Scientific Papers. Series E. Land Reclamation, Earth Observation & Surveying, Environmental Engineering. Vol. XIV, 2025
Print ISSN 2285-6064, CD-ROM ISSN 2285-6072, Online ISSN 2393-5138, ISSN-L 2285-6064

# GROUND AIR MICROFLORA STUDY USING A CASCADE IMPACTOR

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***Abstract***

*Urban air pollution poses significant public health risks, with airborne microorganisms contributing to air quality concerns and respiratory illnesses. This study investigates the distribution of airborne microflora using a six-stage cascade impactor. Samples were collected from three urban hotspots, three park areas, and a natural control site in Sofia, Bulgaria. Microbial counts were analyzed across six particle size fractions to determine spatial distribution patterns. Hotspots showed significantly higher microbial loads, especially in larger particle fractions (>7 µm, 4.7- 7 µm), whereas densely forested green areas exhibited lower microbial levels with a shift toward finer fractions (2.1- 3.3 µm, 1.1-2.1 µm). Parks with minimal vegetation showed microbial patterns similar to hotspots. These findings underscore the role of urban vegetation in mitigating microbial air pollution and highlight the importance of incorporating forested green spaces into urban planning to enhance air quality and public health.*

***Key words****: air microorganisms, air microflora, cascade impactor, green zone, hotspots.*

## INTRODUCTION

Clean air is essential for human and environmental health. Urbanization and anthropogenic activities have significantly degraded air quality, with particulate matter (PM) serving as an indicator of complex pollutant mixtures. Recent studies suggest a correlation between PM levels and airborne microbial content (Mansour et al., 2014; Palladino et al., 2021). Historically, air quality studies focused on inorganic contaminants due to their abundance; however, interest in organic components, including airborne microbial biota, has grown (Friedlander et al., 2000).

Airborne microorganisms - including bacteria, micromycetes, actinomycetes, and viruses - can remain suspended either freely or attached to dust particles (Stetzenbach, 2009; Lighthart, 1997). Larger dust particles tend to harbor more microorganisms (Meklin et al., 2002). Research conducted by Tignat-Perrier et al. (2019) has shown that airborne microbial communities are influenced by ecosystem types and meteorological conditions. Their concentrations vary with air pollution levels, influenced by factors such as dust storms, fog, biomass burning, and traffic emissions. Seasonal and diurnal patterns in bioaerosol levels often reflect human activity, and studies have found correlations between microbial concentrations and the Air Quality Index (AQI). Shammi et al. (2021) observed distinct seasonal and diurnal variations in bioaerosol concentrations that closely reflect patterns of human activity in densely populated urban areas. Similarly, Yan et al. (2019) reported a strong correlation between bacterial and micromycete concentrations and the Air Quality Index (AQI). Their findings indicated that micromycete concentrations gradually increased when the AQI was below 200, whereas bacterial concentrations rose when the AQI exceeded 200. However, the mechanisms underlying the increase in bioaerosols - particularly aerophilic microorganisms - in response to air pollution remain unclear. One hypothesis is that elevated or extreme pollution levels primarily influence the diversity and composition of airborne microbial communities (Fan et al., 2019). The viability of microorganisms within bioaerosols depends on various environmental factors, including climate and atmospheric chemistry (Gong et al., 2020). Consequently, understanding the microbiological structure and composition of particulate matter is essential for identifying potential disease transmission pathways and assessing risks to human health (Wang et al., 2019).

The primary objective of this study is to examine the dynamics of airborne microflora across three urban hotspots and three park areas in Sofia, Bulgaria, along with one control site located in the Vitosha Mountain. Specifically, the study quantifies the total number of airborne microorganisms in each sample and assesses their distribution across six particle size fractions, as determined by a cascade impactor.

## MATERIALS AND METHODS

The study was conducted in early November 2024 at various locations in Sofia, Bulgaria, and the Vitosha Mountain region. Three urban park areas, their adjacent hotspots, and one site in Vitosha Mountain were selected to compare the composition of airborne microorganisms across different environments. The Vitosha Mountain site was chosen based on Kadinov's research, which highlighted its exposure to anthropogenic pollution - particularly during summer, when slope winds transport pollutants from Sofia (Kadinov, 2021a). However, Kadinov (2019) also reported that the fine dust in Vitosha originates mainly from background sources, with less than 1% attributed to Sofia, and that particulate matter (PM) deposition remains below critical levels. Seasonal PM trends show summer peaks in Vitosha Mountain, in contrast to the winter peaks observed in Sofia (Kadinov, 2021b).

