IMPACT OF AMBIENT TROPHOSPHERIC O_3, CO_2 AND PARTICULATES ON THE EPICUTICULAR WAXES OF ASPEN CLONES DIFFERING IN O_3 TOLERANCE

BLANKA MAŇKOVSKÁ, KEVIN PERCY, DAVID FITZERALD KARNOSKY

Forest Research Institute, Zvolen, Slovakia
Canadian Forest Service, E3B Fredericton, Canada
Michigan Technological University, Houghton, Michigan, USA

Abstract

Maňkovská B., Percy K., Karnosky D.F.: Impact of ambient trophospheric O_3, CO_2 and particulates on the epicuticular waxes of aspen clones differing in O_3 tolerance. Ekológia (Bratislava), Vol.18, No.2, 200-210, 1999.

Epicuticular waxes of trembling aspen (*Populus tremuloides* M i c h x.) clones differing in O_3 tolerance were examined for three growing seasons (1995-1997) at three localities (Rhinelander, Wisconsin -clean and control site; Kalamazoo, Michigan -moderate pollution loading and Kenosha, Wisconsin - high pollution loading) in the Lake States region of the USA. Statistical difference was found in the concentration of Al, As, Ca, Cd, Cu, Fe, Mn, N, Ni, P, Pb, S, and Zn in the aspen foliage. Particulates on the leaves surfaces and in the stomata at the three sites include Al, Ca, Fe, Si, Mg, Na, S, Th, Ti and Y. These elements are typical for particles from industrial sources. Fungi effect was observed on leaves from all localities. Differences in epicuticular wax structure, as determined by scanning electron microscopy were found between these sites with the most severe impact being found at the Kenosha site. These include severe erosion of waxes, and occlusion of stomata. The results suggest that O_3 pollution of the Kenosha and Kalamazoo site show significant negative impact on epicuticular waxes of aspen and that these impacts are the most severe on the O_3 sensitive clones.

Introduction

Global atmospheric and preindustrial CO_2 concentrations are expected to double by the end of the next century. Trophospheric ozone (O_3), a secondary pollutant generated from nitrogen oxides and hydrocarbons, is also increasing globally. At the same time forest tree species are exposed to the effect of CO_2, O_3 and other pollutants. While CO_2 generally stimulates tree growth and O_3 and other air pollutants generally decrease tree growth, there is little information available on the impacts of intereaction of CO_2, O_3 on forest tree growth and productivity. Trembling aspen (*Populus tremuloides* M i c h x.) is a good model species to examine the effects of these two pollutants, as it is highly responsive to both CO_2 and O_3. Furthermore, we have identified a wealth of genetic variation in the response of trembling aspen to air pollutants, and we have isolated O_3-sensitive and tolerant clones (Karnosky et al.,1998).

The objective of the work was to find the response of foliage surface of aspen clones differing in O_3 sensitivity and to find out elemental composition of solid particles on vegetative surface of aspen. The aim of this paper is to present also the actual data about concentration of Al, B, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, N, Ni, Pb, S, V and Zn in the foliage of aspen based on the results for the three USA localities in 1996 and 1997 years. We are also interested in the difference between element concentration of 2 year old spruce and pine needles from two USA localities and two Slovak localities.

Material and methods

Samples of leaves of *Populus tremuloides* M i c h x. issued from three aspen clones (216, 259 and 271) differing in O_3 sensitivity and located at three sites differing in ambient O_3 (low O_3-Rhinelander, WI; intermediate O_3-Kalamazoo, MI and high O_3-Kenosha, WI) (Karnosky et al.,1998). At the site Rhinelander, Kenosha and for the comparison from industrial region of Central Spiš and Low Tatras National Park (NAPANT) in Slovakia we sampled spruce and pine needles. Sampling of 2 year old needles was performed in 1997 according to the international methodology (ICP,1994). Needles came from lightened part of crown and were taken from the 7th whorl for spruce (*Picea abies* K a r s t.) and for pine (*Pinus sylvestris* L.) from the top whorl or the 2nd whorl. Collective sample was mixed from 15 samples after their drying. Foliage samples were analyzed unwashed.

Atomic absorption spectrometry was applied to determine the concentrations of Cd, Co, Cr, Cu, Fe, Mn, Ni, V, Zn (AAS SpectrAA 300 Varian); Al, B, Ca, K, Mg, P (ICP-AES PLASMARAY 3000, LECO) and Hg (AMA 254 Mercury analyzer Praha). Elemental analysis was applied to determine the concentration of S (LECO SC 132) and N (LECO SC 228). The accuracy of data published in paper was verified by 109 separate laboratories and tested by the IUFRO programme (Hunter, 1994).

