Evaluating seasonal variation in trace element content of savory

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Research Article

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Abstract

Background and aims

Satureja kitaibelii Wierzb. ex Heuff. (savory) is one of the most popular herbs in Serbia, used as a culinary plant, as well as tea in traditional medicine. The objective of this study was an analysis of seasonal variation in trace element contents in the soil and *S. kitaibelii,* at the Kravlje village, southeastern Serbia, with emphasis on potential aspects of health promotion.

Methods

We studied the total content of B, Si, Cr, Mn, Ni, Cu and Zn in the soil and savory using inductively coupled plasma-optical emission spectrometry. The obtained results were analyzed by chemometric methods: hierarchical cluster analysis (HCA) and principal component analysis (PCA).

Results

Chemical, statistical and chemometric analysis confirmed a variation in trace element content of studied soil and plant samples. The lowest contents of the studied elements in the soil, except for silicon, were recorded in the vegetative stage. In the plant, the boron content is the highest: 10.5-14.9 mg/kg and the chromium content is the lowest: 0.17-1.2 mg/kg. The highest values of soil-to-plant transfer factor were recorded for five elements (except Si and Cr) in the vegetative stage.

Conclusion

The present study revealed that savory from Serbia can be considered an accumulator of boron and a potential source of valuable trace elements. A significant percentage of daily intake of B, Cr and Ni, can be provided with three cups of tea per day of plants collected in the vegetative and flowering stages.

1. Introduction

Trace elements (TE) take part in vital biochemical and physiological functions, which are necessary for life maintenance. Some TE are essential for plants and animals, and they are responsible for the medicinal properties of herbs (Jungová et al., 2022). According to the World Health Organization, traditional medicine based on herbal remedies represents an important part of health services (WHO 2013). Related to these facts, medicinal plants are also used as food ingredients to combine adequate amounts of nutrients and chemical elements that are essential to a normal diet.

Satureja kitaibelii Wierzb. ex Heuff. is one of the most popular herb in Serbia (also known as Rtanj tea), used as a culinary plant, as well as tea in traditional medicine (Miladinović et al., 2014). While the chemical composition of secondary metabolites and their biological activities have been investigated (López-Cobo et al., 2015; Stanojković et al., 2013; Dodoš et al., 2019; Gopčević et al., 2019; Đorđević et al., 2014), there is not enough data on TE content in *S. kitaibelii* along with soil-to-plant transfer factors.

Having in mind these facts, also given importance of savory as a favorite medicinal and spice plant, the objectives of this study were chemical and chemometric analysis of seasonal variation in seven trace element contents, in the soil and *S. kitaibelii*, at the Kravlje village, southeastern Serbia, with emphasis on potential aspect of health promotion.

2. Material And Methods

2.1 Chemicals and reference materials

All reagents were analytical-reagent grade. Nitric acid (65%), hydrochloric acid (36%), and hydrogen peroxide (30%) were purchased from Merck (Darmstadt, Germany). For all dilutions, deionized water was used.

iTEVA software from Thermo Scientific (Cambridge, UK) was used to collect and analyze the data (Pavlović et al., 2020). Multi-element standard solution IV of the microelements Al, As, Ba, Be, B, Cd, Cr, Co, Cu, Fe, Pb, Mn, Ni, Se, Tl, V and Zn, standard solution III of the macroelements Ca, K, Mg and Na, as well as individual standard solutions of Si, P and Hg (Trace CERT, Fluka Analytical, Switzerland) were used for calibration.

The accuracy of our analytical method was determined using the Certified reference material (CRM LGC7162): K, Ca, Mg, P, Cr, Mn, Fe, Ni and Zn. The found value is reported as value ± standard deviation (SD) (Table S1). Accuracy was expressed as percentage differences between the measured concentration and the certified value to CRM (%). Method precision was evaluated as repeatability and is expressed through the relative standard deviation as a percentage (%). Differences between certified values and quantified concentrations were below 10%. The recovery values were in range of 93.6 and 106.2%. All the results are presented in Table S1.

2.2 Sample collection

The aerial parts of *Satureja kitaibelii* Wierzb. ex Heuff. family Lamiaceae were collected during 2020 from a natural population at the Kravlje village, southeastern Serbia at three different stages of development: vegetative stage (June, M1); flowering stage (July, M2 and August, M3); after flowering stage (September, M4; October, M5 and November, M6). The plants were collected on the fifteenth in the months mentioned. Dr. Marija Marković did identification of plant material, and the voucher specimen (accession number 13220) is deposited at the Herbarium of the Department of Biology and Ecology, Faculty of Science and Mathematics, University of Niš (Herbarium Moesiacum Niš – HMN). Basic characteristics of the locality are given in Table S2.

Seven sample locations were selected, and seven trace element contents were studied: B, Si, Cr, Mn, Ni, Cu, and Zn. The topsoil (0–20 cm) of the sample mixture, consisting of three small samples, was collected 10 m apart at each sampling point. Seven examples of *S. kitaibelii* in the same growth phase were taken at each sample site within 20 × 20 × 20 cm soil blocks, cut using a stainless steel spade. The soil dust and other materials in the savory samples were removed with a plastic brush, washed repeatedly with distilled water, and then stored in pre-cleaned polythene bags. The collected samples were then brought to the laboratory for further processing.

2.3. Soil sampling and preparation

Each soil sample was carefully mixed and external materials such as stones and pebbles, were extracted. The sample was then heated in an electric oven at 60°C until a constant weight was obtained. Dried soil samples are ground into fine powder. Weighted soil sample mass (1,00 g) was placed into an Erlenmeyer and treated with 16 mL mixture of conc. HCl and conc. HNO_3 (3:1) (v/v). The mixture was heated to 190 ^{o}C for about an hour, then 5 mL of H_2O_2 (30%) were added and evaporated to a small volume. Then, it was cooled, filtered (grade 589/3 blue ribbon) and diluted with 0.5% HNO_3 (in ultra-pure deionized water, 0.05 μS/cm) up to the volume of 25 mL. A blank sample was also prepared using a similar experimental procedure (Addis and Abebaw, 2017).

