioaerosol levels and NO_2 was strong for fungi (−0.888, $p < 0.05$) and moderate for bacteria (−0.450, $p < 0.05$). In spring statistically significant negative correlation with O_3 was observed only for fungal aerosols, which was consistent with previous surveys [18, 19]. For the same period only, bacterial aerosols showed moderate negative correlation with PM_{10} (0.457, $p < 0.05$). The rest of atmospheric environmental factors (RH, wind, $PM_{2.5}$, SO_2, had no statistically significant relationship with bioaerosol concentrations during the spring. In summer, the major factors affecting the abundance of bacterial aerosols in the investigated area were RH (0.491, $p < 0.05$), wind velocity (−0.545, $p < 0.05$), UV radiation (−0.429, $p < 0.05$), NO_2 (−0.864, $p < 0.05$), CO (0.653, $p < 0.05$) and SO_2 (−0.490, $p < 0.05$). For the same period, fungal aerosol concentrations were statistically correlated only with CO (0.726, $p < 0.05$) and NO_2 (−0.518, $p < 0.05$). Positive correlation of bacterial aerosols and RH during summer found in this study was consistent with the results reported by other scientific groups [11]. The correlation between fungal aerosol presence and RH was not statistically significant in summer. The correlation analysis results also indicated that a higher solar radiation during summer was associated with a reduction of bioaerosol concentrations, which was statistically significant only for bacterial aerosols ($p < 0.05$). In contrast to other studies [4], we found a moderate, statistically significant correlation between bacterial aerosol presence and wind speed in summer, which was consistent with the findings of another research [19]. Similar to other scientific teams [20] we also observed a strong positive correlation between both bioaerosol levels and CO, but in contrast we found negative relationship with NO_2 and SO_2.

Conclusions. This study provides information about the variations in the concentration and size distribution of microbial aerosols in highly urbanized central area of Sofia city, capital of Bulgaria, where similar studies were not reported. The highest concentrations of fungal aerosols were recorded in June, July, while bacterial aerosol contamination reached maximum values in May, July. The examination of size distributions of bioaerosol particles also indicated a distinct seasonal pattern for bacterial and fungal aerosols. During the spring, both types of bioaerosols showed a significant correlation with a greater number of environmental factors (temperature, solar radiation, presence of co-pollutants). As a primary study on variations in concentrations and size distribution of microbial aerosols in the central part of Sofia during the spring-summer months, the obtained results represent an important piece of information that can be used for risk assessment and future air-quality control measures.

REFERENCES

[1] Walser S. M., D. G. Gerstner, B. Brenner et al. (2015) Evaluation of exposure-response relationships for health effects of microbial bioaerosols – A systematic review, Int. J. Hyg. Environ. Health, **218**(7), 577–589.

[2] Lang-Yona N., K. Dannemiller, N. Yamamoto et al. (2012) Annual distribution of allergenic fungal spores in atmospheric particulate matter in the Eastern Mediterranean; a comparative study between ergosterol and quantitative PCR analysis, Atmos. Chem. Phys., **12**, 2681–2690.

[3] Bowers R., N. Clements, J. Emerson et al. (2013) Seasonal variability in bacterial and fungal diversity of the near surface atmosphere, Environ. Sci. Technol., **47**(21), 12097–12106.

[4] Bragoszewska E., J. S. Pastuszka (2018) Influence of meteorological factors on the level and characteristics of culturable bacteria in the air in Gliwice, Upper Silesia (Poland), Aerobiologia, **34**(2), 241–255.

[5] Dueker M. E., G. D. O'Mullan, A. R. Juhl et al. (2012) Local Environmental Pollution Strongly Influences Culturable Bacterial Aerosols at an Urban Aquatic Superfund Site, Environ. Sci. Technol., **46**(20), 10926–10933.

[6] Nevalainen A., J. Pastuszka, F. Liebhaber, K. Willeke (1992) Performance of bioaerosol samplers: Collection characteristics and sampler design considerations, Atmos. Environ. Part A Gen. Top., **26**(4), 531–540.

[7] Weather forecast and conditions in Sofia, Bulgaria – Weather.sinoptik.bg (n.d.), Weather.sinoptik.bg – Weather forecasts and conditions for 80 000 locations in the whole world, retrieved January 26, 2025, from `https://weather.sinoptik.bg/sofia-bulgaria-100727011`.

[8] AIRTHINGS (n.d.), retrieved January 26, 2025, from `https://platform.airthings-project.com/`.

[9] Trenberth K. E. (1983) What are the seasons?, Bull. Am. Meteorol. Soc., **64**(11), 1276–1282.

[10] Sarda-Estève R., D. Baisnée, B. Guinot et al. (2020) Atmospheric Biodetection Part I: Study of Airborne Bacterial Concentrations from January 2018 to May 2020 at Saclay, France, Int. J. Environ. Res. Public Health, **17**(17), 6292.

[11] Li Y. P., H. L. Fu, W. Wang et al. (2015) Characteristics of bacterial and fungal aerosols during the autumn haze days in Xi'an, China, Atmos. Environ., **122**, 439–447.

[12] Xu C. H., M. Wei, J. M. Chen et al. (2017) Bacterial characterization in ambient submicron particles during severe haze episodes at Ji'nan, China, Sci. Total Environ., **580**, 188–196.

[13] National institute of meteorology and hydrology (n.d.), retrieved January 26, 2025, from `https://bulletins.cfd.meteo.bg/`.

[14] Filipello-Marchisio V., C. Cassinelli, V. Tulloi, A. Piscozzi (1992) Outdoor airborne dermatophytes and related fungi: A survey in Turin (Italy), Mycoses, **35**(9–10), 251–257.

[15] Haas D., H. Galler, J. Luxner, G. Zarfel (2013) The concentrations of culturable microorganisms in relation to particulate matter in urban air, Atmos. Environ., **65**(2), 215–222.

[16] Takatori M., T. Shida, K. Akiyama, K. Takatori (1994) Airborne fungi during the last ten years in Sagamihara, Arerugi, **43**(1), 1–8.
[17] Zhong X., J. Qi, H. Li et al. (2016) Seasonal distribution of microbial activity in bioaerosols in the outdoor environment of the Qingdao coastal region, Atmos. Environ., **140**(Suppl. 1), 506–513.
[18] Ho H. M., C. Y. Rao, H. H. Hsu et al. (2005) Characteristics and determinants of ambient fungal spores in Hualien, Taiwan, Atmos. Environ., **39**(32), 5839–5850.
[19] Montero A., M. E. Dueker, G. D. O'Mullan (2016) Culturable bioaerosols along an urban waterfront are primarily associated with coarse particles, Peer J, **4**, e2827.
[20] Dong L., J. Qi, C. Shao et al. (2016) Concentration and size distribution of total airborne microbes in hazy and foggy weather, Sci. Total Environ., **541**, 1011–1018.

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