Oecologia Montana 2022, **31**, 48-57

Chemical elements accumulated in the mosses (*Sphagnum* sp., *Hylocomium splendens*, *Palustriella commutata*) collected in the Belianske Tatra Mountains

L. LACKOVIČOVÁ and J. SOLÁR

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

Abstract. Mosses are often used for the analysis of chemical elements and air pollutants, due to their high ability to accumulate in tissues. In this study, we focused on moss species such as *Palustriella commutata*, *Hylocomium splendens* and *Sphagnum* sp. These species can be suitable indicators of the accumulation of selected elements such as S, Cl, K, Ca, Ti, Cr, Mn, Fe, Cu, Zn, Rb, Sr, Zr, Mo, Cd, Sb, Ba and Pb, due to their wider occurrence (water environment, decaying wood, waterlogged soil) in the Tatras. We collected a total of 278 samples (*Hylocomium splendens* - 76, *Palustriella commutata* - 115 and *Sphagnum* sp. - 87) at six locations (1083-1566 m a. s. l.), at regular monthly intervals during two years between October 2019 and December 2021. Individual elements were measured by an XRF spectrometer and levels of Cd by AAS. The results confirmed the difference in accumulation between species, wherein *Palustriella commutata* accumulated the most elements. The elements S, K and Ca, which are the main plant nutrients, showed the highest values. Differences between sites were also confirmed, which can be influenced by many factors, including rainfall, geology, and altitude, whereas Cd exhibited a clear positive cumulation trend with altitude. Significant seasonal differences were confirmed only in the species *Hylocomium splendens* and *Sphagnum* sp. which can be considered a suitable species for tracking seasonality and the cycle of elements in the ecosystem. Levels of Cl and K were the highest in summer, and Fe, Pb and Zn gradually accumulated during the spring and summer. The lowest values were measured in fall (Zn and Pb) and winter (Cl, Ti, and Cr).

Key words: chemical elements, heavy metals, mosses, Tatra mountains

Introduction

Interactions between humans and natural components represent an open system that is constantly evolving, so it is important to monitor. Generally, as urbanization and industrialization have increased, emissions from anthropogenic activities have increased as well. Mountain ecosystems are vulnerable and can be more exposed to pollution due to their barrier effect on air masses and the higher amounts of precipitation transported over great distances (Miśkowiec 2022). Though mountain ecosystems are protected (national parks), it is impossible to shield them from anthropogenic influences that occur outside of this specific region (Maňkovská *et al.* 2003; Bodiš *et al.* 2011). The biggest anthropogenic pollution sources in the atmosphere or on the ground include mining, burning fossil fuels, metallurgical industries, coal ash deposits, traffic deposits, and the application of chemicals such as fertilizers and pesticides in agricultural activities (Sandeep *et al.* 2019). However, the most dangerous pollutants are heavy metals (HM), which are mostly classified into non-essential and essential heavy metals based on their influence on organisms. When these elements increase beyond their allowed limits, they become harmful to organisms. Some heavy metals such as Cu, Co, Fe, Mn, Mo, Ni and Zn are essential for biota (Wintz *et al.* 2002) and are thus classified as essential micronutrients (Baker *et al.* 2020). On the other hand, elements like As, Cd, Cr, Hg, and Pb are considered non-essential and can be potentially toxic to plants. Additionally, these metals can be harmful to the ecosystem as a whole as they persist in the food chain for a long time (Szyczewski *et al.* 2009). Other elements in the environment like calcium, potassium and sulphur are also essential nutrients for plant life (Tripathi *et al.* 2014), but they can also be connected to pollution (Šoltés *et al.* 2014).

Due to unique morphological and physiological characteristics, species of the Bryopsida class have been found to be quite effective in both absorbing HM and monitoring the environment (Brown and Buck 1985). They are helpful in investigation of biomonitoring of various environmental characteristics due to their varying sensitivity to airborne pollutants (Ruhling and Tyler 1970). Mosses have a high ability to absorb and retain heavy metals through rainfall as well as dry deposition. As they lack a root system or skin layer, mineral absorption occurs throughout their whole surface area (Rfihling and Tyler 1968). There are many studies focused on the use of mosses in biomonitoring chemical elements worldwide (Frontasyeva *et al.* 2004; Bing *et al.* 2019; Oishi 2022) as well as in Europe specifically (Achotegui-Castells *et al.* 2013; Vuković *et al.* 2015; Kłos *et al.* 2018; Shetekauri *et al.* 2018).

Since 1990, several European countries have used a moss biomonitoring technique on a regular basis every 5 years (Frontasyeva *et al.* 2014; Harmens *et al.* 2015; Schröder *et al.* 2016). Results showed that mosses with higher heavy metal loads are in Eastern and South-eastern Europe (Harmens *et al.* 2013, 2015). These studies are not limited to individual moss families (Bing *et al.* 2019) but the most investigated chemical elements are As, Cd, Cr, Pb, Sb, Zn. Mosses are often used to study atmospheric deposition in Mountain environments (Gerdol 2002; Frontasyeva *et al.* 2004; Shetekauri *et al.* 2015; Oishi 2022; Bozau and Zupančič 2019). In the Tatra National Park, studies on concentrations of elements present in mosses have also been conducted (Šoltés 1992; Samecka-Cymerman *et al.* 2007; Barančoková *et al.* 2009; Šoltés and Gregušková 2013; Maňkovská *et al.* 2017; Korzeniowska *et al.* 2021).

In this study we analysed seasonal accumulation of elements (S, Cl, K, Ca, Ti, Cr, Mn, Fe, Cu, Zn, Rb, Sr, Zr, Mo, Cd, Sb, Ba and Pb) in three species of Bryophyta (*Palustriella commutata*, *Hylocomium splendens* and *Sphagnum* sp.) in the Belianske Tatra Mountains, Slovakia. Hypothesize that species experience differing abilities to accumulate elements, due to the influence of water environment, decaying wood, waterlogged soil, or elevation; and that accumulation of elements is seasonally different.

Material and Methods

Investigated moss species and study sites

For purpose of this study, we focused on three moss species: *Palustriella commutata*, *Hylocomium splendens* and *Sphagnum* sp., which are easily identifiable, commonly widespread in the study area, and representatives of different environmental conditions, including water environment, decaying wood, and waterlogged soil.

The research was done in six locations starting with the Javorova valley in the Tatra Mountains. Javorinka stream continues through Meďodolský potok stream in the Zadné Meďodoly valley to the shore of tarn Kolové pleso (Fig. 1 and 2). The research sites were carefully chosen according to the presence of the investigated species in areas with diverse geology and terrain. This allowed us to element accumulation in relation to different environments.

First location – L1 (N49.2503061°, E20.1560308°) is situated in Javorova valley, in montane zone at 1,083 m a.s.l., and *Palustriella commutata*, grows mainly in the stream on wet rocks. In the second location – L2 (N49.2435478°, E20.1614089°) at 1,134 m a.s.l. the stream bed is wider and *Hylocomium splendens* can be found on decaying wood, while *Palustriella commutata* is found on wet rocks. The third location – L3 (N49.2421589°, E20.1627122°) at 1,148 m a.s.l. is a spring with waterlogged soil. All three types of moss can be found here. The fourth location – L4 (N49.2360147°, E20.1802686°) at 1,325 m a.s.l. is situated in a dry, dead forest (caused by bark beetle) near Meďodolský stream. In this area all species of studied moss can be found. The fifth location – L5 (N49.2338058°, E20.1912122°) at 1,390 m a.s.l. is a spring, but the occurrence of species is different, with only *Palustriella commutata* present. The sixth location – L6 (N49.2205692°, E20.1927411°) in the alpine zone at 1,566 m a.s.l. is on the shore of Kolové pleso lake, where *Sphagnum* sp. can be found.

