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Elemental analysis and ecological safety assessment of nutritive herb *Ziziphora clinopodioides Lam*. cultivated and wild growing in the South-Caucasian flora

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ABSTRACT

Background: Today, the use of mathematical modeling is crucial in assessing the ecological safety of nutritive herbs. The aim of our research was to examine the mineral composition and assess the ecological purity of raw materials from *Ziziphora clinopodioides* Lam., collected across various natural and climatic zones in the South Caucasus region.

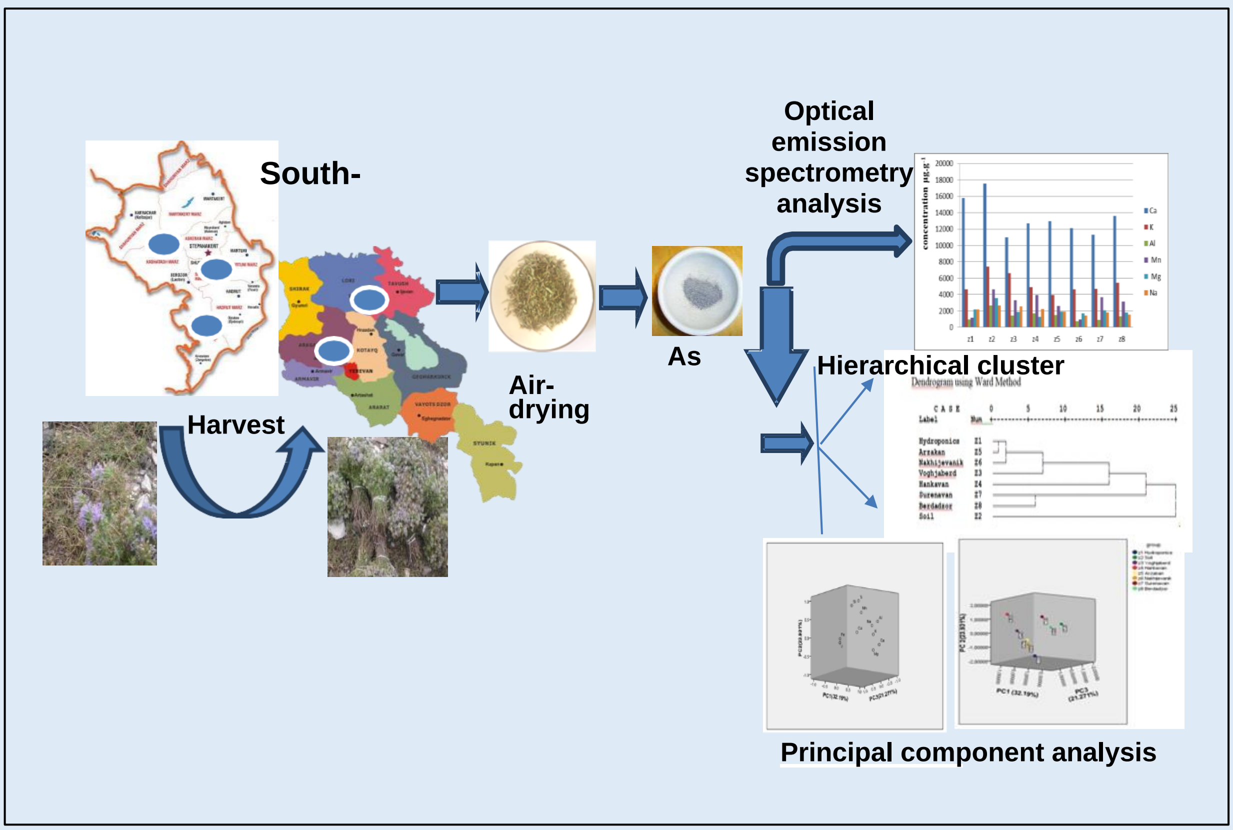
Objective: To conduct an elemental analysis of the mineral composition and assess the ecological safety of nutritive plant raw materials derived from *Ziziphora clinopodioides Lam*.

Methods: The analysis was performed using an Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) instrument, with results reported in micrograms per gram. A multi-standard approach with various dilutions was employed to investigate the quantities of elemental content. The elemental analysis of the obtained ash mass was conducted utilizing the Agilent 5800 VDV ICP-OES instrument (USA). Mathematical modeling techniques, including Correlation Analysis, Principal Component Analysis, and Hierarchical Cluster Analysis, were used in the study.

Results: Experimental data unequivocally demonstrated that the concentration of heavy metals in all analyzed *Z. clinopodioides* samples falls within the established limits of maximum permissible concentrations. Notably, no toxic metals such as Lead (Pb), Arsenic (As), or Cadmium (Cd) were detected in any of the samples, affirming the ecological safety of the raw materials. Principal component analysis yielded four predominant components, collectively accounting for 91.934% of the overall variability among 13 variables, as based on the variation in elemental concentrations. Furthermore, hierarchical cluster analysis (HCA) enabled the categorization of plant raw material samples into three distinct groups, contingent on the geographical origins of the areas and the methods of cultivation.

Conclusions: Chemometric analysis conclusively demonstrates that the variation in elemental composition within the examined samples, as well as the plant's capacity to accumulate biologically active compounds, is contingent not solely upon the altitude of the growth locations above sea level but also by the conditions of cultivation and introduction.

Keywords*:* *Ziziphora clinopodioides Lam.,* nutritive herbs, ash, chemometric approaches, correlation analysis, hierarchical cluster analysis, optical emission spectrum



Graphical Abstract: Elemental analysis and ecological safety assessment of nutritive herb *Ziziphora clinopodioides Lam*. cultivated and wild growing in the South-Caucasian flora.

INTRODUCTION

With advancements in food science and technology, there has been a growing demand for better and more natural food ingredients [1]. Over the past decade, scientific studies have increasingly focused on the impact of environmental factors on the accumulation of macro, micro, and ultra-microelements in nutritive plant raw materials. Additionally, the presence of biologically active substances, particularly minerals such as potassium, calcium, magnesium, and phosphorus, plays a key role in the development of functional products derived from plants. It should be noted that the accumulation of certain biologically active compounds in plants significantly depends on the variety and conditions of their cultivation. It is well-established that trace elements, alongside primary and secondary metabolites, significantly impact the biological activity of plants and plant-derived medicinal products. The quantity of trace elements in food plants is of paramount importance due to theiressential role in the human body. Imbalances in trace element homeostasis can lead to the development of pathological conditions and illnesses [2-4].

