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Original Research

Evaluation of Phytoremediation Potential of Vetiver Grass (*Chrysopogon Zizanioides L.*) Grown on Contaminated Soils in Bulgaria

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Abstract: This research investigated the potential for using vetiver grass (*Chrysopogon zizanioides L.*) to remediate metal-polluted sites in Bulgaria. In the second year of the experiment, the vetiver grass was gathered. The heavy metal (Pb, Cd, Hg), micro (Fe, Cu, Zn, Mn), and macro (P, K, Ca, Mg) element contents in vetiver roots and shoots were determined by ICP after microwave mineralisation. The essential oil of the ground vetiver roots was obtained by steam distillation in laboratory conditions. Gas chromatography-mass spectrometry identified fifteen compounds in the oil, mainly sesquiterpenes. The vetiver grass is tolerant to heavy metals with no signs of toxicity (chlorosis and necrosis) and can be grown on heavy metal polluted soils (37.7 $mg.kg^{-1}$ Cd, 1238.7 $mg.kg^{-1}$ Pb and 1676.4 $mg.kg^{-1}$ Zn). Bioaccumulation factor and translocation factor values (BAF and TF < 1) were less than one suggesting low accumulation in the shoot. This crop can be referred to as a non-accumulating plant for Pb, Cd and Zn and can be used for the phytostabilisation of contaminated soils in situ. Cultivating vetiver on soils contaminated with heavy metals has a beneficial effect on the yield and production of oil of high commercial value (high in khusimol).

Key Words	Heavy metals; vetiver; volatile organic compounds; essential oil; translocation factor
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INTRODUCTION

Vetiver (*Chrysopogon zizanioides*, (family Poaceae) is a perennial herbaceous plant that is mainly cultivated in tropical and subtropical countries (India, Indonesia, Haiti, China, Japan, Brazil and the Reunion Islands) (Chomchalow 2000, Maffei 2002, Pandey & Tiwari, 2024). Vetiver is cultivated mainly to produce essential oil, obtained by steam distillation from the plant's roots (Massardo et al 2006). The oil is amber, brown or dark golden, with high viscosity and extremely low volatility, with a strong and persistent woody odour characterised by different nuances, depending on the geographical origin, cultivation and extraction process. The oil is used in medicine, aromatherapy and perfumery (Maffei 2002, Massardo et al. 2006).The oil balances the hormonal system, nourishes and moisturises the skin, and promotes faster wound healing. The roots can be used to heal burns, improve blood circulation, as an immunity booster (Duke 2002), the shoots are used to remove parasitic infections in animals (Pareek & Kumar 2013), and the whole plant is used as a painkiller for rheumatoid arthritis, lumbago, and sprains (Grover et al. 2021). Vetiver can be used to construct green belts to reduce erosion risks, landslides and flooding (Kim et al. 2022).

Phytoremediation is an inexpensive and environment-friendly method used for cleaning up heavy metal contaminated soils and waters (McGrath et al. 2002, Khoiriyah et al. 2024, Hemani et al. 2024). This technology uses plants to extract contaminants from the environment or to reduce their toxicity. The plants are planted in metal-contaminated soils and grown according to established agronomic practices. Being a plant-based technology, the success of phytoextraction will depend on the extent of uptake of metals by the root system, shrinkage and accumulation in the shoots. Factors such as short growing cycles, rapid growth, large biomass production, disease resistance, and tolerance to heavy metals are also essential in plant selection (Baker et al. 1994). Plants used for phytoextraction must be tolerant of metals, translocate them from the roots to the aerial parts (Reeves and Baker 2000), tolerate difficult soil conditions (i.e., soil pH, soil salinity, soil texture, water content), form a dense root system, and be easy to grow.

Vetiver is a perennial crop (can be grown for 50 years) and has an advantage over annual plants that require annual planting, significantly reducing farmers' costs. Vetiver can be used for remediation and rehabilitation due to its extraordinary and unique morphological and physiological characteristics. The time to extract metals from the soil will depend on the root system's depth and the medium's degree of contamination (Truong et al. 2008, Danh et al.2009). In contrast to grasses, which are characterised by a horizontal root system, vetiver has a deeply penetrating root system (up to 3-4 m deep), making it a suitable plant species for erosion control and rice terrace stabilisation (Truong 2000, Danh et al. 2009). The plant is highly tolerant to elevated levels of heavy metals such as As, Cd, Cu, Cr, Pb, Hg, Ni, Se and Zn in the soil without affecting its growth (Truong et al. 2008, Roongtanakiat et al. 2008, Danh et al. 2009). Vetiver has excellent potential for nutrient (nitrogen and phosporous) and organic pollutant uptake (Truong 2000, Truong et al. 2010). Furthermore, vetiver has a high tolerance and adaptability to extreme conditions such as prolonged drought, flooding, temperature extremes (-14 to 55°C), high soil acidity and alkalinity (pH 3.3 to 9.5), saline soils (electrical conductivity up to 4.0 (Danh 5et al. 2009) and aluminum and Mn toxicity (Truong et al. 2010).

In recent years, interest in vetiver has been steadily increasing, as has research related to vetiver's potential to prevent soil erosion (Oshunsanya et al. 2017), heavy metal soil cleaning (Roongtanakiat & Chairojy 2001, Chen et al. 2004, Antiochia et al. 2007), wastewater (Roongtanakiat et al. 2008) and tailings (Yang et al. 2003, Roongtanakiat et al. 2008). Vetiver tolerates high concentrations of individual heavy metals in soils and combinations of several heavy metals. Typically, highly contaminated soils are contaminated with more than one element (polymetal contamination) with different dominance of the various metals (Ernst et al. 2000, Walker & Bernal 2004). . The pot experiments showed that vetiver could survive and grow well on contaminated soils with Pb contents ranging from 1155 to 3281.6 $mg.kg^{-1}$, Zn from 118.3 to1583 $mg.kg^{-1}$ and Cu from 68 to 1761.8 $mg.kg^{-1}$(Wide et al. 2005, Chiu et al. 2006).

Under field conditions, it has been found that vetiver can grow on soils containing Pb from 2078 to 4164 $mg.kg^{-1}$, Zn, from 2472 to 4377 $mg.kg^{-1}$, Cu from 35 to 174 $mg.kg^{-1}$ and Cd from 7 to 32 $mg.kg^{-1}$ (Shu et al. 2002, Yang et al. 2003, Shu et al. 2004).

Vetiver cultivation is minimal in Europe due to less favorable growing conditions (cold winters and long hot summers) than in tropical and subtropical regions. The first field study of Vetiver in Europe was carried out in 1994 in southern Spain to determine the possibilities for growing vetiver under Mediterranean conditions. In 1996, trials were carried out in Portugal with vetiver to protect dam walls and to stabilize the banks of a heavily eroded watercourse that flows down a cultivated valley. In 1998, studies were carried out in Italy on biomass production and the tolerance of vetiver to salinity, and a private nursery was established near Milan, which was later moved to near Pisa, where the climatic conditions are much more favorable for vetiver production. In Albania, an attempt was made in 1997 to grow vetiver to address erosion problems in small plots. Still, the climatic conditions and low temperatures in winter did not allow satisfactory results. Vetiver cultivation is possible in southern Europe and Mediterranean countries such as Greece, Syria, Turkey, Morocco, Portugal and Spain (Pease 2000). Vetiver is not cultivated in Bulgaria. Climate change and global warming are the reasons for growing non-traditional, more heat-loving crops in Bulgaria. Due to climate change and rising winter temperatures, it has become possible to grow vetiver under field conditions as a perennial crop without the danger of frost. The present study is the first attempt to establish vetiver cultivation possibilities in Bulgaria's industrially polluted areas.