Sampling and processing

Moss species were collected monthly from October 2019 until December 2021. All samples were collected in plastic bags. The total number of samples collected was 278, including 76 samples of *Hylocomium splendens*, 115 samples of *Palustriella commutata* and 87 samples of *Sphagnum* sp.

In the laboratory, samples were cleaned with water to remove debris (e.g., needles, soil, sand, rocks and other natural materials), then stored in Petri dishes to dry at room temperature. The green, upper parts of the plants were used and the rest was removed. Every sample was milled into powder using a Cryomill (Retsch GmbH, Germany) for 40 seconds at 30 Hz. For non-destructive analysis of chemical elements an ED-XRF spectrometer DELTA Professional with XRF WorkStation was used (Olympus, Innov-X, Woburn, Massachusetts, USA). Every sample was placed in a plastic vial in a thin layer of 1 cm, measured three times for five minutes and then averaged. The results were in ppm (parts per million) units. The study was focused on the following elements: S, Cl, K, Ca, Ti, Cr, Mn, Fe, Cu, Zn, Rb, Sr, Zr, Mo, Cd, Sb, Ba and. Pb. As well as P, Co, Ni, As, Se, Ag, Sn and Hg but their concentrations were under the detection limits. Additionally, samples of *Sphagnum* sp. from locations L2, L4 and L6 were selected for measuring of Cd by Atomic Absorption Spectrometry. This procedure included the Microwave Digestion System MARS 6 (CEM Corporation, USA). Samples were weighed in PTFE containers and sample weights varied from 0.0995

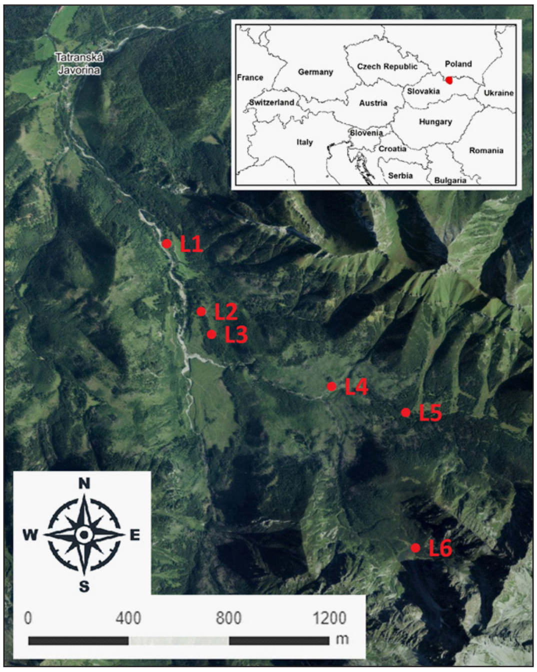

Fig. 1. Study sites in northeast part of Tatra Mountains (Source: GKÚ 2022).

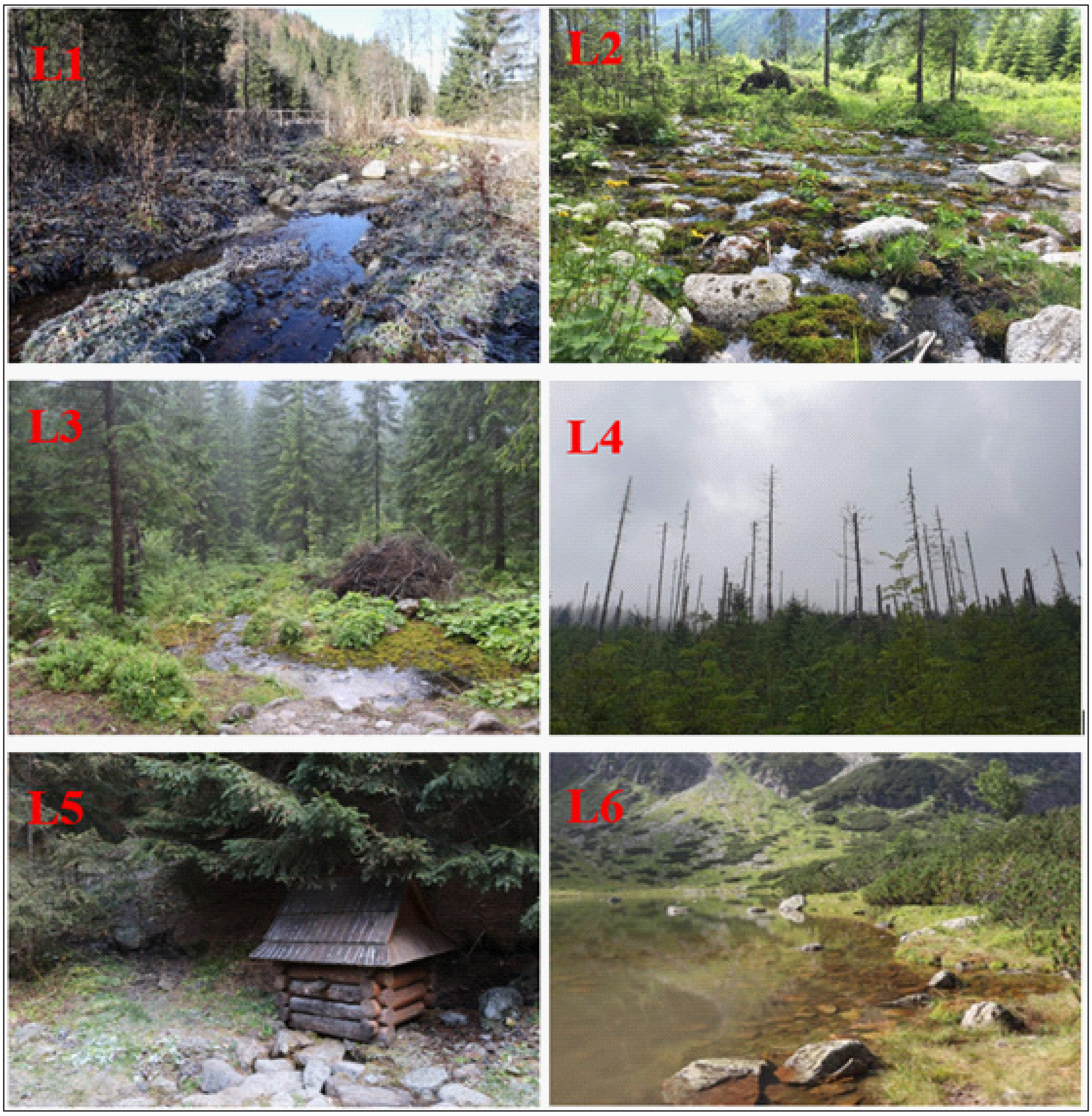

Fig. 2. Study sites. L1 and L2 are sites in valley Javorova dolina. L2- L5 are sites in valley Zadné Meďodoly. L6 is in valley Kolová dolina (Foto: L. Lackovičová, 2022).

g to 3.050 g depending on availability. Next we added 10 ml of 65% HNO_3 and left it to dissolve over a second day in closed PTFE containers. The following day the PTFE containers were stored in the Microwave at 200 °C at 800 psi for 15 minutes, then the temperature slowly decreased for another 15 minutes. Samples were diluted with ultrapure water (Elga PureLab, VWS Deutschland, Germany) to 50 ml. Samples were measured in AAS ZEEnit 650 (Analytik Jena, Germany). Certified reference material Polish Virginia Tobacco Leaves (INCT-PVTL-6) was used as an analytical control. All laboratory glassware and equipment were cleaned in HNO_3.

