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HEAVY METAL ACCUMULATION AND CHEMICAL COMPOSITION OF THE ESSENTIAL OILS OF YARROW (*ACHILLEA MILLEFOLIUM 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 the chemical composition of yarrow oils, as well as to identify the possibility of yarrow 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 the flowering stage, the yarrow plants were gathered. The content of heavy metals in flowers was determined by microwave mineralization and analyzed by ICP. The oils were obtained from the flowers of the plant by hydro distillation and analyzed by gas chromatography-mass spectrometry (GC-MS) technique. Heavy metals do not affect the development of yarrow and the quality and quantity of oil obtained from it. The heavy metal content of yarrow essential oil is very low and is not affected by the degree of heavy metal contamination of the soil. Twenty components were identified in oils. In yarrow oil, the highest content was oxygenated sesquiterpenes (36-38%), followed by sesquiterpene hydrocarbons (24-25%), oxygenated monoterpenes (20-21%) and monoterpene hydrocarbons (17-19%). For the first time, it is reported yarrow oil with mixed α-bisabolol oxide B, α-bisabolol, and β-caryophyllene chemotype. The essential oil of yarrow can be a valuable product for the farmers from polluted regions.

Keywords: contaminated soils, essential oil composition, heavy metals, yarrow

INTRODUCTION

Yarrow (*Achillea millefolium L.*) (family Asteraceae) is a perennial herbaceous plant with white flowers and a strong specific sweetish aroma. It is found almost everywhere in Bulgaria up to about 1600 m above the sea level, mainly in dry grassy places and meadows, by roadsides and bushes. Since ancient times, yarrow has been used in folk medicine for various medicinal purposes, including healing wounds, gastrointestinal disorders, and rheumatic pains (Barda et. al., 2021). Yarrow is used as a raw material for the production of medicines in the food and cosmetic industries (Mohammadhosseini et al. 2017; Gawel-Baden et al. 2020; Strzepek-Gomolka et al. 2021). The whole stalk is harvested for essential oil extraction, with the oil concentration being at-highest in the flower, then in the leaves and least in the stem. In recent years, the demand and price of yarrow essential oil have been increasing globally, with one kilogram reaching a price of 1400 Euros (Akterian, 2022). For this reason, in recent years, the interest in the cultivation of medicinal and essential oil crops in the country has been growing, and the areas of yarrow, St. John's wort, and hyssop have been increasing. The yarrow oil is dark blue, blue-green or olive-green and has a fresh, sweetish-grassy aroma with a faint camphor tinge. Many studies have been done on the composition of yarrow oil (Kocak et al., 2010; Smelcerovic et al., 2010; Raal et al., 2011). The chemical composition of the oil depends on the number of chromosomes. Diploid and tetraploid plants contain pro-azulene sesquiterpenes, which are mainly transformed into hamazulene (up to 25%) as a result of distillation, the main substances found in hexaploid plants are camphor (18%), sabinene (12%), 1,8-cineole (10%), and the main substance found in octaploid plants is linalool (Raal et al., 2011).

The main constituents of yarrow oils vary depending on the place from which the plants are collected, e.g. sabinene, 1,8-cineole, cis-chrysanthenol (Iran, Amin et al., 2008) or germacrene D, α-copaene, borneol (Iran, Rahimmalek et al., 2009) or 1,8-cineole and germacrene D have been found in yarrow oil ((Iran, Barghamadi et al., 2009), D-cadinene, limonene, aloaromadendron (Turkey, Kocak et al., 2010), 1,8-cineole and camphor (Serbia, Smelcerovic et al., 2010).

Medicinal plants can accumulate more significant amounts of heavy metals such as Cd, As, Pb and Hg compared to other plants (Kabata Pendias, 2011). The essential oil and heavy metal content of medicinal plants can be influenced by environmental conditions (Foroozeh & Mirdeylami, 2019), soil geochemical characteristics and plant habitat (Chan, 2003). Some medicinal plants, such as mint, St. John's wort, sage, calendula, marsh marigold, caraway, garlic, garden sorrel, hemp, and others, can accumulate large amounts of toxic heavy metals in their tissues, can be used for phytoremediation, and can replace food crops grown under the same conditions. The concentrations of various plant by-products depend highly on the growing conditions and affect the metabolic pathways responsible for synthesizing associated natural products. 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 the natural plant products (Akula & Ravishankar 2011).

