Oecologia Montana 2015,
24, 50-59

In situ monitoring of air pollutants in the Ružomberok area - vascular plants as indicators

R. KORČEKOVÁ, E. GREGUŠKOVÁ, M. KMEŤOVÁ and M. JANIGA
Institute of High Mountain Biology, Tatranská Javorina 7, SK-059 56 Slovak Republic, e-mail: eva.greguskova@uniza.sk

Abstract. In our study we focused on the usage of *Pinus sylvestris*, more specifically on needles as the bio-indicators of air pollution in the town of Ružomberok. Samples were collected from the Mních Hill locality research area. We created 19 network points and 4 main locations from which we took in the period of autumn months (October, November) 2014 and spring months (March, April) 2015 the samples of *Pinus sylvestris* twigs. After that the samples were dried, split into one-year and two-year needles, these were milled and X-rated in the XRF spectrometer Delta. The results were processed again in Excel and evaluated in the statistical programme STATISTICA 10.1. From the results obtained, we wanted to highlight the degree of contamination by heavy metals and other pollutants and identify the storage capability of a selected bio-indicator. We processed the results indicating an increased concentration of air pollutants. Increased levels of Cl, Pb and Rb were measured mainly in the southern and south-western exposure of the Mních Hill.

Key words: plant bio-indicators, *Pinus sylvestris*, air pollutants, Mondi SCP

Introduction

Atmospheric pollution constitutes one of the major problems in urban environment. The sources of this pollution are represented by natural processes (volcanoes, erosion etc.) and human activity such as industry, combustion of traffic fossil fuels and energy production. Pollutants from many different anthropogenic sources contain especially heavy metals and sulphur oxides (Sawidis 2011; Kuang 2007). This pollution increases locally the concentration of heavy metals as such in soil, plants and animals (Said *et al.* 2005), and can be a genuine health hazard to human beings as well as to the vegetation of any many horticultural plants (Yilmaz and Zengin 2004).

The determination of plant chemical analysis is one of the most frequently used methods to monitor air pollution. It is now well accepted that many groups of plants and evergreen trees can be effectively used as the bio-monitoring of environmental pollution. Various plants such as mosses (Makinen 1987; Ruhling *et al.* 1985), lichens and fungi (Svoboda *et al.* 2000) have shown a marked ability to accumulate certain pollutants (Kuhn *et al.* 1995; Kurczynska *et al.* 1997). Among the most useful conifers for their wide distribution and easy identification has become Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*). These coniferous species, specifically their needles, are used as bio-indicators for atmospheric pollutants in industrial areas. Sulphur dioxin as a major pollutant in industrial areas (Pfanz *et al.* 1987) and the heavy metals deposition have also been found to affect the nutrient status of conifer needles (Yilmaz and Zengin 2004). The plant's main barrier to the SO entry is the stomate (Pfanz *et al.* 1987) and its response towards pollutants is largely dependent on leaf age, concentrations and the combination of pollutants (Kainulainen 1995). It was further found out that the concentrations of different nutrients vary in needles with the phase of the annual physiological cycle (Fife and Nambiar 1982; 1984), needle age (Florence and Chuoung 1974; Madgwick *et al.* 1983) and sampling position (Helmisaari 1992).

Our research was carried out on the peak of the Mních Hill in the town of Ružomberok, situated 2 km north-east of the pulp mill factory complex. For the research we chose bio-indicator species (*P. sylvestris*), specifically needles of pines in which we examined the content of pollutants. The chosen species *P. sylvestris* is the second most widespread conifer in Slovakia and is a pioneer species that readily regenerates, after major natural or human disturbances, whether the weed competition and grazing pressure are low (Mátyás *et al.* 2003). Needle-like leaves grow on the branches in pairs from one place, and they are stiff and pointy. Needles grow 1-8 cm long, 1-1, 8 mm broad, they are spiky, slightly oblong twisted, on the flat ventral side are grey-green, on the gibbous dorsal side are dark green (www.botany.cz 2015). Needles bloom in May and fall off after about 2-3 years (Gilmar and Watson 1994).

The following aims are addressed in this paper:

1. By the means of using *P. sylvestris* needles, as one of the best bio-indicators, to notice the global trend of the increasing contamination of air pollutants.
2. To detect the content of heavy metals in *Pinus* needles and other pollutants on the research area.
3. To point out the accumulated storage capability of species, its physiology (we compare the content of substances in one and two-year needles).

4. To identify the main source of the air pollution and direction spreading of these pollutants in the research area.

Material and Methods

Sampling

The samples of *P. sylvestris* were collected on the peak of the Mních Hill (657 m) on four main points of supply during the period after the growing season (October, November) 2014, and in the growing season (March, April) 2015. Four main sites were chosen deliberately because of the orientation of the slope, prevailing winds in the potential pollution sources (Mondi SCP) and its distance from the sampling sites. In addition to the four main sampling sites, the nineteen point network was also created to determine the spatial distribution of pollutants. To determine the spatial distribution of nineteen sampling sites, at each site three random twigs from a single subject were collected. The criterion for the selection of suitable samples - twigs was the presence of a sufficient quantity of 1 and 2 years old needles. On the four main points of supply mixed samples were taken. As to the mixed sample, three different individuals were selected, spaced three to five meters while one branch was removed in each case. Samples were processed and stored at the Institute of High Mountain Biology in Tatranská Javorina.

Instrumental analysis

Needles were dried in the biological thermostat Memmert IF 160 plus and subsequently crushed into fine powder by a ball mill cryogenic Cromill, Retsch. The powder in an amount of 1.5 g - 2 g was placed into special pellets with a protective prolene folium. Then, the samples were analysed by X-ray fluorescence, using the hand – held XRF spectrometer DELTA CLASSIC (USA). The following elements were determined: S, Pb, Fe, Mn, Cu, K, Ca, Zn, Mo, Cr, Ba, Rb, Sr, Ti, Zr, Cl, As, Co, Cd, Zb, I.

Statistics

To evaluate the data, we used the software Statistics Version 10.1, and we chose the methods of principal components (PCA). The object of the method is, from the obtained data as quantitative variables, to create new, which are a linear combination of foregoing variables - the main components. In our case, we used it to identify the different effects, which may affect the appearance of elements presented in the samples. Vectors (eigenvalues) that we calculated refer to a link between the measurand and also show the influences that affect the data. We calculated factor scores for each measurement and then they were compared with the selected category. The data are different at p = 0.05, One Way ANOVA.

Results

As a result we could take into consideration five factors ranging from 46.7 % to 5 % (Table 1), of which we subsequently transferred a further analysis. In the analysis, we used our factor score and correlation of the age of needles, exposure and the month in which the samples were collected. Outputs from the analysis were transferred to the chart together with descriptions.

