REVIEW

on a dissertation work entitled: "Characteristics of the microbiome during a complex study of fine particulate matter (PMP) in the atmosphere of urbanized urban areas and risk assessment", presented for the acquisition of the educational and scientific degree "Doctor" in the doctoral program "Microbiology", in a professional direction 4.3. Biological sciences of a PhD student Boyanka Nikolaeva Angelova at the Department of "General and Industrial Microbiology" in the Faculty of Biology of the SU "St. Kliment Ohridski" - Sofia

Reviewer: **Prof. Dr. Elena Ivanova Georgieva – IFRG-BAS**

The constantly growing demands of modern society for more accurate and reliable results for the protection of the environment and the health of the population not only in Bulgaria, but also in the world, as well as for improving the weather, air quality and climate, impose the need to improve the used methods for complex monitoring of atmospheric pollution, especially in complex terrains where the majority of the population is concentrated. A new trend for complex air monitoring studies aims to track the presence of not only physical and chemical pollutants, but also the composition and concentration of microorganisms in highly urbanized areas. Interest in microorganisms found in the air dates back to the time of Darwin and Pasteur. For example, at the end of the 19th century it was found that the concentration of spores in the air over certain places in France was higher and the death rate increased as the concentration of microorganisms in the air increased. Microbial bioaerosol contamination of air has been studied in many countries, but the diversity, distribution and interactions of these microorganisms are poorly understood, even though they are a constant element of the airspace. Air is found to be full of life, like soil and water, and is a habitat for a huge variety of microorganisms belonging to different taxonomic groups, some of which have not yet been described due to difficulties in being cultivated in laboratory conditions.

Regardless of the technological and methodological progress, at the moment no full-scale monitoring studies have been conducted regarding the dynamics of microbial bioaerosol contamination in the air of the city of Sofia, where large parts of the city are exposed to sources of severe atmospheric pollution due to accelerated urbanization rates of development. The thesis developed in the dissertation work of Boyanka Angelova is focused on an important problem, with a fundamental and practical orientation, related to the ecological problems of the city of Sofia. A large part of Bulgaria's population is concentrated in our capital, as well as industry, transport, energy production, etc., the tasks developed in this scientific work include a complex study on abiotic and bioaerosol air pollution in the highly urbanized central part of the city. Since recently fine dust particles are considered carcinogens, such a study is extremely relevant, and the results of the polluted atmosphere over the city of Sofia can contribute to the prevention of diseases and improve human health, and can be used in the preparation of future recommendations to prevent crisis situations, as well as to help in making the right management decisions to protect the cleanliness of the city. The developed dissertation is timely and in close compliance with the standards of the European Green Deal of the EU and with the recommendations of the World Health Organization.

The scientific work presented to me for review is written in 300 pages, according to the accepted standards for obtaining the educational and scientific degree "Doctor", and contains: Introduction, Literature review, Aims and tasks, Materials and methods, Results and discussion, Conclusions, Contributions and Recommendations, as well as a list of participation in scientific events and publications. The literary sources used are 641 titles in Latin and 1 in Cyrillic and are reflected on 38 pages. The presented material is richly illustrated with 112 informative figures and 44 tables. A very good impression is made by the recommendations given to the authorities for the introduction of regular microbiological monitoring, which will allow taking into account the changes occurring over time. The published data from this dissertation can also be used for comparison in subsequent air monitoring studies in the city of Sofia. The topic and content of the dissertation fully cover the announced nomenclature specialty in professional direction 4.3 Biological Sciences, Scientific specialty "Microbiology" for the award of the ONS "Doctor". Substantial parts of the obtained results of the dissertation work were reflected in 5 scientific articles, two of which were published in refereed journals on Scopus and Web of Science with quartiles Q2 and SJR 0.244. In two of the publications, Angelova is in first place, and in the others, she is in third, fourth and fifth position. The PhD student presented a list of 8 presentations with posters and sections reports at national and international forums, as well as participation in three scientific projects. The literature review is competently written in 64 pages with high scientific potential, showing the depth and magnitude of this problem, as well as the subsequent experimental work. It occupies approximately a quarter of the entire work. It is written in extremely good scientific language, covers the published literature in a controlled sequence, is easy to read and understand. It discusses more than 600 literary sources and data directly related to research from the development of the scientific problem worldwide. Historical data on air pollution is professionally reflected. All monitoring studies on the composition and number of microorganisms in the air are skillfully and in detail described; the sources, distribution and composition of bioaerosols; the transport and transformations of bioaerosols; ambient air quality standards; bioaerosol collection methods and approaches to their study. Civil air quality monitoring networks are shown in figures. The possibilities and advantages of metagenomic and quantitative PCR analyzes for the study of airborne bioaerosols are discussed. It can be seen that the PhD student knows the state of the problem very well and presents it thoroughly and comprehensively. The accelerated urbanization rates of development of the city of Sofia are the reason and impose the need for in-depth research related to the complex nature of the formation of the general pollution of the atmosphere. The assessment of air pollution in different situations and locations is of interest from both a scientific and a practical point of view.

