Oecologia
Montana 2024,
33, 62-79

Concentration of chemical elements in willow gentian (*Gentiana asclepiadea*), Javorová valley, Tatra mountains

Š. ŠTEFANCOVÁ and M. JANIGA

Institute of High Mountain Biology, Žilina University, Tatranská Javorina 7, SK-059 56, Slovak Republic; e-mail: janiga@uniza.sk

Abstract. Plants, as a basic component of the trophic pyramid, are a source of nutrients and health-promoting substances, but also of dangerous or toxic substances such as heavy metals. Chemical elements were studied in the plant *Gentiana asclepiadea* in the Javorová Valley, in the High Tatras. Plant samples were regularly collected at sampling sites varying in altitude and habitat. Concentrations of biogenic and potentially toxic elements in flowers, leaves, stems, roots, and soil were detected by an INNOV-X spectrometer. In *G. asclepiadea*, more pronounced concentrations of Rb, Hg, and Pb were measured in leaves and flowers. A tendency for the elements to accumulate in plant tissues from the beginning to the end of the growing season was observed. Significant contamination of the surface soil and dry plants was observed in the spring months. This phenomenon is observed in the behaviour of Rb, Sr, Ba, and Hg, as well as biogenic or trace elements such as Zn, Cu, Cr, Ca, Cl and S. In the mountain vegetation stage, there is a tendency to more strongly sequester Rb, Ba, Pb and Hg. This research illustrates the distribution and contamination of chemical elements in the high mountain environment.

Key words: willow gentian, uptake of chemical elements, accumulation, potential pollutants

Introduction

The functioning of the ecosystem is the result of many processes, including the flow of energy (solar radiation) and the cycling of substances. The cycling of substances involves the continuous exchange between organisms and the environment and the transformation of substances within the bodies of organisms. Approximately 20 biogenic and trace elements are involved in the body structure of organisms and many others that are not vital to the functioning of the organism. According to the magnitude of the concentration of elements in the body of organisms, they can be divided into macroelements, microelements and trace elements. Macronutrients make up the largest proportion in the body of organisms and include C, O, H, N and P. Micronutrients include S, Cl, K, Ca, Mg, Fe, Cu, and in smaller amounts, B, Mn, Zn, Si, Co, and F. Trace elements such as Se and Cr are the least abundant. These elements perform important functions in plant organisms. Trace elements also include several heavy metals essential for the plant in minimal quantities. Both deficiency and excess (toxicity) of biogenic elements have serious negative effects on the plant (Orolínová 2009; Vatansever *et al.* 2017).

In metallurgy, the term "heavy metals" is used to refer to 37 elements from the periodic table that have an atomic density greater than 5 g/cm^3. Heavy (toxic or risky) metals are microelements that are not biologically degradable or removable and have the ability to accumulate. They can be toxic to biota, including Cd, Cr, Hg, Co, and Pb, but some are considered nutrients, such as Cu, Mn, Mo, Fe, and Zn (Chmielewská and Bedrna 2007; Ducsay *et al.* 2009).

Heavy metals are a natural component of the environment, and their origin can be found in rocks and minerals in the geological environment. These elements (trace elements) play an indispensable role in the functioning of metabolic processes in organisms, but they have toxic effects when the threshold limit is exceeded. Some metals are toxic to organisms in general. Despite the risks posed by these toxic elements, they are frequently used in industries and agriculture, as well as in the food and pharmaceutical sectors. Anthropogenic contamination significantly increases the risk of their transfer to organisms (Boriová *et al.* 2014; Asati 2016).

Natural sources of heavy metals are primary (e.g., rock weathering) and secondary (e.g., atmospheric deposition, dust storms, volcanic activity). Large sources of anthropogenic origin include mining and metallurgy, as well as certain industries, atmospheric deposition capturing emissions from incinerators, waste dumps, vehicle exhausts, and other substances released into the air. Agriculture is also a significant source through the use of a variety of artificial and organic fertilizers and pesticides, which further contaminate both water, soil, and biota. Upon entry into soil, water, and to a lesser extent, the air, heavy metals undergo physico-chemical changes and become part of the biogeochemical cycle. Heavy metals tend to remain in soils longer than in the hydrosphere and atmosphere (Ross 1994; Lánczos *et al.* 1998; Alloway 2013).

One of the most critical factors determining the risk of heavy metal contamination is their bioavailability to plants in relation to their mobility in soil. The solubility and mobility of heavy metals in soil can be increased by decreased pH, higher salt concentrations, the presence of biological agents (bacteria), or even due to the synergistic (amplifying) effect of multiple elements (Cejpek 2000; Boriová *et al.* 2014).

Chemical elements, both biogenic and toxic, occur naturally in the soil and come from the parent rock (i.e., the mineral, rock part of the soil). Other heavy metals and elevated concentrations of pollutants are the result of anthropogenic activities. The composition of the parent rock is a key factor in determining the amount of chemical elements in the soil. The basic elements make up 99% of the soil: oxygen (O), silicon (Si), aluminium (Al), iron (Fe), calcium (Ca), sodium (Na), potassium (K), magnesium (Mg), phosphorus (P) and titanium (Ti). The remaining 1% is made up of trace elements, the concentration of which does not normally exceed 1000 mg/kg^{-1}, excluding ore materials. Trace elements are, for example, cobalt (Co), copper (Cu), manganese (Mn), and zinc (Zn), which are classified as essential elements. Non-essential, toxic elements are also present in rocks, including: arsenic (As), beryllium (Be), cadmium (Cd), lead (Pb), mercury (Hg), thallium (Th), and others. The parent rock forms the mineral base of the soil. The mineralogical composition also determines the structure of the soil and has an impact on its chemical and physical properties. An important chemical property of soil is its adsorption capacity, which has a significant effect on the bioavailability of metals (Steinnes and Friedland 2006; Purves 2012; Alloway 2013).

Heavy metals and potential pollutants can enter soils in the form of atmospheric aerosols. Aerosols consist of particles smaller than 0.05 microns. From the soil, metals in the form of aqueous solutions are absorbed onto the surface of roots, from where they are translocated trough the root system to various parts of the plant. Another way heavy metals enter plants is through atmospheric deposition, via leaves (foliar). This allows heavy metals to enter aboveground parts of plants, including plant surfaces, from where they can be translocated to different parts of plants, including roots. Due to the submicron size of aerosol particles, they can remain in the atmosphere for several days. During this time, particles can be transported thousands of kilometers from emission sources (Chmielewská and Bedrna 2007; Lehndorf and Schwark 2008; Dadová 2014).

Plants absorb heavy metals from soil solutions and can also take up solid soil particles through contact. The amount of a heavy metal absorbed by plants under specific conditions depends on its concentration in the soil. Most heavy metals accumulate in the root system. Plants have specific mechanisms to retain toxic substances in their roots, reducing the risk of contamination in aboveground parts of the plant. Soil concentration can be affected by the natural content in parent rock and anthropogenic activities (mainly surface layers). The content, solubility, mobility, and bioavailability of heavy metals in plants depend on the specific properties of the metals themselves and the soil. Analyzing different plant parts has generally shown that roots contain the highest metal concentrations, followed by leaves and stems, with lower concentrations present in seeds, fruits, tubers, and bulbs (Chreneková 1983; Ďurža and Khun 2002).

Through the penetration of heavy metals into plants, these metals become part of the food chain, eventually contaminating both animals and humans. The bioaccumulation of heavy metals in plants and animals is a metabolically controlled process of their accumulation in living organisms. This process occurs when an organism uptakes heavy metals faster than it can eliminate them, resulting in the immobilization of the heavy metal within the biomass. Accumulation in plants is also influenced by the nature of the plant itself, the size of the root surface, the capacity for uptake, or the degree of transpiration (Lourie *et al.* 2003).

Material and Methods

Site characteristics and sample collection

The research site, Javorová dolina, is located in the northern part of the Tatra Mountains. It starts in the settlement Tatranská Javorina (1000 m) and ends with the saddle Sedielko (2376 m). It is bordered from the northeast by the ridge of the Belianske Tatras. The Tatras are located in a typical inland climate with warm summers and cold winters. However, the steep rise in altitude greatly impacts the duration of climatic factors (air temperature, cloud cover, sunshine, precipitation and others). The Tatra Mountains rise steeply and surpass the surrounding landscape by up to 2000 metres. As a rule, every 100 metres the temperature drops by about 0.6° C, and the amount of precipitation increases at the same time (Kucharík 2019; Kollár 2020; Sloboda 2021). The surface of the High Tatras has been shaped primarily by glaciers. In the valleys, the youngest remnants of the glacier are preserved in tarns. In the Javorová dolina valley there are four tarns: Čierne Javorové Pleso, Zelené Javorové Pleso, Malé Javorové Pleso, and Žabie Javorové Pleso (Nemčok 1994; Piscová *et al.* 2018; Kollár 2020). The Tatra Mountains have a well-developed river network with a large number of smaller side tributaries that converge into a common main river (Kucharík 2019).

Samples were collected along the hiking trail leading from the point Pod Muráňom towards the mountain saddle Sedielko. 7 sampling points were marked out according to altitude – with altitudes of 1137, 1213, 1259, 1391, 1473, 1562, 1630 m a.s.l. Due to the ecology of gentian, the last sampling point was located at the tarn Žabie Javorové pleso. This section of the Maple Valley is 7.6 km long (Kucharík 2019; Kollár 2020; Lacika 2020; Gurník 2021). At each site, whole plants were collected along with the soil in the immediate vicinity and put in a resealable plastic bag.

Laboratory analyses

In the laboratory, samples were separated into individual plant parts (flower, leaf, stalk, root), placed one by one in Petri dishes and air dried at room temperature. After drying, the samples were crushed in a cryogenic ball mill (CryoMill, Retsch, Germany) to a fine powder. The soil samples were sieved then crushed to remove excess impurities.

Heavy metal analysis was carried out on INNOV-X spectrometers. In the program, the method selected for the measurement of plant material was plant material. The accuracy of the measurement was verified by the analysis of the standard reference material (CRM) for plant samples Polish Virginia Tobacco Leaves (IC-INCT-PVTL-6), (ICHTJ, Poland). For soil measurements, the method "CC141" was selected. The accuracy of the measurement was verified by the analysis of the standard reference material (CRM) for the soil sample Loam Soil (ERM-CC141), (IRMM, Belgium). Afterward, the sample was poured into a vial. For correct calculations of heavy metal concentrations, as much sample as possible was placed in the vials. The program was set to take 3 replicate measurements of the sample, from which it then calculates the average value. For a single measurement of one sample, the time range was set from 80 to 110 seconds.

A direct mercury analyzer (DMA 80, Milestone, Italy) was used to measure Hg concentration value. The combustion of the sample was carried out at 650° C. The program was set to measure plant material - Standard method. Individual samples were poured into metal trays and weighed on a laboratory digital analytical balance (KERN 770, Kern & Sohn, Germany). For the accuracy of the measurements, the smallest possible amount of sample was loaded into the trays, up to a maximum of 0.01 gram. The trays were then loaded into the DMA 80. The mercury was evaporated at 650° C and then rapidly cooled, condensed and collected in the next part of the analyzer. From the measured amount of Hg in the sample, an average value was automatically calculated, for a specific sample. The accuracy of the measurement was verified by analysis of standard reference material (CRM) for the plant sample Polish Virginia Tobacco Leaves (IC-INCT-PVTL-6), (ICHTJ, Poland) and for the soil sample Loam soil (ERM-CC141), (IRMM, Belgium).

Statistical analyses

Several methods were used for visual display and statistical evaluation of measured values of chemical elements. If the concentration of a given element was sufficiently high (the XRF was able to measure the value), the values were plotted on graphs as a function of seasonality and altitude. To calculate the significance of the test we used the Kruskal-Wallis test.

In the case of a significantly increased or decreased concentration of an element in a part of the plant or in the soil, results are shown on a separate graph for better visualization. The graphs of means and standard deviations showed the increasing and decreasing tendency of the individual elements. The line connecting the averages of the values represents the behaviour of the elements in the plant. In cases of insufficient data, the line is broken. In graphs showing element concentrations versus altitude, the line is indicated by a dashed line. In this case, the line is an auxiliary indicator for better readability of the graphs; it does not represent the continuity of the elements as a function of altitude, because the collection points have no logical continuity. Winter months, between December and April have been accumulated into one common category called "Dec/Apr", due to the limited amount of data (climatic conditions).

Chemical elements that were not present in sufficient amounts in the plant (there was a large amount of <LOD (unmeasured data) in the measured results from XRF) were evaluated using the Contingency table. Chi-square test (X^2) was used to calculate the significance of the differences between the measured values in each organ and in the soil, from which the significance level p value was then calculated.

Results

Biogenic elements occurring in the plant were measured at the highest levels. Concentrations measured in plant organs and in soil were similar in most macroelements. The concentration of heavy metals in plant organs was significantly lower compared to the concentration of heavy metals occurring in soil (shown in separate graphs).

