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INORGANIC ELEMENTS CONTENT IN ALL PARTS OF COMFREY (*SYMPHYTUM* SPECIES): IS COMFREY A SAFE RAW MATERIAL FOR MEDICINAL USE?

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ABSTRACT. This study systematically investigated the inorganic elemental composition (Ca, K, Mg, Na, P, Al, As, B, Ba, Be, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, Si, V, and Zn) of individual plant parts of comfrey, a medicinal plant collected from three locations in Serbia. Inductively coupled plasma optical emission spectrometry (ICP-OES) was employed to determine the elemental composition of gavez. Statistical evaluation was performed using Spearman's rank correlation. Potassium was the most abundant macroelement (39.5 mg/g) in the *Symphytum tuberosum* (flower F2), while Na was the least abundant (0.112 mg/g) microelements in the *Symphytum officinale* (flower F3). The levels of lead and cadmium were below the maximum limits prescribed by EU Commission regulations for medicinal plants. Toxic elements correlated more strongly with microelements than macroelements. Overall, the results showed that the plant analyzed do not pose a risk to human health.

Keywords: medicinal plant, elemental composition, ICP-OES, comfrey, WHO.

INTRODUCTION

People have used plants for healing since ancient times until today. The quality of plants is very important due to the risk of contamination with heavy metals because of industrial development and anthropogenic activities. The genus *Symphytum* currently comprises between 28 to 40 accepted species, including *S. officinale* and *S. tuberosum* (STAIGER, 2012; POWO, 2025). Extracts from the roots of *S. officinale* contain phenolic acids (e.g., rosmarinic, chlorogenic, and caffeic acids) and have demonstrated antioxidant and proliferative effects on human dermal fibroblasts (STAIGER *et al.*, 2012).

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The medicinal use of *Symphytum* genus is a largely confined to a small number of species, among which *S. officinale* is the most notable (LUCA *et al*., 2024). Comfrey preparations from roots, leaves, or aerial parts are traditionally used externally (compresses, ointments) and internally (infusions, tinctures) for treating injuries such as fractures, burns, bruises, swellings, hematomas, rheumatic pain, and for certain gastrointestinal, respiratory, and genitourinary disorders (SALEHI *et al*., 2019). Comfrey is a species with various characteristics (antioxidant, antimicrobial, anti-inflammatory) and, as such, represents a source for research in various scientific disciplines. In people, consuming comfrey has been linked to isolated instances of hepatotoxic reactions: fibrosis of the liver, veno-occlusive disorders, and portal hypertension (MAZZOCCHI and MONTANARO, 2012). Comfrey leaves can be considered as animal feed for pigs (OSTER *et al*., 2021). *S. officinale* and *S. tuberosum* have been used to treat skin issues in the USA, gastritis and ulcers in Brazil, and rheumatism in Mexico. The external use of *S. officinale* and *S. tuberosum* for osteoarticular disturbance in Navarra has been documented by ethnographic investigations. Liver diseases are treated with tea. In Lithuania, the root's tea, alcoholic extract, or ointment is used to treat osteoarticular pain; in Jamaica, it's utilized as a tonic (CAMERON and CHRUBASIK, 2013).

In the UK, comfrey can only be bought over the counter for external uses and with a prescription from a certified medical herbalist for internal or external use. In the US, Canada, and some European countries like Germany, Denmark, and Austria, comfrey use is prohibited. Commission EU recommends using it for no more than four to six weeks per year (COLEGATE *et al*., 2012). Because of its photoprotective properties, *S. offcinale* leaf extract-loaded silver nanoparticles have been proposed as a treatment for skin photoaging (SINGH *et al*., 2018). One of the *Symphytum* species that has been studied the most is *S. officinale*. In particular, it has been shown that *S. offcinale* roots are a good source of phenolic chemicals (SAVIĆ *et al*., 2015).

