Oecologia Montana 2018, **27,** 32-42

Ecotoxicological assessment of *Juncus trifidus* in the Dolina Bielej vody Valley, High Tatras

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Abstract. This work focuses on detection of toxic elements in *Juncus trifidus*. The research took place in the Dolina Bielej vody Valley, in the eastern part of the High Tatras. Sampling was carried out in July, August and September. During collection the plant was divided into four parts, each to be analysed separately: the upper green part (leaves, blade, flower or spike); sheath (part of plant at the ground level); roots; and soil. Collected and dried samples were then processed and analysed in the laboratory. The samples were ground in a hand mill and then put into the roentgen which identified specific toxic elements in particular parts of the plant. This research was partially related to that on the snow vole (*Chionomys nivalis*). If toxic elements are detected in the snow vole, it is probable that this toxicity is caused in part by its diet, including *Juncus trifdus*. We observed important differences between processes of accumulation of individual elements in plant tissues. Calcium levels were highest in the green parts of plants in their germinative tissue (likely due to production of seeds). Similarly, zinc and manganese were found in the samples, as calcium, manganese and zinc are biogenic elements. We found variation in the amount of lead in soil versus in the green parts of the plant. This shows that *Juncus trifidus* doesn't accumulate this element in the tissues in high amounts.

Key words: Juncus trifidus, toxic elements, heavy metals

Introduction

Today, pollution of the environment - especially through anthropogenic activity - has a greater impact than ever before. In today´ s society there is a common misconception that natural environments that appear untouched by humans - such as the Alpine - are largely protected from these unfavourable impacts. However, environmentalists have discovered that these zones are actually some of the least protected from environmental impacts as evidenced by the relatively high amount of lead in Alpine ecosystems (Šoltés *et al.* 1992). High altitude works as a natural barrier, retaining precipitation and transmissions of various natures. As such, the altitude is a deciding factor influencing the amount of polluting substances found in an alpine environment. Thus, we decided to study the concentration of toxic elements in *Juncus trifidus*, which is one of the dominant species in the high alpine, (Zeidler and Banaš 2013) and is found in almost all high mountains in Europe.

As *Juncus trifidus* is in abundance throughout the Tatras, it was necessary to concentrate on a sample area; Dolina Bielej vody Valley in the eastern High Tatras was chosen. Additionally, the snow vole (*Chionomys nivalis*) can be found in Dolina Bielej vody Valley, and our research is bound to this rodent as *Juncus trifidus* constitutes part of its diet. Our aim is to find out whether toxic substanceds found in this animal come from the food it feeds on. The aim of this work is to prove or disprove the presence of specific polluting substances or elements in alpine environments by means of sampling and analysing *Juncus trifidus*. The main goals were:

- Identification of possible appearance of heavy metals and other toxic substances in tissues of the plant. Our goal is to determine where the concentration of these substances is highest, as well as how it is transferred from one part of the plant to another.
- Developing an explanation for deposition processes of toxic elements in plant tissues from the surroundings.
- Explain potential relationship between the content of toxic elements in *Juncus trifidus* and content of identical elements in the body of snow vole.

Societies of strongly blown (so called deflationary) slopes and peaks of alpine degree (in accelerating peak part of anemo-orographic systems (A-O systems). They are low vegetation (ca. up to 20 cm) of so called alpine grasses with *Juncus trifidus* and *Festuca supina*, which according to the relief shape and wind force, are unconnected patches with discontinuous areas of bare soil and screes with bryophytes and lichens or bare rocky bedrock (strongly exposed peak parts) to compact vegetation (less exposed slopes and areas with longer lasting snow cover). Nature and development of societies of windward positions is determined by several limiting factors. Firstly, there is vegetation exposed to strong disturbance from the abiotic factors, mainly wind. As a result of constantly strong air flow, there is insufficient deposition of snow cover in deflationary peak plains, ridges and peaks of alpine degree (there is often only weak ice crust in winter season). Due to insufficient insulation of snow layer there occurs intensive pergelation of upper layer of soil profile in these conditions. Therefore the snow melts early and in short time horizon in spring resulting in moisture deficit in case of rainfall shortage in this season (Zeidler and Banaš 2013).

Material and Methods

The research took place in Dolina bielej vody Valley situated in the eastern High Tatras, Slovakia. It is a valley of unusual glacial cirque type, lying at an altitude of 1600 metres, with an area of 1,8 km^2. Biely potok brook flows through it.

Work in the field consists of *Juncus trifidus* sampling. We set 10 sampling points placed evenly to occupy as much area as possible (Point 1: N: 49° 13. 541°, E: 20° 13.329°; Point 2: N: 49° 13.538°, E: 20° 13.309°; Point 3: N: 49° 13.532°, E: 20° 13.271°; Point 4: N: 49° 13.519°, E: 20° 13.237°; Point 5: N: 49° 13.509°, E: 20° 13.201°; Point 6: N: 49° 13.496°, E: 20° 13.240°;Point 7: N: 49° 13.494°, E: 20° 13.258°; Point 8: N: 49° 13.495°, E: 20° 13.285°;Point 9: N: 49° 13.504°, E: 20° 13.285°; Point 10: N: 49° 13.506°, E: 20° 13.311°). It was necessary to dig plants out of the rocky soil as we wanted to collect and retain samples of roots and soil, which needed to be collected.

The samples were taken from July until September. Specific plants were put into separate bags together, and labelled with the sampling point and date of sampling. Later, the samples were taken out and dried. All dried plants were divided into four parts that had to be analysed separately: upper green parts (leaves, blade, flower or spike); ground level parts; roots (thoroughly cleaned); and soil. After dividing the plant, all samples were ground in the hand mill to fine dust. In order to prevent contamination and results distortion it was necessary to wash the drum and metal ball under running water after each grinding and then to thoroughly dry it. Ground samples were inserted into labelled resealable bags. There were 120 samples in total. We inserted sample into special small vessel (min. 3 mm) which was then inserted into X-ray (hand ED-XRF spectrometer DELTA with setting to mode 3 beam (80-80-80) light mode).

Results and Discussion

Our research focused on toxicity in the plant *Juncus trifidus*, and we confirmed the presence of elements that appeared in varying concentrations in the plant. On the whole, we managed to confirm the presence of nineteen elements, out of which two were highly toxic; namely lead and arsenic. Arsenic could be found in trace amounts, while lead was found in much higher amounts. Analysis confirmed the presence of elements such as chromium and chlorine, which can be a burden for the ecosystem when their values reach higher levels. Remaining elements in *Juncus trifidus* were manganese, molybdenum, strontium, rubidium, calcium, copper, barium, zirconium, titanium, potassium, antimony, zinc, iron and sulphur. However, several of these elements are naturally occurring in *Juncus trifidus*, as well as many other plants.