Factor 1

In the first factor we could see the expositionally complex pollution of needles. The contamination of *P. sylvestris* varies depending on the period in which the samples were taken, irrespective of the age and individual needles exposure.

The influence of seasonality on the needles can be observed in Fig. 1. In the first factor we could see the elements S, K, Cr, Mn, Fe, Zn, Mo, Pb. Remarkably higher values were measured in the autumn months,

	FACTOR 1	FACTOR 2	FACTOR 3	FACTOR 4	FACTOR 5
S	-0.389	-0.070	0.171	0.125	-0.212
Cl	0.102	0.071	0.678	0.429	0.473
K	-0.349	-0.147	0.213	0.223	-0.339
Ca	-0.253	0.487	-0.122	0.005	-0.010
Cr	-0.379	-0.124	0.194	-0.105	-0.172
Mn	-0.302	-0.084	-0.245	0.335	-0.215
Fe	-0.365	-0.015	0.217	-0.039	0.173
Zn	-0.280	0.357	0.050	-0.005	0.184
Rb	-0.079	-0.440	-0.406	0.547	0.299
Sr	-0.117	0.581	-0.299	0.284	0.104
Mo	-0.312	-0.136	-0.010	-0.423	0.202
Pb	-0.292	-0.169	-0.215	-0.253	0.577
% total variance	46.7	14.8	10.7	7.4	5

Table 1. Eigenvectors of the correlation matrix *Pinus sylvestris*

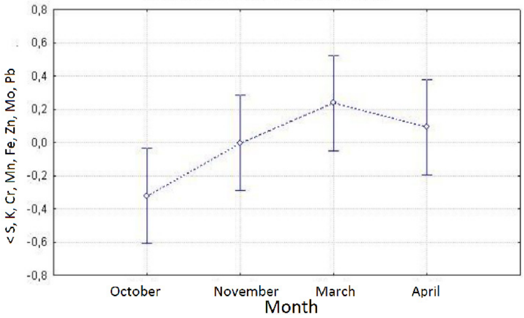

Fig. 1. Elements of S, Cr, Mn, Fe, Zn, Mo, Pb measured in each month. Month, LS Means (F (3,180)=2.6371, p=0.05).

Factor-Month	Mean	Std. Err.	N
October	-0.32a	0.14	46
November	-0.00ab	0.14	46
March	0.23b	0.14	46
April	0.09ab	0.14	46

Table 2. The comparison of mean principal component scores on the component 4 in different seasons. Two - way Month-F = 2.6, P = 0.05, Slope N- F = 18, P = 0.127, Age NS- F = 0.2, P = 0.62.

not in the growing season, compared with the spring months when the growing season begins (Table 2).

Factor 2

The second factor denotes the inverse relationship between Rb and Zn, Ca, Sr. While in case of the factor 1 there was found complex pollution of *P. sylvestris* needles depending on seasons, which indicates local microclimate pollution (higher ranked contamination in the autumn months of local inversions), the second factor probably reflects a biogenic cycle Ca, Zn, Sr in *Pinus sylvestris*. The amount of Ca, Zn, Sr increased relatively to Rb in case of the 2 - year needles on the contrary to the 1 - year needles. The phenomenon of the increasing levels of Ca, Zn and Sr is also depen-

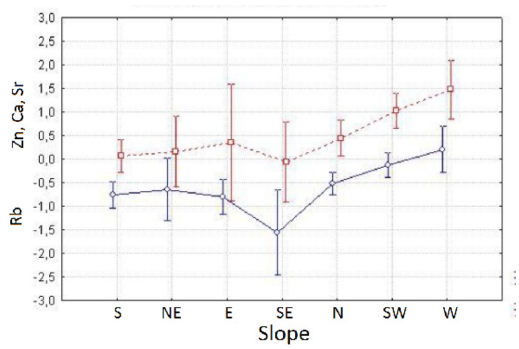

Fig. 2. Mutual inverse relationship between Zn, Ca, Sr and Rb in pine needles depends on needle age and slope.Slope year. (F (6,170)=0.45709, p=0.82)

Factor-Slope	Mean	Std. Err.	N
S	-0.34a	0.13	48
NE	-0.24ab	0.23	16
E	0.23abc	0.32	8
SE	-0.81a	0.32	8
N	-0.04ab	0.12	56
SW	0.45bc	0.19	24
W	0.84c	0.19	24

Table 3. Comparison of mean principal component scores on the component 7 in a different slope. Two - way ANOVA, Slope F = 9.7, P = 0.000, Slope- Year F = 0.46, P = 0.8, Year F = 57.4, P = 0.000.

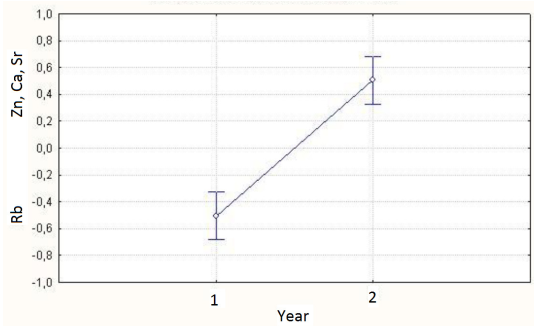

Fig. 3. Value Rb, Zn, Ca, Sr dependencies in the age of needles (F (1, 182)=63.088, p=0.000).

Factor-Year	Mean	Std. Err.	N
1.st.year	-0.5a	0.09	92
2.nd.year	-0.5b	0.09	92

Table 4. Comparison of the average sub-score of the principal components 2 by the age of needles.

dent on exposition, thus the local light and temperature conditions of *P. sylvestris* were growing (Fig. 2, Table 3). However, locating itself is a less significant factor compared to the age of needles. Both factors are important, but they are independent. It is very likely that the accumulation process is an endogenous phenomenon; independent of the seasons like the NS factor, F = 2.0, P = 0.1. It is a biogenic process of *P. sylvestris* in the fact that the other elements such as Pb, Mo in the phenomenon are not manifested significantly. Factors behave independently from exposure, there's no interaction (Fig. 3, Table 4).

Factor 3

This phenomenon is not related to the age of needles (F = 0.005, P = 0.943). The third factor is a factor of the accumulation of Cl in needles in relation to Rb. The ratio of Cl to Rb increased mainly in November, and at southerly located slopes (Fig. 4, Table 5). In con-

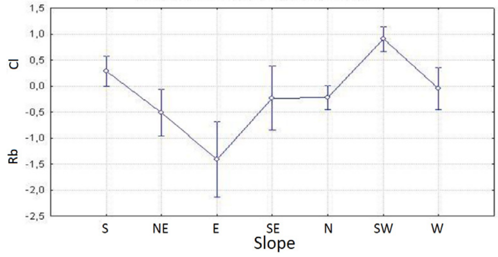

Fig. 4. The ratio of the measured elements Rb to Cl according to the exposure (F (6, 156)=11.648, p=0.000).

