OPINION

by Prof. Petya Koycheva Hristova

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Dissertation Title: "Structural and Functional Characteristics of Soil Microbial Communities in Response to Long-Term Heavy Metal Pollution" for the award of the educational and scientific degree "Doctor" in Professional Field 4.3. Biological Sciences, scientific specialty Molecular Genetics.

Author of the dissertation: Radina Nikolaeva Nikolova

Supervisors: Assoc. Prof. DSc. Galina Radeva

Prof. DSc. Anelia Kenarova

By Order No. 143-OB/28.08.2025 of the Director of the Institute of Molecular Biology, I have been appointed as a Member of the Scientific Jury for the defense procedure of the dissertation of Radina Nikolaeva Nikolova. The submitted documents and materials meet the minimum requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria (LDASRB) and the Regulations for its implementation.

1. Brief Information about the Doctoral Candidate

Radina Nikolaeva Nikolova holds a Master's degree in Microbiology and Microbiological Control from the Faculty of Biology of Sofia University "St. Kliment Ohridski". Since 2020, she has been a regular doctoral student in Professional Field 4.3. Biological Sciences, doctoral program "Molecular Genetics" at the Institute of Molecular Biology, Bulgarian Academy of Sciences. Since 2019, she has been working as an Assistant under a permanent employment contract. Between 2017 and 2022, she was an intern in various programs at Bulgarian and foreign institutions.

2. Relevance and Significance of the Dissertation Topic

Soil pollution with heavy metals poses a serious threat to ecological balance and human health. On one hand, the presence of heavy metals in soil alters microbial diversity and the functioning of the soil microbiome. On the other hand, the physicochemical properties of soil

modify the toxicity of heavy metals and influence the dynamics of microbial communities. Thus, as a result of the interaction between heavy metal pollution and local soil characteristics, a selective pressure is formed, which determines the dominant taxa in a given soil environment. The most suitable method for studying the composition of the formed soil microbiome is metagenomic analysis. This approach allows not only an in-depth study of soil biodiversity but also the revelation of the metabolic capabilities of bacterial communities by predicting their functional potential and specific adaptive mechanisms.

A review of the scientific literature shows that to date, there are no studies in Bulgaria related to the application of a metagenomic approach for investigating the microbiome in permanently heavy metal-polluted soils. This makes the present dissertation particularly relevant and significant.

The dissertation is aimed at investigating the influence of heavy metals and specific environmental factors on the structure and function of microbial communities, applying modern molecular, bioinformatic, and statistical approaches.

3. Assessment of the Dissertation Structure

The dissertation is written on 197 standard A4 pages; the results are summarized and presented in 33 tables and 27 figures. It is structured according to the classical scheme and is balanced in terms of the volume of each section: Introduction (2 pp.), Literature Review (31 pp.), Aim and Objectives (1 p.), Materials and Methods (25 pp.), Results (53 pp.), Discussion (20 pp.), Conclusions (2 pp.), Contributions (1 p.), References (25 pp.) and Appendices (22 pp.). The appended lists of used abbreviations make a very good impression.

The dissertation is written in a good scientific style, with precise use of terminology, indicating that the doctoral candidate has an in-depth knowledge of the subject. It is necessary to note that the presented literature review offers a serious analysis of the state of the problem. The doctoral candidate has acquainted herself very well with the literature data and skillfully uses it in interpreting the results.

3.1. Literature Review

The literature review is properly structured and directly relevant to the aim of the dissertation and the set objectives. The review in the dissertation covers two main aspects of the research topic, which are, however, closely interrelated. In the first part, the doctoral candidate presents the soil microbiome as an indicator of soil health and the influence of abiotic and biotic factors on it. Local seasonal environmental factors are also reflected in this part. In the second part, an in-depth review of the microbiome of soils polluted with heavy metals is made. The approaches

for studying the microbiome, the structure and function of microbial communities in heavy metal-polluted soils are presented.

3.2. Aim and Objectives

The aims of the present dissertation are clearly formulated and include both establishing the taxonomic composition and functional activity of soil microbiomes in permanently heavy metal-polluted soils, and determining the key environmental factors that have a significant effect on the characteristics of the microbial communities.

To achieve the formulated aims, the doctoral candidate sets 6 main objectives, which include: collecting soil samples along the pollution gradient of heavy metals (zinc, lead, cadmium, copper) and the metalloid arsenic from the area of KCM 2000 near Plovdiv city; determining the physicochemical and mechanical properties of the soils, the concentration of heavy metals/metalloids and their bioavailable forms; determining the functional diversity and enzymatic activity of the soil microbial communities; determining the microbial diversity and taxonomic composition of the communities; and establishing the interrelationship between the physicochemical properties of the soils, the heavy metal concentration, and the characteristics of the microbiome.