Samples of foliage of forest tree species were treated on surface by JEOL Ionsputtering. They were assessed by scanning microscope JEOL 840 A and X-ray analyser LINK 10000. The wax surface SEM was done at the Canadian Forest Service's Fredericton, New Brunswick and X-ray analyses at the Forest Research Institute, Zvolen. Particles deposited in stomata of foliage were assessed as to their morphology and EDX spectra. The deposited particles were divided into six basic groups (Table 1) (Maňkovská, 1992).

Fungi infect was classified according 5 class [1-absent of fungi particles (spore and mycelium); 2 lower than 10 particles; 3 of 11 to 25 particles; 4 more than 25 particles with covering less than 25% of surface and 5 covering more than 25% of foliage surface].

For assessment of vegetative material we used usual statistical methods as follows: the calculation of basic statistic characteristics and correlation analysis. For the assessment of total loading of the sites studied by air pollutants loading (Kz). Kz gives exceeding of limit values of the elements studied for the foliage of forest tree species (Maňkovská, 1996).

Results and discussion

The comparison of the concentrations of Al, As, B, Cd, Co, Cu, Cr, Fe, Hg, K, Mn, N, Ni, P, Pb, S, V and Zn in the leaves of *Populus tremuloides* from three US localities for 1996 and 1997 years is in Table 2. Statistically significant difference between the years 1996/

T a b l e 1. Classification of particles deposited in surface and stomata of aspen leaves

Category Type	Morphology of particles	Presence of elements
Biologic	Characteristic shape with a low spectre – pollen, pores, plant and animal remains and waxes	Si,S,Ca,K,P
Mineral	Nonspherical irregular shape, fairly big particles, origin: soil, calcite [$CaCO_3$], dolomite [Ca,Mg $(CO_3)_2$], SiO_2, $CaSO_4$ and more complex mixtures of alkaline origin	Al,Si,S,K, Ca,Fe,Na,Mg,Ti
Fuel-oil ash	Small spherical shapes, rich in Al, Si, S; cenospherical Al-Si particles with V and Ni; sulphates rich in Cr, Fe, Ni, of black-metallic luster	Al,Si,V,Ni, Cr
Coal ash	Small spherical glassy particles dominated by Al-Si, with various admixtures	Al, Si
Coal and fuel-oil ash	Small porous particles with carbon along with categories C and D	Al, Si
Industrial	Very variegated reflecting technologies used	
	Aluminium plant	Al
	Cement and lime plants	Ca
	Magnesite plants	Mg
	Iron	Fe
	Base metals	Mn,Ni,Zn, Br,Rb,Sr
	Other	As,Be,Cd,Co, Cr,Cu,Mo,Ni, Pb,Se Sb,V, Zn

1997 and the concentrations of elements in aspen leaves in Rhinelander were found for Cd, Mn, P, Pb and S, in Kenosha for Al, Cd, Cu, Fe, N, Ni, Pb a S and in Kalamazoo for Cd, N, P, Pb, S a Zn. Statistically significant difference for both years in the concentration of Al and Ca were found for Rhinelander and Kenosha; Ca, Mn, N and S between Rhinelander and Kalamazoo and Mn, and N between Kenosha and Kalamazoo. Finding of high values of S in aspen leaves in Rhinelander, in spite of an absence of the source of SO_2 emissions is surprising. This increase can be explained by the uptake of SO_2 emissions from long-distance transfer in the form of wet fallout.

Total concentration of 19 individual elements in the 2 year old spruce and pine needles from two US (Rhinelader and kenosha) and two Slovak localities (Low Tatras National Park [NAPANT] and Spiš) were ascertained. The highest values of Al, Cd, Cu, Fe, Hg, Mn, Ni, Pb and S in pine needles and Al, Fe, Hg, Mn and S in spruce needles were found in the industrial zone of central Spiš. The maximal value of Co and V in pine needles and of Cr, Cu and Ni in spruce needles were registered at the site Kenosha. The highest value of Cd, Co and Pb was found in spruce needles at the site Rhinelander. The highest value of Cr was found in pine needles in NAPANT.