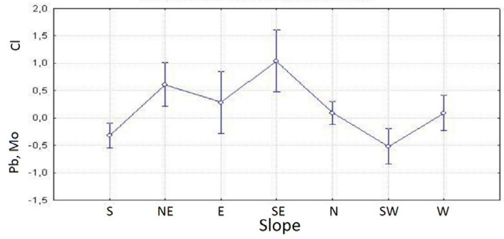

Fig. 6. The elements Pb and Mo Cl compared to the exposure and its implications moving volume element in *Pinus sylvestris* needles (F (6, 156)=6.997, p=0.000).

Factor-Slope	**Mean**	**Std. Err.**	**N**
S	0.29b	0.13	48
NE	-0.51a	0.22	16
E	-1.4c	0.31	8
SE	-0.23ab	0.31	8
N	-0.23a	0.12	56
SW	0.9d	0.18	24
W	-0.05ab	0.18	24

Table 5. Comparison of mean principal component scores on component 7 in a different slope. Two – way ANOVA, F = 11.6, P = 0.000.

Factor-Slope	**Mean**	**Std. Err.**	**N**
S	-0.32a	0.14	48
NE	0.61b	0.23	16
E	0.28ab	0.33	8
SE	1.04b	0.33	8
N	0.09ab	0.13	56
SW	-0.52a	0.19	24
W	0.09ab	0.19	24

Table 7. Comparison of mean principal component scores on the component 7 in a different slope. Two – way ANOVA, F = 7.000, p = 0.000.

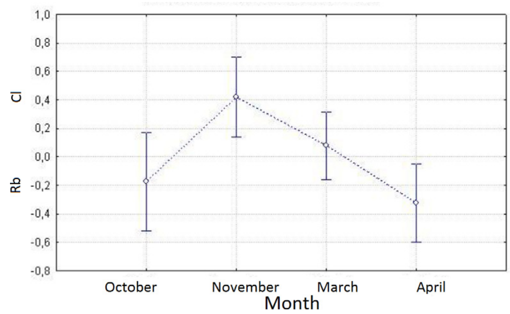

Fig. 5. Rb against Cl dependencies in the months when the samples were collected (F (3, 156)=4.2659, p=0.006).

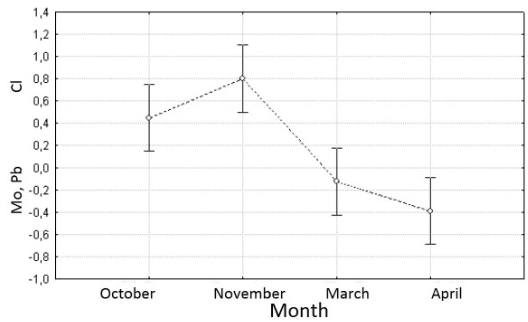

Fig. 7. The movement of the concentration of elements Mo, Pb Cl due to the actions of spring and autumn months (F (3, 156)=12.605, p=0.000).

Factor-Month	**Mean**	**Std. Err.**	**N**
S	-0.17a	0.14	46
NE	0.42b	0.14	46
E	0.08ab	0.14	46
SE	-0.33a	0.14	46

Table 6. Comparison of mean principal component scores on component 4 in different seasons. Two – way ANOVA, F = 4.3, p = 0.006.

trast, in the east, the relation was opposite. These two effects, i.e. season and exposure, are unrelated to each other, although they have a significant impact on the relative Cl accumulation in the needles of *P. sylvestris* (Fig. 5, Table 6). The accumulation of Cl is expressly an exogenous phenomenon, since the impact of one- and two-year needles had no effect. It can be assumed that the southwest slopes of the phenomenon relate to the metabolism of *Pinus sylvestris*. This phenomenon is conditioned by a better light and temperature conditions.

Factor 4

It is a more complex factor of pollution. It identifies the situation of Pb pollution, caused by transport and the Cl dust from Mondi SCP, Inc. In the autumn month of November particularly, the amount of Pb on the needles is relative to the amount of Cl in either one or two-year pins (Fig. 6, Table 7, Fig. 7,

Factor-Month	Mean	Std. Err.	N
OCTOBER	0.25ab	0.13	46
NOVEMBER	0.57b	0.13	46
MARCH	-0.14a	0.13	46
APRIL	-0.69b	0.13	46

Table 8. Comparison of mean principal component scores on the component 4 in different seasons. Two – way ANOVA, F = 8.1, P = 0.000.

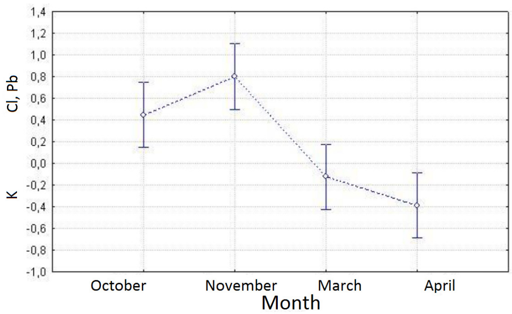

Fig. 8. K to Cl and Pb due to months (F (3, 156)=12.605, p=0.000).

Factor-Month	Mean	Std. Err.	N
OCTOBER	-0.24a	0.13	46
NOVEMBER	-0.42a	0.13	46
MAREC	0.26b	0.13	46
APRIL	0.40b	0.13	46

Table 9. Comparison of mean principal component scores on the component 4 in different seasons. Two - way ANOVA, F = 3.5, P = 0.02.

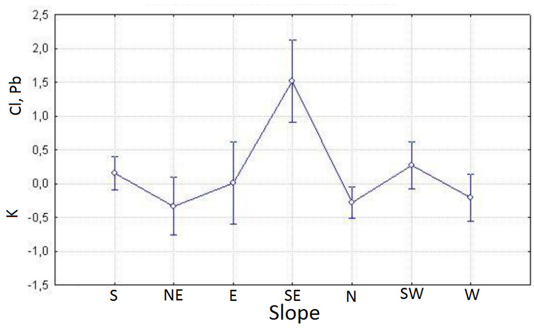

Fig. 9. The effect of exposure to Pb and Cl. (F (6, 156)=6.2931, p=0.00001).

Table 8). In the spring month, the amount of Cl inversions in the town of Ružomberok tends to decrease, but the traffic remained relatively the same, increasing the proportion of Pb to Cl. This phenomenon is likely to be monitored by monitoring stations in the vicinity of the town of Ružomberok. It is

Factor-Slope	Mean	Std. Err.	N
S	0.15a	0.13	48
NE	-0.33a	0.23	16
E	0.01a	0.33	8
SE	1.51b	0.33	8
N	-0.28a	0.12	56
SW	0.27a	0.19	24
W	-0.2a	0.19	24

Table 10. Comparison of mean principal component scores on the component 7 in a different slope. Two – way ANOVA, F = 6.3, P = 0.000.

not related to the age of *P. sylvestris* needles (F= 0.029, p = 0.864).

