EFFECTS OF AIR POLLUTION ON KEY TREE SPECIES OF THE CARPATHIAN MOUNTAINS

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Abstract

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Concentrations of Al, B, Ca, Cu, Fe, K, Mg, Mn, N, Na, P, S and Zn in the foliage of white fir (*Abies alba*), Norway spruce (*Picea abies)* and common beech *(Fagus sylvatica)* from 26 sites of the Carpathian Mts forests are discussed in a context of their limit values. S/N ratio was different higher from optimum in 90% of localities when compared with the European limit values. Likewise we found increase of Fe and Cu concentrations compared with their background levels in 100% of locations. Mn concentrations were increased in 76% of localities. Mn mobilization values indicate the disturbance of physiological balance leading to the change of the ratio with Fe. SEM-investigation of foliage waxes from 25 sites in the Carpathian Mts showed that there is a statistically significant difference in mean wax quality. Epistomatal waxes were damaged as indicated by increased development of net and amorphous waxes. The most damaged stomata in spruce needles were from Yablunitsa (UA); in fir needles from Stoliky (SK), and in beech leaves from Malá Fatra (SK). Spruce needles in the Carpathian Mts had more damaged stomata than fir needles and beech leaves. Spruce seems to be the most sensitive tree species to environmental stress in Carpathian Mts forests. Foliage surfaces of three forest tree species contained Al, Si, Ca, Fe, Mg, K, Cl, Mn, Na, Ni, and Ti in all studied localities. Presence of nutrition elements (Ca, Fe, Mg, K and Mn) on foliage surface hinders opening and closing stomata and it is not physiologically usable for tree species.

Key words: spruce, beech, fir, atmospheric deposition, nutrition elements, heavy metals, epicuticular waxes

Introduction

Air pollution sooner or later deposites on the Earth´s surface. A residential time of pollutants in the atmosphere depends on the physical and chemical properties of both the atmosphere and pollution. Between many pollutants heavy metals are the most toxic component for all living organisms. As biological objects can respond more or less specifically to the influence of environmental factor intensities, including atmospheric deposition levels (sensitive, tolerant, bioaccumulative responses of organisms) suitable biotas are searched for use as biometers. Generally, passive and active methods of plant bioindication are used. Several surveys of biomonitoring methods and useable bioindicators are available in the literature (Buse et al., 2003; Maňkovská et al., 2003; Markert et al., 2003; Manning et al., 2002; Suchara et al., 2007).

Forests of the Carpathian Mountains represent unique reservoir of many endemic, rare and unusual plant and animal species in Central Europe. Norway spruce (*Picea abies* K a r s t.) and common beech (*Fagus sylvatica* L.) are well distributed there. These species have attracted significant attention of scientists because of their decline and reduced genetic diversity being caused by air pollution. The region on the border of Slovakia, Poland and Czech Republic was declared the second "black triangle" in Europe with substantially higher concentrations of heavy metals than in the first "black triangle" on the border of the Czech Republic, Poland and Germany (Markert et al., 1996). Information about the air pollution status of the Carpathian Mts forests is essential for a better understanding of environmental stress. Chemical foliar analysis is a widely used diagnostic and monitoring method in environmental studies (Maňkovská, 1996; Maňkovská et al., 2003). Foliage of forest tree species from contaminated regions can be considered an accumulation monitor where significant amounts of chemical elements are deposited on the surface or in the wax layer (Maňkovská, 1996).

The attention has been paid to the impact of polluted air on forests (Karaoz, 2003; Stefan et al., 1997); forest land (Hovdmand, Bille-Jansen, 1997); surface of foliage (Percy et al., 1995); crown condition and pollution (Solberg et al., 2002); biomonitoring and statistical analysis (Suzuki et al., 1994; Torseth et al., 2001).

Emissions of SO_2 cause a noticeable damage to forest stands. Many authors deal with accumulation of sulphur in foliage (Ayodele, Ahmed, 2001); metabolism of sulphur in plants (Lofgren et al., 2001; Rennenberg, 1994).

Emissions of NO_x belong in addition to SO_2 to important injurious agents. Many papers dealt with nitrogen, its circulation and influence on growth (Lovett et al., 2004). In case of cumulating nitrogen and sulphur in the foliage of forest tree species the ratio of S/N changes and nutrition can be damaged (Kaupenjohan et al., 1989). Simultaneously in addition to the excess of sulphur and nitrogen in the foliage of forest tree species there affect also other injurious agents such as microelements in excess and heavy metals (Hlawiczka et al., 2003; Lomander, Johansson, 2001).

Some long-term studies about an interaction between leaf surface on trees and atmospheric pollution were carried out (Altieri et al., 1994; Sanz et al., 1995). Effects of atmospheric pollution and epicuticular waxes and stomata were reported through the observations on electron scansion microscope and in some studies was reported the role of SO_2 and other pollutants on morphological leaf wax structure (Trimbacher, Weiss, 2004). Alterations in the barrier between tree and atmosphere, the wax cuticle, can have important physiological effects and can therefore be used as a tool to indicate the tree response to abiotic factors. The erosion of epicuticular wax structure and stomatal damage in conifer needles are very widespread phenomena: they are considered important factors in the syndrome of forest decline (Turunen, Huttunen, 1990). SO_2 and NO_x emissions and organic and solid depositions in Slovakia forest area was studied and described previously by Maňkovská (1996).

Primary aim of this paper was to study the effect of polluted air on foliage of three forest tree species in the Carpathian range. It was namely evaluation of total concentration of sulphur and nitrogen (as the most important air pollutants). Furthermore, our aim was to evaluate air pollution impacts on stomatal quality for three important forest tree species on 26 sites in the Carpathian Mts.

Material and methods

Sampling sites were established in the Czech Republic (CZ): 1 – Bílý Kříž, 2 – Javorina; Poland (PL): 3 – Babia Góra, 4 – Bieszczady, 5 – Brenna, 6 – Magura, 7 – Pieniny, 8 – Tatry; Slovakia (SK): 9 – Malá Fatra–Štefanová, 10 – Malé Karpaty–Geldek, 11 – Vihorlat-Morské Oko, 12 – Poľana, 13 – Stoliky, 14 – Kozie chrbty-Východná; Ukraine (UA): 15 – Kryvopilja, 16 – Kuzij, 17 – Vizhnitsa, 18 – Synevir, 19 – Uzhoksky Pass, 20 – Yablunitsa; Romania (RO): 21 – Fudata, 22 – Magura, 23 – Obcina Mare, 24 – Rarau, 25 – Stana de Vale, 26 – Retezat in December 1998 (Bytnerowicz et al., 2002; Fraczek et al., 2002). Altogether 1101 trees (spruce, fir and beech) of dominant or co-dominant class in the second age category (21–40 years old) were sampled using ICP protocol (ICP, 1994).

Samples of foliage were analysed unwashed**.** They were dried at the temperature not exceeding 80 °C for the period of 24 hours. Needles were separated from branches and leaves from stems. Dry samples of foliage were completely homogenized and equal amounts of biomass from 15 trees per plot were mixed. Pressure mineralization was performed in a microwave furnace MDS 2000 (CEM company). Atomic absorption spectrometer (LECO-PLASMA-RAY 2000) was applied to determine concentrations of aluminium (Al), boron (B), calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na) and zinc (Zn). Elementary analyser LECO SC 132 was applied to determine concentration of sulphur. Elementary analyser LECO SP 228 was used to determine total concentration of foliar nitrogen.

JEOL Ion sputtering was used for treating foliar surfaces of the studied trees. Scanning microscope JEOL 840 A and X-ray analyser LINK 10000 were used for their assessment. Quantification changes (Table 1, Figs 1, 2, 3) in the epistomatal wax structures of five distinct classes were defined by two criteria: differences in wax morphology and varying degree of changed wax structures in the stomatal area (Maňkovská, 1996; Trimbacher, Weiss, 2004). We used Q coefficient of occlusion (arithmetical mean of epicuticular wax quality of 200 stomata per leave or needle). Particles deposited in foliar stomata were assessed according to their morphology and X-ray

T a b l e 1. Classification of changes of the epistomatal wax of *Picea abies, Abies alba, Fagus sylvatica.*

Class I	Maximum of 10% of the total stomatal area shows the beginnings of fussion of single wax tubules.
Class II	Some of the atypically aggregated wax tubules fuse to small wax tufts at different parts of the stomatal area. The latter cover 10 to 25% of the total stomatal area.
Class III	In addition to the wax tufts plate-like wax parts can be found which, in total, cover more than 25% and up to 50% of the total stomatal area.
Class IV	More than 50% and up to 75% of the total stomatal area shows small parts of wax tufts as well as large platelet wax forms.
Class V	More than 75% of the total stomatal area is characterized by considerably changed wax microstructures. The stomatal antechamber is almost or completely occluded with an amorphous wax plug.

