

INVESTIGATION OF MICROBIOTA IN ATMOSPHERIC  
AEROSOLS DURING LIDAR MONITORING OF HIGHLY  
URBANIZED AREA IN SOFIA CITY

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**Abstract:** The air quality in fine particle matters polluted areas in Sofia city became a serious problem in the last few years. The microbial fractions (bioaerosols) associated with particulate matter (PM) is also very important for air quality. The purpose of the present study was to conduct microbiological analysis of airborne microbiota in outlined by LIDAR characteristic zones of increased concentration of aerosol pollutants using classical taxonomy and 16S rDNA sequencing analysis.

The LIDAR mapping 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. LIDAR beam was directed to Tsarigradsko shose blvd. and the samples were collected from three location points – Sofia Tech Park, Orlov most and Dragan Tsankov Blvd. A Hygitest 106 (Maimex) for air sampling as well as Koch sedimentation method were used for collection of the samples. Four different filters (Gelatin, Cellulose-ester, Polymer-fibrous and Glass fibrous) and different elective media were used to enumerate and identify the isolates.

The results obtained showed dominant presence of genera *Bacillus*, *Erwinia*, *Acidovorax*, *Enterobacter*, *Arthrobacter*, *Streptomyces*, *Kocuria* and *Rathayibacter*. Opportunistic pathogens were established among the isolates, such as *Bacillus cereus*, *Bacillus pumilus*, *Enterobacter aerogenes* and *Erwinia herbicola*. In all samples, the fungi from genera *Penicillium* and *Aspergillus* were permanently present. The combined approach based on LIDAR mapping and adapted sampling procedures allowed accurate assessment of the microbial aerosols diversity, as well as determination of the target groups of microorganisms posing health risks.

## INTRODUCTION

The problems related to the air quality in urban areas are considered today as being among the most important ones concerning the overall sustainable development of humanity.

PM of diameter 10  $\mu\text{m}$  and 2.5  $\mu\text{m}$  and the microbiota, associated with them are in the focus of many investigators (Goyer et al, 2001, Brooks et al, 2004, Despres et al, 2012). Respiratory problems after short-term exposure to PM 10 may occur, but greater risk to health is represented by PM 2.5.

Bioaerosols are polydispersed cells of a variety of microorganisms, carried over the air, most often in the form of aggregates, coated with a thin moist layer and adsorbed on a carrier. Bioaerosols can contain pollen, bacteria, actinomycetes, fungal spores, drops that occur during sneezing and coughing, as well as endotoxins, mycotoxins and allergens. Due to adverse atmospheric conditions, such as dehydration and UV radiation (Griffiths et al, 1994), not only vegetative forms but also bacterial and fungal spores are present in the ambient air.

The size of the bioaerosol particles can be with aerodynamic diameter of several nanometers (viruses and cellular fragments) up to several hundred micrometers (pollen, plant parts) (Cox et al, 1995; Hinds, 1999; Pöschl, 2005). Soil and sand particles can serve as a 'raft' for attaching microorganisms (Brooks et al., 2004). A number of studies have shown that bacteria in the air most often coexist with dust particles and will be thus transported over long distances by continental wind (Maki et al., 2014).

The global transfer of the aerosol microbiota (including pollen, bacteria, and fungi) is through desert dust (Kellong et al., 2004, Kellong et al, 2006). The average residence time of bioaerosols in the atmosphere can range from a day to several weeks, depending on their size and aerodynamic properties (De Nuntiis et al., 2003; Després et al., 2012).

Many microorganisms, occurring in the air, including bacteria, yeast, molds, can cause diseases in humans, animals and plants (Dowd et al., 1999). Certain infections in humans, such as measles or tuberculosis, can be spread through bioaerosols containing infectious microorganisms (Ijaz et al., 2016; Jones et al., 2015). After sneezing or coughing, the causative agent of pneumonia, *Streptococcus pneumoniae*, may also spread in the form of a bioaerosol (Richter et al., 2013).

The qualitative and quantitative composition of microorganisms varies greatly between cities (Brodie et al., 2007) and therefore there is no exact description of the characteristic bacterial composition in urban areas. Spore-forming bacteria, such as the representatives of the genus *Bacillus* and other Gram-positive bacteria are prevalent in studies on cultivable microbial diversity in the air (Womack et al., 2010). Gram-positive microorganisms are more resistant to drying than Gram-negative ones because of their cell wall thickness and resistance (Madigan et al.,

2009). In addition, endospores are extremely drought-resistant and contribute to the survival of spore-forming bacteria in the air (Goyer et al., 2001). Most of the bacteria found in the air are typical for soil environment. The most common genera are *Micrococcus*, *Staphylococcus*, *Aerococcus* and *Bacillus*. Representatives of the genus *Pseudomonas*, *Xantomonas* and *Escherichia* are the dominant Gram-negative bacteria.