Factor 5

Pb and Cl together contaminate the southeast exposure. Probably, the factor is represented by the factory Mondi SCP, Inc. Subsequently, it causes the increase of dustiness, where Pb, Cl from various sources bind to the particles. The elements Pb and Cl in the needles are guided by prevailing winds that go east to northeast (Fig. 8, Table 9). This method of Cl, Pb accumulation probably affects the metabolism of the biogenic elements of potassium. The phenomenon is independent with respect to the age of needles (F = 1.71, P = 0.7). The exposure phenomenon has an appreciable influence on the accumulation of Cl and Pb in comparison with the months (Fig. 9, Table 10).

Discussion

The bio-monitoring of air quality while using plants has been widely applied to detect and to monitor the effects of pollution (Sawidis *et al.* 1995 a,b,c; Bargagli 1998; Mingorance and Oliva 2006; Gajić *et al.* 2009) and has been practised for many years, in many European countries, especially in urban areas. Trees are very efficient at trapping atmospheric particles. They play a special role in reducing the level of fine, "high risk" respirable particulates, which have the potential to cause serious human health problems. Using the bio-indications of *P. sylvestris*, we have tried to identify the main sources of air pollution in the industrialized loaded area of the town of Ružomberok.

The harmful effect of immission on forest trees is essentially a resulting action of the endogenous properties of wood and exogenous environmental factors (Sobocký 1974). The endogenous factors include cycles and concentrations of the nutrient in plant biomass, which generally influence the balance between the nutrient uptake, plant growth, nutrient retranslocation and loss. These processes are likely to be influenced both by plant genomes and soil fertility as well as by other environmental conditions such as orographic situation, particular exposure, developmental stage of plants, critical phase of growth, age, cover, period of years and health conditions of trees.

The other factors include the growth and habitat conditions such as climate, soil, extreme weather situations, mutual relationship of nutrition, and the status of a plant or vegetation in the whole community (Sobocký 1974).

In *Pinus* needles, by XRF spectrometer DELTA, we detected elements S, Cl, K, Ca, Cr, Mn, Fe, Zn, Rb, Sr, Mo an Pb in various concentrations. These numerous elements are unevenly distributed along the length of pine needles. The element concentration is a dynamic process, which is coupled with other metabolic processes and also influenced by many factors including the foliar age, time of a year, and environmental conditions at the site e.g., edaphic quality and pollution level (van den Driessche 1974; Ostrowska and Szczubialka 1988; Heliövaara and Väisänen 1989). The distribution patterns along the needle can be influenced by pollution, by differences in the mobility of elements and by the distal accumulation of toxic elements, and also by the influence of physiological and biochemical processes (Gierthych 1996).

Most of the detected elements themselves belong to group of macronutrients, which provide calories or energy of trees. For example, potassium in trees is important in the protein formation, photosynthesis, fruit formation and disease resistance; sulphur is necessary in the formation of proteins, enzymes and vitamins and is critical to the formation of chlorophyll; calcium is essential in the formation of cell walls (www.treerx.com 2015).

Several studies have shown that element concentrations in needles can be used to diagnose nutrient deficiency or excess, particularly under the pollution stress (Smidt *et al.* 1988). Elements such as Fe, Cu, Zn, Cd and Pb may render negative impacts on pine trees under the pollution stress since their concentrations were far above their normal ranges for the plant growth. According to Fober (1993), changes caused by the deficiency of N, Ca, Fe or B are visible in whole needles, whereas changes caused by the deficiency of K, P, Mg or Mn are only visible at the needle tip.

Detected elements such as Fe, Zn, Mn, Pb include the group of heavy metals and these elements may render a negative input on pine trees under the pollution stress since their concentration were far above their normal ranges for the plant growth. The study by Kuang *et al.* (2007) confirmed the usefulness of needle sheaths as a good bio-indicator for particular pollution containing Fe, Cu, Zn, Pb, Cr, Cd and Al. In general, elevated concentrations of Cu, Zn and Pb in plant tissues indicate the contamination of air with these elements.

The highest concentration detected elements S, Cl, K and Ca in one-year and two-year needles. According to the authors Tiegs (1930) and Zinkernagel (1958), the normal content of sulphur in the leaves of trees fluctuates throughout the growing season of one day. As with sulphur, also with taking evidence of the chlorine immission, we must bear in mind that there is a natural content of chlorides in variable amounts in the plant, and therefore it is impossible to secure the evidence of harming the plant with chlorine. Zahn (1970) speaks of the so-called detoxification mechanism, thus supplying the plants with sulphur through the leaves in the form of SO_2 in very low concentrations is vital for them.

Our study suggested that small sources of air pollution are represented by households; the highest potential industrial source of air pollution in the study area is represented by the factory Mondi SCP and its location in the Liptovská Mara Dam in the combination with unfavourable meteorological conditions (temperature inversion and certain wind directions) The results of our analysis, however, is that we assume that elements Cl, Pb and S are air pollutants, and Cl and S are from the production of the factory Mondi SCP. Our claim corresponds with several studies carried out in the area of the town of Ružomberok aimed on the environmental pollution. The results of these studies suggest the negative influence by these air pollutants on animals and plants in the study area. Of these conclusions, we can say that Cl is vented to the air by the production of the factory Mondi SCP. As regards the contamination of Pb in the study area, its highest concentration in plants (Šoltés 2014; Mráz 2015; Bartaloš 2015) and animal organisms, e.g. small mammals (Gajdoš 2015) suggest the influence by traffic. These elements were detected in *P. sylvestris* needles, and that does seem to have a negative effect on their vegetation cycles. Emissions of chlorine to the plants are received by vents, and damage primarily the parenchyma of the plants. Increased concentrations of chlorine cause deformation and cell shrinkage. According to Mengel (1968), chloride moves very well in the phloem in plants, which could explain the rapid transport from the assimilation organs to the roots. The loss of chloride takes place by the exchange diffusion or the diffusion of cavities filled with water. Lundegardh (1960) believes that the chloride ion influences the intensity of respiration in the storage process. According to observations by Eaton (1942), chloride ions affect the growth and transpiration at first in a positive way, and after overcoming certain concentrations, which are in case of many plant species different, in a negative way.

