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Total phosphorus and its extractable form in plant drugs. Interrelation with selected micro- and macroelements

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Abstract

Determinations of total phosphorus, its extractable inorganic form and selected essential elements (Ca, Mg, Na, K, Fe, Zn, Mn, Cu) in 27 leaves of medicinal plants supplied from the Polish herbal enterprise – Herbapol, were carried out. After the microwave digestion of plant samples, the total phosphorus was determined spectrophotometrically, using the phosphomolybdenum blue method, whereas contents of metals were established by flame atomic absorption spectrometry (FAAS). Extraction with 2% (v/v) acetic acid solution was done in order to separate the extractable inorganic fraction of phosphorus, which was evaluated by the same method as used for the total elements. The macroelements (P, Ca, Mg, Na, K) were determined in a range of concentration from several hundreds of mg/kg to thousands of mg/kg of dry plant tissue. Microelements (Fe, Zn, Mn, Cu) were found in a range up to several hundreds of mg/kg of dry plant weight. The average level of the inorganic fraction of phosphorus represented 63.2% of the total concentration of that non-metal in leaves, which indicates that a large fraction of phosphorus may be bioavailable for people who often use herbal teas in their everyday diet. Statistically significant correlations between the total and extractable phosphorus and among metals (Ca–Mg, Ca–K, Ca–Fe, Mg–Fe, Cu–K, Cu–Zn, and Mn–Zn) were observed, confirming their indispensable role in activation of the same group of enzymes in medicinal plants. PCA revealed, that the levels of the total, extractable phosphorus and metals, may depend on the origin of the analyzed leaf sample from plants of the same plant species.

Keywords: Leaves of medicinal plants; Total phosphorus; Extractable inorganic phosphorus; Speciation; Essential metals

1. Introduction

The elemental composition of medicinal plants is now very often an object of study (Goldman, 2001; Gomez, Cerutti, Olsina, Silva, & Martinez, 2004). One reason for this is the need to monitor the level of elements, which have a potentially negative effect on human health, such as Hg, Pb or Cd (Mamani, Aleixo, de Abreu, & Rath, 2005). Also, the concentrations of so-called essential elements (Kumar, Nair, Reddy, & Garg, 2005), for example iron, magnesium, calcium, potassium, manganese, zinc, copper and phosphorus are analyzed. Because drugs of plant origin or herbal spices are now commonly used, they can be an additional source of macro- and microelements in the everyday diet. Therefore, in such a sense, certain plant drugs may be treated as food additives (Lemberkovics, Czinner, Szentmihalyi, Balazs, & Szoke, 2002).

The essential elements are involved in many metabolic processes in the human organism, especially as enzymes activators, e.g. Fe, Zn and Mn (Razic, Onjia, Dogo, Slavkovic, & Popovic, 2005). They can also interact with some organic compounds – for instance with flavonoids, introduced into the human organism, after using the plant drugs, influencing their biological activity (Weber, 1988). Taking this into consideration, not only the total level of the essential elements in herbs, but also their interactions with the plant's constituents should be studied.

It is important to recognize the potentially bioavailable fraction of the elements, taken orally by humans. Therefore, the concentration of water-soluble fraction, of the element, present in a plant drug, should be determined. This

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problem can be studied by employing speciation analysis (Apostoli, 1999; Szpunar & Lobinski, 2002; Templeton, 1999) of the essential elements in plant drugs, or by analysis of the water-extractable chemical form of the studied element (Lozak, Soltyk, Ostapczuk, & Fijalek, 2002). This approach was lately applied for studying the bioavailable chemical forms or species of iron, manganese and zinc in medicinal plants and drugs prepared from them, in relation to the plant species by investigation the influence of the solvent, its pH and lipophilicity of the drug form (Li & Deng, 2003; Li, Deng, & Zheng, 2004). In another study, speciation analysis of selected metals in water extracts of medicinal plants revealed that Mg and Mn are probably bound to carbohydrates: however, Zn can interact with polyphenolic compounds (Weber & Konieczynski, 2003).

