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Mineral Composition, multivariate analysis of some oligo-elements and heavy metals in some species of genus *Thymus*

M. Ouknin[1,2], A. Romane[2*], M.Y. Arjouni[2], L. Majidi[1]

[1]*Laboratory of Natural Substances & Synthesis and Molecular Dynamics, Faculty of Sciences and Techniques, Moulay Ismail University, 52000 Errachidia, Morocco.*
[2]*Laboratory of Applied Chemistry, Faculty of Sciences Semlalia BP. 2390, Cadi Ayyad University, Marrakech, Morocco.*

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romane@uca.ma;
Phone: +212 5 24 43 46 49
Fax: +212 5 24 43 74 08

Abstract
The aromatic and medicinal plants are an excellent source of most of nutrients and essential source of vitamins and minerals that play a vital role in ameliorating food deficit and overcoming nutritional problem. This study was conceived to evaluate minerals and heavy metals extracted from the aerial parts of three medicinal species of Moroccan thyme (*Thymus willdenowii*Boiss, *Thymus pallidus* and *Thymus zygis* subsp.gracilis). The oligo-element and heavy metals were investigated using ICP-AES techniques. Investigations of metal accumulation in the screened plants indicated that element accumulation in different plants varied by species and aerial parts. (K, 180.11±39.83; Ca, 156.38±30.29; P, 36.56±10.44; Mg, 35.69±9.6 and Fe 13.97±6.96 mg/kg), were respectively, the major elements of *T. pallidus* stems parts. The level of Zn and Na were the highest in *T. zygis* leaves and flowers parts. The important level of Si was observed in *T. willdenowii*Boiss flower and leaves parts, respectively, 3.47±0.33 and 2.7±0.6 mg/kg. Principal component analysis and cluster analysis, coupled with correlation coefficient analysis, were used to reduce the number of variables into four factors with 89.3% of the total variance.

1. Introduction
Aromatic and medicinal plants are still a source of medical care in developing countries in the absence of modern medical system [1]. In addition, in rural areas, men and women have considerable knowledge and practical know-how gained many years of life in contact with aromatic and medicinal plants in the wild. However their skills are neither identified nor valued while it is very important to translate traditional knowledge into scientific knowledge in order to maintain and enhance the one hand and ensuring rational use of plants on the other hand [2].

Plants are under the continual threat of changing climatic conditions that are associated with various types of abiotic stresses. In particular, heavy metal contamination is a major environmental concern that restricts plant growth. Plants absorb heavy metals along with essential elements from the soil and have evolved different strategies to cope with the accumulation of heavy metals [3]. Heavy metal bioavailability largely depends on soil characteristics and the growing plant species [4-5]. Plants require certain metals, particularly zinc (Zn), iron (Fe), and copper (Cu), for the proper functioning of various metal-dependent enzymes and proteins. However, several metals, such as arsenic(As), mercury (Hg), cadmium (Cd), and lead (Pb), are non-essential and potentially toxic to plants [6].

The plants of *Thymus* genus are among the most popular plants throughout the world, commonly used as herbal teas, flavouring agents (condiment and spice), aromatic, and medicinal plants [7]. These species have also been used as carminative, diuretic, urinary disinfectant and vermifuge [8]. Various *Thymus* species are aromatic plants of the Mediterranean flora, commonly used as spices and as traditional medicine remedies. They are also reported to possess some biological effects such as antispasmodic [9], antibacterial [10], antiviral, expectorating [11] and antioxidant activities [12].

Thymus zygis subsp.gracilis, *Thymus pallidus* and *Thymus willdenowii*Boiss, These species belong to the Lamiaceae family ,they are growing in different regions of Middle mountainous regions Morocco. The goal of the present study was to evaluate minerals and heavy metals extracted from the aerial parts of three medicinal species of Moroccan thyme (*Thymus willdenowii*Boiss, *Thymus pallidus* and *Thymus zygis* subsp.gracilis).

Indeed, Samples of plants were subjected to digestion and heavy metals were determined by inductively coupled plasma mass spectrometry.