2.4 Plant sampling and preparation

Savory samples were dried in an electric oven at 60°C until a constant weight was obtained and then powdered. Powdered soil and savory samples were sieved through a 63 μm sieve shaker.

Digestion of plant samples was realized according to slightly modified procedure of Mosetlha et al. (2007). 1,00 g of each sample was mineralized in an Erlenmeyer flask with 15 mL of conc. HNO_3, covered with a watch glass and left over-night. After that, the mixture was heated up to 150 ^{o}C and H_2O_2 (30%) was added. Digestion procedure was applied to obtained mixtures to reduce the volume and improve decomposition. Another portion of H_2O_2 was added and evaporation continued. After cooling, the mixture was filtered (grade 589/3 blue ribbon) and diluted with 0.5% HNO_3 up to 25 mL. A blank sample was prepared in the same way.

2.5 Measurement

All analysis was carried out on iCAP 6000 inductively coupled plasma optical emission spectrometer (Thermo Scientific, Cambridge, UK) that uses an Echelle optical design and a change injection device solid-state detector. The operating conditions for the ICP-OES instrument were: flush pump rate 100 rpm, analysis pump rate 50 rpm, RF power 1150 W, nebulizer gas flow rate 0.7 L min^{-1}, coolant gas flow rate 12 L min^{-1}, auxiliary gas flow rate 0.5 L min^{-1}, dual (axial/radial) viewed plasma mode and sample uptake delay 30 s.

All measurements were performed in triplicate. Parameters of conducted ICP-OES analysis based on a calibration curve: wavelength of selected emission lines, correlation coefficient (r), limit of detection (LOD) and limit of quantification (LOQ) of the calibration for each element determination are given in Table S3. The LOD and LOQ values were calculated using the 3σ and 10σ criterion (Uhrovčík 2014).

2.6 Statistical Analysis

Statistical analyses were performed with Statistica 8 (StatSoft, Tulsa) software packages. All chemical analyses were carried out in triplicate and the results were expressed as mean ± SD. To determine the statistical significance of variation of accumulation elements in plant and soil during different stages of development, student's t-test was used. It determines whether any observed differences between the content of elements in plant and soil during different stages of development statistically significant or not. The significance of differences was defined at $p < 0.05$. The same software carried out hierarchical cluster analysis (HCA) and principal component analysis (PCA). Correlation and variability were made at a 95% significance level ($P \leq 0.05$).

3. Results

3.1 Elements content and variation in savory and its growing soil

Trace element contents in savory during different stages of development and its growing soil were given in Table 1. The total level of elements in the soil reflects the geological and climatic origin of the soil. In this research contents of selected elements were within the specified soil values (Sparks 2003). The highest concentration values (mg/kg) were recorded for manganese: 135–221, while the lowest values were noted for boron: 6.8–20.7. It is interesting to point out that the lowest contents of the studied elements in the soil, except for silicon, were recorded in June (vegetative stage).

Table 1
Trace elements content (mg/kg) in *S. kitaibelii* and soil

Months	B	Si	Cr	Mn	Ni	Cu	Zn
	PLANT						
M1	14.1 ± 0.8^{b}	3.9 ± 0.2^{c}	0.17 ± 0.01^{c}	5.8 ± 0.4^{b}	2.7 ± 0.1^{a}	2.3 ± 0.1^{c}	7.9 ± 0.7^{e}
M2	14.3 ± 0.8^{b}	1.79 ± 0.09^{e}	0.18 ± 0.01^{c}	4.1 ± 0.3^{d}	0.66 ± 0.05^{c}	2.1 ± 0.1^{d}	8.2 ± 0.6^{d}
M3	10.1 ± 0.7^{e}	5.6 ± 0.4^{b}	1.2 ± 0.1^{a}	3.8 ± 0.3^{d}	0.62 ± 0.05^{c}	2.81 ± 0.2^{b}	20.1 ± 0.9^{a}
M4	13.8 ± 0.8^{c}	6.7 ± 0.5^{a}	0.25 ± 0.02^{b}	5.7 ± 0.4^{b}	0.59 ± 0.05^{c}	2.4 ± 0.1^{c}	10.9 ± 0.7^{c}
M5	14.9 ± 0.8^{a}	2.5 ± 0.2^{d}	0.22 ± 0.02^{bc}	6.7 ± 0.5^{a}	1.3 ± 0.1^{b}	3.0 ± 0.1^{a}	13.8 ± 0.8^{b}
M6	10.5 ± 0.7^{d}	4.9 ± 0.4^{b}	0.32 ± 0.03^{b}	4.5 ± 0.3^{c}	0.55 ± 0.01^{c}	0.70 ± 0.06^{e}	8.2 ± 0.7^{d}
SOIL							
M1	6.8 ± 0.5^{f}	195 ± 12^{a}	4.4 ± 0.4^{e}	135 ± 10^{c}	5.7 ± 0.5^{d}	7.2 ± 0.6^{e}	8.5 ± 0.7^{e}
M2	20.7 ± 0.9^{a}	126 ± 11^{c}	20.9 ± 0.9^{a}	164 ± 12^{b}	16.3 ± 0.8^{a}	10.2 ± 0.7^{b}	28 ± 1^{c}
M3	9.2 ± 0.7^{e}	24 ± 1^{e}	14.4 ± 0.8^{c}	212 ± 14^{a}	10.9 ± 0.7^{c}	9.2 ± 0.7^{d}	30 ± 2^{b}
M4	16.3 ± 0.8^{b}	112 ± 10^{d}	16.9 ± 0.8^{b}	172 ± 12^{b}	12.5 ± 0.7^{b}	10.3 ± 0.7^{b}	32 ± 1^{a}
M5	15.0 ± 0.7^{c}	112 ± 11^{d}	12.2 ± 0.7^{d}	175 ± 13^{b}	11.1 ± 0.7^{c}	9.5 ± 0.7^{c}	25 ± 1^{d}
M6	12.6 ± 0.7^{d}	140 ± 11^{b}	16.7 ± 0.8^{b}	221 ± 14^{a}	13.2 ± 0.7^{b}	11.8 ± 0.7^{a}	30 ± 2^{b}

Values are the mean ± standard deviation (n = 3).