Concentration levels of biogenic elements

Sulfur

The amount of sulphur in the soil remains about the same throughput different months, but the amount of sulphur in plant organs increases during the winter and autumn months. The highest concentrations are recorded in the leaves and flowers (Fig. 1a). At individual sites, sulphur occurs in the soil independently of the sulphur concentration in the plant. The amount of sulphur increases slightly at 1259 m a.s.l., decreases, and then increases again up to 1630 m a.s.l. (Fig. 1b).

Chlorine

Concentrations of chlorine in the soil did not depend on the seasons (Fig. 2a); in the plant, concentrations were higher in the winter months than in the summer months. Chlorine concentrations behave similarly to sulfur concentrations. At each elevation the amount of chlorine in the soil was lower at lower elevations (Fig. 2b), and chlorine concentration was consistent in the plant organs at each site.

Potassium

Due to seasonality, potassium in the plant varies significantly from month to month, except in the flower. In the autumn months, its amount decreases in the leaf and stalk and increases significantly after winter. In the root, potassium has a similar tendency. Conversely, in the soil, the concentration is the same from month to month (Fig. 3a). In the soil, potassium concentration is highly variable from site to site. The elevated amount of K occurs at 1213 m a.s.l. and 1391 m. a.s.l. In the plant, potassium concentration is consistent at each site (Fig. 3b).

Calcium

Calcium concentrations vary from month to month in flowers, leaves and roots. The high-

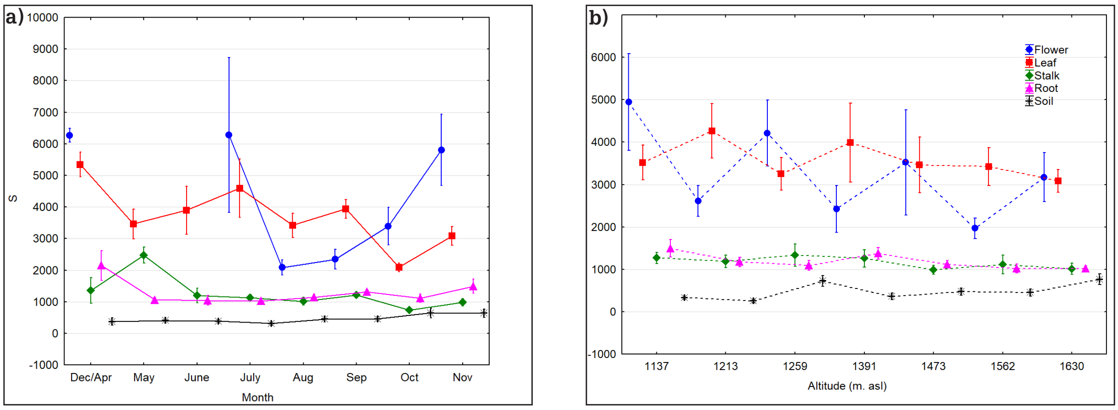

Fig. 1. Mean (±SE) levels of sulphur in *Gentiana asclepiadea* and in the soil in dependence on season a) and on locality b). Locality is indicated by the altitude. The concentration differed significantly among months (K-W test: flower = 21.78, $p < 0.01$; leaf = 22.85, $p < 0.01$; stalk = 32.58, $p < 0.01$; root = 17.45, $p = 0.01$; soil = 6.54, $p = 0.47$) as well as among altitude (K-W test: flower = 8.69, $p = 0.19$; leaf = 1.99, $p = 0.91$; stalk = 3.98, $p = 0.68$; root = 8.63, $p = 0.19$; soil = 26.5, $p < 0.01$).

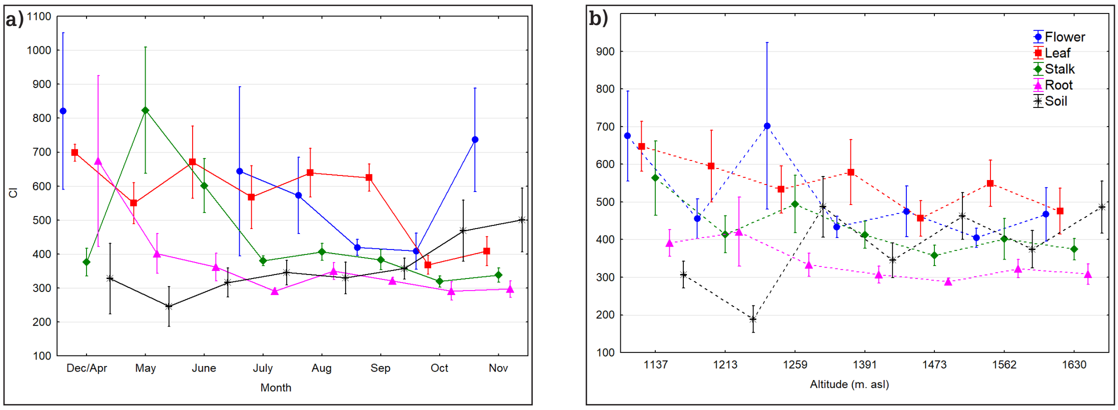

Fig. 2. Mean (± SE) levels of chlorine in *Gentiana asclepiadea* and in the soil according to seasonality a) and altitude b). Values significance was tested in dependence of months (K-W test: flower = 13.81, $p = 0.01$; leaf = 27.32, $p < 0.01$; stalk = 33.69, $p < 0.01$; root = 16.76, $p = 0.02$; soil = 6.6, $p = 0.47$) as well as in the altitude (K-W test: flower = 5.62, $p = 0.46$; leaf = 5.36, $p = 0.49$; stalk = 7.8, $p = 0.25$; root = 7.24, $p = 0.29$; soil = 21.32, $p < 0.0116$).

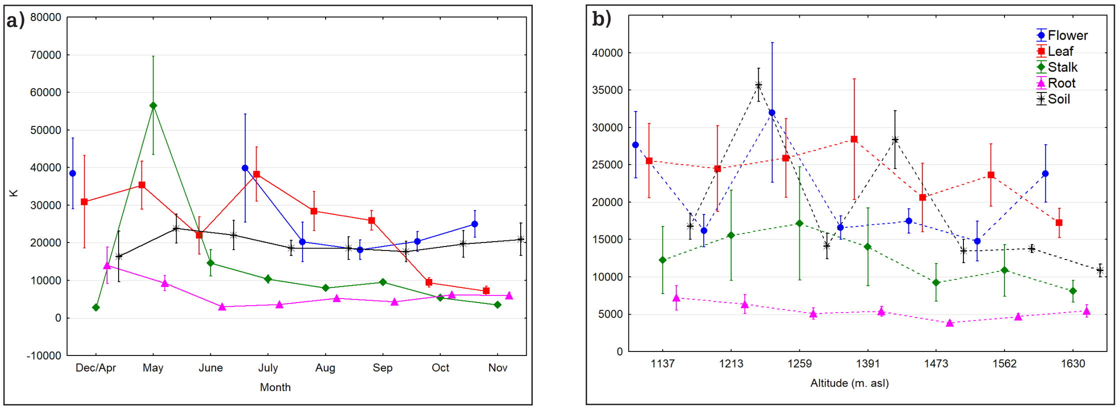

Fig. 3. Mean (± SE) levels of potassium in *Gentiana asclepiadea* and in the soil in dependence on seasonality a) and on altitude b). The significance of values was tested according to seasonality (K-W tests: flower = 9.53, $p = 0.09$; leaf = 38.98, $p < 0.01$; stalk = 46.91, $p < 0.01$; root = 31.57, $p < 0.01$; soil = 3.89, $p = 0.79$) and to altitude (K-W test: flower = 9.24, $p = 0.16$; leaf = 1.21, $p = 0.98$; stalk = 2.39, $p = 0.88$; root = 6.05, $p = 0.42$; soil = 35.7, $p < 0.01$).

est values occurred during autumn and winter months. In the soil, due to seasonality, calcium concentration is consistent (Fig. 4a). Calcium values vary considerably from site to site in the soil, where the calcium concentration decreases with increasing altitude. In the stalk and in the root, the values also vary and have a similar tendency to those in the soil (Fig. 4b).

Chromium

Chromium concentrations vary from month to month in all organs of the plant and in the soil. Values rise in autumn and increase significantly in the winter months and early summer (Fig. 5a). The highest values are in the flower (Fig. 5b), in the leaf and in the soil.

At individual sites, the amount of chromium in the soil occurs independently of the plant. In the soil, the values vary considerably, whereas in the plant they are consistent. Most chromium is found in soil at middle altitudes from 1213 to 1473 m a.s.l. (Fig. 6a,b).

Manganese

The amount of manganese varies significantly in the flower. Concentration increases in autumn and winter months and decreases in summer months. In the root, manganese levels are significant at 0.059. Manganese levels increase slightly toward the autumn months, with a more significant increase in spring (Fig. 7).

Manganese varies considerably in all parts of the plant (Fig. 8a) and in the soil due to altitude. The highest values are in the soil (Fig. 8b). In all parts of the plant and in the soil, the highest manganese values are found at an altitude of around 1259-1391 m a.s.l. Higher values are influenced by

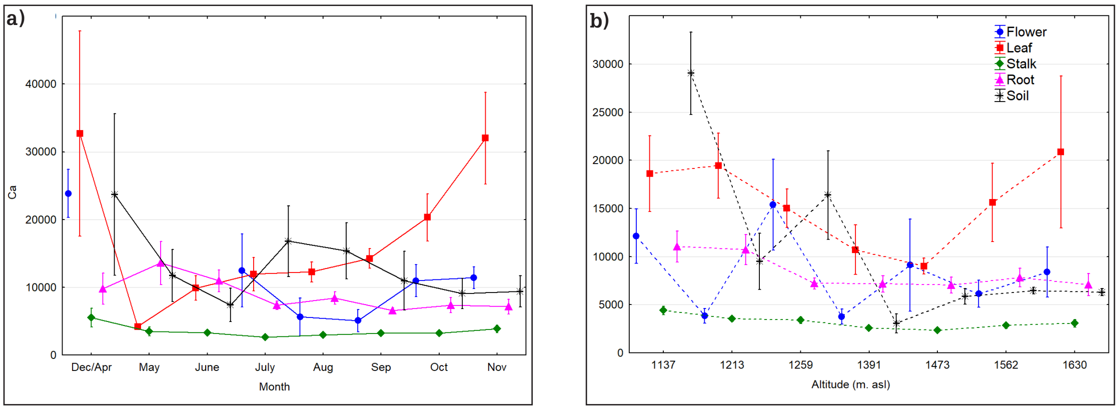

Fig. 4. Mean (± SE) levels of calcium in *Gentiana asclepiadea* and in the soil in dependence on seasonality a) and on altitude b). The concentration differed significantly among months (K-W test: flower = 23.94, $p < 0.01$; leaf = 36.3, $p < 0.01$; stalk = 9.67, $p = 0.21$; root = 15.19, $p = 0.03$; soil = 3.81, $p = 0.27$) and among altitude (K-W test: flower = 11.78, $p = 0.06$; leaf = 8.96, $p = 0.18$; stalk = 22.03, $p < 0.01$; root = 13.04, $p = 0.04$; soil = 42.16, $p < 0.01$).

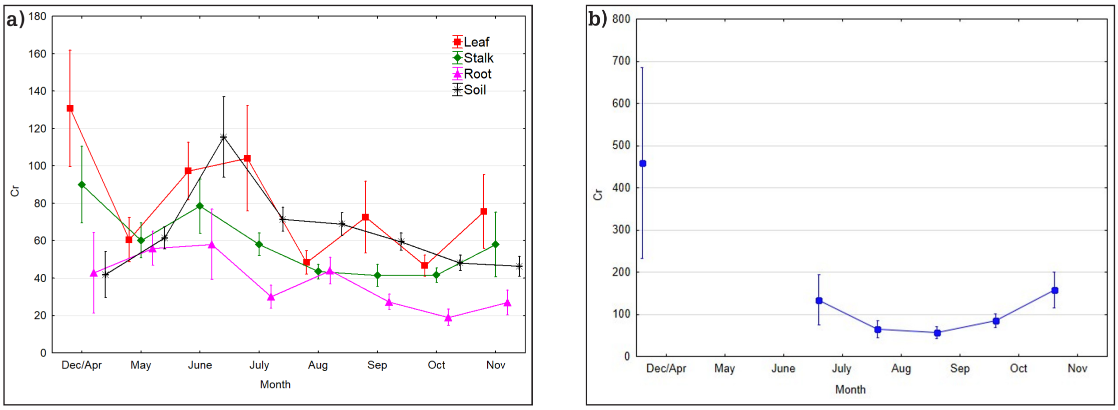

Fig. 5. Mean (± SE) levels of chromium in *Gentiana asclepiadea* and in the soil a) and separately in the flower b) in dependence on seasonality (K-W test: flower = 17.52, $p < 0.01$; leaf = 19.27, $p = 0.01$; stalk = 15.92, $p = 0.03$; root = 20.55, $p < 0.01$; soil = 29.63, $p < 0.01$).

