AGRICULTURAL UNIVERSITY – PLOVDIV

FACULTY OF PLANT PROTECTION AND AGROECOLOGY

DEPARTMENT OF CHEMISTRY, PHYTOPHARMACEUTICALS AND ENVIRONMENTAL PROTECTION

MARIA RADKOVA IHTYAROVA

Phytoremediation of Heavy Metal-Contaminated Soils Using Species of the Family *Asteraceae*

ABSTRACT

of a dissertation for the acquisition of the educational and scientific degree "Doctor" in the scientific specialty " Ecology and Ecosystem Protection ", professional field 4.4.

Scientific leaders

PROF. Dr. VIOLINA ANGELOVA

ASSOC. PROF. DR. STEFAN KRASTEV

Plovdiv, 2026

The dissertation is 148 pages long and contains 21 tables and 11 figures. The cited literature includes 234 sources in Latin and 7 in Cyrillic.

The dissertation work was discussed at an extended departmental council of the Department of Chemistry, Phytopharmacy and EOOS at the Faculty of Plant Protection and Agroecology of the Agricultural University - Plovdiv.

The defense of the dissertation will take place on 06.10.2026 at 13.00 hours in hall 114 of the Faculty of Plant Protection and Agroecology, at an open meeting of the scientific jury, approved by Order of the Rector RD--16-809 of 29.06.2026.

Reviews by: prof. dr. Mariana Genova Doncheva-Boneva
prof. dr. Krasimir Ivanov Ivanov
Opinions from: prof. dr. Diana Atanasova Kirin
prof. dr. Ekaterina Dimitrova Pavlova
doc. dr. Penka Stancheva Zaprianova

The materials for the defense are available to those interested in the library of the Agricultural University - Plovdiv, 12 Mendeleev Blvd.

Please send feedback and comments to: violina@au-plovdiv.bg

1 Introduction

Anthropogenic soil pollution with heavy metals is one of the most serious and long-term environmental problems on a global scale. It affects both industrially developed and agrarian regions, and in Bulgaria, the territories located near metallurgical enterprises, mining complexes and industrial zones are of particular risk. In these areas, prolonged technogenic load is observed, which leads to the accumulation of elements such as Pb , Cd and Zn in the soil profile. Due to their high chemical stability, lack of biological degradation and ability for long-term retention in the soil, heavy metals have a chronic impact on ecosystems.

The accumulation of heavy metals in soils leads to significant disruptions in their ecological functions, including a decrease in microbiological activity, deterioration of soil fertility and changes in the chemical balance. A particularly significant problem is the transfer of metals to plants and their inclusion in trophic chains, which creates a risk to animal and human health. In this regard, the bioavailability of metals is considered a key indicator of the real ecological hazard, since it determines the degree of their absorption by living organisms.

remediation methods (excavation, soil washing, stabilization and chemical immobilization) are effective, but are associated with high costs, technological limitations and the risk of soil structure disruption. This necessitates the development of alternative, sustainable and environmentally compatible approaches for the restoration of degraded terrains.

Phytoremediation is established as a promising technology based on the ability of plants to extract, stabilize or limit the mobility of pollutants in the soil environment. The method is characterized by a relatively low cost, the possibility of application in situ and preservation of natural ecosystem processes, making it particularly suitable for large-scale and mildly to moderately contaminated areas. The effectiveness of phytoremediation depends on the complex interaction between soil characteristics, metal bioavailability and the biological characteristics of the plant species used.

The selection of suitable plants with high tolerance to heavy metal pollution, ability to accumulate or stabilize metals and adaptability to changing conditions is essential. Of increasing interest are representatives of the *Asteraceae* family , which are distinguished by high ecological plasticity and significant species diversity in terms of mechanisms for accumulation and translocation of heavy metals. An additional advantage is the ability of some species to synthesize biologically active secondary metabolites with economic and pharmacognostic value.

There are insufficiently understood relationships between the bioavailability of Pb , Cd and Zn in soil, their accumulation and transfer in *Asteraceae* plants and their subsequent influence on the composition of secondary metabolites. Integrated field studies that simultaneously cover soil geochemistry, plant metabolism and the chemical profile of essential oils under real conditions of industrial pollution are lacking.

The present dissertation work is aimed at clarifying the interrelationships in the "soil - plant - essential oil" system, including the influence of soil pollution on the accumulation of heavy metals and on the chemical composition of terpene compounds. The aim of the study is to evaluate the possibilities for combined application of essential oil plants from the Asteraceae family as phytoremediators and as a source of secondary products with preserved quality and safety.

The scientific novelty of the study lies in the integrated assessment of the interrelationships between the bioavailable forms of Pb , Cd and Zn in the soil, their accumulation and transfer in plants of the Asteraceae family and their influence on the quantitative and qualitative composition of essential oils under field conditions of industrial pollution.

2 Purpose, objectives and working hypotheses of the study

The main goal of the dissertation is to perform a comprehensive assessment of the behavior of Pb , Cd and Zn in the "soil - plant - essential oil" system under conditions of technogenic pollution through analysis of their bioavailability, accumulation and translocation, determination of the phytoremediation potential of the studied plants and assessment of the quality and safety of the obtained essential oils.

To achieve the goal, the following sequential tasks have been set:

1. To conduct a detailed characterization of the soils from selected sites by determining the main physicochemical and agrochemical indicators, the content of macro- and microelements, as well as the total content and bioavailable forms of Pb , Cd and Zn , and to assess the degree of soil contamination.

2. To analyze the accumulation, translocation and organ-specific distribution of heavy metals (Pb , Cd and Zn), as well as essential micro- and macroelements in selected plants of the Asteraceae family .

3. To assess the influence of the bioavailability of heavy metals on their accumulation and translocation in the plant organism.

4. To determine the phytoremediation potential of the studied plants.

5. To investigate the content of Pb , Cd and Zn in essential oils and to assess their safety.

6. To analyze the influence of heavy metal pollution on the chemical composition and chemotypic affiliation of essential oils.

7. To develop and substantiate an integrative conceptual model of the interaction between soil pollution with Pb , Cd and Zn and the “soil – plant – essential oil” system.

The working hypotheses are formulated based on the analysis of literature data and existing scientific concepts about the behavior of heavy metals in the "soil - plant" system and their influence on the synthesis of secondary metabolites.

The present study is based on the following working hypotheses:

1. The accumulation of Pb , Cd and Zn in plants is more closely related to the bioavailable forms of the metals in the soil than to their total content.
2. The accumulation and transfer of heavy metals in the plant organism are species- and organ-specific processes, determined by the physiological and morphological characteristics of plants.
3. With increased soil contamination with Pb , Cd and Zn, no linear relationship is observed between their total content in the soil and their concentration in essential oils, due to selective physiological barriers and limited transfer of metal ions to the volatile fraction.
4. Exposure to Pb , Cd and Zn leads to quantitative changes in the terpene profile of essential oils, associated with induced physiological and biochemical adaptation mechanisms, and these changes do not necessarily lead to a change in the chemotypic affiliation of the species.
5. Some of the studied species from the Asteraceae family exhibit a simultaneous ability to accumulate Pb , Cd and Zn in aboveground biomass and to synthesize essential oils that meet pharmacopoeial requirements for quality and safety.

3 Material and methods of the experimental study

3.1. Study area

The study was conducted in the area of "KCM" AD - Plovdiv, which represents one of the longest-operated industrial sources of heavy metal pollution in Bulgaria. The main activity of the enterprise is related to the production and processing of lead and zinc, which causes increased concentrations of Pb , Cd and Zn in the environment.

The experimental plots are located approximately 0.5 km from the industrial complex in an area with pronounced anthropogenic load. The soils in the area function as a long-term accumulator of heavy metals as a result of prolonged atmospheric deposition of dust emissions. The territory is characterized by a flat relief, typical of the Upper Thracian Lowland. The studied area was chosen as a model system for assessing the behavior of heavy metals in the "soil–plant–essential oil" system under conditions of real industrial pollution.

3.2. Experimental design

The study was conducted as a field experiment on industrially influenced soils in the area of KCM – Plovdiv. Two test sites with different degrees of technogenic pollution were selected, characterized by different content and bioavailability of Pb , Cd and Zn , but with similar soil and climatic conditions. An area in the Training and Experimental Field of the Agricultural University – Plovdiv, characterized by the absence of direct industrial impact, was used as a control plot.

