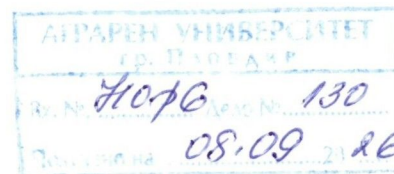


REVIEW



of a doctoral thesis submitted for the award of the academic and scientific degree of 'Doctor' in: the field of higher education 'Natural Sciences, Mathematics and Computer Science', professional area 4.4. 'Earth Sciences', scientific specialisation 'Ecology and Ecosystem Conservation'

Author of the doctoral thesis:

Maria Radkova Ikhtyarova, part-time PhD student at the Department of 'Chemistry, Phytopharmacy, Ecology and Environmental Protection' at the Agricultural University, Plovdiv

Thesis topic:

'Phytoremediation of soils contaminated with heavy metals using plants from the Asteraceae family'

Reviewer:

Prof. Dr Mariana Genova Doncheva–Boneva, Forestry University, Sofia, field of higher education 'Natural Sciences, Mathematics and Informatics', professional field 4.4. Earth Sciences, scientific specialisation 'Ecology and Ecosystem Conservation',

appointed as a member of the academic jury by Order No. RD 16-809/29 June 2026 of the Rector of the University.

1. Brief introduction to the candidate.

Maria Ikhtyarova completed her secondary education in 1999 at the 'Acad. Nikola Obreshkov' Natural Sciences and Mathematics High School in Burgas. Between 1999 and 2004, she continued her studies at the Forestry University, where she obtained a Bachelor's degree in 'Ecology and Environmental Protection', graduating with distinction. At the same time, between 2001 and 2010, he studied at the same university, graduating with a Bachelor's degree in "Forestry", qualifying as a forestry engineer. Between 2005 and 2007, she continued her studies at the University of Burgas 'Prof. Dr Asen Zlatarov', specialising in 'Ecology and Environmental Protection', where she obtained a Master's degree in Ecology, also with first-class honours.

In 2004, the PhD student took part in two seminars – 'Working with Volunteers' and 'Working with the Media' – organised by the Bulgarian Biodiversity Foundation.

His professional experience and the posts he has held are in line with his qualifications. Between 2004 and 2006, he worked as a forester at the State Hunting Enterprise in Gramatikovo. From 2006 to 2015, he held the post of design engineer at 'Agrolesproekt', where he was involved in the development of forest management projects and forestry plans. Since 2015, she has been working as a practical training

teacher at the 'N. Vaptsarov' Vocational Secondary School of Agriculture, Forestry and Tourism in Chepelare. Her professional and design experience, as well as her expertise in the field of environmental protection and forestry, are of vital importance to her teaching work, helping to develop the professional knowledge, skills and competences of her students that are necessary for the conservation and sustainable management of forest ecosystems.

She has a good command of English and Russian, and is proficient in using Microsoft Office (Word, Excel, PowerPoint).

2. Relevance of the issue.

Soil contamination with heavy metals is one of the most serious and long-term environmental problems on a global scale. Despite the measures taken over the years and the introduction of new production technologies, the problem persists.

Various approaches have been developed and used to remediate soil contaminated with heavy metals: primarily physico-chemical technologies – excavation, leaching, stabilisation and chemical immobilisation. These methods involve disruption to soil structure and biological activity. Phytoremediation is a promising approach for improving soil conditions and restoring heavily contaminated sites.

Biological reclamation of land severely disturbed and contaminated by mining and industrial activities has been practised for decades. The focus has been on using plants tolerant to adverse conditions, with the aim of successfully restoring the disturbed areas.

In this thesis, the PhD student explores an alternative approach to the restoration and utilisation of soils contaminated with heavy metals, by planting plants that accumulate the pollutants and can simultaneously be used for the production of essential oils, without posing a risk to human health.

This direction in the development of phytoremediation is not only topical but also economically viable, which makes it highly promising.

