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EXPERIMENTAL PAPER

Interrelationships among selected essential elements in medicinal plant raw materials and their water-extractable forms

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S u m m a r y

In the study, the relationships were investigated among N, P, Fe, Zn, Mn and Cu, in medicinal plant raw materials (herbal bags) and their water-soluble chemical forms in infuses. 42 independent samples of plant materials were chosen, represented by different morphological plant parts as herbs, leaves, flowers and fruits. The elements were determined by UV/Vis spectrometry (non-metals), and FAAS (metallic elements) after previous mineralization of plant samples (total concentrations), and directly in herbal teas (water-extractable forms). Most frequently the correlations between non-metals and Zn and Mn occurred, both between their total contents the water-extractable forms. Statistically significant correlations were also found in pairs: Zn-Mn, Fe-Zn, Mn-Fe, and Cu-Zn. Application of multivariate analysis revealed that cluster analysis grouped the studied samples into clusters with similar levels of the analyzed elements, and principal component analysis allowed the identification of water-extractable Zn, $P\text{-}PO_4$ and water-extractable Cu as the most crucial factors determining the differentiation of the studied plant samples.

Key words: *inter-elemental relationships, essential elements, plantae ad ptisanam, infuses, statistical analysis*

INTRODUCTION

It is well known that medicinal plants are consumed worldwide as home remedies, and they constitute raw materials for herbal industry. The World Health

Organization has estimated that about 80% of global population relies mainly on traditional herbal medicine, and still about 50% of all drug preparations in highly developed countries are of plant origin [1, 2]. Herbs play a significant role for human health, preventing organism from diseases correlated with civilization stress and they are considered as being alternative for overmedication with synthetic drugs [3, 4]. One of the most popular ways to prepare the medicinal herbs at home is making tea or preparation of aqueous extracts (infuses). Infuses obtained in a such a way can be treated as "herbal cocktails" containing all organic chemical compounds and macro- and microelements soluble in hot water. These compounds, with biological activity on living organism, can be stimulated by specific elements, which can enhance or decrease their action, having therefore synergistic or antagonistic interaction with each other. Moreover, accumulation of individual elements in medicinal plants can have influence on the synthesis of physiologically active substances and sometimes can be correlated with their pharmacological properties, too [5].

Interactions between elements in plants have been widely described by Kabata-Pendias and Pendias [6], however there is a rather limited number of reports on this subject in relation to medicinal plants. In our several previous studies it was found that phosphorus and nitrogen are often correlated one to another, both their total levels in different plant parts are used medicinally, as well as in aqueous extracts (herbal teas) obtained from them [7-9]. Also Gallaher and Gallaher [10] reported several statistically significant relationships among total concentrations of essential elements in dry tea material and in herbal tea infusions. In another study, Kalavrouziotis and Koukoulakis [11] have found total 588 interactions between essential elements in roots, leaves and sprouts of Brussel sprouts plants, of which 564 were statistically significant. Also Kumar et al. [3] have analyzed the level and interactions of essential elements in herbal drink, *Pragya-peya* used in India. They discovered a strong correlation between the concentration of two essential metals, Fe and Zn, as well as between Fe and Co. Choudry et al. [12] have studied the interactions of elements in medicinal herbs used in the treatment of diabetes, and found a statistically significant relationship between Cu and Zn. It can be proven that between essential elements synergistic interaction occurs, which can be explained by the participation of these metals in the same biochemical processes, mainly in activation of enzymes important for plant's metabolism.

Keeping all abovementioned in mind, the aim of the investigation is focused on finding the relationships among selected essential elements (N, P, Fe, Zn, Mn, Cu) in raw plant materials and in herbal teas obtained from them. This studies are preliminary prior to further research in future, which will lead to discover interrelationships among essential elements and secondary metabolites in medicinal plants. This aspect of the investigation can be important for human health, since the water-extractable species of essential elements introduced in a form of herbal tea into digestive system can potentially influence the homeostasis of a human organism.

