

HEAVY METAL ACCUMULATION AND CHEMICAL COMPOSITION OF *THYMUS PULEGIOIDES* ESSENTIAL OIL GROWN ON SERPENTINE SOILS IN BULGARIA

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Abstract

This study investigated the heavy metal concentrations and chemical compositions of the essential oils of Thymus pulegioides (Lamiaceae), growing on serpentine soils in the Eastern Rhodopes Mountains, Bulgaria. Elevated Ni content in soils does not affect the development of Thymus pulegioides and the quality and quantity of oil obtained from it. Forty-two components representing 98.38- 98.48% of the total oil were identified. The major compounds were determined β -caryophyllene (14.15-15.54%), germacrene D (16.55-17.11%), Sabinene (10.40-11.54%), caryophyllene oxide (8.41-9.65%), cis-beta-ocimene (6.62 -6.91%), trans-beta-ocimene (5.09- 5.32%), (-)- spathulenol (4.34 - 6.54%), α -cadinol (2.06-5.51%), β -selinene (3.15- 3.30%), δ -cadinene (2.07-2.17%), α -humulene (1.95-2.04%), and β -bourbonene (1.58-1.65%) in thyme oils. Thymus pulegioides can be considered as “accumulator plant”, containing high metal concentrations in the aboveground biomass. Concentrations for toxic elements (Ni and Cr) in herbs were higher than the permissible limits for consumption. The strict control of marketed herbs for toxic metal content, and people should be informed about the risk of collecting and consuming herbs from serpentine soils. Thymus pulegioides found on serpentine soils are recommended to be collected only for pharmaceutical purposes.

Keywords: essential oil composition, macroelements, mountain thyme, trace elements, ultramafic soils

1. INTRODUCTION

Thymus pulegioides (family *Lamiaceae*) is widespread in Europe. It occurs on alkaline or neutral soil on hills, rocky uplands, gravel, sandy places, wastelands, and roadsides (Bani et al., 2009). Mountain thyme is a perennial herbaceous plant, reaching up to 5-25 cm in height, with highly aromatic leaves and lilac-pink flowers in early summer. Thyme is one of the most used herbs in both formal and traditional medicine. The flowering aerial part of the thyme and the essential oil extracted from it is used. Thyme contains various biologically active compounds: tanning substances, mineral salts, resins, essential oil, flavones, etc. Due to the wide variety of thyme species and their polymorphism, the composition of medicinal raw materials varies considerably. The essential oil of thyme contains 40 to 80 substances, including thymol, carvacrol, n-cymene, α -terpinolene, borneol, etc.

The aboveground parts of the herbs (thyme, St. John's wort, yarrow, etc.) are usually collected from natural populations, mainly from central and southern Bulgaria, and used for personal consumption, as offered domestically or exported to the foreign market (Evstatieva et al., 2007). Herbs have valuable medicinal properties, can treat various health problems and are used to produce plant-based medicines. However, herbs may also contain potentially dangerous toxic elements. Herbs growing wild on metallic soils, such as serpentines, can accumulate toxic elements depending on their individual genotypic properties and the concentrations and bioavailability of the elements in the soil (van der Ent et al., 2013). Such medicinal plants can have serious toxic effects on humans (Khan et al., 2013). People in such regions commonly consider the plants pure and use them daily for consumption. The concentrations of various plant by-products strongly depend on the growing conditions and influence the metabolic pathways responsible for synthesizing associated natural products (Akula & Ravishankar, 2011). Metals can significantly alter the chemical composition of secondary metabolites in the aerial parts of plants and thus seriously affect the quality, safety and efficacy of natural plant products produced from them (Pavlova & Karadjova, 2013).

Although the composition of thyme oils is well studied, there is a lack of information on the heavy metal, macro and trace element content of the aboveground mass of thyme growing on serpentine soils in Bulgaria. The present study aimed to conduct a comparative study that would allow us to determine the amounts of heavy metals, macro and microelements in the aboveground mass of mountain thyme and its oil quality growing on serpentine soils.