3. Aim, objectives, hypotheses and research methods.

The aim of this doctoral thesis is to carry out a comprehensive assessment of the behaviour of the heavy metals Pb, Cd and Zn within the 'soil–plant–essential oils and associated terpenoid secondary metabolites' system under conditions of real-world industrial pollution. This objective gives rise to clearly formulated tasks that are logically linked to the aim of the study: to carry out a detailed characterisation of the soils at selected sites and to assess the extent of soil contamination; to analyse the accumulation, translocation and organ-specific distribution of major micro- and macroelements in selected plants of the *Asteraceae* family; to assess the influence of the bioavailability of heavy metals on their accumulation and translocation within the plant organism; to determine the phytoremediation potential of the plants under study; to investigate the content of Pb, Cd and Zn in essential oils and to assess their safety; to analyse the influence of heavy metals on the chemical composition

and chemotypic classification of essential oils; to develop and justify an integrative conceptual model of the interaction between soil contamination with Pb, Cd and Zn and the 'soil–plant–essential oil' system.

The research thesis is well-founded and is based on existing scientific concepts regarding the behaviour of heavy metals in the 'soil–plant' system and their influence on the synthesis of secondary metabolites.

The methodological approach used in the preparation of the thesis is consistent with its objective and the tasks set. The broad scope of the scientific research required the application of numerous methods related to the study, analysis and assessment of soil conditions, as well as the analysis and assessment of the plants used for phytoremediation and the essential oils obtained from them. Specific coefficients and indices have been used to evaluate the results concerning the behaviour of heavy metals in the soil and plants. Statistical methods and correlation analyses have been applied to verify the reliability of the results and to establish interrelationships.

4. Illustration and presentation of the results obtained.

The thesis comprises 135 pages, plus a bibliography containing 241 titles and five appendices, totalling 158 pages. The thesis is structured logically in accordance with its aim and the research objectives set, with a good balance between the individual sections: Introduction – 1.5 per cent; Chapter 1 – Literature Review – 18.5 per cent; Chapter 2 – Aim, Objectives and Working Hypotheses – 1.5 per cent; Chapter 3 – Materials and Methods of the Experimental Study – 13.3 per cent; Chapter 4 – Results and Discussion – 62.2 per cent; Chapter 5 – General Conclusions, Findings, Contributions and Recommendations – 3.0 per cent.

In the thesis, the results of the study are presented in appropriate tables – 21 in total – and are well illustrated with figures and interesting, informative diagrams (11 in total).

5. Discussion of the results and literature reviewed.

The results of the study cover various scientific fields. The thesis includes results, analyses and assessments of:

- soil conditions – mechanical composition; mineral composition; acidity and content of carbon, nitrogen, phosphorus, calcium, potassium and magnesium; content of total forms of copper, iron, manganese, lead, cadmium and zinc; content of mobile forms of lead, cadmium and zinc.
- the elemental composition, accumulation and distribution of the elements studied in various organs of the plants selected for the experiment – content of microelements (lead, cadmium, zinc, copper, iron and manganese) and macronutrients (phosphorus, potassium, calcium, magnesium) in various plant organs of the family *Asteraceae*;
- the phytoremediation potential and tolerance of the plants to heavy metals;

- the content and composition of the essential oils extracted from the plants used in the study – the behaviour of lead, cadmium and zinc in the essential oils;
- the effect of heavy metals on the terpene profile and the chemotypic classification of the plant species.

The PhD student demonstrates an ability to interpret the results obtained, having carried out in-depth analyses, identified links and relationships between the individual indicators, and applied appropriate criteria for evaluating the data obtained. The discussion of the results is carried out in a coherent and well-reasoned manner, in line with the stated objective. High praise is due to the proposed conceptual model, which provides a clear synthesis of the results of the entire study. The study highlights the interrelationships between soil contamination, the accumulation of heavy metals in plants and their limited transfer to essential oils, as well as which of the species studied can combine phytoremediation potential with the production of essential oils.

Although the literature used is extensive, the literature review cites a relatively small number of sources – around 30 per cent.

6. Contributions of the thesis.

The thesis contains scientific and applied scientific contributions that complement existing scientific research in the field of phytoremediation and the behaviour of certain heavy metals in the soil–plant–essential oil system.

Scientific contributions

1. A clearly expressed species- and organ-specific accumulation of lead, cadmium and zinc has been established in plants of the *Asteraceae* family grown under conditions of industrial pollution.
2. Correlations have been established between the accumulation of lead, cadmium and zinc and the distribution of micro- and macroelements in the various plant organs, which is significant for the mineral nutrition and adaptation of plants under conditions of heavy metal pollution.
3. A classification of the plant species studied has been developed based on their ability to uptake, translocate and accumulate heavy metals, enabling a comparative assessment of their phytoremediation potential.
4. A conceptual model of a 'double selective filter' has been developed, involving the combined action of physiological barrier mechanisms and the selectivity of the distillation process, which explains the limited transfer of heavy metals into the essential oils produced by these plants.
5. It has been established that contamination with lead, cadmium and zinc does not alter the chemotypic composition of the essential oils, but is associated with quantitative changes in the terpene profile without any loss of chemical identity.

