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MICROFLORA OF THE GROUND AIR FROM "HOT POINTS" AND PARK AREAS

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Abstract

The present study aims to track the quantity and distribution of microorganisms in the ground air in "hot points" and park areas of the territory of the city of Sofia. For this purpose, 6 "hot points" were selected, on the territory of boulevards and intersections, and six in the park areas of the capital. The study was carried out using the "Open air" method on suitable solid nutrient media. Each measurement was performed in five replicates. The total microbial number was determined, as well as the amount of the main microbiological groups. The potential presence of streptococci and staphylococci was reported. The data show several times increased levels of the total microflora in the hot spots compared to the green areas. There is also a difference in the percentage participation of the different microbiological groups in the two types of areas studied. In the hot points, fungi represent the main group of microorganisms, followed by bacteria. In green areas, bacteria represent the main group of microorganisms.

Key words: *air microorganisms, air microflora, green zone, hot points.*

INTRODUCTION

Air pollution represents a significant concern for public health in Europe, as underscored by Annesi-Maesano (2017). Recent findings highlight not only the profound implications of air pollution on human health but also its detrimental effects on ecosystems and the exacerbation of global warming, according to the European Environment Agency (EEA) in 2020. While particulate matter, specifically aerosols, is commonly associated with air pollution, there has been a disproportionate focus on non-biological pollutants, leading to an oversight of bioaerosols (Penner et al., 2001). Bioaerosols are biological particles suspended in the air - such as bacteria, fungi, fungal hyphae, spores, actinomycetes and other microscopic entities such as viruses for example (Kim et al., 2018). Often these bioaerosols are called airborne microorganisms (Chen et al., 2020), although bioaerosols also include fragments of pollen and other organic components.

It is known that fine dust particles represent concentration nuclei for the so-called airborne microorganisms (Aziz et al., 2018). For this reason, fine dust is a suitable environment for the development and spread of airborne microorganisms (Pepper et al.,2015; Xu et al., 2017). Zhen et al. (2017) established that the air could contain more than 10^6 microbial cells per cubic meter, emphasizing the potential risk posed by fine dust not only through its physical effects but also as vectors for biologically active microorganisms (Klein et al., 2016). These insights highlight the role of fine dust in the attachment and dissemination of microorganisms, including pathogens (Shammi et al., 2021; Chen et al., 2020). Despite their widespread distribution in the atmosphere, the understanding of airborne microbial communities remains limited (Aziz et al., 2018), particularly concerning their concentration in ground air and the influence of environmental factors such as pollution, temperature, climatic conditions, fog presence, vegetation, etc. Ground air is the main part of the atmosphere where airborne microorganisms are concentrated. Studies have been conducted looking for a correlation between the amount of air microflora and various air parameters - such as pollution, temperature, climatic conditions, presence of fog, presence of vegetation, etc. Early studies (Mancinelli & Shulls, 1977) initially suggested a lack of correlation between air microflora and these environmental parameters. However, further research has identified significant correlations between climatic conditions and microbial air concentrations (Cao et al., 2014; Fang et al., 2018; Chatoutsidou et al., 2023).

Comparative studies between urban and park areas regarding microbial composition and concentration are limited. Mhuireach et al. (2016) delineated differences in microbial communities between urban and green spaces, a finding supported by other researchers examining the influence of vegetation on microbial distributions and pathogen prevalence (Lymperopoulou et al., 2016; Li et al., 2021).

This study aims to explore for the first time the dynamics of total microflora across six hot points and six park areas within Sofia, Bulgaria. For the purpose of the study, the total microbial number of each of the studied samples was determined, as well as the percentage participation of the individual studied microbiological groups.

MATERIALS AND METHODS

The present study aims to track the quantity and distribution of microorganisms in the ground air in "hot points" and park areas within the city of Sofia. For this purpose, six "hot points" (HP) were selected, on the territory of boulevards and intersections, and six in park areas (P) of the capital. The sampling locations are as follows:

- Sample 1: Park Zaimov - green area;
- Sample 2: Crossroad of Evlogii and Hristo Georgievi boulevards and Yanko Sakazov boulevard – hotspot;
- Sample 3: Studentski Grad Park - green area;
- Sample 4: Crossroad of 8 Dekemvri St. and Rosario St. - hot zone;
- Sample 5: Park part on the border with Vitosha Nature Park - green zone;
- Sample 6: Crossroad of Cherni Vrah Boulevard and Ring Road Boulevard - hot spot;
- Sample 7: West Park - green zone;
- Sample 8: Crossroad of Cherni vrah boulevard and Ljubotrun street - hot spot;
- Sample 9: Loven Park - green zone;
- Sample 10: Nikola Vaptsarov Blvd. - hot spot;
- Sample 11: park Borisova Gradina - green zone
- Sample 12: Crossroad Orlov most- Hot Spot.

