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HEAVY METAL ACCUMULATION AND CHEMICAL COMPOSITION OF ESSENTIAL OILS OF COSTMARY (*TANACETUM BALSAMITA* L.) CULTIVATED ON HEAVY METAL CONTAMINATED SOILS

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Abstract

Comparative research has been conducted to determine the content of heavy metals and chemical composition of costmary oils, as well as to identify the possibility of costmary (Tanacetum Balsamita L.)| growth on soils contaminated by heavy metals. Costmary is a plant tolerant of heavy metals and can be grown on contaminated soils. Heavy metals do not affect the development of costmary and the quality of oil obtained from it. Twenty-three components were identified, accounting for 98.26-98.99% of the total oil components. The major components (> 1.0%) contained in costmary oil are carvone (42.66-44.12%), alpha-thujone (29.81-30.07%), beta-bisabolene (5.51-6.24%), 1,8-cineole (2.71-3.58%), beta-thujone (2.47-2.60%), cis-para-mentha-1(7),8-dien-2-ol (1,63-1,72%), trans-para-mentha-2,8-dienol (1.34-1.41%), cis-carveol (1.20-1.74%), and gamma-muurolene (1.69-1.78%). The content of oxygen-containing monoterpenes (88.75-89.41%) is the highest in costmary oil, followed by sesquiterpene hydrocarbons (7.74-8.35%), oxygen-containing sesquiterpenes (1.0-1.06%) and monoterpene hydrocarbons (0.58-0.61%). The analysed costmary oils belong to the carvone - α-thujone chemotype.

Key words*: contaminated soils, costmary, essential oil composition, heavy metals.*

INTRODUCTION

Costmary (*Tanacetum balsamita*), also called balsam chrysanthemum, is a herbaceous perennial plant that belongs to the Asteraceae family. The balsam chrysanthemum reaches a height of 45-60 centimetres and blooms in summer with yellow flowers. In spring, it begins its growth with the growth of a leafy rosette and a flower stalk. The leaves are oval, and the flowers are basket-shaped and are grouped in an umbel-like inflorescence.
The species is widespread in southeastern Europe and southwestern Asia but has been naturalised and cultivated in various parts of the world (Hassanpouraghdam et al., 2008a; Hassanpouraghdam et al., 2008b; Oberprieler et al., 2009). It occurs in the botanical gardens of most European countries (Philips and Foy, 1992). It is cultivated in Asia, America, Iran, Turkey and Romania, Germany, Italy, Spain, and England (Bylaite et al., 2000; Marculescu et al., 2001a; Marculescu et al., 2001b; Gallori et al., 2001; Hassanpouraghdam et al., 2008a).
The plants are cold-tolerant. It prefers full sun and well-drained soil but grows well under most conditions (Hassanpouraghdam et al., 2008b). Propagation of this plant is by division or by root cuttings, while propagation by seed is not satisfactory (Marculescu et al., 2001b).
The aboveground part of the plant (leaves and flowers) is used in the Mediterranean, Balkans, South America, and Asia (Bylaite et al., 2000; Abad et al., 2006; Hassanpouraghdam et al., 2008a). Costmary contains biologically active substances (essential oil, phenylpropane derivatives, flavonoids (flavonols, apigenin derivatives, scutellarin derivatives and luteolin derivatives potent antioxidants), tannins and oligo elements (Ca, K, Mg, P and Fe), vitamin C (ascorbic acid). For this reason, it is used as a spice, herbal tea, and to produce essential oil (Hassanpouraghdam et al., 2008a; Venskutonis, 2016). The fresh and dried leaves of the costmary possess a lemon-myrrh solid aroma and a sweet, astringent taste. Fresh leaves can be added to salads, pasta and various beverages. Dried leaves are used as flavourings in soups and meats, sausages and pastries, and refreshing tea (Bylaite et al., 2000; Abad et al., 2006). The flowers can be added to jams, sweets and preserves. Costmary leaves have been used as a hepatoprotective, tonic, sedative, painkiller and astringent