The study included five aromatic and medicinal species from the Asteraceae family : *Achillea millefolium* L. , *Artemisia absinthe* L. , *Artemisia dracunculus* L. , *Tanacetum balsam* L. and *Tanacetum vulgar* L. Sampling of soils and plant material was carried out in the flowering or early flowering phase. Five replications were set for each experimental combination. The scheme of the experimental design is presented in Figure 1.

3.3. Soil samples and soil chemical analysis

Soil samples were taken from the surface soil layer (0–20 cm) according to ISO 18400-102:2017. For each replicate, composite samples were formed by mixing subsamples taken from evenly distributed points within the respective area.

Sample preparation was performed according to ISO 11464:2006 and included air drying, crushing, sieving through a sieve with a mesh size of less than 2 mm and homogenization. The resulting fraction (< 2 mm) was used for subsequent analyses.

The main physicochemical and agrochemical parameters were determined – pH , organic matter content, total nitrogen and mechanical composition. The mineral composition was determined by X-ray diffraction analysis (XRD).

The total (pseudototal) content of Pb , Cd and Zn was determined after extraction with aqua regia (HCl : HNO3 = 3:1) according to ISO 11466:1995. The bioavailable forms of the metals were determined by DTPA -extraction according to the method of Lindsay and Norvell (1978). The concentrations of the elements were measured by inductively coupled plasma optical emission spectrometry (ICP - OES). The analytical procedures were performed under quality controlled conditions, using calibration standards and procedures to control the accuracy and precision of the measurements. The limits of detection and quantification for each element were determined experimentally for the respective matrices.

Figure 3.1. Scheme of the experimental design of the study, including the distribution of plant species from the *Asteraceae family* on two sample plots with varying degrees of industrial pollution in the area of KCM - Plovdiv, and the control plot used.

To assess the degree of contamination and bioavailability of metals, bioavailability coefficient (Availability), contamination coefficient (CF), geoaccumulation index (Igeo), loading index (PLI) and Nemerow pollution index (NPI) were calculated.

3.4. Plant material and analysis

The plant material was obtained from plants grown under field conditions on the experimental plots. After sampling, the plants were separated into roots, stems, leaves and inflorescences, washed, dried to constant mass, ground and homogenized.

The content of Pb , Cd , Zn , Fe , Mn , Cu , Ca , Mg , K and P was determined after microwave mineralization with HNO_3/ H_2O_2 by ICP - OES . The obtained results were used to assess the accumulation and distribution of metals in different plant organs.

To assess the uptake and transfer of metals in the soil-plant system, bioconcentration factor (BCF), translocation factor (TF), biological accumulation factor (BAC) and enrichment factor (EF) were calculated.

3.5. Analysis of essential oils

Clevenger apparatus . The oil yield was determined as a percentage of the dry weight of the plant material used.

The chemical composition of the essential oils was determined by gas chromatography coupled with mass spectrometry (GC – MS). The identification of the components was performed by comparing the mass spectra with spectral libraries (NIST and Wiley), retention indices and literature data. The obtained results were used to determine the terpene profile and chemotype affiliation of the studied oils.

The content of Pb , Cd and Zn in the essential oils was determined after acid digestion with concentrated HNO_3 and H_2O_2 by ICP - OES . To minimize analytical bias during the mineralization of the essential oils, controlled digestion conditions and procedures for controlling possible losses of volatile components were applied. The method was validated for the purpose of determining trace amounts of metals in organic matrix.

The results are compared with the regulatory requirements of the European Pharmacopoeia, the European Medicines Agency (EMA) and the World Health Organization (WHO).

3.6. Statistical analysis

Student 's t-test and one-way analysis of variance (ANOVA) were used to assess the differences between the experimental variants at a significance level of $p \leq 0.05$.

Pearson 's correlation coefficient . For the interpretation of multivariate data, hierarchical cluster analysis (HCA) was applied .

Statistical processing and graphical presentation of the results were performed using Microsoft Excel , SPSS , OriginPro and R.

The methodology used allows for an integrated analysis of the interactions between soil geochemistry, plant metabolism and the chemical composition of essential oils, providing the opportunity for quantitative assessment of the processes of accumulation, transfer and biochemical transformation of heavy metals in the soil-plant system.

4. Results and discussion

The obtained results allow a comprehensive assessment of the behavior of Pb , Cd and Zn in the "soil - plant - essential oil" system. The analysis covers the geochemical characteristics of the soil, the bioavailability of the metals, their accumulation and translocation in plants, the phytoremediation potential of the studied species, as well as the influence of the metal load on the quality and chemical composition of the obtained essential oils.

4.1. Soil characteristics and heavy metal content

The studied soils from the KCM – Plovdiv region are characterized by a neutral to slightly alkaline reaction (pH 7.4–7.6), the presence of carbonates and a relatively high content of organic matter, which creates favorable conditions for sorption and stabilization of heavy metals. The established values of organic carbon (4.1–5.4%), organic matter (7.1–9.3%) and C : N ratio (12.1–13.5) indicate a well-developed humus horizon and stable organic matter. The mineralogical analysis establishes a predominance of kaolinite , quartz, feldspars, calcite and dolomite, as well as the presence of Fe - and Mn -oxides, which form an effective sorption system for retaining metal cations.

The main physicochemical indicators and the content of heavy metals are presented in Table 4.1. The data show a clearly pronounced technogenic load on the soils with Pb , Cd and Zn , with the values many times exceeding the background concentrations and the normative reference levels, which confirms the long-term industrial impact in the area.

Significant differences in the mobility and ecological behavior of the studied elements are established. Pb is characterized by limited mobility due to fixation by carbonates, organic matter and Fe – Mn oxides, Cd exhibits the highest bioavailability and mobility, and Zn occupies an intermediate position with relatively high but controlled mobility.

For a quantitative assessment of the degree of pollution, the pollution coefficient (CF), the geoaccumulation index (Igeo), the loading index (PLI) and the Nemerov index (NPI) were calculated. The index estimates confirm a high degree of soil pollution, with Cd standing out as the main risk element with the highest contribution to the total environmental load.

The integral indices PLI and NPI confirm the presence of significant general pollution of the soil environment. The PLI values are many times above the critical value of 1, which indicates a clearly pronounced anthropogenic load on the studied system. At the same time, the Nemerov index classifies the soils as highly to extremely polluted and emphasizes the key role of cadmium in the formation of the general ecological risk.

Table 4.1. Main physicochemical indicators and heavy metal content in the studied soils

Indicator	Section 1	Section 2	Meaning
pH	7.6	7.4	Controls the mobility of metals
Organic matter (%)	9.3	7.1	Sorption and stabilization of metals
$CaCO_3$ (%)	1.5	2.0	Buffer capacity
Pb (total), mg / kg	2966.7	2509.1	Major pollutant
Pb (DTPA), mg / kg	**1244.7**	849.1	Bioavailable fraction
Cd (total), mg / kg	**92.0**	63.7	Main risk element
Cd (DTPA), mg / kg	61.9	36.7	Bioavailable fraction
Zn (total), mg / kg	3196.1	2423.9	High load
Zn (DTPA), mg / kg	339.1	236.8	Bioavailable fraction

Table 4.2 . Pollution indices of the studied soils

Element	Section 1	Section 2	CF class	Igeo class
Pb	2966.7	2509.1	>6	≥5
CD	92.0	63.7	>6	≥5
Zn	3196.1	2423.9	>6	4–5

CF > 6 - very high degree of contamination
Igeo ≥ 5 – extremely contaminated soils

The comprehensive assessment of the physicochemical characteristics, heavy metal content and index indicators shows that the soils in the area of KCM - Plovdiv represent a highly polluted geochemical system with long-term anthropogenic load. Despite the presence of favorable conditions for fixation of some of the metals, a certain fraction remains in a mobile and bioavailable form, which creates a potential for migration in the "soil-plant" system.

The combined analysis of total content, mobility and contamination indices shows a clear hierarchy of ecological risk, with cadmium emerging as the most hazardous element, followed by zinc and lead. Based on the combined indices and element mobility, the following hierarchy of ecological risk is established: Cd > Zn > Pb .

4.2. Elemental composition, accumulation and organ-specific distribution in the studied plant species

High concentrations of Pb , Cd and Zn in soils create conditions for intensive interaction between the soil and plant components, with the uptake of metals being determined both by their bioavailability and by the species-specific physiological and morphological characteristics of plants. The relationship between the content of metals in the soil and their accumulation in the plant organism is not linear and depends simultaneously on the total concentrations, the proportion of bioavailable forms and the soil physicochemical properties.

