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Original Research

Monitoring of Air Pollution in Košice (Eastern Slovakia) Using Lichens

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Abstract

The influence of air pollution on epiphytic lichens in Košice city has been studied. We observed differences in number of species as well as lichen abundance at sites close to a steel factory south of the city, the city center, and peripheral parts north of the city. For the city center and sites close to steel factory, lichens more tolerant to pollution from Zone 3-4 were typical. However, on the north periphery of the city (site "Alpinka") we found even *Ramalina fastigiata*, a typical member of zone 7, which include lichens very sensitive to air pollution [30].

We demonstrated by chlorophyll analysis of transplanted *Hypogymnia physodes,* that chlorophyll *a* degradation (expressed as ratio of OD 435/OD 415) negatively correlates with degree of lichen diversity and abundance at the studied sites.

Using EDX-microanalysis we determined amounts of elements in lichen thalli of *Lecanora chlarotera*, *Physcia tenella* and bark of the tree *Populus tremula* (lichen substrate) near U.S. Steel in Košice due to determine the chemical nature of air pollution. Similarly, we analyzed the amount of these pollutants in control lichens *Flavoparmelia caperata*, *Ramalina fastigiata* and *Physcia aipolia*, grown in northern peripheral parts of the city. We demonstrated possibilities to parallel the use of several methodologies in assessment of air pollution by lichens in urban areas with intensive industry.

Keywords: air pollution monitoring, chlorophyll degradation, element analysis, transplants

Introduction

Lichens are often and effectively used as monitors of pollution. To date, monitoring of sulfur dioxide [52], smoke and dust [38], fluorides [40], car fumes [25, 39], hydrocarbons [56], heavy metals [19, 20, 45], radionuclides [15, 35], or agrochemicals [50] were documented.

The earliest lichen monitoring studies were related to analysis of the status of single lichen species or lichen communities in studied areas and often are termed "passive biomonitoring with lichens". Differential sensitivity of lichen to air pollutants (mainly sulfur dioxide), the absence or presence of species after prolonged exposure, diversity as well as coverage and abundance, contributed to the pollution scale of Hawksworth & Rose [30]. They defined ten zones based on estimates of sulfur dioxide pollution in England and Wales. Zone 1 was characterized by an absence of epiphytic lichens and mean winter levels of SO_2 in excess of 170 µg . m^{-3}, whereas zone 10 displayed many sensitive lichen species and SO_2 concentra-

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tions less than 10 μg · m^{-3}. Although the number of zones was reduced in some subsequent studies (e.g. a simplified zone scale was used in the Advisory Centre for Education [29]), the scale is still widely used due to the remarkable accuracy of estimating SO_2 concentrations in contaminated zones as well as the low cost. On the other hand, this method requires basic knowledge in lichen taxonomy and ecology and is time consuming. At the same time it may be ineffectual due to subjective assessments.

These studies were related mainly to prolonged exposure to air pollutants (years). When smoke and sulfur dioxide levels decrease, e.g. in Inner London and other urban areas in the British Isles since 1960, lichens successfully recolonize in response to improving atmospheric conditions [31, 49].

A second way to use lichens as biomonitors is to transplant them along with substrates such as twigs [18, 20, 26] or bark [7], from the unpolluted, mainly rural places to polluted, mainly industrial or urban areas. After a certain exposure time (usually months) lichens are compared to control thalli.

In the context of pollution monitoring which uses naturally occurring lichens at monitoring sites as well as lichen transplants, changes in lichen morphology, cytology, metabolism and physiological processes all provide environmental insight. Normal *Hypogymnia physodes* (L.) Nyl., for example, has a smooth, grey thallus with large lobes but when exposed to air pollution, the surface becomes cracked and the coloration brown or black [46]. However, this process takes months and recently more quickly responding parameters have been used instead. Changes in lichen ultrastructure are usually observed in lichen algae after a few weeks [32, 40, 55]. Similarly, metabolic and physiological processes are usually influenced much earlier than morphological damage to whole thalli. Changes in respiratory rate and photosynthesis [4, 13] measured mainly as CO_2 gas exchange and ^{14}C incorporation [47], modulated chlorophyll (Chl) a fluorescence [27], chlorophyll content [10, 23] and decrease of ATP content [22, 34] are all parameters in lichen biomonitoring, as well as concentration of stress-ethylene [14, 27].

The uptake and accumulation of elements in lichens are also extensively studied [11, 19, 20] for monitoring. Such information may also help to reveal the principal sources of local pollution. Energy-dispersive X-ray microanalysis is sensitive to low element concentrations, as detection limits are below 0.1% in the best cases, typically about 0.5%. However, this method is sometimes the only applicable, as requirements for sample preparation are minimal. It was also used in lichen studies, as this method is relatively simple [24, 36, 57].

