

Seasonal concentration changes of trace elements in selected lichen and moss in protected areas in Slovakia

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Abstract. In this study, we focused on trace element changes in the lichen *Hypogymnia physodes* and moss *Sphagnum* sp. during different seasons in two selected protected areas in the Slovak mountains, which represent a characteristic ecosystem type of *Sphagnum* spruce forests. Samples were collected during three summer and winter months. The main objective of this study was to determine the relationship of selected trace elements to the seasons and to compare these concentrations between the plant species studied, between areas (Hruštinka and Pavlova) and according to seasons. The results show that lichens accumulate lower amounts of trace elements than mosses. In most cases, pollution was shown to be higher in the winter season than in the summer season. During the winter period, we were particularly interested in sulphur, whose values were higher in the summer period at the Hruštinka site. Overall, the Pavlová site was more polluted, with trace elements mainly of anthropogenic origin.

Key words: trace elements, moss, lichen, *Sphagnum*, *Hypogymnia physodes*, pollution

Introduction

Environmental trace element pollution is a very important topic to understand (Malaspina *et al.* 2014; Pruves 2016; Chowdhury *et al.* 2018), mainly because of its far-reaching impact on ecosystems, human health and biodiversity. In general, pollutants enter the environment through a variety of pathways and sources (Horowitz *et al.* 2014; Xu *et al.* 2015). In the case of air pollution, dispersion modelling and field monitoring are widely used. However, field monitoring, which is needed also for modelling validation, requires long-term sampling at appropriate numbers of sampling areas (Wolterbeek 2002). This issue was solved using a various biomonitoring technique including bioindicators. Many studies have used mosses and lichens as very effective bio-

indicators of trace element pollution in ecosystems (Conti and Cecchetti 2001; Adamo *et al.* 2003; Basile *et al.* 2008), because mosses and lichens lack affinity to uptake trace elements from substrate (Budka *et al.* 2002; Baczewska-Dąbrowska *et al.* 2023). Studies that used mosses for bioindication mostly deal with the very effective moss-bag method (Adamo *et al.* 2003; Basile *et al.* 2008; Świsłowski *et al.* 2022). In the case of lichens, researchers combine measuring the concentrations of pollutants in native lichen (tissue) or assessing health status of lichens (Budka *et al.* 2002; Abas 2021; Yang *et al.* 2023).

Biomonitoring using mosses is based on the ability of terrestrial carpet species to take up most nutrients directly from wet and dry deposition, thus clearly reflecting atmospheric deposition. Moss species are particularly suitable for monitoring heavy metal pollution over long time scales (Čeburnis *et al.* 2002). Mosses as bioindicators can also reflect elevated concentrations of sulphur dioxide (SO₂) and other contaminants emitted to the atmosphere from both natural and anthropogenic sources (Sinha *et al.* 2021). Their properties have been investigated in many studies, including local investigations and regional surveys in different parts of the world (Čeburnis *et al.* 1999; Giordano *et al.* 2004). Other advantages of mosses include minimal morphological changes during their lifetime, sampling availability, ability to survive in highly polluted environments, and the ability to determine contaminant concentrations in annual growth segments (Čeburnis *et al.* 2002; Cenci *et al.* 2003; Poikolainen 2004; Wang *et al.* 2008; Dragovic and Mihailovic 2009). Bryophytes are resistant to many substances that are highly toxic to other plants. Due to this property, they can survive in diverse and often extreme environments. These sedentary organisms possess a wide range of physiological adaptations. Mosses have been shown to be able to survive complete desiccation and extreme temperatures up to 110 °C (Cenci *et al.* 2003; Fernandez *et al.* 2006; Dragovic and Mihailovic 2009).

Lichen is a very specific species of symbiotic organism, in which thallus consists of fungal tissue where photosynthetic algae are embedded. Precisely because of their body structure, and the fact that lichens do not have true roots but only rhizines, they rely primarily on nutrient uptake by the entire body surface (extracellularly and intracellularly) and are thus very effective indicators of air pollution (Budka *et al.* 2002; Bačkor and Loppi 2009). Lichen have served as well-established biomonitoring tools in the long-term monitoring of pollution (Thakur *et al.* 2024). The use of lichen biomonitoring is a widely adopted

approach in studies predominantly focused on air pollution (including HM, SO₂ or radioactivity) in urban and industrial areas (Abas 2021; Anderson *et al.* 2022). These cost-effective techniques are easy to implement (without requires to electricity for installation and operation) and can be easily used in natural remote areas (Conti *et al.* 2009; Loppi 2014).