The studied representatives of the *Asteraceae family* demonstrate different strategies for the uptake, transport and detoxification of heavy metals, which is manifested in a clearly expressed organ-specific differentiation of accumulation.

4.2.1. Content and accumulation of Pb , Cd and Zn

The content of Pb , Cd and Zn in the different plant organs is presented in Table 4.2.1. The data show significant variability both between species and between individual organs.

Lead shows highly variable accumulation between species. The highest values were found in *Artemisia absinthium* , where the concentration reaches 4414.9 mg / kg in the leaves. High values are also observed in *Achillea millefolium* , especially in the flowers (1170.9 mg / kg).

At *Tanacetum balsam* Pb accumulates mainly in the roots, indicating a dominant phytostabilization strategy. A similar but less pronounced trend is observed in *Tanacetum vulgare* . The lowest concentrations were found in *Artemisia dracunculus* , indicating limited uptake and transport.

Cadmium is characterized by the highest mobility and most intense accumulation in the above-ground organs. Maximum values were recorded in the leaves of *Artemisia absinthium* (225.1 mg / kg), followed by *Achillea millefolium* (133.0 mg / kg). In all species, the leaves are the main storage organ for Cd . *Artemisia dracunculus* shows significantly lower values, which confirms effective mechanisms for limiting the entry of the metal.

Zinc reached the highest absolute concentrations among the elements studied. The highest values were found in *Artemisia absinthium* (2026.6 mg / kg in leaves) and *Achillea millefolium* (1035.3 mg / kg in flowers).

As an essential element , Zn accumulates primarily in physiologically active organs and participates in key metabolic processes, which accounts for its higher mobility compared to Pb .

Comparing the results shows clearly distinguished patterns of behavior:

- Pb – predominant root retention and limited translocation
- Cd – highest mobility and accumulation in aboveground organs

- Zn – intermediate behavior with preferential accumulation in leaves and flowers

Table 4.2.1. Pb , Cd and Zn content (mg / kg dry matter) in different organs of the studied plant species

Type	Authority	Pb	CD	Zn
Tansy balsamita (balsam)	Roots	676.3	58.5	500.7
	Stems	495. 1	77.1	395. 1
	Leaves	566. 1	59.7	664. 7
	Colors	244. 1	18.4	159.7
Tansy vulgare (tante)	Roots	237. 1	56.3	112.8
	Stems	71.8	75.4	74.0
	Leaves	425.0	99.4	508.0
	Colors	310.6	56.5	312.4
Yarrow millefolium (yarrow)	Roots	1000.3	102. 6	506.5
	Stems	230. 9	82.7	196.4
	Leaves	405. 6	133.0	737.6
	Colors	1170.9	89.8	1035.3
Artemisia absinthium (wormwood)	Roots	1913.0	102.7	1495.2
	Stems	123.5	19.9	76.1
	Leaves	4414.9	225.1	2026.6
	Colors	1223.3	163.9	911.5
Artemisia dracunculus (taros)	Roots	91.1	3.9	50.9
	Stems	56.6	15.5	68.5
	Leaves	742.8	39.0	556.1
	Colors	294.3	35.1	304.6

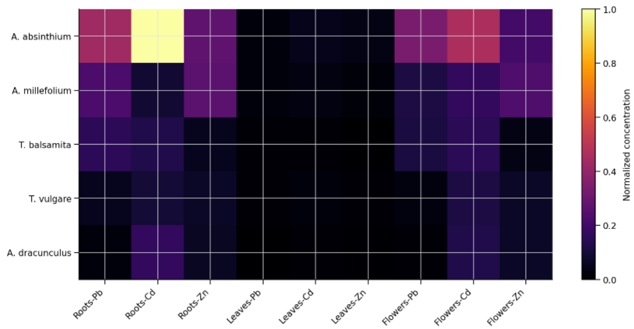

Figure 4.2.1 . Organ-specific distribution of Pb , Cd and Zn in the studied plant species.

Artemisia demonstrates the highest battery potential *absinthium* , followed by *Achillea millefolium* . The lowest concentrations are observed in *Artemisia dracunculus* .

4.2.2. Micro- and macronutrients and their role

Micro- and macroelements play a key role in plant adaptation to heavy metal stress. Analysis shows elevated concentrations of Fe and Mn in *Artemisia absinthium* and *Achillea millefolium* , which correlates with their high storage capacity.

Fe and Mn content supports antioxidant protection and redox homeostasis . Macroelements show a clear organ-specific distribution: Ca predominates in the roots, Mg and K in the leaves and flowers, and P is involved in energy metabolism and detoxification processes.

4.2.3. Organ-specific accumulation

Roots function as a major barrier and primary accumulation zone, especially for Pb and Cd . Stems have a transitional role and show lower concentrations.

The leaves represent the main terminal accumulator organ for the three metals, while the flowers also accumulate significant amounts, which is important from a pharmacological point of view.

4.2.4. Functional strategies of plants

Based on the results, the following strategies are distinguished:

- Artemisia absinthium – hyperaccumulator type
- Yarrow millefolium – high accumulator with organ selectivity
- Tansy spp . – intermediate type with partial root retention
- Artemisia dracunculus – limited accumulation
- The obtained results allow defining a functional gradient of accumulation potential in the studied species: *Artemisia absinthium* → *Achillea millefolium* → *Tanacetum spp* . → *Artemisia dracunculus* . This gradient reflects differences in the ability of plants to absorb, transport and detoxify heavy metals and serves as the basis for their ecological and functional classification.
- A clear relationship was established between the plant species, plant organ and the accumulation of Pb , Cd and Zn . Cd is characterized by the highest bioavailability and mobility, Pb shows predominant root retention, and Zn exhibits intermediate behavior with participation in metabolic processes.
- The studied species demonstrate different strategies of adaptation to metal loading, with the highest accumulation potential observed in

Artemisia absinthium and *Achillea millefolium* , while *Artemisia dracunculus* is characterized by limited absorption and accumulation.

Table 4.2.2. Dominant accumulation of Pb , Cd and Zn and functional strategy

Type	**Pb – dominant organ**	**Cd – dominant organ**	**Zn – dominant organ**	**Functional strategy**
Artemisia absinthium	leaves / colors	leaves	leaves	Battery
Achillea millefolium	Colors	leaves	colors	Selective accumulator
Tanacetum balsamita	Roots	roots	roots	Intermediate type
Tansy	roots / evenly	leaves	leaves	Intermediate type
Artemisia dracunculus	Roots	roots	roots	Restrictive type

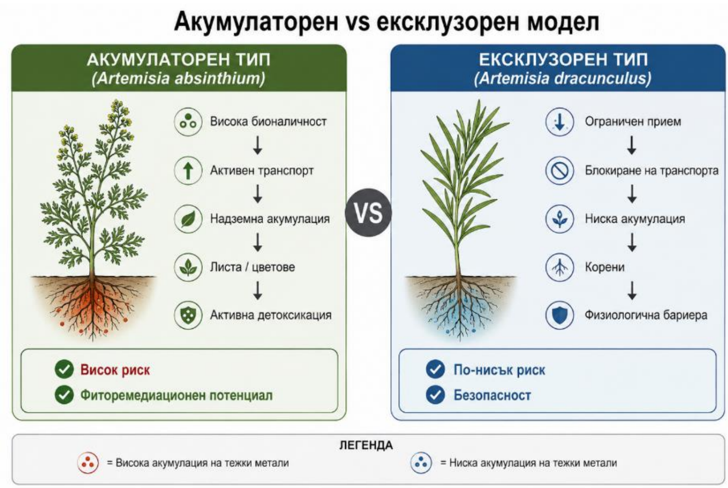

Figure 4.2.2. Functional strategies of plants in heavy metal accumulation – accumulator type and type with limited metal accumulation.

These results confirm the significant ecological risk associated with the transfer of Cd and Zn in the soil-plant system, as well as the key role of species specificity in assessing heavy metal pollution.
Additionally, it was found that the bioavailable forms of Cd and Zn are the main factors determining the intensity of metal transfer in the plant organism.

4.3. Quantitative assessment of the phytoremediation potential of plants

The bioconcentration factor (BCF), translocation factor (TF), bioaccumulation factor (BAC) and enrichment factor (EF) were used to quantitatively assess the phytoremediation potential of the studied species. These indicators allow a comprehensive assessment of the uptake, intrastem transport and accumulation of heavy metals, as well as the differentiation of phytoextraction, phytostabilization and limited accumulation strategies.

