Oecologia
Montana 2016,
25, 33-38

Heavy metals and other elements in the *Pinus sylvestris* needles – Ružomberok

P. VÁCLAVÍK, M. KMEŤOVÁ and M. JANIGA[1]

Institute of High Mountain Biology, University of Žilina, SK-059 56 Tatranská Javorina 7, Slovak Republic, [1]corresponding author: janiga@uniza.sk

Abstract. In our work we examined the use of needles *Pinus sylvestris* as the bioindicator of pollution in Ružomberok. One-year, two-year and three-year needles were collected during the years 2011 and 2012 during autumn, spring and summer months in two sampling locations (Mních and Hrboltová). The samples were dried, ground and X-rated in the XRF spectrometer Delta. The resulting data matrix was evaluated by the program STATISTICA 10. From the analysis made, we tried to determine the level of concentration of pollution and to examine the consequences and causes of contamination and the impact of Mondi SCP on this pollution. Probably, the pollution of needles is unrelated with the impact of local paper factory, but with the global pollution of the area. The concentration of Cr, Pb, Mo, S, K, Zn does not depend on the sample locality. The concentration of K, Cl and S in relation to Mn is influenced by the presence of the paper plant in the Liptov Basin. The accumulation of K, Cl, S is raising when the growing season begins.

Key words: bioindicators, *Pinus sylvestris*, heavy metals, Ružomberok, Mondi SCP

Introduction

During the last decades we have seen an increase of paper production, which can have no other effect than an only adverse impact on air, soil and water (Vall 2006). The location of the factory Mondi SCP, a. s. in the Liptov Basin is very hazardous; due to the lack of winds in the atmosphere, emissions are concentrated in the area and the air pollutants contained in precipitation have an adverse impact on the environment.

In the industrial production, pollutants are getting into the environment. Pollutants such as heavy metals, e.g. Pb, Cu, Cr, Ti, Cd as well as S and Cl have an adverse impact on the environment and biota and their accumulation in plant tissues and animal tissues, diseases of parts (Giertych *et al.* 1999).

Plants which accumulate heavy metals can explore our advanced technology when they are used as bio-indicators (Kuang *et al.* 2006). Pine is an excellent bioindicator because of its wide distribution and easy identification, inter alia, these are evergreen trees, whose needles do not fall off under normal conditions. Most studies have focused on the chemical composition of needles, where most of the elements reached their highest concentrations. The element content in the needles was often studied as part of wider studies concerning various issues, e.g. air and soil pollution (Dmochowski and Bytnerowicz 1995; Rautio *et al.* 1998). It is known that Scots Pine (*Pinus sylvestris*) is a good bioindicator, so this kind of plant is used in the case of research surrounding Mondi SCP, a. s. namely from the top of the Mních Hill and the neighbouring Hrboltová village.

Industrialization and urbanization result in the release of gaseous and particulate pollutants such as sulphur and heavy metals. These have been reported to cause abnormalities in the nutrient status of conifers (Rautio *et al.* 1998) and also to reduce plant growth and development at high concentrations, causing the death of plants in extreme cases (Yilmaz 2004). Changes in calcium concentrations have been found to be a general physiological response of plants to metal toxicity (Nieminen and Helmisaari 1996). Pine is an excellent bioindicator because of its wide distribution and easy identification, inter alia, these are evergreen trees whose needles do not fall off in normal conditions. Most studies have focused on the chemical composition of needles where most of the elements reached their highest concentrations. The element content in the needles was often studied as part of wider studies concerning various issues, e.g. air and soil pollution (Dmochowski and Bytnerowicz 1995; Kurczyńska *et al.* 1997; Rautio *et al.* 1998). It is known that pollutants are in a tree receiving the land, but many times the aerial parts of the plant contain a much larger value of pollutants than the actual soil solution (Ma *et al.* 2001), therefore the plants accumulate heavy metals and the air. To determine the exact source of the contamination is very difficult, practically impossible (Oliva and Espinosa 2007). Changing the concentration of pollutants in needles is directly proportional to the change in concentration in the atmosphere (Pacyna *et al.* 2009). It is known that trees are not the best indicators for monitoring the air pollution when compared to lower plants (fungi, algae, lichens or mosses), but they are widely distributed in many countries as the major plant type of polluted urban areas (Wittig 1993). A huge advantage of trees as bio-indicators is that they live a long time, so they can observe the changes, but these changes can be examined only after a certain time from the beginning of pollution. From leaves and pine needles the wind or rain can wash away pollutants contained (Sawidis 2011), and therefore there should be a noticeable difference between spring and autumn needles.