For statistic evaluation we used Statistica 8 (StatSoft, USA). According to the Shapiro–Wilk normality test, our data did not have normal distribution. Therefore, we used nonparametric statistical methods (such as the Kruskal–Wallis H test (K-W H)) for estimation of accumulation differences between mosses, sites, and seasons. Individual differences for pairs of mosses were calculated using the Mann-Whitney U test (M-W U).

Results

Accumulation differences in investigated mosses

Differences in the accumulation of some elements (except Mn, Cu, Zn and Cd) in *Palustriella commutata*, *Hylocomium splendens* and *Sphagnum* sp. were confirmed (Table 1). Generally, *Palustriella commutata* represented the species in which the elements accumulate the most. The following elements were found to be significantly different in mosses: S, Cl, K, Ca, Ti, Cr, Fe, Rb, Sr, Sb, Ba and Pb. The only element deemed to be non-significant was Mo. The highest mean levels of S were measured in *Sphagnum* (3 098.59 mg/kg), Cl in *Palustriella* (1 526.30 mg/kg), K in *Sphagnum* (16 567.87 mg/kg), Ca in *Palustriella* (42 021.23 mg/kg), Ti in *Palustriella* (470 mg/kg), Cr in *Hylocomium* (176.16 mg/kg), Fe in *Palustriella* (4858.62 mg/kg), Rb in *Sphagnum* (12.57 mg/kg), Se in *Palustriella* (54.86 mg/kg), Sb in *Hylocomium* (20.79 mg/kg), Ba in *Palustriella* (233.35 mg/kg)

Elements	Species	N	Mean	SE	K-W H	K-W H p - value	M-W U 1	M-W U 2
S	*Sphagnum*	87	3098.59	102.72	10.4235	0.0055		
	Palustriella	115	2757.72	105.47			***	
	Hylocomium	76	2948.13	96.45			-	**
Cl	*Sphagnum*	87	1233.47	78.85	70.1127	0.0001		
	Palustriella	115	1526.30	96.41			**	
	Hylocomium	72	691.62	52.87			***	***
K	*Sphagnum*	87	16,567.87	559.94	46.0257	0.0001		
	Palustriella	115	12,117.30	475.06			-	
	Hylocomium	76	12,600.92	516.68			***	-
Ca	*Sphagnum*	87	14,725.13	862.88	179.3915	0.0001		
	Palustriella	115	42,021.23	1813.71			-	
	Hylocomium	76	14,593.28	599.13			-	***
Ti	*Sphagnum*	13	122.77	20.82	49.4542	0.0001		
	Palustriella	90	470.00	36.34			***	
	Hylocomium	34	125.06	12.02			-	***
Cr	*Sphagnum*	87	111.44	4.57	23.9042	0.0001		
	Palustriella	115	155.82	9.74			**	
	Hylocomium	76	176.16	11.06			***	***
Fe	*Sphagnum*	87	1024.57	58.76	102.4834	0.0001		
	Palustriella	115	4858.62	404.05			-	
	Hylocomium	76	1640.87	77.88			***	***
Rb	*Sphagnum*	87	12.57	0.34	88.3172	0.0001		
	Palustriella	115	11.30	0.33			***	
	Hylocomium	76	8.37	0.19			***	***
Sr	*Sphagnum*	84	22.51	3.52	141.8056	0.0001		
	Palustriella	115	54.86	2.72			-	
	Hylocomium	74	8.82	0.54			-	***
Mo	*Sphagnum*	14	1.08	0.02	4.0284	0.1334		
	Palustriella	35	1.17	0.02			*	
	Hylocomium	21	1.14	0.03			-	-
Sb	*Sphagnum*	23	18.43	0.59	12.1425	0.0023		
	Palustriella	38	16.87	0.51			***	
	Hylocomium	14	20.79	1.31			-	***
Ba	*Sphagnum*	87	204.77	12.27	10.6571	0.0049		
	Palustriella	115	233.35	9.84			***	
	Hylocomium	76	184.54	4.38			-	***
Pb	*Sphagnum*	86	18.16	0.47	12.0370	0.0024		
	Palustriella	113	20.85	0.59			***	
	Hylocomium	75	18.29	0.44			-	***

Table 1. Differences of elements accumulation in mosses collected from Tatra Mountains (the highest mean values in bold). K-W H – Kruskal-Wallis H test; significant values $p < 0.05$ in bold; M-W U – Mann-Whitney U Test *** $p < 0.01$; ** $p < 0.03$; * $p < 0.05$; SE – standard error of mean, 1 – *Sphagnum*; 2 – *Palustriella*.

and Pb in *Palustriella* (20.85 mg/kg). Significant differences of accumulation between individual pairs of species were confirmed in Cl, Cr and Rb. Significant differences between *Palustriella* and *Sphagnum* were found with S, Ti, Sb, Ba and Pb. Significant differences between *Palustriella* and *Hylocomium* were found with S, Ca, Ti, Fe, Sr, Sb, Ba and Pb. Significant differences between *Sphagnum* and *Hylocomium* were only found with K and Fe. We can conclude that the most differences were found in accumulation levels in *Palustriella* compared to either *Hylocomium* or *Sphagnum*.

Accumulation site differences

The differences between sites were assessed individually for each species (Table 2). The significant site differences for all investigated moss species were in levels of Mn, Zn and Rb. Elements such as S and Mo did not vary in accumulation based on site. *Palustriella* was the species with the most elements that were different by site. *Hylocomium* differed in its accumulation of K, Cr, Mn, Zn, Rb and Sb based on the site. Whereas *Sphagnum* showed differentiation between sites in accumulation of Cl, Ca, Mn, Zn, Rb, Sr, Ba and Pb. Additional measurements of Cd by AAS in selected *Sphagnum* samples from locations L2, L4 and L6 confirmed significant differences at those sites (Fig. 3 A). The lowest mean value was in L2, and the highest mean value in L6. Thus, it is evident that Cd values in *Sphagnum* increase with increasing altitude. A comparable situation was not observed in the case of Pb (Fig. 3 B). *Hylocomium* cumulated a higher level of Pb in lower-altitude sites (L1-2), and *Palustriella* at site L4. *Sphagnum* had the highest mean value of Pb at L6 and the lowest at L4.

Seasonal trends

Seasonal differences were also assessed individually for each species (Table 3). Significant differences in element accumulation was not found in *Palustriella*. On the other hand, *Hylocomium* and *Sphagnum* showed significant seasonal differences of element accumulation with respect to K, Fe and Zn (Fig. 4 A and 5A, B). *Sphagnum* had significant variation in Cl, and *Hylocomium* showed significant variation in Ti, Cr and Pb.