4.3.1. Phytoremediation potential with respect to Pb

BCF , TF and BAC values for Pb show clear interspecific differences (Table 4.3.1).

Table 4.3.1 . BCF, TF and BAC factors for Pb

Type	**BCF (root/soil)**	**TF (aboveground/roots)**	**BAC**
T. balsamita	0.23	1.20	0.28
T. vulgare	0.08	2.90	0.26
A. millefolium	0.40	1.58	0.64
A. absinthium	**0.76**	**2.94**	**1.05**
A. dracunculus	0.04	4.20	0.41

For Pb, clearly expressed interspecific differences in accumulation potential are observed. The highest BCF , TF and BAC values are found in *Artemisia absinthium* , which defines the species as an effective phytoextractor of lead. *Achillea millefolium* shows good translocation but limited total storage capacity due to lower root uptake.
Artemisia dracunculus was characterized by the lowest BCF , indicating effective limitation of Pb input from the soil. Despite high translocation, total accumulation remained low, emphasizing that transport capacity alone is not a sufficient criterion for phytoextraction efficiency.
Species of the genus *Tanacetum* demonstrate an intermediate behavioral pattern without a clearly expressed phytoextraction strategy.

The highest values of all indicators are observed in *Artemisia absinthium* , *which is characterized by relatively high* Pb uptake from soil (BCF = 0.76), efficient translocation to aboveground organs (TF = 2.94) and the highest total accumulation (BAC = 1.05). This combination defines the species as a significant phytoextractor of lead.
Yarrow millefolium also shows good Pb translocation , but lower BCF and BAC values limit its overall storage potential.
In *Artemisia dracunculus* had the lowest BCF (0.04), indicating effective limitation of Pb uptake from the soil. Despite the high TF value, total accumulation remained low, demonstrating that high transport alone does not guarantee phytoextraction efficiency.
Species of the genus *Tanacetum* show an intermediate pattern of behavior without a clearly expressed phytoextraction strategy.

4.3.2. Phytoremediation potential for Cd

Cadmium shows the highest values of all analyzed coefficients, which reflects its high bioavailability and mobility in the soil-plant system (Table 4.3.2).

Table 4.3.2. BCF, TF and BAC factors for Cd

Type	BCF	TF	BAC
T. balsamita	0.64	1.33	0.85
T. vulgare	0.61	1.82	1.11
A. millefolium	**1.59**	1.09	**1.73**
A. absinthium	**1.60**	**3.00**	**4.79**
A. dracunculus	0.06	6.03	0.59

Artemisia absinthium stands out as the most efficient phytoextractor , characterized by simultaneously high BCF (1.60), TF (3.00) and BAC (4.79), indicating intensive uptake and efficient translocation to the aboveground biomass.
Yarrow millefolium demonstrated stable accumulation at BCF and BAC values above unity, which defines it as a good phytoextractor of Cd at moderate translocation. *Tanacetum vulgare* and *T. balsamita show an* intermediate pattern of behavior with limited efficiency.
Artemisia dracunculus is characterized by a very low BCF (0.06), indicating a very limited uptake from the soil, despite the high TF value . This confirms a dominant control of the root barrier mechanism and a low phytoremediation potential.

In summary, Cd is determined as the most effective element for phytoextraction in the studied system due to its high bioavailability and the reported values of accumulation coefficients.

4.3.3. Phytoremediation potential with respect to Zn

With regard to Zn , an intermediate model of behavior is established, determined by the essential nature of the element and its participation in basic metabolic processes.

Artemisia absinthium shows the highest accumulation potential, combined with relatively high root uptake and efficient translocation to aboveground organs, reaching the highest BAC (1.22) and being determined as the most promising Zn accumulator among the studied species.

Yarrow millefolium demonstrated more pronounced translocation at moderate root uptake, indicating a balanced pattern of uptake and distribution.

Artemisia dracunculus is characterized by the lowest BCF , which confirms effective mechanisms for limiting the entry and accumulation of Zn in plant tissues.

4.3.4. Enrichment Factor (EF)

To determine the influence of the anthropogenic factor on the accumulation of metals, the enrichment factor (EF) with the reference element Fe was used.

Table 4.3.3. BCF, TF and BAC factors for Zn

Type	BCF	TF	BAC
T. balsamita	0.16	1.65	0.27
T. vulgare	0.04	4.61	0.34
A. millefolium	0.21	**2.84**	0.59
A. absinthium	**0.62**	**1.97**	**1.22**
A. dracunculus	0.02	16.9	0.36

The results show that the strongest anthropogenic enrichment is observed for Cd , followed by Pb , while Zn demonstrates a transitional character between anthropogenic and geochemical origins.

The highest EF values were found in *Artemisia absinthium* and *Achillea millefolium*, which is consistent with the results of BCF, TF and BAC analyses and confirms a more intense anthropogenic contribution in these species.

In *Artemisia dracunculus* EF values are below or around unity for most elements, indicating a dominant geochemical background and limited influence of anthropogenically enriched fractions in its elemental composition.

Table 4.3.4. Enrichment factor (EF) using Fe as a reference element

Element	Type	EF	Interpretation
Pb	*Artemisia absinthe*	**≫ 1**	Strong anthropogenic enrichment
	Yarrow millefolium	**> 1**	Anthropogenic influence
	Tansy spp .	~1	Mixed (natural + anthropogenic)
	Artemisia dracunculus	< 1	Geochemical background
CD	*Artemisia absinthe*	**≫ 1**	Pronounced anthropogenic enrichment
	Yarrow millefolium	**≫ 1**	High anthropogenic contribution
	Tansy spp .	> 1	Moderate enrichment
	Artemisia dracunculus	< 1	Lack of anthropogenic influence
Zn	*Artemisia absinthe*	> 1	Anthropogenically influenced
	Yarrow millefolium	~1	Borderline
	Tansy spp .	< 1	Mainly geochemical origin
	Artemisia dracunculus	< 1	Geochemical background

4.3.5. Statistical dependencies in the soil-plant system

The correlation analysis shows a lack of significant positive relationship between the total concentrations of metals in the soil and their content in plant tissues. For all studied elements, low to negative values of the correlation coefficients are observed.

Table 4.3.5. Correlation relationships (Pearson r) between the content of heavy metals and the studied indicators

Element	Soil – Root	Soil – Leaves	Root – Leaves	DTPA– Leaves
Pb	−0.41	−0.43	0.84	~−0.43
CD	−0.17	−0.39	0.83	~−0.39
Zn	−0.36	−0.45	0.97	~−0.45

In contrast, strong positive correlations were found between roots and leaves (r = 0.83–0.97), indicating that th internal transfer of metals in the plant is mainly controlled by physiological and transport mechanisms, rather than by soil concentrations.

The most pronounced relationship is between DTPA- extractable Cd and its content in leaf biomass, which confirms that the bioavailable fraction of the element is a more reliable indicator of its accumulation in aboveground organs compared to the total soil content.

The results emphasize that bioavailability, rather than total soil content, is a determining factor in metal transfer to plant tissues.

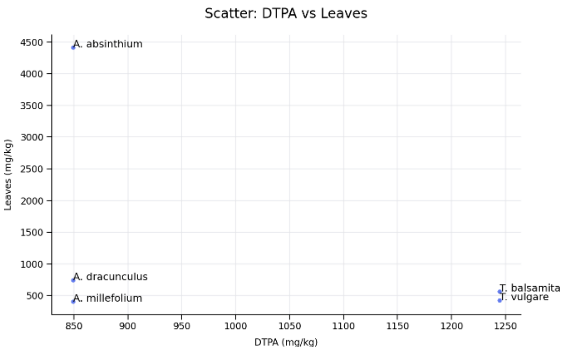

Figure 4. 3.1 . Dependence between DTPA-Cd in soil and the content in the leaves .

4.3.6. Cluster analysis

To summarize the established dependencies, hierarchical cluster analysis was applied, which distinguishes three main groups of species.

Figure 4.3.2 . Hierarchical cluster analysis of the studied species.

Artemisia absinthium stands out as a separate cluster, characterized by the highest storage potential and a clearly expressed phytoextraction strategy. The second group includes *Achillea millefolium* , *Tanacetum balsamita* and *Tanacetum vulgare* , which show a similar, intermediate pattern of metal accumulation and translocation. *Artemisia dracunculus* forms a distinct cluster, reflecting a specific strategy of limited uptake and accumulation of heavy metals.