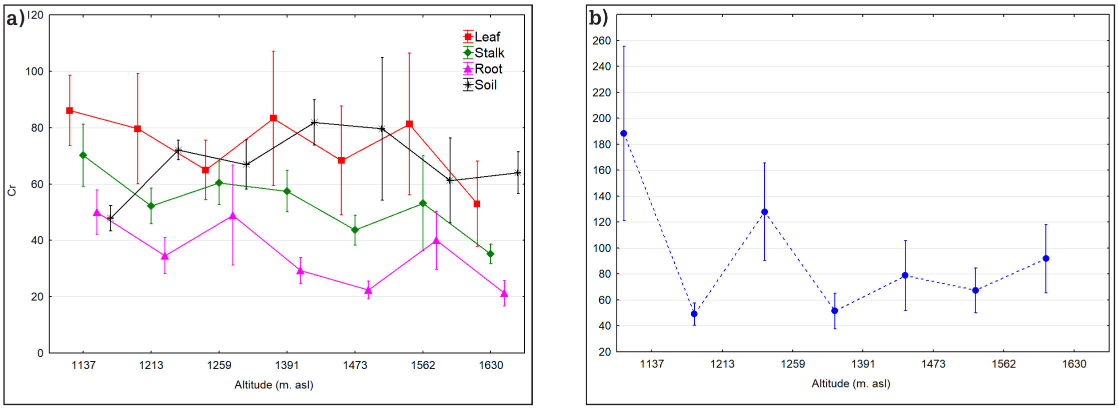

Fig. 6. Mean (± SE) levels of chromium in *Gentiana asclepiadea* and in the soil a) and separately in the flower b) in dependence on altitude (K-W test: flower = 5.73, $p = 0.45$ leaf = 6.92, $p = 0.33$; stalk = 12.08, $p = 0.06$; root = 9.51, $p = 0.15$; soil = 18.96, $p < 0.01$).

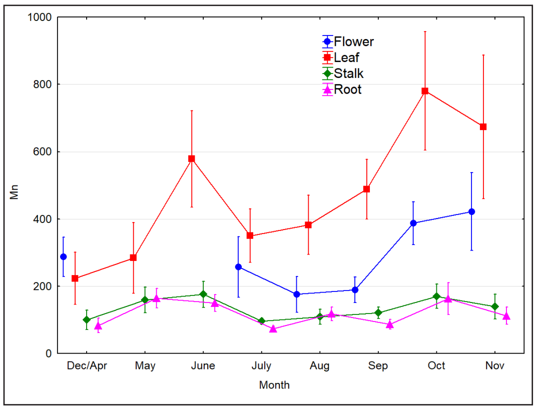

Fig. 7. Mean (± SE) levels of manganese in *Gentiana asclepiadea* and in the soil separately in dependence on seasonality (K-W test: flower = 15.14, p = 0.01; leaf = 11.72, p = 0.11; stalk = 5.21, p = 0.63; root = 13.58, p = 0.059; soil = 3.47, p = 0.84).

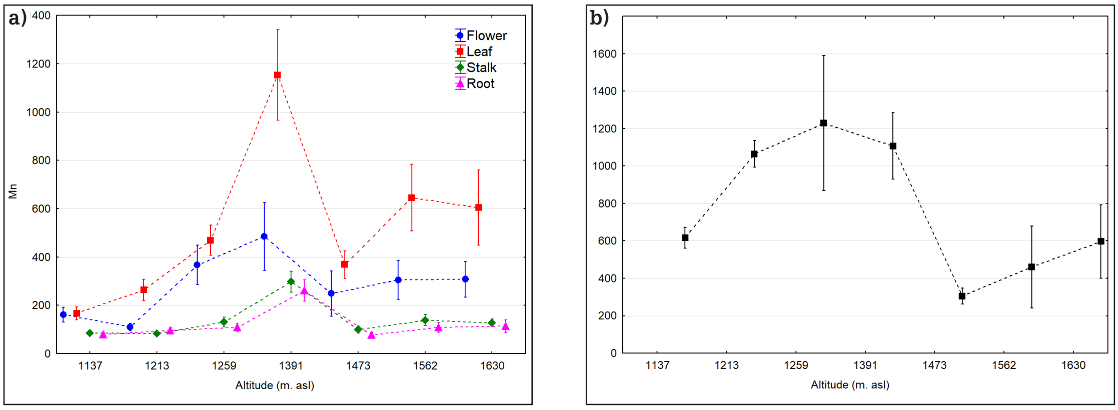

Fig. 8. Mean (± SE) levels of manganese in *Gentiana asclepiadea* a) and in the soil separately b) in dependence on altitude (K-W test: flower = 14.28, p = 0.03; leaf = 40.92, p < 0.01; stalk = 29.82, p < 0.01; root = 23.21, p < 0.01; soil = 37.43, p < 0.01).

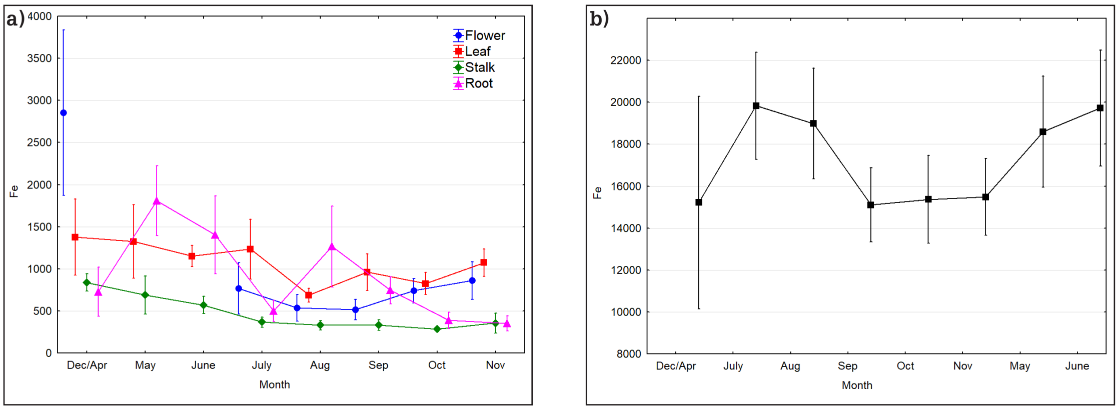

Fig. 9. Mean (± SE) levels of iron in *Gentiana asclepiadea* a) and in the soil separately b) in dependence on seasonality (K-W test: flower = 9.43, p = 0.09; leaf = 9.34, p = 0.23; stalk = 13.91, p = 0.05; root = 17.51, p = 0.01; soil = 5.99, p = 0.54).

the change in the geological profile at these altitudes. There is still a slight increase in manganese concentrations toward the end of the route.

Iron

Significant levels of iron during the season are found in the root. Iron concentration decreases in the autumn and winter months and increases significantly in spring and late summer. In the stem, values vary at 0.05, increasing in the late autumn and winter months (Fig. 9a, b).

The highest iron concentrations are in the soil, where they vary considerably from site to site. The highest values occur around 1213 and 1391 m a.s.l. These high values are likely due to geology; in these places the route moves away from the stream (Fig. 10a). Iron concentration also varies in the root and stalk; values decrease with increasing altitude. Concentrations are significantly higher in the root compared to the stalk, with the highest concentrations at 1213 m a.s.l. (Fig. 10b).

Copper

Copper varies significantly from month to month, but only in the flower. Values rise in the autumn months. In the winter months the line is broken due to insufficient data (climatic conditions) (Fig. 11).

In the flower, copper levels vary in contrast to the rest of the plant. It reaches its highest values at the beginning of the route and at an altitude of about 1259 m a.s.l. (Fig. 12a). Copper varies considerably from site to site in the soil, with the amount of copper increasing with increasing altitude and decreasing sharply at the highest point (Fig. 12b).

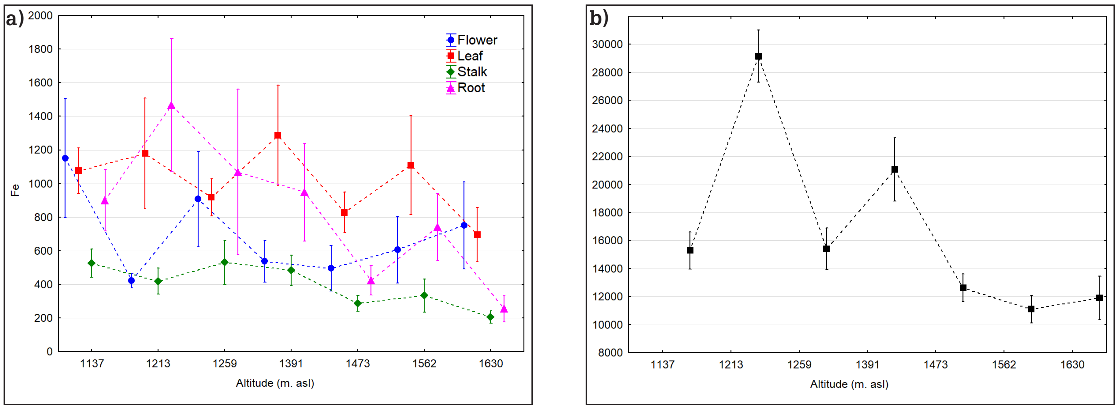

Fig. 10. Mean (± SE) levels of iron in *Gentiana asclepiadea* a) and in the soil separately b) in dependence on altitude (K-W test: flower = 2.33, p = 0.89; leaf = 5.31, p = 0.51; stalk = 15.75, p = 0.02; root = 16.22, p = 0.01; soil = 40.47, p < 0.01).

Zinc

The zinc concentrations in *G. asclepiadea* dependent on altitude were not significant, hence the graph is not shown (K-W test: flower = 8.38; p = 0.21; leaf = 3.8; p = 0.7; stalk = 6.83; p = 0.34; root = 10.67; p = 0.099; soil = 34.1; p < 0.01). The amount of zinc varies from month to month in the flower, leaves, and stalk. The highest values are found in the leaf.

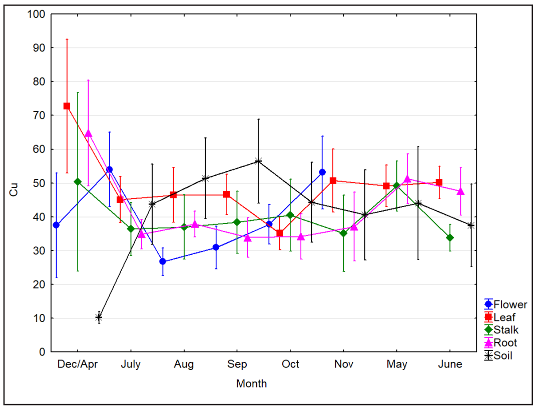

Fig. 11. Mean (± SE) levels of copper in *Gentiana asclepiadea* and in the soil in dependence on seasonality (K-W test: flower = 12.63, p = 0.03; leaf = 8.17, p = 0.32; stalk = 6.97, p = 0.43; root = 10.91, p = 0.14; soil = 8.33, p = 0.3).

In the autumn and winter months, zinc concentration increases in the leaf and flower, rises significantly in the stem following winter, and then decreases (Fig. 13a). The concentration in the soil varies considerably from place to place. High values occur around 1259 m a.s.l., likely due to the geological subsoil (Fig. 13b).

Titan

The highest levels of titanium are found in the soil, measured in all samples (92). Titanium was measured in the root in almost half of the samples (40), found in 29 leaf samples, and only detected in 10 flower and stalk samples, combined. The variation in the amount of titanium in the different organs of the plant and in the soil is significant (Table 1).

Molybdenum

The difference between the amount of molybdenum in the plant and in the soil varies considerably. Most is found in the soil and higher amounts are also found in the leaves. There is less in the flower and the lowest amounts are in the root and stalk (Table 1).

Nickel

There are significant differences in nickel concentration between the different organs and in the soil. The

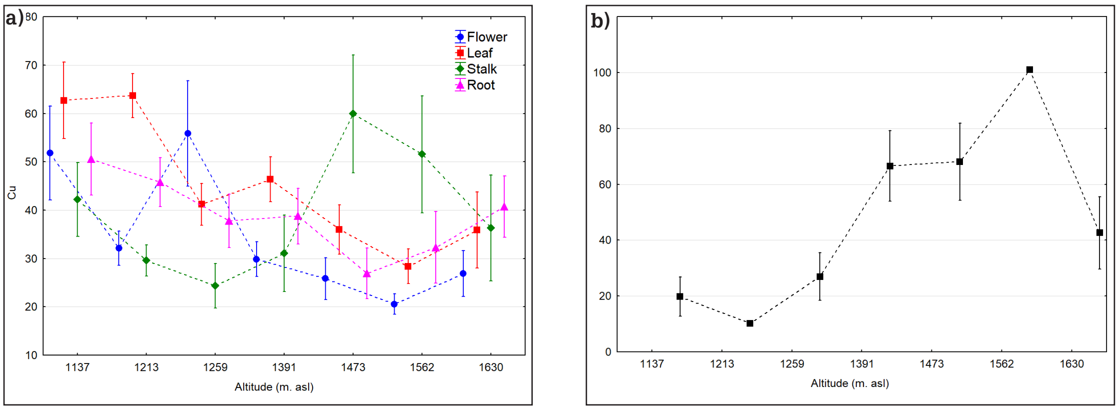

Fig. 12. Mean (± SE) levels of copper in *Gentiana asclepiadea* a) and in the soil separately b) in dependence on altitude (K-W test: flower = 17.03, p = 0.01; leaf = 29.19, p = 0.09; stalk = 9.23, p = 0.16; root = 10.41, p = 0.11; soil = 34.65, p < 0.01).

