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Potential of *Salvia sclarea* L. for Phytoremediation of Soils Contaminated with Heavy Metals

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***Abstract*—**A field study was conducted to evaluate the efficacy of *Salvia sclarea* L. for phytoremediation of contaminated soils. The experiment was performed on an agricultural fields contaminated by the Non-Ferrous-Metal Works near Plovdiv, Bulgaria. The content of heavy metals in different parts of *Salvia sclarea* L. (roots, stems, leaves and inflorescences) was determined by ICP. The essential oil of the *Salvia sclarea* L. was obtained by steam distillation in laboratory conditions and was analyzed for heavy metals and its chemical composition was determined. *Salvia sclarea* L. is a plant which is tolerant to heavy metals and can be grown on contaminated soils. Based on the obtained results and using the most common criteria, *Salvia sclarea* L. can be classified as Pb hyperaccumulator and Cd and Zn accumulators, therefore, this plant has suitable potential for the phytoremediation of heavy metal contaminated soils. Favorable is also the fact that heavy metals do not influence the development of the *Salvia sclarea* L., as well as on the quality and quantity of the essential oil. For clary sage oil obtained from the processing of clary sage grown on highly contaminated soils, its key odour-determining ingredients meet the quality requirements of the European Pharmacopoeia and BS ISO 7609 regarding Bulgarian clary sage oil and/or have values that are close to the limits of these standards. The possibility of further industrial processing will make *Salvia sclarea* L. an economically interesting crops for farmers of phytoextraction technology.

***Keywords*—**Clary sage, heavy metals, phytoremediation, polluted soils.

I. INTRODUCTION

ENVIRONMENTAL pollution with heavy metals is a global problem, and therefore, the development of phytoremediation technologies for plant-based clean-up of contaminated soils is of significant interest. Aromatic and medicinal plants appear to be a good choice for phytoremediation since these species are mainly grown for secondary products (essential oil), and thus, the contamination of the food chain with heavy metals is eliminated. They can replace food crops grown under the same conditions [1]. Aromatic and medicinal plants have shown to uptake and accumulate toxic heavy metals from polluted areas, and could be used as biomonitors or accumulators of pollutants [2], [3]. Some medical plants such as mint, St. John's wort, lavender, marigold, marshmallow, cumin, garlic, garden sorrel, hemp and others can accumulate large amounts of toxic heavy metals in their tissues. However, [4] demonstrated that aromatic crops may not have significant phytoremediation potential, but growth of these crops in metal contaminated agricultural soils is a feasible alternative. Aromatic crops can provide economic return and metal-free final product, the essential oil. The essential oil of aromatic plants does not contain heavy metals, although there is an accumulation in the plant biomass [2], [5], [6].

Salvia sclarea L., also known as clary sage, belongs to the family Lamiaceae. The plants are 60-100 cm high with large hairy leaves and small blue, white or purple flowers [7], [8]. The plant is native to many Mediterranean countries including Italy, southern France, and Morocco and is one of the most significant plants cultivated in the world as a source of essential oils and other perfumery products [7]-[9]. Salvia is grown for the inflorescences, which in fresh state contains 0.15% to 0.20% essential oil. Salvia oil is obtained by steam distillation because water distillation reduces the yield of oil and the content of the esters in it [10]. The analysis of the essential oils of *S. sclarea* inflorescence and leaves has been reported by a number of researchers [10]-[25]. The essential oil of *Salvia Sclarea* is easily movable, transparent, colourless to pale yellow liquid with a characteristic smell of the inflorescences resembling lavender, bergamot and amber [26]. From ancient times, the oil is being used for cleaning eyes and is popularly referred to as “Clary or Clear eye”. The essential oil has a lasting, fine and soft musk and bittersweet aroma. It has a strong fixing property in the composition of aromas, and therefore is used in the perfume and cosmetics industry. The essential oil is used in the food industry to flavour drinks, Rhine wine, beer, etc. The oil has antibacterial, antiseptic, astringent effect – it helps with infections, cramps, flatulence, profuse sweating. With its antispasmodic and anti-inflammatory properties, it helps to relieve muscle pain, headaches and migraines, painful menstruation. Suitable for relaxing the nerves – it has a calming, antidepressant property. Means to treat wounds - boils, acne, inflamed skin, oily skin; for oily hair and dandruff. In aromatherapy it is used for inhalations, baths, massages and compresses [11], [18], [19], [27]-[31]. Tones in mental and physical fatigue, helps the healing process, has a calming effect in nervous tension, it is applied to nourish normal and aging skin, and has an anti-dandruff action [27].

Insufficient information is available on the potential of clary sage for accumulation of heavy metals and its potential for use for phytoextraction. The studies connected with growing the clary sage in polluted soils are also limited and sometimes contradictory. [32] established that Clary sage [*Salvia* sp.] was cadmium excluder, while [33]-[35] came to the conclusion that clary sage can be grown on sites of severe air and soil heavy metal pollution as a substitute for some other edible

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crops. This gave us the grounds to carry out a comparative research, which will allow us to determine the quantities and the centers of accumulation of Pb, Zn and Cd in the vegetative organs of clary sage, the quality of clary sage oil, as well as the possibilities to use the plant for phytoremediation of heavy metal contaminated soils.

II. Material and Methods

The experiment was performed on an agricultural fields contaminated by Zn, Pb and Cd, situated at different distances (0.5, 2.0 and 15.0 km) from the source of pollution, the KCM (Non-Ferrous Metals Processing Plant) near Plovdiv, Bulgaria.

Characteristics of soils are shown in Table I. The soils were slightly acidic to neutral, with moderate content of organic matter and essential nutrients (N, P and K) (Table I). The pseudo-total content of Zn, Pb and Cd is high and exceeds the maximum permissible concentrations (MPC).

The test plant was *Salvia sclare* L., clary sage seeds were sown in each plot; between row and within row distances were 70 cm and 20 cm, respectively. Each hole was 2–3 cm deep, containing three seeds. After clary sage had grown, it was thinned to one plant per hole.