Values with different letters within columns are statistically different at $p < 0.05$ by paired Student t test.

The coefficients of variation (CV) are shown in Fig. 1. CVs of the soil samples decreased in the sequence Si > Cr > B > Zn > Ni > Mn > Cu, while plant samples followed the order Cr > Ni > Si > Zn > Cu > Mn > B.

3.2 Soil-to-plant transfer factor

Soil-to-plant transfer factor (TF) indicates the uptake and accumulation behavior of elements in *S. kitaibelii* (Table 2). TF was calculated as the concentration of TE in plant over that in soil (TF = [TE plant]/[TE soil]). A plant could be considered to be an accumulator of the studied element when TF > 1 (Márquez-García and Córdoba, 2010).

Table 2
S. kitaibelii soil-to-plant transfer factors

Months Elements	B	Si	Cr	Mn	Ni	Cu	Zn
M1	2.05	0.02	0.04	0.04	0.48	0.33	0.93
M2	0.69	0.01	0.01	0.03	0.04	0.20	0.30
M3	1.10	0.23	0.08	0.02	0.06	0.31	0.67
M4	0.85	0.06	0.01	0.03	0.05	0.24	0.34
M5	1.00	0.02	0.02	0.04	0.11	0.32	0.56
M6	0.83	0.04	0.02	0.02	0.04	0.06	0.27

The highest TF values were recorded for five elements (except Si and Cr) in the vegetative stage. TF values of boron in June and August were greater than 1 and higher than those of other elements, during the studied stages of development. Therefore, savory can be considered an accumulator of this element. In addition, with a TF value of 0.93 in vegetative stage, *S. kitaibelii* showed a good tendency to accumulate Zn.

3.3 Correlation analysis between soil and plant elements

Correlation analysis (CA) between the content of elements in the plant and soil samples was conducted to investigate their interaction. The results are shown in Table 3. As can be seen, eight strong negative correlations (Ratner 2009) were identified.

Table 3
Correlation analysis between plant and soil trace elements

	B-S	Si-S	Cr-S	Mn-S	Ni-S	Cu-S	Zn-S
B-P	0.43	0.51	-0.18	**-0.84**	-0.07	-0.34	-0.39
Si-P	-0.39	-0.30	-0.04	0.38	-0.22	0.12	0.32
Cr-P	-0.38	**-0.87**	0.09	0.63	-0.02	-0.02	0.38
Mn-P	-0.05	0.46	-0.53	-0.53	-0.46	-0.32	-0.41
Ni-P	-0.53	0.58	**-0.89**	**-0.81**	**-0.83**	**-0.91**	**-0.96**
Cu-P	-0.05	-0.39	-0.29	-0.39	-0.30	-0.61	-0.16
Zn-P	-0.27	**-0.90**	0.00	0.46	-0.08	-0.13	0.35

Bold values indicate strong negative correlations; P-plant; S-soil

3.4 Hierarchical cluster analysis

The trace elements of savory and soils in three different stages of development were subjected to chemometrics analysis to detect any interactions between them. The similarity of the different stages of savory and similarity between analyzed elements was assessed using hierarchical cluster analysis. HCA is a multivariate technique to classify objects of a system into categories or clusters based on their similarities (Johnson and Wichern, 2002). The distance between the two objects indicates their similarity, i.e., dissimilarity.

HCA was performed by Ward's method using Pearson's correlation as a measure of similarity. When two objects are close, it indicates a significant similarity. The distance will be less and get closer to 0 as the correlation goes to 1. The distance was reported as D_{link}/D_{max}, representing the quotient between the linkage distances for a particular case divided by the maximal linkage distance (Singh et al., 2004). D_{link} is the distance between the variables that are grouped, and D_{max} is the maximum distance between the variables. The results are shown as a dendrogram in Figs. 2 and 3.

3.5 Principal component analysis

The PCA method uses and presents more information, unlike HCA (Patras et al., 2011). The goal of the HCA is to partition the samples into homogeneous groups-clusters, such that the within clusters similarities are large compared to the between-clusters similarities. On the other hand, PCA aims to reduce and extract original variables in a smaller number of underlying variables, to reveal the interrelationships between the variables. Also, to find the optimum number of extracted principal components.

Analyzed elements were correlated with two principal components (PCs) with 74.79% of the total variance. This is an acceptably large percentage. The results are shown in Fig. 4. The first principal component (PC) describes the maximum possible variation that can be projected onto one dimension; the second PC captures the second most and so on (Anderson et al., 1999). In this case, the first component explained 47.20% while the second component explained 27.59% of the total variance. Ni accumulation in plants and Zn content in soil are the most important contributors to the formation of PC1, 14.3% and 13.3%, respectively (Table 4). At the same time, the highest contribution on PC2 had Cr-P (17.5%) and B-S (16.2%).

Table 4
Contribution of variables to the formation of PC1 and PC2 (%)

Variable	PC1	PC2
B-P	6.1	7.9
Cr-P	4.2	17.5
Cu-P	1.5	4.2
Mn-P	6.8	0.7
Ni-P	14.3	0.9
Si-P	1.2	6.6
Zn-P	1.9	15.9
B-S	2.4	16.2
Cr-S	10.7	5.8
Cu-S	9.9	5.5
Mn-S	11.1	2.1
Ni-S	8.9	8.8
Si-S	7.4	7.6
Zn-S	13.3	0.3

3.6 Contribution of elements in S. kitaibelii to recommended dietary intake

We calculated the contribution of all elements to recommended dietary intake (RDI) (U.S. National Academies 2001). The results are presented in the Table 5.

Table 5
Contribution of elements in *S. kitaibelii* to RDI (%)

Months	Elements						
	B	Si	Cr	Mn	Ni	Cu	Zn
RDI (mg/day)	1	33.5	0.025	1.8	0.05	0.9	11
M1	21.2	0.2	10.2	4.8	81.0	3.8	1.1
M2	21.5	0.1	10.8	3.4	19.8	3.5	1.1
M3	15.2	0.3	72.0	3.2	18.6	4.7	2.7
M4	20.7	0.3	15.0	4.8	17.7	4.0	1.5
M5	22.4	0.1	13.2	5.6	39.0	5.0	1.9
M6	15.8	0.2	19.2	3.8	16.5	1.2	1.1

The contribution of the elements was calculated on the assumption of consuming three cups of tea, i.e. 3 x 5 g of dried plant per day. As can be seen, the percentage of potentially possible daily intake of the elements is in the range of 0.1% (Si) to 81% (Ni).