The recognition of the inter-elemental relations between metals and non-metals is also worth investigation. Especially is it important in the case of phosphorus present as the phosphate group, which can render some metals insoluble, Fe or Zn, making these elements unavailable for humans (Duhan, Khetarpaul, & Bishnoi, 2002). Phosphorus has so far been an object of several studies, especially focussed on its bioavailable form, in plants used in agriculture. These studies were done in order to find a correlation between the extractable fraction of phosphorus, mainly inorganic phosphorus, and correct plant growth and development (Bollons & Barraclough, 1997; Sun, Simpson, & Sands, 1992). Phosphorus contained in the plant tissues occurs mainly in the form of inorganic compounds, such as ortho- and pyrophosphates, and in organic forms, especially in phospholipids, sugar-phosphates and nucleoproteins. In most cases organic phosphorus is present in plant cells in the form of a phosphate group (Adelantado, Reig, Garcia, & Martinez, 1983). Extraction with 2% acetic acid allows separation of the water-soluble fraction of phosphorus, which is considered as the inorganic fraction of that element (Bollons & Barraclough, 1997; Sun et al., 1992). Therefore, the aim of the studies was to establish the relationship of the extractable inorganic phosphorus concentration to the total level of that essential element in medicinal plants, as well as to find the relationship between phosphorus and essential metallic (Ca, Mg, Na, K, Fe, Zn, Mn, Cu) elements. It would be interesting to find, whether the water-extractable inorganic phosphorus in plant drugs can be treated as the potentially bioavailable source of that element for humans.

2. Materials and methods

2.1. Plant material

The set of 27 dried leaf samples of medicinal plants was supplied by the Polish herbal enterprise – Herbapol. They are as follows (plant species and sample number is given in brackets): *Folium Betulae*, (Betula species Ehrh., 1), *Folium Farfarae*, (Tussilago farfara L., 2, 3, 4, 5), *Folium Fragariae*, (Fragaria vesca L., 6), *Folium Lauri*, (Laurus nobilis L., 7), *Folium Melissae*, (Melissa officinalis L., 8), *Folium Menthae piperitae*, (Mentha piperita L., 9, 10, 11), *Folium Menyanthidis*,(Menyanthes trifoliata L., 12, 13), *Folium Plantaginis lanc.*, (Plantago lanceolata L., 14), *Folium Ribis nigri*, (Rubus nigrum L., 15, 16, 17), *Folium Rosmarini*, (Rosmarinus officinalis L., 18, 19, 20), *Folium Rubi fruticosi*, (Rubus fruticosus L., 21, 22), *Folium Rubi idaei*, (Rubus idaeus L., 23), *Folium Salviae*, (Salvia officinalis L., 24, 25), *Folium Sennae*, (Cassia senna L., 26), and *Folium Uvae-ursi*, (Arctostaphyllos uva-ursi L., 27).

The plant samples were ground using a Knifetec sample mill (Foss-Tecator, Denmark), and were kept in polyethylene containers prior to analysis.

2.2. Digestion procedure

Microwave digestion of plant samples was done with the use of the mixture H_2O_2/HNO_3 (3:5, v/v) (both from Merck, Germany). Mineralization was performed in the Uniclever BM-1z (Plazmatronika, Poland) unit. After this process, the samples were transferred to 50 ml volumetric flasks and diluted with the bidistilled water obtained from the quartz–glass system (Heraeus, Switzerland).

2.3. Extraction procedure

A solution of acetic acid (2% (v/v), POCh, Poland) was used for extraction. To the accurately weighed amount of plant sample (about 1 g), 30 ml of the solution was added, then stirred on an electromagnetic stirrer for 30 min at room temperature (20 °C) and filtered through a filter paper with medium-sized pores (Filtrak, Germany). The filtrate was collected in a volumetric flask and diluted to 50 ml with bidistilled water.

2.4. Determination of total and extractable phosphorus

The procedure of total and extractable phosphorus determination was based on the spectrophotometric phosphomolybdenum blue method (Marczenko & Balcerzak, 1998). The external calibration graph method was used. The appropriate volume of mineralized sample was put into a 25 ml volumetric flask: next, 10 ml of mixed reagent containing ammonium molybdate and ferrous sulphate (both from POCh, Poland) were added and the absorbance was measured using a UV/ViS spectrophotometer SP 870 (Metertek, South Korea) at $\lambda = 650$ nm and cell path length = 1 cm.