MATERIALS AND METHODS

Plant material

The plant material (42 samples) in a form of herbal tea bags originating from Polish suppliers "Kawon Hurt" and "Herbapol". The medicinal plants were cultivated or collected according to Good Manufacturing Practice (GMP) and ISO 9001 norm. Before the analysis, plant samples were ground using the *Knifetec* (Foss-Tecator, Denmark) sample mill and kept in plastic containers. The samples are listed below:

Herbs*: Euphrasiae* (samples 1 and 2), *Millefolii* (samples 3 and 4), *Equiseti* (samples 5-7), *Hyperici* (8-10), *Violae tricoloris* (11-12); leaves*: Salviae* (samples 13-16), *Sennae* (samples 17-19), *Urticae* (samples 20-21); *Inflorescentia: Tiliae* (samples 22-25), *Crataegi* (26-28); flowers*: Sambuci* (samples 29 and 30); *Calendulae* (samples 31-32), *Chamomillae* (samples 33-36); fruits*: Anisi* (samples 37-38), *Crataegi* (samples 39-40) and *Foeniculi* (samples 41 and 42).

Microwave digestion

The microwave digestion procedure was applied for preparation of samples before determining the total concentrations of P, Fe, Zn, Mn and Cu. Microwave digestion of accurately weighed plant samples (about 1.0 g) was performed by the use of mixture of 30% H_2O_2 (p. a., POChem, Poland) and concentrated 65% HNO_3 (Selectipur, Merck, Germany) (3:5, *v/v*). Digestion was done in the *Uniclever BM-1z* (Plazmatronika, Poland) device, applying the temperature of 250 to 350ºC and pressure from 31 to 45 atm. After this process, the samples were transferred to 50 ml volumetric flasks and diluted with the twice distilled water obtained from the quarz-glass system (Heraeus, Switzerland).

Wet digestion

For total nitrogen determination, wet digestion procedure was applied. The accurately weighed plant sample (about 0.5 g) was set in a Kjeldahl flask, next 5 ml of 30% H_2O_2 solution and 10 ml of concentrated H_2SO_4 solution (pure for analysis, both form POChem, Poland) were added. The sample was digested until the solution became clear. Subsequently, it was transferred to the volumetric flask, and the volume of 100 ml was made up with twice distilled water.

Extraction

Twice distilled boiling water was used for the extraction. To the accurately weighed plant sample (one tea bag) in a glass beaker (from 1.0 to 2.0 g), 100 ml of boiling water was added, then it was covered by a glass plate and kept under cover for 15 min. After this time the tea bag was removed from the beaker and the obtained aqueous extract was transferred into the volumetric flask and diluted to 100 ml with the twice distilled water.

Determination

For the determination of total and extractable P, phospho-molybdenum blue method was used. For total nitrogen the method based on Nessler reaction and for $N\text{-}NO_3$ the nitration reaction were applied. Absorbance was measured by use of the UV/Vis spectrophotometer SP 870 (Metertek, South Korea).

The concentrations of total and water-extractable forms of Fe, Zn, Mn and Cu were determined by flame atomic absorption spectrometry using 250 Plus AA spectrometer (Varian, Australia). The standard conditions in air/acetylene flame were used, applying following analytical wavelengths for the determination of Fe (248.3 nm), Zn (213.9 nm), Mn (279.5 nm) and Cu (324.8 nm).

Precision and accuracy of applied methods was checked by use of certified reference materials, Mixed Polish Herbs (INCT-MPH-2) for P, Fe, Zn, Mn and Cu, and tomato leaves (NIST SRM 1573a) for N, as presented in table 1. It was shown that precision and accuracy of the applied analytical procedures are satisfactory, taking into consideration that the samples of medicinal plant raw materials are characterized by complex organic matrix.

Table 1.

Accuracy and precision of the applied analytical methods

Element (total concentration)	Nitrogen	Phosphorus	Iron	Zinc	Manganese	Copper
Declared contents [mg/kg d.m.]	29.20*	2.50*	460.00	33.50	191.00	7.77
Determined contents ± SD [mg/kg d.m.]	23.75±3.21*	2.16±0.06*	360.00±4.41	37.72±1.39	219.04±8.56	7.59±0.22
Accuracy as recovery [%]	81.34	86.40	78.26	112.60	117.49	97.68
Precision as CV [%]	13.51	2.78	1.23	3.69	3.81	2.90

d.m. – dry mass, * [mg/g d.m.], CV – coefficient of variation

Statistical analysis

Statistical calculations (correlation analysis, cluster analysis (CA) and principal component analysis (PCA) were done by the Statistica 7.1 (Statsoft, Poland) software.

