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HEAVY METAL ACCUMULATION AND CHEMICAL COMPOSITION OF ESSENTIAL OILS OF WORMWOOD (*ARTEMISIA ABSINTHIUM* L.) CULTIVATED ON HEAVY METAL CONTAMINATED SOILS

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Abstract

Comparative research has been conducted to allow us to determine the content of heavy metals and chemical composition of wormwood oils, as well as to identify the possibility of wormwood growth on soils contaminated by heavy metals. The experimental plots were situated at different distances of 0.5 km, and 15 km, respectively, from the source of pollution the Non-Ferrous-Metal Works (MFMW) near Plovdiv, Bulgaria. On reaching flowering stage the wormwood plants were gathered. The content of heavy metals in leaves and flowering tips of wormwood were determined by ICP. The essential oils of the wormwood were obtained by steam distillation in laboratory conditions which were analysed for heavy metals and chemical composition was determined. Wormwood is a plant which is tolerant to heavy metals and can be grown on contaminated soils. Heavy metals do not affect the development of wormwood and the quality and quantity of oil obtained from it. Thirty-two components were identified in the oils. The main compounds of essential oi were as follows: β-Myrcene (2.24-28.20%), Sabinene (24.54-26.53%), Terpinene-4-ol (2.17-8.28%), beta-Linalool (2.24-6.27%), iso-Longifolol (4.40-5.59%), (E)-β-Caryophyllene (1.97-5.45%), Phytol (4.33-5.27%), γ-Terpinene (0.91-3.92%), Cembrene (1.24-2.72%), p-Cymene (0.11-2.50%), α-Terpinene (0.30-2.06%), Caryophyllene oxide (1.82-2.02%), β-Elemene (1.00-2.21%), α-14-hydroxy-Humulene (1.46-1.78%), α-Pinene (0.58-1.18%), (E)-9-epi-Caryophyllene (0.94-1.07%), Lavandulol (0.62-1.11%), cis-Sabinene hydrate (0.17-1.04%). The compounds in the essential oil that decreased as a result of heavy metals pollution are β-Myrcene, Sabinene, beta-Linalool, (E)-β-Caryophyllene, Cembrene, β-Elemene, Lavandulol, and cis-Sabinene hydrate, while the Terpinene-4-ol, Phytol, γ-Terpinene, p-Cymene, α-Terpinene, α-Pinene, and (E)-9-epi-Caryophyllene significantly increased. The essential oil of wormwood can be a valuable product for the farmers from polluted regions.

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

INTRODUCTION

Artemisia absinthium L. is a perennial shrub of the genus *Artemisia* and occurs as a weed in North Africa, Asia, Europe and New Zealand (Wang, 2004). The plant is known with different names such as wormwood (in English), grand absinthe (in French) and wermut (in German) (Wright, 2002). Common wormwood (*Artemisia absinthium*) is a perennial silver grey herbaceous plant, covered with a multitude of hairs with a pleasant aroma and a bitter taste. The stem is 40-120 cm high, branched at the top and woody at the base. Leaves on both sides are covered with silvery silk hairs. The flowers are tubular, yellow, without petioles, placed on a hairy torus. Wormwood blooms from July to September (Goud and Swamy, 2015).

Common wormwood has been known to humans as a spice and healing plant since ancient times. These properties are due to the essential oils, tannins, resins, flavonoids, fatty acids, vitamins (C, B6), minerals (K, Ca, P, Fe, Na) contained in common wormwood (Ahmad et al., 2010; Miron et al., 2014; Rachieru et al., 2017). Common wormwood contains 0.1-1.5% of oil (Orav et al., 2006; Basta et al., 2007; Msaada et al., 2015). The main components of oil are oxygen derivatives of bicyclic terpenes - the alcohol thiol and the ketone thujone, which are considered to be the characteristic ingredients for the wormwood oil (Juteau et al., 2003). The etheric oil also contains compounds such as monocyclic terpene phellandrene, bicyclic sesquiterpene cadinene and azulene sesquiterpene chamazulenogen, which is oxidized in the air to chamazulene. It has been proven that the bitter substances contained in the plant (absinthin) stimulate muscles of digestive organs, increase secretion of digestive juices and improve digestion (Miron et al., 2014). The ingredients contained in the essential oil (thujone, thiol, etc.) excite the central nervous system and have a beneficial effect on neurological diseases (Lachenmeier et al., 2010). Chamazulene has an anti-inflammatory effect and the ability to remove spasms of the smooth muscle organs (stomach, bronchi, etc.). Common wormwood is used to treat gastritis and other inflammations of the stomach, liver and bile diseases (Ahmed et al., 2014).