Some heavy metals such as Cr, Zn, Mo, Co, Mn and V are necessary for the proper functioning of the human body in small quantities. On the other hand, the intake of elements such as As, Cd, Cu, Cr and Pb is harmful to health (MUNIR *et al*., 2022). Given the widespread use of comfrey preparations, as well as its possible contamination with heavy metals caused by industrial development and anthropogenic activities, it is important to monitor heavy metal concentrations in the plant.

In this study were examined *Symphytum* plant species (flowers, leaves, stems, and roots) for the content of 22 bioavailable inorganic elements, macroelements (Ca, K, Mg, Na, and P), and microelements (Al, As, B, Ba, Be, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, Si, V, and Zn). The objectives of this research are to make a significant contribution to the scientific database, given that comfrey (*Symphytum* spp.) is a medicinal plant and that a comprehensive elemental analysis of its various parts, particularly from the Pešter Plateau and Golija regions, has not yet been conducted; to determine whether comfrey can serve as a source of macroelements; to evaluate whether comfrey is a safe raw material for medicinal use, based on the content of potentially toxic elements.

MATERIALS AND METHODS

Samples of all parts of the comfrey (*Symphytum officinale* L. and *Symphytum tuberosum* L.) were collected from three locations on the territory of the Pešter Plateau and Golija during the summer of 2023 (Tab. 1). Collected plant samples (F = flower; L = leaf; S = stem; R = root) were dried in an airy place for 2 weeks. Research conducted in the field revealed that *Symphytum* species are used in folk medicine for local applications related to blunt injuries and musculoskeletal conditions.

Table 1. Geographic data on the locations of studied plant *Symphytum* species, Serbia.

Sample*	Latin name	Location	Geographic coordinates		Altitudes (m)
			Longitude	*Latitude*	
F1, L1, S1, R1	*S. Officinale*	Golija	43°13'15"	20°25'39"	717
F2, L2, S2, R2	*S. Tuberosum*	Golija	43°13'20"	20°24'16"	688
F3, L3, S3, R3	*S. Officinale*	Pešter Plateau	43°16'14"	19°59'35"	1026

*F= flower; L= leaf; S=stem; R=root. 1,2,3 = Location.

Chemicals

For sample preparation, 65% nitric acid (Merck, Germany) and 30% hydrogen peroxide (Fluka, Switzerland) were used. All plastic containers were rinsed with 20% v/v HNO_3 and washed with ultrapure deionized water (0.05 µS/cm) Micro Med high purity water system (TKA Wasseraufbereitungs systeme GmbH) prior to use. Multielement standard solutions III (Ca (1000 ppm), Mg (400 ppm), K (200 ppm) and Na (1000 ppm)), IV (Be, Cd, Co, and Mn (10 ppm); Cr, Cu, and Ni (20 ppm); Al, As, Ba, Pb, and V (40 ppm); B, Fe, Se, Tl, and Zn (100 ppm)) and individual standard solutions of Si and P (1000 ppm) (TraceCERT, Fluka, Analytical, Switzerland) were used for ICP analysis. All chemicals were of analytical grade.

Decomposition of samples

After drying, the samples (flower, leaf, stem) were ground and passed through a 1.5 mm sieve. Root samples were washed with deionized water and dried in an oven at a temperature of (100±2)°C to constant mass and were ground and passed through a 1.5 mm sieve.

Dry method was used for the complete decomposition of the plant samples. Plant samples were prepared according to literature data (MATEOS-APARICIO *et al.*, 2010; AZUE and MUDROCH, 1994). 2 g of the homogenized sample was accurately weighed with an analytical balance and transferred to an annealing crucible. The first annealing was carried out in a flame and then in an annealing furnace for 2 hours at a temperature of 250°C. After that, the temperature gradually increased to 550°C, and the annealing continued for another 12 hours. If the ash was not white, 2 mL of concentrated nitric acid was added and annealed for another 2 hours or until a white ash was obtained. After annealing, the cooled ash is poured over with 5 mL of concentrated nitric acid and filtered through a quantitative filter paper. The crucible is washed several times with deionized water, and the 25 mL volumetric flask is also washed (it is also possible to rinse the crucible with 0.5% nitric acid and top up the 25 mL volumetric flask) (MATEOS-APARICIO et al., 2010; AZUE and MUDROCH, 1994).

