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Original Article

Multi-elemental analysis of *Ziziphora clinopodioides* from different regions, periods and parts using atomic absorption spectrometry and chemometric approaches

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ABSTRACT

In this study, ten trace elements in *Ziziphora clinopodioides* Lam., Lamiaceae, from different regions, periods and parts in Xinjiang were determined by atomic absorption spectrometry following microwave-assisted acid digestion. The decreasing sequence of elements levels was K > Ca > Mg > Fe > Cu > Zn > Na > Mn > Cd > Pb. Chemometric approaches, such as correlation analysis, principal component analysis, and hierarchical cluster analysis were applied to classify *Z. clinopodioides* according to its elements contents. Principal component analysis revealed 83.51% of the variance with the first four principal component variables. Hierarchical cluster analysis indicated five groups from the eighteen regions, and the result of classification can correspond to the geographical distribution for the most regions. Variation in the elements exhibited a decreasing trend, but of different types in the studied periods. Elemental contents distributed in leaves were higher than those in flowers and stems. Therefore, chemometric approaches could be used to analyze data to accurately classify *Z. clinopodioides* according to origins. This study provided some elemental information on chemotaxonomy, diversity, changing pattern, distribution, and metabolism of *Z. clinopodioides* at spatial and temporal levels, and could be used as a reference of planting and quality standards.

Introduction

Ziziphora clinopodioides Lam., a perennial plant belonging to the Lamiaceae, is a well known traditional Uyghur medicinal herb from Xinjiang, China. In folk society, fresh and dry *Z. clinopodioides* has been used as spice in noodle and tea. Moreover, this aromatic plant is rich in essential oil, which is applied in food, perfume, and medical treatments, thus, local people call *Z. clinopodioides* as "wild peppermint." This plant is also used to treat hypertension, fever, edema, heart disease, neurasthenia, insomnia, tracheitis, lung abscess, and hemorrhoids (Liu et al., 1999; Senejouxa et al., 2010). Other ethnobotanical uses of *Ziziphora* species as stomachic, carminative and wound healing material have been recorded in Iranian and Turkish folk medicines (Meral et al., 2002; Ozturk and Ercisli, 2007). Previous pharmacological investigations also revealed that this species exhibits antimicrobial (Ji et al., 2012), antifungal (Behravan et al., 2007), antioxidative (Tian et al., 2011), and anti-hypertensive (Guo et al., 2014) properties attributed to its essential oil. Phytochemical investigations have shown that this genus is a source of flavonoids, polyphenols, polysaccharides, fatty acids and sterols (Yu et al., 2012). The herb also biosynthesizes phenolic compounds, such as caffeic acid, rosmarinic acid, and flavonoid derivatives, including luteolin, linarin, diosmin, and thymonin (Yang et al., 2008; Tian et al., 2012). Moreover, this aromatic plant is rich in essential oil. Pulegone, *trans*-isopulegone, menthol, 1,8-cineole and limonene were found to be the major components of *Z. clinopodioides* essential oil (Ding et al., 2014a,b).

Trace elements play an important role in an organism's biological activities. In plants, trace elements are implicated in several physiological activities, including metabolism and biosynthesis, as well as enzyme cofactors (Maiga et al., 2005). Traditional medicine plays an important role in the general health status of a population, and the demand for medicinal herbs and its products have

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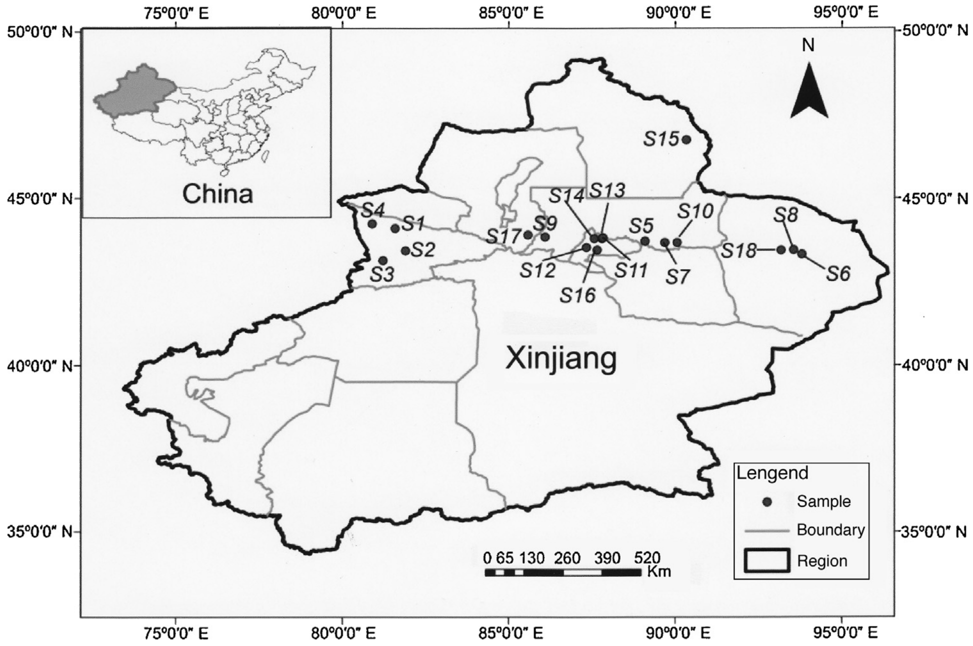

