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EVALUATION OF CHEMICAL COMPOSITION OF ESSENTIAL OIL AND TOXIC METAL ACCUMULATION OF LEMONGRASS (*CYMBOPOGON CITRATUS*) CULTIVATED ON METAL-CONTAMINATED SOILS

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Abstract

The present study determines the chemical composition of lemongrass oil, content of heavy metals and identifies the possibility of lemongrass growth on soil contaminated by heavy metals. The experimental plots were situated 0.5 km from the source of pollution, the Non-Ferrous-Metal Works (MFMW) near Plovdiv, Bulgaria. Lemongrass is a heavy metal tolerant plant that can be grown on heavily heavy metal contaminated soils (45.0 mg/kg Cd, 1917.9 mg/kg Pb and 2273.1 mg/kg Zn). Pb and Cd accumulate mainly in the above-ground mass, Hg - in the root system, while Zn accumulates approximately in equal amounts in the roots and above-ground mass. Oxygenated monoterpenes (85.57%) predominate in lemongrass essential oil, followed by monoterpene hydrocarbons (8.77%) and oxygenated aliphatic hydrocarbons (3.13%), oxygenated sesquiterpenes (0.84%), sesquiterpene hydrocarbons (0.66%) and phenylpropanoid compounds (0.61%). The content of heavy metals in lemongrass essential oil is lower than the accepted maximum values and meets the requirements for an environmentally friendly product.

Key words: *contaminated soils, essential oil, heavy metals, lemongrass.*

INTRODUCTION

Lemongrass (*Cymbopogon citratus*) is a tropical herbaceous perennial plant that belongs to the Gramineae (Poaceae) family and the genus *Cymbopogon*.

In tropical climates, lemongrass is grown as a perennial, while lemongrass is grown annually in countries with cold winters. Lemongrass forms tuft with long and tall stems and can reach 1.5 m in height. The leaves are evergreen bluish-green, linear in shape, 1.3-2.5 cm wide and 0.9 m long.

Lemongrass is adaptable to different soil and climatic conditions. It grows best on sunny sites at 20-35 degrees Celsius temperatures, well-drained, nutrient-rich soils with high organic matter content (Sugumaran et al., 2005). Lemongrass can be harvested 4 to 6 months after planting and subsequent harvests at 2 to 3-month intervals (Joy et al., 2006). Harvesting is done by cutting 20 cm above the ground (Sugumaran et al., 2005).

Lemongrass can also be grown successfully in Bulgaria by propagating the tufts. In autumn, the tufts (which have multiple stems) must be removed, transferred to containers, and stored in light and warm weather (above 15 degrees) during winter.

Lemongrass is a rich source of minerals (Ca, K, Na, P, Mg, Cu, Fe, Zn and Mn), vitamins (vitamin A, vitamin B1 (thiamine), vitamin B2, vitamin B3, vitamin B5, vitamin B6, vitamin C and folic acid) and macronutrients (carbohydrates, protein and small amounts of fat) (USDA National Nutrient Database, 2019).

Lemongrass is cultivated chiefly for its essential oil, mainly biosynthesized in the plant leaves (dijAvila et al., 2016). In the amount of 1-2% (Carlson et al., 2001).

Lemongrass leaves and oil have a typical lemony odor, mainly due to the presence of citral. Lemongrass oil is used in the pharmaceutical, cosmetic, food, and perfume industries (Verma et al., 2014). The oil has antiviral, antimicrobial (Bassole et al., 2011), antioxidant (Lawrence, 2015), antifungal (Rajeswara et al., 2015), anticancer (Kumar et al., 2008), sedative, and anti-inflammatory properties (Figueirinha et al., 2010).

Solving soils contaminated with toxic metals using aromatic plants is a promising and sustainable approach. Recently, interest in aromatic plants for essential oil production and

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phytoremediation has increased. It has been found that many aromatic plants do not absorb heavy metals from the soil and that heavy metals do not affect the chemical composition of the oil (Zheljazkov et al., 2006). It has been shown that there is the least risk of heavy metal contamination of essential oil obtained by the steam distillation process since the heavy metals remain in the plant and the oil can be marketed. Most promising aromatic plants for phytoremediation of toxic metal-contaminated sites have been identified from the families - Poaceae, Lamiaceae, Asteraceae, and Geraniaceae. Some aromatic grasses such as lemongrass, palmarosa, citronella, vetiver etc. are stress tolerant (Das & Maiti, 2009). Lemongrass has the potential for remediation of highly polluted industrial sites such as landfills (Maiti & Maiti, 2015) and chromite-asbestos mines (Kumar & Maiti, 2015). Lemongrass is recommended for phytoremediation of metal-contaminated sites as it can potentially accumulate the metals Cd, Ni, and Pb (Zakka Israila et al., 2015).

