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COMPARATIVE STUDY OF METAL ACCUMULATION IN LICHENS AND TREE LEAVES IN ORDER TO USE THEM AS BIOINDICATORS

***Ladislau KÉKEDY-NAGY*[1], *Katalin BARTÓK*[2]**
[1] "Babeş-Bolyai" University, Faculty of Chemistry and Chemical Engineering, Department of Analytical Chemistry,
11, Arany J. str., **RO-400028 Cluj-Napoca, Romania**
[2] "Babeş-Bolyai" University, Faculty of Biology and Geology, Department of Taxonomy and Ecology,
42, Republicii str., **RO-400015 Cluj- Napoca, Romania**
e-mail: lkekedy@chem.ubbcluj.ro
bartok_katalin@yahoo.com

Abstract: The aim of this work is to study the bioaccumulation of some elements in plants in order to use them as bioindicators by passive monitoring. It was quantified the Na, K, Li, Ca, Mg, Zn, Fe, Cd, Pb and Cu content by flame atomic spectrometry of some lichen samples (*Xanthoria parietina*), walnut (*Juglans regia*), plum (*Prunus domestica*) and canadian poplar (*Populus x canadensis*) collected in the Şărmăşag village area (Sălaj county). The leaves were collected during July and September, the lichen samples only in September 2005. At the same time and in same area, soil samples have been collected from 0 – 10 cm depth. The chemometric processing of the data reveals that the lichen, tree leave and soil samples differ significantly concerning their chemical composition. The discriminant capacity of the metals, on which the classification is based, varies in order Li>Zn>Cu>Mg>Na. The Mg content of leaves is in inverse proportion with that of in the soil. At the same time, the results show similar chemical composition of plum and canadian poplar tree leaves. The tree leaves accumulate selectively the heavy metals in order Zn > Fe = Cu. The Pb and Cd concentration in most of the cases were under the detection limits, fact that suggests the absence of heavy metal pollution in the studied area.

Key words: lichen, tree leaves, bioaccumulation of metals, classification, cluster analysis

Introduction

The inorganic ions (despite of their origin), stemmed from the atmosphere or from the soil, are absorbed and accumulated by the plants [1]. The absorption takes place in different ways, depending on species and environmental conditions. The ions from the atmosphere, mainly as aerosols, are first adsorbed by dry or wet deposition, then are absorbed and deposited by the plants [13]. In the soil the ions are present in three different forms: as low solubility compounds, as adsorbed species (even by ion exchange) on the surface of soil colloids and as free mobile solutes in the groundwater. The absorption of inorganic ions in the plant depends on several physical and chemical environmental factors, as: the temperature, the light intensity, pH, and the concentration of O_2, CO_2 and HCO_3^-. It also depends on the nature and concentration of the ions, the nature and magnitude of interactions in the soil [3].

The vascular plants absorb the mineral substances, in ionic form, mainly by their root system and in lesser extent by leaves. The ions absorbed by the roots are accumulated mostly in the cellular saps, those absorbed by leaves are assimilated by the local tissues or are driven toward the roots. The young leaves absorb more intensely the inorganic ions than the older ones; cations are easier retained than the anions. It is considered that the ions enter the leaves through the epidermal cuticle by diffusion and by ion exchange [3].

In the absence of roots, the lichens exhibit a major dependence on atmospheric sources for nutrients instead of the soil pool exploited by vascular plants. The surface of lichens does not have the waxy cuticle and stomata, found in vascular plant leaves. So the elemental exchange occurs across the entire lichen surface through ion exchange, intracellular uptake and by particulate entrapment mechanisms. In the same time, as long-lived perennial organisms, the lichens are exposed to air all year long, missing the deciduous parts of the vascular plants. Due to their biological features, the lichens are recognized as being very sensitive to air pollution; they could be used for studies of regional atmospheric deposition of metals and other atmospheric contaminants [11].

Flame atomic spectrometry is a fast, robust, sensitive method for the determination of metals in biological samples [14, 15]. As atomization and excitation source usually the high temperature C_2H_2-air or C_2H_2–N_2O premixed flames are used. The low temperature (~ 2000 K) propane-butane-air (PB-A) flame was used in few cases for the determination of metals, mostly in early stage of the flame atomic spectrometry development. The methane-air (M-A) flame has similar properties (temperature, burning velocity etc) with the PB-A flame; the quantification of the different metals was recently reported using this flame, as Zn [8]; Rb [9] ; K [10] and Li [7]. The vegetal samples are digested prior to be introduced as fine liquid aerosol droplets into the flame by pneumatic pulverization. Two main mineralization procedures are currently applied: the wet digestion using oxidizing concentrated acid or acid mixtures, as HNO_3, HNO_3 – $HClO_4$ or HNO_3 – H_2O_2, respectively [2]. The other accomplishment adopts the dry ashing of the vegetal sample in the muffle furnace at 500 – 520 °C for several hours followed by the dissolution of the ash in diluted HCl or HNO_3 acid. [4, 6, 12].

