Mineral composition of different basil (*Ocimum* spp.) genotypes

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Abstract

This experiment investigated mineral composition of 13 basil genotypes (*Ocimum* spp.) in order to find varieties supporting human dietary intake of essential minerals and to evaluate basil genotypes which could serve for herbal production as raw material in pharmaceutical or food processing industry. In addition, this study tested a potential risk of the accumulation of heavy metals during the commercial production of basil on agricultural soil. Mineral composition of basil genotypes was found to be in association with its genetic potential, where some of them can be used in human nutrition as an additional source of several minerals, particularly micronutrients (Fe, Mn and Zn), which generally improve human immune system. Iron-rich basil genotypes were identified in this experiment, like Compact (3576.0 mg/kg), with Lattuga (1585.6 mg/kg) and Blue spice (1167.9 mg/kg) genotypes, containing more than 1000 mg/kg of Fe in herbal part on dry basil (d.m.). This attract a special attention as a source of iron, especially for humans with low Fe intake, and consequently, for people with low level of hemoglobin. Basil grown on agricultural soil was tested on the accumulation of heavy metals (Cu, Co, Cu, Ni, Cr and Pb), which were not found to be excessive in herbal parts of the plants. Cluster analysis (CA) distinguished *Ocmium* spp. genotypes in two separate groups. Despite of significant differences among the genotypes, content of Fe, Mn, Co, Cr, Ni and Pb made a clear distinction between the clusters.

Keywords: basil, genotyp, mineral composition, iron, heavy metals, cluster analysis.

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Basil (*Ocimum* spp.) falls within Labiates mint family plant that usually grows in tropical and subtropical countries and it is widely used in human nutrition as a spice, improving aroma in meat, vegetable and other meals. However, the primary culinary interest in this herb has decreased to be substituted by its medicinal properties, since it is widely used in health treatments [1–3]. Recently, basil studies get all the more attention due to organic compounds containing aroma compounds which are to be found in volatile extracts exhibiting potent antioxidant activity comparable to the known antioxidants [4]. In considering the abundance of these aroma chemicals in basil plants, we can say that the total activity is either comparable or higher to the known antioxidants [5]. In fact, the obvious antioxidant potential of basil are based on the presence of polyphenolic compounds and essential oils extracted from the above ground herbal parts, in which rosmarinic acid is the most prevalent basil phenol [3,6–8]. Therefore, in recent years a great commercial interest in this crop increased after such assigned antioxidant health benefit of basil for humans [9], so that basil is now globally grown as a medicinal plant species. Its commercial cultivation usually tends to sustain growth originating from its natural eco system [10], thus limiting fertilizer use, especially the use of nitrogen which affects its antioxidant potential [11,12], while increased potassium application can alter antioxidant capacity and phenolic concentration of basil in a positive way [13].

Despite the fact that people's interest in basil as a medicinal plant increases all the time, all around the world there is also a strong interest in aromatic plants due to their dietary minerals [14–16]. Green parts of basil can provide additional amount of several minerals in human nutrition, particularly micronutrients [17] which play a critical role in some protein synthesis and essential enzyme system which can improve resistance to diseases in people [18]. The importance of mineral content of basil has been recognized in several recent studies, imposing environmental factors (locality) as the one playing an important role in accumulation of mineral nutrients [19–22]. However, genetic diversity of basil species may also significantly change the mineral and chemical composition of the plant, especially in secondary metabolite products of herbal parts, sometimes strictly related to its taxonomy species [23]. This is usually related to basil morphological variability, expressed as differences in steam and leaves shape, making it difficult to use morphological traits as a reliable indicator for its selection in commercial use.

This study, which included 13 different basil genotypes grown in the same soil substrate under the iden-

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tical nutritional regimes, gives an opportunity to evaluate each genotype according to the presence/excess of essential elements in herbal tissue and to select basil species recommendable for further growth. Besides, concerning other important parameters like a basil herbal yield and its morphological appearance, this study can significantly contribute to its selection for pharmaceutical and food processing industry purposes. Although many studies have focused on the characterization of volatile oils and phenol compounds of different genotypes within this species [24–26], the obtained results could be also used to describe genotype diversity and to indicate to chemical polymorphism in mineral composition of this herbaceous and medical plant. Apart from the fact that they contain essential minerals, the main constraint of each plant species of wild origin is the presence of heavy metals in their herbal tissues, varying among the species and growing conditions [27,28], so that this study also highlights the specific heavy metal (Cu, Cd, Co, Cr, Ni and Pb) accumulation in each tested genotype during its growth for commercial purpose.

Mineral composition may serve to identify similarities or differences between basil cultivars, therefore one can draw certain conclusions regarding the classification of individual samples. In order to obtain useful biological information from a complex spectral data set, mineral composition can be combined with cluster analysis. Previously, CA has been used to evaluate different basil collections [29–31].