The level of aluminium in aspen leaves can be considered balanced one. Increased values were found in Rhinelander equally for aspen and pine in 1996. Maximal values were

T a b l e 2. Concentracion of elements in the leaves of aspen [in mg.kg^{-1}]

Element	Rhinelander		Kenosha		Kalamazoo		Literature value[a] range
	1996 X(SD)	1997 X(SD)	1996 X(SD)	1997 X(SD)	1996 X(SD)	1997 X(SD)	
Al	160(84)	142(71)	81.7(12.1)	31.9(17.2)	95.1(20.6)	91.7(15.8)	50-150
As	0.42(0.21)	–	0.49(0.28)	–	0.33(0.12)	–	<0.2
B	–	2.8(0.3)	–	43.2(7.3)	–	38.4(5.3)	11-100
Ca	9947(2428)	8457(1987)	14787(4173)	14377(2983)	13461(3572)	11667(2597)	4000-8000
Cd	4.9(1.2)	0.36(071)	5.3(1.1)	0.9(0.8)	6.0(1.7)	0.7(0.4)	<0.5
Co	–	2.92(0.41)	–	1.24(0.28)	–	1.50(0.17)	<1.0
Cr	–	2.14(0.31)	–	0.68(0.24)	–	0.61(0.18)	<1.0
Cu	12.5(3.4)	14.1(1.3)	10.7(5.2)	16.5(4.8)	15.0(14.6)	7.3(4.6)	6-14
Fe	137(57)	94(62)	65.6(7.1)	82.2(11.5)	106(34)	81(28)	200-2000
Hg	–	0.02(0.01)	–	0.03(0.01)	–	0.02(0.01)	<0.06
K	–	1254(350)	–	9400(623)	–	12338(459)	5000-10000
Mn	78.6(18.4)	47.7(22.3)	79.8(18.0)	66.4(21.7)	133(37)	131(41)	1000
N	22505(4328)	35118(4052)	19583(7170)	27206(3996)	29434(1931)	21420(2420)	18000-25000
Ni	2.6(0.6)	2.86(0.8)	3.0(0.7)	4.9(1.2)	3.0(0.4)	3.1(0.5)	1-2
P	1711(198)	2288(112)	1989(294)	2104(155)	2009(384)	1603(301)	120-3000
Pb	0.47(0.12)	1.66(0.09)	0.49(0.13)	3.10(0.24)	0.52(0.13)	1.86(0.11)	2-6
S	4150(136)	2542(147)	4566(646)	2681(113)	4583(207)	2148(105)	1300-2000
V	–	1.27(0.09)	–	1.18(0.14)	–	0.66(0.07)	<1.0
Zn	136(213)	51(42)	199(68)	143(98)	166(84)	97(34)	20-80
Kz	2.80(0.80)	1.50(0.90)	3.20(0.40)	2.10(1.40)	3.20(0.80)	1.40(1.00)	1

Note: X – arithmetical mean, SD – standard deviation, Literature value[a] – for broadleaves (Maňkovská, 1996)

found for pine needles in Spiš. As limit values for foliage of forest tree species are given the concentration from 120 to 180 mg.kg^{-1}. Aluminium is non-essential element. It is being deposited into stomata and on the surface of the foliage of forest tree species (Wyttenbach et al., 1995). Aluminium is emitted also from the earth dust. Aluminium is a potential factor in current forest damage as it is released with acid deposition.

Arsenic is essential element. It is typical pollutant originating in coal combustion. Markert (1993) gives for forest tree species as a limit value the concentration 0.214 mg.kg^{-1}. Arsenic was recorded in aspen leaves only in 1996 and it represents increased content at all three localities in the USA. Finding of the highest value of As at unpolluted localit Rhinelander is interesting.

Boron is essential element. In comparison with data of Bowen (1979) the recorded concentration in the foliage of aspen, spruce and pine is not exceeding limit value.

Calcium is essential element for higher plants for its physiological, regulation and electrochemical functions. It is an activator of enzymes. In plant Ca is not such mobile as Mg and therefore it is accumulated in older plant tissues. Innes (1995) found in 2 year old spruce needles the values 2200-8600 mg.kg^{-1} and in 2 year old pine needles 1700-5000

$mg.kg^{-1}$. The contents of Ca in aspen leaves can be considered balanced. Increased contents were found in NAPANT what is obviously connected with liming of forest stands.

Cadmium is non-essential element for higher plants and its increased values are important from environmental viewpoint. Damage by cadmium can be passive and first of all metabolic cadmium is in plants very mobile and it is transported into different part of plants. High concentrations were found in leaves but also in roots. As a limit value for cadmium the content 0.5 $mg.kg^{-1}$ can be considered (Maňkovská, 1996). In aspen foliage the cadmium concentration decreased when comparing the years 1997/1996. In 1997 the highest values of cadmium were found at the site in Kenosha. The content of Cd in spruce and pine needles has not increased.