(Hassanpouraghdam, 2009). The oils can be used as natural additives in many foods due to their antibacterial, antifungal, antioxidant and anticarcinogenic properties (Juknevicine et al., 1973; Hüsnü et al., 2001; Jaimand & Rezaee, 2005), and to alleviate inflammatory diseases (Bagci et al., 2008; Yousefzadi et al., 2009; Venskutonis, 2016; Ivashchenko, 2017).
The essential oil is extracted by steam distillation of the aerial parts (leaves and flowers) and is a colourless to pale yellow liquid (Hassanpouraghdam et al., 2008a). The oil is secreted in the glandular trichomes, with the highest essential oil content in the leaves before flowering. For the essential oil of the costmary to be of high quality, the plants must be harvested at the beginning of flowering (Bylaite et al., 2000; Gallori et al., 2001; Hassanpouraghdam et al., 2008a, 2008b). The essential oil contains up to 186 compounds (Bylaite et al., 2000), which are dominated by monoterpenes and sesquiterpenes. The oil greatly affects skin diseases such as eczema, rashes, pimples, and acne. The essential oil is used in medicine, pharmacology, food, perfume and confectionery industries. The essential oil can also be used in aromatherapy.
It is known that some medicinal plants, such as mint, St. John's wort, sage, and others, can accumulate large amounts of toxic heavy metals in their tissues, be used for phytoremediation, and replace food crops grown under the same conditions. The concentrations of various plant by-products are highly dependent on the growing conditions and affect the metabolic pathways responsible for synthesising associated natural products. Metals can significantly alter the chemical composition of secondary metabolites in aerial parts of plants and thus seriously affect the quality, safety and efficacy of natural plant products (Akula & Ravishankar, 2011).
Although there is data on the composition of costmary oil, there needs to be more information on the heavy metal content of the aerial mass and oil when costmary is grown on soils contaminated with heavy metals.
The present work aims to conduct a comparative study that will allow us to determine the amounts and deposition of Pb, Zn, Cd and Hg accumulation in the vegetative organs of costmary, the quality of costmary oil, as well as to establish the feasibility of growing costmary on soils contaminated with heavy metals.

MATERIALS AND METHODS

The experiment was performed on an agricultural field contaminated by Zn, Pb, and Cd, situated at different distances (0.5 and 15.0 km) from the source of pollution, the NFMW (Non Ferrous Metal Works) near Plovdiv, Bulgaria.
The study was conducted with a costmary test plant. Costmary seedlings were purchased and planted in spring on areas located at different distances (0.5 km and 15 km) from the source of contamination NFMW-Plovdiv. In the second and third years after planting, samples of plant material (roots, stems, leaves, and flowers) were taken for analysis from each experimental plot. Costmary was hand-harvested at the flowering stage in June. After transporting the plants to the laboratory, scissors were used to divide them into their individual organs (roots, stems, leaves and inflorescences). Samples of the roots, stems, leaves and flowers were dried at room temperature until an air-dry mass was obtained, after which they were dried at 45ºC. The content of heavy metals in different parts - roots, stems, leaves and flowers were determined. The essential oil of the costmary was obtained in laboratory conditions from the flowers by steam distillation for 2 h using a Clevenger-type apparatus.
The pseudo-total content of metals in soils was determined in accordance with ISO 11466. The available (mobile) heavy metal contents were extracted by ISO 14870 by a solution of DTPA. The contents of Pb, Zn, Cd and Hg in the plants and in the costmary's essential oil were determined by microwave mineralisation method. The quantitative measures were carried out by ICP (Jobin Yvon Emission - JY 38 S, France). The Hg content of the samples was determined without preliminary sample preparation with a Hg analyser. Digestion and analytical efficiency of ICP and Hg analyser were validated using a standard reference material of apple leaves (SRM 1515, National Institute of Standards and Technology, NIST). The chemical composition of the oils in hexane

(1:1000) was analysed on Agilent 7890A Gas Chromatography system equipped with FID detector and Agilent 5975C mass spectrometer. The oil's chemical constituents were determined on a 7890A gas chromatograph (Agilent Technologies) and a 5975C mass spectral detector (Agilent Technologies). Compounds were identified by comparing retention times and Kovacs relative indices (RI) with those of standard substances and mass spectral data from the NIST'08 library (National Institute of Standards and Technology, USA).