Scientific and applied contributions

1. Plant species from the family *Asteraceae* with potential for phytoremediation have been identified, providing a basis for their application in technologies for

- the stabilisation and/or extraction of heavy metals from contaminated soils.
2. The safety of essential oils extracted from plants grown on soils contaminated with heavy metals has been assessed, and it has been established that high concentrations of lead, cadmium and zinc in the soil and plant biomass do not lead to contamination of the final product.
 3. Practical guidelines have been proposed for the use of plants from the *Asteraceae* family on contaminated sites, combining ecological soil restoration with the production of pharmacognostically valuable products.
 4. A practical conceptual model has been proposed for assessing the risk and potential of plant systems in contaminated environments, which can be used as a methodological basis for planning ecological, agro-ecological and phytotechnological interventions.

7. Critical comments and questions.

I have the following comments and recommendations for the PhD candidate:

1. The literature review provides a good introduction to the issue under consideration, but a relatively small number of authors from those listed in the bibliography have been cited. I would recommend that the PhD student, given her sound knowledge of the literature, include most of the authors from the bibliography in the literature review.
2. Very few Bulgarian authors are included in the bibliography, yet studies on heavy metal soil contamination and phytoremediation in areas surrounding mining and metallurgical processing plants in Bulgaria have been conducted for decades. I recommend that the PhD candidate familiarise herself with the works of these authors in her future work.
3. In the 'Results' chapter, many authors are cited; in some cases, these clarify the results obtained by the PhD candidate. In other cases, the citations are not linked to specific data from the studies conducted – e.g. section 4.2.6.3. and others.

I have the following questions for the PhD candidate:

1. When preparing the plant samples, it is stated that the individual fractions were washed with water. Is there not a risk that some of the macronutrients might be leached out during washing – e.g. potassium? Will this affect your results?
2. What quantity of essential oil is obtained from 1 tonne of above-ground biomass from the plants you have studied?
3. Do you have any information on the uses of the essential oil from the species you have studied?
4. What is the economic impact of essential oil extraction when cultivating the plants with a high phytoremediation rating that are the subject of the study?
5. Wormwood and white yarrow are characterised by a high capacity for accumulation and tolerance to heavy metals; how should the above-ground biomass from these plants be handled if there is no interest in essential oil production?

6. How should the biomass remaining after distillation be treated?

8. Published articles and citations.

PhD student Maria Ikhtyarova has submitted five scientific papers relating to her thesis (one in press), which were developed in collaboration with her supervisor.

The articles have been published in prestigious journals – four of which are indexed in Web of Science, including two in the Q4 category (Scientific Papers. Series E. Land Reclamation, Earth Observation & Surveying, Environmental Engineering), one in Agricultural Sciences/Agrarni Nauki and one in the proceedings of the 20th International Symposium on Agriculture in Croatia. One of the articles was published in Scientific Works of the Agricultural University, Plovdiv. The publications are thematically linked to the research in the doctoral thesis and reflect specific aspects thereof.

The publications meet the requirements for the award of the academic and scientific degree of 'Doctor'.

The doctoral candidate has provided a list of four citations of two of the publications, which demonstrates interest from researchers both in Bulgaria and abroad and confirms the significance of the results obtained from the study.

The abstract submitted objectively reflects the structure and content of the doctoral thesis.

CONCLUSION:

On the basis of the various research methods studied and applied by the PhD candidate, the correctly conducted experiments, and the generalisations and conclusions drawn, I consider that the thesis presented meets the requirements of the Law on Scientific Research and Academic Degrees and the Regulations of the Agricultural University governing its application, which gives me grounds to assess it **POSITIVELY**.

I would like to propose that the esteemed Academic Jury also vote in favour and award *Maria Radkova Ikhtyarova* the academic and scientific degree of '**Doctor**' in the scientific discipline of 'Ecology and Ecosystem Conservation'

Date: 31.07.2026
Plovdiv

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