In this context, the investigation of the mineral composition of plant raw materials becomes a subject of considerable relevance. In today's world, environmental pollution stands as a universal concern, prompting extensive scientific research into how environmental factors affect the accumulation dynamics of macro, micro, and ultra-trace elements within herbal raw materials. Given that humans derive essential and conditionally essential vital elements from plant-based food sources, the study of the mineral composition of herbal raw materials assumes a pivotal role. Furthermore, the mineral composition of plants not only underscores their medicinal value but also opens avenues for analyzing the corresponding mineral composition in herbal raw materials, facilitating the development of novel medicines. To address this concern, it becomes imperative to evaluate the degree of pollution in plants, as heavy metals accumulated in plants can pose harm to the human body. The accumulation of these metals is influenced not only by climatic factors but also by the conditions of growth [5].

Over the years, the genus Ziziphora, particularly the species *Z. clinopodioides Lam*., has garnered significant interest in scientific research across various climatic zones. Ziziphora species represent a quintessential example within the Lamiaceae family. Z*. clinopodioides* is an annual or perennial herb, or subshrub, with wide distribution spanning from the Mediterranean to central Asia and Afghanistan. Notably, in Xinjiang, China, the entire herb has found application in traditional Kazakh and Uygur medicines for its anti-febrile and detoxicating properties, while in Iranian traditional medicine, it is recognized for its antioxidant, antimicrobial, antibacterial, sedative, and stomach-soothing attributes [6-10]

Z. clinopodioides ranks among the most significant medicinal plants in traditional medicine within the natural mountainous regions of Iran. Extensive investigations into its ecological characteristics, such as climate preferences, vegetation studies, and physical and chemical soil analyses, have revealed its inclination towards loamy textures, lime, alkaline pH, and non-saline soils [6].

Phytochemical analysis of *Z. clinopodioides* essential oils has unveiled a rich content of monoterpene and sesquiterpene compounds, while the extracts contain flavonoids, a subgroup of phenolic compounds. This composition has led to the utilization of Ziziphora species in Kazakh traditional medicine for ailments related to the cardiovascular system and various infections [11]. Some studies have also indicated that the application of Ziziphora in traditional medicine is attributed not only to biologically active phenolic substances but also to the presence of macro and trace elements. Research has demonstrated that *Z. clinopodioides* growing in China boast relatively high concentrations of macro and microelements, including calcium, magnesium, and potassium. Furthermore, chemometric approaches have been employed to discern information on chemotaxonomy, diversity, and the plant's metabolic processes across spatial and temporal dimensions [12-13].

In the 1990s, Armenian scientists notably directed their attention toward *Z. clinopodioides*. Subsequent studies underscored the prevalence of flavonoids, particularly thymonin, as the primary biologically active substances within Ziziphora, demonstrating pronounced antimicrobial activity [14-16]. A review of the scientific literature has revealed *Z. clinopodioides Lam*. to be an endemic plant within the floras of South-Caucases. Resource assessments have identified wild-growing Ziziphora populations in Armenia, primarily as small-scattered semi-shrubs inhabiting rocky slopes in mountainous areas to subalpine elevations [17].

The chemical constituents of essential oils and the biological activity of Ziziphora raw materials, whether cultivated in hydroponic conditions or growing wild in the floras of South- Caucasian have undergone extensive examination. The results have shown the antimicrobial activity of essential oils containing over 60 components, as well as relatively strong antioxidant properties in the extracts. These findings emphasize the potential of Ziziphora as a valuable source of essential oils and flavonoids, as well as antimicrobial and antioxidant agents [18-19].

Notably, in recent years, considerable attention has been drawn to chemometric research approaches that play a pivotal role in analyzing plant raw materials, particularly in elucidating the link between composition and biological activity [13,20].

Given the contemporary importance of assessing the ecological safety of plants in the South- Caucasian floras, our research aims to investigate the mineral composition and evaluate the ecological purity of raw materials collected from diverse natural and climatic zones within these regions. We employ chemometric approaches in elemental analysis to achieve these objectives.

MATERIALS AND METHODS

Plant Materia: Numerous samples of *Ziziphora clinopodioides Lam*., both from natural populations and hydroponically cultivated herbs, along with associated soil samples, were collected during the months of June and July in 2021 from various locations.

The samples were meticulously identified following the guidelines outlined by the registry for species identification. Specifically, the identification was performed in accordance with the taxonomy of *Z. clinopodioides Lam*. as described by Takhtajyan (1987) and Grossgeym (1949), along with adherence to the Good Agricultural and Collection Practices (GACP) established by the World Health Organization (WHO) in 2003 [19,21-23]

Voucher specimens of the wild-growing plants in the South-Caucasian floras (cataloged as ERE N194583 and ERE N201407) have been securely deposited in the Institute of Botany after

A.L. Takhtajyan of the National Academy of Sciences of Armenia. This meticulous documentation ensures the traceability and authenticity of the plant material used in our scientific research.

Cultivation Method: To commence the cultivation of *Ziziphora clinopodioides*, approximately 50 plant bushes were harvested from the vicinity of Voghjaberd and Hankavan, located in the Kotayk region, in mid-April (2021). These plants were subsequently transplanted to both hydroponic and soil areas, each covering a space of 5 square meters.

In the cultivation process, black slag with a diameter ranging from 3-15 mm was employed as a nutrient filler. Prior to use, this material underwent disinfection with a 0.05% solution of potassium permanganate ($KMnO_4$). Throughout the growth cycle, the plants received regular nutrition using Davtyan's method, which involved the application of a nutrient solution with a pH range of 5.5-6.5, 1-2 times daily administration [17,24].

The initial harvest of raw materials was conducted in early July, coinciding with the onset of the flowering phase, to the Good Agricultural and Collection Practices (GACP) outlined by WHO [23]. This systematic cultivation process ensures the production of high-quality plant materials for further scientific research and analysis.