4. Discussion

4.1 Elements content variation

The content of trace elements in the plant depends on several factors such as plant species, factors of soil, stage of maturity and seasonal and temperature effects (Kabata-Pendias 2011). In savory from Serbia sufficient contents of studied elements were determined, except chromium (Watanabe et al., 2007). In the plant, the boron content is the highest: 10.5–14.9 mg/kg and the chromium content is the lowest: 0.17–1.2 mg/kg. In an investigation related to content of macroelements and trace elements in two species of *Satureja* genus, a very similar content of elements was established, with a note that the content of chromium was slightly higher (Dunkić et al., 2012).

If we accept the criterion that the CV is acceptable in the range of 20–30% (Gomes 2009), it can be concluded that Mn concentrations in the soil and in the plant were stable, and the CVs were < 23%. Concentrations of Cu in savory were unstable, and the CV was large. By contrast, its concentrations in the soil were very stable, and the CV was small. Therefore, the absorption of this element might be affected by the climatic factors in the growth location of savory. On the other hand, concentration of boron is the most stable in the plant, compared to all other elements, CV was 16.4%. By contrast, its concentrations were not stable in the soil samples, and the CV was large. So, it can be assumed that the absorption of B is related to biological characteristics of *S. kitaibelii*, rather than its soil conditions (Filip and Tack, 2010). It is an interesting fact that the three elements with the highest coefficient of variation in the plant are Cr, Ni, and Si. These three elements are not classified as essential in plants, unlike the other four examined elements.

4.2 Relationships between elements content in savory and its growing soil

The results of the correlation analysis indicate that silicon, manganese, and copper in the plant samples were not related to any element in the soils. Also, boron in the soils was not related to any element in the plant samples. Obviously, that the total particular element content in soil negatively affects the element content in plant samples. It can be assumed that the contents of the elements in the savory are affected by available forms of trace elements in the soil (Kabata-Pendias 2011).

4.2.1. Chemometric analysis

For a quality comparison, hierarchical cluster analysis was applied to group months based on the accumulation of elements in savory and its growing soil. HCA yields a dendrogram (Fig. 2), suggesting two statistically significant clusters at $(D_{link}/ D_{max}) \times 100 < 50$, cluster A and cluster B. Cluster A is divided into two sub-clusters (A_1 and A_2). The strongest clustering is observed for September (M4) and November (M6), with minimal distance, which showed close association with October (M5). This observation indicates a significant similarity among these samples. In addition to the existence of subclusters in this cluster, M2 (July) separation from other months was observed. Mentioned samples belong to subcluster A_1. The second subcluster (A_2) was composed of June (M1). The most significant distance between the samples was recorded in clusters A and B, indicating the differences. Cluster B was constituted by August (M3).

Figure 3 shows a dendrogram of cluster analysis for the mean of element contents in plant and soil in different stages of development. The main objective of HCA is to investigate similarities between the accumulated elements and indicate the reason for their clustering. Division to three clusters, A, B and C, (condition: $(D_{link}/D_{max}) \times 100 < 50$) indicates a different content in the soil and accumulation of elements by the plant. Elements content in soil (Mn-S, Zn-S, Cu-S, Ni-S, Cr-S and B-S) are associated in cluster A. This cluster is divided into two subclusters. Cr-S, Ni-S and B-S form a separate subcluster (A_1), while Cu-S, Zn-S and Mn-S constitute the second subcluster (A_2). The smallest distance was recorded between Cr-S and Ni-S, indicating the significant correlation between these two elements in the soil. Nickel concentrations are frequently associated with high concentrations of iron, zinc, and chromium in soil (Barker and Pilbeam, 2007). Cluster B contains only elements accumulated in the plant, such as Cr-P, Zn-P, Cu-P and Si-P. The strongest clustering within this cluster is between Cr and Zn. Within the cluster C, there are two subclusters (C_1 and C_2), one of which is important to point out because it indicates a correlation between manganese and boron in the plant. The second sub-cluster was composed of Ni-P and Si-S.

The PCA results are in accordance with the HCA analysis but using this method, we raised our study to the next level to display the connection between the accumulation of elements and different stages of plant development. The number of principal components is determined (Kaiser 1960). The PCA pointed out M2, M4, and M6 on the plot's left side, suggesting that Zn-S, Cu-S, Cr-S, Ni-S, and B-S, which were found in the same quadrant, are dominant elements in the soil in these months. This grouping corresponds to cluster A (Fig. 3). As illustrated in Fig. 4, the vectors of the variables Cu-S and Cr-S are parallel, indicating a strong correlation (https://analyse-it.com/docs/tutorials/correlation/creating-correlation-monoplot) [27].

The vectors of variables Cr-P and Zn-P occupy an acute angle, indicating a significant correlation. These elements and Si-P and Mn-S are co-located in the higher left-hand quadrant of Fig. 4, together with M3, suggesting that they have a high content in this stage of development. Ni-P is co-located in the higher right-hand quadrant, in the immediate vicinity with M1, suggesting high content of Ni-P in this month. M5 (October) occupied a location in the fourth quadrant of the figure. In this month we have the highest concentration of manganese in the plant, which is also located in this area.

4.3 Potential aspects of health promotion

As we have said, trace elements are helpful for proper growth, development, preservation, and recovery of organism health. They are important components of enzymes that donate or accept electrons, regulating important biological processes through actions such as assisting the binding of molecules to receptor sites on cell membranes. Additionally, some trace elements provide structural stability to important biological molecules (Anal and Chase 2016).

