



A potential relationship between fine particulate matter and airborne microorganisms in Sofia, Bulgaria

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Abstract: This preliminary study presents the results from a measurement campaign conducted in Sofia on particulate matter concentrations (PM₁₀, PM_{2.5}, and PM₁) and airborne microorganisms (total microbial number, TMN) carried out at seven preselected sampling sites on January 15 and 16, 2025. Meteorological variables such as temperature, humidity, and wind speed were recorded alongside the measurements. The sampling sites were organised in pairs to include urbanised and nearby green areas, as well as major crossroads in Sofia. The PMs concentrations were measured with Envea PM Cairsense, while the airborne microorganisms were sampled with the IUL Spin Air 360°. The cultivation of microorganisms was carried out according to the requirements of each studied microbial group, and a total microbial number (TMN) for each sampling site was estimated. The results revealed that PM₁₀ concentrations peaked at 48.54 µg/m³ at sampling site SS6, followed by PM_{2.5} at 38.01 µg/m³ and PM₁ with a maximum concentration of 27.23 µg/m³ at the same site. In contrast, SS1 exhibited the lowest PM concentrations, with PM₁₀ levels at 22.50 µg/m³ and PM_{2.5} at 20.12 µg/m³, nearly two times lower than SS6 and the nearby hotspot (SS2), which recorded PM₁₀ levels of 44.13 µg/m³ and PM_{2.5} levels of 32.45 µg/m³. PMs concentrations were lower in the green areas than in the hotspots, except for SS3 (31.58 µg/m³), which exhibits higher levels compared to the nearby hotspot SS4 (28.06 µg/m³). The highest total microbial number (TMN) across all sites was observed at SS2, reaching 730 CFU/m³, almost twice as high as the second-highest TMN (340 CFU/m³) at SS6. Notably, SS7, despite being a less anthropogenised zone, recorded PM₁₀ concentrations at 38.0 µg/m³ and TMN

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at 270 CFU/m³, levels that approached the highest values observed at other sites, indicating the impact of solid fuel heating on air quality. A strong correlation ($r=0.99$) was established between particulate matter levels and airborne microflora abundance in green areas. In contrast, hotspots displayed a weaker correlation ($r=0.49$) influenced by multiple urban variables that could be attributed to the traffic volume, crossroad sizes, waiting times at traffic lights, and construction activities, if they exist.

Keywords: measurement campaign, fine particulate matter, airborne microorganisms, air quality, Sofia

1. INTRODUCTION

Nearly 3.3 million people die prematurely from polluted air every year worldwide (Lelieveld et al., 2015). In the last decades, particulate matter has become one of the most serious environmental issues in cities worldwide, especially in big cities with a population of over a million people. Although there has been a decline in air pollution emissions in the EU in recent years and a 45% reduction in premature deaths caused by PM_{2.5}, still, 239,000 people die annually because of excessive exposure to PM_{2.5}, which makes it the single most dangerous air pollutant (EEA, 2024).

As the European Environment Agency points out, air pollution poses a potential risk to human health. According to the Agency, 96% of the urban population in the EU is exposed to unhealthy concentrations of PM_{2.5} (EEA, 2024). According to the National Program for Improving Ambient Air Quality 2018-2024, the excess of the maximum annual number of exceedances of the daily PM₁₀ limit value, set to protect human health, is a significant issue (NPIAAQ, 2019). The program reports that only two out of 37 air monitoring sites across the country comply with PM₁₀ standards for daily mean concentrations during 2011-2016. The situation has been improving in recent years, only two monitoring sites exceed the maximum number of exceedances per year (35 times) of the daily PM₁₀ limit value (50 µg/m³).