Although the composition of yarrow essential oil is well studied, there needs to be collected more information on the heavy metal content of flowers and oils when growing yarrow on contaminated soils and its potential use for phytoremediation.

This study aims to conduct a comparative study that will allow us to determine the amounts of heavy metals in the flowers of yarrow, the quality of yarrow essential oil, and the possibilities of using the yarrow for the phytoremediation of heavy metal-contaminated soils.

MATERIALS AND METHODS

The experiment was performed on an agricultural field contaminated by Zn, Pb, Cd, and Hg, situated at different distances (0.5 and 15.0 km) from the source of pollution, the NFMW near Plovdiv, Bulgaria.

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

Table 1. Content of Pb, Zn, Cd and Hg ($mg.kg^{-1}$) in the soils sampled from the NFMW

Parameter	S1 (0.5 km)	S2 (15 km)
pH	7.4	7.5
Pb	2509.1±6.5	49.4±0.2
Cd	2423.9±6.	172.7±2.1
Zn	64.3±0.2	1.0±0.1
Hg	2.2±0.1	0.5±0.1

x- average value($mg.kg^{-1}$) from 5 repetitions; sd - mean standard deviation
MPC (pH 6.0-7.4)-Pb-100 $mg.kg^{-1}$, Cd-2.0 $mg.kg^{-1}$, Zn-320 $mg.kg^{-1}$, Hg -1.5 $mg.kg^{-1}$
MPC (pH >7.4) – Pb-100 $mg.kg^{-1}$, Cd - 3.0 $mg.kg^{-1}$, Zn -400 $mg.kg^{-1}$, Hg -1.5 $mg.kg^{-1}$

The study included yarrow (variety “Proa”) grown on areas located at different distances (0.5 km and 15 km) from the source of contamination – the NFMW Plovdiv. Yarrow is grown according to conventional technology (Doncheva & Baltov, 2017). Upon reaching the stage of flowering, yarrow was harvested. The plants were dried at room temperature, and the flowers were detached from the stems and leaves before drying. The content of Pb, Zn, Cd and Hg in the flowers was determined. The essential oil of yarrow was obtained in laboratory conditions by steam distillation for 3 h using a Clevenger-type apparatus following the European Pharmacopoeia method.

The pseudo-total content of metals in the soils was determined by ISO 11466. The available (mobile) heavy metal contents were extracted by ISO 14870 by a solution of DTPA. The contents of Pb, Zn, and Cd in the flowers and the essential oil of yarrow were determined by the method of microwave mineralisation. The quantitative measures were carried out by ICP (Jobin Yvon Emission - JY 38 S, France). The Hg content of the samples was determined without a preliminary sample preparation with a mercury analyzer MA-3000 (Nippon Instruments Corporation). The digestion and analytical efficiency of ICP and the mercury analyzer were validated using a standard reference material of the apple leaves (SRM 1515, National Institute of Standards and Technology, NIST). 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 DISCUSSION

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 the NFMW), the reported values for Pb exceeded MPC approved for Bulgaria and reached 2509.1 mg.kg^{-1}. In the area located at a distance of 15 km, the contents of Pb were significantly reduced to 49.4 mg.kg^{-1}. Similar results were obtained for Cd, Zn, and Hg (Table 1).

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 57,2%, followed by Pb with 33.8 %, Zn with 9.8% and Hg to 1.3%. In the soil located at a distance of 15 km from the NFMW, the mobile forms of Cd are the most significant part of it.

Table 2. DTPA-extractable Pb, Zn, Cd (mg.kg^{-1}) and Hg (ng.g^{-1}) in the soils sampled from the NFMW

Element		S1	S2
Pb	mg.kg^{-1}	849.1	21.5
	%	33.8	43.5
Cd	mg.kg^{-1}	36.8	0.7
	%	57.2	70.0
Zn	mg.kg^{-1}	236.8	38.9
	%	9.8	22.5
Hg	ng.g^{-1}	24.0	1.3
	%	12.6	2.5

*DTPA -extractable / total content

Content of heavy metals in yarrow flowers

Significant Pb accumulation has been found in the yarrow flowers. The content of this element reaches 405.6 mg.kg^{-1} in yarrow flowers grown at a distance of 0.5 km from the NFMW. A portion of the accumulated heavy metals in the aboveground mass of yarrow may be due to aerosol pollution. The Cd content in the yarrow flowers grown at a distance of 0.5km from the NFMW reaches up to 89.8 mg.kg^{-1}, respectively, values considered toxic to plants. According to Kabata Pendias (2011), 5.0 mg.kg^{-1} is a toxic value for plants. The obtained results show the ability of yarrow to accumulate Cd in the aboveground mass.

