EXPERT OPINION

By: Assoc. Prof. Dr. Trayana Nedeva, Department of General and Industrial Microbiology, Faculty of Biology, Sofia University “St. Kliment Ohridski”

Re: PhD thesis presented for defence to a scientific jury for acquiring the educational and scientific degree "Doctor"

PhD Thesis author: RADINA NIKOLAYEVA NIKOLOVA

PhD Thesis title: „Structural and functional characteristics of soil microbial communities in response to long-term heavy metal contamination”

Soil is a universal source of vital services for all ecosystems on Earth due to its importance as the largest repository of nutrients and water for plants, the most powerful regulator of gas emissions, and the main participant in the processes of circulation and recycling of biogenic elements and molecules. The negative effects of climate change, such as intense flooding or long drought periods, as well as extensive anthropogenic activities related to industry and agriculture, are leading to accelerated soil fragmentation. Urgent measures are needed to minimise these negative impacts on soil structure and protect soil health. One strategy that includes an effective set of protective measures is the use of bioindicators, which characterise fluctuations in soil health and provide valuable information that complements that gathered from its physical and chemical characteristics. Ecological studies examine various bioindicators. Among them, microorganisms have promising potential thanks to the regulated methods established by the International Organisation for Standardisation (ISO) for their use in soil quality analyses. However, only one of these methods (ISO 11063:2020) involves the application of genomic techniques for direct DNA extraction from soil. Therefore, it is necessary to acquire knowledge and practical skills in the use of metagenomic techniques to assess soil quality and condition by identifying microbial soil communities in different samples.

Soil contamination with heavy metals poses a significant risk to the environment and health, originating from both natural and anthropogenic sources, and requires effective strategies to mitigate its impact. Soil microorganisms, which are crucial for soil functions/functioning, are affected by heavy metal contamination. Microbial activity, including enzyme activity, microbial community composition and diversity, and microbial biomass/abundance, which reflect the impact of heavy metals, shows diverse microbial responses influenced by both heavy metal contamination and soil properties. In light of these facts, Mag. Radina Nikolova's research reflects current trends in the study of biodiversity and changes in the structure and function of microbial communities in soils permanently affected by heavy metal contamination. The topic of the Ph.D. thesis and the tasks formulated therein highlight the relevance of the scientific problem in the field of microbial ecology.

The Ph.D. thesis is written in a correct scientific style; the description of the experiments and the presentation of the results obtained are logical, clear, and consistent. The discussion of the experimental data is comprehensive, well-argued, and creative in the context of the available scientific information on the influence of heavy metals on microbial communities.

The literature review is structured in a way that fully reflects the Ph.D. thesis topic with an internal balance between its four subsections. It is both in-depth and specific in nature, demonstrating Mag. Radina Nikoloca's acquired skills in analysing scientific information and making scientific generalizations. This helps her formulate a clear working hypothesis, positioned in the concluding part of the review and focusing on the unresolved problems and the rational approach to testing the validity of the hypothesis.

Mag. Radina Nikolova has conducted impressive experimental work, logically linked to the continuum of sampling, physicochemical, and microbiological analyses of soils, molecular biological approaches (metagenomic analysis through sequencing at the Illumina MiSeq platform and construction of 16S rDNA clone libraries), and bioinformatic analyses for microbial communities' characterization. The information in figures indicates 37 different physicochemical, biochemical, and molecular biological analyses, structured in a logical implementation scheme that covers all research tasks in their sequence.

As a result of this combined approach, significant and valuable results have been accumulated in relation to the study of the structure and function of the soil microbiome in an industrially polluted habitat, exposed to heavy metal stress, and the assessment of this impact on microbial diversity and function through the application of modern ecological approaches and methods. This wide-ranging research, the scientific data generated by it, and its analysis and interpretation allow Mag. Radina Nikolova to propose a model for conducting a comprehensive study of the microbiome in soils contaminated with heavy metals, by identifying bioindicator bacterial species and microbial taxa along the heavy metal contamination gradient and demonstrating key microbial taxa in terms of adaptation to heavy metals.