Therefore, the analyzed data can be useful in the selection of varieties, where excessive heavy metal accumulation is one of the most limiting factors when it comes to the use thereof by humans, while the goal of cluster analysis was to detect relations between the genotypes.

EXPERIMENTAL

Material

The experimental plot was located in Belgrade at the “City nursery” in NE part district – Zemun (longitude 44.8500° N, latitude 20.4000° E) throughout 2011. The nursery was used for city’s decorative and herbal plants production, covering 4 ha area. The field experiment was established based on the system of a random block arrangement with 4 repetitions. The experimental crop was established through the nursery. Plants were transplanted at the distance of 50 cm in the basic plot, so that plants were set at the distance of 50 cm (50 cm×50 cm). The size of the basic plot was 6 m^2 and it contained 35 plants in each. The total number of basic plots was 52, and the surface covered by the experiment was 312 m^2. They used basil genotypes labelled by numbers 01 to 13 in which: Lime, Hollander, Purple Ruffles, Lattuga, Cinnamon, Osmin, Blue Spice, Siam Queen, Fino Verde, Purple Opal, Compact, Genovese, belong to *Ocimim basilicum* L., while Holy Red belongs to *Ocimim sanctum* L. The used basil genotypes have been derived from the national collection of germ-plasma (Plant Gene Bank of the Republic of Serbia) and its genotype collection of Institute of Crop Science (Faculty of Agriculture, University of Belgrade) as described by Beatović *et al*. [32].

Soil analysis

Representative soil sampling was carried out to the depth of 30 cm, with 3 sub-samples from each experimental block merged in a single sample, the quantities thereof necessary for the analyses were brought to the laboratory in appropriate polyethylene bags. For the analyses, a representative sample of each 4 replicate was made upon the mixing and homogenization of 20 spade probes, which was thereafter air dried (20 °C), and sieved through a 2 mm stainless-steel mesh.

Particle-sized distribution combined wet sieving (coarse, medium and fine sand) and pipette sedimentation technique (silt and clay). Prior to separation, 1:4 soil/water suspension was ultrasonically treated (40 MHz, 120 W) for 30 min following the procedure described by Hereter *et al*. [33]. Collected fractions were dried at 40 °C and weighed.

Basic chemical properties of soil were analyzed using standard soil analysis methods: pH was measured in a suspension characterized by a residue/water ratio of 1:2.5. Inorganic $CaCO_3$ was quantified by Scheibler method [34], organic C by dichromate method. Total N was determined using a Kjeldahl method [35], available N forms (NH_4^+ and NO_3^-) were extracted with 2M KCl and determined by hot distillation with MgO and Devarda alloy for NO_3^- determination [36]. Available P was determined using spectrophotometry upon the extraction in AL solution (0.1 M ammonium lactate and 0.4 M HOAc) [37], Available K was quantified by flame photometry from the same extraction solution as K. Determination of available boron was done after its extraction with hot water and used cucurmin method for colorimetric determination [38].

Pseudo-total metal concentrations (Fe, Mn, Cu, Zn, Co, Pb, Ni and Cd) in nursery soil were quantified using atomic absorption spectrophotometry AAS (Varian SpectrAA 202 FS), in flame acetylene/air, after digestion using HNO_3 (70%) + H_2O_2 (33%) [34]. This digestion is considered useful if complete metal recovery is not essential [35] where metals in residuum are not expected to be released in a solution over a reasonable time span under the conditions normally encountered in nature [36]. Detection limits, calculated as analytic concentration greater than three times standard deviation, obtained after eight measurements of the blank solution, were (μg/ml): 0.010 Fe, 0.003 Mn, 0.001 Cu, 0.015 Zn, 0.002 Co, 0.002 Pb, 0.002 Ni, 0.007 Cr and 0.001 Cd. The certified reference material, BCR No. 141, was analyzed to ensure accuracy. Labile pool of heavy metals in soil samples was determined by flame AAS after extraction with 0.005 M DTPA solution (diethylenetriaminopentaacetic acid), as proposed by Lindsay and Norvell [37].

Plant analysis

The yield of fresh and dry basil herb was determined on the sample of plants per genotype (4×10). Plants were cut at the height of 5 cm above the soil surface, and the fresh herb yield (g/plant) above the soil surface was measured on the scale (KERN MH5K5) for field measurements. Dry herb yield was determined upon drying fresh herb at the temperature of 40 °C.

