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Assessment of some oligo-elements and heavy metals in different parts of the *Thymus broussonettii* growing in Morocco.

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Abstract
In order to valorize natural substances, inductively coupled plasma atomic emission spectroscopy (ICP-AES) is employed for the determination of essential and nonessential elements in different parts (leaves, woods, and flowers) of *thymus broussonettii* medicinal plants largely used in Morocco generally as remedies. This endemic was analyzed for Ca, K, Na, Mg, Fe, Mn, Zn, Cu, Co, Cr, Mo, Se, Si, B, Ni and Pb. Principal component analysis was performed to provide a data structure study.

Keywords: Thymus broussonettii, medicinal plant, mineral elements, ICP-AES.

Introduction
It is known that serious systemic health problems can develop as a result of excessive accumulation of dietary heavy metals such as Cd, Cr, and Pb in the human body [1]. Heavy metals are extremely persistent in the environment; they are non-biodegradable and non-thermo-degradable and thus readily accumulate to toxic levels. Medicinal and aromatic plants are mainly used for their flavor and odor.
Last years, the importance of anti-oxidants and oligo-elements on the human health was increased. Many others have studied the role of the oligo-elements in traditional medicine in India [2,3], china [4,5], Nigeria [6,7], Greece [8] that intakes of mineral element due to the consumption of herbal infusion by a weighting person are of 500mg of Ca, 300mg of Mg, 15mg of Fe, 5mg of Al, 2.8mg of Mn, 15mg Zn, 2.5 mg of Cu, 1.6mg Sr, 1.1mg of Ba, 0.025mg of Ni, 0.05 to 0.2 mg of Cr, 0.04mg of Co, 0.415mg of Pb and 0.05mg of Cd.
In order to prevent many diseases for men, a significant increase in the use of herbal medicine is noticed. Many studies showed that intakes of mineral elements could reduce risk factor of individual. Iron (Fe), for instance, plays an important role to favorite the transport of oxygen (hemoglobin) and electrons (cytochrome), muscle metabolism and immunity.
Manganese (Mn) is an enzymatic activator, hydrolase, kinases, carboxylases, transferases. Zinc (Zn) plays an important role in reinforcing immunity, endocrinology (insulin, thyroid…); it is an anti-oxidant, dismulase. Cobalt is a constituent of B12 vitamin. Chromium is a mineral that humans require in trace amounts, although its mechanisms of action in the body, it was identified as the active ingredient in this so-called "glucose tolerance factor" in 1959[9]. Molybdenum (Mo) intervenes in the function of xanthine, aldehyde and sulfite involved in purine metabolism and sulfur excretion.
Selenium (Se) is an antioxidant which is involved in the peroxidase glutathione, thyroid (deiodinase) metabolism and hepatic metabolism. These could play an important role in the alimentation system, source of oligo-element [8-10].
Thyme is a medicinal plant used in the countries of Mediterranean basin, fresh or dried, or an aromatic herb in cooking, traditional medicine as infusion against, fever, hypertension, and gastritis or as antiseptic and antimicrobial properties [11].
The purpose of this work is to study the variability of the mineral composition in different part of thymus.

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2. Materials and methods

2.1. Sampling and analytical methods

Different thyme samples have been collected from south-west of morocco near the Ounarha town situated at 20km from city of Essaouira (N31° 25' 0' W 9° 30' 0'). The identified samples were dried at ambient temperature. A specimen is deposited in the Natural History Museum of the Cadi Ayyad University-Marrakech.

In the order to mineralize vegetal matter, 2 mL of sulfuric acid (H_2SO_4), 6 mL of nitric acid (HNO_3) and 6ml of oxygenized water (H_2O_2) were added to 0.5g of dried and pounded thyme vegetal water. This mixture is heated during 30min. The residue is cooled and filtered then a 25ml of nitric acid is added.

The analyses of "mineralisate" is realized by metallic "dosage" in the obtained solution by using introductive coupled plasma spectrometry (JOBIN-YVON 70 ICP).

2.2. Statistical analysis

All analyzes of samples were made in triplicate. Data are expressed as mean value ± standard deviation. HCA classifying asset of observations described by numerical variables in groups, was performed. Principal component analysis (PCA) was used to reduce data and to extract a smaller number of independent factors (principal components) for analyzing relationships among heavy metals and to investigate the multivariate structure of the dataset and to highlight possible trends among the data. The statistical analyzes were performed by SPSS program.