The sampling sites included:

- TA1: Borisova Gradina – green area; coordinates: 42°41′15″N, 23°20′16″E
- TA2: Orlov Most intersection – hotspot; coordinates: 42°41′23″N, 23°20′17″E
- TA3: Voenna Academia Park – green area; coordinates: 42°41′30″N, 23°20′42″E
- TA4: Voenna Academia intersection – hotspot; coordinates: 42°41′33″N, 23°20′39″E
- TA5: Studentski Grad Park – green area; coordinates: 42°39′09″N, 23°21′09″E
- TA6: 8 Dekemvri St. and Rosario St. intersection – hotspot; coordinates: 42°39′06″N, 23°21′16″E
- TA7: Vitosha Mountain – nature park; coordinates: 42°36′34″N, 23°19′34″E

The geographic distribution of all sampling locations is illustrated in Figure 1, providing a visual overview of the selected green areas, urban hotspots, and the control site in Vitosha Mountain.

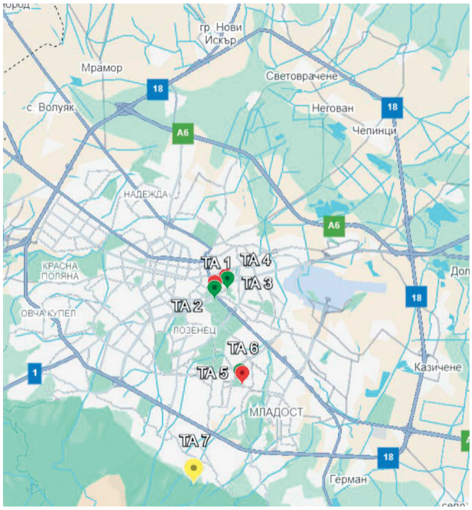


Figure 1. Map of the sampling locations

### Air microflora sampling and analysis

Airborne microbial abundance was analyzed using a six-stage cascade impactor, which separates particles by aerodynamic diameter as follows:

- Stage 1: > 7 µm;
- Stage 2: 4.7-7 µm;
- Stage 3: 3.3-4.7 µm;
- Stage 4: 2.1-3.3 µm;
- Stage 5: 1.1-2.1 µm;
- Stage 6: 0.65-1.1 µm.

Petri dishes with appropriate nutrient media were placed on each stage. One cubic meter of air passed through the impactor at a constant flow rate. The microorganisms collected on each plate were then cultured and counted.

### Microbial cultivation and identification

Microbial colonies were cultured under controlled conditions using the following nutrient media:

- Meat-Peptone Agar (MPA): bacterial growth at 28°C for 48 hours.
- Starch-Ammonia Agar (SAA): bacterial growth at 28°C for 10 days.
- Czapek Dox Agar Medium: fungal growth at 28°C for 7 days.

### Sampling conditions and data processing

Sampling was conducted on a dry day following five consecutive precipitation-free days to minimize the influence of weather on airborne microbial concentrations.

Average atmospheric conditions during sampling:

- temperature: 4°C;
- relative humidity: 92%;
- atmospheric pressure: 1009 millibars;
- wind speed: 9 km/h (southwest direction).

Statistical analysis of the microbiological data was performed using StatSoft Statistica 12. Results are presented as the means of three replicates with corresponding standard deviations. A 95% confidence level was used to determine statistical significance.

## RESULTS AND DISCUSSIONS

The results of the experiments are presented in colony-forming units (CFU) × 10²/m³. The total levels of airborne microorganisms are shown in Figure 2, while Figure 3 illustrates the distribution of microorganisms across the different stages of the cascade impactor. Unlike our previous studies (Peshev et al., 2024), the current study revealed that not all green areas exhibited lower microbial loads compared to their adjacent hotspots.
Table 1 provides a summary of microbial abundance across various particle size fractions for each location. The overall microbial load observed in this study is notably lower than that reported in studies conducted during the winter season. This difference is likely related to the increased concentration of fine particulate matter (PM) in winter. As Azis et al. (2018) noted, fine PM serves as a core for the accumulation of airborne microorganisms. Accordingly, microbial levels are expected to increase during the winter months, driven by increased PM concentrations due to the continued widespread use of solid fuels for heating in Sofia.