2. MATERIALS AND METHODS

2.1. Materials

Mountain thyme growing on serpentine soils in the Eastern Rhodopes was included in the study. The serpentine soils are collected near the village of Kazak and the village of Golyamo Kamenyane. The location and geographic coordinates of the soil and plant sampling sites are as follows: Kazak (K) Latitude (N) 41°24'37.2", Longitude (E) 25°53'10.0", Golyamo Kamenyane (GK) Latitude (N) 41°24'06.8", Longitude (E) 25°42'23.1"

Five plants were collected from each site at flowering stage in late June and early July. Aboveground plant parts were cleaned of soil particles, washed with distilled water, air-dried to constant weight and then ground. Three parallel samples of each composite sample were used for analysis. Oils were extracted from aboveground mass of thyme with a Clevenger apparatus by steam distillation for 2 hours. At the end of each distillation, the oil was separated from the water, collected in glass vials, and stored until gas chromatographic analyses were performed.

Simultaneously, three soil samples were collected at 0-20 cm depth from each site. The soils were air-dried and ground.

2.2. Methods

Soil samples were prepared for analysis by treatment with aqua regia (ISO 11466). The mobilizable heavy metals contents in soils, considered a "potentially bioavailable metal fraction", were extracted by a solution of DTPA (ISO 14870). The plant and oil samples were treated by the method of microwave mineralization. To determine the content of heavy metal, macro and microelements in the plant and soil samples, an inductively coupled emission spectrometer (Jobin Yvon Horiba "ULTIMA 2", France) was used.

The oils were analyzed for heavy metals, and their chemical composition was determined. Determining the oil's chemical constituents was performed 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).

3. RESULTS

The soils have a neutral reaction (pH 6.6-6.8) and an average organic matter content (3.35-4.48%). Table 1 presents the results obtained for the content of heavy metals, macro and microelements in soil samples (total amount and DTPA-extractable) and their content in the aboveground mass of mountain thyme growing on serpentine soils in the Eastern Rhodopes. The soils of the Kazak and Golyamo Kamenyane areas are characterized by elevated levels of Fe, Ni, Cr, Co and Mn, typical for serpentine soils (Table 1).

	Pb	Zn	Cu	Fe	Mn	P	Cr	Ni	Ca	Mg	K	Co
K												
st	19,9	67,5	10,7	67442,8	2019,0	266,3	1876,8	1481,3	2767,6	63512,8	999,5	314,4
se	0,88	3,0	3,2	58,1	56,5	1,9	-	99,4	655,0	378,9	13,0	2,74
p	4,1	46,1	7,75	7739,1	423,80	613,37	46,57	249,2	5556,5	7046,8	5902,8	68,19
GK												
st	6,7	45,1	30,2	61351,5	1493,4	60	493,7	2397,2	2938,7	49507,6	565,8	233,6
se	0,52	0,56	1,8	35,6	19,3	1,52	-	30,4	988,3	1062,54	12,6	0,68
p	2,3	35,3	6,28	3810,1	130,2	716,28	14,28	118,3	6029,6	4138,91	8883,5	20,83

st- soil total content, sa- DTPA –extrated, p- plant thyme

Table 1. Content of heavy metals, macro and microelements (mg/kg) in soils (total content and DTPA-extractable) and aboveground mass of thyme from serpentine soils in Kazak and Golyamo Kamenyane

The composition of thyme oil was also determined. To clarify the issues related to the influence of heavy metals on the composition of oil from serpentine soils, samples of thyme oil obtained in the laboratory by steam distillation were analyzed. Table 2 presents the results obtained for the composition of essential oils. Forty-two components belonging to the classes of monoterpenes, sesquiterpenes, diterpenes, triterpenes and hydrocarbons were identified in the thyme oils (Table 2). The composition of thyme oil was dominated by β -caryophyllene (14.15-15.54%), germacrene D (16.55-17.11%), sabinene (10.40-11.54%), caryophyllene oxide (8.41-9.65%), cis-beta-ocimene (6.62-6.91%), trans-beta-ocimene (5.09- 5.32%), (-)- spathulenol (4.34 - 6.54%), α -cadinol (2.06-5,51%), β -selinene (3,15-3,30%), δ -cadinene (2,07-2,17%), α -humulene (1,95-2,04%), and β -bourbonene (1,58-1,65 %).