The accuracy and precision of the used method were checked by determination of total phosphorus in the Polish Certified Reference Material-Oriental Tobacco Leaves 1 (CTA-OTL-1). The results are shown in Table 1.

2.5. Determination of metals

The concentrations of Ca, Mg, Na, K, Fe, Zn, Mn, Cu were determined by FAAS using a 250 Plus Atomic

Table 1
The accuracy and precision of phosphorus and metal determination procedures; the result are the arithmetic means of six measurements ($n = 6$)

Element	Certified reference material	Certified value (mg/kg)	Determined value (mg/kg)	Recovery (%)	Relative standard deviation (%)
P	IC-CTA-OTL-1	2892 ± 134	2810 ± 60	97.2	2.2
Ca	INCT-MPH-2	10,800 ± 700	11,210 ± 953	104	8.5
Mg	INCT-MPH-2	2920 ± 180	2720 ± 49	93.2	1.8
K	INCT-MPH-2	19,100 ± 1200	22,200 ± 530	116	2.4
Na	INCT-MPH-2	350[a]	306 ± 38	87.6	12.4
Fe	IC-CTA-VTL-2	1083 ± 33	997.0 ± 50	92.1	5.0
Mn	IC-CTA-VTL-2	79.7 ± 2.6	67.7 ± 2.5	84.9	3.7
Zn	INCT-MPH-2	33.5 ± 2.1	32.0 ± 0.5	95.6	1.5
Cu	IC-CTA-VTL-2	18.2 ± 0.9	17.5 ± 1.6	96.1	9.0

[a] "Information" value.

Absorption Spectrometer (Varian, Australia). The standard analytical parameters (air/acetylene flame) were used and analytical wavelengths in nm, were as follows: Ca, 422.7; Mg, 285.2; Na, 589.0, K, 766.5; Fe, 248.3; Zn, 213.9; Mn, 279.5 and Cu, 324.8.

The accuracy and precision of the metal determination procedures were checked by use of the certified reference materials: INCT-MPH-2 (Mixed Polish Herbs) and IC-CTA-VTL-2 (Virginia Tobacco Leaves), both produced and certified by the Institute of Nuclear Chemistry and Technology, Warsaw, Poland. The results are shown in Table 1.

2.6. Calculations

Correlation analysis and principal component analysis (PCA) were done by using *Statistica* (StatSoft, Poland) software.

3. Results and discussion

3.1. Total and extractable phosphorus

The concentration range, in which the total phosphorus was determined, is from 850 mg/kg of plant dry weight (d. wt.) in one sample of *Folium Rosmarini* (sample number 19), to 4950 mg/kg d. wt. in the sample of *Folium Ribis nigri* (16). Analyzing the content of total phosphorus in the leaves of medicinal plants originating from the same plant species, it is possible to notice some similarities. For example, in all leaves of *Folium Farfarae* (samples numbered 2–5) the total phosphorus was determined to be from 2360 to 3330 mg/kg d. wt.; also, in *Folium Menthae piperitae* (9–11), total amount of the analyzed element was at a similar level from 2810 to 3260 mg/kg d. wt. It is worth noting that, among four samples of *Folium Farfarae*, one represented the leaves of a plant collected in southern Poland (sample numbered 5), and the other three samples were taken from the plants growing in the north part of the country. However, the average concentration of the total phosphorus, detected in the sample numbered 5, remains almost at the same level as that determined in the other leaves of that plant. There are other samples, e.g. *Folium Rosmarini* (18–20) or *Folium Salviae* (24, 25), where a similar concentration of total and extractable phosphorus, occurs. In general, the concentration of total phosphorus in the analyzed plant material is in agreement with the level of that non-metal, reported by others (Kumar et al., 2005; Lemberkovics et al., 2002) confirming fact, that it depends, to a great extent, on the plant species.

