

Original Scientific paper

UDC 622.333:[582.683.2 582.929.2

DOI: 10.7251/afts.2018.1019.085B

COBISS.RS-ID 7713304

## MINERAL COMPOSITION OF PLANT EXTRACTS FROM THE FAMILY BORAGINACEAE

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### ABSTRACT

The mineral composition of plant extracts from the Boraginaceae family was examined in this paper. The research includes the plants: *Anchusa officinalis* L., *Echium vulgare* L. and *Echium italicum* L. Content analysis of the elements was done using inductively coupled plasma with a mass spectrophotometer. The results of the study showed that the extracts tested are rich in mineral composition, while the concentration of toxic elements is below the corresponding limit maximum permitted concentration according to the recommendation of the World Health Organization.

Key words: *Boraginaceae*, *extract*, *mineral composition*

### INTRODUCTION

Plants for growth and reproduction requires different amounts of mineral matter. While some plant species require the presence of certain elements in soil at higher concentrations, others are more tolerant to a higher concentration of essential elements, which would be toxic to most other plant species [1]. The ability of plants to selectively accumulate essential elements varies in different species, and mainly depends on the geochemical characteristics of the soil type [2].

The study of mineral plant composition aims to quantitatively analyze their nutritional value, as well as the analysis of potential contamination with heavy metals. The general risk of heavy metals is conditioned by their ability to bioaccumulate and concentrate when moving along trophic chains because they can not be degraded or transformed during chemical processes. In addition, the removal of heavy metals from the organisms is difficult because they are firmly bound to proteins and other components of cellular structures [3]. Consequently, the detection and quantification of elements in plants, primarily intended for human consumption, is a very important stage in examining their quality.

The Boraginaceae family originated in Asia and includes a family of flowering plants with about 2700 species and 147 genera. Plants from the Boraginaceae family are traditionally used in the treatment of fever, asthma, kidney stones, diuretics and wound healing [4]. The plant species *Anchusa officinalis* L., *Echium vulgare* L. and *Echium italicum* L. prefer sunny, warm and dry habitats, and they are found

them in the pastures, grasslands, untreated land and the edges of the road [5]. Plants thrive on low quality soils, and since they have well-developed root system tolerate drought.

The plant material was collected from Brđanska gorge near Gornji Milanovac. Brđanska gorge is characterized by serpentine base and specific steppe flora with a large number of endemic species [6]. The proximity of the road and the local landfill indicates that anthropogenic influence has been performed at this site.

A significant contribution of this research is that this is the first report on the mineral composition of the extracts of the plants tested.

## MATERIAL AND METHODS

The plant species *Anchusa officinalis* L., *Echium vulgare* L. and *Echium italicum* L. were collected in 2013 at Brđanska gorge locality near Gornji Milanovac. Plants are crushed using a cylindrical crusher. The plant material, previously defatted with petroleum ether (40 ° C), was extracted with a series of solvents in Soxhlet's apparatus. The resulting solutions were left to cool and after 24 h their evaporation on a rotary vacuum evaporator at 40 ° C was carried out.

## EXAMINATION OF MINERAL COMPOSITION OF PLANT EXTRACTS

The element content analysis was performed using inductively coupled plasma with mass spectrophotometer (ICP-MS) "iCap Q" (Thermo Scientific, Bremen, Germany), which contains a collision cell and works in KED mode. The analysis was done at the Faculty of Agriculture in Cacak. The determination was based on the measurement of the concentration of the following isotopes:  $^{23}\text{Na}$ ,  $^{24}\text{Mg}$ ,  $^{39}\text{K}$ ,  $^{44}\text{Ca}$ ,  $^{52}\text{Cr}$ ,  $^{55}\text{Mn}$ ,  $^{57}\text{Fe}$ ,  $^{59}\text{Co}$ ,  $^{60}\text{Ni}$ ,  $^{63}\text{Cu}$ ,  $^{66}\text{Zn}$ ,  $^{75}\text{As}$ ,  $^{77}\text{Se}$ ,  $^{111}\text{Cd}$ ,  $^{118}\text{Sn}$ ,  $^{202}\text{Hg}$  and  $^{208}\text{Pb}$ . Before to reading the contents of the elements in the ICP-MS samples, the physical and electrical parameters of the instrument were used by using the Thermo Scientific Tune B (calibration solution). The calibration curve contained five points (including zero) for each isotope:  $^{23}\text{Na}$ ,  $^{24}\text{Mg}$ ,  $^{39}\text{K}$ ,  $^{44}\text{Ca}$ ,  $^{52}\text{Cr}$ ,  $^{55}\text{Mn}$ ,  $^{57}\text{Fe}$ ,  $^{59}\text{Co}$ ,  $^{60}\text{Ni}$ ,  $^{63}\text{Cu}$ ,  $^{66}\text{Zn}$  and  $^{77}\text{Se}$  in the range 0.1-2.0 mg / L;  $^{75}\text{As}$ ,  $^{111}\text{Cd}$ ,  $^{202}\text{Hg}$  and  $^{208}\text{Pb}$  - in the range of 0.1-2.0 mg / L;  $^{118}\text{Sn}$  in the range 0.1-0.4 mg / L. All solutions were prepared in 2% nitric acid.