In Bulgaria, about 61% of the areas contaminated with heavy metals above the maximum permissible content of harmful substances in soil are located in the areas of metallurgical plants. In the regions of smelters, combined contamination of plants is observed - (i) uptake of accumulated heavy metals in the soil by the root system of plants and (ii) by aerosols from the atmosphere on the leaf surface (Kabata-Pendias & Pendias, 2001). The aerosols' concentration varies with distance from the source, weather conditions and particle size. Larger particles settle quickly to the ground surface, in contrast to smaller particles which can be lifted by air currents to high altitudes and disperse over considerable distances. The height of smelter stacks, wind speed, rainfall and the terrain's character also influence. Aerosols cling to plant shoots, and trace elements may be absorbed. Pb remains mainly as a surface deposit, while Zn and Cd penetrate partially into the shoots (Kabata-Pendias & Pendias 2001).

The area around a Non-Ferrous Metal Works-Plovdiv (NFMW) is one of the "hot" ecological spots of the country. The plant's production activities are associated with the emission of industrial gases and dust (aerosols), the region's primary pollutants. These emissions are dominated by aerosols of Pb and Zn, accompanied by Cd and Hg. The metals in the emissions are predominantly in the form of oxides. In recent years, there has been a downward trend in heavy metal and sulphur oxide emissions to the air due to improvements in Pb and Zn production technology at NFMW. However, accumulated heavy metals from previous years remain in the surface soil layer (Dimitrov et al. 2019). Finding suitable plant species that can be grown on these contaminated soils is necessary.

The present work aimed to conduct a comparative study to determine the heavy metal (Pb, Cd, Hg), micro (Fe, Cu, Zn, Mn), and macro (P, K, Ca, Mg) element contents in vetiver, the composition of vetiver oil, as well as the possibilities to use the vetiver for phytoremediation of heavy metal contaminated soils.

MATERIALS AND METHODS

Plant material

The study was conducted with a vetiver test plant. Vetiver seedlings were purchased from Spain. The seedlings were planted in areas spaced at different distances from the pollution source NFMW - Plovdiv (0.1 km, 3.5 km and 15 km).The study area is located in southern Bulgaria and falls within a transitional-continental climatic region. The winters are relatively mild, and the summers are long and hot. Summers are hot, autumns warm and long, and spring cool, with frequent frosts. The average daily maximum is 36°C in July and August, the average daily minimum is 0°C in January, and rainfall is 56 mm. Nearly 90% of the precipitation falls during the winter months from December to April. The field trials were laid out using the block method in four replications with an experimental plot size of 100 m^2. Vetiver seedlings were planted in spring on the previously prepared plots at each sampling location. The plots were ploughed to a depth of 40 cm to break up the soil and eradicate the rhizomes of perennial weeds. After the initial tillage, the land was disked, and planting holes were dug. Vetiver seedlings were planted in a 50 x 50 cm scheme. After planting, the plants were watered regularly, and in the second year, they were watered mainly during the hot months of the year. In the second year after planting, samples of plant material (roots and above-ground mass) of vetiver were taken for analysis. For this purpose, plants were removed with the roots from each experimental plot in October. The roots were separated from the above-ground mass. Vetiver oil was extracted from the roots in the 19th month of planting. The roots were washed, dried, cut and soaked in water. The oil was obtained under laboratory conditions by steam distillation for 16 h using a Clevenger-type apparatus.

Roots and shoots from each experimental plot were analysed to determine the vetiver's heavy metal and nutrient contents. The analysis was performed on pre-dried at 75ºC root and leaf samples.

Soil samples

Soil samples were also collected from individual plots before vetiver seedlings were planted. Soil sample 1 (S1) is located at the 0.5 distance from NFMW - Plovdiv (0.5 km). Sample 2 (S2) was taken at a distance of 3.5 km from NFMW - Plovdiv (from the experimental field of the Agricultural University-Plovdiv in Brestnik village), and sample 3 (S3) from the experimental field of the Agricultural University-Plovdiv (15 km from NFMW - Plovdiv).

Soil samples were collected using a hand probe at 0 - 30 cm depth. One average sample was taken from each plot, formed from 10 stitches taken diagonally. Soil from all stitches from each plot was homogenised, air-dried and sieved through a 2 mm sieve.

A soil subsample was characterised for soil pH (H_2O) in deionised water suspension of 1:5 (w/v); total nitrogen by the Kjeldahl method (N Kjeldahl); total oxidisable organic carbon according to the Tube digestion method (with titration) (Sparks 1996).

Heavy metals analysis

The total and DTPA-extractable concentrations of metals in the soils were determined. Total content of metals in soils was determined by ISO 11466. 3 g of air-dry soil to the nearest 0.001 g was weighed and moistened with 1 mL H_2O. Add 28 mL of a mixture of $HCI:HNO_3$ (3:1). The sample is left for 16 hours at room temperature, then placed in a sand bath and heated for 2 hours at 180 to 200ºC. The residue was quantitatively transferred to a volumetric flask by filtration through a blue band filter and the volume was made up to 100 mL.

The available (mobile) metals ((Pb, Zn, Cd, Hg) contents were extracted by a solution of DTPA (ISO 14870). To prepare 1 L of DTPA extraction solution, 14.91 g of TEA, 1.957 g of DTPA and 1.47 g of CaCl2 were used and dissolved in 1 L of deionised water. The solution was adjusted to pH 7,3 by the addition of 6 $mol.L^{-1}$ HCl. 10 g of soil (sieved through a 2-mm sieve) was weighed into a 100 mL container and added 20 mL of DTPA solution. The samples were placed on a shaker and shaken for 120 minutes, then centrifuged, decanted and filtered through a pleated filter. Before measuring elemental content by ICP-OES, samples were diluted 1:25 with 2% HNO_3.

The concentrations of heavy metals and nutrients (Pb, Cd, Hg, Fe, Cu, Zn, Mn, P, K, Ca, Mg) in roots, shoots, and oils were determined by the method of microwave mineralisation. Milestone 1200 MEGA microwave system with 10 MRD 300 rotor (10 positions) was used. 0.2 g of the dried and well-homogenised sample was weighed to the nearest 0.001 and transferred to Teflon bombs, and 5 mL HNO_3 and 1 mL H_2O_2 were added to each sample. The mineralisation program includes three stages: (i) 5 minutes of 250 W non-pulsed irradiation; (ii) 5 minutes of 400 W pulsed irradiation; and (iii) 5 minutes of 600 W pulsed irradiation. After ventilation for 1 minute, the sample was cooled and transferred quantitatively to a 25 mL volumetric flask.

