Metal content in common daisy (*Bellis perennis* L.) and correspond soils from Niš city area (Serbia)

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ABSTRACT

Bellis perennis L. is a common species of daisy, of the Asteraceae family. It is usually found in grasslands, meadows, gardens, urban areas and areas near the roadsides. Emission of heavy metals from traffic activities is an important pollution source to roadside ecosystems. This study focused on ICP OES quantification of some metals of common daisy samples and their growing soils. Plant material (*B. perennis* L.) and belonging soils from 16 different locations of Niš city area (South-East Serbia) were used for the ICP OES determination of metal content. The concentrations of Al, Ba, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, V, and Zn were calculated on a dry weight basis. The pseudo-total metal content of equivalent growing soils was also determined. The study showed that heavy metal content of plant material and growing soils was below the maximally allowed concentrations or below the limit of detection, so we can say that contamination was detected neither in *B. perrenis* plant material nor in the growing soils.

Keywords: daisy, metals, ICP OES, contamination, soil

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Introduction

Bellis perennis L., also known as common daisy, is a plant species from *Bellis* genus and Asteraceae family. Asteraceae is a family of herbaceous plants, rarely bushes, lianas or low trees. They are mostly ground halophytes or epiphytes, rarely wetland and aquatic plants. The family currently has more than 30000 accepted species names, in around 1900 genera and 13 subfamilies. The *Bellis* genus consists of dicotyledonous species native in Europe, Mediterranean region and North Africa. Common daisy is perennial plant species native to western, central and northern Europe. It generally blooms from early to midsummer producing spherical blooms in a range of sizes (approx. 2–3 cm) with white ray florets (often tipped red) and yellow disc florets. The daisy blossom (rarely leaf or whole plant) is used in folk medicine for tea preparation (Kojić et al., 1998).

According to their biological function, elements can be divided into essential and non-essential. The essential elements are further classified into two categories: macro and microelements. Microelements, such as copper, iron, zinc, manganese, molybdenum, nickel, and cobalt are essential for the healthy growth and development of plants. The lack or complete absence of these metals can lead to severe disorders in the plant organism, even to death. Non-essential metals include lead, cadmium, mercury, arsenic, and chromium. They do not have any known beneficial role and only show a toxic effect on the plant organism (Alagić et al., 2013; Nagajyoti et al., 2010).

Heavy metals represent one of the contaminants in the environment. Besides natural activities, almost all human activities also have a potential contribution to produce heavy metals as side effects. Heavy metals are constituents of the Earth's crust, but concentrations of some of them, in many ecosystems, have reached toxic levels, primarily as a result of anthropogenic activity. Heavy metals directly cause harm by entering the body *via* soil and dust (Abrahams, 2002). In ecological terms, any metal, or metalloid causing a problem in the environment, or one that cannot be biologically destroyed, should be regarded as heavy metal. Metal pollution shows a negative effect on biological systems and does not undergo biodegradation. Toxic heavy metals (*e.g.*, lead, cobalt, cadmium) can be distinguished from other pollutants since they cannot be biodegraded but can be accumulated in living organisms (causing various diseases and disorders even in relatively lower concentrations) (Pelhivan et al., 2009; Tangahu et al., 2011). The typical elements cadmium, lead, zinc, and copper, which can be found in the roadside soils, can be transported through the food chain, and thus be very toxic to people. A total of 53 elements have been classified as heavy metals, which is more precisely defined as a group of elements whose density is higher than 5 g·mL^{-1} (Kastori et al., 1997). Their abundance in the place of work or the

environment can be a serious health and environmental risk because they are toxic, remain in the soils for a long time and accumulate in live systems through the food chain (Sarma, 2011).

The determination of some metal contents in the *B. perennis* L. samples and its growing soil, as well as the correlation between the concentration of metals in plant and soil samples, were objectives of our study. Particular interest is devoted to the potential accumulation of metals in plant tissue, due to the proximity of major roads in the urban area of Niš.