2. Material and methods

2.1. Study area and sampling plant

Three species of *Thymus*, are collected during the flowering period in May 2015 from the Khenifra region (Figure 1), in order to be analysed. *Thymus willdenowii* Boiss, *Thymus pallidus* and *Thymus zygis* subsp.gracilis are collected in Lahri, Kerrouchane and Ait Ishak respectively. The samples were placed in individual plastic bags, transported to the laboratory and stored at 4°C until processing. The inflorescences, leaves and stems were separated by hand. Voucher specimen was deposited in the herbarium of the Faculty of Sciences of Marrakech (Morocco).

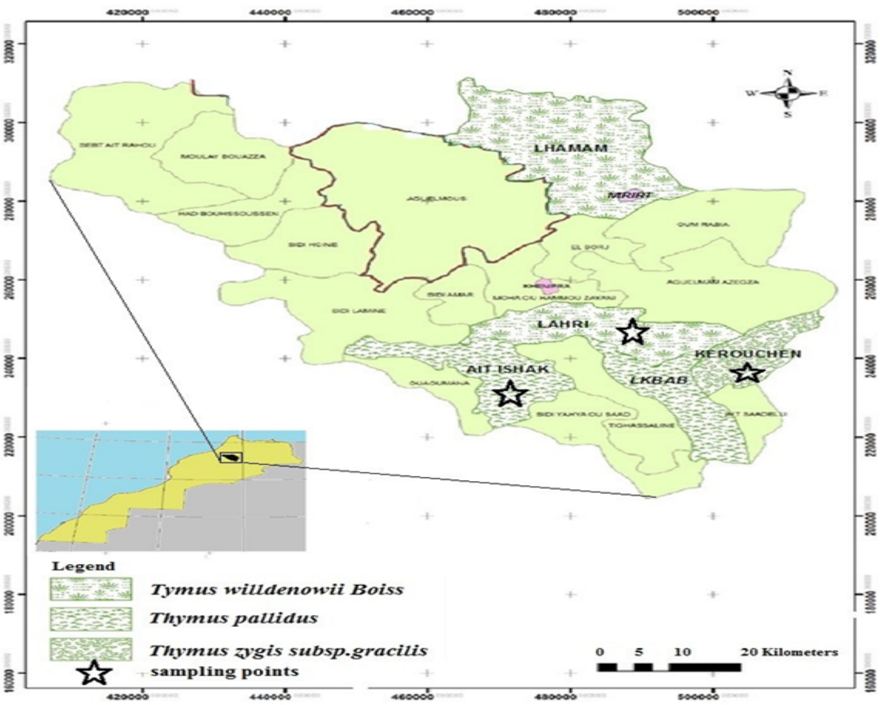

Figure 1: Study area and climatic characteristics of each region sampling.

2.2. Plant analysis

A small quantity (0.5g) of dry thyme matter was mineralised with 2mL of sulphuric acid (H_2SO_4), 6mL of nitric acid (HNO_3) and 6mL of oxygenated water (H_2O_2). This mixture was heated for 30 min. The mineral deposit was cooled and filtered by Whatman ashless filter and then supplemented to 25mL of 0.1M HNO_3 [13]. All procedures of handling were carried out without contact with metals, to prevent cross-contaminations.
The analysis of the samples was realised by metallic dosage in the obtained solution by using inductively coupled plasma mass spectrometry (Jobin-Yvon 7O ICP) ULTIMA AND JY70. All experiments were carried out in triplicate.

2.3. Statistical analysis

SPSS 22 for Windows was used to perform statistical analysis. Descriptive data analysis, including mean, standard deviation (S.D), was carried out. In addition, Pearson correlation coefficients were calculated to determine relationships among different metals. Principal component analysis (PCA) and cluster analysis (CA) were used to discover the factors that could explain the correlation model between metals. PCA is a multivariate statistical method used for data reduction and for an effective representation of the multivariate dataset, it is also performed using the correlation matrix to identify possible associations and evaluate the extent of association between metals. Cluster analysis was used to identify homogenous groups. The Ward method was used and the Euclidean distance applied for the regrouping and identification of the distribution model of essential metals. Results of CA were represented in a dendogram which depicts the levels of similarity between the different variables. The Kaiser-Meyer-Olkin (KMO) and Bartlett's test were introduced to evaluate the validity of PCA.All tests were carried out in triplicate.

3. Results and discussion

3.1. Descriptive statistics

Concentration of heavy metals and their mean, standard deviation, minimum and maximum values in different plants are listed in Table 1.