The samples (filtrate) were stored in plastic vials at 4°C. For the preparation of samples for analysis by the ICP-OES method, samples were filtered using a 0.45 µm syringe filter. Sample blanks and CRM were prepared in the same way as samples. All analyses were performed in triplicate.

Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)

Multielement analysis was performed using ICP-OES to determine the concentrations of 22 bioavailable elements, including macroelements (Ca, K, Mg, Na, and P) and microelements (Al, As, B, Ba, Be, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, Si, V, and Zn). The measurements were conducted with an iCAP 6000 series inductively coupled plasma optical emission spectrometer (Thermo Scientific, Cambridge, UK), equipped with a sample introduction system, concentric spray nebulizer, and a CID detector.

The operating conditions for the ICP-OES instrument were as follows: flush pump rate of 100 rpm, analysis pump rate of 50 rpm, RF power of 1150 W, nebulizer gas flow rate of 0.7 L/min, coolant gas flow rate of 12 L/min, auxiliary gas flow rate of 0.5 L/min, dual-view plasma mode (axial/radial), and a sample uptake delay of 30 seconds. High purity argon gas 5.0 was used as plasma gas.

Analytical response characteristics

The validation procedure includes the selection of analytical wavelengths, linearity, precision, accuracy, as well as the limit of detection and quantification of the analyzed elements. Calibration curves for all elements have a good linearity, with correlation coefficients higher than 0.9991 (Table S1). The linearity of the method was tested in the concentration levels from 0 μg/g to 100 μg/g. The precision of the method was expressed as the realtive standard deviation (%RSD) (n=3), and the accuracy of the method was evaluated using strawberry leaves (LGC7162), as certified reference material (Table S2). The limit of detection (LOD) and the limit of quantification (LOQ) were calculated using 3σ and 10σ criterion, respectively (UHROVČÍK, 2014; NAOZUKA *et al*., 2011).

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics software (22.0). Prior to analysis, data were tested for normality using the Shapiro–Wilk test. Since most of the variables did not follow a normal distribution, non-parametric tests were applied. The relationships between toxic elements and macro- and microelements were examined using Spearman’s rank correlation coefficient. The significance of correlations was evaluated at the $p < 0.05$, $p < 0.01$, and $p < 0.001$ levels (two-tailed test).

RESULTS AND DISCUSSION METHODS

Identification and quantitation of inorganic elements

ICP-OES was used to determine the concentrations including 22 bioavailable elements, including macroelements (Ca, K, Mg, Na, and P), and microelements (Al, As, B, Ba, Be, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, Si, V, and Zn), species *Symphytum* (flowers, leaves, stems, roots) that were collected from three different places in Serbia. Quality of the analytical procedures was assured using certified reference material, strawberry leaves (LGC7162). The results are given in Supplementary, Tab. S1.

The analytical response characteristics were determined for each of the elements using multielement standard solutions. Good linearity and satisfactory coefficient of correlation ($r>0.999$) were observed for each of the elemental response functions. The limits of detection and determination were calculated from each calibration response (Tab. S2).

Macroelements

The concentrations of Ca, K, Mg, Na, and P in all parts of the medicinal plant comfrey, collected at two locations on the Golija mountain and one urban location in Sjenica, Serbia, are presented in Tab. 2. The results showed notable variations in the concentrations of these elements among different parts of the plant and across various locations.

The macroelement contents in the samples of flower (F), leaf (L), stem (S), and root (R), at all locations, are in the following order: K > Ca > P > Mg > Na. The measured highest and lowest macroelement concentrations per part comfrey were as follows: for Ca, the highest is 13.7 mg/g in L3 and the lowest is 3.41 mg/g in R1; for K, the highest is 39.5 mg/g in F2 and the lowest is 14.8 mg/g in R3; for Mg, the highest is 2.95 mg/g in F2 and the lowest is 0.55 mg/g in R3, S2; for Na, the highest is 0.58 mg/g in L3 and the lowest is 0.112 mg/g in F3; for P, the highest is 7.1 mg/g in F2 and the lowest is 1.45 mg/g in R1.