Wormwood is an important ingredient of the absinthe liquor, and is also used for flavouring in some other alcoholic beverages and wines, such as vermouth (Judzentiene et al., 2009). Thujone levels in foodstuffs and beverages are limited by the EU (Council Directive 88/388/EEC), with the maximum levels (α- and β-thujones) 0.5 mg/kg.

Many studies have been done on the composition of *A. absinthium* oil (Chiavla et al., 1983; Tucker et al., 1993; Wright, 2003). Significant differences in the composition of wormwood oil were found. Thujones (*cis* and *trans*) were the principal components of wormwood oils from Argentina, Estonia, Greece, Italy, Spain, Ukraine, France, Iran, Serbia and Montenegro, USA, Poland, Croatia and Russia (Blagojevic et al., 2006; Chiavla et al., 1983; Khalilov et al., 2001; Lawrence, 2003; 2006; Morteza-Semnani and Akbarzadeh, 2006; Orav et al., 2006; Rezaeinodehi and Khangholi, 2008; Sacco and Chialva, 1988; Wright, 2003). *cis*-chrysanthenol (69.0%) is predominant in the oil from central France (Carnat et al., 1992), *cis*-epoxyocimene (49.7%) and *cis*-chrysanthenyl acetate (36.7%) in the oils from southern French Alps (Juteau et al., 2003), *cis*-epoxyocimene (76.3%) or *cis*-epoxyocimene (39.9%)/*cis*-chrysanthenyl acetate (33.4%) predominate in Spanish oils (Arino et al., 1999a, 1999b). Oils from Estonia, Russia and Italy also contain high levels of epoxyocimenes (59.7% in Estonia oil, 22.1% in Russian oil, 23.1-56.6% in Italian oil) (Chiavla et al., 1983; Orav et al., 2006). Monoterpene hydrocarbons (β-pinene - 34.0%, p-cymene - 14.6%, α-phelandrene - 25.5%, β-myrcene - 8.9% and sabinene - 14.4%) predominate in Iranian oils (Rahimizadeh et al., 2001; Sefidkon et al., 2003). High amounts of sabinene and β-myrcene are characteristic of oils from Estonia, Hungary, Scotland and Moldova (Orav et al., 2006). Oils from Estonia, France, Belgium, Russia, Armenia, Latvia, Italy and France are rich in sabinyl acetate (Chiavla et al., 1983; Lawrence, 2003; 2006; Orav et al., 2006; Wright, 2003). Turkish oils contain chamazulene (17.8%), nuciferyl butyrate (8.2%) and propionate (5.1%) (Kordali et al., 2005). Cuban oil contains bornyl acetate (23.0%) (Pino et al., 1997). Oils from Spain and Italy contain significant amounts of 1,8-cineole (18.0%), carvone (18.5%), thymol (10.8%) and carvacrol (9.7%), while *trans* verbenol is predominant in oils from Latvia and Lithuania (9.2% and 11.7%, respectively) (Orav et al., 2006). Greek oil contains caryophyllene oxide (25.3%), p-cymene (16.8%) 1,8-cineole (8.9%) and (Z)-lanceol acetate (7.3%) (Basta et al., 2007), whereas US oil contains 33.1% *trans*-thujone and 32.8% *cis*-sabinyl acetate (Tucker et al., 1993).

It has been found that the medicinal plants can accumulate larger amounts of heavy metals such as Cd, As, Pb and Hg compared to other plants (Kabata Pendias and Pendias, 2011). It is known that plants of the *Artemisia* family can accumulate metals from soils (Alirzayeva et al. 2006). The plants of the *Artemisia* family are also widespread in Bulgaria and have a large biomass production capacity. However, there are no studies on the possibilities of heavy metals accumulation in *Artemisia absinthium* L. when grown on contaminated soils, as well as the composition of wormwood oil in Bulgaria.