	Name	RI	K	GK
1	α -Pinene	939	0,24	0,25
2	Camphene	954	0,11	0,12
3	Sabinene	969	11,54	10,40
4	β -Pinene	979	0,26	0,27
5	2-Octanone	988	0,43	0,45
6	β -Myrcene	991	1,24	1,29
7	α -Terpinene	1018	0,35	0,36
8	p-Cymene	1025	1,17	1,22
9	Limonene	1030	0,45	0,47
10	Eucalyptol	1031	0,25	0,26
11	β -Phellandrene	1032	0,61	0,64
12	cis-beta-Ocimene	1040	6,62	6,91
13	trans-beta-Ocimene	1050	5,09	5,32
14	γ -Terpinene	1055	0,90	0,94

15	Terpinolene	1086	0,14	0,15
16	β -Linalool	1092	0,59	0,62
17	Terpinene-4-ol	1179	1,68	1,76
18	α -Terpineol	1189	0,30	0,31
19	Linalool acetate	1254	1,20	1,25
20	Neryl acetate	1342	0,26	0,27
21	Geranyl acetate	1350	0,75	0,78
22	β -Bourbonene	1388	1,58	1,65
23	β -Elemene	1390	0,43	0,45
24	β -Caryophyllene	1419	15,54	14,15
25	α -Humulene	1454	1,95	2,04
26	Allo-Aromadendrene	1458	0,81	0,84
27	γ -Muurolene	1477	0,34	0,36
28	Germacrene D	1484	16,55	17,11
29	β -Selinene	1490	3,15	3,30
30	γ -Cadinene	1513	0,27	0,29
31	δ -Cadinene	1524	2,07	2,17
32	Caryophyllene oxide	1581	8,41	9,65
33	(-)-Spathulenol	1619	4,34	6,54
34	α -Cadinol	1652	5,51	2,06
35	Phytol	2105	0,49	0,52
36	n-Tricosane	2300	0,13	0,14
37	n-Tetracosane	2400	0,26	0,27
38	n-Pentacosane	2500	0,36	0,38
39	n-Hexacosane	2600	0,16	0,17
40	n-Heptacosane	2700	0,12	0,12
41	n-Octacosane	2800	0,68	0,71
42	Squalene	2817	1,02	1,51
Total			98,38	98,48

Table 2. Chemical composition (%) of thyme oil

4. DISCUSSION

4.1. Serpentine soils

Ultramafic (serpentine) soils are considered unfavourable habitats for plants due to low nutrient content, calcium deficiency, magnesium toxicity and high concentrations of potentially toxic elements such as Cr, Ni, and Co. High concentrations of Mg relative to Ca and elevated concentrations of Ni are considered important factors affecting plant growth and survival on serpentine soils.

The Ni content ranges from 1481.3 mg/kg to 2397.2 mg/kg and is considered typical (500-5000 mg/kg) for serpentine and other Mg- and Fe-rich soils. Ni is known to be toxic in soils above 500 mg/kg (Allen et al., 1974), although toxicity depends on Ni bioavailability. The total Cr content of Kazak soils reaches 1876.8 mg/kg and significantly exceeds the upper limit given for soils by Allen et al. (1974) (500 mg/kg).