Table 2. Macroelements content (c_{sr} ± SD, mg/g)* in samples of *Symphytum* species, Serbia.

Sample**	Ca	K	Mg	Na	P
F1	6.4±0.1	16.9±0.1	1.54±0.03	0.23±0.01	2.70±0.03
L1	9.3±0.3	21.1±0.4	1.38±0.05	0.40±0.01	2.08±0.05
S1	5.4±0.1	22.6±0.6	0.89±0.02	0.144±0.005	1.86±0.03
R1	3.41±0.02	14.9±0.2	0.59±0.01	0.27±0.01	1.45±0.02
F2	10.9±0.1	39.5±0.3	2.95±0.01	0.30±0.02	7.1±0.1
L2	12.6±0.2	25.3±0.5	1.31±0.02	0.165±0.003	3.7±0.1
S2	4.5±0.2	19.6±0.6	0.55±0.02	0.25±0.01	2.6±0.1
R2	8.0±0.2	16.8±0.6	0.66±0.03	0.32±0.02	2.9±0.1
F3	13.4±0.1	38.6 ±0.4	1.96±0.04	0.112±0.001	6.10±0.02
L3	13.7±0.5	24.1±0.4	0.83±0.03	0.58±0.02	3.7±0.1
S3	7.03±0.04	27.1±0.6	0.68±0.02	0.250±0.002	2.9±0.1
R3	5.9±0.2	14.8±0.6	0.55±0.03	0.116±0.001	4.2±0.2

*mean value ± standard deviation, n=3
**F= flower; L= leaf; S=stem; R=root; 1= Golija; 2= Golija; 3= Pešter Plateau

The leaves contained the most Ca and Na (*S. officinale* is the leader) and the flowers contained the most K, P and Mg (*S. tuberosum* is the leader). Comfrey from location 3 were contained more of all macroelements than comfrey from location 1 (both *S. officinale*).

A study from Istanbul, Turkey (OZYIGIT *et al*., 2018), reported comparable concentrations of Ca and Mg in comfrey, but significantly lower K and higher Na concentrations compared with results obtained in this study. Generally, the macroelement concentrations in the studied samples were comparable to or higher than those in Turkish medicinal plants (OZYIGIT *et al*., 2018). Preliminary findings suggest that comfrey may be a valuable source of macroelements; however, further studies are required to confirm these results. Caution is advised due to the presence of pyrrolizidine alkaloids, which are hepatotoxic and potentially carcinogenic (MEI *et al*., 2010).

Microelements

The concentrations of Al, As, B, Ba, Be, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, Si, V, and Zn in all parts of the medicinal plant comfrey, collected at two locations on the Golija mountain and one urban location in Sjenica, Serbia, are presented in Tab. 3 and 4.

In general, Al, Si, and Fe were the most abundant in all parts of *Symphytum* plants. The elements Cr, Cu, Fe, Mn, Ni, Pb, and V, were the scarcest in the stem, while the elements B, Se, and Zn were the scarcest in the roots. Aluminum and As were least present in flowers, while Be and Co were least present in flowers and stems. Barium was least abundant in roots and stems, while cadmium was less abundant in flowers, leaves, and stems than in roots.

The elements Al, As, Be, Cd, Co, Fe, Pb, and V were most abundant in roots, while Ba, Se, Si, and Zn predominated in flowers. Cupper and Cr were highest in both flowers and roots, Ni in leaves, B in flowers and leaves, and Mn in flowers, leaves, and roots.