Ash Content Determination Method: The work we've completed involved the determination of ash content in the material through the following steps: We measured a 2-gram quantity of ground, air-dried material. The material was carefully placed into a previously ignited and tared crucible, typically made of silica. Ensuring even distribution, the material was spread uniformly within the crucible.

Gradually, we increased the heat to a temperature range between 500-600°C. This gradual heating process is crucial for accurate results. The material was heated until it turned white, indicating the absence of carbon. Following the heating process, we allowed the crucible and its contents to cool within a desiccator. Once the crucible and material reached room temperature, we determined the weight of the sample. The residue, which now contained the ash, was placed back into a suitable desiccator for an additional 30 minutes to ensure complete cooling. After this cooling period, we weighed the crucible with the ash immediately. We then calculated the total quantity of ash present in milligrams per gram of air-dried substance [25].

This method yielded precise results and is crucial for various analytical and quality control purposes.

Optical emission spectrometry: All the reagents, as well as the standard solutions of Ca, K, Mn, Mg, Na, Si, Al, Fe, Ti, Cu, Mo, V, Ni, Cd, Pb, and As, were provided by the Scientific and Production Center "Armbiotechnology" of the National Academy of Sciences, Republic of Armenia.

Analytical Samples: Ash mass samples from eight raw materials were prepared for the study, each designated as follows: (analytic samples of the raw material) №1-sample from the hydroponics (z1), №2-sample from the soil (z2), №3 -sample from the Voghjaberd (z3), №4-sample from the Hankavan (z4),

№5-sample from the Arzakan (z5), №6-sample from the Nakhijevanik (z6), №7-sample from the Surenavan (z7), №8-sample from the Berdadzor (z8).

Analytical Sample Preparation: Sample preparation was conducted using a microwave instrument (Anton Paar Multiwave GO Plus, Austria). The process involved weighing the obtained ash mass, approximately 0.8 grams, into microwave vessels. Subsequently, 5 ml of concentrated (69%) HNO_3 was added to the vessels. The dissolution process was carried out in the microwave, employing a temperature of 180°C for a duration of 10 minutes. Following dissolution, the volume of the resulting solution was adjusted to 50 mL with deionized water in preparation for analysis.

The analysis itself was performed using an Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) instrument, with results reported in micrograms per gram (µg/g). A multi-standard with various dilutions was employed to investigate the quantities of elemental content [26].

The elemental analysis of the obtained ash mass was conducted utilizing the Agilent 5800 VDV ICP-OES instrument (USA) [27-28]. This analytical approach allows for the precise determination of elemental composition in the samples.

Data Analysis: The results of the study were processed by mathematical statistics using the MS application package "Excel 2010," "SPSS" ® for Windows (Version 19.0, Chicago, IL, USA).

Normal Distribution Assessment: To verify whether the sample data adhered to the normal distribution rule, the Kolmogorov-Smirnov test was employed. Descriptive Statistics: The results were presented as mean ± standard error of the mean (S.E.M) ($\bar{x} \pm E_s$), calculated by the t-test.

Statistical Comparison: The data were evaluated by one-way analysis (ANOVA) variance followed by a Tukey's test. Mean differences were considered statistically significant if $P \le 0.05$. Chemometric approaches were employed to perform a comprehensive assessment of the element content data. These approaches included:

Correlation Analysis (CA): This method was used to examine the relationships between different elements within the composition of *Ziziphora clinopodioides* raw material ash. It quantified the linear relationships between pairs of elements, with the correlation coefficient (cc) as the measure. A correlation coefficient near +1 or -1 indicates strong similarity or dissimilarity between two elements, while a value near 0 suggests a weak or potentially unrelated connection.

Principal Component Analysis (PCA): PCA was used to reduce the dimensionality of the data and identify underlying patterns or groupings in the element content dataset.

Hierarchical Cluster Analysis (HCA): HCA, employing Ward's method, was utilized to explore distinctions in the element composition among various *Ziziphora clinopodioides* samples. Ward's clustering method was applied using the square of the Euclidean distance as the measure [29-30].

These chemometric techniques provided a comprehensive and insightful analysis of the element content data, helping to reveal patterns, associations, and groupings within the dataset.

Correlation Analysis: Correlation analysis was utilized to explore the relationships between elements within the composition of *Ziziphora clinopodioides* raw material ash. The correlation coefficient (cc) served as a quantitative measure of the linear relationships between pairs of data columns. A correlation coefficient close to +1 or -1 indicated a strong similarity (positive or negative correlation, respectively) between the two elements. Conversely, a value near 0 suggested a weak or potentially unrelated connection between the two elements.

Hierarchical Cluster Analysis (HCA): Hierarchical cluster analysis was conducted using Ward's method to examine differences in the composition of elements across various *Ziziphora clinopodioides* samples. Ward's clustering method was applied using the square of the Euclidean distance as a measure [30]. This analysis aimed to identify patterns and groupings in the elemental composition data, shedding light on the diversity and relationships among the samples.

RESULTS AND DISCUSSION

Elemental Content of the Raw Material Wild Growing and Cultivated Plants: The elemental content of *Ziziphora clinopodioides* raw materials, collected from both wild- growing plants and those cultivated under different conditions in various regions of South-Caucasian, was analyzed using optical emission spectrometry. The analysis revealed the presence of 12-13 biogenic elements, which collectively constituted 75.78% of the ash. These elements included 7 essential elements (Na, Ca, Mg, Fe, Cu, Mo, Mn) and 5 conditionally essential elements (Si, Ti, V, Ni, Al) (see Table 1 and Figure 1). The mineral composition of *Z. clinopodioides* raw ash samples were categorized based on the geochemical parameters and cultivation area. On average, the elemental content of the samples decreased in the following order: Ca > K > Mn > Mg > Na > Si > Al > Fe > Ti > Cu > Mo > V > Ni (see Table 2 and Diagram 1). It's worth noting that this elemental sequence differs from the results of previous studies conducted by Chinese scientists (K > Ca > Mg > Fe > Cu > Zn > Na > Mn > Cd > Pb) [26, 30].