An inductively coupled emission spectrometer (Jobin Yvon Emission - JY 38 S, Paris, France) was used to determine the element content in the samples. The mercury (Hg) content of the soil and plant samples was determined without prior sample preparation using an MA-3000 mercury analyser (Nippon Instruments Corporation). The quality and analytical performance of the ICP and the Hg analyser were validated using standard reference materials from contaminated brickyard soil, ERM CC135a, and apple leaves (SRM 1515, National Institute of Standards and Technology, NIST). The results showed acceptable agreement between the established and certified values for the elements.

Evaluation of the phytoextraction ability of vetiver plants

The phytoremediation ability of the plants was evaluated by calculating the translocation factor (TF), and the bioconcentration factor (BCF).

The translocation factor (TF), which is the ability of a plant to translocate metals from its roots to shoot was calculated by the following formula

$$TF = \frac{\text{Cshoots}}{\text{Croots}}$$

where Cshoots represent the element contents (in $mg.kg^{-1}$) in the plant shoots and Croots shows the element concentration in the roots (in $mg.kg^{-1}$)

Bioaconcentration factor (BCF) is also used to calculate metals transfer from soil to various plant parts. BCF was calculated by the following formula

$$BCF = \frac{\text{Cplant parts}}{Csoil}$$

where Cplant parts represent the element contents (in $mg.kg^{-1}$) in the plant organs (roots, shoot) and Csoil (in mg/kg) shows the element concentration in the corresponding soils.

Essential oil extraction

Vetiver essential oil is produced in secretory cells located in the first cortical layer outside the endodermis of mature vetiver roots, and vetiver oil is distilled from the roots of this monocotyledonous plant. Vetiver roots do not readily release the oil because the essential oils are located in hard-to-reach root tissues. To be extracted, these oils must diffuse (a relatively slow physical process) from the interior of the fibrous tissues outward to the surface. In addition, vetiver oil consists of a high percentage of sesquiterpenes (which have high molecular weights with low vapor pressures), contributing to the long extraction time required. The most valuable fractions of vetiver oil have the highest boiling points and constitute the high-specific-gravity oil fraction. They are separated in the most significant volume at the end of distillation. These fractions are rich in vetivones and vetiverol (Chomchalow 2001).

Essential oils from the roots of plants were obtained in laboratory conditions by steam distillation for 16 h in a Clevenger-type apparatus. Before distillation, air-dried roots were blended to a fine powder and soaked overnight in water. The oils were dried over anhydrous sodium sulphate and, after filtration, stored in a freezer at -20ºC until tested and analysed.

Gas chromatography/mass spectrometry (GC/MS) analysis of essential oils

GC-MS analysis was carried out on a 7890A gas chromatograph interfaced with a 5975 C mass selective detector (Agilent Technologies). Separations were performed using a 30 m × 0.25 mm (i.d.) DB-5ms silica-fused capillary column coated with 0.25 µm film of poly (dimethylsiloxane) as the stationary phase. The flow rate of carrier gas (helium) was maintained at 1.0 $ml.min^{-1}$. The injector and the transfer line temperature were kept at 250°C. The oven temperature program used was 100°C for 2 min then 15°$C.min^{-1}$ to 180°C for 1 min then 5°$C.min^{-1}$ to 300°C for 10 min. The injection volume was 1 µl. The injections were carried out in a split mode 20:1. The mass spectrometer was scanned from 50 to 550 m/z. All mass spectra were acquired in electron impact (EI) mode with 70 eV.

A mixture of aliphatic hydrocarbons (C8-C40) (Sigma) was injected into the system under the above temperature programme in order to calculate the relative retention index of each compound. Identification of components was obtained by comparing the RI and the spectral data from the NIST'08 (National Institute of Standards and Technology, USA).

Statistical analysis

The SPSS for Windows program was used in the statistical processing of the data.

RESULTS

Content of heavy metals in soils

To elucidate the extent of soil contamination with heavy metals and their localisation in the vegetative organs of vetiver, soil samples were collected from the areas located at different distances from MFMW (0.5, 3.5 and 15 km). The physical and chemical properties of the soil samples are presented in Table 1. The soils are characterised by a slightly alkaline reaction, medium organic carbon content and medium to high nutrient (N, P, K) availability.

The results presented in Table 1 show that with distance from NFMW - Plovdiv, there is a well-defined tendency for a decrease of the total content of heavy metals in the soil. In soil samples collected from the area 0.1 km and 3.5 km away from NFMW, values for Pb exceeding the maximum permissible concentrations (MAC) (100 mg/kg) and the accepted values of 100 mg.kg-1for toxic effect on plants were recorded (Kabata-Pendias & Pendias 2001).

Table 1: Soil characteristics of the study areas

Parameter	S1 (0.1 km) x±sd	S2 (3.5 km) x±sd	S3 (15 km) x±sd
pH	7.5	7.8	8.0
Organic C,%	1.56	1.52	1.54
N Kjeldal,%	0.34	0.22	0.13
Pb, mg.kg^{-1}	1238.7±14.5	214.4±2.1	33.3±0.5
Cd, mg.kg^{-1}	37.7±0.5	3.1±0.2	0.5±0.05
Hg, mg.kg^{-1}	2.2±0.1	0.5±0.01	0.06±0.01
Zn, mg.kg^{-1}	1676.4±13.5	329.8±2.3	70.0±0.8
Cu, mg.kg^{-1}	222.1±2.1	78.8±1.1	7.6±0.3
Fe, mg.kg^{-1}	27388±16.0	50329±20.0	15566±14.5
Mn, mg.kg^{-1}	1045.3±5.2	1683.0±6.3	884.2±2.4
K, mg.kg^{-1}	8029.5±17.9	10555±20	6780.0±16.4
Ca, mg.kg^{-1}	243556±20	13152±18	10061±17
Mg, mg.kg^{-1}	12574±18	8412.3±16	7926.8±16
P, mg.kg^{-1}	607.2±8.8	783.9±9.1	604.8±8.9

x- average value(mg.kg^{-1}) from 5 repetitions; sd - mean standard deviation
MAC (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}

In the samples taken from S1 (0.1 km away), the Pb values reached 1238.7 mg.kg^{-1}. The Pb content in S2 (3.5 km away from the NFMW) reached 214.4 mg.kg^{-1}, while in the area 15 km away (S3), the Pb content decreased significantly to 33.3 mg.kg^{-1}. Similar results were obtained for Cd, Zn and Hg. At S1, 1676.4 mg.kg^{-1} Zn, 37.7 mg.kg^{-1} Cd, and 2.1 mg.kg^{-1} Hg was recorded, at S2 – 329.8 mg.kg^{-1} Zn, 3.1 mg.kg^{-1} Cd and 0.5 mg.kg^{-1} Hg, and at the more remote area, (15 km from NFMW) 70.0 mg.kg^{-1} Zn, 0.5 mg.kg^{-1} Cd and 0.06 mg.kg^{-1} Hg were detected (Table 1).

Table 2 presents the results for the mobile forms of Pb, Zn, Cd and Hg in the investigated soils. The table also gives the percentage of mobile forms relative to the total amount of the elements in the soil.