The concentration of mercury was below the limit of detection (LOD) for all samples. Regarding the toxic elements (Cd and Pb), according to WHO (2007), the results obtained for herbal medicines, are below recommendations (0.3 mg/kg for Cd and 10 mg/kg for Pb) in all samples. On the other hand, the EU COMMISSION REGULATION (2023) and WHO (2007) have not set proposed limits for As in herbal medicines, respectively. Some examples of national limits for As (5 ppm, Canada; 2 ppm, China) are also given in WHO (2007) guidelines for assessing the quality of herbal medicines with reference to contaminants and residues. When compared to the recommended limits, the concentrations of arsenic determined in the analyzed comfrey samples were found to be substantially lower.

Table 3. Trace microelements content (c_{sr} ± SD, μg/g)* in samples of *Symphytum* species, Serbia.

Sample**	**Al**	**B**	**Ba**	**Be**	**Co**
F1	82±1	20.8±0.6	71±7	0.0010±0.0003	0.115±0.002
L1	224±6	21.4±0.4	112±5	0.0061±0.0001	0.290±0.006
S1	159±1	13.9±0.1	<LOD	0.0056±0.0002	0.284±0.009
R1	601±8	10.8±0.2	77±2	0.036±0.001	0.424±0.006
F2	170±6	39.8±0.5	284±12	<LOD	0.42±0.01
L2	244±4	27.5±0.9	58±2	0.011±0.001	0.340±0.003
S2	171±5	11.0±0.4	20.1±0.3	0.007±0.001	0.186±0.001
R2	480±2	13.5±0.3	19.0±0.8	0.024±0.001	0.650±0.007
F3	84±5	27.8±0.1	124±6	<LOD	0.433±0.006
L3	121±1	37±2	29.3±0.2	0.0070±0.0001	0.24±0.01
S3	113±3	15.5±0.6	31.5±0.4	<LOD	0.221±0.006
R3	665±9	11.2±0.1	14.9±0.2	0.038±0.001	0.58±0.02
Sample**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**
F1	1.45±0.06	18.2±0.1	75±1	21.7±0.2	4.9±0.2
L1	1.36±0.03	18.5±0.8	170±3	70±1	11.5±0.2
S1	1.44±0.02	12.8±0.1	158±3	35±1	1.76±0.01
R1	25.4±0.3	23.7±0.5	401±1	25.4±0.3	2.68±0.06
F2	6.8±0.1	50±2	167±4	44±1	6.4±0.1
L2	2.6±0.1	14.8±0.1	234±1	41±1	11.7±0.5
S2	1.20±0.01	13.0±0.2	132±1	13.2±0.2	1.40±0.04
R2	4.42±0.03	22.8±0.4	381± 8	44±2	3.69±0.07
F3	8.1±0.5	35±1	130±2	31±1	10.7±0.2
L3	3.22±0.09	16.4±0.8	64±1	30±1	3.66±0.05
S3	1.06±0.01	9.6±0.1	40±1	19±1	2.17±0.03
R3	6.03±0.05	24±1	395±17	42±1	4.2±0.2
Sample**	**Se**	**Si**	**V**	**Zn**	
F1	0.141±0.005	337±15	3.37±0.07	27.7±0.8	
L1	0.107±0.004	121±3	3.29±0.07	21.7±0.8	
S1	0.020±0.001	644±31	2.2±0.1	37±2	
R1	<LOD	1077±26	3.00±0.03	22±1	
F2	0.212±0.004	1872±87	5.5±0.1	57±1	
L2	0.096±0.002	105±14	3.21±0.06	25±1	
S2	0.030±0.001	768±26	1.28±0.06	18.5±0.2	
R2	<LOD	475±13	4.63±0.02	22±1	
F3	0.309±0.002	2513±143	3.90±0.08	83±3	
L3	0.215±0.003	541±23	2.1±0.1	40±2	
S3	0.129±0.001	1250±16	1.26±0.06	44±2	
R3	0.029±0.001	1089±17	5.7±0.3	32±1	

*mean value ± standard deviation, n=3

**F = flower; L = leaf; S = stem; R = root; 1 = Golija; 2 = Golija; 3 = Pešter Plateau.

Table 4. Toxic microelements content (c_{sr} ± SD, µg/g)* in samples of *Symphytum* species, Serbia.