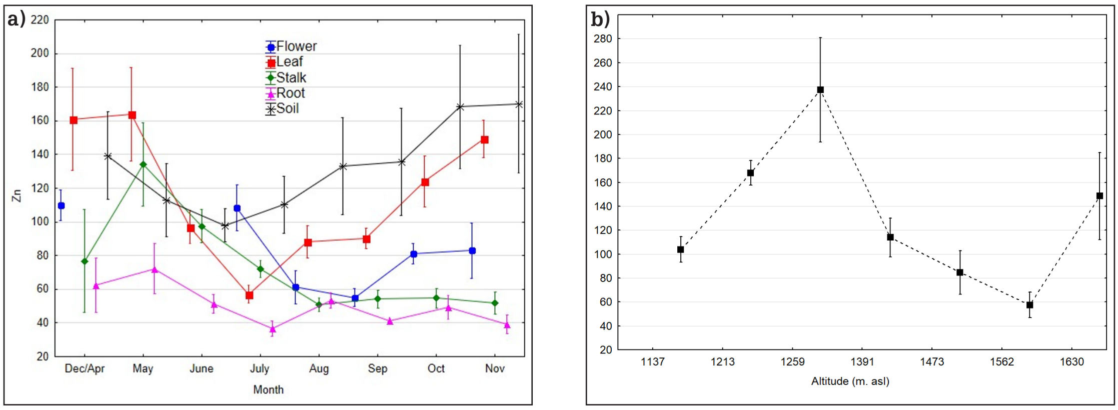

Fig. 13. Mean (± SE) levels of zinc in *Gentiana asclepiadea* and in the soil a) in dependence on seasonality (K-W test: flower = 17.64, p < 0.01; leaf = 38.52, p < 0.01; stalk = 30.80, p < 0.01; root = 11.75, p = 0.12; soil = 3.13, p = 0.87) and concentration of zinc in the soil in dependence on altitude (b).

		Plant organ					
		Flower	**Leaf**	**Root**	**Soil**	**Stem**	**Sum**
Ti	Measured	4	29	40	92	6	171
	Unmeas.	49	62	51		84	246
	Sum	53	91	91	92	90	417
Mo	Measured	9	20	1	74	3	107
	Unmeas.	44	71	90	18	87	310
	Sum	53	91	91	92	90	417
Ni	Measured	16	11	1	8		36
	Unmeas.	37	80	90	84	90	381
	Sum	53	91	91	92	90	417
Se	Measured				7		7
	Unmeas.	53	91	91	85	90	410
	Sum	53	91	91	92	90	417
Cd	Measured		1	10	2	4	17
	Unmeas.	53	90	81	90	86	400
	Sum	53	91	91	92	90	417
Sn	Measured	5	18	37	17	30	107
	Unmeas.	48	73	54	75	60	310
	Sum	53	91	91	92	90	417
Sb	Measured	23	40	78	26	61	228
	Unmeas.	30	51	13	66	29	189
	Sum	53	91	91	92	90	417
As	Measured				54		54
	Unmeas.	53	91	91	38	90	363
	Sum	53	91	91	92	90	417
Zr	Measured		2	22	92	1	117
	Unmeas.	53	89	69		89	300
	Sum	53	91	91	92	90	417

Table 1. Contingency table showing the number of measured and unmeasured values of Ti, Mo, Ni, Se, Cd, Sn, Sb, As and Zr in the *Gentiana asclepiadea* and in the soil. Chi-square test was used to determine the significance and the difference between the values Ti ($X^2 = 204.22$; $p < 0.01$), Mo ($X^2 = 199.74$; $p < 0.01$), Ni ($X^2 = 47.65$; $p < 0.01$), Se ($X^2 = 25.15$; $p < 0.01$), Cd ($X^2 = 16.32$; $p < 0.01$), Sn ($X^2 = 24.96$; $p < 0.01$), Sb ($X^2 = 24.96$; $p < 0.01$), As ($X^2 = 219.14$; $p < 0.01$), Zr ($X^2 = 319.77$; $p < 0.01$).

amount of nickel measured is higher in the flower and in the leaf, while it is almost absent in the root and in the stalk. In the soil there is a smaller amount. Most nickel is found in the flower (Table 1).

Selenium

Significant differences are found between the different organs and in the soil. Selenium was only measured soil samples, and only in 7 samples out of 92 (Table 1).

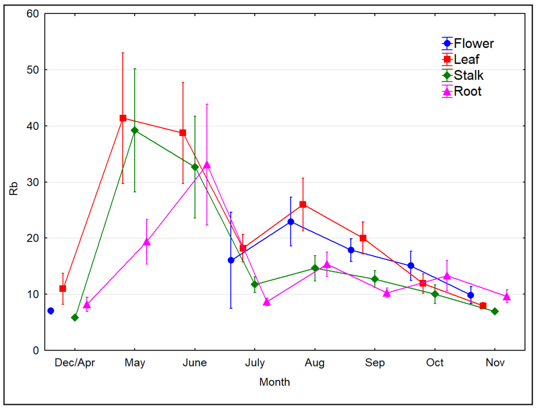

Fig. 14. Mean (± SE) levels of rubidium in *Gentiana asclepiadea* in dependence on seasonality (K-W test: flower = 11.25, $p = 0.05$; leaf = 29.39, $p < 0.01$; stalk = 31.93, $p < 0.01$; root = 23.85, $p < 0.01$; soil = 6.67, $p = 0.46$).

Concentration levels of potential pollutants

Rubidium

Rubidium concentrations vary considerably from month to month in the different organs of the plant. The amount of rubidium rises sharply after winter snowmelt, and continues to fall throughout the year until the following winter. There is a slight increase in values in August (Fig. 14).

In plant organs, values are similar in character, increasing from 1259 - 1391 m a.s.l., with values decreasing thereafter and increasing slightly towards the end of the route. The highest concentration is found in the leaves (Fig. 15a). Rubidium levels vary significantly in both plant organs and soil. In soil, the highest values are found at altitudes around 1213 and 1391 m a.s.l. (Fig. 15b).

Strontium

Mean (± SE) levels of strontium in *G. asclepiadea* and in the soil depend on seasonality (K-W test: f = 6.69; $p = 0.24$; l = 4.73; $p = 0.69$; s = 6.85; $p = 0.44$; r = 10.72; $p = 0.15$; so = 2.31; $p = 0.94$). The values are not significant. The difference between strontium concentrations at each site is found in the stalk, the root, and the soil. In the stalk, values increase significantly at 1213 m a.s.l.; in the root, values increase at higher altitudes (Fig. 16a). Strontium values in soil

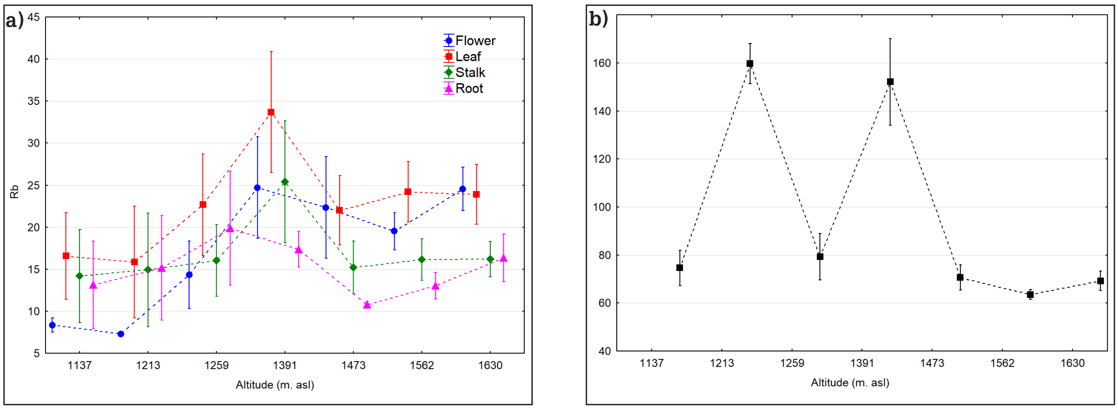

Fig. 15. Mean (± SE) levels of rubidium in *Gentiana asclepiadea* a) and in the soil separately b) in dependence on altitude (K-W test: flower = 33.09, $p < 0.01$; leaf = 21.03, $p < 0.01$; stalk = 19.81, $p < 0.01$; root = 26.91, $p < 0.01$; soil = 32.99, $p < 0.01$).

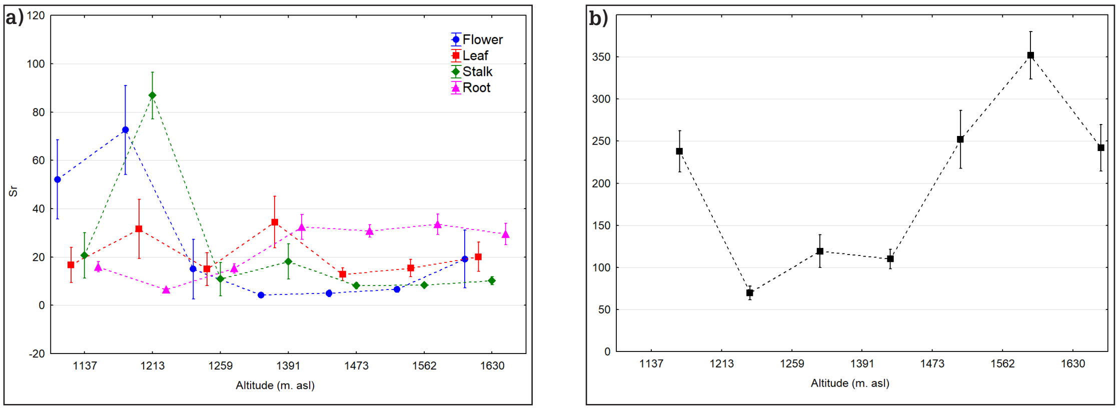

Fig. 16. Mean (± SE) levels of strontium in *Gentiana asclepiadea* a) and in the soil separately b) in dependence on altitude (K-W test: flower = 8.06, $p = 0.23$; leaf = 11.6, $p = 0.07$; stalk = 30.84, $p < 0.01$; root = 54.01, $p < 0.01$; soil = 53.41, $p < 0.01$).

are highest at high altitudes. At 1137 m, at the beginning of the route, the strontium concentration is also relatively high. Values decrease further, then increase again from 1259 m a.s.l. (Fig. 16b).

Barium

Significant differences are found in the leaves and in the root over the months. Concentrations of barium in the leaves increase in the autumn and winter months, then increase in the root after winter snowmelt (Fig. 17a). There are significant differences in the soil with respect to altitude, with the highest values at 1213 and 1391 m a.s.l. The high values are likely to be influenced by the change in geological bedrock (Fig. 17b).

Lead

Due to an insufficient number of flower data, the K-W test was not performed. In the leaf and stem, the values are nonsignificant, p > 0.05. In the root, the values vary significantly, and a sharp rise is seen in May and June (Fig. 18). Due to seasonality, Pb values in soil do not differ.

Due to an insufficient number of flower data, the K-W test was not performed. The amount of lead in the plant is likely affected by the amount of lead in the soil. In the root, the values vary. They decrease slightly at the beginning of the trail and then increase significantly at mid-elevations at 1213-1391 m a.s.l. (Fig. 19a). The amount of lead in the soil is highly significant with the highest values reached at

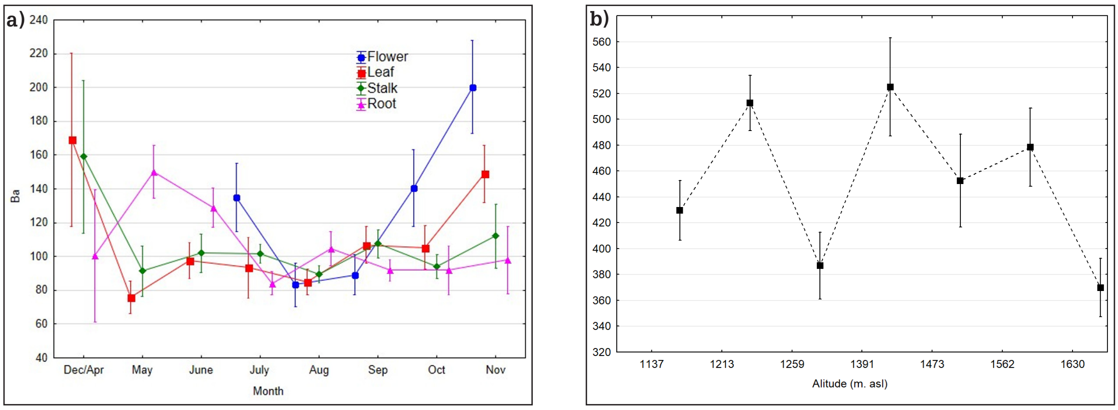

Fig. 17. Mean (± SE) levels of barium in *Gentiana asclepiadea* a) in dependence on seasonality (K-W test: leaf = 17.77; p = 0.01; stalk = 6.42; p = 0.49; root = 21.07; p < 0.01; soil = 1.5; p = 0.98). Due to insufficient number of flower data, the K-W test was not performed. Mean levels of barium in the soil b) in dependence on altitude (K-W test: flower = 4.99; p = 0.54; leaf = 3.16; p = 0.79; stalk = 4.72; p = 0.58; root = 10.14; p = 0.12; soil = 26.48; p < 0.01).