The plant material (basil herbs) collected from field was brought into the lab and then prepared for analyses: washed with tap water, rinsed with distilled water, submerged in 0.1 M HCl to ensure that leaf surface was free of adhering soil particles, and finally carefully rinsed with de-ionized water. Prior to mineral and metal analysis, all plant material was oven-dried (80 °C) for 15 h, weighed and grounded into powder in a plant lab sample grinder (Retsch). The analytical method applied for macronutrient and B determination was the same method applied for the soil, while metal content in the plant material was also determined by AAS, after the digestion in concentrated acids: HNO_3 and $HClO_4$, with the addition of 33% H_2O_2.

Statistical analyses

Analysis of variance (ANOVA) was carried out. All ANOVAs were performed using treatments as statistical parameters at a significance level of $P \leq 0.05$. The least significant difference (*LSD*), if necessary, was used to determine whether the difference between two accessions was big enough to be considered real at a fixed level of confidence (*LSD* 0.05 = 95% confidence and *LSD* 0.01 = 99% confidence). Data referring to the content of elements in the plant material, as well as the weight of fresh and dry basil herb, were expressed in the average value and the standard deviation value.

All the properties were used to cluster accessions into similarity groups using the unequal pair group method with arithmetic mean (UPGMA). Statistical analyses were conducted using STATISTICA for Windows 6.0 (StatSoft Inc., Tulsa Okla).

RESULTS AND DISCUSSION

Soil properties and metal concentration

The intended experimental nursery plot, stipulated for basil growth, is located on chernozem soil type [38], and it obtains seedling production of different agricultural and decorative plant species. Main properties of the soil have been analyzed, as shown in Table 1, in order to determine physical, chemical and nutritional properties thereof. Soil texture is sandy loam, with a strong predominance of silt fraction (41.65%), and significant presence of fine sand (36.52%). Investigated chernozem is of slightly alkaline pH (7.66) reaction, generally in optimal values for the growth of the most plants. A slight amount (0.33%) of $CaCO_3$ was present in this soil. In comparison with the total nitrogen, the total organic matter is not so high for this soil type (2.98 and 0.174%), while available phosphorus is low (< 10 mg/100 g) and available potassium in the medium range (18.4 mg/100 g). In the analyzed samples, the available nitrogen content (14.0 mg/kg) is also low, as a result of missed fertilization, which should keep the herbal quality of basil as good as possible.

Table 1. Soil properties with pseudo-total (aqua regia) and DTPA-extractable metal concentrations (means ± standard deviation) collected from experimental field used for growth of different basil (Ocimum spp.) genotypes

Soil property	Values
pH_{H_2O}	7.66
pH_{KCl}	6.47
Organic matter, %	2.98
$CaCO_3$, %	0.3
Coarse sand (0.2–2.0 mm), %	1.4
Fine sand (0.05–0.2 mm), %	36.52
Silt (0.05–0.002 mm), %	41.65
Clay (<0.002 mm) , %	21.33
Total N, %	0.174
NH_4+NO_3, mg kg^{-1}	14.0
P_2O_5, mg/100 g	6.6
K_2O, mg/100 g	18.4
B, mg/kg	0.5±0.05
Fe, mg/kg	1440±51.0[a], 11.5±1.8[b]
Mn, mg/kg	183.4±10.12[a], 3.9±0.8[b]
Cu, mg/kg	10.9±0.31[a], 2.53±0.42[b]
Zn, mg/kg	46.2±7.11[a], 0.51±0.02[b]
Cd, mg/kg	–[c], 0.08±0.01[b]
Co, mg/kg	8.9±0.03[a], 0.05±0.01[b]
Ni, mg/kg	28.1±6.4[a], 0.23±0.01[b]
Pb, mg/kg	22.8±2.16[a], 2.39±0.05[b]
Cr, mg/kg	23.9±5.43[a], –[d]

[a] Pseudo-total (*aqua regia*); [b] DTPA-extractable concentrations; [c] HNO_3 is an uppropriate media for pseudo total Cd detection; [d] DTPA extraction of available Cr under detection limit (0.007 µg Cr/ml)

Analysis of pseudo-total (*aqua regia*) metal content is usual for this soil type [39]. The level of heavy metals was mainly below the critical values for soils and substrates, and analyzed chernozem should be treated unpolluted. Heavy metal content (mg/kg) slightly varied among collected samples, according to applied block system, with the average of 10.9 Cu, 46.2 Zn, 8.9 Cd, 28.,1 Ni, 22.8 Pb and 23.9 Cr, while HNO_3 was an appropriate media for pseudo total Cd detection. At all sampling locations, the average content of heavy metals in each soil sample was under the critical threshold background.