	***Thymus zygis* subsp.gracilis**						***Thymus pallidus***						***Thymus willdenowii*Boiss**					
	Flowers		Leaves		Stems		Flowers		Leaves		Stems		Flowers		Leaves		Stems	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Al	9.47	2.36	2.11	1.03	2.27	0.36	3.83	1.31	1.16	0.11	5.21	1.96	2.20	0.71	2.86	0.71	1.31	1.14
B	0.32	0.02	0.22	0.09	0.19	0.02	0.30	0.07	0.14	0.03	0.37	0.08	0.19	0.05	0.23	0.05	0.14	0.11
Ba	0.12	0.03	0.21	0.04	0.10	0.02	0.12	0.01	0.20	0.13	0.14	0.05	0.22	0.05	0.20	0.12	0.14	0.04
Ca	118.73	9.19	92.13	29.93	90.54	5.51	139.12	24.39	53.59	1.92	156.38	30.29	100.25	15.24	81.37	26.36	55.75	49.02
Cr	0.43	0.11	1.86	1.15	0.65	0.30	0.73	0.06	0.07	0.04	0.37	0.07	0.58	0.06	0.14	0.05	0.07	0.06
Fe	15.15	2.83	3.30	0.90	4.31	0.47	9.08	4.38	1.95	0.07	13.97	6.96	4.21	1.05	6.24	1.18	2.85	2.48
K	151.51	22.26	150.67	43.94	91.36	7.23	149.52	30.50	102.43	4.85	180.11	39.83	134.09	30.36	100.50	17.35	70.19	57.26
Mg	32.86	3.76	22.17	6.58	18.03	1.18	30.24	7.10	13.06	0.87	35.96	9.60	20.50	4.11	19.98	3.37	11.29	9.78
Mn	0.78	0.09	0.42	0.13	0.40	0.03	0.74	0.20	0.22	0.02	0.96	0.34	0.46	0.10	0.54	0.10	5.44	8.68
Na	3.19	0.93	5.27	1.81	2.78	0.33	2.75	0.06	2.80	0.58	4.71	3.44	3.47	0.33	2.08	0.37	2.16	0.21
P	21.96	2.95	17.00	5.35	15.53	0.77	28.27	6.71	10.65	0.80	36.56	10.44	18.74	3.81	23.22	4.11	12.87	11.13
Si	1.58	0.68	2.56	1.10	1.50	0.02	3.09	0.38	2.61	0.09	3.90	0.15	3.36	0.93	2.70	0.60	1.71	1.44
Sn	0.10	0.02	0.14	0.01	0.10	0.03	0.11	0.01	0.10	0.01	0.13	0.01	0.11	0.02	0.10	0.02	0.93	1.45
Zn	0.95	0.18	3.44	3.39	1.20	0.15	0.53	0.07	0.20	0.04	0.56	0.17	0.93	0.25	0.08	0.08	0.07	0.08

Table 1: Descriptive statistics for heavy metal content and oligo-element in some species of *Thymus*.

Variable concentrations of heavy metals were recorded in all samples analyzed. Samples of *Thymus pallidus* present extremely high levels in K, Ca and P in stems (180.11±39.83, 156.38±30.29, 36.56 ±10.44 mg/kg) respectively, compared to *Thymus willdenowii* Boiss and *Thymus zygis* subsp.gracilis. in the other hand *Thymus willdenowii* Boiss present extremely high levels in Mg and Al in flower (32.86 ± 3.76, 9.47 ± 2.36 mg/kg) respectively compared to others.

The nutrient concentration (Potassium, Magnesium, Calcium, Phosphorus, Aluminum, Iron, Sodium and Silicium) for the different components shows that K (151.51 ± 22.26)> Ca (118.73 ± 9.19)> Mg (32.86 ± 3.76)> P (21.96 ± 2.95)> Fe (15.15 ± 2.83)> Al (9.47 ± 2.36)> Na (3.19 ± 0.93)> Si (1.58 ± 0.68) in flowers over parts of the plant of *Thymus zygis* subsp.gracilis.

Regarding *Thymus willdenowii* Boiss we note that the contributions of individual metals were in this order: K (134.09 ± 30.36)> Ca (100.25 ± 15.24)> Mg (20.50 ± 4.11)> P (18.74 ± 3.81)> Fe (4.21 ± 1.05)> Na (3.47 ± 0.33)> Si (3.36 ± 0.93)> Al (2.20 ± 0.71), in the flowers over parts of the plant.