Table 2: Mobile forms (DTPA- extractable) of Pb, Zn, Cd (mg/kg) and Hg (ng/g) in soils from the study area

Distance from NFMW, km	Pb, mg.kg^{-1} x±sd	Zn, mg.kg^{-1} x±sd	Cd, mg.kg^{-1} x±sd	Hg, µg.kg^{-1} x±sd
S1 (0.1 km)	617.9±3.2	223.2±1.0	21.8±0.5	24.0±0.1
	49.9%*	13.3%	47.6%	1.3%
S2 (3.5 km)	33.3±1.5	23.8±0.4	1.6±0.3	12.6±0.1
	9.2%	7.2%	51.6%	2.5%
S3(15 km)	5.1±0.5	4.6±0.2	0.2±0.05	2.1±0.01
	15.1%	7.0%	40.0%	3.4%

*%DTPA -extractable/total content

The results for the mobile forms of the metals determined by DTPA show that the mobile forms of Cd in the contaminated soils are the most significant fraction of its total content, followed by Pb, Zn and Hg. In terms of Cd, they ranged from 47.6% (S1) to 51.6% (S2). Similar results were obtained for Pb and Zn. The mobile forms of Pb ranged from 9.2% (S2) to 49.9% (S1), Zn from 7.0% to 13.3% and Hg from 1.3 to 2.5%. In uncontaminated soils, again, mobile forms of Cd accounted for the largest proportion of the total content and reached up to 40%, followed by Pb with 15.1%, Zn with 7% and Hg with 3.4%.

Content of heavy metals and nutrients in vetiver roots and shoots

Soil conditions and plant type influence the uptake of heavy metals into plants, and their accumulation in plant organs depends on their content in the soil. Plants grown on contaminated soils accumulate heavy metals and get them in their vegetative organs. In most cases, most of them accumulate in the roots of plants as they come into contact with the contaminated soil. However, some plants accumulate a substantial fraction of heavy metals in the shoots (Kabata-Pendias & Pendias 2001).

Table 3 presents the results obtained for heavy metal, micro- and macro element contents of roots in the aerial mass of vetiver.

Table 3**:** Content of the heavy metal (Pb, Cd, Hg), micro (Fe, Cu, Zn, Mn), and macro (P, K, Ca, Mg) element in vetiver roots and shoots

Element	Roots (S1)	Leaves (S1)	Roots(S2)	Leaves (S2)	Roots (S3)	Leaves (S3)
Pb, $mg.kg^{-1}$	709.6±5.3	190.3±2.4	19.4±0.5	3.8±0.2	5.6±0.2	2.6±0.1
Cd,$mg.kg^{-1}$	36.0±1.0	5.8±0.2	2.3±0.1	1.7±0.1	0.35±0.05	0.21±0.05
Hg, $\mu g.kg^{-1}$	2870±10	696.2±2	915.7±2	235.0±1	223.6±1	66.4±1
Zn, $mg.kg^{-1}$	442.0±1.2	113.9±0.7	28.0±0.3	7.7±0.2	15.1±0.3	6.7±0.2
Cu,$mg.kg^{-1}$	55.0±0.5	17.5±0.3	5.7±0.2	1.28±0.1	5.2±0.1	1.4±0.1
Fe, $mg.kg^{-1}$	725.7±0.6	226.4±0.5	991.5±0.9	116.0±0.6	907.7±0.8	108.1±0.5
Mn,$mg.kg^{-1}$	37.9±0.2	11.1±0.1	86.4±0.4	22.6±0.1	45.4±0.1	31.3±0.1
P, $mg.kg^{-1}$	472.5±3.0	558.2±3.5	622.5±3.5	1331.7±5.1	643.1±3.6	1150.9±5.0
Ca,$mg.kg^{-1}$	2101.2±7.8	2855.7±8.6	1095.0±5.6	875.5±3.8	1311.8±5.9	720.3±2.8
Mg,$mg.kg^{-1}$	449.2±2.0	504.1±2.4	527.4±2.3	366.4±2.5	732.0±3.8	497.7±2.6
K, $mg.kg^{-1}$	3169.3±3.8	10601.7±5.6	4224.5±4.0	8788.8±5.3	2940.6±2.9	9735.1±5.4

Heavy metal content in the essential oil

The ability of vetiver to absorb heavy metals in the roots can lead to heavy metal contamination of the oil, as the oil is extracted from the roots of the plant. The results show that most of the heavy metals contained in the roots of the vetiver 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 vetiver when grown on S1 and S2 ranged from 0.57 to 0.58 $mg.kg^{-1}$, Zn from 11.3 to 13.2 $mg.kg^{-1}$, Cd from 0.05 to 0.07 $mg.kg^{-1}$, while the range of Hg is below the limits of the quantitative measurement of the method used (Table 4). Significantly lower results in the essential oil of vetiver grown at a distance of 15 km from NFMW - 0.02 $mg.kg^{-1}$ Pb and 5.5 $mg.kg^{-1}$ Zn, while the content of Cd is below the limits of the quantitative measurement of the method used. The Pb, Cd and Hg amounts in the vetiver 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)(European Pharmacopoeia 2021).

Table 4: Content of heavy metals in vetiver essential oil

Element	**S1 (0.5 km)**	**S2 (3.5 km)**	**S3 (15 km)**
Pb, mg.kg^{-1}	0.58±0.1	0.54±0.1	0.24±0.01
Cd, mg.kg^{-1}	0.07±0.01	0.05±0.01	nd
Hg, µg.kg^{-1}	Nd	nd	nd
Zn, mg.kg^{-1}	13.2±0.2	11.3±0.2	5.5±0.1

nd- not detected

The results confirm the ones established by Angelova et al. (2015), which found that the heavy metal content in essential oil is very low and is not affected by the level of soil contamination with heavy metals. Essential oils contain only traces of heavy metals in distilled oils because these metals have too heavy and large molecules to be volatilised enough and to be concentrated by the distillation process.

Essential oil content and composition of vetiver essential oil

The results of the chromatographic analysis of essential oils obtained by processing the roots of vetiver grown at a different distance from NFMW-Plovdiv are presented in Table 5. Figure 1 shows the chromatograms from the GC MS analysis of vetiver oil.