Importantly, all analytical samples displayed no detectable levels of Pb, As, and Cd, indicating the environmental purity of the raw materials (see Table 1) [25]. This finding diverges from the results of studies on the mineral composition of *Z. clinopodioides* native to China [12-13].

These differences can be attributed to variations in soil composition in South-Caucasian as well as certain technological factors.

Furthermore, the experimental data demonstrated that the concentrations of heavy metals (Fe, Cu, Mo, Mn, Ni, V, Ti, Pb, As, Cd) in all analytical samples of *Z. clinopodioides* fell within the maximum permissible concentrations for heavy metals. These results provide evidence of the ecological purity of *Z. clinopodioides* raw materials, reinforcing their safety for various applications (see Table 1) [25].

Table1. Concentrations of elements in *Z.clinopodioides* raw material wild growing in the South Caucasus flora, in soil and in the hydroponic conditions[a].

	Ca(μg · g-1)	K(μg · g1)	Al(μg · g1)	Mn(μg · g-1)	Mg(μg · g-1)	Na(μg · g-1)	Fe(μg · g-1)	Mo(g · g-1)	Si(μg · g-1)	Ti (μg · g-1)	Cu (μg · g-1)	V(μg · g-1)	Ni(μg · g1)
z1	15782.2± 192.57	4609.89± 98.31	941.83± 30.7	1163.5± 39.26	2189.4± 20.46	2154.3± 16.99	287.4± 10.37	3.6± 0.09	1664.8± 1.41	28.89± 0.9	2.72± 0.1	1.84± 0.11	ND
z2	17534.2± 119.04	7412.91± 123.38	2638.7± 9.33	4626.59± 37.4	3551.96± 48.12	2653.84± 26.8	462.52± 9.21	4.29± 0.12	2603.25± 14.61	82.4± 1.64	6.59± 0.12	2.17± 0.12	ND
z3	10965.04± 267.33	6618.36± 21.23	1414.04± 7.51	3295.79± 37.53	1880.19± 27.61	2530.19± 40.78	326.56± 6.64	0.99± 0.07	1855.27± 6.76	88.4± 1.36	3.24± 0.11	1.85± 0.083	1.89± 0.109
z4	12688.76± 189.93	4876.9± 98.44	1716.38± 14.45	3932.04± 30.29	1254.91± 19.16	2229.27± 22.93	294.29± 6.8	2.95± 0.08	3378.87± 21.4	227.42± 5.66	3.96± 0.1	1.692± 0.08	ND
z5	12978.41± 254.16	3932.04± 77.66	1470.67± 7.36	2603.04± 57.3	1897± 23.98	1934.98± 26.7	339.1± 5.29	ND	1958.48± 1.12	91.25± 1.69	2.58± 0.1	1.9± 0.097	3.38±0.09
z6	12118.21± 281.94	4623.36± 80.18	718.85± 24.98	958.89± 21.85	1671.87± 41.95	1436.93± 16.32	398.6± 6.35	ND	2275.05± 6.73	39.75± 1.03	3.92± 0.11	2.26± 0.08	ND
z7	11279.67± 247.36	4706.53± 131.82	897.73± 6.24	3694.79± 41.54	2077.52± 27.19	1818.93± 23.7	938.37± 44.36	ND	2800.92± 11.9	66.3± 1.51	4.91± 0.1	3.73± 0.11	2,1± 0,12
z8	13584.72± 306.74	5418.77± 112.27	1349.5± 32.28	3133.69± 33.14	1804.09± 45.36	1511.47± 19.52	789.36± 30.34	ND	2386.2± 16.63	54.96± 0.73	8.4± 0.34	2.4± 0.095	ND
Mean	13366.40	5274.85	1393.46	2926.04	2040.88	2033.75	479.53	2.96	2365.36	84.92	4,54	2,23	2,46

Table 2. The content of macro, micro, and ultramicroelements in the ash of the analytical samples z1, z2, z3, z4, z5, z6, z7, z8 of the *Z. clinopodioides* raw material in the descending order.

z1	Ca> K > Mg = Na > Si >Al > Fe>Ti >Mn > Mo>Cu>V
z2	Ca> K >Mn> Mg > Si=Na=Al> Ti > Fe > Cu>Mo>V
z3	Ca> K > Mn> Na > Mg = Si >Al > Fe>Ti > Mo>Cu>V =Ni
z4	Ca> K >Mn> Si >Na>Al > Mg > Fe>Ti >Cu> Mo >V
z5	Ca> K > Mn > Mg = Si =Na > Al > Fe>Ti > Ni>Cu>V
z6	Ca> K > Si > Mg >Na >Mn >Al > Fe=Ti >Cu>V
z7	Ca> K>Mn> Si > Mg >Na >Al =Fe>Ti >Cu>V > Ni
z8	Ca> K>Mn > Si > Mg > Na > Al > Fe>Ti >Cu>V

