

REVIEW

by Prof. **Petya Koycheva Hristova**, PhD

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on Ph.D. Thesis of **Boyanka Nikolaeva Angelova**

Entitled: "*Characterization of the microbiome in a complex study of fine dust particles (FDP) in the atmosphere of urban areas and risk assessment*" presented for the acquisition of the educational and scientific degree "Doctor" Scientific area 4.3. Biological Sciences (Microbiology)

Scientific supervisors: Assoc. Prof. Dr. Mihail Iliev and Prof. Ivan Nedkov, DSc

By order of the Rector of SU "Kliment Ohridski" No. RD 38-388/13.07.2022, I am appointed as a Member of the Scientific Jury to ensure a procedure for the defense of the PhD thesis of Boyanka Nikolaeva Angelova. The set of documents and materials presented meets the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB) and the Regulations for its implementation.

1. Brief presentation of the candidate

Doctoral student Boyanka Angelova is a graduate of the Faculty of Biology at the University of St. Kliment Ohridski", where she graduated in 2017 with honors "Bachelor" degree, specialty "Molecular Biology". She continued her education at the Master program of Microbiology and Microbiological Control at the Department of General and Industrial Microbiology, successfully graduating in 2019.

Immediately after completing her bachelor's degree, she began working as a technical assistant at the Department of General and Industrial Microbiology, and then as a microbiologist. In the period 15.07.2019-15.07.2022, Boyanka Angelova is a full-time PhD student in the Department of "General and Industrial Microbiology" and at the same time a biologist - a part-time.

2. Relevance and significance of the dissertation

The dissertation proposed for review is dedicated an important and current problem related to the health of the population in the EU. According to the WHO, air pollution is one of the most significant environmental risk factors. The topic stems from the EU's declared

priorities for "Environmental Protection" and the results obtained have important fundamental and applied contributions.

The understanding of the complex nature of air pollution imposed in recent years directs the efforts of researchers not only to establish the qualitative and quantitative composition of the microbial component associated with dust pollution, but also to determine the multifactorial conditioning of its dynamics. This leads to the emergence of a new approach for assessing the degree of air pollution by means of complex monitoring studies. Tracking the dependence of air quality on the presence of various physical, chemical and microbiological pollutants gives more in-depth information about the problems especially in highly urbanized areas. The results of such complex studies can support the making of correct management decisions for environmental protection.

The presented facts clearly motivate the choice of the topic, its relevance and practical importance. The content of the dissertation fully covers the announced nomenclature specialty in professional area 4.3 Biological Sciences, Scientific specialty "Microbiology" for the award of the ESD "Doctor".

3. Characteristics and evaluation of the PhD thesis

The dissertation is written on 300 standard pages - A4 format, the results are summarized and presented in 44 tables and 112 figures. It is structured according to the classical scheme and is balanced in terms of the volume of each section. The first 9 sections include Introduction (1 p.), Literature Review (63 p.), Aim and Objectives (1 p.), Materials and Methods (22 p.), Results and Discussion (150 p.), Conclusions (2 p. .), Contributions (1 p.), References (1 p.) and References (30 p.). A very good impression is made by the grouping of some of the data in a separate Appendix, as well as the attached lists of figures, tables and abbreviations used.

The dissertation is written in a good scientific style, with accurate use of terminology, which shows that the doctoral student knows the subject matter professionally. It should be noted that the presented literature reference (641 publications on the subject) offers a thorough analysis of the state of the problem. The dissertation student has become very familiar with the literature data and skilfully uses it in interpreting the results.

3.1. Literature review

The literature review is structured correctly with direct reference to the purpose of the dissertation and the tasks set. The thesis overview covers the main aspects of aeromicrobiology and air biogeography. Conditionally, several main topics are distinguished, which, however, are closely related to each other. In the first part, the doctoral student presents the air as a living

environment for microorganisms and the factors that condition their existence in this ecological niche. The second part is aimed at presenting the qualitative and quantitative composition of the air microbiome and the methods for its research. The third topic reveals the nature of pollutants in the air - physical and chemical and their role in the emergence of risk to the health of the population. In the fourth topic, the doctoral student presents the quality of atmospheric air in urbanized areas, the accepted standards for air quality assessment, the Bulgarian national pollution monitoring network, as well as the air monitoring system in Sofia. The fifth topic discusses in detail the methods of collecting aerosols and the devices that are used. The sixth topic presents the advantages of metagenomic analysis for the study of bioaerosols and for conducting microbiological monitoring of air in urbanized urban areas. The conclusion of the literature review clearly summarizes the knowledge achieved to date, outlines the need for a new approach to profile microbial bioaerosol contamination in the metropolitan city, and ably points to the need to conduct a full comprehensive study on abiotic and bioaerosol air pollution in the highly urbanized central part of Sofia.