3.3. Materials and Methods

This is an important part of the dissertation, showing the level of the conducted scientific research. A wide range of modern physicochemical, molecular-genetic, bioinformatic, microbiological, and statistical methods were used, the development and successful application of which is already an undeniable success and demonstrates the serious methodological preparation of the doctoral candidate. The multidisciplinary nature of the research has allowed the doctoral candidate to acquire new methodological and practical skills.

3.4. Results, Discussion, and Conclusions

The main results are presented according to the set objectives and the chosen methodological approaches. The experimental part is executed very precisely and presented reliably. All results are analyzed and compared with literature data in a separate section.

The research quite logically begins with soil sampling along the heavy metal concentration gradient and assessment of the physicochemical and mechanical properties of the soils. The doctoral candidate establishes that soil moisture, organic carbon, and phosphates are important for shaping the characteristics of the soil samples as they modify the effect of heavy metal intoxication on the microbial communities. Furthermore, the taxonomic profile of the soil microbiome is strongly influenced by heavy metal pollution. In this study, specific bioindicator taxa were

identified, such as *Chloroflexia*, *Gemmatimonadetes*, *Planctomycetia*, and *Bacillicladium*, for different levels of pollution. These are bacterial communities that have developed a high potential for resistance under the specific pollution conditions and form the basis for carrying out ecological processes in anthropogenically polluted soils. The doctoral candidate proves that the metabolic profiles of heterotrophic bacteria shift towards more active degradation of complex carbohydrates with the accumulation of more energy for their resistance needs. Also, the obtained data from measuring dehydrogenase and beta-glucosidase activity can serve as indicators for assessing the functional activity of the soil microbiome in soil processes under high levels of heavy metal pollution.

Based on the obtained results, 7 conclusions are formulated, which correctly reflect the essential part of the research.

4. Scientific and Applied Contributions

In the conducted research, contributions of an original and confirmatory nature can be outlined, which prove the significance of the scientific work.

I accept the contributions of the dissertation as formulated by the doctoral candidate. Of essential importance is the fact that this is the first in-depth comprehensive study of soil microbiomes in ecological niches polluted with heavy metals. This is the first documented presence of bioindicator species – *Neobacillus niacini*, *Massilia neuiana*, and *Bacillus pseudomycoides*, associated with resistance to heavy metals, and *Agromyces ramosus* for low pollution in soils from the area of KCM 2000, Plovdiv. Key taxa in the soil microbiome regarding adaptation to heavy metals were proven. Representatives of the bacterial classes *Gemmatimonadetes*, *Chloroflexia*, and *Bacilli* and the fungal class *Eurotiomycetes* were determined as resistant. Representatives of the bacterial classes *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Actinomycetia*, *Vicina mibacteria* and the fungal classes *Dothideomycetes* and *Sordariomycetes* were identified as tolerant. The most sensitive are representatives of the bacterial classes *Thermoleophilia* and *Bacteroidia* and the fungal class *Ascomycota*_cls_Incertae_sedis.

5. Participation of the Doctoral Candidate in the Preparation of the Dissertation

I believe that the execution of the dissertation work is entirely the work of the doctoral candidate.

6. Publications Related to the Dissertation

The doctoral candidate has presented a list of three scientific publications related to the topic of the dissertation and one with the published sequences. Two are in Q3 and one in Q4

journals, total IF $_{2024}$ = 2.062. Two of the articles already have 24 citations, which is evidence of the scientific value of the dissertation.

7. Abstract (Autoreferat)

The abstract is prepared according to the requirements and accurately reflects the results of the dissertation, presenting the most important elements from all sections (except the literature review) in a concise form.

8. Recommendations, Remarks, and Questions

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

9. Acquired Competence and Compliance with the Requirements for the Educational and Scientific Degree "Doctor"

In the course of performing the experimental work and preparing the dissertation, Radina Nikolova has acquired competencies and skills that can be grouped as follows:

- She has expanded her theoretical competence in a specific field of microbiology;
- She has acquired skills for working with scientific literature, analyzing and summarizing scientific information;
- She has enriched her methodological skills, particularly in the application of modern bioinformatic methods;
- She has acquired the skills to independently present and discuss the results obtained during the development of the dissertation, as well as to draw conclusions based on them.

10. Conclusion

The presented dissertation is a comprehensive and complete study that provides significant scientific information and reveals possibilities for practical application. The very good theoretical preparation has enabled the doctoral candidate to select and combine a set of methods that ensure the fulfillment of the set objectives and the achievement of the aims.

Based on the stated arguments, I evaluate positively the dissertation proposed for review, which satisfies the requirements of the LDASRB, the Regulations for its implementation, and the Regulations of the Institute of Molecular Biology, and I propose to the Scientific Jury to award Radina Nikolaeva Nikolova the educational degree "Doctor".

17.11.2025 Prepared the opinion:

Prof. Petya Hristova

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