The major objective of the present study was the use of lichens in biomonitoring of Košice city and show advantages and disadvantages of this type of monitoring in the industrial-urban complex. The steel factory with about 15,000 workers is one of the most important producers of sulfur dioxide in Slovakia, thus lichen communities were studied to determine air pollution levels in selected parts of the city as well as to discover the other possible sources of pollution, e.g. other industrial plants or traffic. The elemental contents of lichens naturally occurring near the steel factory and lichens from northern parts of the city, with higher lichen diversity, were also determined in order to characterize the chemical nature of emissions.

A third goal of this study was to investigate damage to chlorophyll in the transplanted lichen *Hypogymnia physodes* (L.) Nyl. in relation to different levels of air pollution at selected monitoring sites in the city.

Experimental Procedures

Study Area, Passive Monitoring Sites, Transplantation

The present study was conducted near Košice, a city in east Slovakia, with a population of 240,000 (area 24,382 ha). Košice, Slovakia's second largest city, lies in the valley of the river Hornád in the Košice basin, and is encircled by the spurs of the Čierna Hora mountains to the north and Volovské Vrchy hills to the west. Košice is industrially active with metallurgy being the principal basis for industry. The company United States Steel (formerly VSŽ) has more than 15,000 workers and is one of the most important producers of atmospheric sulfur dioxide in Slovakia.

Twelve sites for passive monitoring of air pollution by lichens were selected for this preliminary study (Table 1). For additional orientation, the distances (km) to two of the main polluting areas are recorded. This study was conducted mainly with lichens which grew on several genera of deciduous trees: *Populus* sp., *Quercus* sp., *Acer* sp. and *Tilia* sp. At one site (Chata Alpinka), lichens on a few fruit trees were included as well. Leprose and crustose lichens were recorded only as present or absent on each site. For foliose and fruticose species additional data were also taken: abundance (classes 1-5, [31]), size of largest thalli (if possible to determine) and whether the species was found only at the base of a tree (mark: < 50). Five to ten trees were usually inspected in each site.

The foliose lichen *Hypogymnia physodes* (L.) NYL. was selected for transplantation. The lichen was collected in October 2000 in Prakovce, Zimná dolina (valley) about 470 m a.s.l., 2 km SSE of the village (7191/d), about 30 km from the center of Košice city. The site is characterized by a great abundance of lichen epiphytes from zone #6-7 [30]. About 50 twigs of spruce (*Picea abies*) of 30-60 cm in diameter bearing lichens were pruned and transferred to sixteen sites in and near Košice city. Site descriptions are in Table 2 where distance (km) is shown to two general sources of pollution, as for sites used for passive monitoring. The lichen-covered twigs were nailed 1.5 to 2 m above ground level on trunks of local trees. At the end of the exposure period (16 weeks), in February 2001, lichen material was retrieved from monitoring sites as well as from the similarly treated original (control) site and transported to the laboratory.

Table 1. Description of the passive monitoring sites (from north to south) in Košice.

Site number	Site name	Remarks	Distance from city center (km)	Distance from U.S. Steel (km)
1.	Kavečany	peripheral part of city, NW from center, deciduous forest	7.7	16.6
2.	Chata Alpinka	peripheral part of city, NW from center, mainly deciduous trees	6.7	14.5
3.	Ťahanovce	urban area, nearest part of housing estate to city center, without well developed deciduous trees, NE from city center, near frequent road	3.2	14.4
4.	Sídl. Darg. Hrdinov A	urban area, nearest part of housing estate to city center, NE from city center near frequent road	3.0	.14.1
5.	Botanical Garden	large and closed area with high diversity of trees, NW from center	1.8	11.5
6.	Sídl. Darg. Hrdinov B	urban area, peripheral part of housing estate, out of city (E50), near major road	3.0	14.1
7.	Natural Sci. Dean's Office	urban area in city center (in this study considered as city center)	0.0	10.6
8.	Bukovec	suburban close area near water reservoir, west from city center	7.5	9.9
9.	Západ	urban area, west from city center	1.7	9.9
10.	Nad Jazerom	urban area, near major road, south from city center	4.6	9.2
11.	Poľov	urban, SW from city center, near E571 road	7.8	3.5
12.	US Steel	industrial area, south from city center	10.8	0.0

Analysis of the Photobiont Chlorophyll in the Lichen Thallus

The ratio of optical density at wavelengths of 435 and 415 nm of lichen chlorophyll samples dissolved in dimethyl sulfoxide (DMSO) is considered a reliable parameter for estimating the degradation of photobiont chlorophyll [48]. In this study we examined chlorophyll *a* and *b* concentrations, total chlorophylls, chlorophyll *a/b* ratio and chlorophyll *a* degradation.

