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„СВ. КЛИМЕНТ ОХРИДСКИ“
БИОЛОГИЧЕСКИ ФАКУЛТЕТ

SOFIA UNIVERSITY
St. KLIMENT OHRIDSKI
FACULTY OF BIOLOGY

ABSTRACT OF PhD THESIS

for acquiring the educational and science degree “Doctor“
in the professional field 4.3. Biological sciences,
Scientific specialty: Microbiology

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CHARACTERIZATION OF THE MICROBIOME IN A COMPLEX STUDY OF PARTICULATE MATTER (PM) IN THE ATMOSPHERE OF URBAN AREAS AND RISK ASSESSMENT

Supervisors: Assoc. Prof. Mihail Iliev
Prof. Ivan Nedkov

Sofia 2022

The full volume of the dissertation is **300** pages. It consists of an **Introduction** (1 page), **Review** (64 pages), **Purpose and main tasks** (1 page), **Materials and Methods** (22 pages), **Results and Disscussion** (153), **Conclusions** (2 pages), **Contributions** (1 page), **Recommendations** (1 page). The list of references contains **641** titles. The text of the dissertation includes **112 figures** and **44 tables**. These lists are given at the beginning of the dissertation.

The experimental work was carried out in the Laboratory of Geological Microbiology at the Department of General and Industrial Microbiology. The lidar monitoring was carried out with lidars of IE-BAS, and the physicochemical characterization of PM - in IC-BAS.

The PhD thesis was discussed and allowed to be defended during an extended session of the Department of General and Industrial Microbiology, Faculty of Biology, Sofia University, which was held on 10.06.2022. It is scheduled for defense before a scientific jury formed by order …… of the Rector of Sofia University "St. Kliment Ohridski".

Scientific jury:

1. **prof. Dr. Petya Koycheva Hristova**
2. **assoc. prof. Dr Anna Atanasova Tomova**
3. **prof. Dr. Elena Ivanova Georgieva**
4. **Prof. Maria Bogomilova Angelova, DSc**
5. **Prof. Margarita Stoyanova Kamburova, DSc**

The defense of the PhD thesis will be on …… at …… at the Faculty of Biology.

The materials related to the defense are available in the Department of General and Industrial Microbiology and on the website of the Faculty of Biology.

INTRODUCTION

According to the World Health Organization, air pollution is the most significant environmental risk factor for the population of the European Union.

At the same time, the main scientific interest for decades has remained focused mainly on physical and chemical air pollutants, which severely limits our knowledge of microbial contamination in the outdoor air. The exploration of the complex nature of air pollution has become apparent in the recent years, and efforts are focused on research related to establishing the qualitative and quantitative composition of the microorganisms associated with particulate matter (PM) pollution, as well as the multifactorial nature of its dynamics. As a result of these efforts, a relatively new trend for complex monitoring studies is being laid, which aims to trace the relationship between air quality, the presence of various pollutants (physical and chemical), and the composition and concentration of microorganisms in the studied areas. Studies of outdoor air over highly urbanized areas are particularly relevant and important, as air pollution usually affects urban dwellers much more than rural one. In the microbial concentration, composition, size distribution and taxonomic status of the detected predominant microbial species in the atmosphere in populated urban areas, changes are observed depending on the urbanization specifics of the settlement, geographical location and climate. At the same time, each of the currently available studies has been conducted in places that differ dramatically in their geographical, climatic and anthropogenic characteristics. Therefore, the accelerated urbanization of Sofia city necessitates in-depth research related to the complex nature of air pollution, which can help in making the right management decisions to preserve its cleanliness. The research in the present dissertation is a step in this direction.

PURPOSE AND MAIN TASKS

The main purpose of the dissertation is to conduct a comprehensive study of the air microbiota in a highly urbanized central part of Sofia, including quantitative monitoring of bioaerosol contamination levels for year in the chosen location, identification of dominant microbial species and full characterization of associated particulate matter pollution.

The main task is to accomplish annual monitoring of quantitative variations of culturable bacterial and fungal bioaerosols for the period May 2020 – April 2021 in the central part of Sofia city.

Tasks

1) Quantitative monitoring of the seasonal, weekly and daily variations in the number of microbial concentration in outdoor air in the selected location.
2) Qualitative analysis of the microbial content and identification of the dominant microbial species in the collected air samples.
3) Taxonomic identification of the dominant microbial species by the methods of the classical taxonomic identification scheme.
4) Metagenomic analysis of the microbial composition in urban air.
5) Characterization of the fractions of PM in lidar-localized aerosol fields and analyze their chemical structure, size distribution and morphology.

MATERIALS AND METHODS

The object of the present study is the microbial bioaerosols and the associated particulate matter pollution in the outdoor air over the highly urbanized central part of Sofia city. The sampling location is Faculty of Biology, Sofia University. The exact location of the selected area and its coordinates are as follows: Sofia, Dragan Tsankov 8 Blvd., GPS coordinates: 42 ° 41'01.9 "N, 23 ° 19'58.3" E.

Qualitative analysis of bioaerosols in urban air

Airborne samples were collected during the period May 2020 - April 2021, that covers 54 weeks. Four days from each annual season were selected for monitoring the diurnal variation in the concentration of microbial bioaerosols. Weekly dynamics in the concentration of bioaerosols was studied during selected weeks, from each season. During the experimental work, monitoring was performed on selected days, with certain meteorological phenomena - mist, rain, snowfall and transboundary dust pollution.

Meteorological conditions and concentration of the main chemical pollutants were obtained during each of the samplings (https://www.sinoptik.bg/sofia-bulgaria-

100727011?location; https://platform.airthings-project.com/, Nova PM sensor SDS011). The lidar monitoring was performed by the two-wavelength lidar system developed at the Laser Radar Laboratory (LRL) of the IE-BAS that is capable of scanning the horizontal aerosol distributions and the vertical long-distance transport of air masses.

In situ sampling was achieved by Six-Stage Viable Cascade Impactor (FSC-A6 (Honri Air Clean Technology Co., Ltd), for size-selectively enumeration of culturable heterotrophic bacteria and fungi in the range from >7μm to 0.65μm.

Quantitative analysis of bioaerosols in urban air

248 pure bacterial cultures and 35 fungal cultures were obtained during the experimental work. The isolates were identified on the base of classic microbiological scheme for identification of bacteria and fungi.

Metagenomic analysis of bioaerosol samples

High-throughput sequencing was performed using the purified total DNA from samples collected during the spring and winter. The Next-generation sequencing and the bioinformatics analysis were performed by Novogene Company Ltd (Cambridge, UK). The effective tags were analyzed by software Uparse v7.0.1001. Operational Taxonomic Units (OTUs) were obtained by clustering with 97% identity on the Effective Tags of all samples, and then identified. KRONA was used for visualization of the result from the taxonomic annotation.

Physicochemical characterization of PM

Physicochemical characterization of PM was performed at the Institute of Catalysis-BAS. The following equipment was used: Emission spectrometer ICP-OES "Spectro Arcos"; TUR-M62" with PC control of HZG-3 goniometer for X-ray phase and X-ray diffraction analysis; Mössbauer spectrometer "Wissenshaflishe Elektronik GMBN"; IR spectrophotometer "Nicolet 6700", Thermo Electron Corporation; X-ray photoelectron spectroscopy with ESCALAB MkII -VG Scientific; Scanning Electron Microscope JEOL JSN 5510 and Analyzer for particle size Nano Brook 173.

RESULTS AND DISCUSSION

1. Quantitative analysis of the culturable microbiota in air samples from the selected location

Up to date, information from monitoring studies related to the quantitative presence of microorganisms in the outdoor air over highly urbanized urban areas in global scale are extremely scarce. The conducted quantitative analysis on the microbial presence in outdoor air in the central part of the city of Sofia is the first monitoring research in Bulgaria and one of the longest for the territory of Europe.

1.1. Monthly variations in the concentration of bacterial and fungal bioaerosols in outdoor air at the selected location

The dynamics in the concentration of bacterial and fungal bioaerosols in the air at the selected location for each month of the year was established. The results can be summarized as follows:

→ There is a pronounced monthly dynamic in the levels of bioaerosols, which is determined by the geographical and climatic features of the region. The city of Sofia, in the center of which the sampling site (Faculty of Biology) is located, is distinguished by its specific geographical and climatic profile. What most distinguishes the city of Sofia is its altitude (550 m) and its location in the flat area of the Sofia valley, which determines the frequent temperature inversions, winds and long-term retention of mist.

→ There are no similar monitoring studies in cities with such geographical, climatic and anthropogenic characteristics, which makes it difficult to compare the results obtained during the monitoring study on the monthly dynamics in the concentration of air microbial contamination.

1.2. Seasonal variations in the concentration of bacterial and fungal bioaerosols in outdoor air at the selected location

a) Results from quantitative analysis of bioaerosols during the winter (December 2020, January 2021, February 2021)

During the first month of the period (December, 2020) bacterial bioaerosol concentration was greatly reduced, with no significant dynamics in the values over the

month - maximum values for the month were reported in the fourth week of December - 141.0 CFU/m^3. The maximal fungal concentration was detected in the third week (21.0 CFU/m^3).

Reduction in the levels of bacterial concentration was also observed during the second month of the winter period. The fungal concentration is low, but a distinct peak in the values was observed during the third week – 131.0 CFU/m^3.

The last month of the winter season is characterized by a slight increase in the bacterial concentration, with reported maximum values in the 4th week - 40.0 CFU/m^3. Fungal bioaerosol levels during this month were also reduced, with a decrease in the concentration in the 2nd and 3rd weeks (resp. 14.0 and 16.0 CFU/m^3) and an increase in the last week - 48.0 CFU/m^3.

The results of percentage distribution of bioaerosol particles (bacterial and fungal) for the respective reporting period are shown in fig. 1.

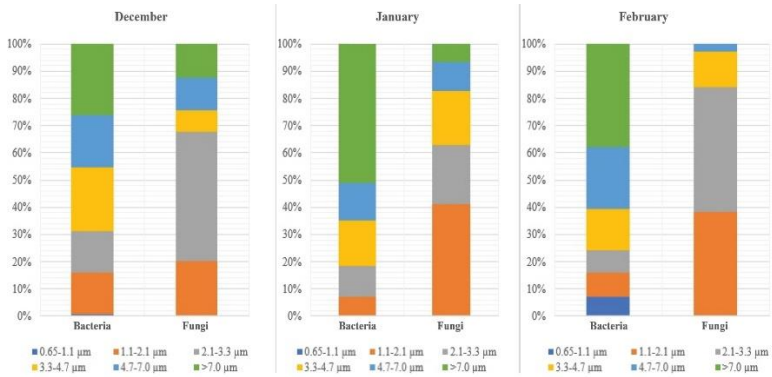


Figure 1. *Size distribution of bacterial and fungal bioaerosols – winter months.*

During the winter months of the year, a number of monitoring studies reported low but distinct values of the detected bioaerosol presence - Qingdao, China - 168.0 CFU/m^3 (Li et al., 2011), Gdansk, Poland -> 20.0 CFU/m^3 (Kruczalak et al., 2002); Gliwice, Poland - 49.0 CFU/m^3 (Bragoszewska and Pastuszka, 2018).

b) Results from quantitative analysis of bioaerosols during the spring (March 2021, April 2021, May 2020)

The spring season is characterized by an increase in the observed bacterial and fungal bioaerosol levels. In February the bacterial concentration demonstrated a gradual increase with maximum values in the 4th week - 44.0 CFU/m^3. The fungal concentrations reached a maximum for the month in the 1st week (192.0 CFU/m^3). In the second month of the period - April, the bacterial concentration slightly increased compared to the previous month - maximum reported values in the 4th week - 47.0 CFU/m^3. During the first week of April, maximum values for the month were also reported for fungal bioaerosols - 177.0 CFU/m^3. During the last month for the period - May, there is a significant increase in the amount of bacterial and fungal contamination, with maximum concentration in the 3rd week - 720.0 CFU/m^3 for bacteria, and respectively - 560.0 CFU/m^3 for fungi. The results from the size distribution of bioaerosol particles by aerodynamic diameter are shown in fig. 2.

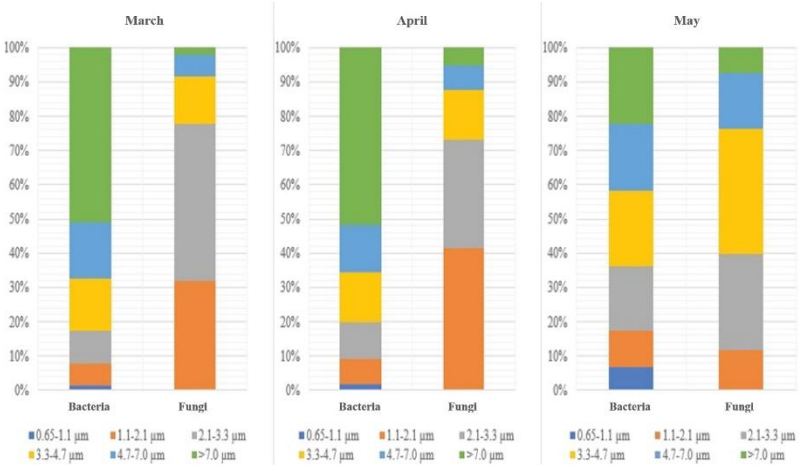


Figure 2*. Size distribution of bacterial and fungal bioaerosols – spring months.*

What significantly distinguishes the spring season from the previous one is the active vegetation of the plants. The negative correlation between the degree of afforestation and the amount of bioaerosols in the air has been reported by a number of authors (Song, 1999; Ju, 2003). On the one hand, large green areas maintained on

the territory of settlements contribute to the increase of microbial presence by emission of microorganisms dwelling their leaf surfaces, but on the other hand they have a certain inhibitory effect on airborne microorganisms, probably due to volatile products from their metabolism (Xie, 1999). This is confirmed by the data obtained in the selected location, during the experimental work - the location is adjacent to a large park area, which has a similar effect during the seasons with active vegetation.

c) Results from quantitative analysis of bioaerosols during the summer (June 2020, July 2020, August 2020)

There was a significant increase in the microbial concentration, during the summer period, compared to the previous seasons. In the first month from the period (June), the increase in fungal concentration was significant - with maximum values observed in the 3rd week - 1579.0 CFU/m^3. The increase in bacterial levels is also significant compared to previous months with a maximal value of 269.0 CFU/m^3 reached in the 2nd week. Peak values for fungal bioaerosols in the period were reported in July - reaching a maximum of 2666.0 CFU/m^3. During the month, bacterial bioaerosol concentration also reached maximum values for the summer period - 1260.0 CFU/m^3.

In August, there was a decrease in the levels of both types of bioaerosols compared to the previous month – the maximum levels of 120.0 CFU/m^3 for bacteria were reached in the 1st and 4th week, and respectively for fungal bioaerosols -451.0 CFU/m^3 during the first week of the month. The size distribution pattern of bioaerosol particles is shown in fig. 3.