Studies related to the assessment of the phytoremediation potential of lemongrass are limited. There are no studies related to lemongrass cultivation and oil composition.

The present work aims to conduct a comparative study that will allow us to determine the content of heavy metals in the vegetative organs of lemongrass and the quality of essential oil and establish the feasibility of growing it on soils contaminated with heavy metals.

MATERIALS AND METHODS

The experiment was carried out on an agricultural field contaminated with heavy metals, located at a distance of 0.5 from the source of contamination - a Non-ferrous metals plant (NFMW) near Plovdiv, Bulgaria.

The study was conducted using lemongrass as a test plant. Six months after planting, samples of plant material (roots and aerial mass) were collected for analysis. After transporting the plants to the laboratory, scissors separated them into their organs (roots and aerial mass). Samples of roots and above-ground mass (stems and leaves) were dried at room temperature until an air-dry mass was obtained, after which they were dried at 45ºC. The heavy metal content of the roots and above-ground mass was determined. Using a Clevenger-type apparatus, Lemongrass essential oil was obtained under laboratory conditions by steam distillation for 2 hours.

The total metal content of the soils was determined according to ISO 11466. The microwave mineralization method determined the elemental content of the lemongrass and essential oil. Quantitative measurements were carried out by ICP (Jobin Yvon Emission - JY 38 S, France). The Hg content of the samples was determined without prior sample preparation with a mercury analyzer.

Digestion and analytical efficiency of ICP and mercury analyzer were validated using a standard reference material of apple leaves (SRM 1515, National Institute of Standards and Technology, NIST). The chemical composition of the oils in hexane (1:1000) was analyzed on an Agilent 7890A Gas Chromatography system equipped with an FID detector and Agilent 5975C mass spectrometer. The oil's chemical constituents were determined on a 7890A gas chromatograph (Agilent Technologies) and a 5975C mass spectral detector (Agilent Technologies). Compounds were identified by comparing retention times and Kovacs relative indices (RI) with those of standard substances and mass spectral data from the NIST'08 library (National Institute of Standards and Technology, USA.

The SPSS for Windows program was used to process the statistical data.

RESULTS AND DISCUSSIONS

Soils

The total content Pb, Zn, Cd, and Hg in soil sampled from NFMW-Plovdiv are presented in Table 1.

The soil used in this experiment was slightly alkalic (pH 7.6), with moderate organic matter content (2.5%). The total content of Zn, Pb, and Cd is high (2273.1 mg/kg Zn, 1917.9 mg/kg Pb, and 40.5 mg/kg Cd, respectively) and exceeds the maximum permissible concentrations (MPC) (400 mg/kg Zn, 100 mg/kg Pb and 3.0 mg/kg Cd). The Hg content in soils was lower than the MPC.

Table 1. The total content of Pb, Zn, Cd (mg/kg) and Hg (ng/g) in soils sampled from NFMW-Plovdiv

Element	Pb	Zn	Cd	Hg
x ± sd	1917.9±4.5	2273.1±3.5	45.0±0.8	574.8±10

x- average value (mg/kg) from 5 repetitions; sd - mean standard deviation
MPC (pH > 7.4) – Pb - 100 mg/kg, Cd - 3.0 mg/kg, Zn - 400 mg/kg, Hg - 1.5 mg/kg

Heavy metal content in lemongrass

Table 2 presents the results obtained for the heavy metal contents in the organs of the essential oil crop studied.

Table 2. Content of Pb, Zn, Cd (mg/kg) and Hg (μg/kg) in lemongrass

Plant organ	Pb, mg/kg x ± sd	Cd, mg/kg x ± sd	Zn, mg/kg x ± sd	Hg, μg/kg x ± sd
Roots	57.2±0.8	11.6±0.5	258.5±1.2	579.9±10
Stems + leaves	149.3±2.4	23.6±1.0	248.8±1.5	96.8±5
Oil	0.11±0.01	nd	0.52±0.05	nd

x- average value(mg/kg) from 5 repetitions; sd - mean standard deviation; nd- non detectable

Pb and Cd accumulate mainly in the above-ground mass, Hg - in the root system, while Zn accumulates approximately equal amounts in the roots and above-ground mass (stems and leaves). The Pb content in the roots of lemongrass grown at a distance of 0.5 km from the NFMW reached 57.2 mg/kg, Zn – 258.5 mg/kg, Cd – 11.7 mg/kg, and Hg – 515.6 μg/kg. The values obtained for the heavy metals (Cd, Pb, and Zn) in the roots were significantly higher than the values considered toxic to plants, 1 mg/kg Cd, 30 mg/kg Pb, and 100 mg/kg Zn (Kabata-Pendias, 2001).