Natural remote areas, which are usually strictly protected, are critical for biodiversity conservation, and often serve as benchmarks for natural conditions to help us understand trace element dynamics in nature and estimate their possible impacts or distinguish between natural and anthropogenic sources. Despite their naturality, and the lack of local emission sources (Vannini *et al.* 2021), the most well-conserved areas of Europe are often near industrial areas (Šakalys *et al.* 2009), or may be affected by atmospheric long-range transport of emissions (Camarero *et al.* 2017). Despite this complexity, dynamic mechanisms of trace element uptake by mosses or lichens can reflect environmental conditions like local climate (wind strength, temperature) and seasons, particularly through precipitation rate, which is more likely to result in high deposition (Lovett and Kinsman, 1990; Mahapatra *et al.* 2019). Many studies focused on seasonal variations were conducted predominantly during vegetation periods and data from winter are lacking in data.

It is important for the natural environment to be aware of the cycling of trace elements, how they got there and also whether and in what amounts they are able to be retained and accumulated in the context of seasonal change. In this study we focused on two natural well-preserved forest ecosystems called the 'Bog woodland' (*Abieto-Piceetum*), including ground mosses (*Sphagnum*) and epiphytic lichens growing on the bark of Norway spruce (*Picea abies*). The main aim of the study was to compare seasonal changes (between summer and winter) in trace element concentrations and compare these changes between selected moss species (*Sphagnum* sp.) and lichen (*Hypogymnium physodes*).

We hypothesis that these changes will be not similar, even while both species remain well-established biomonitors of environmental pollution.

Material and Methods

Study sites were selected based on their similar habitat classification and status of protection. The sites are both classified as Bog woodland (*Abieto-Piceetum*) on the basis of their characteristic plant composition. We selected two sites in different mountain ranges. The first collecting site was in the Oravská Magura, specifically the northern slope of Kubínska hoľa (Fig. 1). The site is a strictly protected area of European importance, included in the NATURA 2000 network. The pollution that may affect it is likely a combined effect from the nearby ski and recreation resorts, located 600 metres away as the crow flies (or as close as 1.8 km away), but may also be largely related to atmospheric deposition. The second site is also a NATURA 2000 site - the Pavlova Nature Reserve (Fig. 1) at the foot of the Belianske Tatras, which corresponds to the first site in terms of altitude and the natural character of the forest cover. Close to second site, only household heating by firewood, and traffic from the nearby road (connection road 66 in Slovakia-Poland border) affect pollution.

Samples of peat moss (*Sphagnum* sp.) and lichen *H. physodes* were collected monthly from November 2023 to January 2024, and between June 2024 and August 2024, in both areas at the same location according to the coordinates. Lichen samples were collected from the trunk and branches of the tree and moss samples were collected from the site directly under the tree. Sampling was followed during the entirety of sample collection. Samples were placed in zip-lock bags and labelled with the appropriate sample number. GPS coordinates of the sampling location were also obtained and recorded using the A-GPS Tracker application.

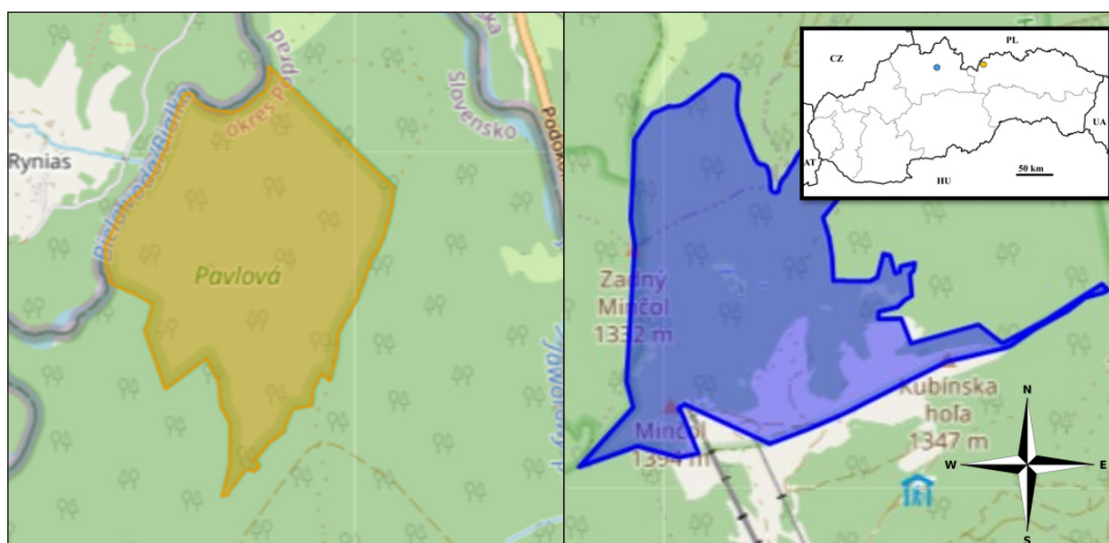


Fig. 1. Map of the studied areas. The orange marked site is the Pavlova Nature Reserve in the Belianske Tatras. The site marked in blue is the NATURA 2000 area Springs of Hruštinky located in Orava Magura. Both sites are located in the north of Slovakia at an altitude of approximately 1000 to 1200 m a.s.l.

