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CHEMOMETRIC APPROACH TO THE ELEMENTAL COMPOSITION OF ETHEREAL OIL PLANT MATERIALS

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Abstract

The aim of the present study is the effective application of chemometric approach to the elemental composition of a data set of ten ethereal oil plant materials. The contents of 28 essential and toxic mineral elements at main, minor and trace level are determined in each sample by means of direct solid sampling electrothermal vapourization inductively coupled plasma optical emission spectrometry (ETV-ICP-OES) and are subjected to hierarchical and non-hierarchical cluster analysis and principal component analysis. Z-transformation of the raw data, Ward's method of linkage and squared Euclidean distance as similarity measure are used in order to discover appropriate linkage between the plant materials and between the chemical parameters characterizing them. Thus, the investigated plant samples are classified, depending on their chemical composition. On the other hand, the mineral elements are categorized into several patterns which are related to the specific soil characteristics.

Key words: ethereal oil plant materials, essential and toxic mineral elements, cluster analysis, principal components analysis

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Introduction. Earth is a reservoir of 92 elements, and about 82 elements can be utilized by many plant species [1]. Elements that plants need to survive are called "plant nutrients". The "non-mineral elements" are three macronutrients that plants can obtain from water, air, or both – carbon, hydrogen and oxygen. They are also called "structural elements" and represent the main part (more than 95%) of the plant mass. All other elements, called "mineral elements" are soil-derived thus the main path of element uptake in plants is via the roots [2,3].

In the analysis of plant tissues, it is possible to see almost all the elements found in nature. Although the plants are selective about taking nutrient ions, as the rate of nutritious form of nutrient elements found in the growth medium increases, some toxic metals that can pass into the body of plants by passive means can also enter involved in the food chain. As a result, this can toxically affect plants and the humans and animals who feed on these plants [4–7].

Most of the ethereal oil plants are applied in traditional medicine and are considered non-toxic. Usually they are consumed in raw form, either as a powder or a decoction. In fact, soil factors and climatic conditions, in which plants grow, serve as a source of elements thus to quantify the essential elements of plants, as well as to quantify the toxic inorganic hazardous elements which is an important analytical task. Certain elements such as iron, zinc, copper, chromium and manganese are considered essential because of their vital role in physiological and biological pathways. These elements could even be toxic if taken above their permissible limits. Hence, in order to ensure the beneficial properties of medicinal and ethereal oil plants, respectively, it is essential to quantify their elemental composition along with other phytochemical analyses [1].

Many of the elements occur at such low concentrations in plant materials that they are quite difficult to determine. Therefore many papers focus on very few elements, often only one, of interest for pollution studies. However, multi-element analyses are needed to fully describe inter-element relations and to substantiate that all relevant characteristics of certain plant species are understood and taken into consideration when interpreting analytical results [3,8–10]. The great variety of plants with respect to their location and mineral elements content often requires a specific approach for elucidation of experimental data. Chemometric data classification, modelling and interpretation seems to be the most reliable assessment procedure [11,12].

In the present study chemometric approach was applied to the results obtained for the content of 28 essential and toxic mineral elements in ten plant materials by means of direct solid sampling via electrothermal vapourization inductively coupled plasma optical emission spectrometry (ETV-ICP-OES). Multivariate statistical techniques such as cluster analysis and principal component analysis were used for the analysis of data. The results obtained can give information on the element uptake by plants depending on plant species and soil characteristics.

Experimental. The present study deals with the application of cluster analysis and principal components analysis (PCA) to a data set of ten plant materials previously subjected to ETV-ICP-OES analysis.

The investigation is based on commercial samples of eight Bulgarian ethereal oil plants namely *Mentha spicata* L. (MS), *Ruta graveolens* L. (RG), *Thymus vulgaris* L. (TV), *Hypericum perforatum* L. (HP), *Achillea millefolium* L. (AM), *Anethum graveolens* L. (AG), *Melissa officinalis* L. (MO), and *Lavandula angustifolia* L. (LA), as well as two waste products obtained after the extraction of the ethereal oils from the corresponding plants MO and LA and denoted as M and L, respectively.