Sample**	As	Cd	Pb	Hg
F1	0.142±0.007	0.0149±0.0004	0.56± 0.01	<LOD
L1	0.029±0.001	0.023±0.001	1.03±0.03	<LOD
S1	0.043±0.002	0.031±0.001	1.00±0.03	<LOD
R1	0.270±0.004	0.058±0.002	2.04±0.02	<LOD
F2	<LOD	0.043 ±0.001	1.79±0.03	<LOD
L2	0.239±0.003	0.033±0.001	1.40± 0.02	<LOD
S2	0.050±0.001	0.026±0.001	1.00±0.05	<LOD
R2	0.845±0.001	0.105±0.003	2.24±0.04	<LOD
F3	<LOD	0.090±0.003	2.13±0.03	<LOD
L3	0.41±0.02	0.025±0.001	0.98±0.01	<LOD
S3	<LOD	0.029±0.001	0.78±0.01	<LOD
R3	0.35±0.02	0.049±0.001	1.35±0.06	<LOD

*mean value ± standard deviation, n=3

**F = flower; L = leaf; S = stem; R = root; 1 = Golija; 2 = Golija; 3 = Pešter Plateau.

A study from Istanbul, Turkey (OZYIGIT *et al*., 2018) reported high concentrations of Cd, B, and Fe in comfrey, with significantly lower concentrations of Cr and Cu compared with results obtained in this study. Values for Cd and Pb in comfrey from Serbia (KANDIĆ *et al*., 2023) were higher than those reported in the present study at all three locations, while values for Cr and Ni were lower. Research conducted in Obrenovac, Serbia, 5 km from the power plant, revealed significantly higher levels of trace elements (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn, and As), with concentrations ranging from 10 to 200 times higher than the results obtained in this study (STANOJKOVIĆ-SEBIĆ *et al*., 2017). These findings support the conclusion that comfrey exhibits exceptional bioaccumulation potential.

The results showed significant differences in the content of inorganic elements in different parts of the plant and different locations. The highest and lowest concentrations of microelements measured in each part of comfrey (flower (F), leaf (L), stem (S), and root (R)) across all locations are shown in Fig. 1. For Al, the highest is 665 µg/g in R3 and the lowest is 82 µg/g in F1; for As, the highest concneration of 0.845 µg/g was found in R2, while it was below the limit of detection (LOD) in F2, F3 and S3; for B, the highest concentration of 39.8 µg/g was found in F2 and the lowest is 10.8 µg/g in R1; for Ba, the highest concentration of 284 µg/g was observed in F2, while it was below the LOD in S1; for Be, the highest concentration of 0.038 µg/g was detected in R3, while it was below the LOD in F2, F3 and S3; for Cd, the concentration ranged from a maximum of 0.105 µg/g in R2 to a minimum of 0.0149 µg/g in F1; for Co, the highest is 0.650 µg/g in R2 and the lowest is 0.115 µg/g in F1; for Cr, the highest is 25.4 µg/g in R1 and the lowest is 1.06 µg/g in S3; for Cu, the highest is 50 µg/g in F2 and the lowest is 9.6 µg/g in S3; for Fe, the highest is 401 µg/g in R1 and the lowest is 40 µg/g in S3; for Mn, the highest concentration of 70 µg/g was in L1 and the lowest is 13.2 µg/g in S2; for Ni, concentrations ranged from 11.7 µg/g in L2 to lowest 1.40 µg/g in S2; for Pb, the highest concentration of 2.24 µg/g was observed in R2 and the lowest is 0.56 µg/g in F1; for Se, the highest concentration of 0.309 µg/g was found in F3, while it was below the LOD in R1 and R2; for Si, the highest concentration of 2513 µg/g was found in F3 and the lowest is 105 µg/g in L2; for V, the highest is 5.7 µg/g in R3 and the lowest is 1.26 µg/g in S3; for Zn, the highest is 83 µg/g in F3 and the lowest is 21.7 µg/g in L1.