Table 5: Chemical composition of vetiver essential oil (%)

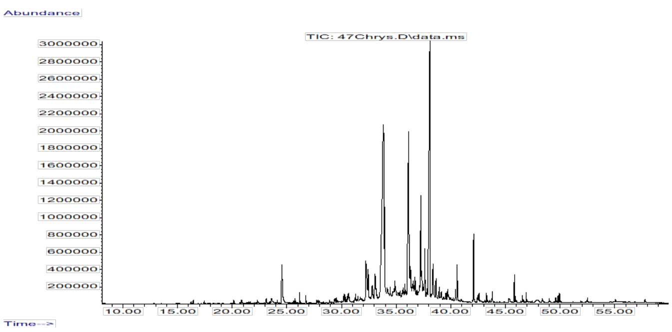
	Compound	RI	S1	S2	S3
	Oil content,%		1.26	0.98	0.78
1	α-Copaene	1378	1.05±0.1	1.13±0.1	1.08±0.1
2	(E)-Caryophyllene	1419	1.34±0.1	1.44±0.1	1.39±0.1
3	α-Amorphene	1485	2.30±0.1	2.44±0.1	2.38±0.1
4	β-Vetispirene	1495	3.14±0.1	3.39±0.1	3.26±0.1
5	β-Vetivenene	1557	3.38±0.1	3.55±0.1	3.50±0.1
6	10-epi-γ-Eudesmol	1619	3.85±0.1	4.15±0.1	3.99±0.1
7	Junenol	1621	6.36±0.1	5.87±0.1	6.59±0.1
8	7-epi-α-Eudesmol	1658	3.12±0.1	3.37±0.1	3.23±0.1
9	Valerianol	1662	10.33±0.1	9.07±0.1	9.67±0.1
10	Guaiol acetate	1724	7.83±0.1	8.15	8.12±0.1
11	Vetiselinenol	1728	7.27±0.1	7.84±0.1	7.53±0.1
12	Khusimol	1736	30.25±0.1	29.40±0.1	28.23±0.1
13	(E)-Isovalencenol	1794	5.95±0.1	6.42±0.1	6.16±0.1
14	β-Vetivone	1809	5.21±0.1	5.62±0.1	5.40±0.1
15	α-Vetivone	1847	6.79±0.1	7.12±0.1	7.03±0.1
		HC	11.20	11.95	11.61
		Alchohols	67.14	66.12	65.40
		Esters	7.83	8.15	8.12
		Ketones	12.00	12.75	12.43
		Bicyclic	66.88	68.44	68.24
		Triciclic	31.30	30.53	29.31
		Total	98.18	98.97	97.55

RI - Kovacs relative indices

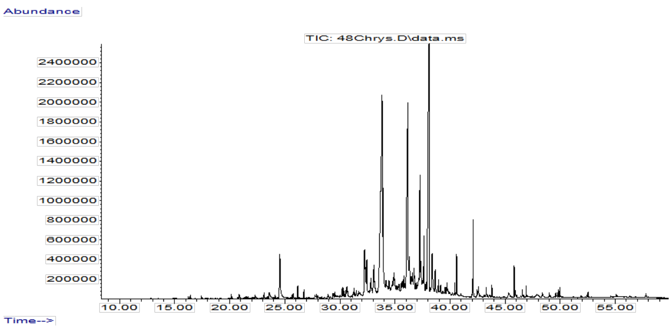
Vetiver essential oil has a very complex chemical composition, with many constituents (mainly sesquiterpenoids) showing a substantial structural diversity (Prasad et al. 2014). More than 150 components have been identified in vetiver oil produced in different countries (Chowdhury et al. 2002, Champagnat et al. 2006).

Fifteen components were identified in vetiver oil, accounting for 97.55-98.9% of the total oil components (Table 5). The bicyclic sexviterpenes from the Caryophyllanes, Cadinane, Guaianes, Eudesmane, Eremophilane Nootkatane, Spirane and the tricyclic sesquiterpenes from the group of Copaanes and Zizaanes were contained in the studied oils. No monocyclic sesquiterpenes of the group of Bisobalane and Elemol were detected.

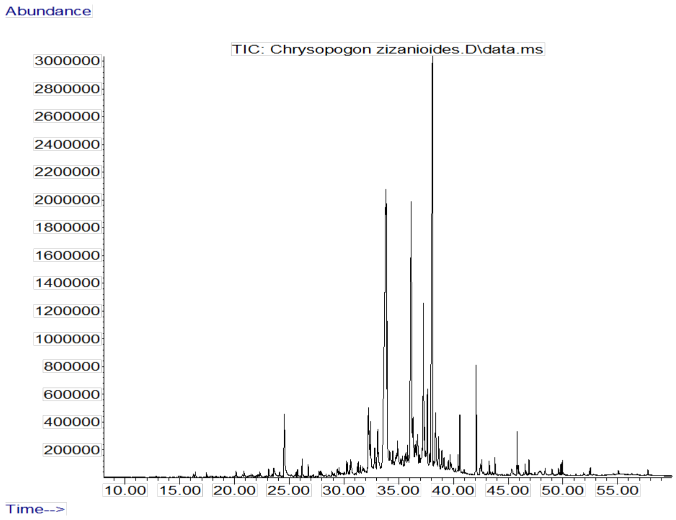
The terpene hydrocarbons (α-Copaene, (E)Caryophyllene, α-Amorphene, β-Vetispirene, β-Vetivenene) the terpene alcohols (-epi-γ-Eudesmol, Junenol, 7-epi-α-Eudesmol, Valerianol, Vetiselinenol, Khusimol, (E)-Isovalencenol), terpene ketones (β-Vetivone, α-Vetivone) and ester (Guaiol acetate) are contained in vetiver oil.



(a)



(b)



(c)

Fig. 1: GC-MS chromatogram of essential oil of vetiver: (**a**) S1; (**b**) S2; **(c)** S3

DISCUSSION

Content of heavy metals and nutrients in vetiver roots and shoots

Significant differences were found in the contents of the elements in different parts of the vetiver. Most of the heavy metals accumulated primarily in the root system of vetiver, which is consistent with the results of other authors. The higher accumulation of heavy metals in the roots is due to the fact that vetiver has a dense and deep root system which can create an ideal environment for microbial processes, and contribute to the uptake of contaminants from the soil (Truong 2000). As the source of contamination becomes more distant, there is a clear tendency for the heavy metal content of vetiver roots and above-ground mass to decrease. The amount depends primarily on the distance to the pollution source and the heavy metal content of the soil. The obtained results are similar to Roongtanakiat & Chairojy (2001), Antiochia et al. (2007), Roongtanakiat et al. (2008), and Hasan et al. (2017), who found that heavy metal uptake by plant roots depends on their soil content. Pb content in vetiver roots ranged from 19.4 mg.kg^{-1}(S2) to 709.6 mg.kg^{-1} (S1), Zn from 28.0 mg.kg^{-1} (S2) to 442.0 mg.kg^{-1} (S1), Cd from 2.3 mg.kg^{-1} (S2) to 36.0 mg.kg^{-1} (S1) and Hg from 915.7 to 2870 µg.kg^{-1} (Table 3). Significantly lower values of 5.6 mg.kg^{-1} Pb, 15.1 mg.kg^{-1} Zn and 0.35 mg.kg^{-1} Cd and 223.6 µg.kg^{-1} Hg were found in vetiver roots grown on uncontaminated soil (S3). The values obtained for Cd, Pb and Zn in vetiver roots from the highly contaminated soil were significantly higher than the values considered toxic to plants by Kabata-Pendias & Pendias (2001)(5 mg.kg^{-1}Cd, 30 mg.kg^{-1} Pb, 100 mg.kg^{-1} Zn).

The Pb content in vetiver shoots grown at a distance of 0.1 km (S1) reached up to 190.8 mg.kg^{-1}, Zn up to 113.9 mg.kg^{-1}, Cd up to 5.8 mg.kg^{-1} and Hg up to 696.2 µg.kg^{-1}. Significantly lower concentrations were found in the shoots of vetiver than in the less contaminated and uncontaminated soils. Pb contents ranged from 2.6 to 3.8 mg.kg^{-1}, Zn from 5.7 to 7.6 mg.kg^{-1} and Cd from 0.21 to 1.7 mg.kg^{-1} (Table 3). The differences in heavy metal contents in vetiver shoot grown at different distances from the NFMW indicate significant aerosol contamination in the NFMW area, which decreased with increasing distance from the source of contamination.

