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The effect of altitude on accumulation of heavy metals in *Vaccinium myrtillus* organs in the Low Tatra mountains (from the Veľká Chochuľa mountain to the Prašivá mountain)

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Abstract. Bilberry samples were collected in the Low Tatras (the Carpathian mountain range) for analysis of heavy metals in roots, stems and leaves. We focused on heavy metal concentrations, which indicate pollution in alpine environments. Collection of samples was performed in two transects to compare samples from different altitudes. Each transect was divided into two parts based on the collection area. We have hypothesized that pollution in bilberries has contributed to the decline in marmot population in the area. Therefore, we decided to verify the presence of pollution in bilberries, which could also be related to escalating precipitation at higher altitudes. We found significant differences between accumulation of some elements in different plant organs as well as between the eastern and western parts of the mountain ridge. Elevation had a significant effect on the accumulation of several elements in bilberries.

Keywords: bilberry, heavy metals, accumulation, bioindicator

Introduction

Bilberries are dwarf shrubs that constitute one of the major drivers in ecosystem dynamics of boreal forests. They provide food, regulate species, and affect seedling regeneration and soil nutrient and carbon cycles. Bilberry grows primarily in acidic soils and is not very demanding with respect to nutrients or water; but long-term nitrogen deposition often leads to a decline in bilberry and replacement by ferns (Höcke *et al.* 2016). Bilberry is capable of growing on sites contaminated by heavy metals, and therefore, is useful as a bioindicator (Mikkonen and Huttunen 1981; Uhlig *et al.* 2001) as well as for the efficient colonization of contaminated sites (Białońska *et al.* 2007; Kandziora-Ciupa *et al.* 2013; Kukla and Kuklová 2008; Reimann *et al.* 2001; Salemaa *et al.* 2004; Sheppard 1991; Taulavuori *et al.* 2013). Experimental germination indicates that copper is more toxic to bilberry seeds than nickel. It has been found to delay germination and suppress seedling growth by inhibiting cell expansion and division (Lyanguzova 1999).

Heavy metals (HM), when present in higher than optimal concentrations, affect different cellular components, thereby interfering with the normal metabolic functions of plant cells (Tkalec *et al.* 2014). As metals cannot be broken down, when concentrations within the plant exceed optimal levels, they adversely affect the plant both directly and indirectly, and some of the direct toxic effects caused by high metal concentration include inhibition of cytoplasmic enzymes and damage to cell structures due to oxidative stress (Assche and Clijsters 1990; Jadia and Fulekar 1999). The negative influence of heavy metal on the growth and activities of soil microorganisms also indirectly affect the growth of plants. Reduction in the number of beneficial soil microorganisms due to high metal concentration may lead to a decrease in organic matter decomposition leading reduced soil fertility. Enzyme activities are useful for plant metabolism, but can be hampered due to interference of HM with activities of soil microorganisms. These toxic effects (both direct and indirect) lead to a decrease in plant growth, ultimately resulting in the death of plant (Schaller and Diez 1991). With an excessive amount of heavy metals, the surface of the plant is disturbed, and manifestations vary (e.g., leaf necrosis begins to reduce plant biomass. Metals that do not play any beneficial role in plant growth, such as Pb, Cd, Hg, and As, can cause adverse effects at very low concentrations in the growth medium (Kibra 2008). Plants on land tend to absorb HM from the soil and retain the majority in their roots. There is some evidence that plant foliage may also take up HM (and it is possible that this lead is moved to other parts of the plant). Pb is one of the ubiquitously distributed but most abundant toxic elements in soil. Uptake of lead by roots of the plant may be reduced with the application of calcium and phosphorus to the soil. Lead has an adverse effect on morphology, growth and photosynthetic processes of plants (Morzck and Funicclli 1982), and also induces proliferative effects on the vascular plant repair process (Kaji *et al.* 1995). Conversely, iron toxicity is not common. Iron as an essential element for all plants, and has many important biological roles in processes such as photosynthesis, chloroplast development, and chlorophyll biosynthesis. Although most mineral soils are rich in iron, the expression of iron toxicity symptoms in leaf tissues occurs only under flooded conditions, which involves the microbial reduction of insoluble Fe^{3+} and insoluble Fe^{2+} (Becker and Asch 2005). High chromium concentration can induce lipid peroxidation in plants and disturb the chloroplast ultrastructure, thereby disturbing the photosynthetic process (Peralta *et al.* 2001).