The aim of this work is to quantify the Na, K, Li, Ca, Mg, Zn, Fe, Cd, Pb and Cu content of different plants; to compare the metal content of different vegetal species; to follow the variation of the element-content of the plant tissues in time; to identify a possible heavy metal pollution of the studied area as well as to classify the plants by their cation content using nonparametric chemometric approach.

Experimental

Instrumentation

The measurements were carried out with a single beam HEATH-701 (Heath Co., Benton Harbor, MI, USA) spectrophotometer using a HEATH EU-700 scanning monochromator, a HEATH EU-700-30 type photomultiplier module fitted with 1P28A (Hammamatsu, Japan) and M12FC51 (NARVA, Germany) photomultipliers. The photomultiplier signal was introduced via a home made I/U and A/D converter (National Instruments) into a Pentium 2 PC and processed using homemade software written in Quick Basic language. The data acquisition speed was of 100 ms, one data represents the mean value of 50 measuring points, each point being the average of 300 individual measurements. The pneumatic nebulizer-spray chamber-burner system was from an AAS-1 (Carl Zeiss Jena) atomic absorption spectrophotometer. The original slot type burner-head (for C_2H_2-air flame) was replaced with a similar, Mecker type, developed by us for atomic absorption measurements in the M-A flame [5]. The air flow-rate was constant, 500 L/h, the flow rate of the methane being varied as a function of the gas mixture wanted. As CH_4 source, the city gas of 99 % purity was used, from the pipe.

Procedure

The clear, digested sample solutions were sprayed into the methane-air flame via pneumatic nebulizer. Using the standard calibration method was carried out the quantification of the elements. Four replicate measurements were made in each case; the mean, the standard deviation and the S/N ratio were calculated. The optimal experimental conditions for each element are summarized in Table 1.

Table 1: The experimental conditions for the metal quantification in the M-A flame

Element	Method	λ (nm)	Calibration range (mg.L^{-1})	Observations
Na	FAES	589.5	0.5 – 4	sample dilution: 1: 150
K	FAES	766.4	0.5 – 8	sample dilution: 1: 250
Li	FAES	670.7	0.5 – 10	–
Ca	FAAS	422.7	5 – 100	i = 4 mA sample dilution: 1:10
Mg	FAAS	285.2	0.5 – 10	i = 3 mA sample dilution: 1:21
Zn	FAAS	213.9	0.5 – 4	i = 4 mA
Pb	FAAS	217.0	0.5 – 10	i = 6 mA
Cu	FAAS	324.7	0.5 – 10	i = 3 mA
Fe	FAAS	271.9	0.5 – 20	i = 6 mA sample dilution: 1:5
Cd	FAAS	228.8	1 – 10	i = 5 mA

Legend: FAES – Flame Atomic Emission Spectrometry
FAAS – Flame Atomic Absorption Spectrometry

Chemicals

Stock standard solutions (1000 mg.L^{-1}) were prepared by dissolving the appropriate amount of metals (Zn, Mg, Cu, Cd (Specpure, Johson Matthey Chemicals Limited, England)) and compounds ($CaCO_3$, Li_2CO_3 Specpure, Johnson Matthey Chemicals Limited, England) in corresponding acid. KCl, NaCl, (analytical grade, Reactivul, Bucuresti, Romania), Fe, Pb, HCl, H_2SO_4, $HClO_4$ and H_3PO_4 (analytical grade, Merck, Darmstadt, Germany)) were dissolved and diluted in double distilled water. For further dilutions, double distilled water was used in all cases.

Sampling, sample handling and preparation

The leaves of *Juglans regia*, *Prunus domestica* and *Populus x canadensis* were collected in July and in September, after two weeks of dry season. The leaves were dried first at room temperature than in the drying oven at 90 °C. Lichen samples of *Xantoria parietina* were collected from the cortex of trees by cutting with a sharp knife. After removal the rest of cortex, the lichens were dried in oven at 90 °C. The topsoils were collected in 5 - 10 cm depth; the residues of vegetal organic matter were removed (roots, leaves, etc.), dried in oven at 105 °C. The dried samples were grinded and sieved, about 2 g of dry samples were taken for analysis. The samples were digested by treating with 10 mL of concentrated HNO_3, allowed to stand overnight, and heated carefully on hot plate until the production of red NO_2 fumes ceased. After cooling 2 mL of 70% $HClO_4$ were added and heated again to allow evaporating to a small volume. Finally, the clear digests were diluted to a volume of 50 mL with distilled water.

Results and Discussion

The Na, K, Li, Ca, Mg, Zn, Fe, Pb and Cu content of the vegetal and soil samples are summarized in Table 2. The Cd content of all the samples were lower than the detection limit of 0.14 mg/L, therefore it was omitted from the table.