These important environmental problems are the subject of this scientific work, and I can say with great satisfaction that the obtained results will not only be a basis for common initiatives and collaborations, but also for making informed decisions to improve the air in our capital.

In the "Goals and Tasks" section, the main problem of the dissertation is clearly and concretely formulated, namely: carrying out a complex study of the air microbiota in a highly urbanized central part of the city of Sofia and conducting year-round quantitative monitoring of the levels of bioaerosol contamination in a selected location, identification of dominant

microbial species and full characterization of associated dust contamination. To achieve the set goal, the implementation of one main task helps: conducting a full-scale year-round study for the period May, 2020 - April, 2021 on the quantitative dynamics of culturable bacterial and fungal bioaerosol contamination in a selected location from the central part of the city of Sofia. The successful implementation of this task was achieved by solving 5 well-thought-out, specifically formulated and logically following tasks, entirely related to the protection of air quality in a densely populated metropolitan area. I must note that the tasks set in this way cover and even exceed the volume of a doctoral thesis.

In the "Materials" section, from the "Materials and methods" part, the research object is huge, namely: microbial bioaerosol contamination and dust pollution in the air over the highly urbanized central part of the city of Sofia, located at Dragan Tsankov Blvd. 8 and GPS coordinates: 42°41'01.9"N, 23°19'58.3"E. The wide range of classical microbiological, genetic, physicochemical, molecular, microscopic, cell biological and statistical methods of the methods used, as well as their competent description, confirms my impression of the excellent theoretical and experimental training of the doctoral student. For a period of 54 weeks, beginning in May 2020 and ending in April 2021, Boyanka Angelova, using labor-intensive methods, performed a qualitative and quantitative analysis of the bioaerosol presence. For a qualitative analysis of the bioaerosol presence, the doctoral student examined 248 pure bacterial and 35 pure fungal cultures isolated from 324 samples, and the object of her quantitative analysis was the total number of heterotrophic bacteria and mold fungi. These analyses were performed according to the classical scheme for the identification of bacteria and fungi. To track the diurnal course in the dynamics of microbial air pollution, 4 days belonging to the four annual seasons in the sampled period were selected: spring-summer-autumn-winter. A six-stage Andersen cascade impactor was used to collect bioaerosols from the air and quantify them. Through next-generation sequencing (NGS [Next-Generation Sequencing]) analysis of isolated DNA from two samples collected during the warm and cold part of the year, an accurate assessment of bioaerosol presence results was made. Sequencing and bioinformatic analysis of the obtained sequences was performed by Novogene Company Ltd (UK).

The main body of research has focused on the microbiological monitoring of bioaerosol pollution, but for the better theoretical understanding and future practical application of the results, the doctoral student also carried out a physicochemical characterization of FPC (Fine Powder Particles), carried out at the Institute of Catalysis of the BAS. Modern equipment for high-quality measurements such as X-ray phase and X-ray structural analysis, scanning electron microscope, particle size analyzer and many others were used for these studies, with which the doctoral student became familiar.

One of the most important sections for evaluating the level of results obtained is the "Results and Discussion" section. The candidate received a lot of evidence from the tasks set for resolution, which are shown in representative figures and tables. The first task performed is a quantitative analysis of the culturable microbiota found in the air of the selected location. The dynamics in the levels of bacterial and fungal bioaerosols in the air of the selected location were determined for each month of the surveyed full calendar year. The data showed pronounced monthly dynamics in the levels of tracked bioaerosols, which is determined by the geographical and climatic features of the city of Sofia. The recorded

maximums for bacterial contamination (May 2020; July 2020 and September 2020) and fungal contamination (June 2020; July 2020 and October 2020) do not exactly coincide in time with the first peak of reported dust pollution maximums in November and December, 2020. A partial overlap was observed at the beginning of a marked increase in the concentration of dust pollution - in October, and in February and March, due to the unfavorable values of abiotic factors temperature, humidity, etc., relatively low levels of dust pollution values were recorded. The results obtained from the annual monitoring of the levels of bioaerosol contamination detected in the air at the studied location prove that the annual dynamics in the numbers of the monitored bacterial and fungal bioaerosol component is extremely dynamic and is a reflection of the specifics of the geographical and climatic characteristics of the city of Sofia. Peak values for fungal contamination were reported in the months of June, 2020; July 2020 and October 2020, (1181.0 CFU/m3; 1482.0 CFU/m3 and 1491.0 CFU/m3). Low levels in the quantitative share of fungi were reported in the months of December, 2020, January, 2021 and February, 2021, resp. 16.0 CFU/m3; 20.0 CFU/m3 and 31.0 CFU/m3. Bacterial contamination recorded maximum values in the months of May 2020; July 2020 and September 2020, (401.0 CFU/m3, 495.0 CFU/m3 and 379.0 CFU/m3). Minimum levels of bacterial contamination in the air of the studied location were reported in the months of January and February, 2021, respectively 58.0 CFU/m3 and 25.0 CFU/m3.