About 20 mg fresh weight (fw) of lichens were directly extracted in 5 ml of DMSO in the presence of polyvinyl-polypyrrolidone (2.5 mg $\cdot$ ml^{-1}) to minimize chlorophyll degradation during extraction in the dark for 1 h at 65°C. After incubation, extracts were allowed to cool to ambient temperature, diluted 1:1 with fresh DMSO, and the turbidity of the extract was checked at 750 nm to ensure it was always less than 0.01. The absorbance of the extracts was then read at 665, 648, 435 and 415 nm. Chlorophyll *a*, chlorophyll *b* and chlorophyll *a*+*b* were calculated using equations derived from specific absorption coefficients for pure chlorophylls *a* and *b* [3]. Chlorophyll *a/b* was used as an indicator of the measurement of physiological activity of algal cells. The ratio of optical densities at 435 and 415 nm (OD 435/OD 415) was interpreted as the phaeophytinization quotient, which reflects the ratio of chlorophyll *a* to phaeophytin *a*.

Determination of the Chemical Nature of Pollution

For determination of the chemical nature of pollution in Košice, crustose (*Lecanora chlarotera*) and foliose (*Physcia tenella*) lichens were selected, as both grow close to the United States Steel factory, the most apparent source of air pollution (site number 12, see Table 1). Crustose lichens are in general considered more tolerant to pollution than species with more complex morphology (e.g. foliose and fruticose lichens). Lichen substrate (bark of *Populus tremula*) from the same site was used as control. It was demonstrated [16] that *Lecanora conizaeoides* was useful for monitoring atmospheric pollution by metals in Armadale (Scotland). For control, lichens more sensitive to pollution from northern peripheral localities (*Flavoparmelia caperata* from site n. 1, *Ramalina fastigiata* from site n. 2, and *Physcia aipolia* from urban site n. 6, see Table 1) were used for EDX-microanalysis.

An ISI-DS130 conventional scanning electron microscope (SEM) equipped with a Gresham light element detector and a Quartz Xone energy dispersive X-ray (EDX) analysis system was used in our study.

Statistical Analysis

Differences in chlorophyll response to air pollution and elemental content were compared using one-way analysis of variance and Tukey's pairwise comparisons (software MINITAB).

Results

Passive Monitoring by Lichens

The diversity of epiphytic lichen at twelve monitoring sites in Košice city is presented in Table 3. We observed 17 different species of lichens, although four had only limited presence at only one site. Lichen diversity, in general, increased with greater distance from city center, and was greatest at Kavečany (11 lichen species), Alpinka (8 lichen species), Bukovec (6 lichen species) and Sídl. Darg. hrdinov B (5 lichen species). Botanická záhrada (Botanical Garden) with 11 discovered lichen species was the only one of those sites with higher lichen diversity near the center of the city.

As is evident from Table 3, we did not observe in Košice city the so-called "lichen desert" or zone 0 according to Hawksworth and Rose [30] scale. The most pollution-tolerant lichens collected within the monitored area were those typical for zones 3-4, characteristically occurring where mean winter sulfur dioxide levels are 70-125 $\mu g \cdot m^{-3}$. Zone 3-4 species included crustose and leprose lichens as *Lecanora* sp. (frequency 11), *Lepraria* sp. (frequency 4) and *Lecidea* sp. (frequency 4) and foliose species, e.g. *Physcia adscendens* (frequency 8). In Košice there were also frequent lichens typical for zones 4-5 areas with mean winter sulfur dioxide 60-70 $\mu g \cdot m^{-3}$. These included the foliose lichens *Hypogymnia physodes* (frequency 6), *Xanthoria parietina* (frequency 6), *Parmelia sulcata* (frequency 4) and other members of genus *Parmelia,* newly included in genus *Melanelia* (frequency 4) often associated with lichens from zones 3-4, but generally with lower abundance. Except for Botanical Garden these were generally absent from sites near city center. Lichens typical of zones 5-6 were observed mainly on the periphery of the city (sites Kavečany and Alpinka). The only locality near the city center with these lichens was Botanical Garden, but their abundance was low and thalli were minute. We observed only one lichen (*Ramalina fastigiata*) belonging to zone 7 in the Hawksworth and Rose scale. This was found only in one locality (Chata Alpinka) on an old maple tree with lowest abundance (1).

Table 2. Description of Košice transplant sites (from north to south).