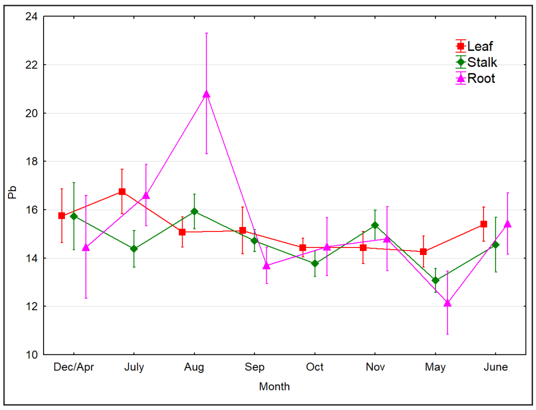

Fig. 18. Mean (± SE) levels of lead in *Gentiana asclepiadea* and in the soil separately in dependence on seasonality (K-W test: leaf = 7.79; p = 0.35; stalk = 13.73; p = 0.056; root = 19.63; p = 0.01; soil = 5.23; p = 0.63).

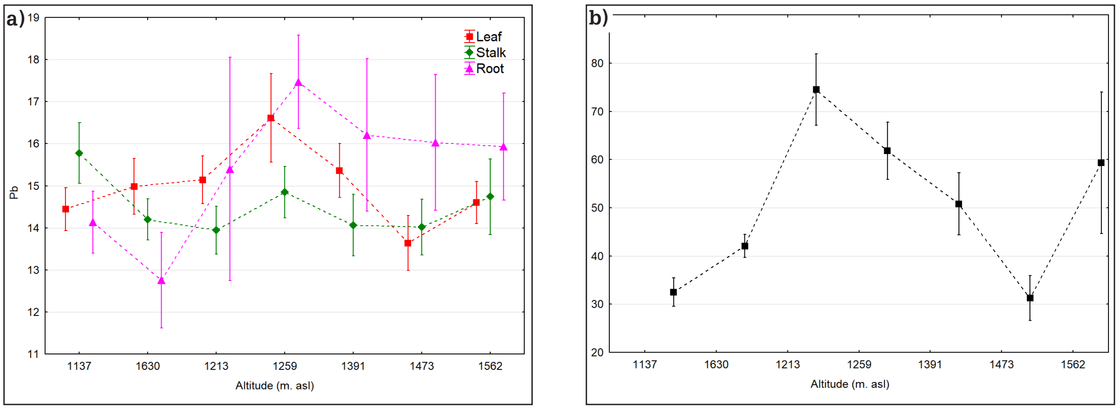

Fig. 19. Mean (± SE) levels of lead in *Gentiana asclepiadea* a) and in the soil separately b) in dependence on altitude (K-W tests: leaf = 6.47; p = 0.37; stalk = 5.78; p = 0.45; root = 12.94; p = 0.04; soil = 35.42; p < 0.01).

1259-1391 m a.s.l. At this altitude, the route moves significantly away from the stream, which changes the composition of the bedrock, affecting the concentration of lead in the soil (Fig. 19b).

Mercury

Significant differences in the organs of the plant are found in the leaves, stalk, and root in different months. Mercury concentration is the highest in the leaves. While there is a low concentration evident during May and June, Hg levels rise sharply during the autumn months. The amount of Hg in the stalk rises in the 6th and 7th months and decreases towards autumn. Based on test results, mercury is taken up into the plant through the root. Hg enters the root in spring when the snow melts in the 4th and 5th months. From there, it is absorbed and moves into the stem, where values increase in the 7th and 8th months, and from the stem it moves into the leaves, where we see the greatest increases in the 8th, 9th, 10th, and 11th months. In leaves, Hg accumulates throughout the year, resulting in significantly higher values compared to other plant organs. Towards the end of the year, it reaches the highest concentrations. Thus, autumn leaves are significantly contaminated with Hg in contrast to young leaves in spring (Fig. 20a). The amount of Hg in the soil varies significantly with altitude. It reaches the highest amounts at 1259-1391 m a.s.l. The type of subsoil is likely to have a significant effect on the Hg concentration in soil. At lower and higher elevations, the route follows a mountain stream. The bedrock is composed of alluvial sediments and is strongly influenced by fluvial activity. The middle zone moves away from the stream for some time, so the bedrock changes to mineral bedrock. Total Hg in the soil consequently affects the total amount of Hg in the plant (Fig. 20b).

Cadmium

The greatest number of measured values occurred in the root, less in the stalk, a very few values in the soil and in the leaf. Cadmium was not measured in the flower (Table 1).

Tin

Tin levels are measured in all parts of the plant and in the soil. The values differ significantly. The highest number of tin values occur in the root, followed by the stalk. Tin was found in a similar number of leaf and soil samples, with the lowest number of samples found in the flower (Table 1).

Antimony

Antimony behaves similarly to tin. The values are significant in both plants and soil. The highest amounts are found in the root and in the stalk. However, significant amounts are also found in the leaf. Antimony appears the least frequently in the flower and has approximately the same occurrence in soil. Overall, concentrations of antimony were relatively high (Table 1).

Arsenic

The differences in the plant and in the soil are significant. Arsenic is not present in the plant organs, but in the soil it is present in more than half of the samples (Table 1).

Zircon

There are significant differences in both the plant and the soil. In the soil, zircon was measured in all samples. Relatively high amounts are found in the root and few in the leaf and stalk (Table 1).

Discussion

Biogens in Gentiana asclepiadea

Sulphur

The amount of sulphur in plant organs varies considerably from month to month, with sulphur concentrations decreasing from the beginning of the growing season and increasing more markedly in the autumn and winter months. Similar

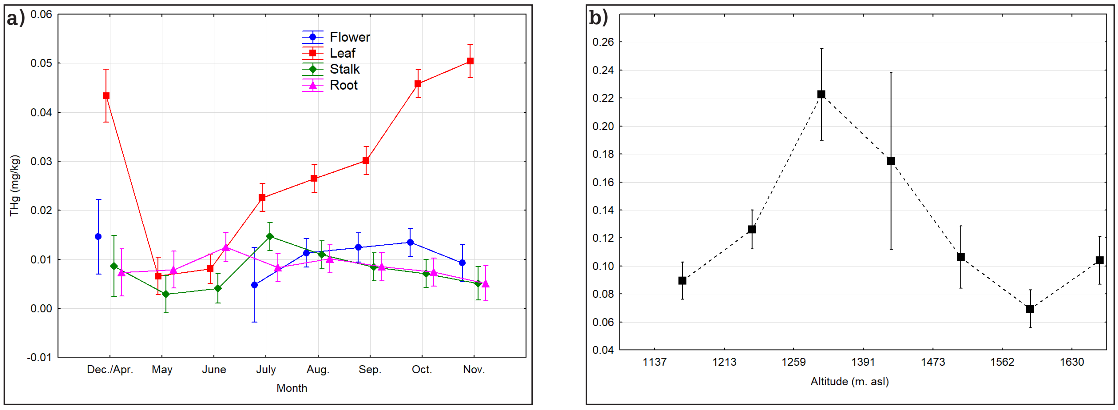

Fig. 20. Mean (± SE) levels of mercury in *Gentiana asclepiadea* a) in dependence on seasonality (K-W test: flower = 9.6; p = 0.08; leaf = 52.5; $p < 0.01$; stalk = 23.6; $p < 0.01$; root = 14.3; p = 0.04; soil = 11.82; p = 0.11) and mean (± SE) levels of mercury in the soil separately b) in dependence on altitude (K-W test: flower = 11.23; p = 0.08; leaf = 3.3; p = 0.77; stalk = 4.05; p = 0.7; root = 6.14; p = 0.4; soil = 19.6; $p < 0.01$).

results were measured in the study by Grešíková and Janiga (2017). The highest S concentrations in *G. asclepiadea* are measured in leaves and in flowers (Fig. 1a), where the effect of wet atmospheric deposition is evident. In autumn and winter months, the value of daily precipitation is generally higher than during the year. In areas with increased precipitation (mountainous areas), there is a high probability of sulphur being washed out by precipitation (or snow) (Brown 1982). S accumulates in the root under natural conditions, but when plants are exposed to atmospheric SO_2 uptake, sulphur accumulates significantly in plant shoots (De Kok 1990) and less in roots.

The occurrence of sulphur in the plant, based on the results, is not dependent on altitude. This claim is supported by the study of Grešíková and Janiga (2017). In soil, we observe a directly proportional dependence with altitude. As the altitude increases, the sulphur concentration also increases. Organic sulphur, readily available to plants, is bound to organic material in the soil (Brown 1982), which reaches its highest amount in soils of high-altitude meadows and coniferous forests. As elevation increases, we also observe an increase in the amount of organic material contained in the soil, which influences increasing sulfur concentrations in the soil (Bu *et al.* 2012). At 1259 m. a.s.l., there is a more pronounced increase in S concentration (Fig. 1b), where we see the effect of the increased amount of organic matter in the forest ecosystem.

Chlorine

There are no significant changes in soil Cl concentrations over the seasons. In plant organs of *G. asclepiadea*, chlorine concentrations are higher in winter months and slightly elevated in summer months (Fig. 2a). Higher Cl values in winter were also demonstrated in another study (Grešíková and Janiga 2017). Precipitation is one of the main factors influencing chlorine uptake by the plant. This statement is supported by the accumulation of Cl in winter and summer months when precipitation is more frequent (in the form of snow in winter) (White and Broadley 2001). The highest chlorine concentrations were observed in leaves and flowers. Chlorine is mainly taken up by the plant through the roots, but shows a high mobility within the plant, which is reflected by its increased concentration in the higher parts of the plant (White and Broadley 2001).

Chlorine concentrations behave similarly to sulphur concentrations. Cl concentrations in plant organs, unlike in soil, are not dependent on altitude, but on different locations of occurrence. As altitude increases, Cl concentrations also increase (Fig. 2b). Under natural conditions, chlorine enters the soil through rainwater (White and Broadley 2001). In mountainous areas, as altitude increases, the amount of precipitation also increases, which can be attributed to the increasing trend of chlorine concentrations in soil.

Potassium

In their study, Britto and Kronzucker (2006) report a similarity in potassium and chlorine intake. Our results support the claim (Fig. 3a). Over the course of the year, potassium accumulated most in the leaves and in the flowers. Concentrations in leaves increased in winter and summer months, and in stem and root mainly in winter months. Plants take up potassium directly from the soil solution (Asher and Ozanne 1967). In the winter and summer months, potassium uptake is affected by increased soil moisture as reported by Mackay and Barber (1985), increasing soil moisture influences increased potassium uptake by the plant. Through the respiratory process of plants, K is more bound to the green parts of the plant (Krendželák *et al.* 2018), which explains its higher content in leaves and flowers.

In the soil, the potassium concentration is significant at individual sites (Fig. 3b). An elevated amount of K occurs at 1213 m a.s.l. and 1391 m a.s.l. Soil potassium concentration is significantly affected by soil pH. Geologically, we observed similar geological compositions at these sampling sites, with deltaic-proluvial sediments occurring at both sites. At the second site, bedrock is also associated with glacigenic sediments, which occur in proximity to the fourth site. Acidic pH releases potassium from complexes and thus increases its bioavailability to plants. There is a tendency for higher K levels in these soils (Mengel and Kirkby 1982; Brady *et al.* 2010). At elevations of 1213 m a.s.l., the trail rises more steeply above the Javorinka River and enters the forest. Acidic forest soils greatly increase the mobility of elements in the soil, making it more accessible to plants (Zöttl 1985). Additionally, the proportion of organic matter in forest ecosystems provides a prerequisite for a higher K content in these areas (Hinsinger 2005).

Calcium

In plants, Ca moves unidirectionally from the roots upwards to the leaves, where it accumulates. Calcium taken up by leaves, by external deposition, is immobile and stored in leaf tissues (Hanger 1979). Accumulation in leaves is also observed in *G. asclepiadea*, where Ca concentrations gradually increase from the beginning of the growing season until the end of autumn (Fig. 4a). Ca is taken up by the plant from the soil via the root, where increased concentrations occur after winter. Calcium, despite being a macronutrient, is one of the most immobile elements in the plant. It is stored in the root, from where it is slowly translocated to the leaves, but its distribution in other parts of the plant is very poor (Hanger 1979).