Experimental

Plant and soil material

Aerial parts of *B. perennis* and corresponding soil materials were collected in May 2017 from the 16 different location in Niš city area. The sampling locations are graphically represented in Figure 1.
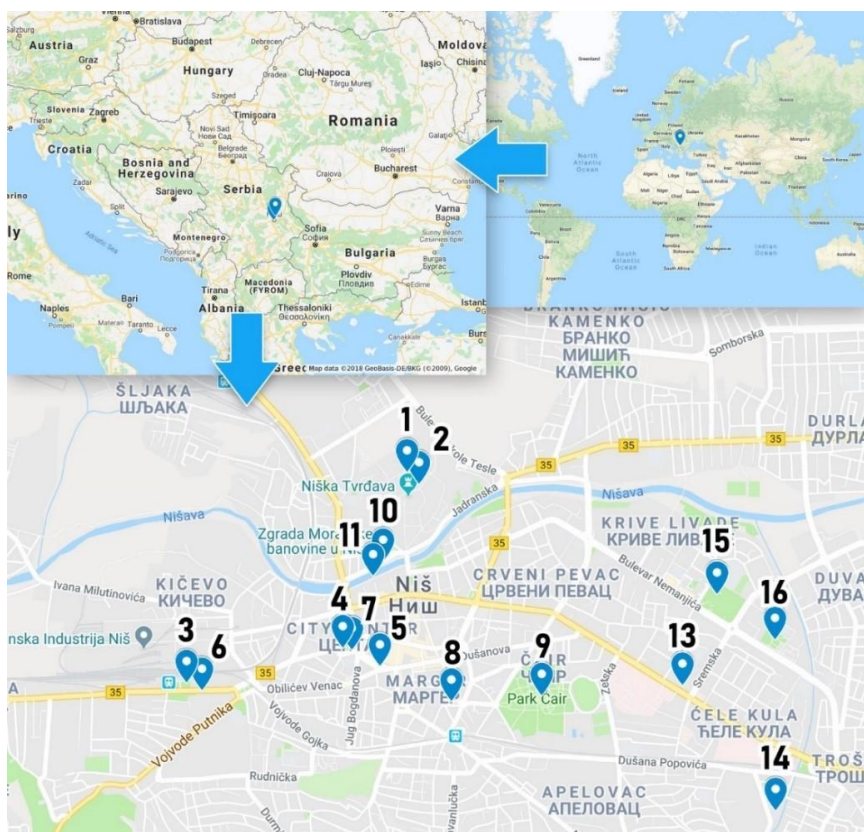

Figure 1. Graphical representation of sampling locations of common daisy from Niš city area

Reagents

Nitric acid (65%, m/m), hydrochloric acid (36%, m/m) and hydrogen peroxide (30%, m/m) were purchased from Merck (Darmstadt, Germany). Ultrascientific (USA) ICP multi-element standard solution $(20.00 \pm 0.10 \ \mu g \cdot mL^{-1})$ was used as a stock solution for calibration. Deionized water $(0.15 \ \mu S \cdot cm^{-1})$, obtained using MicroMed high purity water system (TKA Wasser auf bereitungs systeme GmbH, Germany), was used to prepare all standards and sample solutions. The plasma torch argon, with purity greater than 99.999%, was obtained from Messer (Messer Tehnogas AD, Serbia).

Instrumentation

The iCAP 6000 inductively coupled plasma optical emission spectrometer (Thermo Scientific, Cambridge, United Kingdom) with Echelle optical design and a Charge Injection Device (CID) solid state-detector was used for all mineral determinations. The analytical lines used for each element and the instrumental conditions are given in Table 2 and Table 3. Dry ashing method was carried out in the electric furnace (VIMS, Serbia) equipped with a microprocessor's program for the temperature $(\pm 1°C)$.

Table 1. Operating ICP OES parameters

Flush pump rate	100 rpm
Analysis pump rate	50 rpm
RF power	1150 W
Nebuliser gas flow	$0.7 \ L \cdot min^{-1}$
Cooling gas flow	$12 \ L \cdot min^{-1}$
Auxiliary gas flow	$0.5 \ L \cdot min^{-1}$
Plasma view	Axial

Plant samples preparation

The samples of the plant were put in the oven at 105 °C to remove the water content. The dried material was crushed with an electric mill into a fine powder. Around 5 g of the obtained powder was weighted with an analytical balance. Afterward, samples were ashed in a furnace for 20 h. The furnace was programmed to raise the temperature from starting 50 °C to 450 °C in the first 8 h, after which it was kept at constant 450 °C until the end of the process. The ash was dissolved in 4 mL of $HNO_3:H_2O$ (1:1 v/v), filtered and diluted to 50 mL using HNO_3 (5%, v/v) (Radojevic and Bashkin, 1999).