Table 3. Content of Pb, Cd, Zn ($mg.kg^{-1}$) and Hg ($ng.g^{-1}$) in the flowers and essential oil of yarrow

Element	Distance, km	Flowers, x ± sd	Essential oil x ± sd
Pb	0.5 km	405.6±0.8	0.11+0.01
Pb	15 km	5.9±0.5	0.02±0.01
Cd	0.5 km	89.8±1.4	nd
Cd	15 km	0.3±0.05	nd
Zn	0.5 km	737.6±5.9	2.44±0.09
Zn	15 km	24.2±0.5	0.14±0.02
Hg	0.5 km	126.5	nd
Hg	15 km	50.7	nd

x- average value(mg.kg-1) from 5 repetitions; sd - mean standard deviation, nd- non detected

The Zn content in the yarrow flowers grown at a distance of 0.5 km from the NFMW reaches up to 737.6 $mg.kg^{-1}$, as these values are also higher compared to the values critical for plants - 100-400 $mg.kg^{-1}$. The content of heavy metals in the yarrow flowers grown at 15 km from the NFMW reaches up to 5.9 $mg.kg^{-1}$ Pb, 0.3 $mg.kg^{-1}$ Cd and 24.2 $mg.kg^{-1}$ Zn.

The Hg content of the yarrow flowers ranges from 50.7 to 126.5 $ng.g^{-1}$.

The results show that significant quantities of heavy metals are accumulated in the flowers of yarrow, with their values significantly exceeding the accepted critical toxic levels (Marschner, 1995), but none of the symptoms of heavy metal toxicity has been detected. This shows that yarrow has a high tolerance to heavy metals. The results contradict the findings of Murtic et al. (2019), who found that yarrow has shown a very low capacity to absorb and accumulate heavy metals from soils. The Cd and Pb contents were higher than the maximum limits of 0.5 $mg.kg^{-1}$ for Cd and 5 $mg.kg^{-1}$ for Pb (European Pharmacopoeia) (Council of Europe, 2008).

The heavy metal content in the essential oil from yarrow was also determined. The results show that most of the heavy metals contained in the flowers of the yarrow do not pass into the oil during the distillation. Therefore, their content in the oil is much lower. Pb content in the essential oil of yarrow reaches up to 0.11 $mg.kg^{-1}$ and Zn up to 2.44 $mg.kg^{-1}$, while the range of Cd and Hg is below the limits of the quantitative measurement of the method used. Significantly lower results in the essential oil of yarrow grown at a distance of 15 km from the NFMW – 0.02 $mg.kg^{-1}$ Pb and 0,14 $mg.kg^{-1}$ Zn. The results show that the content of heavy metals in the essential oils is much lower than in the yarrow flowers. The amounts of Pb, Cd and Hg in the yarrow oil are lower than the accepted maximum values and meet the requirements of an environmentally friendly product (5 $mg.kg^{-1}$ Pb, 1 $mg.kg^{-1}$ Cd, 0.1 $mg.kg^{-1}$ Hg) (Council of Europe, 2021).

The results confirm 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 the 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

The oil yield of yarrow inflorescences ranges from 0,35 to 0,4 % (by volume) on a dry flower basis and complies with the European Pharmacopoeia standard (not less than 0,2 %)(Council of Europe, 2013).

The results of the chromatographic analysis of the essential oils obtained from the processing of yarrow flowers grown at different distances from the NMFW-Plovdiv are presented in Table 4. Figure 1 shows the chromatograms from the GC MS analysis of yarrow oil.