The level of the extractable fraction of phosphorus was determined to be in the range from 650 mg/kg d. wt. in the sample of *Folium Rosmarini* (19) to 2770 mg/kg d. wt. in *Folium Ribis nigri* (17). As shown in Fig. 1, the correlation coefficient $r = 0.75$ is statistically significant ($p < 0.05$), and indicates that the pool of inorganic phosphorus is related to the total concentration of that element in a plant.

Taking, as an example, the high total concentration of phosphorus determined in three samples of *Folium Ribis nigri* (15–17), from 3420 to 4950 mg/kg of dry plant tissue, it can be noticed in Fig. 1, that inorganic phosphorus also in these samples, from 2560 to 2770 mg/kg d. wt., was at a high level in comparison with the other leaves of medicinal plants. On the other hand, there are samples of leaves with very low concentrations of total phosphorus, for instance three samples of *Folium Rosmarini* (18–20), where total P was detected from 850 to 1110 mg/kg d. wt., and in which extractable phosphorus was also determined at a low level, from 650 to 790 mg/kg d. wt.

Analyzing the percent share of extractable phosphorus in the total amount of that non-metal in leaves, it is noticeable that this fraction covers a relatively wide range of values, from less than 30% to more than 80% of the total concentration of the analyzed element. These results are in accordance with reports of the extractable fraction of phosphorus in plant leaves (Bollons & Barraclough, 1997; Sun et al., 1992). It can also be concluded that, for most of the analyzed leaves, the inorganic extractable fraction of phosphorus in certain samples is high, indicating destruction of organic compounds of that element during drying and long-term storage of plant materials. Therefore, it can be concluded, that the pool of inorganic phosphorus present in the water extract, obtained with the use of 2% (v/v) acetic acid solution, may be an additional source of bioavailable phosphorus to humans, who often use the leaves of medicinal plants as natural drugs in their everyday therapy.

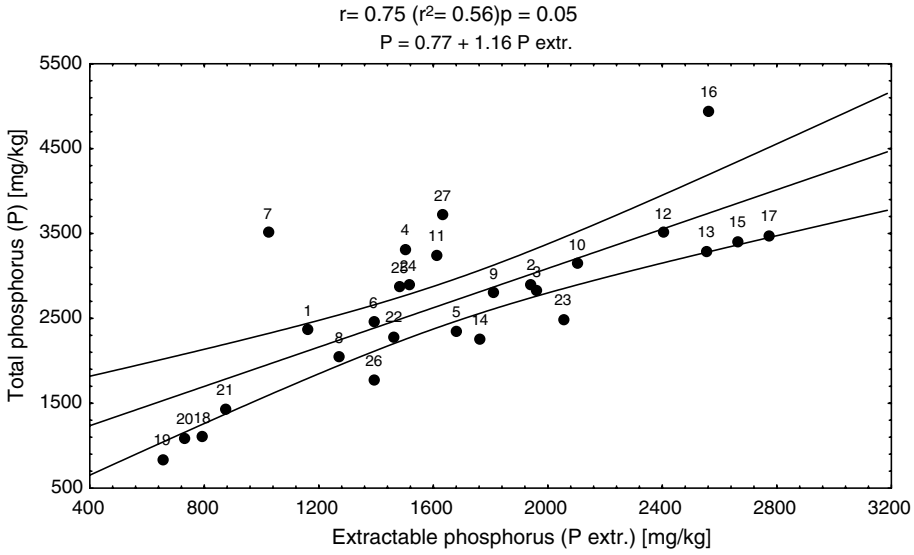

Fig. 1. Relationship of extractable to total phosphorus in the leaves of medicinal plants.

3.2. Metallic elements

The results of the determination of metallic bioelements, compiled in Table 2, indicate that they can be divided into two main groups, the first one including macroelements (Ca, Mg, Na and K) and the second essential microelements (Fe, Zn, Mn and Cu). There are some characteristic plant materials worth comparative analysis. For instance, in *Folium Betulae* (sample number 1) a high amount of Zn was found, almost equal to 130 mg/kg of d. wt. whereas, in other leaves, the content of that essential metal did not exceed 100 mg/kg d. wt. Taking into account the concentration of iron, the plant material extremely rich in that element appeared to be *Folium Sennae* (sample number 26), which had more than 170 mg/kg d. wt. The concentration of iron in other leaves was less than 50 mg/kg d. wt. The plant materials mentioned above may be treated as additional sources of these two essential metals in the diet of humans, who often use *Folium Betulae* and *Sennae* as herbal teas.