3. Results and discussion

The concentration of mineral element in leaves and flowers of *Thymus vulgaris* in comparison with data of obtained for other species of the lamiaceae family is shown in Table 1 [12]. The highest concentration of selenium (1,5mg/kg) and in boron (58mg/kg) was observed in the Thyme plant. Indeed, selenium plays an important role for the human health [13-14] like modulator in the immunology and inflammatory responses [15]. Boron interacts with magnesium, copper, calcium, the D vitamin and estrogen to modify the metabolism by reducing the "osteoporoses" risk, for instance [16].

Table 1: Comparison of the mineral composition of the leaves and flowers of *Thymus vulgaris* with some species of the family Lamiaceae (mg / kg). [12]

	Thymus vulgaris	*Mentha spicata*	*Satureja hortensis*	*Salvia fruticosa*	*Lavandula officinalis*	*Origanum vulgare*	*Ocimum basilicum*
Ca	12455	11326	11333	11131	10622	10473	12363
K	8470.2	24758	13660	11568	17623	19625	24811
Mg	4359	5267	4138	4182	4596	3268	5738
Fe	405	414	203	565	1229.2	159	503
Mn	22.4	97.9	21.90	38.8	50.1	25.5	117
Zn	14.3	18.7	29.1	28.7	25.9	19.3	13.7
Cu	4.88	8.48	5.83	4.67	10.70	6.651	8.05
Cr	8.76	10.0	8.26	10.1	19.1	7.43	7.95
Se	1.50	1.12	0.15	1.44	–	–	–
B	58.8	47.6	33.3	37.8	14.1	16.83	31.75
Pb	–	–	–	0.51	4.73	0.49	2.10
Li	0.38	1.47	0.26	0.46	0.68	0.19	0.73
V	10.1	11.7	9.69	5.08	2.26	4.11	19.7
Cd	–	–	–	–	–	0.02	–

The oligo-elements and heavy metals average concentration found in leaves, stems and flowers of *Thymus broussonettii* are shown in Table 2, where we can differentiate three mineral groups: elements that are very abundant, elements in a medium concentration, and trace elements.

Table 2: Descriptive Statistical Data for Mineral Content in different parts of *Thymus broussonettii*.

Elements	Leaves	Stems	Flowers
Ca	14990.83±15.12	5770.00±15.39	6015.75±48
K	6953.00±8.33	3421.33±1.09	8586.00±2
Na	2817.33±4.78	2900.33±1.58	2252.00±1
Mg	7.75±0.02	<2.5	7.50±0.03
Fe	271.67±0.18	115.83±0.20	260.25±1.00
Mn	28.67±0.04	12.00±0.00	26.75±0.02
Zn	33.00±0.21	11.33±0.01	20.25±0.01
Cu	7.00±0.01	3.83±0.01	4.50±0.00
Co	2.83±0.06	<0.5	<0.5
Cr	1.50±0.00	1.25±0.01	1.50±0.00
Mo	<0.5	<0.5	<0.5
Se	<5	<5	<5
Si	111.83±0.18	46.83±0.11	69.75±0.09
B	42.50±0.08	14.83±0.01	27.75±0.02
Ni	2.33±0.01	<0.02	3.00±0.01
Pb	<2.5	<2.5	<2.5

The leaves part mainly presented the highest concentrations of the elements analyzed. Ca, Mn, Zn, Cu, Co, Si and B accumulate in greater proportion with 14990.83mg/kg, 28.67mg/kg, 33mg/kg, 7mg/kg, 2.83mg/kg , 111.83mg/kg and 42.50mg/kg respectively. The Highest mean concentration level of Potassium is found in flowers part of thymus (8585mg/kg).

The analysis of oligo-elements and heavy metals distribution in different parts of the plant species shows that certain elements are mainly translocated to aerial part of the plant, while others focus more on stems or flowers.

For the CA, a similarity matrix was constructed from the autoscaled data. The elements of this similarity matrix were the Euclidean distance of one object from the rest. The clustering procedure used was the complete linkage method with the Euclidean distances between objects. The results obtained showed the presence of parts clusters; the data of minerals contained significant information to achieve a two-category classification between flowers thyme and stems leaves thyme. The results of CA are shown in a dendogram (Figure1), two clusters were found for different samples. The first group is composed of flowers and the second group is composed of stems leaves parts thyme.