Both biological and physical factors influence air quality. Biological factors include bioaerosols and airborne microorganisms. It is essential to distinguish between the two terms, given that bioaerosols are particles of non-microbial origin suspended in the air (Chen et al., 2020). The term bioaerosols is often used to refer to airborne microflora (Chen et al., 2020), as these microorganisms make up a large percentage of the composition of bioaerosols. However, particles such as pollen, plant parts and other tiny components of biological origin are also considered bioaerosols (Chen et al., 2020; Kim et al., 2018). Of the physical factors, the most important for air quality is the amount of fine particulate matter (PM). Particles with a diameter below 10 µm (PM₁₀), 2.5 µm (PM_{2.5}) and 1 µm (PM₁) are of most significant importance for air quality and human health. Particles with a diameter of <5 µm, or respiratory particles, are the most dangerous to human health, as they can be deposited in the lower respiratory tract and

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enter the bronchioles and alveoli (Harrison et al., 2014). The aerodynamic diameter of bioaerosols determines their potential to be deposited in the lungs' tracheal, bronchial or alveolar regions. Thus, the effects of bioaerosols on human health are related not only to their concentrations but also to their size distribution.

Physical and biological factors affecting air quality are interrelated, as fine particulate matter serves as the adhesion nucleus for the attachment and transport of aerobic microorganisms (Aziz et al., 2018). Microbial cells in the air can exist both as free cells and as deposited on PM (Fröhlich-Nowoisky et al., 2016; Despres et al., 2012). Desert dust particles carry significant amounts of bacteria on their surface, with more than half of these being viable (Hu et al., 2020). The attachment of bacterial cells to particles can significantly alter their fate in the air, as it provides protection from harsh environmental conditions and enhances gravitational settling (Zhang, 2008; Bowers et al., 2013). Once in the atmosphere, bioaerosols often associate with suspended particulate matter, which helps their transport over long distances through processes such as diffusion and active air mixing (Maki et al. 2018; Soleimani et al. 2013). In this regard, the aerodynamic size of PM and the size of bioaerosols are a fundamental prerequisite for the entry of bacterial and micromycetes populations into the atmosphere (Wang et al., 2019). Bacterial aerosols enter the atmosphere from various sources, including vegetation, water surfaces, soil, and human and animal activities (Brodie et al., 2007; Bowers et al., 2011; Sun et al., 2018; Fan et al., 2019). Raindrops also contribute to microbial aerosolisation, releasing dust particles into the atmosphere upon contact with the soil. In addition, Qi et al., (2020) noted that soil, as one of the primary sources of bioaerosols, is associated with releasing large amounts of micromycetes into the air.

Fan et al., (2019) noted that levels of atmospheric pollutants had a more substantial impact on the structure of the bacterial community than on micromycetes. Li et al. (2020) found that land surface and atmospheric conditions influence the concentration of bacteria in the ground air and the structures of microbial populations. Atmospheric stress factors, such as temperature, solar radiation intensity, air humidity deficit and UV radiation, are unfavourable for microbial growth (Zhen et al., 2017). Ultraviolet radiation has the potential to sterilise the air of bioaerosols and influence the transportation of bacteria (Kowalski and Pastuszka 2018). Brągoszewska et al., (2017) prove that temperature and ultraviolet radiation are some of the most essential meteorological factors for the survival of bacteria in the atmosphere. Temperature, relative humidity, wind speed, and solar radiation are the meteorological factors that significantly affect the transport and abundance of airborne microflora in the ground atmosphere (Dong et al., 2016; Mouli et al., 2005; Zhong et al., 2016). These findings were also proved by Iliev et al. (2025) in their recent study about the seasonal variability of ambient bioaerosol concentrations in Sofia. They also discovered that fungal aerosols peaked in June and July, while bacterial aerosols reached maximums in May and July. Precipitation types (snowfall and rain) can affect airborne microorganisms differently.

Snowfall in winter traps bioaerosols, while rain in summer aerosolises microbes from the ground into the air through splashing and wetting of the topsoil (Uk Lee et al., 2016), so rainfall (1–4 mm) increases the concentration of micromycetes in the environment (Rajput et al., 2017).