While Cu and Zn contents fall within the range of normal soils (Kabata-Pendias, 2001), Pb contents are significantly higher and reach a maximum level of 19.9 mg/kg, which is in agreement with the data reported by Babalonas et al. (1984) from serpentine soils in northern Greece. The Cd content of the soils was below the detection limits of the method used. The Mn content of the soils varied between 1493.4 mg/kg and 2019.0 mg/kg, with higher Mn levels in the Kazak area being atypical and probably due to the soil-forming rocks, topography, vegetation and the high content of limiting substance in the soil. The Fe content in serpentine soils is high and ranges from 61351.5 mg/kg to 67442.8 mg/kg, respectively, which is typical for serpentine soils of the Balkan Peninsula. The total Ca content of serpentine soils ranges from 2767.6 mg/kg to 2938.7 mg/kg. The Mg content is higher than Ca and ranges from 49507.6 mg/kg to 63512.8 mg/kg, and the Ca: Mg ratio is <1.

The chemical composition of serpentine soils from the Eastern Rhodopes is similar to serpentine soils from other parts of the world (Brooks 1987), with high concentrations of metals such as Ni and Cr and relatively low Ca/Mg ratios. Ca/Mg ratios range from 0.044 to 0.059. The Ca and Mg levels found in these Bulgarian serpentine soils are typical of such soils from other areas (Brooks 1987). The total Ni content of the soil is similar to that of Italian, Greek (Babalonas et al. 1984) and Albanian (Shallari et al. 1998) serpentine zones.

4.2. Thyme

The content of Ni in the aboveground mass ranged from 118.3 mg/kg to 249.2 mg/kg. The Ni content of thyme is higher than the area of Kazak, although the total Ni content of the soil is lower than that of Golyamo Kamenyane. This is due to the significantly higher content of mobile forms of Ni, 99.4 mg/kg (6.7%) in Kazak compared to 30.4 mg/kg (1.2%) in Golyamo Kamenyane. Typically, the Ni content of plants from normal soils reaches up to 10 mg/kg. The Ni content in the studied plants was much higher than the permissible values (0.4-4 mg/kg) proposed by the Cadastre of Elemental Concentration in Ecosystems (Anonymous, 1994) and higher than the values presented in Regulation No. 31 (2.0 mg/kg) (Bulgarian Ministry of Health, 2006). Similar values were found by Salihaj et al. (2016) for thyme on serpentine soils from Kosovo (178,21 mg/kg)

According to the ability to accumulate heavy metals, plants are classified into three groups - excluders, indicators and accumulators. Exclusion plants maintain constant levels in aboveground mass over a wide range of metals in the soil. In indicator plants, the heavy metal content increases with increasing soil metal content. In contrast, in accumulator plants, the uptake of metals in the aboveground mass is independent of soil metal content. Thyme can be classified as an accumulator plant because the Ni content of the aboveground mass is independent of the Ni content of the soil.

The Ni content in the aboveground mass of thyme is higher than Cr, which is consistent with their soil bioavailability. Such behaviour has been observed in other plants from serpentine deposits (Naseem et al., 2009). Ni and Cr are metals with potential risks to human health and are classified in Class A by the European Medicines Agency (EMA, 1998). Although Cr content in serpentine soils is generally elevated, bioaccumulation of this element from thyme is low. Cr content in thyme ranges from 14.3 to 46.6 mg/kg, and significantly higher than the values (0.2-1 mg/kg) proposed by the Cadastre of Elemental Concentration in Ecosystems (Anonymous, 1994) and higher than the values presented in Regulation No. 31 (0.5 mg/kg) (Bulgarian Ministry of Health, 2006). Similar results to be obtained for thyme from serpentine soils of Kosovo (16.2 mg/kg) (Salihaj et al., 2016). With higher contents in medicinal plants, Cr can be toxic to humans. It is classified as a significant contaminant in herbs along with Pb, Cd and Hg (WHO, 2007).