Generally, elevated levels of heavy metals are also associated with increased oxidative stress by increasing ROS production in plants (Reddy *et al.* 2005; Emamverdian *et al.* 2015), interfering with various macromolecules and disrupting normal cellular functions and metabolism (e.g., resulting in lipid peroxidation, inactivation or damage of proteins and chlorophyll, DNA injury) (Anjum *et al.* 2016). Once formed, ROS must be detoxified as efficiently as possible to minimize damage. Antioxidant systems in plants are complex and involve an array of non-enzymatic and enzymatic mechanisms capable of preventing the cascades of uncontrolled oxidation (Gratao *et al.* 2005, 2008; Kandziora-Ciupa *et al.* 2013).

Bilberries have a large capability to accumulate Mn. A positive correlation was found between the level of non-protein thiols and Cd and Zn concentrations, as well as between proline and these metals. Increased Mn accumulation caused a decrease in antioxidant response (Kandziora-Ciupa *et al.* 2017). Many other authors have mentioned the large capability of bilberry to accumulate Mn "as a manganese hyperaccumulator". As a hyperaccumulator of manganese, bilberry has detoxification mechanisms and shows no symptoms of phytotoxicity (Korcak 1989; Reeves 2006). Manganese occurs unevenly in the soil environment (Fiala *et al.* 2013), and its presence is associated with its relative content in the parent rock or with emissions. Manganese is a microelement needed by plants for their normal growth and development. Dominant forms accessible to the plants are Mn^{2+} compounds taken up by means of epidermal cells of plant root parts (Marschner 2006; Pittman 2005). Manganese is a heavy metal needed for plant growth, and if it is not present in a given amount for a given plant, the plant will become stunted and overgrown by others, resulting in Mn deficient plants being pushed out, unless in the presence companion plants (i.e., *Vaccinium vitis-idaea*) with different nutrient or soil requirements. Plants can suffer from lack and or surplus of Mn. Excessive uptake occurs in more acidic soils with a pH < 5.5 (Bergmann 1988; Kabata-Pendias 2011; Mengel and Kirkby 2001; Xue *et al.* 2004). The general hypothesis points to the differentiated change of manganese content in the assimilatory apparatus of *Vaccinium myrtillus* over the years, as well as the effect of manganese concentrations in soil during the growing season, as influenced by precipitation levels. In the second half of the vegetation period, Mn content in leaves is generally higher than in branches. Although manganese content in branches and leaves was above toxic limits, evidence of leaf damage from toxicity was not detected (Reeves 2006). Bilberry, as a medium accumulator of manganese, has suitable mechanisms for Mn elimination. Antioxidant systems in plants may be used as early indicators of environmental stress on target organisms preceding morphological or ultrastructural damage, and as warning indicators for the ecosystem (Białońska *et al.* 2007; Kandziora-Ciupa *et al.* 2016).

Our paper is a part of wider study focused on decline in marmot populations in the Low Tatra Mountains. The main aim of this study was analysis of heavy metals in roots, stems and leaves of *Vaccinium myrtillus* close to abandoned marmot burrows in selected parts of a mountain ridge in the Low Tatra Mountains. This research is intended to answer two basic questions:

1. Are bilberries contaminated in alpine meadows above the tree line in the Low Tatras?
2. How does altitude affect pollution?

Material and Methods

Sample collection

This study was conducted during summer seasons between 2018 and 2019 in a mountain ridge of the Low Tatra Mountains (between Prašivá Mountain and Veľká Chochuľa Mountain). Samples of bilberry were collected along two horizontal transects using 1 × 1- metre plots. The first transect was located at the upper line of the occurrence of abandoned marmot (*Marmota marmota latirostris*) burrows, and in parallel with the upper line of dwarf shrubs (1545 – 1743 m a.s.l.). Based on our previous research (unpublished), it is likely that marmots have abandoned this location due to cessation of livestock grazing and subsequent spreading of the dwarf shrub species *Vaccinium myrtillus*, *Vaccinium vitis-idaea* and *Empetrum nigrum*. The second horizontal transect was located 100 altitudinal meters below the first transect. Quadrat plots were situated each 100 m along both horizontal transects. For each 1 × 1-metre plot, environmental variables (altitude, aspect) and data on the coverage of dominant plant species were recorded. Localization, altitude, and aspect of each vegetation plot was measured using Garmin Oregon 300 equipment. The tallest plant of bilberry was collected from each plot. Plants were cut at ground level, labelled, and transported to the laboratory in zip-sealed plastic bags.

Laboratory work

The first transect was not specifically divided. Samples consist of whole stems with leaves. The second transect was divided into three groups: leaves marked as A; stems as B; and roots as C. These samples were dried in a laboratory incubator (IF 160 Plus) (Memmert, Germany). Temperature was set to 80° C with the air circulation fan set at 30 %. Samples were dried for 8 hours. Dried samples were crushed in a cryomill (Retsch, Germany). Crushing time depended on sturdiness of samples, particularly roots, but took approximately one minute for leaves and stems with a frequency of 30 Hz. Roots were cut into small pieces for better results.