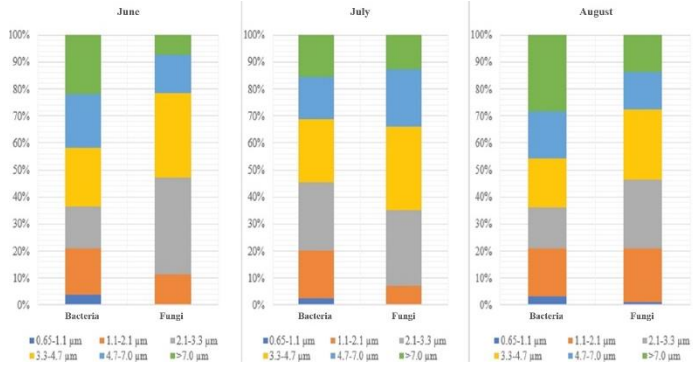


Figure 3*. Size distribution of bacterial and fungal bioaerosols – summer months.*

An increase in bacterial and fungal concentration in outdoor air during the summer months has been reported too by a number of authors (Gupta et al., 1993; D'Amato et al., 1983; Jones and Cookson, 1983; Takatori et al., 1994), but in the same time in some of the seasonal monitoring studies, was observed a decrease in bacterial and fungal bioaerosol concentrations (Filipello-Marchisio et al., 1992; Mullius et al., 1984).

d) Results from quantitative analysis of bioaerosols during the autumn (September 2020, October 2020, November 2020)

During the autumn there was an increase in microbial contamination compared to the previous months. The maximal values of 740.0 CFU/m^3 for bacterial contamination in September were reported in the 3rd and 4th week. There was an increase in fungal contamination (712.0 CFU/m^3 in the 3rd week). compared to August. In October, a gradual reduction in the amount of bacterial contamination was registered - maximum reported values were 300.0 CFU/m^3. For the same period, peak values of fungal concentration were reported - 4774.0 CFU/m^3 in the 2nd week of October. This was the observed maximal fungal concentration during the whole year. During the next month, there was a reduction in the concentration of both types of bioaerosols. Bacterial concentration was slightly reduced with maximal values of 328.0 CFU/m^3 in the 4th week. Whereas the reduction in fungal bioaerosol levels was significant – maximum of 190.0 CFU/m^3 in the 1st week. The size distribution profile of bioaerosols by aerodynamic diameter for the observed period of the year is presented in fig. 4.

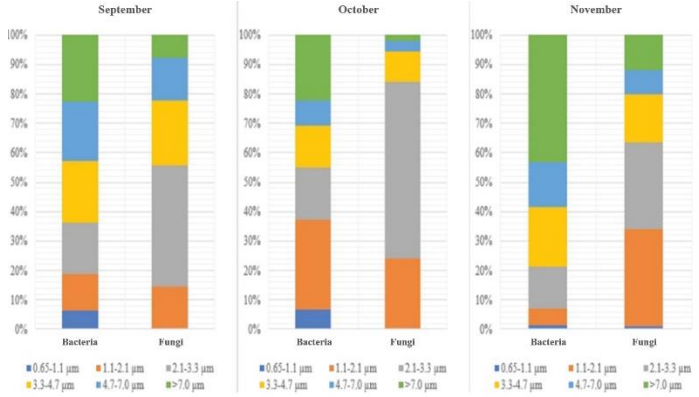


Figure 4. *Size distribution of bacterial and fungal bioaerosols – autumn months.*

The following summary can be made, based on the results of the seasonal monitoring of the concentration of bacterial and fungal bioaerosols, observed in the samples from outdoor air at the location:

→ The spring season is characterized by an increase in the reported levels of microbial air contamination, with particularly pronounced variations in the amount of the fungi.

→ There is a significant increase in the levels of microbial contamination during the first half of the summer season, compared to the previous spring months.

→ There is a decrease in the levels of fungal and bacterial bioaerosol concentration at the end of the summer season.

→ There is an increase in microbial air concentration at the beginning of the autumn season, compared to the end of the summer season. This rise at the concentration at the first half of the season is followed by a significant reduction in the last autumn month.

→ Microbial bioaerosol contamination is greatly reduced during the winter season, and no significant variation in the values for the whole period is observed.

1.3. Annual variations in the concentration of bacterial and fungal bioaerosols in outdoor air at the selected location

Average monthly values for the bacterial and fungal concentration were obtained, based on the weekly results of the monitoring study for the whole period. This allows us to determine the annual variations in the concentration of bioaerosols in outdoor air at the selected location (Fig. 5).

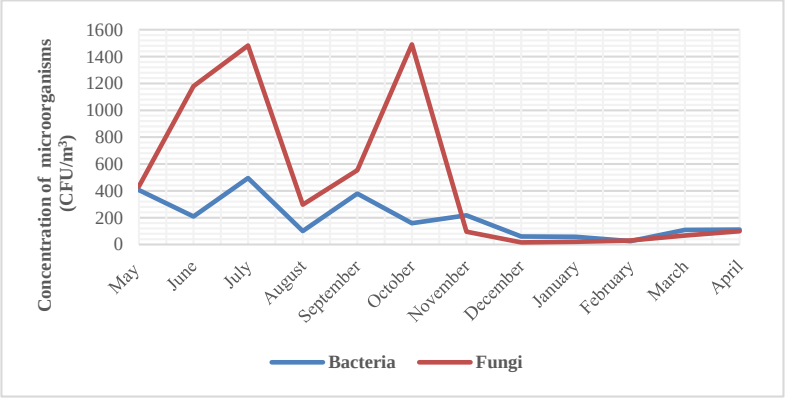


Figure 5. *Averaged monthly bacterial and fungal (CFU/m^3), in outdoor air.*

A distinctive course in the annual variation of bacterial and fungal concentration is observed. Peak value for fungal concentration were reported in June, 2020; July 2020 and October 2020 (respectively 1181.0 CFU/m^3; 1482.0 CFU/m^3 and 1491.0 CFU/m^3). Minimal fungal contamination in outdoor air is registered in December, 2020, January 2021 and February, 2021, respectively. 16.0 CFU/m^3; 20.0 CFU/m^3 and 31.0 CFU/m^3.

Maximum values for bacterial concentration were observed in May 2020, July 2020 and September 2020 (401.0 CFU/m^3; 495.0 CFU/m^3 and 379.0 CFU/m^3). Lowest bacterial contamination in the outdoor air at the study location is reported in January and February, 2021 - 58.0 CFU/m^3 and 25.0 CFU/m^3.

The annual variation in the bacterial and fungal concentration is compared with the reported annual dynamics in the concentration of particulate matter pollution (PM2.5/10.0), registered in the location (Fig. 6).

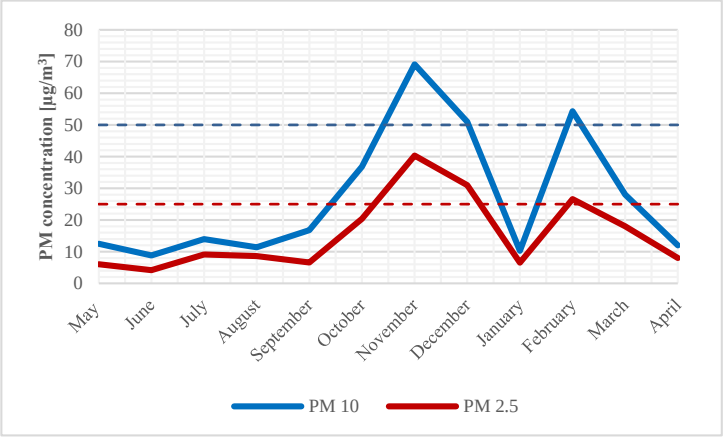


Figure 6. *Annual variations in the concentration of PM2.5/10 in the location.*

The annual variations in the concentration of PM2.5/10, maximum values are reported for the months November and December, 2020, as well as the second peak in the months of February and March, 2021, respectively. 69.12 µg/m^3, 51.0 µg/m^3 and 54.35 µg/m^3, 28.0 µg/m^3.

From comparison of the results from average monthly values of the bacterial and fungal contamination and the average monthly values of the PM pollution in the location, the following conclusions can be made:

→ The observed bacterial contamination maximums (May, 2020, July 2020 and September 2020) and fungal contamination maximums (June, 2020; July 2020 and October 2020) do not coincide completely with the first peak in PM pollution (November and December, 2020). Partial overlap is observed at the beginning of the increase in the concentration of PM concentration in October.

→ At the second maximum in the values of PM pollution (February and March) the reported levels of bioaerosol presence in outdoor air are relatively low due to the unfavorable condition in the atmosphere (temperature, humidity, etc.).

Abiotic parameters of the environment were reported, during the microbiological monitoring at the site. The averaged monthly values for the different environmental parameters are shown in Table 1.

Table 1. *Averaged monthly values of the environmental abiotic parameters in the location for the period May 2020 – April 2021.*

	May	June	July	August	September	October	November	December	January	February	March	April
T, °C	15.5	22.4	27.3	24.8	20.5	12.0	3.5	0.8	2.8	0.5	3.0	8.8
Wind, m/s	5.2	2.9	3.1	4.5	1.9	0.8	0.8	1.2	6.3	1.7	1.9	4.6
Relative humidity,	60.0	58.3	61.5	53.5	52.3	78.8	79.5	91.8	69.5	80.3	62.0	61.5
CO, mg/m^3	0.7	0.7	0.7	0.5	0.4	10.6	0.6	0.4	0.3	0.4	0.4	0.3
NO_2, µg/m^3	21.8	31.0	31.1	46.3	62.9	46.2	49.5	44.8	30.6	45.4	48.6	33.0
SO_2, µg/m^3	11.7	12.4	12.8	16.4	9.4	11.5	11.7	11.5	11.2	11.7	11.3	14.5
O_3, µg/m^3	39.4	67.3	68.5	67.9	279.6	36.8	19.7	16.2	17.3	21.1	22.5	33.1
UV index	6.5	7.3	9.5	8.0	6.0	3.5	2.0	1.5	1.0	2.3	3.0	4.5

The annual variations in the concentrations of the bacterial and fungal bioaerosols detected in the air of the location depending on the change of the observed abiotic parameters of the environment are presented in Table 2.

The average values for each month are presented. The increase/decrease (↑ / ↓) of the values compared to the previous month is marked.

Table 2. *Annual variations in the concentrations of bioaerosols depending on the change of the abiotic parameters.*

Increase/decrease in the concentration of bioaerosols (↑ ↓) when the values of the abiotic factors of the environment (↑ ↓) change compared to the previous month												
Bacterial bioaerosols (CFU/m³)	372,0	↓	↑	↓	↑	↓	↑	↓	↓	↓	↑	↑
Fungal bioaerosols (CFU/m³)	517,0	↑	↑	↓	↑	↑	↓	↓	↑	↑	↑	↑
PM10 (µg/m³)	12,5	↓	↑	↓	↑	↑	↑	↓	↓	↑	↓	↓
PM2.5 (µg/m³)	6,0	↓	↑	↓	↓	↑	↑	↓	↓	↑	↓	↓
Temperature (°C)	15,5	↑	↑	↓	↓	↓	↓	↓	↑	↓	↑	↑
Relative humidity (%)	60,0	↓	↑	↓	↓	↑	↑	↑	↓	↑	↓	↓
O_3 (µg/m³)	39,4	↑	↑	↓	↑	↓	↓	↓	↑	↑	↑	↑
CO (mg/m³)	0,7	—	—	↓	↓	↑	↓	↓	↓	↑	—	↓
NO_2 (µg/m³)	21,8	↑	↑	↑	↑	↓	↑	↓	↓	↑	↑	↓
SO_2 (µg/m³)	11,7	↑	↑	↑	↓	↑	↑	↓	↓	↑	↓	↑
Wind, (m/s)	5,2	↓	↑	↑	↓	↓	—	↑	↑	↓	↑	↑
UV index	6,5	↑	↑	↓	↓	↓	↓	↓	↓	↑	↑	↑
Month	V* 2020	VI 2020	VII 2020	VIII 2020	IX 2020	X 2020	XI 2020	XII 2020	I 2021	II 2021	III 2022	IV 2022

↑ - maximal measured value of the factor; ↓ - minimal measured value of the factor; * - values measured during the first month of the monitoring study

The following summary can be made, based on the results of the annual monitoring of the levels of bioaerosol contamination detected in the outdoor air at the location:

→ The annual variations in the bacterial and fungal bioaerosol concentration is extremely dynamic and reflects the specifics of the geographical and climatic characteristics of the city of Sofia..

→ Peak fungal contamination is observed in June, 2020; July 2020 and October 2020, (1181.0 CFU/m^3; 1482.0 CFU/m^3 and 1491.0 CFU/m^3). Low levels in the quantitative share of the fungi were reported in December, 2020, January 2021 and February, 2021, respectively. 16.0 CFU/m^3; 20.0 CFU/m^3 and 31.0 CFU/m^3.

→ Maximum values in the dynamics of bacterial contamination are registered in May, 2020, July 2020 and September 2020 (401.0 CFU/m^3, 495.0 CFU/m^3 and 379.0 CFU/m^3). Minimum levels of bacterial bioaerosol contamination in the outdoor air at the study area were reported in January and February, 2021 - 58.0 CFU/m^3 and respectively 25.0 CFU/m^3.

1.4. Diurnal variations in the concentration of bacterial and fungal bioaerosols in outdoor air at the selected location

In parallel with the annual microbiological monitoring, studies on the daily course of microbial concentration were conducted. Four days from all of the seasons with typical seasonal abiotic parameters of the environment were selected.

The selected days were: **1)** 30.07.2020 (summer); **2)** 06.11.2020 (autumn); **3)** 30.12.2020 (winter); **4)** 31.03.2021 (spring).

a) Daily monitoring of microbial bioaerosol concentration at the selected location in summer (30.07.2020)

The maximal fungal concentration during the whole year is observed in the summer months with the peak of 2666 CFU/m^3. The maximal bacterial concentration is also observed during July - 1260.0 CFU/m^3.

The daily course in the concentrations of the bioaerosols registered in the outdoor air at the location is presented in fig. 7.

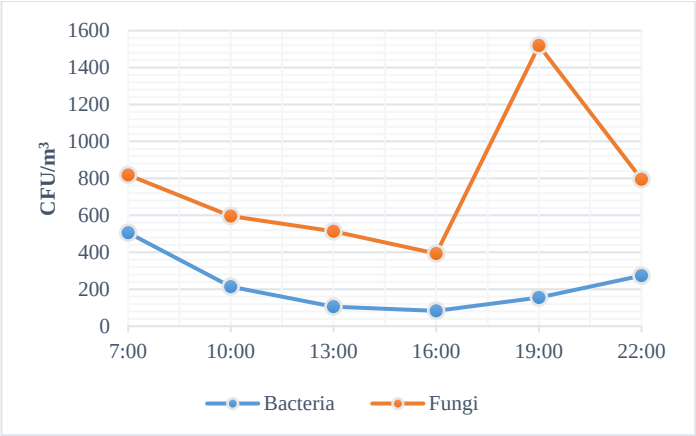


Figure 7. *Daily course in the concentrations of the bioaerosols (30.07.2020)*

Relatively high microbial concentration is reported during the first sampling interval (7:00 AM). The registered bacterial bioaerosol contamination is 506.0 CFU/m^3. This level of bacterial bioaerosol presence is close to the reported average values for July, 2020 - 465.0 CFU/m^3. During the same interval the reported fungal bioaerosol concentration was 819.0 CFU/m^3 (monthly averages of 1482.0 CFU/m^3). During the next three reported intervals (10:00AM; 13:00 and 16:00) the variations in the concentrations of the two types of studied bioaerosols is similar. With the increase of temperature, ozone concentration and UV index, as well as the decrease of relative humidity and the intensity of traffic, a gradual reduction is observed for fungal and bacterial bioaerosols with minimum values for the day at 16:00. An increase in the bioaerosol concentration is observed during the next two intervals in the afternoon and evening. The increase in the concentration of fungal bioaerosols is particularly pronounced, reaching a daily maximum at 19:00 - 1520.0 CFU/m^3. In the last time interval, coinciding with the onset of night, a decrease in fungal concentration is observed, taking into account values of 795.0 CFU/m^3. An increase is also reported for bacterial bioaerosols, which continues until the last sampling interval (22:00), when values reached 273.0 CFU/m^3 in the outdoor air of the site during the summer season.

b) Daily monitoring of microbial bioaerosol concentration at the selected location in autumn (06.11.2020)

The daily monitoring for the autumn season was conducted on November 6, 2020. In November, a decrease in the reported levels of both types of studied bioaerosols was observed. The decrease is less pronounced for bacterial bioaerosols, while in fungal bioaerosols the reduction in the concentration is significant. The daily course in the concentrations of the bioaerosols monitored in the outdoor air at the location is presented in fig. 8.