Insufficient is also the information available on the potential of wormwood for accumulation of heavy metals and its potential use for phytoremediation. There are no comprehensive studies on the relationship between the total content of heavy metals in soil, their uptake by the wormwood leaves and flowering tips and quality of oil.

The purpose of this work is to conduct a comparative study, which allows us to determine the quantities and deposits for accumulation of Pb, Zn, and Cd in the leaves and flowering tips of wormwood, the quality of wormwood oil, as well as the possibilities to use the wormwood for phytoremediation of heavy metal contaminated soils.

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 near Plovdiv, Bulgaria.
Characteristics of soils are shown in Table 1. The soils were slightly neutral to alkalic with moderate content of organic matter and essential nutrients (N, P and K) (data are not shown). The pseudo-total content of Zn, Pb and Cd is high and exceeds the maximum permissible concentrations (MPC) in soil 1 (S1) (Table 1).

Table 1. Content of Pb, Zn and Cd in soils sampled from NFMW

Distance	pH	Pb x ± sd	Zn x ± sd	Cd x ± sd
Soil 1 (S1) 0.5 km	7.4	2509.1±6.5	2423.9±6.3	64.3±0.2
Soil 2 (S2) 15 km	7.5	49.4±0.2	172.7±2.1	1.0±0.1

x- average value (mg/kg) from 5 repetitions; sd - mean standard deviation
MPC (pH 6.0-7.4) - Pb -100 mg/kg, Cd-2.0 mg.kg, Zn-320 mg/kg
MPC (pH > 7.4) - Pb -100 mg/kg, Cd - 3.0 mg/kg, Zn -400 mg/kg

The study included wormwood grown on areas located at different distances (0.5 km and 1 km) from the source of contamination NFMW Plovdiv. Wormwood is grown according to conventional technology. Five plants of each area were used for the analysis. Upon reaching the stage of flowering, wormwood was harvested and the content of Pb, Zn and Cd in leaves and flowering tips was determined. The essential oil of the wormwood was obtained in laboratory conditions by steam distillation for 3 h using a Clevenger-type apparatus in accordance with European Pharmacopoeia method.
Pseudo-total content of metals in soils was determined in accordance with ISO 11466. The available (mobile) heavy metals contents were extracted in accordance with ISO 14870 by a solution of DTPA. The contents of heavy metals (Pb, Zn and Cd) in the plant material (leaves and flowering tips) and in the essential oil of sage were determined by the method of the microwave mineralization. The quantitative measures were carried out by ICP method (Jobin Yvon Emission - JY 38 S, France). Digestion and analytical efficiency of ICP was 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) were analysed on Agilent 7890A Gas Chromatography system equipped with FID detector and Agilent 5975C mass spectrometer.

RESULTS AND DISCUSSIONS

Soils
The results presented in Tables 1 and 2 show that in the soil samples S1 (taken from the area situated at the distance of 0.5 km from NFMW), the reported values for Pb were exceeding MPC approved for Bulgaria and reached to 2509.1 mg/kg. In the area located at a distance of 15 km, the contents of Pb significantly reduce to 49.4 mg/kg. Similar results were obtained for Cd and Zn.
The results for the mobile forms of the metals extracted 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 57.2%, followed by Pb with 33.8 % and Zn with 9.8%.
In the soil located at a distance of 15 km from NFMW the mobile forms of Cd are the most significant part of its.

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

Soils	Pb		Cd		Zn	
	mg/kg	%*	mg/kg	%	mg/kg	%
S1	849.1	33.8	36.8	57.2	236.8	9.8
S2	21.5	43.5	0.7	70	38.9	22.5

*DTPA -extractable/total content

Content of Heavy Metals in Wormwood
A significant accumulation of Pb is found in the leaves of the wormwood. The content of this element reaches up to 4414.9 mg/kg in leaves of the wormwood grown at a distance of 0.5 km from NFMW. Probably a portion of the accumulated heavy metals in the above-ground mass of the wormwood is also due to aerosol pollution, which can be explained by the anatomical and morphological characteristics of the crop. The greater accumulation of Pb in the above-ground mass is probably due to that the leaves and stems of the plant are covered

with fine silky hairs which favours the attachment of the aerosols and their accumulation therein. The content of Cd in the leaves of the wormwood grown at a distance of 0.5 km from NFMW reaches up to 225 mg/kg, respectively, values considered to be toxic to plants. According to Kabata Pendias and Pendias (2011), 5.0 mg/kg is considered to be a toxic value for the plants. Our results show the ability of the wormwood to accumulate Cd in the above-ground mass.