The clustering reflects distinct physiological and ecological differences between species related to the mechanisms of uptake, intracellular transport and detoxification of heavy metals, which confirms the species-specific nature of phytoremediation strategies.

4.3.7. Interactions between heavy metals and nutrients

Negative relationships were found between Cd and Ca , as well as between Pb and Fe , indicating the presence of competitive and sorption interactions in the soil-plant system. Fe and Mn limit the mobility of Pb through adsorption and precipitation processes on oxides, while Ca and Mg reduce the uptake of Cd and Zn due to ionic competition. Zn exhibits a more complex behavior, with its mobility and uptake being influenced simultaneously by several elements.

Table 4.3.7. Main relationships between heavy metals and micro- and macroelements in the soil-plant system.

Connection	Type dependence	Explanation
Cd–Ca	Negative	competition for absorption
Cd–Mg	weak negative	similar ion effect
Pb–Fe	Negative	sorption on Fe- oxides
Pb–Mn	Negative	retention in soil
Zn–Fe	weak negative	competitively absorption
Zn–Mn	Moderate	similar behavior
Pb–P	Negative	precipitation as phosphates
Cd-K	Weak	lack of a pronounced relationship
Zn–K	Moderate	participation in metabolic processes

These results confirm the key role of nutrients as regulators of heavy metal behavior in the soil-plant system.

4.3.8. Integral assessment of phytoremediation potential

The integral analysis of all indicators (BCF , TF , BAC and EF) shows that *Artemisia absinthium* possesses the highest and most stable

phytoremediation potential with respect to Pb , Cd and Zn . The species combines high values of BCF , TF , BAC and EF and is characterized as a universal accumulator with high phytoextraction potential.
Yarrow millefolium ranks second and shows particularly high efficiency with respect to Cd .

Table 4.3.8. Integral assessment of phytoremediation effectiveness

Element	Most -effective type	Strategy type	Key criteria	Practical applicability
Pb	*Artemisia absinthe*	Strong phytoextractor	BCF ↑, TF ↑, BAC > 1	Suitable for active phytoextraction
	Yarrow millefolium	Moderate phytoextractor	BCF ~ 0.4, TF > 1	Limited phytoextraction
	Artemisia dracunculus	Restrictive type	BCF ≪ 1, TF ↑	Not suitable for phytoremediation
CD	*Artemisia absinthe*	**Very strong phytoextractor**	BCF > 1, TF ≫ 1, BAC ≫ 1	Excellent for phytoextraction
	Yarrow millefolium	Strong phytoextractor	BCF > 1, BAC > 1	Suitable for Cd -remediation
	Tansy spp .	Intermediate	BCF ~ 0.6, TF ~ 1–2	Limited effectiveness
Zn	*Artemisia absinthe*	Restrictive type	BCF ~ 0.6, TF ≈ 2, BAC > 1	Suitable for control
	Yarrow millefolium	Translocator	TF ↑, BCF < 1	Partial efficiency
	Artemisia dracunculus	Restrictive type	BCF ≪ 1	Unsuitable
Total (Pb+Cd +Zn)	*Artemisia absinthe*	**Universal battery**	All odds high	Most -suitable for phytoremediation

Figure 4.3.5. Conceptual scheme for classification of the studied plant species according to their phytoremediation potential, based on BCF , TF and BAC indicators for Pb , Cd and Zn .

Species of the genus *Tanacetum* exhibit intermediate phytoremediation potential and can be used primarily for phytostabilization.

Artemisia dracunculus demonstrates consistently low values of accumulation indicators and is characterized as a species with limited accumulation of heavy metals.

The results obtained allow a clear functional classification of the studied species according to their phytoremediation potential, such as *Artemisia absinthium* is defined as the most effective phytoextractor , *Achillea millefolium* as a secondary accumulator type, *Tanacetum spp* . as an intermediate group with limited effectiveness, and *Artemisia dracunculus* as a species with low phytoremediation potential.

4.4. Tolerance to heavy metals in members of the Asteraceae family

Table 4.3.9. Integral phytoremediation assessment by elements

Type	**Pb**	**CD**	**Zn**	**Strategy type**	**Phytoremediation suitability**	**Practical assessment**
Artemisia absinthe	Very high	**Extremely high**	High	Strong battery	Phytoextraction	very high
Yarrow millefolium	Moderate	High	Moderate	Battery (Cd -specific)	Phytoextraction (limited)	high
Tansy balsam	Low–moderate	Moderate	Low	Intermediate	Phytostabilization	in the yard
Tansy vulgar	Moderate (TF↑)	Moderate	Moderate	Intermediate	Combined	moderate
Artemisia dracunculus	Low	Low	Low	Restrictive type	Unsuitable	low

The results obtained show that the studied representatives of the *Asteraceae family* exhibit varying degrees of tolerance to Pb , Cd and Zn . It was found that tolerance does not depend solely on the degree of accumulation, but on the ability of plants to regulate the uptake, transport and intracellular distribution of metals.

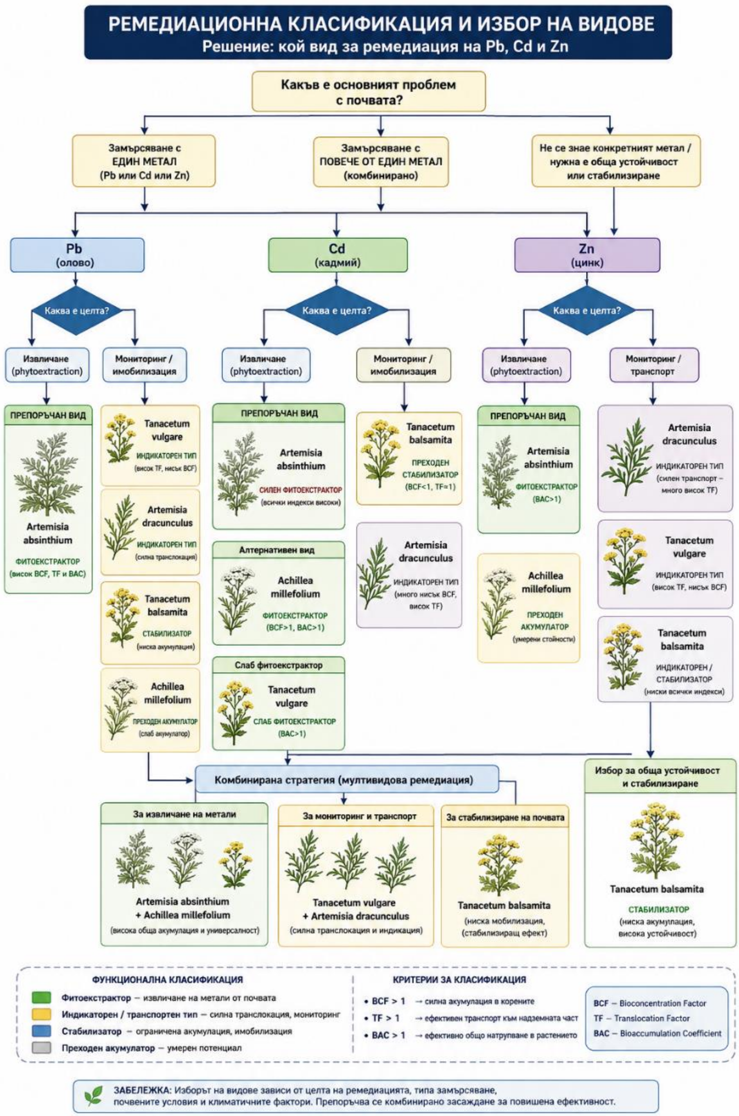

Figure 4.3.5 . Conceptual scheme for classification on the researched vegetable types according to phytoremediation them potential , on basis of BCF, TF and BAC indicators for Pb, Cd and Zn **.**

The highest tolerance was found in *Artemisia absinthium* , which combines high accumulation with effective mechanisms for detoxification and internal regulation. *Achillea millefolium* shows good resistance, especially to Cd . Members of the genus *Tanacetum* are characterized by moderate tolerance, mainly related to limited translocation and retention of metals in the roots. *Artemisia dracunculus* demonstrates a different adaptation strategy based on limited metal uptake at the root level.

