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Analytical Methods

Evaluation of trace metal concentrations in some herbs and herbal teas by principal component analysis

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ABSTRACT

Sixteen trace metallic analytes (Ba, Ca, Ce, Co, Cr, Cu, Fe, K, La, Mg, Mn, Na, Ni, P, Sr and Zn) in acid digests of herbal teas were determined and the data subjected to chemometric evaluation in an attempt to classify the herbal tea samples. Nettle, Senna, Camomile, Peppermint, Lemon Balm, Sage, Hollyhock, Linden, Lavender, Blackberry, Ginger, Galangal, Cinnamon, Green tea, Black tea, Rosehip, Thyme and Rose were used as plant materials in this study. Trace metals in these plants were determined by using inductively coupled plasma-atomic emission spectrometry and inductively coupled plasma-mass spectrometry. Principal component analysis (PCA), linear discriminant analysis (LDA) and cluster analysis (CA) were used as classification techniques. About 18 plants were classified into 5 groups by PCA and all group members determined by PCA are in the predicted group that 100.0% of original grouped cases correctly classified by LDA. Very similar grouping was obtained using CA.

1. Introduction

Herbal tea has been imbibed for nearly as long as written history extends. Also known as a tisane or herbal infusion, herbal tea is simply the combination of boiling water and dried fruit, flowers or herbs. Documents have been recovered dating back to as early as ancient Egypt that discusses the enjoyment and uses of herbal tea. Herbal teas can be made with fresh or dried flowers, leaves, seeds or roots, generally by pouring boiling water over the plant parts and letting them steep for a few minutes. Seeds and roots can also be boiled on a stove. Herbal teas are often consumed for their physical or medicinal effects, especially for their stimulant, relaxant or sedative properties.[1]

At present, there are many herbal tea products widely consumed in Turkey and worldwide. Among these products, black tea, green tea, linden, sage and rosehip are the most popular herbal tea products consumed for medical purposes or for maintaining good health. The mineral contents of some herbal teas have been determined in several previous publications (Gallaher, Gallaher, Marshall, & Marshall, 2006; Nookabkaew, Rangkadilok, & Satayavivad, 2006; Özcan & Akbulut, 2008; Özcan, Ünver, Uçar, & Arslan, 2008). These studies usually used univariate methods such as analysis of variance (ANOVA), i.e. compared with the concentration of one element with another or one sample with another. However, multivariate methods such as principal component analysis (PCA) can provide further interpretation. PCA is a data reduction technique that aims to explain most of the variance in the data whilst reducing the number of variables to a few uncorrelated components (Anderson, 2003; Sharma, 1996). This method enables us to identify groups of variables or individuals. PCA is used to identify groups of variables, based on the loadings, i.e. correlations between the variables and the principal components, and groups of individuals based on the principal component scores (Boruvka, Vacek, & Jehlicka, 2005). Generally the output of a PCA package is a graph which are called "scores" (equivalent to the variables) that are estimated in bilinear modelling methods where information carried by several variables is concentrated onto a few underlying variables. Each sample has a score along each model component. The scores show the locations of the samples along each model component, and can be used to detect sample patterns, groupings, similarities or differences. One of the other graphs produced using PCA are called "loadings" that are estimated in bilinear modelling methods where information carried by several variables is concentrated onto a few components. Each variable has a loading along each model component. The loadings show how well a variable is taken into account by the model components. They can be used to understand how much each variable contributes to the

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[1] <http://en.wikipedia.org/wiki/Herbal_tea>.

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meaningful variation in the data, and to interpret variable relationships. They are also useful for interpreting the meaning of each model component (CAMO Software AS, 1998). Many computational algorithms have been developed for PCA. Some methods compute all components simultaneously, whereas others find the most significant component first and then the next component and so on (Brereton, 1990). Principal component analysis was used to evaluate teas (green and black tea) collected from different parts of the world and their metal contents (Marcos, Fisher, Rea, & Hill, 1998; Moreda-Piñeiro, Fisher, & Hill, 2003; Fernández-Cáceres, Martín, Pablos, & González, 2001).

The aim of this study is to demonstrate the application of this data reduction technique to evaluate whether or not there is a relationship between the metal contents in the different herbal teas. Since many people are now consuming these herbal supplements, it is important to determine their nutrient composition so that their effect on human health can be understood. It is also important to try to elucidate whether or not there are any relationships between different plant types and the uptake of metals from the soils. Before any of this can be studied in any great detail, it is important to obtain accurate analytical data and then to insert this data into a chemometrics package in an attempt to produce a working model that is a reliable template. Only when a reliable working model has been produced can much larger studies be undertaken in which different plant types may be grown under identical conditions in the same soil etc. About 18 different herbal teas (Rose, Cinnamon, Lavender, Galangal, Thyme, Hollyhock, Blackberry, Rosehip, Linden, Sage, Black tea, Senna, Lemon balm, Nettle, Ginger, Green tea, Camomile and Peppermint) were evaluated for their content of 16 elements (Ba, Ca, Ce, Co, Cr, Cu, Fe, K, La, Mg, Mn, Na, Ni, P, Sr, Zn). One tea reference material (Chinese reference material GBW 08505) was analysed to demonstrate the accuracy of the analytical procedure. The evaluation of whether or not there is a relationship between the metals in plants was done using PCA and other data manipulation techniques such as linear discriminant analysis (LDA) and cluster analysis (CA).

2. Experimental

2.1. Reagents and solutions

Doubly de-ionized water (18.2 MΩ cm), obtained from a Primar water system (Elga, Buckinghamshire, UK) was used throughout the experiment. Plant digests were prepared using HNO_3 (Merck, UK). Stock standard solutions of individual metals (1000 or 10,000 mg L^{-1}) were supplied by Merck. A certified reference material (Chinese reference material GBW 08505, obtained from the Bureau of Analysed Samples, Middlesbrough, UK) was used to verify the accuracy of the results.