Figure 1. Geographical distribution of the collected *Z. clinopodioides* samples in Xinjiang, China.

continuously increased. However, health risk is posed by various medicinal herbs and their products because of the presence of toxic elements. The human body requires metallic and nonmetallic elements within certain permissible limits for growth and good health. As such, various trace element concentrations should be quantitatively estimated to evaluate the effectiveness of medicinal plants for the treatment of various diseases and understand pharmacological activities (Tokalıoglu, 2012). Superior quality control for medicinal herbs should be implemented to protect consumers from contamination. Various techniques, such as atomic absorption spectrometry (AAS) (Wang and Liu, 2010), inductively coupled plasma optical emission spectrometry (ICP-OES) (Liu et al., 2014), inductively coupled plasma-mass spectrometry (ICP-MS) (Bu et al., 2013), and X-ray fluorescence (XRF) (Kierdorf et al., 2014), are used to analyze trace elements in medicinal herbs. AAS as rapid and accurate technique for this purpose was chosen for the presented work.

This study aimed to determine ten trace elements (Ca, Cd, Cu, Fe, K, Mg, Mn, Na, Pb, and Zn) in *Z. clinopodioides* samples from different regions, periods and parts in Xinjiang, China. These elements were determined by flame and graphite furnace atomic absorption spectrometry, after microwave-assisted acid digestion was performed. To provide better insights into the patterns of elements, we performed common chemometric approaches, such as correlation analysis (CA), principal component analysis (PCA), and hierarchical cluster analysis (HCA), as classification techniques to evaluate data. Semi-monthly variation of elemental concentrations and the distribution of elements in leaves, flowers and stems were also investigated.

Materials and methods

Reagents and materials

All of the reagents used throughout the experiments were of analytical-reagent grade. Ultrapure grade HNO_3 (65%) and H_2O_2 (37%) were purchased from Tianjin Chemical Co., Ltd., China. Double-deionized water was produced using MilliQ water purification system (Millipore, Bedford, MA, USA). The standard solutions of Ca, Cd, Cu, Fe, K, Mg, Mn, Na, Pb, and Zn were obtained from the National Research Center for Certified Reference Material (CRM) of China (NRCCRM), and used to prepare the stock solution. Glassware and Teflon digestion vessels were cleaned by soaking overnight in diluted HNO_3 (1/9, v/v) and by subsequently rinsing with double-deionized water and drying before use.

Instruments

A Hitachi Z-2000 atomic absorption spectrometer (Hitachi Co., Ltd., Japan) equipped with a multi-elemental hollow cathode lamp and a Deuterium and Zeeman background correction system were used to determinate of trace elements. This equipment was operated using an air/acetylene flame and transverse-heated graphite furnace controlled by a personal computer. Operating parameters of the working concentrations of elements were set according to manufacturer's recommendation. Each sample was read three times to obtain a mean value. Concentration was corrected on the basis of volume of acid used in sample preparation.

A Sineo MDS-6 closed vessel microwave digestion system (Shanghai EU Analytical Instrument Co., Ltd., China) was used for extraction. Teflon reaction vessels were used for all digestion procedures. Operating parameters were set according to the manufacturer's recommendations.

Procedure

Eighteen samples of *Ziziphora clinopodioides* Lam., Lamiaceae, were collected in eighteen different regions in Xinjiang (Fig. 1) in July 2013. Nine samples were collected semi-monthly during May to August from one stationary place, one of the samples was divided into three parts (flowers, stems, and leaves) for study. All of the samples were identified by Yonghe Li, a chief apothecary of the Chinese Medicine Hospital of Xinjiang, China. These samples were washed with double-deionized water and then oven-dried at 60 °C. Dried samples were grinded and homogenized using a homogenizer. Afterward, these samples were screened with a 200 mesh sieve and stored in plastic bags.

A microwave-assisted digestion procedure was carried out to achieve a shorter digestion time. Two grams of herbal samples was accurately weighed into a Teflon digestion vessel. Afterward, 8 ml of a freshly prepared mixture of concentrated HNO_3–H_2O_2 (7,1, v/v) was added to the vessel and kept at room temperature for 10 min. The digestion conditions for the microwave system were applied as follows: (1) 400 W at 90 °C for 2 min, and (2) 400 W at 120 °C for 8 min. After ambient temperature was reached, the reactors were opened to eliminate nitrous vapors. The undissolved parts were separated by centrifugation at 1788.8 × g for 5 min. The extract was transferred to a volumetric flask and final volume of 50 ml was obtained by adding double-deionized water. Blank experiments (n = 3) were carried out in the same way.