Soil sample preparation

The soil samples were dried in thin layers in an oven at 105 °C to remove all moisture and prepared according to a method for the acid digestion of sediments, sludges, and soils (EPA method 3050B). The dried material was passed through a 1 mm sieve, eliminating stones and roots. The obtained material was measured on an analytical balance (1 g) and transferred into the round bottom boiling flask. Afterward, 10 mL of diluted HNO_3 (1:1, v/v) was added and treated in reflux at 95±5 °C for 15 min. The sample was allowed to cool, 5 mL of concentrated HNO_3 was added, and reflux was continued for 30 min. Subsequently, 10 mL of $H_2O_2:H_2O$ (4 mL H_2O_2 and 6 mL H_2O) was added, and reflux proceeded for another 15 min. Another 10 mL of concentrated HCl was added, and reflux was continued for 15 min. After that, the solution was cooled, filtered through Whatman No. 41 filter paper and diluted to 100 mL with deionized water. Three replicates of each dried sample were analyzed.

Validation

Method for each element was created by selecting four wavelengths with the highest relative emission intensity. The calibration curve was constructed using three standard solutions. Two of them, a concentration of 2 mg·L^{-1} and 5 mg·L^{-1}, were prepared diluting the reference multi-standard while the third standard was deionized water. The working wavelength was selected based on the relative emission intensity, the standard deviation of the calibration parameters, the correlation coefficient, and the interference at wavelengths left and right of the selected wavelength.

The validation of the measurements based on ICP OES technique was checked using the linearity of the calibration curve, the limit of detection (LOD) and the limit of quantification (LOQ) (Table 2), and spike recovery test. The correlation coefficients of calibration curves assessed the linearity.

Quantification wavelengths for each element, the calibration parameters (k-slope and n-intercept), LOD, LOQ, and the coefficients of determination (r^2) are represented in Table 3.

Table 2. Emission wavelengths (λ), coefficients of determination of calibration graphs (r^2), limit of detection (LOD), limit of quantification (LOQ), and calibration curve parameters (n-intercept and k-slope) for each element analyzed

Element	λ (nm)	r^2	LOD (mg·L^{-1})	LOQ (mg·L^{-1})	N	k
Al	309.271	0.99914	0.00441	0.01470	5964	2548
Ba	455.403	0.99902	0.00004	0.00013	22930	390821
Cd	226.502	0.99992	0.00021	0.00071	36	5673
Co	228.616	0.99972	0.00030	0.00100	35	4816
Cr	267.716	0.99948	0.00068	0.00228	47	6765
Cu	324.754	0.99982	0.00043	0.00142	1742	17377
Fe	259.940	0.99930	0.00042	0.00142	11389	10302
Mn	257.610	0.99926	0.00011	0.00035	15615	36963
Ni	221.647	0.99926	0.00078	0.00259	-120	2443
Pb	220.353	0.99978	0.00200	0.00667	14	585
V	309.311	0.99920	0.00080	0.00266	-3554	24141
Zn	213.856	0.99880	0.00011	0.00038	5072	9134

Statistical analysis

Pearson's correlation analysis and Agglomerative Hierarchical Cluster Analysis-(AHC) were used on original variables – metal concentration in soils and plant materials. In the case of AHC analysis, Dissimilarity matrix, and Euclidean distance as a measure of diversity were used. Complete linkage was used as the grouping criterion. Results were expressed as the mean±standard deviation.

Results and Discussion

The metal content of soil samples and *B. perennis* L. plant samples from 16 different locations was determined by the ICP OES method. The concentrations of twelve elements (Al, Ba, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, V, and Zn) determined in the plant, and soil samples are summarized in Tables 3 and 5. The results are represented as milligram of metal per kilogram of dry weight (mg·kg^{-1} dry weight).

Content of metals found in soil samples from sixteen different locations is represented in Table 3. Aluminum (0.61-1.36%) and iron (0.38-0.63%) were the most abundant elements. It is known that aluminum is the third most abundant element in the Earth's crust (about 8.2%), and it is naturally expected high concentration to be found in the analyzed soil samples. Nevertheless, the content of aluminum is lower than the average values for typical soils, ranged between 0.5% and 5% (Radojević and Bashkin, 1999). This phenomenon is because aluminum is constituent of aluminosilicate rocks, which are insensitive on the treatment applied.

