

# Potential of Lavender (*Lavandula vera* L.) for Phytoremediation of Soils Contaminated with Heavy Metals

Violina R. Angelova, Dimitar F. Grekov, Veselin K. Kisyov, Krasimir I. Ivanov

**Abstract**—A field study was conducted to evaluate the efficacy of lavender for phytoremediation of contaminated soils. The experiment was performed on an agricultural fields contaminated by the Non-Ferrous-Metal Works near Plovdiv, Bulgaria. The concentrations of Pb, Zn and Cd in lavender (roots, stems, leaves and inflorescences) and in the essential oils of lavender were determined. Lavender is a plant which is tolerant to heavy metals and can be grown on contaminated soils, and which can be referred to the hyperaccumulators of lead and the accumulators of cadmium and zinc, and can be successfully used in the phytoremediation of heavy metal contaminated soils. Favorable is also the fact that heavy metals do not influence the development of the lavender, as well as on the quality and quantity of the essential oil. The possibility of further industrial processing will make lavender economically interesting crops for farmers of phytoextraction technology.

**Keywords**—Heavy metals, lavender, phytoremediation, polluted soils.

## I. INTRODUCTION

A large area of land is contaminated with heavy metals due to use of sludge or municipal compost, pesticides, fertilizers and emissions from municipal wastes incinerators, car exhausts, residues from metalliferous mined, and smelting industries. Excessive metal concentrations in the contaminated soils can result in soil quality degradation, crop yield reduction, and poor quality of agricultural products [1]. Heavy metal contamination of soils can be of concern for human and animal health, as the metals may be transferred and accumulated in the bodies of animals or human beings through food chain. The clean-up of soils contaminated with heavy metals is one of the most difficult tasks for environmental engineering. Use of green plants to remove heavy metals from the contaminated soils, known as phytoremediation, is an emerging technique that offers the benefits being in situ, low cost and environmentally sustainable [2], [3]. As a technology based on the use of plants, the success of phytoextraction will mainly depend on the proper selection of plants. Plants used for phytoextraction should have rapid growth and should have the ability to accumulate significant amounts of metals in their stems [1]. Many plant species have been studied in order to

determine their potential for phytoextraction. Researchers initially used hyperaccumulators for purification of soils contaminated with metals. So far, about 400 hyperaccumulators have been known [4], but most are not suitable for phytoextraction due to their slow growth and small size. Many researchers examine the rapidly growing, high biomass plants, including agronomic crops for their ability to tolerate and accumulate metals in their shoots [5]-[10]. Many metal tolerant plant species, especially wheat plants of the family *Gramineae*, avoid toxicity through the mechanisms of exclusion and therefore are more suitable for phytostabilization than phytoextraction [5], [11]. However, barley (*Hordeum vulgare* L.) and oat (*Avena sativa* L.) are tolerant to metals such as Cu, Cd, and Zn, and accumulate from medium to high levels of these metals in their tissues [11]. Many types of plants can accumulate medium quantities of metals in their above-ground mass.

There is a growing opinion, however, that the use of food crops for phytoremediation is not environmentally safe as heavy metals can enter the food chain upon consumption of these cultures by humans or animals. Medical and aromatic plants can be a better choice for phytoremediation as these species are grown primarily for processing (essential oils) and thus contamination of the food chain with heavy metals is eliminated. Aromatic and medicinal plants also have the ability to accumulate heavy metals [12], [13]. Studies show that heavy metals accumulated by those species are not included in the essential oils [12], [14].

Aromatic plants are grown for the production of essential oils and are used in the food processing industry. The essential oil of the aromatic plants is used in cosmetics, perfumery and food industry. These plants are not consumed directly by humans or animals, such as grains, legumes, vegetables, etc. The essential oil of aromatic plants does not contain heavy metals, although there is an accumulation in the plant biomass. Heavy metals do not pass on the food chain when aromatic plants are used for phytoremediation. For this reason, aromatic plants can be used in phytoremediation of heavy metal polluted soils. Aromatic plants such as vetiver (*Vetiveria zizanioides*), palmarosa (*Cymbopogon martinii*), lemon grass (*Cymbopogon eleuosus*), citronella (*Cymbopogon winterianus*), geranium and mint (*Mentha* sp.), basil (*Ocimum basilicum*) may be used for this purpose. Some aromatic herbs such as lemon grass, palmarosa, citronella, vetiver are perennial plants that are tolerant to contaminants and are

V. R. Angelova is with the Agricultural University – Plovdiv Bulgaria (corresponding author to provide phone: 357 888 578126 ; fax: 357-32-633-157; e-mail: vileriz@abv.bg).

D. F. Grekov, V. Kisyov, and K. I. Ivanov is with the Agricultural University–Plovdiv Bulgaria (e-mails: rector@au-plovdiv.bg, veselin\_kisyov@abv.bg, kivanov1@abv.bg).

resistant to stress. Herbs can be collected for years and then to receive essential oil.

Herbs can be collected for years and to receive essential oil from them. These plants have economic value with low production costs. Therefore, in recent years an increasing attention is paid to the aromatic plants as an alternative for conducting environmentally safe and cost-effective phytoremediation. The obtaining of oil and its use in the food and perfume industry will allow farmers to recover the costs.