Ten elements (Ca, Cd, Cu, Fe, K, Mg, Mn, Na, Pb, and Zn) were determined in this study. Ca, Cu, Fe, K, Mg, Mn, Na, and Zn ions were determined in an air/acetylene flame. Cd and Pb ions were determined using a graphite furnace atomic absorption spectrometer (GFAAS). The operating parameters for the working concentrations of the elements were set as recommended by the manufacturer. Each sample was read three times to obtain a mean value. Concentration was corrected for the volume of acid used in the sample preparation.

Data analysis

Statistical data analysis was performed using SPSS 19.0 software for Windows. Chemometric approaches were used to evaluate elemental data widely (Wang and Liu, 2010), such as CA, PCA, and HCA, to analyze differences in trace element patterns in *Z. clinopodioides* samples.

Results and discussion

Elemental content of different geographical regions

Table 1 lists the average results of the elemental contents obtained from triplicate analyses by AAS method. The average content of the elements in the studied *Z. clinopodioides* decreased in the following order, K > Ca > Mg > Fe > Cu > Zn > Na > Mn > Cd > Pb. This result was similar to that in a previous study (Tian et al., 2009). In general, trace elements in plants are mainly obtained from soil (Russell, 1979). Different *Z. clinopodioides* samples varied on the basis of elemental content. A total of eighteen samples were collected from different regions along Tianshan Mount in Xinjiang, and each of them was placed away from one another at a long distance. The soils on this mountain differ because of the complex structure and geographical environment of Tianshan Mountain (Ma et al., 2014). Various reports have revealed the same conclusions as the result of this study (Shi et al., 2009). Our results were also affected by other factors, such as climate, humans, or other plants. For example, sample 4 contained Cd and Pb levels higher than the other samples. To our knowledge, the major reason is because an important highway that crosses the distribution of sample 4. Excessive amounts of automobile exhaust induced the accumulation of heavy metals in soil and this exhaust was absorbed by *Z. clinopodioides*. On the contrary, samples 7, 10, 11, 13, 15, and 17 contained lower levels of Cd and Pb because of less human activity.

Correlation analysis

Correlation coefficient (c.c.) is a measure of the linear relationship between two attributes or columns of data. A coefficient close to +1 or −1 indicates that a similarity is observed between two elements (positive and negative c.c. indicate positive and negative correlations, respectively, between the two elements). A value close to 0 shows that two elements are weakly related or probably not related. The results of the correlation matrix analysis of 10 trace elements are presented in Table 2 showing moderate to strong correlations for all of the elements included in this study.

Table 1
Concentrations of elements in *Ziziphora clinopodioides* samples from different geographical regions.[a]