The elements Ag, Al, As, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Sb, Se, Si, Sn, Ti, Zn, and Zr were determined by means of direct solid sampling ETV-ICP-OES as described in [13]. The data obtained

Table 1

Mean values (in µg g^{-1}) for the mineral elements in the investigated plant materials

Sample	MS	RG	MO	TV	HP	AM	AG	LA	M	L
Ag	0.050	0.052	0.042	0.090	0.053	0.062	0.046	0.046	0.161	0.039
Al	625	2400	414	3709	416	1140	1559	199	3229	1222
As	0.084	0.101	0.075	0.163	0.061	0.083	0.063	0.066	0.125	0.090
B	20.1	20.9	35.2	14.0	40.4	36.0	22.0	37.5	15.1	16.7
Ba	50.1	38.9	70.9	120.9	11.6	14.9	17.5	17.7	98.8	86.9
Be	0.222	1.489	0.159	1.489	0.205	0.289	0.386	0.071	1.239	0.382
Ca	23150	21150	20318	13080	6255	10392	20701	9739	16263	14833
Cd	0.042	0.125	0.018	0.168	0.643	0.064	0.045	0.035	0.045	0.015
Co	0.231	0.670	0.188	6.280	0.469	0.539	0.961	0.123	1.506	0.509
Cr	2.69	12.34	1.12	82.16	3.58	13,04	6.78	2.84	18.34	8.50
Cu	61.9	44.7	21.7	17.4	11.2	17.6	12.1	12.3	27.5	21.8
Fe	388	1722	284	6554	317	781	1934	245	2823	858
K	14788	11359	12989	13789	10275	15232	16137	9590	10683	6838
Mg	4172	3392	4513	4737	2728	2683	3944	4080	5254	4525
Mn	45.0	70.5	38.3	464.8	355.6	76.6	78.7	29.4	129.5	59.6
Mo	1.609	0.555	4.366	0.371	0.795	1.161	1.391	0.361	0.316	0.135
Na	190.3	2048.3	135.7	659.9	129.4	156.5	10368.8	62.0	751.1	185.4
Ni	1.32	5.37	0.73	84.24	3.69	5.78	4.09	2.45	10.55	3.79
P	3553	2498	3264	1686	2823	3002	2206	1913	3312	2662
Pb	5.64	7.24	5.74	6.01	0.78	3.85	1.85	1.47	4.36	3.59
S	7077	3485	5822	1032	2541	2124	7980	1942	3343	1960
Sb	0.122	0.142	0.046	0.671	0.109	0.123	0.179	0.152	0.172	0.115
Se	0.683	0.460	0.341	0.469	0.360	0.239	0.248	0.234	0.252	0.173
Si	3507	12488	2089	19754	1991	11542	5943	1837	20021	5459
Sn	0.839	1.341	0.482	0.827	0.337	1.086	0.496	0.630	0.950	0.863
Ti	61.9	126.9	45.9	312.8	108.5	140.5	245.7	32.3	261.8	107.0
Zn	88.9	43.5	112.7	92.4	67.7	46.0	45.0	44.8	73.9	43.9
Zr	1.94	6.31	2.86	7.89	2.60	6.62	5.79	5.41	32.24	3.68

for the 28 mineral elements (Table 1) in each sample were treated by means of hierarchical and non-hierarchical cluster analysis (z-transformation of the raw data, Ward's method of linkage, squared Euclidean distance as similarity measure, K-means clustering) and PCA (z-transformed data, Varimax rotation mode) in order to discover appropriate linkage between the investigated plant materials and between the chemical parameters characterizing them [8–12, 14].

Results and discussion. ***Hierarchical cluster analysis (z-transformed input data, Ward's method of linkage, squared Euclidean distances as similarity measures, Sneath's index of cluster significance).*** The input data set subjected to multivariate statistical treatment has dimensions $[10 \times 28]$ (10 plant materials treated for 28 chemical parameters – Ag, Al, As, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Sb, Se, Si, Sn, Ti, Zn, and Zr). A hierarchical dendrogram for clustering of the 28 chemical variables from the data set is presented in Fig. 1a. As can be seen, five clusters (denoted as K1, K2, K3, K4, and K5, respectively) are formed at the significance level of 33.3 % $D_{\max}$.

