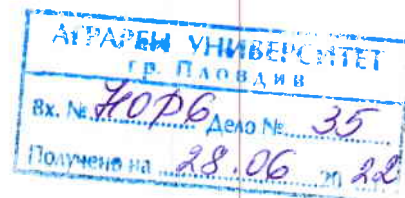


REVIEW



on a dissertation for the degree of Doctor of Science in: field of higher education 4 Natural Sciences, Mathematics and Informatics professional field 4.4. Earth Sciences, scientific specialty Ecology and Ecosystem Conservation

Author of the dissertation: Ivelina Dimitrova Neykova, full-time PhD student at the Department of Microbiology and Ecological Biotechnologies, Agricultural University-Plovdiv, Bulgaria.

Thesis: Phytoremediation of heavy metals in contaminated soil by composts and beneficial microorganisms in vegetable plants

Reviewer: prof. PhD Violina Angelova Rizova, Department of Chemistry and Phytopharmacy, Agrarian University-Plovdiv, field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field 4.4. Earth Sciences, scientific specialty "Ecology and Ecosystem Conservation" (Ecochemistry of Heavy Metals),

appointed as a member of the scientific jury by Order No. 16-611/31.05.2022 of the Rector of the Agricultural University - Plovdiv.

1. Brief introduction of the candidate.

Ivelina Dimitrova Neykova was born on 26. 06. 1988 r. She graduated with a Bachelor's degree in Plant Protection from the Agricultural University - Plovdiv in 2011. She successfully obtained a Master's degree in Business Communications and Public Relations at P. Hilendarski" – Plovdiv in 2013.

By order RD 26-19/26.02.2017 she was enrolled as a full-time PhD student in the Department of Microbiology and Ecological Biotechnologies at the Faculty of Plant Protection and Agroecology at Agricultural University-Plovdiv, with a study period of three years (01. "Immobilization of heavy metals in contaminated soils by composts and beneficial microorganisms in vegetable crops" with scientific supervisors Assoc. Prof. Dr. Stefan Shilev and Assoc. Prof. Dr. Todor Babrikov. By order RD 26-17/16.02.2017 the period of study was extended by 6 months until 1.09.2017. She was dismissed with the right to protection by order RD 26-62/13.10.2017, By order RD 26-63/13.10.2017 the topic of the dissertation was changed from "Immobilization of heavy metals in contaminated soil by composts and beneficial microorganisms in vegetable crops" to "Phytoremediation of heavy metals in contaminated soil by composts and beneficial microorganisms in vegetable crops". During her studies, she passed PhD examinations in the following subjects included in the individual training plan: 1) Ecology of microorganisms, 2) Experimental methodology and mathematical processing of collected

numerical information, 3) Databases and statistical software in agricultural sciences and practices, and 4) Methodology for PhD thesis preparation and development.

According to the presented biographical data, in the period 2015-2017 Ivelina Neykova taught the courses "Microbiology" and "Ecology of Microorganisms" on a civil contract at the Agricultural University, Department of Microbiology and Ecological Biotechnologies.

From 18.04. 2016 to 12.07.2021 he works in the company Sopharma Trading AD. From 30.09.2021 to the present she has been working as a medical representative at Sevex Pharma Ltd.

2. Relevance of the problem.

The topic of the dissertation is extremely relevant as it concerns a significant problem related to phytoremediation of heavy metal contaminated soils. Anthropogenic heavy metal pollution is a problem in many countries around the world, including Bulgaria. Increased uptake of heavy metals by plants can lead to contamination of food products and cause acute and chronic diseases in humans. Cleaning contaminated soils of heavy metals is extremely difficult. Unlike most organic pollutants, heavy metals cannot be eliminated from the environment by chemical or biological transformation. Although it is possible to reduce the toxicity of some metals by affecting their nature, their content does not decrease and they remain unchanged in the environment. Phytoremediation is an alternative to conventional technologies. It uses the natural ability of plants (and associated micro-organisms) to extract, transform and inactivate heavy metals from the soil. In Bulgaria, research in the field of phytoremediation is episodic and mainly focused on the search for hyperaccumulators or contaminant tolerant crops that can find application for non-food purposes. There is still a lack of information on the co-use of compost and populations of beneficial bacteria in the phytoremediation of heavy metal contaminated soils and on the processes occurring in the rhizosphere of vegetable plants.

All this gives me reason to define the present study as topical, significant and of scientific and applied nature.

3. Purpose, tasks, hypotheses and research methods.

The main purpose of the thesis is to investigate the possibilities of improving the growth and development of vegetable plants grown on heavy metal contaminated soil.

In order to achieve the mentioned aim, 6 tasks were set, the sequential solution of which is presented in the experimental part of the dissertation. These include the isolation of microorganisms tolerant to heavy metals, selection of isolates capable of promoting the growth of vegetable crops; the species identification of the isolates; the study of the development of vegetable crops on contaminated soil using composts and selected populations of beneficial microorganisms; the study of the behaviour of heavy

metals in contaminated soil and the study of changes in microbial communities in the rhizosphere and non-rhizosphere of the vegetable plants used. The methodological part is well planned to realize the set aims and objectives. A large amount of work has been carried out, including precisely derived laboratory experiments. A large number of indicators have been tracked and analysed with very well chosen statistical analyses.

