REVIEW

by **Prof. DSc. Krasimir Ivanov Ivanov**, Department of General Chemistry, Agricultural University of Plovdiv (currently retired), on a dissertation submitted for the award of the educational and scientific degree **Doctor (PhD)**.

Pursuant to Order No. **RD-16-809 dated 29 June 2026** of the Rector of the Agricultural University of Plovdiv (AU), Prof. Boryana Ivanova, PhD, I was appointed as a member of the Scientific Jury for the procedure concerning the public defense of the dissertation entitled**"Phytoremediation of Heavy Metal Contaminated Soils Using Plants of the Asteraceae Family"** for the award of the educational and scientific degree **Doctor (PhD)** in the field of higher education: **4. Natural Sciences, Mathematics and Informatics**, professional field: **4.4. Earth Sciences**, scientific specialty: **Ecology and Ecosystem Protection**.

The author of the dissertation, **Maria Radkova Ihtyarova**, is a part-time doctoral student in the Department of **Chemistry, Phytopharmacy, Ecology and Environmental Protection** at the Faculty of Plant Protection and Agroecology, Agricultural University of Plovdiv, under the supervision of **Prof. Violina Angelova, PhD** and **Assoc. Prof. Stefan Krustev, PhD**.

1. General Presentation of the Submitted Materials

The set of materials submitted by the doctoral candidate complies with Article 48(1) of the Regulations for the Implementation of the Academic Staff Development Act at the Agricultural University of Plovdiv. The dissertation consists of **148 pages** and contains **21 tables** and **11 figures**. The cited literature comprises **234 references in Latin script** and **7 references in Cyrillic script**. The abstract is **41 pages** long and accurately reflects the research conducted and the results obtained.

Candidates seeking the educational and scientific degree **of Doctor (PhD)** must meet the minimum national requirements established in the Regulations for the Implementation of the Academic Staff Development Act in the Republic of Bulgaria, as amended by Council of Ministers Decree No. 26 of 13 February 2019. The Agricultural University of Plovdiv has not introduced any additional requirements for obtaining the Doctor (PhD) degree. The list of publications submitted by doctoral candidate Ihtyarova demonstrates that she exceeds the minimum requirements for the degree sought.

2. Brief Biographical Information about the Candidate

Doctoral candidate **Maria Ihtyarova** possesses an extensive professional background and scientific and professional qualifications across a broad range of activities within the professional field **4. Earth Sciences**. In 2004, she graduated from the **University of Forestry in Sofia**, earning a Bachelor's degree in **Ecology and Environmental Protection**. In 2010, she earned an additional Bachelor's degree in **Forestry Engineer**ing from the same university. In 2007, she obtained a Master's degree in **Ecology and Environmental Protection** from **Prof. Dr Assen Zlatarov University, Burgas**.

Her professional experience includes a variety of activities, including work as a **forester** (planning and reporting on forestry activities), a **design engineer** (developing forest management projects and forestry plans), and **a practical training teacher** at the Nikola Yonkov Vaptsarov Vocational High School of Forestry and Woodworking in Chepelare.

In 2017, she was enrolled as a part-time PhD student at the Department of **Chemistry, Phytopharmacy and Ecology and Environmental Protection**. In 2023, she was officially released with the right to defend her dissertation.

I have no personal impressions of the doctoral candidate's work, and my assessment is based exclusively on the results presented in the dissertation under review.

3. Evaluation of the Dissertation

The topic of the dissertation submitted by the doctoral candidate is a continuation of the traditional research conducted by the Department of **Chemistry, Phytopharmacy and Ecology and Environmental Protection** at the Agricultural University of Plovdiv in the field of **phytoremediation and phytostabilisation techniques**. The seven research objectives formulated by the author logically follow from the literature review. They are subordinated to the overall aim of conducting a **comprehensive assessment of the behaviour of the heavy metals Pb, Cd, and Zn within the "soil-plant-essential oils" system under conditions of anthropogenic contamination**. This was achieved by investigating their bioavailability in soil, accumulation and translocation in plants, evaluating the phytoremediation potential of the studied species, and analysing their influence on the chemical composition, quality, and safety of the essential oils obtained.

The relevance of such studies is beyond dispute, as they have been identified as a priority area in numerous national and European Commission strategic documents. Since the beginning of 2020, environmental protection issues and their solutions have become increasingly prominent topics in Bulgaria and have remained among the leading subjects in the public information space.