Dried and crushed samples were analysed using DELTA Environmental Handheld XRF Analyzer (Olympus, Innov-x Systems, USA). Measurements ran in a closed Delta XRF portable workstation. The spectrometer was calibrated using a certificated reference material of INCT-PVTL-6 Virginia tobacco leaves (ICHTI, Poland). Samples were analysed in plastic cuvettes with plastic foil on the bottom for 240 seconds in three equal measurements. From these the arithmetic average was calculated.

Statistical analyses

Principal component analysis (PCA) was used to determine main components, the component weights, and the percentage of variation. We examined a total of 399 samples. From these we considered eleven factors, of which six constituted more than 5% of the total variability. One-way ANOVA of principal component scores and linear regression was used to compare the effects of environmental variables (elevation, locality, combination of locality and month) or different plant organs (stems, leaves, roots).

Results

Effect of altitude on concentrations of elements in stems of bilberries from the first transect

Principal component analysis (PCA) shows correlations between elements in bilberry samples using five factors (Table 1). The first factor indicates the negative correlation between S, Cl, K, Ca, Cr and Ba. The second factor shows the positive correlation with Pb. The third factor represents the negative correlation with Rb (Fig. 1). The fourth factor showed a decline in Mn. The fifth factor indicates a negative correlation with Zn. For factor 3 the correlation of Rb increases with altitude (Fig. 1). Linear regression for factors 1, 2, 4, 5 is shown in Table 2. Differences in Pb accumulation (factor 2) by mountain are show in Fig. 2. A complete overview of Pb accumulation from both transects is shown in Fig. 3.

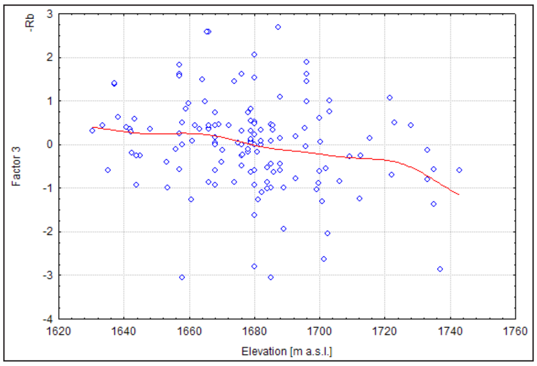

Fig. 1. Concentration of Rb increases with elevation (y = 17.892 - 0.0107*x, r = 0.24, n = 142, F (1,140) = 8.3711, p < 0.0044) in stems (with leaves) of *V. myrtillus* in the first transect from October. The line is calculated as distance weighted least squares.

Element	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
S	**-0.970**	-0.076	0.013	0.064	0.023
Cl	**-0.878**	-0.308	-0.017	0.192	-0.041
K	**-0.894**	-0.339	-0.153	0.011	-0.001
Ca	**-0.938**	-0.144	0.111	0.093	-0.101
Cr	**-0.956**	0.023	0.071	0.164	0.068
Mn	-0.512	-0.492	0.389	**-0.572**	0.120
Fe	-0.791	0.519	0.089	-0.048	0.120
Zn	-0.490	0.693	-0.143	-0.276	**-0.419**
Rb	-0.246	-0.190	**-0.920**	-0.186	0.135
Ba	**-0.944**	0.048	-0.015	0.038	-0.042
Pb	-0.302	**0.873**	0.033	-0.030	0.330
Eigenvalue	6.5	2.0	1.1	0.5	0.4
Total variance [%]	58.9	18.4	9.7	4.7	3.2

Table 1. Eigenvectors with the percentage of variance in principal component analysis of elements in the *Vaccinium myrtillus* from the first transect. The highest correlations are in bold.

Factor	Regression formula	R	Result
Factor 1	y = 25.3298 - 0.0151*x	0.14	F (1,140) = 2.6597, p < 0.10517
Factor 2	y = -9.0674 + 0.0054*x	0.08	F (1,140) = 0.86857, p < 0.35296
Factor 4	y = -3.6617 + 0.0022*x	0.07	F (1,140) = 0.68353, p < 0.40978
Factor 5	y = 2.27 - 0.0014*x	0.05	F (1,140) = 0.38918, p < 0.53374

Table 2. Results of linear regression analyses of factors from PCA with elevation (n = 142).