Lemongrass anatomical and biological features can explain the results obtained. The root system of lemongrass has rhizomes and densely furrowed fibrous roots, relatively compact near the soil surface, between 0-20 cm deep, which develop horizontally and gradually penetrate to depth.

Significant translocation and accumulation of Pb, Cd, and Zn have been detected in lemongrass aerial mass. Pb content reached 149.3 mg/kg in the leaves, Cd up to 23.6 mg/kg, and zinc up to 248.8 mg/kg. This is probably due to the anatomical and morphological features of the plant. The significant accumulation of Pb, Cd, and Zn in the above-ground mass is perhaps because the stems and leaves are covered with spiny hairs (trichomes), which contribute to the fixation of aerosol pollutants and their accumulation there.

Hg contamination of plants is due to aerosol pollution (Kabata-Pendias, 2001). The main uptake pathway for Hg is leaves, and the uptake of this element by soil is of secondary importance. Lower levels of Hg were found in the above-ground mass of lemongrass compared to the roots.

The heavy metal content of the essential oil of lemongrass has also been determined. The results showed that most heavy metals in the yellow flowers did not pass into the oil during distillation. Their content in the oil is, therefore, much lower. The Pb content in the essential oil of lemongrass reaches up to 0.11 mg/kg, and the Zn content up to 0.52 mg/kg. The results strongly indicate that most of the Pb, Cd, and Zn in the above-ground mass of lemongrass grown 0.5 km from the NFMW do not pass through into the resulting essential oil. The Pb, Cd, and Hg amounts in the lemongrass essential oil are lower than the accepted maximum values and meet the requirements for an environmentally friendly product (5 mg/kg Pb, 1 mg/kg Cd, 0.1 mg/kg Hg) (Council of Europe, 2021). The extraction of essential oil by steam distillation process may be the main reason for the low concentration of heavy metals in the oil (Zheljazkov et al., 2006). Our result agrees with the finding of Pandey et al. (2020) that despite the high concentration of heavy metals in plant tissues, the content of heavy metals (Cr, Cd, Pb, and Ni) in the essential oil of lion grass is within the WHO recommended limits. Similar results were reported by Khajanchi et al. (2013) and Gautam et al. (2017) in essential oils extracted from lemongrass grown under different irrigation regimes with wastewater and groundwater and treated with red mud, respectively).

Determination of the phytoremediation potential of lemongrass

To definitively answer the question of the ability of lemongrass to absorb heavy metals from soil and to assess its potential for phytoremediation, the bioconcentration factor (BCF) and translocation factor (TF) were calculated. The results obtained for BCF and TF are presented in Figure 1.