RESULTS AND DISCUSSION

Total and water-extractable forms of elements

The results presented in table 2 show total level of N, P, Fe, Zn, Mn and Cu in herbal tea bags and their concentration in aqueous extracts obtained from them. The macro-elements were found in the range of concentration from several to several tens of mg/g of dry mass (d.m.), both in total N and of total P. The average level of total P was statistically significantly lower than the contents of total N in all samples, which confirms the data obtained in previous studies [7-9]. The hot water extracted yield was different depending on the element studied, and on the analyzed morphological part of a plant, as shown in figure 1. Analyzing the extraction yield of nitrate nitrogen to total concentration of this non-metal, from 1.7% of total N in fruits to 7.5% of total N in herbs was found. As for the water-extractable inorganic form of phosphorus, this form was determined in the range from 20% of total P in fruits to 68% of total P in herbs.

Table 2.

Content of N, P [mg/g d.m.], Fe, Zn, Mn and Cu [mg/kg d.m.] in the herbal bags and herbal teas (the range; the mean concentration ±SD)

Element	Herbs (*n = 12*)		Leaves (*n = 9*)		Flowers (*n = 15*)		Fruits (*n = 6*)	
	T	E	T	E	T	E	T	E
N	10.77–18.10 13.78±2.10	0.50–2.05 1.03±0.51	14.75–30.43 19.79±6.09	0.47–2.09 1.18±0.55	8.97–25.74 15.88±5.10	0.19–2.44 0.89±0.65	3.62–28.52 16.96±10.12	0.21–0.38 0.28±0.06
P	1.79–3.56 2.40±0.51	0.98–2.42 1.64±0.46	0.73–4.58 2.23±1.31	0.17–1.46 0.90±0.45	1.93–4.50 2.99±0.87	0.82–3.57 1.31±0.72	1.16–4.01 2.73±1.18	0.33–0.82 0.55±0.17
Fe	52.97–352.71 153.61±81.05	3.38–15.30 7.55±3.80	74.23–408.64 201.40±116.31	3.03–9.93 6.18±2.22	44.94–373.46 131.43±86.17	1.78–13.35 5.98±3.85	27.49–127.56 63.81±34.58	1.80–31.39 7.43±11.76
Zn	33.99–85.36 52.81±16.75	4.73–28.65 16.66±9.19	14.97–62.53 32.57±15.95	4.90–26.75 13.10±8.06	28.18–63.01 46.31±11.59	2.37–49.35 13.34±14.81	14.72–54.49 36.12±15.97	2.68–7.93 5.18±2.08
Mn	38.54–243.01 133.36±69.15	16.00–92.05 49.50±29.40	28.49–171.97 69.80±47.06	7.54–91.11 24.75±27.13	26.79–231.22 89.98±67.13	2.99–49.00 17.41±12.23	7.41–37.84; 20.59±10.88	2.53–14.83 4.17±5.81
Cu	5.30–27.30 10.60±6.30	0.75–5.93 3.20±2.37	4.79–11.29 7.44±1.91	0.50–2.33 0.87±0.99	8.44–14.77 10.49±1.71	0.33–13.00 2.63±3.58	5.48–56.85 17.93±19.46	0.25–23.93 4.39±9.61