The concentration of airborne microorganisms exhibits a significant negative correlation with temperature and ozone concentration (Yan et al., 2019), as well as a positive correlation with the Air Quality Index (AQI) and ground-level concentrations of SO_x, NO_x, and CO (Dong et al., 2016). Iliev et al., (2025) discovered, during the spring and summer seasons, a strong positive correlation between CO and bacteria, and a moderate correlation for fungal aerosols. They also found a negative correlation between NO₂ and O₃, as well as between bacteria and fungi aerosols. The amount of microorganisms positively correlates with the concentration of particulate matter. Li et al. (2012) noted that the correlation between PM_{2.5} and bacteria was higher than between PM_{2.5} and micromycetes. Amarloei et al. (2020) found that increasing PM concentrations increased the number of bacterial colonies. Airborne bacteria are mainly found in particles larger than 2.1 μm (Li et al., 2011). Gao et al. (2016) proved that PM₁₀ and PM_{2.5} have a statistically significant relationship with some characteristics of bioaerosols, respectively and with airborne microflora. According to them, PM_{2.5} has a negative correlation with the concentrations of cultivated airborne microorganisms. A possible reason for this is that PM_{2.5} usually contains soot, metals and secondary inorganic components, which have an adverse effect on the cultivation and survival of airborne microorganisms. However, the impact of PM₁₀ on airborne microorganisms is different.

The present study examines potential relationships between fine particulate matter and aerobic microorganisms, with a focus on the impact of green areas and hotspots on these microorganism communities. This type of study, with an emphasis on the various areas where particles and microorganisms enter the lower atmosphere, is being conducted for the first time in Bulgaria. Furthermore, the results will be used for a more comprehensive study.

2. MATERIALS AND METHODS

2.1. Study area

The study area is the capital city of Sofia, the largest and most densely populated city in Bulgaria, which suffers from high PM concentrations, especially during the winter. This is due partly because to its geographical location, a valley surrounded by the Balkan, Vitosha, Lyulin, Plana, and Lozenska mountains, which contributes to accumulation of air pollutants, and primarily because of PM emissions from the transport and residential heating. Considering all factors, we can easily conclude that the city's citizens may be exposed to harmful levels of PM pollution at certain times of

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the year. This pollution can be even a greater threat to sensitive people with respiratory and cardiovascular diseases.

The following hotspots and green areas were identified for the study's purpose (Figure 1). As hotspots were labelled crossroads with dense traffic, they were identified as the source of PM emitters. For green areas, parks near hotspots were selected for comparison of PM and airborne microorganism loading between the two types of areas. Therefore, there is a corresponding green area for each hotspot. The hotspot sampling sites (SS) are Eagle Bridge, the Military Academy crossroad, and the crossroad at Studentski Park (8 December Street). The sampling sites in green areas are Borisova Gradina Park, the Military Academy, and Studentski Park. One sampling site was selected in Vitosha Nature Park, a mountain area (978 MASL) unaffected by extensive urbanisation. It is situated in an open area, known as “Danova polyana”, surrounded by a thick forest, near the old Hotel Montana. The sampling site is 700 meters straight-line distance from the Simeonovo district and 164 meters above it. Leading in identifying SS were the types of green areas – predominantly low vegetation, as grass and sparse trees (SS5), woody (SS1), and dense woody vegetation (SS3). Another criterion was to be situated in densely populated areas where many people could be affected, and finally, areas known for their intensive traffic and poor air quality. Based on these main criteria, the sampling site emerged. The exact coordinates of the sample sites are presented in Table 1.

The sampling campaign was carried out on January 15 and 16, 2025, which are typically among the busiest days of the workweek in terms of human activity. Another important factor was the air temperature: these dates were among the coldest of the month, marked by overcast winter skies and daily temperatures ranging from -2.0 to -6.5 °C, along with light winds. Such conditions generally lead to increased contributions to particulate matter (PM) pollution from common sources like heating and transportation.