The results of the monitoring of the daily trend in the levels of bioaerosol contamination detected in the air at the location have shown that the concentrations of the studied bioaerosols change hourly, in direct relation to the abiotic parameters of the environment and reflect the cyclicity of the anthropogenic pressure in the location. The daily kinetics is markedly seasonal, determined by climatic factors typical for each season, as well as the activities of the population. The monitoring data on the weekly trend in the levels of bioaerosol contamination detected in the air at the location show that the weekly dynamics in the concentrations of the studied bioaerosols, regardless of the season, are determined to a large extent by anthropogenic activities - an increase in the values at the beginning of working days, reaching of maximum values in the middle of the week and a reduction towards the end of the working week. A secondary peak in the daily concentration was observed on the last working day and the first of the weekends. Taxonomic analyzes of the cultivable bacterial microbiota in the air of the location showed that the dominant bacterial isolates belonged to 25 genera, with the most isolates assigned to the genera Bacillus, Arthrobacter, Micrococcus, Enterobacter and Paracoccus. The fungal presence in the air of the location is of significantly less pronounced taxonomic diversity. The isolates were assigned to four genera of the Ascomycota division – Penicillium, Aspergillus, Alternaria and Cladosporium. With the results of the metagenomic analysis, the doctoral student revealed an extremely high taxonomic diversity in the studied samples - representatives of the phyla Firmicutes, Proteobacteria, Bacteroides, Actinobacteria, Cyanobacteria, Ascomycota and Basidiomycota were found. The next task performed by the doctoral student was physicochemical analyzes of the FPCH. She found that fine dust particles are not stable, do not have a constant composition and size, and their chemical nature can be modified by various physicochemical processes. Although some particles enter the atmosphere directly from natural and anthropogenic aerosols, a large number are formed

secondarily in the atmosphere. The collected FFPs are mostly aggregates of a solid core and a liquid surface. Aggregates are composed of small particles, including those of nanometric size. Increased dust content (FPC10/2.5) in the air is associated with an increase in microbial presence, while rain and snow precipitation lead to its reduction. The data obtained from this large-scale study have shown that, while the peaks of PMF pollution are mainly associated with increases in car traffic, industry and heating, the change in the quantitative and qualitative composition of the microbiota is seasonal. The study of the morphology of FPCH in the area of the location 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. Regarding the chemical composition of the established dust pollution (FPCH10/2.5) in the air of the area, the main phases are silicate, aluminosilicate and sulfate compounds, as well as organic and inorganic (carbonate and coal) carbon phases. A high content of iron oxides was also recorded. In conclusion of this section, I can summarize that a huge number of microbiological materials, adequate controls and number of experiments were used, which achieved high reliability in the statistical processing of the results. The reliability and credibility of the obtained results are validated with properly documented, highly informative and representative figures and tables. Information was obtained regarding the daily, weekly, monthly and annual dynamics of microbial bioaerosol air pollution in the city of Sofia, as well as information on the taxonomic diversity of air microbial contamination, and the dominant bacterial and fungal taxa were determined. The first of its kind on a national scale, a complex study of fine dust particles in the atmosphere by lidar localization of their time-space distribution, characterization by composition, structure, and morphology was carried out. The obtained data are a significant contribution to the development of a strategy for control and assessment of air pollution in the territory of the city of Sofia. A part of the obtained scientific data has been reflected in 5 publications, 2 of them are in indexed journals and 8 prestigious international and national scientific forums. The doctoral student participated in the development of 3 scientific projects. I accept the 14 main conclusions, 7 contributions and 3 recommendations presented and analytically argued by the doctoral student. The results in Angelova's dissertation are a prerequisite for future extended studies in the field. The abstract is prepared according to the requirements and correctly reflects the presentation of the dissertation work and the formulated conclusions and contributions.

CONCLUSION:

Boyanka Angelova has done extensive research. From the huge amount of work invested and numerous experiments performed with modern methodology, she has obtained important results and attempted their evaluation and interpretation within the framework of existing hypotheses. The dissertation work is up-to-date, with clear goals and tasks, with significant conclusions and achievements, and has indisputable scientific and scientific-applied contributions. Determining the spatio-temporal variability of the characteristics of the bacterial and fungal microbiota, as well as the FFP, requires conducting high-quality long-term measurements by a well-trained specialist. These processes are characterized by great complexity and extreme diversity in the conditions of complex terrain in a crowded urban environment. From everything stated, I definitely believe that Boyanka Angelova has

in-depth knowledge, not only in microbiology, but also in molecular and classical genetics, and shows the ability to implement independent ideas. The dissertation work of Boyanka Angelova fully meets the requirements of the ŽRASRB and the Regulations for the terms and conditions for acquiring scientific degrees and for occupying academic positions in the Faculty of Arts of SU "Kl. Ohridski". Considering the professional qualities and scientific achievements of the doctoral student, I strongly recommend the scientific jury to award Boyanka Angelova the educational and scientific degree "Doctor".

Reviewer:

/prof. Dr. Elena Georgieva/