As the herbal ingredients of this world accumulate elements that are not always desirable only for their growth, they introduce some threat to humanity in the form of plant food. From plant food, which is without heat treatment, pollutants can directly enter into the receiving bodies of animals (Gergen and Harmanescu 2012). Of course, some elements are also important for our body, but, for example, Cd, Pb and Hg are toxic already in low amounts (Gergen and Harmanescu 2012). Toxicology also suggests that even the elements, which our body needs in small amounts for proper functioning, are not beneficial in higher doses and they are even harmful. These are elements such as Cu, Ni, Mn, Fe, Cr, Mo, most of which also occur in pine needles. Trace elements of animal body (Fe, Mn, Zn and Cu) have a central role in numerous metabolic processes of the body. They are essential as a catalyst in many enzyme and hormone systems that influence the growth and development of bones, feathers, enzyme structure and function. On the other hand, it is established that these metals, in large quantities, can cause oxidative stress in the body of animals, which can on one side be beneficial, kill cancer cells, but on the other hand, they can have a negative impact causing the cancer oxidative DNA damage (Valko *et al.* 2006).

Epidemiological literature of the 1990's revealed surprisingly large public health impacts associated with the pollution levels in European cities (Kousa *et al.* 2002). A report of the World Health Organization, which focused on vehicular emissions of particles in three European countries, revealed that more people were prematurely killed by the effects of these pollutants than by the means of car accidents.

The main aims of this study are:

- To determine the presence and concentration of pollutants in needles.

- To compare accumulation of pollutants in one-year, two-year and three-year needles.

- To compare the content of pollutants in spring, summer and autumn during a year.

- To compare the concentration of pollutants in the years 2011,2012, 2014, 2015.

Material and Methods

Sampling

Samples were collected from two different locations. The first location is the Mních Hill at an altitude of 657 m above sea level, which is oriented NW of the company Mondi SCP, a. s. The second location is the Hrboltová village, also with the NW orientation from Mondi SCP, a. s., but further away (Fig. 1). In these locations, we have identified 25 points of delivery, and from these we have collected 1-year, 2-year and 3-year needles *Pinus sylvestris*. The samples were collected during the years 2011 and 2012, during the months of April, July and October. Needles were collected, embedded in polythene bags and transferred to the laboratory.

Samples were separately inserted into Petri dish and then dried for 24 hours in the oven Memmert IF160plus. After complete drying, we made them a fine powder while using the device - bead mill - CryoMill-in, Retsch. The device has the auto precooling function and to choose one of three modes, we chose the dry mode. CryoMill works to the high-frequency horizontal vibration, which oscillates in the cylinder durable steel ball that crushed the needles into fine powder (RETSCH GmbH 2015). Subsequently, the powder determined the values of heavy elements and other pollutants while using the X-ray equipment spectrometer DELTA CLASSIC (US). A small container - cell - transparent bottom flask was charged with the powder needle in a volume of 15 ml, i. j. 1.5 g - 2 g of the fine powder. Processing a single sample needle-like powder in the device lasts 13 minutes and includes the 3x transition X-ray beam pattern. Three measured values are then averaged. The Spectrometer DELTA CLASSIC is able to detect the following elements: S, Pb, Fe, Mn, K, Cr, Ca, Zn, Mo, Sr, Rb, Cl, and not detected elements are: In, Cu, Ba, Ti, Zr, I, As, Cd and Co.

Final concentrations of the measured elements - averaged values were saved in Microsoft Excel, and further processed in the statistical program STATISTICA 10. The method of principal components PCA was performed. The result of this analysis was the correlation matrix data. Factor scores are calculated while using the One Way ANOVA, where the level of significance is <=0,05.