TABLE I
Characterization of the Soils

Parameter	Soil 1 (S1) 0.5 km	Soil 2 (S2) 2 km	Soil 4 (S4) 15 km
pH	7.4	7.5	7.5
EC, dS/m	0.15	0.14	0.15
Organic C,%	2.20	5.4	1.54
N Kjeldal,%	0.22	0.29	0.12
P, mg/kg	625.6	840.0	387.3
K, mg/kg	6960.0	5467.6	6780.0
Pb, mg/kg	913.5	5865,6	24.6
Zn, mg/kg	1903.8	5566.0	33.9
Cd, mg/kg	26.9	178.6	2.7

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 analyses were made in the first year of the growing of the plants. The plants were gathered in for analysis on ripening of the seeds in the central raceme. Under laboratory conditions, essential oil was extracted from the racemes of *Salvia sclarea* L. by means of steam distillation.

The contents of heavy metals (Pb, Cu, Zn and Cd) in the plant material (roots, stems, leaves and inflorescences) and in the essential oils of *Salvia sclarea* L. was determined by the method of the microwave mineralization. The total content of heavy metals in the soil was determined in accordance with [35]. The mobilisable heavy metals contents in soils, considered as a "potentially bioavailable metal fraction", were extracted by a solution of DTPA [36]. The quantitative measurements were carried out with inductively coupled plasma emission spectrometry (ICP) (Jobin Yvon Emission - JY 38 S, France).

The resulting essential oil is analysed under [37]. The determination of the main ingredients of the oil is performed on a gas chromatograph PYE UNICAM under the following working conditions: capillary column Econo -CapTM EC TM -1, length 30 m, internal diameter of 0.32 mm. Temperature programming: from 70 °C to 230 ºC at a speed of rise of 8 °C per minute. Injector temperature: 300 ºC, detector temperature 300 ºC, carrier gas - hydrogen, injected volume 0.1μl, carrier gas rate - 1.3 ml/min. In the resulting chromatograms, identified representative and typical ingredients were obtained. Identification of the oil constituents was based on the retention time of standard compounds (linalool, linalyl acetate, sclareol, etc.). Statistical analyses were conducted with Statistica v. 7.0.

III. Results and Discussion

A. Soils

The results presented in Tables I and II show that the soil contamination in the area of KCM-Plovdiv is on the torch of contamination and with the moving away from KCM - Plovdiv a notable trend to reduce the total content of heavy metals in the soil is observed. In the soil samples S1 and S2 (taken from the area situated at the distance of 0.5 and 2.0 km from KCM), the reported values for Pb were exceeding MPC approved for Bulgaria and varied from 913.5 mg/kg to 5856.6 mg/kg. In the area located at a distance of 15 km, the contents of Pb significantly reduce to 24.6 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 range from 29.0 to 34.2%, followed by Pb with 12.4 to 21.3% and Zn with 8.4 to 15.6%. It is notable that the content of the mobile forms of heavy metals is significantly higher in soil located at a distance of 0.5 km from KCM (S1), although it is characterized by slightly alkaline reaction.

TABLE II
DTPA - Extractable Pb, Zn and Cd (mg/kg) in Soils Sampled from KCM

Soils	Pb		Cd		Zn	
	mg/kg	%*	mg/kg	%	mg/kg	%
S1	194.7	21.3	9.2	34.2	297.0	15.6
S2	728.4	12.4	51.8	29.0	467.4	8.4
S4	2.1	8.5	0.67	24.8	2.4	7.1

*DTPA -extractable / total content

B. Clary Sage

The results presented in Tables I and II show that the soil contamination in the area of KCM-Plovdiv is on the edge of contamination and with the moving away from KCM - Plovdiv a notable trend to reduce the total content of heavy metals in the soil is observed. In the soil samples S1 and S2 (taken from the area situated at the distance of 0.5 km and 2.0 km from KCM), the reported values for Pb were exceeding MPC approved for Bulgaria and varied from 913.5 mg/kg to 5856.6 mg/kg. In the area located at a distance of 15 km, the contents of Pb significantly reduce to 24.6 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 range from 29.0 to

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34.2%, followed by Pb with 12.4 to 21.3% and Zn with 8.4 to 15.6%. It is notable that the content of the mobile forms of heavy metals is significantly higher in soil located at a distance of 0.5 km from KCM (S1), although it is characterized by slightly alkaline reaction.

The content of Pb in the roots of clary sage grown on contaminated soils (S1-S2) ranged from 281 mg/kg to 471 mg/kg, Zn – from 501.2 mg/kg to 657.7 mg/kg and Cd – from 17.5 mg/kg to 23.2 mg/kg. The obtained values for the heavy metals (Cd, Pb and Zn) in the roots are much higher than the values considered by [38] as toxic for the plants (0.1 mg/kg Cd, 30 mg/kg Pb, 100 mg/kg Zn). The obtained results could be explained with the anatomic and biologic peculiarities of the plants. The greater part of the heavy metals, that had entered the soil, are fixed and accumulated in the roots of *Salvia sclarea* L., as clary sage formed a powerful root system with strong absorbing ability. Considerably lower values were established in the in the roots of clary sage grown on uncontaminated soil (S3). The content of Pb in the roots reached to 0.82 mg/kg, Zn – 7.6 mg/kg and Cd – 0.12 mg/kg.

The heavy metal contents in the stems of the clary sage were considerably lower compared to those in the root system. The content of Pb in the stems of clary sage grown on contaminated soils (S1-S2) ranged from 80.7 mg/kg to 244.0 mg/kg, Zn – from 195.5 mg/kg to 369.3 mg/kg and Cd – from 1.6 mg/kg to 6.0 mg/kg. Considerably lower values were established in the stems of clary sage grown on uncontaminated soil (S3). The content of Pb in the stems reached to 0.42 mg/kg, Zn – 4.2 mg/kg and Cd – 0.04 mg/kg. The higher values, reached in *Salvia sclarea* L., could be explained with the anatomic structure of the stems of the studied plant. *Salvia sclarea* L. was distinguished for its stem, covered with plenty of pappus, contributing to the fixing of the aerosol pollutants and their accumulation there.

The content of Pb, Zn and Cd in leaves is higher compared to the root system, which shows absorption and movement of metals in the vascular system. According to [4], the sage plant accumulates a moderate amount of heavy metals in its aboveground biomass. Our results confirm found by [39] that the accumulation of metals in shoot was higher than roots.