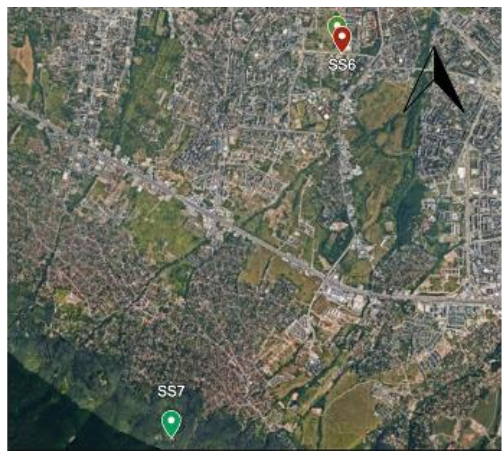
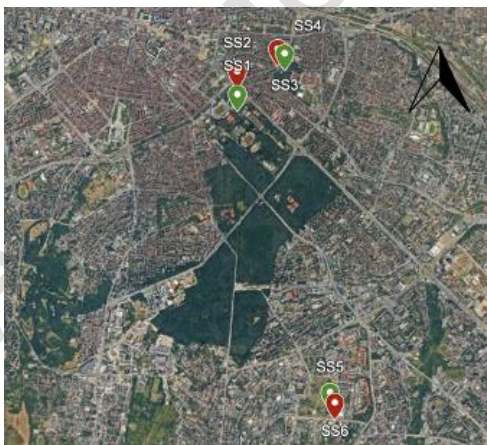


Fig. 1. Locations of the sampling sites**Table 1.** Sampling sites coordinates

№	Sample site	Type	Altitude (m)	Longitude	Latitude
SS1	Borisova Gradina Park	Green area	550	42°41'14"N	23°20'16"E
SS2	Eagle Bridge	Hot spot	540	42°41'23"N	23°20'16"E
SS3	Military Academy Park	Green area	536	42°41'31"N	23°20'43"E
SS4	Military Academy crossroad	Hot spot	542	42°41'33"N	23°20'39"E
SS5	Studentski Park	Green area	594	42°39'09"N	23°21'09"E
SS6	Studentski Park crossroad	Hot spot	591	42°39'05"N	23°21'12"E
SS7	Vitosha Nature Park	Green area	978	42°36'30"N	23°19'37"E

2.2. Airborne microorganisms sampling

We used an enriched AGAR medium to sample bacteria and a Czapek-Dox AGAR growth medium to propagate fungi. We sampled the air with the IUL Spin Air 360°, which enhances air sampling accuracy, precision, and resolution even when sampling in medium-to-highly contaminated environments. It uses 100% of the Petri plate agar surface to plate microorganisms. This dramatically improves data statistical significance, avoiding the use of colony count correction tables. During the sampling period, the sampler was positioned 1.5-2.0 m above the ground in the human-breathable heights. The sampling time was set to 120 sec. for each airborne microorganism group subject to this survey. Sampling volume was determined as 100 L/min. These sampling characteristics were chosen as the best for cultivation and countability of the microorganisms after a series of experiments. The sampling was conducted in this order: first, bacteria were sampled on Nutrient agar, then fungi on Czapek Dox agar and last, actinomycetes on Starch ammonia agar. After sampling, the samples were transported to the laboratory and incubated. The bacteria were incubated for 48 hours at 28°C. The fungi were cultivated for 7 days at 30°C, and the actinomycetes were cultivated at 35°C for 10 days. Then the colonies were counted with a digital colony counter.

2.3. Particulate matter sampling

Particulate matter sampling was carried out with the Envea Cairsense® micro-sensors for PM, which comply with the European Directive 2008/50 EC for indicative measurements. This particular sensor can measure simultaneously PM₁₀, PM_{2.5}, and PM₁ fraction sizes. The measurement principle is based on light scattering. The particles in the measuring chamber pass through a continuous laser beam and emit light in all directions according to the light scattering principle. This scattered light is analysed to calculate a mass concentration in $\mu\text{g}/\text{m}^3$ for each particle size fraction: PM₁, PM_{2.5} and PM₁₀. The sampling is carried out by a micro-fan controlled to maintain the flow rate at 2.5 L/min. It operates without any influence from moisture in ambient air. A sample heating system maintains the relative humidity in the chamber below the 60% threshold, beyond which the optical characteristics of the particles are altered and the sensor measurement is distorted. This system avoids the undesirable effects of high relative humidity when measuring PM₁₀, PM_{2.5}, and PM₁. The sensor comes calibrated from the manufacturer, and no further calibration is required during the sensor's lifespan. The sampling height is the same as the microorganism sampler. Sampling duration is 10 min. During the PM's sampling, the atmospheric conditions were monitored with a Davis Vantage Pro 2 mobile meteorological station. Meteorological variables like air temperature, relative humidity, atmospheric pressure, solar radiation, wind speed and direction were recorded. Meteorological variables were measured continuously during the sampling and recorded every three minutes.