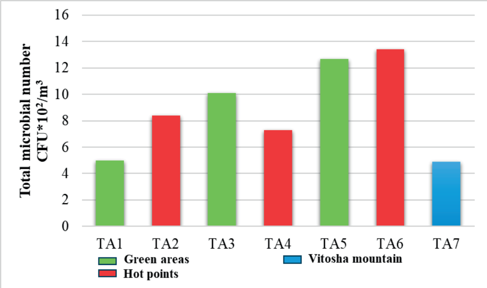


Figure 2. Total number of airborne microorganisms (CFU × 10²/m³) measured across all sampling sites

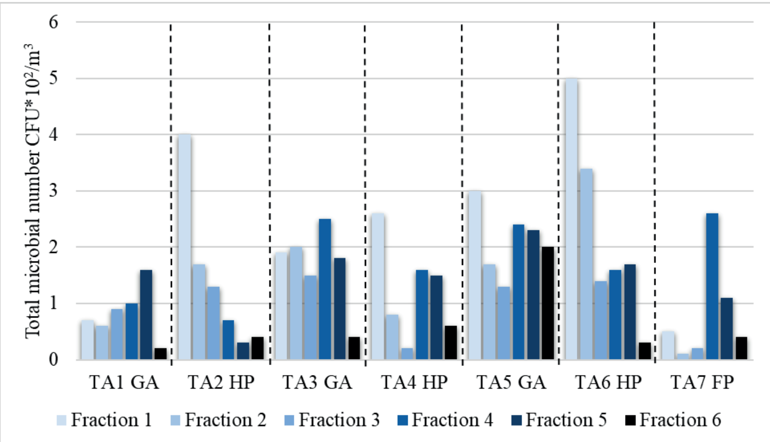


Figure 3. Total microbial number of airborne microorganisms from each of the stage of air Separator

According to the World Health Organization (WHO), air pollution is the greatest environmental health risk globally, affecting both developed and developing countries (WHO, 2017). However, no current WHO guideline specifically addresses the health impacts of airborne microorganisms in outdoor environments. Most WHO documentation on air quality and respiratory health focuses on non-organic hazards such as ozone, carbon dioxide, and fine particulate matter.

Table 1. Microbial abundance at different sites (CFU ×10²/m³)

| **Location** | **Microbial count (CFU x $10^2/m^3$)** | **Dominant particle size fraction** | **Observations** |
|---|---|---|---|
| Hotspot (TA2-Orlov most) | 8.4 ± 3.25 | >7 µm, 4.7-7 µm | Hight traffic, PM accumulation |
| Hotspot (TA4-Voenna Academia | 7.3 ± 3.25 | >7 µm, 4.7-7 µm | Hight vehicle emissions |
| Hotspot (TA6 – Studentski grad | 13.4 ± 3.25 | >7 µm | Construction activities contributing to PM |
| Green Area (TA1 – Borisova Gradina) | 5.0 ± 3.92 | 2.1-3.3 µm, 1.1-2.1 µm | Dense tree cover, reduce PM levels |
| Green Area (TA3 – Voenna Academia Park | 10.1 ± 3.92 | 3.3-4.7 µm, 2.1-3.3 µm | Lower elevation, direct PM exposure from traffic |
| Green area (TA5 – Studentski grad Par) | 13.4 ± 3.92 | > 7 µm, 4.7-7 µm | Minimal tree cover, resembling hotspots |
| Vitosha mountain – Nature Park | 4.9 | 2.1-3.3 µm, 1.1-2.1 µm | Nature forest, the lowest load with microorganisms |

When microorganisms are considered, the emphasis is typically on indoor air quality. Nevertheless, the 2021 WHO guidelines on particulate matter acknowledge the importance of analyzing the effects of organic aerosols on human health, alongside combustion particles and secondary inorganic aerosols (WHO, 2021). Many studies highlight the role of green spaces in mitigating air pollution (Givoni, 1991; Cohen et al., 2014). Chiesura (2004) found that urban parks improve air quality, particularly in large cities, while Dadvand et al. (2023) documented the positive health effects of tree vegetation.

Our study clearly shows that the location and forest cover of park areas matter.