The highest was the accumulation of Pb, Zn and Cd in the leaves of clary sage grown on contaminated soils, where Pb ranged from 1571.3 mg/kg to 1978.0 mg/kg, Zn – from 2169.8 mg/kg to 2630.0 mg/kg, and Cd – from 45.3 mg/kg to 57.2 mg/kg. There were no signs of Cd and Pb toxicity despite high concentrations of cadmium and lead in leaves. Considerably lower values were established in the leaves of clary sage grown on uncontaminated soil (S3), where Pb reached up to 2.2 mg/kg, Zn – up to 16.4 mg/kg, and Cd – up to 0.2 mg/kg.

The results from our previous studies were proved - Salvia sclarea L. accumulate extraordinarily high concentrations of Pb, Zn and Cd in the leaves. Their stronger accumulation in *Salvia sclarea* L. was probably due to the fact that the leaves of *Salvia sclarea* L. were rich in pappus and with a very large surface, which contributed to the fixing of the aerosol pollutants and for their accumulation there. According to [40] the relatively high amount of Pb may be caused by the morphological characteristics of the plant. Trichomas (grandular and covering hair) of the plant surface characterize muscat sage and is a common morphological sign of the Lamiaceae family. Trichomes of the sage may stick to the pollutant, which could not be removed by washing. Therefore, sage samples frequently contain Pb in higher concentration than 2 mg/kg, which is confirmed from our results for Pb in the leaves from uncontaminated soil S3 (2.2 mg/kg).

The accumulation of heavy metals in inflorescences is high, but lower compared to those in the leaves. The content of Pb in inflorescences of clary sage grown on contaminated soils (S1-S2) ranged from 703.8 mg/kg to 1077.9 mg/kg, Zn – from 870.0 mg/kg to 1314.5 mg/kg, and Cd – from 12.0 mg/kg to 19.5 mg/kg. Considerably lower values were established in the inflorescences of clary sage grown on uncontaminated soil (S3). The concentrations of heavy metals in the inflorescences were: Pb – up to 0.56 mg/kg, Zn – up to 6.3 mg/kg, and Cd – up to 0.08 mg/kg. Our results were in contradiction with the found from [32], who stated that the inflorescences showed relatively high Cd uptake independent of provenance or soil Cd content. The higher values, reached in *Salvia sclarea* L., could be explained with the anatomic structure of the racemes and the inflorescences of the studied plants. The racemes of *Salvia sclarea* L. were very big and the inflorescences covered with pappus, contributing to the fixing of the aerosol pollutants and their accumulation there.

The ratio of the heavy metals in the above-ground mass and the roots of the clary sage grown in heavy metal polluted soils under field conditions is higher than 1, which means that clary sage is an accumulator of heavy metals and can be used for the phytoextraction of heavy metals from the soils.

The content of heavy metals in essential oils of *Salvia sclarea* L. was also determined. The obtained results showed that the main part of the heavy metals contained in the inflorescences of *Salvia sclarea* L. was not transferred to the oil during inflorescences' processing, due to which their content in the oil was considerably lower. In the process of oil extraction by distillation, heavy metals remain in the extracted plant residues, limiting the quantities of heavy metals in the commercial oil product [5], [41]. Pb in the essential oil of *Salvia sclarea* L. grown on contaminated soils (S1-S2) ranged from 2.7 mg/kg to 4.2 mg/kg, Zn – from 1.7 mg/kg to 3.4 mg/kg, and Cd – from 0.08 mg/kg to 0.11 mg/kg (Fig. 2). Considerably lower values were established in the essential oil of *Salvia sclarea* L. grown on uncontaminated soil (S3). The concentrations of heavy metals in the essential oil were: Pb – up to 0.03 mg/kg, Zn – up to 0.16 mg/kg, and Cd – up to 0.005 mg/kg. These results strongly suggest that the main part of Pb, Cd and Zn, contained in the inflorescences of *Salvia sclarea* L., cultivated 0.5 km and 2.0 km from the KCM, does not pass into the oil obtained, its content in the oil are lower according the Directive 76/768/EEC for plant extracts (20 mg/kg Pb and 2 mg/kg Cd) and it can be used for cosmetics. This result is very important, given the commercial value of the essential oil extracts of the aromatic and medicinal plants, and it agrees with corresponding results by [33] that the contents of heavy metals in the essential oils of *Salvia sclarea* L. were very low and were not affected by the level of soil pollution with heavy metals. The results of [42] were proved - the accumulation of heavy metals in the end products, obtained after the processing of the floriferous stems of *Salvia sclarea* L., was within the limits of the permissible values and was getting close to the normative requirements for ecologically clean product.

The content of heavy metals in the wastes after essential oil distillation was also determined. The content of Pb in clary sage wastes from contaminated soils (S1-S2) ranged from 927.1 mg/kg to 1440.5.9 mg/kg, Zn – from 115.8 mg/kg to 1691.9 mg/kg, and Cd – from 15.4 mg/kg to 26.1 mg/kg.