2.2. Instrumentation

An ICP-MS instrument (VG PlasmaQuad, PQ2+ Turbo, Thermo Elemental, Winsford, Cheshire, UK) was used for the determination of Co, Cr, Ce and La. Operating conditions for the ICP-MS instrument were: forward power 1.35 kW, coolant gas flow rate 12 L min^{-1}, auxiliary gas flow rate 1 L min^{-1}; nebulizer gas flow rate 0.9 L min^{-1}. An ICP-OES instrument (Varian 725-ES, Melbourne, Australia) was used for the determination of Ba, Ca, Cu, Fe, K, Mg, Mn, Na, Ni, Sr, P and Zn in the plant digests. Operating conditions for ICP-OES instrument were: forward power 1.4 kW, coolant gas flow rate 15 L min^{-1}. Auxiliary gas flow rate 1.5 L min $^{-1}$; nebulizer gas flow rate 0.68 L min^{-1}; the viewing height was 8 mm above the load coil.

2.3. Procedure

2.3.1. Sample preparations

Herbal tea products (Rose (*Rosa*), Cinnamon (*Cinnamomum casia*), Lavender (*Lavandula officinalis*), Galangal (*Alpinia officinarium*), Thyme (*Thymbra spicata*), Hollyhock (*Alcea rosea*), Blackberry (*Rubus allegheniensis*), Rosehip (*Rosa canina*), Linden (*Tilia* spp.), Sage (*Salvia fruticosa*), Senna (*Cassia acutifolia*), Lemon balm (*Melissa officinalis*), Nettle (*Urtica dioica*), Ginger (*Zingiber officinale*), Green tea and Black tea (*Camelia sinensis*), Camomile (*Matricaria chamomilla*) and Peppermint (*Mentha piperita*) were purchased from a supermarket in Balikesir, Turkey. They included both imported and locally made products. The samples were ground using a pestle and mortar. The pulverised and powdered herbal tea samples were transferred into plastic bags. All herbal teas were treated in an identical manner. For acid digestion, herbal tea (0.2500 g) was weighed into a pre-cleaned beaker. Concentrated nitric acid (10 ml) was added, the beaker covered with a watch-glass and the sample boiled gently on a laboratory hot-plate until digestion was complete. This process took approximately 3 h. The digested sample was then allowed to cool before being transferred quantitatively into clean 25 ml volumetric flasks. The samples were then diluted to volume by the addition of ultrapure water. Four replicate digestions were made for each herbal tea type. To ensure that the results obtained for the analyses were accurate, a certified reference material (Chinese reference material GBW 08505, obtained from the Bureau of Analysed Samples, Middlesbrough, UK) was prepared in the same way.

2.3.2. Sample analysis

In the herbal tea acid extracts, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, Ni, Sr, P and Zn were determined by ICP-OES and Ce, Co, Cr and La were determined by ICP-MS. Indium (as an internal standard for ICP-MS measurements) was added to each digest to give a concentration of 100 μg L^{-1} after dilution to 25 ml. All results are the mean of the four replicates and are quoted on a dry weight basis. All statistical calculations were made using SPSS 10 (SPSS 10 & Release 10.0.1, 1989–1999) and Statistica (Statistica 99 edition, 1984–1999) packages.

3. Results and discussion

The average results taken from the ICP-OES and ICP-MS analyses are shown in Table 1. The relative standard deviations (RSD %) are given below the mean values. In general, the RSD was less than 10%. The results for the analysis of the certified material are also shown in Table 1. The results given in Table 1 are the average concentration of four replicate analyses. Metals were classified using correlation analysis and principal component analysis. The plants were classified using principal component analysis, cluster analysis and linear discriminant analysis.

3.1. Correlation analysis

Correlation analysis of total element contents (Table 2) shows moderate to strong correlations in six groups of elements. The negative correlation coefficients show a negative correlation whilst the positive correlation coefficients show a positive correlation between the two variables. The closer this coefficient is to 1 the more similar the two variables are. If this coefficient is close to 0, it means that there is a very weak or perhaps even no relation between the two variables. Cu, La and P is positively correlated with all the elements in Table 2. Magnesium is positively correlated with all elements except Mn; zinc is positively correlated with

Table 1
Concentrations of elements in different plants (mean and RSD %)