No.	Ca ($\mu g\,g^{-1}$)	Cu ($\mu g\,g^{-1}$)	Zn ($\mu g\,g^{-1}$)	Fe ($\mu g\,g^{-1}$)	K ($\mu g\,g^{-1}$)	Mg ($\mu g\,g^{-1}$)	Mn ($\mu g\,g^{-1}$)	Na ($\mu g\,g^{-1}$)	Cd ($ng\,g^{-1}$)	Pb ($ng\,g^{-1}$)
S1	2980.17 ± 63.61	54.01 ± 1.12	36.18 ± 0.57	497.05 ± 6.12	11,553.67 ± 40.80	958.83 ± 22.77	17.99 ± 1.73	9.46 ± 0.35	0.73 ± 0.02725	0.03 ± 0.00094
S2	3152.59 ± 57.66	67.51 ± 1.87	79.61 ± 0.60	538.27 ± 12.76	9943.50 ± 37.09	3346.24 ± 38.34	19.07 ± 1.32	23.72 ± 0.36	0.11 ± 0.00403	0.02 ± 0.00070
S3	2911.21 ± 17.86	60.76 ± 0.96	46.05 ± 0.26	778.72 ± 11.08	8333.33 ± 13.43	2700.99 ± 22.38	20.16 ± 0.91	24.19 ± 0.77	0.73 ± 0.03925	0.02 ± 0.00013
S4	3575.00 ± 29.87	74.26 ± 5.42	117.11 ± 0 94	1067.26 ± 30.18	12,090.40 ± 52.28	2926.83 ± 46.78	20.16 ± 0.81	26.09 ± 0.64	4.69 ± 0.13336	0.03 ± 0.00069
S5	3497.41 ± 54.36	60.76 ± 1.27	37.50 ± 0.49	552.01 ± 16.93	11,045.20 ± 56.94	2507.42 ± 27.73	14.74 ± 0.33	17.54 ± 0.83	0.11 ± 0.00503	0.01 ± 0.00002
S6	1643.97 ± 28.38	83.26 ± 1.47	63.82 ± 0.14	1218.40 ± 30.79	13,361.58 ± 22.18	2120.27 ± 39.31	21.24 ± 1.33	105.91 ± 0.49	3.65 ± 0.20757	0.02 ± 0.00016
S7	2023.28 ± 20.28	63.01 ± 0.73	62.50 ± 0.16	421.48 ± 4.18	9378.53 ± 19.06	2507.42 ± 2.79	13.65 ± 1.10	15.64 ± 0.66	ND	0.01 ± 0.00019
S8	1083.62 ± 13.87	78.76 ± 1.09	53.95 ± 0.23	2674.84 ± 16.97	11,807.91 ± 59.67	3249.45 ± 36.21	60.28 ± 1.94	70.76 ± 0.66	4.07 ± 0.19586	0.03 ± 0.00048
S9	1626.72 ± 34.41	72.01 ± 1.09	44.74 ± 0.33	1726.78 ± 10.16	13,192.09 ± 48.98	2410.63 ± 16.79	31.00 ± 0.72	68.38 ± 2.24	ND	0.02 ± 0.00020
S10	3135.34 ± 43.44	58.51 ± 0.87	32.89 ± 0 42	421.48 ± 6.01	8983.05 ± 27.78	1991.22 ± 1.87	17.99 ± 1.54	18.97 ± 0.29	ND	0.01 ± 0.00021
S11	3118.10 ± 36.82	69.76 ± 1 79	44.74 ± 0.16	1445.11 ± 11.24	8926.55 ± 49.67	5604.59 ± 38.37	37.51 ± 1.73	58.40 ± 0.89	ND	0.01 ± 0.00042
S12	2721.55 ± 38.74	65.26 ± 1.50	38.82 ± 0.34	778.72 ± 19.60	8474.58 ± 55.95	3055.88 ± 8.85	36.42 ± 2.64	26.57 ± 0.47	1.36 ± 0.13932	ND
S13	2704.31 ± 75.54	56.26 ± 3.04	59.87 ± 0.45	476.44 ± 5.42	9943.50 ± 35.20	2700.99 ± 15.18	28.83 ± 2.17	27.04 ± 1.59	0.53 ± 0.01022	ND
S14	3075.00 ± 25.74	74.26 ± 1.13	54.61 ± 0.69	1184.05 ± 9.85	7937.85 ± 25.32	4281.84 ± 61.45	49.44 ± 1.20	138.70 ± 1.13	ND	0.04 ± 0.00091
S15	2307.76 ± 16.17	69.76 ± 1.53	13.16 ± 0.19	991.69 ± 11.17	10,480.23 ± 24.96	3055.88 ± 21.96	32.09 ± 0.74	32.75 ± 0.28	0.32 ± 0.01472	ND
S16	2756.03 ± 14.79	47.25 ± 1.38	29.61 ± 0.08	579.49 ± 6.35	9039.55 ± 44.11	2249.32 ± 56.68	29.92 ± 1.92	33.22 ± 1.08	3.86 ± 0.09100	ND
S17	3040.52 ± 39.78	83.26 ± 2.17	44.74 ± 0.23	2008.45 ± 14.65	9124.29 ± 15.80	5507.81 ± 91.30	72.21 ± 1.54	80.73 ± 1.45	ND	0.01 ± 0.00022
S18	2307.76 ± 46.22	76.51 ± 3.70	71.71 ± 0.53	2441.26 ± 24.64	10,564.97 ± 26.68	3443.02 ± 72.32	67.87 ± 0.23	85.96 ± 1.30	ND	ND
Mean	2647.80	67.51	51.75	1100.08	10, 232.27	3034.37	32.81	48.00	1.12	0.01

[a] Results were mean ± standard deviation obtained from three replicates of one sample. ND: not detected.

Table 2
Correlation matrix for the elemental concentrations in *Ziziphora clinopodioides*.[a]

	Ca	Cu	Zn	Fe	K	Mg	Mn	Na	Cd	Pb
Ca	1.000									
Cu	−0.357	1.000								
Zn	0.113	0.371	1.000							
Fe	−0.510[*]	0.753[**]	0.147	1.000						
K	−0.471[*]	0.341	0.283	0.292	1.000					
Mg	0.174	0.537[*]	0.092	0.491[*]	−0.412	1.000				
Mn	−0.256	0.578[*]	−0.027	0.842[**]	−0.140	0.652[**]	1.000			
Na	−0.335	0.712[**]	0.113	0.645[**]	0.088	0.477[*]	0.643[**]	1.000		
Cd	−0.239	0.115	0.360	0.154	0.410	−0.242	−0.042	0.046	1.000	
Pb	−0.050	0.294	0.435	0.137	0.247	−0.034	−0.078	0.317	0.186	1.000

[a] *p* values.
[*] Means $p < 0.05$.
[**] Means $p < 0.01$.