Micronutrients (boron, copper, iron, manganese, molybdenum, and zinc) are essential elements. These elements are used in small quantities and despite low requirements, plants functions, growth, and yields could be limited if those are unavailable for plant uptake (Wiedenhoeft, 2006). Some authors also consider Ni as micronutrient (Berker and Pilbeam, 2007). The same authors claim that Co and V are useful elements that enhance the plants' growth. Besides iron, which is the most abundant element in soil, of the other micronutrients detected, manganese was the most prevalent (80-387 $mg \cdot kg^{-1}$). Zinc was the next most abundant micronutrient (1.77-369 $mg \cdot kg^{-1}$), with the highest content in the soils from the Niš fortress 1 and Niš fortress 2 sampling locations (369 $mg \cdot kg^{-1}$ and 139 $mg \cdot kg^{-1}$, respectively).

The contamination of soils is caused by the accumulation of heavy metals from various sources (Khan et al., 2008; Zhang et al., 2010), and those most commonly found at contaminated sites are lead, chromium, arsenic, cadmium, copper, mercury and nickel (Raymond et al., 2011). The content of analyzed pollutants in this study ranged from 0.54-1.53 $mg \cdot kg^{-1}$ for cadmium, 5.2-72 $mg \cdot kg^{-1}$ for copper, 10.1-20.9 $mg \cdot kg^{-1}$ for chromium, 12.6-47.8 $mg \cdot kg^{-1}$ for lead and 17.3-35.1 $mg \cdot kg^{-1}$ for nickel. The allowed levels of toxic elements, cadmium, and lead for agricultural soils approved by the EPA Clean Water Act are 39 ppm and 300 ppm, respectively. Accordingly, toxic metals concentrations in the analyzed soil samples from this study were below the maximal allowed values. The obtained results were compared to those for the metal content of the typical soil obtained by the Radojević and Bashkin (1999) and Aloway (1995). Those results are represented in Table 4, and they are following the previous studies.

Table 3. Metal contents[*] in the soil samples

Sample	Al	Ba	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
1	0.61±0.04	80±2	0.75±0.03	0.39±0.11	10.1±0.5	72±1	0.38±0.01	138±2	21.8±1	47.8±1.4	34±1	369±58
2	0.75±0.01	86±3	0.89±0.02	4.71±0.11	12.1±0.3	33±1	0.44±0.01	171±7	22.8±0.4	37.6±0.7	38.1±0.4	139±4
3	0.83±0.01	66±1	1.16±0.03	5.76±0.05	15.4±0.3	19.9±0.3	0.52±0.01	131±2	27.3±0.2	28.2±0.1	41.3±0.3	37.6±0.6
4	0.73±0.01	65±2	0.99±0.03	5.19±0.08	12.3±0.1	14.6±0.1	0.47±0.01	125±6.	23.9±0.3	26.8±0.5	38.6±0.4	34.0±0.9
5	0.71±0.03	65±2	0.95±0.02	4.32±0.03	11.2±0.3	49±1	0.44±0.02	106±5	20.1±0.2	45.2±0.3	34.1±0.9	42.7±0.7
6	1.07±0.02	67±2	1.08±0.03	5.56±0.13	15.9±0.4	12.6±0.3	0.51±0.01	141±3	27.1±0.6	18.1±0.4	41±1	14.8±0.1
7	1.11±0.03	61±1	1.23±0.02	6.66±0.09	19.5±0.3	10.6±0.2	0.56±0.01	159±3	32.5±0.5	14.1±0.3	46±1	20.3±0.6
8	0.92±0.02	55±1	0.92±0.02	5.03±0.04	12.9±0.2	10.6±0.3	0.46±0.01	118±2	21.7±0.2	13.1±0.1	41±1	56.7±0.8
9	1.15±0.03	68±3	1.28±0.01	5.93±0.06	17.1±0.3	10.5±0.2	0.54±0.01	131±3	27.4±0.2	13.0±0.2	43±1	18.7±0.3
10	0.86±0.03	57±2	1.05±0.02	5.73±0.11	16.3±0.4	8.8±0.3	0.50±0.01	128±3	29.7±0.6	26.3±0.5	38±18	79±2
11	1.23±0.02	61±1	1.23±0.02	6.86±0.15	20.2±0.5	7.4±0.2	0.53±0.01	145±3	35.1±0.7	15.6±0.3	44.5±0.5	18.3±0.7
12	1.36±0.05	51±2	1.07±0.03	8.93±0.34	20.2±0.6	5.2±0.2	0.58±0.03	372±19	32.2±1.2	15.1±0.1	45±2	41.8±0.5
13	1.13±0.03	62±1	1.53±0.03	9.01±0.02	20.9±0.3	9.2±0.2	0.63±0.03	3879±13	32.6±0.4	29.9±0.4	52±1	32±1
14	0.91±0.01	50±1	0.54±0.02	4.45±0.13	12.1±0.2	5.9±0.2	0.40±0.01	80±2	21.9±0.4	6.5±0.2	38.1±0.9	<LOD[**]
15	0.89±0.02	50±3	0.73±0.02	5.95±0.14	16.0±0.2	5.65±0.01	0.46±0.01	126±1	29.6±0.7	12.6±0.3	36±2	1.77±0.09
16	1.30±0.01	57±3	0.84±0.01	6.50±0.04	19.5±0.2	6.26±0.07	0.50±0.02	135±2	17.3±0.1	13.0±0.1	44.8±0.3	18.0±0.2