At individual sites, Ca concentrations vary significantly in stem, root and soil (Fig. 4b). Ca decreased in all plant parts and in the soil. At the first (1137 m a.s.l.) and third (1259 m a.s.l.) sampling sites, elevated soil Ca concentrations were recorded, which may be due to the similar geological composition at these sites (Fig. 2). Ca is taken up by the plant from the soil. The Ca content of the soil is related to the parent rock, as it enters the soil naturally, for example, by weathering feldspar, apatite, limestone, and gypsum, or as a component of many compounds. Limestones are not typically found in the Javorová Valley area, so much of the Ca from the soil is likely to come from compounds in the soil (Barker and Pilbeam 2015). Ca deficiency for plants can be caused by low soil pH, moisture, cold, and low transpiration. As altitude increases, temperature decreases and rainfall increases, and the amount of organic matter contained in the soil, which has lower pH values, also increases, resulting in lower Ca concentrations at higher altitudes (Demarty *et al.* 1984; Bu 2012).

Chromium

Chromium concentrations vary from month to month in all *G. asclepiadea* organs and in the soil. They reach their highest values in the winter months and during early summer (Fig. 5a, b). The plant receives Cr from the soil through the roots. The translocation activity of Cr varies markedly for specific plant species. Cr is a very immobile element in both the soil and the plant. Translocation activity like that of Pb has been measured in many plants (Chatterjee and Chatterjee 2000; Zayed and Terry 2003; Małkowski *et al.* 2019). If we proceed from the hypothesis of a low Cr translocation factor in *G. asclepiadea*, the high amounts of Cr in leaves and flowers could be due to atmospheric deposition. Cr is one of the elements that accounts for a high proportion of the emissions. These are subsequently leached out by precipitation and accumulated in leaf tissues (Isinkaralar *et al.* 2022), resulting in elevated amounts of Cr in winter and summer months when precipitation is more frequent (also in the form of snow).

With increasing altitude, the amount of Cr concentration in the plant does not differ significantly. Nevertheless, the highest values are again measured in the leaves and in the flowers, indicating the influence of atmospheric deposition. The amount of Cr in the plant is influenced by the Cr content in the soil, where the values vary significantly with different altitudes. At middle altitudes, the soil Cr content increases (Fig. 6a). In soil, the highest Cr concentrations occur naturally mainly on volcanic rocks (Utermann *et al.* 2006), which also form the geological bedrock of the Tatra Mountains. Acidification can cause Cr solubility, leading to elevated Cr concentrations in the mid-elevation, forest zone (Seigneur and Constantinou 1995).

Manganese

The amount of manganese varies significantly in the flower (Fig. 7) and the concentration increases in the autumn and spring months. Mn concentrations are highest in the leaves. The Mn cycle resembles the Ca cycle in that Mn also accumulates to the higher parts of the plant, mainly to the leaves (Krendželák *et al.* 2018). Mn tends to accumulate in leaves and higher parts of the plant, thus its rapidly increasing tendency throughout the growing season until the winter months (Marschner 1995).

Manganese varies considerably in all parts of the plant and in the soil due to altitude (Fig. 8a, b). The highest values are measured in the soil and in the leaves. In all parts of the plant and in the soil, the highest manganese values are found at an altitude of around 1259-1391 m a.s.l. At this altitude, coniferous forest soils are typically acidic. The low pH of the soil increases the amount of soluble Mn^{2+}, which is readily available to plants. Increased soil moisture accelerates oxidation and thus contributes significantly to increasing bioavailable Mn in the soil. Mn is very rapidly translocated from the roots to the leaves, where it is subsequently accumulated (Marschner 1995). In alkaline environments, availability to the plant may be affected by increased organic material or water, hence, Mn toxicity is possible even in soils with higher pH (Hue 1998).

Iron

A significant amount of iron during the season is found in the root (Fig. 9a). The concentration of iron decreases in the autumn and winter months and increases significantly in spring and late summer. In these months we can consider the influence of snowmelt and increased precipitation in summer. The influence of precipitation on Fe concentration has been found for moderately rainy sites (930-1380 mm/year), when significant Fe enrichment of the soil occurs (Teutsch *et al.* 1999). Although Fe acts as an essential element in the plant, it does not reach such high concentrations in the leaves. Brown (1978) states that Fe deficiency in the plant can often be due to the plant's inability to move it from the root to the higher parts of the plant, which often leads to its accumulation in the root.

The highest iron concentrations are found in the soil, where they vary considerably from site to site (Fig. 10b). Fe is one of the essential components of the rock environment, and is widespread in the earth's crust, yet its availability to plants is very low. Fe in soil is very poorly soluble, which can cause oxidative stress to plants. Plants take it up from the soil in the form of Fe^{2+}, which they can absorb through their roots (Orolínová 2009). High soil Fe values are measured at sites 2 and 4, where there is potential for higher soil acidity due to the forest ecosystem. These sampling sites are also characterized by similar geological composition, which influences their similar physical and chemical properties. Fe solubility is good in very acidic soils (with $pH < 4.0$). Fe is very difficult for plants to access in calcareous soil (Latimer 1952).

Copper

Copper varies significantly from month to month in the flower (Fig. 11). Copper values increase in the autumn months. In the winter months the line is broken due to insufficient data (climatic conditions). Concentrations in the flower may be due to atmospheric deposition. The effect of precipitation on soil copper concentrations is verified (Teutsch *et al.* 1999).

In the flower, copper values vary in contrast to the rest of the plant (Fig. 12a). It reaches its highest values at the beginning of the route and at an altitude of about 1259 m a.s.l. Copper varies considerably from place to place in the soil, with the amount of copper increasing with increasing altitude and decreasing sharply at the highest point (Fig. 12b). Barančíková (1998) states that Cu belongs to the group of metals whose highest mobility in soil is at pH lower than 4.5 and higher than 7. It is also characterized by a high affinity for soil organic matter. Organic matter also tends to increase with increasing altitude, which may influence the increasing tendency of Cu in soil as a function of altitude (Teutsch *et al.* 1999; Ciriaková 2009).

Zinc

Unlike Pb and Cd, Zn is an essential element for the plant. Its translocation activity from root to shoot is much higher (Małkowski *et al.* 2019). Zn is taken up by the plant through the root and subsequently translocated to higher parts (Marschner 1995). It is finally accumulated in the shoots and in the leaves, where the highest concentrations are measured in *G. asclepiadea* (Fig. 13a). An extremely low Zn translocation factor was measured in a study by Barrameda-Medina *et al.* (2014). However, the plants were grown in Hoagland's solution, which contained high concentrations of phosphate ions. He hypothesized that such a high concentration resulted in a reduction of Zn translocation from the root to the shoots.

With increasing altitude, the Zn concentration in the soil varied significantly (Fig. 13b). High Zn values are measured at around 1259 m and then decrease significantly. Kubica *et al.* (2007) reported in their study that the Zn concentration in soil increases directly in proportion to increasing altitude and soil organic matter content (Heinrichs and Mayer 1980). A study by Makovníková *et al.* (2006) also reported that Zn is most available in acidic, mineral-poor soils.

Titanium

The amount of Ti in the measured plant and soil samples was not sufficient for statistical analysis, so the evaluation of the data is done by means of a contig table and X^2 test. The differences in the amount of titanium in the different organs of the plant and in the soil are considerable (Table 1). Titanium is relatively abundant in soil, and in our measurements it was measured in all soil samples (92). The concentration of Ti in soil is strongly influenced by soil pH. As pH decreases, Ti concentration increases (Dumon and Ernst 1988). Titanium preferentially accumulates in the roots and only small amounts move into the leaves. With a high concentration of Ti in the soil, it accumulates relatively rapidly in the roots, from where it moves slowly to the shoots (Nautsch-Laufer 1974), consistent with results measured in *G. asclepiadea*. In the root, Ti was measured in almost half of the samples (40 out of 91), with a relatively high number of measurements in the leaf (29 out of 91) and very few in the flower and stem.

Molybdenum

The presence of Mo in the samples was evaluated using the contig table and the X^2 test as there was insufficient concentration of the element in the samples. The difference between the amount of molybdenum in the plant and in the soil varies considerably (Table 1). In soil, the concentration of Mo is generally quite abundant, as shown in soil samples from Javorová Valley, where sufficient Mo concentration was measured in 74 samples (out of 92). However, it is often bound in insoluble compounds, which severely limits its availability to plants. Sorption of Mo by plants is also severely limited by low pH (Zimmer and Mendel 1999). Mo is a highly mobile compound that is taken up by the plant from the soil via the roots and is subsequently transported very rapidly to the leaves (Kannan and Ramani 1978). Our research confirms this statement, as most Mo was measured in leaves (in 20 samples out of 91), while it was only measured in one root sample (out of 92).

Nickel

Ni in *G. asclepiadea* was assessed for lack of concentration in the samples using the contig table and X^2 test. Nickel is more mobile than most essential elements. Its translocation factor is significantly higher than that of Pb, Cd, or Cr (Broadley *et al.* 2012). In *G. asclepiadea* there is a similar tendency, with the highest amount of Ni accumulated in the flower and in the leaf (Table 1). In the root, the number of measured values was significantly lower. No Ni values were measured in the stem. The stem likely functions as a kind of transition site in the translocation of elements from the root to the shoots, due to which Ni does not accumulate in it. A significant effect of atmospheric deposition on Ni deposition in the higher parts of plants has also been found, which may account for the increased accumulations in flowers and leaves (Heinrichs and Mayer 1980).

Selenium

Due to the lack of Se concentration in the samples, the contig table and X^2 test were used to evaluate the data. Selenium was measured in *G. asclepiadea* only in 7 soil samples out of 92 (Table 1). There is an apparent lack of bioavailable selenium in the soil for plants. It is toxic to most plants, but there are plants known to respond as accumulators in the event of Se toxicity; these include some plants in the legume family (Wilber 1980). In soil, Se is mostly found in insoluble complexes that plants are unable to absorb, so it does not pose a danger to them (Harborne 1993).

Heavy metals in Gentiana asclepiadea

Rubidium

Rubidium concentrations vary considerably from month to month in different organs of the plant. The amount of rubidium rises sharply following winter snowmelt, and continues to fall throughout the year until the following winter. In August there is a slight increase in values (Fig. 14). We can assume some influence of atmospheric precipitation on the Rb concentration in the plant during these months, but this influence has not yet been sufficiently investigated.

Rb has a similar chemical composition to K, so it is toxic because it is often confused by plants, although it cannot replace the function of K (Brenchley 1934). We see similar behavior of Rb and K in soil as a function of elevation (Fig. 15b). The highest Rb concentrations are measured at the second and fourth sampling sites, which have similar geological composition, we observed the same behavior for K (Fig. 3b). Rb in soil is relatively widespread, but its bioavailability to plants is closely related to the soluble K content of the soil. The uptake of Rb by plants increases with decreasing K uptake. A probable influence of an antagonistic relationship between K and Rb uptake is likely in our case. The K content of the plant ranges from 1000 - 10000 mg/kg, while the Rb content is in the range of up to 40 mg/kg. Soil pH also has a significant effect. In the case of significantly low pH, K uptake decreases and Rb uptake increases (Tyler 1976; Drobner and Tyler 1998). In *G. asclepiadea*, Rb accumulates most in the plant at mid-elevations, where we can consider a more pronounced effect of low pH in the forest zone (Fig. 15a). We observe a similar behaviour in soil (Fig. 15b).

Strontium

The values of strontium concentration in *G. asclepiadea* organs and in soil are not significantly different seasonally. The difference between strontium concentrations at different sites is in the stem, root, and soil. In the stem, values increase significantly at 1213 m a.s.l. and decrease further; in the root, values increase at higher altitudes (Fig. 16a). At 1137 m a.s.l., at the beginning of the route, the strontium concentration is also relatively high; values decrease further and increase again from 1259 m a.s.l., reaching the highest values at about 1600 m a.s.l. (Fig. 16b). According to the research of Rediske and Selders (1953), the highest bioavailability of Sr is at pH 6,. We can consider a similar soil pH at the first site when Sr concentrations in the plant were highest. As acidity increases, the solubility of Sr in the soil is higher and thus its availability to plants is higher, which is likely to account for the higher Sr concentrations at higher elevations in the topsoil and in the root (Rediske and Selders 1953). According to Salt *et al.* (1995), Sr accumulates most in the root of plants.

Barium

Significant differences in leaves and roots were found over the months. Barium concentrations in leaves increase in autumn and winter months, and in roots after winter snowmelt (Fig. 17a). The presence of Ba has been detected in emissions produced by transport (Monaci and Bargagli 1997); thus, we can consider the influence of atmospheric deposition in the form of snow. In soil, the highest values are measured at 1213 and 1391 m a.s.l. (Fig. 17b), where the influence of pH on the forest ecosystem is possible. The second (1213 m a.s.l.) and fourth (1391 m.) sampling sites are characterized by similar geologic compositions; the geologic substrate is deluvial-proluvial sediments with glacigenic sediments at the second site and in proximity at the fourth site. Geological composition has a significant influence on soil composition and properties. In general, Ba is poorly mobile in soils, but its mobility and bioavailability increases in acidic soils (Madejón 2013). Bowen (1966) reported an average soil Ba content of 500 mg/kg; the study site in Javorová Valley has the highest soil Ba content at site 4 at approximately 520 mg/kg. Thus, we do not anticipate Ba contamination in this area.