Plants	Element concentration (μg g^{-1} dry weight)							
	Mg	Zn	Cu	Fe	Mn	Ba	Na	Ni
Reference tea (certified value)	2240	38.7	16.2	373	766	15.7	142	7.61
	(8.5)	(10.1)	(11.7)	(6.2)	(3.7)	(12.1)	(9.2)	(6.3)
Reference tea (found value)	2278	36.2	16.3	375	682	10.9	142	7.60
	(4.79)	(2.5)	(2.5)	(1.6)	(5.3)	(3.7)	(7.7)	(6.2)
Rose (no. 1)	1897	11.8	4.9	106	70.6	9.0	79.5	2.6
	(4.5)	(2.1)	(3.4)	(3.1)	(3.5)	(3.9)	(2.6)	(5.5)
Cinnamon (no. 2)	852	17.9	3.3	56.7	104	14.1	65.7	0.6
	(4.9)	(6.5)	(1.4)	(9.5)	(7.9)	(4.9)	(4.1)	(6.5)
Lavender (no. 3)	4573	13.2	5.7	680	48.9	12.7	86.7	3.6
	(5.3)	(5.9)	(5.9)	(0.4)	(2.5)	(3.7)	(3.6)	(2.4)
Galangal (no. 4)	802	9.7	2.1	337	281	3.6	438.4	1.7
	(6.9)	(5.5)	(6.5)	(1.9)	(4.8)	(3.9)	(7.9)	(8.2)
Thyme (no. 5)	2115	22.4	6.1	440	116	81.6	106.5	1.5
	(6.2)	(2.3)	(1.9)	(1.4)	(8.7)	(6.7)	(4.9)	(9.2)
Hollyhock (no. 6)	4538	17.4	5.7	164	31.7	33.0	125.5	2.2
	(8.8)	(2.6)	(6.9)	(5.9)	(5.7)	(6.5)	(2.4)	(4.1)
Blackberry (no. 7)	2786	12.4	6.6	165	54.6	9.7	44.0	0.7
	(1.2)	(5.6)	(6.4)	(4.2)	(8.9)	(2.9)	(3.2)	(4.7)
Rosehip (no. 8)	2931	3.2	3.0	27.5	47.5	8.2	44.3	1.6
	(9.7)	(6.7)	(6.7)	(2.9)	(2.1)	(2.8)	(7.3)	(6.7)
Linden (no. 9)	2822	20.9	9.5	109	113	14	78.1	3.6
	(7.4)	(8.2)	(5.5)	(3.9)	(4.2)	(3.3)	(8.1)	(2.3)
Sage (no. 10)	4631	28.0	5.6	1106	66.4	32.8	34.4	6.0
	(1.4)	(7.1)	(5.8)	(2.0)	(3.1)	(2.3)	(5.6)	(7.7)
Black tea (no. 11)	1992	18.6	13.1	243	580	18.9	139	4.0
	(5.3)	(6.3)	(4.3)	(4.5)	(6.5)	(3.9)	(2.7)	(7.5)
Senna (no. 12)	6503	15.1	5.6	270	46.5	64.4	1233	0.8
	(2.3)	(2.5)	(4.7	(5.6)	(2.3)	(5.0)	(5.4)	(2.7)
Lemon balm (no. 13)	5636	24.5	8.4	530	47.9	32.4	54.9	1.8
	(1.8)	(2.3)	(1.4)	(1.4)	(6.2)	(5.2)	(7.0)	(2.5)
Nettle (no. 14)	7324	22.0	11.2	999	66.5	37.5	128	2.0
	(5.3)	(4.9)	(3.5)	(6.8)	(1.7)	(4.6)	(7.8)	(3.5)
Ginger (no. 15)	2006	13.5	4.0	86.8	127	18.7	103	1.9
	(8.8)	(6.2)	(6.7)	(6.6)	(8.7)	(0.3)	(2.9)	(2.8)
Green tea (no. 16)	2095	21.4	11.1	231	786	21.7	52.6	4.9
	(0.3)	(9.1)	(3.5)	(4.4)	(4.7)	(0.8)	(1.8)	(3.4)
Camomile (no. 17)	2319	24.4	8.2	521	96.4	9.8	2132	1.5
	(5.9)	(7.1)	(1.3)	(3.7)	(1.7)	(6.6)	(4.2)	(2.8)
Peppermint (no. 18)	2929	17.9	17.7	975	112	13.9	3467	1.0
	(7.8)	(1.4)	(3.5)	(2.9)	(3.6)	(1.2)	(4.9)	(7.2)

Plants	Sr	P	K	Ca	Co	Cr	Ce	La
Reference tea (certified value)	10.8	4260	19 700	2840	0.2	0.8	0.686	0.458
	(16.7)	(5.4)	(6.6)	(7.4)			(13.4)	(4.4)
Reference tea (found value)	9.85	3890	18 125	2429	0.122	0.80	0.758	0.405
	(1.0)	(0.5)	(3.8)	(3.1)	(1.6)	(7.5)	(2.8)	(4.7)
Rose (no. 1)	11.6	1584	11536	8109	0.11	0.33	0.16	0.11
	(7.7)	(2.9)	(2.9)	(8.3)	(5.2)	(6.4)	(6.4)	(4.5)
Cinnamon (no. 2)	60.9	595	7010	10978	0.11	0.21	0.065	0.030
	(6.6)	(5.1)	(2.4)	(8.9)	(4.8)	(4.5)	(4.7)	(4.2)
Lavender (no. 3)	27.5	1093	14315	14330	0.26	1.26	1.37	0.65
	(4.9)	(3.8)	(0.8)	(3.0)	(1.7)	(6.8)	(3.4)	(6.2)
Galangal (no. 4)	6.23	863	8491	762	0.21	0.61	0.84	0.72
	(5.2)	(1.3)	(6.3)	(4.6)	(3.0)	(6.2)	(5.7)	(1.5)
Thyme (no. 5)	45.6	1199	14708	7759	0.15	0.57	1.40	0.71
	(5.3)	(5.9)	(4.2)	(6.8)	(2.6)	(7.9)	(7.1)	(4.0)
Hollyhock (no. 6)	85.6	3126	15815	21749	0.23	0.44	0.21	0.093
	(3.3)	(3.7)	(3.4)	(2.9)	(5.5)	(2.4)	(6.2)	(2.8)
Blackberry (no. 7)	19.2	1848	9474	4414	0.10	0.51	0.23	0.11
	(2.8)	(5.5)	(1.2)	(5.1)	(6.5)	(3.5)	(2.1)	(5.8)
Rosehip (no. 8)	39.2	939	13519	8020	0.10	0.23	0.10	0.022
	(6.9)	(4.7)	(3.7)	(3.0)	(2.9)	(3.1)	(4.6)	(4.3)
Linden (no. 9)	38.7	2295	13993	14162	0.19	0.60	0.19	0.12
	(4.2)	(4.9)	(4.2)	(2.9)	(3.0)	(5.8)	(4.2)	(3.5)
Sage (no. 10)	18.3	1580	18594	9299	0.12	0.66	0.84	0.44
	(1.8)	(2.8)	(4.5)	(2.6)	(3.2)	(6.2)	(4.7)	(6.7)
Black tea (no. 11)	12.1	2225	14313	3153	0.14	0.88	0.51	0.2
	(4.1)	(6.7)	(5.8)	(1.9)	(3.8)	(6.3)	(4.5)	(2.6)
Senna (no. 12)	411	1217	96640	26908	0.26	0.75	0.35	0.26
	(5.7)	(7.5)	(6.6)	(3.3)	(3.3)	(3.8)	(2.4)	(5.1)
Lemon balm (no. 13)	22.5	2234	18737	12905	0.31	1.16	0.96	0.39
	(7.7)	(1.4)	(1.5)	(0.9)	(2.5)	(2.7)	(5.3)	(6.6)
Nettle (no. 14)	134	3365	17472	38401	0.50	1.77	1.57	0.70
	(4.0)	(7.5)	(5.7)	(7.0)	(4.7)	(5.4)	(4.5)	(2.9)