[*]The metal content, mean value ± standard deviation, is given in mg·kg^{-1} (dry weight), except for the Al and Fe which values are given in %;

[**]<LOD-below the limit of detection.

Table 4. Normal content range[*] of different elements in soil and plants (ppm)

Element	Typical soils[1]	Normal level in the soils[2]	Normal level in the plants[2]	Uncontaminated agricultural soils[3]
Al	5000-50000	-	-	-
Cd	<0.01-8	0.01-2.0	0.1-2.4	0.27
Co	-	0.5-65	0.02-1	-
Cr	0.9-1500	5-1500	0.03-14	-
Cu	<1-390	2-250	5-20	-
Mn	<1-18300	20-10000	20-1000	-
Ni	0.1-1520	2-750	0.02-5	-
Pb	<1-890	2-300	0.2-20	0.1-5
V	0.8-1000	3-500	0.001-1.5	-
Zn	1.5-2000	1-900	1-400	-

[*1]Radojevic i Bashkin, 1999 ; [2]Adapted from Aloway, 1995; [3]Wiersma, 1986 and Holmgren et al., 1993

Comparing the results for the content of individual metals from different locations, obtained in this study, it was noted that concentrations are of the same order of magnitude, with the exception of zinc content from the Niš fortress soil samples.

Content of metals found in *Bellis perennis* L. samples from sixteen different locations is represented in Table 5.

The most abundant elements in *B. perennis* L. samples from different locations were found to be aluminum and iron. Content of aluminum and iron ranged from 102-856 mg·kg^{-1} and 9.1-231 mg·kg^{-1}, respectively. Micronutrients content in the plant samples ranged from 102-231 mg·kg^{-1} for iron, 0.37-18.5 mg·kg^{-1} for manganese, and 0.6-1.92 mg·kg^{-1} for copper while the zinc content in all analyzed samples was below the LOD.

The lead content in the plant samples ranged from 0.57-4.96 mg·kg^{-1}, while the cadmium content was below the detection limit. According to the Joint Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) (1999), the maximum allowed concentrations of cadmium and lead in fresh vegetables are 0.2 and 1.0 μg per gram of fresh plant, respectively. The concentrations of both elements are below the allowed concentrations. Lead content calculated on raw vegetable mass is about 0.20 mg·kg^{-1}.