RESULTS AND DISCUSSIONS

Soils

To elucidate the extent of soil contamination with heavy metals and their localisation in the vegetative organs of costmary, soil samples were collected from the areas at different distances from the NFMW (0.5 and 15 km). The physical and chemical properties of the soil samples are presented in Table 1. The soils are characterised by a slightly alkaline reaction, medium organic carbon content and medium to high nutrient (N, P, K) availability.

Table 1. Characterization of soils sampled from NFMW-Plovdiv

Parameter	Soil 1 (S1) 0.5 km from NFMW	Soil 2 (S2) 15 km from NFMW
pH	7.6	7.5
Organic content,%	5.4	1.5
N Kjeldal,%	0.29	0.12
P, mg/kg	703.1	387.3
K, mg/kg	7782.5	6780.0
Ca, mg/kg	22746	7927.3
Mg, mg/kg	14046	8428.4
Cu, mg/kg	307.4	35.2
Fe, mg/kg	32340	25282
Mn, mg/kg	957.6	879.0
Pb, mg/kg	2966.9	24.6
Zn, mg/kg	3196.1	177.9
Cd, mg/kg	92.0	2.7
Hg, ng/g	488.1	41.0

MPC (pH >7.4) - Pb -100 mg/kg, Cd - 3.0 mg/kg, Zn -400 mg/kg, Hg - 1.5 mg/kg

The results presented in Table 1 show that with distance from NFMW-Plovdiv, there is a well-defined trend of decreasing total heavy metal content in the soil. In the soil samples taken from the area 0.5 km away from KCM (S1), values for Pb exceeding the MPC (maximum permissible concentrations, approved for Bulgaria) (100 mg/kg) were recorded - 2966.9 mg/kg. In the area 15 km away, the Pb content decreased significantly to 24.6 mg/kg. Similar results were obtained for Cd and Zn. In the area 0.5 km away from the NFMW, 3196.1 mg/kg Zn and 92.0 mg/kg Cd were recorded, with values significantly exceeding the MPC. In the more distant area (15 km from NFMW), 177.9 mg/kg Zn and 2.7 mg/kg Cd were detected. The Hg content in soils from both regions was lower than the MPC.

The results for the mobile forms of the metals determined by DTPA show that the mobile forms of Cd in the contaminated soils are the most significant portion of its total content and reached to 67.2%, followed by Pb with 42.0%, Zn with 10.6% and Hg to 1.3%.

In the uncontaminated soils, the mobile forms of Cd were the most significant fraction of its total content and reached 24.8%, followed by Pb at 8.5%, Zn at 7.1% and Hg at 2.7% (Table 2).

Table 2. DTPA-extactable Pb, Zn, Cd (mg/kg) and Hg (ng/g) in soils sampled from NFMW

Soils		S1	S2
Pb	mg/kg	1244.7	21.5
	%*	42.0	43.5
Cd	mg/kg	61.9	0.7
	%	67.2	70
Zn	mg/kg	339.1	40.0
	%	10.6	22.5
Hg	ng/g	6.3	1.1
	%	1.3	2.7

*DTPA -extractable/total content

Costmary

To elucidate the uptake, accumulation and distribution of heavy metals in the vegetative organs of the costmary, samples of roots, stems, leaves and flowers were analysed.

In Table 3 are presented the results obtained for the heavy metal content in the organs of the studied essential oil crop. Significant differences were found in the elemental contents of the different parts of the costmary. The main part (Pb, Cd, Zn and Hg) accumulated in the aerial parts of the costmary (leaves and flowers).

The content of Pb in the roots of costmary grown on contaminated soils (S1) reached up to 648.0 mg/kg, Zn - up to 500.7 mg/kg, Cd - up to 67.3 mg/kg, and Hg - up to 365.4 ng/g. The values obtained for the heavy metals (Cd, Pb and Zn) in the roots were much higher than the values considered toxic to plants by Kabata Pendias (2001) (0.1 mg/kg Cd, 30 mg/kg Pb, 100 mg/kg Zn). Significantly lower values were found in the roots of the costmary grown on uncontaminated soil (S2). The Pb content in the roots reaches 1.1 mg/kg, Zn - 30.7 mg/kg, Cd - 0.08 mg/kg and Hg - 81.8 ng/g. Costmary is distinguished by a shallow root system, with many thin horizontally arranged feeding roots (Philips & Foy, 1992). This is probably the reason why it accumulates significant amounts of heavy metals in the roots.