(*continued on next page*)

Table 1 (*continued*)

Plants	Element concentration (μg g^{-1} dry weight)							
	Sr	P	K	Ca	Co	Cr	Ce	La
Ginger (no. 15)	7.19	1692	8808	944	0.067	0.61	0.38	0.21
	(4.1)	(4.5)	(3.2)	(6.1)	(7.9)	(2.8)	(2.9)	(5.0)
Green Tea (no. 16)	15.4	2055	13327	3668	0.14	0.75	0.59	0.30
	(4.5)	(3.1)	(4.4)	(5.4)	(3.2)	(2.2)	(6.0)	(2.1)
Camomile (no. 17)	49.0	2428	18399	6959	0.20	1.70	0.80	0.35
	(2.5)	(4.4)	(0.9)	(1.1)	(4.6)	(5.5)	(4.3)	(7.0)
Peppermint (no. 18)	150	2666	17216	11749	0.42	2.34	1.59	0.54
	(1.9)	(4.2)	(0.6)	(3.8)	(5.7)	(3.8)	(4.5)	(7.7)

Table 2
Correlation matrix for the element concentrations in plants (figures in bold indicate that the higher correlations are between two metals)

	Mg	Zn	Cu	Fe	Mn	Ba	Na	Ni	Sr	P	K	Ca	Co	Cr	Ce	La
Mg	1.00															
Zn	0.27	1.00														
Cu	0.18	0.45	1.00													
Fe	0.51	0.53	0.45	1.00												
Mn	−0.39	0.14	0.36	−0.17	1.00											
Ba	0.45	0.41	0.01	0.20	−0.13	1.00										
Na	0.00	0.10	0.54	0.39	−0.11	−0.07	1.00									
Ni	0.05	0.40	0.16	0.26	0.48	−0.07	−0.35	1.00								
Sr	0.56	−0.01	0.14	0.13	−0.25	0.51	0.43	−0.38	1.00							
P	0.43	0.46	0.68	0.35	0.05	0.03	0.26	0.11	0.06	1.00						
K	0.38	0.60	0.52	0.65	−0.07	0.08	0.21	0.38	−0.23	0.63	1.00					
Ca	0.84	0.20	0.18	0.37	−0.41	0.42	0.05	−0.16	0.65	0.44	0.21	1.00				
Co	0.64	0.26	0.57	0.65	−0.19	0.17	0.46	−0.19	0.44	0.58	0.44	0.72	1.00			
Cr	0.36	0.37	0.74	0.73	−0.03	−0.02	0.73	−0.08	0.23	0.56	0.57	0.32	0.80	1.00		
Ce	0.34	0.39	0.47	0.83	−0.04	0.30	0.38	0.05	0.06	0.27	0.55	0.26	0.70	0.77	1.00	
La	0.24	0.29	0.20	0.72	0.01	0.32	0.22	0.08	0.04	0.06	0.33	0.18	0.57	0.56	0.92	1

all elements except Sr, iron is positively correlated with all elements except Mn and finally cerium is positively correlated with all elements except Mn. Nickel and manganese are moderately correlated with each other whereas there is not a significant correlation for these analytes with other metals. The relationships between the elements appear complex and difficult to explain individually. In general, interpretation of correlation analysis was done using correlation coefficients values higher than 0.5. However, some values close to 0.5 were also included to produce grouping such as 0.48 for Mn and Ni; 0.45 for Ba and Mg and 0.42 for Ba and Ca. Interpretation of correlation analyses enabled the groupings below to be obtained:

Group 1: Mg, Ca, Sr, Ba
Group 2: Fe, Co, Cr, Ce, La
Group 3: Mn, Ni
Group 4: Zn, Fe, K
Group 5: Na, Cr, Cu
Group 6: Cr, Cu, P, K.

Further elucidation may be obtained using more powerful chemometric techniques such as PCA.

From the listed elements, P, K, Ca, Mg, Fe, Mn, Zn, Cu, Ni, Cr are essential whilst Na and Co are beneficial elements for plants (Bohn, McNeal, & O'Connar 2001). P, K, Ca, Fe and Mg are macro-elements whilst Mn, Zn, Cu, Ni, Cr, Co, Ba, Sr are micro-elements in plants (Jeffrey, 1987). The first group includes alkaline earth cations in which Mg and Ca are macro-nutrients in soils and plants and observed very high correlations coefficient (0.84) between these two metal ions in Table 2. Group 3 elements (Mn and Ni), group 4 elements (Zn, Fe and K), group 6 elements (Cr, Cu, P and K) are all essential elements for plants and a correlation was also shown between these metals.