Site number	Site name	Remarks	Distance from city center (km)	Distance from U.S. Steel (km)
1.	Prakovce	relatively air unpolluted area, valley near village, rich on epiphytes from zone 6-7 (Hawksworth and Rose 1970), estimated mean winter SO_2 40-50 $\mu g/m^3$, transplantation material, collected in October 2000	30.0	32.5
2.	Prakovce	as site n. 1, control, collected in February 2001	30.0	32.5
3.	Družstevná	village outside of city, rural, suburban area, north of city center	8.2	18.2
4.	Kavečany	peripheral part of city, NW of center	7.1	16.3
5.	Sídl. Darg. Hrdinov B	urban part of city, NE of center	2.8	13.1
6.	Botanical Garden	large and close area with high diversity of trees, NW of center	1.8	11.5
7.	President's residenec	urban area, very close to city center	0.7	11.3
8.	Natural Sci. Dean's Office	urban area in city center (in this study considered as city center)	0.0	10.6
9.	Bukovec	suburban closed area near water reservoir, west of city center	7.5	9.9
10.	Západ	urban area, west of city center	1.7	9.9
11.	Južná Trieda	urban, industrial area, south of city center	2.3	9.2
12.	Pereš	urban area, SW of city center	4.6	6.3
13.	Krásna nad Hornádom	urban, SE of city center	7.4	9.9
14.	Poľov	urban, SW from city center	7.4	3.9
15.	Šebastovce	urban, near road, south of city center	7.4	5.6
16.	Ludvíkov Dvor	sparsely inhabited area, south from city center	9.9	1.1
17.	Šaca	urban area, south of city center	11.5	1.9
18.	US Steel	industrial area, south from city center	10.8	0.0

Changes of Lichen Photobiont Chlorophyll Content in Transplanted Thalli

Table 4 presents chlorophyll analysis of *H. physodes* transplants after 16 week exposure to air pollution at different sites in Košice city and control (n.1, 2, according to Table 2). Localities with corresponding numbers from Table 2 (sites numbers = s.no.) are ordered according to the mean values of parameter, from highest to lowest.

The highest values for chlorophyll *a* concentration were found in control sites 1 and 2 and we did not observe significant differences between chlorophyll *a* content

Table 3. The composition of epiphytic lichen communities at twelve monitoring sites in Košice city, distribution, abundance and frequency (+ = presence at site; number in first row = abundance estimated on a 1-5 scale: 1, 1-5 thalli; 2, 6-10 thalli; 3, 11-100 thalli, 4, 101-1000 thalli; 5, over 1000 thalli for 10 investigated trees; number in parenthesis = maximal size of thallus in cm; < 50 = presence confirmed on the tree bases only). The localities are arranged in descending order of lichen diversity, lichen species are arranged in descending order of frequency.

Frequency	Sídl. Darg. hrdinov A	Nad jazerom	Staré mesto	Ťahanovce	Poľov	US Steel	Západ (trieda SNP)	Sídl. Darg. hrdinov B	Bukovec	Alpinka	Botanická záhrada	Kavečany	Site name
11	+	+	+	+	+	+	-	+	+	+	+	+	*Lecanora* sp. , *L. chlarotera* Nyl. mainly
8	-	-	1	2	5	5	5	2	-		3	4	*Physcia adscendens* (Fr.) H. Olivier
6	-	-	1 (4.6)	1 (5.7)	-	-	-	-	4 (6.8)	5 (8.4)	4 (6.1)	5 (7.8)	*Hypogymnia physodes* (L.) Nyl.
6	2 (1.1)	-	-	-	1 (2.6)	3 (13.5)	2 (6.7)	1 (1.2)	-	-	1 (1.1)	-	*Xanthoria parietina* (L.) Th. Fr.
4	-	+	-	-	-	-	-	-	+	+	-	+	*Lecidea* sp.
4	-	-	-	-	-	-	+	-	+	<50 -	<50 +	+	*Lepraria* sp.
4	-	-	-	-	-	-	-	-	3 (4.6)	3 (3.6)	1 (2.1)	2 (2.1)	*Melanelia* Essl.
4	-	-	-	-	-	-	2 (5.6)	-	-	3 (5.4)	1 (5.2)	3 (7.5)	*Parmelia sulcata* Taylor.
4	-	-	-	-	4	3	5	-	-	-	4	-	*Physcia tenella* (Scop.) DC.
3	-	+	-	-	-	-	-	+	-	<50 -	+	-	*Candelariella vitellina* (Hoffm.) Müll. Arg.
3	-	-	-	-	-	-	-	-	-	2 (2.5)	1 (2.0)	3 (3.7)	*Evernia prunastri* (L.) Ach.
2	-	-	-	-	-	-	-	<50 -	1 (1.3)	-	<50 -	2 (0.8)	*Cladonia digitata* (L.) Schaer.
2	-	-	-	-	-	-	-	1 (4.7)	-	-	1 (2.1)	-	*Parmelia tiliacea* (Hoffm.) Ach.
2	-	-	-	-	-	-	-	-	<50 -	1 (0.9)	-	3 (1.6)	*Vulpicida pinastri* (Scop.) Mattson et Lai
1	-	-	-	-	-	-	-	-	-	-	-	1 (8.7)	*Flavoparmelia caperata* (L.) Hale
1	-	-	-	-	-	-	-	1 (3.4)	-	-	-	-	*Physcia aipolia* (Ehrh.) Hampe
1	-	-	-	-	-	-	-	-	-	1 (1.9)	-	-	*Ramalina fastigiata* (Pers.) Ach.
	13	12	11	10	9	8	7	6	5	4	3	2	**Number of taxa**