Table 3. Content of Pb, Cd and Zn (mg/kg) in leaves, flowering tips and essential oil of wormwood

Element	Distance km	Leaves x ± sd	Flowering tips x ± sd	Oils x ± sd
Pb	0.5 km	4414.9± 10.8	1223.3± 6.8	0.11+ 0.01
Pb	15 km	7.2±0.8	4.0±0.5	0.02± 0.01
Cd	0.5 km	225.1±3.5	163.9± 2.4	Nd
Cd	15 km	0.7±0.05	0.3±0.05	Nd
Zn	0.5 km	2461.8± 9.7	871.6± 6.9	0.44± 0.05
Zn	15 km	114.0±3.4	67.7±1.0	0.14± 0.02

x- average value (mg/kg) from 5 repetitions; sd - mean standard deviation, Nd- non detected

The content of Zn in the leaves of the wormwood grown at a distance of 0.5 km from NFMW reaches up to 2461.8 mg/kg, as these values are also higher than the critical values for plants: 100-400 mg/kg.

The content of heavy metals in the leaves of the wormwood grown at 15 km from NFMW reaches up to 7.2 mg/kg Pb, 0.7 mg/kg Cd and 114.0 mg/kg Zn.

The results obtained show that significant quantities of heavy metals are accumulated in the above ground part of wormwood, with their values significantly exceeding the widely accepted critical toxic levels (Marschner, 1995; Lobnik, 2004), but none of the symptoms of heavy metal toxicity have been detected. This shows that common wormwood has high tolerance to heavy metals. The results are in line with the findings of Alirzayeva et al. (2006), that in the *Artemisia* species studied, larger amounts of Zn are accumulated in their above ground parts. Zn concentrations vary from 600 to 900 mg/kg in the above ground mass, and from 100 to 330 mg/kg in the roots. The results we obtained are much higher than the results of Badea (2015) and Fischer et al. (2017) (5.37-14.42 mg/kg Pb, 0.118-0.434 mg/kg Cd and 0.005-0.012 mg/kg Zn) as well as the results of Chizola (2012) and Steff et al. (2009) (0-0.5 mg/kg Cd, 0-2.25 mg/kg Pb, and 41.3- 41.9 mg/kg Zn).

According to Rachieru et al. (2017), *Artemisia absinthium* L. has the ability to accumulate heavy metals mainly in the above ground parts (stems and leaves), when grown on a landfill. According to the authors, the high values of heavy metals in the above ground parts cannot be explained only by their absorption by the soil, but are also due to the adsorption of heavy metal aerosols from the air. Similar results were found by Imelouane et al. (2011), according to whom significant amounts of Pb and Cd in wormwood leaves are due to road traffic (Pb - 1036.1 mg/kg, Cd - 20.6 mg/kg, Zn - 798.1 mg/kg)

The heavy metal content in the flowering tips is considerably lower in comparison to the above-ground mass of the plants. The contents of Pb, Zn and Cd in the flowering tips of the wormwood grown at a distance of 0.5 km from NFMW reaches up to 1223.3 mg/kg, 871.6 mg/kg and 163.0 mg/kg, respectively. With increasing the distance from NFMW, a clear trend is seen towards reducing the content of heavy metals in the flowering tips of the studied crop. Significantly lower is the content of heavy metals in the flowering tips of the wormwood grown at 15 km from NFMW. The content of Pb in the flowering tips reaches up to 4.0 mg/kg, Zn - up to 67.7 mg/kg and Cd - up to 0.3 mg/kg. Probably a portion of the accumulated heavy metals in the flowering tips of the wormwood is due to the aerosol pollution (Table 4).