Our study clearly demonstrates that both the location and forest cover of park areas significantly influence airborne microbial levels. TA1 – Borisova Gradina, one of the largest parks in Sofia with dense tree and shrub vegetation, recorded the lowest levels of airborne microorganisms. These findings are consistent with previous research (Mhuireach et al., 2016). The timing of the sampling - late autumn - likely contributed to these results, as trees retained a substantial portion of their leaf mass, aiding in particle filtration.

Interestingly, TA3, another green area, showed higher microbial concentrations than its adjacent hotspot, TA4. This discrepancy underscores the importance of both vegetation and location. TA3 is situated at a lower elevation than the nearby boulevard, making it more susceptible to direct dust and PM exposure from traffic. This highlights the need for careful urban planning that incorporates principles of "Environmental Hygiene". As Robinson et al. (2019) suggest, expanding research in this area is vital given the growing recognition of urban green spaces as essential to public health.

The data for TA5 further emphasizes the importance of vegetation type. This green area, dominated by grass with minimal tree cover, exhibited microbial levels comparable to its nearby hotspot, TA6. These findings, supported by the trends in Figure 2, suggest that grassland-type parks do not effectively reduce PM or associated microbial loads.

In hotspots, microorganisms were primarily concentrated in the upper stages of the cascade impactor (Stage 1: >7 µm; Stage 2: 4.7-7 µm), indicating association with larger PM particles. In contrast, green areas with substantial woody vegetation exhibited a greater concentration of microorganisms in the finer particle stages. TA5 again deviated from this trend due to its lack of trees, resulting in microbial distributions more similar to those observed in hotspots.

Moreover, notable differences in microbial loads among hotspots were observed. TA2, despite being one of the busiest intersections in Sofia, had lower microbial concentrations than TA6, a residential neighbourhood. This can be attributed to the influence of wind and air mass movement. Additionally, ongoing excavation work in the TA6 area at the time of sampling likely increased dust levels, contributing to higher microbial counts. These findings support the conclusion that construction and renovation activities significantly deteriorate ambient air quality by increasing both PM and microbial content.

Our study reinforces the need for more urban green spaces that mimic natural forests, rather than purely aesthetic, meadow-type landscapes. Tree vegetation, even with reduced leaf mass during autumn, remains effective in capturing particulate matter and the microorganisms it carries. In hotspots, microbial concentrations were highest in the coarser PM fractions, whereas in green areas with tree cover, microorganisms were more prevalent in finer fractions (Stages 4 and 5). The control site in Vitosha Mountain demonstrated the lowest microbial levels, comparable only to Borisova Gradina, further affirming the role of dense forest vegetation in limiting airborne microflora.

## CONCLUSIONS

The main objective of this study was to conduct a preliminary analysis of airborne microbial abundance across selected urban and natural locations and to assess the distribution of microorganisms in relation to different particulate matter (PM) size fractions. The results revealed that green areas do not always exhibit lower microbial loads than their adjacent urban hotspots. This variation is largely influenced by the type of vegetation cover and the specific location of the green spaces.

Park areas dominated by grass and with limited woody vegetation displayed microbial patterns similar to nearby high-traffic zones. In contrast, parks with dense tree covers showed a more favorable profile, with lower microbial concentrations. Additionally, areas with strong air mixing due to moving air masses demonstrated a reduction in microbial presence, even in busy hotspots.

Our findings suggest that both busy intersections and green spaces situated near active construction or renovation zones should be approached with caution, as they pose elevated health risks due to increased microbial and particulate loads. In hotspots and grass-dominated green areas, microorganisms were mainly associated with larger PM fractions. Conversely, in tree-rich parks, microbial abundance shifted toward finer particle fractions.

These insights underscore the importance of integrating principles of "Environmental Hygiene" into urban planning. Efforts should focus on minimizing particulate and microbial exposure to improve respiratory health among urban populations. This study provides a foundation for further research and can inform the development of detailed urban design strategies aimed at optimizing air quality and public health outcomes.

## ACKNOWLEDGEMENTS

This research work was carried out with the support of Research Fund of Ministry of Education and Science in Bulgaria, project №: КП-06-Н74/5 „Relationships between the amount of fine particulate matter and airborne microorganisms in "hot spots", park areas, urban background areas and control forest areas’’.

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