It was found that the content of the essential oil obtained from the fresh plant mass of the lavender is not affected by the level of heavy metal soil contamination [15]. However, due to the decreased yield of plant mass the yield of oil reduces by up to 14% in the cultivation of lavender on highly contaminated soils. Zheljzkov [15] found that peppermint can extract significant quantities of heavy metals from the soil. It was found that the tested varieties of mint can be successfully grown on soils highly polluted with heavy metals (in the region of KCM – Plovdiv (Non-ferrous metals plant)), without contamination of the final product - the essential oil. Despite the reduction in the yield (14%) due to pollution with heavy metals, mint still remains very profitable crop and can be used as an alternative to food crops. 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. They can also be successfully used in phytoremediation and can replace food crops grown under the same conditions [16].

Insufficient is the information available on the potential of lavender for accumulation of heavy metals and its potential for use for phytoextraction. There are no comprehensive studies on the relationship between the total content of heavy metals in the soil, the amount of their exchange forms and their absorption by the lavender, as well as their distribution in the vegetative organs.

The purpose of this work is to undertake a comparative study, which allows us to determine the quantities and the deposits for accumulation of Pb, Zn and Cd in the vegetative organs of lavender, the quality of lavender oil, as well as the possibilities to use the plant for phytoremediation of heavy metal contaminated soils.

## II. MATERIAL AND METHODS

The study included lavender grown on areas located at different distances from the source of contamination KCM-Plovdiv (0.3 km and 1 km). Lavender is one of the main essential oil crops for the conditions in Bulgaria. Plantations of lavender in the area of KCM were made in 2001-2002 and respectively occupy an area of 165 decares (distance 0.3 km) and 51 decares (1 km).

Lavender is grown according to conventional technology. 5 plants of each of the areas were used for the analysis. Upon reaching the stage of flowering, lavender was harvested and the content of Pb, Zn and Cd in different parts - roots, stems, leaves and flowers was determined. The essential oil of the lavender was obtained by steam distillation in industrial

conditions which was analyzed for heavy metals and its chemical composition was determined.

Before the analyses root and shoot samples were thoroughly washed by distilled water to remove all adhering soil particles. Samples were then oven dried to constant weights at 105°C. Each dried sample was ground to powder using a blender. One gram of each sample was used for analysis.

Pseudo-total content of metals in soils was determined in accordance with [17]. The available (mobile) heavy metals contents were extracted by a solution of DTPA (1 M  $\text{NH}_4\text{HCO}_3$  and 0.005 M DTPA, pH 7.8). The contents of heavy metals (Pb, Zn and Cd) in the plant material (roots, stems, leaves and inflorescences) and in the essential oils of lavender were determined by the method of the dry mineralization. The quantitative measures were carried out by ICP method (Jobin Yvon Emission - JY 38 S, France). Digestion and analytical efficiency of ICP was validated using a standard reference material of apple leaves (SRM 1515, National Institute of Standards and Technology, NIST).

The chemical composition of the oil was determined on a gas chromatograph PYE UNICAM series 204, equipped with a flame ionization detector and a capillary column CARBOWAX 20 M with hydrogen carrier gas.

## III. RESULTS AND DISCUSSION

### A. Soils

To clarify the degree of soil contamination with heavy metals and their localization in the vegetative organs of lavender, soil samples were taken from the areas located at different distances from KCM (0.3 and 1 km). The physical and chemical properties of the soil samples are presented in Table I.

The results presented in Table II show that with receding further from KCM - Plovdiv a notable trend to reduce the total (overall) content of heavy metals in the soil is observed. In soil samples taken from the area, located at 0.1 km from KCM, values for Pb were reported to exceed the threshold limit value (80 mg/kg) - 2112.5 mg/kg in layer 0-20 cm. In the area located at the distance of 1 km from KCM the content of Pb decreases about 20 times and reaches up to 94.1 mg/kg.

TABLE I  
SOIL PROPERTIES FOR SOIL SAMPLED FROM THE NON-FERROUS METALS  
PLANT (KCM) NEAR PLOVDIV

Classification	Distance from KCM, km	Depth, cm	pH (H <sub>2</sub> O)	Org.C %	CaCO <sub>3</sub> %	Clay %
Alluvial soil	0.3	0 – 20	7.7	2.77	3.85	37.60
Alluvial soil	1.0	0 – 20	8.3	1.54	8.70	12.71

Similar results were obtained for Cd and Zn. In the area of KCM (0.3 km) 2512.5 mg/kg of Zn and 43.6 mg/kg of Cd were recorded, whose values exceed significantly the threshold limit value, while at a distance of 1 km 171.1 mg/kg and 1.97 mg/kg were found respectively.

Table III presents the results for the mobile (available) forms of Pb, Zn and Cd in the soils studied. The results for the mobile forms of metals determined by DTPA show that the mobile forms of Cd in highly contaminated soils (0.3 km) are

the most significant part of the total content and reach up to 70.6%, followed by Pb - 38.7% and Zn - 13.5%. In the soil located at a distance of 1 km from KCM the mobile forms of Pb are the most significant part of its total content and reach up to 52.3%, followed by Cd - 49.7% and Zn -22.0%.