Comparison of the metal content of the vegetal samples

For this study 5-tree leave and lichen samples were selected, pair wise collected from the same site. In order to study the metal intake of the plants soil samples were taken and analyzed too, originated from the same area as the vegetal ones. The tree leaves considered were those, collected in summer. The analytical data show, that as expected, the soil contains the greatest amount of metal ion. The tree leaves contain fewer amounts; the lichen's cation content is the poorest. Among the cations Na, K, Ca, Mg and Fe were present in the highest level in the vegetal tissues, whereas Li, Pb and Cu were present in trace levels. Lichens contain in average higher quantity of Fe, Pb and Cu than the tree leaves (in brackets), their mean concentration are (expressed in mg/kg): 1226 (189) of Fe, 20 (1.15) of Pb and 23 (14) of Cu, respectively. The results confirm the fact that the metal absorption in the plants from the soil takes place selectively by their root system; the high heavy metal content of the lichens is mainly the result of the outfall from atmosphere. The mean cation content of tree leaves and soil samples originated from the same area are not correlated, with exception of Mg; the mean Mg concentration of the tree leaves (4553 mg kg^{-1}) is half from that found in soils (9003 mg kg^{-1}).

Table 2: Results of analysis of vegetal and soil samples (n=4)

Sample	The metal content of vegetal and soil samples (mg/kg)								
	Na	K	Li	Ca	Mg	Zn	Fe	Pb	Cu
L1	188	9308	0.0	200	1840	66	1430	42	20
L2	164	4722	0.0	9	2172	56	878	0.0	14
L3	228	12616	0.0	241	2335	70	931	21	21
L4	246	9719	0.0	100	1909	68	819	17	34
L5	184	9078	0.3	539	2662	72	2073	21	25
W1s	160	348104	1.0	29424	4882	29	66.7	5.8	15
Cp1s	7247	12309	1.3	12784	4527	101	64.2	0.0	9.1
Cp2s	149	14515	1.9	12818	5440	44	79.2	0.0	5.8
P1s	201	32813	0.7	186	3705	41	417	0.0	20
P2s	221	26005	0.0	4530	4210	150	317	0.0	19
P3s	5726	20967	0.8	5998	4844	150	59	4.5	13
Cp3s	6986	16845	1.4	9264	4258	32	59	0.0	5.4
W2s	105	11885	0.3	145230	4926	36	45	0.0	12
W1a	144	14988	0.9	6379	5640	44	166	0.7	17
Cp1a	5345	17621	0.2	12820	6240	160	47	0.0	13
Cp2a	151	18004	1.7	13999	5550	61	66	29.2	6.4
P1a	207	32717	0.6	588	3627	43	651	0.0	25
P2a	4181	24001	1.8	3248	4244	194	277	0.0	19
P3a	2092	12348	1.0	3891	3214	212	175	0.0	13
Cp3a	188	26765	1.6	20722	3086	24	52	0.0	6.1
W2a	135	9976	0.7	21852	6543	33	48	0.0	14
S1	6939	3000	6.9	2133	6789	76	14728	46	451
S2	4757	7011	11.4	435.6	7506	76	27236	39	477
S3	9937	7319	8.7	11877	10706	131	34824	59	1980
S4	5924	9522	9.6	10295	11175	126	31180	64	2057
S5	8976	7006	8.7	141	8854	129	26547	50	761

Abbreviations: L – lichen; S – soil; T – tree; W – walnut; P – plum; Cp – canadian poplar; s – summer; a – autumn

In the next step, the classification of tree, lichen and soil samples was carried out using the cluster analysis and the principal component design. For simplicity and transparency, the following coding was made: W1s = T1; Cp1s = T2; Cp2s = T3, P1s = T4 and P2s = T5, respectively. First, the classification was carried out, using 9 cations (elements) as variables, choosing the complete linkage and euclidean distances. The tree diagram is presented in Figure 1.