The heavy metal content in the essential oil from wormwood was also determined. The results obtained show that the majority of the heavy metals contained in the flowering tips of the wormwood do not pass into the oil during the distillation, therefore their content in the oil are much lower. Pb content in the essential oil of wormwood reaches up to 0.11 mg kg, Zn up to 0.44 mg/kg, while the content of Cd is below the limits of the quantitative measurement of the method used. Significantly lower are the results in the essential oil of wormwood grown at a distance of 15 km from NFMW - 0.02

mg/kg Pb and 0.14 mg/kg Zn. The results obtained shows that the content of heavy metals in the essential oils is much lower compared to the leaves and flowering tips of the wormwood, and the amounts of Pb, Zn and Cd in the oil of wormwood are lower than the accepted maximum values and meet the requirements of an environmentally friendly product (0.1 mg/kg Pb, 0.05 mg/kg Cd). The results are confirming the ones established by Angelova et al. (2015), which found that the heavy metal content in the essential oil is very low and is not affected by the level of soil contamination with heavy metals. Essential oils contain only traces of heavy metals in distilled oils because these metals have too heavy and large molecules to be volatilized enough and to be concentrated by the distillation process.

Essential oil content and composition

Oil content of wormwood from different European countries was reported as 0.1-1.1% (Orav et al., 2006). Evaluating our results of oil content measurements, it can be established that our results are in accordance with those of previous reports and corresponded to the European Pharmacopoeia standard (not less than 0.2%).

The results of the chromatographic analysis of essential oils obtained by processing the leaves of wormwood grown at a different distance from NFMW-Plovdiv are presented in Table 4.

Figure 1 shows the chromatograms from the GC MS analysis of wormwood oil.

The results show that wormwood oil is a complex mixture of monoterpenes, monoterpenoids, sesquiterpenes, and 32 compounds (Table 4) were identified that represent 97.51-97.95% of total oil content.

The main compounds of essential oils were as follows: β-myrcene (26.24-28.20%), sabinene (24.54-26.53%), terpinene-4-ol (2.17-8.28%), beta-linalool (2.24-6.27%), iso-longifolol (4.40-5.59%), (E)-β-caryophyllene (1.97-5.45%), phytol (4.33-5.27%), γ-terpinene (0.91-3.92%), cembrene (1.24-2.72%), p-cymene (0.11-2.50%), *α*-terpinene (0.30-2.06%), caryophyllene oxide (1.82-2.02%), β-elemene (1.00-2.21%), *α*-14-hydroxy-humulene (1.46-1.78%), *α*-pinene (0.58-1.18%), (E)-9-epi-caryophyllene (0.94-1.07%), lavandulol (0.62-1.11%), *cis*-sabinene hydrate

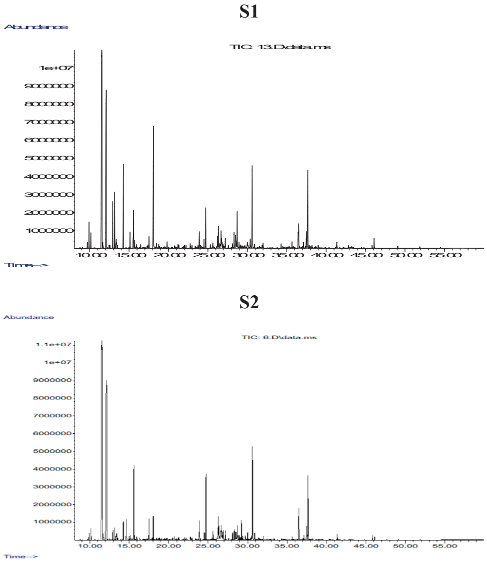

Figure 1. Chromatograms of the essential oil from the wormwood (*Artemisia absinthium* L.) grown in the region of NFMW-Plovdiv

(0.17-1.04%). There are significant compositions differences for most components between oils obtained from areas located at different distances from NFMW-Plovdiv, which have varying degrees of pollution.

The results obtained show that monoterpenic hydrocarbons (sabinene, β-myrcene, cambrene, p-cimene, α-terpinene, α-pinene) are predominant in oil, but significant differences in their content are observed in oils from contaminated and uncontaminated area.

The content of sabinene ranges from 24.54% in the contaminated soil (S1) to 26.53% in the uncontaminated area (S2); β-myrcene from 26.24% (S1) to 28.21% (S2), cambrene from 1.24% (S1) to 2.72% (S2); α-terpinene from 0.299% (S2) to 2.06% (S1); p-cimene from 0.11% (S2) to 2.5 (S1); and α-pinene from 0.58% (S2) to 1.18% (S1).