TABLE II  
CONTENT OF PB, ZN AND CD (MG/KG) IN SOIL

Distance from KCM, km	Depth, cm	Pb x±sd	Zn x±sd	Cd x±sd
0.3	0-20	2112.5±10.2	2512.5±8.7	43.6±1.8
1.0	0-20	94.1±1.8	171.1±1.5	1.97±0.4
MPC		80	340	2.5

x- average value(mg/kg) from 5 repetitions; sd - mean standard deviation; MPC -maximum permissible concentration (approved for Bulgaria)

TABLE III  
DTPA – AVAILABLE FORMS OF PB, ZN AND CD (MG/KG) IN SOILS

Distance from KCM, km	Pb		Zn		Cd	
	mg/kg x±sd	%	mg/kg x±sd	%	mg/kg x±sd	%
0.3	816.9± 6.0	38.7	340.0±1.3	13.5	30.8± 0.5	70.6
1.0	49.2±0.5	52.3	37.7±0.6	22.0	0.975±0.1	49.7

x- average value(mg/kg) from 5 repetitions; sd - mean standard deviation, %DTPA -available/ total content

### B. Content of Heavy Metals in Lavender

The soil conditions and the type of plants affect the entry of heavy metals in plants and their accumulation in the bodies of plants depends on their content in the soil. Plants grown on contaminated soils accumulate heavy metals and accumulate them in vegetative organs.

Plants grown on contaminated soils accumulate heavy metals and deposit them in their vegetative organs. In most cases, most of them are accumulated in the roots of the plants, as they are in contact with contaminated soil. In some plants, however, the substantial portion of the heavy metals is accumulated in the above-ground mass [18].

To clarify the issues of absorption, accumulation and distribution of heavy metals in the vegetative organs of

lavender samples of roots, stems, leaves and inflorescence (flowering stalks) were analyzed.

Table IV presents the results obtained for the content of heavy metals in the organs of studied essential oils crop. Substantial differences were discovered in the content of the elements in different parts of the lavender. Most of them (Pb, Cd and Zn) are accumulated in the aboveground parts of the lavender (the leaves). The content of Pb in the roots of the lavender grown at 0.3 km from KCM reaches up to 1566.9 mg/kg, Zn - 1755.9 mg/kg and Cd - 160.9 mg/kg. The obtained values for the heavy metals (Cd, Pb and Zn) in the roots are much higher than the values considered by [19] as toxic for the plants (0.1 mg/kg Cd, 30 mg/kg Pb, 100 mg/kg Zn).

The studies of [18] lead to the conclusion that lavender accumulates heavy metals primarily in the roots. The roots are the main body of the plant, which is in contact with the contaminated soil and the body which accumulates heavy metals. Root growth is a good indicator for the plant tolerance to metals.

Our results do not match what was found by [18]. The reason for this is that in growing plants under field conditions, the roots penetrate deeply and easily avoid areas with contaminated soil [20], while with pot plant the roots are in contact only with the contaminated soil, which is probably the reason why the roots of the lavender accumulates significantly greater amounts of heavy metals. It is well known that the lavender forms a strong root system, which is composed of highly developed adventitious roots, some of which reach up to 300-400 cm depth, and in the side – up to 100-140 cm. The major portion of the roots of the lavender is located in the soil layer from 30 to 50 cm. This is probably why the lavender accumulates fewer amounts of heavy metals in the roots and develops normally on contaminated soils during growth under field conditions.

TABLE IV  
CONTENT OF PB, ZN AND CD (MG/KG) IN LAVENDER

Element	Distance, km	Roots, x±sd	Stems, x±sd	Leaves, x±sd	Flowering stalks, x±sd	Oil, x±sd
Pb	0.3	1566.9±8.7	2157.9±9.6	5784.7± 10.8	1147.3± 6.8	0.28± 0.01
	1	20.5± 0.2	71.2± 0.6	89.97± 0.9	31.4± 0.5	0.14± 0.01
Cd	0.3	160.9± 2.1	27.1± 0.7	113.2± 2.0	15.6± 0.5	nd
	1	2.3± 0.03	3.1± 0.03	28.4± 0.8	0.62± 0.01	nd
Zn	0.3	1755.9±7.5	683.2±6.8	2881.9±9.7	349.3± 5.3	0.84± 0.05
	1	46.8± 0.6	74.9± 0.9	80.2± 0.9	57.1± 0.8	0.24± 0.02

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

The content of heavy metals in the above-ground mass is greater than that of the root system.

A significant accumulation of Pb is found in the leaves and stems of the lavender. The content of this element reaches up to 2157.9 mg/kg in stems, and 5784.5 mg/kg in leaves of the lavender grown at a distance of 0.3 km from KCM. Probably a portion of heavy metals absorbed by the conduction system moves from the roots to the above-ground parts of the lavender and are accumulated predominantly there. Probably a

portion of the accumulated heavy metals in the above-ground mass of the lavender is also due to aerosol pollution, which can be explained by the anatomical and morphological characteristics of the crop. The greater accumulation of Pb in the above-ground mass is probably due to the fact that the lavender is distinguished by a woody stem, with a rough surface, which favors the attachment of the aerosols and their accumulation therein. The content of Cd in the stems and leaves of the lavender grown at a distance of 0.3 km from

KCM reaches up to 27.1 mg/kg and 113,2 mg/kg, respectively, values considered to be toxic to plants. According to [21] 5.0 mg/kg is considered to be a toxic value for the plants. Our results show the ability of the lavender to accumulate Cd in the above-ground mass.

The content of Zn in the stems and leaves of the lavender grown at a distance of 0.3 km from KCM reaches up to 683.2 mg/kg, and 2881.9 mg/kg, as these values are also higher than the critical values for plants - 100-400 mg/kg.