Co content rarely reaches 10 mg/kg in plants on normal soils, and even on serpentine soils, this level is not commonly exceeded. However, our results show that the Co content is high and ranges from 20.8 to 68.2 mg/kg. These values are much higher than the values (0.2-1 mg/kg) suggested by the Cadastre

of Elemental Concentration in Ecosystems (Anonymous, 1994). Significant Co content was found in the mash of serpentine soils in Kosovo (21.97-25.98 mg/kg) (Salihaj et al., 2016)

Trace elements Mn, Fe, Cu, Co and Zn are essential for plants, are involved in redox reactions, and act as enzymes. However, these metals can also be toxic at high concentrations. Generally, the Cu and Zn contents of serpentine soil plants are low, and their accumulation depends on the plant species and the bioavailability of the elements in the soil. The Cu content of thyme ranges from 6.28 to 7.75 mg/kg and Zn from 35.32 to 46.1 mg/kg. The toxicity of zinc is lower than that of copper. The toxic concentration of zinc was manifested at 200 mg/kg for dry plant mass (Golubovic & Blagojevic, 2013), much higher than the concentrations found in the studied plant samples. The content of both elements was below the maximum permissible levels in food (25 mg/kg for copper and 50 mg/kg for zinc) (Bulgarian Ministry of Health, 2006).

The Mn content in thyme leaves ranged from 130.2 to 423.8 mg/kg, and these values were below and within the norms of the critical level (300-500 mg/kg dry weight). The Fe content in thyme leaves is exceptionally high and ranges from 3810.1 to 7739.1 mg/kg and is higher than the 500 mg/kg limit suggested by Allen et al. (1974) and (>200 mg/kg) according by the Cadastre of Elemental Concentration in Ecosystems (Anonymous, 1994). Iron is an essential element for plant growth. Iron uptake by plants is influenced by abiotic factors such as soil, climate and biotic conditions such as plant growth stages and their ability to accumulate Fe (Imelouane et al., 2011).

Similar values for Fe have been found for other plants growing on serpentine soils (above 2000 mg/kg for *T. chamaedrys*, *T. polium* and *C. officinarum*) (Pavlova & Karadjova, 2013). This may be due to the ability of the plants to acidify the rhizosphere and increase the solubility of ferrous ions and their reduction to even more soluble ferrous ion. Extremely high values have also been found in other thyme species growing in Turkey (up to 3479 mg/kg) (Kucukbay & Kuyumcu, 2010). Probably, the high Fe content of the plants is not only a unique feature of the serpentine flora of the Balkans (Babalonas et al., 1984).

On the other hand, the high Fe content may also be due to sample contamination. The usual cleaning procedures by washing with water are not sufficiently effective for plants that are densely covered with hairs, such as thyme. Higher values often indicate sample contamination from serpentine soils, which are not always easily removed by conventional washing procedures (Reeves et al., 1999).

The high Mg content of serpentine soils also determines the high Mg content of plant tissues (4138.9 - 7046.8 mg/kg). Notably, the Ca uptake by plants is significant (ranging from 5556.5 to 6029.6 mg/kg), although the amount of Ca in serpentine soils is low. According to Proctor (1971), Ca is one of the elements influencing the reduction of heavy metal toxicity. Probably plants growing on serpentine soils absorb more Ca to compensate for the toxic action of various toxic metals. Karataglis et al. (1982) suggested that plants have a mechanism that allows them to assimilate significant amounts of Ca. The Ca: Mg ratio in plants is generally consistently above 1. Plants of serpentine soils maintain a ratio greater than one despite the minimum Ca levels found in soils. The Ca/Mg ratio in thyme from Golyamo Kamenyane is > 1 (1.46), while in plants from Kazak the ratio is less than 1 (0.79). They either have very efficient Ca uptake systems or the ability to exclude Mg despite high soil concentrations.

Pb and Cd are non-essential trace elements that can cause various toxic effects at low doses. Both elements are not part of the "serpentine syndrome"; these elements in most serpentine soils are at relatively low levels, close to typical ranges for uncontaminated regions. Lead content in the aboveground mass of mountain thyme ranged from 2.3 to 4.1, and cadmium was below the detection limit with the method used. The Cd and Pb contents were below the maximum limits of 0.5 mg/kg for Cd and 5 mg/kg for Pb (European Pharmacopoeia) (Council of Europe, 2008), WHO (2000) requirements (0.3 and 10 mg/kg), and above the permissible levels according to the Bulgarian food (herbs) legislation (0.2 mg/kg for Cd and 0.3 mg/kg for Pb) (Bulgarian Ministry of Health, 2006).