T – total contents, E – water-extractable form, n – number of samples

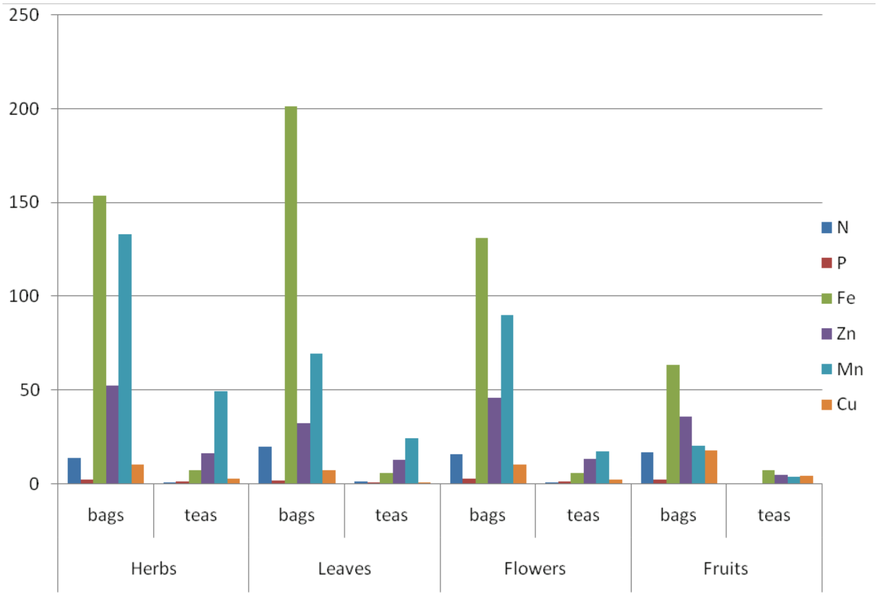

Figure 1

Results of total contents and water-extractable levels of N, P [mg/g d.m.] and Fe, Zn, Mn and Cu [mg/kg d.m.]

Analyzing the results of microelements' determination shown also in table 2, it can be noticed that the highest average total level of elements in the case of Fe was found, next of Mn, Zn and the lowest of Cu. The range of concentration in mg/kg d.m. of these metallic elements is comparable to those determined by other researchers in medicinal herbs [2-4, 12]. However, the extracted yield is different, depending on the microelement analyzed. The lowest extraction yield was obtained in the case of water-extractable Fe, ranging from 3% of total Fe in leaves to 11% of total Fe in fruits. Water-extractable fractions of Mn and Zn were determined in the ranges from 19% of total Mn in flowers to 37% of total Mn in herbs, and from 14% of total Zn in fruits to 40% of total Zn in leaves. The water-extractable form of Cu was found in the range from 12% of total Cu in leaves to 30% of total Cu in herbs. The low extraction yield in Fe present in medicinal plants has been reported previously [13, 14] and this can prove the major part of Fe in medicinal plant material is bonded organically in compounds non-soluble in water. The results obtained in this study concerning total and water-extractable level of Fe, Mn, Zn and Cu point out, that the level of Cu is the lowest one, both in dry samples of medicinal plant material and in aqueous extracts. Similar results were obtained by other researchers [4, 15-18].

Interactions of elements

In order to detect the interrelationships among total and water-extractable forms of N, P, Fe, Zn, Mn and Cu, a correlation analysis was applied [19]. The results of calculations are shown in table 3. Among 66 pairs of elements, 22 were statistically significant. As it is known from the literature [5], between elements synergistic or antagonistic interaction are possible in plants. In the study, only positive statistically significant relationships were obtained, and some of them are strong, with the correlation coefficient higher than 0.7, the other relations were lower. In two cases, between Mn total and water-extractable Mn, as well as between Cu total and water-extractable Cu, the correlation was strong and the correlation coefficients calculated for these pairs were equal or higher than 0.8, respectively. Also quite high correlation occurred between total N and total P, which is justified by their synergistic participation in the biosynthesis of important for plants metabolites, as reported in the literature [20].

Table 3.

Results of correlation analysis

	N total	N-NO_3	P total	P-PO_4	Fe total	Fe extractable	Zn total	Zn extractable	Mn total	Mn extractable	Cu total
N total	1										
N–NO_3	**0.42**	1									
P total	**0.62**	0.25	1								
P-PO_4	–0.22	0.16	0.23	1							
Fe total	–0.03	0.20	–0.24	0.01	1						
Fe extr.	–0.11	0.13	–0.06	0.21	0.18	1					
Zn total	0.19	0.12	**0.43**	**0.50**	0.13	0.08	1				
Zn extr.	0.26	**0.39**	**0.46**	**0.68**	0.06	**0.33**	**0,52**	1			
Mn total	–0.18	0.19	0.04	**0.32**	**0.45**	0.15	**0,56**	0.27	1		
Mn extr.	–0.20	**0.31**	–0.20	**0.34**	**0.48**	0.24	**0,49**	**0.40**	**0.80**	1	
Cu total	0.18	–0.19	0.26	0.01	–0.14	–0.18	0,26	0.07	0.03	0.03	1
Cu extr.	0.12	0.03	**0.35**	**0.35**	–0.10	–0.01	0,29	**0.40**	0.06	0.13	**0.83**

Correlation coefficients statistically significant ($\alpha < 0,05$) are in bold font

Interesting interaction between water-extractable Zn and inorganic phosphate phosphorus was obtained, since the correlation coefficient for this pair equaled to 0.68. This suggests a synergistic interaction between this metal and phosphate phosphorus in metabolic transitions in plant organism, probably activated by Zn of some enzymes. Phosphate phosphorus is also positively correlated with total Zn, total Mn and water-extractable Mn as well as with water-extractable Cu.