2.4. Statistical data processing

The data were analysed statistically to determine the potential relationship between fine particulate matter and the reported amount of microorganisms in each sample. The StatSoft Statistica 12 software was used to process the results, with a significance level of 95%.

3. RESULTS AND DISCUSSION

Based on the measurements, the quantities of each PM fraction and the main microbiological groups: bacteria, micromycetes, and actinomycetes were estimated, and the total microbial number was determined.

3.1. Meteorological parameters

As mentioned, meteorological data, including wind speed, temperature, and air humidity, were recorded during the sampling campaign (January 15 and 16, 2025). On the first day, data were collected for the SS from one to four. On the second day, data were recorded for the remaining SS. Figure 2 provides a visualization of the mean values

of the meteorological parameters at the different SS. Regarding the wind speed at the sampling sites, it was expected to be similar for SS1 to SS4, around 1 m/s, due to their proximity (average distance of approximately 650 m). At SS5 and SS6, the wind was slightly stronger, with a difference of 1 m/s. The highest winds were measured at SS7 – 3 m/s. Overall, if excessive emitter sources exist, the low wind speeds favour the accumulation of pollutants, especially particulate matter. The relative humidity varied slightly around the sampling sites, ranging from 80% to 86%. The latter was measured in SS7. Surprisingly, the lowest air temperatures were measured at SS6 and SS5 (-6.3 °C), not at SS7 as could be expected. However, the difference is only 0.3 degrees Celsius. The highest temperatures were recorded at the Military Academy sampling sites.

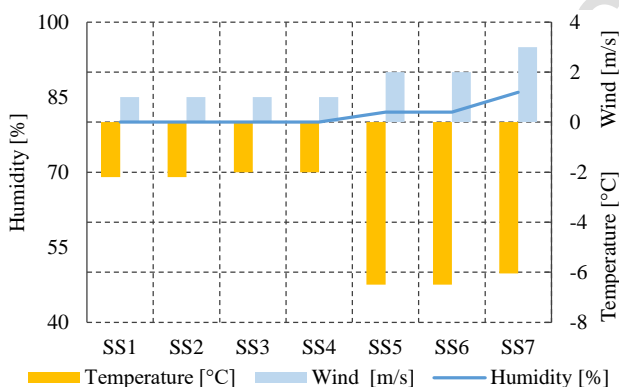


Fig. 2. Mean values of the meteorological parameters at the different SS

3.2. Particulate matter

Regarding PM10 concentrations measured at the sampling sites, a clear difference is observed between the couples (Figure 3). The most prominent distinction is between SS1 and SS2, where PM10 concentrations at Eagle Bridge are twice as high as those at Borisova Gradina Park. Additionally, they exhibit the most variability, with concentrations of PM10 ranging from 32 to 61 $\mu\text{g}/\text{m}^3$, which is the highest PM10 concentration. In contrast, SS7 is the least variable, with a tiny margin between the lowest and highest recorded values. The highest average concentrations were measured at SS6 (48.54 $\mu\text{g}/\text{m}^3$) and SS5 (42.71 $\mu\text{g}/\text{m}^3$) for hotspots and green areas, whereas the lowest were observed at SS4 (31.58 $\mu\text{g}/\text{m}^3$) and SS1 (22.50 $\mu\text{g}/\text{m}^3$), respectively. It is interesting to notice that SS3, SS5 and SS7 exhibit higher PM10 loads than the hotspot SS4.