Micronutrients and heavy metals are phytoavailable as DTPA extracted values in Table 2. DTPA-extractable proportion of heavy metals pseudo-total concentration decreases in order Cu > Pb > Zn > Ni > Co, while DTPA extractable Cr was under the detection limit (0.007 µg Cr/ml). A very low phytoavailable proportion of pseudo total content was found in all samples for Ni (0.23%), Zn (0.51%) and Co (0.05%), possibly influenced by relatively high pH (>6.5) [40] and high organic content (2.98%) [41], while Cu and Pb had considerably higher available amounts (2.53 and 2.39%, respectively). However, positive correlation between pseudo-total and available amounts of heavy metals in soil was not found.

Yield and morphology issue

The taxonomy of *Ocimum* was complex due to the interspecies hybridization and polyploidy of the species in genus, but now we can speak about 65 basil species, where the *Ocimum basilicum* L. should be treated as a major genus aimed as crop and grown in many countries [42]. There was no matching in genotype and habitus appearance among investigated basil genotypes (13). In fact, there was no clear connection between any available shape of basil aboveground part and its genotype status. However, the obtained basil biomass yield significantly varied between genotypes, both as a fresh or dried biomass, pointing out that primary basil yield depended on its genotype origin (Table 2).

Fresh basil herb yield was calculated at the sample of 40 plants per genotype (4×10). In the experiment, two genotypes had the biggest herbal yield: Fino verde (65.85 g) and Hollander (67.0 g), both as a linalool chemotype, however with different habitus, intermediarius and upright, respectively. The biomass variation among investigated genotypes were rather high, sometimes double or even tree times (3.78) higher (e.g., Holy Red: 17.7 g *versus* Holandjanin: 67.0 g), which provided useful information for basil selection for cultivation.

Comparing our results to those of other researchers, regarding the same genotypes and different agroecological conditions, we can say that the experiment was set up at the advantageous conditions for basil production. Namely, the experiments carried out in Europe, on the same genotypes, have resulted in basil herb yields somewhat below the average values [43–46].

Macronutrient issue

Since there was no significant interaction between macronutrient and micronutrient accumulation in examined basil genotypes, the presence of this minerals in dry tissue was discussed separately. Two approaches could be used to summarize a review about plant tissue macronutrient accumulation: their percentage in tissue or their uptake by plant yield (g/plant) (Table 3).

A significant variation has been found between the content of elements in the plant of each investigated basil genotype, showing the substantial mineral diversity of examined species. Two elements (N and Ca) participate with the highest amount in basil biomass, where the level of the former could be easily changed

Table 2. Herbal biomass yield (g/plant of fresh and dried biomass) of different basil (Ocimum spp.) genotypes with chemotype classification grown in field experiment

Genotype	Chemotype	Habitus	Fresh herba, g/plant	Dried herba, g/plant
Holy Red	Caryophyllene	Upright	85.5±2.14	17.7±0.32
Lime	Geranial – neral	Intermediarius	106.75±3.66	20.05±0.41
Hollander	Linalool	Upright	318.5 ±3.56	67.0±0.70
Purple Ruffles	Linalool	Upright	138.75±2.71	19.85±0.41
Lattuga	Linalool	Intermediarius	208.0±2.92	31.0±0.52
Cinnamon	Linalool – methyl cinnamate	Upright	242.5±4.21	43.2±0.73
Osmin	Linalool	Intermediarius	168.75±2.17	25.5 ±0.74
Blue Spice	Bisabollene	Intermediarius	205.75±2.06	35.15±0.43
Siam Queen	Methyl chavicol	Intermediarius	232.5±3.53	43.65±0.65
Fino Verde	Linalool	Intermediarius	311.0±2.85	65.85±0.60
Purple Opal	Linalool	Intermediarius	141.75±4.16	22.3±0.39
Compact	Linalool	Roundish	276.0±3.45	55.2±0.70
Genovese	Linalool	Upright	260.0±3.64	44.9±0.32
LSD = 0.05			5.35	2.75
LSD = 0.01			7.45	4.25

Table 3. Macronutrient content (%) and their uptake by plant yield (g/plant) of different genotypes of basil (Ocimum spp.) grown in field experiment