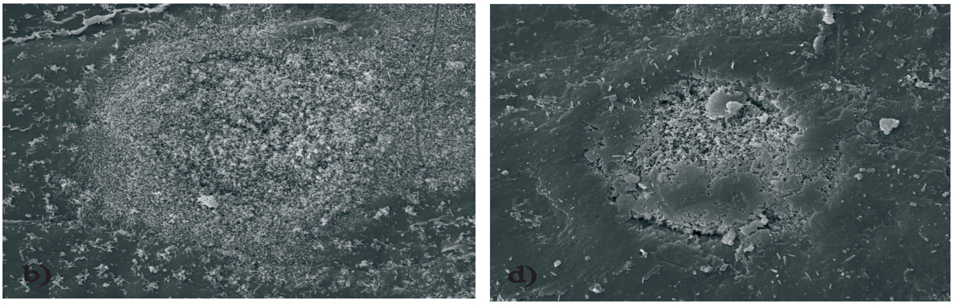

Fig. 1. Microstructure epicuticular waxes in epistomatal area *P. abies* K a r s t.
Note: classes: b) classification class I , d) classification class IV, abscisa represents 20µm

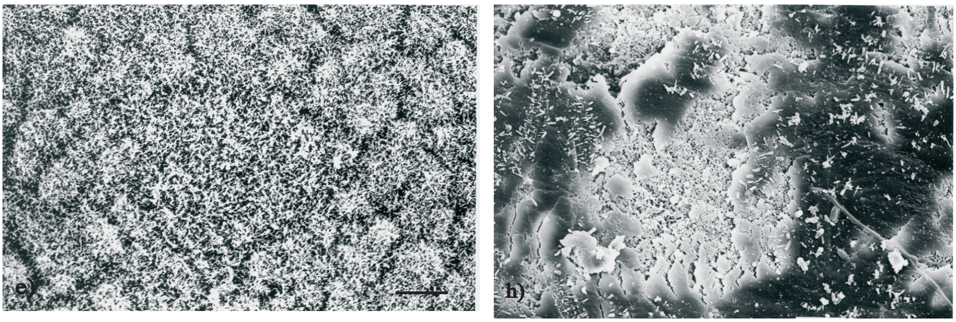

Fig. 2. Microstructure epicuticular waxes in epistomatal area *A. alba* M i l l.
Note: classes: e) classification class I, h) classification class IV, abscisa represents 10 µm.

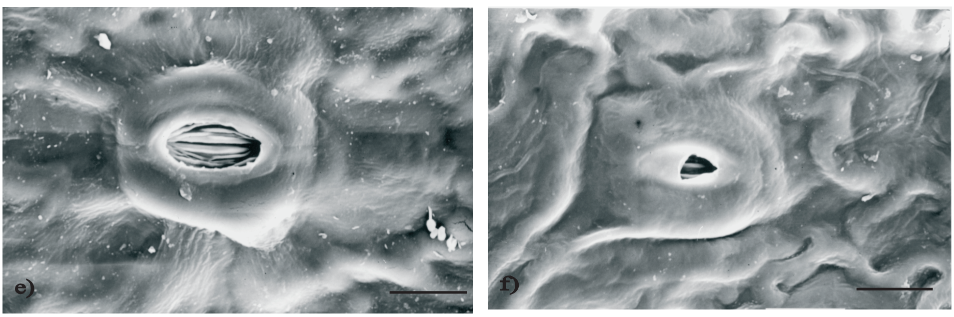

Fig. 3. Microstructure epicuticular waxes in epistomatal area of *F. sylvatica* L.
Note: e) classification class I; f) classification class III, abscissa represents 10 µm

T a b l e 2. Classification of particles deposited in surface and stomata of foliage.

Category	Morphology of particles		Major EDX spectrum
Biologic (A)	Characteristic shape with a low spectre–pollen, spores, plant and animal remains and waxes		Low peak for background ratios of the elements: Si,S,Ca,K,P
Mineral (B)	Nonspherical irregular shape, fairly big particles, origin: soil, limestone [$CaCO_3$]; dolomite [Ca,Mg $(CO_3)_2$], SiO_2, $CaSO_4$ and complex mixtures of alkaline origin		High peak for background ratios of the elements Si or Ca and others as Al, K, Ti, Fe, Na
Fly ash from black oil (C)	Small oval particles rich in Al, Si, S; cenospheric with Al-Si, V and Ni; sulphates rich in Cr, Fe, Ni with black-metallic luster		Al, Si, S, V, Ni, Cr
Fly ash from coal (D)	Small oval particles similar to the ones from B group with Al-Si, with various admixtures		Similar to mineral particles with Al-Si
Fly ash from black oil and coal (E)	Small oval particles similar to the ones from C group with Al-Si; small porous particles containing carbon together with D category		Al, Si, V, Ni,Cr
Industrial (F)	Very variegated reflecting Technologies used:		
	Aluminium plant	F 1	Al
	Cement and lime plants	F 2	Ca
	Magnesite plants	F 3	Mg
	Iron	F 4	Fe
	Base metals	F 5	Mn,Ni,Zn
	Other	F 6	Br,Rb,Sr, As,Be,Cd,Co,Cr,Cu,Ge Mo,Ni,Pb,Se Sb,V,Zn

spectra (Table 2). The deposited particles were divided into four basic groups: 1 – biological; 2 – mineral; 3 – coal and fuel oil ash and 4 – industrial (cement and lime plant, iron plant and other technologies) (Maňkovská, 1996). Fungal infection was classified to 5 classes: 1 – absence of fungal particles (spore and mycelium); 2 – < 10 particles; 3 – 11 to 25 particles; 4 – > 25 particles with covering less than 25% of surface, and 5 – > 25% of foliage surface covered. The epicuticular wax was evaluated by Kolmogorov- mirnov statistical non-parametric comparisons test for quality.

The accuracy of data was verified by an analysis of standard plant samples and by a comparison with the results obtained in 109 laboratories within the IUFRO working group for quality assurance (Hunter, 1994). For an assessment of vegetation material we used current statistical methods, correlation analysis and analysis of main components (PCA), which is included into factor analysis. Factor rotation was performed by Varimax method in a way to define the factors with the slope either very high (close to 1.0 or -1.0) and to eliminate medium loading. The number of factors was set at 8; the percent of explained variability was 90%.

Results and discussion

Foliar analyses

Foliar analyses (Table 3) show that European limit values for tree foliage (Stefan et al., 1997) were exceeded for S, Fe, Cu, and Mn. For better understanding we processed the results separately for 26 sites. In this way it is possible to differentiate better on studied tree species for geographic regions such as Western Carpathian Mt., Eastern Carpathian Mts and Southern Carpathian Mts. Statistically significant differences between concentrations of all studied elements in the foliage of forest tree species were found between all studied sites. Nutrition ratios (S/N, N/P, N/K, N/Mg, K/Ca, K/Mg, Ca/Mg, Fe/Mn) in beech leaves, spruce and fir needles are given in Table 4. Unbalanced ratios for S/N for all tree species in all sites were found. The ratio of Fe/Mn was unbalanced for spruce needles in the 66% sites and for fir needles in the 40%. Similarly this ratio was unbalanced for beech leaves in the 63% sites. Other elemental ratios were balanced. Balance of single elements in plant organisms is a basis for normal growth. Similar chemical properties, derived from approximately the same ionic radicals and charges, probably cause that the interactions of single elements occur inside of the tree. Synergistic and antagonistic relationships may occur between the studied elements and these may be modified by the presence of air pollutants. Markert (1993) as the first researcher explained mutual correlation of P, N, K, Ca and Mg in 54 higher and lower plant species. P and N are important during protein biosynthesis, and Ca and Mg as common enzymatic activators during metabolic physiological processes (Markert et al., 1996, 2003) also found high correlation between P, N, Ca, Mg and Sr concentrations and Co/Mo, Cr/Co ratios in Scots pine needles. The same author listed Al/Ca, Mn/Ca and B/Sb as typical antagonistic elementary pairs. We did not find either positive or negative correlation with the correlation coefficient r higher or equal to ± 0.9 (Table 5) between single pairs of elements in the foliage of all studied tree species in the Carpathian Mts. Mutual correlation with r higher or equal to ± 0.5 existed for the following pairs of elements: beech leaves (S/N, S/P, Cu/P, Cu/K and Cu/Mg); spruce needles (S/N, Fe/P, Fe/K, Mn/B, Na/Cu, Na/Mn and Zn/Cu) and fir needles (S/N, Mg/Ca, Zn/K, Zn/Mg and Zn/Fe).

Aluminium is a metallic non-essential element in foliage of forest trees. The concentrations of Al ranged for 1-year-old spruce needles between 39 and 156 $mg.kg^{-1}$, for 1-year-old fir needles between 50 and 301 $mg.kg^{-1}$ and for beech leaves between 59 and 237 $mg.kg^{-1}$. For the entire Carpathian Mts range the highest Al concentrations were determined in Tatry, PL (spruce); Magura PL (fir needles) and in the Kozie chrbty, SK (beech leaves). According to Maňkovská (1996), the allowable limit value for Al in the foliage of forest tree species is 100 mg kg^{-1}. Al was present in 97.2% of the evaluated stomata.

Boron is an essential element for trees. The concentrations of B ranged for 1-year-old spruce needles between 11 and 22$mg.kg^{-1}$, for 1-year-old fir needles between 14 and –21 $mg.kg^{-1}$ and for beech leaves between 17 and –35 $mg.kg^{-1}$. For the entire Carpathian Mts range the highest concentrations were determined for spruce in Stana de Vale (RO), for fir in Stoliky (SK) and for beech foliage in Malé Karpaty, Geldek (SK). According to Stefan

T a b l e 3. Concentration of elements in the foliage of *Fagus sylvatica, Picea abies* and *Abies alba* (median in $mg.kg^{-1}$).