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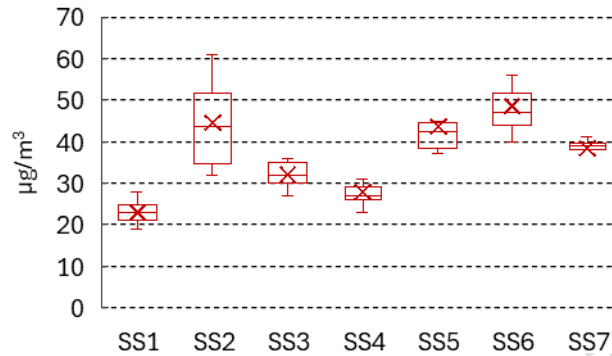


Fig. 3. Box-plots of PM10 concentrations ($\mu\text{g}/\text{m}^3$) at the sampling sites, 15 and 16 January

Figure 4 depicts the dynamics of PM2.5 concentrations at the sampling sites, similar to those in Figure 3. The highest average ($38.01 \mu\text{g}/\text{m}^3$) and absolute ($44.13 \mu\text{g}/\text{m}^3$) values of PM2.5 were recorded at SS6. The margin between the concentrations of PM2.5 at SS1 and SS2 is similar to that of the PM10 levels. The lowest variation and concentration ($18.50 \mu\text{g}/\text{m}^3$) of PM2.5 was recorded at SS1. The average levels of PM2.5 at Vitosha Nature Park ($35.58 \mu\text{g}/\text{m}^3$) and Studentski Park ($33.78 \mu\text{g}/\text{m}^3$) are higher compared to the hotspot sites at Eagle Bridge ($32.00 \mu\text{g}/\text{m}^3$) and the Military Academy intersection ($24.06 \mu\text{g}/\text{m}^3$).

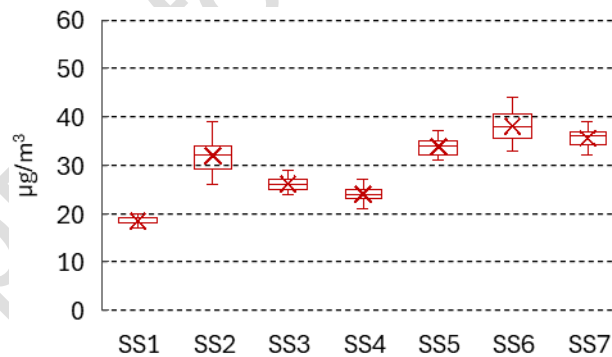


Fig. 4. Box-plots of PM2.5 concentrations ($\mu\text{g}/\text{m}^3$) at the sampling sites, 15 and 16 January

The situation regarding PM1 levels is similar to that of PM2.5 levels at Borisova Gradina Park (Figure 5). The lowest measured PM1 concentrations ($13.93 \mu\text{g}/\text{m}^3$) and variation were recorded at SS1. On the other hand, the most significant variability was observed at SS2. The green areas SS5 and SS7 manifested higher PM1 loads than hotspots SS2 and SS4, with a greater margin than was observed in Figure 4. Even more, concentrations at SS7 ($26.83 \mu\text{g}/\text{m}^3$) were similar to those of SS6 ($27.23 \mu\text{g}/\text{m}^3$), the site that suffers the most from PM pollution.

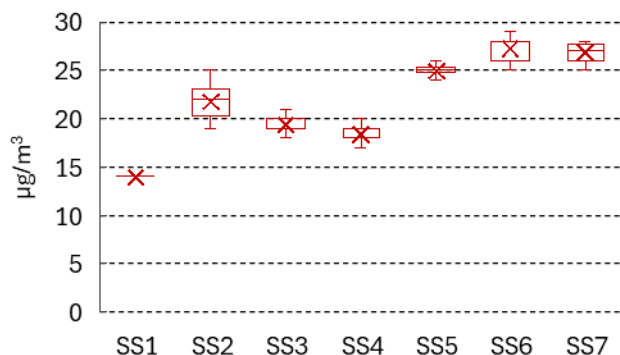


Fig. 5. Box-plots of PM1 concentrations ($\mu\text{g}/\text{m}^3$) at the sampling sites, 15 and 16 January

3.3. Total microbial number

Figure 6 represents the amounts of the recorded PM by fractions and the estimated total microbial number for each sampling site. The studied points are grouped in pairs (hot spot-green zone) to reflect the specifics of the closely located zones. The highest level of TMN was estimated at SS2 ($7.3 \times 10^2 \text{ CFU}/\text{m}^3$), which is almost twice as high as the second-highest TMN ($3.4 \times 10^2 \text{ CFU}/\text{m}^3$) at SS6, although the latter is the site with the highest PM loads. The lowest TMN was estimated at SS4 ($0.9 \times 10^2 \text{ CFU}/\text{m}^3$).