Literature Review

The literature review may conventionally be divided into two parts and encompasses **307 scientific references**, with emphasis placed on publications from the last decade.

In the **first part**, the author analyses issues related to heavy metal contamination and its effects on environmental protection and human health. A comprehensive review is presented of the sources of heavy metal pollution, including those occurring in Bulgaria. Special attention is given to the **bioavailability of heavy metals**, a key characteristic that determines their environmental behaviour, their impact on living organisms, and their transfer within the **soil-plant-final product** chain.

The **second part** of the literature review focuses on **phytoremediation** as a biologically based approach for the management, containment, and restoration of contaminated soil, water, and sediment ecosystems. Considerable attention is devoted to plant responses to heavy metal contamination and to the classification of plants by their heavy-metal accumulation capacity and tolerance, with particular emphasis on **hyperaccumulator species**. The review of scientific

information on plants in the ***family Asteraceae*** demonstrates that its representatives exhibit high ecological plasticity, good adaptability to heavy metal contamination, and include economically important species. This provides sufficient justification for the investigations conducted within the dissertation.

The analysis of the most recent scientific literature has led to the logical conclusion that an **integrated approach** is needed, combining the soil geochemical characteristics of contaminated environments, the mechanisms of metal accumulation and distribution within plants, and the changes occurring in secondary plant metabolites. This conclusion is convincingly supported by the methodological approach adopted in the dissertation. It has resulted in a noticeable advancement in the quality of research conducted in this scientific field at the Department of Chemistry, Phytopharmacy and Ecology and Environmental Protection.

Experimental Design

The experimental design includes a model system to study the behaviour of heavy metals within the **soil-plant-final product** system under conditions of actual industrial pollution in the vicinity of the **KCM-Plovdiv** industrial complex.

Two experimental sites were selected in proximity to each other and to the contamination source. They were characterised by similar geographical, climatic, and agroecological conditions but differed in their degree of Pb contamination. The objective was to conduct a comparative analysis of heavy metal behaviour and plant responses across different pollution levels while minimising the influence of external factors.

To some extent, this concept is undermined by the insufficiently justified selection of plant species cultivated on Soils 1 and 2. Nevertheless, the control site was located at an adequate distance from the pollution source, and preliminary investigations confirmed the absence of additional contamination.

The selected representatives of the ***Asteraceae*** family were: *Achillea millefolium* L. (Yarrow), *Artemisia absinthium* L. (Wormwood), *Artemisia dracunculus* L. (Tarragon), *Tanacetum balsamita* L. (Costmary) and *Tanacetum vulgare* L. (Tansy).

These species are characterised by their ability to grow in technogenically disturbed habitats and to accumulate varying amounts of heavy metals in both aboveground and belowground biomass, making them suitable for evaluating species-specific responses to industrial contamination.

Experimental Procedures and Analytical Methods

The experiments were conducted in accordance with generally accepted principles of reproducibility and representativeness. Established **ISO standards** were applied for sampling, sample preparation, and analytical measurements. A notable aspect of the work is the extensive use of modern analytical methods alongside classical approaches commonly employed in accredited laboratories.

The mineralogical composition of soils was determined using **X-ray diffraction (XRD)** based on reference data from international databases (**ICDD-PDF**). Total concentrations and mobile (bioavailable) forms of heavy metals were analysed using **Atomic Absorption**

Spectrometry (AAS) and/or **Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)**. In addition to Pb, Cd, and Zn, the contents of major macroelements (Ca, Mg, K, and P) and microelements (Fe, Mn, and Cu) were determined.

The same analytical techniques were applied in the examination of plant material. The analytical workflow concluded with the isolation and characterisation of essential oils using **hydrodistillation with a Clevenger apparatus**, followed by **Gas Chromatography-Mass Spectrometry (GC-MS)**, AAS, and ICP-OES analyses.

Assessment of Heavy Metal Transfer and Phytoremediation Potential

To evaluate heavy metal bioavailability, soil contamination levels, and the transfer of Pb, Cd, and Zn from soil to plants and subsequently to different plant organs, the author employed a combined assessment framework incorporating both bioavailable metal fractions and widely used geochemical and biological indices, including: Bioavailability Coefficient, Contamination Factor (CF), Geoaccumulation Index (Igeo), Pollution Load Index (PLI), Nemerow Pollution Index (NPI), Bioconcentration Factor (BCF), Translocation Factor (TF) and Enrichment Factor (EF).