Lead

Over 95% of plant species accumulate Pb in the root, but only a small amount of the Pb concentration is subsequently translocated to the shoots. No clear difference in Pb translocation activity has been demonstrated between monocotyledonous and dicotyledonous plants (Małkowski *et al.* 2019).

Significant values of Pb concentration were measured in the root of the plant dependent on seasonality (Fig. 18). Pb concentrations in the root are similar compared to other plant parts. According to the research of Pourrut *et al.* (2011), Pb accumulates most in the root, which is due to the low translocation activity of Pb from the root to the shoots. In June, a significantly increased Pb concentration is recorded in the root, which is believed to be due to atmospheric precipitation in these months. Precipitation also has a significant effect on the high Pb concentrations measured in the leaves of Gentiana asclepidea. With increasing precipitation, the amount of Pb in the ecosystem also increases significantly (Teutsch *et al.* 1999). Beneš (1993) reported that up to 80% of accumulated Pb comes from atmospheric deposition. It enters the atmosphere mainly from industrial activities, including from emissions (Svičeková and Havránek 1993; Krendželák 2018).

The concentration of lead in the soil reaches its highest values at 1259-1391 m. a.s.l. (Fig. 19b). At this altitude, the route moves significantly away from the stream, changing the composition of the bedrock, which is likely to influence the lead concentration in the soil. According to Alloway (2013), the mobility of Pb in soil is low, hence its bioavailability to plants. The main factors affecting Pb uptake by plant roots and its translocation in the plant, according to a study by Makovníková *et al.* (2006), are low pH and low phosphorus content, with which Pb forms insoluble compounds. Low pH results in increased mobility of metals in soil (Widyatmoko 1994). Pb is one of the elements that are also characterized by high affinity for soil organic matter (Ciriaková 2009). In the middle altitudes, the route passes through a forest zone and samples were collected from forest areas. Forest ecosystems are often a reservoir of heavy metals, which they capture from the air. After these organisms die, Pb re-enters the soil. Acidic forest soils with low pH increase the mobility of elements in the soil, which further increases the availability of metals to plants (Zöttl 1985).

Mercury

In leaves, depending on seasonality, mercury concentration is highest. From the beginning of the growing season, concentrations rise sharply and reach their highest values in autumn. A significant accumulation of Hg in leaves is evident throughout the year. Hg enters the plant through the root in spring when the snow melts. Some studies report Hg accumulation in plant roots, which form a kind of absorption barrier for transport to higher parts of the plant (Lindberg *et al.* 1979). Only a very small amount of Hg taken up by the plant through the root is transported into the shoots. The extremely high concentrations in leaves at the end of the growing season are the result of the influence of atmospheric precipitation, which is very heavy in the summer months (Steinnes 2013). Precipitation has the most important influence on Hg accumulation in leaves (Lindberg *et al.* 1979). Thus, autumn leaves are significantly contaminated with Hg in contrast to young leaves in spring (Fig. 20a).

The amount of Hg in the soil varies considerably with altitude. It reaches its highest values at 1259-1391 m a.s.l. (Fig. 20b). Atmospheric deposition of Hg is the largest source of contamination of surface soils, so we can consider the increased influence of precipitation in the rain zone. Concentration is also tied to organic material in acidic soils (Varshal *et al.* 1999; Steinnes 2013), which occurred in elevated amounts in the forest ecosystem at these elevations. According to Barančíková (1998), Hg mobility in soil is not dependent on soil reaction.

Cadmium

The contig table and X^2 test were used to analyze Cd in *G. asclepiadea* and in soil due to insufficient concentration. Cd is classified as an element with higher mobility in soil (Alloway 2013). Yong *et al.* (1992) reported that cadmium mobility, and thus its bioavailability, is highest in acidic soils with pH 4.5 to 5.5. Conversely, cadmium is less mobile in alkaline soils.

The amount of Cd concentration in *G. asclepiadea* was measured in the root, where it was measured the most frequently. Less was found in the stem, with only small amounts found in the soil and in the leaves (Table 1). According to research by Pourrut *et al.* 2011, Cd accumulates in the root and is poorly mobile in the plant, resulting in negligible contamination of the higher parts of the plant. According to a study by Kabata-Pendias and Pendias (1992), Cd is mobile in plants, but at high concentrations it can accumulate in the root.

The presence of chlorine in the soil increases the uptake of cadmium by plants due to the formation of Cd chlorocomplexes, which are very soluble. On the other hand, zinc inhibits cadmium uptake by plants (Makovníková *et al.* 2006). Increased Cd concentration leads to a decrease in Zn, Mn, and Cu concentrations in barley and *Salix vinimalis* plants (Vassilev *et al.* 2002, 2005; Cuypers *et al.* 2013).

Tin

Due to insufficient tin concentrations in the samples, the contingency table and X^2 test were used for the analysis. The values of tin concentrations in both plant and soil differed significantly (Table 1). Tin is taken up by plants from the soil via the root. It is best absorbed in acid soils with a larger mineral layer (Huang and Matzner 2004). Most Sn is measured in the root, where it accumulates in most plants (Alloway 2013). Significant amounts of tin are also measured in the stem and less in the leaves and soil. Through atmospheric deposition, organic forms of tin can reach the soil or be trapped on the aerial parts of the plant (Alloway 2013), which may be a significant factor influencing the relatively high levels of tin in *G. asclepiadea* in the stem and in the leaves.

Antimony

For the analysis of antimony in *G. asclepiadea* and in soil, a contingency table and X^2 test were used due to insufficient concentrations in the samples. Antimony has low mobility in soil and bioavailability to plants in most forms, thus, it does not pose a high toxicity hazard to plants (Clemente 2013). However, in the case of *G. asclepiadea*, relatively high amounts were measured throughout the plant and in the soil (Table 1) Most Sb was measured in the root and in the stem. Higher amounts were measured in the leaves, with less in the soil and in the flower. The bioavailability of Sb to plants may increase with increasing soil acidity (Nakamaru *et al.* 2006), which is typical for our site in many locations. Studies also show that if Sb is present in the soil in a more mobile form, it can be accumulated by plants relatively easily (Tschan *et al.* 2009). It is taken up from the soil by the root, where it is deposited in larger amounts. This is also observed in *G. asclepiadea*. Further, a smaller amount is transported into the leaves. Atmospheric deposition also forms an important pathway for Sb transfer to the plant surface (Tschan *et al.* 2009), which is likely to be the reason for the high amount of Sb in stems, leaves and flowers.

Arsenic

The contigency table and X^2 test were used for As analysis due to insufficient concentration in the samples. The concentration of As in *G. asclepiadea* was not measured in the plants. On the contrary, the amount of As was higher in the soil; measured in more than half of the soil samples (Table 1). According to Alloway (2013), As shows significantly low mobility in soil, likely resulting in the absence of As in the plant organs, even as significant amounts were measured in soil.

Zircon

Zr content was evaluated in both plant parts of *G. asclepiadea* and soil using a contingency table and X^2 test, due to insufficient concentration in the samples. There are significant differences in concentration in both plant parts and soil (Table 1). In soil, Zr was measured in all samples. Relatively high amounts are found in the root. The measured data confirm the relatively low mobility of Zr in soil and its poor bioavailability to plants (Kabata-Pendias 1993). The translocation activity from the root to the shoots is also very low, as can be seen in the very low Zr content in the higher parts of the plant (Maria and Cogliatti 1988).

References

Alloway, B.J. 2013: Heavy metals in soils: trace metals and metalloids in soils and their bioavailability. Springer, Dordrecht.

Asati, A., Pichhode, M. and Nikhil, K. 2016: Effect of heavy metals on plants: an overview. *IJAIEM*, **5**: 56–66.

Asher, C.J. and Ozanne, P.G. 1963: Growth and potassium content of plants in solution cultures maintained at constant potassium concentrations. *Soil Sci*, **103**: 155–161.

Barančíková, G. 1998: Návrh účelovej kategorizácie pôd SR z hľadiska citlivosti k znečisteniu ťažkými kovmi. *Rostlinná výroba*, **44**: 117–122.

Barker, A.V. and Pilbeam, D.J. 2015: Handbook of plant nutrition. CRC press, Boca Raton.

Barrameda-Medina, Y., Montesinos-Pereira, D., Romero, L., Ruiz, J.M. and Blasco, B. 2014:Comparative study of the toxic effect of Zn in *Lactuca sativa* and *Brassica oleracea* plants: I. Growth, distribution, and accumulation of Zn, and metabolism of carboxylates. *Environ. Exp. Bot.*, **107**: 98–104. https://doi.org/10.1016/j.plaphy.2014.03.007

Beneš, S. 1993: Obsahy a bilance prvku ve sférách ŽP, I. část. Ministerstvo zemědělství ČR, Praha.

Boriová, K., Čerňanský, S., Matúš, P., Bujdoš, M. and Šimonovičová, A. 2014: Bioaccumulation and biovolatilization of various elements using filamentous fungus *Scopulariopsis brevicaulis*. *Lett. Appl. Microbiol.*, **59**: 217–223. https://doi.org/10.1111/lam.12266

Bowen, H.J.M. 1966: Trace elements in biochemistry. Academic Press, New York.

Brady, N.C., Weil, R.R. and Brady, N.C. 2010: Elements of the nature and properties of soils. Pesarson Education International, New Jersay.

Brenchley, W.E. 1934: The effect of rubidium sulphate and palladium chloride on the growth of plants. *Ann. Appl. Biol.*, **21**: 398–417. https://doi.org/10.1111/j.1744-7348.1934.tb06686.x

Britto, D. T. and Kronzucker, H. J. 2006: Futile cycling at the plasma membrane: A hallmark of low-affinity nutrient transport. *Trends Plant Sci.*, **11**: 529–534. https://doi.org/10.1111/10.1016/j.tplants.2006.09.011

Broadley, M., Brown, P., Cakmak, I., Rengel, Z. and Zhao, F. 2012: Function of nutrients: micronutrients. In: *Marschner's mineral nutrition of higher plants* (ed. P. Marschner), pp. 191–248. Academic Press, New York.

Brown, J.C. 1978: Mechanism of iron uptake by plants. *Plant Cell Environ.*, **1**: 249–257. https://doi.org/10.1111/j.1365-3040.1978.tb02037.x

Brown, K.A. 1982: Sulphur in the environment: a review. *Environ. Pollut. B*, **3**: 47–80. https://doi.org/10.1016/0143-148X(82)90042-890042-8)

Bu, X., Ruan, H., Wang, L., Ma, W., Ding, J. and Yu, X. 2012: Soil organic matter in density fractions as related to vegetation changes along altitude gradient in the Wuyi Mountains, southeastern China. *Appl. Soil Ecol.*, **52**: 42–47. https://doi.org/10.1016/j.catena.2012.01.013

Cejpek, K. 2000: Chémia bežného života a životné prostredie. Metodické centrum, Bratislava.

Ciriaková, A. 2009: Heavy metals in the vascular plants of Tatra Mountains. *Oecologia Montana*, **18**: 23–26.

Clemente, R. 2013: Antimony. In: *Heavy metals in soils: Trace metals and metalloids in soils and their bioavailability* (ed. B.J. Alloway), pp. 497–506. Springer Science & Business Media, Dordrecht.

Cuypers, A., Remans, T., Weyens, N., Colpaert, J., Vassilev, A. and Vangronsveld, J. 2013: Soil-plant relationships of heavy metals and metalloids. In: *Heavy metals in soils: Trace metals and metalloids in soils and their bioavailability* (ed. B.J. Alloway), pp. 161–193. Springer Science & Business Media, Dordrecht.

Dadová, J. 2014: Geochemická charakteristika ortuti (výskyt. ťažba. výroba. toxicita). UMB, Banská Bystrica.

De Kok, L.J. 1990: Sulfur metabolism in plants exposed to atmospheric sulfur. In: *Sulfur nutrition and sulfur assimilation in higher plants: Fundamental, environmental and agricultural aspects* (eds. H. Rennenberg, C. Brunold, L.J. de Kok and I. Stulen), pp. 111–130. SPB Academic Publishing, Hague.

Demarty, M., Morvan, C. and Thellier, M. 1984: Calcium and the cell-wall. *Plant Cell Environ.*, **7**: 441–448. https://doi.org/10.1111/j.1365-3040.1984.tb01434.x

Drobner, U. and Tyler, G. 1998: Conditions controlling relative uptake of potassium and rubidium by plants from soils. *Plant and Soil*, **201**: 289–293. https://doi.org/10.1023/A:1004319803952

Ducsay, L., Kočík, K. and Kulich, J. 2009: Zmeny tvorby poľnohospodárskej fytomasy pri expozícii vybranými rizikovými prvkami. TU, Zvolen.