During reading, in parallel with the samples, the multielementary internal standard ( $^6\text{Li}$ ,  $^{45}\text{Sc}$ -10 ng/mL;  $^{71}\text{Ga}$ ,  $^{89}\text{Y}$ ,  $^{209}\text{Bi}$ -2 ng/mL) is introduced into the system. The data processing software automatically reads the values for the elements from the internal standard and expresses them as % of the first reading. Based on this, the software performs an automatic correction of the observed sample concentration for a percentage of the reduced or increased intensity of the internal standard. The quality control of the test was carried out using the ERM-CE278k certified reference material.

## RESULT AND DISCUSSION

In soil and air, many contaminants and residues are present, such as microorganisms, radionuclides and toxic metals, which plants can adopt during growth and development and which pose a global threat to human health. Therefore, many control measures in plant medicine have been developed, and the first and most important step is to control the quality of plants and herbal materials. In this regard, the World Health Organization has developed a strategy on the application of traditional herbal remedies, with a set of technical guidelines and documents relating to the safety, efficacy and quality of medicinal herbs and plant material [7].

The origin of heavy metals in the soil is primarily geochemical, which means that they originate from the lithosphere and their concentration in the soil depends on the contents in the rocks from which the parent substrate has undergone [8]. However, the development of industry and the intensification of agriculture caused the application of various contaminating materials to the soil, so the concentration

of heavy metals on some surfaces increased due to anthropogenic impact. The behavior of heavy metals in soil is conditioned by many factors that can affect their mobility and accumulation by plants, and the most important are the soil reaction and the content of organic matter [9]. In addition to these, other factors may also affect their mobility and harmful effects, such as humidity, calcium carbonate content, hydrated oxides of iron and aluminum, exchange capacity of cations, redox potentials and groundwater levels [10]. Therefore, the detection and quantification of elements in plants is a very important stage in examining their quality.

The results of the analysis of the elements content in the tested extracts of the *A. officinalis* L. plant, using inductively coupled plasma with a mass spectrophotometer, are shown in Table 1. The most common macroelements in all the examined extracts are potassium, calcium and magnesium. The potassium concentration ranged from 32234.63  $\mu\text{g/g}$  in the ethyl acetate extract to 89897.18  $\mu\text{g/g}$  in petroleum extract, calcium from 637.86  $\mu\text{g/g}$  in chloroform extract to 14942.21  $\mu\text{g/g}$  in acetone extract and magnesium from 2552.31  $\mu\text{g/g}$  in chloroform extract to 7409.54  $\mu\text{g/g}$  in acetone extract. While sodium was the most prevalent in the *A. officinalis* L. ethyl acetate extract with 2420.0  $\mu\text{g/g}$ . The content of the microelement in the examined extracts of the *A. officinalis* L. plant ranged from 0.076  $\mu\text{g/g}$  of selenium in an acetone extract to 477.86  $\mu\text{g/g}$  of the magnesium content in the chloroform extract of the *A. officinalis* plant.

The most prevalent microelements in all tested samples are Mn, Cu, Fe, Zn and Ni. In the analyzed extracts of the *A. officinalis* plant, Mn is most concentrated in the chloroform extract (477.86  $\mu\text{g/g}$ ) and Cu (106.26  $\mu\text{g/g}$ ) in the ethyl acetate extract of *A. officinalis*. While Co, Cr, Sn and Se are elements that are found in explicit extracts at very low concentrations. The study of the concentration of macro- and microelements of the extracts of the daze root from the Serbian confirmed that they are rich in mineral composition [11].

Table 1. The concentration of elements ( $\mu\text{g/g}$ ) in extracts of the plant *Anchusa officinalis* L.