The combined use of these indices enabled a reliable assessment of soil contamination status, an analysis of heavy metal transfer to plants, and, ultimately, a well-justified evaluation of the phytoremediation behaviour of the investigated plant species.

This is the section where the most significant advancement beyond previous studies conducted at the Agricultural University in this field has been achieved.

Statistical Analysis

To assess the reliability, reproducibility, and significance of the results and to examine relationships among variables within the **soil-plant-essential oil** system, appropriate statistical methods were applied.

Obtained Results

The results obtained provide clear answers to all questions raised in the literature review and fully address the objectives and tasks set in the dissertation. In general, they may be grouped into three major categories.

1. Analytical Results

A. Soil Samples

A detailed analysis of **Soil 1** and **Soil 2** was conducted from nearly all possible perspectives. The results presented in Tables 4.1.1 and 4.1.2 indicate that the soils are **alluvial-meadow and deluvial-meadow**, formed on carbonate alluvial deposits and characterised by the dominant presence of calcium and magnesium cations within the soil sorption complex. The neutral-to-slightly alkaline soil reaction, together with the presence of carbonates, organic matter, and Fe-Mn oxides, creates a significant geochemical buffering capacity that restricts the mobility of heavy metals and promotes their long-term retention within the soil system.

An enormous volume of data has been presented, which complicates the interpretation of the results. Some of the data on the roles of macro- and microelements in soil are extremely

interesting and useful; however, they do not substantially support the main conclusions and overload the principal concept with unnecessary details. The interactions of macro- and microelements with heavy metals in soils and plant organs, as well as their roles in stress compensation mechanisms, could serve as the basis for a separate dissertation in which the physiological aspect is examined in greater depth.

B. Plant Samples

Table 4.2.1 presents the concentrations of **Pb, Cd, and Zn** in the organs of the studied plant species. Comparative analysis reveals a distinct gradation in the behaviour of Pb, Cd, and Zn with respect to mobility, bioavailability, and physiological effects on plants. Clear differences are observed in the accumulation of these elements among plant organs, indicating well-defined patterns in mobility, accumulation potential, and physiological impact. These patterns result from species-specific mechanisms of uptake, transport, and detoxification.

The species analysis clearly distinguishes the accumulator species ***Artemisia absinthium*** (wormwood) and ***Achillea millefolium*** (yarrow) from ***Artemisia dracunculus*** (tarragon), which is characterised by limited accumulation and restricted translocation of metals to aboveground organs.

Table 4.2.2 presents the predominant accumulation patterns of Pb, Cd, and Zn in plant organs, which allowed the author to classify the investigated species as follows: **Accumulator:** *Artemisia absinthium* (wormwood), **Selective accumulator:** *Achillea millefolium* (yarrow), **Intermediate type:** *Tanacetum vulgare* (tansy) and *Tanacetum balsamita* (costmary) and **Restrictive type:** *Artemisia dracunculus* (tarragon)

A clear organ-specific and species-specific pattern of macroelement and microelement uptake was also established (Tables 4.2.3 and 4.2.4). It was found that, unlike the leaves, the stems do not serve as the primary storage compartment for toxic elements.

C. Flowers and Essential Oils

Essential oils represent a specific plant matrix in which the concentration of heavy metals is not a direct function of their concentration in the plant material from which the oils are obtained. This is clearly demonstrated by the results, which reveal a pronounced contrast between the high concentrations of Pb, Cd, and Zn detected in the roots, leaves, and flowers of the investigated species and the substantially lower concentrations measured in the corresponding essential oils.

A well-reasoned explanation has been provided for each of the observed phenomena associated with the individual elements. This finding represents an important pharmacological and practical result and constitutes one of the principal arguments supporting the study's usefulness and significance.

2. Analysis and Interpretation of the Analytical Results

The modern and reliable analytical instrumentation employed in the study provides sufficient grounds for accepting the obtained results with full confidence. The interpretation of the results has been carried out in depth and demonstrates an excellent command of the scientific literature.

The complexity of biological processes always leaves room for additional questions, and I expect that future research by the team will further develop these findings by involving a plant physiologist, particularly in interpreting certain physiological responses.

3. Conclusions and Findings

The results obtained enabled the author and her supervisors to formulate **nine conclusions** grouped into three principal areas.