The content of heavy metals in the stems and leaves of the lavender grown at 1 km from KCM reaches up to 71.2 mg/kg and 89.97 mg/kg (Pb), 3.1 mg/kg and 28.4 mg/kg (Cd) and 74.9 mg/kg and 80.1 mg/kg (Zn).

The heavy metal content in the flowering stalks is considerably lower in comparison to the root system and the above-ground mass of the plants. The contents of Pb, Zn and Cd in the flowering stalks of the lavender grown at a distance of 0.3 km from KCM reaches up to 1147.3 mg/kg, 349.3 mg/kg and 15.6 mg/kg, respectively. With increasing the distance from KCM a clear trend is seen towards reducing the content of heavy metals in the inflorescences of the studied crop. Significantly lower is the content of heavy metals in the flowering stalks of the lavender grown at 1 km from KCM. The content of Pb in the flowering stalks reaches up to 31.4 mg/kg, Zn- up to 57.1 mg/kg and Cd – up to 0.62 mg/kg.

Probably a portion of the accumulated heavy metals in the flowering stems of the lavender is due to the aerosol pollution. In the lavender the inflorescences are densely located on the highest part of the tufts covering a significant part of the leaves and stems of the plants, making them easily accessible for aerosol pollutants.

The heavy metal content in the essential oil from lavender was also determined. The results obtained show that the majority of the heavy metals contained in the flowering stalks of the lavender do not pass into the oil during the distillation, therefore their content in the oil are much lower. Pb content in the essential oil of lavender reaches up to 0.28 mg/kg, Zn up to 0.84 mg/kg, while the content of Cd is below the limits of the quantitative measurement of the method used. Significantly lower are the figures in the essential oil of lavender grown at a distance of 1 km from KCM - 0,14 mg/kg Pb and 0,24 mg/kg Zn. The results obtained show that the content of heavy metals in the essential oils is much lower compared to the flowering stalks of the lavender, and the amounts of Pb, Zn and Cd in the oil of lavender are lower than the accepted maximum values and meet the requirements of an environmentally friendly product. Our results are inconsistent with the ones established by [14] and [15], which found that the heavy metal content in the essential oil of the lavender is very low and is not affected by the level of soil contamination with heavy metals.

The distribution of heavy metals in the organs of the lavender has a selective nature, which reduces in the following order: leaves> roots> stems> flowering stalks.

There is a distinct feature in the accumulation of heavy metals in the vegetative organs of the lavender. The lavender 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 flowering stalks). The results obtained show that the lavender 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 flowers makes the lavender a very lucrative for the purposes of phytoremediation.

To determine the extent to which lavender accumulates heavy metals and to which group of plants it can be assigned (hyperaccumulators or accumulators) we used the existing criteria for determination of heavy metal accumulation in plants. Plants have the ability to accumulate metals and some of them can accumulate them in large quantities (100 times more than other plants in the same conditions, without showing any adverse effects). Plants that have the ability to tolerate and accumulate heavy metals in quantities greater than the toxic levels are known as hyperaccumulators. In recent years, the number of studies on the use of hyperaccumulators for remediation of contaminated areas, owing to their capacity of these plants to absorb heavy metals from contaminated soil and accumulate them in their aboveground biomass, has been steadily increasing.

The classification of plants as hyperaccumulators, accumulators, indicators and non-accumulator species is based on precise and clear criteria [22]. Plants that contain high quantities of heavy metals in their root biomass but whose aboveground biomass/root biomass ratio is less than 1, belong to the non-accumulator species. According to [23], indicator species are those whose heavy metal content is proportionate to the heavy metal content in the soil. Plants can be classified as hyperaccumulators if they meet the following criteria: (a) have an aboveground biomass/root biomass ratio > 1; (b) have an extraction ratio (the ratio of the heavy metal content in the aboveground biomass to the heavy metal content in the soil) > 1; (c) have a heavy metal content which is 10-500 times higher than that of plants grown in non-contaminated soils; and (d) contain more than 1000 mg/kg of copper, lead, nickel, chromium; more than 100 mg/kg of cadmium or more than 10,000 mg/kg of zinc [24].

The values obtained for BCF and TF, are the most important test that can be used to assess the potential of the plants for phytoremediation. Our results indicate that in the lavender both factors EF and TF are higher than 1 and by this indicator it can also be classified as an accumulator in terms of Pb, Zn and Cd (Table V).

TABLE V  
UPTAKE OF HEAVY METALS FROM LAVENDER

Element	BCF		TF		BAC	
	0.3 km	1 km	0.3 km	1 km	0.3 km	1 km
Pb	1.92	0.42	3.69	4.38	9.72	3.27
Cd	5.22	2.35	0.70	1.24	4.56	5.34
Zn	5.16	1.24	1.64	1.71	10.49	4.11

$$\text{BCF} = \frac{[\text{Metal}]_{\text{roots}}}{[\text{Available metal}]_{\text{soil}}}, \text{TF} = \frac{[\text{Metal}]_{\text{shoots}}}{[\text{Metal}]_{\text{roots}}},$$

$$\text{BAC (EF)} = \frac{[\text{Metal}]_{\text{shoots}}}{[\text{Available metal}]_{\text{roots}}}$$

Comparing the criteria shows that in terms of Pb all criteria necessary for referral of the lavender to the hyperaccumulators are covered. In terms of Cd and Zn only 1 of the criteria is not covered - sufficient accumulation of Zn in the above-ground mass (less than 10000 mg/kg Zn) and movement of Cd from the soil to the above-ground mass. Therefore, the lavender can be attributed to the group of accumulators of Cd and Zn.