3.2. Purpose and tasks

The aim of the dissertation is clearly and precisely formulated as including conducting year-round quantitative monitoring of bioaerosol contamination levels in a selected location, identification of dominant microbial species and full characterization of associated dust pollution. To achieve the formulated goal, the doctoral student sets herself five main tasks - tracking the seasonal, weekly and all-day dynamics in the number of microbial presence, qualitative analysis and identification of the dominant species, establishing the taxonomic affiliation of the dominant species both by classical methods and by metagenomic analysis and systematic and complex characterization of the fractions of FDP in lidar-characterized aerosol fields.

3.3. Materials and methods

This is one important part of the dissertation that shows the level of research. Modern microbiological, biochemical, molecular, physical and microscopic methods are presented, so their development and successful application is already an indisputable success and shows the serious methodical preparation of the doctoral student. For a period of 54 weeks, a qualitative and quantitative analysis of bioaerosols was conducted, during which 324 samples were processed and 248 pure bacterial cultures and 35 fungal cultures were isolated. The PhD student applies both classical microbiological methods for taxonomic characterization of the isolates, as well as next-generation sequencing (NGS [Next-Generation Sequencing]) analysis of isolated DNA from samples collected during the warm and cold part of the year. This approach

allows full characterization of the bioaerosol microbial component. A six-stage Andersen cascade impactor was used to collect bioaerosols from the air and quantify them. It should be emphasized that the application of the specific methods such as X-ray phase and X-ray structural analysis, scanning electron microscope, particle size analyzer provides in-depth information about the structure and type of bioaerosols, which was limited in the literature before the present study. The multidisciplinary nature of the research allowed the doctoral student to acquire new methodological skills.

3.4. Results and discussion

The main results are presented according to the set tasks and the chosen methodical approaches, and in their nature are an in-depth characterization of the microbiome of bioaerosols in an urbanized central area of the city of Sofia. The experimental part is made very precisely and presented reliably. Through daily, weekly and monthly monitoring, the quantitative and qualitative composition of the air microbiota was tracked. In order to determine the factors having a specific impact on the levels of bioaerosol contamination, studies were conducted on certain days with different meteorological phenomena (fog, rain, snowfall, wind).

From all 54 weekly samplings, 648 samples were processed, from which representatives of different phylogenetic groups were isolated and identified. Bacterial isolates were characterized according to the methods of classical taxonomy and assigned to 25 genera, while fungal isolates were assigned to 4 genera of the Ascomycota department – *Penicillium*, *Aspergillus*, *Alternaria* and *Cladosporium*. Most bacterial isolates were assigned to the genera *Bacillus*, *Arthrobacter*, *Micrococcus*, *Enterobacter* and *Paracoccus*. All results are presented in a comparative plan regarding seasonal dynamics, species diversity and extent of distribution. To overcome the shortcomings of culture-dependent studies, the PhD student applied metagenomic analysis, which examines the total microbial genomes in samples obtained directly from the environment. Through this approach, the eubacterial, archaeal and fungal diversity, as well as the dominant species, which are representatives of the phyla Firmicutes, Proteobacteria, Bacteroides, Actinobacteria, Cyanobacteria, Ascomycota and Basidiomycota, were determined. This is the first study of the air in Bulgaria and represents an important fundamental and practical contribution to the work.