Seasonal mean concentrations of K were higher in *Sphagnum* during all seasons (Fig. 4 A). Values decreased between fall and spring both for *Sphagnum* and for *Hylocomium*. Between spring and summer, all mean concentrations increased again, which is why the values were highest in summer for both species. The mean Cl concentrations in *Sphagnum* appeared highest during summer, gradually decreased in autumn, and the lowest mean levels were measured in winter. In the spring, their mean level rose again (Fig. 4 B). The mean concentrations of Fe were higher in *Hylocomium* in all seasons (Fig. 5 A). *Sphagnum* has the lowest values in autumn, the concentration gradually increases from autumn to spring, and decreases again in summer. *Hylocomium* has the lowest value in winter, the mean concentration increases during spring and is the highest in summer. The trend of the mean concentration of Zn during the seasons is relatively the same for both monitored species (Fig. 5 B). For both, the lowest value occurred in the fall, the concentration increased during the winter, and the values were the highest in the spring. During the summer, Zn values decreased again in both cases. The mean concentrations of Ti in *Hylocomium* show that the highest values occurred during spring and gradually decreased, with the lowest value present in winter (Fig. 6 A). The mean values of Cr in *Hylocomium* show that Cr accumulates the most during summer, decreases in autumn and has the

Elements	*Sphagnum* sp.	*Palustriella commutata*	*Hylocomium splendens*
S	0.1688	0.2020	0.8031
Cl	**0.0007**	**0.0001**	0.9951
K	0.0602	**0.0001**	**0.0062**
Ca	**0.0001**	**0.0001**	0.4010
Ti	0.3084	**0.0001**	**0.0152**
Cr	0.2821	**0.0001**	**0.0001**
Mn	**0.0001**	**0.0001**	**0.0014**
Fe	0.1673	**0.0001**	0.5909
Cu	1.0000	**0.0326**	0.0768
Zn	**0.0001**	**0.0001**	**0.0484**
Rb	**0.0001**	**0.0001**	**0.0196**
Sr	**0.0001**	**0.0001**	1.0000
Zr	1.0000	**0.0033**	0.3424
Mo	0.5300	0.1332	1.0000
Sb	0.3999	0.1252	**0.0244**
Ba	**0.0001**	**0.0001**	0.0709
Pb	**0.0189**	**0.0001**	1.0000
Cd AAS	**0.0001**	-	-

Table 2. Site differences of elements accumulation in mosses collected from Tatra Mountains. Values represent level p of Kruskal-Wallis H test; significant values $p < 0.05$ in bold.

Elements	*Sphagnum* sp.	*Palustriella commutata*	*Hylocomium splendens*
S	0.1248	0.9286	0.2083
Cl	**0.0474**	0.3645	0.1765
K	**0.0019**	0.2277	**0.0015**
Ca	0.2526	0.4332	0.7102
Ti	0.3305	0.1448	**0.0093**
Cr	0.0709	0.2446	**0.0013**
Mn	0.1709	0.2869	0.6872
Fe	**0.0051**	0.8800	**0.0005**
Cu	-	0.7025	0.2567
Zn	**0.0199**	0.9814	**0.0022**
Rb	0.3885	0.7787	0.6281
Sr	0.6810	0.3352	0.4059
Zr	-	0.5469	-
Mo	0.2063	0.1522	0.3952
Sb	0.3163	0.2433	0.3096
Ba	0.3471	0.4751	0.2145
Pb	0.2341	0.9984	**0.0336**
Cd AAS	0.4084	-	-

Table 3. Seasonal differences of elements accumulation in mosses collected from Tatra Mountains. Values represent level p of Kruskal-Wallis H test; significant values $p < 0.05$ in bold.

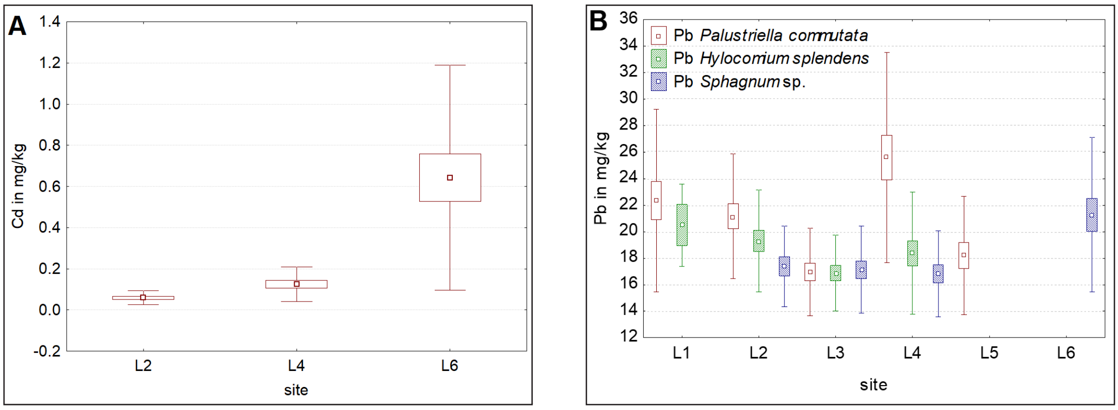

Fig. 3. **A** - Concentrations of Cd (in mg/kg) in *Sphagnum* sp. in relation to study sites (L2, 4 and 6). K-W H (2, 62) = 23.2501, p = 0.0001; **B** - Concentrations of Pb (in mg/kg) in *Palustriella commutata* (K-W H (4, 113) = 30.3965, p = 0.0001), *Hylocomium splendens* (K-W H (2, 71) = 5.2944, p = 0.0709) and *Sphagnum* sp. (K-W H (4, 113) = 30.3966, p = 0.0001) in relation to study sites (L1-L6). Point: Mean; Box: Standard error of mean; Whisker: Standard deviation.

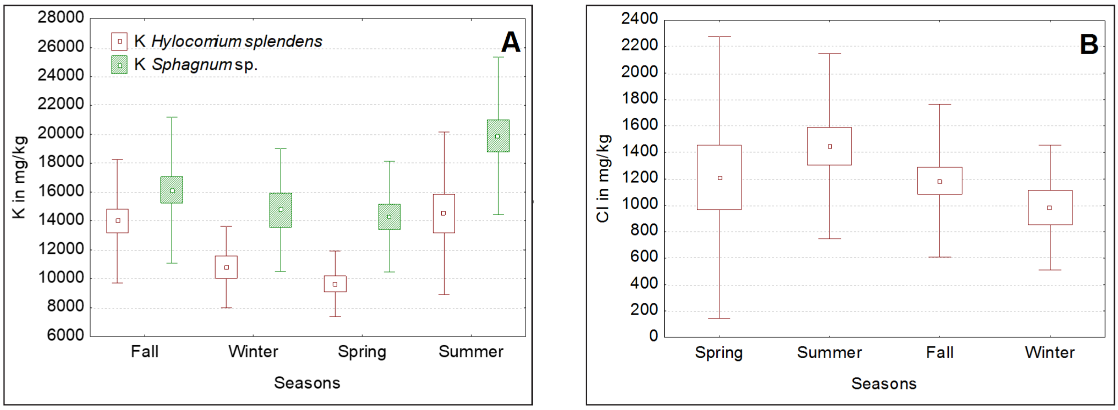

Fig. 4. A - Concentrations of K (in mg/kg) in *Hylocomium splendens* (K-W H (3, 76) = 15.4215, p = 0.0015) and *Sphagnum* sp. (K-W H (3, 87) = 14.9255, p = 0.0019) depending on the seasons. **B** - Concentrations of Cl (in mg/kg) in *Sphagnum* sp. (K-W H (3, 87) = 7.9329, p = 0.0474) depending on the seasons. Point: Mean; Box: Standard error of mean; Whisker: Standard deviation.