C.c. > 0.5 (or <−0.5) was used to interpret correlation analysis results. However, some values close to ±0.5 were also included to produce groupings such as 0.491 for Mg and Fe and −0.471 for Ca and K. Significant correlation between two or more elements indicates similar ability to enter *Z. clinopodioides* or the presence of the same source. High correlations were found among Na, Cu, Fe, Mg, and Mn with c.c. between 0.491 and 0.842. Dependency between these elements can be explained by the common origin of the soil minerals. In addition, the strongest correlation was observed between Mn and Fe (c.c. = 0.842). However, Ca was negatively correlated with Fe and K (c.c. = −0.510 and c.c. = −0.471, respectively).

Correlation coefficient method provides information that could only indicate the same source or behavior for two or more elements, whereas information on the differences between samples from the different regions cannot be provided. Further interpretations between elements and regions may be obtained using more powerful chemometric techniques, such as PCA.

Principal component analysis

In order to highlight the relationships between elements, we subjected the entire data set to PCA. PCA could also be performed on the concentrations of elements to enable a discrimination of the samples. The first four principal components were extracted and explained 83.51% of the total variability among the 10 variables. Fig. 2A shows the loading plot (PC 1 *versus* PC 2) where Cu, Fe, Mn, and Na predominated in the higher positive values of PC 1 and explained 38.15% of the total variance. On the contrary, Ca exhibited considerably negative values in the PC 1 because Ca was negatively correlated with these elements. K and Cd elicited the highest effect on PC 2 and accounted for 22.82% of the total variance. Mg negatively correlated with PC 2. The third component was correlated with Zn and Ca. Based on the scores on the first principal component, Cu, Fe, Mn, and Na contents on the first principal component loadings were higher in sample 8, 9, 14, 17, and 18 than in the other samples; by contrast, Cu, Fe, Mn, and Na contents on the first principal component loading were lower in samples 1, 10, 13, and 16 than in the other samples. Based on the scores of the second principal component, K and Cd concentrations were higher in samples 4, 6, and 8 but lower in samples 10, 11, and 14 than in the other samples.

The first two varimax rotated principal component loadings and scores are shown in Fig. 2. The loadings indicated the similarities and correlations between the two elements. Elements with high loadings greatly influenced on the grouping and the separation of samples; by contrast, elements with small loadings slightly influenced data structure. In Fig. 2A, an association between Cu, Fe, Mn, and Na was apparent. Another association was observed between K and Cd on the PC 2, whereas Ca and Mg appeared more dispersed into the component space and showed a more individualized characteristic. The scores in Fig. 2B indicated the similarities and the correlations between two samples. The points in the anomalous positions 6, 8, 9, 14, 17, and 18 were outliers, in particular, the sample 6 and 17 contained the highest Cu, whereas samples 6 and 9 contained the lowest Ca (Table 1); Sample 8 and 18 contained the highest Fe, whereas sample 14 contained the highest Na (Table 1).

Hierarchical cluster analysis

HCA is one of the most widely used unsupervised pattern recognition techniques in chemometrics. In this technique relationships between samples are determined without using prior information pertaining to these relationships. HCA was applied using SPSS 19.0. In this study, Ward's method of clustering was applied to the squared Euclidean distance as a measure. Similar groupings to those described previously were obtained from the results of HCA, as shown in the dendrogram (Fig. 2C). These groups were listed as follows,

Group 1, Samples 2, 3, 7, 10, 12, 13, 15, and16.
Group 2, Samples 1, 4, and 5.
Group 3, Samples 11, 14, and 17.
Group 4, Samples 6 and 9.
Group 5, Samples 8 and 18.

Considering geographical distance (Fig. 1), we found that our classification was similar to the geographical distribution in most regions. The samples in Group 1 were not far from each other in geographical distance, but sample 15 was slightly far from other samples. Sample 1 and 4 in Group 2 were close to each other, however, these samples are far from sample 5. In Group 3, samples 11 and 14 were near to each other, but these samples were far from sample 17. Group 3 was around Group 1, but was not to assigned in the same cluster. Sample 6 was closer to samples 8 and 18 which were in Group 5, but far from sample 9. Most clusters could correspond to geographical distribution, but not for several samples. Soil is the major source of elements in plants. Different nutrient uptakes of plant from roots are attributed to the type of soil (Malaspinam et al., 2014). As Fig. 1 shows the collected *Z. clinopodioides* samples from the different areas of nearly the same latitude along the Tianshan Mountains, which is a complex topography. This complex structure provides different environments with great diversity. Environmental diversity induces differences in soil type and plant growth, thus, clusters differ based on trace element concentrations, which did not correspond to geographical factors (Xu et al., 2015).