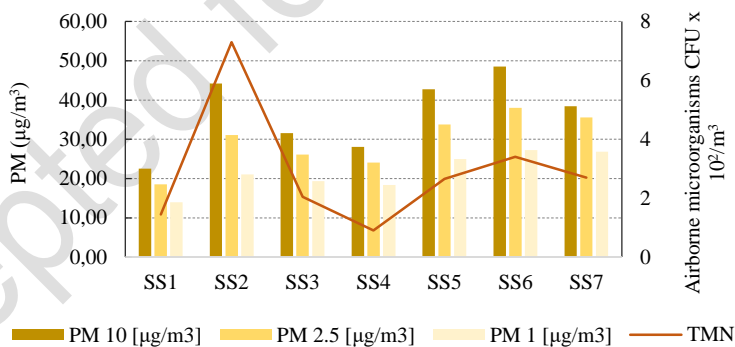


Fig. 6. PM by fractions compared to TMN

Figure 7 shows the percentage of the PM fraction compared to the total microbial number. As can be seen from the graph, regardless of the point under consideration, the distribution of the individual fractions is similar. The total microbial number is dynamic. However, in each pair, the number of microorganisms is lower in green areas compared to hotspots. In their study, Li et al., (2021) also established the influence of vegetation, its amount, percentage of foliage, and others on minimizing the amount of

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microorganisms in the air, including the authors reporting a decrease in the number of pathogenic species in the studied green areas compared to open urban areas.

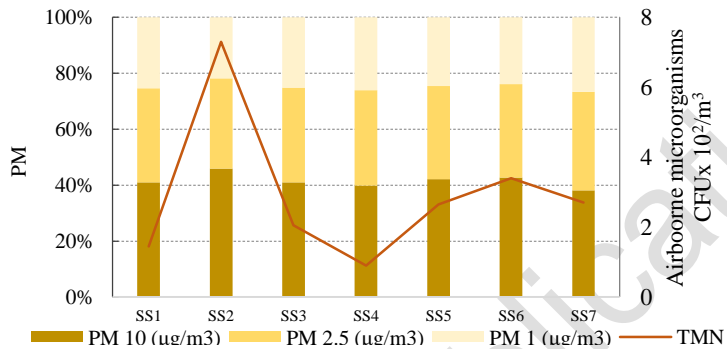


Fig. 7. Percentage of PM fractions compared to TMN

Several significant trends emerge from the results:

All green sites indicate lower microbial load and PM concentrations compared to their adjacent hotspots. The Studentski Park crossroad (SS6) stands out as having the highest load due to the construction and repair activities carried out, as well as the longer retention of car traffic. The adjacent green area (SS5) also shows atypically high levels of particulate matter and microorganisms. This is likely due to the specificity of landscaping, which predominantly features grassy vegetation. The Eagle bridge (SS2) results also show very high levels of particles and microbial abundance. Given that this is a heavily trafficked crossroad and the peak hour of sampling, the data are expected. The adjacent green area, Borisova Gradina Park (SS1), has the lowest recorded values of PM and airborne microorganisms. Our other studies also confirm those data (Peshev et al., 2024a). Borisova Gradina Park (SS1) features a landscape design that simulates natural forest vegetation. As our previous studies have shown, the amount of PM and microorganisms decreases with a higher percentage of woody vegetation in a given green area (Peshev et al., 2024b).

The data for pairs SS3 and SS4 are interesting. The amount of PM and airborne microorganisms in this pair differs, although the greatest proximity between them is the tiniest among the pairs, less than a hundred meters. In this pair, the amount of PM and airborne microorganisms is higher in the green area than in the hotspot. These data show vegetation vitality and the location of the green areas in urbanized cities. The green zone is located at a lower level than the Military Academy crossroad (SS4). For this reason, many of the generated particles and the microorganisms adhering to them are dispersed in the lower park area.