Statistical data

The statistical data (Tab. 5) showed a strong positive correlation between As and Be concentrations, as well as between Pb and Co. A slightly strong positive correlation was detected between Cd and Cr, and between Pb and Cr, while moderate correlations were found between Pb and Cu, as well as between Pb and Fe. Overall, the concentrations of toxic elements were more strongly correlated with the levels of microelements, whereas macroelement content did not have a significant influence on the concentration of toxic elements. Among the macroelements, a strong negative correlation was observed between K and As, indicating that an increase in K content is associated with a decrease in As concentration.

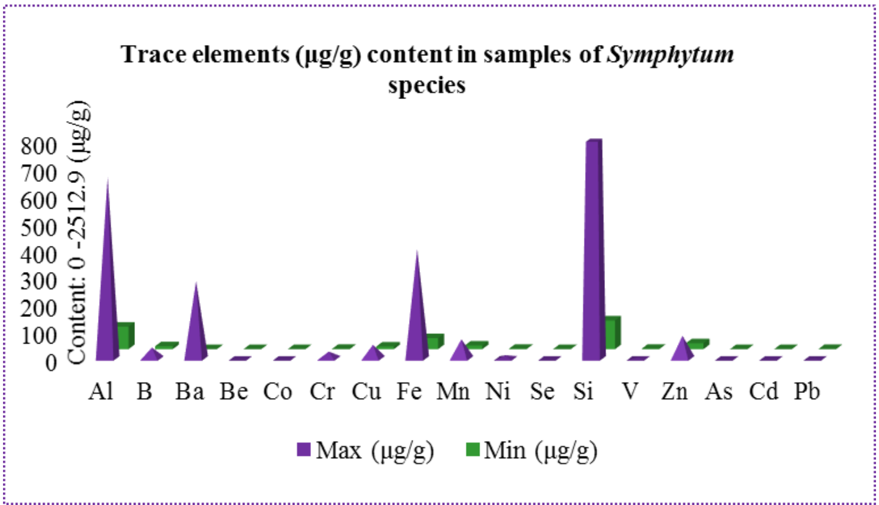

Figure 1. Trace elements (µg/g) content in samples of *Symphytum* species, Serbia

Table 5. Spearman's correlation coefficient.

	As	Cd	Pb	Hg
Al	0.535	0.462	0.532	-0.044
B	-0.373	-0.203	-0.046	0.480
Ba	-0.535	-0.014	0.249	0.480
Be	0.856***	0.286	0.295	-0.397
Co	0.268	0.295	0.900***	0.131
Cr	0.246	0.713**	0.729**	0.306
Cu	0.021	0.545	0.683*	0.480
Fe	0.437	0.566	0.683*	0.044
Mn	0.113	0.322	0.546	0.306
Ni	-0.127	0.070	0.347	0.218
Se	-0.497	-0.350	-0.281	0.306
Si	-0.472	0.455	0.221	0.393
V	0.120	0.475	0.567	0.393
Zn	-0.430	0.196	-0.025	0.393
Ca	-0.113	-0.007	0.144	0.218
K	-0.704*	-0.070	-0.035	0.480
Mg	-0.571	-0.165	0.033	0.481
Na	-0.247	-0.270	-0.044	0.219
P	-0.131	0.312	0.257	0.482

* Correlation is significant at the 0.05 level (2-tailed)
** Correlation is significant at the 0.01 level (2-tailed)
*** Correlation is significant at the 0.001 level (2-tailed)

CONCLUSIONS

Comfrey was found to be a significant source of macronutrients. The tested samples contained high concentration macroelements, in the following order (mg/g): K, 39.5 (F2) > Ca, 13.7 (L3) > P, 7.1 (F2) > Mg 2.95, (F2) > Na, 0.58 (L3). Monitoring heavy metal concentrations in medicinal plants remains essential to ensure their safety for therapeutic use. Considerable variations in elemental concentrations were observed among different plant parts and collection sites, indicating that environmental factors and soil composition strongly influence the accumulation of both minerals and heavy metals. The highest detected levels of Cd (0.105 µg/g, R2) and Pb (2.24 µg/g, R2) were below board according to WHO (0.3 mg/kg for Cd and 10 mg/kg for Pb), confirming that all tested parts of comfrey are safe regarding heavy metal content. However, current data are insufficient for a precise risk assessment of comfrey use. These results provide a preliminary foundation for future studies, which should include a more comprehensive analysis of larger and more representative sample sizes.