Higher levels of monoterpenic hydrocarbons α-terpinene, α-pinene, camphene, and p-cimene are observed in the oils from the contaminated area

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Table 4. Composition of wormwood oil (%) obtained by processing fresh leaves and flowering tips of wormwood grown in the region of NFMW

№	Composition	RI	S1 (0.5 km)	S2 (15.0 km)
	Oil content, %			
	Compound		% of TIC	
1	α-Pinene	939	0.58	1.18
2	Camphene	953	0.33	0.73
3	Sabinene	969	26.53	24.54
4	β-Pinene	979	0.57	0.26
5	β-Myrcene	991	28.20	26.24
6	α-Terpinene	1014	0.30	2.06
7	p-Cymene	1026	0.11	2.5
8	Eucalyptol	1031	0.58	0.22
9	γ-Terpinene	1054	0.907	3.93
10	*cis*-Sabinene hydrate	1065	1.039	0.17
11	Terpinolene	1086	0.23	0.73
12	beta-Linalool	1097	6.27	2.24
13	n-Nonanal	1102	0.19	0.38
14	Lavandulol	1165	1.11	0.62
15	Terpinene-4-ol	1177	2.17	8.28
16	α-Terpineol	1189	0.31	0.16
17	*trans*-Piperitol	1207	0.42	0.15
18	p-Cumic aldehyde	1238	0.22	0.18
19	Geranial	1270	0.58	0.25
20	Perillaldehyde	1273	0.97	0.78
21	Lavandulyl acetate	1288	0.17	0.13
22	β-Elemene	1389	2.21	1.01
23	(E)-β-Caryophyllene	1419	5.45	1.97
24	α-Caryophyllene	1454	0.38	0.51
25	(E)-9-epi-Caryophyllene	1465	0.94	1.07
26	*trans*-Nerolidol	1567	0.43	1.04
27	Caryophyllene oxide	1581	1.82	2.02
28	α-14-hydroxy-Humulene	1700	1.46	1.78
29	Longifolol	1713	0.83	1.51
30	iso-Longifolol	1728	5.59	4.40
31	Cembrene	1937	2.72	1.24
32	Phytol	1942	4.33	5.23
	Total		97.95	97.51

RI - Relative Index; TIC - Total Ion Current compared to the oil from the uncontaminated area.

Significant amounts of oxygenated monoterpenes were also found in the oils: β-linalool, which ranges from 2.24% (S1) to 6.27% (S2); phytol - from 4.33% (S2) to 5.27 (S1); terpinene-4-ol - from 2.17 (S2) up to 8.28% (S1), perillaldehyde from 0.78 (S1) to 0.97% (S2). Higher levels of oxygenated monoterpenes terpinene-4-ol, phytol, and n-nonanal are observed in the oils from the contaminated area compared to the oil from the uncontaminated area.

The content of sesquiterpene hydrocarbons ((E)-B-caryophyllene, α-caryophyllene, (E)-9-epi-caryophyllene, α-14-hydroxy-humulene, longifolol and iso-longifolol, β-elemene) in the oils has been determined, their quantity being higher in oils from uncontaminated area (S2).

The oils also contain oxygenated sesquiterpenes (*trans*-nerolidol and caryophyllene oxide), their quantity being higher in oil from contaminated area (S1).

Chialva et al. (1983) describe six different chemotypes of European *A. absinthium*, three “pure” chemotypes: (a) (Z)-6,7-epoxyocimene (Italy), (b) sabinyl acetate (France), and (c) β-thujone (Italy), and three “mixed” chemotypes: (d) β-thujone/(Z)-6,7-epoxyocimene, (e) β-thujone/sabinyl acetate, and (f) (Z)-6,7-epoxyocimene/chrysanthenyl acetate/sabinyl acetate (Italy, Siberia and Romania). The content of sabinene and myrcene in these chemotypes was low (0.1-6.3%).

Orav et al. (2006) found three additional chemotypes of *A. absinthium*: (g) sabinene/myrcene (Estonia), (h) neryl butanoate (France) and (i) 1,8-cineole (Spain).