Sulphur (S), Calcium (Ca), Manganese (Mn), Zinc (Zn)

The highest amount of sulphur was found in soils. With regard to the plant itself, the green part contained a higher amount of sulphur than dry sheaths and roots. The sulphur cycle in the plant was more or less the same in all months, and it is likely that the contamination of aboveground parts was a little higher in September (Fig. 3).

The sulphur cycle is a biogeochemical cycle. To a certain extent, there are participating organisms that decompose or synthesize various sulphuric compounds. The complexity of the sulphur cycle varies due to the level of oxidation of sulphur. Sulphur occurs naturally in the body of organisms and makes up part of the protein structure (amino acids cysteine and methionine or metalloproteins) and coenzyme structure (Stránská 2013).

High amounts of calcium can be found in aboveground parts of plants in September when the plant is already dry. Since calcium can be found in dry sheaths only in small amounts, there is high probability that calcium gets into dry parts of plants in autumn and thus is contained mainly in seeds created by plant in the spring-summer (Fig. 4). Calcium is one of the main building elements in plants and participates in various biological processes.

Calcium is quite widespread in Earth's crust: 3.5% Ca (limestones $CaCo_3$, dolomites $CaCo_3 \cdot MgCO_3$, magnesites $MgCO_3$, gypsums $CaSO_4 \cdot 2H_2O$). Ca^{2+} gets in water by digestion from minerals. Its solubility depends on content of CO_2 in water (Orolínová 2009).

The manganese cycle is similar to the calcium cycle, with higher content in the green parts of plants after they are dried. As the amount of manganese is low in the dry sheath, we suppose that increased amounts can be found in green part of plants (Fig. 5). Manganese is relatively evenly distributed in ecosystems. It occurs in iron ores and it gets to water mostly by digestion of dead parts of plants and by digestion of minerals. Manganese can get into the environment through anthropogenic activities, including waste waters from metallurgy and metal processing. Manganese is not distributed uniformly in soil substrata and, in addition to various nodules, is also concentrated in certain spots and veins (Kabata-Pendias and Mukherjee 2007).

Zinc amounts did not differ over the sampling period. The highest amount of zinc was found in the green parts of plants. Smaller amounts of zinc were found in the dry part, and the smallest amount in root systems. Zinc content in the dry part was similar to calcium and manganese. High amounts of Zinc were found in the seeds, while a small amount of zinc was found in the soil (Fig. 6).

Plants pull zinc from the soil in the form of microelements such as the divalent cation Zn^{2+}. Its concentration in most of the plants is between 20 and 100 ug/g (Horník 2010). A shortage of zinc

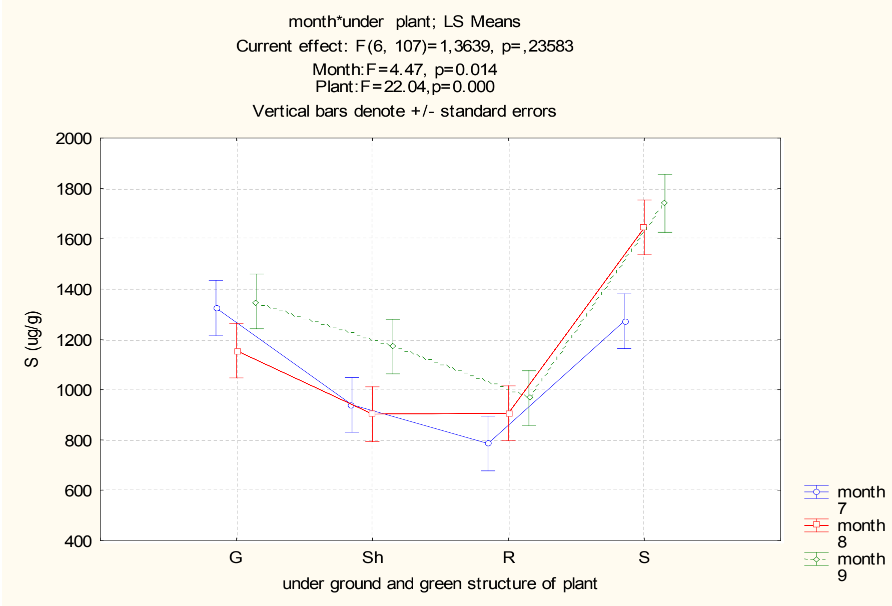

Fig. 1. The amount of sulphur was highest in the soils. The green parts of the plants contained higher levels of sulphur than the dry sheaths or roots. The cycle and distribution of sulphur was more or less equal in all months, the external contamination of habitats was significantly higher in September than in summer (July). (G – green medium and apical part of the plant, Sh – sheath, R – roots, S – soil).

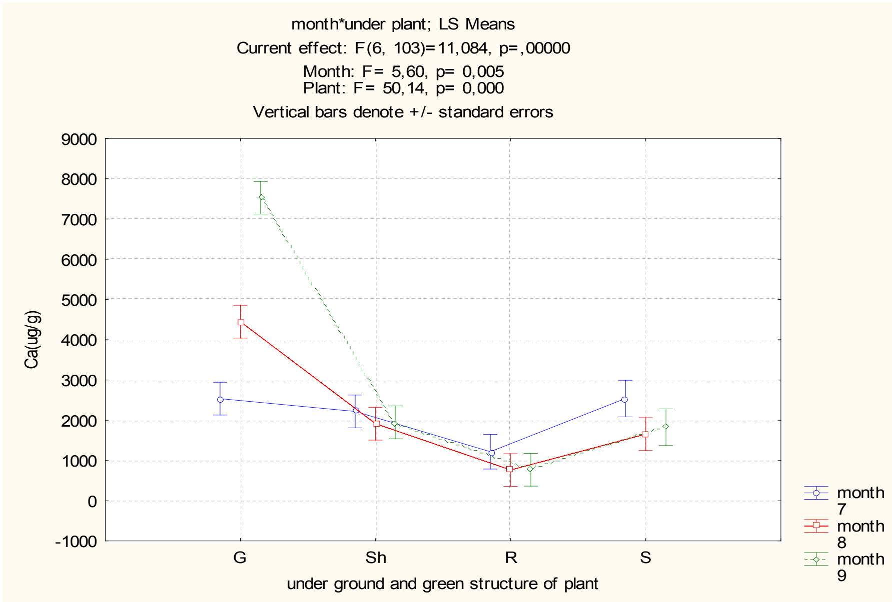

Fig. 2. The amount of calcium was highest in green parts of plants, and probably mainly in their germinative parts (seeds – September). The sheaths and roots contained less calcium than green parts. Calcium is a biogenic element. In green parts of the plant the levels of calcium were significantly higher than in the surrounding soils. (G – green medium and apical part of the plant, Sh – sheath, R – roots, S – soil).

can result in weakening of the stem and dwarfed growth (Marschner 1995).