It could be assumed that the chemical composition of the ten plant materials in consideration is substantially influenced by five major soil factors related to the soil quality – soil pollution level, fertilizers used, soil salt content, toxic ingredients and earth crustal composition, as follows:

K1: (Sn, Pb, Se, Cu) – reflects the soil pollutant impact;

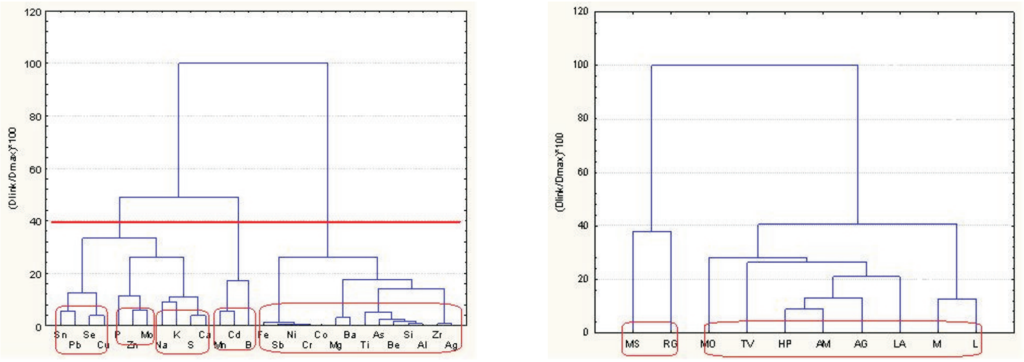
K2: (P, Zn, Mo) – reflects the soil fertilizers impact;

K3: (Na, K, S, Ca) – reflects the soil salt impact;

K4: (Mn, Cd, B) – reflects the soil toxic impact;

K5: (Fe, Sb, Ni, Cr, Co, Mg, Ba, Ti, As, Be, Si, Al, Zr, Ag) – reflects the geological impact.

It is supposed that the elements from clusters K1, K2 and K4 are more or



(a) (b)

Fig. 1. Hierarchical dendrogram for clustering of the 28 chemical variables (a) and for clustering of the 10 plant objects (b) using Ward's method of linkage and squared Euclidean distances

less of anthropogenic origin and can be attributed to different fields of human activities, while the elements forming the clusters K3 and K5 are mainly of non-anthropogenic origin, both related to naturally occurring ores and minerals. The elements forming clusters K1 and K4 are reflecting the toxic pollution impact due to industrial activities such as non-ferrous metallurgy (K1) or coal-fired power plants (K4) while the elements from cluster K2 are probably due to fertilizers used in agricultural production.

Cluster K3 contains only plant nutrients – three essential macronutrients: Ca, K, S, and sodium, which is also regarded as a plant nutrient [15,16]. This group could be described as "soil salt impact".

Cluster K5 consists of altogether 14 toxic, non-toxic and essential elements at minor and trace levels, probably originating from rocks, ores and minerals, which could be related to the earth crust composition.

The hierarchic dendrogram for clustering of the 10 plant samples (Fig. 1b) from the data set [10×28] presents two major clusters – Cluster 1 and Cluster 2 (2/3 D_{max} test) as follows:

Cluster 1: (MO, TV, HP, AM, AG, LA, subcluster [M, L])

Cluster 2: (MS, RG)

Probably, these patterns are due to the biological specificities of the plant samples and therefore are called provisionally "plant families". As expected, both samples M and L, obtained after the extraction of the ethereal oils from the corresponding plants, form a separate subcluster [M, L].

Non-hierarchical cluster analysis (K-means clustering). The non-hierarchical clustering was carried out in order to prove the a priori hypothesis that the linkage of the chemical variables causes formation of five clusters (due to the soil quality indicators) and, respectively, the clustering of the plant objects – two clusters (due to the "plant families" involved).