Elements/ Extras	Ethanol	Ethyl acetate	Chloroform	Petroleum ether	Acetone
<i>Macroelements</i> (ppm= $\mu\text{g/g}$ )					
Na	230.68	2420.0	122.02	739.65	307.02
Mg	3552.40	7506.03	2552.31	6100.65	7409.54
K	83875.16	32234.63	34465.23	89897.18	61722.99
Ca	8232.41	4156.32	637.86	13976.00	14942.21
<i>Microelements</i> (ppm= $\mu\text{g/g}$ )					
Cr	0.352	0.200	0.298	0.401	0.700
Mn	54.03	2.94	477.86	52.61	21.05
Fe	61.12	6.53	11.00	36.32	9.39
Co	0.345	0.189	0.482	0.383	0.129
Ni	3.610	26.975	9.876	6.622	5.549
Cu	18.09	106.26	21.43	36.19	38.50
Zn	21.01	7.28	10.15	12.85	21.90
Se	0.376	0.982	0.352	0.982	0.076
Sn	<0.4	<0.4	<0.4	<0.4	<0.4
<i>Toxic elements</i> ( $\mu\text{g/g}$ )					
As	0.1075	0.0212	0.2786	0.2008	0.0625
Cd	0.0025	0.1308	0.0083	0.0055	0.00092
Hg	0.00088	0.00096	0.0168	0.1077	0.0022
Pb	1.5614	0.9973	0.6835	1.773	0.7843

Table 2 shows the obtained results of the analysis of the contents of the elements in the tested extracts of the *E. vulgare* L. plant. From the examined macroelements, potassium, calcium and magnesium are the elements that dominate the tested extracts, unlike the less dominant sodium. The potassium content was the highest in chloroform 91818.24  $\mu\text{g/g}$  and ethyl acetate extract with 87049.80  $\mu\text{g/g}$ , calcium 18589.73  $\mu\text{g/g}$  in ethanol and magnesium 7893.25  $\mu\text{g/g}$  in acetone extract. The obtained values of the

microelements in the examined extracts of the *E. vulgare* L. plant ranged from 0.089 µg/g of selenium in an ethyl acetate extract to 89 µg/g Fe in the acetone extract of the *E. vulgare* L. plant. From the examined microelements, manganese, iron, copper and zinc are elements that are present at significantly higher concentrations, unlike Co, Cr, Sn and Se, which is in agreement with the obtained concentrations of microelements of selected plant species from the same site [12].

The significantly higher concentration of copper in relation to zinc is due to the antagonistic interaction of the adoption of the elements of Zn and Cu, which is reflected in the fact that the adoption of one reduces the acceptance of another element, which was confirmed by other authors in their research [13]. Iron with 89 µg/g and 82 µg/g manganese in the acetone extract are microelements with the highest measured values.

Table 2 The concentration of elements (µg/g) in extracts of the plant *Echium vulgare* L.

Elements/ Extras	Ethanol	Ethyl acetate	Chloroform	Petroleum ether	Acetone
<i>Macroelements (ppm= µg/g)</i>					
Na	150.52	318.95	602.57	241.18	254.90
Mg	5845.06	6273.74	4882.01	3563.40	7893.25
K	30936.51	87049.80	91818.24	83834.16	50113.22
Ca	18589.73	7828.78	7273.24	8222.41	14362.40
<i>Microelements (ppm= µg/g)</i>					
Cr	0.232	0.400	0.932	0.368	0.322
Mn	49.76	15.32	9.88	56.21	82.00
Fe	26.12	21.34	42.34	62.35	89.00
Co	0.227	0.161	0.108	0.345	0.166
Ni	0.320	26.319	7.516	3.615	2.661
Cu	23.23	34.31	42.23	32.00	14.32
Zn	17.69	32.17	28.47	28.01	10.39
Se	0.242	0.089	0.352	0.362	0.249
Sn	<0.4	<0.4	<0.4	<0.4	<0.4
<i>Toxic elements (µg/g)</i>					
As	0.0826	0.0491	0.0619	0.1175	0.1008
Cd	0.0001	0.00098	0.0090	0.0095	0.0014
Hg	0.0084	0.0210	0.0197	0.0112	0.0208
Pb	0.4498	2.4611	1.0202	1.5744	51.1802

The content of the macro-, micro- and toxic elements of the tested extracts of the plant *E. italicum* L. are shown in Table 3. The relatively high concentration of potassium, calcium and magnesium is characteristic for all investigated extracts. The chloroform extract had the highest value of potassium 89907.18 g/g, petroleum ether extract had the highest value of calcium 18595.73 g/g and ethanol extract had the highest value of magnesium 7506.03µg/g. Of the investigated microelements, Mn, Cu, Fe, Zn and Ni are the most represented, while the Co, Cr, Sn and Se elements are significantly less represented.