P. sylvestris disposes with the length of needles while the structure of wax on the surface of needles varies in the age of needles and responds to the pollution of air by exogenous influences (Knapp 2010). In our results of principal components (PCA), we found several phenomena related to the accumulation of detected elements in needles in relation to environmental variables (age of needles and slope exposure). The first phenomenon showed that significantly the highest values of the elements S, K, Cr, Mn, Fe, Zn, Mo and Pb were measured in the autumn period (October, November), when the needles were not growing. On the contrary, the lowest values of these elements were measured in the spring period (March, April). This phenomenon applies to 46.7 % of cases (eigenvector). As to the results of the correlation matrix, we can say that the more of S, Ca, Cr, and Fe, the less of Cl, Rb, Sr, Pb. In our study we confirmed the fact that the pine accumulation of various heavy metals in different periods (one or two years of age needles) varies. Recent studies (Knapp 2010) present findings that due to the growth of new needles concentrations of elements in the needles *P. sylvestris* are lower than they were in previous years. It follows the same theory as by Nikolajevskij (1974) that researched the contamination of *Pinus* needles by acid gases in Ural. In this study he found out that one-year needles begin their normal photosynthetic activities only in the middle of summer, while the two-year needles are already intoxicated by acid gases. This means that the coniferous trees suffer from "carbon hunger", which actually causes their death. Our results further support the fact that with the increasing age of needles, the content of contamination by heavy metals increases in the early developed needles, which can be susceptible to various elements, and in particular to the accumulation of SO_2. The authors Pietrzykowski *et al.* (2013) claimed that the effects of SO_2 and heavy metals persisting for many years have inflicted significant damage on trees. Although air pollution caused by SO_2 creates mainly a threat to vegetation, pollution with heavy metals is more dangerous to animals and humans. However, heavy metals at high concentrations may reduce the plant growth and development, and in extreme cases may lead to the death of plants (Greszta 1982). Heavy metals may reach terrestrial ecosystems as wet or dry deposition (Pacyna 1990). Our deposition has also been found to affect the nutrient status of conifer needles (Hutchinson and Whitby 1974). Sulphur compounds measured in the present study confirm the trend of increasing concentration of S in different parts of the needles. This theory was confirmed by Kuang (2007) when his measured values of S were different in each part of the needles, from the tip to the housing needles. The consequence of destroying the waxy layer on pine needles, changing the content elements permeated into individual needles. The statistical difference of P between the sheath and tip section in C was found in needles, while significant differences were found between the sheath and the middle, as well as between the sheath and tip section in C needles (Kuang 2007). Rautio (1998) in their study confirmed the fact that the level concentration of S contained in the spile may significantly increase S in the needle.

The second phenomenon in results described the inverse relationship between Rb and Zn, Ca, Sr. While one factor depends on the seasons, the second factor probably reflects the endogenous physiology of *P. sylvestris*, where the age of needles affects the accumulation of Zn, Ca, Sr to Rb. The amount of Ca, Zn, Sr increased relatively to Rb in two - year needles on the contrary to one - year needles. This phenomenon also depends on the slope exposition of the Mních Hill as well as on the local light and temperature conditions were *P. sylvestris* growing. The same relates to one - year and two - year needles. On the southeast slopes, one - year a two - year pine needles have proportionally more Zn, Ca, Sr to Rb than on the northern slopes. As to the result of the correlation matrix, we can say that more Ca, Zn and Sr decrease the amount of Rb, with the total variance of 14.8%. In general, elevated concentrations of Cu, Zn and Pb in plant tissues indicate the contamination of air with these elements.

Zinc is an essential element in all organisms and it plays an important role in the biosynthesis of enzymes, auxins and some proteins. The plant with symptoms of Zn deficiency experiences a retarded elongation of cells (Raven and Johnson 1986). It is known that there is more Zn in the roots of plants than in the areal parts, and thus it can be assumed that some of the contamination may come from the present variation. Our measurements indicate the occurrence of Zn together with Ca against Rb in an alternative relationship. In the situation when the concentration of Rb rises, Zn and Ca declined.

The measured values of Rb pointed to the presence of greater concentrations in one-year needles of *P. sylvestris*. It is caused by the translocation of the elements from the old to younger needles (Wyttenbach *et al.* 1995). The normal concentrations of Zn in plants generally range between 10 and 100 ppm (Allen *et al.* 1974). In other words, the contamination of environment with Zn does not seem to have negative effects on Scots pine needles.

Copper, similar to Zn, is a microelement essential for all organisms and is an important constituent of many enzymes of oxidation-reduction reactions (Raven and Johnson 1986). Disturbances in Cu supply (both deficiencies and excessive) can cause significant modification of biochemical processes in plants leading to lower yields and quality of agricultural crops. An excessive supply of Cu causes symptoms of chlorosis that are similar to the symptoms of iron deficiency (Bergman 1983).

The third phenomenon reflects the accumulation of Cl in needles in relation to Rb. The ratio of Cl to Rb mainly increased in November and at southerly located slopes. In contrast, in the east, the relation was opposite. The accumulation of Cl in needles on the southwest slopes is related to the metabolism of *P. sylvestris*. It is an exogenous phenomenon. The height of Rb content in plant tissues might inhibit the activity of different enzyme systems or interfere with the hormonal balance in the plant due to the competition with K in the plant metabolism. Berry and Smith (1969) found that gas can negate the inhibition induced by high Rb concentrations, suggesting that the level of gibberellins in the plant is reduced when treated with high Rb. Na and Rb may affect the growth of plants by at least three modes of action. First, under low K conditions they may cause a redistribution of K from old to young leaves or from petioles to blades. Second, they may maintain a favourable cation balance in the cell for various enzymatic reactions. Third, they may have an essential role in the growth of plants.

Toxicity of Rb is prevented by either increasing the K concentration of the culture solution or by supplying Rb in small doses, so that it gradually increases in concentration in the plant tissues. Under low K conditions, the growth of tops and fibrous roots is increased even with large amounts of Rb, if Rb is supplied in small doses. The extra growth from Rb at low K supply is due to the redistribution of K within the plant.

Plant physiologists have generally assumed that only 16 elements are indispensable to the growth of higher green plants. Other chemical elements, for example, Na and Rb are also frequently recognized as being beneficial for the growth of some plant species, but this fact alone does not establish the essentiality of these elements. Generally, Rb increased K concentration of the mature blades and decreased K in the corresponding petioles, indicating that K was displaced from the mature petiole to the mature blade tissues. When Rb was added in split doses to a medium low in K and Na, growth increased, and therefore the K displaced from the petioles to the blades had a less pronounced effect on blade K (El-Sheikhi and Ulrich 1970).