The Vitosha Nature Park (SS7) results show a worrying trend related to the influence of solid fuel used for heating on ambient air quality. Considering that previous studies have shown this site to have the lowest load during the warmer months (Peshev et al.,

2024a; Peshev et al., 2024b), the relationship between the particle components released from the stoves and the concentration of microorganisms on them is evident. Often when using solid fuel, the released particle components serve as adhesion nuclei for the concentration of microbial cells (Aziz et al, 2018).

3.4. Correlation analysis

A correlation analysis of the data for the hotspots and the studied green areas was performed (Figures 8 and 9). The results reveal specific differences between the green areas and the hotspots. In the case of green areas, we have environmental dynamics concerning two main parameters – the size of the park area and the type of afforestation (predominance of woody or grassy vegetation). This is likely the reason for the reported high direct correlation ($r = 0.99$). When considering hotspots, more variables are available — traffic volume, crossroad sizes, waiting time at traffic lights, congestion, construction activities, etc. The results did not reveal a high-order correlation ($r = 0.49$), indicating the need for additional in-depth research.

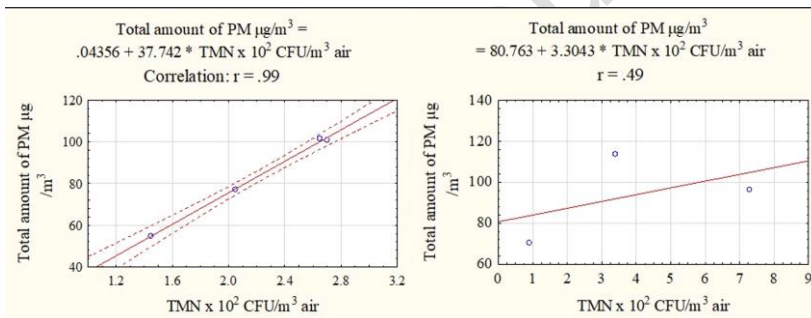


Fig. 8. Relationship between PM and TMN in green areas (left); hotspots (right)

Figure 9 presents data on the percentage participation of individual microbiological groups. The generated results clearly show the specificity in the distribution depending on the type of the analysed sampling site. Thus, in hotspots, the participation of micromycetes and actinomycetes is low. In contrast, in green areas, the higher percentage of the involvement of micromycetes is visible, which is related to the adjacent vegetation. Similar conclusions were reached by (Mhuireach et al., 2016).

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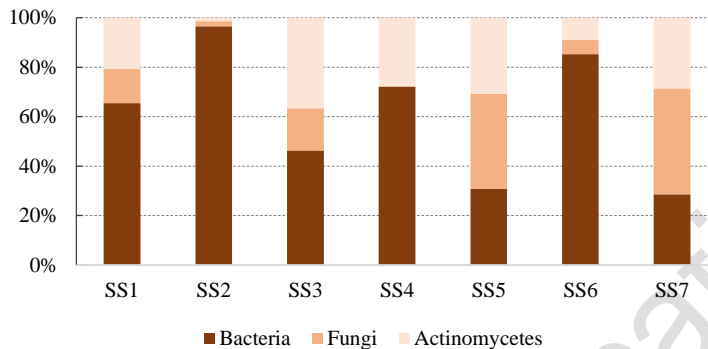


Fig. 9. Percentage of the microbial groups' populations at sample sites

3. CONCLUSIONS

This pilot study aims to provide basic information on the potential relationships between the amount of PM and airborne microorganisms in the region of Sofia. Based on the measurements taken during the sampling campaign on 15 January, it was found that the sites need to be considered in pairs (hotspot - green area) to assess their specifics. A correlation between PM and airborne microorganisms has been established for green areas. Green areas have lower particle concentrations and microbiological loads. Vegetation affects the amount of microorganisms and the percentage of the main groups. The use of solid fuel negatively affects the air load with both PM and airborne microorganisms. When planning the construction of green zones, they need to be considered in the direction of "Environmental Hygiene". The predominant vegetation and the location of green areas are essential for the amount of PM and airborne microorganisms. Additional measurements in several daily dynamics are needed to elucidate stable interrelationships between the studied parameters. This study can serve as a basis for future studies and as a starting point for the construction of new residential and park areas on the territory of the Sofia Municipality, Bulgaria.

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