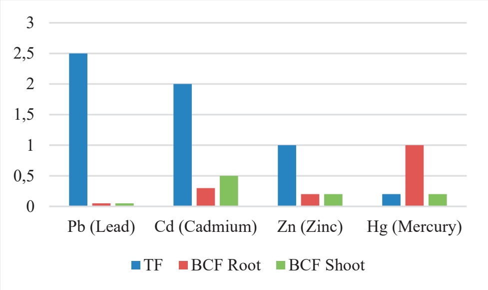

Figure 1. Translocation and bioconcentration factors for lemongrass

The translocation factor (TF) gives information on the ability of plants to uptake heavy metals through the roots and move them to the above-ground mass (leaves). TF values more significant than 1 indicate that the plant is a potential accumulator of heavy metals and can translocate metals efficiently from the roots to the above-ground mass. For Pb and Cd, TF values are more significant than 1. for Zn around 1, and for Hg lower than 1.
Bioconcentration factor (BCF) is the ratio of the heavy metal content in plant organs to the content in the soil. It measures the plant's ability to accumulate metals from the soil. The bioconcentration factor (BCF) is one of the most important indicators for measuring the effectiveness of the phytoremediation process (Adriano, 2001). A plant is an excluder if BCF < 1, an accumulator if 1 < BCF < 10, and if BCF > 10, the plant is a hyperaccumulator. Plants with a BCF value > 1 are suitable for phytoextraction. Plants with TF<1 and BCF root -soil >1 is ideal for phytostabilisation.
For Pb, Cd Zn, BCFroot, and BCFshoot were <1, indicating that their content in the lemongrass does not exceed the amount in the soil. Concerning Hg, BCFshoot was <1, while BCFroot were 1 (Figure 1).
The BCFroot and TF factors of Hg indicate that this element accumulates in the underground mass of lemongrass.
Based on the BCF values, lemongrass can be referred to as an exclusionary plant of lead, cadmium, and zinc and can be used for phytostabilization for Hg. However, according to Pandey et al. (2019), TF>1 for Pb and Ni, while TF was less than 1 for Cr and Cd, and BCFsshoot mass was more significant than 1 for Pb and Ni, indicating that metals moved into the aerial parts of lemongrass. According to Pandey et al. (2020), lemongrass can accumulate Pb and Ni in its plant parts and be exploited as a phytoextractant for the same metals. Soil properties such as pH, EC, and organic carbon content influence the translocation of heavy metals in lemongrass plant parts (Pandey et al., 2020).

Essential oil quality

The results of the chromatographic analysis of the essential oils obtained from the processing of lemongrass leaves grown within 0.5 km of the NFMW are presented in Table 3.
Twenty-nine compounds were identified, constituting 99.58% of the oil (Table 3). The major compounds in the oils were geranial (40.06%), neral (34.45%), myrcene (8.17%), geranyl acetate (2.33%), 6-Methyl-5-heptene-2-one (2.25%), geraniol (2.01%), β-linalool (1.15%). The essential oil mainly contains aldehydes, ketones, alcohol, and esters (Table 3). Table 3 also gives the classification of the identified compounds based on functional groups. Oxygenated monoterpenes (85.57%) were dominant in the oil, followed by monoterpene hydrocarbons (8.77%) and oxygenated aliphatic hydrocarbons (3.13%), while oxygenated sesquiterpenes (0.84%), sesquiterpene hydrocarbons (0.66%) and phenylpropanoid compounds (0.61%) were present in lesser amounts (Table 3).
The major component of lemongrass essential oil is citral. It is a mixture of two geometric isomers. The E-isomer is geranial or citral A, and the Z-isomer is neral or citral B. The content of geranial in the essential oil reaches up to 40.06% and neral up to 34.45% (Table 3), with geranial dominating over neral. The geranial content of lemongrass essential oils is known to range from 20 to 50%, and the neral from 30 to 40% (Schaneberg and Khan (2002); Pandey et al., 2003; Chandrashekar and Joshi, 2006).
The quality of lemongrass essential oil is usually assessed by its citral content.
According to the ISO 3217 (1974) standard, lemongrass oil must contain at least 75% citral to be considered a high-quality product (Barbosa et al., 2008). The quality and quantity of lemongrass essential oil is highly dependent on the time of harvesting of the plants, as the

composition and content of essential oil are related to the stage of development of the whole plant, plant organs, and cells (Verma et al., 2015). The geranial content of lemongrass essential oil increased from 37.58% to 45.95% when lemongrass was harvested at 6.5 months after planting. At later harvest (7.5 months after planting), its content slightly decreased to 42.95%.

Table 3. Composition of lemongrass oil (%)

	Component	RI	% of TIC
1	6-Methyl-5-heptene-2-one	975	2.25
2	Myrcene	990	8.17
3	cis-β-Ocimene	1034	0.4
4	trans-β-Ocimene	1045	0.2
5	6.7-Epoxymyrcene	1088	0.68
6	α-Pinene oxide	1093	0.15
7	β-Linalool	1099	1.15
8	Citronellal	1146	0.7
9	β-Citronellol	1218	0.52
10	Nerol	1227	0.99
11	Neral	1236	34.45
12	Geraniol	1249	2.01
13	Geranial	1266	40.06
14	Methyl nonyl ketone	1274	0.75
15	Methyl nerolate	1283	0.27
16	Geranyl formate	1294	0.49
17	Citronellic acid	1312	0.98
18	Methyl geranate	1320	0.79
19	Geranyl acetate	1377	2.33
20	β-Caryophyllene	1422	0.16
21	β-Copaene	1429	0.22
22	α-Bergamotene	1434	0.17
23	(Z.E)-α-Farnesene	1441	0.11
24	2-Tridecanone	1497	0.13
25	Elemicin	1556	0.1
26	Caryophyllene oxide	1584	0.29
27	Selina-6-en-4-ol	1619	0.43
28	Isoelemicin	1655	0.51
29	Intermedeol	1668	0.12
Oxygenated aliphatic hydrocarbons			3.13
Monoterpene hydrocarbons (MH)			8.77
Oxygen monoterpenes (OM)			85.57
Sesquiterpene hydrocarbons (SH)			0.66
Oxygenated sesquiterpenes (OS)			0.84
Phenylpropanoids			0.61
Total			99.58

RI - Kovacs relative indices

A significant amount of citral indicates high antimicrobial potential and antioxidant activity of lemongrass essential oil.