Boron is not an essential trace element in human health, but its biochemical function is very important in numerous biological functions, including calcium metabolism, growth and maintenance of bone tissue. Also, boron reduces the risk of certain types of cancer, the development of arthritis, and associated heart disease symptoms. Further, it accelerates wound healing, reduces pain in gynecological diseases, and kidney stones by reducing cytokines (Rondanelli et al., 2020). Our research has determined that *S. kitaibelii* is the accumulator of this element. The conclusion is that a significant percentage of daily intake of B, Cr and Ni, can be provided with three cups of tea per day of plants collected in the vegetative and flowering stages. It should be said that the greatest contribution of boron to RDI was recorded in October (22,4%). The value was insignificantly lower in July (21,5%). Considering that it is better, i. e. healthier, to use a younger plant, we recommend for savory collection the flowering stage.

5. Conclusions

In the present study, combined chemical and chemometric analysis of seasonal variation in trace element contents, in the *Satureja kitaibelii* Wierzb. ex Heuff. and its growing soil, over the course of six months was done, with emphasis on potential aspects of health promotion. On the base statistical and chemometric analysis, it can be concluded that there is a variation in trace element content of studied soil and plant samples, caused by seasonal variation. The lowest contents of the studied elements in the soil, except for silicon, were recorded in June (vegetative stage). In this stage of development, the highest values of soil-to-plant transfer factor were recorded for five elements (except Si and Cr). Savory can be considered an accumulator of boron. Significant percentage of daily intake of B, Cr and Ni, can be provided with three cups of tea per day of plants collected in the vegetative and flowering stages.

Abbreviations

CA - Correlation analysis

CRM - Certified reference material

CV - Coefficients of variation

HCA - Hierarchical cluster analysis

PCA - Principal component analysis

SD - Standard deviation

TE - Trace elements

TF - Transfer factor

Declarations

Conflicts of Interest All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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Author contributions

DM **-** conceived and wrote the study, MD - did statistical and chemometrics analysis and interpretation of the data, JM – works on ICP-OES, MM did on the taxonomy and botany, AP - did critical revision of the manuscript. All authors read the manuscript and approved the final version.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. Addis W, Abebaw A (2017) Determination of heavy metal concentration in soils used for cultivation of *Allium sativum* L. (garlic) in East Gojjam Zone, Amhara Region, Ethiopia. Cogent Chem 3:1–12. https://doi.org/10.1080/23312009.2017.1419422
2. Anal JMH, Chase P (2016) Trace elements analysis in some medicinal plants using graphite furnace-atomic absorption spectroscopy. Enviro Eng Res 21:247–255. https://doi.org/10.4491/eer.2016.007
3. Anderson KA, Magnuson BA, Tschirgi ML, Smit B (1999) Determining the Geographic Origin of Potatoes with Trace Metal Analysis Using Statistical and Neural Network Classifiers. J Agric Food Chem 47:1568–1579. https://doi.org/10.1021/jf980677u
4. Barker AV, Pilbeam DJ (2007) Handbook of plant nutrition. CRC Press Taylor & Francis Group, USA
5. Dodoš T, Rajčević N, Janaćković P, Vujisić L, Marin P (2019) Essential oil profile in relation to geographic origin and plant organ of *Satureja kitaibelii* Wierzb. ex Heuff. Ind Crops Prod 139:111549. https://doi.org/10.1016/j.indcrop.2019.111549
6. Đorđević A, Palić I, Stojanović G, Ristić N, Palić R (2014) Chemical Profile of *Satureja Kitaibelii* Wierzb. Ex Heuff. Essential Oils: Composition of *Satureja Kitaibelii* Essential Oils. Int J Food Prop 17:2157–2165. https://doi.org/10.1080/10942912.2013.784333
7. Dunkić V, Kremer D, Dragojević Müller I, Stabentheinerd E et al (2012) Chemotaxonomic and Micromorphological Traits of *Satureja montana* L. and *S. subspicata* Vis. (Lamiaceae). Chem Biodivers 9:2825–2842. https://doi.org/10.1002/cbdv.201100376
8. Filip M, Tack G (2010) Trace Elements: General Soil Chemistry, Principles and Processes. In: Hooda PS (ed) In Trace Elements in Soils. Blackwell Publishing Ltd.
9. GOMES FP (2009) Curso de estatística experimental. 15th Edition, FEALQ, Piracicaba
10. Gopčević K, Grujić S, Arsenijević J, Karadžić I, Izrael-Živković L, Maksimović Z (2019) Phytochemical Properties of *Satureja kitaibelii*, Potential Natural Antioxidants: a New Insight. Plant Foods Hum Nutr 74:1–6. https://doi.org/10.1007/s11130-019-0716-3
11. Johnson RA, Wichern DW (2002) Applied multivariate statistical analysis. Prentice-Hall, London, UK
12. Jungová M, Asare M, Jurasová V, Hejcman M (2022) Distribution of micro- (Fe, Zn, Cu, and Mn) and risk (Al, As, Cr, Ni, Pb, and Cd) elements in the organs of Rumex alpinus L. in the Alps and Krkonoše Mountains. https://doi.org/10.1007/s11104-022-05440-2. Plant Soil
13. Kabata-Pendias A (2011) Trace elements in soils and plants. CRC Press Taylor&Francis Group, Boca Raton London New York
14. Kaiser HF (1960) The application of electronic computers to factor analysis. Educ Psychol Meas 20:141–151. https://doi.org/10.1177/001316446002000116
15. López-Cobo A, Gómez-Caravaca AM, Švarc-Gajić J, SeguraCarretero A, Fernández-Gutiérrez A (2015) **() Determination of phenolic compounds and antioxidant activity of a Mediterranean plant**: **the case of** Satureja montana **subsp**. kitaibelii. **J Funct Foods 1167–1178**. :*//doi.org/10.1016/j.jff.2014.10.023*