However, the correlation coefficients are low and do not exceed 0.7. The literature data [21] confirm the interaction between P and metals, since these elements are involved in their metabolic processes. Analyzing the relationships among metals, the statistically significant correlations between Zn and Mn, Fe and Zn, Mn and Fe as well as Cu and Zn were found, which indicates their synergistic interactions in plant metabolism.

Statistical analysis of the data

In order to find similarities and dissimilarities between the analyzed samples of herbal teas, and to group them in categories with common properties, cluster analysis (CA) [19] was applied for the obtained data. For CA, the Word's method and the Euclidean distance for distance calculation, were used. As shown in figure 2, the samples of herbal teas with similar contents of elements studied were clustered together. In general, the studied material can be divided into two major classes designed by Roman numerals in figure 2. First cluster, in the upper area of the plot, contains all samples with high water-extractable Zn content, and with relatively high water-extractable P-PO_4 level. Among them are the samples of *Herba Millefolii* (numbered 3 and 4), *Violae* (numbered 11 and 12), *Hyperici* (8-10), *Flos Sambuci* (31 and 32), and also *Flos Calendulae* (31 and 32) and many others. The second large cluster in the lower area of the dendrogram, contains in general the samples of herbal teas with low level of total and water-extractable Cu. As examples of plant materials, which can be located in this cluster, the samples of *Herba Equiseti* (5-7), *Folium Sennae* (17-19), *Fructus Anisi* (37-38), can serve.

Principal component analysis (PCA) reduces the number of experimental variables into 2-3 new variables, principal components with the highest eigenvalues [19], as shown in table 4. PCA allows also to present the distribution of the analyzed samples in two-dimensional plot (fig. 3). Characteristic samples are circumscribed by lines. Therefore, it is possible to find in the left area of the scatterplot the samples of *Flos Calendulae* (31 and 32) with high P-PO_4, water-extractable Zn and Cu, also the group containing three samples of *Herba Hyperici* (8–10) and *Herba Euphrasiae* (1 and 2) in the central area, characterized by high concentration of water-extractable Zn and Mn. In the upper part of the plot there are the samples of *Herba Violae* (11 and 12), in which high amounts of N-NO_3 and water-extractable Mn were determined. Two samples of *Herba Millefolii* (numbered 3 and 4), can also be located in the central zone of the plot. On the other hand, one of two samples of *Fructus Foeniculi* (41) is located in the right down corner of the plot, which is caused with very high level of water-extractable Cu in it. Also one of two samples of *Fructus Crataegi* (40) is characteristically located in the upper area of the plot and contains the highest level of water-extractable Fe in comparison with the other plant samples.