Fig. 1. Locations of the sampling of *Pinus sylvestris*; red polygon - Mondi SCP; yellow circles - sample plots of the Mních Hill and Hrboltová village (source: Google Earth, 2015).

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8	Factor 9	Factor 10
S	-0,740	0,077	0,322	-0,208	0,059	0,265	0,302	-0,020	-0,354	0,094
CI	-0,191	0,026	0,353	0,798	0,412	0,142	-0,086	-0,032	0,010	0,043
K	-0,467	-0,271	0,562	-0,449	0,192	0,176	-0,158	0,019	0,311	0,020
Ca	-0,258	0,814	0,046	-0,011	0,155	-0,256	0,221	0,316	0,174	0,003
Cr	-0,855	-0,026	-0,206	0,104	-0,027	0,051	0,121	-0,159	0,094	-0,403
Mn	-0,253	-0,097	-0,614	-0,234	0,691	-0,026	-0,091	-0,011	-0,061	0,065
Zn	-0,466	0,680	0,127	-0,104	-0,096	-0,244	-0,392	-0,231	-0,131	0,019
Rb	-0,212	-0,559	0,288	0,017	0,101	-0,729	0,059	0,034	-0,107	-0,039
Mo	-0,741	-0,252	-0,192	0,130	-0,254	0,106	-0,276	0,414	-0,102	-0,001
Pb	-0,772	-0,142	-0,294	0,174	-0,250	-0,113	0,134	-0,178	0,212	0,312
% Total variance	30,700	16,100	11,900	10,100	8,600	8,100	4,500	3,800	3,500	2,800

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

Results

As a result, we can consider factors with percentages from 30.7% to 8.6% of total variance (Table 1). These factors were transferred through a further analysis. By the analysis, we use our factor score and the correlation of locality (Mních, Hrboltová), age of needles and month, in which the samples were collected.

Factor 1

In this factor, we could see the accumulation of pollutants in *Pinus sylvestris* needles during the autumn, spring and summer in the followed and control area (M - Mních, H - Hrboltová). Probably, the pollution of needles is unrelated to the impact of the local paper factory, but to the global pollution of the area. The comparison between collection areas and the influence of seasonability we can see in Fig. 2.

In the first factor we can see Cr, Pb, Mo, S, K, Zn. The concentration of these elements is higher in the autumn month and in relation to spring and summer it decreases. The concentration does not depend on the sample locality. The contamination of needles did not differ according to the age of needles $F = 2.2$; $p = 0.11$.

Factor 2

This factor is probably influenced by the physiology of *Pinus sylvestris*. In Factor 2 we can see the accumulation of Ca, Zn in relation to Rb. In the sample locality of the Mních Hill, there are better light conditions because of its SE orientation of slopes (where we made the sampling) than the Hrboltová village, which has the SW orientation of slopes, so needles accumulate more Ca and Zn at the expense of Rb because of their growth. Two-way ANOVA: Locality/Month: $F = 0.23$; $p = 0.79$. (Fig. 3, M - Mních, H - Hrboltová).

We expect that *Pinus sylvestris* needs Ca and Zn for its growth. The Mních Hill locality has a better light condition, so it accumulates more Ca and Zn in relation to Rb. The Hrboltová village does not have light conditions as in the case of the aforementioned, so it accumulates Rb in relation to Ca and Zn, because needles do not grow too much.

Pinus sylvestris needs Ca and Zn for its growth, so it accumulates these elements more during some years. One-way ANOVA: Age of needles: $F=79.5$; $p=0.00$ (Fig. 4).

Factor 3

In Factor 3 we can see the accumulation of K, Cl and S in relation to Mn. This factor is influenced by the presence of the paper plant in the Liptov Basin. The accumulation of K, Cl, S is raising when the growing season begins. Two-way ANOVA: $F = 2.37$; $p = 0.09$. (Fig. 5, M - Mních, H - Hrboltová).