By against *Thymus pallidus* is observed that the rods is the richest respectively respectively in potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P), aluminum (Al) and iron (Fe) in the stems over parts of the plant. Furthermore, *T. pallidus* is the richest specie in Fe(423 mg/kg) [14]. It appears that the strong concentration of calcium in all tissues participates in the neutralization of top soil acidity. And iron is an essential element which acts as an important cofactor for the functioning of several enzymes [15]. Variability of the mineral composition of plants is due to several factors: condition of growth, genetic factors, geographical variations and analytical procedures. According to [16], *T. broussonetii* and *T. maroccanus* are characterized by a highest level of calcium (Ca) 14,990 mg/kg and 14,830 mg/kg, respectively.

The content of essential elements in plants seems to be conditional, being affected by the geochemical characteristics of the soil and by the ability of plants to accumulate some of these elements selectively [17].

It is well known that some elements, depending on their concentrations, can play different roles in plant life. Zn, Mn and Fe are important co-enzymes; Cu is bound to amino acids while Mn and Ce bind to some bio-macromolecules forming coordination compounds [18].

Variations in the element concentrations of plants can also be caused by various growth conditions and differences in the developmental stages. As was reported, the surrounding environment may have a greater influence on the concentrations of elements in leaves compared with the effect of belonging of the plant to one or another taxonomic group [19].

3.2. Correlation coefficient analysis results

The Pearson's correlation coefficients for elements investigated in the thymus plants are presented in Table 2. K, B, Ca, Mg, Al, and Fe concentrations were significantly positively correlated one to other, with correlation coefficient values of 0.56 to 0.97 ($P<0.01$).Na is also significantly positively correlated with Zn and Cr at $P<0.01$, this indicates possible same sources for these elements. In addition, the correlation matrix shows that only Sn correlate with the element Mn (Pearson r = 0.99P<0.01), indicating a common origin.On the other hand, a negative correlation was found between K and Mn (r=-0.49), K and Zn (r=0.53) at 0.01 level.

Table 2: Pearson's correlation matrix among the variable.

	Al	B	Ba	Ca	Cr	Fe	K	Mg	Mn	Na	P	Si	Sn
B	0.75**	1											
Ba	-0.19	-0.09	1										
Ca	0.62**	0.91**	-0.28	1									
Cr	0.01	0.22	0.2	0.27									
Fe	0.91**	0.85**	-0.27	0.76**	-0.07	1							
K	0.62**	0.88**	0.06	0.86**	0.41*	0.70**	1						
Mg	0.80**	0.97**	-0.15	0.93**	0.23	0.90**	0.92**	1					
Mn	-0.18	-0.40*	0.04	-0.43*	-0.17	-0.17	-0.49**	-0.39*	1				
Na	0.08	0.28	0.33	0.29	0.60**	0.01	0.43*	0.25	-0.16	1			
P	0.58**	0.92**	-0.14	0.91**	0.08	0.80**	0.82**	0.91**	-0.36	0.14	1		
Si	0.10	0.55**	0.34	0.58**	0.21	0.26	0.69**	0.51**	-0.46*	0.38	0.66**	1	
Sn	-0.24	-0.46*	0.07	-0.49*	-0.15	-0.25	-0.53**	-0.45*	0.99**	-0.15	-0.43*	-0.47*	1
Zn	0.08	0.23	0.23	0.22	0.93**	-0.04	0.41*	0.21	-0.12	0.56**	0.05	0.19	-0.11

3.3. Principal components analysis

In this present study, a principal component analysis (PCA) was used to identify the sources of the selected elements in the thymus samples collected from Morocco. The first four principal components (PC) with eigenvalues higher than 1 (before and after rotation) explained 89.3% of the total variance. The relations among these metals based on the four principal components are illustrated in three-dimensional (3D) space (Figure 2).