Table 4. Chlorophyll analysis (a - chlorophyll *a*, b - chlorophyll *b*, a + b – chlorophyll *a + b*, a / b – chlorophyll *a / b*, 435 / 415 – optical density 435 nm / 415 nm) of *Hypogymnia physodes* transplants after 16-week exposure to air pollution at different sites in Košice city and control (n. 1, 2, Prakovce village) site (s.no. = Site number, see Table 2, n = 5; X = mean values; SD = standard deviations). Values in each vertical column followed by the same letter do not differ significantly at P < 0.05 by Tukey's pairwise comparisons.

A ($mg.g^{-1}$)		**B** ($mg.g^{-1}$)		**A+B** ($mg.g^{-1}$)		**A/B**		**435/415**	
s. no.	**X±SD**	**s. no.**	**X±SD**	**s. no.**	**X±SD**	**s. no.**	**X±SD**	**s. no.**	**X±SD**
1	2.761 ± 0.340**a**	**3**	0.813 ± 0.191**a**	**1**	3.373 ± 0.377**a**	**2**	4.827 ± 0.823**a**	**1**	0.963 ± 0.051**a**
2	2.625 ± 0.373**a**	**4**	0.622 ± 0.169**ab**	**2**	3.175 ± 0.382**ab**	**7**	4.741 ± 0.669**a**	**2**	0.956 ± 0.039**ab**
4	2.012 ± 0.330**b**	**1**	0.611 ± 0.069**ab**	**4**	2.633 ± 0.401**bc**	**1**	4.541 ± 0.555**ab**	**4**	0.887 ± 0.044**bc**
13	1.789 ± 0.219**bc**	**16**	0.611 ± 0.447**ab**	**3**	2.367 ± 0.408**cd**	**14**	4.302 ± 0.530**ab**	**9**	0.879 ± 0.045**cd**
9	1.786 ± 0.122**bcd**	**10**	0.558 ± 0.283**ab**	**9**	2.324 ± 0.114**cd**	**12**	4.231 ± 0.607**ab**	**13**	0.875 ± 0.018**cd**
10	1.747 ± 0.151**bcde**	**2**	0.549 ± 0.067**ab**	**10**	2.306 ± 0.275**cd**	**8**	4.196 ± 0.202**abc**	**6**	0.871 ± 0.044**cde**
16	1.609 ± 0.140**bcdef**	**9**	0.538 ± 0.058**ab**	**13**	2.279 ± 0.232**cd**	**11**	4.020 ± 0.635**abc**	**14**	0.870 ± 0.021**cde**
14	1.573 ± 0.065**bcdefg**	**5**	0.514 ± 0.067**ab**	**16**	2.219 ± 0.415**cd**	**18**	4.001 ± 0.514**abc**	**10**	0.845 ± 0.022**cdef**
12	1.555 ± 0.157**cdefg**	**15**	0.499 ± 0.084**ab**	**5**	1.945 ± 0.064**de**	**13**	3.863 ± 1.077**abc**	**7**	0.836 ± 0.063**cdef**
3	1.555 ± 0.265**cdefg**	**13**	0.490 ± 0.135**ab**	**14**	1.942 ± 0.059**de**	**10**	3.720 ± 1.576**abc**	**12**	0.834 ± 0.023**cdef**
11	1.472 ± 0.059**cdefg**	**17**	0.441 ± 0.115**b**	**12**	1.928 ± 0.193**de**	**6**	3.585 ± 0.544**abc**	**3**	0.832 ± 0.025**cdef**
6	1.463 ± 0.189**cdefg**	**6**	0.415 ± 0.075**b**	**6**	1.878 ± 0.236**de**	**17**	3.509 ± 1.332**abc**	**11**	0.827 ± 0.014**cdef**
17	1.435 ± 0.129**cdefg**	**11**	0.376 ± 0.083**b**	**17**	1.876 ± 0.159**de**	**16**	3.437 ± 1.392**abc**	**18**	0.812 ± 0.008**defg**
5	1.431 ± 0.037**cdefg**	**12**	0.373 ± 0.061**b**	**11**	1.848 ± 0.126**de**	**4**	3.388 ± 0.848**abc**	**16**	0.812 ± 0.020**defg**
18	1.431 ± 0.076**cdefg**	**14**	0.369 ± 0.035**b**	**15**	1.844 ± 0.110**de**	**9**	3.355 ± 0.460**abc**	**17**	0.799 ± 0.030**efg**
15	1.345 ± 0.064**defg**	**18**	0.364 ± 0.062**b**	**18**	1.794 ± 0.115**de**	**5**	2.818 ± 0.349**bc**	**15**	0.790 ± 0.010**fg**
7	1.318 ± 0.066**efg**	**7**	0.283 ± 0.046**b**	**7**	1.601 ± 0.105**e**	**15**	2.762 ± 0.491**bc**	**5**	0.784 ± 0.019**fg**
8	1.157 ± 0.178**g**	**8**	0.276 ± 0.043**b**	**8**	1.433 ± 0.220**e**	**3**	1.956 ± 0.319**c**	**8**	0.752 ± 0.024**g**
ANOVA									
F = 23.96		**F** = 3.92		**F** = 19.73		**F** = 4.11		**F** = 14.40	
P = 0.000		**P** = 0.000		**P** = 0.000		**P** = 0.000		**P** = 0.000	