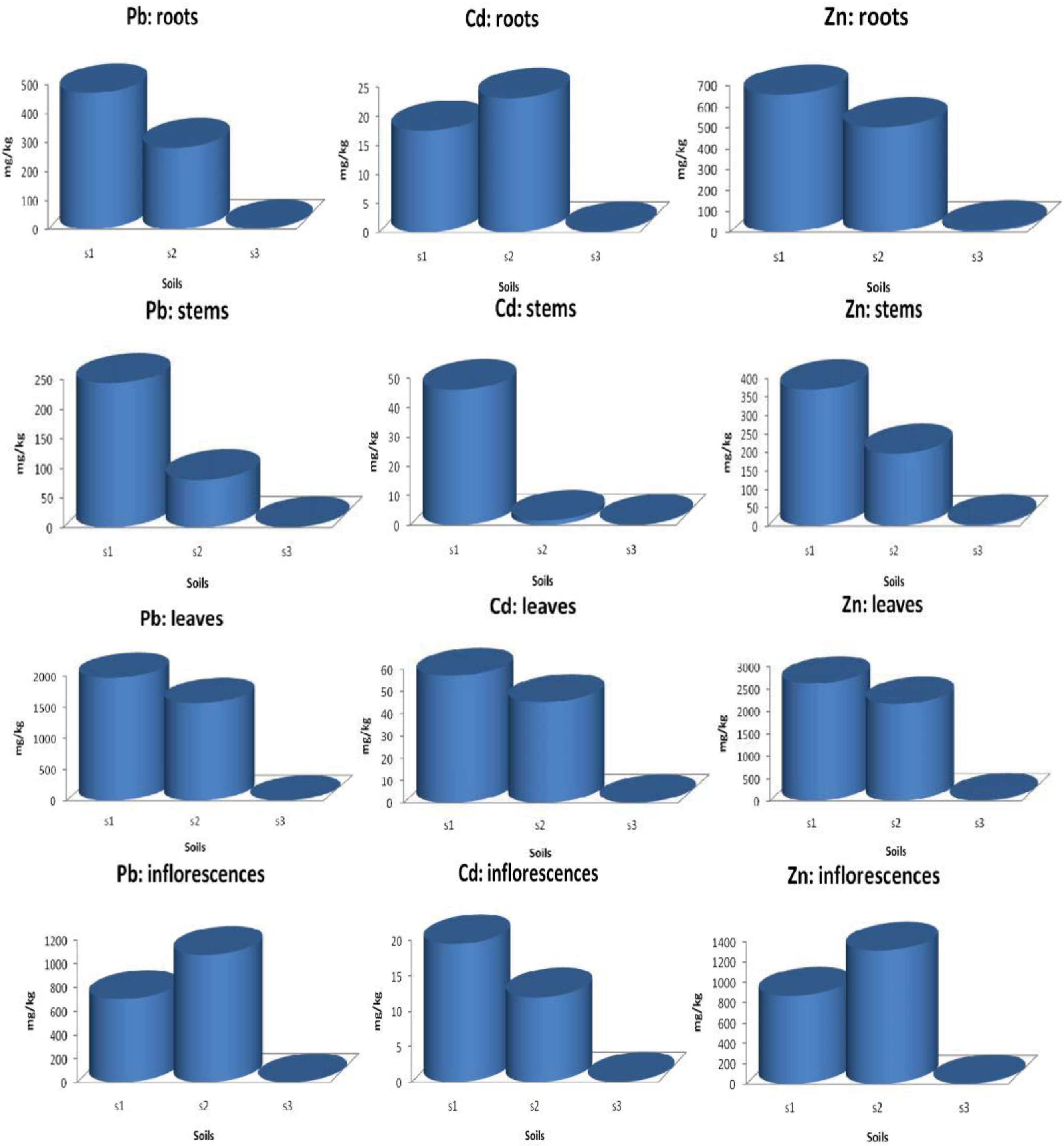

Fig. 1 Content of heavy metals in vegetative organs of clary sage

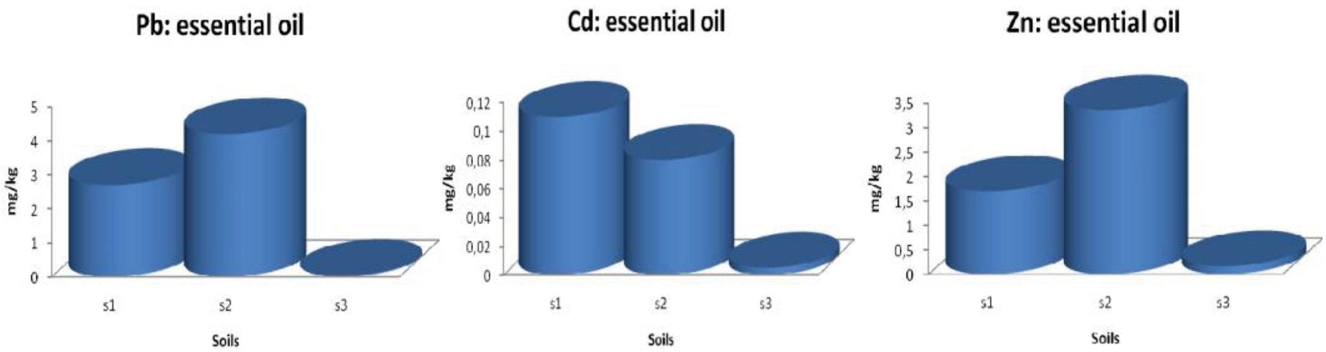

Fig. 2 Content of heavy metals in essential oils of *Salvia sclarea* L.

Considerably lower values were established in the clary sage wastes from uncontaminated soil (S3), where Pb reached to 0.72 mg/kg, Zn – to 8.1 mg/kg, and Cd – up to 0.11 mg/kg (Fig. 3). In the process of oil extraction by distillation, heavy metals remain in the extracted plant residues, limiting the quantities of heavy metals in the commercial oil product [33], [41]. Thus, significant amounts of heavy metals could be removed from the soil through proper disposal of the metal contaminated plant residues, while the metal-free, extracted oils could be safely marketed. Remaining after the extraction of essential oil waste is dried and can be used as feed for livestock, mulching and fertilizing. Our results suggest that heavy metals are accumulated in shoots and wastes from distillation above the maximum permissible concentrations for these elements in animal feed [43], making these waste products unsuitable as animal feed. The remaining after the extraction of essential oil waste should be treated with caution. They can be utilized in the preparation of compost or be destroyed by incineration.