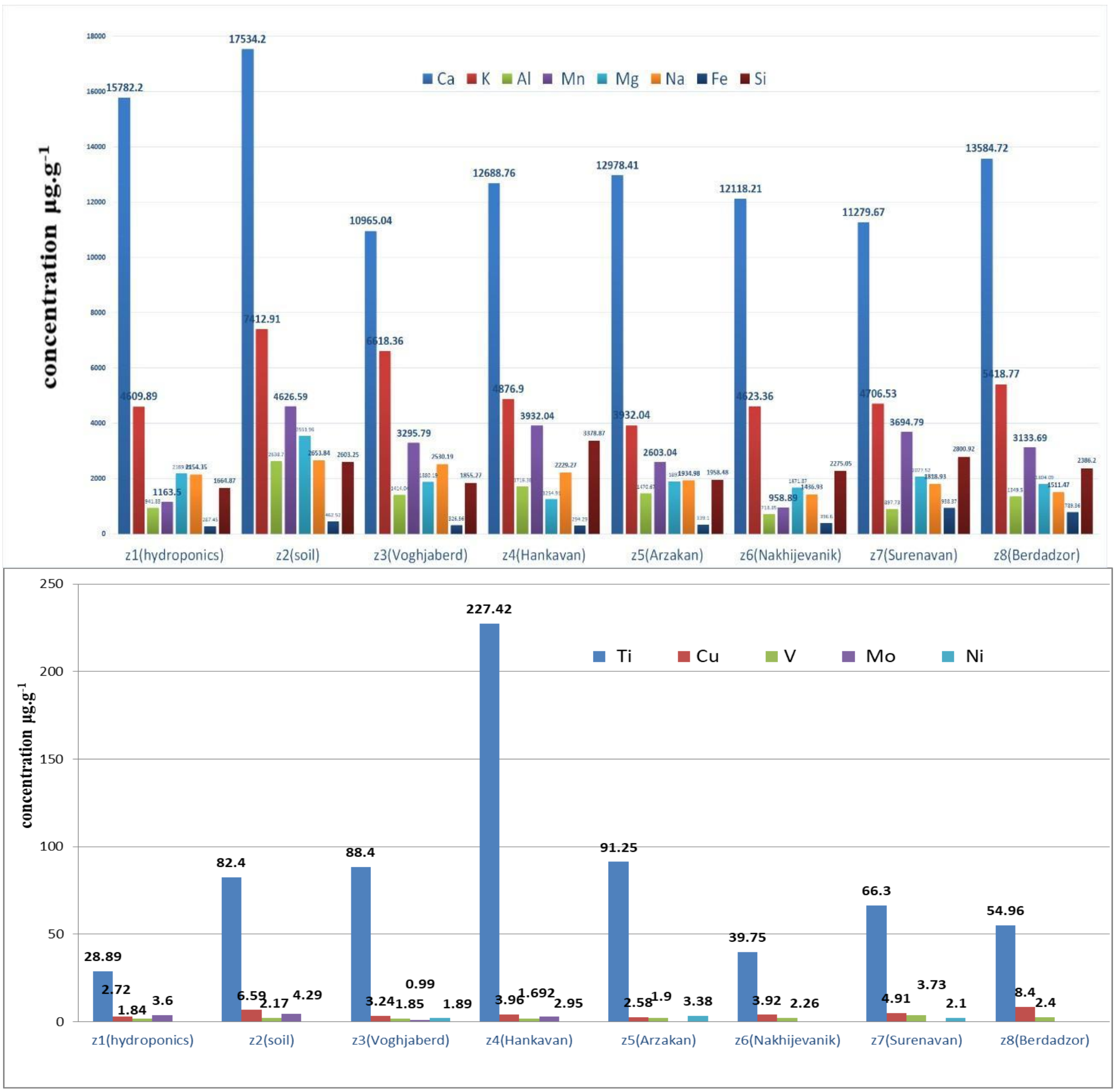
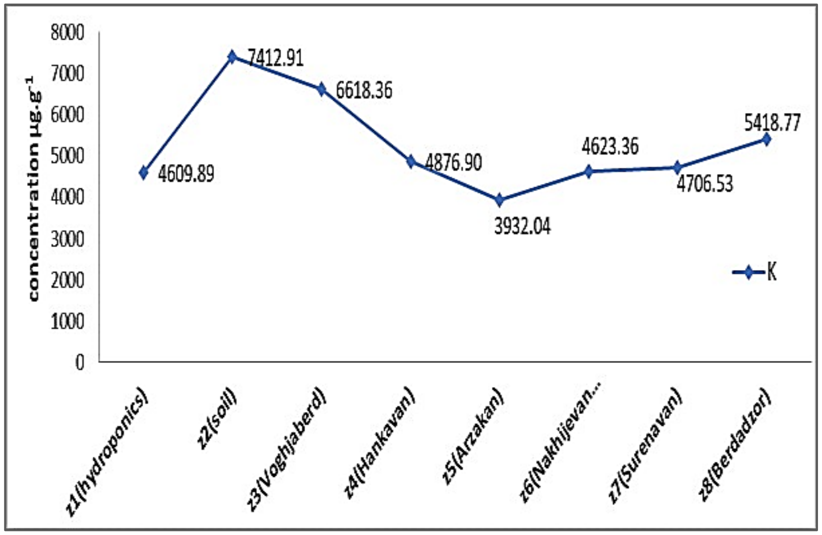
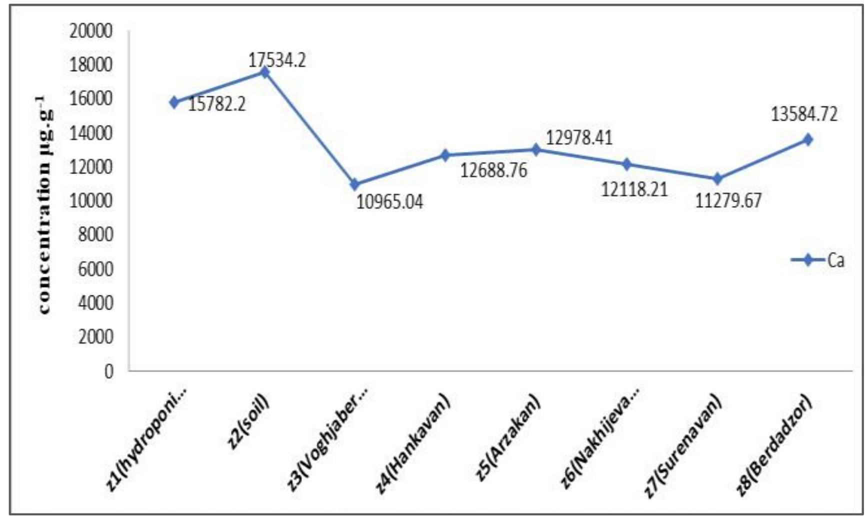
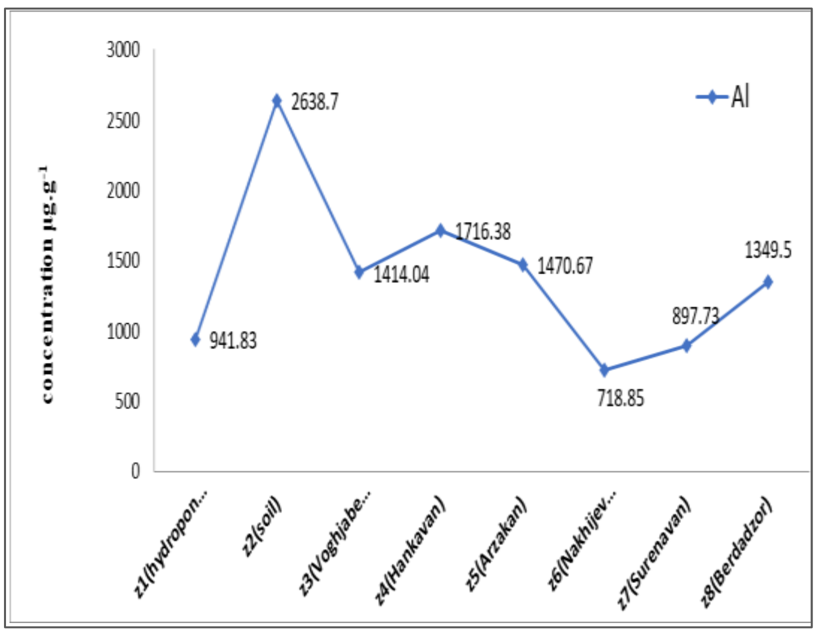
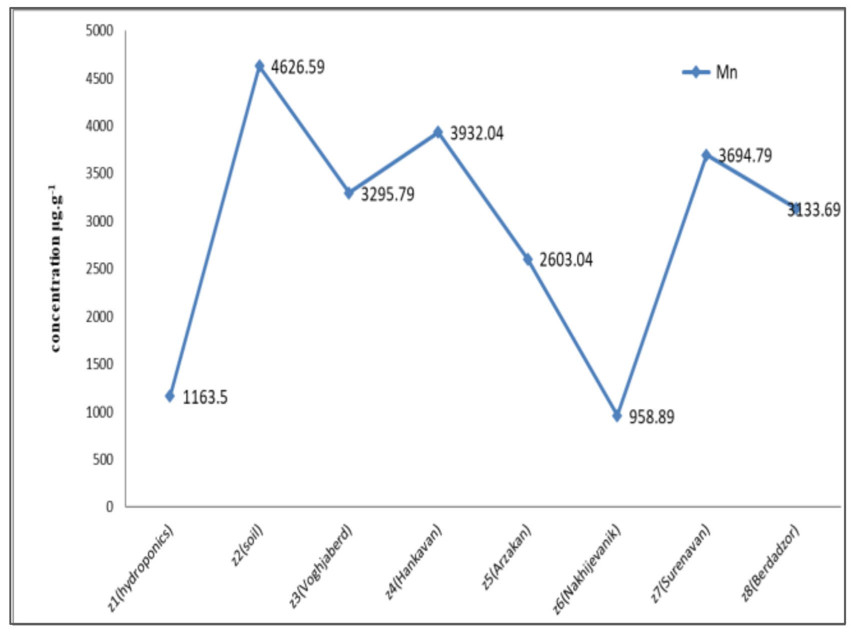