Although phytoremediation is a new technology, in recent years many studies have been conducted in an attempt to determine how plants absorb metals, the mechanisms of movement of translocation of metals from the roots to the above-ground mass, the storage and their detoxification. One of the main principles of phytoremediation is to find suitable plant species that can be grown on contaminated soils. Our results strongly suggest that the lavender is a crop that is tolerant to heavy metals and can be grown on heavy metals polluted soils. It can be attributed to hyperaccumulators of Pb and to accumulators of Cd and Zn and can be successfully used for phytoremediation of soils contaminated with Pb, Zn and Cd.

### C. Effect of Heavy Metals on the Quality of the Oil

The results of the chromatographic analysis of essential oils obtained by processing of flowering stalks of lavender grown at a different distance from KCM are presented in Table VI. The values of the main components of the essential oil of lavender are compared with the requirements of ISO3515:2002 [25] for the Bulgarian lavender oil and French lavender oil of seed population. According to the standards for composition and quality of lavender oil set by the International Organization for Standardization [25], the content of linalool, linalyl acetate, cymene, terpinen-4-ol and camphor should be between 25.0-38.0%, 25.0-45.0%, 4.0-10.0%, 2.0-6.0% and 0-0.5%, respectively.

Identified are 5 terpene alcohols (linalool, alpha terpineol, borneol, lavandulol and terpinen-4-ol), of which linalool is the dominant component (33.76-34.17%). Two ester components (linalyl acetate and lavandulyl acetate) were identified. Identified were 5 mono terpinen hydrocarbons (camphene, myrcene, alpha pinene, cis ocimene and trans-β-ocimene). In oil only one sesquiterpene (caryophyllene) was discovered. Two mono terpinen ketones were identified (camphor and 3-octanol) as well as mono terpinen ether (eucalyptol). Hexyl ester was also discovered in the oil.

The main components (> 1.0%) contained in lavender oil are linalyl acetate, linalool, lavandulyl acetate, β-caryophyllene, α-terpineol, cis ocimene, trans ocimene, borneol and 1,8-cineole. A trace of the following components (minor components (in the range of <1.0 and> 0.10%): lavandulol, camphene, myrcene, pinene, camphor, 3-octanol, and hexyl are identified in oils. There are no significant composition differences between oils obtained from areas located at different distances from KCM - Plovdiv, which have varying degrees of pollution. It is well known that the lavender oil is a mixture of many compounds. It includes more than 200 components, belonging to the terpene and non-terpene hydrocarbons, alcohols, aldehydes, ketones, esters,

ethers, acids, lactones, phenols, and sulfur-containing compounds. All these components have a content in the essential oil of the individual branches or offsprings which are more or less influenced by heredity, which they carry, the specific soil and climatic conditions, time of harvest, type of distillation of essential oil, etc. [26]. Even within one species of Lavender the essential composition of the oil varies greatly depending on where and under what conditions the plant was grown [27]. Differences in the base oil composition may be due to the process of distillation - duration, temperature and pressure of distillation [28].

TABLE VI  
COMPOSITION OF OIL OF LAVENDER (%) OBTAINED BY PROCESSING FRESH FLORAL STALKS OF LAVENDER GROWN IN THE REGION OF KCM

Oil content	Distance from KCM, km		ISO3515:2002	
	0.3	1	Bulgaria	France
Linalool	33.76	34.17	22.0-34.0	25.0-38.0
α-terpineol	2.96	2.98	0.6-2.0	
Borneol	1.23	1.22	-	
Lavandulol	0.47	0.48	Min 0.3	
Terpinen-4-ol	3.67	3.68	2.0-5.0	
Linalyl acetate	35.64	35.97	30.0-42.0	25.0-45.0
Lavandulyl acetate	1.37	1.38	2.0-5.0	2.0-6.0
Camphene	0.11	0.11	-	
Myrcene	1.07	1.06	-	
α-pinene	0.11	0.11	-	
Cis ocimene	2.87	2.86	3.0-9.0	4.0-10.0
Trans ocimene	1.31	1.31	2.0-5.0	1.5-6.0
1,8 cineol	1.22	1.21	Max 2.0	
β-caryophyllene	3.83	3.88	-	
Camphor	0.29	0.32	Max 0.60	Max 0.50
3-octanone	0.55	0.54	0.2-1.6	
Hexylacetate	0.42	0.41	-	

According to literature data the content of the main components in the lavender oil from the different parts is linalool (27.3-42.2%), linalyl acetate (27.2-46.6%), (Z)-β-ocimene (0.2-11.6%), terpinen-4-ol (0.70-4.6%), lavandulyl acetate (0.50-4.8%), β-caryophyllene (1.8--5.1%), (E)-β-ocimene (0.30-3.8%), α-terpineol (0.30-2.0%) and 1,8-cineol (0.10-1.2%) [29].

As basic and defining nature of the smell of the oil are the following: linalyl acetate, linalool, terpinen-4-ol, cineol, lavandulol and lavandulyl acetate, camphor, cis- and trans-β-ocimene. The assessment of the lavender oil is made, reflected in the standards of the countries concerned, based on the organoleptic, physical and chemical indicators, paying attention mainly to the ester content [30]–[33].

The content of 1,8-cineole in the tested oils is in the range characteristic of the typical Bulgarian lavender oil, and do not exceed in value (from 0.5 to 1.6%) the requirements of the standard [25] for the French lavender oil.