F. sylvatica

Site	Al	B	Ca	Cu	Fe	K	Mg	Mn	N	Na	P	S	Zn
2	**73**	25	**11385**	**13.9**	**841**	**10719**	**1991**	536	**25200**	**70**	1489	1840	38
4	**64**	27	**11826**	**6.0**	**319**	7447	1382	337	18000	59	1030	1550	36
6	**103**	27	**8874**	**13.4**	170	**11631**	**1864**	808	**28000**	**113**	1241	**2160**	32
9	**59**	23	**9699**	**10.8**	113	9646	**2535**	47	19800	**85**	1000	1730	27
10	**99**	35	**9553**	**10.8**	138	6321	**3680**	39	**25400**	**100**	1324	**2310**	25
11	**159**	31	6453	**12.0**	163	**11372**	**1517**	**2741**	**27900**	**84**	1287	**2650**	28
12	**102**	17	**8374**	**14.0**	99	**11593**	**1848**	652	**29700**	50	1671	**2650**	25
14	**237**	24	**10554**	**11.2**	**479**	8081	**2099**	**2075**	22800	**168**	**1887**	**2330**	34
5	**119**	19	**13813**	**12.4**	**533**	6049	**2000**	208	**25100**	**87**	1542	**2590**	36
16	**100**	22	**7055**	**15.1**	**578**	**10893**	**1643**	**2167**	23100	**103**	**1937**	**2590**	**46**
17	**67**	19	**10526**	**10.6**	**1039**	7406	**2264**	311	**27300**	**96**	1360	**2540**	**59**
19	**109**	22	**9158**	**12.9**	**1052**	**11624**	**2174**	**1407**	**29000**	**87**	1571	**2720**	**63**
20	**115**	28	**8206**	**12.9**	**1031**	7792	**2398**	**1939**	**28900**	**102**	1375	**2540**	44
21	**118**	17	7452	**10.1**	**1020**	8717	**1943**	**1356**	**25300**	**103**	1295	**2390**	**48**
22	**80**	18	**12479**	**12.0**	**1090**	6934	**2715**	454	22600	**106**	1444	**2730**	**53**
25	**145**	21	**10990**	**11.3**	**286**	**10390**	**2074**	999	**25800**	**75**	1363	**2500**	**33**

P. abies (1-year-old needles)

Site	Al	B	Ca	Cu	Fe	K	Mg	Mn	N	Na	P	S	Zn
1	**89**	19	2931	**5.3**	**213**	5456	1092	188	12900	44	1463	1420	33
3	**56**	14	3627	**7.4**	90	**7725**	1332	67	11700	84	1005	**1518**	30
5	**55**	13	4289	**7.6**	90	**8203**	1354	38	11700	84	1005	**1518**	**48**
8	**156**	15	1978	**5.5**	92	5004	717	633	13700	60	1389	**1580**	11
9	**54**	13	4756	**7.6**	90	**9653**	1443	32	11700	82	1186	**1540**	**49**
12	**95**	11	**7960**	**10.9**	**306**	**7698**	1229	507	12900	80	1516	**1520**	**48**
13	39	16	**6153**	3.0	76	4213	1316	**1310**	13100	48	1345	1290	34

Table 3. (Continued)

14	**62**	11	4623	**4.4**	141	**9075**	1007	28	11900	49	1258	1140	44
15	**78**	17	3650	**9.0**	**3135**	**7161**	1170	798	16500	67	1591	**1780**	**101**
18	**143**	20	**12237**	**9.9**	**251**	**7416**	**1663**	**1063**	17700	49	1369	**1650**	37
19	**64**	18	4837	**8.1**	**2042**	**7358**	1151	415	16200	72	1690	**1530**	**86**
20	**94**	15	4342	**7.6**	**288**	**8254**	1185	925	15900	42	1594	1370	43
23	**116**	13	2406	**5.5**	92	5760	717	693	13700	68	1398	**1613**	13
24	**86**	18	**6639**	**7.1**	**3482**	**8748**	919	237	13100	**154**	1269	**1630**	**70**
25	**112**	22	**4892**	**13.0**	**816**	**10844**	**1721**	**1389**	-	**139**	1882	-	**71**

A. alba (1-year-old needles)

6	**301**	15	**9693**	**7.8**	71	6836	1124	**2424**	15500	56	1047	**1890**	27
7	**156**	17	**9749**	**5.8**	101	4757	**1857**	626	15150	42	995	1460	33
12	**244**	17	**7306**	**5.8**	**323**	**9959**	**1536**	322	12400	75	1733	1340	41
13	**112**	21	**8295**	**4.1**	105	3382	**1628**	**2856**	12200	61	1610	1420	33
14	50	14	**9579**	**3.8**	**290**	**6419**	1443	13	11700	38	1190	1230	40

Limit *, **

FS	50–100	15–50	4000–8000	2.0–5.0	50–200	5000–10000	1000–1500	200–1000	18000–25000	30–60	1000–1700	1000–2000	30–45
PA, AA	50–100	15–50	4860	2.0–5.0	50–150	3500–6000	600–1500	200–1000	12000–20000	< 100	1000–2000	1000–1500	30–45

Note: Site: (CZ): 1 – Bílý Kříž; 2 – Javorina; (PL): 3 – Babia Góra; 4 – Bieszczady; 5 – Brenna; 6 – Magura; 7 – Pieniny; 8 – Tatry; (SK): 9 – Malá Fatra–Štefanová; 10 – Malé Karpaty–Geldek; 11 – Vihorlat–Morské Oko; 12 – Poľana; 13 – Stoliky; 14 – Kozie chrbty–Východná; (UA): 15 – Kryvopilja; 16 – Kuzij; 17 – Vizhnitsa; 18 – Synevir; 19 – Uzhoksky Pass; 20 – Yablunitsa; (RO): 21 – Fudata; 22 – Magura; 23 – Obcina Mare; 24 – Rarau; 25 – Stana de Vale; 26 – Retezat
FS: *F. sylvatica;* PA: *P. abies;* AA: *A. alba*. *(Maňkovská, 1996); **(Stefan et al., 1997) – European arithmetical mean. Values in bold face are exceed limit val-

T a b l e 4. Ratio of elements in the foliage of *Fagus sylvatica, Picea abies* and *Abies alba.*

Site	S/N	N/P	N/K	N/Mg	K/Ca	K/Mg	Ca/Mg	Fe/Mn
F. sylvatica								
2	**0.077**	17.7	2.2	12.6	0.909	5.5	6.0	**1.48**
4	**0.073**	19.3	2.4	12.4	0.601	6.2	8.3	**0.94**
6	**0.077**	20.9	2.3	13.9	1.428	5.8	4.8	0.22
9	**0.088**	20.1	1.9	7.9	0.992	3.7	3.6	**2.41**
10	**0.091**	19.1	4.0	7.0	0.662	1.7	3.2	**3.45**
11	**0.092**	21.5	2.5	17.9	1.762	8.4	4.4	0.06
12	**0.094**	17.8	2.6	16.1	1.384	6.3	4.5	0.14
14	**0.111**	11.1	2.6	10.6	0.724	3.9	5.4	0.27
15	**0.100**	14.2	4.0	11.7	0.449	2.9	6.5	**2.95**
16	**0.110**	13.4	2.2	14.1	1.497	6.3	4.3	0.27
17	**0.093**	20.1	3.7	12.1	0.704	3.3	4.6	**3.34**
19	**0.094**	18.5	2.5	13.3	1.269	5.3	4.2	**0.75**
20	**0.088**	21	3.7	12.1	0.950	3.2	3.4	**0.53**
21	**0.094**	19.5	2.9	13.0	1.170	4.5	3.8	**0.75**
22	**0.121**	15.7	3.3	8.3	0.556	2.6	4.6	**2.40**
25	**0.098**	19.2	2.4	12.7	0.927	5.0	5.6	0.29
P. abies								
1	**0.118**	8.82	2.18	12.7	2.41	7.08	2.7	**1.01**
3	**0.116**	10.18	1.45	8.94	1.95	6.69	3.45	**2.29**
5	**0.116**	10.18	1.42	8.78	1.95	6.69	3.45	**2.29**
8	**0.115**	9.37	2.11	19.11	2.53	6.76	3.28	0.15
9	**0.118**	9.87	1.34	8.78	1.94	6.69	3.45	**2.29**
12	**0.118**	8.51	1.57	10.41	0.93	6.26	6.66	**0.63**
13	**0.101**	10.11	3.04	9.79	0.76	3.22	4.0	0.06
14	**0.105**	9.02	1.33	11.08	1.77	8.33	4.46	**5.06**
15	**0.108**	10.37	2.30	14.10	1.96	6.12	3.12	**3.93**
18	**0.093**	12.93	2.39	10.64	0.61	4.46	7.36	0.24
19	**0.094**	9.59	2.2	14.08	1.52	6.39	4.2	**4.92**
20	**0.086**	9.98	1.93	13.42	1.9	6.97	3.66	0.31
23	**0.115**	9.37	2.11	12.99	2.45	6.56	3.28	0.15
24	**0.125**	10.42	1.50	17.3	1.35	9.72	7.51	**12.8**
25	-	-	-	-	2.28	6.97	3.07	**0.63**
A. alba								
6	**0.114**	15.68	2.32	15.04	0.75	6.49	8.29	0.03
7	**0.090**	16.04	2.97	8.76	0.56	2.87	5.46	0.16
12	**0.101**	7.82	1.40	9.05	1.36	6.48	5.03	**1.09**
13	**0.103**	8.21	3.73	8.5	0.45	2.08	5.10	0.04
14	**0.105**	9.44	1.82	8.32	0.63	4.21	6.64	**22.16**
Limit *,**								
FS	0.052	10.6-25.0	1.8-5.0	12.0-25.0	0.6-2.5	3.3-10.0	3.7-8.0	0.50
PA,.AA	0.065	6.0-17.0	1.3-4.9	8.0-28.3	0.6-6.0	2.3-15.0	1.0-10.0	0.50

Notes: explanation see in Table 3

T a b l e 5. Correlation between elements in the foliage of *Fagus sylvatica, Picea abies* and *Abies alba* (26 sites in all countries).