PCA was performed to provide a data structure study in a reduced dimension, covering the maximum amount of information present in the data. PCA represents the original data matrix as a product of two matrices, the score matrix and the loading matrix. This corresponds to the projection of the data matrix onto a few-dimensional space. Table 3 shows the factor-loading matrix obtained for the two factors and the variance explained by each of them.

The first principal component accounts for 75.50% of the variance and the second for 24.50%, the cumulative variance being 100%. The differences among the honey types were highlighted by the PCA analysis. The first two components were chosen to examine the dataset and they explained 100% of the total variance. Figure 1 show the loadings of the variables (the element concentrations) in the plane defined by the two principal components (F1 and F2). F1 has a negative correlation with most elements has as Mn, Cr, Fe, Mg, Ni and K and has positive correlation with Co, Ca, Cu, Si, and Na, while F2 has a negative correlation with the Na concentrations and a positive correlation with a rest of the elements. Figure 2 represents also the score plot of the parts of thyme plant. There is a clear separation of the leaves, stems and flowers. The stems are situated on the left side of the plot, as the Mostly have negative values of F1. This means that these samples had the highest concentrations of the Sodium. The leaves and flowers are placed on the right side of the plot, since these samples had higher element contents except Na, the leaves are characterised by Na, Co, Ca, Cu, B and Si. The flowers are mostly characterised by Mn, Cr, FE Mg, Ni and k.