The last two phenomena describe the relationship of Mo, Pb to Cl and the relationship of Cl, Pb to K. The first phenomenon depends on the age of needles. In the autumn period (November) the amount of Cl to Mo, Pb was increasing, and on the contrary, in the spring period (March, April), the ratio Cl to Mo, Pb decreased, but the amount of Pb, Mo to Cl proportionally increased. As to the result of the correlation matrix, we can say that when there is more Cl, Mn, Rb, the amount of Mo and Pb decreases. The last phenomenon described the relationship of Cl, Pb to K, and it is independent of the age of needles, but is dependant on the slope. In this phenomenon we see that, Pb and Cl together contaminate the southeast exposure of the slope. We suppose that the reason is air pollution from the factory Mondi SCP. Elements Pb and Cl in needles are guided by prevailing winds that go east to northeast slopes. The accumulation of Cl and Pb affects the amount K in needles. The highest ratio of Cl, Pb to K was on the southeast slope of the Mních Hill. As far as the result of the correlation matrix is concerned, we can say that the more Cl and Pb, the less K. Increased attributes of Cl that we measured mostly on the southern and south-western slopes of the Mních Hill show the negative impact of emissions from the company Mondi SCP. The greatest accumulations of Cl in *Pinus sylvestris* are precisely in pine needles, however, the contamination of aged needles (one and two-year needles) was irrelevant. The higher attributes of CI in leaves may be partially caused by transportation, and Cl from the roots (from the soil) gets to the leaves of the plant (Xu et al., 1999). The biggest factor affecting the concentration of Cl was the exposition of the slope to Mondi SCP and its distance from the research area. It was confirmed that on the eastern lee side slopes of the Mních Hill we recorded reduced concentrations of Cl in needles. Pb translocation from roots to areal parts is limited and therefore we confirmed the theory by Šoltés (2014) where he claims that Pb is connected with industry/traffic and also with discharging aerosols into the atmosphere. Nearby the researched, a few main roads and the pulp mill of Mondi SCP are located, which affect the trend of Pb concentrations. From those polluting sources of Pb comes the dust, particles get by air on the needles of *P. sylvestris*. Increasing concentrations of Pb were recorded in the spring period due to the shrinking temperature inversion.

Lead is one of the heavy metals and is considered to be the one of dangerous environmental pollutants. It is emitted from industries, motor vehicles, stationary fuel, road dust composition and traffic roads. Lead is not only a toxic element but also can be accumulated in plant organs and agricultural products (Burzynski 1987; Mahmoud and El-Beltagy 1998), consequently enters the human food chain (Wagner 1993). Pb is not an essential nutrient for plants, majority of lead is easily taken up by plants from the soil and accumulated in root while only a small fraction is translocated upward to the shoots (Patra *et al.* 2004). Normal concentrations of Pb in plants are less than 10 ppm (Kabata-Pendias and Piotrowska 1974). Lead pollution on a local scale is caused by industrial emissions, and a larger scale is caused by emissions from motor vehicles using leaded gasoline (Koeppe 1981). Soils contaminated with Pb cause sharp decreases in crop productivity thereby posing a serious problem for agriculture (Johnson and Eaton 1980). Pb affects several metabolic activities in different cell compartments. The effect of Pb depends on concentration, type of soil, soil properties and plant species. Pb toxicity leads to decreases in germination percentage, length and dry mass of root and shoots (Munzuroglu and Geckil 2002), disturbed mineral nutrition (Paivoke 2002), reduction in cell division (Eun *et al.* 2000). According to Yilmaz and Zengin (2004), twigs with needles are better bio-indicators than the roots of deciduous and coniferous trees. Green plants are always a stable potential source of food for herbivores throughout the year, but they are protected by high concentration of antiherbivore defensive compounds in pine needles (Chapin 1980). One of the elements of the food chain is also all green plants. Some of them, due to the increase of heavy metals in air, soil or water, will die, but some of them are more durable. Linden and pine survive in the conditions of the increased pollution. So that Cu, Pb and possibly many other metals can enter food chain through the ether oils and teas (Serbula *et al.* 2013).

Conclusions

The study was carried out in the period of October 2014 - April 2015. In the view of 184 samples that were collected and processed, we could work with a good data matrix. The high number of data allowed us to define more precisely the sources of pollution and their share of emissions on the observed Mních Hill. Using the bio-indications of *P. sylvestris*, which is one of the most appropriate methods to detect concentrations of air pollutants, we were able to reach the objectives of this study.

Thanks to using several methods, we were able to identify factors that affect pollution research on the Mních Hill and to specify reasons for the increase in the measured component dependencies in various aspects (offtake month, exposure, age of needles). The surprising finding was that the higher ranked concentration of air pollutants which da been measured on the northern slopes in previous studies was not confirmed in our studies. Our results clearly indicate the southern and southwestern exposition of the slope. The sources of contamination show mixed effects of air emissions. These emissions come in part from the nearby industrial park Mondi SCP and also from nearby villages. A large share refers to transport, which is very frequent in Ružomberok, since the town stretches across the main east - west road. For that reason we suggest to continue intensively in the study, but with supplementing the other methods that could further specify or exclude the origin of the contamination and amounts. The regular sampling needles of *P. sylvestris* should be also enlarged by the collection of roots and soil. With these two indicators we will be able to confirm with absolute precision that harmful substances contained in the needles get into plants through air pollutants and to eliminate doubts about any other contamination, for example soil or ground water.

References

Allen, S. E, Grimshow, H. M., Parkinson, J. A. and Quarmby, C. 1974: Chemical analysis of ecological materials. Blackwell Scientific Publications, Oxford.

Bargagli, R. 1998: Trace Elements in Terrestrial Plants: An Ecophysiological Approach to Biomonitoring and Biorecovery. Springer-Verlag, Berlin.

Bartaloš, T. 2015: Metal contamination of apple fruit in experimental study area Ružomberok. Bachelor thesis, IHMB ŽU, Tatranská Javorina.

Bergman, W. 1983: Farbatlas Ernährungsstörungen bei Kulturpflanzen für den Gebrauch im Feldbestand. VEB Gustav Fischer Verlag, Jena.

Berry, W. L. and Smith, O.E. 1969: Effects of rubidium, potassium and gibberellic acid on plant growth. *Plant and Cell Physiology*, **10**: 161-170.

Burzynski, M. 1987: The uptake and transpiration of water and the accumulation of lead by plants growing on lead chloride solutions. *Acta Societatis Botanicorum Poloniae*, **56**: 271-280.

Eaton, 1942: Niektoré výsledky výskumu poškodenia rastlín zlúčeninami chlóru. In: *Les a priemyselné imisie* (ed. J. Sobocký), pp.84. Veda, Banská Bystrica.

El-Sheikhi, A. M. and Ulrich, A. 1970: Interactions of Rubidium, Sodium, and Potassium on the Nutrition of Sugar Beet Plants. *Plant Physiology*, **46**: 645-649.

Eun, S. O., Youn H. S. and Lee, Y. 2000: Lead distrubs microtubule organization in the root meristem of *Zea mays*. *Physiologia Plantarum*, **110**: 357-365.

Fife, D. N. and Nambiar, E. K. S. 1982: Accumulation and retranslocation of mineral nutrients in developing needles in relation to seasonal growth of young Radiata pine trees. *Annals of Botany*, **50**: 817-829.