There is no Bulgarian legislation and guidelines for assessing the quality of the outdoor ground air in relation to the presence of bioaerosols. The study was carried out using the "Open air" method (Nustorova & Malcheva, 2020). The method is based on physical sedimentation of microbial cells under the action of their own weight. The method is based on the axiom that 10 liters of air flow through 100 cm^2 in 5 minutes, i.e. the microorganisms contained in 10 liters of air settle. For each of the samples, a recalculation to 1 m^3 of air was performed. Petri dishes were used with suitable selective nutrient agar media for the studied microorganisms. Meat-Peptone Agar (MPA) was used for the cultivation of bacteria. Cultivation was carried out at a temperature of 2°C, for 48 hours, aerobically; Determination of actinomycetes - on selective medium for actinomycetes: Starch-ammonia agar (SAA). Cultivation was carried out at 28°C for 10 days, aerobically; Capek Dox Agar medium was used for the cultivation of micromycetes. Cultivation was carried out at 28°C for 7 days, aerobically. For the isolation of staphylococci and streptococci, *Staphylococcus* selective agar (48 hours at 35°C, aerobically) and Selective strep agar (48 hours at 35°C, aerobically) were used, respectively.

Each measurement was performed in five replicates, the average value was calculated. The samples were collected during the winter season of 2023-2024. All samples were collected at the same time in all locations at 1 m above the ground. Samples were collected on a non-precipitation day preceded by five non-precipitation days. The reported average air temperature is 11.6°C, relative air humidity-78%, atmospheric pressure 1009 millibars and wind speed 14 km/h from the southeast. The total microbial number was determined, as well as the amount of the main microbiological groups. The potential presence of streptococci and staphylococci was reported. Statistical processing of the microbiological results included calculation of the mean value of five replicates and standard deviation, using the StatSoft Statistica 12 program at significance thresholds of 95%.

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RESULTS AND DISCUSSIONS

The results of the microbiological analysis are detailed in Table 1, quantified as colony-forming units (CFU*10^3/m^3). The study did not detect the presence of streptococci, a genus often linked to a variety of respiratory and other health conditions, typically more prevalent indoors than outdoors (Torrey & Michael, 1941). Staphylococci were found in the lowest concentrations among the analyzed microbial groups. Despite their association with densely populated indoor environments, research has demonstrated a correlation between the levels of fine particulate matter, such as black carbon, and the presence, distribution, and activity levels of specific staphylococci species (Hussey et al., 2017).

The analysis presents clear trends, underscoring that urban hotspots exhibit significantly higher microbial numbers compared to green areas, as depicted in Figure 1. This finding is consistent with previous research (Mhuireach et al., 2016). Our study examined green areas of varying sizes: a large park exceeding 5 km² near the Vitosha Natural Park border (sample 5), three medium-sized parks ranging from 1 to 2 km² (samples 7, 9, and 11), and two smaller urban parks under 0.5 km² (samples 1 and 3). Additionally, the maintenance and visitation rates of these parks were considered - whether they were well-maintained and frequently visited (samples 1, 3, 7, and 11) or more akin to natural forest settings with minimal human activity (samples 5 and 9). The study found that parks with fewer visitors and more natural, forest-like vegetation exhibited a markedly lower concentration of airborne microorganisms than their urban counterparts. These observations suggest that the size of the park, coupled with its maintenance level, significantly influences the reduction of fine dust particles and their associated microorganisms. Supporting literature emphasizes the role of specific vegetation types in modulating the distribution and density of airborne microorganisms (Lymperopoulou et al., 2016), as well as vegetation's capacity to lower pathogen levels (Li et al., 2021).

When comparing the total microbial count of only the green areas, the above statement is again confirmed.