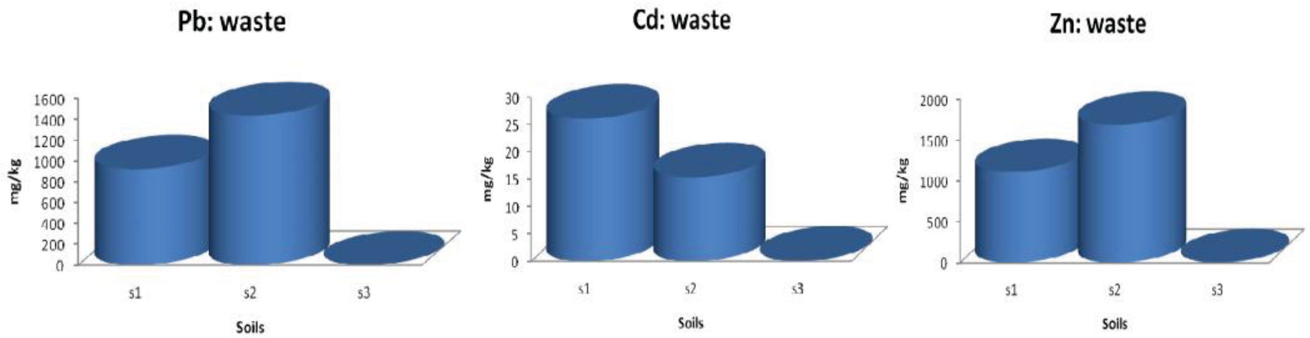

Fig. 3 Content of heavy metals in clary sage wastes

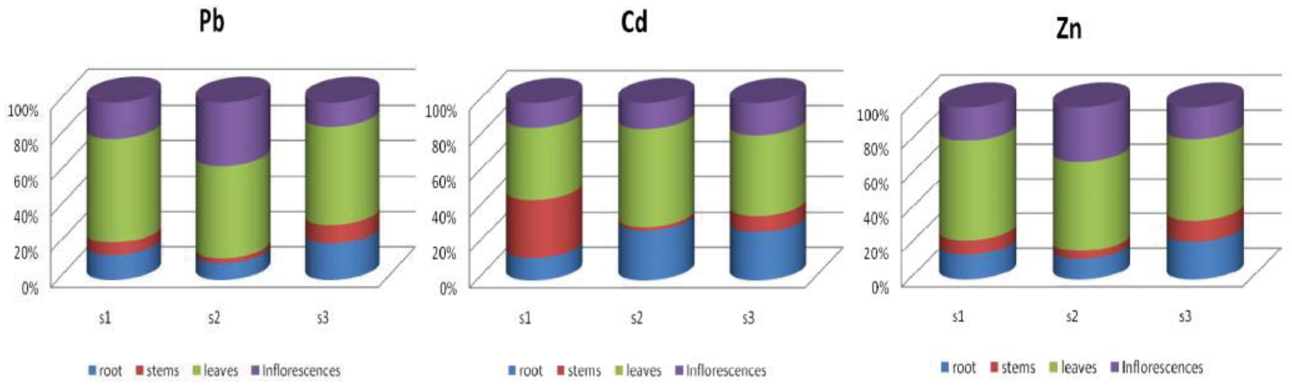

Fig. 4 Distribution of heavy metals in vegetative organs of clary sage

The distribution of the heavy metals in the organs of the clary sage has a selective character that in clary sage decreases in the following order: leaves > roots> stems > seeds (Fig. 4).

The obtained from us results showed that the distribution of the heavy metals in the plants' organs had a selective nature and depended above all on the parts of the plant and their surface that was examined. Pb, Zn and Cd distribution in *Salvia sclarea* L. decreased in the following order: leaves> inflorescences> roots> stems. The finding of [33] was not confirmed, who stated that heavy metal concentration in plant parts of *Salvia sclarea* L. decreased in order: leaves> roots> inflorescences>stems.

There is a distinct feature in the accumulation of heavy metals in the vegetative organs of the clary sage. The clary sage accumulates heavy metals through its root system, but a very small portion of the heavy metals are retained by the roots, and most of them move and accumulate in the above-ground parts (stems, leaves and inflorescences). The results obtained show that the clary sage can be successfully grown on heavy metals areas. On the one hand, that may reduce the risk of the use of these areas for growing food crops. On the other hand, the selective accumulation of Pb, Zn and Cd in stems, leaves and inflorescences makes the clary sage a very lucrative for the purposes of phytoremediation.

C. Clary Sage – Accumulator of Hyperaccumulator?

To be able to give a categorical answer to the question what are the abilities of the clary sage to extract heavy metals from the soil and to assess the potential of clary sage for phytoextraction, the translocation factor (TF=Cshoots/Croots) [44] and enrichment factor (EF=Cshoots/Csoils) [45] were calculated. Our results show that the translocation factor for all metals is greater than 1, regardless of the degree of contamination of the soil (Table III).

In terms of Pb the enrichment factor in clary sage grown on contaminated soils (0.5 km and 2.0 km from KCM) ranged from 9.3 to 15.0, in Zn from 7.9 to 13.0, and in cadmium from 1.1 to 13.3. Both TF and EF coefficients are greater than 1 in the cultivation of the clary sage on heavy metal polluted soils. It can be claimed that by increasing the level of contamination the potential of clary sage to translocate and accumulate Cd, Pb and Zn in its above-ground parts increases as well. The clary sage may be referred to the group of hyperaccumulators of Pb, and the accumulators of zinc and cadmium, as the content of lead in the above-ground mass exceed 1000 mg/kg Pb. The above-ground mass of the clary sage in its cultivation on heavy metal polluted soils accumulates Zn and Cd, but does not reach the values of 10,000 mg/kg for Zn and 100 mg/kg of Cd so that the clary sage can be referred to the Zn and Cd accumulators.

TABLE III
TRANSLOCATION (TF) AND ENRICHMENT (EF) FACTORS IN CLARY SAGE

Soils	Pb		Cd		Zn	
	TF	EF	TF	EF	TF	EF
S1	6.2	15.0	5.89	13.3	4.6	13.0
S2	9.7	9.3	2.0	1.1	4.7	7.9
S3	3.9	1.51	2.0	0.48	2.7	11.2

The obtained from us results were in contradiction with the found from [32], who stated that Clary sage [*Salvia* sp.] was cadmium excluder. According to [39] sage could be a suitable accumulator of heavy metals for phytoremediation of metal polluted soils.

The accumulation and distribution of metals in plant tissue are important aspects to evaluate the role of plants in remediation of heavy metal contaminated sites [46]. The success of phytoremediation process depends on adequate plant yield and the plant having an ability to take up significantly high amount of heavy metals in their shoots [39]. Our results strongly suggest that *Salvia sclarea* L. is a crop which is tolerant to heavy metals and can be grown in contaminated soil. It can be assigned to the hyperaccumulators of lead and to the accumulators of zinc and cadmium, and therefore, it can be successfully used for phytoremediation of soils contaminated with Pb, Cd and Zn. The clary sage could be used for cleaning the toxic metals from polluted soils, and as well as the essential oil could be used in the perfumery and cosmetics and tobacco industries.