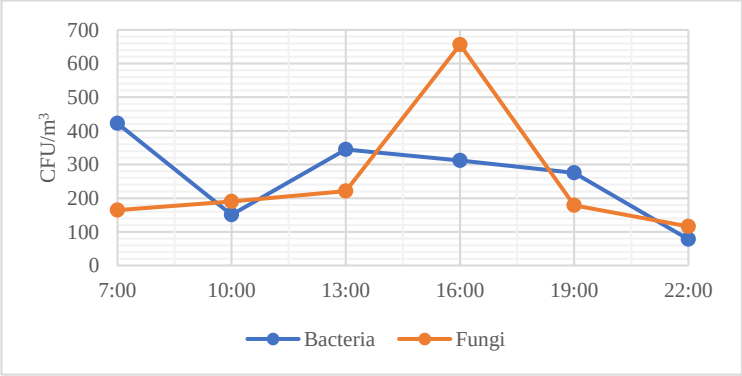


Figure 8. *Daily course in the concentrations of the bioaerosols (06.11.2020).*

Relatively high values of microbial levels, exceeding the average monthly values (average monthly value 217.0 CFU/m^3 for bacteria; 95.0 CFU/m^3, for fungi) were reported during the sampling day in November.

The reported bacterial bioaerosol contamination during the first interval in the morning (7:00 AM) was 422.0 CFU/m^3. In the second interval at 10:00 AM a significant reduction was registered - 151.0 CFU/m^3 During noon an increase is observed again, as the reported levels remain relatively constant until 19:00 With the onset of the evening there is a reduction, reaching a minimum for the day of 78.0 CFU/m^3 in the last sampling time at 22:00 PM.

The reported daily variations in the quantitative presence of fungal bioaerosols demonstrates a distinctive course during the autumn day. After relatively low levels in the morning (165.0 CFU/m^3 at 7:00 AM and 190.0 CFU/m^3, at 10:00 AM), in the

early afternoon there was an increase in the concentration of fungal presence in the outdoor air of the location, reaching maximum daily value of 656.0 CFU/m^3 at 16:00. During the evening period, a significant decrease in the concentration of fungal bioaerosols was reported, which reached a daily minimum of 78.0 CFU/m^3 at 22:00.

c) Daily monitoring of microbial bioaerosol concentration at the selected location in winter (30.12.2020)

The daily monitoring aimed to reveal the variations in the microbial concentration during the winter period was conducted on 30.12.2020. A significant increase in the levels of PM pollution was registered in December, whereas there was observed a significant reduction in the concentrations of the fungal and bacterial bioaerosols

Bacterial concentration in the early morning (7:00AM) was 18.0 CFU/m^3. This value is lower than the average monthly values of 85.0 CFU/m^3 During the next two intervals, a significant increase in the concentration of bacterial bioaerosols was observed, reaching a peak value for the day at 13:00 (80.0 CFU/m^3). The daily maximum was followed by a decrease during the next periods, reaching a minimal daily concentration of 6.0 CFU/m^3 at 19:00. A slight increase in the concentration of bacterial bioaerosols (20.0 CFU/m^3) was observed during the last sampling at 22:00 (Fig. 9).

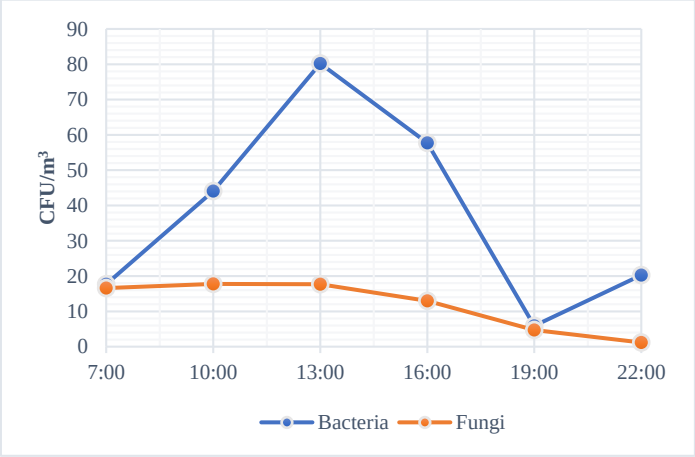


***Figure 9**. Daily course in the concentrations of the bioaerosols (30.12.2020)*

The daily variations in the fungal concentration showed a different pattern. The initial level of fungal contamination reported at the first sampling at 7:00AM coincides with the average monthly values - 18.0 CFU/m^3. During the day there is no change in the amount of reported fungal contamination. During the last two reporting intervals, a decrease was registered, reaching a minimum daily value of 1.0 CFU/m^3 at 22:00.

d) Daily monitoring of microbial bioaerosol concentration at the selected location in spring (31.03.2021).

The monitoring of the daily variations in the quantities of bacterial and fungal bioaerosols was carried out on 31.03.2021. During this month there is an increase in the levels of both types of bioaerosols compared to the previous winter months of the monitoring study. The daily course in the concentrations of the bioaerosols registered in the outdoor air at the location is presented in fig. 10.

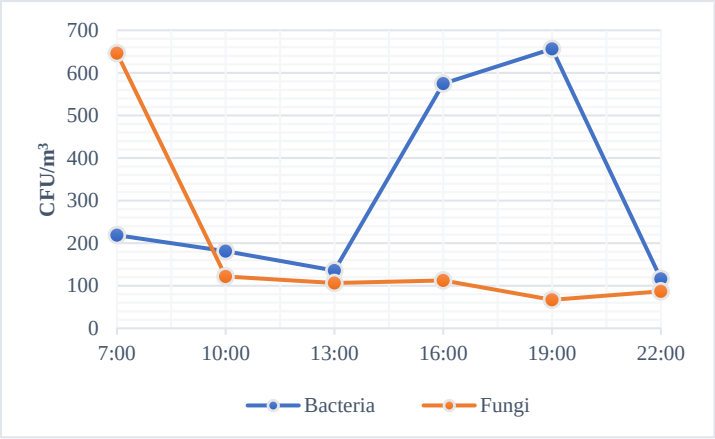


Figure 10*. Daily course in the concentrations of the bioaerosols (31.03.2021)*

At the early hours of the day (7:00 AM), the fungal concentration was 646.0 CFU/m^3 whereas, bacterial levels were lower – 219.0 CFU/m^3. At 10:00AM there was a significant decrease in the concentration of fungal bioaerosols (122.0 CFU/m^3), while the decrease in the bacterial concentration was significantly less pronounced - 181.0 CFU/m^3). The microbial concentration remains constant in the next period

(13:00). In the afternoon the variations in the fungal and bacterial concentration show different patterns. Bacterial levels increased during the next samplings, reaching a maximum of 657.0 CFU/m^3 at 19:00. The fungal concentration remains steady during the end of the day. A daily minimum for bacterial bioaerosols of 116.0 CFU/m^3 was reported at the evening at 22:00

The reported daily dynamics in the bacterial and fungal bioaerosol concentrations during the seasons of the year demonstrates a different profile (Table 3).

Table 3. *Comparison of the daily dynamics of the concentration of bacterial and fungal bioaerosols during the different seasons of the year*

	CFU/m^3	7:00	10:00	13:00	16:00	19:00	22:00
Summer	Bacteria	506.0	↓	↓	↓	↑	↑
	Fungi	819.0	↓	↓	↓	↑	↓
Autumn	Bacteria	422.0	↓	↑	↓	↑	↓
	Fungi	165.0	↑	↑	↑	↓	↓
Winter	Bacteria	18.0	↑	↑	↓	↓	↑
	Fungi	17.0	↑	↑	–	↓	↓
Spring	Bacteria	219.0	↓	↓	↑	↑	↓
	Fungi	646.0	↓	↓	↑	↓	↑

During noon (13:00 PM) the simultaneous decrease in the concentrations of bacterial and fungal bioaerosols was observed during the summer and spring seasons. At the same time in autumn and winter there is an increase in both types of bioaerosols (with maximum daily values reached during the winter season).

During the summer season daily minima for both microbial bioaerosols were registered at 16:00. During the autumn season, the levels of bacterial bioaerosols decrease, while the maximum daily concentration of fungi are reported. The dynamics is similar during the same interval in the winter season, while in the spring there is an increase in both types of bioaerosols in the afternoon.

During the summer season at 19:00 o'clock there is an increase in the reported values for microbial bioaerosols in outdoor air, with a daily maximum for fungi. For the same interval during the autumn season, an increase was registered for bacterial bioaerosols, while there was a decrease in fungal concentration. There was a significant decrease during the winter at 19:00, compared to the previous interval for both types of bioaerosols, as the fungal bioaerosols reached a minimum daily value. For the same spring interval, bacterial bioaerosols have a daily maximum, while fungal bioaerosols reach minimum levels for the day.

During the summer season at the last sampling interval the increase in the levels of bacterial bioaerosols continues, while the fungal bioaerosols decrease after their maximum presence in the previous reporting interval. During the autumn season at 22:00, minimum daily values of concentration are reported for both types of bioaerosols. During the winter season, at 22:00, bacterial bioaerosols rise slightly, while fungal bioaerosols reach minimum daily values. During the spring season at 22:00 minimum daily values are reported for bacterial bioaerosols, whereas there was a slight increase in fungal concentration compared to the previous reporting interval.

Based on the results of the monitoring of the daily course in the levels of bioaerosol contamination detected in the air of the site, the following summary can be made:

→ The concentrations of the fungal snd bacterial bioaerosols in the outdoor air of the location change hourly, in direct connection with the abiotic parameters of the environment.

→ Changes in bioaerosol concentrations reflect the shift in anthropogenic activity at the location. Significantly high levels are found in the morning, followed by reduction and retention in the values at noon. In the afternoon there is another increase in bioaerosol concentrations.

→The daily variation is determined by the season, climatic factors, as well as anthropogenic activities during the course of the day.

1.5. Variations in the concentration of bacterial and fungal bioaerosols in outdoor air at the selected location during the weekdays

The complex nature of abiotic and biotic factors in the highly urbanized areas, in which the main sampling location is positioned, is directly related to the quantitative profile of airborne microbial contamination. This necessitated the monitoring of the weekly course in the variations of the bacterial and fungal bioaerosol concentrations due to the different intensity of urban life (road and pedestrian traffic, household, construction and repair activities) on different days of the week.

Four full weeks of the all annual seasons (spring, summer, autumn and winter) were selected in order to clarify the seasonal differences in the weekly course of

microbial contamination. All four weeks of monitoring followed the daily seasonal monitoring. The selected weeks for sampling are as follows: **1)** Winter season: 02.01 - 08.01.2021; **2)** Spring season: 03.04 - 09.04.2021; **3)** Summer season: 01.08 - 07.08.2020; **4)** Autumn season: 07.11 - 13.11.2020

a) Weekly monitoring of microbial bioaerosol concentration at the selected location in winter - 02.01 – 08.01.2021.

In January, a significant reduction in the concentration of both bacteria and fungi was observed at the sampling site. The weekly monitoring for winter season was carried out from 02.01.2021 to 08.01. 2021 at 14:00. The results are presented in fig. 11.

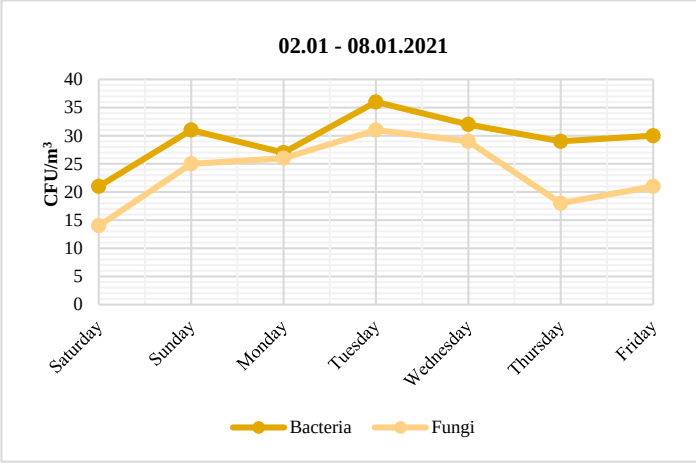


***Figure 11**. Weekly variations in the microbial concentration in winter*

During the first sampling day (02.01., Saturday) the registered levels of microbial contamination were relatively low, below the average monthly values of 57.0 CFU/m^3 for bacteria and 20.0 CFU/m^3 for fungi. The bacterial bioaerosol contamination on Saturday was 21.0 CFU/m^3, and the fungal was 14.0 CFU/m^3. During Sunday (03.01.) there is an increase in the concentrations of bacterial and fungal bioaerosols, which reach 31.0 CFU/m^3, and respectively. 25.0 CFU/m^3. The day coincides with the end

of a long series of weekends related to the New Year holidays and intensive vehicle traffic during the day.

In the first of the working days (Monday, 04.01) there is a decrease in the levels of microbial contamination, but the values remain higher than those established during the first of the weekend day - 27.0 CFU/m^3, respectively. 26.0 CFU/m^3. Microbial concentration weekly maximums were observed on Tuesday (05.01). The bacterial concentration was 36.0 CFU/m^3 and the fungal – 31.0 CFU/m^3. During the next days (Wednesday and Thursday) there was a decrease in the concentrations of the bioaerosols, which is more pronounced for fungal contamination. On Friday, 07.01. the levels of bacterial and fungal contamination are increasing, which could be related to the intensive outgoing traffic on the occasion of the upcoming weekend.

b) Weekly monitoring of microbial bioaerosol concentration at the selected location in spring - 03.04 – 09.04.2021

With the onset of the spring season, in April the levels of microbial bioaerosol contamination detected in the outdoor air at the location increased compared to the previous winter months - average monthly values for bacteria - 111.0 CFU/m^3, respectively for fungi 99.0 CFU/m^3. The daily monitoring carried out on 31.03.2021 shows maximum values of bioaerosol concentrations in the early afternoon. For this reason, the sampling time for weekly monitoring in spring was 14:00. The results are presented in fig. 12.

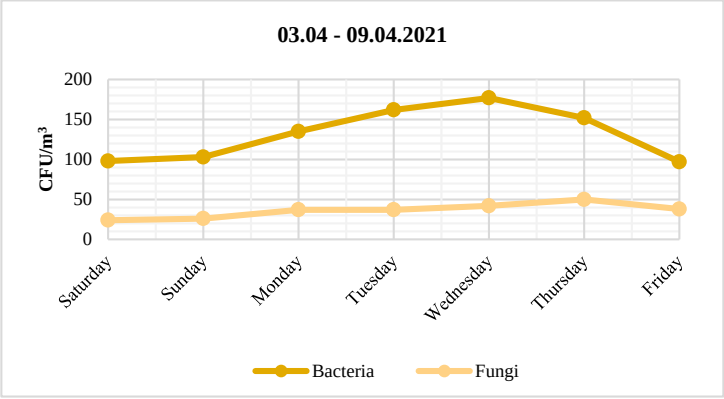


Figure 12. *Weekly variations in the microbial concentration in spring*

The quantitative profile of the bacterial and fungal bioaerosols has a relatively similar dynamic. The lower levels were observed on weekends and a gradual increase was registered during working days. This tendency is more pronounced for bacterial bioaerosols. On Saturday (April 3), there was a significant difference in bacterial and fungal concentration. The bacterial concentration was close to the monthly averaged values (98.0 CFU/m^3), whereas fungal concentration was lower – 24.0 CFU/m^3. A slight increase was registered during the second day off (04.04., Sunday) for both types of bioaerosols - 103.0 CFU/m^3 for bacteria, respectively 26.0 CFU/m^3 for fungi. At the beginning of the working week there was an increase in both bacterial and fungal contamination, and also in PM concentration. The increase continues, with bacterial contamination reaching a peak on Wednesday, April 7 (177.0 CFU/m^3). Maximum fungal bioaerosol contamination is reported on Thursday, (08.04) - 50.0 CFU/m^3. During the last working day (09.04, Friday) there is a slight decrease in the values, but they are higher compared to the levels of the weekends.

c) Weekly monitoring of microbial bioaerosol concentration at the selected location in summer - 01.08 – 07.08.2020

There was a decrease in the levels of both types of bioaerosols in August, compared to the previous month due largely to unfavorable abiotic parameters such as high daily temperatures, high UV index, low relative humidity. The daily monitoring showed an increase in the concentrations of the bioaerosols in the evening hours, so the selected time for weekly monitoring was 19:00. The results are presented in fig. 13.