Taking into consideration the contents of all essential metallic elements, it is possible to notice, that plant materials rich in metals are *Folium Farfarae* (2–5), *Sennae* (26) and *Salviae* (24, 25). In the latter plant material, the highest concentration of Ca of all samples, was determined, almost 31,000 mg/kg d. wt. The same sample has the highest level of iron. Also, *Folium Betulae* (1) is characterized by high contents of Zn and Mn, but by average levels of the other metallic elements. In all sets of analyzed leaves of medicinal plants, it is not easy to unambiguously identify plant samples with a low concentration of a given element. The reason for this is the fact, that most of the samples with low amounts of microelements, have high levels of macroelements. Examples, which confirm that conclusion, are the samples of *Folium Ribis nigri* (15–17) or *Salviae* (24, 25), which are characterized by low levels of Zn, Cu and Mn, but high contents of Ca, Mg and Fe.

After elimination of the sample of *Folium Sennae* (sample number 26) as the result of the Q-test, from the analyzed material, the mean and the median were again calculated, as shown in Table 2. This sample was removed because the very high concentrations of Ca and Fe made the statistical evaluation of the data inaccurate, significantly changing the values of the mean concentration. In

Table 2
Results of the element determinations in leaves of medicinal plants

Element	Range ($n = 27$)	Mean ($n = 27$)	Median ($n = 27$)	Mean ($n = 26$)	Median ($n = 26$)
P	850–4950	2690	2830	2720	2860
P–PO_4	650–2770	1650	1610	1670	1620
Ca	3340–30,950	12,470	11,120	11,760	11,040
Mg	830–5260	2460	2570	2350	2530
K	2220–51,440	16,700	13,010	15,360	12,150
Na	6.31–4030	522	131	153[a]	111[a]
Fe	3.73–174	27.2	19.9	21.5	19.8
Mn	4.98–67.21	23.1	18.3	22.4	18.0
Zn	0.67–129.47	28.1	23.8	24.2	23.7
Cu	0.49–10.59	3.22	2.44	3.16	2.41

Concentrations of elements are in mg/kg dry weight.
[a] $n = 23$.

the case of Na, more samples were discarded, because of the very high concentration of that element in *Folium Menyanthidis*, (samples numbered 12 and 13), *Plantaginis lanceolatae* (14), and *Folium Sennae* (26). Fig. 2 also illustrates the statistical evaluation of the macro- and microelement determinations in the analyzed plant material.

As shown in Table 3, statistically significant correlations were found between several metals, but no correlation was determined between total phosphorus or extractable phosphate phosphorus and metallic elements. It is known from the literature, that significant correlation exists between Fe, Zn and Co in medicinal plants (Kumar et al., 2005). This can be explained by the fact that these elements are enzyme activators. Also, correlations between the following pairs of metals: Ca–Mg, Cu–Mg, Cu–K and Zn–K, in several herbal drugs, was recently reported (Razic et al., 2005). Therefore, our results on inter-element relationships confirm these observations in herbal drugs.

3.3. Principal component analysis of the results

In order to extract the information on relationships between metals and phosphorus in the medicinal plant leaves, one of the pattern recognition methods, PCA, was used. This method allows separation of samples of leaves, characterized by similar elemental contents, as well as discovery of factors, which are responsible for the differentiation of samples. PCA was used previously (Razic et al., 2005; Wesolowski & Konieczynski, 2003) for such an interpretation of the thermoanalytical and chemical data for medicinal plants. In this analysis, an experimental matrix was created, based on the results of the determination of Ca, Mg, Na, K, Fe, Zn, Mn, Cu, P and P–PO_4 in 27 samples of leaves.