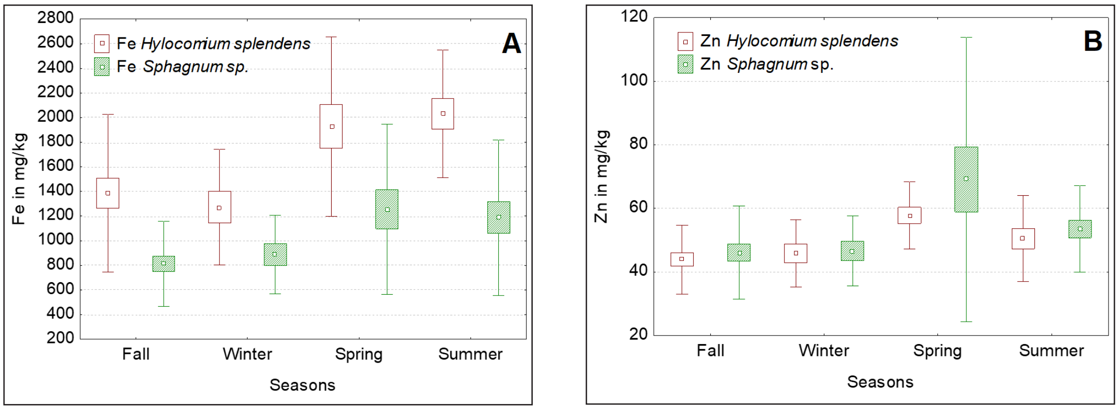

Fig. 5. **A** - Concentrations of Fe (in mg/kg) in *Hylocomium splendens* (KW-H (3, 76) = 17.8494, p = 0.0005) and *Sphagnum* sp. (K-W H (3, 87) = 12.8026, p = 0.0051) depending on the seasons. **B** - Concentrations of Zn (in mg/kg) in *Hylocomium splendens* (K-W H (3, 76) = 14.5731, p = 0.0022) and *Sphagnum* sp. (K-W H (3, 87) = 9.8529, p = 0.0199) depending on the seasons. Point: Mean; Box: Standard error of mean; Whisker: Standard deviation.

lowest values in winter (Fig. 6 B). In the spring, the concentration starts to increase again. The mean concentrations of Pb in *Hylocomium* show that lead accumulates most in spring, decreases during summer, then continues to decrease to its lowest levels in the autumns, then begins to accumulate again in the winter (Fig. 7).

Discussion

The chemical elements S, Cl, K, Ca, Ti, Cr, Mn, Fe, Cu, Zn, Rb, Sr, Zr, Mo, Cd, Sb, Ba and Pb were measured in this study. The highest mean values were measured for calcium (Ca), potassium (K) and sulphur (S). These elements are among the essen-

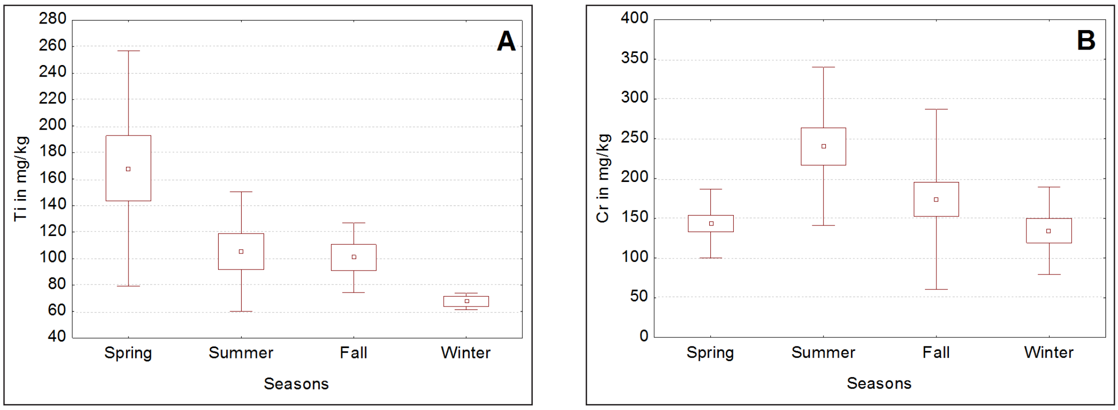

Fig. 6. A - Concentrations of Ti (in mg/kg) in *Hylocomium splendens* (K-W H (3,34) = 11.5085, $p = 0.0093$) depending on the seasons. **B** - Concentrations of Cr (in mg/kg) in *Hylocomium splendens* (K-W H (3,76) = 15.6931, $p = 0.0013$) depending on the seasons. Point: Mean; Box: Standard error of mean; Whisker: Standard deviation.

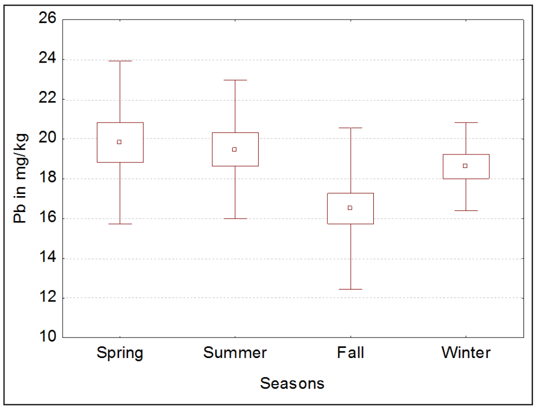

Fig. 7. Concentrations of Pb (in mg/kg) in *Hylocomium splendens* (K-W H (3, 75) = 8.6948, $p = 0.0336$) depending on the seasons. Point: Mean; Box: Standard error of mean; Whisker: Standard deviation.

tial nutrients of plants (Tripathi *et al.* 2014) and that is why they can occur in such enormous quantities. Similarly, high values of these elements were also recorded in studies by Šoltés and Gregušková (2013) and Tuchyňa (2022). The study by Šoltés and Gregušková (2013) that the highest concentrations occurred in 1-year leaves, which in Bryophyta grow over the years. On the other hand, the concentration of these elements in stems were highest in the first year and then lowered. Tuchyňa (2022) found that algae in aquatic environments had a growing concentration of Ca over the course of the years. In our samples of *Palustriella*, an aquatic moss, we recorded a similar phenomenon of high calcium concentrations. Concentration of Ca and K are can be predicted by water chemistry (Hájek *et al.* 2014) due to bedrock. The Belianske Tatra Mountains are mainly composed of limestone and dolomite (Sedláková 2008) and these rocks are rich in Ca and K (Bodiš *et al.* 2011). *Hylocomium* did not exhibit site differences as it was collected mainly on dead and decaying wood (Andersson and Hytteborn 1991) where there is no connection with bedrock.

Potassium (P) levels showed significant differences between sites in both *Palustriella* and *Hylocomium*. Among the main nutrients, P is mainly present in soil. According to Kobza *et al.* (2008) there is about 2% of potassium in rendzinas. The amount of P in the soil also depends on its thickness and overall soil moisture. In general, the wetter the soil is, the higher a plant's ability to absorb P (Mouhamad *et al.* 2016). Likewise, significant differences were also shown in seasonal trends in *Sphagnum* and *Hylocomium*. This may be influenced by the mobility of the element from the soil to the moss and its water solubility in the spring. In the spring, there is a larger amount of water in the mountains from melting snow and spring precipitation, and it is because of this that P could have been washed out and stored during the summer in the intercellular space. Experimental studies (Vázquez *et al.* 1999, 2000; Figueira and Ribeiro 2005) show that intracellular accumulation of P is higher than extracellular in mosses and decreases in more acidic environments with higher metal content. Significantly different P accumulations within seasonality with an increase during summer coincide with the study of Bargagli *et al.* (1995), where element values were compared in *Hylocomium splendens* in Italy and Antarctica. In *Sphagnum* sp., seasonal differences could also be influenced by the physiological status of plant parts (ageing segments), as older segments lose most of their P accumulation (Hájek and Adamec 2009).