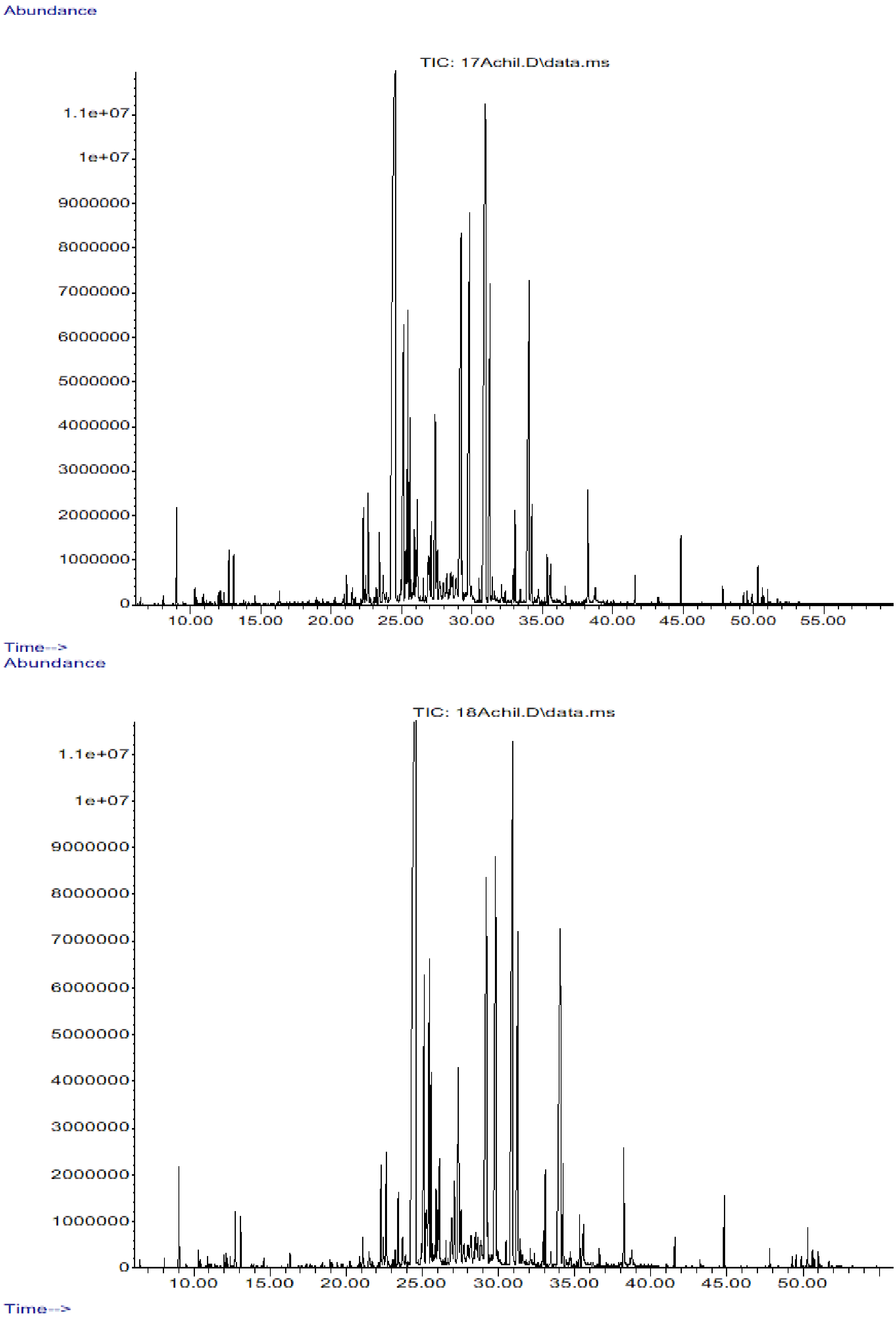

Fig.1. Chromatogram of essential oils from the yarrow grown at a distance of 0.5 km and 15 km from the NFMW

Table 4. Composition of the yarrow essential oil (%) obtained by processing the flowers

№	Compound	RI	S1	S2
			% of TIC	
1	α-Pinene	939	1.40	1.55
2	β-Pinene	979	8.01	8.9
3	β-Phellandrene	1031	5.58	6.19
4	Eucalyptol	1032	9.56	7.52
5	cis-beta Ocimene	1040	0.55	0.61
6	trans-beta-Ocimene	1050	1.02	1.13
7	Borneol	1165	9.16	10.17
8	α-Terpineol	1189	2.09	2.32
9	β-Caryophyllene	1419	12.82	12.25
10	β-Farnesene	1443	0.67	0.74
11	Humulene	1454	0.69	0.77
12	β-Bisabolene	1510	2.02	2.24
13	Ledol	1565	2.51	2.8
14	Caryophyllene oxide	1581	1.32	1.87
15	Viridiflorol	1590	2.23	2.47
16	Humulene epoxide	1606	2.44	2.71
17	α-Bisabolol oxide B	1644	12.42	11.58
18	α-Bisabolol	1683	14.16	12.74
19	Chamazulene	1725	4.87	5.41
20	Bisabolol oxide A	1744	5.01	4.46