The results obtained from this study agree with the findings of Roongtanakiat et al. (2008), who reported that roots contained more metals than shoots. However, the results of Hasan et al. (2017) showed that the accumulation of metals was higher in the above-ground mass than in the roots due to the movement of heavy metals from roots to shoots.

The selective accumulation of heavy metals in vetiver roots indicates that the plants should be used primarily for phytostabilisation rather than phytoextraction (Banerjee et al. 2016).

The trace element content of the vetiver root system was higher than that of the above-ground mass, while the opposite trend was observed for nutrients. The values recorded were as follows - in roots - 5.2 - 54.7 $mg.kg^{-1}$Cu, 725.7 - 991.5 $mg.kg^{-1}$ Fe, 37.9- 86.4 $mg.kg^{-1}$ Mn, 472.5- 643.1 $mg.kg^{-1}$ P, 1095.0-2101.2 $mg.kg^{-1}$Ca, 449.2- 527.4 $mg.kg^{-1}$ Mg, 2940. 6.- 4224.5 $mg.kg^{-1}$ P, in shoots 1.3 to 17.5 $mg.kg^{-1}$ Cu, 108.1 to 226.4 $mg.kg^{-1}$ Fe, 11.1 to 31.3 $mg.kg^{-1}$ Mn, 558.2 to 1331.7 $mg.kg^{-1}$P, 720.3 to 2855.7 $mg.kg^{-1}$ Ca, 366.3 to 504.1 $mg.kg^{-1}$ Mg, 8788.8 to 10601.7 $mg.kg^{-1}$ P.

High Pb content in the soil can lead to an imbalance in plant nutrient uptake. It is known that in most cases, Pb can block the uptake of K, Ca, Mg, Mn, Zn, Cu, and Fe into the root system (Kabata-Pendias & Pendias 2001). It has been found that P, Mg, Fe and Mn contents are lower in vetiver roots than in contaminated soil.

The accumulation of elements by plants mainly depends on the content of mobile/immobile forms of the elements in the soil (Kabata-Pendias & Pendias 2001). The amount of mobile forms is related to the geochemistry of elements in the soil and the accumulation of heavy metals in plants strongly correlates with soil contamination. The comparative analysis between the accumulation of metals in plants and the amount of mobile forms of metals in soil shows that heavy metal uptake and movement in vetiver depends on the amount of mobile forms in soil, and the mechanism of metal accumulation is the concentration gradient.

The distribution of heavy metals and micro and macro elements in the vetiver organs has a selective character, and is specific to the individual elements, which follows the order: roots - K > Ca > Fe > Pb > Mg > Zn > P > Cu > Cd > Hg, above-ground mass – K > Ca > Mg > P > Fe > Pb >Zn > Cu > Cd > Hg.

Bioconcentration factor (BCF) and translocation factor (TF)

To be able to give a definite answer to the question of what is the ability of vetiver to extract heavy metals from soil and to evaluate the potential of vetiver for phytoremediation, bioconcentration factors (BCFroots and BCFshoots) and translocation factor (TF) were calculated. The bioconcentration and translocation factors (BCF and TF) are beneficial parameters for studying the movement pattern and accumulation of heavy metals from soil to plant (Baker 1981). Table 6 presents the results obtained for BCFroots, BCFshoots and TF.

Bioconcentration Factor (BCFroots) represents the ratio of heavy metal content in plant roots to the content in soil (Yoon et al. 2006). BCF values concerning Pb were <1, indicating that the concentration of the element does not exceed its content in the soil. Similar results were obtained for Zn. For cadmium, these values were around 1 (0.96) for vetiver from the highly contaminated soil (S1).

The translocation factor (TF) gives information about the ability of plants to assimilate heavy metals through the roots and move them to the shoots. TF showed the same trend as BCF (values less than 1). Most of the absorbed Pb in plants accumulates in the roots and only a small fraction of Pb moves to the shoots. It is known that when Pb enters the plant roots, it immediately interacts with phosphates and carbonates present in high concentrations in the intercellular spaces and precipitates as phosphates or carbonates (Boonyapookana et al. 2005). The formation of insoluble Pb compounds leads to decreased Pb translocation in plants (Berti & Cunningham 2000). Data from Banerjee et al. (2016) for TF showed that except for Cu, other metals (Fe, Al, Zn, Cr, Mn and Ni) assimilated by vetiver were retained mainly in the roots when vetiver was grown for three months on ore soils. Similar results were also obtained by Rotkittikhun et al. (2000). It was found that when vetiver was grown for 120 days, the heavy metal content was higher in the roots. In contrast, when vetiver was grown for 60 days, the heavy metal concentrations were higher in the shoots than in the roots (Roongtanakiat et al. 2001). Vetiver has been found to be a hyperaccumulator of Pb in some studies (Antiochia et al. 2007, Roongtanakiat et al. 2008). It was found that 4940 mg.kg-1Pb accumulated in roots and 359 $mg.kg^{-1}$ Pb in shoots, or over 0.22% Pb. Pb content reached in vetiver up to 1390-1450 $mg.kg^{-1}$ after nine months of cultivation on polygon soils (Wilde et al. 2006). It was established vetiver to be a hyperaccumulator of Pb and Zn, accumulating over 0.4% Pb accumulated in shoots and 1% Pb in roots, as well as 1% in Zn in roots and above-ground mass, respectively when irrigated daily with of $PbCl_2$ and $ZnCl_2$ solution for 30 days (Antiochia et al. 2007). The higher uptake and translocation of Pb and Zn to the shoots are the available Pb and Zn content (Chiu et al. 2006, Antiochia et al. 2007).

Table 6**:** TF and BCF coefficients in vetiver grass

Element	Coefficient	S1 (0.1 km)	S2 (3.5 km)	S3 (15 km)
Pb	BCFroots	0.57	0.09	0.17
	BCFshoots	0.15	0.02	0.08
	TF	0.27	0.20	0.46
Zn	BCFroots	0.26	0.08	0.22
	BCFshoots	0.07	0.02	0.10
	TF	0.26	0.27	0.44
Cd	BCFroots	0.96	0.74	0.70
	BCFshoots	0.15	0.54	0.42
	TF	0.16	0.74	0.6
Hg	BCFroots	1.36	1.88	3.60
	BCFshoots	0.33	0.48	1.07
	TF	0.24	0.26	0.3

The phytoextraction efficiency is also determined by the bioconcentration factor. The bioconcentration factor is defined as the ratio of the metal concentration in the above-ground mass of the plant to the soil and is a measure of the plant's ability to uptake and move metals to the above-ground mass (Baker et al. 1994). Metal concentration in plants can indicate the effectiveness of plants for remediation of heavy metal-contaminated soils (Chen et al. 2004). The results of Chen et al. (2004) showed that the ratio of Pb in shoots in vetiver to total Pb content in soil was very low (0.002 - 0.009).