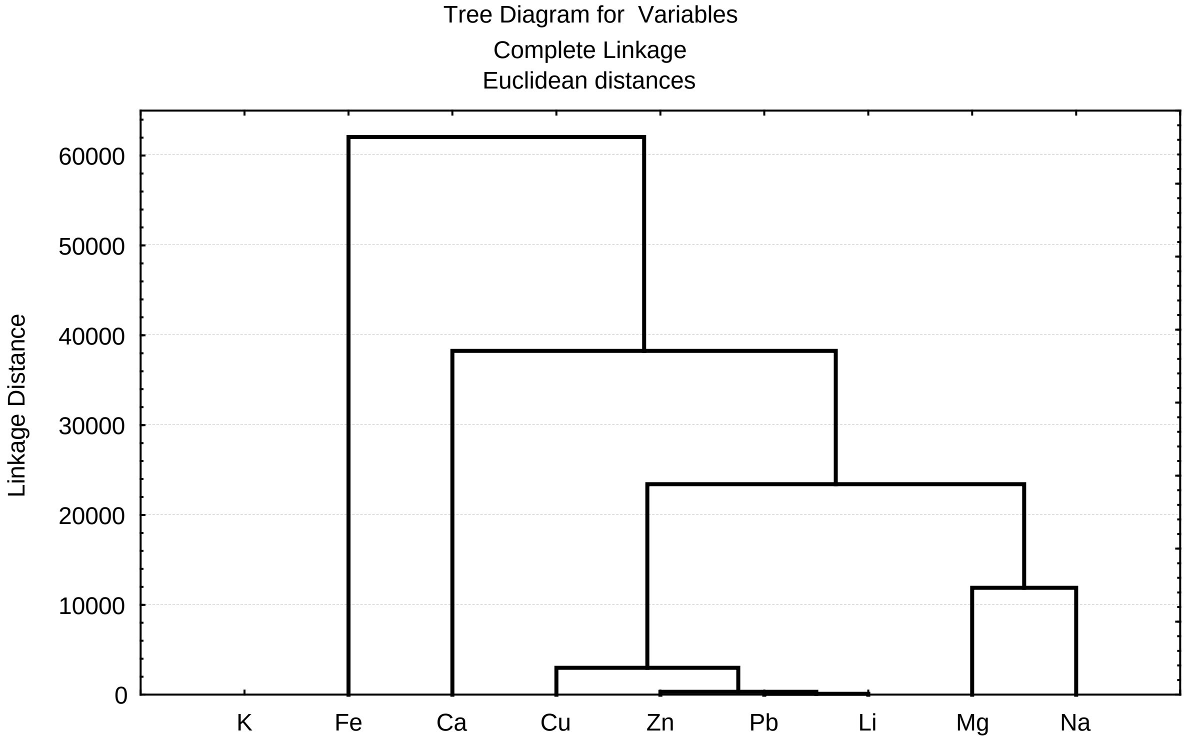

Fig. 1: The tree diagram for 9 variables (cations)

As the diagram shows, the best correlation among the variables present, in decreasing order, Li < Pb < Zn< Cu. From this list Li and Pb could be excluded, because most of the lichens and tree leaves samples are missed of Li and Pb, respectively. Looser coupling exists between Mg, Na and Ca, Fe. The high linkage distance of K with the former groups (over 35000 units) shows that K is the less significant element for the classification. K is almost in the same concentration level in all the vegetal samples, being the osmotic pressure regulator of the vegetal cells. Considering all the nine cations, the tree diagram for 15 cases (the samples) choosing the complete linkage and euclidean distances is presented in Figure 2. The results show a distinct chemical composition of the sample groups, based on their origin. The best similitude concerning the metal composition exhibits the lichens, despite of the provenance. The tree leaves seems to have similar composition, the most likely being pair-wise the Canadian poplars (T2, T3) and plums (T4, T5). The T1 sample composition differs significantly from all vegetal ones, emphasizing the proper and differentiated biological activity of the walnut in the foliage period. The soils represent a separate group with well-defined proper chemical composition.

In conclusion, by quantification of cation content of the plant tissues and by using the cluster analysis the samples can be unequally classified by their origin, the base of classification presents the Zn, Cu, Na, Mg, and Fe content of the samples. Lichens accumulate Fe, Pb and Cu in higher concentration, so they could be efficient bioindicators for these elements.

To emphasize the conclusions formulated earlier and to extract the principal components, the factor analysis was carried out too. For the calculations all experimental data were considered. Two factors were selected and the normalized varimax rotation of the axis was applied. Factor F1 contains the following elements (for which loadings are greater than 0.7): Na (0.874), Li (0.925), Mg (0.955), Fe (0.972), Pb (0.848), and Cu (0.923). They are considered the principal components. Factor F2 contains K and Ca with the loadings of 0.9261 and 0.9481, respectively. These results are in concordance with the previous ones, which suggest the pertinence of former conclusions. Extraction of the principal component concerning the cases the factor loadings relations, calculated in the same way, are represented in Figure 3.