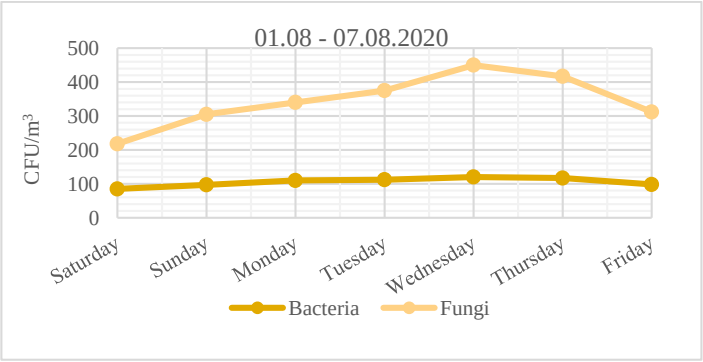


Figure 13. *Weekly variations in the microbial concentration in summer*

The conducted weekly monitoring demonstrates a different quantitative profile for the two types of bioaerosols with significantly more pronounced variations in fungal concentration than the bacterial.

During the first weekend day (Saturday, 01.08) the observed bacterial bioaerosol contamination was significantly lower than the average monthly: (50.0 CFU/m^3, with an average value for the month of 101.0 CFU/m^3). In the case of fungal bioaerosol contamination, the reported values are also lower than the monthly average (218.0 CFU/m^3, with a monthly average of 298.0 CFU/m^3). In the second of the weekends (August 2, Sunday) there was a slight increase in both types of bioaerosols - 77.0 CFU/m^3 for bacteria, respectively. 305.0 CFU/m^3 for fungi. During the rest of the week, the profile of bacterial and fungal contamination in the outdoor air at the location differs significantly. Bacterial bioaerosols reached a weekly maximum of 120.0 CFU/m^3 on Monday (03.08), while on other working days there was a retention of the level and a slight decrease on the last working day (Friday, 07.08), when the reported concentration was 98.0 CFU/m^3. In the case of fungal bioaerosol contamination, a more significant weekly dynamics is observed: an increase is registered during the first working days of the week, which reaches a maximum value on Wednesday (05.08) - 450.0 CFU/m^3. In the last two working days of the week, a reduction of 312.0 CFU/m^3 was observed again on Friday. The whole week of testing coincides with the beginning of the summer vacation period, during which the city's vehicle and pedestrian traffic is greatly reduced, at the expense of intensive repairs of the city's outdoor infrastructure.

d) Weekly monitoring of microbial bioaerosol concentration at the selected location in autumn - 07.11 – 13.11.2020.

In November there was a reduction in the reported levels of microbial bioaerosols. The decrease is less pronounced for bacterial bioaerosols, while the reduction in fungal bioaerosol concentration is significant. During the first week of the month selected for weekly monitoring, the registered levels of microbial contamination are above the average monthly values. The daily monitoring carried out on 06.11.2020 shows reaching maximum values of bioaerosol concentrations in the early afternoon.

For this reason, a sampling time in the weekly monitoring was accomplished at 14:00. Throughout the week, there was an increase in the concentration of PM in the air. The results are presented in fig. 14.

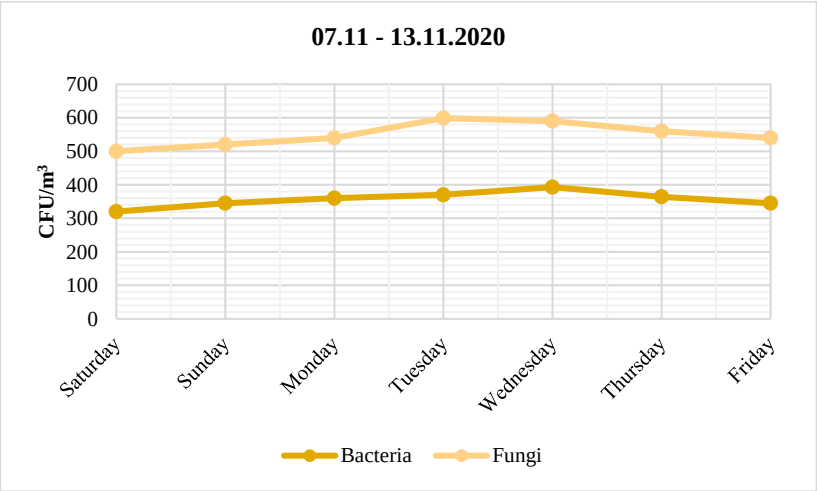


Figure 14. *Weekly variations in the microbial concentration in autumn*

On the first day off (07.11. Saturday) relatively high levels of microbial contamination in the air of the site are reported: 320.0 CFU/m^3 bacteria, respectively 500.0 CFU/m^3 fungi. A slight increase was noted on the second day off (08.11., Sunday) - 345.0 CFU/m^3, respectively. 520.0 CFU/m^3. During the working days of the week the dynamics in the course of the concentration is weakly expressed in both types of studied bioaerosols. A weekly maximum of 393.0 CFU/m^3 for bacterial bioaerosol contamination was reached on Tuesday, and on Wednesday the weekly maximum in the level of fungal bioaerosol contamination was registered - 599.0 CFU/m^3. At the end of the working week there was a decrease in both types of bioaerosols, but the levels remained higher than those reported on weekends. The week coincides with a period of relatively cold weather, due to which the incoming/outgoing traffic from the capital in connection with the weekends is relatively weak.

Based on the results of the monitoring of the weekly course in the levels of bioaerosol contamination detected in the outdoor air of the location, the following summary can be made:

→ The weekly variation in the concentrations of the microbial bioaerosols, regardless of the season, is largely determined by anthropogenic activities - increase in values at the beginning of working days, reaching maximum values in the middle of the week, followed by a reduction towards the end of the working week. On the last working day and the first of the weekends, a secondary peak in the daily concentration is observed.

→ Bacterial and fungal bioaerosols show a different pattern in terms of daily concentrations during the observed weeks. In summer and autumn, bacterial bioaerosols reach their maximum concentration on Tuesday, while the peak of the fungal presence in the air at the location is on Wednesday and Thursday.

→ Due to the unfavorable conditions for travelling during the winter season, the established weekly dynamics of both types of bioaerosols is different from that demonstrated during the other seasons.

1.6. Monitoring of bioaerosol concentration in the outdoor air at the location in selected days, with certain meteorological phenomena - mist, rain, snowfall and transboundary dust pollution.

The weather is the set of meteorological phenomena that occur in the atmosphere of a place and at a given time. Certain meteorological phenomena can have a significant impact on the quantitative share of the microbial air component and are directly related to its increase, or respectively reduction. For this reason, in the course of the annual monitoring study, days with a pronounced specific meteorological phenomena were selected as follows:

1.6.1. Mist (22.02.2021)

For February 22, 2021, on the territory of the city of Sofia an yellow code for dense fog was announced. Visibility in the area of the location is extremely limited - up to 50.0 m. During the day there is an increase in dust pollution, significantly exceeding the measured average monthly values for PM10 - 147.6 μg/m^3 (average

monthly value - 54.4 µg/m^3), respectively 63.7 µg/m^3 for PM2.5 (average monthly value - 22.6 µg/m^3). Regarding the detected microbial presence: an increased value of bacterial bioaerosol contamination (48.0 CFU/m^3) is reported, compared to the average monthly values from the monitoring study - 25.0 CFU/m^3. Fungal bioaerosol contamination is absent in the air at the site, which is observed throughout the last week of February. The data for the distribution of the bioaerosol particles by aerodynamic diameter for 22.02.2021 are presented in fig. 15.

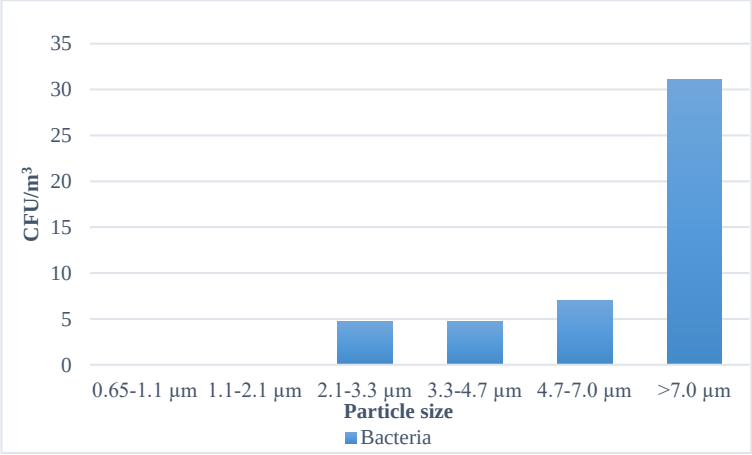


Figure 15. *Size distribution of bioaerosol particles by aerodynamic diameter during a mist*

On that day, the size distribution profile of the bacterial bioaerosol particles shows a predominant share of particles with sizes over 7.0 µm (40%), followed by particles with sizes over 4.7 - 7.0 µm. No bioaerosol particles in the range of 0.65 - 1.1 µm and 1.1 - 2.1 µm were captured. 15% of all captured bacterial bioaerosol particles were with aerodynamic diameter 2.1 - 3.3 µm.

1.6.2. Rainfall (21.01.2021)

Cloudy weather with significant rainfalls prevailed on the territory of the city of Sofia in the period 19.01 - 21.01.2021. The sampling was carried out on 21.01.2021 at 12:00, one hour after the rain had stopped.

Very low levels of PM pollution in the air of the location were reported during the sampling - PM10 concentration - 1.59 µg/m^3 (average monthly value - 10.3 µg/m^3), resp.

- PM2.5 concentrations - 0.57 µg/m^3 (monthly average value - 6.6 µg/m^3). At the same time, the wind speed was 7.2 m/s.

The reported levels of microbial bioaerosol contamination for the sampling after rainfall are as follows:

- Bacterial bioaerosol contamination - 38.0 CFU/m^3. This measured concentration is lower than the average concentration in the January samples (57.0 CFU/m^3). There is a significant reduction compared to the levels from the previous sampling week (before the onset of rainfall in the site area) (131.0 CFU/m^3).
- Fungal bioaerosol contamination - 14.0 CFU/m^3. There is an increase compared to the previous week of the monitoring study for the month (2.0 CFU/m^3), but at the same time the reported concentration is lower than the monthly average values (20.0 CFU/m^3).

The data for the size distribution of the bioaerosol particles by aerodynamic diameter for 21.01.2021 are presented in fig. 16.

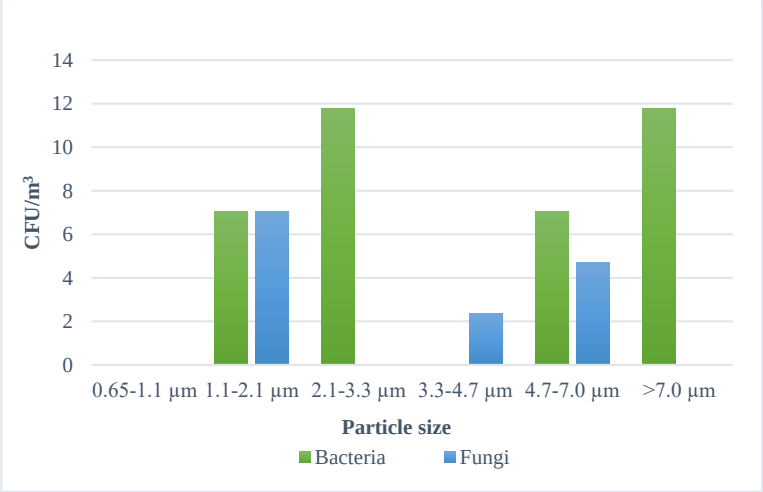


***Figure 16**. Size distribution of bioaerosol particles by aerodynamic diameter during after rainfall.*

Despite the significant amount of rain in the hours before sampling, the size distribution profile of the bacterial bioaerosol particles did not differ significantly from the distribution profile observed in January. Particles with sizes over 7.0 µm and

2.1 - 3.3 μm have a dominant share, followed by particles with sizes 4.7 - 7.0 μm. There were no bacterial bioaerosol particles with sizes in the range of 0.65 - 1.1 μm and 3.3 - 4.7 μm.

The particle size distribution profile of fungal bioaerosols also did not differ from the observed distribution profile in January. There are no particles with dimensions over 7.0 μm, as well as those with dimensions 2.1 - 3.3 μm and 0.65 - 1.1 μm. The share of particles with size 1.1 - 2.1 μm dominates, followed by particles with dimensions 4.7 - 7.0 μm.

1.6.3. Snowfall (13.01.2021)

The sampling was carried out on 13.01.2021 one hour after the snowfall stopped in the area of the location.

During the sampling the concentration of PM was relatively. The concentration of PM10 was 23.97 μg/m^3 (average monthly value - 10.3 μg/m^3) and PM2.5 - 9.04 μg/m^3 (average monthly value - 6.6 μg/m^3). The day is from the series of cold days at the beginning of the month, as the reported daily temperature value is 0 ° C. The wind in the area of the location is relatively weak - 5.7 m/s. Low, dense clouds were observed throughout the day.

The reported levels of microbial bioaerosol contamination in that sampling day are as follows:

- Bacterial bioaerosol contamination - 25.0 CFU/m^3. This concentration is lower than the average concentration of other samples from January (57.0 CFU/m^3). There is a significant reduction compared to the levels from the previous sampling week (before the onset of rainfall in the site area) (131.0 CFU/m^3).
- Fungal bioaerosol contamination - 34.0 CFU/m^3. There is a significant increase compared to the previous week of the monitoring study for the month (2.0 CFU/m^3), as the detected concentration is higher than the average monthly concentration (20.0 CFU/m^3).

The results from size distribution of the bioaerosol particles by aerodynamic diameter for 13.01.2021 are presented in fig. 17.

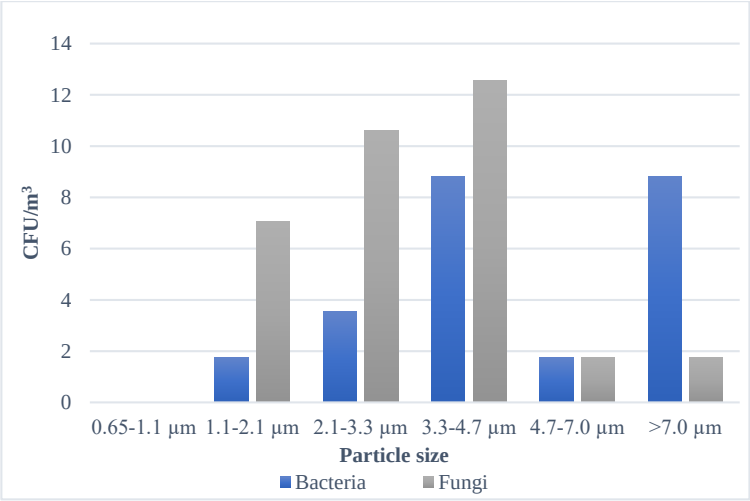


***Figure 17**. Size distribution of bioaerosol particles by aerodynamic diameter during after snowfall.*

The size distribution profile of bacterial bioaerosols demonstrates a similar pattern for the second week of January. Particles with sizes 3.3 - 4.7 µm and particles over 7.0 µm predominate. No particles with dimensions 0.65 - 1.1 µm were captured. The share of particles with sizes 1.1 - 2.1 µm and 4.7 - 7.0 µm is relatively low.