Needles accumulate K, Cl, S in relation to Mn mainly in the first year of their growth and during the next years they lose K, Cl, S. One-way ANOVA: $F = 4.5$; $p = 0.01$ (Fig. 6).

The highest content of K, Cl, S in relation to Mn is

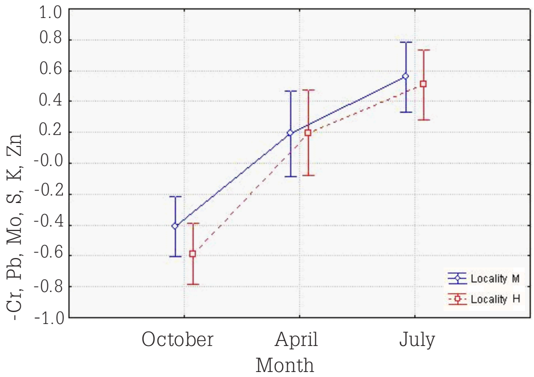

Fig. 2. Elements Cr, Pb, Mo, S, K, Zn measured in each month in two localities. (F (2.354)=0.336, p=0.714).

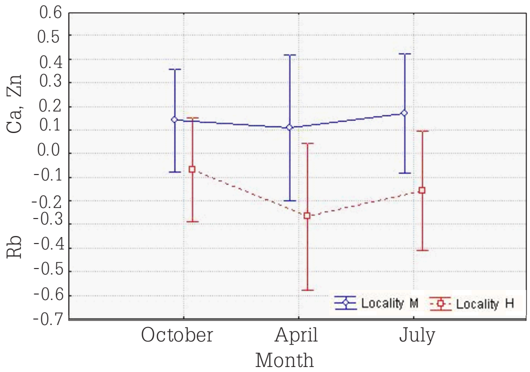

Fig. 3. Elements Ca and Zn versus Rb during a month in locality. (F (2, 354)=0.228, p=0.796).

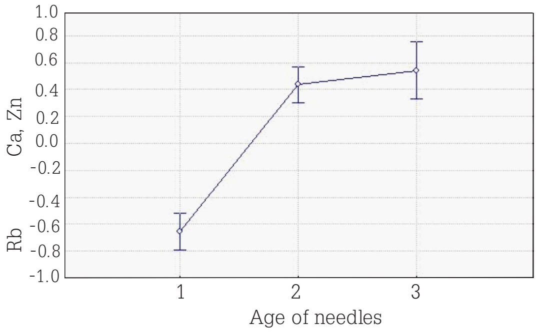

Fig. 4. In 2-year and 3-year needles, the content of Ca and Zn was bigger than in 1-year needles. (F (2, 357)=79.479, p=0.000).

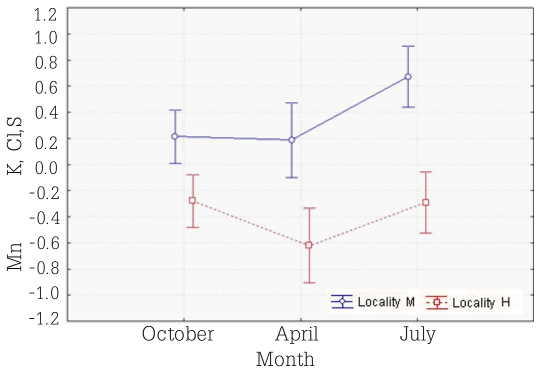

Fig. 5. Comparison of accumulation K, Cl, S in relation to Mn. The content of K, Cl, S increases in growing season mainly in the Mních Hill locality, which is next to Mondi SCP, a.s. (F (2, 354)=2.369, p=0.095).

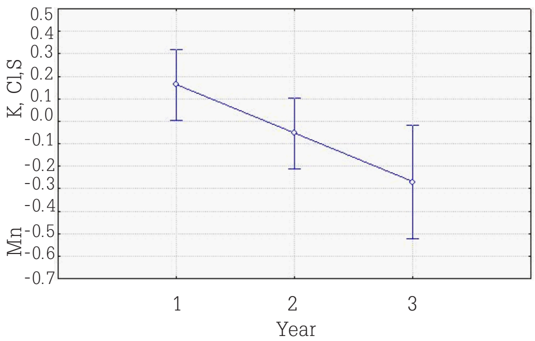

Fig. 6. K, Cl, S versus Mn due to the age of needles. (F (2, 357)=4.457, p=0.012).

in the first year of the growth of needles, because at the beginning of the growth needles absorb nutrients from water and soil where contaminants accumulate.