Table 3. Element contents in costmary grown 0.5 and 15 km from NFMW-Plovdiv

Element	Roots	Srems	Leaves	Flowers	Oils
S1 Pb, mg/kg	648.0	217.0	566.0	244.0	2.7
S1 Cd, mg/kg	67.3	18.7	83.1	23.9	0.08
S1 Zn, mg/kg	500.7	121.1	768.0	190.0	1.7
S1 Hg, μg/kg	365.4	215.3	412.2	348.6	nd
S2 Pb, mg/kg	1.1	1.5	2.7	0.8	0.03
S2 Cd, mg/kg	0.08	0.1	0.3	0.1	0.005
S2 Zn, mg/kg	30.7	13.6	11.5	62.4	0.16
S2 Hg, μg/kg	81.8	54.5	99.3	76.3	nd

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The Pb content in the stems of costmary grown on contaminated soil (S1) reaches 217.0 mg/kg, Zn up to 121.1 mg/kg, Cd up to 18.7 mg/kg, and Hg up to 215.3 ng/g. Significantly lower values were found in the stems of costmary grown on uncontaminated soil (S2). The content of Pb in stems reached up to 1.5 mg/kg, Zn - 13.6 mg/kg, Cd - 0.1 mg/kg, Hg - 54.5 ng/g. The anatomical structure of the stems of the tested plant can explain the higher values found in the costmary grown on the contaminated soil. Costmary is distinguished by a stem covered with hairs, contributing to the fixation of aerosol pollutants and their accumulation.

The content of heavy metals is higher in the aboveground mass of the costmary than the roots, indicating uptake and movement of metals along the conducting system. The results obtained were similar to Jasion et al. (2013), who found that metal accumulation in leaves, were higher compared to roots.

The Pb, Zn, Cd and Hg contents in the leaves of costmary from the contaminated soil reached 566.0 mg/kg, 768.0 mg/kg, 348.6 mg/kg and 412.2 ng/g, respectively. Despite high concentrations of Cd and Pb in the leaves, there was no evidence of Cd and Pb toxicity. Significantly lower values were found in the leaves of costmary grown on uncontaminated soil (S2), where Pb reached up to 2.7 mg/kg, Zn up to 11.5 mg/kg, Cd up to 0.3 mg/kg and Hg up to 99.3 ng/g.

The higher accumulation of heavy metals in the leaves of the costmary is probably due to the fact that the leaves of the costmary are mossy, which contributes to the fixation of aerosol pollutants and their accumulation there. A characteristic feature of the costmary is the presence of trichomes (hairs) on the plant's surface, a typical morphological part of the Asteraceae family.

The accumulation of heavy metals in the flowers is high but lower than in the leaves. The Pb content in the flowers of costmary grown on contaminated soil reaches up to 244.0 mg/kg, Zn - up to 190 mg/kg, Cd - up to 23.9 mg/kg and Hg - 348.6 ng/g. Significantly lower values were found in the flowers of costmary grown on non-contaminated soil (S2): Pb - up to 0.8 mg/kg, Zn - up to 62.4 mg/kg, Cd - up to 0.1 mg/kg, Hg - up to 76.3 ng/g.

The content of heavy metals in the essential oils of costmary was also determined. The obtained results show that the main part of heavy metals contained in the flowers of the costmary does not pass into the oil during the processing of the flowers. Therefore their content in the oil is significantly lower. When the oils are obtained by distillation, the heavy metals remain in the plant residues, so the heavy metals in oils remains low.

Pb in the essential oil of costmary grown on contaminated soil (S1) reaches up to 2.7 mg/kg, Zn up to 1.7 mg/kg and Cd up to 0.08 mg/kg. Significantly lower values were found in costmary essential oil grown on uncontaminated soil (Pb up to 0.03 mg/kg, Zn up to 0.16 mg/kg and Cd up to 0.005 mg/kg). The Hg content of the oil is below the limits of the method used.