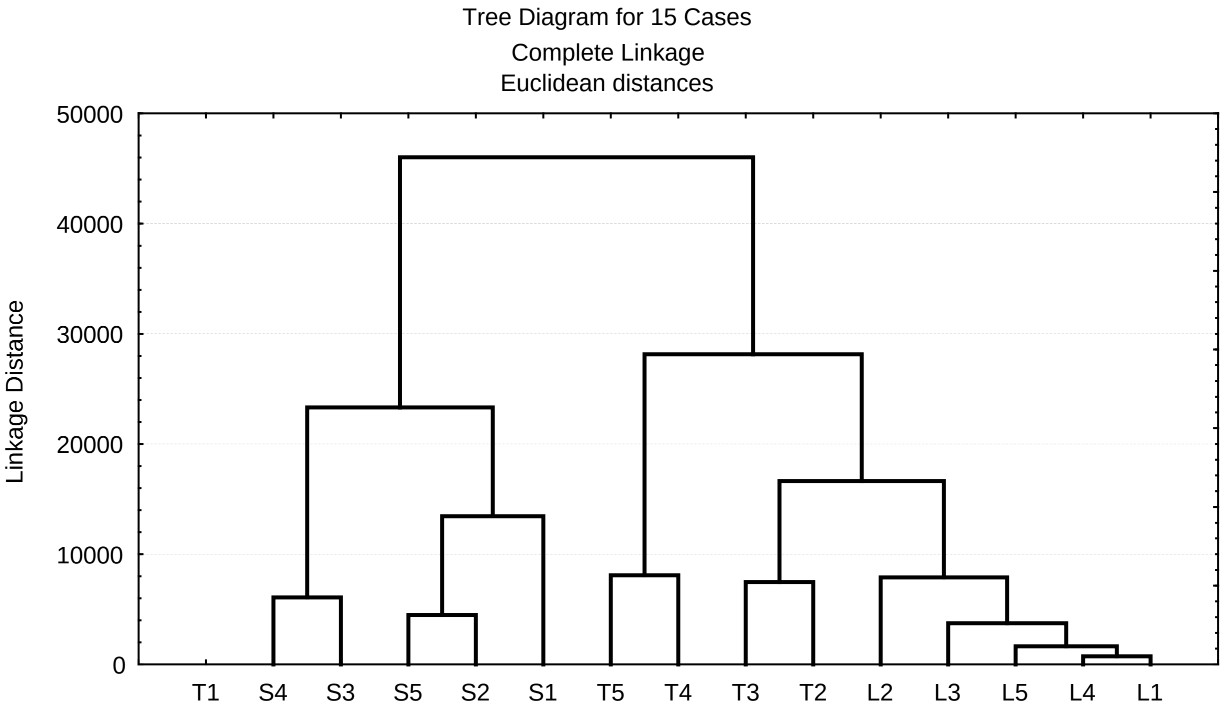

Fig. 2: The tree diagram for 15 cases (samples)

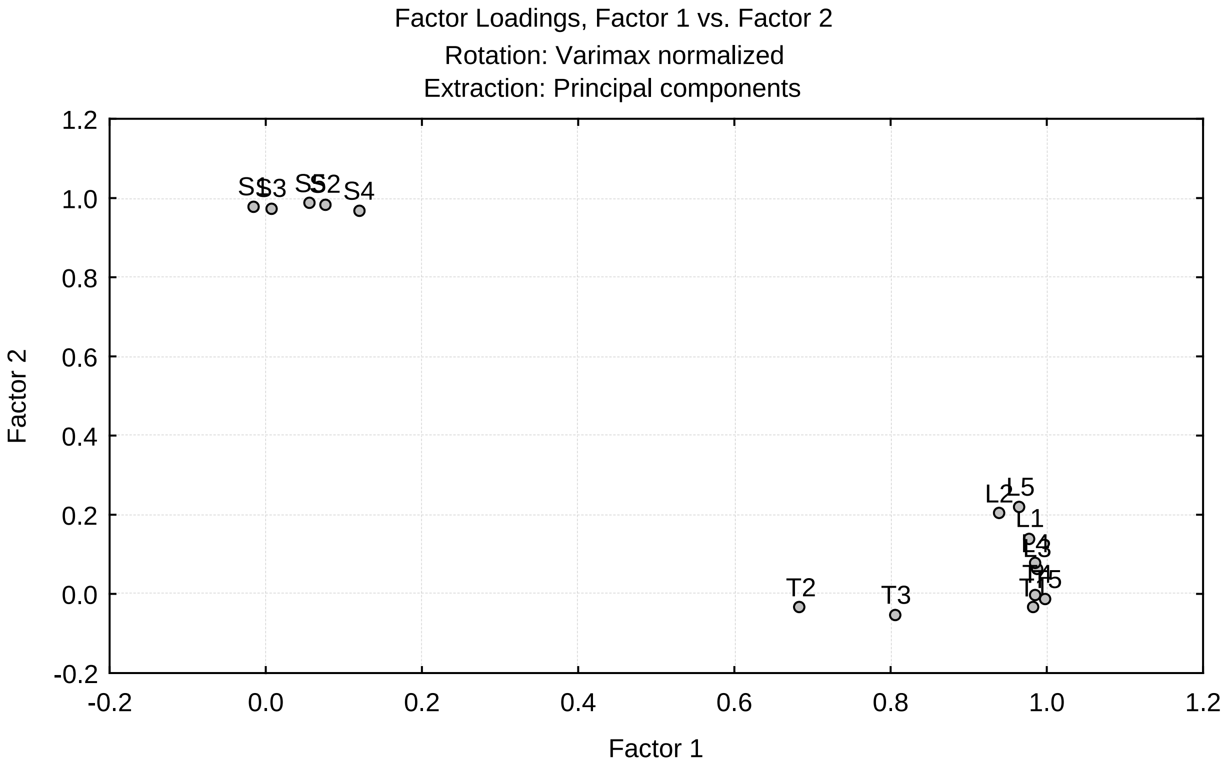

Fig. 3: The factor loadings for the cases

The factor analysis shows that factor F2 is significant for all of soil samples, they being tightly grouped. The vegetal samples represent another tight group (with exception of samples T2 and T3), being included in the factor F1. The results emphasize the conclusion that the soils have similar chemical composition, which differ significantly from the relative homogeneous chemical composition of the vegetal tissues.