Table 5a-b. Elemental analysis of lichen samples (LEC – *Lecanora chlarotera*, PHT – *Physcia tenella*, PHA – *Physcia aipolia*, PAR – *Flavoparmelia caperata*, RAM – *Ramalina fastigiata*) and substrate (SUB - bark of *Populus tremula*) collected from selected sites in Košice (in wt %, ND = not detectable amount of element by EDX-microanalysis, N=3-6). Values in each vertical column followed by the same letter do not differ significantly at P < 0.05 by Tukey's pairwise comparisons.

5a

C	X±SD	O	X±SD	Na	X±SD	Mg	X±SD	S	X±SD	Cl	X±SD
LEC	37.9 ± 2.13**a**	**RAM**	60.8 ± 0.42**a**	**LEC**	0.63 ± 0.18**a**	**LEC**	0.46 ± 0.22**a**	**SUB**	0.71 ± 0.35**a**	**LEC**	0.91 ± 0.35**a**
PAR	36.6 ± 2.39**a**	**PAR**	59.1 ± 2.03**ab**	**SUB**	ND ± 0.00**b**	**PHT**	0.36 ± 0.33**a**	**LEC**	0.39 ± 0.13**ab**	**SUB**	ND ± 0.00**b**
SUB	36.3 ± 0.96**a**	**PHT**	57.7 ± 2.18**ab**	**PHT**	ND ± 0.00**b**	**SUB**	0.35 ± 0.05**a**	**PHT**	0.14 ± 0.24**bc**	**PHT**	ND ± 0.00**b**
PHA	36.0 ± 3.44**a**	**PHA**	56.5 ± 1.93**b**	**PAR**	ND ± 0.00**b**	**PHA**	0.29 ± 0.14**a**	**PAR**	ND ± 0.00**c**	**PAR**	ND ± 0.00**b**
RAM	35.5 ± 0.61**a**	**SUB**	53.9 ± 0.29**bc**	**RAM**	ND ± 0.00**b**	**RAM**	0.20 ± 0.09**a**	**RAM**	ND ± 0.00**c**	**RAM**	ND ± 0.00**b**
PHT	22.1 ± 8.83**b**	**LEC**	50.6 ± 3.01**c**	**PHA**	ND ± 0.00**b**	**PAR**	0.07 ± 0.06**a**	**PHA**	ND ± 0.00**c**	**PHA**	ND ± 0.00**b**
ANOVA											
F = 8.09		**F = 14.32**		**F = 39.74**		**F = 2.20**		**F = 12.12**		**F = 23.78**	
P = 0.000		**P = 0.000**		**P = 0.000**		**P = 0.000**		**P = 0.000**		**P = 0.000**	