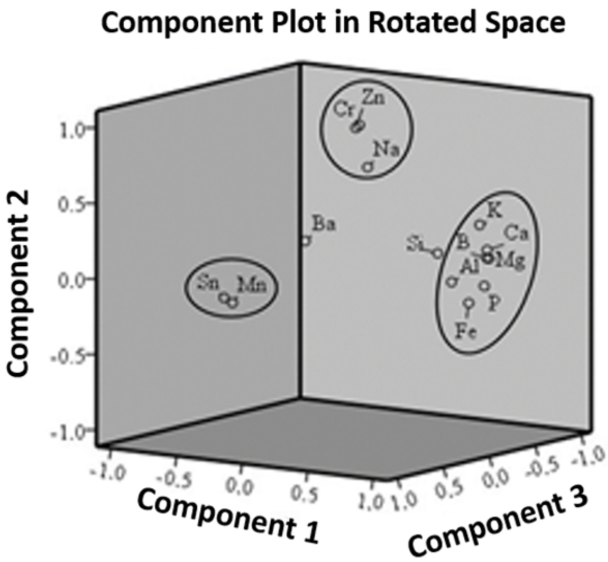

Figure 2: Plot of loading of three principal components in PCA results.

The first principal component (PC I) explains the 42.75% of the total variance and presents strong positive values for Al, B, Ca, Fe, K, Mg and P. The second principal component (PC II) accounts for 18.53% of the variance and loads heavily on Zn, Na and Cr. The third principal component (PC III) dominated by: Mn and Sn, accounts for 17.21% of the total variance. The fourth principal component (PC IV) is correlated very strongly with Ba, which has loading value (0.68), and moderately by Si, accounting for 10.76% of the total variance.

3.4. Cluster analysis

Cluster analysis (CA) is coupled with PCA to provide grouping and confirm results of variables [20]. The CA results for the elements studied are illustrated in Figure 3 as a dendrogram. There were five distinct clusters with different associations were distinguished from the figure. The first cluster included Mn and Sn. The second cluster composed by Al, Fe, Mg, B, Ca, P and K. The third cluster contained only Si. The fourth cluster contained Cr, Na and Zn. Thus, CA suggests at least five different sources of oligo-elements and heavy metals.

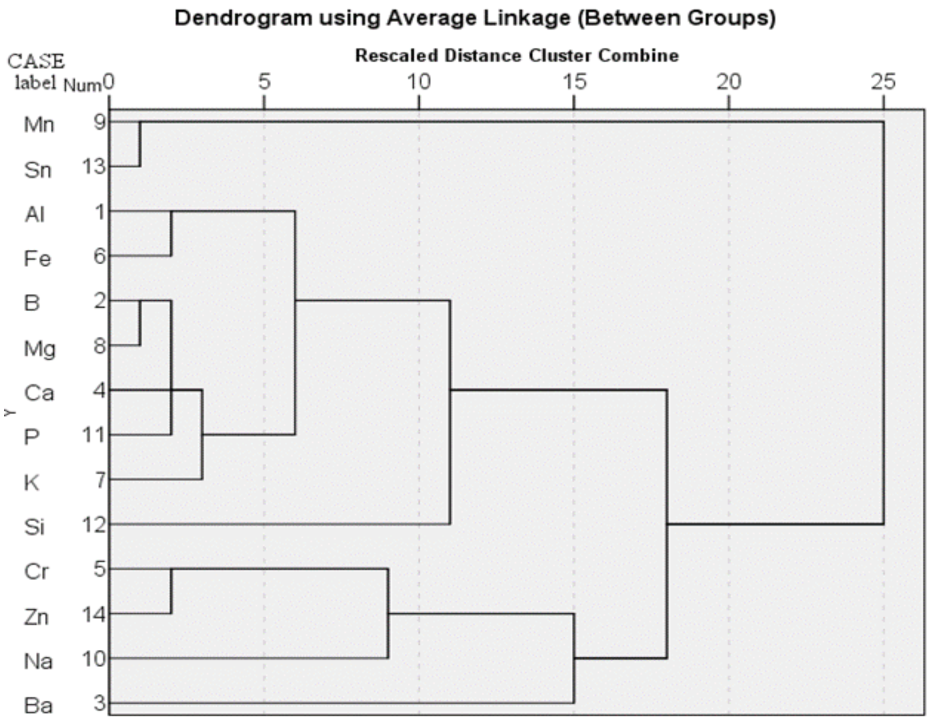

Figure 3: Hierarchical dendrogram for metal elements in plants using average linkage between groups and Pearson correlation as measure interval.