The obtained results show that the content of Ni, Cr, Co and iron in the aboveground mass of thyme from serpentine soils is much higher than the values proposed in the cadastre, and the content of Ni and Cr is also higher than the maximum permissible values specified in Regulation No. 31 for food

(Bulgarian Ministry of Health, 2006). Consumption of thyme and herbal tea prepared from thyme may pose a health risk to consumers if taken over a prolonged time. The risk will depend on the dose, age, sex and duration of exposure (Khan et al, 2013). Since heavy metals pose a hazard to human health, their concentrations in medicinal plants should be limited. Medicinal herbs should not be grown and harvested from contaminated soils. Harvesting herbs from serpentines is a risk due to elevated levels of heavy metals in soil and plants. For this reason, strict controls on medicinal plants and people should be made aware of the risk of collecting herbs from such places. Consuming such herbs in the form of tea or decoctions poses a risk of human exposure to heavy metals.

The heavy metal content of thyme essential oil has also been determined. The results show that most of the heavy metals contained in the thyme leaves do not pass into the oil during the processing of the leaves, so their content in the oil is significantly lower. Pb in thyme essential oil reaches up to 0,05 mg/kg, Zn up to 0,01 mg/kg, and Cd is below the detection limit with the method used. The heavy metal content in the essential oils is lower than the aboveground part of the thyme, the amounts of Pb, Zn and Cd in the thyme oil are lower than the accepted maximum permissible values, and the oil meets the criteria for an environmentally friendly product.

4.3. Composition of oils

Sesquiterpenes, which include sesquiterpene hydrocarbons (SH) and oxygen-containing sesquiterpenes (OS), are the predominant chemical class of oil constituents, ranging from 60.61 to 60.97% of the total oil composition (table 2, fig.1). The amount of sesquiterpene hydrocarbons ranges from 42.71 to 42.36%. The main components of this group are germacrene D (16.55-17.11%), β -caryophyllene (14.15-15.54%), β -selinene (3.15-3.30%), δ -cadinene (2.07-2.17%), α -humulene (1.95-2.04%), β -bourbonene (1.58-1.65%). The amount of oxygenated sesquiterpenes reaches 18.26% of the total oil composition. caryophyllene oxide (8.41-9.65%), (-)-spathulenol (6.54-14.34%), α -cadinol (2.06-5.51%) predominate in the oil,

Monoterpenes, which include monoterpene hydrocarbons (MH) and oxygen monoterpenes (OM), belong to the second major chemical class of oil constituents. Their amount varies from 33.61 to 33.75% of the total oil composition. The oil is dominated by monoterpene hydrocarbons sabinene (10.41-11.54%), cis-beta-ocimene (6.62-6.91%), trans-beta-ocimene (5.09-5.32%), β -myrcene (1.24-1.29%), p cymene (1.17-1.22%) and γ -terpinene (0.90-0.94%). Overall, sabinene has the highest content of monoterpene hydrocarbons in the oil. Significantly lower was the content of oxygen-containing monoterpenes. The main oxygen-containing monoterpenes are terpinene-4-ol (1.68-1.76%), linalool acetate (1.20-1.25%), geranyl acetate (0.75-0.78%), β -linalool (0.59-0.62%).

The oil also contains the acyclic diterpene alcohol phytol (0.49-0.52%), and the polyunsaturated triterpene hydrocarbon squalene (1.02-1.51%). The data published by other authors on the composition of thyme oil do not report the presence of diterpenes and triterpenes. The hydrocarbon content of the oil ranges from 1.72 to 1.80%.

The time of harvesting and especially environmental conditions such as temperature, rainfall, altitude, solar radiation, soil chemistry, etc., may also influence the quantitative composition of the essential oils and the percentage of chemical compounds in them. No significant differences were observed in the amount of individual components in the oils from Kazak and Golyamo Kamenyane. The content of sabine, β -caryophyllene and α -cadinol is higher in the oil from Kazak. In comparison, the content of germacrene D, caryophyllene oxide, (-)-spathulenol and squalene is higher in the oil from Golyamo Kamenyane.