Table 5. Metal contents[*] in the *B. perennis* L. samples

Sample	Al	Ba	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
1	542±2	6.23±0.14	<LOD[**]	0.187±0.003	0.88±0.01	1.02±0.01	139±1	0.37±0.07	5.16±0.02	4.84±0.11	11.4±0.2	<LOD
2	655±8	7.1±0.1	<LOD	0.04±0.01	1.20±0.05	<LOD	149±3	18.5±0.1	5.25±0.04	3.06±0.06	11.1±0.1	<LOD
3	599±19	6.06±0.03	<LOD	0.040±0.003	1.07±0.03	0.79±0.01	231±7	14.5±0.1	5.46±0.11	3.33±0.03	11.6±0.1	<LOD
4	795±18	5.5±0.1	<LOD	0.067±0.003	4.08±0.14	1.14±0.02	264±9	9.6±0.3	8.40±0.07	4.16±0.09	12.6±0.1	<LOD
5	346±17	4.2±0.1	<LOD	0.06±0.01	0.42±0.02	1.23±0.07	88±1	13.3±0.6	4.72±0.12	2.12±0.11	11.5±0.2	<LOD
6	770±1	8.74±0.06	<LOD	0.12±0.01	1.99±0.09	1.92±0.01	173±2	4.83±0.10	5.83±0.04	4.96±0.05	11.80±0.04	<LOD
7	755±14	4.9±0.1	<LOD	0.19±0.01	1.38±0.04	0.60±0.02	114±4	7.38±0.64	4.61±0.07	2.10±0.05	11.0±0.1	<LOD
8	288±15	1.6±0.3	<LOD	0.03±0.01	0.49±0.05	<LOD	54±6	<LOD	3.99±0.03	0.76±0.02	9.5±0.1	<LOD
9	289±12	1.7±0.2	<LOD	<LOD	0.62±0.05	<LOD	35.7±0.4	<LOD	3.94±0.01	0.25±0.02	10.6±0.3	<LOD
10	757±18	2.1±0.1	<LOD	0.09±0.01	0.41±0.01	<LOD	119±5	<LOD	4.98±0.11	2.47±0.07	11.43±0.03	<LOD
11	856±27	4.4±0.1	<LOD	0.057±0.003	3.35±0.03	<LOD	163±8	7.46±0.10	6.09±0.06	1.20±0.03	11.0±0.3	<LOD
12	233±2	0.34±0.02	<LOD	0.15±0.04	0.21±0.01	1.40±0.03	16.5±0.1	1.65±0.43	4.03±0.02	0.58±0.04	9.8±0.3	<LOD
13	299±8	3.26±0.01	<LOD	0.157±0.003	0.47±0.02	1.04±0.05	58.2±0.9	4.53±0.12	4.20±0.19	1.17±0.07	11.3±0.1	<LOD
14	161±6	3.7±0.1	<LOD	0.18±0.01	0.42±0.03	1.13±0.04	9.1±1.9	11.4±0.6	3.94±0.02	0.57±0.04	10.9±0.1	<LOD
15	158±6	1.52±0.04	<LOD	0.15±0.01	0.11±0.01	1.01±0.01	9.3±1.8	12.9±0.1	3.68±0.03	0.68±0.02	10.5±0.1	<LOD
16	102±5	1.91±0.03	<LOD	0.12±0.01	0.12±0.01	1.00±0.02	12.8±0.7	7.24±0.28	3.76±0.05	0.91±0.06	10.5±0.1	<LOD

[*]The metal content, mean value ± standard deviation, is given in mg·kg^{-1} (dry weight); [**]<LOD - below the limit of detection

Correlation analysis was performed in order to determine the correlation between the concentrations of the metals in the soil and plant tissue in analyzed samples. A statistically significant positive correlation between the concentration of barium in the soil and the concentration of barium in plant samples ($r = 0.696$; $p = 0.002$) was observed.