Regarding the content of cis-β-ocimene significant differences are found between the oils obtained from areas located at different distances from KCM. The content of cis-β-ocimene in oils in areas located at a distance of 0.3 km and 1 km from KCM is with values below the requirements of both standards (2.87%) under requirements from 3% to 9% for the

Bulgarian and from 1% to 10% for the French lavender oil. The results show that the content of heavy metals in the soil does not affect the content of cis-ocimene in the lavender oil.

Similar are the results for the content of trans-ocimene. The values obtained reach up to 1.31% for the oil from the area located at a distance of 0.3 km and 1 km from KCM, as these values are lower than the standard requirements (2-5%).

Regarding the content of linalool no significant differences are found in the values obtained for oils from areas located at a distance of 0.3 km and 1 km from KCM. The content of linalool varies from 33.76 to 34.17%, and these values are within the limits of the standard (22.0-34.0%).

The low content of camphor is a mandatory requirement for quality lavender oil. The camphor content in the oil ranges from 0.29 to 0.33%, and is significantly lower than the requirements of the standards (0.6%). The content values of lavandulol and lavandulyl acetate can also be defined as typical for the Bulgarian lavender oil, meeting the standard.

Regarding the content of terpinen-4-ol in the oil, the values are very similar (3.67-3.68%), and are within the normal limits, and do not exceed the requirements of the standard (2 to 5%). The selection of varieties of lavender in the last 30 years is aimed at lowering the values of terpinen 4-ol. As a result of this trend the contemporary Bulgarian varieties have a very low content (with the exception of the variety Jubilee), which is inherited by their seed offsprings [34]. Probably the content of heavy metals in the soil does not have a significant impact on the content of terpinen-4-ol.

Linalyl acetate is the main ingredient which determines the quality of lavender oil. The content of linalyl acetate is high (35.64-35.97%) and is within the standards for quality lavender oil (30-42%). The results show that the content of heavy metals in the soil does not influence the content of linalyl acetate in lavender oil. An important point in the study is to see whether heavy metals influence the quality of the essential oil. Lavender oil is used in a wide range of products for perfumery, pharmacy and food industry and must have guaranteed indicators for cleanliness and safety, i.e. to meet the pharmacopoeial requirements. This means that it must not contain heavy metals and/or contain traces of metals and must not have changes in its composition. In the oil itself the biggest share is occupied by the terpene oxygen compounds, as major components are linalyl acetate and linalool. They determine the type of smell, but for the quality of the latter, significant are other elements and their relationship.

According to [35] the high-quality lavender oils have linalool:linalyl acetate ratio from 0.7:1 to 0.8:1. Terpinene-4-ol and cineole give unwanted grassy note in the smell and the aim is to reduce them [36]. It was found that mono terpene hydrocarbons of limonene and ocimene are also important for the quality. Data show that the cultivation of lavender on soils contaminated with heavy metals does not have negative effect on the composition of the lavender oil. Heavy metals in the soil have a negligible effect on yield (data not shown) and do not affect the quality of the oil.

In the lavender oil the amount of linalool and linalyl acetate is key factor to the overall smell of the oil. Linalool is sweeter,

but the smell of its ester, linalyl acetate is more refreshing. The ratio of linalool and linalyl acetate may change depending on the time of distillation and can affect the final smell of the oil [37]. According to literature data, in high quality lavender oils, the ratio of linalool and linalyl acetate must be greater than one unit. The content of linalool is lower compared to linalyl acetate in the oils studied by us, which meets the requirements for high quality lavender oil. According to [38], however, the content of linalool is higher in the lavender oil compared to linalyl acetate in all sorts of lavender.

According to [39]-[42] the essential oils should be distilled at the right time to release all active ingredients. Distillation can determine the value of the oil or destroy the value of the oil. Oils obtained from areas located at a distance of 0.3 km and 1 km from KCM contain more  $\alpha$ -terpineol (2.96-2.98%) compared with the standard (0.6-2.0%). The higher content of  $\alpha$ -terpineol, as well as the lower content of linalyl acetate may be explained by the molecular rearrangement and hydrolysis which can occur during the distillation. Geraniol and various terpineol isomers can be obtained from linalyl acetate during the process of distillation, and may alter the smell of the lavender oil in geranium, which is not observed in the oils obtained by us.

Medicinal and olfactory properties of the lavender are mainly due to the proportional composition of linalool, linalyl acetate, eucalyptol (1.8-cineol),  $\beta$ -ocimene, 1-terpinen-4-ol and camphor [42].

The best quality oil is used in perfumery. It must contain large amounts of linalool and linalyl acetate and traces of camphor. The use of the oil in the medicine is determined by the ratio of monoterpenes of desired biological activity [43].

In medicine, preferred is the use of oils with a higher content of camphor,  $\alpha$ -terpineol and 1-terpinen-4-ol (with antibacterial properties), as well as with a content of linalool (29.92%) and linalyl acetate (12.35%) which have a sedative or anesthetic effect [44].

Our results indicate that in the cultivation of lavender on soils contaminated with heavy metals an oil with very good quality is produced – with low content of camphor (0.24 - 0.33%) and high content of linalool (22.32 -34.17%) and linalyl acetate (27.41-35.97%), which may be suitable for perfumery. Our results show that heavy metals do not affect the development of lavender and the quality and quantity of oil obtained from it. The possibility of processing inflorescences of lavender into oil and its use in perfumery makes lavender very suitable for phytoremediation of heavy metal polluted soils.