F. sylvatica

Elem.	P	K	Ca	Mg	S	Al	B	Cu	Fe	Mn	Na	Zn
N	0.170	0.461	-0.439	-0.129	**0.479**	0.120	0.057	0.501	-0.058	0.176	-0.196	0.065
P	1.000	0.134	-0.024	-0.105	**0.499**	0.414	-0.162	**0.496**	0.254	0.393	0.435	0.242
K		1.000	-0.402	-0.424	0.137	0.109	0.094	**0.493**	-0.186	0.386	-0.074	-0.002
Ca			1.000	0.300	-0.406	0.059	0.139	-0.368	0.191	-0.370	0.053	0.027
Mg				1.000	-0.041	-0.101	0.240	-0.061	-0.018	-0.400	0.249	-0.133
S					1.000	0.197	-0.261	**0.503**	0.179	0.435	0.202	0.253
Al						1.000	-0.050	0.001	-0.115	0.446	0.441	-0.041
B							1.000	0.010	-0.190	0.071	0.009	-0.336
Cu								1.000	0.102	0.311	-0.023	0.088
Fe									1.000	0.108	0.144	0.659
Mn										1.000	0.384	0.162
Na											1.000	0.153

A. alba

N	-0.195	-0.164	0.186	0.082	**0.605**	0.513	0.252	0.470	-0.352	0.285	0.027	-0.162
P	1.000	0.277	-0.388	-0.011	-0.126	-0.067	0.051	-0.404	**0.540**	-0.011	-0.123	0.536
K		1.000	-0.300	-0.195	-0.199	0.513	-0.414	0.328	**0.526**	-0.486	0.759	0.089
Ca			1.000	0.151	0.241	0.174	0.275	-0.086	0.022	0.014	0.029	0.056
Mg				1.000	0.025	-0.145	0.062	-0.207	-0.098	-0.361	-0.125	0.123
S					1.000	0.330	0.003	0.325	-0.271	0.323	0.110	0.086
Al						1.000	-0.118	**0.611**	0.285	-0.202	0.747	-0.133
B							1.000	0.078	-0.119	**0.506**	-0.466	-0.103
Cu								1.000	-0.255	0.174	**0.494**	**-0.516**
Fe									1.000	-0.556	0.438	0.486
Mn										1.000	**-0.494**	-0.176
Na											1.000	0.022

P. abies

N	0.012	-0.262	0.007	-0.293	**0.856**	0.035	-0.172	-0.361	0.043	-0.263	-0.235	-0.191
P	1.000	0.331	0.143	0.423	0.048	0.064	-0.089	0.203	0.176	0.403	0.082	0.414
K		1.000	0.150	0.226	-0.270	0.174	-0.087	0.244	0.249	-0.177	0.494	**0.539**
Ca			1.000	**0.493**	-0.046	-0.147	-0.011	0.367	0.239	0.259	0.052	0.481
Mg				1.000	-0.288	-0.348	-0.048	0.290	-0.052	0.395	-0.079	**0.495**
S					1.000	0.107	-0.210	-0.383	0.145	-0.268	-0.056	-0.185
Al						1.000	0.205	0.276	0.026	-0.014	0.511	-0.198
B							1.000	0.132	0.178	0.385	0.242	0.030
Cu								1.000	0.190	0.195	0.421	0.444
Fe									1.000	-0.004	0.480	**0.669**
Mn										1.000	-0.030	0.101
Na											1.000	0.305

Note: **Marked correlations** are significant at $p < 0.005$; Elem. – element.

at al. (1997) and Maňkovská (1996), the allowable limit value for B in the foliage of forest trees ranges between 15 and 50 $mg.kg^{-1}$.

Calcium is an essential element and concentrations of this element in tree foliage depend on its availability in soil. In principle, the Ca concentrations correlate with Mg levels. Variation range of Ca in foliage (in $mg.kg^{-1}$) was as follows: 1978 – 12 237 for 1-year-old spruce needles; 7306–9749 for 1-year-old fir needles, and 6453–13813 for beech leaves. In plants Ca is not as mobile as Mg and thus it is being accumulated in older plant tissues. The highest concentrations were determined for beech in Krivopilja (RO) for fir in Pieniny (PL) and for spruce in the Synevir (RO). According to Stefan et al. (1997) the allowable limit value for Ca in the foliage of beech ranges between 4000 and 8000 $mg.kg^{-1}$. The European arithmetical mean value for Ca in foliage is 4860 $mg.kg^{-1}$ for spruce and fir.

Copper is an essential microelement. The concentration of Cu ranged for 1-year-old spruce needles between 3 and 11 $mg.kg^{-1}$, for 1-year-old fir needles between 4 and 8mg kg^{-1} and for beech leaves between 6 and 15 mg kg^{-1}. For the entire Carpathian Mts range the highest concentrations were determined for spruce in Poľana (SK), for fir in Magura (PL) and for beech in Kuzij (RO). According to Maňkovská (1996), the allowable limit value for copper in the foliage of forest tree species is between 2 and 3 $mg.kg^{-1}$. Stefan et al. (1997) gives a value 2.5–3 $mg.kg^{-1}$ for spruce needles; Maňkovská (1996) considers foliar Cu concentration < 5 $mg.kg^{-1}$ as limit level and values >100 $mg.kg^{-1}$ as indicative of extreme load by air pollutants. Total copper concentrations > 5 $mg.kg^{-1}$ is present on about 2/3 of the Slovak territory. Copper was present only in 0.4% of the studied stomata of fir needles.

Phosphorus is an essential element and its limit values range from 1000 to 2000 $mg.kg^{-1}$ (Stefan et al., 1997; Maňkovská, 1996). Foliar concentrations of P ranged for 1-year-old spruce needles between 1005 and 1882 $mg.kg^{-1}$; for beech leaves between 1000 and 1937 $mg.kg^{-1}$ and in 1-year-old fir needles between 995 and 1733 $mg.kg^{-1}$. For the entire Carpathian Mts range the highest concentrations were determined in Romania for spruce (Stana de Vale) and beech (Kuzij – UA), and for fir in Poľana (SK).

Iron is a typical essential element with physiological enzymatic function. In higher concentrations its effects on plants are toxic. According to Stefan et al. (1997) normal concentrations in healthy spruce needles are close to 50 $mg.kg^{-1}$ and in healthy beech leaves about 129 $mg.kg^{-1}$. Innes (1995) found in 2- years-old Norway spruce needles concentrations in a range of 40–169 $mg.kg^{-1}$ and in Scots pine needles (*Pinus sylvestris* L.*)* values in a range of 77–373 $mg.kg^{-1}$. Optimal value for Fe in foliage is within the range 50–200 $mg.kg^{-1}$ (Maňkovská, 1996). In this study, Fe concentrations in 1year old spruce needles were 76–3482 $mg.kg^{-1}$; in beech leaves 99–1090 $mg.kg^{-1}$, and in 1-yearold fir needles 71–323 $mg.kg^{-1}$. Concentration of Fe is elevated in all studied tree species and it is obviously connected with the presence of Fe in fly ashes emitted from heating plants. The highest Fe concentrations were determined in spruce needles – Rarau (UA); in beech leaves – Magura (RO) and fir needles in Poľana (SK).

Potassium is an essential element. It can be replaced by rubidium, caesium, barium, lead and thallium. Essential to all organisms, it has electrochemical, catalytic and enzymatic (enzyme activation) functions, and supports osmoregulation and hydration. Oxygen deficiency disturbs water balance (withering tips of leaves, contorted older leaves, premature loss of older needles, rotting roots). Variations in potassium concentration in foliage of forest tree species are controlled by soil. The concentration of K ranged for 1-year-old spruce needles between 4213 and 10844 $mg.kg^{-1}$, for 1-year-old fir needles between 3382 and 9959 $mg.kg^{-1}$ and for beech leaves between 6453 and 13813 $mg.kg^{-1}$. According to the data K concentration from 5000 up to 10000 $mg.kg^{-1}$ is optimal and sufficient. Markert et al. (1996) found in Scots pine 4400 $mg.kg^{-1}$. The highest concentration was determined in Stana de Vale, Romania (spruce), Magura, Poland (beech) and Poľana, Slovakia (fir).

Magnesium concentration in foliage of forest trees depends on its soil content. Optimal nutritional values for Mg range from 600 to 1500 $mg.kg^{-1}$ (Stefan et al., 1997). The Mg concentrations are higher in older needle classes of healthy trees. In regions affected by air pollutants, the Mg concentrations increased up to the 3rd year needle class, and then dropped. Low values of Mg correlate with needle yellowing. Maňkovská (1996) showed that concentration of Mg in the needles of older trees (4000 $mg.kg^{-1}$) was lower in comparison with younger individuals (6200 $mg.kg^{-1}$). The range of Mg found in this study are as follows: 717–1721 $mg.kg^{-1}$ for spruce needles, 1382–3680 $mg.kg^{-1}$ for beech leaves, and 1124–1857 $mg.kg^{-1}$ for fir needles. The Ca concentrations in principle correlate well with the Mg levels. The highest Mg concentrations were determined in Stana de Vale, Romania (spruce); Malé Karpaty–Geldek, Slovakia (beech) and Pieniny, Poland (fir).