The results are presented in 23 well-designed and high-quality figures and 17 tables. This is evidence that convincingly illustrates the experimental achievements. Overall, the Ph.D. thesis is written with skill, precision, and professionalism.

The conclusions follow the logical sequence of the main problems in the thesis. They correspond both to the scope of the research conducted and to its significance in fundamental and applied terms. Complex in nature, the seven conclusions truly reflect the achievements of the work.

The Ph.D. thesis submitted for review has undoubted merits, the most important of which are as follows:

1. The main significance of the work is the comprehensive approach applied to solving a problem of fundamental and practical importance, such as soil contamination with heavy metals. This approach comprised generating and applying a procedure for a multifaceted assessment of the microbiome in a soil matrix loaded with heavy metals and predicting, based on microbial bioindicators, the adaptive capacity of the microbiome to respond to specific abiotic stress while maintaining soil health.

2. The established model of changes in the taxonomic profile of microbial communities along the heavy metal contamination gradient, the identified microbial bioindicators, and the influence of abiotic soil factors on the toxic effect of heavy metals are original.
3. The high potential of the studied microbiome for resistance to heavy metals, recorded using biochemical and molecular-genetic approaches, which permit the implementation of basic ecological processes in anthropogenically contaminated soils, is of a contributory nature.
4. From a methodological point of view, the methodological possibility of indicating the effects of heavy metal pollution on soil microbial communities through the established bioindicator taxa should be noted as original.

Mag. Radina Nikolova's scientific achievements are summarized in three scientific publications in specialized refereed scientific journals (two with quartile Q3 and one with Q4, total IF2025 = 2.062, SJR = 0.17), in which she is the lead author. She has deposited 166 annotated, reliable 16S rRNA gene sequences in GenBank. Mag. Radina Nikolova has participated in three national and six international scientific forums, presenting three posters and six oral presentations, one of which was awarded the best presentation award. She has also presented 2 publications outside the scope of her Ph.D. thesis.

The prepared abstract fully corresponds to the content of the Ph.D. thesis and the state requirements for this type of work.

The above-mentioned facts entitle me to assess Mag. Radina Nikolova's work as a high-quality research study dedicated to an important fundamental problem with achieved results and contributions that can serve as a reliable basis for further scientific research.

The Ph.D. thesis fully meets the criteria for obtaining an educational and scientific degree of "doctor." Mag. Radina Nikolova has acquired knowledge and skills in microbial ecology: studying the structure and function of the soil microbiome in an industrially polluted habitat exposed to heavy metal stress and assessing this impact on microbial diversity and its functions. She has mastered the application of a combined scientific approach to studying these issues at the biochemical and molecular level. Through the application of specialized techniques, analysis, interpretation, and synthesis of the results, Mag. Radina Nikolova generated new scientific data and confirmed established trends while creating a model for conducting a comprehensive study of the microbiome in soils contaminated with heavy metals. Regarding the qualification descriptor independence and responsibility, she expressed an interest in modern scientific ideas, independence and creativity in their development, good theoretical background and methodological skills, and high potential for high-quality performance of various academic-style duties.

Based on the above arguments, I appreciate the Ph.D. thesis, which fully meets the requirements of the Academic Staff Development Act in the Republic of Bulgaria, the Rules for its application, and the rules of the Institute of Molecular Biology “Acad. Roumen Tsanev” – Bulgarian Academy of Sciences. Hence, I propose the respected members of the scientific jury, assigned by the Ordinance of the Director of the IMB-BAS No. 143-ОБ/28.08.2025, to award RADINA NIKOLAYEVA NIKOLOVA the educational and scientific degree Doctor.

Sofia — Assoc. Prof. Dr. Trayana Nedeva:

19.11.2025