Analyses of elements in Vaccinium myrtillus from the 2nd transect from June

PCA shows correlations between elements in bilberry using six factors (Table 3). The first factor indicates a positive correlation between S, Cl, K and Ca. The second factor shows a positive correlation between Cr, Fe and Ba. The third factor shows the antagonistic relationship between Fe and Zn. While Fe levels increase, Zn levels decrease and vice versa. The fourth factor shows a decline in Rb. The fifth factor indicates a positive increase in Mn. The sixth factor captured the positive accumulation of Pb. Factor 1 for bilberry leaves showed a positive correlation with S, Cl, K and Ca, as they are mostly accumulated in leaves

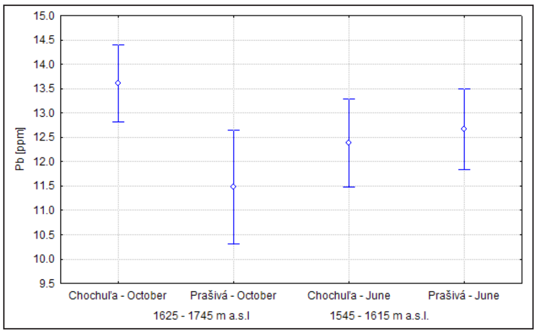

Fig. 2. Concentrations of Pb are higher in stem samples of *V. myrtillus* (with leaves) from the area of Veľká Chochuľa and Malá Chochuľa than from the area of Prašivá from an elevation of 1625 – 1745 m a.s.l. from the first transect from October (One-way ANOVA, n = 142, F (1, 140) = 16.280, p = 0.00009).

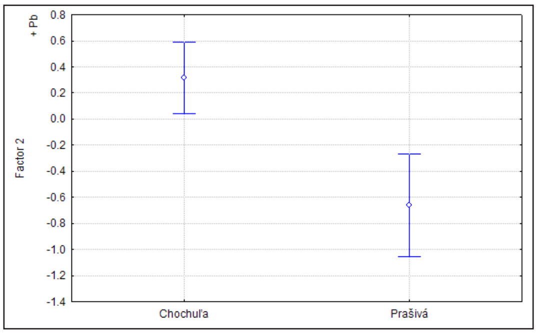

Fig. 3. Differences in Pb distribution of stem and leaf samples of *V. myrtillus* between ridge areas of two transects from autumn and early summer months (One-way ANOVA, n = 314, F (3, 310) = 3.3105, p = 0.02042).

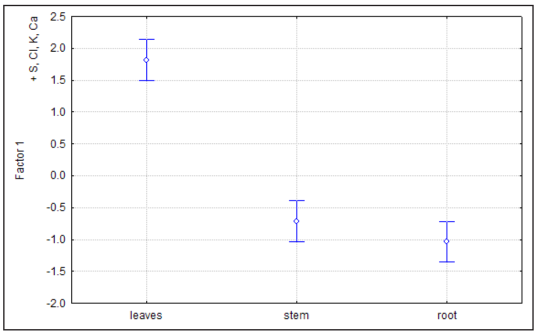

Fig. 4. Comparison of differences in effects of factor 1 in the different parts of *V. myrtillus* from June (One-way ANOVA, F (2, 249) = 90.965, p = 0.0000). LS means with 0.95 confidence limits of scores of factor 1. Vertical bars denote ± standard errors.

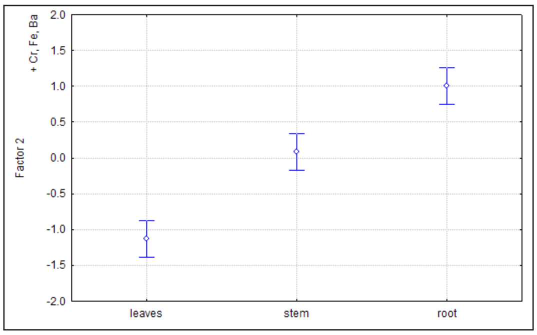

Fig. 5. Comparison of differences in effects of factor 2 in the different parts of *V. myrtillus* from June (One-way ANOVA, F (2, 249) = 67.511, p = 0.0000). LS means with 0.95 confidence limits of scores of factor 2. Vertical bars denote ± standard errors.

Element	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
S	**0.822**	-0.182	-0.067	0.315	-0.242	0.134
Cl	**0.860**	-0.397	0.019	-0.087	0.075	-0.020
K	**0.891**	-0.385	0.056	-0.075	0.001	-0.009
Ca	**0.839**	-0.034	-0.420	0.043	-0.097	-0.142
Cr	0.494	**0.629**	0.493	0.211	0.096	-0.192
Mn	0.274	0.264	-0.473	0.079	**0.689**	0.388
Fe	0.356	**0.631**	**0.626**	-0.018	0.207	-0.090
Zn	-0.167	0.432	**-0.636**	-0.433	-0.013	-0.298
Rb	0.417	-0.218	0.246	**-0.788**	0.169	-0.072
Ba	0.469	**0.615**	-0.470	0.142	-0.148	-0.168
Pb	0.222	0.584	0.067	-0.374	-0.440	**0.514**
Eigenvalue	3.8	2.2	1.7	1.1	0.8	0.6
Total variance [%]	34.8	19.6	15.8	10.3	7.5	5.6