The results obtained strongly indicate that most of the Pb, Cd, Zn and Hg contained in the flowers of a costmary grown 0.5 km from the NFMW do not pass into the resulting oil. The amounts of Pb, Cd and Hg in the costmary oil are lower than the accepted maximum values and meet the requirements for an environmentally friendly product (5 mg/kg Pb, 1 mg/kg Cd, 0.1 mg/kg Hg) (Council of

Europe, 2021). The results of previous studies by Angelova et al. (2015) are confirmed, according to which the heavy metal content of the essential oil is very low and is not affected by the level of soil contamination with heavy metals. Essential oils contain only trace amounts of heavy metals in distilled oils because these metals have molecules too heavy and oversized to volatilise and concentrate in the distillation process sufficiently.

In order to provide a definitive answer to the question of the mudflat's ability to absorb heavy metals from the soil and assess its potential for phytoremediation, the Bioconcentration Factor (BCF), and Translocation Factor (TF) were calculated.

The results obtained for BCF and TF are presented in Figure 1.

Transport factor (TF) gives information on the ability of plants to uptake heavy metals through the roots and move them to the aboveground mass (leaves). TF values more significant than 1 indicates that the plant is a potential accumulator of heavy metals and can translocate metals efficiently from the roots to the aboveground mass. For Pb, Zn, Cd and Hg, TF values are greater than 1, regardless of the degree of soil contamination.

Bioconcentration factor (BCF) is the ratio of heavy metal content in plant organs to that in soil. Depending on this value, plants can be classified as hyperaccumulators (BCF > 1) or exclusion (BCF < 1).

For Pb and Zn, all coefficients (root BCF, aboveground BCF and plant BCF) are <1, indicating that the elemental content in the mudflat does not exceed the amount in the soil. For Cd, the BCF root values were <1, while BCF aboveground mass and BCF plant reached 1.37 and 2.09.

In terms of Hg, BCF aboveground mass and BCF plant were >1, while BCF roots were <1 (Fig. 1). The Hg content of the studied soils was relatively low, suggesting that the primary source of soil and plant contamination was the aerosol deposition of Hg.

The bioconcentration factor (BCF) and translocation factor (TF) values of Cd and Hg indicate that these elements accumulate in the aboveground part of the costmary. Considering the TF values for the capacity of costmary to move pollutants out of the soil and the BCF value, costmary can be referred to indicator plants for Hg and Cd and partially for Pb where the heavy metal content is similar to that in soil. Similar results were obtained by Jasion et al. (2013), who found that T. vulgare can be used as a bioindicator of Cd, Mn, and Zn in areas of lignite industry.

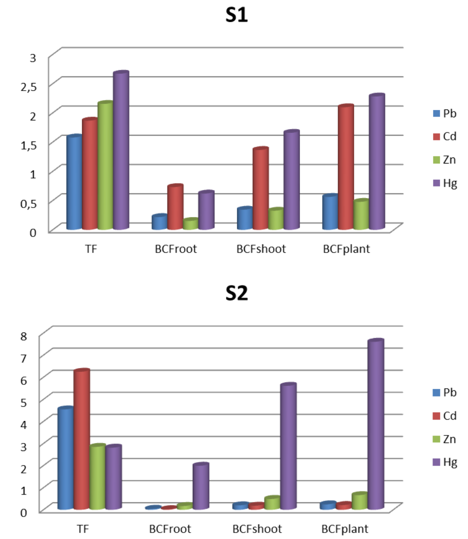


Figure 1. Translocation and bioconcentration factors for costmary

The results show that the distribution of heavy metals in the costmary organs is selective and depends primarily on the parts of the plant under study and their surface area. The distribution of heavy metals in the organs of costmary has a selective character, which decreases in the following order: Pb - roots≥ leaves>flowers>stems, Cd, Zn and Hg - leaves>roots>flowers>stems (Figure 2).

There is a distinct pattern in the accumulation of heavy metals in the vegetative organs of the costmary. The costmary accumulates heavy metals through its root system, much of which the roots retain. However, most of the metals move and get in the aboveground parts (stems, leaves and flowers). The results show that costmary can be successfully grown in areas contaminated with heavy metals.