Manganese is an essential element that becomes toxic in higher concentrations. Its mobilization indicates the disturbance of a physiological balance leading to a change of the Mn/Fe (normal ratio should be 1:2) (Maňkovská, 1996). According to Markert et al. (1996), Mn concentrations in spruce needles correlate well with needle loss. Therefore Mn is being used as an indicator of tree damage (Maňkovská, 1996). Manganese mobilization indicates an unstable state in the regime of mineral substances of forest stands as well as trees. According to Stefan et al. (1997), needles the limit of insufficiency of Mn is about 20 $mg.kg^{-1}$ for spruce with an optimal content **>** 50 $mg.kg^{-1}$. Stefan et al. (1997) found in healthy 1-year-old spruce needles 320 $mg.kg^{-1}$ and in damaged needles 1300 $mg.kg^{-1}$, and in healthy beech leaves 940 $mg.kg^{-1}$. Innes (1995) found in 2-years-old needles of spruce 100–5540 $mg.kg^{-1}$ and in pine 150–1740 $mg.kg^{-1}$. In this study, Mn concentration in tree foliage ranged from 28 to 1389 mg. kg^{-1} for spruce (1-year-old needles); from 39 to 2741 $mg.kg^{-1}$ for beech and from 13 to 2856 mg kg^{-1} for fir (1-year-old needles). The highest values of Mn in spruce needles were found in Stana de Vale (RO), in beech leaves in Vihorlat-Morské Oko and fir needles in Stoliky (SK).

Sulphur and nitrogen are essential plant nutrients. S and N air pollutants as SO_2, H_2S, NO_2, NH_3, or HNO_3 can cause increased foliar concentrations of both elements in plants. With regard to damage to forest ecosystems, three main reasons of S toxicity should be considered: damage to roots from elevated concentrations of S in humus complex, damage to foliage by S metabolites resulting from excessive SO_2 and H_2S uptake and redistribution and accumulation of S in older organs (older leaves, wood, etc.). Sulphur is an important nutrient limiting the growth of plants – both excess and deficiency of S may cause growth reduction (Innes, 1995).

Concentrations of S in 1-year-old spruce needles ranged from 1140 to 1780 mg.kg^{-1}, in beech leaves from 1550 to 2730 mg.kg^{-1}, and in fir needles from 1230 to 1890 mg.kg^{-1}. According to Innes (1995) S concentrations in spruce needles range from 800 to 1000 mg.kg^{-1}, what corresponds with our data (Maňkovská, 1988). In polluted regions the S concentration in spruce and fir needles increases markedly up to 5000 mg.kg^{-1}. The values from 1000 to 1500 mg.kg^{-1} for coniferous trees and 1000 to 2000 mg.kg^{-1} for broadleaved trees are considered as sufficient. Higher concentrations should be considered undesirable. These observed high values confirm marked impact of sulphur oxides in the entire range of the Carpathian Mountains. The highest values of S were found in spruce in Kryvopilja (UA), in beech in Magura (RO) and in fir in Magura (PL).

Concentrations of N in 1-year-old spruce needles in the Carpathian Mts ranged from 11700 to 17700 mg.kg^{-1}, in beech leaves from 18000 to 29700 mg.kg^{-1}, and in 1-year-old fir needles from 11700 to 15500 mg.kg^{-1}. Maňkovská (1996) considers 13500–17000 mg.kg^{-1} as sufficient foliar concentrations for tree species. The S/N ratio in the foliage of spruce needles ranged from 0.086 to 0.125, in beech leaves from 0.073 to 0.121, and in 1-year-old fir needles from 0.09 to 0.114 (Table 4). The S/N ratio is a sensitive indicator of S accumulation in the foliage of forest trees subjected to atmospheric pollution. Molar ratio of protein S and protein N ranges from 0.05 to 0.15 (Stefan et al., 1997) and it is relatively constant for all tree species. The S/N ratio is no optimally balanced (Table 2) in all study tree species when compared with the limit ranges. The highest values of N were found for spruce in Synevir (UA), for beech in Poľana (SK), and for fir in Magura (PL).

Sodium concentrations (in mg.kg^{-1}) in 1-year-old spruce needles ranged from 42 to 154 mg.kg^{-1}, in beech leaves from 50 to 168 mg.kg^{-1}, and in fir needles from 38 to 75 mg.kg^{-1}. Markert (1993) reported in pine needles 101 mg.kg^{-1} and Maňkovská (1996) found values lower than 100 mg.kg^{-1} in forest trees. Sodium is an essential element for higher plants and it has an important electrochemical function. The highest values of Na were found for spruce in Rarau (RO), for beech in Kozie Chrbty–Východná (SK) and for fir in Poľana, (SK).

Zinc is an essential element for plants. Zinc is a constituent of chlorophyll, activates enzymes, and takes part in dehydrogenase, protein degradation and formation of growth agents (Maňkovská, 1996). In this study, foliar Zn concentrations ranged as follows: 11–101 mg.kg^{-1} for 1-year-old spruce needles; 25–63 mg.kg^{-1} for beech leaves and 27–41 mg.kg^{-1} for 1 year old fir needles. The highest Zn concentrations were determined for spruce in Kryvopilja (UA); for beech in Uzhodsky pass (UA) and for fir in in Poľana (SK). According to Stefan et al. (1997) optimum for Zn concentrations for the spruce needles are about 50 mg.kg$^{-1.}$

The principal component analysis (PCA) was used for data processing and determination of interrelations of the concentrations of elements accumulated in the foliage of studied tree species (Table 6). 95.3% of total variability was explained by means of 9 factors. All weights of components in PC1 (the first principal component) up to PC9 (the last principal component) are comparable for the Carpathian Mountains. Individual components PC1 up to PC8 have different significances. PC1 accounts for 29.6% of commonality and shows the highest negative values: for sites, K, Cu, Na, Zn, partially for P, Ca, Mg, Fe (beech leaves); for Zn partially for sites (spruce needles); and for sites, partially for N, P, S and Cu (fir

T a b l e 6. Percentage of explained variance for night factors obtained in the PCA (Varimax analysis).

Fagus sylvatica

Cumul. variability (%)	**29.6**	**44.9**	**59.7**	**69.8**	**78**	**84.5**	**88.8**	**92.8**	**95.3**
Factor variable	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Site	**-0.804**	0.051	0.314	0.023	0.121	0.143	-0.389	-0.110	-0.016
N	0.460	0.369	**0.660**	0.283	-0.093	-0.140	0.143	-0.071	-0.070
P	**-0.509**	-0.024	0.295	0.222	**-0.623**	0.342	0.104	0.182	-0.089
K	**-0.595**	0.327	-0.068	-0.493	-0.251	0.174	0.159	-0.343	-0.155
Ca	**-0.573**	-0.150	0.355	0.155	0.140	**-0.570**	-0.073	-0.281	-0.175
Mg	**-0.607**	**-0.548**	0.220	-0.017	-0.199	-0.109	0.240	-0.130	0.346
S	0.428	0.480	**0.637**	0.290	-0.114	-0.069	0.035	-0.030	0.198
Al	0.029	**0.605**	**-0.483**	0.257	-0.443	-0.190	-0.055	-0.072	-0.115
B	-0.142	0.050	-0.493	**0.603**	0.355	0.145	0.404	-0.201	-0.030
Cu	**-0.621**	0.074	-0.336	0.046	-0.134	-0.518	0.068	**0.390**	0.006
Fe	**-0.525**	**0.549**	0.241	0.124	**0.454**	0.187	-0.012	0.230	-0.035
Mn	-0.405	-0.449	-0.118	**0.688**	-0.117	0.175	-0.240	-0.031	-0.028
Na	**-0.487**	0.671	-0.357	0.007	-0.002	0.001	-0.155	-0.127	0.340
Zn	**-0.840**	0.122	0.281	-0.138	0.176	0.039	0.261	0.158	-0.054

Picea abies

Site	**-0.395**	0.035	0.218	0.019	0.114	0.149	**-0.505**	-0.148	-0.027
N	0.226	0.252	**0.458**	0.238	-0.087	-0.146	0.185	-0.095	-0.116
P	-0.250	-0.016	0.205	0.186	**-0.584**	0.356	0.135	0.244	-0.148
K	-0.293	0.224	-0.047	**-0.414**	-0.235	0.181	0.207	**-0.460**	-0.258
Ca	-0.281	-0.103	0.246	0.130	0.131	**-0.594**	-0.094	-0.377	-0.291
Mg	-0.298	-0.374	0.153	-0.014	-0.187	-0.113	0.311	-0.174	**0.576**
S	0.211	0.328	**0.442**	0.243	-0.107	-0.072	0.045	-0.040	0.329
Al	0.014	**0.413**	-0.335	0.215	**-0.415**	-0.198	-0.071	-0.097	-0.192
B	-0.070	0.034	-0.342	**0.506**	0.333	0.151	0.525	-0.269	-0.049
Cu	-0.305	0.050	-0.233	0.039	-0.126	**-0.539**	0.089	0.522	0.010
Fe	-0.258	**0.375**	0.167	0.104	**0.425**	0.195	-0.016	0.308	-0.059
Mn	-0.199	-0.307	-0.082	**0.577**	-0.110	0.182	-0.312	-0.041	-0.046
Na	-0.239	**0.459**	-0.248	0.006	-0.002	0.001	-0.202	-0.170	**0.565**
Zn	**-0.413**	0.083	0.196	-0.115	0.165	0.040	0.339	0.211	-0.089