The extremely high concentration of manganese is read in acetone (483.86 µg/g) and copper (117.12 µg/g) in the ethanol extract of the *E. italicum* plant. Since the content of the toxic elements is below the MDK value, it can be concluded that the plant extracts can be used for human use. The obtained results are partially in agreement with the study of the concentration of metals of selected plant species from Brđanska gorge [12], which indicated the high content of Ca and Mg, but also significantly higher contents of Cr, Co, Cd, Pb, Ni, Zn and Fe in relation to this research.

The type of plant, the type of extrusion and the geological basis were influenced on the content of the investigated elements. Calcium, phosphorus and manganese are the main antagonistic elements that prevent the absorption and metabolism of many minerals [12]. Extremely low concentrations of Cd in

all three investigated plant species are probably due to the existence of antagonism in the adoption of Cd and Ca elements, which Bolan et al. confirmed in their study [14].

Table 3 The concentration of elements ( $\mu\text{g/g}$ ) in extracts of the plant *Echium italicum* L.

Elements/ Extras	Ethanol	Ethyl acetate	Chloroform	Petroleum ether	Acetone
<i>Macroelements (ppm= <math>\mu\text{g/g}</math>)</i>					
Na	2432.06	312.02	752.15	156.22	160.60
Mg	7506.03	7419.44	6110.65	5842.09	2561.01
K	32244.63	61732.99	89907.18	30941.51	34475.23
Ca	4154.32	14953.21	13981.00	18595.73	643.86
<i>Microelements (ppm= <math>\mu\text{g/g}</math>)</i>					
Cr	0.202	0.701	0.407	0.243	0.300
Mn	12.90	32.85	72.49	63.52	483.86
Fe	16.99	10.88	30.29	32.12	13.43
Co	0.199	0.179	0.463	0.287	0.492
Ni	26.972	5.53	6.621	0.329	9.879
Cu	117.12	38.59	36.21	23.20	21.49
Zn	11.28	42.90	12.85	16.69	10.15
Se	0.929	0.077	0.100	0.242	0.352
Sn	<0.4	<0.4	<0.4	<0.4	<0.4
<i>Toxic elements (<math>\mu\text{g/g}</math>)</i>					
As	0.0212	0.0625	0.2048	0.0866	0.2836
Cd	0.1528	0.0052	0.0105	0.0021	0.0163
Hg	0.0016	0.0092	0.1117	0.0134	0.0208
Pb	1.0003	0.7723	1.7710	0.4528	0.6895

Plants acquire a certain amounts of metal, some of which are important for human consumption, others are even necessary, but some can also be toxic. Contamination of soil and plants is conditioned on one side by air pollution, exhaust gases, climatic factors, and on the other hand by using pesticides in agricultural production. Pesticides containing arsenic and mercury in the last few years have long been used in agricultural production, while in some underdeveloped countries they are still in use [10]. High concentrations of heavy metals in natural environments have a toxic effect on animals, plants and microorganisms, which contributes to the prevention of biotope development, reduction of biodiversity and productivity of the ecosystem [15,16,17,18].

Therefore it is very important to determine the content of metals in plant material, primarily in the material used for medical, nutritional and cosmetic purposes. Examination of the contents of elements of plant species *Anchusa officinalis*, *Echium vulgare* and *Echium italicum* showed that their extracts are rich mineral composition, with the highest concentrations of potassium, calcium and magnesium, which is to be expected, because the macroelements such plants necessary in large quantities. The content of all the micronutrients in the examined extracts is at a lower concentration, and the content of the toxic elements in the plants is below the appropriate limit maximum permitted concentration according to the recommendation of the World Health Organization [7].

## CONCLUSION

The plant species *Anchusa officinalis* L., *Echium vulgare* L. and *Echium italicum* L. were collected at Brđanska gorge near Gornji Milanovac. On the basis of the analysis of the extracts of the investigated plants, it can be concluded that the relatively large content of K, Ca and Mg is characteristic of all investigated plant extracts. The most prevalent microelements in all tested samples are Mn, Cu, Fe, Zn and Ni. Cobalt, chromium, tin and selenium are elements that are in very small quantities in the

extracts examined or their content is below the detection limit of the applied method. The low content of toxic elements shows that extracts of satisfactory quality for human use, that they do not contain toxic elements above the permitted limits. The type of plant, the type of extrusion and the geological basis were influenced on the content of the metals.

(Received March 2018, accepted August 2018)

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