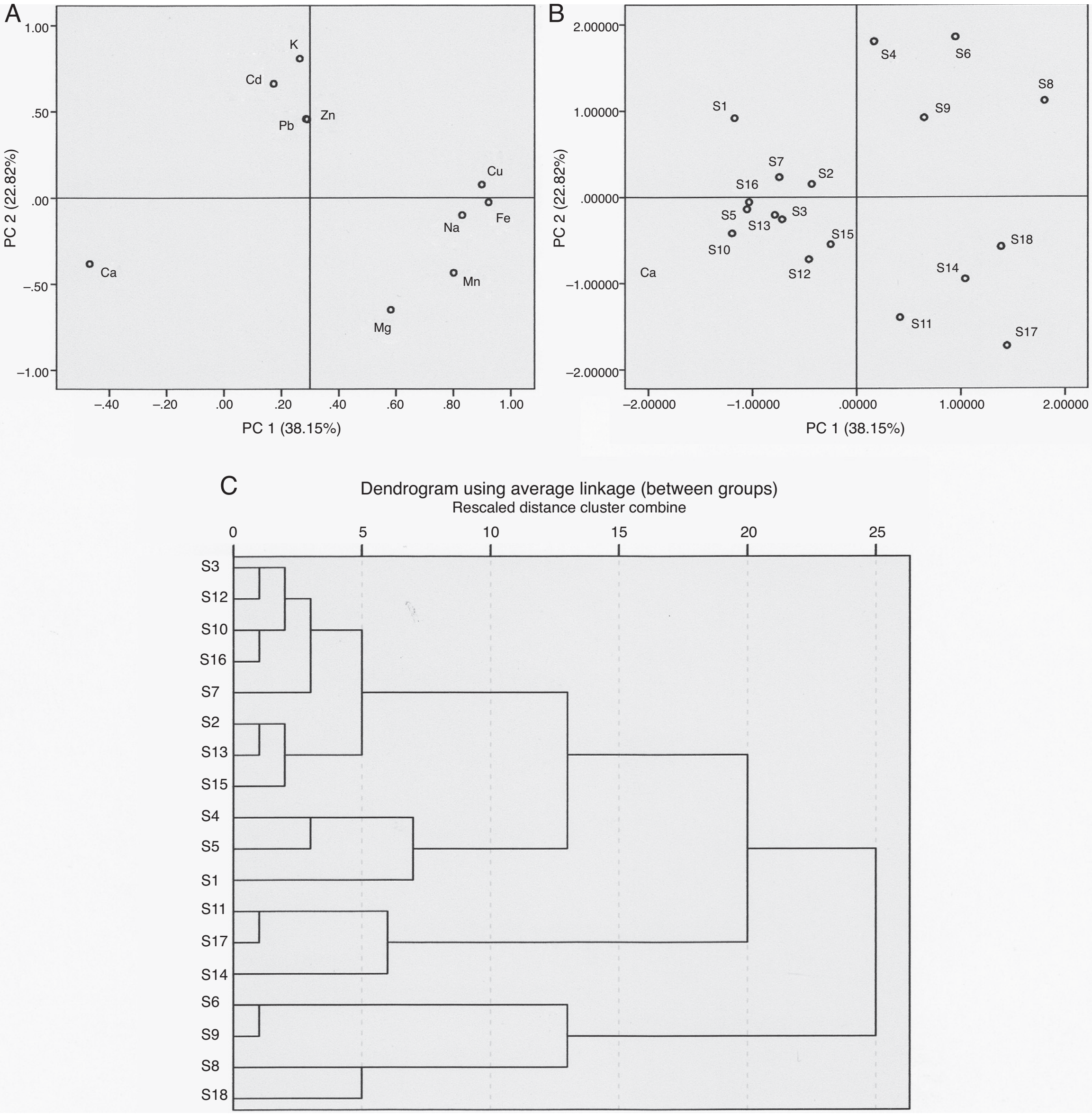

Figure 2. Principal component analysis and hierarchical cluster analysis on elemental concentrations based on 10 variables in *Z. clinopodioides.* Panel A and B illustrate the corresponding loading plot and the score plot. C is the dendrogram of cluster analysis of the 18 *Z. clinopodioides* samples from different geographical origins in Xinjiang.

Trace elements variation in different periods

A similar trend that is mainly characterized by a decline from the beginning time to the final time, was observed in the highest trace element concentrations in *Z. clinopodioides* samples from nine different collection time points but with a minimal difference between elements. In Fig. 3, the eight elements (except Cd and Pb) could be grouped into one major type, the “W” type (Ca, Fe, Mg, Na, and Zn), and three individual types, namely, “N” type (Mn), “Oblique” type (Cu), and “Wave” type (K).

For the “W” type, a reducing trend was observed in early collection period and then an uncertain peak appeared during the summer time. Ca, Fe, and Zn reached the peaks in early summer, whereas Na and Mg reached the peaks in middle and late summer, respectively. An increase was appeared in early autumn (Fig. 3) for the “N” type, indicating an increase in early summer; a decrease in Mn concentration was then observed until middle summer. No more fluctuations occurred in the following time (Fig. 3).