The chemical classification of chemotypes is based on the chemical composition of the essential oils, with the names of the chemotypes determined by the major chemical compound (or compounds) in the essential oils. There are different chemotypes in which the predominant compounds are monoterpenes (geraniol, terpineol, linalool, etc.) and aromatic compounds (thymol and carvacrol) (Loziene & Venskutonis, 2005.; Zeljkojic & Maksimovic, 2014). Sesquiterpenes are thought to be present in low concentrations in species of the genus *Thymus* (Stahl-Biskup, 2002). Still, several sesquiterpenoid chemotypes have been found in representatives of the genus growing on the Balkan Peninsula (Pavel et al., 2009). The carvacrol and thymol chemotypes have been found in Norway, the UK and

Germany, Italy, Slovakia, Croatia, Romania, Yugoslavia and Lithuania (Martonfi et al., 1992, Senatore, 1996, Stahl-Biskup, 2002, Loziene et al., 2002, Loziene et al., 2003, Mockute & Bernotiene, 2005). Geraniol and geraniol/linalool have been identified as chemotypes in the UK, Slovakia, Croatia, Bosnia and Herzegovina and Lithuania and citral/geraniol chemotypes in Slovakia and Lithuania. Plants biosynthesizing linalool as a significant constituent of oil have been collected in the UK, Slovakia, Croatia and Lithuania. A phenolic chemotype has been found in Slovakia, while an α -terpinylacetate chemotype has been identified in Lithuania (Stahl-Biskup, 2002, Mockute & Bernotiene, 2005).

Thyme essential oil is characterized by a spicy, pungent and pleasant coniferous-citrus aroma. The aroma of thyme oil is mainly determined by the components contained in the oil. For example, citral-geraniol chemotype oil is characterized by a 'lemony' odour. In this chemotype, citral (neral + geranial, 18.4-30.2%), geraniol (14.4-23.2%), β -caryophyllene (9.4-13.1%) and nerol (5.5-12.1%); predominate. A "sweet" odour distinguishes thyme of the geraniol chemotype, with the amount of geraniol in the essential oil of plants with a "sweet" odour containing geraniol (23.1-25.5%), β -caryophyllene (10.8-13.5%) and β -bisabolene (9.5-10.2%) (Mockute and Bernotiene, 2005). The essential oil of the geraniol chemotype contains a smaller amount of citral and oxygen-carrying monoterpenes and a larger amount of monoterpene hydrocarbons than that of the citralgeraniol chemotype.

Thyme of the γ -terpene-p-cymene chemotype is characterized by a "hydrocarbon" odour. It has a refreshing grassy-citrusy smell, not as lemony as the oil of the citral-geraniol chemotype. The γ -terpinene/p-cymene chemotype oil ("hydrocarbon" odour) contains γ -terpinene (15.5-17.2%), p-cymene (7.7-17.0%), carvacrol (4.6-13.5%) and β -caryophyllene (9.6-10.5%) (Mockute and Bernotiene, 2005).

In the thyme of the caryophyllene chemotype ('spicy' odour), about half of the essential oil from the 'spicy' odour plant consists of sesquiterpene hydrocarbons. 3) caryophyllene chemotype ("spicy" odor): β -caryophyllene (18.0-19.7%), β -bisabolene (14.5-15.9%), germacrene D (12.5-14.6%) and (E)-nerolidol (8.2-13.8%)(Mockute and Bernotiene, 2005).

Thyme (*T. Pulegioides*) with the carvacrol chemotype (phenolic odour) has phenolic and pungent phenolic odours. The oil contains carvacrol chemotype ("phenolic" odor): carvacrol (24.4-33.3%), β -caryophyllene (9.5-14.9%) and β -bisabolene (10.5-12.9%) (Mockute and Bernotiene, 2005).