Based on the comprehensive assessment, the species can be ranked by degree of tolerance in the following order:

Artemisia absinthium > *Achillea millefolium* > *Tanacetum spp* . > *Artemisia dracunculus* .

4.5. Essential oils – content and composition

Essential oils are a specific plant fraction, significantly different from raw biomass in their physicochemical composition and formation mechanism. They contain mainly volatile terpene compounds, while heavy metals are localized mainly in the non-volatile structural components of tissues. Therefore, the content of Pb , Cd and Zn in essential oils does not directly depend on their concentration in plant biomass, but is determined by a combination of biological and technological factors, including tissue localization and the selectivity of the distillation process.

Total heavy metal content in essential oils

A clear distinction is established between the high concentrations of Pb , Cd and Zn in the plant biomass and their much lower values in the obtained essential oils. This effect is most pronounced in *Artemisia absinthium* and *Achillea millefolium* , which despite the high accumulation of metals in the above-ground organs form essential oils with low content of heavy metals Table 4.5.1.

Table 4.5.1. Pb , Cd and Zn content in essential oils (mg / kg)

Type	Pb	CD	Zn	Pharmacognostic assessment
T. balsamita	2.7 0	0.08	1.70	Low concentrations, no regulatory risk
T. vulgare	0.27	0.01	1.74	Trace amounts, safe oil
A. millefolium	5.9	0.02	2.90	Higher Pb , without exceeding permissible limits
A. absinthium	0.11	0.01	0.44	Very low values, high safety
A. dracunculus	0.27	0.01	1.09	Low concentrations, suitable for practical application

The results show that steam distillation functions as a selective process, in which mainly volatile organic compounds pass into the oil phase, while metal ions remain associated with the structural and insoluble fraction of the plant tissue.

The concentrations of Pb , Cd and Zn in essential oils are low in all studied samples. Pb demonstrates minimal transfer to the oil phase despite its high content in the biomass. Cd also remains limited to trace amounts, despite its high mobility and accumulation in the aboveground organs. Zn shows relatively higher values compared to Pb and Cd , but they remain many times lower than the concentrations in the plant matrix and do not pose a toxicological risk.

Mechanisms on limited transfer

The limited passage on heavy metals to the ethereal oils is causes from totality from physiological and physicochemical factors .

First , Pb, Cd and Zn are localize mainly in cellular walls , vacuoles and others structural components that no participate in biosynthesis on volatile terpene compounds . This spatially separation between metals and zones on formation on the ethereal oils limits the possibility for co-extraction .

Second , it is missing specific chemical affinity between the metal ones ions and the dominant terpene components , which excludes their inclusion in the lipophilic oily phase .

Third , the steam distillation acts as selective technological process , at which is evaporate and condense mainly volatile organic molecules , while inorganic ions and their complexes remain in the plant remainder .

As a result on this the high accumulation on heavy metals in plant biomass no is reflects proportionally on composition on the ethereal oils .

Pharmaceutical and regulatory evaluation

The established concentrations of Pb, Cd and Zn in all analyzed ethereal oils are low and not they assume toxicological risk at practical use . The obtained results correspond on the approaches defined in the European Pharmacopoeia (Ph. Eur.), EMA and WHO, according to which the ethereal oils is are considering as low matrix risk from pollution with heavy metals metals , because their volatility and the specific physicochemical characteristics , as well as the technological way on receiving .

The received data confirm that the combined action on physiological detention on metals in plants fabrics and selectivity on the distillation process effectively limits the transmission of Pb, Cd and Zn to the final product . Table 4.5.2 **.**

After analyzing the results obtained, compliance with the principles set forth in the European Pharmacopoeia (Ph . Eur .), the documents of the European Medicines Agency (EMA) and the guidelines of the World Health Organization (WHO) is established. In accordance with these regulatory frames the ethereal oils is are considering as low matrix risk by attitude on content on heavy metals , conditioned from their volatility and specific physicochemical properties .

Table 4.5.2. Regulatory requirements for heavy metals in essential oils and compliance of the test results

Regulatory body / document	**Approach to heavy metals in essential oils**	**Requirements for Pb and Cd**	**Compliance with results**
European Pharmacopoeia (Ph . Eur .) – General monograph " Essential oils " (2098) and info chapter 5.30	Essential oils are volatile preparations with generally low risk of metal contamination; heavy metal testing is not routinely required unless in doubt	Risk control; no universal limits for all essential oils	All oils show low Pb and Cd ; no regulatory risk identified
EMA (HMPC) – Reflection paper EMA/HMPC/84789/2013	Quality depends on the raw material and extraction method; heavy metals are assessed contextually	There are no fixed universal limits for EO; low content is expected	The low values obtained confirm correct distillation and GMP
WHO – Guidelines for assessing quality of herbal Medicine (2007)	Limits are defined mainly for herbal drugs and extracts; for EO the risk is low due to -their physicochemical properties	Pb and Cd limits for herbal materials ; EO considered as a low-risk matrix	The results are fully consistent with the WHO approach

The obtained results confirm that the steam distillation process, in combination with the physiological mechanisms for metal retention in plant

tissues, provides effective limitation of the transfer of Pb , Cd and Zn to the oil phase.
Despite the high concentrations of heavy metals in plant biomass, essential oils are characterized by a much lower content of Pb , Cd and Zn , which confirms that they represent a selectively enriched volatile fraction in the "soil - plant - essential oil" system.
Therefore, the distillation process functions as an effective physicochemical barrier against the transfer of heavy metals to the final product, thus ensuring its quality, safety and compliance with current pharmacopoeial and regulatory requirements.

4.6. Chemical composition and chemotypic affiliation of essential oils

The analysis of the chemical composition of essential oils was carried out in order to assess the impact of soil contamination with heavy metals on the secondary metabolism of plants and the quality of the resulting products.

Table 4.6.1. Summary of essential oils

Plant species	Essential oil yield (%)	Dominant class of compounds	Main components (>10%)	Hemotype
Artemisia dracunculus L.	1.10	Phenylpropanoids	Sabine , elemicin , isoelemicin , methyleugenol	mixed sabinene / elemicin / isoelemicin / methyleugenol
Artemisia absinthium L.	0.25–0.40	Monoterpene hydrocarbons	Sabinene , β -myrcene	sabine - myrcene
Tansy balsamita L.	1.28–1.45	Oxygenated monoterpenes	Carvone , α -thujone	carvone –α -thujone
Tansy vulgare L.	0.35–0.50	Monoterpene esters	trans -chrysanthenyl acetate, β -thujone	trans -chrysanthenyl acetate
Yarrow millefolium L.	0.35–0.40	Sesquiterpenes	α -bisabolol , α -bisabolol oxide B, β -caryophyllene	mixed α -bisabolol / β -caryophyllene

The results obtained show that despite the high concentrations of Pb , Cd and Zn in some of the studied species, the essential oils retain the chemical profile characteristic of the respective species and a clearly defined chemotypic affiliation. The observed changes mainly affect the relative amounts of the individual terpene groups, without leading to a change in the chemical identity of the oils (Table 4.6.1.).

The species are characterized by stable and specific *chemotypes* : A. *dracunculus* – phenylpropanoid type , *A. absinthium* – *sabinene* – myrcene type, *T. balsamita* – carvone – α - thujone type , *T. vulgare* – chrysanthenyl - acetate type , *A. millefolium* – *sesquiterpene* bisabolol type.

General terpenic profile and chemotype characteristic

The analyzed ethereal oils show clear expressed species differences as by yield , as well as chemical composition .

• *Artemisia dracunculus* is characterized by dominance on phenylpropanoids and partially monoterpene hydrocarbons .

• *Artemisia absinthium* belongs to to sabinene –β- myrcene dominant chemotype on monoterpene hydrocarbons .

• *Tanacetum balsamita* is characterized by carvone –α- thujone chemotype and dominance on oxygenated monoterpenes .

• *Tanacetum vulgare* belongs to to trans- chrysanthenyl acetate hemotype with high content on monoterpene esters .

• *Achillea millefolium* is distinguishes with sesquiterpene dominance and belonging to α- bisabolol /β -caryophyllene hemotype .