Myrcene content in the essential oil from the NFMW - Plovdiv reaches 8.17%. Similar is the content of β-myrcene (7.68%) in the essential oil from Vietnam. Studies have shown that myrcene content varies significantly from 0% in essential oil from Benin (Kpoviessi, 2014) and 0.8% in essential oil from Egypt (Mansour et al., 2015) to 25% in essential oils obtained in Brazil (Farias et al., 2019) and Nigeria (Kasali et al., 2001).

Of the alcohols contained in lemongrass essential oil, geraniol has the highest content, ranging widely (from 1.34% in essential oil from South Africa (Mbili et al., 2017) to 21.86% in essential oil from Nigeria (Kasali et al., 2001). The geraniol content in the essential oil we studied reached up to 2.01%.

Of the esters, geranyl acetate predominated (2.33%). Studies have shown that amounts of geranyl acetate vary considerably (0.24% in essential oil from Kenya (Matasyoh et al., 2011) to 3.42% in essential oil from Nigeria (Kasali et al., 2001).

Habitat, genetic variability, climatic conditions, harvesting time, and oil extraction methods can explain these variations in essential oil composition.

The chemical composition of lemongrass essential oil is related to its geographical origin. *C. citratus* essential oil from Africa contains a high amount of myrcene, while the essential oil from Ethiopia contains geraniol (40%), citral (13%), and oxobisal (12%) (Ekpenyong et al., 2014). The major components identified in essential oil from Egypt were geranial (20.90-40.72%), neral (16.20-34.98%), geraniol (8.30%) and linalool (5.60%) (Hana et al., 2012; Mansour et al., 2015), while significant amounts of geranial (37.80%) and neral (33.60%) were found in essential oil from Saudi Arabia (Mansour et al., 2015). The essential oil obtained from Nigeria was poor in citral (0.99%) but rich in geraniol (21.86%), limonene (7.90%), and camphene (7.89%) (Kasali et al., 2001).

Schaneberg and Khan (2002) suggested that geranial, neral, limonene, citronellal, myrcene, and geraniol could be used as marker compounds in lemongrass essential oil. However, some essential oils do not always present limonene, citronellal, and geraniol. In the oil we have studied, limonene is not present. Some essential oils are rich in limonene (Nigeria 7.90%) (Kasali et al., 2001), while the essential oil from Kenya does not contain this terpene (Matasyoh, 2011).

According to Majewska et al. (2019), citral (neral and geranial) content and β-myrcene content are to be used to distinguish the origin of essential oil. Marker compounds can be used to verify the identity of the essential oil.

The lemongrass essential oils from Brazil, Asia, and West and East Africa have a high geranial content and belong to the geranial chemotype. Two chemotypes of C. citratus have been found in Congo - a citral-type and a geraniol-type (Majewska et al., 2019). The oil we studied belongs to the citral chemotype.

CONCLUSIONS

There is a clear pattern in the accumulation of heavy metals in the vegetative organs of lemongrass. Pb and Cd accumulate mainly in the above-ground mass, Hg - in the root system, while Zn accumulates approximately equal amounts in the roots and above-ground mass.

Lemongrass is a heavy metal tolerant plant that can be grown on heavily heavy metal contaminated soils and shows no symptoms of toxicity (chlorosis and necrosis) at a soil content of 45.0 mg/kg Cd, 1917.9 mg/kg Pb and 2273.1 mg/kg Zn.

Lemongrass is an indicator plant and can be used for monitoring of soil and aerosol contamination with heavy metals.

The content of heavy metals Pb, Cd, and Hg in lemongrass essential oil is lower than the accepted maximum values and meets the requirements for an environmentally friendly product. The highest content of oxygenated monoterpenes (85.57%) was found in lemongrass essential oil, followed by monoterpene hydrocarbons (8.77%) and oxygenated aliphatic hydrocarbons (3.13%), oxygenated sesquiterpenes (0.84%), sesquiterpene hydrocarbons (0.66%) and phenylpropanoid compounds (0.61%). Lemongrass essential oil belongs to the citral chemiotype.

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