Cobalt is essential element for higher plants. It is accumulated in relatively lower concentrations in storage organs of plants or seeds compared to the ones in vegetative part. Markert (1993) gives for *Pinus sylvestris* 0.124 $mg.kg^{-1}$. The values of cobalt in aspen leaves can be considered slightly increased, the values of Co in pine and spruce needles are balanced.

Chromium is non-essential for higher plants. Translocation of chromium from roots to other parts of plants is low. Markert (1993) gives for *Pinus sylvestris* as a limit value the content 1.72 $mg.kg^{-1}$. The concentrations of chromium at all American and Slovak localities are balanced. The chromium concentration in aspen leaves at the locality Rhinelander must be considered slightly increased value.

Cooper is essential element, but simultaneously it is emitted, and in higher concentration harmful. Jochheim et al. (1993) considers the content 5 $mg.kg^{-1}$as limit one and the values above 100 $mg.kg^{-1}$ as the ones indicating an extreme air pollution loading. Recorded values of cooper are slightly increased in the foliage of aspen, spruce and pine. The highest values were found for pine in Spiš where cooper is produced.

Iron is typical element with physiological enzymatic function. In higher concentrations it affects the plants toxically. Kaupenjohan et al. (1989) give for healthy spruce the value 50 $mg.kg^{-1}$ and Markert (1993) gives for *Pinus sylvestris* 118 $mg.kg^{-1}$. Recorded concentrations of iron are balanced with the exception of pine in Spiš what is connected with industrial production.

For higher plants mercury is non-essential and very toxic. Its toxicity is increasing from elemental Hg, ionic Hg to organomercuric compounds. As a limit value for mercury the content 0.06 $mg.kg^{-1}$ can be considered (Maňkovská, 1996). The concentration of mercury is not exceeding at the American localites limit values. Maximal values were found for pine in Spiš where also mercury plant is situated.

Potassium is for higher plants essential with electrochemical and catalytic function. Its enzymatic function activates enzymes. It participating in osmoregulation and supports hydration. Innes (1995) found in 2 year old needles of *P. abies* the values 4900-11000 $mg.kg^{-1}$ and of *Pinus sylvestris* 5200-8100 $mg.kg^{-1}$. The level of potassium is balanced at all studied localities with the exception of the locality Rhinelander, where the content of potassium in aspen leaves can be considered insufficient.

Magnesium is for all plants essential and it is a constitutional component of enzymes and chlorofyll. It has electrochemical and catalytic function, it regulates hydratation and photosynthesis. The values for optimal magnesium nutrition range from 600-1500 $mg.kg^{-1}$(Maňkovská, 1996). The content of Mg is balanced at all studied localities. Manganese is essential element but in higher concentrations is harmful. Mobilization of manganese indicates the disturbance of physiological balance leading to the change of ratio with iron (the ratio should be 1:2) (Kaupenjohan et al., 1989). Markert (1992) gives that from studied parameters only the contents of manganese in spruce needles correlate with its loss of needles. Therefore manganese is being assessed as an indicator of damage to tree species. Already the phase of manganese mobilization indicates instable status in the regime of mineral substances of forest and trees and for spruce needles gives the threshold of Mn storage 20 $mg.kg^{-1}$, optimal content >50, for beech >100 $mg.kg^{-1}$. The highest values of manganese were found in Spiš, where a decline of spruce as well as pine is going on.

Nickel is essential element. It is a typical emission component originating in the combustion of heavy oils. As a limit value can be considered for nickel the amount 1 $mg.kg^{-1}$ (Maňkovská, 1996). The level of nickel is slightly increased at all studied localities with the maximum at the locality Kenosha for the foliage of all study trees.

Sulphur and nitrogen are structural elements. Increased amounts of both elements in plant material are caused by polluted air. The ratio S/N is a sensitive indicator of sulphur accumulation in the foliage of forest tree species that are exposed to atmospheric pollution. Materna, Mejstřík (1987) give that the contents of sulphur in the needles of spruce range 800-1000 $mg.kg^{-1}$ what corresponds to our data (Maňkovská, 1996). Recorded levels must be considered as increased ones for aspen leaves in all American localities. Nitrogen is essential element for higher plants. Simple compounds of nitrogen represent at present an extensive ecotoxicological problem, for example the problem of nitrites for animals, NO_2-emissions, N_2O and NH_4^+. For broadleaves tree species Bublinec (1990) considers the values 19000-26000 $mg.kg^{-1}$ sufficient ones. Innes (1995) has found in 2 year old needles of *Picea abies* the values 11010-16400 $mg.kg^{-1}$ and in *Pinus sylvestris* 12500-23200 $mg.kg^{-1}$. The content of nitrogen was balanced with the exception of its increase in 1997 in the locality Rhinelander in aspen leaves. The coefficient of molar ratio S/N in aspen leaves ranged from 0.072 to 0.233, in spruce needles from 0.094 to 0.142 and in pine needles from 0.060 to 0.142. The molar ratio of protein sulphur and protein nitrogen ranges from 0.025 to 0.032 and it is relatively constant for all tree species. Obtained results showed that the ability of sulphur to increase its content exceeded in all cases the need of plants for protein synthesis. In aspen leaves the content of sulphur decreased markedly comparing the years 1997/1996.