It is necessary to carry out further research in order to clarify the real potential of clary sage when grown on larger areas. Experts will need to offer additional management practices in order to increase the potential and efficiency of sage for cleansing soil of heavy metal contaminants. The cultivation of the plant, planting, irrigation, pests and diseases, crop rotation, cycle duration and harvesting should be further studied. These issues need further clarification and a significant amount of research.

D. Quality of Essential Oil

The yields of essential oil of clary sage are listed in Table IV. The results show that essential oil content in clary sage grown on uncontaminated soil (S3) was higher than that grown on contaminated soils (S2-S3). According to the literature data, the essential oil content ranges from 0.1% to 0.34% of fresh clary sage inflorescences. Some heavy metals were reported to affect the essential oil yields of aromatic medicinal plants [4], [47]. Reference [33] established that the simultaneous contamination of soil with excessive amounts of Cd and Zn decreased the yield of fresh inflorescences of *Salvia sclarea* L. by 14% to 19%, and the yield of oil by 12% to 18%. Moreover, the reduction in yield with increasing heavy metal content in soil might be due to inhibition in the photosynthetic rate by the toxic concentration of heavy metals adversely affecting plant growth [48], [49]. Reference [50] reported that heavy metals, especially Cr, Pb and Cd are translocated to the shoots of the plant and negatively affected the cellular metabolism of the shoots, resulting in reduction in plant biomass. Reduction in oil yield was due to reduction in shoot biomass, as oil yield is a function of biomass yield x oil content in biomass.

The results of the chromatographic analysis of essential oils obtained by processing of racemes of *Salvia sclarea* L. grown at a different distance from KCM are presented in Table IV. The values of the main components of the essential oil of clary sage are compared with the requirements of EU Pharmacopea [51] and Bulgarian standards [52] for the *Salvia sclarea* L. and the data obtained from all cited references (The presented variation in essential oil composition was calculated from data obtained from all cited references). According to the EU Pharmacopea [51], the content of linalool, linalyl acetate, germacrene D, sclareol and α-terpinenol in clary sage oil should be between 6.5% to 24.6%, 56% to 78%, 1.2% to 12.0%, 0.4% to 2.60% and <5.0, respectively. According to the Bulgarian standard, the content of linalool and linalyl acetate should be between 15.0% and 45.0%, -25.0%, and -65.0% [52].

The results of chemical analysis of *S. sclarea* L. essential oil are presented in Table IV. The terpene alcohols (linalool, alpha terpineol, borneol, terpinen-4-ol, 1-8 cineole and sclareol) have been identified, of which linalool is the dominant component (10.97%-12.75%). Two ester components (linalyl acetate and bornyl acetate) have been identified. The following monoterpene hydrocarbons have been identified (myrcene, α-pinene, cis β-ocimene, trans-β-ocimene, limonene and terpinolene). Sesquiterpenes (β-caryophyllene, bourborene, germacrene D and α -copaene) have also been found. A monoterpene ketone (camphor) has also been identified. The main components in sage oils from our experiment were linalyl acetate (54.91%), linalool (10.97%), germacrene D (9.59%), borneol (2.295%), bourbonene (2.62%), β-caryophyllene (1.92%), α-copaene (1.45%) and β- mircene (1.12%). A minor component (in the range of (1.0 and 0.10%) trans β-ocimene – (0.99%), terpinolene (0.92%), sclareol (0.78%), cis β-ocimene (0.55), 1.8 cineole and limonene (0.25%), terpinen-4-ol (0.47%), α-terpineol (0.65%), bornylacetate (0.16%), are identified in oils. A trace of the following components (<0.1) pinene and camphor are identified in oils. The analysed oil sample belongs to the group of clary sage oils of linalyl acetate/linalool chemotype [27], [31], [53]. According to its chemical composition essential oil of *S. sclarea* L. growing in Bulgaria was comparable to commercial clary sage essential oils [18], [27]. Similar are the results obtained for the composition of the essential oil by other authors.

Clary sage oil is produced in several countries and as a result may have varying chemical compositions. The quality of essential oil is dependent on the relative composition of different oil constituents, which are greatly influenced by the agro-climatic condition as well as nutrient availability and abiotic stress [54]. However, it is known to have high concentrations of esters (linalyl acetate), alcohols and sesquiterpenes [20]. The diterpene sclareol and sesquiterpene caryophyllene are also found in the essential oil. Gas chromatographic and mass spectrometric analysis of commercial clary sage essential oils from Russia, Bulgaria, USA, China, France, Serbia, Poland, Hungary, Ukraine, Kashmir and Tajikistan led to identification of linalool and linalyl acetate as major constituents of the oil [18], [19], [28], [53], [55], [56]. Notable differences in the linalool/linalyl acetate ratio among oil samples from different geographic regions were detected. In the composition of commercial clary sage oil from Russia, United States, China, France, and Bulgaria, prevails linalool (10-20%) and linalyl acetate (45-75%) [12].

According to [13], the component composition of the US oil is much richer, but the amount of linalyl acetate is lower. The Russian oil is distinguished by a high amount of linalyl acetate (45-61%) and linalool (10-21%), followed by β-caryophyllene (1.1-1.8%) and α-terpineol (1.2-2.5%), the remaining components are less than 1%, and traces. The French type of oil besides linalyl acetate (49-73%), linalool (9-16%) contains a larger amount of germacrene D (1.6-2%) [20]. Commercial Serbian clary sage oil had 53% linalyl acetate and 18% linalool [8]. According to [18], chemical composition of Bulgarian clary sage essential oil is linalyl acetate (56.88%), linalool (20.75%), germacrene D (5.08%) and β-caryophyllene (3.41%). According to [53], the oil from Tajikistan was dominated by the linalyl acetate (39.21%) and the linalool (12.5%), followed by α-terpineol (5.5%) and germacrene D.

The main chemical component of clary sage is linalyl acetate, part of the esters group, which has antispasmodic and sedative properties, making it one of the most relaxing, soothing, and balancing essential oils. The content of linalyl acetate in the oil obtained from Salvia grown on contaminated soils is high (54.91-55.05%) and is within the standards of quality clary sage oil (45-65%) and was just below the EP 5 lower limit for linalyl acetate (56.0%) requirements of the European Pharmacopoeia [51]. The content of linalyl acetate in the oil produced from the Salvia grown on uncontaminated soil reaches up to 56.05% and does not differ significantly from the results obtained by other authors for the Bulgarian oil (55.01-56.88%). The results show that the content of heavy metals in the soil does not affect the content of linalyl acetate in the Salvia oil. Our values are close to the ones obtained by [8], [57] (respectively, 52.83% and 57.9% linalyl acetate). According to some authors the content linalyl acetate in clary sage oil can reach up to 72.4% in Kashmir oil [15].