Twenty components belonging to the classes of monoterpenes and sesquiterpenes were identified in the yarrow oil samples (Table 4). The sesquiterpenes, which include the sesquiterpene hydrocarbons (MH) and oxygenated sesquiterpenes (OS), were the predominant chemical class of the oil constituents, ranging from 60.0% to 61.2% of the total oil composition. The amount of sesquiterpene hydrocarbons varies from 23.51 to 24.12%. The major components of this group are β-caryophyllene (12.25-12.82%), chamazulene (4.87- 5.41%), humulene epoxide (2.44-2.71%), β-bisabolene (2.02-2.24%), β-farnesene (0.67-0.74%), and humulene (0.69-0.77%).

The amount of oxygenated sesquiterpenes varies from 35.9 to 37.7% of the total oil composition. The oil contains α-bisabolol (12.74-14.16%), α-bisabolol oxide B (11.58-12.42%), bisabolol oxide A (4.46-5.01%), ledol (2.51-2.8%), viridiflorol (2.23-2.47%) and caryophyllene oxide (1.32-1.87%).

Monoterpenes, which include monoterpene hydrocarbons (MH) and oxygen monoterpenes (OM), belong to the second major chemical class of oil constituents, which range from 37.37 to 38.39% of the total oil composition. The oil is dominated by monoterpene hydrocarbons β-pinene (8.01-8.9%), β-phellandrene (5.58-6.19%), α-pinene (1.4-1.55%), trans-beta-ocimene (1.02-1.13%). The amount of oxygen-containing monoterpenes varies from 20.0 to 20.8% of the total oil composition. The main oxygen-containing monoterpenes are eucalyptol (7.52-9.56%), borneol (9.16-10.17%), α-terpineol (2.09-2.32%).

The European Pharmacopoeia specifies four requirements for yarrow: plant origin (*A. millefolium L.*), appearance (whole or cut, flowering tops), and two limits for the essential oil - not less than 2 $ml.kg^{-1}$ essential oil (0.2%), and not less than 0.02% proazulenes expressed as hemazulenes, both calculated for dried material (Council of Europe, 2013). Hamazulene, the constituent in the oil that is the cause of the oil's antiphlogistic action, belongs to a class of sesquiterpene lactones (proazulenes). Hamazulene determines the essential oil's dark blue colour and is considered a marker of its high quality (Rauchensteiner et al., 2004). Hamazulene content in the yarrow oil ranges from 4.87 to 5.41% (Table 4) and is higher than the values found in the oil from Norway (0.8 to 1.2%) (Raal et al., 2011). Higher values of chamazulene are contained in the oil from Austria (15.7%), and in the oils from Germany, Estonia, the Czech Republic, Ukraine, United Kingdom, the values are significantly higher (23.3-44.3%) (Raal et al., 2011).

Numerous studies have shown significant differences in the composition of the yarrow essential oil (Raal et al., 2011, Mohammadhosseini, et al, 2017). The main constituents in yarrow oil have been found to be

1, 8-cineole, α/β-pinene, sabinene, camphor, linalool, α-terpineol, borneol, α/β-thujone, caryophyllene oxide and chamazulene (Lawrence, 2005). Orav et al. (2006) found that the main components in the yarrow oil from Estonia were 1,8-cineole, hamazulene, β-pinene, (E)-β-caryophyllene, sabinene and germacrene D. In the oil of *A. millefolium* from Italy, (Falconieri et al. (2011)) the main components are α-asarone, β-bisabolene and α-pinene, in the oil from Portugal trans-tujon, β-pinene and trans-chrysanthenol acetate predominate (Figueiredo et al., 1992), while in the oils from Turkey - 1,8-cineole, α-terpineol, camphor, borneol and β-pinene predominate (Candan et al., 2003).

Figure 2 shows the classification of the identified compounds based on functional groups. The highest content of oxygen-containing sesquiterpenes (36-38%) is found in yarrow oil, followed by sesquiterpene hydrocarbons (24-25%), oxygen-containing monoterpenes (20-21%) and monoterpene hydrocarbons (17-19%).