For the “Oblique” type, a totally reducing trend was displayed during the investigation periods for the Cu concentration (Fig. 3); “Wave” type was presented in the semi-monthly variation in K. with three peaks occurring in early, middle, and late summer, Therefore, three increase and decrease, like a wave, were found in the variation in K during the investigation periods (Fig. 3).

Trace elements play an important role in plant growth and metabolism. The variation in the elemental contents was correlated with the growth characteristics and biosynthesis of secondary metabolites. Our previous work focused on the chemical components (including volatile and non-volatile components) variation in the 9 periods of the same *Z. clinopodioides* samples (Ding et al., 2014a,b). Varying results in the elements were similar to those of the chemical components. For the volatile components, the essential oil content (%) showed a decreasing-increasing-decreasing trend, which was the same as variation in Ca, Fe, Mg, Na, and Zn. The major compounds, such as pulegone, displayed the same result. Other volatile chemical compounds, such as *p*-menthanone,

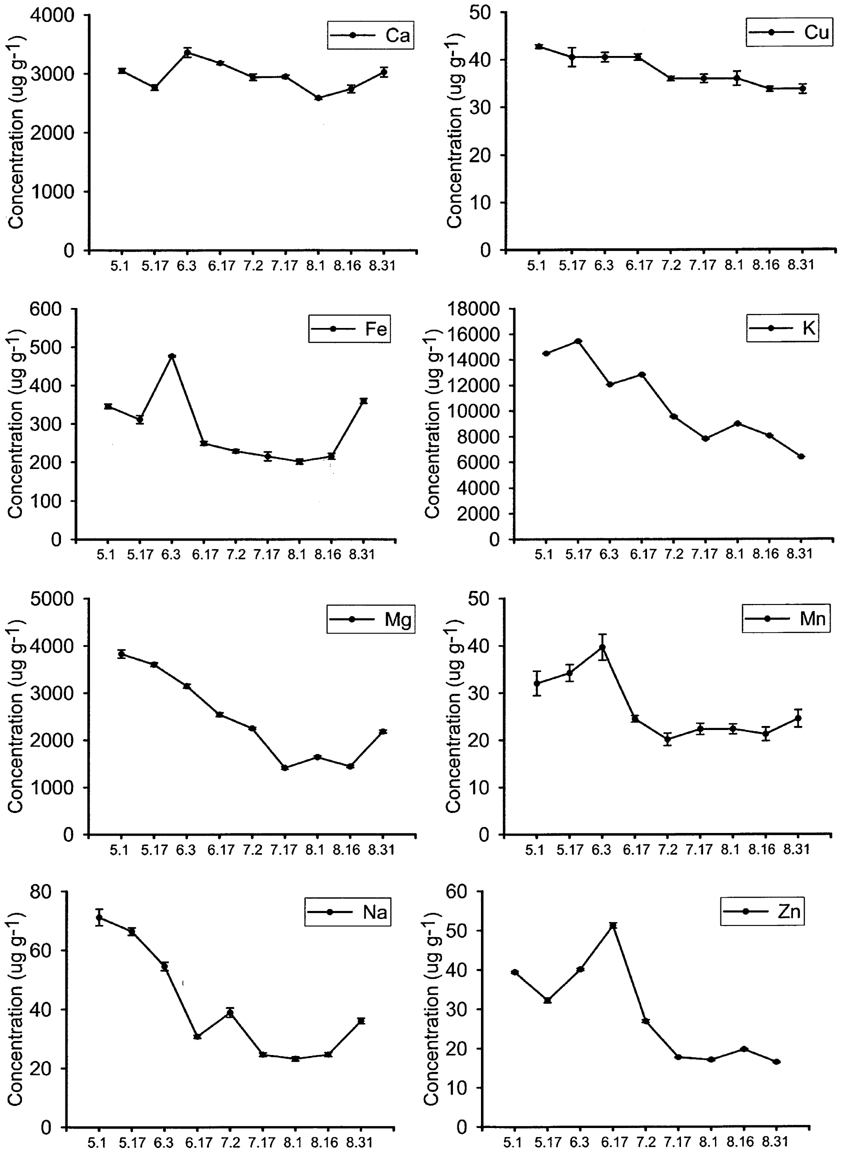

Figure 3. Semi-monthly variation of elemental concentrations in *Z. clinopodioides*.

trans-isopulegone, eucarvone, α-pinene, and β-pinene, showed a “Wave” type variation. On the contrary, non-volatile components, except flavonoid compounds, showed an increasing trend; phenolic and triterpenoid compounds exhibited a “W” type similar to most elements, free amino acids, and K. In polysaccharides, higher contents are also detected in the early growth stage (Ding et al., 2014a,b).