1. Soils in the KCM-Plovdiv Area

In this area, it is difficult to identify entirely new findings given the large number of prior investigations. Nevertheless, such studies are essential for the correct interpretation of the results and for assessing the potential transfer of contaminants within the **soil-plant system**.

2. Plants

These investigations are entirely original and demonstrate organ-specific accumulation of Pb, Cd, and Zn in the studied species of the ***Asteraceae*** family. The roots function as the primary barrier structure, whereas the leaves and flowers serve as the principal organs of accumulation.

It was established that the accumulation of heavy metals in plant biomass does not substantially increase their concentrations in essential oils or alter the chemotypic characteristics of the oils. Instead, it leads only to quantitative changes in the terpene profile.

3. Phytoremediation Potential

The investigated species occupy different positions along the gradient between high accumulation potential and restricted metal accumulation. Specifically:

- ***Artemisia absinthium*** (wormwood) exhibits the highest accumulation potential.
- ***Achillea millefolium*** (yarrow) demonstrates intermediate behaviour.
- Species of the genus ***Tanacetum*** (costmary and tansy) predominantly retain metals within the root system.
- ***Artemisia dracunculus*** (tarragon) effectively limits the uptake and accumulation of heavy metals.

Contributions of the Dissertation

The contributions of the dissertation are indisputable, and some of them can be considered pioneering for Bulgaria. They may be grouped into two categories.

1. Scientific Contributions

The dissertation advances scientific knowledge of plants in the **family Asteraceae** through a systematic research approach. A functional classification of the investigated species has been developed according to their capacity for heavy metal uptake, translocation, and accumulation.

2. Applied Contributions

It has been demonstrated that ***Artemisia absinthium*** (wormwood) and ***Achillea millefolium*** (yarrow) possess potential for application in phytoremediation technologies for soils contaminated with Pb, Cd, and Zn. Furthermore, the safety of essential oils from plants cultivated under heavy-metal-contaminated conditions has been evaluated. I expect these contributions to attract considerable attention both in the scientific literature and, hopefully, in practical remediation activities.

4. Assessment of the Candidate's Personal Contribution

The doctoral candidate's publication activity comprises **five full-text scientific papers**, four of which have already been published, two of which are in journals ranked in **the Q4 quartile**. A fifth paper has been accepted for publication and is also assigned to the **Q4 quartile** (*Scientific Papers, Series E: Land Reclamation, Earth Observation & Surveying, Environmental Engineering, Vol. XV, 2026*). In two of these publications, Ms Ihtyarova is the first author, while in three she is the second author.

Two of the publications, including one in which Ms Ihtyarova is the first author, have received a total of **five citations**, four of which are from foreign authors.

Doctoral candidate Ihtyarova has also participated in **three scientific conferences**, at which portions of the dissertation results were presented. All this provides sufficient evidence for me to conclude that her contribution to the planning and execution of the dissertation research, the interpretation of the results, and the presentation of the results in scientific publications has been substantial.

5. Critical Remarks and Recommendations

In terms of scope, scientific justification, experimental Implementation, and originality of the results, the submitted dissertation exceeds the requirements for the award of the educational and scientific degree **Doctor (PhD)**.

I recommend that the doctoral candidate and her supervisors exercise greater precision in the planning of future experiments. In future studies focusing on interactions among macro- and microelements and heavy metals, as well as physiological stress under conditions of severe heavy metal contamination, greater attention should be paid to the selection and analysis of background samples.

Conclusion

The dissertation submitted for review fully complies with all the requirements of the **Academic Staff Development Act in the Republic of Bulgaria**, the **Regulations for its Implementation**, and the **Regulations of the Agricultural University of Plovdiv**. In terms of both scope and quality of the research, it exceeds the generally accepted criteria for a doctoral dissertation.

The development of this dissertation has contributed substantially to establishing Ms Ihtyarova as an independent researcher capable of obtaining, analysing, and presenting original

scientific results. This provides sufficient grounds for me to give a **positive evaluation** of the research conducted and confidently recommend that the esteemed Scientific Jury award the educational and scientific degree of **Doctor (PhD)** to **Maria Radkova Ihtyarova** in the field of higher education **4. Natural Sciences, Mathematics and Informatics**, professional field **4.4. Earth Sciences**, scientific speciality: **Ecology and Ecosystem Protection**.

05 August 2026

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