Figure 1. Distribution and concentration of elements in *Z. clinopodioides* raw material of depending on the growing areas and conditions of introduction


concentration µg.g-1
K
4609.89
7412.91
6618.36
4876.90
3932.04
4623.36
4706.53
5418.77
z1(hydroponics)
z2(soil)
z3(Voghjaberd)
z4(Hankavan)
z5(Arzakan)
z6(Nakhijevan...
z7(Surenavan)
z8(Berdadzor)


concentration µg.g-1
Ca
15782.2
17534.2
10965.04
12688.76
12978.41
12118.21
11279.67
13584.72
z1(hydroponi...
z2(soil)
z3(Voghjaber...
z4(Hankavan)
z5(Arzakan)
z6(Nakhijeva...
z7(Surenavan)
z8(Berdadzor)


concentration µg.g-1
Al
941.83
2638.7
1414.04
1716.38
1470.67
718.85
897.73
1349.5
z1(hydropon...
z2(soil)
z3(Voghjabe...
z4(Hankavan)
z5(Arzakan)
z6(Nakhijev...
z7(Surenavan)
z8(Berdadzor)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4626.59
3295.79
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958.89
3694.79
3133.69
z1(hydroponics)
z2(soil)
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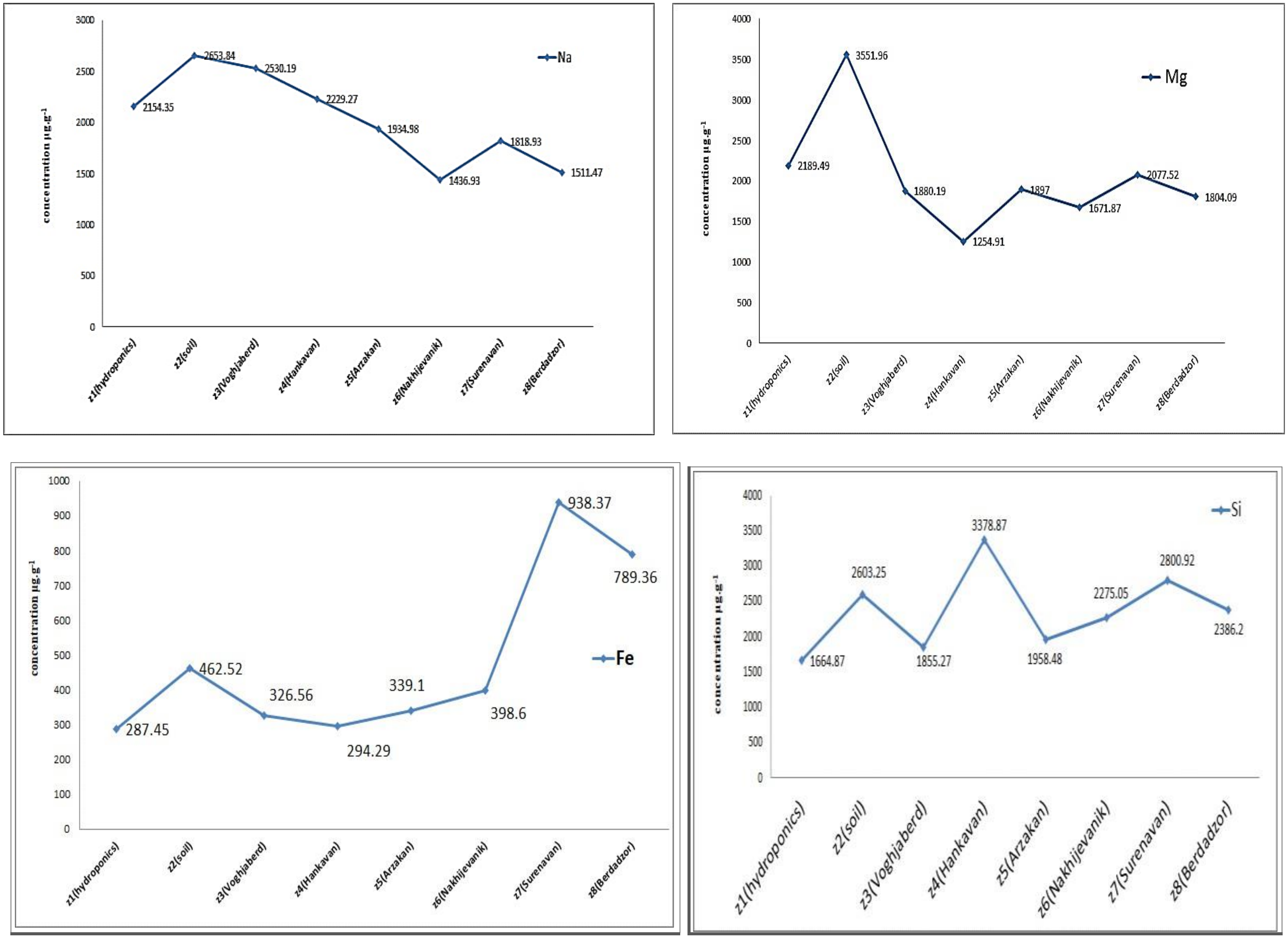