In the case of fungal bioaerosols, there is also no significant difference in the distribution profile of their particles by size - particles with sizes 3.3 - 4.7 µm dominate, followed by particles with sizes 2.1 - 3.3 µm. The proportion of particles with sizes 1.1 - 2.1 µm is also high. There are no fungal particles in the range of 0.65 - 1.1 µm.

1.6.4. Transboundary dust pollution, 13.05. – 27.05.2020

During the period 13 - 27 May 2020 on the territory of the Republic of Bulgaria there was an intensive transport of air masses moving in the direction of North Africa to Balkan Peninsula. The warm air flow is associated with the transport of aerosol particles and sand from the Sahara. The most pronounced effects of transboundary pollution in the country were reported on 14 and 15 May. Sampling was conducted in 5 days in the period 13.05 - 27.05. During the surveyed period there is a pronounced

dynamic in the abiotic parameters in the location. The increase in the concentration of PM10 reached its maximum (22.0 µg/m^3) on the third day - 14.05, while maximum values for the concentration of PM2.5 (10.77 µg/m^3) were registered later on 20.05. This PM concentration exceeded the registered average values for the month in the monitoring study - 22.0 µg/m^3 for PM10.0 and 6.0 µg/m^3 for PM2.5. The movement of dust pollution is accompanied by a strong southwest wind, associated with an increase in daily temperatures - with a maximum reached for the period of 27°C on 15.05. On this day, the lowest relative humidity in the location was reported - 26%.

Figure 18 represents the variations in the concentrations of the bacterial and fungal bioaerosols.

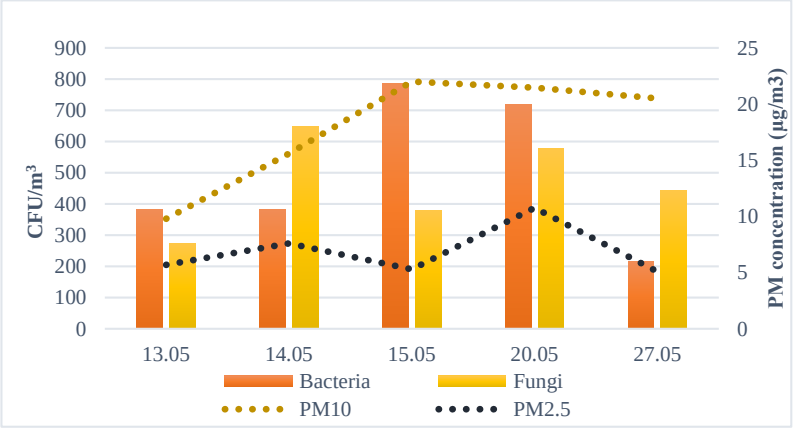


***Figure 18**. Variations in the bioaerosol concentration in outdoor air during transboundary dust pollution*

During the first two days of the sampling period, the concentration of bacterial bioaerosol contamination was close to the average for the month reported value - 383.0 CFU/m^3 (average monthly value of 372.0 CFU/m^3). An increase in the concentrations of particulate matter was registered, which is more pronounced for PM10: 10.0 µg/m^3 on 13.05 and 5.6 µg/m^3 on 14.05. During the testing on 15.05 the maximum in the concentration of the bacterial bioaerosol contamination was established - 785.0 CFU/m^3, which coincides with the reported maximum of the dust pollution (PM10.0) - 22.0 µg/m^3. At 20.5 there was a slight increase in bacterial

bioaerosol contamination - 785.0 CFU/m^3 (reported maximum of PM2.5 - 10.77 μg/m^3). At the last sampling for the specific period (27.05) the reported levels of bacterial contamination were below the average values for the month - 785.0 CFU/m^3. The values of PM10 in the air of the site remain relatively high - 21.0 μg/m^3, while the concentration of PM2.5 decreases below the average monthly values - 5.0 μg/m^3. The dynamics regarding the concentration of fungal bioaerosol contamination is similar: on the first day a value was reported - 274.0 CFU/m^3, significantly lower than the average monthly value (517.0 CFU/m^3). On 14.05 a maximum was reached for the period 649.0 CFU/m^3, followed by a decrease on the next day - 380.0 CFU/m^3. With the registration of the maximum for the period dust pollution from PM2.5, an increase in the concentration of fungal contamination is again observed - 577.0 CFU/m^3. At the last test on 27.05 the reported fungal contamination was lower than the average for the month.

Based on the results of the monitoring carried out on days with meteorological phenomena (fog, rain, snow, transboundary dust pollution) the following summary can be made:

→ The increased content of PM concentration during foggy days affects the levels of microbial and fungal presence in the air, which is expressed in an increase in the concentration of bacterial bioaerosols

→ Snowfall and rainfall are associated with reductions in reported levels of both types of bioaerosols, with values lower than the monthly average.

→ On days with transboundary movement of dust pollution, the amount of bacterial and fungal bioaerosols in the outdoor air in the sampling location increases.

2. Identification of dominant microorganisms in bioaerosol samples from the location, using classical taxonomic methods

An essential element of the research of the microbial diversity in the outdoor air at the location is the isolation of pure cultures from cultivated microorganisms (bacteria and fungi) and the determination of their taxonomic status. Samples collected by the six-stage cascade impactor throughout the monitoring period

(05.2020 - 04. 2021) were used to isolate pure cultures from the dominant airborne microorganisms (Figs. 19 and 20).

The subjects of the study were samples from a total of 54 weekly samples (6 Petri dishes with Nutrient agar and 6 Petri dishes with YGC agar for each sample). A total of 648 samples were processed for the entire time interval of the study.

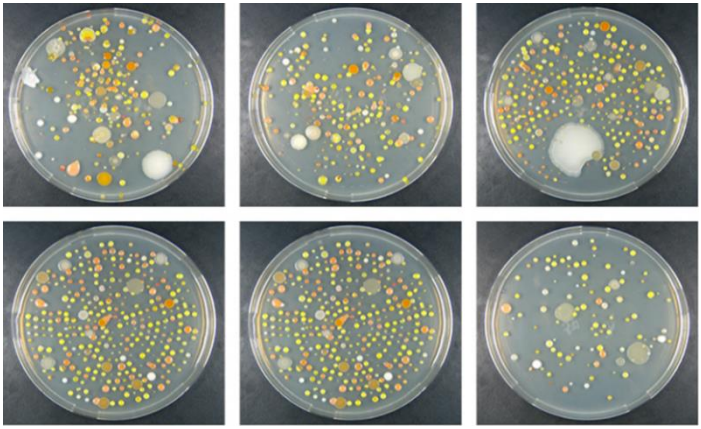


Figure 19. *View of Petri dishes with Nutrient agar (cascade impactor, levels 1-6, 07.2020) used to isolate pure bacterial cultures.*

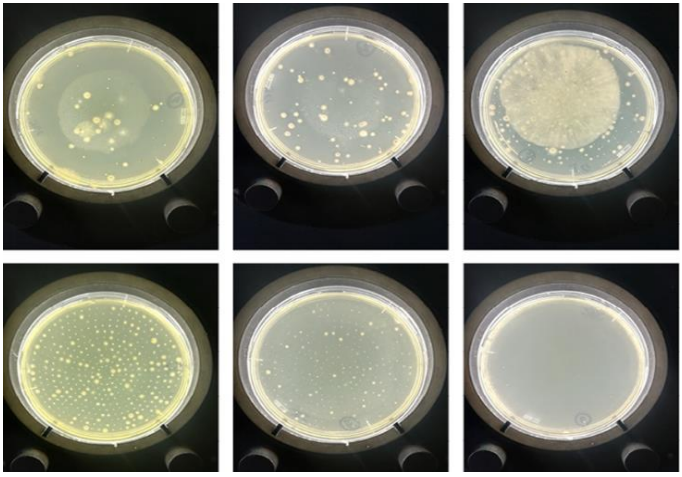


Figure 20. *View of Petri dishes with YGC agar (cascade impactor, levels 1-6, 07.2020) used to isolate pure bacterial cultures.*

A total of 283 pure microbial cultures (248 bacterial and 35 fungal) were isolated by applying the methods for isolating pure cultures from the selected single colonies and checking their purity.

2.1. Identification of bacterial isolates

The taxonomic status of the isolated pure cultures was carried out according to a scheme from the latest edition of *Bergey's Manual of Determinative Bacteriology* (2000). The current taxonomic status has been determined based on *Bergey's Manual of Systematics of Archaea and Bacteria* (2015).

a) ***Winter season, months: December 2020, January 2021, February 2021***

A total of 65 isolates are subject to identification: December (24 isolates marked D1-D24); January (20 isolates marked Z1-Z20); February (21 isolates marked F1 - F21). Regarding the Gram status, 44 of the isolated isolates were defined as Gram-positive (+) and 21 of the isolates were Gram-negative (-).

45 of the isolates were rod-shaped, 8 were staphylococci (D11, D12, D13, D14, D20, Z7, F14, F15). Isolates D4, D15, Z2, Z8, Z20, F7 were defined as diplococci (6 isolates in total). In three isolates, branching cell morphology was found (D5, D7 and F16). Based on the results of the taxonomic analysis, the selected bacterial isolates from the winter season can be systematically classified as follows - the results are presented in Tables 4 and 5:

Table 4. *Taxonomic status of Gram-negative bacteria, winter season.*

Gram negative bacteria		
Isolate	**Genus**	**Taxonomic status**
D1, D8, Z10, F8, F21	***Pantoea***	*Proteobacteria/ Gammaproteobacteria/ Enterobacteriales/ Enterobacteriaceae*
D2, Z9, F18	***Acinetobacter***	*Proteobacteria/ Gammaproteobacteria/ Pseudomonadales/ Enterobacteriaceae*
D6	***Ralstonia***	*Proteobacteria/ Betaproteobacteria/ Burkholderiales/ Burkholderiaceae*
D19, F6	***Sphingomonas***	*Proteobacteria/ Alphaproteobacteria/ Sphingomonadales/ Sphingomonadaceae*
D17, Z5, Z13, F10, F20, Z14	***Pseudomonas***	*Proteobacteria/ Gammaproteobacteria/ Pseudomonadales/Pseudomonadaceae*
F19	***Enterobacter***	*Proteobacteria/ Gammaproteobacteria/ Enterobacteriales/ Enterobacteriaceae*
D15, D4, F15	***Paracoccus***	*Proteobacteria/ Alphaproteobacteria/ Rhodobacterales/ Rhodobacteraceae*

Table 5. *Taxonomic status of Gram-positive bacteria, winter season.*

Gram-positive bacteria		
Isolate	**Genus**	**Taxonomic status**
D3, D16, D23, Z3, Z6, Z15, Z17, Z19, F2, F5, F9, F12	***Bacillus***	*Firmicutes/ Bacilli/ Bacillales/ Bacillaceae*
D10, D24, F3	***Microbacterium***	*Actinobacteria/ Actinobacteria/ Micrococcales/ Microbacteriaceae*
D18, D21, Z4, F1, F17	***Arthrobacter***	*Actinobacteria/ Actinobacteria/ Micrococcales/ Microbacteriaceae*
D22, Z11, Z18	***Cellulomonas***	*Actinobacteria/ Actinobacteria/ Micrococcales/ Cellulomonadaceae*
Z12, Z16,	***Gordonia***	*Actinobacteria/ Actinobacteria/ Corynebacteriales/ Nocardiaceae*
F11, F13	***Aeromicrobium***	*Actinobacteria/ Actinobacteria/ Propionibacteriales/Nocardioidaceae*
D9, D13, F14	***Staphylococcus***	*Firmicutes/ Bacilli/ Bacillales/ Staphylococcaceae*
D11, D12, D20, F4, F7	***Micrococcus***	*Actinobacteria/ Actinobacteria/ Micrococcales/ Micrococcaceae*
Z1, Z7, Z2, Z20	***Aerococcus***	*Firmicutes/ Bacilli/ Lactobacillales/ Aerococcaceae*
D14, Z8	***Enterococcus***	*Firmicutes/ Bacilli/ Lactobacillales/ Enterococcaceae*
D5	***Streptomyces***	*Actinobacteria/ Actinobacteria/ Streptomycetales/ Streptomycetaceae*
D7, F16	***Actinomyces***	*Actinobacteria/ Actinobacteria/ Actinomycetales/ Actinomycetaceae*

b) ***Spring season, Months: March 2021, April 2021, May 2020***

To identify the dominant bacterial contamination in the air at the site during the spring season of the monitoring study, a total of 58 isolates were selected as follows: March (24 isolates, designations MT1-MT24); April (14 isolates, designations A1 - A14); May (20 isolates, designations M1 -M20).

42 of the studied bacterial isolates are Gram-positive, and 16 of the isolates are Gram-negative. A rod-shaped form was found in 30 of the studied isolates. 16 of the isolates are cocci. Isolates MT1, MT23, M1, M6 and M15 have a filamentous cell morphology. Regarding microformations: 5 of the isolates (MT16, MT21, M2, M8, M20) are diplococci, 3 of the isolates (MT22, M3 and M16) are tetracocci, 3 of the isolates (A2, M17 and M19) are defined as staphylococci.

Based on the results of the taxonomic analysis, the selected bacterial isolates from the spring season can be systematically classified as follows - the results are presented in Tables 6 and 7.

Table 6. *Taxonomic status of Gram-negative bacteria, spring season.*

Gram negative bacteria		
Isolate	**Genus**	**Taxonomic status**
MT4, MT11, MT19, APR9, M14	***Pseudomonas***	*Proteobacteria/Gammaproteobacteria/ Pseudomonadales/Pseudomonadaceae*
MT16, MT21, APR7	***Paracoccus***	*Proteobacteria/Alphaproteobacteria/ Rhodobacterales/Rhodobacteraceae*
MT7, MT17	***Sphingomonas***	*Proteobacteria/Alphaproteobacteria/ Sphingomonadales/Sphingomonadaceae*
MT18, MT20	***Pantoea***	*Proteobacteria/Gammaproteobacteria/ Enterobacteriales/Enterobacteriaceae*
MT14, M18	***Ralstonia***	*Proteobacteria/Betaproteobacteria/ Burkholderiales/Burkholderiaceae*
MT24, APR6	***Enterobacter***	*Proteobacteria/Gammaproteobacteria/ Enterobacteriales/Enterobacteriaceae*

Table 7*. Taxonomic status of Gram-positive bacteria, spring season.*

Gram positive bacteria		
Isolate	**Genus**	**Taxonomic status**
MT3, MT5, MT8, MT9, MT15, APR4, A4, M11	***Bacillus***	*Firmicutes/ Bacilli/ Bacillales/ Bacillaceae*
MT6.APR2, APR3, A3, M4, M5, M8	***Kocuria***	*Actinobacteria/Actinobacteria/ Micrococcales/Micrococcaceae*
MT13, APR8, M2, M7, M17	***Micrococcus***	*Actinobacteria/Actinobacteria/ Micrococcales/Micrococcaceae*
A1, M2, M19, M20	***Staphylococcus***	*Firmicutes/Bacilli/ Bacillales/Staphylococcaceae*
M10, M9, M13	***Gordonia***	*Actinobacteria/Actinobacteria/ Corynebacteriales/Nocardiaceae/*
MT22, M3, M16	***Aerococcus***	*Firmicutes/Bacilli/ Lactobacillales/Aerococcaceae*
MT2, MT12, APR1	***Arthrobacter***	*Actinobacteria/Actinobacteria/ Micrococcales/ Microbacteriaceae*
MT23, M1	***Streptococcus***	*Firmicutes/Bacilli/ Lactobacillales/Streptococcaceae*
MT1, M6	***Rhodococcus***	*Actinobacteria/Actinobacteria/ Corynebacteriales/Nocardiaceae*
A4	***Rathayibacter***	*Actinobacteria/Actinobacteria/ Micrococcales/ Microbacteriaceae*
APR5	***Exgiobacterium***	*Firmicutes/Bacilli/Bacillales/ incertae Sedis - Family III*
M12	***Cellulomonas***	*Actinobacteria/Actinobacteria/ Micrococcales/Cellulomonadaceae*
M10	***Enterococcus***	*Firmicutes/Bacilli/ Lactobacillales/Enterococcaceae*
M15	***Nocardia***	*Actinobacteria/Actinobacteria/ Corynebacteriales/Nocardiaceae*

c) Summer season, Months: June 2020, July 2020, August 2020

For the summer season a total of 54 bacterial cultures were isolated predominantly detected during the air monitoring in the location. For the month of June, 24 isolates were selected, marked J1-J24. In July, 24 isolates were selected with designations JL1 to JL 24, respectively for August 6 isolates (AT1 -AT6).