3.2. Principal component analysis

PCA is a bilinear modelling method which gives an interpretable overview of the main information in a multi-dimensional data table. The information carried by the original variables is projected onto a smaller number of underlying ("latent") variables called principal components. The first principal component covers as much of the variation in the data as possible. The second principal component is orthogonal to the first and covers as much of the remaining variation as possible, and so on. By plotting the principal components, one can view inter-relationships between different variables, and detect and interpret sample patterns, groupings, similarities or differences (CAMO Software AS, 1998).

Principal component analysis was applied to the whole set of data. The principal components which have eigenvalues higher than 1 were extracted. This led to the formation of five principal components. The first component accounted for 39.5%, the second for 17.5%, the third for 11.9%, the fourth for 10.0% and the fifth for 7.3% of the total variation of the data. The first five components account for 86.2% of variances for all of the data. The first component represents the maximum variation of the data set. The components were rotated using Varimax rotation. There are various rotational strategies that have been proposed. Probably the best known approach (available in most commercial factor analysis software) is called Varimax rotation. The principal components are rotated so that the total sum of squares of the loadings along each new axis is maximised (Brereton, 1990). The goal of all of these strategies is to obtain a clear pattern of loadings, that is, factors that are somehow clearly marked by high loadings for some variables and low loadings for others. This general pattern is also sometimes referred to as simple structure (a more formalised definition can be found in most standard textbooks). The higher the loading of a variable implies a larger contribution to the variation, accounting for

Table 3
The loadings and the scores of the first five rotated principal components

The loadings						The scores					
Element	PC1	PC2	PC3	PC4	PC5	Plant	PC1	PC2	PC3	PC4	PC5
Mg	0.18	0.74	0.19	0.18	0.48	Rose	−0.46	0.16	−0.21	−0.85	−0.78
Zn	0.31	0.020	0.65	0.30	0.26	Cinnamon	−0.80	−0.41	−0.92	−0.84	0.064
Cu	0.89	−0.14	0.22	0.19	0.082	Lavender	−0.82	0.57	−0.047	1.45	−0.63
Fe	0.32	0.29	0.25	0.79	0.055	Galangal	−1.09	−1.08	−1.12	1.15	−0.72
Mn	0.21	−0.78	0.40	−0.11	0.064	Thyme	−1.34	−0.86	0.28	1.53	1.31
Ba	−0.17	0.10	0.17	0.24	0.84	Hollyhock	0.32	1.40	0.52	−1.32	0.18
Na	0.70	−0.11	−0.48	0.32	0.020	Blackberry	−0.20	0.15	−0.70	−0.84	−0.69
Ni	−0.050	−0.19	0.84	0.052	−0.10	Rosehip	−0.82	0.57	−0.84	−0.91	−0.95
Sr	0.26	0.23	−0.42	−0.030	0.79	Linden	0.44	0.30	0.74	−1.12	−0.25
P	0.76	0.36	0.37	−0.032	−0.002	Sage	−0.93	0.46	1.99	0.91	−0.15
K	0.44	0.34	0.56	0.41	−0.22	Black tea	0.90	−1.48	1.12	−0.70	0.043
Ca	0.25	0.73	0.010	0.071	0.54	Senna	−0.054	0.26	−1.42	−0.63	3.17
Co	0.59	0.45	−0.093	0.50	0.24	Lemon balm	0.14	1.03	0.69	0.40	−0.16
Cr	0.76	0.14	−0.071	0.60	−0.019	Nettle	0.91	1.96	0.57	1.02	0.79
Ce	0.27	0.081	0.090	0.93	0.10	Ginger	−0.61	−0.43	−0.42	−0.45	−0.47
La	0.009	−0.015	0.057	0.95	0.15	Green tea	0.64	−2.10	1.54	−0.51	0.56
						Camomile	1.17	−0.066	−0.34	0.38	−0.93
						Peppermint	2.61	−0.45	−1.43	1.33	−0.38

the Varimax rotated principal components. Table 3 gives the rotated loadings and communality for each element. The loadings were large for Cu, P, Cr, Na, Co and K on the first component, for Mg, Ca and Co on the second component, for Ni, Zn, K on the third component, for La, Ce, Cr and Fe on the fourth component and for Ca, Ba, Mg and Sr on the fifth component. A very similar classification of the analytes was achieved using classification analysis, above. Table 3 also gives the score values for each principal component for each plant sample. From the scores on the first principal component it can be interpreted that the concentrations of Cu, P, Cr, Na, Co and K on the first principal component loadings are higher for Peppermint, Camomile, Nettle, Black tea and Green tea than the other plants and are lower for Thyme, Galangal, Sage, Rosehip, Lavender and Cinnamon than the other plants. When the second principal component is interpreted, Mg, Ca and Co concentrations are higher for Nettle, Hollyhock and Lemon balm and are lower for Green tea, Black tea, Galangal and Thyme than for the other plants investigated. On the third principal component, Ni, Zn and K concentrations are higher for Sage, Green tea, Black tea and Linden and are lower for Peppermint, Senna, Galangal, Cinnamon, Rosehip and Blackberry than for the other plants. La, Ce, Fe and Cr concentrations in the fourth principal component are higher for Thyme, Galangal, Lavender, Peppermint, Nettle and Sage and lower for Hollyhock, Linden, Rosehip, Rose, Cinnamon and Blackberry. Finally, Ca, Ba, Mg and Sr concentrations on the fifth principal component are higher for Thyme and Senna and lower for Rosehip, Camomile, Rose, Galangal and Blackberry.