Lead has not biological function and its higher concentrations can be explained by emissions from industry. As allowed amounts of lead from non-contamined regions the values from 2 to 6 $mg.kg^{-1}$ are given for broadleaved and coniferous tree species (Maňkovská, 1996). Recorded concentrations of lead in all tree species studied are not exceeding limit values, with the exception of a slight increase in pine in the industrial region Spiš.

Vanadium is essential for higher plants. Vanadium inhibits the synthesis of chlorophyll. Markert (1993) gives as a limit value of vanadium for *Pinus sylvestris* 0.65 and Maňkovská (1996) gives for broadleaved tree species the value 1 $mg.kg^{-1}$. The content of vanadium in all tree species can be considered balanced.

Zink is essential element, but at the same time it is being emitted and its higher concentrations are harmful. According to Bergman (1986) teh optimal level of zink ranges from 16 to 38 $mg.kg^{-1}$. Slightly increased values were recorded in aspen leaves in all studied localities.

The equilibrium of individual elements in plant bodies is the precondition of their normal growth. Similar chemical properties due to roughly equal ion radicals and charges probably cause interactions between individual elements in plant organisms. Markert (1993) identified high correlations among P, N, Ca, Mg and Sr as well as Co/Mo and Cr/Co in needles of *Pinus sylvestris*. He has also stated that Al/Ca, Mn/Ca and B/Sb are typical antagonistic pairs of elements. Mutual correlation among individual pair of elements in spruce and pine needles have been investigated separately in 2 USA areas and 2 Slovak areas. In clean region Rhineland we found with r>0.9 following pair of elements: Al/Fe, Al/Hg, B/Ca, B/Zn, Ca/Cd, Ca/Zn, Cd/Mg, Cd/N, Cd/S, Cd/Zn, Co/Mg, Mg/S, MG/Zn, N/S a N/Zn and in the region Kenosha loaded by air pollutants we found with r>0.9 the pairs as follows: B/Zn, Cu/K, Cu/N, Cu/S, K/Mg, N/P a N/S. Suprisingly, in contrast to data put forward by Markert (1993), only highly positive correlable pairs of locally emitted elements were observed in Spiš region, namely Ni/As, in the Southern part of Low Tatras a positive correlation between Cd/Mn, Al/Co a Pb/ Cr a Cr/Fe was found. No negative correlation was found among pairs of elements whose exceeded or equalled -0.9.

It is proved in a lot of works that foliage of forest tree species from contaminated regions can be considered as an accumulation monitor, at the same time it can be stated that a large number of elements is located on surface in stomata or in a wax layer (Maňkovská, 1992; Wyttenbach et al., 1995). Qualitative analysis of air-borne pollutant particles from the surface and stomata of foliage are in Table 3. The particles of biological and mineral origin, fly-ash and industrial particles were present on the surface of all aspen clones. The maximum of particles that – by their form and chemical composition – represent the category E- particles of fly-ash were present in 100% of leaves from all localities with the exception of the 1995 in Kalamazoo. The industrial particles (category F_6) were present on all clones and all localities, but in 1996 in a substantially greater extent.

Evaluation of element concentration (EDX spectrum) in particles settled in stomata of foliage is in the Table 3. Foliage stomata of observed clone of aspen (Table 3) contained Al and Si as a part of minerals and ashes; Fe was a part of particles of minerals, ashes and Fe_2O_3 in the vicinity of metallurgy complex and thermal power plant; Ca, Mg, K and Cl was found as a part of mineral and biological particles. Other elements such as Mn, Na, Ni, Ti, were found in all studied localities. In the locality Rhinelander the particles with the content of Th and Y were found in Kalamazoo the particles with the content of Ba. 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 stomata of foliage (Wytenbach et al., 1995).

Total view on leaves surface from locality Rhinelander, Kenosha i Kalamazoo is in Fig. 1-3. It can be seen in figures a different damage to epicuticular waxes. The highest damage was found for sensitive clones of aspen in the industrial zone Kenosha.