Chlorine (Cl), Potassium (K), Rubidium (Rb)

Chlorine (Fig. 7) appears neither in water nor in the air. Chlorine is a common halogen element in terrestrial and aquatic environments (Kabata-Pendias and Mukherjee 2007). However, it can make its way into ecosystems through anthropogenic activities or unique natural processes such as volcanic activity. Sometimes chlorine enters the environment through the air by weathering of cryolite, apatite, aluminium fluoride or sodium fluoride. Chlorine is absorbed more often as a result of anthropogenic activity, such as chlorination or whitening processes or by combustion of plastic emissions.

Potassium is naturally found in feldspars and micas, which are prevalent in the soil. The underlying rock at the sample site was granite, which is composed of these two minerals for the most part (Fig. 8). These minerals are characterized by banded structure and are able to accumulate cations K^{+} (Čurlík 2003). Potassium, similar to chlorine, likely binds to green parts of plants through their respiratory process.

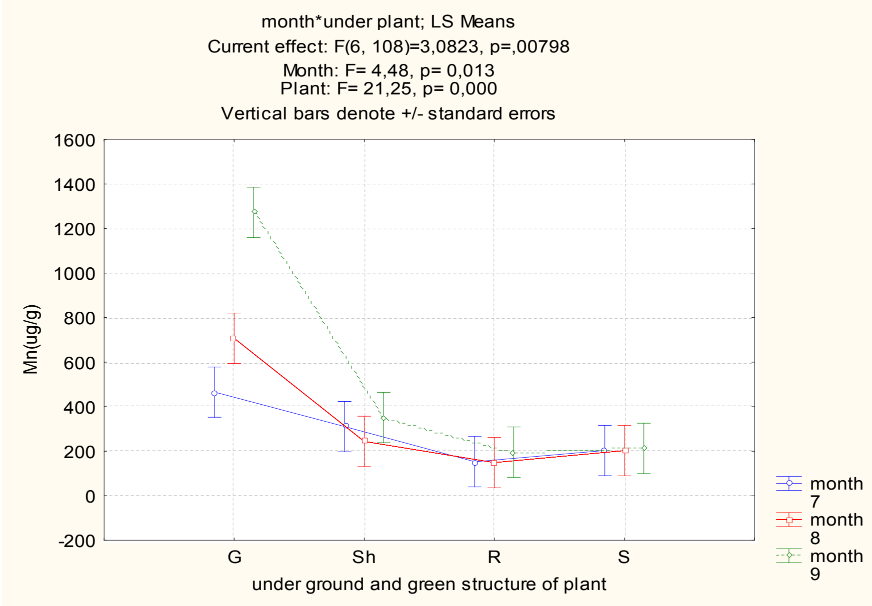

Fig. 3. Manganese cycle is similar to calcium cycle. Its amount is higher in green parts of plants after they are dried (G – green medium and apical part of the plant, Sh – sheath, R – roots, S – soil).

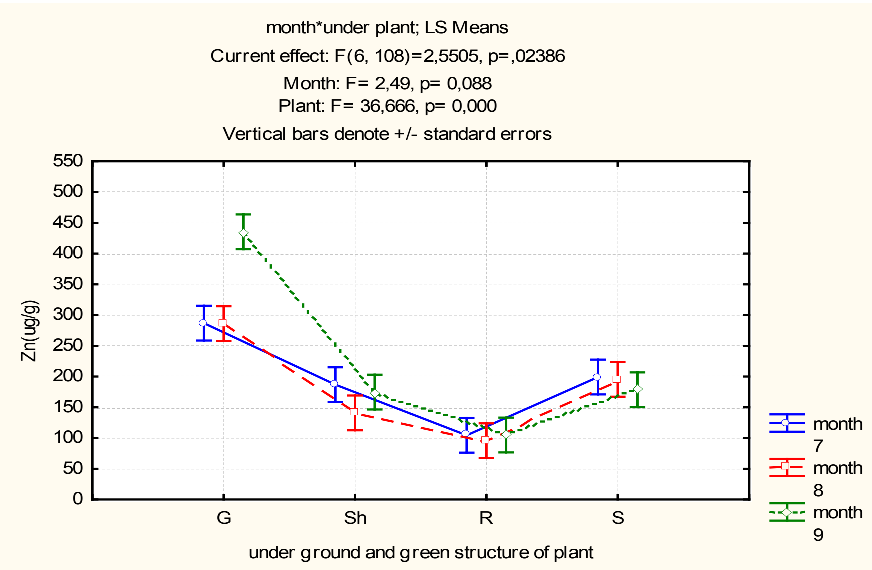

Fig. 4. Accumulation of zinc in the soils (S), roots (R), sheaths (Sh) and green (G) above ground parts of *Juncus trifidus* (for detailed explanation see text).

Rubidium has a similar cycle to potassium and chlorine in plants (Fig. 9). It was found in much higher amounts in the soil than in the roots or dry parts. Higher concentrations of rubidium in the green parts of plants indicates that rubidium concentrates there during photosynthesis. When dried, rubidum decreases in aboveground parts and sheaths. Potassium and sodium accompany rubidium in small concentrations. Minerals containing these elements are the cause of their usual concentration increase in water. Rubidium in small concentrations forms part of the plants structure (Bencko *et al.* 1995).

Iron (Fe), Lead (Pb)

Iron (Fig. 10) is found in nature as a compound in rocks and minerals such as limonite, pyrite, magnetite, siderite or aluminosilicates. In our case, granite with a high concentration of aluminosilicates containing iron is the cause of high iron content in soil. However, iron gets in water and then in the environment only minimally by means of mineral digestion. CO_2 sulfuric acid or humic substances help iron to dissolve but it was found that the highest concentration of iron in soil occures as a result of anthropogenetic factors (Ciriaková 2009).

Lead (Fig. 11) appears in nature only as a compound in minerals such as galenite, cerusite, and anglesite. In the past, lead pollution was mainly as a result of burning fuel from transport vehicles. Today lead gets into the environment mainly from industrial activities, namely, emissions. Much lead is thus collected from the plant leafs. The smallest amount is found in fruit and seeds (Svičeková and Havránek 1993).