Vascular experiments were carried out with spinach (*Spinacea oleracea* L.) cv 'Matador', radish (*Raphanus sativus* L.) cv 'Regal' and pea (*Pisum sativum* L.) cv 'Ran 1'.

4. Visualization and presentation of the results.

The dissertation is 200 pages long, well structured and balanced, and contains all the commonly accepted sections for this type of research. The research results are very well illustrated and presented in 29 tables and 61 figures. The literature cited is related to the problem under consideration and includes 508 sources, 2 of which are in Cyrillic and 508 in Latin.

The literature review corresponds to the topic and the main directions of the dissertation. The literature review is divided into subsections, which address issues related to the classification and impact of heavy metals, phytoremediation of heavy metal contaminated soils, classification of plants according to their capacity for uptake and distribution of heavy metals in their organs, application of compost in the process of phytoremediation of heavy metal contaminated soils and the role of microbial communities in the context of soil bioremediation. The conclusion at the end of the section determines the necessity of conducting the present study.

Ivelina Neykova shows a good awareness of the achievements on the problem treated in the thesis, and has developed skills for the purposeful use of previous scientific achievements in her work. The dissertation is written in a high scholarly style and reflects the doctoral student's ability to independently analyze and summarize results.

5. Discussion of results and literature used.

The results of the research, their analysis and discussion occupy an essential part of the thesis. They are presented in a well-structured logical sequence. The analysis of the results is carried out thoroughly, in a logical sequence and in a high scientific style. The PhD student shows a good theoretical background and mastery of modern methods of analysis. The literature used is well selected and gives an idea of the PhD student's level of knowledge on the issues under consideration. The comparison of the data obtained by Ivelina Neykova with those of other authors show the personal contribution to the development. All literature sources are correctly cited and described bibliographically.

The research carried out is in four main directions (i) isolation of microorganisms tolerant to heavy metals and characterization of isolates, (ii) study of plant development on soils contaminated with heavy metals (iii) study of microbial communities in the

rhizosphere and non-rhizosphere of vegetable plants and (iv) study of changes in the soil-rhizosphere-plant system.

The conclusions are consistent with the experimental results.

6. Contributions of the thesis

The dissertation contains scientific, scientific-applied and applied contributions, which in terms of content, significance and usefulness for science and practice are fully sufficient for obtaining the educational and scientific degree "Doctor".

Scientific contributions

1. A comprehensive study was conducted on the effect of compost and beneficial bacterial populations on the development, growth and heavy metal accumulation in spinach, pea and radish.
2. For the first time in Bulgaria, a study of soil microbial communities based on their metabolic profile in phytostabilization of heavy metal contaminated soils was conducted.

Scientific and applied contributions

1. The application of compost to soil contaminated with heavy metals leads to an improvement in the overall condition of the vegetable plants studied.
2. Addition of beneficial bacteria populations and compost leads to improved soil health, both in the rhizosphere and non-rhizosphere.
3. The addition of compost leads to improved soil microbial community development and reduced concentrations of bioavailable fractions of heavy metals.
4. The applicability of ecoplaques as a suitable tool to characterize the metabolic capacity of microbial communities in a given soil habitat has been demonstrated.

Applied contributions

1. Spinach can be successfully used as a test crop to detect heavy metal contamination of agricultural soils.
2. The use of quality organic additives in conjunction with populations of beneficial bacteria is a suitable, promising approach for phytostabilization of soils contaminated with heavy metals. The application of compost to soil contaminated with heavy metals leads to an improvement in the overall condition of the vegetable plants studied.

7. Critical comments and questions.

I have no significant critical comments regarding the thesis and the abstract presented by Ivelina Neykova.

I have the following questions for the PhD student:

1. One of the conclusions in the dissertation is that the approach used is a type of phytostabilization. What is this conclusion based on?
2. What are the most appropriate studies to characterize changes in soil microbial communities during phytoremediation of heavy metal contaminated soils?

8. Published articles and citations.

Ivelina Neykova has presented 4 scientific publications related to the development of her dissertation. In two of the publications she is the first author. One of the submitted publications is in a reputable journal with impact factor (Journal of chemical technology and biotechnology, 3,174). One of the publications is a published book chapter (Singh, D.P., Gupta, V.K., Prabha, R. (Eds.) Microbial Interventions in Agriculture and Environment, Volume 1: Research Trends, Priorities and Prospects, Springer Singapore). The information in these articles is fully consistent with the data presented in the thesis.

According to the Regulations for the Application of the PhD Law, the submitted publications account for 34.67 points, which exceeds the minimum requirements (30 points) for admission to the defence of a thesis for the degree of Doctor of Education and Science?

No citations of the published results are given in the prepared reference.

The presented abstract objectively reflects the structure and content of the dissertation.

CONCLUSIONS:

On the basis of the different research methods learned and applied by the PhD student, the correctly derived experiments, the made generalizations and conclusions, I consider that the presented dissertation meets the requirements of the Agricultural University Regulations for its application, which gives me the reason to evaluate it **POSITIVE**.

I take the liberty to propose to the esteemed Scientific Jury also to vote positively and to award to **Ivelina Dimitrova Neykova** the scientific degree "**Doctor**" in the scientific specialty "Ecology and Conservation of Ecosystems".

Data: 26.06.2022
Plovdiv

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