Table 4.6.2. Distribution on the main ones terpene groups (%)

Plant species	**Monoterpene hydrocarbons**	**Oxygenated monoterpenes**	**Sesquiterpene hydrocarbons**	**Oxygenated sesquiterpenes**	**Phenylpropanoids**
A. dracunculus	35.05	5.57	7.25	2.21	48.98
A. absinthium	60.47–63.39	17.71–18.50	12.24–16.86	2.21–3.06	–
T. balsamita	0.58–0.61	88.75–89.41	7.74–8.35	1.01–1.06	–
T. vulgare	1.12–1.50	80.67–85.25	0.83–1.12	8.66–12.08	–
A. millefolium	17–19	20–21	24–25	36–38	–

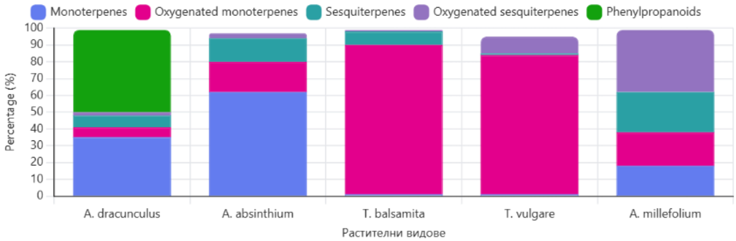

Figure 4.6.1. Distribution of terpene groups in essential oils

Influence of heavy metal pollution on chemical composition

The comparison on samples from contaminated and control sections shows that heavy metal load shows **quantitatively , but not good quality influence** on composition on the ethereal oils .

Table 4.6.3. Main changes in the composition of essential oils under conditions of soil contamination with heavy metals

Plant species	Compounds with reduced content	Compounds with increased content	Main trend
A. dracunculus	–	Oxygenated monoterpenes	Metabolic adaptation
A. absinthium	Sabinene , β -myrcene , β -linalool	Terpinene -4ol, phytol , γ -terpinene	↑ oxygenated derivatives
T. balsamita	Ketones, esters	Alcohols, ethers	OM restructuring
T. vulgare	α -thujone	trans -chrysanthenyl acetate	Enhanced ester fraction
A. millefolium	Hamazulene , β -pinene	α -bisabolol , bisabolol oxides	↑ protective sesquiterpenes

The most pronounced the trend is increasing on the relative share on oxygenated mono - and sesquiterpenes at the plants developing is on contaminated soils . This restructuring is interprets as adaptive metabolic activation - related response on protective mechanisms against oxidative stress induced from the heavy ones metals .

Despite these changes **no is establishes change in hemotype affiliation at neither one from the researched species** , which shows high stability on the main one secondary metabolic profile .

Integrated interpretation

The integrated analysis shows that the heavy ones metals act as abiotic stressful factor that induces quantitative amendments in the secondary metabolism on the plants .

Observed changes are targeted basically to increase on oxygenated terpene compounds that participate in antioxidant protection and detoxification on reactive oxygen forms .

At the same time time the secondary metabolism is characterized by high structural stability , so as the species hemotypic affiliation remains unchanged . This shows balance between metabolic plasticity and genetic determined chemical identity .

Especially it is important that despite the high one accumulation of Pb, Cd and Zn in plant biomass , essential oils oils keep its pharmaceutical value and typical chemical profile .

The results show that the heavy ones metals influence basically on quantitative composition on the secondary metabolites , but no change the chemical identity on the ethereal oils . Despite the high one accumulation of Pb, Cd and Zn in plant biomass , the obtained oils is characterized by low content on metals and preserved pharmaceutical profile , which confirms their safety and technological stability .

Conceptual model

The received results allow formulation on conceptual model for interaction in the system " soil - plant - etheric" oil " .

The model shows that soil pollution influences on the accumulation of heavy metals in plant biomass and the quantitative composition of secondary metabolites, but has a limited impact on the final product - the essential oil, due to: physiological barriers in the plant and selectivity of the distillation process

As a result, a clear distinction is observed between: metal accumulation in biomass and the chemical stability of essential oils.

In summary, the results show that the behavior of Pb , Cd and Zn in the soil-plant-essential oil system is determined by both the geochemical characteristics of the soil and the species-specific physiological characteristics of the plants. Significant differences in the phytoremediation potential and tolerance of the studied species, such as *Artemisia absinthium* stands out as the most promising accumulator and phytoextractor . Despite the high accumulation of heavy metals in plant biomass, essential oils retain their characteristic chemical profile and are characterized by a low content of Pb , Cd and Zn , which confirms their safety and practical applicability.

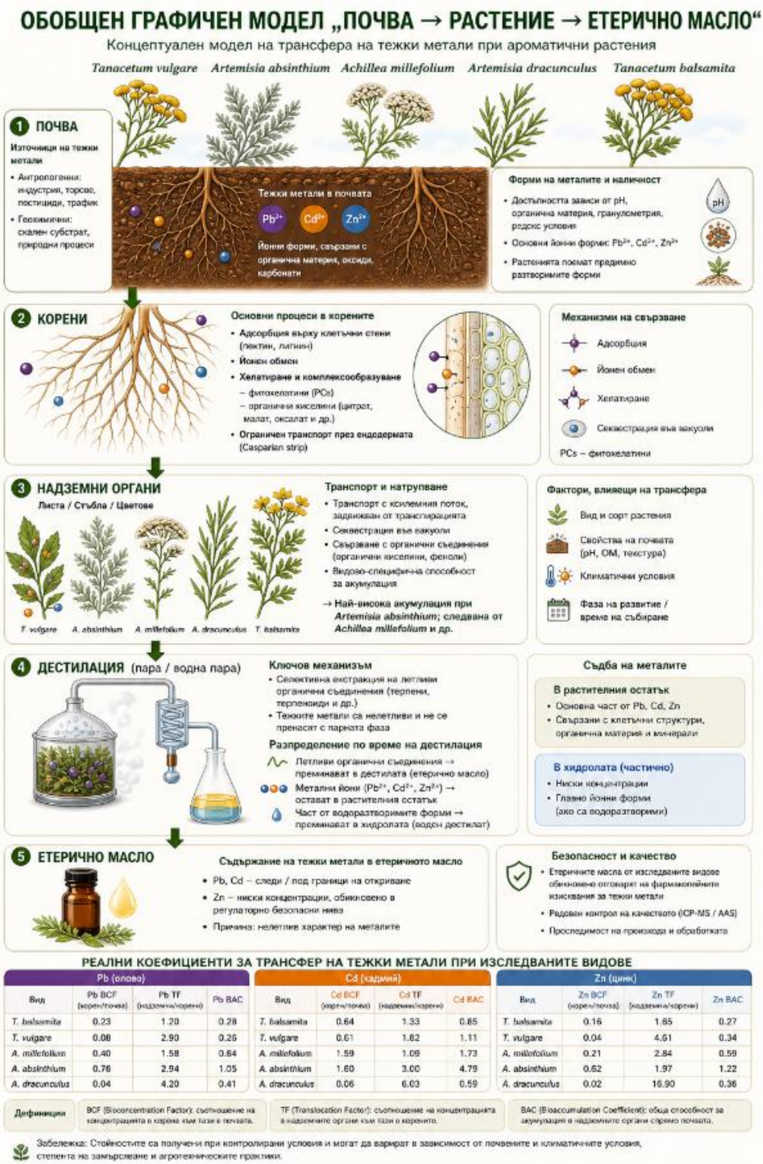

Figure 4.6.2. Conceptual model of the interaction in the “soil – plant – essential oil” system

5.1. GENERAL CONCLUSIONS

This dissertation study presents an integrated analysis of the behavior of the heavy metals Pb , Cd and Zn in the "soil - plant - essential oil" system through a combined soil chemical , phytoremediation and pharmacognostic approach.

The results show that the accumulation and transfer of heavy metals in plants is a complex process that is determined by the interaction between the geochemical characteristics of the soil, the physiological characteristics of the plants, and the technological parameters of essential oil extraction.

It was found that the studied species of the Asteraceae family exhibit a clearly pronounced species- and organ-specific accumulation of heavy metals. The root system functions as the main barrier and detoxification structure, while the above-ground organs represent the main accumulation compartments and a source of pharmacognostically significant biomass.

It has been shown that high concentrations of Pb , Cd and Zn in plant biomass do not lead to a corresponding accumulation in essential oils. This is explained by the action of a “double selective filter”, including biological immobilization of metals in tissues and selective extraction of volatile organic compounds during distillation.

It has been established that heavy metal contamination does not change the chemotypic identity of essential oils, but mainly leads to quantitative changes in the terpene profile, without loss of chemical specificity.

Based on the results obtained, it is confirmed that essential oil plants can simultaneously participate in phytoremediation processes and provide safe pharmacognostically valuable products under conditions of anthropogenic stress.