#### IV. CONCLUSIONS

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

1. There is a clear distinction in the accumulation of heavy metals in vegetative organs of lavender. Lavender accumulates heavy metals through its root system, but a very small part of the heavy metals are retained by the roots and most of them move and are accumulated in the above-ground parts (stems, leaves and petals).

2. Lavender is a crop that is tolerant to heavy metals; it can be attributed to hyperaccumulators of Pb and accumulators of Cd and Zn and can be successfully used in phytoremediation of heavy metal polluted soils. Processing of inflorescences to oil and the use of the obtained oil in perfumery will significantly reduce the cost of phytoremediation.
3. The main odor-determining components of the lavender oil obtained from the processing of lavender grown on highly contaminated soils meet the requirements of ISO 3515:2002 for the quality of the Bulgarian and French lavender oil and/or have values close to the limits of the standards.

## REFERENCES

- [1] S.P. McGrath, J. R. Sanders and M.H. Shabaly, "The effects of soil organic matter levels on soil solution concentrations and extractabilities of manganese, zinc and copper", *Geoderma*, vol. 42, pp.177-188, 1988.
- [2] D. E. Salt, R. D. Smith and I. Raskin, "Phytoremediation", *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, vol. 49, pp. 643-668, 1998.
- [3] L. Q. Ma, K. M. Komar and C. Tu, "A fern that accumulates arsenic", *Nature*, vol. 409, pp. 579-584, 2001.
- [4] D. E. Salt, and U. Kramer, "Mechanisms of metal hyperaccumulation in plants", in *Phytoremediation of toxic metals: using plants to clean-up the environment*, I. Raskin and B.D. Ensley, Eds. John Wiley and Sons, Inc., New York, 2000, pp. 231-246.
- [5] S. D. Ebbs, M. M. Lasat, D.J. Brandy, J. Cornish, R. Gordon, and L. V. Kochian, "Heavy metals in the environment: Phytoextraction of cadmium and zinc from a contaminated soil", *Journal of Environmental Quality*, vol. 26, pp. 1424-1430, 1997.
- [6] S. D. Ebbs, and L. V. Kochian, "Phytoextraction of zinc by oat (*Avena sativa*), barley (*Hordeum vulgare*), and Indian mustard (*Brassica juncea*)", *Environmental Science and Technology*, vol. 32, no. 6, pp. 802-806, 1998.
- [7] S. Kirkova, Y. Dylugerski and T. Milanova, "Properties of new varieties and lines Burley tobacco investigation on consumables", *Bulgarian Journal of Agricultural Science*, vol. 20, pp. 643-646, 2014.
- [8] H. Bozukov, S. Kirkova and P. Zaprianova, "Study of technologically unusable tobacco waste and practical solutions for its recovery", *Tymyn/Tobacco*, vol. 62, pp.109-114, 2014.
- [9] Y. Dylugerski, S. Kirkova and T.Milanova, "Comparative analysis of Expert evaluation and Chemical composition of Varieties and lines Burley Tobacco", *Agricultural Science*, vol. 46, pp.47-53, 2013.
- [10] S. Pandeliev, L. Angelov, B. Stalev, M. Papanikolau, "Szanse ekologicznej uprawy winorosli w poludniowych winiarskich regionach bulgarii", in *Proc. IV Ogólnopolska Konferencja Winiarska. Nowosci w uprawie winorosli i produkcji win*, Sulechów, 2010, pp. 99-109.
- [11] A. J. M. Baker, S. P. McGrath and C. M. D. Sidoli, "The possibility of in situ heavy metal decontamination of polluted soils using crops of metal-accumulating plants", *Resour Conserv Recycl.*, vol.11, pp. 41-49, 1994.
- [12] M. S. Abu-Darwish, "Essential Oils and Heavy Metals Content of Some Aromatic Medicinal Plants Grown in Ash-Shoubak Region, South of Jordan", *Journal of Food, Agriculture and Environment*, vol. 7, 3-4, pp. 920-924, 2009.
- [13] V. Angelova, "Potential of some medicinal and aromatic plants for phytoremediation of soils contaminated with heavy metals", *Agricultural Science*, vol. 11, pp. 61-66, 2012.
- [14] V. D. Zheljzkov, L. E. Craker, and X. Baoshan, "Effects of Cd, Pb and Cu on growth and essential oil contents in dill pepper mint, and basil", *Environ. Exp. Bot.*, vol. 58, pp. 9-16, 2006.
- [15] V. D. Zheljzkov, and N. E. Nielsen, "Studies on the effect of heavy metals (Cd, Pb, Cu, Mn, Zn and Fe) upon the growth, productivity and quality of lavender (*Lavandula angustifolia* Mill) production". *J. Essential Oil Res.*, vol. 8, no.3, pp. 259-274, 1996.
- [16] R. B. Bağdat, and E. M. Eid, "Phytoremediation behaviour of some medicinal and aromatic plants to various pollutants", *Journal of Field Crops Central Research Institute*, vol. 16, no.1-2, pp. 1-10, 2007.
- [17] Anonymous, ISO 11466, *Soil quality - Extraction of trace elements soluble in aqua regia*, 1995.