16. Márquez-García B, Córdoba F (2010) Antioxidative system in wild populations of *Erica andevalensis*. Environ Exp Bot 68:58–65. https://doi.org/10.1016/j.envexpbot.2009.10.008
17. Miladinović D, Ilić B, Kocić B, Miladinović M (2014) **An *in vitro* antibacterial study of savory essential oil and geraniol in combination with standard antimicrobials**. Nat Prod Commun 9:1629–1632**)**
18. Mosetlha K, Torto N, Wibetoe G (2007) Determination of Cu and Ni in plants by microdialysis sampling: Comparison of dialyzable metal fractions with total metal content. Talanta 71:766–770. https://doi.org/10.1016/j.talanta.2006.05.020
19. Patras AN, Brunton P, Downey G, Rawson A, Warriner K, Gernigon G (2011) Application of principal component and hierarchical cluster analysis to classify fruits and vegetables commonly consumed in Ireland based on *in vitro* antioxidant activity. J Food Compost Anal 24:250–256. https://doi.org/10.1016/j.jfca.2010.09.012
20. Pavlović AN, Mrmošanin JM, Jovanović S, Mitić SS, Tošić SB, Krstić JN, Stojanovića GS (2020) Studia UBB Chemia 65:69–83. https://doi.org/10.24193/subbchem.2020.2.06
21. Ratner B (2009) The correlation coefficient: Its values range between + 1/−1, or do they? J Target Meas Anal Mark 17:139–142
22. Rondanelli M, Faliva MA, Peroni G, Infantino V et al (2020) Pivotal role of boron supplementation on bone health: A narrative review. J Trace Elem Med Biol 62:126577. https://doi.org/10.1016/j.jtemb.2020.126577
23. Singh KP, Malik A, Mohan D, Sinha S (2004) Multivariate statistical techniques for the evaluation of spatial and temporal ariations in water quality of Gomti River (India): a case study. Water Res 38:3980–3992. https://doi.org/10.1016/j.watres.2004.06.011
24. Sparks DL (2003) Environmental soil chemistry. Academic Press, San Diego
25. Stanojković T, Kolundžija B, Ćirić A, Soković M, Nikolić D, Kundaković T (2013) Cytotoxicity and antimicrobial activity of *Satureja kitaibelii* Wierzb. Ex Heuff (Lamiaceae). Dig J Nanomat Bios 8:845–885
26. National Academies US (2001) Institute of Medicine, Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. The National Academies Press, Washington, USA,
27. Uhrovčík J (2014) Strategy for determination of LOD and LOQ values – Some basic aspects. Talanta 119:178–180. https://doi.org/10.1016/j.talanta.2013.10.061
28. Understanding the relationship between variables, available at: https://analyse-it.com/docs/tutorials/correlation/creating-correlation-monoplot
29. Watanabe T, Broadley MR, Jansen S, White PJ, Takada J, Satake K et al (2007) Evolutionary control of leaf element composition in plants. New Phytol 174:516–523. https://doi.org/10.1111/j.1469-8137.2007.02078.x
30. World Health Organization (2013) WHO Traditional Medicine Strategy 2014–2023