Factor 4

This is a factor of pollution cause by transport and traffic in the town of Ružomberok. It deals with the accumulation of Cl (Pb) in relation to K. In the Hrboltová village, Cl (Pb) reaches proportionally greater values, regardless of the season. The reason is probably bigger traffic. In spring, plants start their growth season and draw nutrients from soil and water. The peak value of Cp (Pb) is in the spring. Two-way ANOVA: F = 2.9; p = 0.05. (Fig. 7, M - Mních, H - Hrboltová). Between one-, two- and three-year needles there were no significant differences in the proportional accumulation of Cl (Pb) to potassium, the phenomenon can therefore be seen as a phenomenon of external contamination rather than a physiological phenomenon of internal laws with *Pinus sylvestris* (One-way ANOVA: F = 2.6; p = 0.76). One-way ANOVA separately for LOCALITY: F = 74.7; p = 0.00, MONTH: F = 27.9; p = 0.00

Factor 5

Factor 5 discloses a bipolar vector, on the one hand, the accumulated Mn and Cl, or on the other hand, Mo and Pb. In the control area of the Hrboltová village there were no differences between October, April and July, however, there was proportionally more Mn and Cl in relation to Mo and Pb. The location of the Mních Hill meant that in July the proportion of Cl and Mn increased at the expense of Mo and Pb and get to the level of the trees from the site of the Hrboltová village. Two-way ANOVA: F = 6.43; p = 0.001. (Fig. 8, M - Mních, H - Hrboltová).

This probably reflects the fact that localization processes to the Mních Hill highly influenced this phenomenon, as these CI, the only explanation seems to be the positioning of the paper factory in the region and especially in July. There is the proportional accumulation of Cl and Mn on the one hand, on the other, Mo and Pb does not vary according to the age of needles. One-way ANOVA, F = 1.1; p = 0.12. Results of One-way ANOVA separately for LOCALITY: F = 20.20; p = 0.00, MONTH: F = 2.65; p = 0.07.

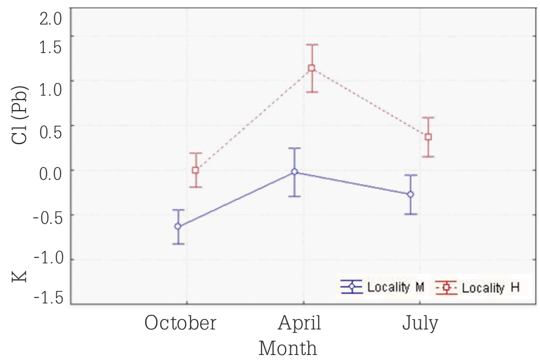

Fig. 7. The contamination with Cl (Pb) in relation to K during autumn, spring and summer in two localities. (F (2. 354)=2.888, p=0.056).

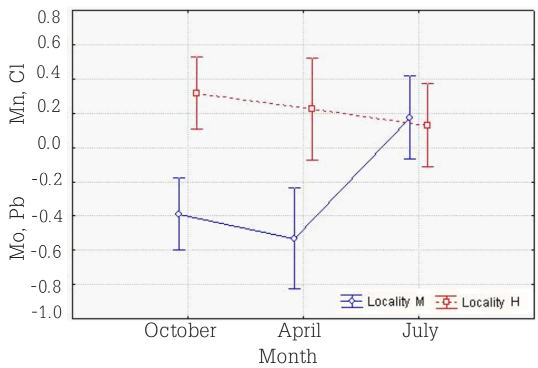

Fig. 8. The physiological accumulation of Cl and Mn in relation to Mo and Pb independently of age. (F (2. 354)=2.888, p=0.056).