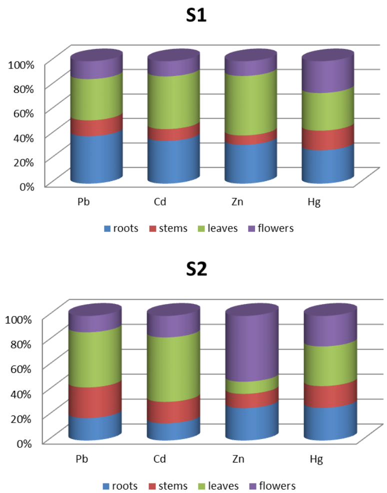


Figure 2. Distribution of heavy metals in the vegetative and reproductive organs of costmary

Oil

The yield of essential oil from the costmary is presented in Table 4. The oil yield depends on the origin of the plants, the anatomical part used to obtain the oil and the harvesting period. The oil content has been found to vary from 0.05% in stems to 2.2% in flowers (Jukneviciene et al., 1973), while in leaves from 0.31 to 1.25% (Bylaite et al., 2000). Significantly lower scores were found by Jaimand & Rezaee (2005) in the essential oil of leaves (0.25%), flowers (0.15%) and stems (0.05%) of costmary. During intense flowering, the yield decreases from both leaves and flowers (Bylaitė et al., 2000).

According to some authors, the oil content of flowers is higher than that of leaves (Jukneviciene et al., 1973; Bylaitė et al., 2000), while according to others it is lower (Jaimand & Rezaee, 2005).

The results obtained in this experiment showed that the essential oil content of costmary ranged from 1.28% to 1.45%, with higher contents in costmary grown on uncontaminated soil. Some heavy metals have been reported to affect essential oil yield from aromatic medicinal plants. The decrease in oil content with increasing soil heavy metal content may be due to the inhibition of photosynthetic rate by the toxic concentration of heavy metals which adversely affect plant growth. Das (1997) reported that the heavy metals Cr, Pb and Cd move to the aboveground mass and negatively affect the plant metabolism, resulting in decreased plant biomass. The decrease in essential oil yield is due to the reduction of biomass of the plant, as the oil yield is calculated by the formula (biomass yield x oil in biomass).

The results of the chromatographic analysis of the essential oils obtained from the processing of the flowers of costmary grown at different distances from the NFMW are presented in Table 4. The volatiles identified in the essential oil of *T. balsamita*, their retention indices and percentages are presented in Table 4. Table 4 also shows the detailed distribution for each class of organic compounds.

Twenty-three components were identified in the costmary oil samples belonging to the monoterpenes and sesquiterpenes classes, which accounted for 98.68-98.82% of the total number of oil components (Table 4).

The major components (>1.0%) present in the costmary oil are carvone (42.66-44.12%), α-thujone (29.81-30.07%), β-bisabolene (5.51-6.24%), 1,8-cineole (2.71-3.58%), β-thujone (2.47-2.60%), cis-para-mentha-1(7),8-dien-2-ol (1.63-1.72%), trans-para-mentha-2,8-dienol (1.34-1.41%), cis-carveol (1.20-1.74%), gamma-muurolene (1.69-1.78%). Trace minor components (in the range < 1.0 and > 0.10%) myrtenol (0.80-0.85%), cis-para-mentha-2,8-dienol (0.77-0.81%), α-cadinol (0.62-0.65%), p-cymene (0.58-0.61%), delta-cadinene (0.43-0.45%), trans-pinocarveol (0.42-0.44%), cis-verbenol (0.32-0.50%), eudesm-7(11)-en-4-ol (0.39-0.41%), trans-verbenol (0.36-0.38%), cis-carvyl acetate (0.37-0.39%), pinocarvone (0.35-0.3%), cis -chrysanthenyl acetate (0.33-0.35%), and terpinen-4-ol (0.27-0.29%). The predominant class of compounds in the costmary oil is oxygenated monoterpenes, whose content ranges from 88.75% in the oil from S1 oil to 89.41% in the oil from S2.

Of the oxygenated monoterpenes, the highest content was monoterpene ketones, which ranged from 75.29 to 77.14%, consistent with the results of Bylaite et al. (2000) (71.6%). Carvone (monoterpene ketone) is the significant component identified in the oils of costmary grown in the NFMW area. Its content ranged from (42.66-44.12%), with higher content in the costmary from the uncontaminated area. It is known that carvone is an antiseptic, central nervous system stimulant, antitumor agent, and insecticide.