Fife, D. N. and Nambiar, E. K. S. 1984: Movement of nutrients in Radiata pine needles in relation to the growth of shoots. *Annals of Botany*, **54**: 303-314.

Florence, R. G. and Chuoung, P. H. 1974: The influence of soil type on foliar nutrients in *Pinus radiata* plantations. *Australian Forest Research*, **6**: 1-8.

Fober, H. 1993: Mineral nutrition. In *Biology of Scots Pine* (eds. S. Bialobok, A. Boratynski and W. Bugala), pp. 182-193. Sorus, Poznan. (In Polish with English summary).

Gajdoš, M. 2015. Contamination of *Apodemus flavicollis* in the experimental study area - Ružomberok. Bachelor thesis, IHMB ŽU, Tatranská Javorina.

Gajić, G., Mitrović, M., Pavlović, P., Stevanović, B., Djurdjević, L., and Kostić, O. 2009: An assessment of the tolerance of *Ligustrum ovalifolium Hassk.* to traffic-generated Pb using physiological and biochemical markers. *Ecotoxicology and Environmental Safety*, **72**: 1090-1101.

Giertych, M. J. and Werner, A. 1996: Phenolic compounds in needles of Scots pine (*Pinus sylvestris* L.) damaged by industrial pollution. *Arboretum Kórnickie*, **41**:165-172.

Gilman, E. and Watson, E. 1994: *Pinus sylvestris* Scotch Pine. *Fact Sheet ST*, **477**: 3.

Greszta, J. 1982: Correlation between content of copper, zinc, lead and cadmium in the soil and the content of these metals in the seedlings of selected forest tree species. *Fragmenta Floristica et Geobotanica*, **28**: 29-52.

Heliövaara, K. and R. Väisänen, 1989: Quantitative variation in the elemental composition of Scots pine needles along a pollutant gradient. *Silva Fennica*, **23**:1-11.

Helmisaari, H. S. 1992: Spatial variation in nutrient concentrations of *Pinus sylvestris* needles. *Silva Fennica*, **26**:145-153.

Hutchinson, T. C. and Whitby, L. M. 1974: Heavy metal pollution in the Sudbury mining and smelting region of Canada: I. Soil and vegetation contamination by nickel, copper and other metals. *Environmental Conservation*, **1**: 123-132.

Chapin, F. S. 1980: Influence of air pollution on the foliar nutrition of conifers in Great Britain. *Environmental Pollution*, **88**: 183-192.

Johnson, M. S. and J. W. Eaton, 1980: Environmental contamination through residual trace metal dispersal from a derelict lead-zinc mine. *Journal of Environmental Quality*, **9**: 175-179.

Kabata-Pendias, A., Bolibrzuch, E., Galczynska, B., Piotrowska, M., Tarlowski, P. and Wiacek, K. 1974: Spektrometryczne Metody Oznaczania Zawartosci w Glebach i Roslinach Nastepujacych Pierwiastkow: As, B, Cd, Cr, Cu, F, Fe, Hg, Mn, Mo, N, Pb, S, Se, Zn. Instytut Uprawy I Nawozenia, Poland.

Kainulainen, B. P., Holopainen, J.K. and Oksanen, J. 1995: Effect of SO_2 on the concentrations of carbohydrates and secondary compounds in Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L). Karts.) seedlings. *New Phytologist*, **130**: 231-238.

Knappová, M. 2010: Využití jehličí jako pasivního vzorkovače pro sledování kvality ovzduší: diplomová práca. Brno: MUNI, 2010. 72 s.

Koeppe, D. E. 1981: Lead: understanding the minimal toxicity of lead in plants. In: *Effects of trace metals on plant function* (ed. N.W. Lepp), pp. 55-76. Applied Science Publ, London.

Kuang, Y. W., Wen, D. Z., Zhou, G. Y., and Liu, S. Z. 2007: Distribution of elements in needles of *Pinus massoniana* (Lamb.) was uneven and affected by needle age. *Environmental Pollution*, **145**: 146-153.

Kuhn, E., Hooper, R., Donohue, D. L. 1995: Application of environmental monitoring to IAEA safeguards. In: *Proceedings of the International Symposium on Environmental Impact of Radioactive Release* (eds. members of IAEA), pp. 35-43. IAEA, Vienna.

Kurczynska, E. U., Dmuchowski, W., Wloch, W., Bytnerowicz, A., 1997: The influence of air pollutants on needle and stems of Scots pine (*Pinus sylvestris* L.) trees. *Environmental Pollution*, **98**: 325-334.

Lundegardh, H. 1960: Niektoré výsledky výskumu poškodenia rastlín zlúčeninami chlóru. In: *Les a priemyselné imisie* (ed. J. Sobocký), pp.84. Veda, Banská Bystrica.

Mahmoud, W. H. and A. El-Beltagy, 1998: Isolation, Identification and potential use of lead reduction from heavy metal polluted soil. *Menufiya Journal of Agricultural Research*, **23**: 1461-1473.

Madgwick, H. A. I., Beets, P. N., Sandberg, A. M. and Jackson, D. S. 1983: Nitrogen concentration in the foliage of *Pinus radiata* as affected by nitrogen nutrition, thinning, needle age and position in the crown. *New Zealand Journal of Forestry Science*, **13**: 197-204.

Makinen, A. 1987: Use of *Hylocomium splendens* for regional and local heavy metal monitoring around a coal-fried power plant in southern Finland. *Symposia Biologica Hungarica*, **35**: 777-794.

Mátyás, C., Ackzell, L., Samuel, C.J.A. 2003: Euforgen Technicl guidelines for genetic conservation and use for Scots pine (*Pinus sylvestris*). International Plant to metal contaminated soil. In: E*nvironmental change and sustainability* (ed. Steven, L.), pp.1-6. EUFORGEN, Rome.

Mengel, R. 1968: Niektoré výsledky výskumu poškodenia rastlín zlúčeninami chlóru. In: *Les a priemyselné imisie* (ed. J. Sobocký), pp.84. Veda, Banská Bystrica.

Mingorance, M. D. and Oliva, S. R., 2006: Heavy metals content in N. oleander eaves as urban pollution assessment. *Environmental Monitoring and Assessment*, **119**: 57-68.

Mráz, M. 2015: Invasive vascular plants as bioindicators of pollution in the experimental study area - Ružomberok. Bachelor thesis, IHMB ŽU, Tatranská Javorina.

Munzuroglu, O. and Geckil, H. 2002: Effects of metals on seed germination, root elongation and coleoptile and hypocotyl growth in *Triticum aestivum* and *Cucumis sativus*. *Archives of Environmental Contamination and Toxicology*, **43**: 203-213.