Plants grow fast in early growth stages to accomplish plant development and organ formation. Amounts of trace elements are absorbed from soil and then become accumulated to complete the growth process. Elements are more significant in plant metabolism and slightly participate in bioavailability after plant organs are formed. Lesser amounts are needed in early growth stage than during plant development. However, different plants show different patterns of metabolic changes in trace elements (Niu et al., 2014).

Trace elements distribution in different parts of Z. clinopodioides

The concentrations of eight elements (Ca, Cu, Fe, K, Mg, Mn, Na, and Zn) in flowers, stems and leaves from one plant of *Z. clinopodioides* were investigated. The concentrations of the examined elements were different (Fig. 4). The following concentrations were observed in flowers, stems, and leaves,

Flowers, K > Ca > Mg > Fe > Cu > Zn > Na > Mn.
Stems, K > Ca > Mg > Fe > Cu > Na > Zn > Mn.
Leaves, K > Ca > Mg > Fe > Cu > Zn > Mn > Na.

The same ranking was observed in K, Ca, Mg, Fe, and Cu in the three parts. The difference was observed in the sequence of Na, Mn, and Zn. Na, Mn, and Zn were also in the same level but showed few differences, thus, no great influence was observed. However, certain gaps were detected in K, Ca, Mg, and Fe (Fig. 4). The concentration of K was the most abundant of all the examined elements, with approximately 10 mg g^{-1} followed by Ca, Mg, and Fe, which were approximately 3, 1, and 0.1 mg g^{-1}, respectively.

For the distribution, leaves accumulate more concentration of elements than those of the flowers and stems. Leaves play a major role in plant photosynthesis, energy production, and respiration.

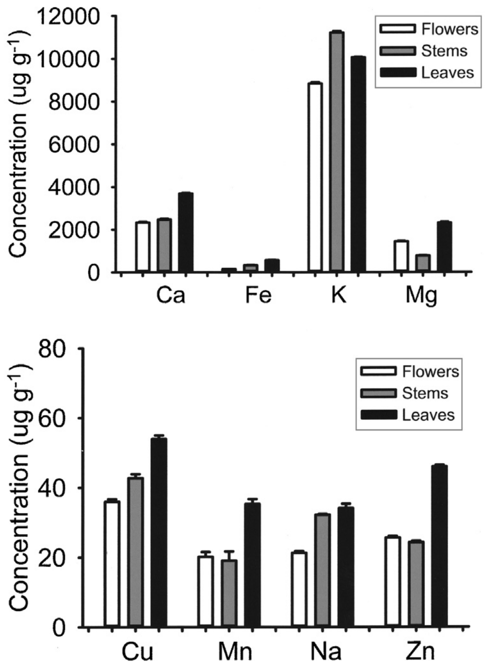

Figure 4. The distribution and concentrations of elements in flowers, stems, and leaves of *Z. clinopodioides*.

Many elements are necessary to stimulate physiological activities in leaves. However, K content in the stems was more than that in leaves. Ca, Mn and Zn concentrations are approximated between the flowers and stems. The stems contains higher Cu, Fe and Na contents than the flowers, by contrast, Mg concentration was higher in the flowers than stems.

Conclusion

In the present study, 18 *Z. clinopodioides* samples collected from different regions in Xinjiang were characterized according to their metal contents. The concentrations of the 10 elements (Ca, Cd, Cu, Fe, K, Mg, Mn, Na, Pb, and Zn) were determined by flame and graphite furnace atomic absorption spectrometry following microwave-assisted acid digestion. The average content of the elements in the studied *Z. clinopodioides* decreased in the following order, K > Ca > Mg > Fe > Cu > Zn > Na > Mn > Cd > Pb. Chemometric methods, such as PCA and HCA techniques were used to classify these samples based on their concentrations. The results obtained by PCA and HCA confirmed that elemental content data support adequate information to differentiate *Z. clinopodioides* samples according to their type. PCA yielded four principal PCs that accounted for 83.51% of the total variance in the data. HCA revealed five groups of *Z. clinopodioides* samples based on their trace element concentrations. This study revealed that PCA and HCA analysis are useful tools for the authentic differentiation of *Z. clinopodioides* samples by using the profile of elemental contents and they highlighted the diversity of elements. Variations in the elements in the studied periods showed a decreasing trend, but with different types. This change was associated with the chemical components reported previously. Among the three plant parts, the leaves showed the highest amount of accumulated elements. This study provided some elemental information on content, diversity, chemotaxonomy, changing pattern, distribution, and metabolism of *Z. clinopodioides* at spatial and temporal levels; this result could be used as a reference of planting and quality standards.

Authors' contributions

XJZ, JL and FL contributed in running the laboratory work and XJZ carried out data analyses and wrote the manuscript. WHD, XJZ, JL and XYZ contributed in collecting plant samples. SGT designed the study, supervised the laboratory work and gave critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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