Table 4. Composition of costmary oil (%)

	Component	RI	S1	S2
1	p-cymene	1025	0.58	0.61
2	1,8-cineole	1030	3.58	2.71
3	alfa-thujone	1103	29.81	30.07
4	beta-thujone	1115	2.47	2.60
5	trans-para-mentha-2,8-dienol	1124	1.34	1.41
6	cis-para-mentha-2,8-dienol	1139	0.77	0.81
7	trans-pinocarveol	1141	0.42	0.44
8	cis-verbenol	1143	0.50	0.32
9	trans-verbenol	1145	0.36	0.38
10	pinocarvone	1166	0.35	0.36
11	terpinen-4-ol	1177	0.27	0.29
12	trans-para-mentha-1(7),8-dien-2-ol	1190	1.35	1.42
13	myrtenol	1197	0.80	0.85
14	cis-carveol	1230	1.74	1.20
15	cis-para-mentha-1(7),8-dien-2-ol	1232	1.63	1.72
16	carvone	1244	42.66	44.12
17	cis-chrysanthenyl acetate	1265	0.33	0.35
18	cis-carvyl acetate	1370	0.37	0.39
19	gamma-muurolene	1480	1.69	1.78
20	beta-bisabolene	1507	6.24	5.51
21	delta-cadinene	1525	0.43	0.45
22	alfa-cadinol	1653	0.62	0.65
23	eudesm-7(11)-en-4-ol	1702	0.39	0.41
Monoterpene hydrocarbons (MH)			0.58	0.61
Oxygenated monoterpenes (eters)			3.58	2.71
Oxygenated monoterpenes (ketones)			75.29	77.14
Oxygenated monoterpenes (alcohols)			9.19	8.83
Oxygenated monoterpenes (esters)			0.70	0.73
Sesquiterpene hydrocarbons (SH)			8.35	7.74
Sesquiterpene alcohols			1.01	1.06
Total			98.68	98.82

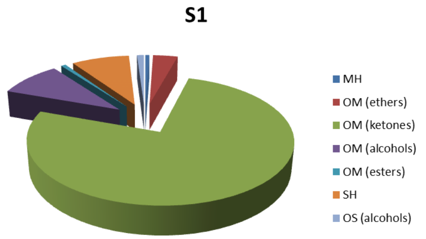
Carvone has been found to be a significant component in the essential oil of costmary from Lithuania (56-80%, Bylaite et al., 2000), Turkey (52%) and Spain (57%) (Basher et al., 2001; Perez Alonso et al., 1992; Hassanpouraghdam, 2009), with no significant difference between the carvone content of the leaf (52.1%) and flower oils (54.2%) (Bylaite et al., 2000; Vukic et al., 2022). Carvone, camphor and α-thujone predominate in oils from Romania, Poland, Germany and Russia (Gallori et al., 2001). In oils from Iran, the main components are bornyl acetate, pinocarvone, camphor and terpineol (Jaimand & Rezaee, 2005). The content of monoterpene ketone α-thujone was high (29.81-30.07%) in oils studied, with a higher content in the oil from the non-polluted area (S2). The content of β-thujone was significantly lower (2.47-2.60%) in the oil. Many studies have found that the highly toxic monoterpenes α-thujone and β-thujone are present in costmary oil. The content of α-thujone and β-thujone in costmary oil varies widely: α-thujone 1% (Serbia, Vutic et al., 2022), 15.93% (Gallori et al., 2011), 24.6% (Iran, Hassanpouraghdam, 2009); β-thujone - 2.68% (Iran, Hassanpouraghdam, 2009), 6.4% (Serbia), 9.0 to 16.1% (Bylaite et al., 2000), 84% (Baczek et al., 2017). Although, thujone has antibacterial, pesticidal, and insecticidal effects. care should be taken in the internal use of α-thujone and β-thujone rich essential oils of costmary (Bylaite et al., 2000; Hassanpouraghdam et al., 2008). The EMA (European Medicines Agency) and the EC (European Commission) recommend a maximum intake of between 3 and 7 mg of thujone/day (EMA, 2012).The second class of compounds identified in the oil are the monoterpene alcohols (9.19- 8.83%), of which cis-carveol (1.74-1.20%), cis-p-mentha-1(7),8-dien-2-ol (1.63-1.72%) and trans-p-mentha-1(7),8-dien-2-ol (1.35-1.42%) predominate.