Some data about the dominant microorganisms in the bioaerosols in the air of the locations with high traffic in Sofia town are present in this paper.

## MATERIALS AND METHODS

LIDAR (Light Detection and Ranging) sounding of the atmosphere was based on the available sophisticated equipment. It allowed remote sensing for efficient mapping of the aerosol fields distribution over large urban areas. Since 2002, IE-BAS, through the Laser Radars Laboratory, has been part of the EARLINET European LIDAR Network for aerosol distribution monitoring. The LIDAR mapping was performed by the two-wavelength LIDAR system (Fig.1) 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 with a spatial resolution of 15 to 30 m and an angular resolution of  $\sim 1^\circ$  at operational distances exceeding 25 km. LIDAR beam was directed to the locations mentioned to probe the near surface atmosphere in a constant horizontal direction.



**Fig. 1** Two-wavelength LIDAR system

The samples were collected in three location points, situated near one of the most important traffic lines in Sofia – Sofia Tech Park, Orlov most and Dragan Tsankov Blvd.

A Hygitest 106 (Maimex), a high – efficiency portable device for air sampling (Fig. 2a) as well as Koch sedimentation method were used for collecting air

samples. Four different filters (Gelatin (Fig. 2b), Cellulose-ester, Polymer-fibrous and Glass fibrous) were tested. Different elective media were applied for conducting quantitative and qualitative analysis.



**Fig. 2a.** Portable device for air sampling



**Fig. 2b.** Gelatin filters

DNA isolation from pure cultures was performed by a GeneMATRIX Tissue & Bacterial DNA Purification Kit. Agarose gel (1%) was used for electrophoresis. The visualization of the isolated DNA was done by the SimplySafe™ dye.

The PCR reaction was performed by a Ready-To-Go PCR kit (GE Healthcare). Universal eubacterial primers 27F (sense) and 1492R2 (antisense) were used. The reactions were performed at Eppendorf Thermocycler. PCR products were verified on 2% agarose gel (Agarose low EEO, AppliChem, Germany). DNA ladder (Gene Ruler™, 100 bp) was used to determine the size of the amplified fragments.

The sequencing of PCR amplicons was performed by Macrogen, Netherlands. Specialized BLAST software was applied for bioinformatics analysis.

## RESULTS AND DISCUSSION

Intensive pedestrian and road traffic at selected points is a good prerequisite for using LIDAR monitoring as a method for characterization of PM pollution. The duration of the LIDAR survey in the chosen direction allowed the accumulation of sufficient statistics that, after computer processing, can be used in air quality monitoring systems. The data provided by IE-BAS showed the presence of a large amount of PM sized between 2.5  $\mu\text{m}$  and 10  $\mu\text{m}$  (Fig. 3). The maps are color-coded, with the areas of lowest aerosol concentration marked in deep blue, and those with the highest, in deep red.

Fifty six pure bacterial cultures were isolated during the microbiological investigation of the samples. Twenty eight of them were typical Gram positive, rod-shaped, whereas Gram negative rod shaped isolates were 15. The other isolates were recognized as staphylococci and micrococci.

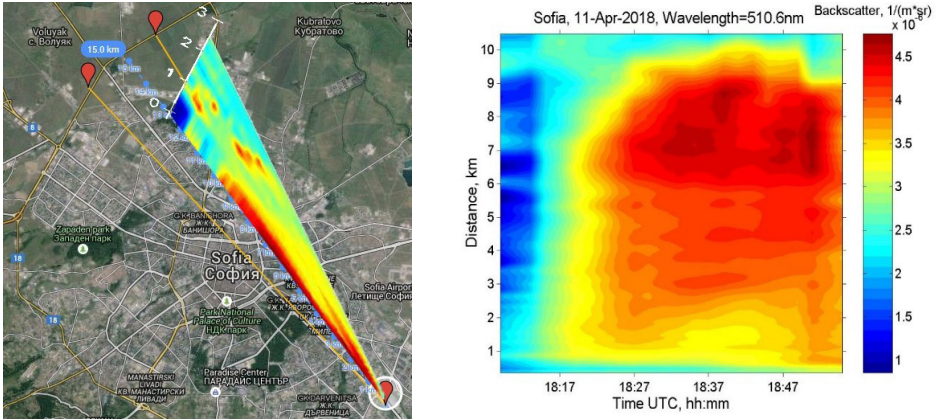


Fig. 3. LIDAR maps

In spite of the microbiological diversity in the samples, the obtained results using classical taxonomy showed dominant presence of rod-shaped, Gram positive, spore-forming bacteria referred to genus *Bacillus*. The second prevailing group of bacteria was from the family Enterobacteriaceae. In all samples the fungal isolates of genera *Penicillium* and *Aspergillus* dominated.