Genotype	N		P_2O_5		K_2O		Ca		Mg	
	%	g/plant	%	g/plant	%	g/plant	%	g/plant	%	g/plant
Holy Red	3.22±0.11	0.57	0.63±0.08	0.11	1.11±0.05	0.20	1.92±0.03	0.34	0.46±0.11	0.08
Lime	2.75±0.18	0.55	0.76±0.04	0.15	1.52±0.17	0.31	1.94±0.04	0.39	0.84±0.08	0.17
Hollander	3.33±0.10	2.23	0.84±0.03	0.57	1.45±0.06	0.97	2.56±0.11	1.71	0.54±0.04	0.36
Purple Ruffles	3.33±0.22	0.66	0.81±0.01	0.16	1.64±0.08	0.33	2.78±0.09	0.55	0.87±0.08	0.17
Lattuga	3.52±0.13	1.09	0.99±0.12	0.31	1.76±0.34	0.55	3.60±0.10	1.12	0.75±0.15	0.23
Cinnamon	3.81±0.10	1.64	1.08±0.07	0.47	2.07±0.03	0.89	2.46±0.22	1.06	0.70±0.07	0.30
Osmin	3.29±0.01	0.84	0.85±0.19	0.22	2.03±0.17	0.52	3.18±0.14	0.81	0.62±0.04	0.16
Blue Spice	2.40±0.34	0.86	0.80±0.03	0.28	1.86±0.05	0.65	1.92±0.12	0.68	1.53±0.19	0.54
Siam Queen	2.91±0.49	1.27	0.93±0.06	0.40	2.15±0.15	0.94	2.64±0.67	1.15	0.81±0.04	0.35
Fino Verde	2.74±0.12	1.81	0.68±0.02	0.45	1.40±0.25	0.92	3.65±0.07	2.41	0.67±0.12	0.44
Purple Opal	2.27±0.16	0.51	0.97±0.12	0.22	1.55±0.10	0.35	3.29±0.05	0.73	0.84±0.20	0.19
Compact	1.97±0.33	1.09	0.80±0.03	0.44	1.94±0.03	1.07	3.22±0.11	1.78	0.73±0.04	0.40
Genovese	3.45±0.11	1.55	0.81±0.01	0.36	1.13±0.08	0.51	3.42±0.09	1.54	0.84±0.08	0.38
LSD = 0.05	0.07		0.01		0.05		0.06		0.05	
LSD = 0.01	0.05		0.005		0.04		0.04		0.04	

by fertilization [47], but the uptake of the latter is genetically controlled [48]. In addition, potassium fertilization during the basil production is still necessary and considered desirable [49]. The "dilution effect" in mineral analyses [50] could be successfully overcome by the calculation of nutrient uptake by the formed biomass. This usually provides more obvious data about the potential and capability of each genotype to accumulate these minerals from the soil. Therefore, formed plant biomass sometimes is crucial for nutrient accumulation, where low or high nutrient content in tissues does not portray a realistic picture of the plant's needs. This is illustrated by our results: certain genotypes with the smallest growth between tested species (Holy red, Lattuga and Osmin), have had a relatively small accumulation of nitrogen, dispute N high concentration in analyzed tissues (3.22, 3.52 and 3.29%, respectively) (Table 3). Among others, two genotypes stand out for the highest N accumulation in tissues (Hollander and Fino Verde), assuming that this genotype feature has the biggest impact on organic compound synthesis.

Micronutrient issue

Monitored medicinal, aromatic and spice plants grown in different regions usually show differences in the concentration of elements, as a consequence of soil composition and the climate in which the plant is grown [51]. However, the presence of micronutrients in green parts of the plants support a synthesis of organic compounds in plant tissues [52], providing organic and mineral substances which act as an enzyme activator in human body [53]. So far, in recent years, with enhanced awareness of the importance of trace elements on health, an increasing number of reports on the role of trace elements in medicines has been published in different countries [54–56]. According to the results obtained from this investigation, some genotypes of examined basil should be regarded as a rich source of micronutrients for humans (Table 4).

A significant variation in microelement content was identified among basil genotypes, which can be related to different biomass growth of tested basil species, or to its genotype adaptation to the growing conditions. Consequently, this could possibly induce the presence of different chemical compounds in herbal tissues [57]. In fact, some elements were detected in substantial amounts, like Fe and Mn in Compact and Zn in Lattuga variety, qualifying these genotypes as extraordinary for human use. The ability of plants to make Fe organic chelating in tissues [52] is another basil advance for medical use. Therefore, found Fe-rich basil genotypes (Blue Spice – 1167.9 mg/kg, Lattuga – 1585.6 mg/kg and especially Compact, with 3576.0 mg/kg of Fe on dry basis) could be of special interest as iron source for humans with extreme hemoglobin deficit. Dispute that basil is classified as a spice and usually used in human nutrition in very small amounts, its effects as a Fe source in human food can be illustrate on the basis of its daily intake by salad. The result of Fe fresh tomato salad use could be taken into account. If dry mater of tomato fruits contain the highest 2.41±0.34 mg/kg of Fe according to investigation [59], due to its very high water content (92.84–94.76%) [58], the amount of 1000 g of this vegetable will provide 0.1446 mg of iron in nutrition. The addition of only 1 g of d.m. of basil spice (Compact variety) to this salad, however, can

Table 4. Micronutrient and heavy metal content (mg/kg) in basil of different basil genotypes (Ocimum spp.) grown in field experiment