Table 2

Members of Cluster 1 and Cluster 2 and distances from respective Cluster centre

Cluster Number,	Member	Distance
Cluster 1	MO	0.74
Cluster 1	TV	1.52
Cluster 1	HP	1.01
Cluster 1	AM	0.62
Cluster 1	AG	0.90
Cluster 1	LA	0.78
Cluster 1	M	1.00
Cluster 1	L	0.62
Cluster 2	MS	0.44
Cluster 2	RG	0.44

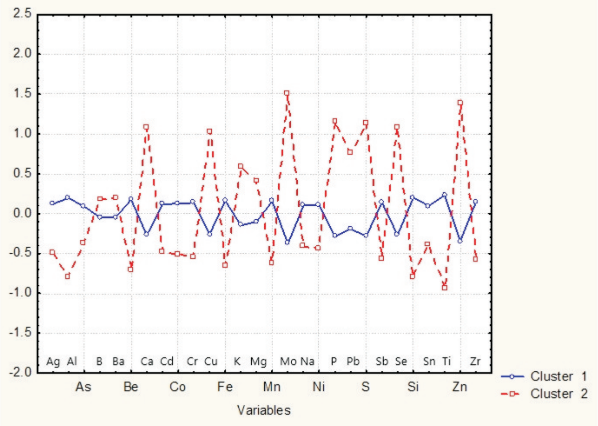


Fig. 2. Mean values for each variable for each identified cluster (standardized values)

The members of clusters Cluster 1 and Cluster 2 (plant objects) and the distances from respective cluster centre are presented in Table 2.

The mean values (standardized values) of each variable for each identified cluster with respect to the chemical indicators (variables) are presented in Fig. 2.

The two "plant families" are well separated from each other. All chemical indicators for the bigger "family" (Cluster 1) show an average level for all indicators since the levels for the indicators for the smaller cluster (Cluster 2) differ significantly (higher levels for 12 components [B, Ba, Ca, Cu, K, Mg, Mo, P, Pb, S, Se, Zn] and lower levels for 16 components [Ag, Al, As, Be, Cd, Co, Cr, Fe, Mn, Na, Ni, Sb, Si, Sn, Ti, Zr]) from the levels in Cluster 1. For Cluster 1 the soil crustal impact is diminished since the influence of the other four identified by hierarchical

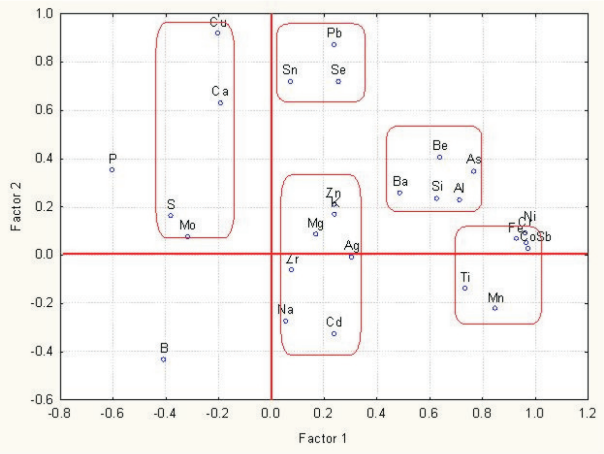


Fig. 3. Factor loadings plot Factor 1 vs. Factor 2

clustering impacts are enhanced. Cluster 2 is characterized by constant impact of all soil quality patterns.

Principal component analysis (PCA). The non-hierarchical clustering of the 28 variables followed the a priori hypothesis for the effect of five soil quality patterns on the plant objects. This data structure is additionally verified by the PCA, where five latent factors appear to be responsible for it. These five latent factors explain over 85% of the total variance. The separation of the factors is well indicated. The plane projection for the first two latent factors (Fig. 3) confirms in general the results from hierarchical and non-hierarchical clustering of the plant objects. Some minor differences are negligible since this plot gives the projection of only two latent factors.

Conclusions. In the present study a chemometric approach was applied to the data obtained for 28 mineral elements in ten ethereal oil plant materials. It could be assumed that the chemical composition of the ten plant samples in consideration is substantially influenced by five major soil factors related to the soil quality. The elements from clusters K1, K2 and K4 are more or less of anthropogenic origin and can be attributed to different fields of human activities, while the elements forming the clusters K3 and K5 are probably of non-anthropogenic origin, both related to naturally occurring ores and minerals. The clustering of the plant objects causes formation of two clusters. It is supposed that these patterns are due to the plant features. Both samples M and L, obtained after the extraction of the ethereal oils from the corresponding plants, form a separate subcluster. The non-hierarchical clustering of the 28 variables followed the a priori hypothesis for the effect of five soil quality patterns on the plant objects. This data structure is additionally verified by the PCA, where five latent factors appear to be responsible for it.

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