The results of the PCA calculation are shown in Table 4. Based on these data, the first two principal components were extracted, which were characterized by the eigenvalues above 2. As illustrated in Fig. 3, the distribution of samples in a two-dimensional plot of principal compo-

Table 4
The results of PCA calculations; principal components are numbered consecutively

Principal component	Eigenvalue	Variance (%)	Cumulated variance (%)
PC1	2.29	22.88	22.88
PC2	2.24	22.38	45.26
PC3	1.71	17.08	62.34
PC4	1.37	13.71	76.05

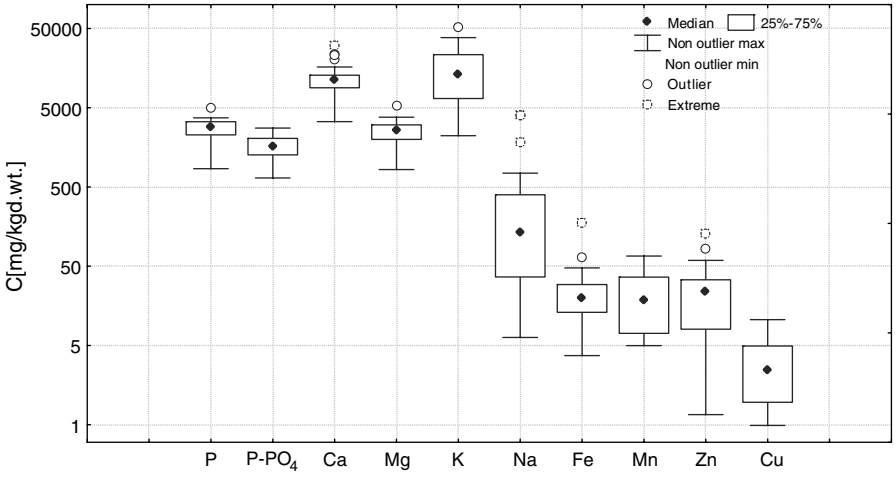

Fig. 2. The statistical evaluation of the macro- and microelement determinations.

Table 3
The linear correlation coefficients between the elements in leaves of medicinal plants

	P	P–PO_4	Ca	Mg	K	Na	Fe	Mn	Zn
P–PO_4	**0.75**								
Ca	0.04	0.17							
Mg	0.00	0.24	**0.62**						
K	0.16	0.23	**0.41**	0.25					
Na	0.12	0.35	−0.10	−0.14	0.10				
Fe	−0.24	−0.18	**0.47**	**0.41**	−0.29	0.08			
Mn	0.05	−0.06	−0.03	0.00	−0.32	−0.14	0.18		
Zn	−0.08	−0.19	−0.10	−0.03	0.02	−0.09	−0.06	**0.51**	
Cu	0.01	−0.13	0.37	0.02	**0.43**	−0.22	0.12	0.29	**0.46**

Statistically significant ($\alpha < 0.05$) values are denoted by bold numbers.

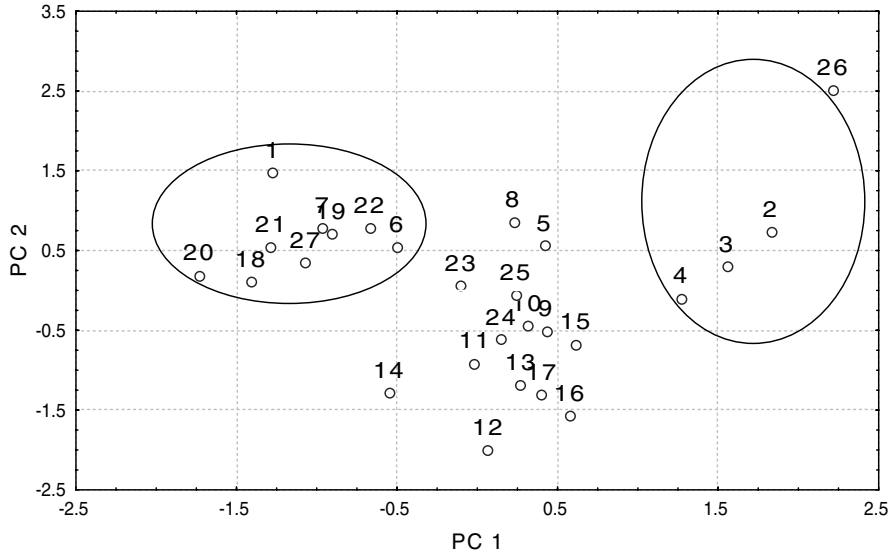

Fig. 3. The principal component scores plot of PC1 vs. PC2.