Anthropogenic loading of the foliage of individual species in all localities, being expressed by the coefficient of loading by air pollutans K_z for the years 1995 and 1996 is in Table 2. Limit values of S content are exceed the most in all studied localities.

T a b l e 3. Categories of air–borne particles from the surface and stomata of foliage [%], fungi effect [%], elements and number of particles [%]

Locality clones		Fungi effect [%]				Category of particles [%]						Presence of elements (EDX spectrum)		No of particles [%]			State of epicutic waxes
		1	2	3	4	A	B	E	F2	F4	F6	>50%	<50%	1	2	3	
Rhinelander																	
216	1995	33	67	–	–	67	100	100	17	50	50	Al,Ca,Fe,Mg,Si	Cl,K,Mn,Ti	–	67	33	
	1996	67	–	–	33	33	100	100	–	–	100	Al,Ca,Cl,Fe,Si,Ti	K,Mg,Mn, Na	33	67	–	Fig.1
259	1995	33	17	50	–	67	100	100	50	17	67	Al,Ca,Fe,K,S,Si	Mg,Na,Ti	–	67	33	
	1996	–	100	–	–	100	100	100	67	–	100	Al,Ca,Fe,Ni,Si,Ti	K,Mg	–	100	–	
271	1995	50	50	–	–	50	100	100	33	83	17	Al,Ca,Cl,Fe,K,Mg,Si,Ti	Mn,Na	–	100	–	
	1996	67	33	–	–	33	100	100	67	33	100	Ag,Al,Ca,Cl,Fe,K,P,Si, Ti	Mg,Na,Th, Y	–	100	–	
Kenosha																	
216	1995	17	66	17	–	83	100	67	17	–	100	Al,Ca,Cl,K,Mg,S,Si	Fe,Na,Ti	–	83	17	
	1996	–	67	–	33	100	100	100	–	67	33	Al,Ca,S,Si,Sr	Cl,K,Mg,Ti	–	100	–	Fig.2
259	1995	17	83	–	–	83	100	100	–	17	100	Al,Ca,Cl,Cu,Fe,Mg,S,Ti	K,Mn,Na	–	83	17	
	1996	–	33	67	–	100	100	100	–	33	100	Al,Ca,Fe,Mg,S,Si	Cl,K,Ti	–	100	–	
271	1995	50	33	17	–	50	100	100	33	17	50	Al,Ca,Cl,Fe,Mg,S,Si	K,Na	–	100	–	
	1996	–	67	33	–	100	100	100	–	33	100	Al,Ca,Fe,Mg,S,Si	K,Mn	–	67	33	
Kalamazoo																	
216	1996	–	33	33	34	100	100	100	33	100	100	Al,Ca,Fe,K,Mg,Si,Ti	Cl,Mn,Na	–	100	–	Fig.3
259	1996	–	–	75	25	100	100	100	–	50	75	Al,Ca,Fe,Mg,S,Si,Ti	Ba,Cl,K	–	100	–	
271	1996	–	33	67	–	100	100	100	33	33	100	Al,Ca,Cl,Fe,K,S,Si	Mg,Mn,Na, Ti	–	67	33	

Note: Clones 216(O_3–tolerant), 259(O_3– sensitive), 271(O_3 tolerant)

Conclusion

1. Foliage surface of three aspen clons contained Al, Si, Ca, Fe, Mg, K, Cl Mn, Na, Ni, Ti in all studied localities. In the locality Rhinelander the particles with content of Th and Y were found and in Kalamazoo the particles contained Ba.

2. Fungi effect was observed on leaves from all localities, but it was not too important for surface of leaves.

3. Epicuticular waxes were damaged, that mean beginning creation of net and amorfen waxes. Clone 259 was always more damaged than clones 216 and 271, which are tolerant opposite to O_3. Clon 259 from Kenosha locality was damaged the most.

4. In the leaves of *Populus tremuloides* it was found the content of Al, As, B, Cd, Co,

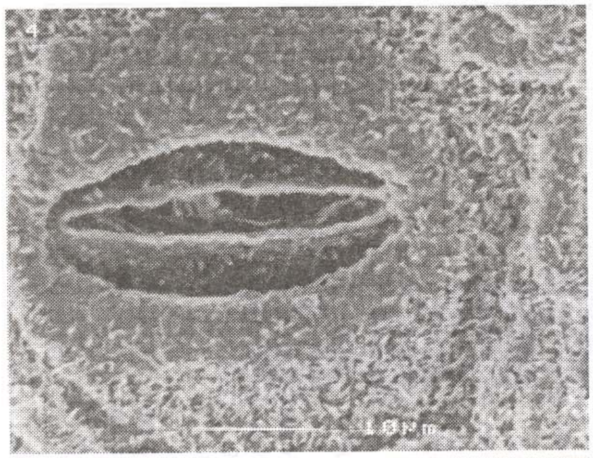

Fig.1. Stomata of *Populus tremuloides*, clone 216 (tolerant O_3) from clean locality Rhinelander, magn. 2500.