Genotype	Fe	Mn	Cu	Zn	B	Cd	Co	Cr	Ni	Pb
Holy Red	766.5±40.0	73.2±9.5	13.78±1.01	28.60±3.19	25.83±1.26	0.21±0.02	1.75±0.38	3.11±0.13	1.97±0.06	1.08±0.03
Lime	477.4±8.7	74.4±14.6	15.64±2.44	44.36±5.59	19.67±0.76	0.17±0.02	1.78±0.40	4.19±0.13	1.62±0.17	0.69±0.17
Hollander	202.1±17.7	78.5±9.6	24.19±3.66	52.87±5.68	20.67±2.57	0.21±0.08	1.90±0.05	2.15±1.02	2.77±0.83	0.51±0.10
Purple Ruffles	719.1±92.8	92.6±31.8	16.91±3.29	42.28±7.06	27.00±3.77	0.07±0.03	2.55±0.44	5.82±0.45	2.40±0.24	0.30±0.02
Lattunga	1126.0±101.6	126.4±36.3	19.14±4.61	124.6 ±21.6	30.33±1.26	0.23±0.05	2.97±0.28	4.50±0.71	3.39±0.60	0.85±0.18
Cinnamon	290.1±61.7	77.5±5.3	19.36±1.92	47.89±3.59	23.17±2.02	0.35±0.08	2.20±0.15	3.23±0.10	1.65±0.14	0.73±0.08
Osmin	686.0±62.8	87.4±8.3	18.92±2.89	54.35±5.50	30.67±2.25	0.25±0.02	2.68±0.03	3.22±0.29	2.82±0.33	0.72±0.07
Blue Spice	1507.2±251.7	94.5±15.1	15.72±4.31	36.82±4.07	21.67±3.55	0.16±0.05	2.10±0.28	4.97±1.11	2.87±0.24	0.29±0.04
Siam Queen	508.4±15.5	88.6±2.9	22.60±3.92	78.57±10.8	27.17±1.61	0.23±0.03	2.33±0.20	2.62±0.50	2.53±0.38	0.14±0.11
Fino Verde	540.5±146.1	130.8±22.2	21.71±3.06	68.32±2.52	24.50±0.87	0.08±0.10	2.72±0.28	2.20±0.22	3.42±0.20	0.53±0.13
Purple Opal	740.3±125.2	114.2±12.0	18.88±4.42	70.76±1.90	30.33±3.40	0.32±0.07	2.50±0.20	3.08±0.09	2.50±0.45	1.01±0.15
Compact	3576.0±275.6	234.9±44.8	24.75±5.19	60.91±3.91	22.67±4.86	0.20±0.02	5.07±0.98	8.42±0.77	10.2±1.06	2.84±0.23
Genovese	1010.9±140.8	126.2±10.6	23.31±2.73	57.21±11.61	25.83±4.75	0.16±0.05	3.05±0.17	3.17±0.10	1.96±0.50	0.55±0.08
LSD = 0.05	0.051	7.02		7.67	3.14	0.03	0.11	0.07	0.33	0.05
LSD = 0.01	0.037	5.09		5.52	2,28	0.02	0.08	0.05	0.24	0.03

enrich this food with additional 0.2794 mg of Fe. Such high content of iron (10814, 9043 and 2465 mg/kg) in dray weight of basil leaf has been found in some basil genotypes (*O. basilicum*, *O. sanctum*, *O. minimum*, respectively) grown in Indian arid areas [59], but this results should be accepted with reserve, because this analytical material was obtained using a manual grinder. Similarly, high iron content in basil with over 1000 mg/kg of d.m., should be found only in the seed, if this species is grown in arid areas as a wild plant [60].

The tree-plot obtained by the cluster procedure (UPGMA) showed basil genotypes grouped in the clusters with their respective distances. Cluster analysis classified 13 *Ocimum* genotypes into two distinct groups (Figure 1). In fact, one cluster included almost all genotypes studied, while the second one included only one. The average genotype distance (*D*) among the osmium cultivars based on the examined traits was 799, ranging from 36.4 (the most related accessions, Purple Ruffles and Osmin) to 3377.6 (the most distantly related, Hollander and Compact).

