

SCIENTIFIC OPINION

from **Prof. Maria Bogomilova Angelova-Dyankova, DSc,**

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on a dissertation presented to a scientific jury, formed by order No. RD-38-388/13.07.2022 of the Rector of Sofia University "St. Kliment Ohridski" for obtaining the educational and scientific degree "Doctor" in Professional field: 4.3. Biological Sciences (Microbiology)

Doctoral candidate: **Boyanka Nikolaeva Angelova**

Dissertation topic: **Characterization of the microbiome in a complex study of fine particulate matter (FNP) in the atmosphere of urbanized urban areas and risk assessment**

Scientific supervisors: **Assoc. Prof. Mihail Iliev, PhD**

Prof. Ivan Nedkov, DSc

The adverse consequences of environmental changes and active anthropogenic activity are one of the most serious problems of the 21st century. This includes the impact of polluted air on human health. Although the air is not the most favorable environment for the development of microorganisms due to the low content of nutrients, low humidity, and high levels of solar radiation, various representatives of the microbiota inhabit the atmosphere and form the main component of the so-called bioaerosols. There is much research on the effects of chemical and physical pollutants, but studies on the atmospheric microbiome are extremely rare. Even less attention is paid to the complex nature of airborne pollutants, especially in highly urbanized areas. Any detailed study of the relationship between abiotic and bioaerosol air pollution in the largest cities can provide results to substantiate an effective pollution risk prevention strategy. This is the main scientific direction in the development of Boyanka Angelova, and this is an indication of its relevance and significance. The dissertation thesis is focused on a complex study of the air microbiota in a highly urbanized central part of the city of Sofia and thus for the first time includes the state of the problem in Bulgaria in the international databases. In addition, it outlines the contribution of Bulgarian microbiology to solving contemporary environmental problems and the commitment of the doctoral candidate and her scientific supervisors to them. This is an unexplored niche, which is a prerequisite for original contributions.

The dissertation is constructed in a traditional form with appropriate sections. It is written in 300 standard computer pages, including 260 pages of text, 30 pages of literature, and 10 pages of appendices. The section Literature Review is purposefully and specifically prepared and takes into account all aspects of the study. It contains 641 literary sources (639 are in Latin) and 9 sites that correspond to each of the tasks set. The Review of literature presents the state of the problem at the moment, correctly considering the historical development and what has been achieved so far in Aeromicrobiology. The available literature data on the quality and changes in the atmospheric

microbiota, the types of air pollutants and their impact on human health, the state of the air in urban areas, and the standards for its monitoring are discussed in great detail. Considering the importance of approaches to bioaerosol sampling collection, the Ph.D. student includes a detailed examination of the methods used. At the end of the overview, a subsection "Conclusion" is included, which highlighted the unresolved questions and the necessity of such research. In my opinion, this is a very good idea that helps the reader perceive the relevance of the dissertation thesis. The text is presented clearly, it is read with ease and interest, which is contributed by the included tables and figures.

The purpose of the dissertation corresponds to the relevance of the problem, emphasizes the innovative nature of the study, and directs attention to a modern level of research in Microbial Ecology. It is clear, well formulated, and brings together all strands of experimental work. To realize this goal, 5 specific, interrelated and logically following tasks have been formulated, which include all mandatory stages of such a study. Even here one can see the serious volume of the experimental work.

The section "Materials and methods" corresponds to the multidisciplinary character of the work, routine and modern microbiological, biochemical, molecular biological, and physicochemical methods are used. All of them are adequate for the research and create a prerequisite for correct results. Furthermore, the methods are presented understandably, complete enough to be reproduced. The illustrative material helps a lot in this direction. Here I would like to highlight the molecular identification of the composition of the bacterial and fungal community, metagenomic analysis, physicochemical characterization by X-ray phase analysis and Mössbauer spectra, infrared and X-ray photoelectron spectroscopy, scanning electron microscopy, etc. All of them are sufficient grounds for credibility and precision.

In the section "Results and Discussion", a vast experimental material is included, which is characterized by a logical sequence and outlines a serious scientific study. The individual stages are elaborated on in detail. At the same time, each of them is a starting point for the next direction of scientific research. This gives the dissertation a characteristic of completeness. It should also be noted the very well-chosen research area - the roof space of the building of the Faculty of Biology of Sofia University, i.e. the ideal center of the capital with its adjacent key road routes and urban infrastructure.

The main part of the research is related to proving the quantitative and qualitative composition of the air microbiota, the changes that occur during different research periods (days, weeks, months), or as a result of the change of seasons and atmospheric conditions. Pure cultures of bacteria and fungi were isolated, which were identified by the methods of classical taxonomy. The presented study is the first quantitative analysis of samples from the central part of the city of Sofia on a national scale and one of the longest for the territory of Europe. Of particular interest is the coincidence between the time frame of the monitoring study and the epidemiological situation due to Covid-19. This poses new challenges for the doctoral student, which she overcomes and gives an explanation for the results

obtained. Here I want to emphasize that the data from microbiological studies are interpreted depending on the data on the size and distribution of bioaerosol particles in the atmosphere, as well as on the parameters of abiotic factors at the relevant time. It should be noted the precision in presenting the results for the taxonomic status and genus affiliation of the isolated bacteria and fungi depending on the season, atmospheric conditions, and their dynamics for a day, a week, or a month. The profile of the dominant genera was determined, which was compared with data from similar studies in large cities in Greece, Turkey, Poland, China, Egypt, etc.