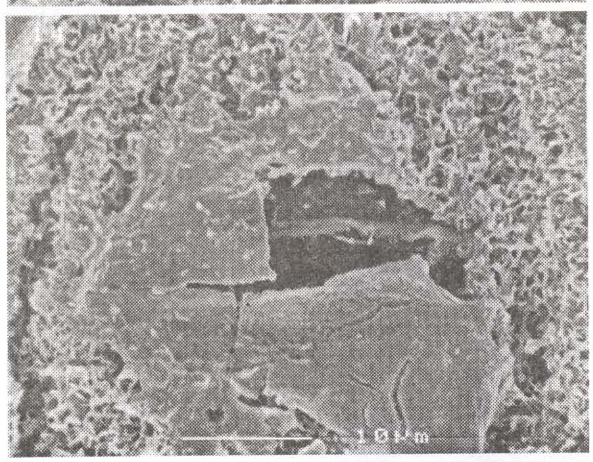

Fig.2. Stomata of *Populus tremuloides*, clone 216 (tolerant O_3) from industrial locality Kenosha, magn. 2500.

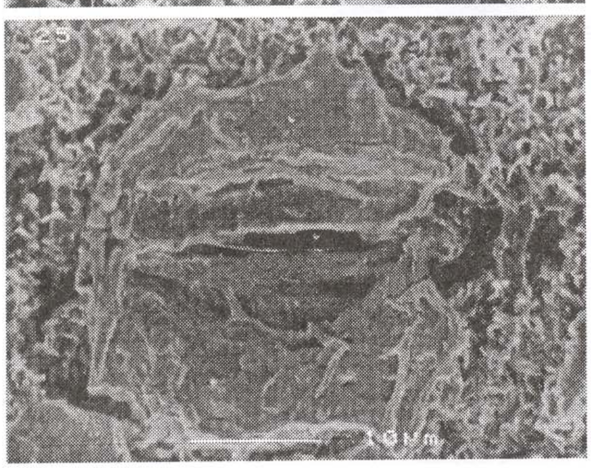

Fig.3. Stomata of *Populus tremuloides*, clone 216 (tolerant O_3) from industrial locality Kalamazoo, magn. 2500.

Cu, Cr, Fe, Hg, K, Mn, N, Ni, P, Pb, S, V and Zn in all localities. Statistically significant difference between the years 1996/1997 and the concentrations of elements in aspen leaves in Rhinelander was found for Cd, Mn, P, Pb and S, in Kenosha for Al, Cd, Cu, Fe, N, Ni, Pb and S, in Kalamazoo for Cd, N, P, Pb, S and Zn. Statistically significant difference for both years in the concentration of Al and Ca was found between Rhinelander and Kenosha; for Ca, Mn, N and S between Rhinelander and Kalamazoo, and for Mn and N between Kenosha and Kalamazoo. Finding of high values of S in aspen leaves in Rhinelander, in spite of absence of the source of SO_2 emissions, is surprising.

5. In spruce and pine needles from two American localities (Kenosha, Rhinelander) and from two Slovak localities (NAPANT, SPIŠ) it was found the concentration of Al, Cd, Co, Cu, Cr, Fe, Hg, K, Mn, N, Ni, P, Pb, S, V and Zn. The highest values of Al, Cd, Cu, Fe, Hg, Mn, Ni, Pb and S in pine needles and of Al, Fe, Hg, Mn and S in spruce needles were found in the industrial region of Central Spiš. Maximal values of Co and V in pine needles and of Cr, Cu and Ni in spruce needles were registered at the site Kenosha. The highest values of Cd, Co and Pb were found in spruce needles at the site Rhinelander. The highest value of Cr was found in pine needles in NAPANT. Molar coefficient S/N in spruce and pine shows in all cases the exceeding of the need of sulphur for plants in all cases.

6. Anthropogenic loading of aspen in all three localities, being expressed by the coefficient of loading by air pollutants K_z represented in 1997 1.5 times higher loading of the locality Rhinelander, 2.1 times higher loading of the locality Kenosha and 1.4 times higher loading of the locality Kalamazoo. In 1996 the loading by air pollutants was higher, mainly due to sulphur compounds.

Translated by the authors

References

Bergman, W., 1986: Farbatlas Ernährungsstörungen bei Kulturpplanzen. Fischer, Jena, 124 pp.