Cluster I. This group included 12 genotypes (all but Compact) this cluster was split off into three distinct sub-groups, defined as cluster IA, IB and IC. Sub-

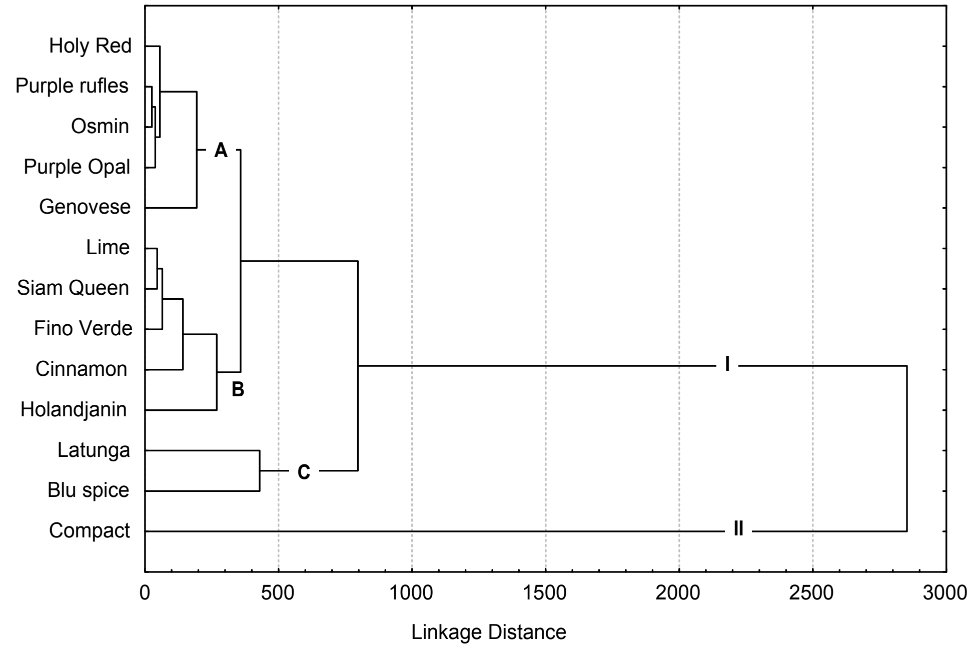

Figure 1. Cluster analyses of present minerals in herbs in basil genotypes.

clustering was done according to the Fe content. Subgroup (IA) consisted of five genotypes (Holy Red, Purple Ruffles, Osmin, Purple Opal and Genovese) with higher content of Fe than in the following genotypes: Lime, Siam Queen, Fino Verde, Cinnamon and Hollander, which formed sub-group (IB). IC sub-group separated Latunga and Blu Spice, with relatively high content of Fe.

Cluster II. It consisted only of one genotype, Compact. This cultivar is characterized by a very high content of Fe, Mn, Co, Cr, Ni and Pb. Cluster analysis pointed out to a considerable diversity in the *Ocumum* cultivars where the content of some heavy metals (Fe, Mn, Co, Cr, Ni and Pb) was a determinant criterion for genotypes clustering. This points out that even different genotypes of plant species accumulate a different amount of heavy metals, which should be of concern [61,62].

Heavy metal issue

Like all other plant species, medicinal plant growing in nature can accumulate heavy metals through complex absorption process, governed by numerous mutually influencing factors. Accumulation to certain extent depends on individual properties and genotype, while the total concentration of heavy metals in various soil is less important than their availability and mobility, which greatly depends on soil properties such as: pH, organic matter, clay content, etc. [41]. As heavy metals pose a hazard to human health, dispute that some of them are essential for people and plants (Fe, Mn, Cu, Co and Zn), monitoring of these metals in herbal parts is of great importance for public protection against the hazards with possibly toxic effects [63]. Heavy metal contamination of agricultural soil should especially be of concern as one of major environmental problems that can reduce a plant production and safety of plant products used as food [64]. Therefore, it is of crucial importance that the commercial production of this plant species take place at unpolluted or potentially uncontaminated agricultural soil, and it should be tested to the level of presence of heavy metals (Table 1) [65].

Heavy metal concentrations in basil plants grown on agricultural soil (chernozem) are shown in Table 4. Presented results reveal considerable variations in concentration depending on the element and basil genotype, simultaneously demonstrating that metal absorption significantly depends on genetic properties of tested types.

Heavy metals accumulate in plant tissue by the following order: Cu > Cr > Ni > Co > Pb > Cd, while the total uptake greatly depends on genotype biomass. Estimated concentration, mg/kg, in investigated genotypes varied for Cu 13.78–24.75, Cr 2.15–8.42, Ni 1.6–10.2, Co 1.75–5.07, Pb 0.14–2.84 and Cd 0.07–0.35, having values in the range as was proved in earlier basil studies [66]. Since these plants were grown under field conditions, the contamination level with the toxic heavy metals, Cr, Ni, Co, Pb and Cd, can be classified as very low, as expected. This investigation of contamination level is very important because some of herbal plants have a phytoremediation potential [67] where the genotype variation of heavy metal accumulation in herbal plants exists as well [68]. Hence, as recommended by the World Health Organization, all medicinal/herbal plant material should be tested to the presence of heavy metals and other pollutants, regardless of the place they have been grown [69].