The thyme from the serpentine soils of the Kazak and Great Kamenyane area belongs to the caryophyllene chemotype ('spicy' odour): about half of the essential oil from the plant with the 'spicy' odour consists of sesquiterpene hydrocarbons. The predominant sesquiterpene hydrocarbons are germacrene D (16.55-17.11%) and β -caryophyllene (14.15-15.54%) and the oxygen-containing sesquiterpene caryophyllene oxide (8.41-9.65%) (-) spathulenol (4.34-6.54%), α -cadinol (2.06-5.5%1). Caryophyllene oxide is a metabolite of β -caryophyllene (a product of caryophyllene oxidation that occurs during crop maturation).

However, the oil also contains the monoterpene hydrocarbon sabinene (10.40-11.54%) High content of the monoterpene bicyclic hydrocarbon Sabinene in the oils we studied, which has a warm spicy-peppery smell.

Thyme oil from the serpentine soils of the Kazak and Golyamo Kamenyane area does not belong to any of the chemotypes described in the literature. A mixed caryophyllene/sabinene chemotype characterizes the oil. It has a 'spicy' odour, which is attributed to the components germacrene D (16.55-17.11%), β -caryophyllene (14.15-15.54%), sabinene (10.40-11.54%) and caryophyllene oxide (8.41-9.65%) contained in it. No such chemotype has been detected so far.

The biological activity of thyme oil was related to the presence of the compounds in it. The anti-inflammatory, anticarcinogenic, antimicrobial, antibacterial, antioxidant and analgesic activities are due to the high content of β -caryophyllene and β -caryophyllene oxide.

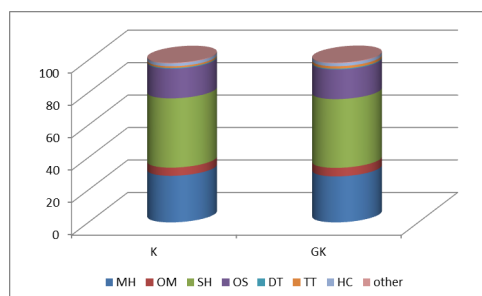


Fig. 1. Classification of identified compounds based on functional groups in thyme oil

5. CONCLUSIONS

1. The chemical composition of serpentine soils from the Eastern Rhodopes is similar to that of the Balkan Peninsula. It is characterized by high contents of Ni (1481.3 -2397.2 mg/kg), Cr (493.7-1876.8 mg/kg) and Mg (49507.6 - 63512.8 mg/kg).
2. The unfavourable soil characteristics do not affect the development of the thyme or the quality of the oil obtained.
3. Thyme is an accumulating plant which contains Ni (118.3 - 249.2 mg/kg), Cr (14.3 – 46.6 mg/kg), and Co (20.8 - 68.2 mg/kg) in its aerial parts. Ni, Cr, and Co content in the aboveground mass of thyme from serpentine soils is much higher than the maximum permissible values. Thymus should not be grown and harvested from serpentine soils. The strict control of marketed herbs for toxic metal content and people should be informed about the risk of collecting and consuming herbs from serpentine soils
4. The composition of thyme oil is dominated by sesquiterpene hydrocarbons (42.36-42.71%), followed by monoterpene hydrocarbons (28.61-28.98%), oxygen-containing sesquiterpenes (18.25%), oxygen-containing monoterpenes (5.03--5.26%), diterpenoids (0.49-0.52%), triterpenes (1.02-1.51%), hydrocarbons (2.74-3.30%) and other organic compounds (0.43-0.45%) were found in the oil composition.
5. The *Thymus pulegioides* L. oil belongs to a mixed caryophyllene/sabinene chemotype with a 'spicy' odour. No significant differences were observed in the amount of individual components in oils from Kazak and Golyamo Kamenyane. The content of sabinene, β -caryophyllene and α -cadinol is higher in the oil from Kazak. In contrast, the content of germacrene D, caryophyllene oxide, (-)-spathulenol and squalene is higher in the oil from Golyamo Kamenyane.

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