Table 3. Eigenvectors with the percentage of variance in principal component analysis of elements in the *Vaccinium myrtillus* from the second transect. The highest correlations are in bold.

while stems have lower amounts and roots exhibit the lowest (both with negative cumulation) (Fig. 4). Leaves do not accumulate Cr, Fe and Ba, but bilberry root accumulates these elements the most (Fig. 5). In factor 3, roots and leaves show a positive correlation between Zn, Fe, while stems show a negative correlation (Fig. 6). In June, Rb accumulates the most in stems of bilberries, whereas lower levels of Rb are found in leaves and roots (Fig. 7). We did not find any significant effect in bilberries organs from Mn accumulation (Fig. 8). Factor 6 shows Pb concentration, with which leaves and roots correlate almost equally; they show a slightly positive correlation, while stems show a negative correlation (Fig. 9).

Effect of altitude on concentrations of elements in stems of bilberries from the second transect

For the first factor from the second transect we have recorded a positive accumulation of the following elements: S, Cl, K, Ca. The accumula-

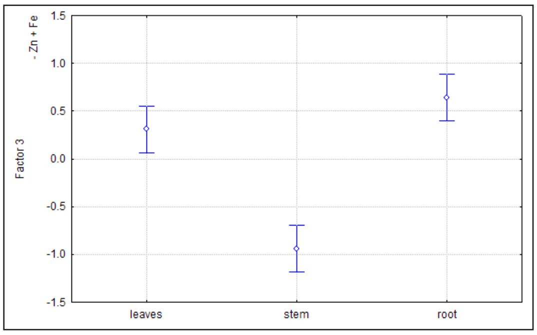

Fig. 6. Comparison of differences in effects of factor 3 in the different parts of *V. myrtillus* from June (One-way ANOVA, F (2, 249) = 46.168, p = 0.0000). LS means with 0.95 confidence limits of scores of factor 3. Vertical bars denote ± standard errors.

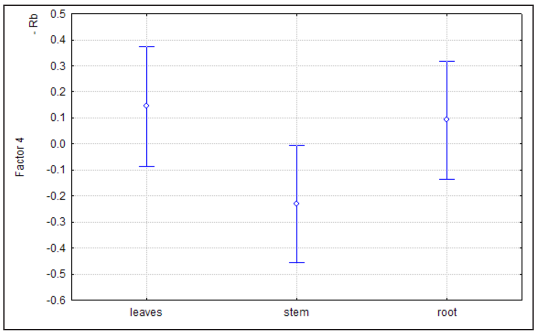

Fig. 7. Comparison of differences in effects of factor 4 in the different parts of *V. myrtillus* from June (One-way ANOVA, F (2, 249) = 3.1195, p = 0.04591). LS means with 0.95 confidence limits of scores of factor 4. Vertical bars denote ± standard errors.

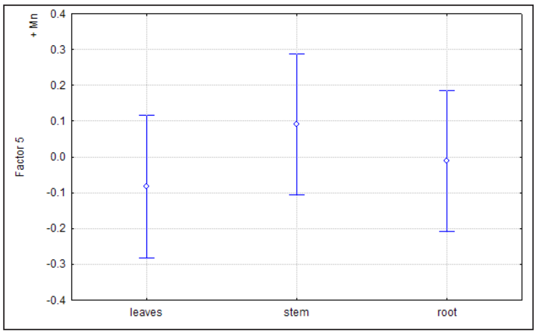

Fig. 8. Comparison of differences in effects of factor 5 in the different parts of *V. myrtillus* from June (One-way ANOVA, F (2, 249) = 0.74197, p = 0.47723). LS means with 0.95 confidence limits of scores of factor 5. Vertical bars denote ± standard errors.

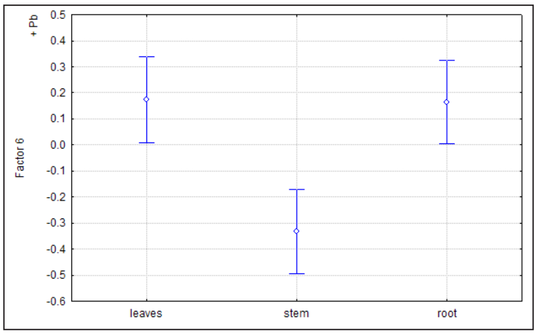

Fig. 9. Comparison of differences in effects of factor 6 in the different parts of *V. myrtillus* from June (One-way ANOVA, F (2, 249) = 12.488, p = 0.00001). LS means with 0.95 confidence limits of scores of factor 6. Vertical bars denote ± standard errors.