Abies alba

Site	**-0.470**	0.067	0.051	-0.086	0.085	0.024	-0.224	0.204	-0.029
N	**0.373**	0.046	**0.335**	-0.112	-0.190	-0.218	0.472	-0.241	0.003
P	**-0.315**	-0.079	0.233	**-0.455**	-0.174	-0.267	0.128	0.291	0.311
K	-0.043	**-0.480**	-0.113	-0.156	0.011	-0.130	-0.215	-0.121	0.432
Ca	0.116	0.083	**0.416**	0.368	**0.520**	0.294	-0.030	0.092	0.420
Mg	-0.055	0.075	0.246	0.517	-0.307	**-0.572**	-0.302	0.096	0.176
S	0.278	0.039	**0.421**	-0.114	-0.367	0.306	-0.310	**0.460**	-0.272
Al	0.280	**-0.370**	0.227	-0.097	0.133	-0.098	0.250	0.153	0.124
B	0.060	0.295	0.217	-0.219	**0.512**	-0.464	-0.225	-0.131	-0.239
Cu	**0.405**	-0.174	-0.150	-0.172	0.058	-0.191	**-0.424**	-0.103	-0.190
Fe	-0.273	**-0.342**	0.244	-0.086	0.310	-0.040	0.142	0.178	-0.416
Mn	0.163	0.353	-0.002	**-0.476**	0.054	0.163	-0.229	-0.004	0.387
Na	0.127	**-0.491**	0.027	0.068	0.061	0.114	-0.284	-0.042	0.007
Zn	-0.288	-0.070	0.475	-0.083	-0.200	0.232	-0.174	**-0.697**	-0.062

Note: Characteristic elements for the factors are marked in **bold type.**

needles). PC2 accounts for 44.9% of variability and shows: the highest positive values for Al, and Fe (beech leaves), partially for Mg negative values; the highest positive values for Al, Fe, Na (spruce needles); the highest negative values for K, Al, Fe and Na (fir needles). PC3 accounts for 59.7% of variability and has the highest positive values for N and S (beech leaves, spruce and fir needles). PC4 (69.8% of variability) has: the highest positive values for B and Mn (beech leaves and spruce needles), partially negative values for K (spruce needles); and negative values for P and Mn (fir needles). PC5 (78% of variability) has: the highest negative values for P and partially positive values for Fe (beech leaves); the highest negative values for P, partially negative value for Al and partially positive values for Fe (spruce needles); the highest negative values for Ca and B, e (spruce needles); PC6 (84.5% of variability) has: the highest negative values for Ca (beech leaves); the highest negative values for Ca and Cu (spruce needles); the highest negative values for Mg (fir needles). PC7 up to PC9 account for less than 5% of total variability: for site, B, Cu, Na (beech leaves); for site, K, Mg, Na (spruce needles); for N, Cu, S, Zn, Fe (spruce needles).

Epicuticular waxes

The epicuticular waxes (EW) provide a physical barrier to protect foliage from pathogen infection (Mengen, 1996). It is well known that air pollutants can alter seriously the appearance of these waxes (Kerstien, 1996) and that these changes are dramatically manifested in leaves of aspen growing under elevated O_3 (Maňkovská et al., 1998; Karnosky et al., 1999, 2002). Differences in EW structure were quantified by a coefficient of occlusion (Q) (arithmetical mean of wax quality of 200 stomata per leave). Percentage of EW in wax tube distribution classes (Table 1) for spruce is shown in Fig. 1, fir in Fig. 2, and for beech in Fig. 3. Evaluation of EW quality by Q for all forest tree species and all sites is presented in Table 7. The Kolmogorov–Smirnov test revealed statistically significant differences in the quality of stomata between 25 studied localities (Fig. 1) for 83% spruce, 66% fir, and 63% beech (Table 8). Statistically significant differences between the qualities of stomata of other samples were not found. The highest Q for spruce needles had the value 3.80 for Yablunitsa (Ukraine) (approx. 45% damage of EW) comparing to 2.29 in Východná (Slovakia). It is interesting that the worst state of EW was found in Ukraine (Yablunitsa), where at the same time we found increased concentration of all studied elements in spruce needles. On the contrary in Slovakia (Východná), where we found least damaged EW, also the concentration of studied elements was lower in spruce needles as well the lowest concentration of SO_2 were found. It appeared to be connected with increased presence of fungi and mycelia, which get on disturbed surface of spruce needles much easier (Maňkovská, 1996), as well as with the occurrence of various particles deposited in the stomata (Table 9).

The highest Q for fir needles had value 2.47 for Stoliky (Slovakia) (approx. 30% damage of EW) comparing to 1.31 in Východná (Slovakia). The Stoliky site is one of the most polluted ones in the Carpathian Mts and at the same time it is being subjected to the impact of heavy metals emission and SO_2 from three smelter complexes. The Východná site is subjected to SO_2 emission the least (Maňkovská, 1996).

T a b l e 7. Evaluation of epicuticular waxes by coefficient of occlusion „Q“.

Fagus sylvatica		*Picea abies*		*Abies alba*	
Site	x(SD)	Site	x(SD)	Site	x(SD)
2	1.65(0.82)	1	2.42(1.04)	6	2.06(0.89)
4	2.14(0.82)	3	3.29(1.08)	7	2.23(1.00)
6	2.11(0.93)	9	2.71(0.91)	9	2.46(1.31)
9	2.74(1.01)	12	3.07(0.81)	12	1.78(0.74)
10	1.70(1.00)	13	2.90(1.06)	13	2.47(1.16)
11	2.58(1.00)	14	2.29(1.11)	14	1.31(0.80)
12	1.92(0.77)	15	3.36(0.92)		
14	2.27(1.01)	16	3.69(0.85)		
15	1.85(0.78)	19	3.36(0.92)		
16	1.85(0.70)	20	3.80(0.83)		
17	2.37(1.00)	23	2.81(1.04)		
19	1.76(0.84)	24	2.86(1.12)		
20	2.58(1.03)	25	2.51(0.99)		
21	2.06(0.95)				
22	2.03(0.93)				
23	1.97(0.93)				

Notes: explanation see in Table 3

The highest Q for beech leaves had value 2.74 for Štefanová (Slovakia) (approx. 30% damage of EW) comparing to 1.65 in Javorina (Czech Republic). Štefanová is being influenced mainly by emissions from heating plants (Považská Bystrica and Žilina) (Maňkovská, 1996). The Javorina (CZ) site is influenced by emissions the least (Suchara et al., 2007).

Qualitative analysis of air-borne pollutant particles from the surface and stomata of foliage and fungi effects is presented in Table 9. Characteristics of particles deposited in the stomata of the studied foliage are given in Table 2. Particles of E and B (ash) category were present in 100% of foliage from all localities. Stomata of the observed trees contained Al and Si of mineral and ash origins; Fe from mineral particles, ash and Fe_2O_3 found in a vicinity of metallurgy complex and thermal power plant; Ca, Mg, K, and Cl originating from mineral and biological particles. The industrial particles were present almost in all localities, with the exception of category F_2 in Babia Gora, Pieniny (Poland), Štefanová and Poľana (Slovakia); category F_4 in Fundata (Romania); category F_6 in Morské oko, Poľana (Slovakia) and Vizhnitsa (UA). Ba was found at Brenna (PL), Poľana (SK), Synevir, Yablunitsa (UA). Cu was found at Poľana (SK), Vizhnitsa (RO), Magura (RO). Cr was found at Magura (PL). Mn was found at Babia Gora, Brenna, Magura (PL), Vizhnitsa, Uzhodsky pass (UA), Rarau (RO) and V in Brenna (PL). Zn was found at Brenna, (PL), Vizhnitsa,Uzhodsky pass, Yablunitsa (UA), Fundata Magura (RO), Ni Vizhnitsa (UA). Particles containing Ge were found only in Magura (RO).

T a b l e 8. Kolmogorov –Smirnov test of stomata quality.

Fagus sylvatica

Site	4	6	9	10	11	12	14	15	16	17	19	20	21	22	25
2	**4.07**	**3.20**	**5.85**	0.90	**5.10**	**2.77**	**2.23**	1.88	0.69	**2.96**	1.56	**3.41**	**3.04**	**2.53**	**2.34**
4	-	0.85	**4.24**	**5.04**	**3.46**	1.12	1.14	1.43	1.11	1.27	2.18	**2.17**	1.04	1.60	1.80
6	-	-	**4.21**	**4.15**	**3.44**	1.21	1.19	0.92	1.09	1.27	1.58	**2.17**	0.56	0.73	0.93
9	-	-	-	**6.07**	0.78	**5.37**	**3.00**	**4.01**	**4.12**	1.81	**4.08**	0.90	**4.78**	**4.34**	**4.89**
10	-	-	-	-	**5.57**	**3.71**	**4.49**	**2.24**	**2.54**	**3.65**	1.34	**4.10**	**3.99**	**3.49**	**3.29**
11	-	-	-	-	-	**4.62**	**2.23**	**3.45**	**3.57**	1.24	3.54	0.33	**4.01**	**3.60**	**4.11**
12	-	-	-	-	-	-	**2.38**	0.51	0.25	0.63	2.16	**3.05**	0.67	1.12	0.63
14	-	-	-	-	-	-	-	**1.80**	1.95	0.40	1.96	1.30	1.75	1.31	1.82
15	-	-	-	-	-	-	-	-	0.29	1.82	0.68	**2.57**	0.68	0.86	0.50
16	-	-	-	-	-	-	-	-	-	1.95	0.29	**2.68**	0.70	1.03	0.67
17	-	-	-	-	-	-	-	-	-	-	1.97	0.75	1.68	1.36	1.73
19	-	-	-	-	-	-	-	-	-	-	-	**2.69**	1.46	1.09	0.95
20	-	-	-	-	-	-	-	-	-	-	-	-	**2.58**	**2.27**	**2.63**
21	-	-	-	-	-	-	-	-	-	-	-	-	-	0.54	0.75
22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.51