Variation of the metal content of the tree leave tissues in time

For this survey only the tree leaves samples were taken into account. The experimental data show, that the metal content of the leaves varies in time, despite of the origin and species. The magnitude and sign of the change for the metal ions (accumulation or lose) furthermore depends on the metabolism of the given tree species. For example, the K content increases in Canadian poplar in time (with about 29 %) while in the plum and walnut leaves tissues decreases with 22.1 % and 11.2 %, respectively. An unequivocally increase of the heavy metal content (Cu and Zn) was observed despite of the species studied. Copper was accumulated in average in walnut of 10.5%, in Canadian poplar of 17.6 % and in plum of 3.9 % while Zn accumulates in walnut in average of 11.9 %, in Canadian poplar of 10.4 % and in plume of 18.9 %. Iron accumulates in the walnut (32.9 %) and in the plum (29.3 %), Canadian poplar looses about 22.8% of Fe in time. In conclusion, the studied tree species could be used unambiguously as biomonitors for Cu, Zn and Fe (less the Canadian poplar).

In the followings, the nonparametric chemometric analysis was carried out in the same way, as described earlier. The tree diagram of classification using 9 cations (elements) as variables is presented in Figure 4.

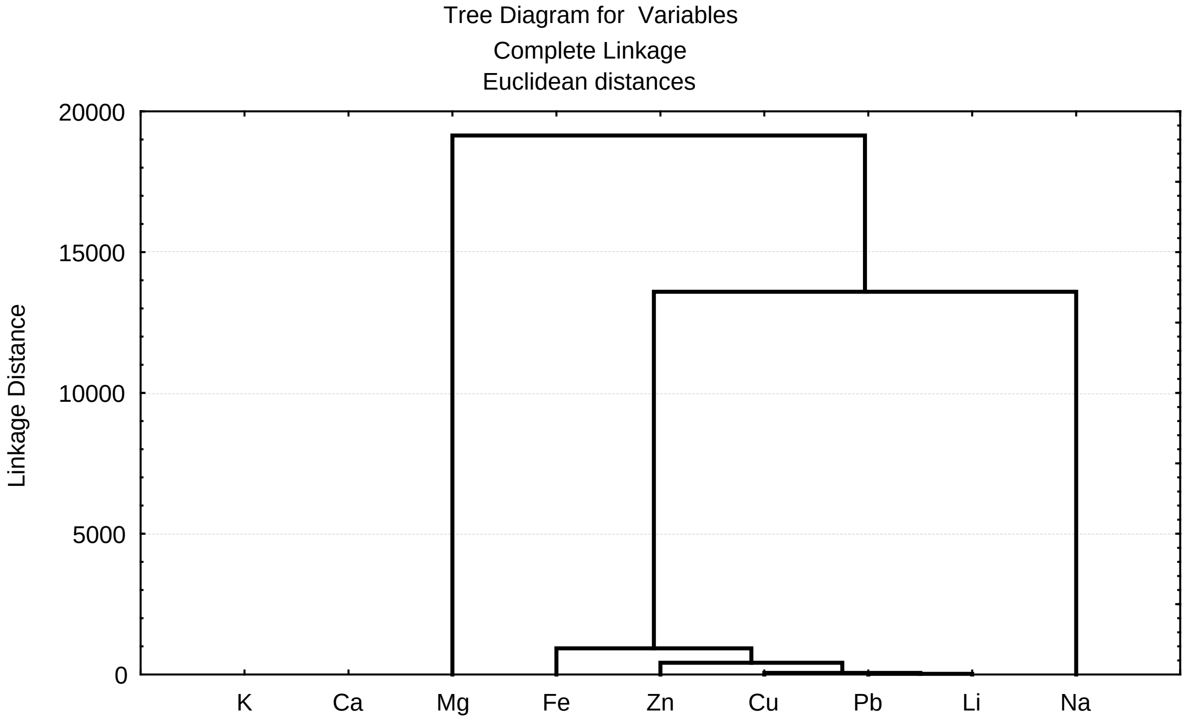

Fig. 4: The tree diagram for 9 elements (variables)

The tree diagram for the elements shows that the macro elements, as K, Ca, Mg, are not suitable for the classification, the linkage distances for them being very high: over 35000 units for K, over 15000 units for Ca and over 14000 units for Mg, respectively. Low linkage distances are between, in ascending order, Pb < Cu < Zn < Li < Fe. As in the former case, the closest correlation exists among the same elements, the ranking differs only. Thence that most of the leaves samples are missing of Pb, this element could be omitted from the classification. The factor analysis was carried out in the same conditions, as earlier: two factors were selected and the normalized varimax rotation of the axis was applied. The calculations show that Factor 1 includes the metals: Fe with the loading of 0.889 and Cu with the loading of 0.929; and factor 2 groups Na, K and Ca with the corresponding loadings of -0.754, 0.463 and 0.572, respectively. Considering all the nine cations, the tree diagram for the 16 cases (the samples) choosing the complete linkage and euclidean distances is presented in Figure 5.