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SUPPLEMENTARY

Kragujevac Journal of Science

Electronic Supplementary Information associated with the paper

INORGANIC ELEMENTS CONTENT IN ALL PARTS OF COMFREY (*SYMPHYTUM* SPECIES): IS COMFREY A SAFE RAW MATERIAL FOR MEDICINAL USE?

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Table S1. The results of analysis of LGC7162 Strawberry Leaves certified reference material.

Element	Accuracy			Precision RSD (%)[***]
	Certified values[*] (mg/kg)	Found values[*] (mg/kg)	Recovery (%)[**]	
As	0.28	0.23±0.02	82.1	5.0
Ba	107	99±8	92.5	4.7
Ca	1.53[d]	1.46±0.08	95.4	3.2
Cd	0.17	0.18±0.01	105.9	3.3
Co	0.47	0.42±0.03	89.4	4.1
Cr	2.15	1.94±0.11	90.2	3.3
Cu	10	10.7±0.8	107	4.3
Fe	818	641±43	78.3	3.9
K	1.96[****]	1.85±0.12	94.4	3.7
Mg	0.377[****]	0.362±0.018	96.0	2.9
Mn	171	166±8	97.1	2.8
Ni	2.6	2.8±0.2	107.7	3.6
P	0.260[d]	0.245±0.016	94.2	3.8
Pb	1.8	1.6±0.1	88.9	3.6
Zn	24	23±1	95.8	2.5

[*] c_{sr}±u, $u = \frac{3 \times s}{\sqrt{n}}$, u-standard uncertainty, n=3, s-standard deviation
[**] [1-(found value-certified value)/certified value]×100%
[***] RSD=s/c_{sr}×100%
[****] g/100g

Table S2. Selected analytical emission wavelengths and characteristics of the ICP-OES method.

Element	Wavelength λ (nm)	Plasma view mode	*r	**LOD (ng/g)	**LOQ (ng/g)
Al	396.152	Axial	0.999822	52.5	175.85
As	189.042	Axial	0.999707	128.8	429.3
B	249.773	Axial	0.999345	39.85	132.85
Ba	455.403	Axial	0.999142	2.15	7.15
Be	234.861	Axial	0.999770	3.7	13.5
Ca	422.673	Radial	0.999265	1.31265***	4.37545***
Cd	228.802	Axial	0.999414	9.75	32.45
Co	238.892	Axial	0.999346	36.1	120.3
Cr	267.716	Axial	0.999768	34.65	115.5
Cu	324.754	Axial	0.999209	33.25	110.8
Fe	259.940	Axial	0.999603	24	80
Hg	184.950	Axial	0.999988	33.2	110.75
K	766.490	Radial	0.999616	2.7978***	9.326***
Mg	279.553	Radial	0.999890	11.45	38.16
Mn	257.610	Axial	0.999653	5.5	18.25
Na	589.592	Radial	0.999887	0.4602***	1.53395***
Ni	221.647	Axial	0.999379	16.55	55.2
P	213.618	Radial	0.999954	0.15575***	0.15925***
Pb	220.353	Axial	0.999209	82.15	273.9
Se	196.090	Axial	0.999232	86.5	695.5
Si	251.611	Axial	0.999872	65.1	217
V	309.311	Axial	0.999901	17.9	53.7
Zn	213.856	Axial	0.999338	7.3	24.25

*r-correlation coefficient; **LOD-limit of detection; LOQ-limit of quantification;***μg/g.