Sulphur (S) accumulated the most in *Sphagnum* sp. Seasonal differences and site differences were not noted, but it accumulated differently between individual moss species. There is a strong assumption that *Sphagnum* sp. can accumulate more S because it can absorb nutrients from water, sludge, and soil, since it grows in waterlogged locations. In the past, high S concentrations were recorded from emissions in the Tatras, as reported by Šoltés *et al.* (2014). Along with heavy metals, S remains in the ecosystem for an exceptionally long time and their permissible values are still exceeded. S emissions are often a result of industrial activity such as metallurgy and engineering production. In *Palustriella*, S concentrations were the lowest as it occurs in an aquatic environment that is constantly dynamic and can wash out S from its tissue structures.

Zinc (Zn) came out to be significantly different between locations in all types of moss. The seasonal assumption was confirmed for *Sphagnum* and *Hylocomium*, where in both cases the lowest value was in autumn and the highest was in spring. It is possible that the increase in the Zn level was influenced by temperature. Comparable results were reported in a study by Martins *et al.* (2004), which compared Zn levels in samples of *Fontinalis antipyretica*. The moss showed higher levels of Zn with increasing temperature and the maximum value reached was at 30°C. It is possible that *Palustriella* did not show this trend because the aquatic environment provides a stable and dynamic place where deposits do not accumulate. It can also be influenced by the difference in locations and how much Zn is in the environment. Low precipitation rates can decrease levels of Zn in environments and mosses. Similar results were recorded in Chopok during the years 1987-2009, where the analysis of Zn concentration in precipitation revealed a significant decreasing trend (Sitár *et al.* 2016). Based on a study by Čeburnis *et al.* (1999), it was found that Zn levels are not as affected by air pollution and dust particles as leaching from the canopy of trees, whereas mosses take up trace metals from soil through several pathways, including upward capillary movement of soil water, raindrops, and windblown soil particles (Bargagli *et al.* 1995). In a study by Say *et al.* (1981), the highest Zn amounts were found in mosses collected downstream. The relationship between Zn and altitude was also confirmed by Šoltés *et al.* (2014).

Iron (Fe) is a micronutrient necessary for metabolic activities (Nagajyoti *et al.* 2010). Significant differences in Fe accumulation between sites were found in *Palustriella*, while significant differences in terms of seasonality were confirmed in *Sphagnum* and *Hylocomium*. Compared to the study of Šoltés and Gregušková (2013), which compared one-year leaves and older leaves of the moss *Polytrichum commune*, the values measured in *Palustriella* were notably higher. Fe can also be related to the geological bedrock, with aquatic environment likely to be richer in Fe. Samples from sites L1, L4 and L5 were collected directly at the mountain stream. The differences between these sites are mostly attributed to altitude. Based on the results, Fe values in *Palustriella* gradually increased from L5, L4 to L1, from which it can be assumed that the lower-altitude the location, the more iron-rich the water is. Although there are mainly limestones here, which are characterized by a lower Fe content, in the Belianske Tatras there is a Carpathian Keuper layer, which is rich in Fe, as observed by Barančoková *et al.* (2009). In a study that points to the content of Fe in sediments in Javorova dolina valley (Zacher 2022), Fe concentrations appeared higher in the parts where the Meďodolský stream flowed into Javorinka. It can therefore be assumed that iron in the stream is gradually collected and washed out from higher-altitude positions to lower ones. On the other hand, the increased iron content observed by Berg and Steinnes (1997) is most likely due to dry deposition of soil particles on the mosses, instead of better uptake capability of these elements in the mosses.

The highest mean lead (Pb) value was noted in *Palustriella*. Significant site differences were noted in *Sphagnum* and *Palustriella*. In terms of seasonality, significant differences were found in *Hylocomium*. It is likely that *Hylocomium* had the lowest Pb values in autumn samples due to reduced precipitation ratios. In September and November 2020 and in September, October and November 2021, precipitation values were below the long-term normal (SHMÚ 2023), indicating that atmospheric deposition could influenced these seasonal differences. In spring, Pb concentrations were the highest, which may be due to an increase in precipitation. Seasonal differences were not reflected in the aquatic environment in *Palustriella*. The lowest Pb concentrations in *Palustriella* were recorded at sites L3 and L5, which represent a spring and a well, and thus the water is probably cleaned of deposits and atmospheric lead. The highest mean value for the highest deviations were recorded for *Palustriella*, sampled in the Meďodolský stream at a higher altitude. The effect of elevated Pb with altitude was noted by Šoltés (1992) in various moss species in Tatra Mountains. On the other hand, geological composition of parent rock could influence Pb levels in aquatic mosses as mentioned by Samecka-Cymerman *et al.* (2007). When compared to Šoltés and Gregušková (2013), the measured Pb values were lower.

According to the XRF spectrometer, cadmium (Cd) was not measured in any monitored moss due to its detection limit, but after using the AAS ZEEnit 650 absorption spectrophotometer in the additional measurement of *Sphagnum* sp. in L2, L4, L6 sites its presence was confirmed. A dependence with increasing altitude was confirmed, with L2 and L4 representing waterlogged sites with flowing water and L6 representing a site with stagnant water. The effect of elevated Cd with altitude was also observed by Šoltés (1992) in the Tatra Mountains. Thera are some indications from comparative heavy metal values from across Europe (Harmens *et al.* 2004), that elevated Cd values were influenced by industrial areas such as the Silesian Black Coal Mine and Ostrava. In Slovakia, Cd values were higher in the High Tatras and Beskydy Mountains because of the influence of industrial zones and the long-distance transmission of pollution. Concentrations of heavy metals were also increased in areas without emission sources in the Scandinavian region, which confirms long-distance transport of substances.

From the observed concentrations of metals in selected species of mosses in the Tatras, it can be concluded that they are useful and effective for monitoring for heavy metal pollution and that their impact is affected by air pollution over long distances (Harmens *et al.* 2004; Šoltés *et al.* 2014) even in areas far from the source. However, on the other hand, it is also likely that soil particles trapped in the mosses resulted in an increased concentration of elements (Bargagli *et al.* 1995). As the species used in this work are common throughout Europe (Berg *et al.* 1995; Berg and Steinnes 1997; Harmens *et al.* 2004; Cowden and Aherne 2019) the values measured can be used for further comparison.

Acknowledgements

We would like to thank doc. RNDr. R. Šoltés, CSc., who helped us with the identification of mosses samples. We would like to thank also to Mgr. T. Pitoňáková for performing analysis of Cd by AAS.

References

Achotegui-Castells, A., Sardans, J., Ribas, A. and Penuelas, J. 2013: Identifying the origin of atmospheric inputs of trace elements in the Prades Mountains (Catalonia) with bryophytes, lichens, and soil monitoring. Environ. *Monit. Assess.*, **185**: 615-629.

Andersson, L.I. and Hytteborn, H. 1991: Bryophytes and decaying wood–a comparison between managed and natural forest. *Ecography*, **14**(2): 121-130.

Baker, A.J., McGrath, S.P., Reeves, R.D. and Smith, J.A.C. 2020: Metal hyperaccumulator plants: a review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soils. In: *Phytoremediation of Contaminated Soil and Water.* (eds. N. Terry and G.S. Banuelos), pp. 85-107. CRC Press, Boca Raton, FL USA.

Barančoková, M., Barančok, P. and Mišovičová, D. 2009: Heavy metal loading of the Belianske Tatry Mts. *Ekológia (Bratislava)*, **28**(3): 255-268.