The results show that wormwood oil from contaminated and uncontaminated area belongs to sabinene and myrcene rich chemotype. Orav et al. (2006) found that monoterpenes predominate with high amounts of sabinene and β-myrcene (21.2% and 25.6%) in some of the oils from Estonia. Comparatively large contents of sabinene and β-myrcene (9.2-38.9%) were also found in the oils from wormwood growing in Hungary, Scotland and Moldova).

Judzentiene et al. (2004) found that monoterpenoids comprised a large part (55.7-80.2%) of wormwood oils, as well as oxygenated monoterpenes (47.1-66.7%) formed about a half of oil content. A high content of oxygenated sesquiterpenes (11.9-29.8%) characterized the samples from Italy, Latvia, Lithuania and Germany (Orav et al., 2006). Msaada et al. (2015) found that in oils from Tunisia, monoterpene hydrocarbons (45.31-62.08%) predominated, with the main component chamazulene (39.93%).

The compounds in the studied essential oils that decreased as a result of heavy metals pollution are β-myrcene, sabinene, beta-linalool, (E)-β-caryophyllene, cembrene, β-elemene, lavandulol and cis-sabinene hydrate, while the terpinene-4-ol, phytol, γ-terpinene, p-cymene, α-terpinene, α-pinene, and (E)-9-epi-caryophyllene significantly increased.The observed differences in the profile of the essential oils of wormwood when grown on contaminated and uncontaminated soils may be related to soil contamination.

The chromatographic profile shows a complex mixture of components contained in wormwood oil. Figure 2 shows the classification of the identified compounds based on functional groups. The highest is the content of monoterpenic hydrocarbons (60.47-63.39%), followed by oxygenated monoterpenes (17.71-18.50%), sesquiterpene hydrocarbons (12.24-16.86%), oxygenated sesquiterpenes (2.21-3.06%) and non-terpene derivates (0.14-033%).

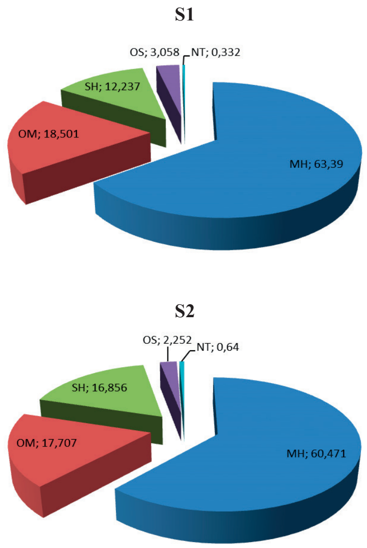

Figure 2. Classification of the identified compounds based on functional groups (OM - oxygenated monoterpenes; MH - monoterpenic hydrocarbons; OS - oxygenated sesquiterpenes;SH - sesquiterpene hydrocarbons; NT - and non-terpene derivates)

CONCLUSIONS

Based on the results obtained the following conclusions can be made:

1. *Artemisia absinthium* L. is a plant which is tolerant to heavy metals and can be grown on contaminated soils.
2. The amounts of Pb, Zn and Cd in the oil of wormwood grown on contaminated soil (Pb - 2509.1 mg/kg, Zn - 2423.9 mg.kg, Cd - 64.3 mg/kg) are lower than the accepted maximum values and meet the requirements of an environmentally friendly product.
3. Processing of the leaves and flowering tips to oil and the use of the wormwood oil will significantly reduce the cost of phytoremediation.
4. Wormwood oil from contaminated and uncontaminated area belongs to sabinene and myrcene rich chemotype.
5. The highest is the content of monoterpenic hydrocarbons in essential oils of wormwood (60.47-63.39%), followed by oxygenated monoterpenes (17.71-18.50%), sesquiterpene hydrocarbons (12.24-16.84%), oxygenated sesquiterpenes (2.21-3.06%), and non-terpene derivates (0.14-033%).
6. The compounds in the essential oil that decreased as a result of heavy metals pollution are β-Myrcene, Sabinene, beta-Linalool, (E)-β-Caryophyllene, Cembrene, β-Elemene, Lavandulol and *cis*-Sabinene hydrate, while the Terpinene-4-ol, Phytol, γ-Terpinene, p-Cymene, α-Terpinene, α-Pinene and (E)-9-epi-Caryophyllene significantly increased.
7. The essential oil of wormwood can be a valuable product for the farmers from the polluted areas.

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