The use of one of the most modern methods for determining the composition of non-cultivable eubacteria, archaea, and fungi, namely metagenomic analysis, makes an exceptional impression. This method enables the author to obtain reliable results for the number of operational taxonomic units and the dominant representatives determined to genus and species. Among the fungal representatives identified are several pathogens and causes of seasonal allergies, as well as potential producers of biologically active substances. The study found marked seasonality in taxonomic diversity in air samples and a clear relationship between this diversity and airborne dust pollution.

As an innovative highlight in the dissertation, I would like to highlight the characterization of the abiotic component of air pollution in the region of the selected location, which is proof of the complex nature of the research. The physical parameters of the aerosol load in the area are determined, and the spatial and temporal characteristics of the established aerosols and their mass concentration were evaluated. Through lidar monitoring with horizontal scanning at different times of the day, dense aerosol clusters are detected and localized.

After the experiments carried out for the detailed physicochemical characterization of FCHP2.5, their structure and morphology, the type of elements located on the surface, their concentrations and ratios, as well as their phase composition, are established. The combined analysis of the results proves that the main phases registered in the sample are silicate, aluminosilicate, sulfate, carbonate, and coal. The obtained data are also interpreted in a comparative aspect regarding the season and year of sampling.

In this section, along with the correct reflection of the obtained data, a thorough and professional discussion has been made based on appropriate literary sources. The discussion is cleverly interwoven with the own data, which gives an impression of confidence and commensurability with what other authors have published. This, of course, is possible thanks to a very good knowledge of the problem and thorough scientific development.

I would like to emphasize with pleasure the excellent arrangement of the dissertation, the excellent scientific style, the correct presentation of the results in tables and figures, as well as their professional design. The photographic material is also of excellent quality and contributes to the correct perception of the results.

The doctoral student presents 14 conclusions, which at first glance are a lot, but considering the large volume of experimental work, this does not disturb the balance of the dissertation work. In my opinion, they are a logical consequence of the data obtained and provide the necessary information about the value of the research conducted. I also accept the wording of the contributions and want to highlight the most important of them, namely:

1. For the first time on a national scale, one-year microbiological monitoring was carried out on the quantitative microbial presence in the air of the central part of the city of Sofia, which includes Bulgaria in the international database.

2. For the first time in Bulgaria, a combined complex study of fine dust particles in the atmosphere has been realized by lidar localization of their distribution depending on time and space. Data were obtained on their composition, structure, and morphology.

3. For the first time, detailed information was obtained on the taxonomic diversity of the air microbiota in a highly urbanized area, on the dominant species, and the changes depending on the temporal dynamics.

4. The obtained information is a significant contribution to the development of a strategy for control and assessment of air pollution and the making of correct decisions for the protection of air purity in a highly urbanized area of the capital city.

One of the merits of Boyanka Angelova's dissertation is the formulation of recommendations based on the results obtained. All of them can be very useful for optimizing the air pollution control system existing in the city of Sofia with FFP.

The data from the dissertation have been included in 2 journal scientific articles with impact factor and quartile Q2 and 8 participations in national and international forums. In addition, Angelova participated in a scientific team for the development of 3 projects related to the topic of the dissertation.

I have the following questions and recommendations for the doctoral student:

1. Is it possible to continue the work on this problem and make a more detailed characterization of the dominant species as potential pathogens, allergens, or producers of biologically active substances?

2. How do you explain the lack of representatives of the *Archaea* domain in sample № 2 (January) in contrast to sample № 1 (May)?

3. I recommend the doctoral student publish the Review of literature, which would be very useful for a wide range of specialists.

4. I recommend avoiding the use of abbreviations in the titles.

In conclusion, I want to emphasize that the experimental material is very well presented, and the very current topic has been developed, which offers innovative approaches to solving problems from Environmental Microbiology. At the same time, it creates conditions for a more effective air

pollution control strategy in a metropolitan city or other highly urbanized areas. The experiments are set methodically correctly, the results obtained are reliable, and are a solid basis for further scientific and applied developments. A huge amount of experimental work has been carried out, and the problem has been multifaceted and studied in detail at a modern level, significant contributions have been made, and practice recommendations have been formulated. To this characteristic of the dissertation work, I would like to add that, in my opinion, Boyanka Angelova leaves her doctoral studies as a well-prepared specialist in the field of Aeromicrobiology, she has mastered a large number of modern methods, and she has become familiar with a problem important for science and practice, she has gained experience in interpreting scientific data and all this gives her the qualification of a young scientist, a worthy competitor to colleagues from foreign laboratories.

Based on the analysis made and the proven growth of the doctoral student, I propose to the respected members of the scientific jury, formed by order No. RD-38-388/13.07.2022 of the Rector of the Sofia University "St. Kliment Ohridski", to award Boyanka Nikolaeva Angelova the educational and scientific degree "Doctor" in scientific direction 4.3 Biological Sciences (Microbiology).

September 7, 2022

Signature:

/Prof. Maria Angelova, DSc/