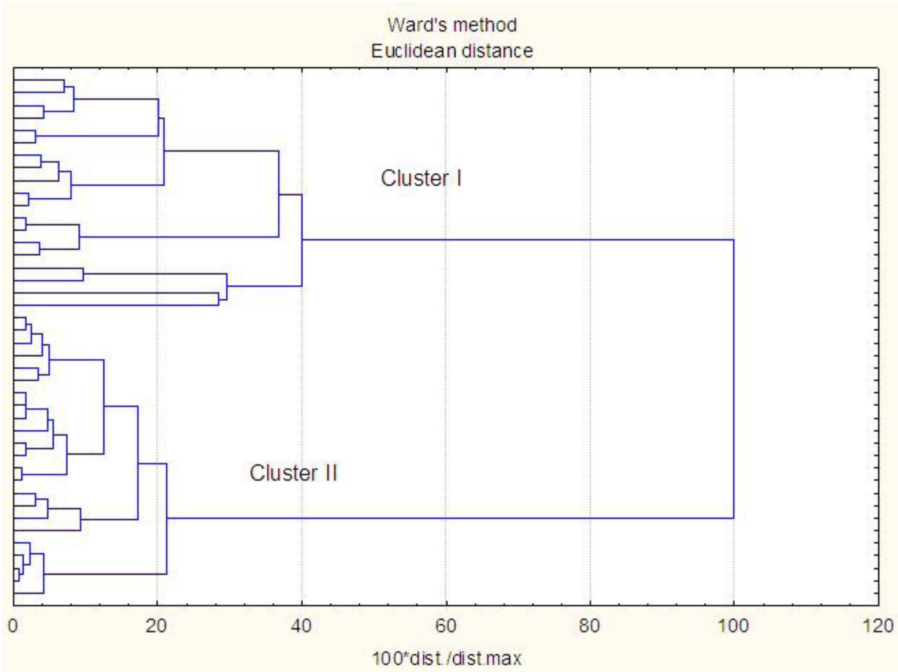

Figure 2.

Dendrogram obtained after use of cluster analysis for the studied herbal tea samples

Table 4.

PCA results for the studied samples

Principal component	Eigenvalue	Variance [%]	Cumulated variance [%]
PC 1	2.49	41.5	41.5
PC 2	1.11	18.5	60.0
PC 3	0.88	14.7	74.7
PC 4	0.66	11.1	85.8
PC 5	0.59	9.9	95.7
PC 6	0.25	4.3	100.0

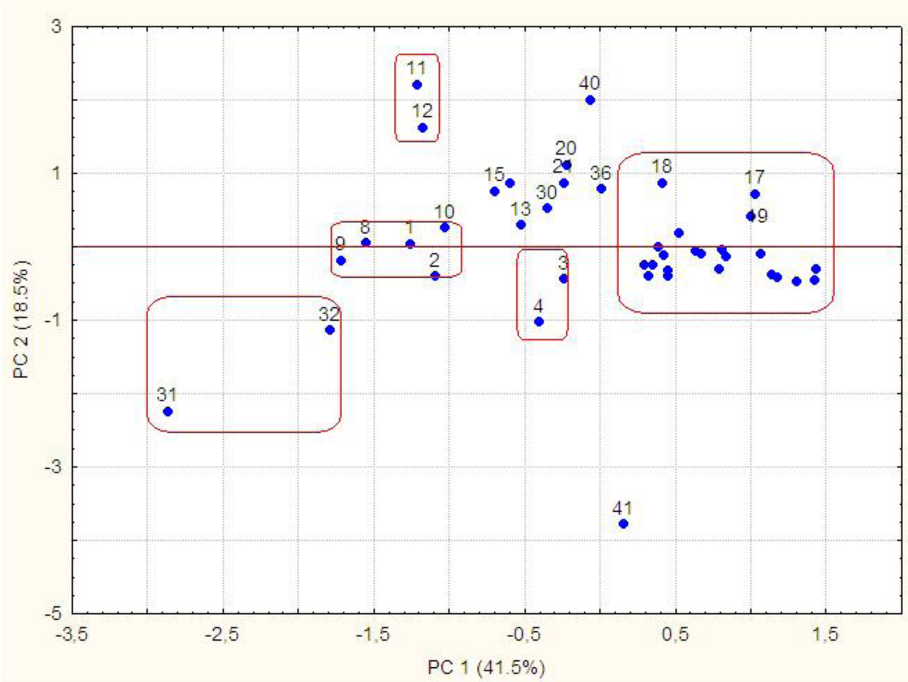

Figure 3.

Scatterplot of the studied herbal tea samples as two-dimensional plot of PC1 vs PC2

The results of PCA confirmed those obtained by the use of CA, indicating the same characteristic samples or groups of samples. Also, PCA helped in the identification the concentrations of water-extractable Zn and P-PO_4, correlated to PC1, and that of water-extractable Cu, correlated to PC2, as the most crucial factors determining the differentiation of the studied plant material, as shown in figure 4.