Bargagli, R., Brown, D.H. and Nelli, L. 1995: Metal biomonitoring with mosses: procedures for correcting for soil contamination. *Environ. Pollut.*, **89**(2): 169-175.

Berg, T. and Steinnes, E. 1997: Use of mosses (*Hylocomium splendens* and *Pleurozium schreberi*) as biomonitors of heavy metal deposition: from relative to absolute deposition values. *Environ. Pollut.*, **98**(1): 61-71.

Berg, T., Røyset, O. and Steinnes, E. 1995: Moss (*Hylocomium splendens*) used as biomonitor of atmospheric trace element deposition: estimation of uptake efficiencies. *Atmos. Environ.*, **29**(3): 353-360.

Bing, H., Wu, Y., Li, J., Xiang, Z., Luo, X., Zhou, J., Sun, H. and Zhang, G. 2019: Biomonitoring trace element contamination impacted by atmospheric deposition in China's remote mountains. *Atmos. Res.*, **224**: 30-41.

Bodiš, D., Rapant, S., Khun, M., Klukanová, A., Lexa, J., Mackových, D., Marsina, K., Pramuka, S. and Vozár, J. 2011: Geochemický atlas Slovenskej republiky, časť VI: Riečne sedimenty. (Geochemical atlas of the Slovak Republic. Part VI Stream sediments). Ministerstvo životného prostredia Slovenskej republiky, Štátny geologický ústav Dionýza Štúra, Geologická služba Slovenskej republiky, Bratislava. Online: http://apl.geology.sk/atlasrs (retrieved 22.9.2022).

Bozau, E. and Zupančič, N. 2019. Trace and rare earth elements of *Sphagnum* mosses from the Upper Harz Mountains (Germany). *Geophys. Res. Abstr.*, **21**: 1-1.

Brown, D.H. and Buck, G.W. 1985: The cellular location of metals in two bryophytes and a lichen. *Cryptogamie. Bryologie, lichénologie*, **6**(3): 279-286.

Čeburnis, D., Steinnes, E. and Kvietkus, K. 1999: Estimation of metal uptake efficiencies from precipitation in mosses in Lithuania. *Chemosphere*, **38**(2): 445-455.

Cowden, P. and Aherne, J. 2019: Interspecies comparison of three moss species (*Hylocomium splendens*, *Pleurozium schreberi*, and *Isothecium stoloniferum*) as biomonitors of trace element deposition. *Environ. Monit. Assess.*, **191**: 1-13.

Figueira, R. and Ribeiro, T. 2005: Transplants of aquatic mosses as biomonitors of metals released by a mine effluent. *Environ. Pollut.*, **136**(2): 293-301.

Frontasyeva, M., Harmens, H., the participants of the ICP Vegetation 2014: Heavy metals, nitrogen and POPs in European mosses: 2015 survey. Monitoring Manual. Online: https://icpvegetation.ceh.ac.uk/sites/default/files/ICP%20Vegetation%20moss%20monitoring%20manual%202020.pdf (retrived 1 9. 2022).

Frontasyeva, M., Smirnov, L., Steinnes, E., Lyapunov, S. and Cherchintsev, V. 2004: Heavy metal atmospheric deposition study in the South Ural Mountains. J. *Radioanal. Nucl. Ch.*, **259**(1): 19-26.

Gerdol, R., Bragazza, L. and Marchesini, R. 2002: Element concentrations in the forest moss *Hylocomium splendens*: variation associated with altitude, net primary production and soil chemistry. *Environ. Pollut.*, **116**(1): 129-135.

Hájek, M., Plesková, Z., Syrovátka, V., Peterka, T., Laburdová, J., Kintrová, K., Jiroušek, M. and Hájek, T. 2014: Patterns in moss element concentrations in fens across species, habitats, and regions. *Perspect. Plant Ecol.*, **16**(5). 203-218.

Hájek, T. and Adamec, L. 2009: Mineral nutrient economy in competing species of *Sphagnum* mosses. *Ecol. Res.*, **24**(2): 291-302.

Harmens, H., Norris, D. and Mills, G. 2013: Heavy Metals and Nitrogen in Mosses: Spatial Patterns in 2010/2011 and Long-Term Temporal Trends in Europe. NERC/Centre for Ecology & Hydrology: Bangor, Maine.

Harmens, H., Buse, A., Büker, P., Norris, D., Mills, G., Williams, B., Reynolds, B., Ashenden, T.W., Rühling, A. and Steinnes, E. 2004: Heavy metal concentrations in European mosses: 2000/2001 survey. *J. Atmos. Chem.*, **49**: 425-436.

Harmens, H., Norris, D.A., Sharps, K., Mills, G., Alber, R., Aleksiayenak, Y., Blum, O., Cucu-Man, S-M., Dam, M., De Temmerman, L., Ene, A., Fernández, J.A., Martinez-Abaigar, J., Frontasyeva, M., Godzik, B., Jeran, Z., Lazo, P., Leblond, S., Liiv, S., Magnússon, SH., Maňkovská, B., Pihl Karlsson, G., Piispanen, J., Poikolainen, J., Santamaria, J.M., Skudnik, M., Špirić, Z., Stafilov, T., Steinnes, E., Stihi, C., Suchara, I., Thöni, L., Todoran, L., Yurukova, L. and Zechmeister, H.G. 2015: Heavy metal and nitrogen concentrations in mosses are declining across Europe whilst some "hotspots" remain in 2010. *Environ. Pollut.*, **200**: 93-104.

Harmens, H., Norris, D.A., Sharps, K., Mills, G., Alber, R., Aleksiayenak, Y., Blum, O., Cucu-Man, S.M., Dam, M., De Temmerman, L. and Ene, A. 2015: Heavy metal and nitrogen concentrations in mosses are declining across Europe whilst some "hotspots" remain in 2010. *Environ. Pollut.*, **200**: 93-104.

Kłos, A., Ziembik, Z., Rajfur, M., Dołhańczuk-Śródka, A., Bochenek, Z., Bjerke, J. W., Tømmervik, H., Zagajewski, B., Ziółkowski, D., Jerz, D., Zielińska, M.,Krems, P., Godyń, P., Marciniak, M. and Świsłowski, P. 2018: Using moss and lichens in biomonitoring of heavy-metal contamination of forest areas in southern and north-eastern Poland. *Sci. Total Environ.*, **627**: 438-449.

Kobza, J., Barančíková, G., Bezáková, Z., Dodok, R., Hrivňáková K., Makovníková, J., Styk, J. and Širáň, M. 2008: Tvorba a hodnotenie poznatkov o vývoji vlastností pôdneho krytu SR pre efektívnu ochranu pôdy v poľnohospodárskej krajine. Priebežná správa za rok 2008. Výskumný ústav pôdoznalectva a ochrany prírody, Bratislava, Slovakia.

Korzeniowska, J., Krąż, P. and Dorocki, S. 2021: Heavy Metal Content in the Plants (*Pleurozium schreberi* and *Picea abies*) of Environmentally Important Protected Areas of the Tatra National Park (the Central Western Carpathians, Poland). *Minerals*, **11**(11): 1231-1248.

Maňkovská, B., Florek, M., Frontasyeva, M.V., Ermakova, E., Oprea, K. and Pavlov, S.S. 2003: Atmospheric deposition of heavy metals in Slovakia studied by the moss biomonitoring technique. *Ekológia (Bratislava)*, **22**(1): 211-217.

Maňkovská, B., Izakovičová, Z., Oszlányi, J. and Frontasyeva, M.V. 2017: Temporal and spatial trends 1990-2010 of heavy metal accumulation in mosses in Slovakia. *Biyolojik Çeşitlilik ve Koruma*, **10**(2): 28-32.