Table 1. Main microbiological parameters of investigated airborne microorganism

Samples		Coordinate	Total microbial number	Bacteria	Fungi (molds)	Actinomy-cetes	Staphylo-coccus	Streptococcus
1	P	42°41′01″ N 23°20′20″ E	2.36 ± 10.93	1.57 ± 4.17	0.47 ± 1.36	0.31 ± 2.05	0.00 ± 0.13	--
2	HP	42°41′47″ N 23°20′47″ E	11.48 ± 8.60	4.09 ± 3.83	7.08 ± 4.34	0.31 ± 1.34	0.00 ± 0.17	--
3	P	42°39′16″ N 23°21′05″ E	15.90 ± 10.93	8.23 ± 4.17	1.89 ± 1.36	5.46 ± 2.05	0.31 ± 0.13	--
4	HP	42°39′06″ N 23°21′11″ E	30.04 ± 8.60	9.91 ± 3.83	16.20 ± 4.34	3.77 ± 1.34	0.16 ±0.17	--
5	P	42°38′28″ N 23°18′48″ E	1.89 ± 10.93	0.94 ± 4.17	0.63 ± 1.36	0.31 ± 2.05	0.00 ± 0.13	--
6	HP	42°37′29″ N 23°18′19″ E	30.51 ± 8.60	11.80 ± 3.83	17.77 ± 4.34	0.79 ± 1.34	0.16 ± 0.17	--
7	P	42°40′07″ N 23°18′34″ E	11.80 ± 10.93	10.85 ± 4.17	0.47 ± 1.36	0.47 ± 2.05	0.00 ± 0.13	--
8	HP	42°40′05″ N 23°19′09″ E	20.38 ± 8.60	5.82 ± 3.83	12.99 ± 4.34	1.42 ± 1.34	0.16 ± 0.17	--
9	P	42°39′59″ N 23°20′09″ E	2.88 ± 10.93	2.40 ± 4.17	0.16 ± 1.36	0.31 ± 2.05	0.00 ± 0.13	--
10	HP	42°40′09″ N 23°19′52″ E	17.41 ± 8.60	6.25 ± 3.83	10.85 ± 4.34	0.31 ± 1.34	0.00 ± 0.17	--
11	P	42°41′03″ N 23°20′26″ E	12.41 ± 10.93	7.82 ± 4.17	3.72 ± 1.36	0.87 ± 2.05	0.00 ± 0.13	--
12	HP	42°41′25″ N 23°20′14″ E	32.82 ± 8.60	13.87 ± 3.83	18.05 ± 4.34	0.42 ± 1.34	0.47 ± 0.17	--

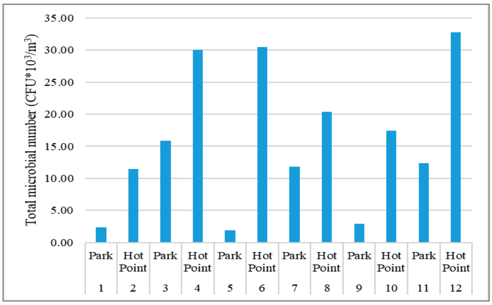

Figure 1. Total microbial number

The study identifies that the samples from the vicinity of Vitosha Nature Park (sample 5) and sample 9 exhibit notably low levels of airborne microorganisms. Similarly, Zaimov Park (sample 1) also shows reduced microbial counts in comparison to other parks. Contrarily, the microbial concentration in the air of the remaining parks is, on average, quintupled. These data show a potential relationship between the number of airborne microorganisms in the ground air of park areas and their anthropogenic load. The specific data here is for Park Zaimov (sample 1), which shows significantly lower levels than the other maintained parks. This data requires additional analyzes of the park territory.

One of the possible reasons for the lower levels of microorganisms in the ground air is that this park is located in the most affluent Municipality of the capital and is frequently cleaned. Part of the cleanup activities include washing the park's walkways, which further minimizes the possibility of lifting dust particles such as condensation nuclei for microorganisms from the ground surface. However, it should not be forgotten that the park is surrounded by boulevards, and it is necessary to analyze other specific parameters that may justify the indicated results.

In sample area 3, the highest total microbial concentration was observed, a finding that can be directly linked to extensive renovation and construction activities in the vicinity, including the development of new residential structures and the execution of infrastructural enhancements within the park itself. This correlation underscores the interaction between elevated dust levels and microbial spread in the atmosphere, where dust particles serve as aggregation nuclei for microbes (Aziz et al, 2018). It is also important to note the low degree of afforestation of the park. Both in our study and in other similar ones, the positive impact of woody plants on the cleanliness and hygiene of the air is shown (Mhuireach et al., 2016).

Analyzing urban crossroads, identified as significant microbial hotspots, we noted the highest microbial densities at the Orlov Most crossroad (sample 12) - the most traffic-congested point in Sofia. This likely contributes to heightened levels of fine particulate matter, serving as a ground for airborne microorganisms. Intersection points represented by Sample 4 and Sample 6 similarly exhibited pronounced microbial counts, reflecting their heavy traffic congestion. In contrast, Sample 2, located in the vicinity of Park Zaimov, demonstrated markedly lower microbial quantities, with levels significantly reduced compared to other locations under scrutiny. This is due to the cleaning and maintenance regime implemented in this area, underlining the critical role of environmental cleanliness in mitigating microbial presence.