Regarding the content of linalool, there are no significant differences in the values obtained for oils from areas located at 0.5 km and 2 km from KCM. The content of linalool ranges from 10.97 to 11.25% and does not exceed in value (from 6.5 to 24.0%) the requirements of the European Pharmacopoeia [51] for Salvia oil. Our results are significantly lower than the norm for linalool specified in the standard [52] (15.0-25.0) and the data obtained by [18] and [15] (20.75% and 23.8%, respectively). Our values come close to the values of linalool (12.4%) obtained by [53].

Regarding the content of α-terpineol, there are significant differences between the oils tested by us. The content of α-terpineol in oil from areas located at 0.5 km and 2 km from KCM (S1 and S2) reaches up to 0.65-0.85%, while the oil from the uncontaminated soil is significantly higher, reaching 4.1%. Similar are the results obtained for the content of α-terpineol in the oil by other authors (2.64% [18], (3.5%) [57] and 4.5% [15]. Our values for oils from contaminated and uncontaminated soils are within the standard (less than 5.0%).

Regarding the content of ß-myrcene, there are significant differences between the oils from contaminated areas (S1 and S2) and uncontaminates areas (S3) tested by us. The content of ß- myrcene in oil from areas located at 0.5 km and 2 km from KCM (1.12-1.25%) is with values higher than the oil obtained from uncontaminated soil (0.5%). Our results are in accordance with the literature values for β-mircene in oil (0.2 до 2.3%).

TABLE IV
CHEMICAL COMPOSITION OF *SALVIA SCLAREA* OIL (%)

Composition	Soils		Reference		Standards	
	S1-S2	S3	Bulgaria	other	1	2
Oil content, %	0.24 -0.26	0.32		0.1-0.34		
α-pinene	0.01	0,01	0,01	0.03-4.57		
β-pinene	0.03	0.02	0.02-0.1	0.01-3.0		
Myrcene	1.12-1.25	0.50	0.48-2.3	0,05-2.82		
p-Cymene	0.01	0.02	0.02	0.01-1.01		
1.8 cineol+Limonene	0.25-0.35	0.20	0.20-0.70	0.1-2.29		
Cis β-ocimene	0.50-0.60	0.25	0.21-0.8	0.1-1.70		
Trans β-ocimene	0.99-1.02	0.50	0.40-1.40	0.06-1.75		
Terpinolene	0.92-1.05	0.20	0.13-0.30	0.10-0.60		
Linalool	10.97-11.25	12.75	20.75-23.8	8.5-31.0	15.0-25.0	6.5-24.6
Camphor	0.02	0.02		0.01-0.88		
Borneol	2.20-2.40	0.65		0.01-0.64		
Terpinen-4-ol	0.47-0.58	0.05	0.04-0.1	0.03-1.19		
α-terpineol	0.65-0.85	4.1	2.64-4.5	0.20-8.01		<5.0
Lynalyl acetate	54.91-55.05	56.05	55.01-56.88	35.9-74.18	45-65	56-78
Bornylacetate	0.16-0.20	0.20		0.01-0.83		
α− copaene	1.45-1.56	0.40	0.4-0.87	0.01-1.0		
α− bourbonene	2.62-2.80	0.1	0.01-0.1	0.01-0.94		
β-caryophyllene	1.92-1.98	1.80	1.80	0.40-9.3		1.0-12
Germacrene D	9.59-9.87	1.80	1.80-5.08	0.36-11.4		
Sclareol	0.50- 0.78	0.20	0.20-0.21	0.06-14.62		0.4-2.6

1- BDS ISO 7609, 2-EU pharmacopeia.

Similar are the results for the content of terpinolene and terpinen-4-olin the oil from contaminated areas (S1 and S2) and uncontaminates areas (S3). The resulting values reach, respectively, for terpinolene up to 0.92% -1.05% and up to 0.47% -0.58% for terpinen-4-ol in oil from areas located at 0.5 km and 2 km from KCM. These values are higher than the values for terpinolene and terpinen-4-olobtained oil from uncontaminated soil (respectively 0.2% and 0.05%). According to some authors the content of terpinolene in the oil may vary from 0.1 to 0.6%, and terpinen-4-ol - from traces to 1.19%. The content of terpinolene in oils from polluted soils is considerably higher than those of literature values, which is probably due to may be due to heavy metal stress as reported in case of mint [54].

Regarding the content of bourbonene, there are also significant differences between oils obtained from areas located at a different distance from KCM. The content of bourbonene in oil from areas located at 0.5 km and 2 km from KCM has values of 2.62%-2.80%. These values are higher than the values obtained for oil from uncontaminated soil (0.4%), as well as than those data for oil obtained by other authors (0.08-0.94%)

The content of germacrene D in the oil varies from 9.59% to 9.87% and does not exceed in value (from 1.2 to 12.0%) the requirements of the European Pharmacopoeia for clary sage oil. These values are higher than the values obtained for the oil from uncontaminated soil (1.8%), as well as the results indicated by [18], [15] (1.8% and 5.08%, respectively).

Regarding the content of ß-caryophyllene, compounds that have antimicrobial activities, there are no significant differences between oils obtained from areas located at a different distance from KCM. The content of ß- caryophyllene in oils from areas located at 0.5 km and 2 km (1.92-1.98%) from KCM has values similar to the oil obtained from uncontaminated soil (1.8%). Our values are similar to the values obtained from [15] for β-caryophyllene - (1.83%)

The content of sclareol, compounds that have antimicrobial activities, in oil from areas locate at a distance of 0.5 km and 2 km from KCM varies from 0.5% to 0.78% and does not exceed in value (from 0.4% to 2.6%) the requirements of the European Pharmacopoeia for clary sage oil. According to some authors the content of sclareol in the oil can reach up to 1.2%, as well as there is data on the content of sclareol up to 11%. These values are higher than the values obtained for the oil from uncontaminated soil (0.2%), as well as than the results obtained by [18] (0.21%).