Fig. 1 shows the two way loadings and score plots. Every principal component was plotted against PC1 to show high percentage of the total variance (57–46.8). Fig. 1b shows the behaviour of variables on the PC1 and PC2. As can be seen, there is an association between Cu, Cr, P, Na, Co and K. There is also another association between Mg and Ca on the PC2 whereas the rest of the metals appear more dispersed into the components space, showing a more individualised behaviour. The superposition of the loading (Fig. 1b) and score (Fig. 1a) plots for PC1 and PC2 show manganese concentrations are higher for Black tea and Green tea. This information can also be found on the internet where tea is described as the richest source of manganese in plants.[2] Mg and Ca concentrations are higher for Nettle, Lemon balm and Hollyhock and the concentrations of Cu, Cr, P, Na, Co and K are higher for Peppermint, Camomile, Green tea and Black tea.

It can be interpreted from Fig. 1c and d from PC1 and PC3 that the Sr concentration is highest for Senna, Na concentration is highest for Peppermint and Ba concentration is highest for Thyme. The concentrations of Zn, Ni and K that have higher loadings on the PC3 are higher for Sage, Green tea, Black tea and Linden.

Fig. 1f shows a cluster of elements with large positive loadings on PC4. It includes La, Ce and Fe. It can be interpreted from the score and loading plots for PC1-PC4 (Fig. 1e and f) that La, Ce and Fe concentrations are higher for Lavender, Galangal, Thyme, Peppermint, Sage and Nettle. Extra information can be obtained from the score and loading plots of PC1 and PC5 (Fig. 1g and h), which indicate that Sr, Ba and K have highest values for Senna, Thyme and Camomile, respectively.

The classification of the herbal teas from the view point of metal contents can be made using three way PC score graphs. Fig. 2a, b and c shows PC 1-2-3, PC 1-2-4 and PC 1-2-5. The PC 1-2-3 graph shows the highest percentage of total variance of about 68.9. It can be seen from the PC 1-2-3 graph (Fig. 2a) that the herbal teas can be classified into four groups. These groups include:

Group 1: Black tea, Green tea
Group 2: Camomile, Peppermint
Group 3: Sage, Nettle, Lemon balm, Hollyhock, Linden
Group 4: Rose, Cinnamon, Lavender, Galangal, Thyme, Rosehip, Blackberry, Senna, Ginger.

Another similar four groups can be obtained from PC 1-2-4 (Fig. 2b) which shows about 67% of total variance. These groups include:

Group 1: Black tea, Green tea
Group 2: Camomile, Peppermint
Group 3: Lavender, Galangal, Thyme, Sage, Nettle, Lemon balm
Group 4: Rose, Cinnamon, Rosehip, Blackberry, Senna, Ginger, Hollyhock, Linden.

The last three groups were obtained from PC 1-2-5 (Fig. 2c) which shows about 64.3% of the total variance. These groups are:

Group 1: Black tea, Green tea, Camomile, Peppermint
Group 2: Senna, Nettle

[2] <http://www.teaauction.com/home/teanhealth.asp>.