Discussion

The town of Ružomberok is among the most polluted areas of the Slovak Republic. A major polluter company, Mondi Business Paper SCP a.s, released within the production of sulphate pulp specific organosulfur compounds to air, which represent a crucial problem in the issue of local environmental quality (RÚVZ 2006). Industrialization and urbanization result in the release of gaseous and particulate pollutants such as sulphur and heavy metals. These have been reported to cause abnormalities in the nutrient status of conifers (Rautio *et al.* 1998) and also to reduce the plant growth and development at high concentrations causing the death of plants in extreme cases (Greszta 1982). Anatomical changes observed in trees from the polluted sites are caused most likely by toxic effects of pollutants according to Kurczyńska *et al.* (1997). *Pinus sylvestris*, chosen by us as an bioindicator, is very popular (Kuhn *et al.* 1995; Kurczynska *et al.* 1997).

Nutrient concentrations in the plant biomass is a result of the balance between the nutrient uptake, plant growth and nutrient retranslocation and loss. The relative importance of site and species as factors determining nutrient concentrations in the plant biomass may differ depending on the nutrient element and biomass fraction. Foliar nutrient concentrations are most often used for the evaluation of plant nutrient status, according to Augusto *el al.* (2000). We found out that the accumulation of Cr, Pb, Mo, S, K and Zn does not take place evenly by the age of needles, but the concentration is the biggest in autumn, and in spring and summer gradually decreases similar to the findings by Korčeková (2015). According to Chapin *et al.* (1980), in their research K was not effectively back translocated from leaves to stems during the year, so we can found an increased amount of K in the needles in comparison to the whole tree. Several studies have shown that element concentration in needles can be used to diagnose the nutrient deficiency or excess, particularly under the pollution stress (Dmuchowski and Bytnerowicz 1995). Based on this, S deficiency may occur in the 2-year old needles indicated by its relatively lower content. This is nothing different between the followed locality of the Mních Hill and controlled locality of the Hrboltová village.

The concentration of Ca and Zn is in effect dependent on external conditions in relation to Rb, and its concentration is higher on SW and S slopes (Korčeková 2015). We assume that the site of the Mních Hill has better lighting conditions and therefore there is a higher concentration of Ca and Zn. The concentration of Ca and Zn increased during years, because Ca is the main element of cell walls, so when *Pinus sylvestris* is growing, it needs Ca for its development. Concentrations of Ca in the stemwood may depend on water consumption (Arthur *et al.* 1999), and the uptake of this element can be increased by an increasing transpiration rate (Barber 1995). In the soil, the contents of rubidium are inversely correlated with the soil pH, i.e. the highest amount of rubidium can be found in the sour soil. Also, the rubidium intake by plants increases with the lower soil pH, where there is a lack of Ca (Kosla *et al.* 2002). We can observe a minimum effect of the location of Mondi SCP, the accumulation of Cu and Zn, and Rb reduction related to exposure as well as to the age of needles, where mutual interaction between age and exposure to needles was potentiating. Changes in calcium concentrations have been found to be a general physiological response of plants to metal toxicity (Nieminen and Helmisaari 1996). Ca comes from reserves in the root and lower shoot, and is a major source of calcium for the new growth at the beginning of the season (Hanger 1979), this is a reason of the increase of Ca and Zn concentrations from spring to summer. Contrary to this, higher concentrations of Ca and Zn were acquired in two-year needles. The rubidium level in plants decreases with age (Kosla *et al.* 2002). This phenomenon is defined as the endogenous physiology of *Pinus sylvestris*, where the age of needles affects the accumulation of Zn, Ca to Rb. In the herbaceous plant, calcium is rapidly immobilized, whereas in the apple, calcium continues to move up the tree over a number of seasons (Hanger 1979) and every following year its content can raise. The lack of phosphate may also cause an opposite effect of reducing Zn in the plant (Korčeková 2015).