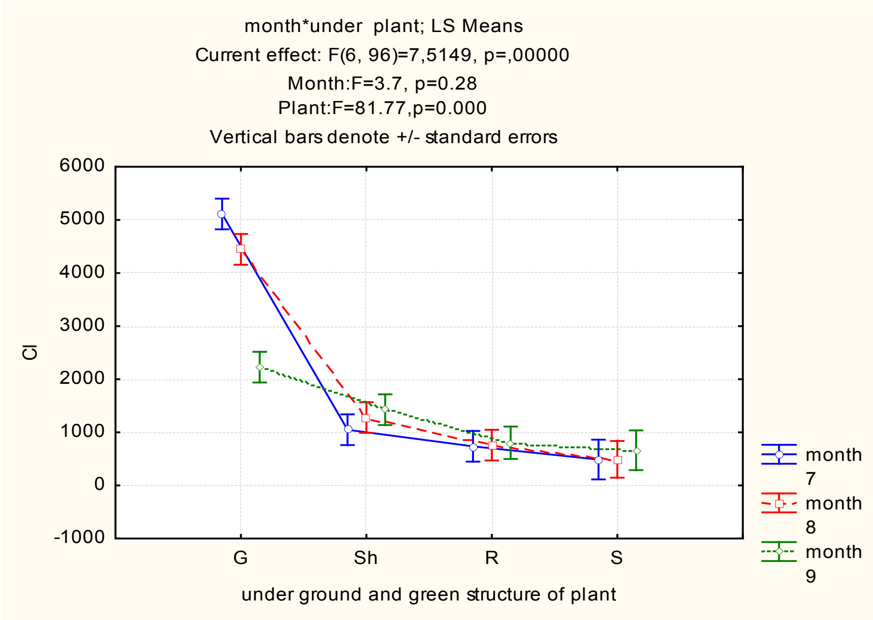

Fig. 5. The highest amount of chlorine was found on the surface of leaves and thus mainly exists in summer months (July and August). When leaves dried in September, the amount of chlorine decreased in sheaths, roots and soil, but still existed in high concentration in green parts. (G – green medium and apical part of the plant, Sh – sheath, R – roots, S – soil).

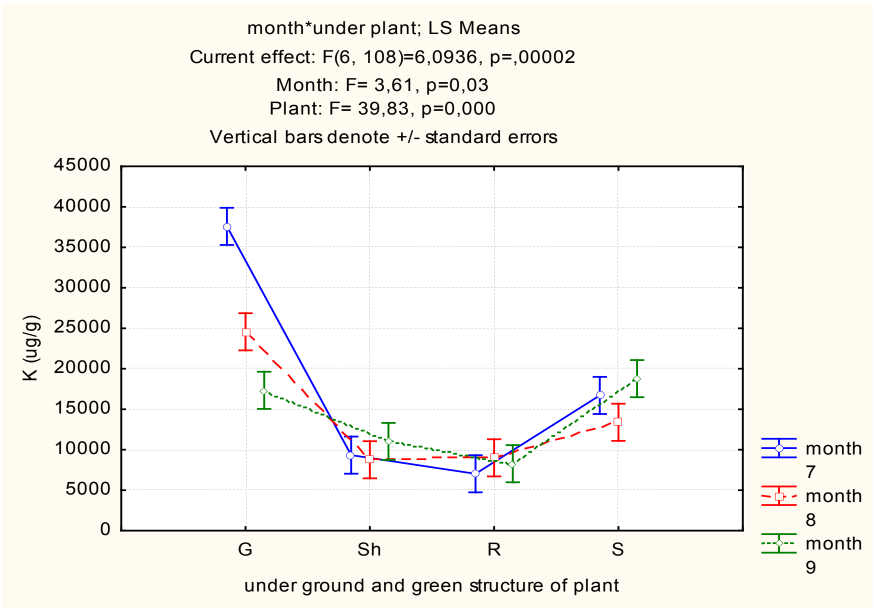

Fig. 6. Potassium, similarly to chlorine, got into green parts of plants in summer season. Potassium amounts decreased in September when plants dried. Minimum amounts were found in sheaths. There was an increased amount of potassium in soil compared to dry parts of plants. Only minimum potassium was found in roots. (G – green medium and apical part of the plant, Sh – sheath, R – roots, S – soil).

Lead has the ability to effectively bind to soil particles (Wu *et al.* 1999; Barona *et al.* 2001; Kos and Leštan 2003). The plant has limited capacity to pull lead into aboveground parts in high quantities, (Woźny 1995; Kumar *et al.* 1995; Sekhar *et al.* 2005). In our research, the highest concentration of lead was found in soils. Thus, the roots also exhibit high lead content. Our research shows a distinct difference in the amount of lead found some parts of the plant compared to others.

Chromium (Cr)

Chromium (Fig. 12) occurs in nature only as the mineral chromite. It can be released into the environment by mineral digestion, but most often gets into an ecosystem through anthropogenic activities such as industrial waste waters or from surface metal processing.

The world median content of Cr in soils has been estabilished as 54 mg kg^{-1}. Its content in soils is determined mainly by its abundance in the parent material. Since soil Cr is inherited from parent rocks, higher content is generally found in soils derived from mafic rocks and argillaceous sediments (Kabata-Pendias and Mukherjee 2007).

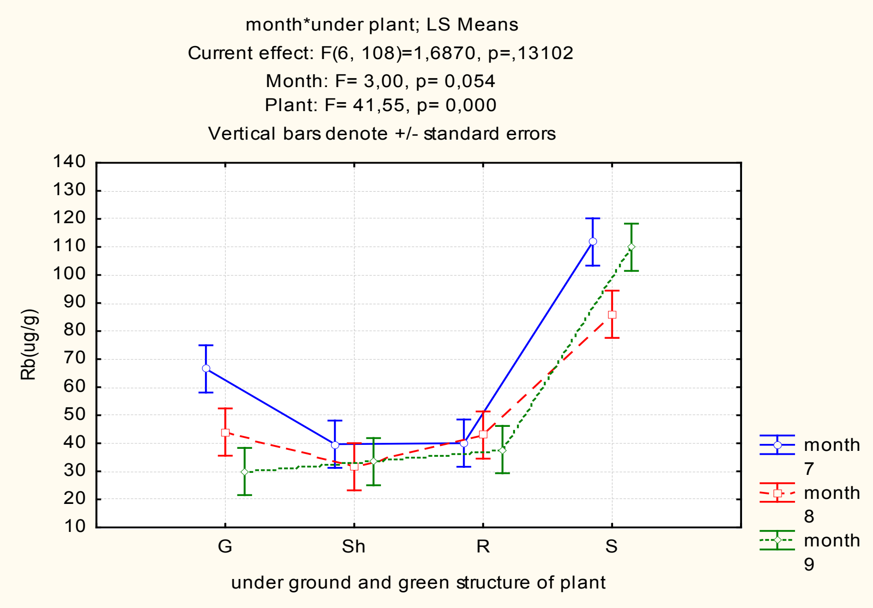

Fig. 7. Accumulation of rubidium in the soils (S), roots (R), sheaths (Sh) and green (G) above ground parts of *Juncus trifidus* (for detailed explanation see text).