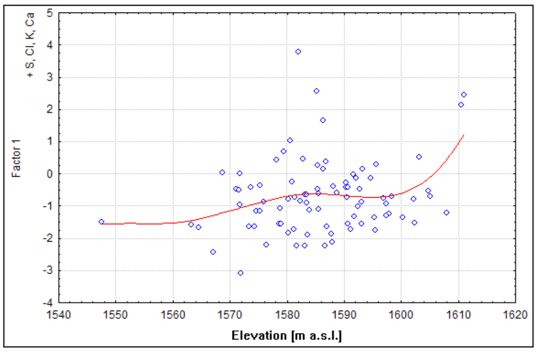

Fig. 10. Concentrations of S, Cl, K, and Ca increase in stems of *V. myrtillus* with elevation (y = -38.5643 + 0.0239*x, r = 0.23, n = 85, F (1,83) = 4.8185 p < 0.03095) in the second transect from June. The line is calculated as distance weighted least squares.

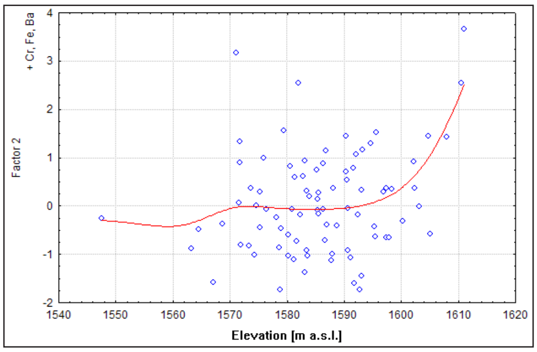

Fig. 11. Concentrations of Cr, Fe and Ba increase in stems of *V. myrtillus* with elevation (y = -37.9224 + 0.024*x, r = 0.25, n = 85, F (1,83) = 5.7164 p < 0.01907) in the second transect from June. The line is calculated as distance weighted least squares.

tion of elements increases with higher elevation (Fig. 10). The second factor shows accumulation of Cr, Fe, Ba. Element concentrations increase with higher altitude (Fig. 11). In factor 3, the inverse effect of (-Zn and +Fe) is not related to elevation (Fig. 12). In factor 4, Rb values appear to increase with altitude. The latter decreasing may be due to fewer samples and so cannot be taken as indicative (Fig. 13). In factor 5, concentration of Mn significantly decreases with elevation (Fig. 14). In factor 6, concentration of Pb decreases with elevation. Lower-lying samples are the most affected by Pb contamination (Fig. 15). In comparison of groups 3 and 4, group 3 represents Chochuľa mountain, and Group 4 represents Prašivá mountain. Significant differences in the second transect were observed only for factors 4 and 6. The concentrations of Rb were higher in the area of Veľká Chochuľa and Malá Chochuľa than from the area of Prašivá (Fig. 16). In factor 6 the amount of Pb was higher on Prašivá (Fig. 17).

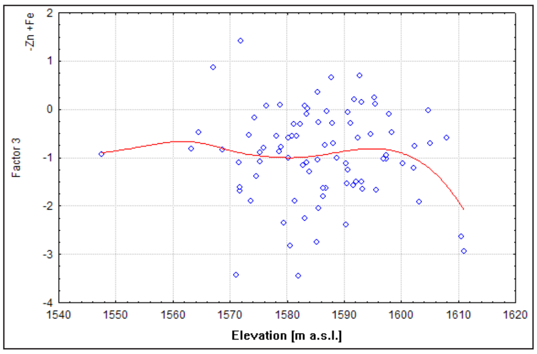

Fig. 12. Inverse effect of Zn and Fe concentrations in stems of *V. myrtillus* is not related to elevation (y = 7.8784 - 0.0056*x, r = 0.07, n = 85, F (1,83) = 0.36568, p < 0.54702) in the second transect from June. The line is calculated as distance weighted least squares.

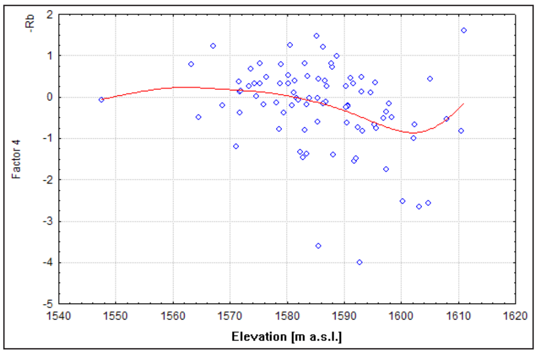

Fig. 13. Concentration of Rb increases with elevation (y = 41.5011 - 0.0263*x, r = 0.29, n = 85, F (1,83) = 7.6663 p < 0.00694) in stems of *V. myrtillus* in the second transect from June. The line is calculated as distance weighted least squares.