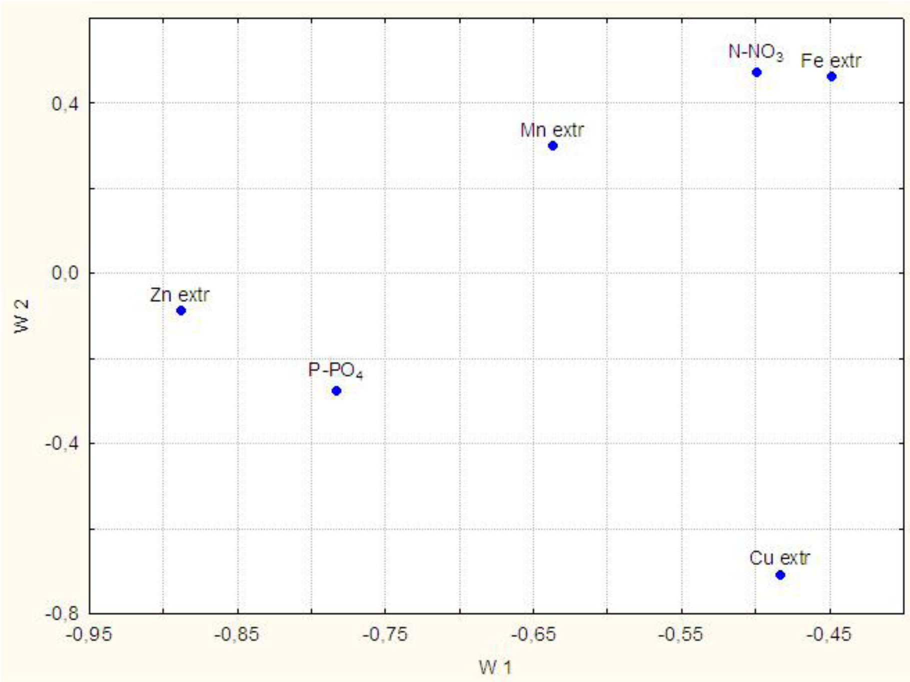

Figure 4.

Loadings of first two PC obtained for the studied herbal tea samples

CONCLUSIONS

By the use of correlation analysis, detection of crucial interrelationships among the essential elements in studied raw plant materials (herbal bags) obtained from different morphological parts of medicinal plants, and their species present in the aqueous extracts (herbal teas) became possible. The most typical examples of these relationships were identified by high correlation coefficients between N, P and their water-extractable forms. Several correlations between non-metals and Zn as well as Mn were also found. This may indicate that these elements take part in mutual biochemical reactions.

Moreover, the use of multivariate statistical techniques was helpful in the reduction of the multidimensionality in the experimental databases (PCA), and divided the samples into two clear clusters (CA). The application of PCA allowed also for the extraction of the information that water-extractable $P\text{-}PO_4$, Zn and Cu appeared to be the most crucial factors which determined the differentiation of the studied plant material.

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RELACJE POMIĘDZY WYBRANYMI PIERWIASTKAMI NIEZBĘDNYMI W ROŚLINNYCH SUROWCACH LECZNICZYCH A ICH FORMAMI EKSTRAHOWANYMI WODĄ

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S t r e s z c z e n i e

W trakcie badań określono relacje pomiędzy N, P, Fe, Zn, Mn i Cu w roślinnych surowcach leczniczych (torebkach) a ich rozpuszczalnymi w wodzie formami chemicznymi w naparach. Wybrano 42 niezależne próbki surowców roślinnych, reprezentowanych przez takie różne części morfologiczne roślin jak ziela, liście, kwiaty i owoce. Pierwiastki oznaczono spektrofotometrycznie w zakresie widzialnym (niemetale) i metodą FAAS (pierwiastki metaliczne) po uprzedniej mineralizacji próbek roślinnych (całkowita zawartość) oraz bezpośrednio w naparach (formy ekstrahowane wodą). Najczęściej występowały korelacje pomiędzy niemetalami a Zn i Mn, zarówno między całkowitą ich zawartością, jak i formami rozpuszczalnymi w wodzie. Statystycznie istotne korelacje stwierdzono również między parami: Zn-Mn, Fe-Zn, Mn-Fe i Cu-Zn. Zastosowanie analizy wieloparametrowej dostarczyło informacji o tym, że analiza skupień pogrupowała badane próbki w klastery o zbliżonym poziomie analizowanych pierwiastków, natomiast analiza głównych składowych pozwoliła na identyfikację Zn ekstrahowanego wodą, P-PO_4 oraz Cu ekstrahowanej wodą, jako najbardziej istotnych czynników wpływających na zróżnicowanie badanych próbek roślinnych.

Słowa kluczowe: *relacje między pierwiastkowe, pierwiastki niezbędne, zioła do zaparzania, napary, analiza statystyczna*