

## **SCIENTIFIC OPINION**

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**"  
to obtain the educational and scientific degree "Doctor"  
in scientific area 4. Natural Sciences, Mathematics, and Informatics, professional field:  
4.3. Biological sciences, scientific specialty: Microbiology

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

From: **Prof. Margarita Kamburova**, DSc,  
"Stefan Angeloff" Institute of Microbiology, BAS

By order of the Rector of SU "Kliment Ohridski" No. OD38-388/13.07.2022, I am appointed as a Member of the Scientific Jury to ensure a procedure for the defense of the dissertation work of Boyanka Nikolaeva Angelova. The set of documents and materials presented by B. Angelova 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**

PhD student Boyanka Angelova graduated with a bachelor's degree in "Molecular Biology" in 2017, and a master's degree in "Microbiology and Microbiological Control" in 2019 at Sofia University "St. Kliment Ohridski", Faculty of Biology, Department of "General and Industrial Microbiology". Meanwhile, after completing her bachelor's degree in 2017, she started working as a technical assistant, and then as a microbiologist until 2019. In the period 07/15/2019-07/15/2022, she is a full-time doctoral student in the Department of General and Industrial Microbiology“, laboratory Geological Microbiology.

### **2. Actuality of the subject, aim and research tasks**

The topic of the dissertation work is dedicated to a very current environmental problem related to the implementation of the declared priorities of the EU and the Republic of Bulgaria - "Protection of the environment" and "Bio-based economy 2030", namely the problem of air pollution as the most essential risk factor for the health of the population in the European Union and in particular in our country. Although Sofia is in the 33rd place in terms of FDP pollution among 157 European cities, no legal analysis of microbial pollution has been done so far. The

relevance of the thesis is particularly evident in the analysis of the impact of pollutants on mortality from a number of diseases and life expectancy of people in large cities in the light of the data from the World Health Organization (WHO) according which more than 91% of the world's population lives in places, where air pollutants exceed WHO permissible values. The conducted quantitative analysis on the microbial presence in the central part of the city of Sofia is the first such study on a national scale and one of the longest for the territory of Europe. The obtained results have a useful practical contribution to the study of the pollution of the atmosphere over the city of Sofia and could serve for the realization of the long-term monitoring program of microbial bioaerosol contamination for the prevention of a number of chronic diseases and increasing the quality of life in Sofia.

### **3. Characteristics and evaluation of the PhD thesis and contributions**

The dissertation contains 300 pages, 44 tables, 112 figures and an appendix consisting of 27 small tables. The reference list includes 641 titles, among which 195 are from the last 10 years. The molecular-biological and microbiological work was carried out in the laboratory "Geological Microbiology" in the Department "General and Industrial Microbiology" of the Faculty of Biology, SU "St. Kliment Ohridski", the physicochemical characterization of the FDP was carried out at the Institute of Catalysis - BAS, the lidars of the Institute of Electronics, BAS were used. The doctoral thesis is written according to the standard requirements with main sections: Literature review (80 pages), Aim and objectives (1 page), Materials and methods (23 pages), Results and discussion (151 pages), divided into four sections, responding generally to the tasks set. The most important results obtained are summarized in 14 conclusions.

In the **Literature review**, a good knowledge of the problem on a global scale is demonstrated in a detailed analysis of the factors influencing microbial air pollution in big cities that drastically differ in terms of their geographical, climatic and anthropogenic characteristics. In accordance with the main hypothesis of the work, the existing reports on the metagenomic and culture-dependent studies of the bacteria, archaea and fungi in the air of large cities are considered. The review of the bioaerosol type, chemical and physical air pollutants and the standards for admissibility in the EU and Bulgaria, as well as the methods and systems for air monitoring in our country and especially in Sofia, covering only the FDP again emphasizes the need to include the biogenic factor in pollution monitoring.

The application of the rich range of methods described in the **Materials and Methods** section, including classical microbiological and modern molecular-biological methods, as well as modern physical and physico-chemical methods, demonstrates the good methodological expertise of the candidate.

The **Results and Discussion** section consists of four chapters, with each subsection usually ending with a summary of the most important achievements. Daily, weekly and monthly monitoring is carried out to fully characterize the contamination with FDP and micro-organisms. The influence of certain meteorological phenomena (fog, rain, snowfall and transboundary dust pollution) has been analysed. As a result of a huge amount of work, representatives of different phylogenetic groups of bacteria and fungi were isolated and identified from the samples of each monitoring. Variations in their representation and dominance are compared. Along with the cultural methods, the metagenomic approach is also used to characterize the phylogenetic diversity and species dominance in the samples. The last chapter presents the results of characterization of the abiotic component of the pollution by their chemical composition, structure and size.

The dissertation ends with the formulation of fourteen conclusions summarizing the most important results obtained and seven contributions that outline the importance of the work for developing a future strategy for improving air quality in the city of Sofia. Some of the conclusions may be edited, most of the contributions are original. The results of the present dissertation work are a solid start in the study of Sofia's microbial pollution with the intention of specific practical applications, typical for the long-standing practice in the laboratory "Geological Microbiology", accompanied by obtained remarkable results and good publications.

#### **4. Questions and notes to the PhD student:**

1. Based on the data obtained from the present work and the demonstrated good knowledge on the problem in other urban areas of the world, what conclusion does the doctoral student reach about the microbial pollution in the city of Sofia?
2. Has there been described in the literature a relationship between the composition and structure of FDPs and their propensity to bind to a certain type of microorganism? Is there any correlation between the size of the FDP and the number of microorganisms captured?
3. Why metagenomic analysis was performed for samples collected in May 2020 and January 2021, while for the characterization of dust pollution - in May and October?

4. Are recommendations based on these data envisaged to become known of Sofia municipality?
5. Some errors should be removed, such as: Page 230 – phylum Oxyphotobacteria. In fact, the phylum is Cyanobacteria, and Oxyphotobacteria is a class within that phylum. Petri dishes 4 and 5 in fig. 71 are repeated. The inscriptions in some figures are illegible, e.g. Fig. 88.

## **5. Evaluation of the publications**

Five publications are presented on the subject of the dissertation work, in two of which B. Angelova is first author. Among the publications, two have an impact factor in Comptes rendus de l'Académie bulgare des Sciences (Q2), one is in an online journal, and two are in conference proceedings. Additionally, she participates in eight conferences; she is first author in three of them. The Abstract of PhD thesis meets the requirements of the ZRASRB and reflects the true and complete content of the dissertation work.

## **CONCLUSION:**

The evaluated PhD thesis devoted to up-to-date topic and including the huge amount of work has served as a school for development of B. Angelova as an expert with theoretical knowledge and professional skills in the field of monitoring ecology. The work sounds contemporary, in tune with the world-wide aspiration to protect air quality in urbanized areas. The publication activity of the doctoral student exceeds the requirements reflected in the Law for the Development of the Academic Staff in the Republic of Bulgaria and the Regulations thereto regarding the criteria for obtaining the scientific degree "Doctor" for the professional field "Biological Sciences". Based on the presented arguments for the dissertation's impact and the original contributions, I give my positive assessment of the research presented in the PhD thesis and recommend the members of the scientific jury to award PhD student Angelova the scientific degree "Doctor" (PhD) in the scientific area 4.Natural Sciences, Mathematics and Informatics, Professional field 4.3 Biological sciences, scientific specialty Microbiology.

August 8, 2022

Signature:

Prof. M. Kamburova, Ph.D.