5.2. MAIN FINDINGS

1. The soils in the area of KCM - Plovdiv are highly contaminated with Pb , Cd and Zn , with the contents significantly exceeding the background and normative values, and the geochemical indices confirm a high degree of anthropogenic load.
2. The neutral to slightly alkaline reaction of the soils, the presence of carbonates, organic matter and Fe – Mn oxides form a significant buffer capacity that limits the mobility of metals and supports their long-term retention.
3. Significant differences in the behavior of metals were found: Cd is characterized by the highest mobility and bioavailability, while Pb and Zn are more strongly fixed by sorption , complexation and precipitation.

4. The bioavailable forms of Pb , Cd and Zn represent a more reliable indicator of transfer in the soil-plant system compared to the total content in the soil.
5. There is a clearly expressed species- and organ-specific accumulation, in which the roots act as a barrier, and the leaves and flowers - as the main accumulation organs.
6. A gradient of accumulation potential is established by the BCF, TF and BAC indicators: *Artemisia absinthium* > *Achillea millefolium* > *Tanacetum spp* . > *Artemisia dracunculus* , with the first species being distinguished by the highest phytoextraction capacity.
7. The accumulation of heavy metals in biomass does not lead to significant contamination of essential oils due to the combined action of biological barriers and the selectivity of distillation.
8. Contamination with Pb , Cd and Zn does not change the chemotypic affiliation of essential oils, but leads to quantitative changes in the terpene profile.
9. The conceptual model of a “double selective filter” explains the limited transfer of heavy metals to essential oils and confirms the possibility of combined ecological and industrial use of the studied species.

5.3. SCIENTIFIC CONTRIBUTIONS

1. For the first time, an integrated analysis of the "soil - plant - essential oil" system has been carried out in representatives of the Asteraceae family , grown under conditions of industrial pollution with Pb , Cd and Zn , combining soil chemical , phytoremediation and pharmacognostic approaches.
2. organ-specific distribution of Pb , Cd and Zn in the studied plant species have been established , which reveal different adaptation strategies to metal stress.
3. A functional classification of the studied plant species was developed based on the indicators BCF , TF , BAC and EF , allowing for a comparative assessment of their phytoremediation potential and distinguishing species with a predominant phytoextraction or phytostabilization strategy.
4. A conceptual model of a “double selective filter” is proposed to explain the limited transfer of Pb , Cd and Zn from soil and plant biomass to essential oils through the joint action of physiological

barrier mechanisms and the selectivity of the steam distillation process.

5. It has been proven that the increased content of Pb , Cd and Zn in plant biomass does not lead to significant contamination of essential oils and does not change their chemotype , but only causes quantitative changes in the relative content of individual terpene components.
6. Scientific knowledge about the interaction between heavy metals, soil characteristics and the secondary metabolism of aromatic and medicinal plants has been expanded, justifying the possibility of combining phytoremediation with the production of safe essential oils.

5.4. SCIENTIFIC AND APPLIED CONTRIBUTIONS

1. Plant species with phytoremediation potential have been identified , which creates a basis for application in phytotechnologies for the stabilization and extraction of heavy metals.
2. The safety of essential oils from plants grown in contaminated soils has been assessed, and it has been proven that no significant contamination of the final product is observed.
3. Guidelines have been formulated for the combined application of Asteraceae species in contaminated sites for the purpose of simultaneous ecological restoration and production of valuable raw materials.
4. An applicable conceptual model for assessing the risk and potential of plant systems in polluted environments is proposed.

5.5. RECOMMENDATIONS

1. ***Artemisia absinthium*** and ***Achillea millefolium*** are recommended for phytoremediation applications due to their high accumulation and translational potential.
2. Phytoextraction technologies require controlled management and safe treatment of contaminated biomass to avoid secondary pollution.
3. Species of the genus ***Tanacetum*** are suitable for phytostabilization, while ***Artemisia dracunculus*** is suitable for cultivation in polluted areas due to low metal accumulation.
4. Future research should focus on long-term dynamics of metal accumulation, multicomponent contaminations and their impact on the quality of essential oils.

Scientific publications

1. **Maria Ihtyarova** , Violina Angelova. Heavy metal accumulation and chemical composition of essential oil of cotsmary(Tanacetum vulgare L.) cultivated him/her heavy metal contaminated soils , Scientific Papers . Series E. Land Reclamation , Earth Observation & Surveying , Environmental Engineering, XII, 320 – 329, 2023, Print ISSN 2285-6064, CD-ROM ISSN 2285-6072, Online ISSN 2393-5138, ISSN-L 2285-6064.
2. Violina Angelova, **Maria Ihtyarova**. Heavy metal accumulation and chemical composition of the essential oil of yarrow(Achillea millefolium L.) cultivated him/her heavy metal-contaminated soils. Agricultural Sciences/Agricultural Nauki, vol. 15(36), 67 – 76, 2023.
3. Maria Ihtyarova, Violina Angelova. Soil metal contamination and pollution indices in the neighborhood of a non-ferrous metal smelter, Plovdiv(Bulgaria). Anniversary Scientific Conference "80 Years Agricultural University – Plovdiv: Traditions Meet Innovations ". Agricultural University – Plovdiv, Scientific Works Vol. LXVII, book 1, 260-269, 2025
4. Violina Angelova, **Maria Ihtyarova**. Evaluation of phytoremediation potential of plants from *Asteraceae* family. Conference paper: 60th Croatian & 20th International Symposium him/her Agriculture, Bol, Brac Island , Croatia , 1-6 June 2025. Proceedings , 2025, 247-251.
5. Violina Angelova, **Maria Ihtyarova** . Potential of Selected Aromatic Asteraceae Species for the Phytoremediation of Heavy Metal – Contaminated Soils. Scientific Papers. Series E. Land Reclamation, Earth Observation & Surveying, Environmental Engineering , Vol. XV, 2026.

Phytoremediation of Heavy Metal-Contaminated Soils Using Species of the Family *Asteraceae*

(Summary)

The present dissertation investigates the behavior of lead (Pb), cadmium (Cd), and zinc (Zn) in the soil–plant–essential oil system in five medicinal and aromatic plant species: *Artemisia absinthium* L., *Artemisia dracunculus* L., *Achillea millefolium* L., *Tanacetum balsamita* L., and *Tanacetum vulgare* L., collected from areas affected by anthropogenic pollution.

The study evaluates the accumulation, translocation, and phytoremediation potential of these species, as well as the influence of heavy metal contamination on the quality, safety, and chemical composition of the obtained essential oils.

The results reveal significant interspecific differences in the uptake and accumulation of Pb, Cd, and Zn. *Artemisia absinthium* exhibits the highest values of the bioconcentration factor (BCF), translocation factor (TF), biological accumulation coefficient (BAC), and enrichment factor (EF), identifying it as the most effective phytoextractor among the investigated species. *Achillea millefolium* shows high accumulation capacity, particularly for cadmium, whereas *Tanacetum* species display intermediate phytoremediation potential. *Artemisia dracunculus* is characterized by limited uptake and accumulation of heavy metals.

Correlation analysis indicates that metal accumulation depends primarily on their bioavailable fraction rather than on their total soil concentration. Hierarchical cluster analysis distinguishes the investigated species according to their phytoremediation potential, with *Artemisia absinthium* forming a separate cluster with the highest accumulation capacity.

The essential oils contain only trace amounts of Pb, Cd, and Zn despite the elevated metal concentrations in plant biomass, demonstrating that steam distillation effectively limits heavy metal transfer into the oil fraction. The obtained values comply with the principles of the European Pharmacopoeia (Ph. Eur.), the European Medicines Agency (EMA), and the World Health Organization (WHO), confirming the safety of the essential oils.

Chemical analysis shows that heavy metal contamination causes quantitative changes in terpene composition without altering the characteristic chemotype of any investigated species. The increase in oxygenated mono- and sesquiterpenes is interpreted as an adaptive response to oxidative stress, while the overall chemical identity of the essential oils remains unchanged.

In conclusion, Pb, Cd, and Zn behavior in the soil–plant–essential oil system is governed by both soil geochemistry and species-specific physiological mechanisms. *Artemisia absinthium* is identified as the most promising species for phytoextraction, whereas the essential oils retain their characteristic chemical profile and very low heavy metal concentrations, confirming their pharmaceutical quality, safety, and practical applicability.