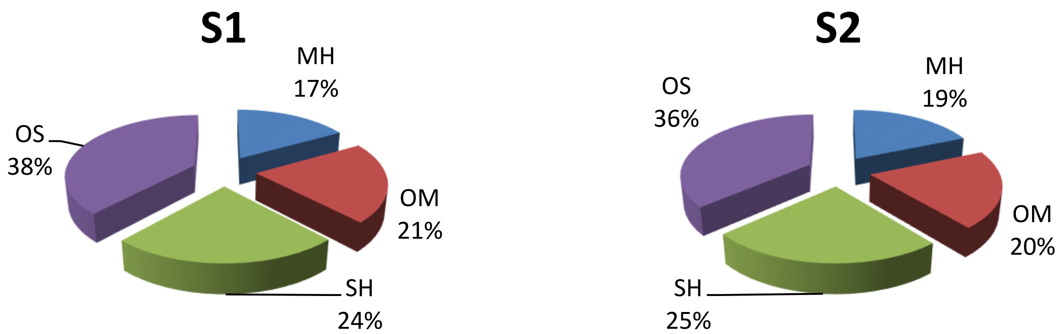

Fig. 2. Classification of identified compounds based on functional groups

Soil contamination affects the individual components of yarrow oil. Higher contents of the oxygen-containing sesquiterpenes α-bisabolol oxide B, α-bisabolol, bisabolol oxide A, the sesquiterpene hydrocarbon β-caryophyllene and the oxygen-containing monoterpene eucalyptol were found in the yarrow oil grown on contaminated soil. Lower values were found for chamazulene, β-pinene, β-phellandrene and borneol, while no significant difference was observed for the other co-constituents. The chemical composition of yarrow essential oil depends on several factors, such as growing medium, development stage and chemotype. The following chemotypes of yarrow oil have been identified: hamazulene (Estonia), sabinene (Norway), hamazulene + bornyl acetate (Germany, Austria), hamazulene + β-pinene + (E)-β-caryophyllene (Estonia, UK, Latvia), sabinene + 1,8-cineole (Norway) (Raal et al, 2011), β-pinene + α-terpinyl acetate (Norway) (Rohloff et al., 2000), 1,8-cineole - in Portugal (Figueiredo et al, 1992], β-pinene in Estonia (Orav et al., 2001) and Lithuania (Mockute & Judzentiene, 2003; Gudaityte & Venskutonis 2007; Judzentiene & Mockute, 2010.), hamazulene and p-cymene in Iran (Jaimand et al., 2006). The essential oil from Serbia and France can be classified as camphor chemotype (12.8% camphor in essential oil). In Lithuania, four chemotypes of yarrow oil have been found: borneol + camphor, chamazulene + β-pinene, nerolidol + β-pinene and β-pinene + 1,8-cineole (Mockute & Judzentiene, 2003). Three chemotypes have been found in Poland: β-pinene, β-pinene + chamazulene and 1, 8-cineole chemotype. β-pinene, β-pinene + hamazulene and 1,8-cineole haemotype. Three essential oil chemotypes of *A. millefolium* were found in Serbia, (1) β-pinene, trans-caryophyllene and hamazulene; (2) lavandulyl acetate, trans-caryophyllene and hamazulene; and (3) germacrene D, trans-chrysanthenol acetate and trans-caryophyllene (Stevanovic, et al., 2015)

The yarrow oil grown in the NFMW-

Plovdiv area does not belong to any chemotypes described in the literature. The oil is characterized by a mixed α-Bisabolol oxide B, α-Bisabolol, β-Caryophyllene chemotype.

CONCLUSION

On the basis of the obtained results the following more important conclusions can be drawn:

1. Yarrow is a plant that is tolerant to heavy metals and can be grown on contaminated soils and used for phytoremediation of heavy metal-contaminated soils. Processing the flowers into essential oil will significantly reduce the cost of phytoremediation.

2. The heavy metal content of yarrow essential oil is very low and is not affected by the degree of heavy metal contamination of the soil.

3. In yarrow oil the highest content is made of oxygen-containing sesquiterpenes (36-38%), followed by sesquiterpene hydrocarbons (24-25%), oxygen-containing monoterpenes (20-21%) and monoterpene hydrocarbons (17-19%).

4. Yarrow essential oil is characterized by a mixed α-Bisabolol oxide B, α-Bisabolol, β-Caryophyllene chemotype, reported for the first time.

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