Picea abies

Site	3	5	8	9	12	13	14	15	18	19	20	23	24	25
1	**3.99**	**6.18**	1.93	1.95	**4.21**	**2.21**	1.26	**4.19**	**4.55**	**3.34**	**4.73**	**2.11**	1.81	0.66
3	-	**2.25**	**3.78**	**3.72**	1.84	**2.72**	**5.27**	1.70	1.88	0.61	**2.14**	**2.49**	**2.21**	**3.06**
5	-	-	**5.44**	**5.26**	**3.12**	**5.00**	**7.46**	0.61	0.78	0.91	1.07	**4.02**	**4.45**	**4.81**
8	-	-	-	1.25	**3.50**	**2.10**	**2.57**	**4.19**	**4.55**	**2.84**	**4.72**	1.41	1.72	1.41
9	-	-	-	-	**2.26**	**2.02**	**3.21**	**4.15**	**4.50**	**2.73**	**4.68**	1.18	1.65	1.09
12	-	-	-	-	-	**2.47**	**5.48**	**2.70**	**2.97**	**1.25**	**3.20**	**2.07**	**2.47**	**3.08**
13	-	-	-	-	-	-	**4.49**	**3.39**	**3.76**	**2.89**	**3.97**	0.50	0.65	1.63
14	-	-	-	-	-	-	-	**4.52**	**5.35**	**4.22**	**5.45**	**3.36**	**3.09**	1.78
15	-	-	-	-	-	-	-	-	0.47	1.20	0.67	**3.33**	**3.05**	**3.75**
18	-	-	-	-	-	-	-	-	-	1.34	0.31	**3.63**	**3.35**	**4.04**
19	-	-	-	-	-	-	-	-	-	-	1.57	1.90	2.11	**2.65**
20	-	-	-	-	-	-	-	-	-	-	-	**3.84**	**3.57**	**4.23**
23	-	-	-	-	-	-	-	-	-	-	-	-	0.49	1.22
24	-	-	-	-	-	-	-	-	-	-	-	-	-	1.23

Abies alba

Site	7	9	12	13	14
6	1.788	1.658	1.421	**3.322**	**7.013**
7	-	1.397	**3.071**	1.655	**6.596**
9	-	-	**2.682**	0.673	**5.027**
12	-	-	-	**4.993**	**5.550**
13		-	-	-	**9.284**

Notes: explanations see in Table 3

λ ($P_{0.05} = 1.358$; $P_{0.01} = 1.628$; $P_{0.001} = 1.950$)

T a b l e 9. Categories of air-borne particles found on the surface and in the stomata of spruce, fir, and beech foliage [%], fungi effect [%] and elements.

Site	Fungi effect in %*				Category of particles in %**						Presence of elements (X ray spectrum)***	
	1	2	3	4	A	B	E	F_2	F_4	F_6	> 50%	< 50%
1	33	67	-	-	33	100	100	17	50	50	Al,Ca,Fe,S,Si	K,Mg,Ti
2	67	33	-	-	67	100	100	33	50	50	Al,Ca,Cl,S,Si	K,Mg,Na
3	67	33	-	-	67	100	100	-	50	100	Al,Ca,Fe,P,S,Si,Ti	K,Mg,**Mn**
4	67	-	33	-	67	100	100	50	83	67	Al,Ca,Fe,K,S,Si	Mg,Na,Ti
5	-	50	50	-	67	100	100	33	67	50	Al,Ca,Fe,Si,Ti,**Zn**	**Ba**,K,Mg,**Mn,V**
6	50	50	-	-	67	100	100	33	33	17	Al,Ca,Fe,K,**Mn,**Si,Ti	**Cr**,K,Mg
7	67	33	-	-	67	100	100	-	33	67	Al,Ca,Fe,Si,Ti	K,Mg
8	50	50	-	-	33	100	100	67	33	100	Al,Ca,Fe,Si,Ti	K,Mg,S
9	67	33	-	-	33	100	100	-	67	33	Al,Ca,Fe,Mg,Fe,Si	K,Ti
10	50	50	-	-	67	100	100	33	67	33	Ca,Cl,Fe,K,Na,Si	K,Al
11	67	33	-	-	33	100	100	67	33	-	Al,Fe,Si	K,Mg,Ti
12	33	17	50	-	33	100	100	-	67	-	Al,**Ba**,Ca,Fe,S,Si,Ti	K,Mg,**Cu**
13	33	67	-	-	33	100	100	67	33	33	Al,Ca,Fe,Si,Ti	K,Mg
14	33	67	-	-	33	100	100	67	33	100	Al,Ca,Fe,Si,Ti	K,Mg
15	17	33	50	-	33	100	100	33	33	33	Al,Ca,Fe,S,Si,Ti	K,Mg
16	33	17	50	-	33	100	100	67	33	33	Al,Fe,K,Si	Ca,Mg,Ti
17	50	-	50	-	33	100	100	67	33	-	Ca,**Cu**,Fe,**Ni**,Ti,**Zn**	Al,K,Mg,**Mn**,Si
18	50	-	50	-	67	100	100	67	33	100	Al,Ca,Cl,Fe,Na,Si,Ti	**Ba**,K,Mg
19	33	17	50	-	33	100	100	67	33	33	Al,Ca,Fe,Si,Ti	K,Mg,**Mn**,Na
20	17	33	50	-	67	100	100	67	33	100	Al,**Ba**,Ca,K	Cl,Fe,Mg,Na,Ti,**Zn**
21	17	33	50	-	67	100	100	67	-	100	Al,Ca,Fe,S,Si	K,Mg,**Zn**
22	33	17	50	-	33	100	100	33	33	100	Al,Ca,**Ge**,Fe,K,Fe,S,Si	Cl,Mg,Na,**Cu,Zn**
23	33	17	50	-	33	100	100	33	33	100	Al,Ca,Cl,Fe,Si	K,Mg,Na,Ti
24	33	17	50	-	33	100	100	33	33	100	Al,Ca,Fe,K,Si	K,Mg,**Mn**
25	17	33	50	-	67	100	100	33	33	100	Al,Ca	K,Mg,Ti
26	33	17	50	-	33	100	100	33	33	100	Al,Ca	K,Mg,Ti

Notes: explanations see in Table 3
* Fungi effect (infection) was classified according to 5 classes: 1 – absence of fungal particles (spore and mycelium); 2 – < 10 particles; 3 – 11 to 25 particles; 4 – > 25 particles with covering less than 25% of surface, and 5 – >25% of foliage surface covered.
** Category of particles were divided into four basic groups: biological (A), mineral (B), coal and fuel oil ash (E), and industrial – category F_2 (cement and lime plant); category F_4 (iron plant) and category F_6 (others technologies).
*** Presence of elements reflects chemical composition of particles shown in Table 2. Elements with the maximal concentration are in bold.

It is theoretically possible to wash away the particles settled on the foliage surface but there is no possibility to wash away the particles deposited in the stomata of foliage (Maňkovská, 1996). The presence of these particles in stomata increases total concentration of studied elements in the foliage. Especially dangerous is if nutrition elements (Fe, Mg, Ca and K) are present in insufficient amount. Moreover their presence in stomata cannot be used from physiological aspect. For example, concentration of Mg was below the limit in all studied spruce sites in Ukraine and Slovakia (Table 3). Mg was present also in particles occurring in foliage stomata on all studied localities of Ukraine and Slovakia (except for locality Štefanová, Malá Fatra Mts) (Table 9). On the basis of this finding we can state a critical depletion of Mg-basic nutrient in spruce foliage in Ukraine and Slovakia. Nutrition deficiency resulted in a discoloration and dysfunction of the assimilation organs. This finding needs detailed and continuous research.

Conclusion

The following conclusions were drawn based on evaluation of foliage of *Picea abies, Abies alba* and *Fagus sylvatica* on 26 sites (1101 trees) in the Carpathian Mts forests:

1. The Carpathian Mts forests are subjected to air pollution impact. We recorded significant effect of SO_2, which resulted in increased S concentrations in forest tree foliage on all sites. Sulphur concentration exceeded the European limit values in all sites. Increased concentration of S in the foliage was also manifested as the disturbed S/N ratio and N/S correlation disturbance.
2. The Carpathian Mts forests have unbalanced concentration of nutrition elements and microelements. We found increased concentrations of Al (50% of sites), Ca (88%), Cu (100%), Fe (94%), K (38%), Mg (75%), Mn (25%), N (56%), Na (88%), P (6%), Zn (31%) in the leaves of beech. Concentration of B has not exceeded European limit values. Spruce needles had increased concentrations of Al (20%), Ca (33%), Cu (93%), Fe (53%), K (67%), Mg (13%), Mn (20%), Na (60%), P (7%), Zn (47%). Concentrations of B and N have not exceeded European limit values. Fir needles had increased concentrations of Al (80%), Ca (100%), Cu (100%), Fe (20%), Mg (60%), Mn (60%), Na (40%), P (20%). Concentrations of B, K, N and Zn have not exceeded European limit values.
3. We found above-limit concentrations of Mn in beech leaves (Štefanová and Geldek), in spruce needles (Babia Gora, Brenna, Štefanová and Východná), in fir needles (Východná). Mn mobilization values indicate the disturbance of a physiological balance leading to the change of the ratio with Fe. Abnormal ratio of Fe/Mn was found in Geldek, Fundata (beech), Rarau, Uzhoksky Pass (spruce) and Východná (spruce, fir). Above-limit and below-limit concentration of nutritionally significant elements cause deterioration of the health condition of the studied forest trees and disturb biogeochemistry of forest stands.
4. The SEM investigation of foliage waxes of 3 forest tree species from 26 sites in the Carpathian Mts showed a statistically significant difference in mean wax quality be-

tween studied tree species in individual localities. Epistomatal waxes were damaged, as detected by creation of net-like and amorphous waxes. The most damaged stomata in spruce needles were from Yablunitsa, Synevir and Brenna; in fir needles from Stoliky and in beech leaves from Malá Fatra, Morské Oko and Yablunitsa. All spruce needles in the Carpathian Mts had more damaged stomata than fir and beech foliage.