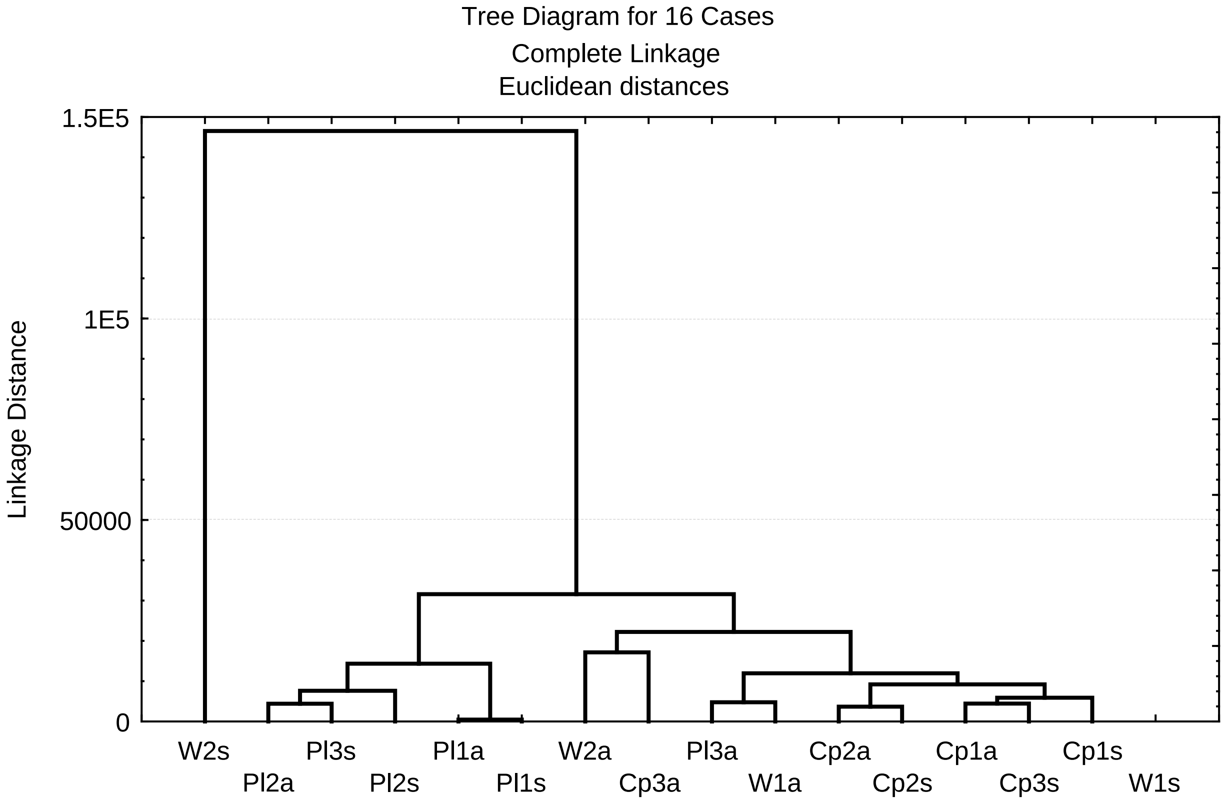

Fig. 5: The tree diagram for samples (16 cases)

The tree leaves tissues exhibit different chemical composition which varies in different way in time. In summer the walnuts leaves chemical composition differ significantly from the others, fact which suggests the species specific metabolism of the walnuts. The poplar and plum tree leaves have very similar chemical composition; they suffer less changes during vegetation. Till autumn the differences in chemical compositions of tissues of different origin emerges, even for the walnut ones. Using cluster analysis only the walnut leaves collected in July could be identified, the others belong to the same cluster, having close similar chemical composition.

The factor analysis for the 16 samples was performed in the same way, as earlier, keeping the same conditions (two factors selected, the normalized varimax rotation of the axis was applied). The results show that for classification only the plum and one of the walnuts sample contribute, being the component part of factor 1, with the following loadings: W1s 0.967; Pl1s 0.989; Pl2s 0.955; Pl3s 0.917; W1a 0.843; Pl1a 0.987; Pl2a 0.971 and Pl3a 0.913. Factor 2 contains the walnut sample number 2: W2s and W2a having the loadings of 0.970 and 0.973, respectively. Canadian poplars exhibit insignificant changes in chemical composition; the factor loadings for them are close; varying between 0.5 and 0.7 for both factor fields. The results of the factor analysis for 16 samples are represented in Figure 6.