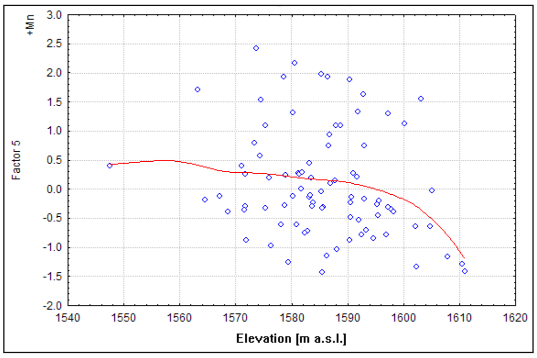

Fig. 14. Concentration of Mn decreases with elevation (y = 30.9601 - 0.0195*x, r = 0.24, n = 85, F (1,83) = 4.9245 p < 0.02921) in stems of *V. myrtillus* in the second transect from June. The line is calculated as distance weighted least squares.

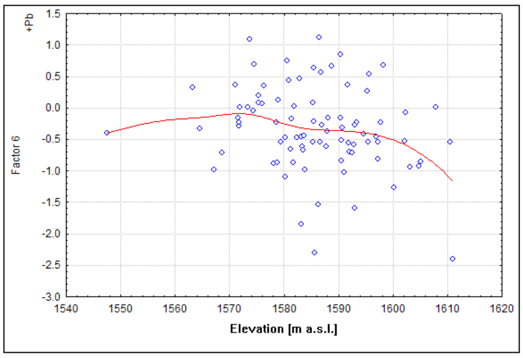

Fig. 15. Concentration of Pb decreases with elevation (y = 21.7749 - 0.0139*x, r = 0.24, n = 85, F (1,83) = 4.9245 p < 0.02921) in stems of *V. myrtillus* in the second transect from June. The line is calculated as distance weighted least squares.

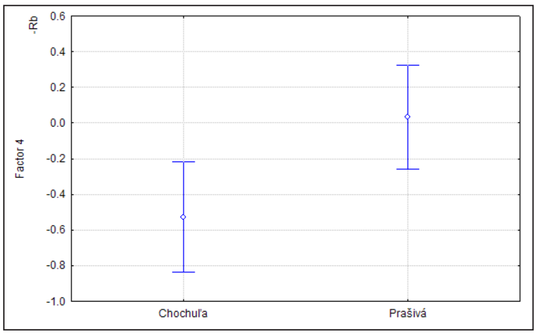

Fig. 16. Concentrations of Rb are higher in stem samples of *V. myrtillus* from the area of Veľká Chochuľa and Malá Chochuľa than from the area of Prašivá in the elevation 1545 – 1615 m a.s.l from the second transect from June (One-way ANOVA, n = 85, F (1, 83) = 6.8, p = 0.01060).

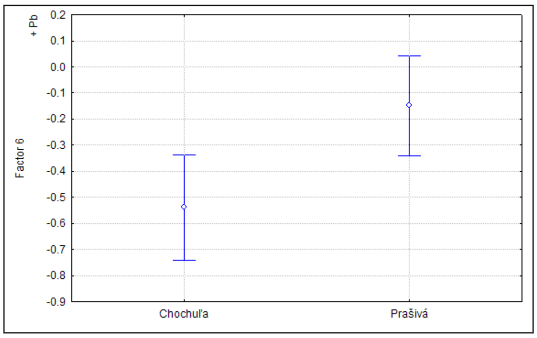

Fig. 17. Concentrations of Pb are higher in stem samples of *V. myrtillus* from the area of Prašivá than from the area of Veľká Chochuľa and Malá Chochuľa in the elevation 1545 – 1615 m a.s.l. from the second transect from June (One-way ANOVA, n = 85, F (1, 83) = 7, p = 0.00648).

Discussion

Pollution

Pollution in alpine zones is caused mainly by rain. Therefore, it is necessary to define where precipitation comes from, and it can be classified into several groups. The first of these is local precipitation affected by domestic production and transportation. There are four larger cities in the vicinity of the Low Tatras, as well as 4 main road connections. Each city has its own production. In Ružomberok, industry mainly produces pulp and paper; Banská Bystrica is known for its metallurgical and mining industry; Brezno is predominanly known for its engineering industry; and Liptovský Mikuláš manufactures leather goods. These cities, together with their roadways, are the largest sources of local pollution. Consequently, precipitation also affects global pollution, as evidenced by the presence of sand from the Sahara Desert in locations far removed. Like sand, various emissions are transmitted. Air flow has a huge effect on this transmission, as well as high and low pressure affecting wind direction.