5. Foliage surfaces of three forest tree species contained Al, Si, Ca, Fe, Mg, K, Cl, Mn, Na, Ni, and Ti in all studied sites. In the site Magura, Romania the particles with a high content of Ge were found. Exceedance of individual elements on surfaces of foliage hinders opening and closing of stomata and it is not physiologically usable for trees. Presence of these metals on foliar surfaces can be used as an indication of environmental pollution in the Carpathian Mts forests.

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References

Altieri, A., Del Caldo, L., Manes, F., 1994: Morphology of epicuticular waxes in *Pinus pinea* needles in relation to seasonal and pollution-climate. Eur. J. For. Path., *2*: 79–91.

Ayodele, J.T., Ahmed, A., 2001: Monitoring air pollution in Kano municipality by chemical analysis of Scots Pine (*Pinus sylvestris* L.) needles for sulphur content. Environmentalist, *21*: 145–151.

Buse, A., Norris, D., Harmens, H., Büker, P., Ashenden, T., Mills, G., 2003: Heavy Metals in European Mosses: 2000/2001 Survey. UNECE ICP Vegetation Coordination Centre, Centre for Ecology and Hydrology, Bangor, UK, 43 pp.

Bytnerowicz, A., Szaro, R., Karnosky, D.F., Manning, W., McManus, M., Musselmann, R., Muzika, R.M., 2002: Importance of international research cooperative programs for better understanding of air pollution effects on forest ecosystems in central Europe. Effects of air pollution on forest health and biodiversity in forests of the Carpathian Mountains. IOS Press, p. 13–20.

Fraczek, W., Postelnicu, D., Bytnerowicz, A., Černý, M., Vasile, C., 2002: GIS as a tool for evaluating air pollution and biological resources of Carpathian forests. IOS Press, p. 121–137.

Hlawiczka, S., Dyduch, B., Fudala, J., 2003: Long-term changes of particulate emission in the industrial region of upper Silesia (Poland) and their effect on the acidity of rainwater. Water, Air, Soil Pollut., *14*: 151–163.

Hovmand, M.F., Bille-Jansen, J., 1997: Atmospheric input to Danish spruce forests and effects on soil acidification and forest growth based on 12 years measurement. Water, Air, Soil Pollut., *116*: 75–88.

Hunter, I.R., 1994: Results from the Interlaboratory sample exchange. IUFRO, Working Group S1.02 –08 Foliar Analysis. Natural Resources Institute, Kent, 18 pp.

Innes, J., 1995: Influence of air pollution on the foliar nutrition of conifers in Great Britain. Environ. Pollut., *88*: 183–192.

Karaoz, M.O., 2003: Air pollution effects on forest trees in Balikeshir, Turkey. Water, Air, Soil Pollut., Focus *3*: 269–279.

Karnosky, D.F., Maňkovská, B., Percy, K.E., Dickson, R.E., Podila, G.K, Sober, J., Noormets, A., Hendrey, G., Coleman, M.D, Kubiske, M., Pregitzer, K.S., Isebrands, J.G., 1999: Effects of tropospheric O_3 on trembling aspen and interaction with CO_2: Results from an O_3-gradient and a FACE experiment. In Sheppard, L.C., Cape, J.N. (eds), Forest growth responses to the pollution climate of the 21st century. Kluwer Academic Publishers, London, *116*, p. 311–322.

Karnosky, D.F., Percy, K.E., Xiang, B., Callan, B., Noormets, A., Maňkovská, B., Hopkin, A.,,Sober, J., Jones, W., Dickson, R.E.,. Isebrands, J.G., 2002: Interacting CO_2-tropospheric O_3 and predisposition of aspen (*Populus tremuloides* Michx.) to infection by *Melampsora medusae* R u s t. Global Change Biol., *8*: 329–338.

Kaupenjohan, M., Zech, W., Hantschlel, R., Horn, R., Schneider, B.U., 1989: Mineral nutrition of forest trees.A regional survey. In Schultze, E.D., Lange, O.L., Oren, R. (eds), Forest decline and air pollution. Ecol. Stud., *77*: 182–294.

Kerstien, G., 1996. Barrier properties of the cuticle to water, solutes and pest and pathogen penetration in leaves of plants grown in polluted atmospheres. In Ynnus, M., Iqbal, M. (eds), Plant response to air pollution. John Wiley and Sons Ltd., New York, p. 167–178.
Lofgren, S., Brngmark, L., Aastrup, M., Hultberg, H., Kindbom, K., Kvarnas, H., 2001: Sulphur balances and dynamics in three forested catchmens in Sweden. Water, Air, Soil Pollut., *130*: 631–636.
Lomander, A., Johansson, M.B., 2001: Changes in concentrations of Cd, Zn, Mn, Cu and Pb in spruce *(Picea abies)* needle litter during decomposition. Water, Air, Soil Pollut., *132*: 165–184.
Lovett, G.M., Weathers, K.C., Arthur, M.A., Schultz, J.C., 2004: Nitrogen cycling in a northern hardwood forest: Do species matter. Biogeochemistry, *67*: 289–308.
Maňkovská, B., 1988: The accumulation of atmospheric pollutants by *Picea abies* K a r s t. Ekológia (ČSFR), *7,* 1: 95–108.
Maňkovská, B., 1996: Geochemical atlas of Slovakia- forest biomass (in Slovak, English). Geologická služba Slovenskej republiky, Bratislava, 87 pp.
Maňkovská, B., Percy, K., Karnosky, D.F.,1998: Impact of ambient tropospheric O_3, CO_2, and particulates on the epicuticular waxes of aspen clones differing in O_3 tolerance. Ekológia, *18*, 2: 200–210.
Maňkovská B., Florek, M., Frontasyeva, M.V., Ermakova, E., Oprea, K., Pavlov, S.S., 2003: Atmospheric deposition of heavy metals in Slovakia studied by the moss biomonitoring technique, neutron activation analysis and flame atomic absorption spectrometry. Ekológia (Bratislava), *22*, Suppl. 1: 157–162.
Manning, W.J., Godzik, B., Musselman, R., 2002: Potential bioindicator plant species for ambient ozone in forested mountain area s of Central Europe. Environ. Pollut., *11*: 283–290.
Markert, B., 1993: Inter-element correlations detectable in plant samples based on data from reference materials and highly accurate research samples. Fresenius J. Anal. Chem., *345*: 318–322.
Markert, B., Herpin, U., Maňkovská, B., 1996: A comparison of heavy metal deposition in selected Eastern European countries using the moss monitoring method, with special emphasis on the “Black Triangle”. Sci. Tot. Environ., *193*: 85–100.
Markert, B., Breure, A.M., Zechmeister, H.G. (eds), 2003: Bioindicators and biomonitors. Principles, concepts and applications. Elsevier, Amsterdam-Tokyo, 997 pp.
Mengen, K., 1996: Fungal attachment and penetration. In Kerstien, G. (ed.), Plant cuticles. Bios. Scientific Publisher Ltd., Oxford, p. 175–188.
Percy, E., Cape, J.N., Jagels, R., Simpson, C.J., (eds), 1995: Air pollutants and the leaf cuticle. NATO ASI Series, Vol. G 36, Springer-Verlag, Berlin Heidelberg, p. 123–138.
Rennenberg, H., 1994: The fate of excess sulphur in higher plants. Ann. Rev. Plant Physiol., *35*: 121–153.
Sanz, M.J., Calatayud, V., Calvo, E., 1995: Diferencias morfologicas en las ceras epiestomaticas de varias poblaciones de *Pinus sylvestris* L. de Castellòn y Teruel. Ecologia, *9*: 201–211.
Solberg, S., Kvindesland, S., Aamlid, D., Venn, K., 2002: Crown condition and needle chemistry of Norway spruce in relation to critical loads of acidity in south-east Norway. Water, Air, Soil Pollut., *140*: 157–171.
Stefan, K., Fűrst, A., Hacker, R., Bartels, U., 1997: Forest foliar condition in Europe. Technical Report. EC and UN/ECE, Brussels, Geneva, 207 pp.
Suchara, I., Florek, M., Godzik, B., Maňkovská, B., Rabnecz, G., Sucharová, J., Tuba, Z., Kapusta, P., 2007: Mapping of main sources of pollutants and their transport in the Visegrád space. Project 11007-2006-IVF, Průhonice, 127 pp.
Suzuki, T., Ohtaguchi, K., Koide, K., 1994: Correlation between flash points and chemical structures of organic compounds, using principal component analysis. Int. Chem. Engin., *34*, 3: 393–402.
Torseth, K., Aas, W., Soldberg, S., 2001: Trends in airborne sulphur and nitrogen compounds in Norway during 1985/1996 in relation to air mass origin. Water, Air, Soil Pollut., *130*: 1493–1498.
Trimbacher, C., Weiss, P., 2004: Norway spruce: A novel methods using surface characteristics and heavy metal concentrations of needles for a large/scale monitoring survey in Austria. Water, Air, Soil Pollut., *15*: 363–386.
Turunen, M., Huttunen, S., 1990: A review of the response of epicuticular wax of conifer needles to air pollution. J. Environ. Qual., *19*: 35–45.