Diagram 1. Variations of elements concentrations in analytical samples of *Z. clinopodioides* raw material

Table 3. Correlation matrix of the elements

	Ca	K	Mn	Mg	Na	Fe	Al	Mo	Si	Ti	Cu	V	Ni
Ca	1,000												
K	0,841**	1,000											
Mn	0,588**	0,763**	1,000										
Mg	0,865**	0,843**	0,637**	1,000									
Na	0,842**	0,893**	0,749**	0,782**	1,000								
Fe	0,354*	0,391**	0,501**	0,399**	0,182	1,000							
Al	0,746**	0,805**	0,845**	0,780**	0,810**	0,162	1,000						
Mo	-0,447**	- 0,546**	- 0,396**	- 0,406**	- 0,715**	0,338*	- 0,565**	1,000					
Si	0,716**	0,707**	0,779**	0,534**	0,699**	0,540**	0,638**	- 0,268	1,000				
Ti	-0,143	-0,062	0,376*	-0,301*	0,143	-0,288	0,282	- 0,332*	0,341*	1,000			
Cu	- 0,889**	- 0,825**	- 0,590**	- 0,700**	-0,843**	0,440**	- 0,587**	0,328*	- 0,807**	0,074	1,000		
V	-0,901**	- 0,842***	- 0,610**	- 0,714**	- 0,842***	- 0,460**	- 0,610**	0,326*	- 0,818**	0,074	0,998**	1,000	
Ni	0,089	-0,266	-0,326*	-0,096	-0,256	-0,414**	0,074	0,071	-0,132	0,089	0,197	0,179	1,000

[a] values of the probability p.

* Correlation is significant at the 0.05 level, means p <0.05 (two-sided).

** Correlation is significant at the 0.01 level, means p <0.01 (two-sided).

*** p >0.05 statistical insignificance of differences noted) Principal Component Analysis (PCA)

Correlation analysis: The correlation analysis unveiled the relationships between the accumulation of elements within Ziziphora clinopodioides raw materials. The results of the 13-element correlation matrix analysis are presented in Table 3. These findings indicate moderate to strong correlations among all elements examined in this study.

High correlations were observed among several pairs of elements: A strong positive correlation was noted between K, Na, and Mg with Ca, with correlation coefficients of 0.841, 0.842, and 0.865, respectively.

There was a substantial positive correlation, with correlation coefficients of 0.843 and 0.893, between Mg and Na with K. A particularly robust correlation of 0.998 was observed between Cu and V (Table 3).

Conversely, a high negative correlation was identified between Na, Ca, and Cu, with correlation coefficients of -0.843 and -0.889, respectively (Table 3).

Moreover, there were strong negative correlations between K, Na, Ca, and V, with correlation coefficients of -0.842 and -0.901, respectively. The most prominent correlations were found between Mg and Ca and between Na and K, with correlation coefficients of 0.865 and 0.893, respectively. The most substantial negative correlations were detected between Ca and Cu and between Ca and V, with correlation coefficients of -0.889 and -0.901, respectively (Table 3). These findings highlight the interdependencies and associations between different elements in

Z. clinopodioides raw materials, providing valuable insights into their elemental composition.

Principal Component Analysis (PCA): Principal Component Analysis (PCA) was employed to elucidate the relationships between element concentrations under varying growth and introduction conditions. This analytical technique identified associations and correlations between two categories of element variability. Notably, heavy load elements played a significant role in grouping and differentiating samples, whereas light load elements had a relatively minor impact on these aspects. The loading plot and group score plot of principal components, subjected to varimax rotation, are depicted in Figure 2 (a, b).

The first four principal components, collectively explaining 91.93% of the overall variance among the 13 variables, are presented in Figure 2 (a, b), where 'a' and 'b' denote the respective elements loading plot and group score plot.

Figure 2a illustrates the loading plot of PC 1 compared to PC 2 and PC 3, revealing that Mg, K, Al, and Ca were the dominant contributors to PC 1 with higher positive values, accounting for 32.19% of the total variance. Additionally, Na exhibited a negative correlation with Fe and Cu within PC 4 (Figure 2a).

PC 2 exhibited substantial variability due to the influence of Mn, Si, and Ti, explaining 23.93% of the total variance. PC 3, on the other hand, displayed variability primarily influenced by Fe and V (as depicted in Figure 2a).