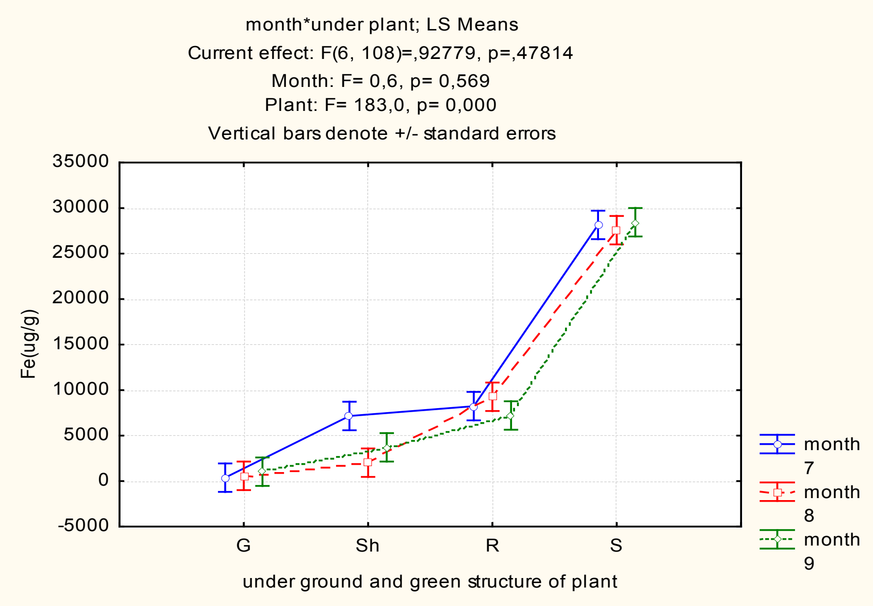

Fig. 8. Iron was found in relatively great amount in soil, but the plants had no tendency to absorb it through roots. There was a little of iron in dry parts of plants, either in summer or in autumn. (G – green medium and apical part of the plant, Sh – sheath, R – roots, S – soil).

Copper (Cu), Molybdenum (Mo), Antimony (Sb)

Small concentrations of copper get into plants though physiological processes (Fig. 13). Concentration of copper in roots and aboveground parts was similar whether they were dry or green. There a high quantity of copper in soil when the terrain was drying up in autumn.

Copper occurs in the Earth's crust at concentrations between 25-75 mg kg^{-1}. Its abundance pattern in rocks shows the tendency for the concentration in mafic igneous rocks and in argillaceous sediment, however, it is mostly excluded from the carbonated rocks (Kabata-Pendias and Mukherjee 2007).

Yong *et al.* (1992) found that optimum amounts of copper are essential to the plant. However, even essential elements necessary for the plant may become toxic if they exceed certain concentrations (Tomáš 2000).

The amount of molybdenum is relatively low in comparison with the amount found in plant bundles (Fig. 14). Molybdenum is not actively absorbed by the plant from she soil. In autumn the difference in the amounts of molybdenum in various parts of the plant increases. Molybdenum concentration is low in July, but high concentrations are found in the roots during autumn. Molybdenum is mined as a primary ore deposit, mainly as molybdenite, as well as a byproduct in copper mines. Annual produc-

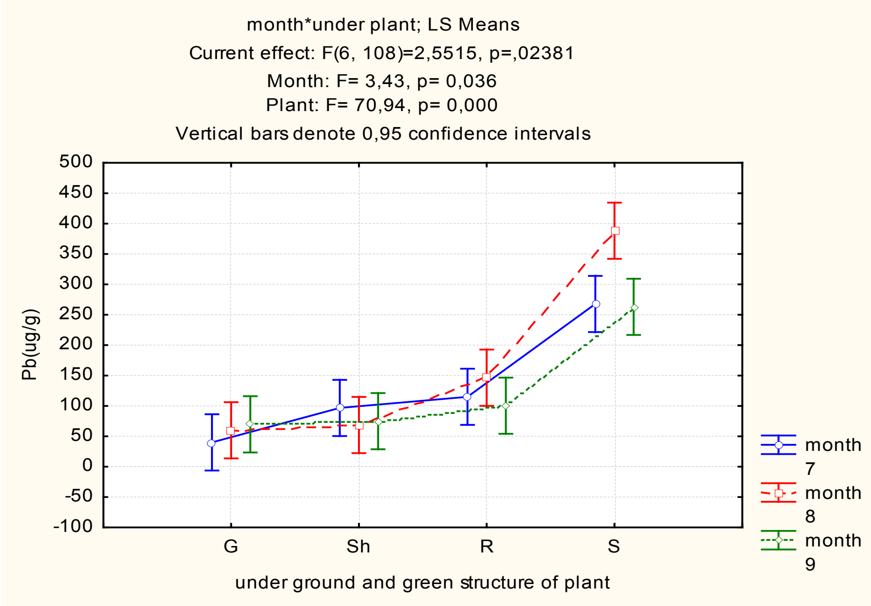

Fig. 9. Content of lead was higher in August than in July and September. Higher amount of lead was found in roots in all months in comparison to the green part of plant. Higher amount of lead in aboveground parts of plants was found in sheaths, which were already dry in July and amount of lead was high in this season. It got into roots early in the spring, and to green parts of plant and sheaths after snow melted. (G – green medium and apical part of the plant, Sh – sheath, R – roots, S – soil).