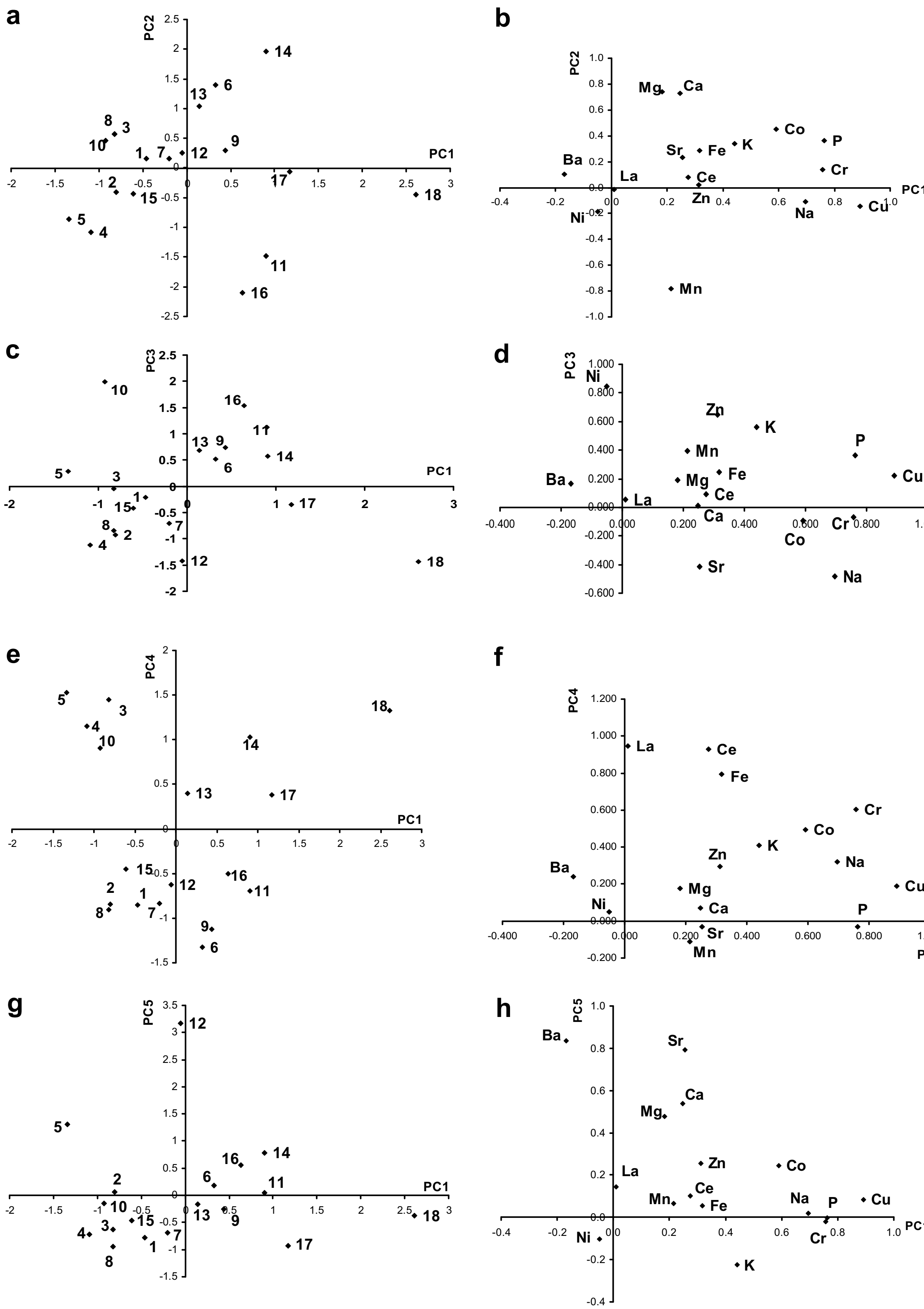

Fig. 1. The score and loading plots (a, c, e and g are the score plots and b, d, f and h are the loading plots) (1, Rose; 2, Cinnamon; 3, Lavender; 4, Galangal; 5, Thyme; 6, Hollyhock; 7, Blackberry; 8, Rosehip; 9, Linden; 10, Sage; 11, Black tea; 12, Senna; 13, Lemon balm; 14, Nettle; 15, Ginger; 16, Green tea; 17, Camomile; 18, Peppermint).

Group 3: Lavender, Galangal, Thyme, Sage, Lemon balm, Rose, Cinnamon, Rosehip, Blackberry, Ginger, Hollyhock and Linden.

When the three PC score plots were investigated together, 5 general groupings were obtained from the point of view of metal contents. The resulting classified groups are:

Group 1: Black tea, Green tea
Group 2: Camomile, Peppermint, Hollyhock, Linden, Sage, Lemon balm
Group 3: Lavender, Galangal, Thyme
Group 4: Nettle, Senna
Group 5: Rose, Rosehip, Blackberry, Ginger, Cinnamon.

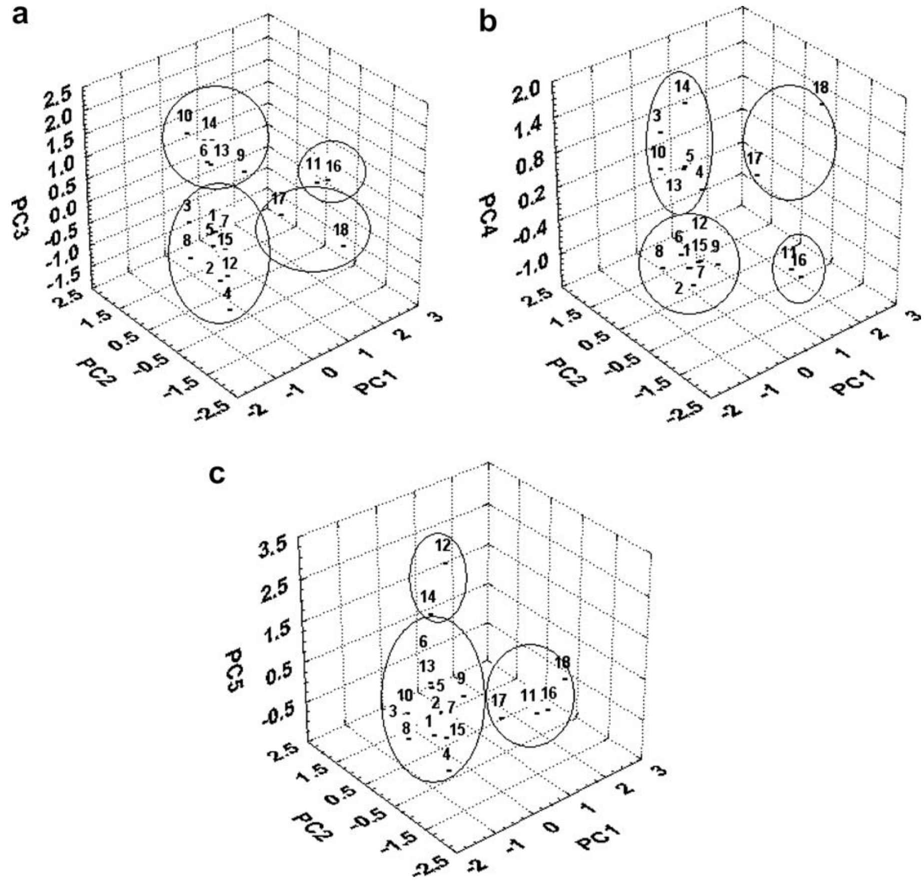

Fig. 2. Three way PCA scores plot (a) PC1-2-3 (b) PC1-2-4 (c) PC1-2-5 (1, Rose; 2, Cinnamon; 3, Lavender; 4, Galangal; 5, Thyme; 6, Hollyhock; 7, Blackberry; 8, Rosehip; 9, Linden; 10, Sage; 11, Black tea; 12, Senna; 13, Lemon balm; 14, Nettle; 15, Ginger; 16, Green tea; 17, Camomile; 18, Peppermint).

It can also be interpreted from the relation between groups and their metal ion concentrations from the Figs. 1 and 2, that the first group plants (Black tea and Green tea) have got the highest concentration of Mn (PC1-PC2) and also higher concentration of Zn, Cu, Ni, P and K (PC1-PC3), comparatively. The second group of plants (Camomile, Peppermint, Hollyhock, Linden, Sage and Lemon balm) has got higher concentration of Mg, Zn, Cu, Fe, P, K, Ca, Cr, Ce, Co, Sr and Na (PC1-PC3). The third groups of plants (Lavender, Galangal and Thyme) have higher concentration of Co, Cr, Ce, La and Fe (PC1-PC4). The fourth group of plants (Nettle and Senna) has higher concentrations of Mg, Ba, Ca, Sr (PC1-PC5). Finally, the fifth group plants (Rose, Cinnamon, Rosehip, Blackberry and Ginger) have lower concentrations of Mg, Ca, Co, P, Sr, Fe, K, Cr, Ce, Zn, Na, Cu, Mn and Ba).