37 of the isolates were Gram-positive. Gram-negative group consists of 17 isolates. 39 of the isolates were rod-shaped, while 13 of the isolates were cocci. Filamentous cell morphology was found in isolates JL13 and JL24. Regarding the observed microformations were tetrads (JL13 and JL23), staphylococci (J3, J19, J22, JL4, JL18, JL20) and streptobacilli (J6).

44% of all isolates were Gram (+) rod-shaped, 28% were Gram (-) rod-shaped. In third place were Gram-positive isolates with a coccoid shape. Gram-negative coccoid isolates as well as filamentous cell-shaped isolates are poorly represented.

Based on the results of the taxonomic analysis, the selected bacterial isolates from the spring season can be systematically classified as follows - the results are presented in Tables 8 and 9.

Table 8. *Taxonomic status of Gram-positive bacteria, summer season.*

Gram positive bacteria		
Isolate	**Genus**	**Taxonomic status**
J1, J2, J5, J6, J9, J14, JL1, JL7, JL16, JL17	***Bacillus***	*Firmicutes/ Bacilli/ Bacillales/ Bacillaceae*
J4, J10, JL2, JL5, JL22	***Gordonia***	*Actinobacteria/Actinobacteria/ Corynebacteriales/Nocardiaceae/*
J7, J16, J22, J23	***Micrococcus***	*Actinobacteria/Actinobacteria/ Micrococcales/Micrococcaceae*
J8, J11, JL8, AT1	***Arthrobacter***	*Actinobacteria/Actinobacteria/ Micrococcales/ Microbacteriaceae*
J17, JL4, JL11	***Kocuria***	*Actinobacteria/Actinobacteria/ Micrococcales/Micrococcaceae*
JL9, JL18, JL20	***Staphylococcus***	*Firmicutes/Bacilli/ Bacillales/Staphylococcaceae*
J18, JL10	***Rathayibacter***	*Actinobacteria/Actinobacteria/ Micrococcales/ Microbacteriaceae*
J13, J24	***Streptomyces***	*Actinobacteria/Actinobacteria/ Streptomycetales/Streptomycetaceae*
JL12	***Exgiobacterium***	*Firmicutes/Bacilli/Bacillales/ incertae Sedis - Family III*
J19	***Aerococcus***	*Firmicutes/Bacilli/ Lactobacillales/Aerococcaceae*
JL3	***Microbacterium***	*Actinobacteria/Actinobacteria/ Micrococcales/Microbacteriaceae*
JL21	***Aeromicrobium***	*Actinobacteria/Actinobacteria/ Propionibacteriales/Nocardioidaceae*

Table 9. *Taxonomic status of Gram-negative bacteria, summer season*

Isolate	Genus	Taxonomic status
JL6, JL14, AT2, AT3, AT5	***Enterobacter***	*Proteobacteria/Gammaproteobacteria/ Enterobacteriales/Enterobacteriaceae*
J15, JL13, JL19	***Acinetobacter***	*Proteobacteria/Gammaproteobacteria/ Pseudomonadales/Moraxellaceae*
J20, J23, AT4	***Pantoea***	*Proteobacteria/Gammaproteobacteria/ Enterobacteriales/Enterobacteriaceae*
JL21, JL24	***Pseudomonas***	*Proteobacteria/Gammaproteobacteria/ Pseudomonadales/Pseudomonadaceae*
JL15, AT6	***Sphingomonas***	*Proteobacteria/Alphaproteobacteria/ Sphingomonadales/Sphingomonadaceae*
J3, J12	***Paracoccus***	*Proteobacteria/Alphaproteobacteria/ Rhodobacterales/Rhodobacteraceae*

d) Autumn season, Months: September 2020, October 2020, November 2020

During the autumn season, 71 bacterial isolates, predominantly detected in the weekly samples, were selected for determination of taxonomic status. The isolates are as follows: September (24 isolates, S1 - S24); October (23 isolates, O1 - O23); November (24 isolates, N1 - N24).

51 of the studied isolates are Gram-positive, whereas Gram-negative bacteria were 20. Rod shape was found in 42 of the isolates, coccoid in 20 and filamentous cell morphology in 9 of the isolates. Regarding microformations - 7 isolates (S21, O8, O16, O20, O22, N8, N14) are staphylococci, 3 isolates (O2, O6 and N3) are diplococci, 2 isolates (N12 and N21) are tetracocci, 2 isolates (O21 and O23) - streptobacilli.

Gram-positive rod-shaped isolates have a predominant share - 39%, followed by Gram-positive cocci - 20% and Gram-negative rods - 20%. Isolates with filamentous morphology are 13%. The share of Gram-negative cocci was the smallest - 8%. Based on the results of the taxonomic analysis, the selected bacterial isolates from the spring season can be systematically classified as follows - the results are presented in Tables 10 and 11.

Table 10*. Taxonomic status of Gram-negative bacteria, autumn season.*

Isolate	Genus	Taxonomic status
S2, S15, S19, S20, O3, O17	***Enterobacter***	*Proteobacteria/Gammaproteobacteria/ Enterobacteriales/Enterobacteriaceae*
S1, O20, N3, N8, N12, N14	***Paracoccus***	*Proteobacteria/Alphaproteobacteria/ Rhodobacterales/Rhodobacteraceae*
S8, O13, N6	***Pseudomonas***	*Proteobacteria/Gammaproteobacteria/ Pseudomonadales/Pseudomonadaceae*
S17, N7, N9	***Sphingomonas***	*Proteobacteria/Alphaproteobacteria/ Sphingomonadales/Sphingomonadaceae*
S14, O4	***Pantoea***	*Proteobacteria/Gammaproteobacteria/ Enterobacteriales/Enterobacteriaceae*

Table 11. *Taxonomic status of Gram-positive bacteria, autumn season.*

Gram positive bacteria		
Isolate	**Genus**	**Taxonomic status**
S3, S5, S11, S13, S22, S24, O10, O11, O14, O15, O21, O23, N1, N16, N23	***Bacillus***	*Firmicutes/ Bacilli/ Bacillales/ Bacillaceae*
S9, S19, O19, N10, N19	***Arthrobacter***	*Actinobacteria/Actinobacteria/ Micrococcales/ Microbacteriaceae*
S6, S7, O2, O22, N2	***Micrococcus***	*Actinobacteria/Actinobacteria/ Micrococcales/Micrococcaceae*
S21, O16, N11, N21	***Staphylococcus***	*Firmicutes/Bacilli/ Bacillales/Staphylococcaceae*
S10, S12, N17, N20	***Streptomyces***	*Actinobacteria/Actinobacteria/ Streptomycetales/Streptomycetaceae*
O5, O12, N4	***Exgiobacterium***	*Firmicutes/Bacilli/Bacillales/ incertae Sedis - Family III*
S4, N15, N22	***Rhodococcus***	*Actinobacteria/Actinobacteria/ Corynebacteriales/Nocardiaceae*
O6, O8	***Streptococcus***	*Firmicutes/Bacilli/ Lactobacillales/Streptococcaceae*
S16, O1	***Gordonia***	*Actinobacteria/Actinobacteria/ Corynebacteriales/Nocardiaceae/*
N5, N18	***Nocardia***	*Actinobacteria/Actinobacteria/ Corynebacteriales/Nocardiaceae*
O18, N13	***Rathayibacter***	*Actinobacteria/Actinobacteria/ Micrococcales/ Microbacteriaceae*
O7	***Enterococcus***	*Firmicutes/Bacilli/ Lactobacillales/Enterococcaceae*
S23	***Microbacterium***	*Actinobacteria/Actinobacteria/ Micrococcales/Microbacteriaceae*
N24	***Aeromicrobium***	*Actinobacteria/Actinobacteria/ Propionibacteriales/Nocardioidaceae*
O9	***Kocuria***	*Actinobacteria/Actinobacteria/ Micrococcales/Micrococcaceae*

As a result of taxonomic studies, all 248 bacterial isolates were classified into 25 genera:

A) Gram-positive isolates are 174 (70.2%) belonging to 18 genera.

B) Gram-negative isolates are 74 (29.8%) belonging to 7 genera.

2.2. Identification of fungi

The objects of taxonomic determination are a total of 35 isolates, divided into four groups by seasons. After a series of procedures to isolate pure cultures, single spore colonies were obtained from all 35 isolates (Fig. 21). Macroscopic and microscopic methods were used to characterize them (Fig. 22).

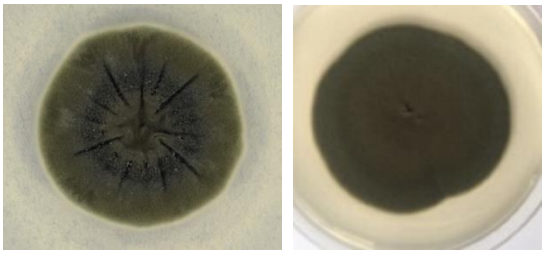


Figure 21. *Macroscopic view of the fungal colonies*

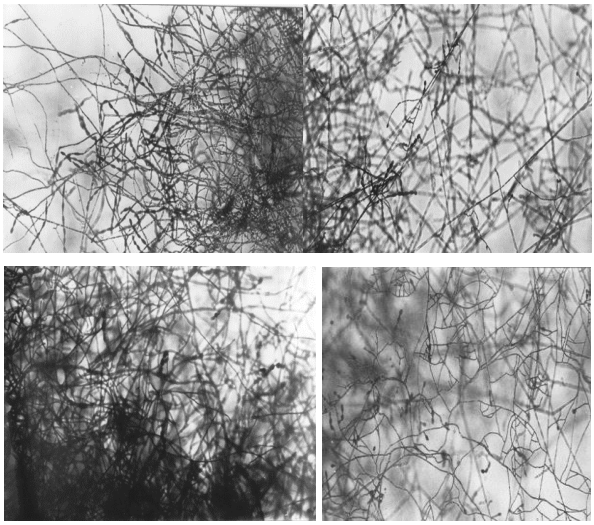


Figure 22. *Microscopic view of the fungal colonies*

Based on the conducted macroscopic and microscopic examinations, the selected dominant fungal isolates can be taxonomicly assigned to 4 genera (Table 12).

The four genera belong to *Ascomycota*. These are mostly soil and airborne fungi. The representatives of the genus *Penicillium* have the highest share in the group of isolated fungi- 12 isolates (34.2%), followed by the isolates belonging to the genus *Aspergillus* - 10 isolates (28.5%). The genus *Alternaria* is represented by 8 isolates (22.8%). The smallest number of isolates belongs to the genus *Cladosporium* - 5 isolates (14.2%).

Table 12. *Taxonomic status of isolated fungi*

Fungi		
Isolate	**Genus**	**Taxonomic status**
ZF1, ZF2, ZF4, P7, E8 (5 isolates)	***Cladosporium***	*Fungi/Ascomycota/ Dothideomycetes/ Capnodiales/Cladosporiaceae*
P3, P1, SF1, SF4, E1, E2, E5, E7 (8 isolates)	***Alternaria***	*Fungi/Ascomycota/ Dothideomycetes/ Pleosporales/Pleosporaceae*
ZF5, ZF6, P2, P4, P5, P8, SF2, SF5, SF7, E3, E4, E9 (12 isolates)	***Penicillium***	*Fungi/Ascomycota/Eurotiomycetes/ Eurotiales/Aspergillaceae*
ZF3, ZF7, P6, SF3, SF6, SF8, SF9, SF10, E6, E10 (10 isolates)	***Aspergillus***	*Fungi/Ascomycota/Eurotiomycetes/ Eurotiales/Aspergillaceae*

From all 5 isolates belonging to the genus *Cladosporium* (5 isolates in total), 3 of them were collected during the winter season, respectively 1 isolate (March, Spring) and 1 isolate (November, Autumn). During the summer season, the dominant presence of fungi of the genus *Cladosporium* in the air of the site is not established. This strongly confirms the psychrophilic potential of this taxon, whose representatives can tolerate extremely low temperatures.

Of the isolates belonging to the genus *Alternaria* (10 isolates in total), half were collected during the autumn season, with no isolated representatives of the genus during the winter sampling. Isolates of the genera *Penicillium* (12 isolates) and *Alternaria* (10 isolates) were obtained during the all seasons, with the largest number of isolates of the genus *Penicillium* collected during the spring season, respectively the largest number of isolates of the genus *Aspergillus* were obtained during the summer season.

3. Metagenomic analysis of microbial bioaerosol presence in the air at the selected location

To evaluate the diversity of the culturable and nonculturable microbiota in the outdoor air at the location in the present dissertation, two samples were collected, during the warm, and respectively the cold period of the surveyed year, namely: Sample 1 (May, 2020) and Sample 2 (January, 2021). (The insignificant fungal presence in the air at the site in January is the reason for the insufficient amount of collected total DNA, which did not allow the study of fungal diversity in Sample 2).