An analysis was conducted to determine the percentage contribution of individual microbiological groups within the total microflora, as illustrated in Figure 2.

The analysis reveals consistent patterns in the distribution of microbial groups within the airborne microflora. Notably, the predominant microbial group differs between high-traffic urban settings and green park areas. Microscopic fungi are the dominant group in urban hotspots, whereas bacterial populations prevail in park environments. This distribution aligns with findings by Barac et al. (2018), who identified fungi as primary agents in various respiratory ailments. This correlation may elucidate the heightened incidence of respiratory issues in populations residing in areas devoid of green spaces. The increased fungi level in the air of urban hot points has been corroborated by additional research (Korneykova, 2021; Pollegioni et al., 2023). Muafa et al. (2024) further established a link between increased vehicular traffic and elevated fungal concentrations in the atmosphere.

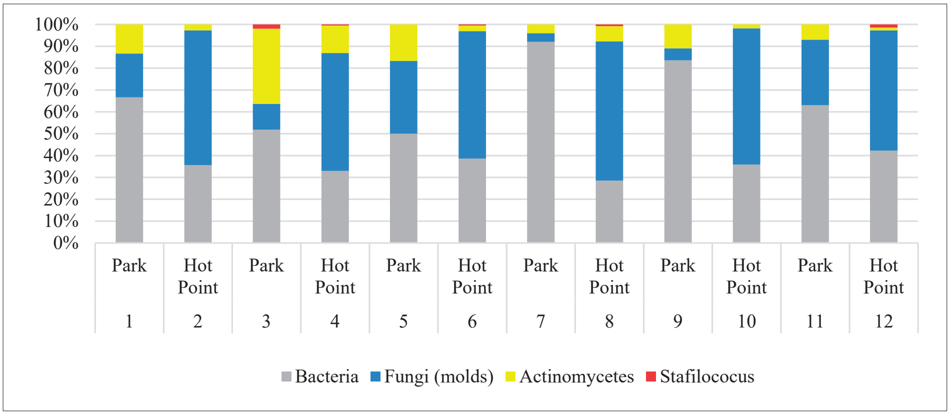

Figure 2. Percentage participation of individual microbial groups to total microbial number

Conversely, studies such as that by Nageen et al. (2021) report no significant disparity in fungal levels between park and urban areas. This discrepancy underscores the necessity for more comprehensive research into the prevalence and intensity of fungal species across various urban contexts, considering their significant impact on respiratory health.

Within all green areas analyzed, bacterial populations were predominant. This dominance, while significant in comparison to the overall microflora composition in these areas, still represents a lower bacterial number than those observed in urban hot points, a finding consistent with the observations made by Gang et al. (2007). Following bacteria, fungi emerged as the secondary microbial group in all park areas, with the exception of sample 3, where actinomycetes were more prevalent. This anomaly in sample 3, showing a heightened presence of actinomycetes, could be attributed to the influence of nearby excavation activities. Lloyd (1996) suggested that such activities might facilitate the migration of actinomycetes into adjacent areas, such as we specified, to be present in the territory of the Student City Park.

CONCLUSIONS

This study was initiated to create a basic analysis concerning the total quantity of cultivable microorganisms and to elucidate the proportional representation of microbial groups within selected places. Six green zones and six hot points were selected. The results demonstrated a higher microbial air load in urban hot points compared to green zones, highlighting the influence of environmental factors such as park size and maintenance practices on microbial prevalence in green spaces. Notably, parks that mimic the conditions of natural forests, both in size and in maintenance approach, exhibited lower microbial numbers in the ground air.

In contrast, the microbial density within urban hot points was significantly shaped by their maintenance standards and the intensity of vehicular traffic. Detailed comparison between two pivotal urban hot points revealed substantial discrepancies in microbial concentrations, with hot point 12 exhibiting microbial levels more than threefold higher than hot point 2. This difference was directly linked to the varying degrees of traffic congestion and the resultant dispersal of fine dust particles, serving as a carrier for microbial dispersal. The data on the distribution of microbial groups show that in hot point the group of fungi predominates, and in park areas the group of bacteria.

The primary goal of this research was to generate a basic dataset that could serve as a reference point for future studies. The outcomes presented herein lay the groundwork for more detailed subsequent investigations into the composition and dynamics of ground-level microflora, offering insights into environmental microbial ecology and its implications for public health and urban planning.

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