Based on the variability estimates, the content of Mg, K, Al, and Ca associated with PC 1 loadings was higher in samples 1, 2, 8, and 5, and lower in samples 3 and 7 (Figure 2a). Meanwhile, concentrations of Mn, Si, and Ti were higher in samples 4, 2, and 7, and lower in specimens 3, 6, and 1, as indicated by PC 2 variability values (Figure 2a). Figure 2b illustrates the similarity and correlation between samples 1 and 2 grown under hydroponic and soil conditions.

Samples 7 and 8 contained higher Fe content, while samples 6 and 7 exhibited the lowest levels of Al. The highest Si concentration was found in Samples 4 and 7, with Sample 4 containing the greatest amount of Ti (as detailed in Table 1).

The existence of significant correlations between two or more elements suggests either a shared propensity to accumulate in the material composition or the presence of similar soil types and introductory conditions.

To assess the interplay between element concentrations, growth regions, and introduction conditions, PCA was employed as a method of analysis. In conclusion, the PCA method was applied to discern relationships between element concentrations, growth areas, and introduction conditions in the acquired dataset

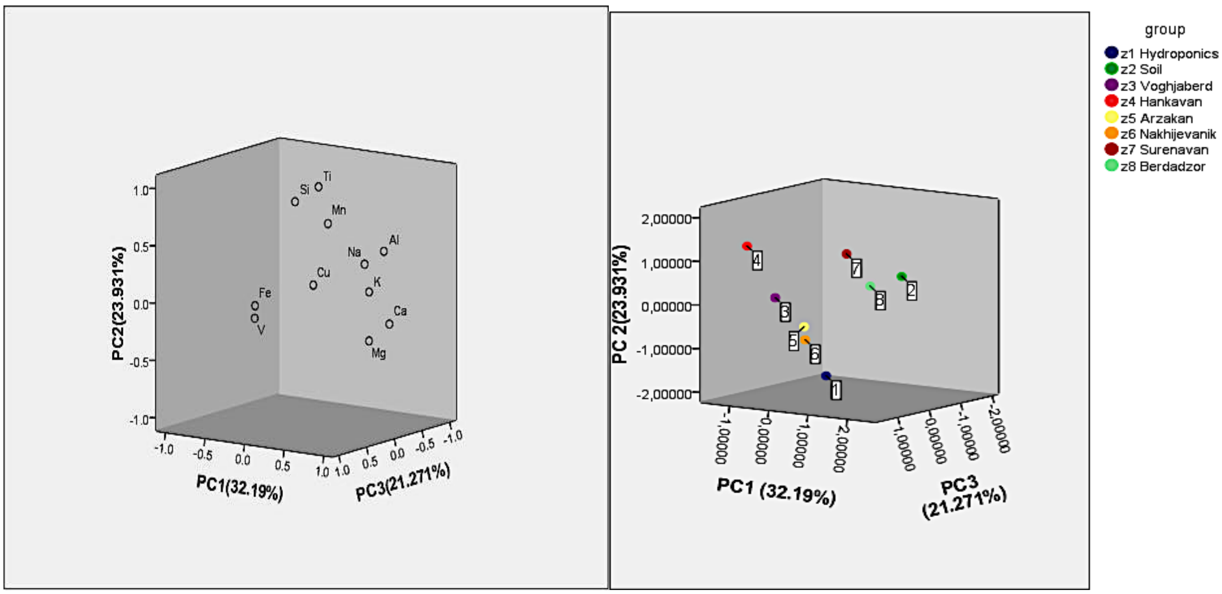

Figure 2. Principal component analysis. (a) the elements loading plot, (b) the groups score plot

Hierarchical cluster analysis (HCA): Hierarchical cluster analysis, known as HCA, is a widely utilized method in chemometrics. This approach helps identify relationships between samples without relying on prior information about these relationships. The results of the HCA in this study were used to categorize and group the samples.

The sample classification appeared to align with the geographic location above sea level, considering the specific elevation of the sites (see Figure 3). The samples were categorized into distinct groups based on this geographic criterion. Group 1 consisted of samples z1, z6, and z5, situated at elevations ranging from 821 to 1700 meters above sea level. These samples exhibited a higher accumulation of essential oil and contained significant amounts of the terpenoid pulegone [18]. Group 2 encompassed samples z3, z4, z7, and z8, which were found at higher elevations, ranging from 1800 to 2650 meters above sea level. These samples displayed lower accumulation of essential oil and contained reduced amounts of the pulegone terpenoid.

Sample z2, derived from soil-cultivated raw materials, constituted Group 3. This sample exhibited the highest concentration of trace elements but had a notably lower yield of essential oil. It also contained the lowest amounts of apigenin and verbascoside [18-19].

In summary, these clusters exhibited differences based on altitude, trace element concentrations, the accumulation of essential oils, and phenolic compounds. These variations could be attributed to disparities in soil composition, geographical location, and methods of introduction. HCA proved instrumental in identifying and understanding these relationships, shedding light on the multifaceted factors that influence the composition of *Ziziphora clinopodioides* raw materials.

CONCLUSIONS

This study represents the first comprehensive report on the growth and cultivation of *Ziziphora clinopodioides* in the floras of South-Caucasian. The results of the research demonstrated that the analyzed raw material samples not only serve as a valuable source of conditionally essential elements crucial for human health but also act as a geochemical indicator for Ziziphora species.

The experimental data revealed that the raw materials of *Z. clinopodioides* exhibit nearly identical qualitative compositions of elements. Differences in the quantity of element content are primarily attributed to the natural climatic growth conditions and the specific methods of cultivation. Importantly, none of the samples investigated contained toxic metals such as Pb, As, or Cd, underlining the ecological safety of *Z. clinopodioides*.

Furthermore, cluster analysis unveiled a correlation between the accumulation of essential oils and biologically active substances, dependent on the growth and introduction conditions. The application of chemometric research methods not only provided insights into the distribution of elements and compound metabolism in raw materials based on geographical location and introduction conditions but also shed light on the chemotaxonomic characteristics of these plants, making them a potential indicator for this species.

The results obtained from mathematical modeling and the developed methods have the potential to contribute to the standardization of medicinal plants. This research opens new avenues for understanding and harnessing the potential of *Ziziphora clinopodioides* in the field of herbal medicine and functional food.

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