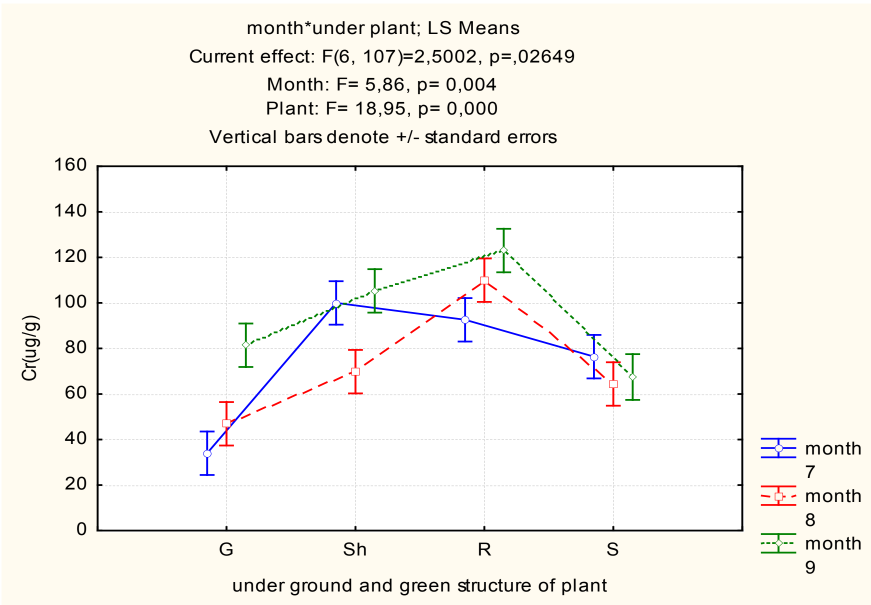

Fig. 10. Chromium amount in plant grows after all green parts have dried up in September. Chromium in sheath and green parts of plant has been balanced gradually. The highest amount is found in roots, which is much higher than in soil. The concentration in all subjects did not differ in different months. This means that plant can actively absorb chromium from soil and cumulates it only in root parts. (G – green medium and apical part of the plant, Sh – sheath, R – roots, S – soil).

tion of Mo in 2003 was 127.4 kt (WMSY 2004). Its main use is in metallurgy for the hardening of alloys (Kabata-Pendias and Mukherjee 2007).

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Antimony exhibits chalcophilic properties, combines readily with sulphides and occurs mainly at 3^+ and 5^+ oxidation stages. Its content in igneous rocks ranges between 0.1 and 0.9 mg kg^{-1} and is likely to increase up to 2 mg kg-1 in argillaceous rocks (Kabata-Pendias and Mukherjee 2007).

Antimony (Fig. 15.) is absorbed by environments as an atmospheric emissio, through anthropogenic activity (mining, technologic and industrial processing), or through natural processes (forest

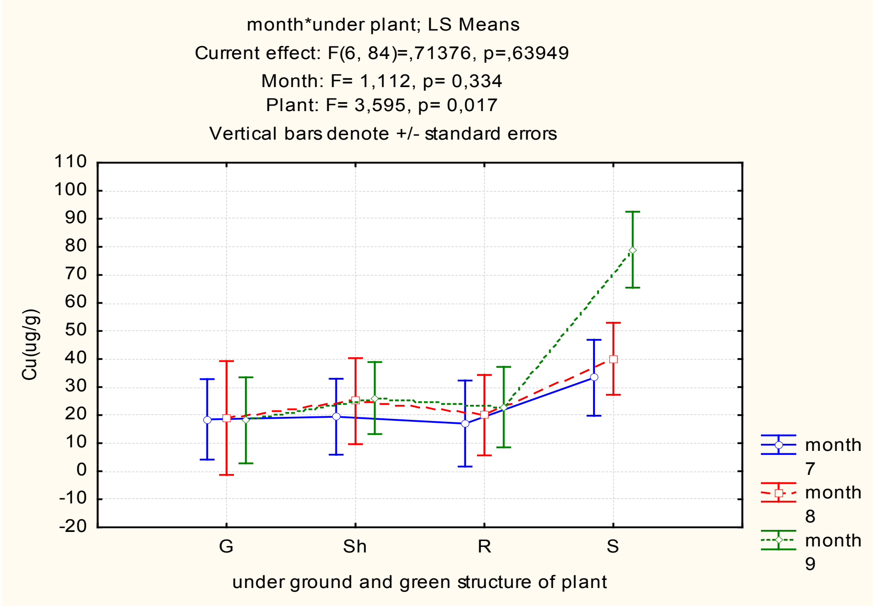

Fig. 11. Accumulation of copper in the soils (S), roots (R), sheaths (Sh) and green (G) above ground parts of *Juncus trifidus* (for detailed explanation see text).

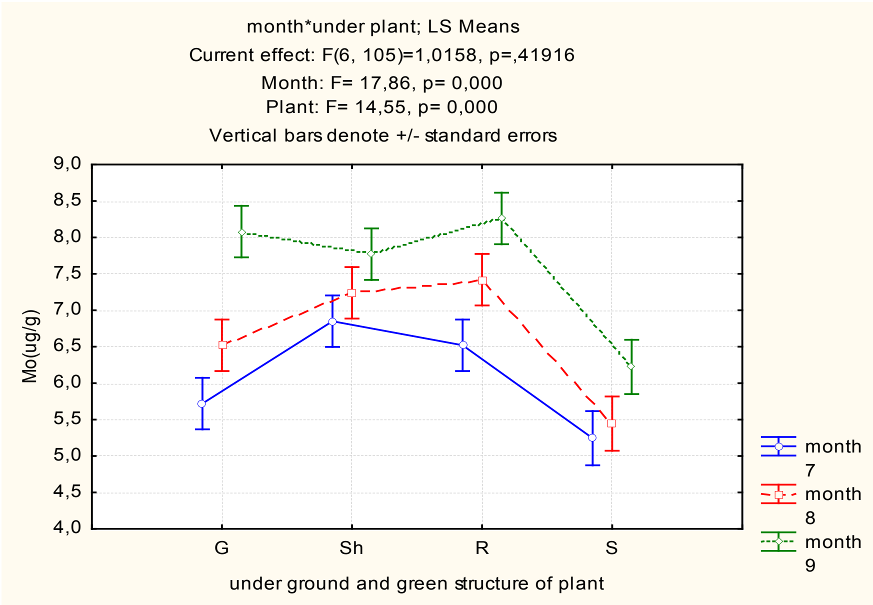

Fig. 12. Accumulation of molybdenum in the soils (S), roots (R), sheaths (Sh) and green (G) above gound parts of *Juncus trifidus* (for detailed explanation see text).

fires, volcanic activity). Yearly, 50 tons of antimony is released into the air from natural processes. In the past 150 tons per year of antimony was released into the air from anthropogenic processes (2005). Nowadays, that amount has increased, totalling up to 1600 tons (Vojteková *et al.* 2013).

Strontium (Sr), Barium (Ba)

Strontium was found in small concentrations in leaves and roots. Higher amounts were found in soil than in the plant iteslf, meaning that the plant did not actively absorb this element (Fig. 16).

Strontium is a relatively common element in the Earth's crust and its prevalence ranges between 260 and 730 mg kg^{-1}. It is likely to concentrate in mafic igneous rocks and in carbonate sediments. Both geochemical and biochemical characteristics of Sr are similar to those of Ca. Strontium possesses lithophilic affinity and is associated with Ca, and to a lesser extent with Mg (Kabata-Pendias and Mukherjee 2007).