The results obtained in this experiment showed that for Pb and Zn, the bioconcetration factor was lower than 1 when vetiver was grown on both contaminated and uncontaminated soils. For cadmium, the bioconcetration factor approaches 1 (0.96) when grown on highly contaminated soil and decreases to 0.7 on lightly contaminated soil (Table 6). For Hg, the bioconcentration factor is higher than 1, irrespective of the degree of soil contamination.

The phytoremediation capacity of plants is assessed by the bioconcetration factor (BCFshoots) and translocation factor (TF). According to Baker et al. (1981), plants are classified as non-accumulators, accumulators or hyperaccumulators if the values of BCF are as follows <1, >1 and >10. The values obtained for BCF for plants grown on highly contaminated soils are less than 1 for Pb, Zn, Cd and Hg. Similar results were obtained by Banerjee et al. (2016) for vetiver when grown on soils from mining activities (mining soils)

BCF and TF values greater than 1 indicate that the plant is a potential accumulator of heavy metals and can efficiently move metals from the roots to the above-ground mass (Baker et al. 1981). The results indicate that in vetiver, both BCF and TF were less than 1; hence, vetiver can be classified as a non-accumulating plant with low metal movement to shoots. Increased concentrations of heavy metals in the roots and low translocation to the aerial parts indicate the suitability of the plants for phytostabilisation (Baker et al. 1981). Since the content of heavy metals in the aboveground mass is low, an opinion can be made for farmers regarding the content of the aboveground mass for animal feed or mulch. An essential advantage of the collected aboveground mass of vetiver is that it is not considered hazardous waste, unlike the residues of hyperaccumulators. It can be safely used for bioenergy production or compost. Obtained results do not support the finding of Antiochia et al. (2007) and Roongtanakiat et al. (2008) that vetiver is a hyperaccumulator of Pb and Zn and can be used for phytoextraction.

Content of heavy metals in vetiver essential oil

Metals in essential oils are associated with plant contamination, which varies depending on the plant species, climate, soil composition, plant age, harvest period, or geographical origin. Therefore, contaminants' sources are anthropogenic, and their establishment in plant organs is mainly from the soil via the root system. The transfer of metals from leaves, bark, roots, seeds, flowers, and fruits to essential oils depends on the extraction technology. According to European Pharmacopoeia (2021) "the risk of contaminants in essential oils is related to the type of contaminants, the origin of the plant material used for production and the production processes." However, contaminants such as heavy metals, pesticides, and aflatoxins are not considered critical for essential oils. They should be considered on a case-by-case basis based on a risk assessment. In particular, the risk is higher for cold-pressed essential oils than for distilled ones.

For vetiver essential oil to be successfully used in perfumery, it must not contain any toxic substances that could adversely affect the health of the users. Studies have shown that vetiver can absorb heavy metals, such as Pb, Zn, and Cd, in its roots. The ability of vetiver to accumulate heavy metals in its roots can lead to contamination of the essential oil with heavy metals. For this reason, essential oils from vetiver grass extracted by steam distillation were further analyzed for heavy metal content using inductively coupled plasma emission spectroscopy (ICP-OES).

When properly distilled, essential oils should not contain the following heavy metals (e.g., mercury, lead, etc.), as these types of molecules are too heavy and large to evaporate under normal distillation conditions. The results show that the content of heavy metals in vetiver essential oil is low and below the maximum permissible limits (5 $mg.kg^{-1}$ Pb, 1 $mg.kg^{-1}$ Cd, 0.1 $mg.kg^{-1}$ Hg)(European Pharmacopoeia 2021) and does not pose a threat if used in perfumery and cosmetics.

Essential oil content and composition of vetiver essential oil

The yield and composition of vetiver essential oil can be influenced by environmental conditions, soil geochemistry and geographical origin (Chowdhury et al. 2002, Champagnat et al. 2006, Prasad et al. 2014). According to Massardo et al. (2006), vetiver oil production is closely related to the metabolic activity of plant roots, which is influenced by changes in ambient temperature. Low temperature (e.g. in winter) leads to a decrease in the metabolic activities of the plants and hence to a reduction of oil production (Massardo et al. 2006).

It has been found that oil yields range from 0.29 to 9.61 % on a dry weight basis (Adams et al 2003). Depending on the method of cultivation, the age of the roots, the method of storage and preparation of the roots before the distillation process, the method and duration of distillation, about 0.42 to 1.29 % (Rotkittikhun et al. 2010) and 0.3 to 2 % (Lavania 2003) essential oil can be obtained on a fresh weight basis. The yield progressively decreases with the storage period, while cutting fresh roots into 2.5-5 cm length increases the yield (Balasankar et al. 2013). Increasing the distillation time in a Clevenger apparatus for 16 h (extraction time); allows to break off of the cells of the vetiver roots and thus removes the heavier components (sesquiterpenes) from the vetiver oil, resulting in an increase in yield to 1.8%(Leite 2012).

Heavy metals were also found to affect oil yield and composition. Pb and Cd influence metabolic processes, including essential oil synthesis (Zheljazkov et al. 2006). Heavy metals in the soils increase the yield of essential oils from vetiver (Sulastri & Tampubolon, 2019). The increase in the percentage yield of essential oil from plants growing in areas contaminated with heavy metals is due to the plant's response to heavy metal stress, leading to an increased release of secondary metabolites such as essential oils. Results obtained showed that vetiver grass grown on contaminated soil (S1) produced more essential oil (1.26%) compared to unpolluted soil (S3)(0.78%) (Table 5). The lowest essential oil yield was obtained from vetiver grown on soils located 15 km from the NFMW (S3), which have the lowest heavy metal content since vetiver is not subjected to metal stress as a stimulating factor for releasing secondary metabolites. Similar results were obtained by Sulastri & Tampubolon (2019), who reported an increase in essential oil content by up to 101.5% in vetiver grown on soils enriched with 100 ppm Cd compared to untreated soils. Prasad et al. (2014) found that applying 50 ppm Cd to uncontaminated soils led to an increase of 33.33% in essential oils from plants grown on such soils.

Elevated levels of Pb in plants have a significant impact on oil content and sometimes lead to changes in the chemical composition of the oil, which can affect the quality and safety of the products produced from it. While for most aromatic plants such as mint, basil, lavender, the oil yield decreases when they are grown on soils contaminated with heavy metals (Champagnat et al. 2006, Zheljazkov et al. 2006), for vetiver the results are ambiguous. It was found that oil yield increased significantly from 0.25 to 0.45 % (v/w), and the number of components in the oil increased from 47 to 143 compounds when soils were treated with Pb (Rotkittikhun et al. 2010). Increased vetiver essential oil production was associated with an increase in root biomass and an increase in root essential oil concentration (Rotkittikhun et al. 2010). Contrary results were obtained from Kotoky et al. (2013), which suggested that adding Cr, Pb and Ni negatively affected root and essential oil yield.

Vetiver essential oil has a very complex chemical composition, with many constituents (mainly sesquiterpenoids) showing a substantial structural diversity (Massardo et al. 2006). More than 150 components have been identified in vetiver oil produced in different countries (Chowdhury et al. 2002, Champagnat et al. 2006).