Our results show that there are minor composition differences between oils obtained from areas located at different distances from KCM - Plovdiv, which have varying degrees of pollution. The compounds in the essential oil that slightly decreased as a result of heavy metals pollution are linalool and α-terpineol. The compounds in the essential oil that increased as a result of heavy metals pollution are myrcene, cis β-ocimene, trans β-ocimene, terpinolene, borneol, terpinen -4-ol, α-copaene, β-cariophyllene, germacren D и sclareol, while camphor, borneol, 1,8-cineole and bornylacetate significantly increased. Regarding the content of α-pinene, β-pinene, camphor, linalylacetate and bornylacetate no significant differences in oils between contaminated and non-contaminated soils was observed. The increase of some of the components of clary sage oil may be due to the stress of heavy metals as reported in case of mint [54].

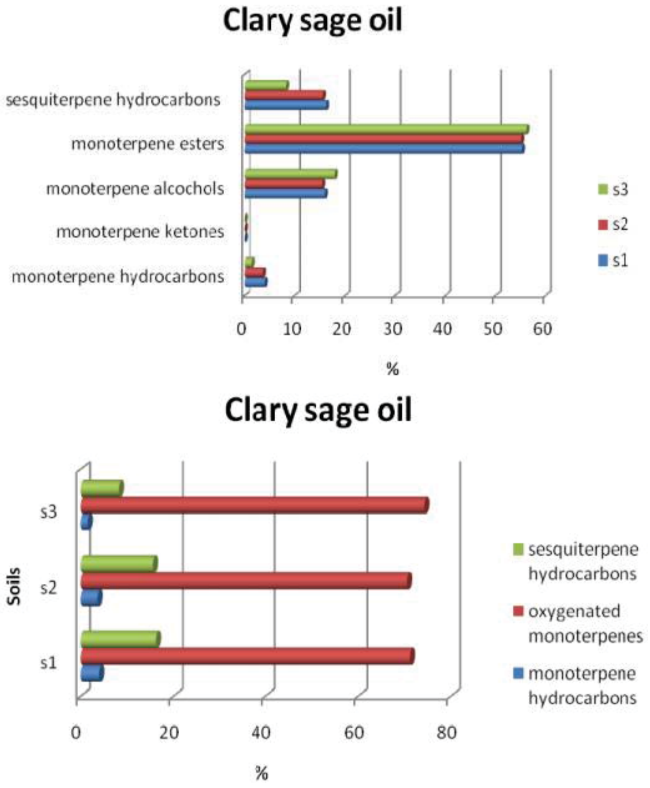

Fig. 5 Distribution of the components in groups of compounds in clary sage oil

The distribution of the components in groups of compounds in clary sage oil is shown in Fig. 5. From the data it is seen that the oil from contaminated soils (S1-S2) oxygen terpene containing compounds (70.53-71.17%) are prevailing, followed by the sesquiterpene hydrocarbons (15.57 to 16.21%) and terpenic hydrocarbons (3.63-3.97%). Our results indicate that there are no significant differences between the mean values of oxygenated monoterpenes in the contaminated soils (S1-S2). By increasing distance from the KCM, the concentration of oxygenated monoterpenes slightly increased from 70.5 to 74 27%. As shown in the figure, no significant differences between the mean values of sesquiterpene hydrocarbons concentration in the contaminated soils (S1-S2). The highest concentration of sesquiterpene hydrocarbons was observed at distances of 0.5 km from the KCM and the lowest value was observed on uncontaminated soil (S3). By increasing distance from the KCM, the concentration of sesquiterpene hydrocarbons decreased from 16.21 to 8.24%. Our results indicate that there are no significant differences between the mean values of monoterpene hydrocarbons in contaminated soils (S1-S2). The highest concentration of monoterpene hydrocarbons was observed at distances of 0.5 km from the KCM and the lowest value was observed on uncontaminated soil (S3). By increasing distance from the KCM, the concentration of monoterpene hydrocarbon decreased from 3.97% to 1.5%.

An important point in the study is to see whether heavy metals influence the quality of the essential oil. Clary sage oil is used in a range of products for the pharmaceutical, perfume and food industries, and must have indicators that can be used to guarantee cleanliness and safety in order to meet the pharmacopoeial requirements. As such, clary sage oil must not contain any traces of heavy metals and there must not be any changes in its composition. Terpene oxygen compounds make up the large proportion of the oil, and its major components are linalyl acetate and linalool. These are primarily what determine the type of smell; however, the quality of the latter is significantly influenced by other elements and their relationships. Our results show that the heavy metal content in the soil has no significant influence on the composition of clary sage oil. In growing clary sage on soils contaminated with heavy metals, oil with very good quality is yielded - high in linalool (10.97-11.25%) and linalyl acetate (54.91-55.05%), which corresponds to the requirements of the Bulgarian standard and the European Pharmacopoeia. The possibility of processing clary sage inflorescences in oil and its use in perfumery makes clary sage very suitable for phytoremediation of soils contaminated with heavy metals.

IV. CONCLUSION

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

1. There is a distinct feature in the accumulation of heavy metals in the vegetative organs of clary sage. Clary sage accumulates heavy metals through its root system, but very small portion of the heavy metals are retained by the roots and most of them move and are accumulated in the aboveground parts (stems, leaves and inflorescences).
2. Clary sage is a culture that is tolerant to heavy metals, it can be attributed to the hyperaccumulators of Pb and the accumulators of Cd and Zn, and it can be successfully used in phytoremediation of soils contaminated with heavy metals.
3. The quantities of Pb, Cu and Cd in the essential oil of *Salvia sclarea* L. grown on contaminated soils were lower than the accepted maximum permissible concentrations, and obtained oil could be used in the perfumery and cosmetics and tobacco industries. The processing of inflorescences to oil and the use of oil in perfumery will significantly reduce the cost of phytoremediation.
4. The studied essential oil of *S. sclarea* L., growing in Bulgaria belongs to the group of linalyl acetate/linalool chemotype. The main odour-determining ingredients of clary sage oil, obtained from the processing of clary sage, grown on highly contaminated soils, meet the requirements of the European Pharmacopoeia and BS ISO 7609 on the quality of the Bulgarian clary sage oil and/or have values close to the limits of the standards.

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