3.3. Linear discriminant analysis

The linear discriminant analysis technique is a supervised pattern recognition method. In supervised pattern recognition, objects are classified into groups (or classes or clusters) with pre-determined models for the class. These approaches differ from unsupervised methods such as cluster analysis where there is no prior class model. The aim of hard-modelling, a form of supervised pattern recognition, is to classify uniquely into a number of pre-determined classes (Brereton, 1990). The linear discriminant analysis was performed on the classified 5 groups resulting from the PCA analyses above for the 16 elements using SPSS 10 statistics software (SPSS 10, 1989–1999). The recognition of these groups was highly satisfactory. All group members determined by PCA are in the predicted group that 100.0% of original grouped cases correctly classified. Five canonical discriminant function that eigen values are bigger than 1 were obtained from the data. The first canonical discriminant function explains 88% of the variance. The discriminant function of the first function is $Z = -59.051 - 0.004\ \mathrm{Mg} + 0.902\ \mathrm{Zn} - 0.106\ \mathrm{Cu} + 0.003\ \mathrm{Fe} + 0.02\ \mathrm{Mn} - 0.408\ \mathrm{Ba} - 0.016\ \mathrm{Na} - 0.030\ \mathrm{Ni} + 0.291\ \mathrm{Sr} + 0.009\ \mathrm{P} + 0.003\ \mathrm{K} - 0.001\ \mathrm{Ca} + 34.218\ \mathrm{Co}$.

3.4. Cluster analysis

Cluster analysis is the most widely used unsupervised pattern recognition technique in chemometrics. This technique involves trying to determine relationships between objects (samples) without using prior information about these relationships. The raw data for cluster analysis consist of a number of objects and related measurements (Brereton, 1990). Objects will be grouped in clusters in terms of their nearness or similarity. The cluster analysis was applied using the SPSS package. The measurement is based on the squared Euclidean distance. In this study, the Ward's method was used as a clustering method (SPSS 10, 1989–1999). Similar results to PCA were obtained after the application of cluster analysis (Fig. 3). Seven groupings were obtained from cluster analysis. These groups contain:

Group 1: Nettle and Senna
Group 2: Camomile, Peppermint, Lemon balm and Sage
Group 3: Hollyhock, Linden and Lavender
Group 4: Blackberry, Ginger and Galangal
Group 5: Cinnamon
Group 6: Green tea, Black tea
Group 7: Rosehip, Thyme and Rose.

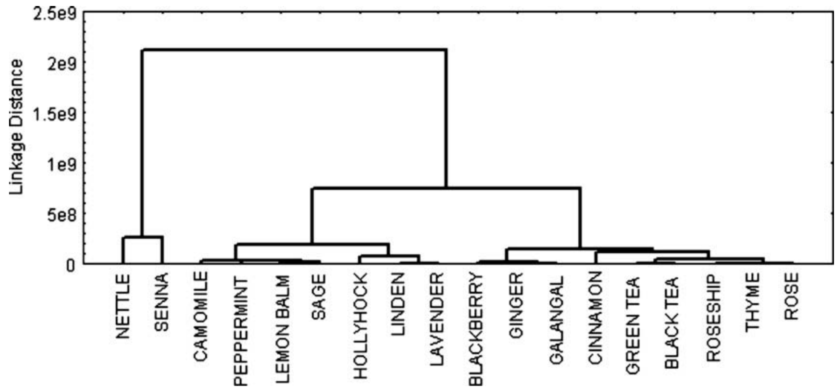

Fig. 3. Dendrogram of cluster analysis.

4. Conclusions

The results obtained show that there is a relationship between plants that are used as herbs or as herbal teas from the perspective of metal concentrations. The plants were classified into five groups by PCA interpretation. The LDA also demonstrated that this grouping is correctly classified as 100.0%. From the point of view of metal contents, the first group contains Black tea, Green tea and second group of metals are Camomile, Peppermint, Hollyhock, Linden, Sage, Lemon balm, the third group of metals are Lavender, Galangal, Thyme, the fourth group of metals are Nettle, Senna and finally, the fifth group are Rose, Cinnamon, Rosehip, Blackberry and Ginger. The first group of herbs (Black tea and Green tea) has got the highest concentration of Mn and also higher concentration of Zn, Cu, Ni, P and K, comparatively. The second group of plants (Camomile, Peppermint, Hollyhock, Linden, Sage and Lemon balm) has got higher concentration of Mg, Zn, Cu, Fe, P, K, Ca, Cr, Ce, Co, Sr and Na. The third group of plants (Lavender, Galangal and Thyme) has higher concentrations of Co, Cr, Ce, La and Fe. The fourth group of plants (Nettle and Senna) has higher concentrations of Mg, Ba, Ca, Sr and finally, the fifth group of plants (Rose, Cinnamon, Rosehip, Blackberry and Ginger) have lower concentrations of Mg, Ca, Co, P, Sr, Fe, K, Cr, Ce, Zn, Na, Cu, Mn and Ba). Cluster analysis also found a similar, but slightly different, grouping. At this stage of the study, it is clear that the different plants may be grouped according their trace element concentrations, but there is insufficient evidence to attribute this to plant physiology or different "external" parameters such as soil type etc. This will be the focus of later studies, where plant types will be grown under identical conditions.

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