Dumon, J.C. and Ernst, W.H.O. 1988: Titanium in plants. *J. Plant Physiol.*, **133**: 203–209. https://doi.org/10.1016/S0176-1617(88)80138-X80138-X)

Ďurža, O. and Khun, M. 2002: Environmentálna geochémia niektorých ťažkých kovov. UK, Bratislava.

Grešíková, S. and Janiga, M. 2017: Analysis of S, Cl, K, Ca, Cr, Mn, Fe, Zn, Rb, Sr, Mo, Ba and Pb concentrations in the needles of *Abies alba* and potential impact of paper mill industry. *Oecologia Montana*, **26**: 47-55.

Gurník, J. 2021: Tatry z chodníka. Dajama, Bratislava.

Hanger, B.C. 1979: The movemen of calcium in plants. *Commun. Soil Sci. Plant Anal.*, **10**: 171–193. https://doi.org/10.1080/00103627909366887

Harborne, J.B. 1993: Introduction to Ecological Biochemistry. Elsevier Academic Press, Oxford.

Heinrichs, H. and Mayer, R. 1980: The role of forest vegetation in the biochemical cycle of heavy metals. *J. Environ. Qual.*, **9**: 111–118. https://doi.org/10.2134/jeq1980.00472425000900010024x

Hinsinger, P. 2005: Potassium. In: *Encyclopedia of soil science* (R. Lal, ed.), pp:1354-1364. CRC Press, Boca Raton.

Huang, J.H. and Matzner, E. 2004: Adsorption and desorption of organotin compounds in organic and mineral soils. *Eur. J. Soil Sci.*, **55**: 693–698. https://doi.org/10.1111/j.1365-2389.2004.00634.x

Hue, N. 1988: A possible mechanism for manganese toxicity in Hawaii soils amended with a low-Mn sewage sludge. *J. Environ. Qual.*, **17**: 473–479. https://doi.org/10.2134/jeq1988.00472425001700030018x

Chatterjee, J. and Chatterjee, C. 2000: Phytotoxicity of cobalt, chromium and copper in cauliflower. *Environ. Pollut.*, **109**: 69–74. https://doi.org/10.1016/S0269-7491(99)00236-500236-5)

Chmielewská, E. and Bedrna, Z. 2007: Rizikové látky a environmentálne hazardy. Cicero, Bratislava.

Chreneková, E. 1983: Vnášanie zvýšených množstiev ťažkých kovov do pôdy a ich príjem rastlinami. *Agrochemia*, **23**: 367–369.

Isinkaralar, K., Koc, I., Erdem, R. and Sevik, H. 2022: Atmospheric Cd, Cr, and Zn deposition in several landscape plants in Mersin, Türkiye. *Water Air Pollut.*, **233**: 120. https://doi.org/10.1007/s11270-022-05607-8

Kabata-Pendias, A. 1993: Behavioural properties of trace metals in soils. *Appl. Geochemistry*, **8**: 3–9. https://doi.org/10.1016/S0883-2927(09)80024-780024-7)

Kabata-Pendias, A. and Pendias, H. 1992. Trace elements in soils and plants, 2nd ed., CRC Press, London.

Kannan, S. and Ramani, S. 1978: Studies on molybdenum absorption and transport in bean and rice. *Plant Physiol.*, **62**: 179–181. https://doi.org/10.1104/pp.62.2.179

Kollár, D. 2020: Najkrajšie pešie okruhy. 1. diel. Dajama, Bratislava.

Krendželák, P., Janiga, M. and Pogányová, A. 2018. Ecotoxicological assessment of *Juncus trifidus* in the Dolina Bielej vody Valley, High Tatras. *Oecologia Montana*, **27**: 32–42.

Kubica, B., Kwiatek, W. M., Stobiński, M., Skiba, S., Skiba, M., Gołaś, J., Kubica, M., Tuteja-Krysa, M., Wrona, A., Misiak, R. and Krzan, Z. 2007: Concentrations of 137Cs, 40K radionuclides and some heavy metals in soil samples of Chochołowska Valley from Tatra National Park. *Pol. J. Environ. Stud.*, **16**: 723–729. https://doi.org/10.1016/j.jenvman.2007.01.019

Kucharík, J. 2019: Tatry Vysoké. Belianske. Západné. Dajama, Bratislava.

Lacika, J. 2020: Vysoké Tatry. Dajama, Bratislava.

Lánczos, T., Mejeed, S. Y. and Milička, J. 1998: Environmentálna geochémia. UK, Bratislava.

Latimer, W.M. 1952: Oxidation potentials. *Soil Sci.*, **74**: 333.

Lehndorf, E. and Schwark, L. 2008: Accumulation histories of major and trace elements on pine needles in the Cologne Conurbation as function of air quality. *Atmospheric Environ.*, **42**: 833–845. https://doi.org/10.1016/j.atmosenv.2007.10.025

Lindberg, S. E., Jackson, D. R., Huckabee, J. R., Janzen, S. A., Levin, M. J. and Lund, J. R. 1979: Atmospheric emission and plant uptake of mercury from agricultural soils near the Almaden mercury mine. Journal of Environmental Quality, 8: 572–578.

Lourie, B., Glenn, W., Ogilvie, K., Everhardus, E., Friesen, K. and Rae, S. 2003: Mercury in the environment. Pollution Probe Foundation, Toronto.

Mackay, A.D. and Barber, S.A. 1985: Soil moisture effects on potassium uptake by corn. *Agron. J.*, **77**: 524–527.

Madejón, P. 2013: Barium. In: *Heavy metals in soils: Trace metals and metalloids in soils and their bioavailability* (ed. B.J. Alloway), pp. 507–514. Springer Science & Business Media, Dordrecht.

Makovníková, J., Barančíková, G., Dlapa, P. and Dercová, K. 2006: Inorganic Contaminants in Soil Ecosystems. *Chemické Listy*, **100**: 424–432.

Małkowski, E., Sitko, K., Zieleźnik-Rusinowska, P., Gieroń, Ż. and Szopiński, M. 2019: Heavy metal toxicity: Physiological implications of metal toxicity in plants. In: *Plant metallomics and functional omics* (ed. G. Sablok), pp. 253–301. University of Silesia in Katowice, Katowice.

Maria, G.E.S. and Cogliatti, D.H. 1988: Bidirectional Zn-fluxes and compartmentation in wheat seeding roots. *J. Plant Physiol.*, **132**: 312–315.

Marschner, H. 1995: Mineral nutrition of higher plants. 2nd ed., Academic Press, New York.

Mayer, R. and Ulrich, B. 1974: Coclusions on the filtering action of forests from ecosystem analysis. *Oecologia Plantarum*, **9**: 157–168.

Mengel, K. and Kirkby, E.A. 1982: Principles of plant nutrition. Intemational Potash Institute, Berne-Worblaufen.

Monaci, F. and Bargagli, R. 1997: Barium and other trace metals as indicators of vehicle emissions. *Water Air Soil Pollut.*, **100**: 89–98.

Nakamaru, Y., Tagami, K. and Uchida, S. 2006: Antimony mobility in Japanese agricultural soils and the factors affecting antimony sorption behavior. *Environ. Pollut.*, **141**: 321–326. https://doi.org/10.1016/j.envpol.2005.08.040

Nautsch-Laufer, C. 1974: Die Wirkung von Titan auf den Stoffwechsel von *Phaseolus vulgaris* und *Zea mays*. Diss. Univ., Munster.

Nemčok J. 1994: Geologická mapa Tatier. GÚDŠ, Bratislava.

Orolínová, M. 2009: Chémia a životné prostredie. TU, Trnava.

Piscová, V. 2018: Využívanie vysokohorskej krajiny a jeho dôsledky na zmenu prostredia: (na príklade Tatier a Nízkych Tatier). VEDA SAV, Bratislava.

Pourrut, B., Shahid, M., Dumat, C., Winterton, P. and Pinelli, E. 2011: Lead uptake, toxicity, and detoxification in plants. *Rev. Environ. Contam. Toxicol.*, **213**: 113–136. https://doi.org/10.1007/978-1-4419-9860-6_4

Purves, D. 2012: Trace-element contamination of the environment. Elsevier, Amsterdam.

Rediske, J.H. and Selders, A.A. 1953: The absorption and translocation of strontium by plants. *Plant Physiol.*, **28**: 594–605. https://doi.org/10.1104/pp.28.4.594

Ross, S.M. 1994: Toxic metals in soil-plant systems. Wiley & Sons Ltd., Chichester.

Salt, D.E., Prince, R.C., Pickering, I.J. and Raskin, I. 1995: Mechanisms of cadmium mobility and accumulation in Indian mustard. *Plant Physiol.*, **109**: 1427–1433. https://doi.org/10.1104/pp.109.4.1427

Seigneur, C. and Constantinou, E. 1995: Chemical kinetics mechanism for atmospheric chromium. *Environ. Sci. Technol.*, **29**: 222–231. https://doi.org/10.1021/es00001a029

Sloboda, M. 2021: Tatry - Portrét regiónu. MS Agency, Bratislava.

Steinnes, E. 2013: Mercury. In: *Heavy metals in soils: Trace metals and metalloids in soils and their bioavailability* (ed. B.J. Alloway), pp. 411–428. Springer Science & Business Media, Dordrecht.

Steinnes, E. and Friedland, A.J. 2006: Metal contamination of natural surface soils from long-range atmospheric transport: Existing and missing knowledge. *Environ. Rev.*, **14**: 169–186. https://doi.org/10.1139/a06-002

Svičeková, M. and Havránek, E. 1993: Stanovenie Pb, Cd, Ni, Zn a Cu vo vzorkách liečivých rastlín metódou diferenčnej pulzovej polarografie. *Farmaceutický obzor*, **62**: 13–17.

Teutsch, N., Erel, Y., Halicz, L. and Chadwick, O. A. 1999: The influence of rainfall in metal concentration and behavior in the soil. *Geochim. Cosmochim. Acta*, **63**: 3499–3511. https://doi.org/10.1016/S0016-7037(99)00152-0

Tschan, M., Robinson, B.H. and Schuli, R. 2009: Antimony in the soil–plant system – a review. *Environ. Chem.*, **6**: 106–115. https://doi.org/10.1071/EN08111

Tyler, G. 1976: Soil factors cotrolling metal ion absorption in the wood anemone, *Anemone nemorosa*. *Oikos*, **27**: 71–80. https://doi.org/10.2307/3543434

Utermann, J., Düwel, O. and Nagel, I. 2006: Contents of trace elements and organic matter in European soils. Background values in European soils and sewage sludges. European Commission, Luxembourg.

Varshal, G.M., Koshcheva, I.Y., Khushvakhtova, S.D., Velyukhanova, T.K., Tatsii, V.N., Danilova, V.N., Tyutunnik, D.N., Chkhtetiya, D.N. and Galuzinskaya, A.K. 1999: Complex formation of mercury with humic acids: An important stage of the biospheric mercury cycle. *Geochemistry Int.*, **37**: 229–234.

Vassilev, A., Lidon, F.C., Matos, M.D., Ramalho, J.C. and Yordanov, I. 2002: Photosynthetic performance and some nutrients content in cadmium- and copper-treated barley plants. *J. Plant Nutr.*, **25**: 2343–2360. https://doi.org/10.1081/PLN-120014699

Vassilev, A., Perez-Sanz, A., Semane, B., Carleer, R. and Vangronsveld, J. 2005: Cadmium accumulation and tolerance of two *Salix viminalis* genotypes hydroponically grown in presence of cadmium. *J. Plant Nutr.*, **28**: 2159–2177. https://doi.org/10.1080/01904160500320806

Vatansever, R., Ozyigit, I.I. and Filiz, E. 2017: Essential and beneficial trace elements in plants, and their transport in roots: a review. *Appl. Biochem. Biotechnol.*, **181**: 464–482. https://doi.org/10.1007/s12010-016-2224-3

White, P.J. and Broadley, M.R. 2001: Chloride in soils and its uptake and movement within the plant: a review. *Ann. Bot.*, **8**8: 967–988. https://doi.org/10.1006/anbo.2001.1540

Widyatmoko, H. 1994: Schwermetalle in quartären Paläoböden im Mittelrhein-Gebiet. Dissertation. Universitaet zu Koeln, Koeln.

Wilber, C.G. 1980: Toxicology of selenium: a review. *Clin. Toxicol.*, **17**: 171–230. https://doi.org/10.3109/15563658008985076

Yong, R.N., Mohamed, A.M.O. and Warkentin, B. 1992: Principles of contaminant transport in soils. Elsevier, London.

Zayed, A.M. and Terry, N. 2003: Chromium in the environment: Factors affecting biological remediation. *Plant and Soil*, **249**: 139–156. https://doi.org/10.1023/A:1022504826342

Zimmer, W. and Mendel, R. 1999: Molybdenum metabolism in plants. *Plant Biol.*, **1**: 160–168. https://doi.org/10.1055/s-2007-978502

Zöttl, H.W. 1985: Heavy metal levels and cycling in forest ecosystems. *Experientia*, **41**: 1104–1113. https://doi.org/10.5555/19851310733

Received 21 May 2024; accepted 10 September 2024.