Figures

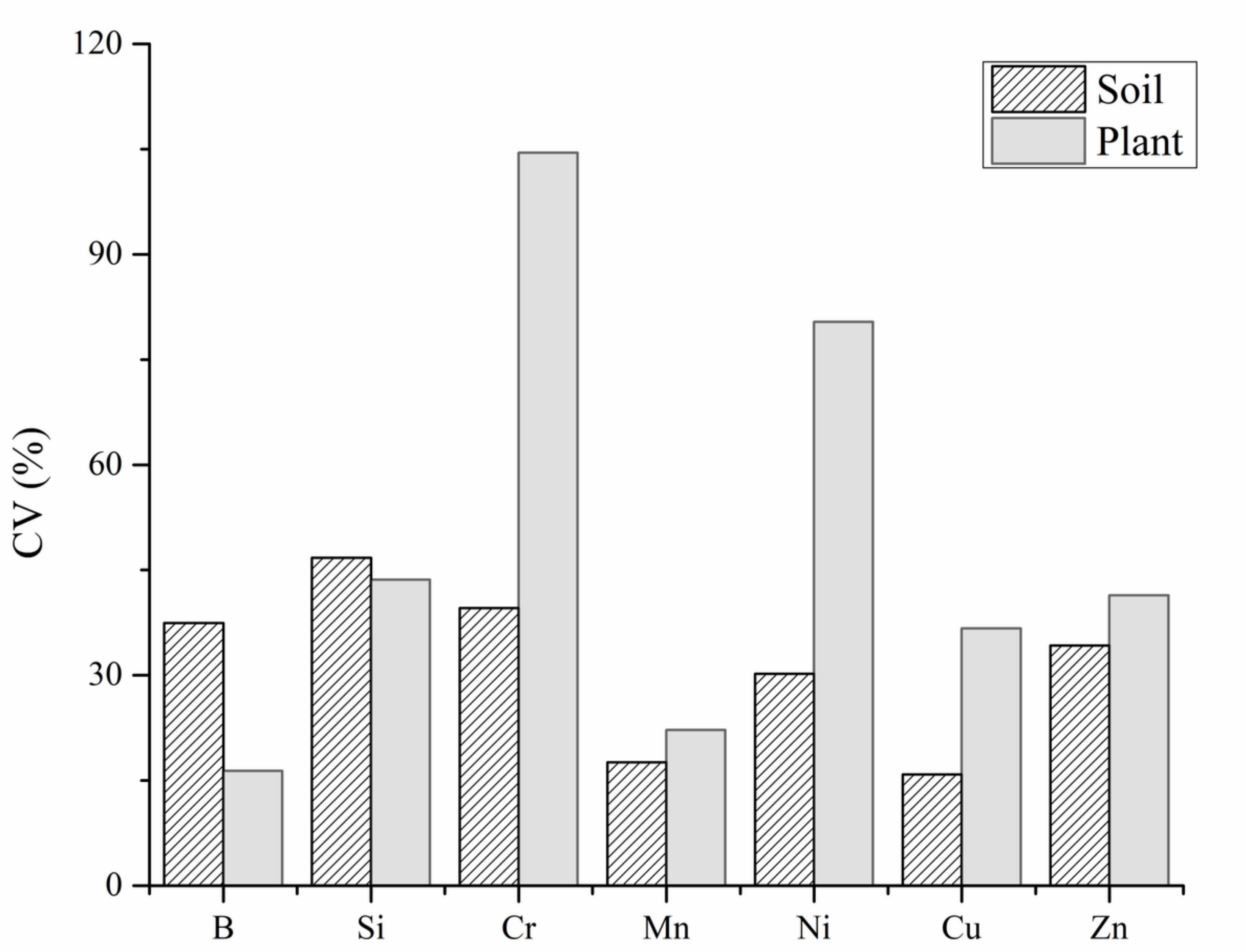

Figure 1

Elements coefficients of variation in *S. kitaibelii* and soil samples

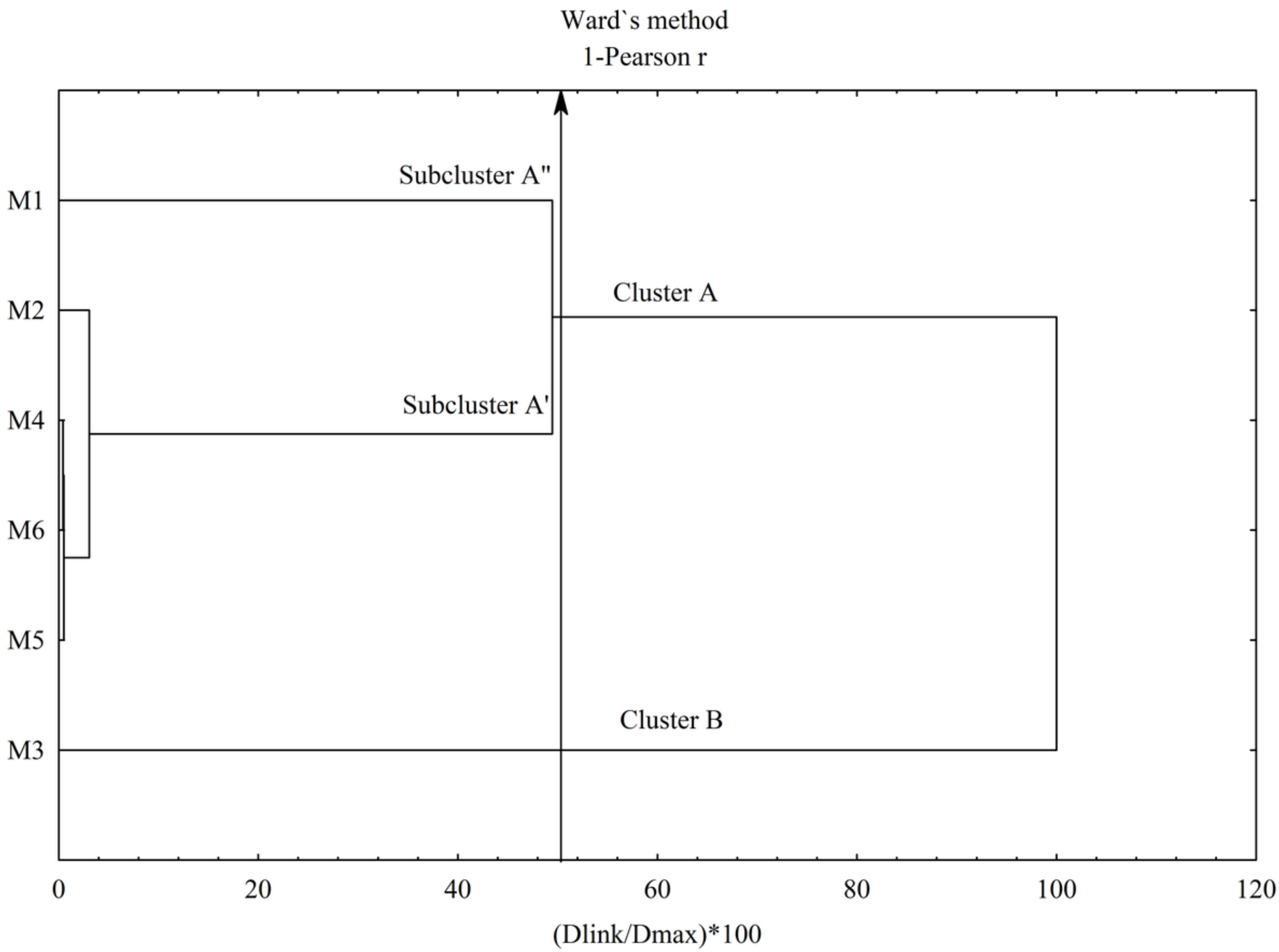

Figure 2

Dendrogram of different stages of development of *S. kitaibelii*

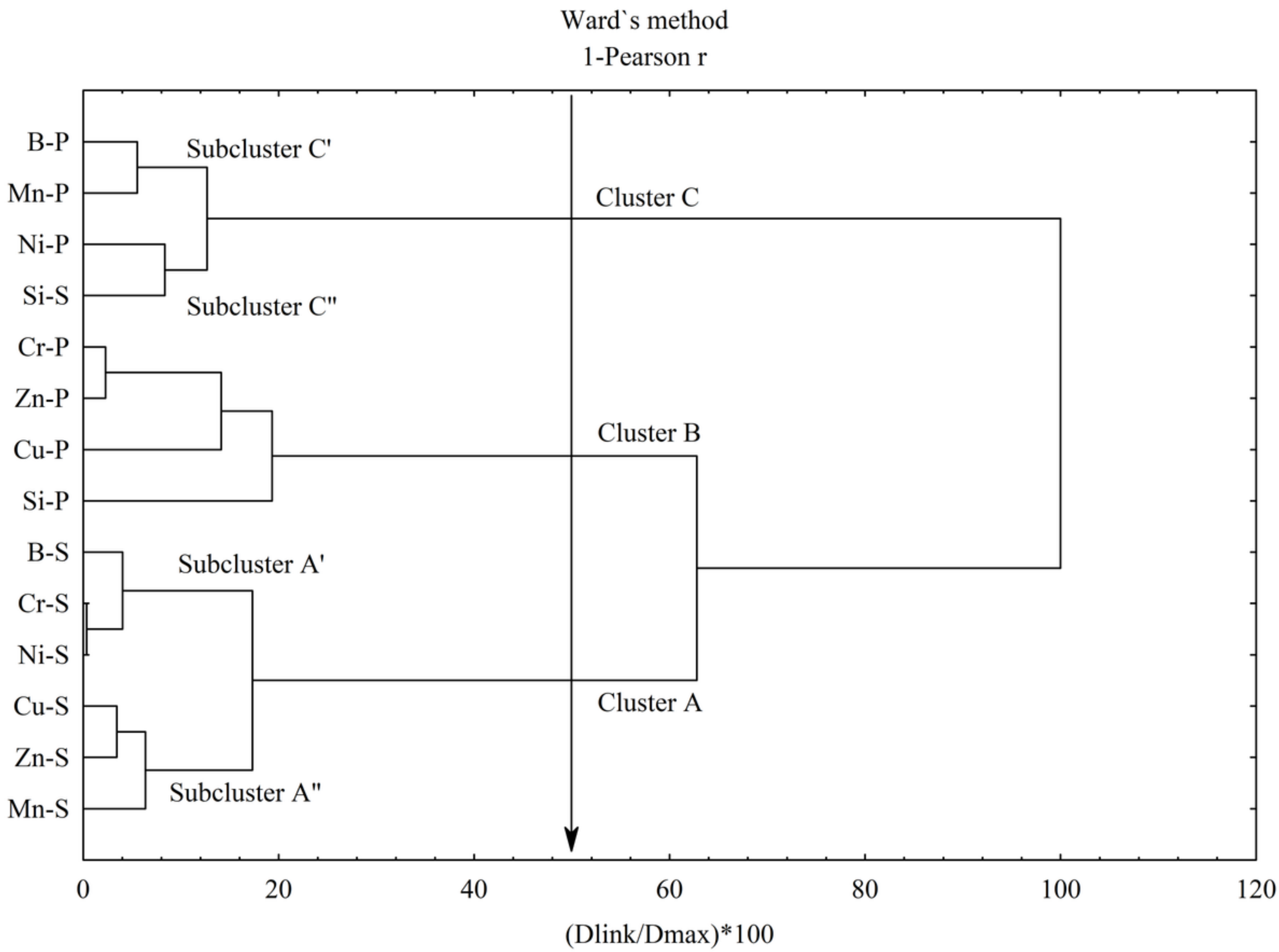

Figure 3