Basil samples contain less than 0.3 mg/kg Cd and less than 3 mg/kg Pb measured in dry weight basis, as recommended for these toxic metals in medicinal plants by EC Regulation [70]. Besides, for this medicinal plant, toxic Ni content in the herb does not depend on soil pH [70], as found in this experiment. The tested genotype variety (Compact), which collects above 5 mg/kg at the soil pH > 7.0, has pointed out that genetic tendency of basil could have a stronger influence on Ni accumulation than soil pH property. The question of toxic Cr is still open; Cr content of analyzed herbal parts of the majority of genotypes is more than 2–3 mg/kg, which is regarded excessive, however non-toxic, concentration for cultivated plants [72]. Chromium content in the investigated basil plant is much lower (3.40–4.46 mg/kg), as found in some medicinal plants, which is considered non-toxic content (Khan 2013). Possible Co toxicity in basil tissues has not been estimated.

CONCLUSION

Mineral compositions of different basil (*Ocimim* spp.) genotypes vary, based on different genetic potential of investigated basil types. There is no reliable connection between shapes of the basil aboveground parts and its mineral content. According to dried herbal biomass, where average of 40 plants per basil genotype was tested, two genotypes should be recommended for further cultivation practice (Hollander and Fino Verde). However, the investigated basil mineral composition, promote some other basil genotypes (Compact, Lattuga and Blue spice) as a good raw material for further processing. According to the content of micronutrients, especially Fe, Mn and Zn, basil can support human dietary intake with this organically bound minerals. Especially the iron concentration in some basil genotypes was proved by statistical evaluation as genotypes with distinct mineral properties. Hence, Fe-rich genotype Compact (3576.0 mg/kg), which is in the cluster II with two other genotypes (Lattuga and Blue Spice) from cluster IC, with more than 1000 mg/kg of Fe (d.m.), could be treated as iron source originating from plant material and used for humans with low level Fe, consequently, with low level of hemoglobin.

Hierarchical cluster analysis allowed the assessment of similarity or dissimilarity and clarified some of the relationships among basil cultivars. Obtained dendrogram had two main clusters, the first one was split-off into three sub-groups. Further accumulation of data across the years might result in better precision of cultivar assessment.

According to the present study, commercial growth of basil was shown as a good practice, which can obtain a constant supply of herbal material for different purposes in pharmaceutical or food processing industry with no excess of heavy metal in herbal parts.

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IZVOD

MINERALNI SASTAV RAZLIČITIH GENOTIPOVABOSILJKA (*Ocimum* spp.)

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(Naučni rad)

U radu je ispitan mineralni sastav 13 različitih genotipova bosiljka (*Ocimum spp.* L.), sa ciljem da se odrede tipovi koji bi mogli da posluže kao dopunski izvori esencijalnih elementa u ljudskoj ishrani, kao i da se odrede genotipovi koji bi poslužili za proizvodnju herbe kao sirovine za farmaceutsku ili prehrambenu industriju. Takođe, u ovom istraživanju je testiran i potencijalni rizik vezan za zagađenje bosiljka teškim metalima pri njegovom komercijalnom gajenju na poljoprivrednom zemljištu. Mineralni sastav ispitivanih genotipova uglavnom je uslovljen njegovim genetskim karakteristikama, ukazujući da u ishrani čoveka ova lekovita biljna vrsta može poslužiti kao značajan izvor nekih od esencijalnih elemenata, naročito mikroelemenata (Fe, Mn i Zn), koji generalno doprinose jačanju ljudskog imuno sistema. Posebno je važno što su u ovom istraživanju identifikovani neki genotipovi bogati gvožđem, kao što je to Compact, kao genotip sa ekstremno visokim nivom Fe u herbi (3576,0 mg/kg), a koji bi zajedno sa genotipovima *Lattuga* (1585,6 mg/kg) i *Blue Spice* (1167,9 mg/kg) koji sadrže više od 1000 mg/kg Fe u suvoj materiji herbe, trebali da privuku posebnu pažnju kao izvori ovog elementa u ishrani ljudi kod kojih je evidentiran njegov nedostatak, načešće ispoljen sa pojavom anemije, odnosno, slabom sintezom hemoglobina. Gajenje bosiljka na poljoprivrednom zemljištu nije uslovilo povećanu akumulaciju teških metala (Cu, Co, Cu, Ni, Cr i Pb) u herbi, pa se može reći da su ovi proizvodi sa aspekta zagađenosti teškim metalima potpuno bezbedni. Klaster analiza je podelila ispitivane genotipove bosiljka (*Ocmium* spp.) u dve grupe. Uprkos različitosti između genotipova, sadržaj Fe, Mn, Co, Cr, Ni i Pb uticao je na jasnu podelu između klastera.

Ključne reči: Bosiljak • Genotip • Mineralni sastav • Gvožđe • Teški metali • Klaster analiza