Barium (Fig. 17) is a common and ubiquitous element. Its mean content in the Earth's crust amounts to 425 mg kg^{-1}, and ranges from 550 to 668 mg kg^{-1} in the upper continental crust. Barium has a lithophilic affinity and is likely to concentrate in acid igneous rocks and argillaceous sediments, ranging widely in various rocks from 250 to 1200 mg kg^{-1} (Kabata-Pendias and Mukherjee 2007).

Barium occurs naturally in minerals such as witherite, celestine, barite, etc. It is easily absorbed into the environment through mineral digestion and

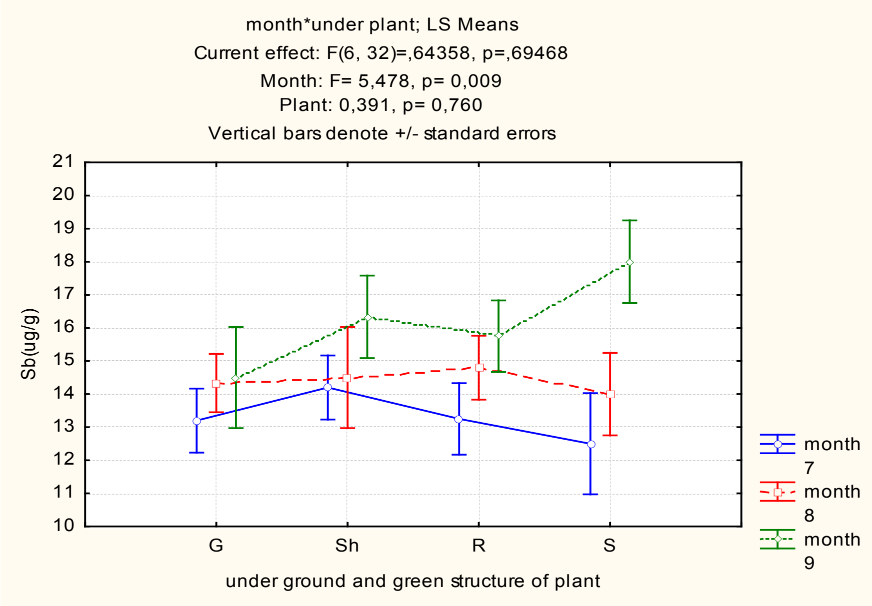

Fig. 13. Amount of antimony was the same in soil and green part of plant. Differences in amount of antimony could be seen during the months, whereas the highest antimony concentrations were found in autumn. (G – green medium and apical part of the plant, Sh – sheath, R – roots, S – soil).

thus occurs in consentrations of up to several tens of micrograms per litre. This element is also absorbed into the environment as a result of anthropogenic factors, (e.g. waste waters of industrial production of paper, glass, dyes, ceramics etc.) (Orolínová 2009).

Arsenic (As), Titanium (Ti)

In some samples, the concentration of arsenic in aboveground parts of the plant was below the detaction limit of the testing device. Consistently high amounts of arsenic were found in the soil over all months. The plant did not pull out arsenic from the soil into its thallus (Fig. 18).

Arsenic is widely distributed in the environment. It occurs in the Earth's crust at levels between 0.5 and 2.5 mg kg^{-1} and is likely to be concentrated, up to 13 mg kg-1, in argillaceous sediments (Kabata-Pendias and Mukherjee 2007).

Titanium concentration in the soil was high over all months, but roots and green parts of the plant do not absorb it. Thus, there is a wide data range on titanium concentration in green parts of plants, as it was under the detection limit in some samples. Titanium is not easily absorbed by the plant, although it can be found in high amounts in soil (Fig. 19).

Titanium shows strong lithophilic characteristics and is a common element in rock, in the range

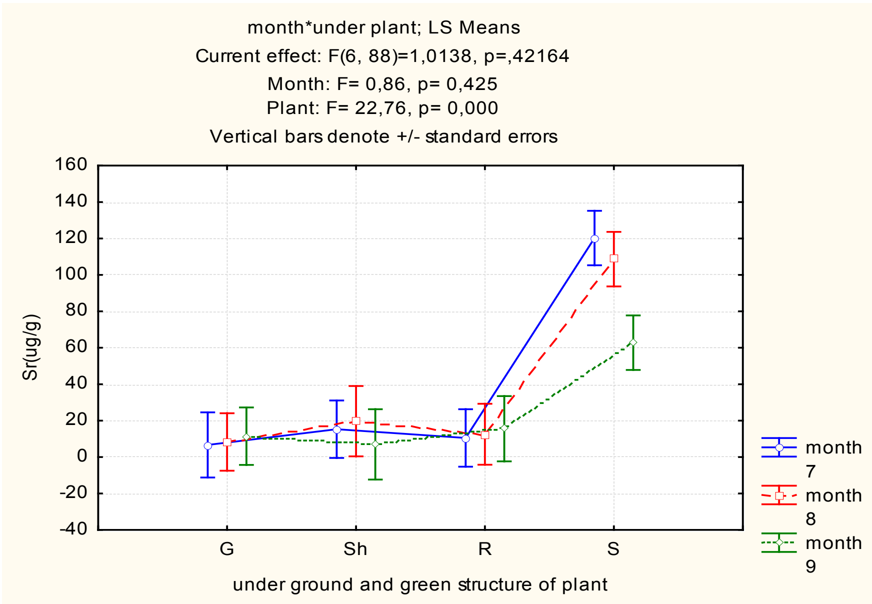

Fig. 14. Accumulation of strontium in the soils (S), roots (R), sheaths (Sh) and green (G) above gound parts of *Juncus trifidus* (for detailed explanation see text).