The sequence analysis confirmed the prevalent presence of *Bacillus* members and representatives of the genera *Erwinia*, *Enterobacter*, *Staphylococcus*, *Streptomyces*, *Arthrobacter*, *Kocuria*, *Plantibacter*, *Gordonia*, *Exiguobacterium*, *Rathayibacter*. (Fig. 4).

Opportunistic pathogens were found among the isolates (*Bacillus cereus*, *Bacillus pumilus*, *Erwinia herbicola*, *Enterobacter aerogenes*). Sequence analysis of fungal isolates confirmed the presence of *Aspergillus*, *Penicillium*, *Cladosporium*, *Botrytis*, and *Symmetrospora*.

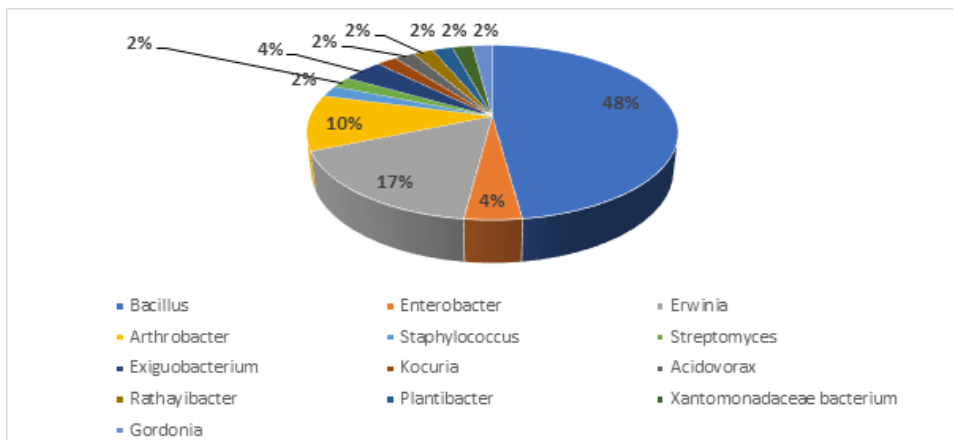


Fig. 4. Percentage of isolated bacteria

The results are similar to those found by other authors (Herr et al., 2003, Kellogg et al., 2006, Griffin et al., 2007, Bragoszewska et al., 2017). In the air over Erdemli, Turkey, during the passage of Saharan dust in March 2002, bacteria belonging to seven genera were isolated and the majority of the species referred to the genus *Streptomyces* (Griffin et al., 2007). In Bamako, Mali, representatives of 20 genera were identified during the passage of a large amount of desert dust, with *Bacillus* species representing 38% of all isolates, followed by genera *Kocuria* - (12.8%), *Saccharococcus* - (7.4%) and *Micrococcus* - (6.4%). From the 95 species of bacteria identified in this study, about 10% are potential pathogens in animals, 5% are phytopathogens, and 25% are opportunistic human pathogens (Kellogg et al., 2004).

Eukaryotic microorganisms from genera *Cladosporium*, *Alternaria*, *Epicoccum* are the dominant species found in open air in different parts of the world, while species of the genera *Penicillium* and *Aspergillus* are more often isolated from enclosed spaces (Akerman et al., 2003). Saharan sandstorms are responsible for the transmission of pathogens associated with widespread coral infections (predominantly *Aspergillus* genus) in the Caribbean region (Shinn et al., 2000; Weir-Brush et al., 2004; Das et al., 2012; Wilken et al., 2015).

## CONCLUSION

In conclusion, it must be noted that the approach used in this study is effective for analyzing the microbiota in bioaerosols during the LIDAR monitoring of particulate matter and it is of great importance for air monitoring and qualitative evaluation of air in urban areas. The content of microorganisms in bioaerosols is an extremely dynamic indicator, influenced by numerous factors such as season, temperature, weather, traffic of cars and people.

The study of fine particulate matter (PM) and the associated microbiota must be a part of evaluation of the negative effect on human health.

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