Nikolajevskij, V. G. 1974: Niektoré závery výskumu odolnosti rastlín proti imisiám na Urale v období rkov 1934-1970. In: *Les a priemyselné imisie* (ed. J. Sobocký), pp.84. Veda, Banská Bystrica.

Ostrowska, A. and Szczubialka, Z. 1988. Comparison of the ion system in the needles of pine of various ages and various growth conditions. *Annals of Warsaw Agri-*

cultural University SGGW-AR Forestry and Wood Technololgy, **37**: 21-30.

Pacyna, J. M. 1990: Source-receptor relationship for atmospheric trace elements in Europe. In: *Acidic precipitation: vol. 3. Sources, deposition and canopy interactions* (eds. A. L. Page and S. A. Norton), pp. 49-71. Springer-Verlag, New York.

Paivoke, A. E. A. 2002: Soil lead alters phytase activity and mineral nutrient balance of *Pisum sativum. Environmental and Experimental Botany*, **48**: 61-73.

Patra, M., Bhowmik, N., Bandopadhyay, B. and Sharma, A. 2004: Comparison of mercury, lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance. *Environmental and Experimental Botany*, **52**: 199-223.

Pfanz, H., Martinoia, E., Lange O-L. and Heher, U. 1987: Flux of SO_2 into leaf cells and cellular acidification by SO_2. *Plant Physiology,* **85**: 928-933.

Pietrzykowski, M., Woś, B. and Haus, N. 2013: Scots pine needles macronutrient (N, P, K, CA, Mg, and S) supply at different reclaimed mine soil substrates-as an indicator of the stability of developed forest ecosystems. *Environmental Monitoring and Assessment,* **185**: 7445-7457.

Rautio, P., Huttunen, S. and Lamppu, J. 1998: Seasonal foliar chemistry of northern scots pines under sulphure and heavy metal pollution. *Chemosphere*, **37**: 271-287.

Raven P. H. and Johnson G. B. 1986: Biology. Times Mirror/ Mosby College Publishing, St. Louis.

Ruhling, A., Rasmussen, L., Pilegaard, K., Makinen, A. and Steinnes, E. 1987: Survey of atmospheric heavy metal deposition in the Nordic countries in 1985 - monitored by moss analyses. *Nord*, **21**:1–10.

Said, T. M., Agarwal, A., Sharma, R. K., Thomas, A. J. and Sikka S. C. 2005: Impact of sperm morphology on DNA damage caused by oxidative stress induced by ß-nicotinamide adenine di nucleotide phosphaate. *Fertility and sterility*, **83**: 95-103.

Sawidis, T., Chettri, M. K., Zachariadis, G. A. and Stratis, J. A. 1995a: Heavy metals in aquatic plants and sediments from water systems in Macedonia, Greece. *Ecotoxicology and Environmental Safety*, **32**: 73-80.

Sawidis, T., Chettri, M. K., Zachariadis, G. A., Stratis, J. A. and Seaward, M. R. D. 1995b: Heavy metal bioaccumulation in lichens from Macedonia in northern Greece. *Toxicological & Environmental Chemistry,* **50**: 157-166.

Sawidis, T., Marnasidis, A., Zachariadis, G. A. and Stratis, J. A. 1995c: A study of fair pollution with heavy metals in Thessaloniki city (Greece) using trees as biological indicators. *Archives of Environmental Contamination and Toxicology,* **28**: 118-124.

Sawidis, T., Breuste, J., Mitrovic, M., Pavlovic, P. and Tsigaridas, K. 2011: Trees as bioindicator of heavy metal pollution in three Europes cities. *Environmental Pollution*, **159:** 3560-3570.

Serbula, S. M., Milljkovic, D. D. J., Kovacevic, R. M. and Ilic, A. A. 2013: Assessment of airborne heavy metal pollution using plant parts and topsoil. *Ecotoxicology and Environmental Safety* **76**: 209–214.

Smidt, S., Mutsch, F., Knoflacher, M.H., Stefan, K. and Herman, F. 1998: Stress on the Northern Tyrolean Limestone Alps by the Deposition of Pollutants. *Phyton*, **36**: 245-270.

Sobocký, E. 1974: Les a priemyselné imisie. VEDA, Banská Bystrica.

Svoboda, L., Zimmermannová, K., and Kalač, P. 2000: Concentrations of mercury, cadmium, lead and copper in fruiting bodies of edible mushrooms in an emission area of a copper smelter and a mercury smelter. *Science of the Total Environment,* **246**: 61-67.

Šoltés, R., Gregušková, E. and Šoltésová, A. 2014: Bioindication of chemical elements deposition in the high Tatra Mts (Slovakia) based on *Calluna vulgaris* (L.) Hull; comparative levels after the improvement of emissions. *Carpathian Journal of Earth and Environmental Sciences*, **9**: 5 -14.

Tiegs, P. 1930: Možnosti indikácie škodlivého účinku imisíí v biologickom materiáli. In: *Les a priemyselné imisie* (ed. J. Sobocký), pp.84. Veda, Banská Bystrica.

Van den Driessche, R. 1974: Prediction of mineral nutrient status of trees by foliar analysis. The Botanical Review **40**: 347-394.

Wagner, G. J., 1993. Accumulation of heavy metals in crop plants and its consequences to human health. *Advances in Agronomy*, **51**: 173-177.

Wyttenbach, A., Bajo, S., Bucher, J., Furrer, V., Schleppi, P. and Tobler, L. 1995: The concentrations of K, Rb and Cs in spruce needles (*Picea abies* Karst.) and in the associated soils. *Zeitschrift für Pflanzenernährung und Bodenkunde*, **158**: 499-504.

Yilmaz, S. and Zengin, M. 2004: Monitoring environmental pollution in Erzurum by chemical analysis of Scots pine (*Pinus sylvestris* L.) needles. *Environment International* **29**: 1041-1047.

Xu, G., Magen, H., Tarchitzky, J., and Kafkafi, U. 1999: Advances in chloride nutrition of plants. *Advances in Agronomy*, **68**: 97-150.

Zahn, U. 1970: Dymové škody v lesnom hospodárstve z hľadiska biológa. In: *Les a priemyselné imisie* (ed. J. Sobocký), pp.84. Veda, Banská Bystrica.

Zinkernagel, R. M. 1958: Možnosti indikácie škodlivého účinku imisíí v biologickom materáli. In: *Les a priemyselné imisie* (ed. J. Sobocký), pp.84. Veda, Banská Bystrica.

www.botany.cz, 2015: *Pinus sylvestris* L. - Scots Pine. Online: http:// botany.cz/en/pinus-sylvestris (retrieved 15.12.2015).

www.treerx.com, 2015: Tree Fertilization - Macro Nutrients. Online: http://www.treerx.com/tree-fertilization-macro-nutrients (retrieved 15.12.2015).