Dendrogram of element contents in *S. kitaibelii* and soil during different stages of development

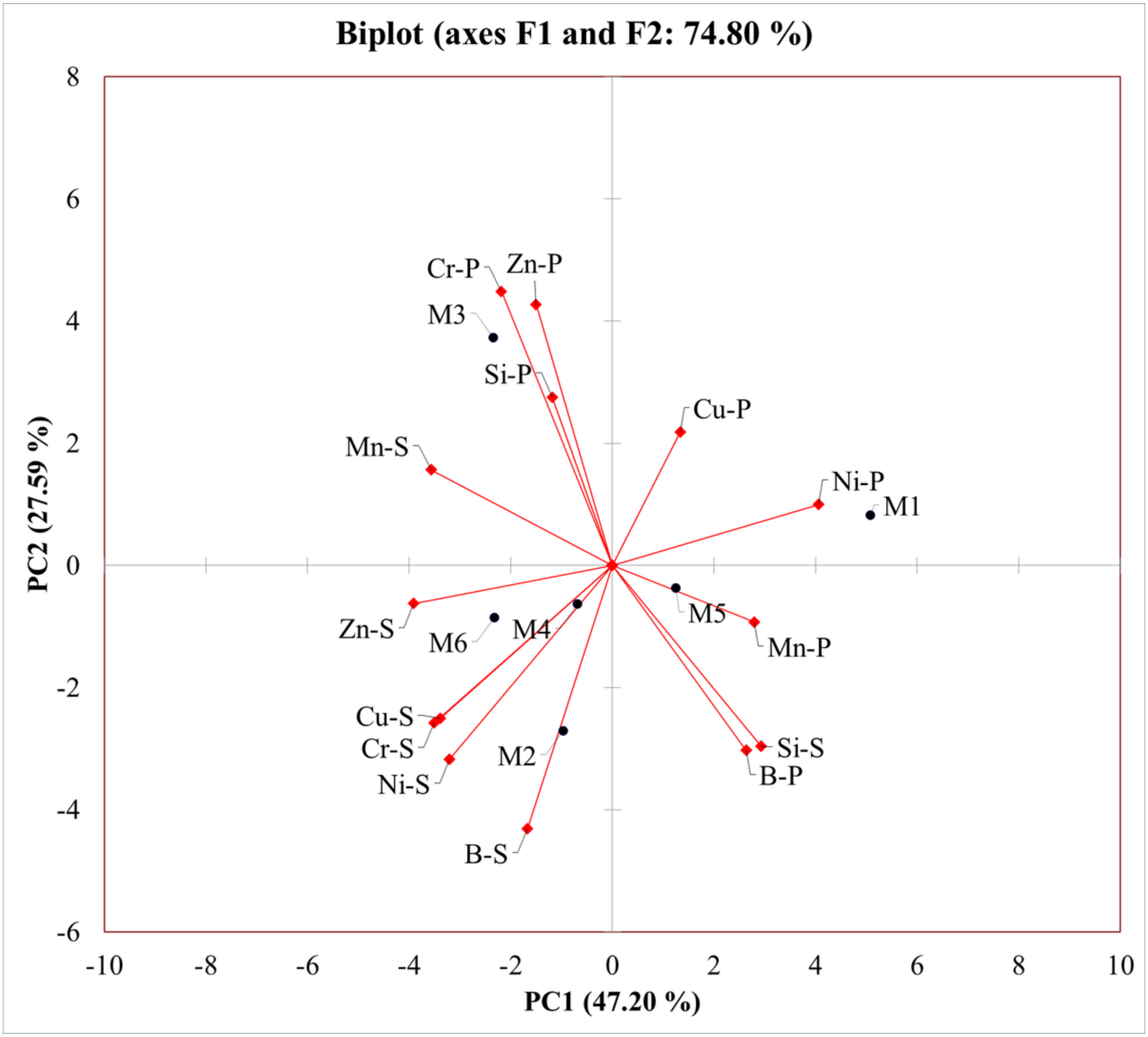

Figure 4

Principal component analysis for elements and different stages of development of *S. kitaibelii*

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