The results show that alcohols are predominant in oil (65.40-67.14%) (Table 6), but significant differences in their content are observed in oils from slightly contaminated and uncontaminated areas. Khusimol and Valerianol were higher in oil from vetiver grown on highly contaminated soil, while the contents of Vetiselinenol, E)-Isovalencenol, 10-epi-γ-Eudesmol and 7-epi-α-Eudesmol were lower.

Hydrocarbons ranged from 11.20 to 11.95% of the total oil composition. There was no significant difference in the hydrocarbon content of the oils from the contaminated and non-contaminated regions.

Ketones ranged from 12.0 to 12.75%, and their content was lower in the oil from the highly contaminated soil. Guaiol acetate ester was also detected in the oil, which ranged from 7.83 to 8.15%.

Khusimol, β-vetivone, α-vetivone are among the major components contained in vetiver oil, and their presence is often considered to be the "fingerprint" of the oil and impart a characteristic odour to vetiver oil (Demole et al.1995).

The values of β-Vetivenene, β-Vetivenene, khusimol, α-vetivenene, (E)-isovalencenol of the essential oil of vetiver from this study are compared with the standard for vetiver oil ISO 4716, in which the values from China, Haiti, Indonesia, Brazil and in Bourbon-type oils are specified).

The content of khusimol and β-Vetivone in vetiver oil grown in Bulgaria is significantly higher than the values specified in the ISO 4716 (Table 7). The β-Vetivenene range of the Bulgarian-grown vetiver oils (3.38 -3.55%) is similar to the values given in the standard for essential oils from Haiti (0.7 to 3%), China (2 to 8%), Bourbon type (3 to 6%). Still, it is significantly lower in essential oils from Indonesia (4 to 9%), and oils from Brazil (6 to 19%). The β-Vetivenene content reaches 9.76% in oils from Turkey (Kirici et al. 2011). The content of isovalencenol in the essential oils of vetiver grown in Bulgaria (5.95 to 6.42%) is in line with the values indicated in the standard (for the oils from China (1 to 11%), from Indonesia (1 to 7%), and Brazil (5 to 11%) and lower than the oils from Haiti (10 to 16%) and Bourbon-type oils (6 to 14%).

Table 7: Comparison of the values of the main components of Bulgarian vetiver oil with ISO 4716:2013 and the literature

Component	Bulgaria	Bourbon type	China	Haiti	Indonesia	Literature
β-Vetivenene	5.21-5.62	3 – 6	2 – 8	0.7 - 3	6 - 9	1.6 – 9.7
β-Vetivone	3.36-3.55	2 – 5	2 – 4	2 - 4	2-4	3.12 - 4.82
Khusimol	28.23-30.25	12 – 18	5 – 15	9 - 15	6 - 11	3.4 – 26.94
α-Vetivone	6.78 - 7.12	3 – 6	2 – 5	2 - 4	2 - 4	2.5 - 6.3
Isovalencenol	5.95-6.16	6 – 14	1 – 11	10 - 16	1 - 7	

The quality of vetiver oil is related to the metabolism of its root system and the agro-climatic conditions under which it is grown. It was found that the content of some of the components in the oil is affected by heavy metal contamination of the soil. e.g. the content of α-vetinone is higher in Pb-contaminated soil, while the amount of beta-vetivone is very low in Pb and Cd contaminated soils (Kotoky et al. 2013). The content of khusimol and valerenol also decreased by 16% and 14% in Cd and 17% and 19% Pb contaminated soils, respectively. Cu, Zn and Ni contamination of soil did not affect the individual components (Kotoky et al. 2013).

Opposite results were obtained from Prasad et al. (2014), who found that khusimol significantly increased due to Pb and Ni application and minor to moderate amounts of Cr and Cd. The significant change in khusimol content may be due to vetiver uptake of essential and phytotoxic metals. Heavy metals may increase the catalytic activity of some enzymes due to enzyme-substrate complex formation with metals and hence affect the synthesis of monoterpene compounds (Prasad et al. 2014).

The results obtained in this study confirm that soil contamination with heavy metals affects the chemical composition of essential oils. Khusimol, the main constituent of vetiver essential oil, varied from 28.23% in plants grown on unpolluted soils to 30.25% in plants from heavily polluted soils.The vetiver oil from contaminated soil was found to have a higher content of alcohols and lower content of ketones and esters and did not significantly affect the hydrocarbon fraction. Soil contamination with heavy metals decreased the total range of bicyclic sesquiterpenes from 68.24 to 66.88 and increased tricyclic sesquiterpenes from 29.31 to 31.30%.

The results confirm that vetiver is a crop tolerant to heavy metals and can be grown on soils contaminated with heavy metals. This crop can be referred to a non-accumulating plant for Pb, Cd and Zn and can be used for phytostabilisation of Pb, Cd, Zn and Hg contaminated soils in situ. The quality of vetiver oil usually depends on the amounts of alcohols (mainly khusimol) that contribute to the desirable woody odour of the oil (Kotoky et al. 2013). Cultivating vetiver on soils contaminated with heavy metals has a beneficial effect on the yield and production of oil of high commercial value (high in khusimol). This can be an economic incentive for farmers to grow vetiver on heavy metal-contaminated soils.

CONCLUSIONS

The present study is the first attempt to establish vetiver cultivation possibilities in Bulgaria's industrially polluted areas. Vetiver grass was tolerant to heavy metals and could be grown on soils contaminated with heavy metals (37.7 $mg.kg^{-1}$ Cd, 1238.7 $mg.kg^{-1}$ Pb and 1676.4 $mg.kg^{-1}$ Zn). Heavy metals (Pb, Cd, Hg) and trace elements (Zn, Cu, Fe and Mn) accumulate mainly in the root system, while macroelements (P, K, Ca, Mg) get in the vetiver shoots. In vetiver, both BCF and TF are less than 1; therefore, vetiver can be classified as a non-accumulating plant and successfully used for the phytostabilisation of heavy metal-contaminated soils. The Pb, Cd and Hg amounts in vetiver oil are lower than the accepted maximum values and meet the requirements for an environmentally friendly product. For the first time, data on the composition of vetiver essential oil are published. The results obtained for the production and composition of the essential oil are auspicious. Vetiver can be cultivated in areas contaminated with heavy metals without the risk of transferring metals from the soil to the essential oil. Cultivation of vetiver on soils contaminated with heavy metals has a beneficial effect on the yield and production of oil of high commercial value (high in khusimol). This study indicates the ability of vetiver grass to produce more essential oil under heavy metal stress making it useful for both essential oil production and important candidate for phytoremediation. The results show that vetiver can be used for soil stabilization in industrial areas of Bulgaria and worldwide. Research is needed to expand the scope of the search with new vetiver varieties or to investigate genetic modifications to improve the phytoremediation capabilities of vetiver. This can be an economic incentive for farmers to grow vetiver on heavy metal-contaminated soils. The advantages of using vetiver for phytoremediation of contaminated soils are the simplicity of the technology, low cost and ease of maintenance. However, further research is needed to clarify the real potential of vetiver when grown in larger areas, as there is still no practical experience in developing this crop in the country and the appropriate treatment of the roots before essential oil distillation to receive high yields and good quality oil.

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