The essential oil also contains the monoterpene ether 1,8-cineole (3.58-2.71%). Significantly lower below 1% is the content of the monoterpene hydrocarbon p-cymene (0.58-0.611%) and the monoterpene esters (cis-hrysanthenyl acetate, 0.33-0.35% and cis-carvyl acetate, 0.37-0.39%).

The next class of compounds identified in the oil were sesquiterpene hydrocarbons (SH) (8.35-7.74%) of which gamma-muurolene (1.69-1.78%) and β-bisabolene (6.24-5.51%) predominated.

The content of sesquiterpene alcohols was significantly lower (1.01-1.06%), with the amount of alpha-cadinol (0.62-0.65%) and eudesm-7(11)-en-4-ol (0.39-0.41%) being less than 1%.

The composition of the oil is affected by heavy metal contamination of the soil. Of the oxygenated monoterpenes, the content of alcohols, ethers, and sesquiterpene hydrocarbons is higher in the costmary oil from the contaminated soil. In comparison, the content of ketones, esters, and monoterpene hydrocarbons is higher in the oil from the non-contaminated soil (S2). No significant difference was observed between the contents of sesquiterpene alcohols.



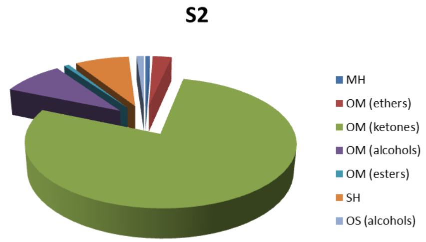


Figure 3. Example text to describe to figure above that should be replaced with your information

The composition of costmary essential oil varies depending on genetic, geographical and climatic factors (Pengelly, 2004). This phenomenon is called chemical polymorphism (Pengelly, 2004).

According to the predominant terpenes in the essential oil, 5 chemotypes have been identified in calophyllum:

1) Carvone type,
2) Camphor type,
3) Camphor-Tunion type,
4) Carvone - α-Tunion chemotype (Bylaite et al., 2000; Marculescuet al., 2001),
5) Bornyl acetate-Pinocarvone chemotype (Jaimand & Rezaee, 2005).

So far, no data are available for the carvone-α-thujone chemotype. The costmary oils analysed in this study belong to the carvone - α-thujone chemotype.

Costmary can be used as a rich source of carvone-like monoterpenes, similar to carvone-containing plants such as peppermint (*Mentha spicata* L.), caraway (*Carum carvi* L.) and fennel (*Anethum graveolens* L.).

CONCLUSIONS

It was found that:

1. There is a distinct pattern in the accumulation of heavy metals in the vegetative organs of costmary. The distribution of heavy metals in the organs of costmary grown on a highly polluted area has a selective character which decreases in the order: Pb–roots>leaves>flowers>stems, Cd, Zn and Hg–leaves>roots>flowers>stems, Zn-leaves>roots>stems>flowers.
2. Costmary is a heavy metal tolerant plant that can be grown on soils heavily contaminated with heavy metals and shows no symptoms of toxicity (chlorosis and necrosis) at a soil content of 92 mg/kg Cd, 2966.9 mg/kg Pb and 3196.1 mg/kg Zn.
3. Costmary can be referred to as indicator plants and used for monitoring soil and aerosol heavy metal pollution.
4. The content of heavy metals Pb, Cd and Hg in costmary essential oil is lower than the accepted maximum values and meets the requirements for an environmentally friendly product.
5. The content of oxygen-containing monoterpenes (88.75-89.41%) is the highest in costmary oil, followed by sesquiterpene hydrocarbons (7.74-8.35%), oxygen-containing sesquiterpenes (1.0-1.06%) and monoterpene hydrocarbons (0.58-0.61%).
6. Soil contamination affects the individual components in the oil of costmary. The monoterpene ethers, monoterpene alcohols are higher, while the concentration of monoterpene ketones and esters as well as monoterpene hydrocarbons is higher in the oil from costmary than the uncontaminated soil. No significant difference was observed with respect to sesquipropane alcohols.
7. This is the first report of a costmary oil with a mixed carvone-α-thujone chemotype.

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