K, Cl, S in the autumn months are about the same at both locations and gradually increase in summer due to Mn, especially in the area of the concerned Mních Hill, which is a noticeable effect of Mondi SCP. Potassium is needed for opening vents, as spring begins to photosynthesize, it needs more (Kmeť 1996). Needles accumulate K, Cl, S to Mn substantially in the first year of their growth and gradually in the second and third year of their growth they are losing to Mn, this is because new needles at the beginning of their growth absorb nutrients from the soil cover and water, where the contaminants accumulate. Two-year old needles had 10 - 100 % lower concentrations of each element of P, S, K, Na and Al than the C needles, but 33 - 120 % higher for Ca, Mn, Zn, Fe and Pb. No significant difference for Mg, Ni, Cd, Cr and Cu was observed between the two needle age classes (Kuang *et al.* 2006).

In comparison with Korčeková (2015), the effect of accumulation of Cl (Pb) in relation to K is opposite. In our case, needles have higher concentration of Cl (Pb) in spring in comparison with autumn. The town of Ružomberok is one of the most polluted areas in Slovakia (RÚVZ 2006) and between polluters the traffic is included. The heavy metals chromium, copper, iron and lead were chosen for the analysis, since they are typical for urban air pollution (Anagnostatou 2008). Pb translocation from roots to aerial parts is limited and, therefore, we confirmed the theory by Šoltés (2014) that Pb is connected with the industry/traffic, and also connected with the discharge of aerosols into the atmosphere. In relation to July the increasing concentration of K due to Cl (Pb) reappears mainly in the Hrboltová village, which can be the effect of physiology of plants, where plants need K from spring for their basic functions (Kmeť 1996).

Conclusions

In our study we examined the Scots pine (*Pinus sylvestris*) and we specifically used it as a bioindicator of polluted atmosphere. We needed its needles for the mentioned bioindication. Our research took place in the Liptov Basin, more precisely around the town of Ružomberok, which is one of the most polluted areas in the Slovak Republic. A share of pollution is represented by the paper production factory Mondi SCP, which is situated in the town of Ružomberok by the Váh River. The transport undoubtedly affects the local pollution, as the frequently used major road from east to west passes through the Liptov Basin. In addition, more significant pollutants are produced by local home heating systems.

The impact of industry (paper production as well) and the burning of fossil fuels has clearly had an effect on the air pollution of the environment and the undermining of human health. Our aim was with the help of *Pinus sylvestris* needles to determine the level of contamination of the air and soil, to identify the culprits of pollution, to compare our results with the results of other similar studies and to show a trend of increasing environmental pollution. Among other things, the study reiterates the *Pinus sylvestris* ability to accumulate heavy metals and other toxic elements, and thus defends its importance of an excellent environmental pollution identifier.

Our findings are generally consistent with the findings of previous studies. For more accurate results, long-term research would be needed, which would have in addition to needles collected samples of soil and rainfall. Only in this way the origin of pollution could be more accurately determined.

Ackonwledgements

We are very grateful to E. Gregušková for the intial help in the laboratory analysis of samples. This work was partly supported by Structural funds of EU - ITMS - 26210120006 and ITMS - 26210120016.

References

Anagnostatou, V. A. 2008: Assessment of heavy metals in central Athens and suburbs using plant material. Dissertation, University of Surrey.

Arthur, M. A., Siccama, T. G. and Yanai, R. D. 1999: Calcium and magnesium in wood of northern hardwood forest species: relations to site characteristics. *Canadian Journal of Forest Research*, **29**: 339-346.

Augusto, L., Ranger, J., Ponette, Q. and Rapp, M. 2000: Relationships between forest tree species, stand production and stand nutrient amount. *Annals of Forest Science*, **57**: 313-324.

Barber, S. A. 1995: Soil nutrient bioavailability: a mechanistic approach. John Wiley & Sons. New York

Dmuchowski, W. and Bytnerowicz, A. 1995: Monitoring environmental pollution in Poland by chemical analysis of Scots pine (*Pinus sylvestris L.*) needles. *Environmental Pollution*, **87**: 87-104.

Gergen, I. and Harmanescu, M. 2012: Application of principal component analysis in the pollution assessment with heavy metals of vegetable food chain in the old mining areas. *Chem Cent J*, **6**:156.