3.5. Variability of ratios of some nutrients

Much evidence has been presented to show that the growth of plants is markedly affected by the proportions of the exchangeable cations which are present in soil. It was demonstrated that ratios of main nutrients in plants remain constant and typical for a particular plant species [21]. In our work, rather significant differences between ratios of chemically similar elements (K/Na and Mg/Ca) in aerial parts (flowers, leaves and stems) of different species studied were observed (Figure 4(a), Figure 4 (b)).

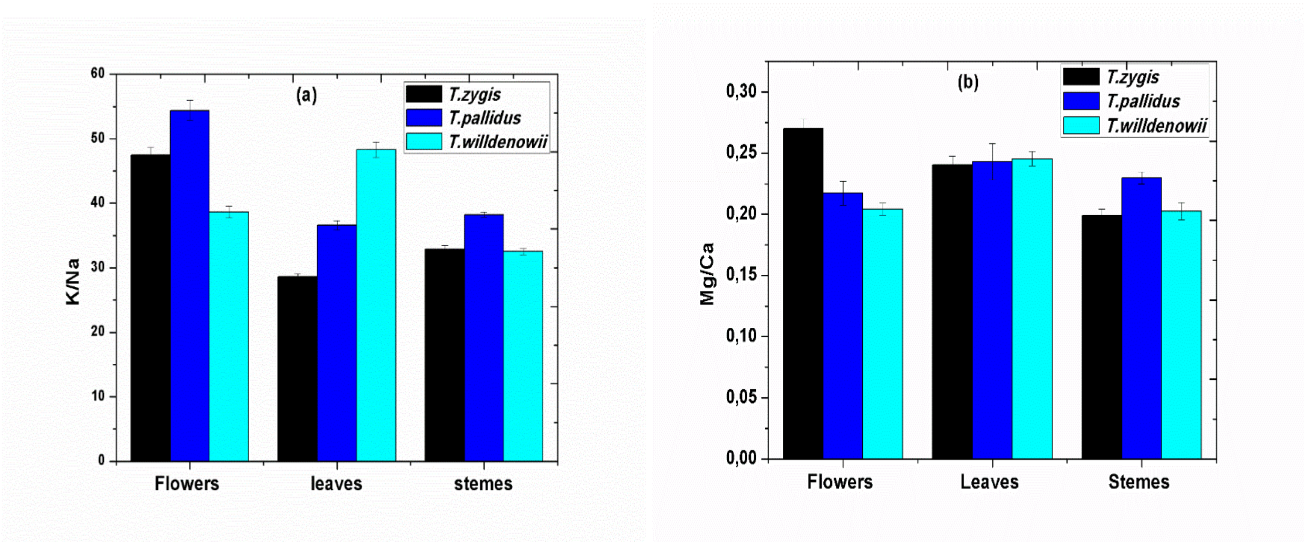

Figure 4: Ratios of K/Na and Mg/Ca in aerial parts (flowers, leaves and stems) of different species studied.

The highest value for K/Na ratio were found for flowers of *Thymus pallidus* and the lowest ratios were observed for leaves of *T. zygis*.However, the value for Mg/Ca ratio remain almost constant for aerial parts (flowers, leaves and stems) of different species studied. Thus, the ratios can also be used as specific indicators of the particular plant species.

Conclusions

Heavy metal toxicity related to the use of traditional medicines has been reported worldwide. Heavy metals may be introduced into medicinal plant products through contaminated agricultural resources and/or poor production practices.

The heavy metal contents in the samples were found at different levels. Therefore, *Thymus pallidus, Thymus willdenowii*Boiss and *Thymus zygis* subsp.gracilis, present important concentration of the mineral elements k, Ca, P, Mg, and Fe, respectively.

This paper provides evidence of the use of medicinal and aromatic plants beings can a source of heavy metals that may have has beneficial or toxic effects for user health. We find that the studied plants may beings as a source of K, Ca, P and Fe that can be used to enrich our needs for nutrients instead of using other sources that may being toxic to health.

Potassium, magnesium, calcium, phosphorus, aluminum, iron, are essentials elements for humans, animals, and plants and is required for growth, development, and maintenance of health.

In concluding their concentrations were low and all meet the national hygiene standards for safely being used by humans.

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