The Agglomerative Hierarchical Cluster Analysis (AHC) of the obtained data sets (concentration of metals in soil and plant material) was performed using the XLSTAT Excel plug-in program, version 2014.4. In the ACH analysis, the Dissimilarity matrix algorithm, and the Euclidean distance as a measure of diversity were applied, and the complete linkage was used as the grouping criterion. It enables to statistically group the analyzed samples of the plant as well as soil samples according to the concentrations of the specified metals in them. The obtained dendrograms are shown in Figures 2 and 3.
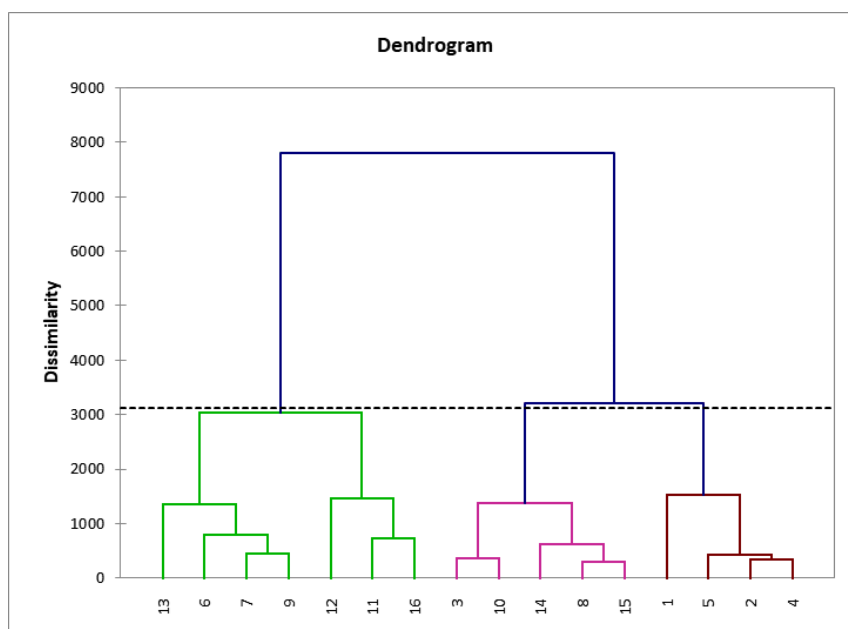

Figure 2. Cluster analysis of soil samples

The results of cluster analysis of soil samples show the existence of three different clusters. The most numerous is the C3 group (samples 6, 7, 9, 11, 12, 13, 16), where the soil samples have been distinguished by the high content of aluminum and iron.
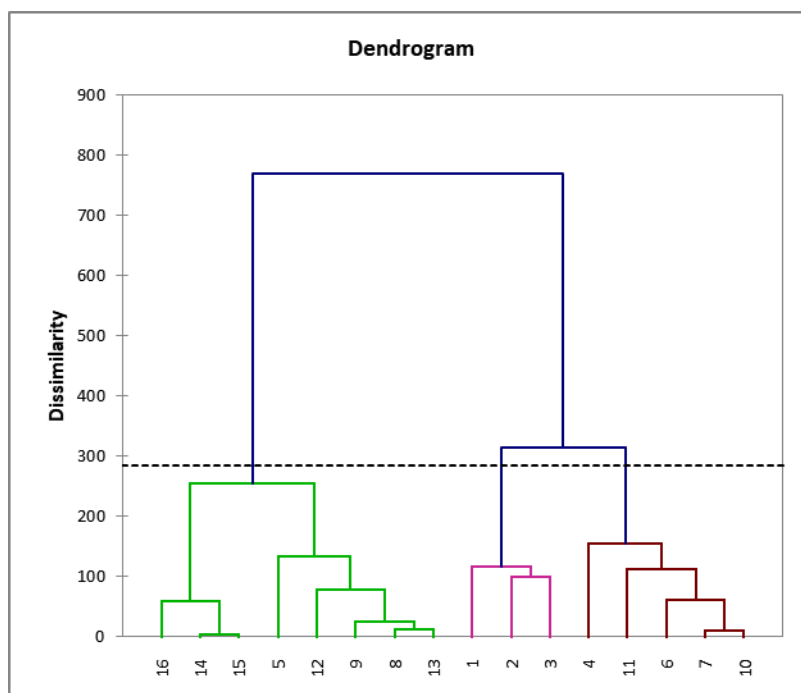
Figure 3. Cluster analysis of plant samples

Grouping the plant material samples, the three different clusters were distinguished. Cluster C3 (5, 8, 9, 12, 13, 14, 15, 16) is the most numerous and includes plant samples with 8 locations. This cluster is characterized by a low concentration of aluminum, barium, chromium, and lead.

Conclusion

The content of 12 metals (Al, Ba, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, V, and Zn) has been determined, in soil samples as well as in the plant samples of species *Bellis perennis* L. from sixteen different locations of the urban area of Niš city. Heavy metals contamination was detected neither in soil nor plant samples analyzed. The mean concentrations of elements determined were within the range of the maximum allowed values given by authorities. No correlation between the heavy metals as the potential pollutants, either in soil or plant samples, and the proximity of the road was found.

Conflict-of-Interest Statement
Declarations of interest: none

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