Giertych, M. J., Karolewski, P. and de Temmerman, L. O. 1999: Foliage age and pollution alter content of phenolic compounds and chemical elements in *Pinus nigra* needles. Water, *Air, and Soil Pollution,* **110**:363-377.

Greszta, J. 1982: Correlation between the 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**: 3-28.

Hanger, B. C. 1979: The movement of calcium in plants. Communications. *Soil Science & Plant Analysis,* **10**: 171-193.

Chapin III., F. S., Johnson, D. A. and McKendrick, J. D. 1980: Seasonal movement of nutrients in plants of differing growth form in an Alaskan tundra ecosystem: implications for herbivory. *The Journal of Ecology,* **68**: 189-209.

Kmeť, J. 1996: Fyziológia rastlín, prednášky. Online: http://www.tuzvo.sk/sk/organizacna_struktura/lesnicka_fakulta/organizacne-clenenie/katedry/katedra-lesnej-tazby-logistiky-melioracii/servis_studentom/hlavna-dreviny/ (Retrieved 18. 05. 2015).

Korčeková, R. 2015: In situ monitoring o fair pollutants in the industrial area of Ružomberok with use of plant bioassays. Bachelor thesis. Institute of High Mountain Biology, Žilinská univerzita v Žiline.

Kousa, A., Kukkonen, J., Karppinen, A., Aarni-o, P. and Koskentalo, T. 2002: A model for evaluating the population exposure to ambient air pollution in an urban area. *Atmospheric Environment,* **36**: 2109-2119.

Kosla, T., Skibniewska, E., Debski, B. and Urbanska-Slomka, G. 2002: Rubidium in the trophic chain soil-plants-animals. *Trace elements and electrolytes,* **19**: 171-176.

Kuang, Y. W. 2006: Distribution of elements in needles of *pinus massoniama* (Lamb.) was uneven and affected by neele age. *Environmental Pollution,* **145**: 146-153.

Kuhn, A. 1995: Prozessketten in der Logistik: Entwicklungstrends und Umsetzungsstrategien. Verlag Praxiswissen. Dortmund.

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

Ma, J. F., Goto, S., Tamai, K. and Ichii, M. 2001: Role of root hairs and lateral roots in silicon uptake by rice. *Plant Physiology,* **127**:1773-1780.

Nieminen, T. and Helmisaari, H. S. 1996: Nutrient retranslocation in the foliage of Pinus sylvestris L. growing along a heavy metal pollution gradient. *Tree Physiology,* **16**: 825-831.

Oliva, S. R. and Espinosa, A. F. 2007: Monitoring of heavy metals in topsoils, atmospheric particles and plant leaves to identify possible contamination sources. *Microchemical Journal,* **86**: 131-139.

Pacyna, J. M., Pacyna, E. G. and Aas, W. 2009: Changes of emissions and atmospheric deposition of mercury, lead, and cadmium. *Atmospheric Environment,* **43**:117-127.

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

RETSCH GmbH 2015: Manual Mixer Mill CryoMill. Haan, Germany. online: http://www.retsch.com/products/milling/ball-mills/mixer-mill-cryomill/ (retrieved 14.3.2015)

RÚVZ 2006: Záverečná správa z hodnotenia zdravotných rizík realizovaného v rámci projektu: Vplyv zapáchajúcich sírnych zlúčenín vznikajúcich pri výrobe celulózy na zdravotný stav pracovníkov celulózky a obyvateľov mesta Ružomberok a blízkeho okolia. (eds. Chobotová, E. *et al.*) RÚVZ, Banská Bystrica.

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

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.

Valko, M., Rhodes, C. J., Moncol, J., Izakovic, M. M. and Mazur, M. 2006: Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-biological interactions,* **160**: 1-40.

Vall, J. 2006: Priemysel a jeho vplyv na životné prostredie v Slovenskej republike k roku 2005. SAŽP, Banská Bystrica.

Wittig, R. 1993: General aspects of biomonitoring heavy metals by plants. Plants as biomonitors, VCH, Weinheim.

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.