- [18] K. Peng, X. Li, C. Luo, and Z. Shen, "Vegetation composition and heavy metal uptake by wild plants at three contaminated sites in Xiangxi area, China", *Journal of Environmental Science and Health Part A*, vol. 40, pp. 65-76, 2006.
- [19] M. S. Liphadzi, and M. B. Kirkham, "Phytoremediation of soil contaminated with heavy metals: a technology for rehabilitation of the environment", *South African Journal of Botany*, vol. 71, pp.24-37, 2005.
- [20] W. Rosselli, C. Keller, and K. Boschi, "Phytoextraction capacity of tree growing on a metal contaminated soil", *Plant Soil.*, vol. 256, pp. 265-272, 2003.
- [21] A. Kabata-Pendias, *Trace Elements in Soils and Plants*. CRC Press LLC, Boca Raton, 2011, pp.505.
- [22] N. Mganga, M. L. K. Manoko, and Z. K. Rulungaranga, "Classification of plants according to their heavy metal content around North Mara gold mine, Tanzania: implication for phytoremediation", *Tanz. J. Sci.*, vol. 37, pp. 109-119, 2011.
- [23] A.J. M. Baker and P. L. Walker, "Ecophysiology of metal uptake by tolerant plants: Heavy metal tolerance in plants", in *Evolutionary Aspects*, A.J. Shaw, Ed. CRC Press, Boca Raton, 1990, pp.150-177.
- [24] S. P. McGrath, and F. J. Zhao, "Phytoextraction of metals and metalloids from contaminated soils", *Current Opinion in Biotechnology*, vol. 14, pp. 277-282, 2003.
- [25] Anonymous, ISO 3515:2012, *Lavander Oil Quality Standarts Oil of lavender (*Lavandula angustifolia* Mill.)*, 2002.
- [26] E. Georgiev, *Distillation and extraction of essential oil processing raw materials and opportunities for intensifying them*. Dissertation DSc., Plovdiv, 1988, pp.189.
- [27] M. D. Guillen, N. Cabo, J. Burillo, "Characterisation of the essential oils of some cultivated aromatic plants of industrial interest", *Journal of the Science of Food & Agriculture*, vol.70, pp.359-363, 1996.
- [28] A. Wesolowska, D. Jadcak, M. Grzeszczuk, "Influence of distillation time on the content and composition of essential oil isolated from lavender (*Lavandula angustifolia* Mill.)", *Herba Polonica*, vol. 6, pp. 24-36, 2010.
- [29] R. P. Adams, and T. Yanke, "Material review: Kashmir Lavender Oil", *Perf. Flav.*, vol. 32, pp. 40-43, 2007.
- [30] H. J. A. Badjah, B. Y. Meklati, H. M. Guermouche, K. Abdeddaim, and H. M. J. Richard. "Analyse quantitative dh'huiles essentielles de lavandes cultivees en Algerie", *Riv. It. Eppos*, vol. 62, no. 6, pp. 293-296, 1980.
- [31] J. Touche, M. Derbesy, and J. R. Linas. "Mailletes et lavandes fines francaises". *Riv. It. Eppos*, vol. 63, no.6, pp.320-323, 1981.
- [32] B. Lavande, "Lavander, lavandin and other french oils", *Perf. Fl.*, vol. 9, no.2, pp. 117-121, 1984.
- [33] B. M. Laurence, "Progress in Essential Oils. Lavender Oil", *Perf. Fl.*, vol.12, no.6, pp. 64-70, 1987.
- [34] S. Stanev, "Qualitative composition of the essential oil obtained from the seed populations of the genus *Lavandula angustifolia* Mill", in *Proc. International Science conference*, Stara Zagora, Bulgaria, pp.125-129, 2009.
- [35] E. Stoyanov, and T. Paunkova, "Comparative assessment of the economic qualities of lavender varieties with a view to their cultivation in industrial lavender array", in *Proceedings II National Conference Problems of perfume and cosmetics production*, Varna, 1976, pp. 377-383.
- [36] D. Dimitrov, "Opportunities for obtaining optimal mixtures of odoriferous substances - fragrance and typified lots of lavender oil", *Thesis*, Plovdiv, 1986, pp.180.
- [37] J. Bowles, *The chemistry of aromatherapeutic oils*. Allen and Unwin, 2003, pp.164-165.
- [38] N. Kara, and H. Baydar, "Determination of lavender and lavandin cultivars (*Lavandula* sp.) containing high quality essential oil in Isparta, Turkey", *Turkish Journal of Field Crops*, vol. 18, no.1, pp.58-65, 2013.
- [39] V. Pitman, *Aromatherapy: a practical approach*. Nelson Thornes, 2004, pp.111.
- [40] Ch.Sell, *The Chemistry of Fragrances. From Perfumer to Consumer*. RSC Publishing, 2006, pp.43.
- [41] G. Zhekova, and N. Nedkov, "Quantitative changes in major components of lavender oil during the distillation process", *Agric. Sci. Technol.*, vol. 2, 26-28, 2010.
- [42] G. Flores, G. P. Blanch, M. L. Ruiz del Castillo, and M. Herraiz, "Enantiomeric composition studies in *Lavandula* species using supercritical fluids", *J. Separ. Sci.*, vol. 28, pp. 2333-2338, 2008.
- [43] R. G. Berger, *Flavours and Fragrances. Chemistry, Bioprocessing and Sustainability*. Springer, 2007, pp.400-403.

- [44] S. D. Cox, C. M. Mann, J. L. Markham, J. E. Gustafson, I. R. Warmington, and S. G. Wylie, "Determining the anti-bacterial actions of Tea Tree Oil", *Molecules*, vol. 6, pp. 87-91, 2001.