3.1. Results from the Next-generation of sequencing

Amplicons were sequenced on Illumina paired-end platform to generate 250 bp paired-end raw reads (Raw PE), and then merged and pretreated to obtain Clean Tags. The chimeric sequences in Clean Tags were detected and removed to obtain the Effective Tags which can be used for subsequent analysis. The summarizations obtained in each step of data processing are shown in table 13:

Table 13. *summarizations of the sequencing of V3-V4 region of 16S rRNA and ITS2 region.*

Sample name	Raw PE	Raw Tags	Clean Tags	Effective Tags	Base (nt)
Sample 1 (May) V3-V4 region	130,004	117,914	116,763	87,652	36,131,624
Sample 2 (January) V3-V4 region	104,356	100,202	98,567	94,838	40,375,719
Sample 1 (May) ITS2 region	101,180	98,942	98,753	70,634	21,318,585
	Average Length (nt)	**Q20**	**Q30**	**G/c (%)**	**Effective (%)**
Sample 1 (May) V3-V4 region	412	98.60	95.26	55.07	67.42
Sample 2 (January) V3-V4 region	426	98.28	94.24	52.19	90.88
Sample 1 (May) ITS2 region	302	98.81	96.58	51.01	69.81

3.2. Operational taxonomic units (OTUs) analysis and taxonomic annotation

In the process of constructing OTUs, basic information of different samples was collected, such as Effective Tags data, low-frequency Tags data and Tags annotation data. The summarization is showed as followed in figure 23 and 24:

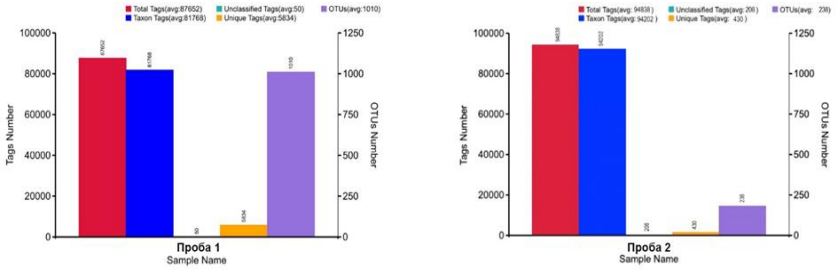


Figure 23. *Summarization of the tags and bacterial OTUs number in Sample 1 and Sample 2.*

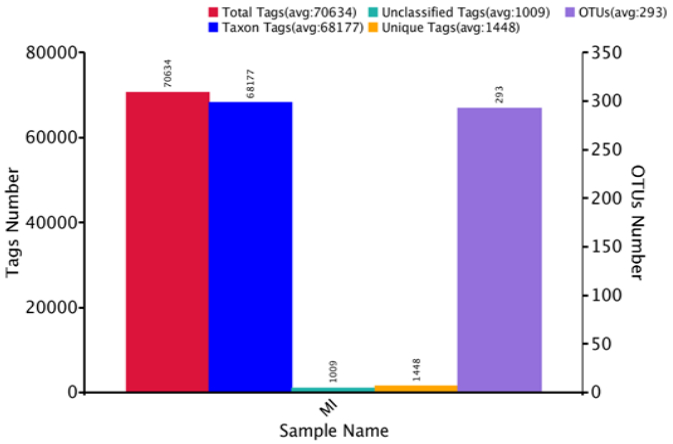


***Figure 24**. Summarization of the tags and fungal OTUs number in Sample.*

For the spring season the indicators are: Effective labels (Total tags) - 87652; Annotated labels (Taxon tags) - 81768; Unclassified tags - 50 and Unique tags - 5834. They are combined into **1010** OTUs. For the winter season the results are: 94838 Effective labels; 94202 Annotated labels; 206 Unclassified labels and 430 Unique labels. They are united in **238** OTUs.

For the fungi in Sample 1, the values after the analysis of the ITS2 region are: Effective labels (Total tags) - 70634; Annotated labels (Taxon tags) - 68177; Unclassified tags - 1009 and Unique tags - 1448. They are clustered in **293** OTUs.

3.3. Relative abundance

The relative abundance of taxa in the phylum and their proportions in different classification levels of each sample are illustrated below:

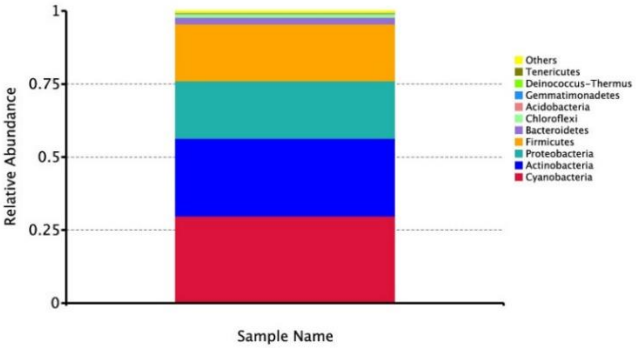


Figure 25. *Bacterial taxa relative abundance in Sample 1.*

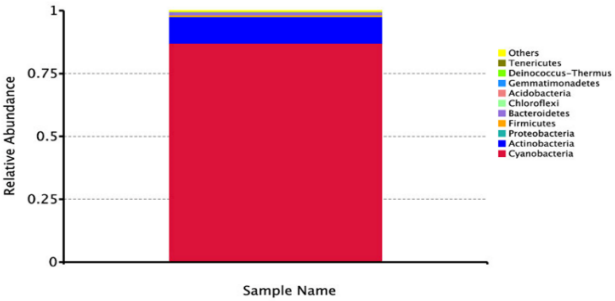


Figure 26. *Bacterial taxa relative abundance in Sample 2.*

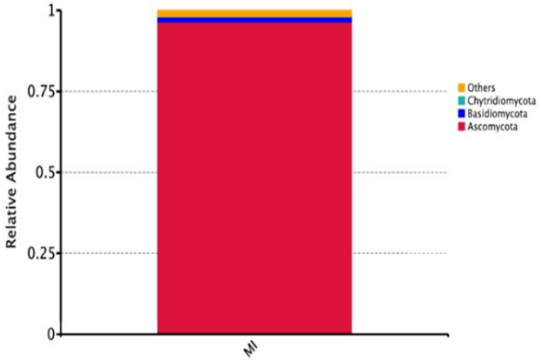


Figure 27. *Fungal taxa relative abundance in Sample 1*

An interactive tool - Krona, was used to visualize and study the result of the analysis of the taxonomic annotation, which is a useful tool for visualization of the composition of metagenomes in a web browser.

a) Bacterial and archeal diversity in Sample 1 (May)

The information from the metagenomic sequencing in Sample 1 shows a dominant presence of domain *Bacteria* (99.93%), and a low share of *Archaea* (0.07%) (Fig. 28).

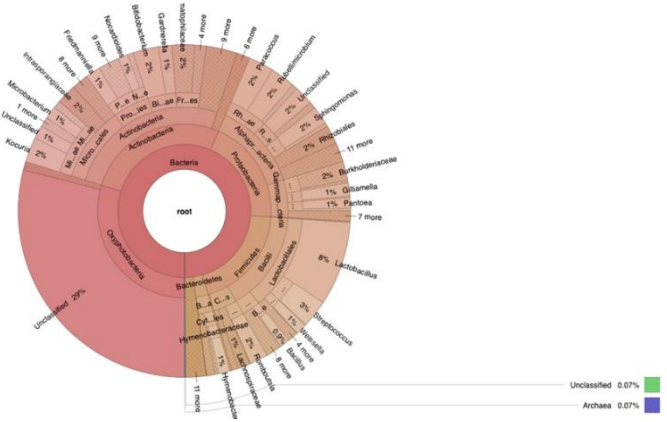


Figure 28. *Taxonomic distribution of airborne bacteria and archaea in Sample 1 (May)*

б) Bacterial diversity in Sample 2 (January)

Only representatives of the domain *Bacteria* are found in Sample 2, in contrast to Sample 1 (Fig. 29).

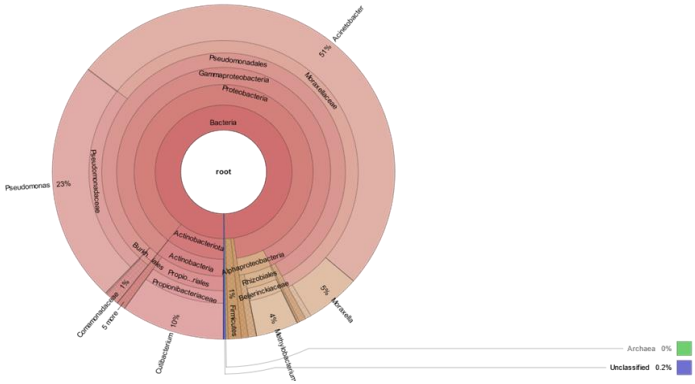


Figure 29*. Taxonomic distribution of airborne bacteria in Sample 2 (January)*

Comparative analysis of the bacterial diversity present in Sample 1 and Sample 2, the ten most prevalent bacterial genera in the outdoor air at the location can be identified in both samples (Table 14).

Table 14. *Relative abundance of the main bacterial taxa in Sample 1 and Sample 2.*

Sample 1 (May) Genus	**Abundance (%)**	**Sample 2 (January) Genus**	**Abundance (%)**
Lactobacillus	8	*Acinetobacter*	51
Streptococcus	3	*Pseudomonas*	23
Kocuria	2	*Cutibacterium*	10
Bifidobacterium	2	*Moraxella*	5
Paracoccus	2	*Methylobacterium-Methylorubrum*	4
Rubellimicrobium	2	*Comamonas*	0.8
Sphingomonas	2	*Corynebacterium*	0.6
Romboutsia	2	*Staphylococcus*	0.6
Friedmanniella	1	*Georgenia*	0.5
Pantoae	1	*Sphingomonas*	0.4

в) Fungal diversity in Sample 1 (May)

Metagenomic analysis of the ITS2 region showed very high abundance of *Ascomycota* phylum (98% of all sequences) (Fig. 30).

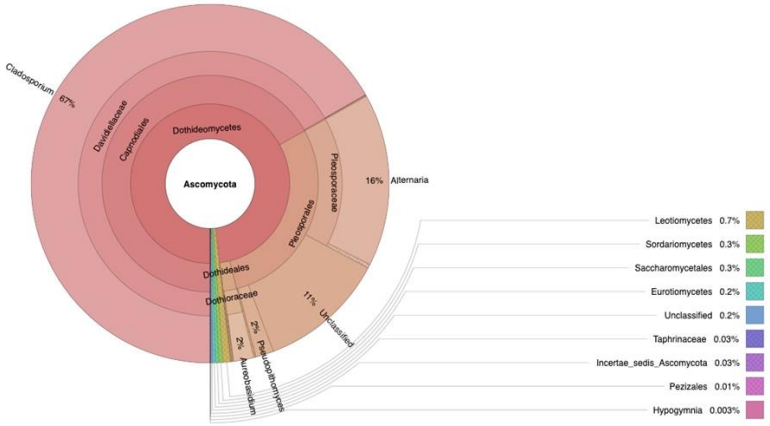


Figure 30. *Taxonomic distribution of airborne fungi in Sample 1 (May)*

The most common fungal genus in Sample 1 was *Cladosporium*, which accounts for 64% of all fungal sequences in the sample. The genus *Alternaria* includes 15% of all sequences, 2% *Pseudopithomyces*, 2% *Aureobasidium*, and 11% unclassified. At the species level the following fungi were identified: *Aureobasidium pullulans*,

Periconia byssoides, Phoma macrostoma, Sawadaea bicornis, Rutstroemia firma, Candida humilis, Saccharomyces cerevisiae, Fusarium solani and Khuskia oryzae.

4. Characterization of the abiotic component of air pollution in the area of the selected location

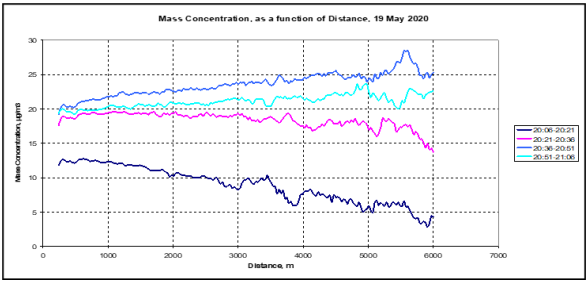
4.1. Lidar investigation of aerosol pollution in air at the location

Adequate and accurate quantitative detection is crucial for the characterization of PM pollution. To date, various approaches and methods for determining the concentration of PM in real time have been developed and exist. One of the most promising methods is lidar mapping. A key point in the experimental scheme is the comparison of the detection potential of the **a)** lidar method; **b)** mobile sensor for PM10/2.5 (type Nova PM sensor SDS011 High precision laser PM10/2.5); **c)** portable aspiration device Higitest; **d)** official station of the EEA in Lozenets district (near the location site – Faculty of Biology).

In the sampling location, comparative study on PM pollution was conducted twice in May (19.05.2020) and in October (07.10.2020). The selected periods correspond to relatively low levels of dust pollution during the warm part of the year and, respectively, the reported increase in dust during the winter.

- ***Sampling 1 (May, 2020)***

Figure 31 illustrates the Lidar measurement data for May, which reflect the spring-summer period.



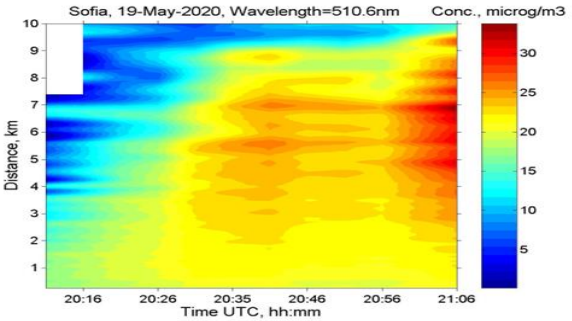


Figure 31. *Lidar measurements data for 19.05.2020 and 3D lidar mapping in the range of 5.5 km.*

- ***Sampling 2, October 2020***

Figure 32 illustrates the Lidar measurement results for October, which reflect the autumn-winter period.

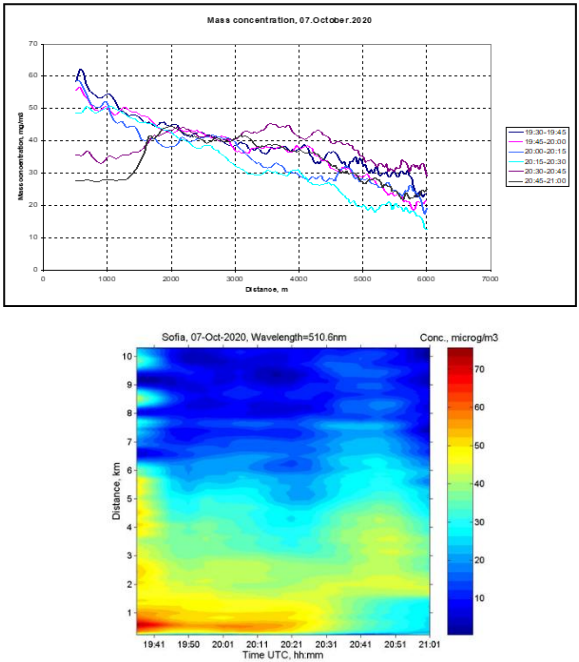


Figure 32. *Lidar measurements data for 07.10.2020 and 3D lidar mapping in the range of 5.5 km.*

Based on the obtained results, the following conclusion can be made: Express detection and localization, at any time of the day, of dense aerosol pollution, is achievable only with the help of lidars, which are systems for remote and contactless active optical drilling of the gas and aerosol phase in the atmosphere, on a large spatial scale (tens and hundreds of kilometers) and with high spatial (meters and decimeters) and temporal (milliseconds) resolution. The existing system in Sofia for the control of PM pollution with the help of official stations of the EEA is accurate, but insufficient in terms of PM2.5. The system is not able to determine the geographical coordinates of the pollution accurately enough.

4.2. Physico-chemical characterization of PM pollution

PM pollution of the air is a prerequisite for the association of a microbial component to it. This requires a detailed analysis of the dust particles detected in the air in terms of: size, elemental and phase composition, structure and morphology of the PM particles, comparative study of the surface/volume of the dust particles.

4.2.1. Structure and morphology of particulate matter

A sample with a fraction of PM2.5 aspirated, through a mobile aspirator type Higitest, collected in the area of BF, Sofia University in October, 2020 was subjected to analysis.

The images obtained with a scanning electron microscope are shown in fig. 33. It can be seen very well that the PM particles vary greatly in size from nanometers to several tenths of a micrometer. Most PMs are actually aggregates of very small particles.