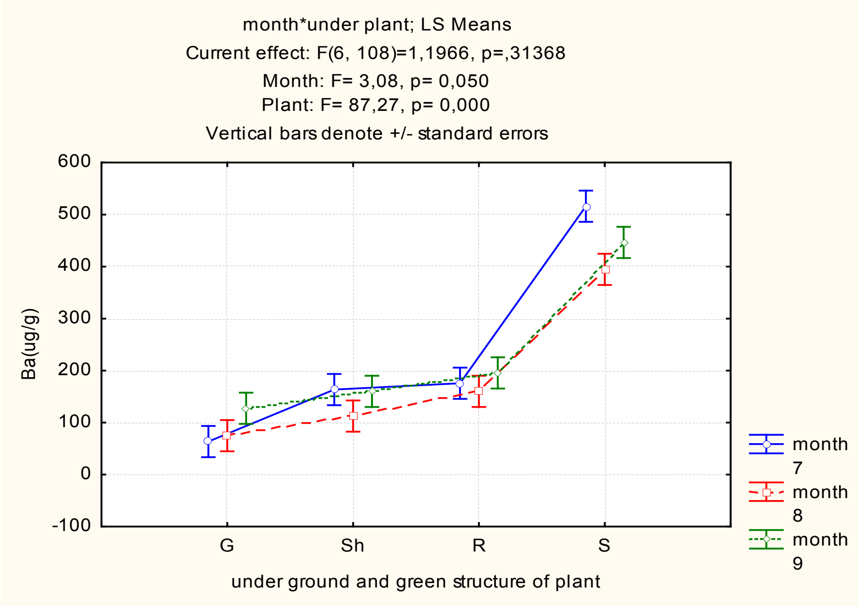

Fig. 15. Barium was found mainly in soil, whereas the plant did not absorb it much into the roots. (G – green medium and apical part of the plant, Sh – sheath, R – roots, S – soil).

of 0.03-1.4%. Its average abundance in the Earth's crust is 0.4-0.6%. Titanium exhibits variable valences, but in minerals occurs mainly in the tetravalent oxidation state as a major component of oxides, titanates, and silicates (Kabata-Pendias and Mukherjee 2007).

Conclusion

Our research started with sampling in July 2016 and concluded in September 2016, as the sample areas became covered by snowfall. Over those three months we collected 30 plants of *Juncus trifidus* from 10 collection sites. In all, there were 120 samples to analyse, as each plant was divided into into 4 parts (aboveground green part, green part, roots and soil). It was necessary to divide the plants into 4 parts to determine in which part there was the greatest concentration of toxic substance. This made it easier for us to determine how these substances may have gotten into the body of the plant.

Our aim was to prove the existence of specific toxic elements that possibly occur in plants of *Juncus trifidus* and to try to determine their mechanism of occurence. We proved the presence of nineteen elements altogether. Discovery of these elements in the ecosystem helped us to understad how vulnerable this ecosystem is, even when it appears untouched by humans at first glance.

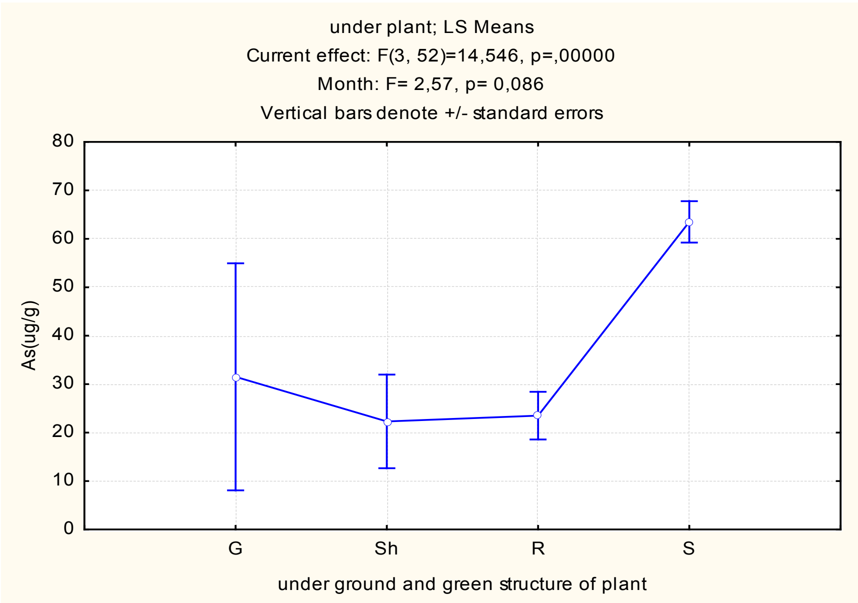

Fig. 16. Accumulation of arsenic in the soils (S), roots (R), sheaths (Sh) and green (G) above ground parts of *Juncus trifidus* (for detailed explanation see text).

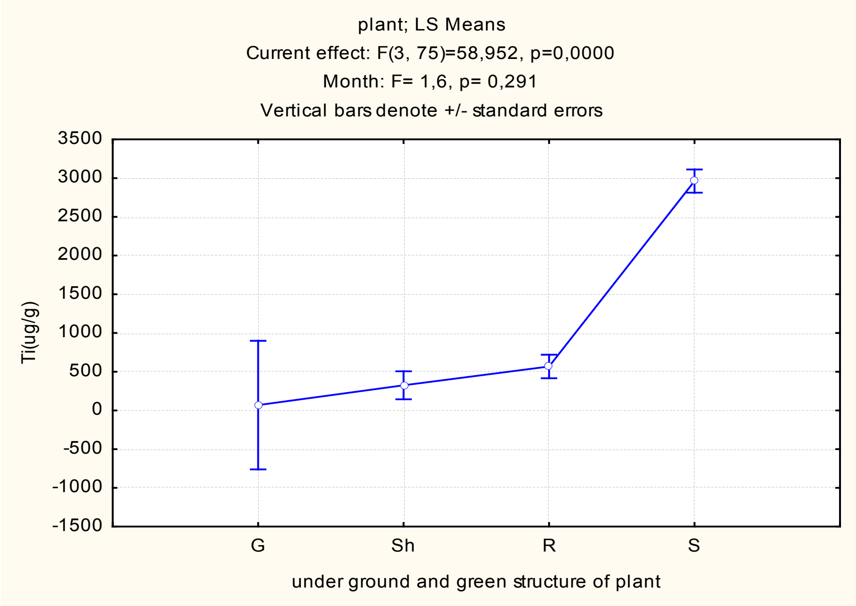

Fig. 17. Accumulation of titanium in the soils (S), roots (R), sheaths (Sh) and green (G) above ground parts of *Juncus trifidus* (for detailed explanation see text).

As previously mentioned, our research coincided with research on toxic substances and the snow vole (*Chionomys nivalis*). As *Juncus trifidus* forms part of its diet, it is reasonable to infer that toxic substances may enter its body through this plant. the Presence of specific elements helped us to better imagine the way these move in an ecosystem, which part of the plant absorbs the highest quantity of which elements, and which elements are important for the plant.

It would be valuable to continue this research and resume collection early in the spring (2017) in order to establish comparative analysis, which could lend weight to our theories regarding mechanism of occurence of these toxic elements and possibly lead to steps toward prevention of the accumulation of these toxic elements in Dolina Bielej vody Valley.

Acknowledgements

We would like to thank Simona Grešíková for a great help at work in the laboratory and also thank Mgr. Lucia Bobková for the professional translation. The research was supported by project ITMS (Grant No. 26210120006).

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Received 24 September 2017; accepted 20 January 2018.