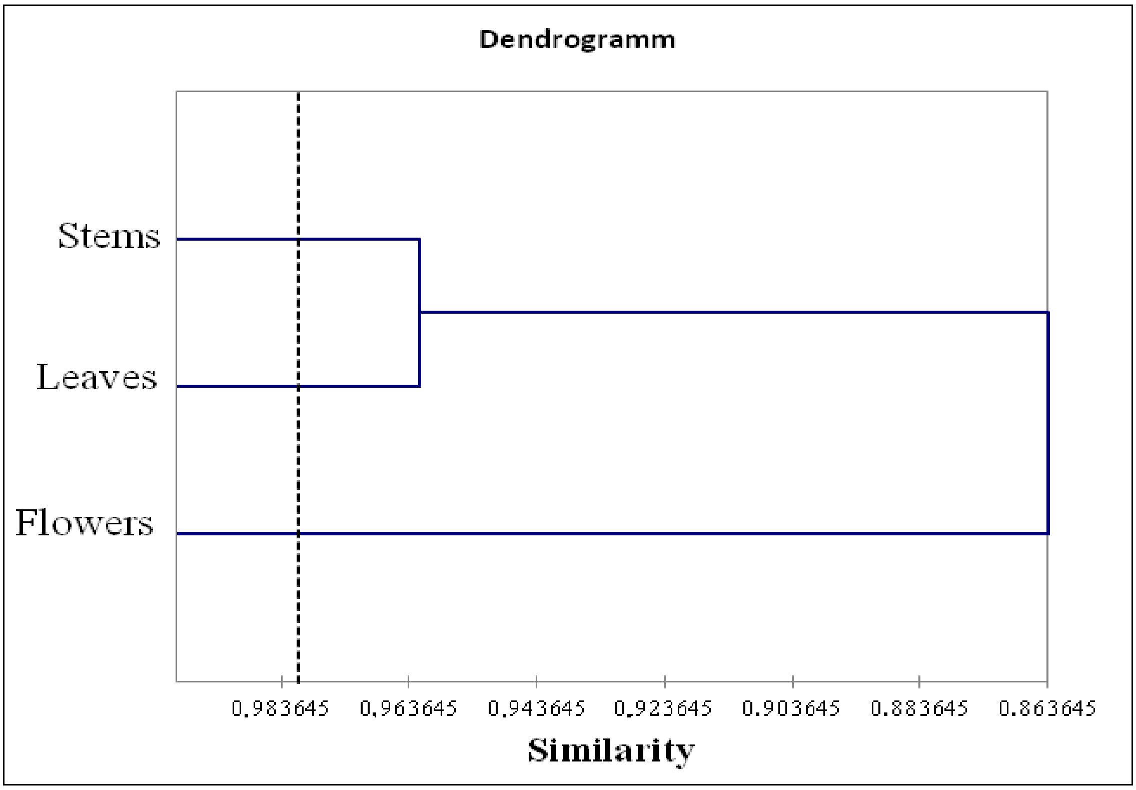

Figure 1: Dendogram for samples thyme: complete linkage with Euclidean distances

Table 3: Results of Principal Component Analysis

	F1	F2
Ca	0.24497	0.35917
K	0.25561	-0.33555
Na	-0.09850	0.53285
Mg	0.30629	-0.15782
Fe	0.30833	-0.14511
Mn	0.31136	-0.12354
Zn	0.30520	0.16420
Cu	0.27742	0.27713
Co	0.24012	0.36912
Cr	0.30222	-0.18036
Mo	0.00000	0.00000
Se	0.00000	0.00000
Si	0.29826	0.19962
B	0.31052	0.12987
Ni	0.27318	-0.28981
Pb	0.00000	0.00000
%total variance	75.50	24.50
Cumulative variance (%)	75.50	100.00

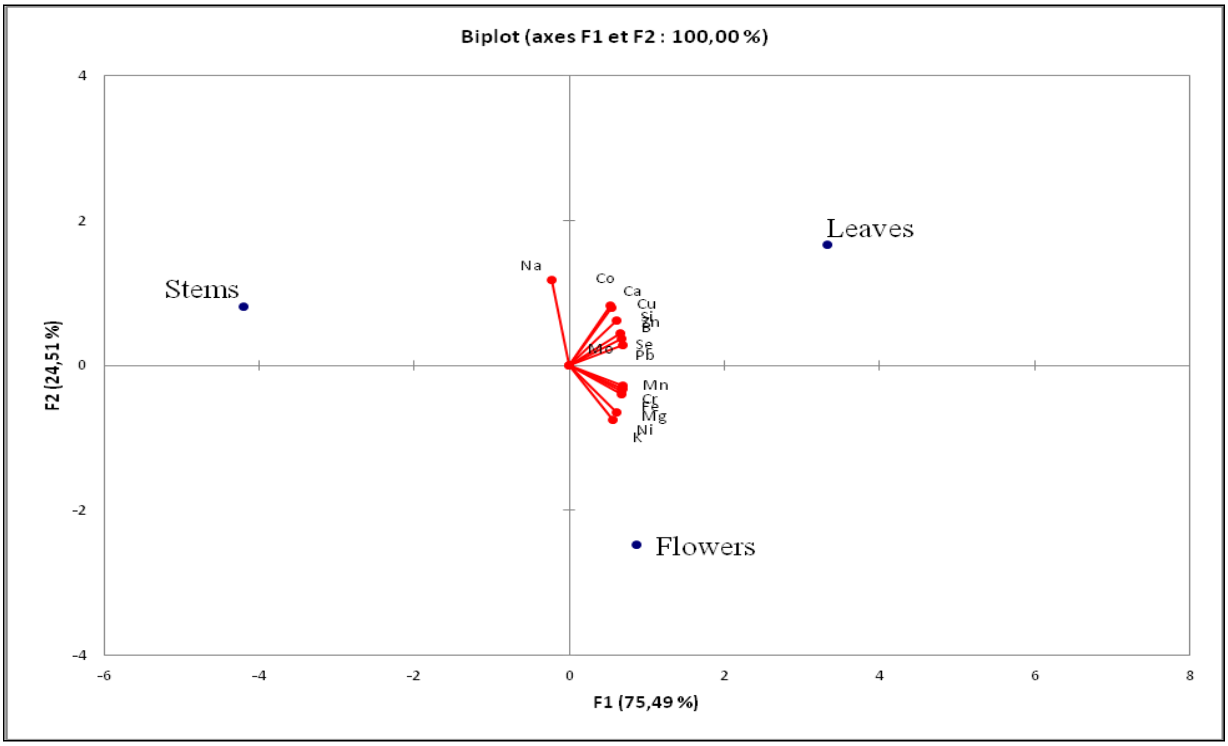

Figure 2: Principal components analysis (F1 vs. F2).

Conclusion

It has been shown by this study that by using ICP-AES we can determine contents of major and trace elements in *Thymus broussonettii* medicinal plant widely used in morocco, important variation of the concentration of the mineral elements were observed in leave, stems and fluorescence of the studied plant. It has been shown that calcium content is higher in leaves than in stem and flowers. Higher Ca, Fe, K, Na, Fe, Zn, Cu, Co, Si, B and Mn were found in leaves wich are more consumed as in herbal infusion and in cooking. It contents of heavy metals were found lower to the World Health Organization (WHO) limits.

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