Martins, R.J., Pardo, R. and Boaventura, R.A. 2004: Cadmium (II) and zinc (II) adsorption by the aquatic moss *Fontinalis antipyretica*: effect of temperature, pH and water hardness. *Water Res.*, **38**(3): 693-699.

Miśkowiec, P. 2022: The impact of the mountain barrier on the spread of heavy metal pollution on the example of Gorce Mountains, Southern Poland. *Environ. Monit. Assess.*, **194**(9): 663-677.

Mouhamad, R., Alsaede, A. and Iqbal, M. 2016: Behavior of potassium in soil: a mini review. *Chemistry International*, **2**(1): 58-69

Nagajyoti, P.C., Lee, K.D. and Sreekanth, T.V.M. 2010: Heavy metals, occurrence and toxicity for plants: a review. *Environ. Chem. Lett.*, **8**: 199-216.

Oishi, Y. 2022: Biomonitoring of transboundary pollutants using moss in Japan's mountains. *Environ. Sci. Pollut. R.*, **29**(10): 15018-15025.

Rühling, Å. and Tyler, G. 1970: Sorption and retention of heavy metals in the woodland moss *Hylocomium splendens* (Hedw.) Br. et Sch. *Oikos*, **21**(1): 92-97.

Ruhling, A. 1968: An ecological approach to the lead problem. *Botanika Notiser*, **121**: 321-342.

Samecka-Cymerman, A., Stankiewicz, A., Kolon, K. and Kempers, A.J. 2007: Self-organizing feature map (neural networks) as a tool in classification of the relations between chemical composition of aquatic bryophytes and types of streambeds in the Tatra national park in Poland. *Chemosphere*, **67**(5): 954-960.

Sandeep, G., Vijayalatha, K.R. and Anitha, T. 2019: Heavy metals and its impact in vegetable crops. *Int J. Chem. Stud.*, **7**(1): 1612-1621.

Say, P.J., Harding, J.P.C. and Whitton, B.A. 1981: Aquatic mosses as monitors of heavy metal contamination in the River Etherow, Great Britain. *Environ. Pollut. B*, **2**(4): 295-307.

Schröder, W., Nickel, S., Schönrock, S., Meyer, M., Wosniok, W., Harmens, H., Frontasyeva, M.V., Alber, R., Aleksiayenak, J., Barandovski, L., Carballeira, A., Danielsson, H., de Temmermann, L., Godzik, B., Jeran, Z., Karlsson, G.P., Lazo, P., Leblond, S., Lindroos, A-J., Liiv, S., H. Magnússon, S.H., Mankovska, B., Martínez-Abaigar, J., Piispanen, J., Poikolainen, J., Popescu, I.V., Qarri, F., Santamaria, J.M., Skudnik, M., Špirić, Z., Stafilov, T., Steinnes, E., Stihi, C., Thöni, L., Uggerud, H.T. and Zechmeister, H.G. 2016: Spatially valid data of atmospheric deposition of heavy metals and nitrogen derived by moss surveys for pollution risk assessments of ecosystems. *Environ. Sci. Pollut. R.*, **23**(11): 10457-10476.

Sedláková, B. 2008: Belianske Tatry bez svišťov. In: *Výskum a ochrana cicavcov na Slovensku VIII. Zborník referátov z konferencie (Zvolen 12.–13. 10. 2007)* (eds. M. Adamec, P. Urban and M. Adamcová), pp. 99-101. ŠOP SR, Banská Bystrica, Slovakia.

Shetekauri, S., Chaligava, O., Shetekauri, T., Kvlividze, A., Kalabegishvili, T., Kirkesali, E., Frontasyeva, M.V., Chepurchenko, O.E. and Tselmovich, V.A. 2018: Biomonitoring air pollution using moss in Georgia. *Pol. J. Environ. Stud.*, **27**(5): 2259-2266.

Shetekauri, S., Shetekauri, T., Kvlividze, A., Chaligava, O., Kalabegishvili, T., Kirkesali, E.I., Frontasyeva, M.V. and Chepurchenko, O.E. 2015: Preliminary results of atmospheric deposition of major and trace elements in the greater and lesser Caucasus Mountains studied by the moss technique and neutron activation analysis. *Annali di Botanica*, **5**: 89-95.

SHMÚ (Slovenský Hydrometeorologický Ústav) 2023: Atmosférické zrážky, Lomincký štít 2020 a 2021. Online: www.shmu.sk (retrived 20.9.2022).

Sitár, M., Hanzelová, M., Minďáš, J. and Škvarenina, J. 2016: Long-term temporal changes of precipitation quality in the mountainous region of Chopok (Low Tatras, Slovakia). In: *Mendel and Bioclimatology* (ed. J. Brzezina), pp. 306-320. Mendel Unversity and Czech Bioclimatological Society, Brno, Czech.

Šoltés, R. and Gregušková, E. 2013: Bryomonitoring of element deposition in three walleys in the Tatra Mts (Slovakia) based on X-ray spectrometry. *Oecologia Montana*, **21**(1): 15-20.

Šoltés, R. 1992: Heavy metal concentrations in the mosses of the Tatra Mountains (Czecho-Slovakia). Multivariate analysis. *Oecologia Montana*, **1**(1): 31-36.

Šoltés, R., Gregušková, E. and Šoltésová, A. 2014: Bioindication of chemical elements deposition in the high Tatra Mts (Slovakia) based on *Calluna vulgaris* (L.) Hull; comparative levels after the improvement of emissions. *Earth Environ. Sci.*, **9**(2): 5-14.

Szyczewski, P., Siepak, J., Niedzielski, P. and Sobczyński, T. 2009: Research on heavy metals in Poland. *Pol. J. Environ. Stud.*, **18**(5): 755-768.

Tripathi, D.K., Singh, V.P., Chauhan, D.K., Prasad, S.M. and Dubey, N.K. 2014: Role of macronutrients in plant growth and acclimation: recent advances and future prospective. *Improvement of Crops in the Era of Climatic Changes*, **2**: 197-216.

Tuchyňa, J. 2022: Seasonal and areal accumulation of heavy metals by algae and cyanobacteria in Javorinka mountain stream. *Oecologia Montana*, **31**(1): 1-12.

Vázquez, M.D., Fernández, J.A., López, J. and Carballeira, A. 2000: Effects of water acidity and metal concentration on accumulation and within-plant distribution of metals in the aquatic bryophyte *Fontinalis antipyretica*. *Water Air Soil Poll.*, **120**: 1-20.

Vázquez, M.D., López, J. and Carballeira, A. 1999: Uptake of heavy metals to the extracellular and intracellular compartments in three species of aquatic bryophyte. *Ecotox. Environ. Safe.*, **44**(1): 12-24.

Vuković, G., Urošević, M.A., Goryainova, Z., Pergal, M., Škrivanj, S., Samson, R. and Popović, A. 2015: Active moss biomonitoring for extensive screening of urban air pollution: Magnetic and chemical analyses. *Sci. Total Environ.*, **521**: 200-210.

Wintz, H., Fox, T. and Vulpe, C. 2002: Responses of plants to iron, zinc and copper deficiencies. B*iochem. Soc. T.*, **30**(4): 766-768.

Zacher, Š. 2022: Analysis of elements of sediments from the mountain stream Javorinka in the High Tatras. Bachelor thesis. Institute of High Mountain Biology, University of Žilina, Slovakia.

Received 28 November 2022; accepted 28 December 2022.