Of particular importance in the dissertation is the interpretation of microbiological studies depending on the parameters and amount of bioaerosol particles in the air. Abiotic dust presence is an essential factor determining to a large extent the dynamics and diversity of the microbial component. Therefore, the determination of the physical parameters of the aerosol load in the sampling area is a significant contribution of the work. The conducted

physicochemical analyzes show that FDPs are mainly aggregates of a solid core and a liquid surface. The aggregates themselves are composed of small particles, including nanosized ones. Most particles are agglomerates, most often enriched with various metals such as iron, copper, zinc and others. Dust particles mainly consist of silicate, aluminosilicate compounds, as well as organic and inorganic carbon phases. Peaks of FDP pollution are mainly associated with increased car traffic, industry and heating, while the change in the quantitative and qualitative composition of the microbiota is seasonal.

3.5. Conclusions and recommendations

The doctoral student summarizes the vast experimental work in 14 conclusions that correspond to each stage of the research done. The annual, seasonal and monthly dynamics of microbial contamination and bioaerosols in the air, which is the result of the climatic, geographical and anthropogenic characteristics of the settlement, were established. The high taxonomic diversity of the culturable bacterial microbiota and the less pronounced diversity in the fungal presence was demonstrated. Metagenomic analysis adds new taxonomic groups to the resulting data. It was found that the type and concentration of FDP in the location area are seasonally dependent. After summarizing the obtained results, the doctoral student formulates three recommendations regarding the optimization of the analyzes for the levels of microbial bioaerosol contamination.

4. Scientific and applied contributions

Both theoretical and original applied contributions can be outlined in the conducted studies, which prove the importance of scientific development. The contributions are as follows:

- The conducted 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.
- Studying the composition of airborne microbiota is a fundamental contribution to aeromicrobiology. Information on the taxonomic diversity of airborne microbial contamination was obtained, and the dominant bacterial and fungal taxa were determined.
- A dependence was established between air quality, the presence of various pollutants (physical and chemical) and the concentration of microbial bioaerosols in the air of the city of Sofia.
- Information was received regarding the daily, weekly, monthly and annual dynamics of microbial bioaerosol air pollution in the city of Sofia.

- 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 the control and assessment of air pollution in the territory of the city of Sofia and the making of correct management decisions for the protection of air purity.

5. Participation of the doctoral student in the development of the dissertation

The contacts made with the doctoral student during the discussion of questions from the dissertation work give me reason to consider that the implementation of the dissertation work is entirely her work.

6. Publications in connection with the dissertation work

The PhD student has submitted a list of two scientific publications (Q2) related to the dissertation. The results of the dissertation have been reported at 8 international and 3 national scientific forums. Participation in three scientific projects related to the topic of the dissertation is presented.

7. Abstract

The abstract is prepared according to the requirements and faithfully reflects the results of the dissertation, as the most important things from all sections are presented in abbreviated form (without the literature review).

8. Recommendations, comments and questions

There are no significant errors in the dissertation regarding the approaches used and the presentation of the results, so I have no recommendations or remarks.

I have the following questions for the doctoral student:

a) What information do operational taxonomic units and labels carry in metagenomic analysis?

9. Acquired competence and compliance with the requirements of the educational and scientific degree "doctor"

In the course of the implementation of the experimental work and the design of the dissertation work, the doctoral student Boyanka Nikolaeva Angelova has acquired competencies and skills that can be grouped as follows:

- expanded her theoretical competence in a specific field of microbiology - aeromicrobiology,
- acquired skills for working with scientific literature, analyzing and summarizing scientific information.

- she has enriched her methodical skills, especially in the part of applying modern methods

- acquired skills to independently shape and discuss the results obtained during the development of the dissertation, as well as draw conclusions based on them.

All of this gives me reason to believe that the doctoral student has acquired the necessary competencies and skills to acquire the educational and scientific degree "Doctor".

10. Conclusion

The presented dissertation work is a completed study that provides valuable scientific information and reveals opportunities for practical application. The very good theoretical preparation has enabled the doctoral student to select and combine a set of methods that ensure the implementation of the tasks and the achievement of the goal. I believe that the volume of this work is impressive, many analyzes have been made, valuable results have been obtained, important conclusions and recommendations have been made, and the research has significant scientific and applied contributions. The abstract is made according to the requirements and faithfully reflects the main results and conclusions of the dissertation work.

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 Sofia University "St. Kliment Ohridski", to award Boyanka Nikolaeva Angelova the educational and scientific degree "doctor" in professional area 4.3 Biological Sciences (Microbiology).

15.09.2022

Reviewer:

prof. Petya Hristova