5b

Al	X±SD	Si	X±SD	K	X±SD	Ca	X±SD	Fe	X±SD	Cu	X±SD
PHA	1.21 ± 0.75**a**	**PHA**	2.56 ± 1.52**a**	**PHA**	1.08 ± 0.41**a**	**PHT**	13.9 ± 9.72**a**	**PHT**	2.08 ± 0.77**a**	**PAR**	0.34 ± 0.15**a**
LEC	1.09 ± 0.35**a**	**LEC**	2.34 ± 0.85**ab**	**RAM**	0.80 ± 0.25**ab**	**SUB**	7.71 ± 0.63**ab**	**LEC**	1.98 ± 0.95**a**	**RAM**	0.31 ± 0.21**a**
PHT	0.78 ± 0.33**a**	**PHT**	1.44 ± 0.51**ab**	**PAR**	0.67 ± 0.20**ab**	**LEC**	2.92 ± 0.82**b**	**PHA**	1.44 ± 0.91**ab**	**LEC**	0.12 ± 0.16**a**
RAM	0.69 ± 0.39**a**	**PAR**	0.87 ± 0.21**ab**	**PHT**	0.55 ± 0.18**ab**	**PHA**	0.70 ± 0.52**b**	**PAR**	0.93 ± 0.39**ab**	**PHA**	0.11 ± 0.17**a**
PAR	0.55 ± 0.27**a**	**RAM**	0.67 ± 0.23**ab**	**LEC**	0.51 ± 0.27**ab**	**RAM**	0.62 ± 0.19**b**	**RAM**	0.29 ± 0.09**b**	**PHT**	ND ± 0.00**a**
SUB	0.34 ± 0.20**a**	**SUB**	0.23 ± 0.19**b**	**SUB**	0.22 ± 0.28**b**	**PAR**	0.49 ± 0.13**b**	**SUB**	0.11 ± 0.19**b**	**SUB**	ND ± 0.00**a**
ANOVA											
F = 1.89		**F = 4.46**		**F = 4.26**		**F = 9.08**		**F = 4.90**		**F = 3.06**	
P = 0.147		**P = 0.008**		**P = 0.010**		**P = 0.000**		**P = 0.005**		**P = 0.036**	

in lichen thalli used for transplantation and the lichen thalli after 16 wks on the control site. However, we observed significant reduction of chlorophyll *a* at all transplanted sites in Košice city. The highest concentration of chlorophyll *a* in transplant sites was measured at Kavečany. Chlorophyll *a* concentration less than 2 mg/g[1] were found at Krásna nad Hornádom, Bukovec, Západ, Ludvíkov dvor and Poľov. In other sites chlorophyll *a* concentration was reduced and the lowest chlorophyll *a* concentrations were recorded at the following sites: U.S. Steel, Šebastovce, the president's residence and the natural sci. dean's office. Chlorophyll *b* content was less influenced (Table 4). The highest content of chlorophyll *b* was measured in the outskirts of Košice (Družstevná) and the lowest at both sites in the city center (president's residence as well as natural sci. dean's office). Chlorophyll *a+b* concentrations corresponded, in general, to chlorophyll *a* concentrations. However, the chlorophyll *a/b* ratio was not changed very dramatically and we did not observe significant differences in this parameter between peripheral parts of the city and sites near apparent sources of air pollution. The ratio of optical densities at 435 and 415 nm (OD 435/OD 415), interpreted as the phaeophytinization quotient, decreased significantly in all transplanted lichens. However, the lowest degree of chlorophyll *a* degradation was observed at sites related to city periphery (Kavečany, Bukovec, Krásna nad Hornádom), while the highest were observed at sites related to U.S. Steel (Šebastovce, Ludvíkov Dvor, Šaca, U.S. Steel) and the city center (natural sci. dean's office). The transplanted lichen collected from site Sídl. Darg. Hrdinov also showed dramatic degradation of chlorophyll *a*.

EDX - Investigation Concerning the Chemical Nature of Pollution

Using EDX-microanalysis we determined the elemental composition of thalli from five different lichen species and lichen substrate from trees grown near U.S. Steel (Table 5a-b).

The carbon content of all samples was relatively stable and significantly less only in the lichen *Physcia tenella*. Oxygen levels of analyzed samples were in interval 50-60 wt %. The amount of some elements (e.g. Na, Mg, S and Cl) was usually near detection limits. Detectable amounts of Na and Cl were only in samples of *Lecanora chlarotera* collected near U.S. Steel. Similarly, detectable amount of S was measured only in both lichens grown near steel factory and in bark of trees from this site. We did not observe significant differences in Mg content of analyzed samples (Table 5a).

We observed relatively high and detectable amounts of Al, Si and K in all samples. The lowest mean values of these elements were detected in the bark of trees grown near the steel factory, in lichens these concentrations were higher. The amount of Ca was highest in samples collected near the steel factory, in both lichen species and their substrate. We observed significantly higher Fe concentrations in lichens collected near the steel factory. The lowest Fe content, however, was detected in lichen substrate samples from the site close to the steel factory. The Cu content of some samples was near detection limit and we did not observe significant differences between samples (Table 5b). The concentrations of other elements were not detected due to detection limits of EDX-microanalysis.

Discussion of Results

In our study we recorded 17 lichen taxa. The increase of lichen diversity and higher abundance of pollution sensitive species on the north and west of the city are probably related to increased distances from the United States Steel factory as well as the city center, although this phenomenon can be partly explained by microclimate and higher altitude (Čierna hora). Local differences in climate and growing conditions can affect biomonitoring [8]. The locality with higher lichen abundance close to the city center is the Botanical garden only, although this locality is also very close to north-west localities, characterized by higher lichen species abundance. The higher lichen diversity is also the result of very high lichen substrate diversity connected by high tree diversity and differences in bark pH.