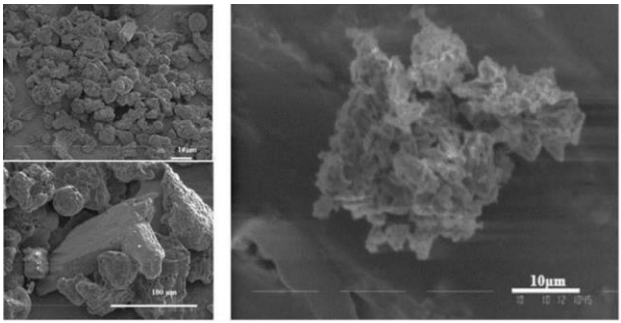


Figure 33*. SEM photographs of particulate matter*

4.2.2. Elemental and phase composition of particulate matter

The X-ray diffraction pattern of the material (Fig. 34) shows the presence of low-intensity and wide X-ray diffraction peaks located on the X-ray amorphous halo. The main crystalline phases registered in the X-ray diffraction pattern are: silicate, aluminosilicate, carbonate and sulfate phases. Characteristic lines of quartz SiO_2 (78-1252), anorthite (73-0264), Mn silicate (12-0215), calcite $CaCO_3$ (03-0596) and K-Mg silicate hydroxide (26-1322) were identified in the analysis. Additionally, smaller amounts of ankerite (79-1348), Fe-Ti sulfate (28-0500) and biotite (42-1414) were found.

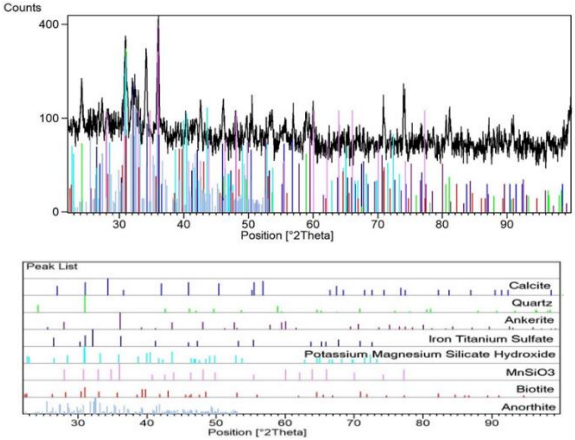


***Figure 34**. X-ray diffraction images of PM (a) analysis of the registered phases (b) with a legend of the identified lines according to JCPDS standards.*

Analysis of the spectra showed the presence of inorganic carbonates (714, 876, 1425 cm^{-1}) and surface carbonate groups (714, 876, 1038, 1441, 1545, 1640, 1660, 1719 cm^{-1}). The presence of inorganic phosphate/phosphate residues can be judged from the bands at 529, 914, 1038, 1080 cm^{-1}, as the range of about 1000 cm^{-1} is typical for the vibrations of the PO_4 group. The sulfur-containing components in the test sample were registered on the characteristic bands of sulphates (621, 1080 cm^{-1}) and organic compounds containing thiol group S-H (664, 697, 1267, 2521 cm^{-1}). Despite the low intensity of the band at 2521 cm^{-1}, it is considered indicative (Fig. 35).

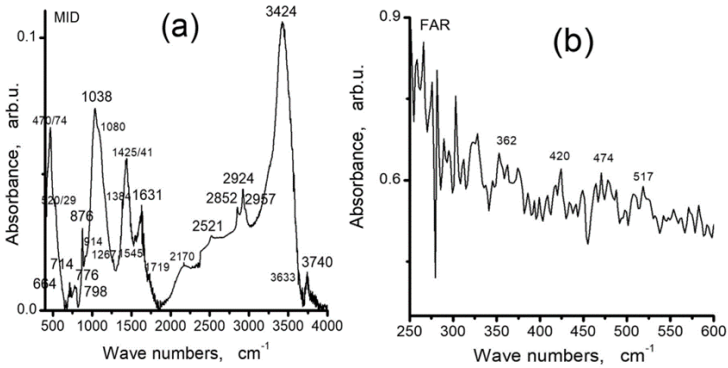


Figure 35. *Infrared spectra of PM particles in the middle (a) and in the far infrared regions*

The analysis shows that the surface of the test sample of particulate matter consists mainly of silicate, aluminosilicate compounds, as well as organic and inorganic carbon phases.

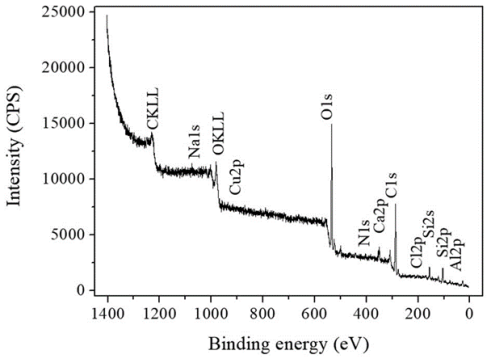


Figure 36*. X-ray photoelectron spectrum of a sample of particulate matter.*

Due to the relatively large amounts of iron determined from the data of the elemental analysis of the sample (Fig. 36), 57Fe Mossbauer spectroscopy was applied for more in-depth study and characterization of PM.

The Mossbauer spectra of the sample (Fig. 37) show the phase composition and dispersion of the available iron-containing phases. Four sextet (Sx) and two doublet components (Db) were used for mathematical processing of the spectrum. The first component Sx1 has ultrafine parameters characteristic of the hematite phase - α-Fe_2O_3.

The calculated Heff value is lower than that of well-crystallized hematite (52 T), indicating that the particle size is about 20 nm. Sextets Sx2 and Sx3 are characteristic of Fe_3O_4 and correspond to iron ions in tetrahedral (sublattice A) and octahedral (sublattice B) configurations characteristic of the spinel phase.

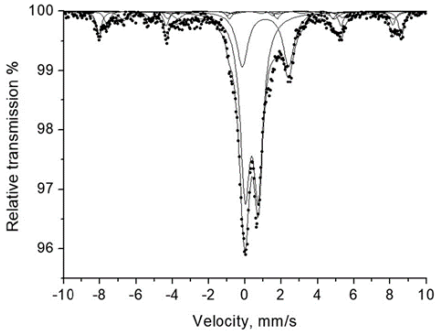


Figure 37. *Mössbauer spectrum of a sample PM.*

TEM images of the sample from the location collected in autumn-winter 2020 were also registered (Figure 38). The obtained images show that using high magnifications it was found that most of the observed particles are aggregates composed of smaller and nanosized crystallites.

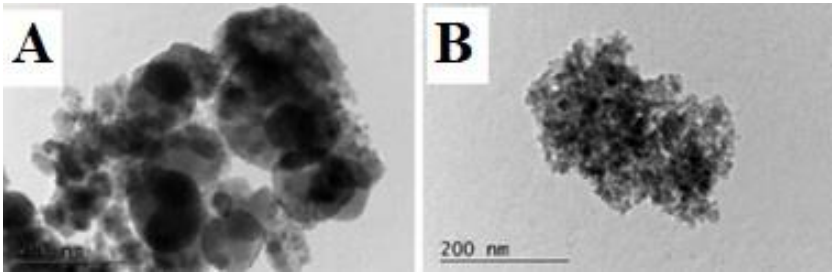


Figure 38. *TEM photographs from samples of PM10 (A) and PM2.5 (B)*

Physicochemical analysis show that PM2.5/10 are not stable, do not have a constant composition and size, and their chemical nature can be modified by various physicochemical processes.

CONCLUSIONS

1. There is a clear monthly dynamics in the levels of microbial contamination in the air of the central part of Sofia, which is complex due to the climatic, geographical and anthropogenic characteristics of the settlement.
2. The spring season is characterized by an increase in the levels of microbial air contamination, with particularly strong variations in the concentration of fungal bioaerosols.
3. In the first half of the summer season there is a significant increase in the concentration of microbial bioaerosol contamination, while in the second half of the summer season there is a decrease in the levels of bacterial and fungal bioaerosols.
4. At the beginning of the autumn season of the year there is an increase in microbial contamination, followed by a significant reduction in the last autumn month.
5. During the winter season, microbial bioaerosol contamination in the outdoor air of the central part of the city is greatly reduced.
6. A different annual course in the variations in the concentration of bacteria and fungi in outdoor air is registered - maximum fungal presence is reported in June, July and October, while bacterial contamination reaches maximum values in May, July and September. Minimum values for both types of bioaerosols are registered in the period December-February.
7. The daily variations in the concentrations of the microbial bioaerosols in the air is different for all of the seasons and follows the changes in anthropogenic activity. Significantly high levels were found in the morning, followed by a reduction in values at noon and a secondary increase in bioaerosol concentrations in the afternoon.
8. The weekly variations in the concentrations of the bioaerosols, regardless of the season, is largely determined by the anthropogenic activities - increase in the values at the beginning of the working days, reaching maximum values in the middle of the week, and reduction towards the end of the working week.

9. The increased PM10/2.5 concentration in the outdoor air is associated with an increase in microbial presence, while precipitation of rain and snow leads to its reduction.
10. The taxonomic diversity of the culturable bacteria isolated from outdoor air at the location is abundant. The predominant bacteria were assigned to 25 genera, with most isolates belonging to the genera *Bacillus*, *Arthrobacter*, *Micrococcus*, *Enterobacter* and *Paracoccus*.
11. The fungal presence in the outdoor air at the location has a significantly less pronounced taxonomic diversity. The isolates were identified as representatives of genera *Penicillium, Aspergillus, Alternaria* and *Cladosporium*.
12. The results form the metagenomic analysis reveal extremely high taxonomic diversity in the air samples - representatives of the phylums *Firmicutes, Proteobacteria, Bacteroides, Actinobacteria, Cyanobacteria, Ascomycota* and *Basidiomycota* were *found.*
13. The study of the morphology of the PM at the location area proves that their type and concentration are seasonally dependent. More than 18% of the particles are aggregates of many small particles, including those in the nanometer scale.
14. With regard to the chemical composition of the detected PM pollution (PM10/2.5) in the area, the main phases are silicate, aluminosilicate and sulphate compounds, as well as organic and inorganic (carbonate and carbon) phases. A high content of iron oxides is also registered.

CONTRIBUTIONS

1. The one-year microbiological monitoring of the quantitative microbial presence in the air of the central part of the city of Sofia is the first study of its kind on a national scale and one of the longest for the territory of Europe.
2. The research has contributed to the exploration of airborne microorganisms, and in particular those found in the air of highly urbanized areas.

3. The relationship between air quality, the presence of various pollutants (physical and chemical), as well as the composition and concentration of microbial bioaerosols in the air of Sofia was observed.
4. Information on the daily, weekly, monthly and annual variations of microbial bioaerosol air pollution in the city of Sofia was obtained.
5. Information on the taxonomic diversity of airborne microorganisms was obtained, and the dominant bacterial and fungal taxa were identified.
6. The first comprehensive national study of fine dust particles in the atmosphere by lidar localization of their spatio temporal distribution, characterization by composition, structure, and morphology was carried out.
7. The obtained results are a significant contribution to the development of a strategy for control and assessment of air pollution in the city of Sofia and making the right management decisions to maintain the air clean.

RECOMMENDATIONS

1. The existing system in the city of Sofia for evaluation of PM pollution with the help of official stations of the Ministry of Environment and Water is accurate, but does not provide data on the associated microbial pollution. It is recommended to optimize it with the inclusion of analyzes of the levels of microbial bioaerosol contamination.
2. It is recommended to use a protocol for combined analysis including lidar mapping and *in situ* sampling to monitor the mass concentration of PM 2.5/10 and the bioaerosol presence along the lidar path.
3. The fast urbanization and growing population in the city of Sofia, it is advisable to introduce regular microbiological monitoring to take into account the changes over time. The data from the present work can be used for comparison in subsequent air monitoring studies in the city of Sofia.

PUBLICATIONS

1. Iliev M., Ilieva R., **Angelova B**., Paneva D., Cherkezova-Zhelev Z., Groudeva V., Nedkov I. (2021) FEATURES OF PARTICULATE MATTER AND MICROBIOTA IN THE LOWEST ATMOSPHERIC LAYER ABOVE DENSELY POPULATED URBAN AREAS. *Comptes Rendus de L'Academie Bulgare des Sciences*, 74 (12), pp. 1739 - 1748, **Q2, SJR 0.244** (2020) DOI: 10.7546/CRABS.2021.12.03.

2. Grigorov I., Ghelev C., Kolarov G., Iliev M., **Angelova B**., Ilieva R., Groudeva V., Cherkezova-Zheleva Z., Stoyanov D., Nedkov I. (2021) LIDAR MONITORING AND *IN SITU* SAMPLING OF PARTICULATE MATTER IN LOWEST ATMOSPHERIC LAYERS OVER URBAN AREA. *Comptes Rendus de L'Academie Bulgare des Sciences*, 74 (9), pp. 1296 - 1304, **Q2, SJR 0.244 (2020).**

PARTICIPATION IN SCIENTIFIC FORUMS

1. Zara P. Cherkezova-Zheleva, Daniela G. Paneva, **Boyanka N. Angelova**, Ralitsa V. Ilieva, Ivan I. Nedkov "MÖSSBAUER INVESTIGATION OF PARTICULATE MATTER IN THE LOWEST ATMOSPHERIC BIOAEROSOL" 36-th International Conference on the Applications of the Mössbauer Effect, ICAME 2021. 05/09-10/09 2021 (poster).

2. Zara Cherkezova-Zheleva, Daniela Paneva, Boris Kunev, **Boyanka Angelova**, Ralitsa Ilieva, Ivan Nedkov "AIR POLLUTION INVESTIGATION OF SOFIA URBAN AREAS DURING THE COVID-19 LOCKDOWN PERIOD: A COMPARATIVE STUDY" 30th International Conference ECOLOGY & SAFETY, 16-19 August 2021. (poster).

3. Stoyanov D, Grigorov I., Kolarov G., Iliev M., **Angelova B.,** Ilieva R., Groudeva V., Cherkezova-Zheleva Z. , Nedkov I. "Possibilities for application of mobile in situ sampling and lidar monitoring of particulate matter in lowest atmospheric bioaerosol" International Black sea coastline countries scientific research symposium VI. April 28-30, 2021/ Giresun, Turkey (oral report).

4. Nedkov I**., B. Angelova**, M Iliev, R.Ilieva, D. Stoyanov,V. Groudeva, Z. Cherkezova-Zheleva. “Atmospheric particulate matter pollution over residential urban areas during COVID-19 Quarantine.” 1st Surface Engineering for Biomedical Applications Workshop – Webinar, 11.11.2020 (oral report)

5. **B. Angelova**, M Iliev, R. Ilieva, Nedkov I., V. Groudeva “Characteristic of airborne microbiota in highly urbanized locations in Sofia city, Bulgaria“ Scientific conference Kliment’s days 2020. (poster)

6. **B. Angelova**, M Iliev, R. Ilieva, Nedkov I., D. Stoyanov, V. Groudeva „AIRBORNE MICROBIOTA IN URBANIZED LOCATIONS IN SOFIA CITY, BULGARIA“ FEMS ONLINE Conference on Microbiology 2020. (poster)

7. Nedkov I., D. Stoyanov, M. Iliev, R. Ilieva, **B. Angelova**, V. Grudeva. “Lidar monitoring and *in situ* sampling of particulate matter in atmosphere”, COST in Dust Training School 27-29 January 2020, Sofia (oral report)

8. **B. Angelova**, R. Ilieva, M Iliev, Nedkov I., D. Stoyanov, V. Groudeva “Investigation of microbiota in atmospheric aerosols during LIDAR monitoring of highly urbanised area in Sofia city” Scientific conference Kliment’s days 2019 (poster)

PROJECTS

1. “Complex study of fine dust particles in the atmosphere by lidar localization of their spatio-temporal distribution, characterization by composition, structure, morphology and microbial content " Contract №: DN18/16 from 20.12.2017 (with extension period until 06.12.2021). Project Manager: Prof. Ivan Nedkov.
2. “Analysis of the culturable microbiota associated with PM pollution in the central part of Sofia and risk assessment", Project Manager: Assoc. Prof. Mihail Iliev
3. “Metagenomic studies of the microflora around the Bulgarian Antarctic base on Livingston Island and determination of different species of invertebrates with molecular genetic methods." Contract № 70-25-72 from 03.08.2021, Project Manager: Assoc. Prof. Dr. Svetoslav Dimov.