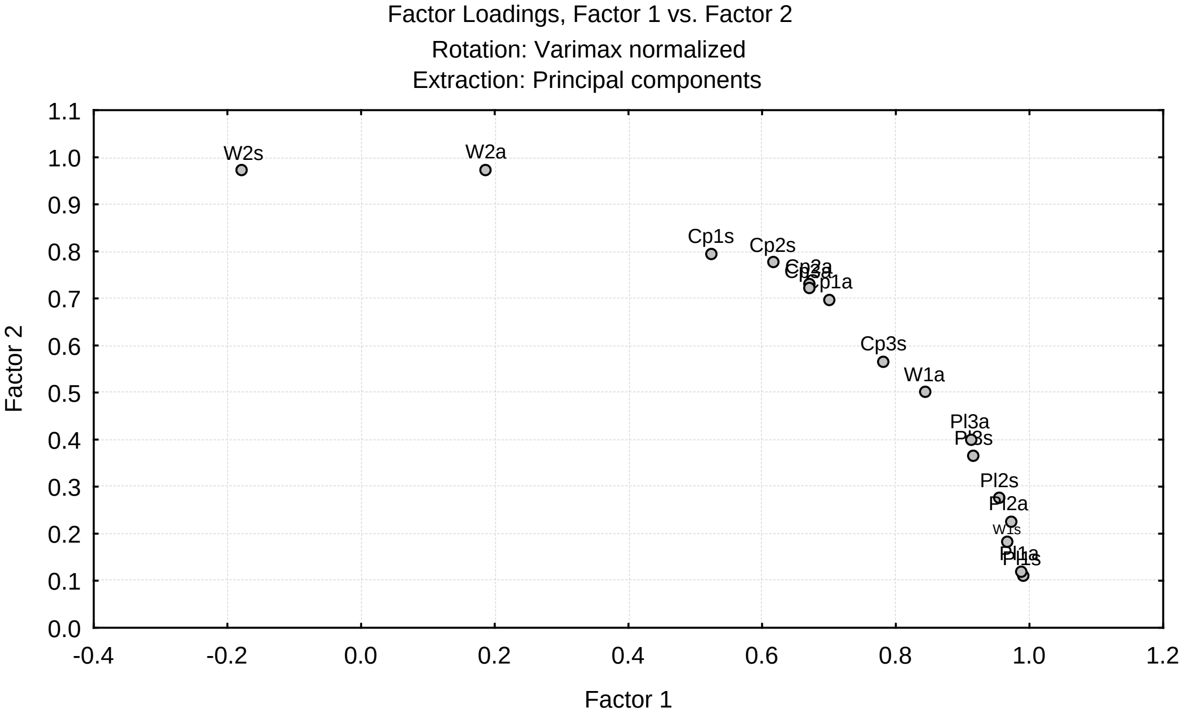

Fig. 6: Factor loadings for 16 samples

Conclusions

1. The plant tissues of different origin exhibit significant disparate chemical composition. The tree leaves contain more metal than lichens.
2. Lichens accumulate Fe, Pb and Cu in higher concentrations; they could be efficient bioindicators for these elements.
3. Quantification of metal ion content of the plant tissues exhibits the unequivocal classification by their origin using the cluster analysis. The base of classification stands the Zn, Cu, Na, Mg, and Fe content of the samples.
4. The metal content of the tree leaves varies in time, Zn and Cu accumulate unequivocally, despite of tree species. Fe accumulates only in walnut and plum, Canadian poplar looses significantly its iron content in time.
5. The studied trees are suitable for biomotinoring the environmental loading of Zn, Cu and Fe (except for the Canadian poplar).
6. The classification of trees is possible based on the quantification of Pb, Cu, Zn, Li and Fe content.
7. As consequence of the similar chemical composition of tree leaves, only the walnut samples collected in summer differ significantly from the other ones.
8. The low concentration levels of Cd and Pb found in the soils suggests that the studied zone is not polluted by these elements.

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STUDIUL COMPARATIV PRIVIND ACUMULAREA UNOR METALE ÎN LICHENI ŞI FRUNZE DE COPACI ÎN VEDEREA UTILIZĂRII LOR CA ŞI BIOINDICATORI

(Rezumat)

Scopul lucrării constă în studiul acumulării unor elemente în plante în vederea utilizării lor ca bioindicatori prin monitorizare pasivă. S-a determinat prin metoda spectrometriei atomice în flacără conţinutul de Na, K, Li, Ca, Mg, Zn, Fe, Cd, Pb şi Cu al unor probe de licheni (*Xanthoria parietina*), de frunze de nuc (*Juglans regia*), de prun (*Prunus domestica*) şi de plop (*Populus x canadensis*), colectate în zona comunei Şărmăşag (jud. Sălaj). Probele de frunze s-au colectat în luna iulie şi septembrie a anului 2005, iar probele de licheni doar în luna septembrie. Din aceleaşi zone s-au colectat simultan şi probe de sol de la 0 – 10 cm adâncime. Prelucrarea chemometrică a datelor arată că lichenii, frunzele de copac şi solul se deosebesc semnificativ privind conţinutul elementelor determinate, clasificarea lor poate fi făcută în ordinea descrescătoare a corelaţiei între elementele: Li>Zn>Cu>Mg>Na. Concentraţia de magneziu din frunze este invers proporţională cu cea din sol. Totodată rezultatele arată că frunzele de plopi şi de prun au compoziţie similară privind conţinutul de elementele determinate. Copacii acumulează selectiv metalele grele, în ordinea Zn > Fe = Cu. Clasificarea frunzelor de copac se poate face pe baza aceloraşi elemente, doar ordinea lor diferă: Li>Cu>Fe>Na>Zn. Cantitatea de Pb şi de Cd în majoritatea cazurilor a fost sub limita de detecţie, fapt ce sugerează absenţa surselor poluante de metale grele în zona studiată.

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