The majority of lichen species found in this study were common epiphytic lichens of Slovakia [42]. The lichens *Evernia prunastri*, *Flavoparmelia caperata*, *Parmelia tiliacea* and *Vulpicida pinastri* are pollution sensitive, and "vulnerable" lichens of Slovakia [43], the last two are new for square 7293 on Slovak Republic net map [42]. *Flavoparmelia caperata* was found in 1970-81 as damaged thalli only within this square [42]. *Ramalina fastigiata*, which belonging to zone 7 [30] was also new for square (7293) and is an endangered lichen species of the Slovak Republic. However, its distribution in Slovakia is much more extensive [42] than some of the more pollution tolerant lichen species found in this study (e.g. *Vulpicida pinastri*).

Effects of transplantation have been assessed by the measuring of many physiological parameters [22, 27, 34, 59] as well as in classical bioaccumulation studies that analyze contaminants in tissue [33]. The lichen *Hypogymnia physodes* has been one of the most frequently studied [19]. The optimal duration of exposure period is 6 to 9 months [20]. A period less than 4 months is usually not enough to accumulate sufficient amount of airborne heavy metals, although an extended period of more than 12 months may cause substantial loss of thalli by wind, rain or hail.

Chlorophyll content and its degradation is often used as one of the cheapest and most accurate methods of biomonitoring [26, 28, 34]. The DMSO as solvent replaces acetone and requires only a small amount of lichen tissue [48]. The ratio of optical densities (OD) chlorophyll samples read at 435 nm and 415 nm (OD 435/415) is the most frequently used parameter for chlorophyll a degradation [23, 27]. This ratio was highly affected at *H. physodes* thalli at all biomonitoring sites in Košice, and the highest amount of degradation was observed at sites previously considered to be near pollution centers or sources. However, this ratio was much less than 1.4, a value that is considered optimum for unaffected chlorophyll *a* in lichens [48] and was measured in axenic cultures of lichen photobiont [1]. However, it was demonstrated previously that the chlorophyll content of lichens is not constant and varies seasonally [54]. Chlorophyll *a* concentrations as well as chlorophyll *a+b* concentrations were significantly affected by pollution in biomonitoring sites in Košice, in accordance with results received for vehicle traffic pollution [37] or for urban emissions [6, 58]. The effect on chlorophyll a/b ratio was not so evident, although chlorophyll b was affected less, similar to results obtained for some metals [10]. In axenic cultures of the lichen photobiont *Trebouxia erici* after copper exposure decreased concentrations of chlorophyll a [1]. Chlorophyll b concentration was less affected and usually increased as copper caused transformation of chlorophyll *a* to chlorophyll *b* [10].

Elemental patterns in lichens reflected atmospheric deposition patterns [5, 12]. Seasonal changes in iron, copper, zinc, lead and some other elements (e.g. K, Na, Ca) were observed, as their levels were higher in summer than in winter [5]. Iron concentrations in lichens are in some cases very high. The epiphytic fruticose lichen *Pseudevernia furfuracea*, for example, collected in Bern (Switzerland) was found to contain more than 10,000 μg/g of iron, although more tolerant foliose lichen *Parmelia sulcata* contained usually less than 1,000 μg/g of iron [21]. It was previously demonstrated that lichen species differed in regard to metal-binding capabilities under laboratory conditions [44]. In foliose lichens iron can reach 90,000 μg/g [51], although it is usually much less [9, 41]. Iron content of *H. physodes* transplants exposed to an iron-contaminated environment depended on the length of exposure [17]. In our previous study we observed growth inhibition of axenic cultures of free-living lichen photobionts caused by increasing some metals in nutritional media [2].

Typical solid waste and slag from the steel industry is composed from iron, carbon, sulfur, Fe_2O_3, SiO_2, Al_2O_3, CaO, and MgO [53], in accordance with elemental composition of analyzed lichens as well as their substrate (bark). Lichens accumulated higher amounts of some elements (e.g. Fe, Si, K and Al) than bark. However, S amount was higher in bark when it was compared with lichens from the same site, although results did not vary significantly. The crustose lichen *Lecanora chlarotera* showed higher variability of detectable elements than the foliose lichen *Physcia tenella*, or bark of *Populus tremula*.

Conclusions

We demonstrated possibilities to parallel the use of several methodologies in assessment of air pollution by lichens in urban areas with intensive industry. By use of passive monitoring, we were able to evaluate the influence of prolonged exposure of steel industry pollutants to lichen diversity distinguished by five typical lichen zones from the ten-zone scale of Hawksworth and Rose [30]. By chemical analyses of lichen thalli and their substrate we analyzed the chemical nature of pollution and distribution of main pollutants in the city. By use of lichen transplants we assessed the influence of local air pollution on physiological status of lichens, measured as changes in lichen chlorophyll content.

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