Bowen, H.J.M., 1979: Environmental chemistry of the Elements. Academic Press. London, New York, Toronto, Sydney, San Francisco, 333 pp.

Bublinec, E., 1990: In Vladovič, V.: Soil component(in Slovak). EKO, Príručka pre prieskum ekológie lesa. Lesprojekt, Zvolen, p.101-141.

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

ICP, 1994: Manual on methods and criteria for harmonized sampling, assessment, monitoring and analysis of the effects of air pollution on forest. 3rd edition, Programme Coordinating Centre West, BHF, Hamburg, 45 pp.

Innes, J. L., 1995: Influence of air pollution on the foliar nutrition of conifers in Great Britain. Environmental Pollution, *88*, p. 183-192.

Jochheim, H. et al., 1993: Effects of altitude on heavy metal accumulation in soils. In Plants as biomonitors. Indicators for heavy metals in the terrestrial environment. B. Markert (ed.). VCH Weinheim, p. 601-611.

Kaupenjohan, M., Zech, W., Hantschel, 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. Ecological studies, *77*, Springer Verlag Berlin, p.182-294.

Karnosky, D.F., Podila, G.K., Pechter, P., Akkapedi, A., Sheng, Y., Riemenscheider, D.E., Coleman M.D., Dickson, R.E., Isebrands, J.G.,1998: Genetic control of responses to interacting trophospheric ozone and CO_2 in *Populus tremuloides*. Chemosphere, *36*, 4-5, p. 807-812.

Maňkovská, B.,1992,: Chemical composition of solid particles on vegetative surface in Slovak forests. Ekológia (ČSFR), *11*, 2, p.205-214.

Maňkovská, B., 1996: Geochemical atlas of Slovakia- forest biomass (Slovak, English). Geologická služba Slovenskej republiky, Bratislava, ISBN 80-85314-51-7, 87 pp.

Markert, B., 1992: Presence and significance of naturally occurring chemical elements of the periodic system in the plant organism and consequences for future investigations on inorganic chemistry in ecosystems. Vegetatio, *103*, p. 1-30.

Markert, B., 1993: Interelement correlations detectable in plant samples based on data from reference materials and highly accurate research samples. Fresenius J. Anal. Chem., *345*, p. 318-322.

Materna, J., Mejstřík, V., 1987: Agriculture and forest management in polluted areas (in Czech). SZN Praha, 152 pp.

Wyttenbach, A., Schleppi, P., Tobler, I., Bajo, S., Bucher, J., 1995: Concentrations of nutritional and trace elements in needles of Norway spruce (*Picea abies* [L.] K a r s t.) as functions of the needle age class. Plant and soil, 168-169, p. 305-312.

Received 15.4.1998

Maňkovská B., Percy K., Karnosky D. F.: **Vplyv troposférického O_3, CO_2 a tuhých častíc na epikutikulárne vosky na O_3 rôzne citlivých klonov amerického topoľa.**

Na troch lokalitách s rozdielnou koncentráciou ozónu (Rhinelander, štát Wisconsin – kontrolné miesto s požadovanou koncentráciou ozónu; Kalamazoo, štát Michigan – stredné znečistenie a Kenosha, štát Wisconsin – silné znečistenie) v regióne Lake States, USA sme počas troch vegetačných sezón (1995-1997) sledovali epikutikulárne vosky troch klonov amerického topoľa (*Populus tremuloides* M i c h x.) s rozdielnou toleranciou na O_3. Medzi koncentráciami Al, As, Ca, Cd, Cu, Fe, Mn, N, Ni, P, Pb, S a Zn v topoľovom lístí sme našli štatisticky významný rozdiel. Častice, ktoré sa nachádzali na povrchu listia a v prieduchoch lístia, na všetkých lokalitách obsahovali Al, Ca, Fe, Si, Mg, Na, S, Th, Ti a Y. Tieto prvky sú typické pre častice pochádzajúce z priemyselných zdrojov. Prítomnosť hubových mycélií a spór sme spozorovali na všetkých lokalitách. Rozdiely v štruktúre epikutikulárnych voskov, ktoré sme určili pomocou rastrovacieho mikroskopu, zistili sme na všetkých troch lokalitách – s najvyšším poškodením na lokalite Kenosha. V prieduchoch sme zaznamenali eróziu až zliatie voskov. Výsledky potvrdili, že znečistenie O_3 v lokalitách Kenosha a Kalamazoo má významný negatívny účinok na stav epikutikulárnych voskov topoľa. Tento účinok je častejší u klonov, ktoré sú citlivé na O_3.