Elevation

In terms of elevation, the samples collected below were visibly older than samples collected on the mountain ridge. This is because bilberry colonies spread from lower altitudes and gradually replaced other vegetation on the mountain ridge, which affected the natural conditions of the alpine ecosystem. One of the biggest consequences of this shift was the decline in marmot populations. The precondition for their disappearance is lack of food, as bilberries do not provide a sufficient nutrient source during spring. It was assumed that samples collected from lower elevations would be more affected by pollution, precisely because of their older age. We found that Mn and PB accumulate in lower altitudes to a greater extent than in higher altitude environments. In terms of Mn, this is natural, as bilberry is a hyperaccumulator of manganese (Korcak 1989; Reeves 2006). Therefore, older samples from lower altitudes were able to store more Mn over their lifespan. Our greatest concern was Pb accumulation at lower altitudes, as the accumulation of heavy metals may affect the food chain (Marques *et al.* 2009). On the other hand, samples from the mountain ridge could also be exposed to more precipitation, causing an increase in elements such as S, Cl, K, Ca, Cr, Fe, and Ba. Some of these are used by bilberries as nutrients and may simply represent a possible difference in soil nutrients, as the younger colony found at higher altitudes is not yet fully developed. For each 100 m in altitude gain, precipitation sum increases. This depends on several factors, and different locations may show variation based on climate, rainfall, or precipitation conditions. According to Shparyk and Parpan (2004), snowfall pollutes the environment more than rainfall. They also identified more chemical elements in snow samples than in soil or moss (e.g., *Pleurozium schreberi*) in the Ukrainian Carpathians.

Distinctive accumulation patterns of selected elements in different parts of bilberry

Many elements found in the environment are essential. Leaves are seasonal parts of the plant that perform photosynthesis and are important for nutrition during the growing season. To some extent, they eliminate heavy metals as they fall off, because pollution is mainly absorbed through soil. Usually, lead is deposited in the roots of plants, but there are cases where it can move into other parts as well. There is some evidence that plant foliage may also absorb lead (and it is possible that this lead has moved between parts of the plant) (Morzck and Funicclli 1982). Our research has clearly shown us that bilberry leaves store Pb in almost the same amount as roots during the month of June. As there is no significant shift in accumulation between June and October, It is likely that some Pb is being eliminated as the bilberry sheds its foliage in the fall. However, the lead from this foliage is ultimately deposited into the soil and may be reabsorbed by the plant or consumed by animals, though distributed over a larger area.

Changes in bilberry

Bilberries exhibited no observable changes in leaves or on the plant, though potential changes would likely only be visible under microscope. Despite lead pollution, bilberry plants have not experienced stunted growth despite the fact that metals such as Pb, Cd, Hg, and As do not play an advantageous role in growth of the plant, and could act adversely even at low concentrations (Kibra 2008). A certain level of resistance to lead is due to the presence of phosphorus and calcium in the soil, which directly influences the absorption of lead into the root of the plant by reducing the amount of lead (Morzck and Funicclli 1982).

The effect of altitude and organ on accumulation of heavy metals in Vaccinium myrtillus in the Low Tatra mountains

The most positive aspect of this research lies in the number of samples studied. As there were a multitude of samples, we were able to eliminate many biases and inaccuracies that would have occurred with a smaller number of samples. However, not all sections of the graphs were free of outliers. This was most evident with Rb, where the smaller number of the highest-altitude bilberry began to influence the results of the others. As a result, we assert that Rb decreases with altitude, as this correlation is only shared by 8 samples. For the other factors, the number of samples is significantly higher. Due to the nature of deposition of this element, we found its values to be quite variable, and thus, we must take into account that variation in these sections that differs from the others. Nevertheless, it can help us in gaining a perspective with regard to the conditions and deposition of heavy metals in bilberries.

Sample season

The period during which samples were collected played a crucial role, due to variation in recorded values. For Chochuľa, the difference was shifted by a quarter. In October the highest amount of Pb was recorded, while in June Pb concentrations were lower. On the other hand, we can see the opposite phenomenon with regard to Pb accumulation on Prašivá, where the amount of Pb in October was less that what was recorded in June. For more consistent results, we recommend collecting samples during the same time period, (even when the variable studied is altitude), as values change during the year due to both weather conditions, and growth and development of the bilberry. In some cases the position of the slope or the location of the snowbank could also play a crucial role in acuumulation (Shparyk and Parpan 2004). Due to changing conditions, the potential for additional research increases, and further studies could be conducted with regard to variation in season on heavy metal accumulation in bilberry, or heavy metal accumulation in soil or as a result of bilberry detritus, that would provide additional information and value to this field of study.

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