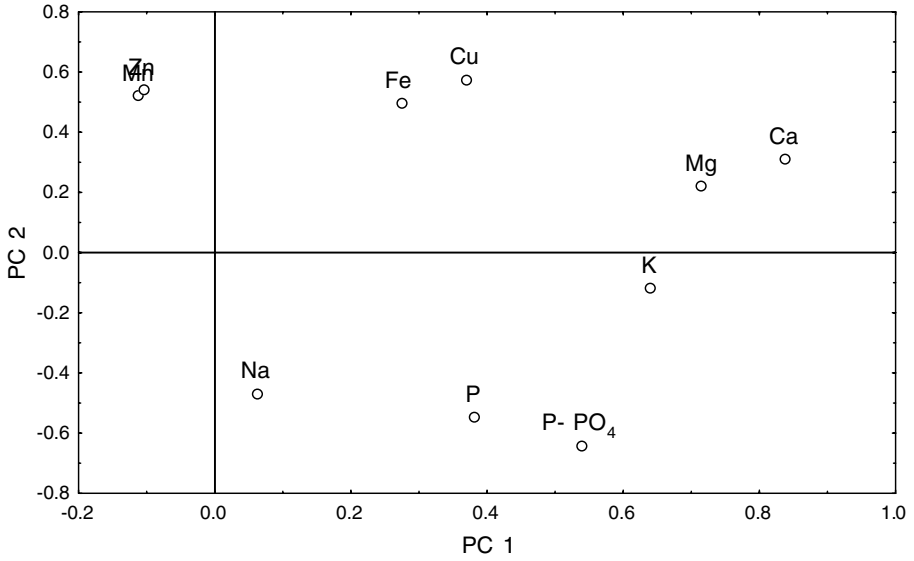

Fig. 4. The principal component loadings plot of PC1 vs. PC2.

nents, revealed some characteristic groups of leaves. The samples are scattered in this way, caused by the differences in their concentrations of metals and phosphorus. They can in general be divided into three groups. The first one, situated on the left area of the plot, contains samples with generally low levels of elements, e.g. *Folium Rosmarini* (samples numbered 18–20), *Rubi fruticosi* (21–22), *Fragariae* (6), *Betulae* (1) and *Lauri* (7). In the central part of the plot there are samples with average contents of bioelements, for example *Folium Ribis nigri* (samples numbered 15–17), *Salviae* (24, 25) and *Menthae* (9–11). On the right side one can find three characteristic samples with high concentrations of elements, e.g. *Folium Farfarae* (2–4) and *Folium Uvae-ursi* (26). Fig. 4 illustrates the relationship between the variables and reveals, that, for the distribution of samples along the PC1 axis, the concentration of Ca and Mg is responsible, but the samples are differentiated along the PC2 because of their P–PO_4, Cu and Fe contents.

4. Conclusions

Extraction with 2% (v/v) acetic acid can be used for the determination of water-extractable phosphorus in the study of the potentially bioavailable for human fraction of inorganic phosphorus present in the leaves of medicinal plants. The fraction of extractable phosphorus determined (as mg/g of dry plant tissue) is covered by the wide range of values, from 30% to 80% of the total phosphorus concentration in leaves. A correlation between the extractable phosphorus and the total level of that element exists. According to the results of our investigation, no significant correlation between the total or extractable phosphate

phosphorus and the metallic elements, in the studied leaves of medicinal plants, exists. However, several statistically significant inter-metal relationships were found. Also, several herbal drugs contain valuable amounts of essential elements, and they can be treated as supplementary food additives. In the case of extractable phosphorus, there is a wide range of its bioavailable fraction, probably depending on the degree of binding of that element with phytic acid, which is known from the literature (Duhan et al., 2002) as an antinutrient agent, decreasing the fraction of phosphorus absorbable by humans using plant drugs.

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