Following fieldwork, samples were transferred to the laboratory, where they were stripped of materials that did not belong in the sample. In the case of diatoms, these were various parts of grasses and other mosses and in the case of lichens, these were bark and needle particles that were caught with the lichen. Excess water was also removed from the peat. Samples were placed in petri dishes labelled with the appropriate sample number and allowed to dry completely at room temperature, which ranged from 20 to 25 °C, for one week. After drying, the samples were ground to powder using a cryomill apparatus (Retsch, Germany) at a frequency of 30 Hz for one minute. However, if the samples were not completely ground, they were further ground until they were completely pulverized.

The DELTA ED-XRF spectrometer (Innov-X Systems, 2018) was used for trace elemental analysis of lichen and selected moss samples. The analysis was carried out in a closed portable Delta XRF workstation, where each sample was processed and examined in the same way. Each sample was measured three times for the most accurate resulting value, obtained by calculating the average. The results were reported in ppm units. Detection limits vary for specific elements that meet the criteria outlined in the DELTA Professional ED XRF Spectrometer Manual (Innov-X Systems, 2018) which were continuously determined for each measurement. The calibration standard (INCT-PVTL-6) and the Compton normalization method were used to obtain the lowest limit of detection (LOD). Detection limits were determined for each element, and the minimum value for a particular element represented the actual detection limit of the spectrometer for the material being measured. The accuracy of this method was verified by analysis of certified standard reference plant material. The elements detected included: S, K, Ca, Cr, Mn, Fe, Zn, Rb, Sr, Mo and P.

Total mercury was determined using a DMA-80 mercury analyzer (Milestone, Italy). The analyser was set at 750 °C for combustion, 125 °C for the cuvette, and approximately 615 °C for the catalyst. Crushed lichen and moss samples were placed in nickel pellets (DMA 8142, part of the DMA-80 accessory) and weighed on a laboratory microbalance (Kern 770; Germany). Mercury values were determined on a dry weight basis. Analysis of certified reference material (INCT-PVTL-6 Virginia tobacco leaves (ICHTI, Poland)) were used to assist in the development of an accurate method for the determination of total mercury in plant material, to control the quality of the measurements, and to validate the measurement method. Two blank runs were run after every three sample measurements to eliminate excess mercury in the instrument. All samples were randomly retested (10%) with standard deviation below 5%.

Differences in the trace elements between locations (Pavlova, Hruštinka) and seasons (Summer, Winter) were statistically evaluated using the independent T-test separately for lichens and mosses. Data are presented in Tables 1 and 2 as a mean and SE (Standard Error of Mean). The 2-way ANOVA test was used to assess the differences in the elements in lichens and mosses according to independent factors: Factor 1: location, Factor 2: season,

with the Holm post-hoc test. The pollution Z-score was calculated separately for lichens and mosses as a sum of Z-scores of all elements, with the following 2-way ANOVA test described above. Data were statistically analyzed in the JASP software, and graphs were created in the GraphPad Prism version 8.0.1 (GraphPad Software, Inc., CA, USA).

Results

At the Pavlova site, we identified several elements in higher abundance in mosses during winter collection compared to summer collection (Table 1). The abundance of Hg, S, Cr, Rb, and Sr in the mosses was significantly higher during winter compared to summer-time collection (all: $p < 0.05$; Table 1). The Fe, Ba, Mn, and Pb content in mosses collected in Pavlova did not differ between collection seasons (Table 1). The content of Hg, Rb, Sr, Mn, and Pb in lichens at the Pavlova site was significantly higher during the winter collection period compared to the summer period (all: $p < 0.05$; Table 1). We did not observe statistical differences between the winter and summer seasons in the abundance of the S, Cr, Fe, and Ba in lichens picked in the site Pavlova (Table 1).

At the Hruštinka site, we determined that Hg and Rb had higher accumulation in mosses during winter than in summer (Hg: $p < 0.001$; Rb: $p < 0.05$; Table 2). On the other hand, S, Fe, and Mn had significantly less accumulation in mosses during the winter collection period compared to the summer (S: $p < 0.05$; Fe: $p = 0.06$; Rb: $p < 0.05$; Mn: $p = 0.06$; Table 2). Moreover, in the lichens collected at the Hruštinka site, the higher accumulation of Hg, Sr, and Pb occurred during the winter, when compared to summer (Hg: $p < 0.001$; Sr: $p < 0.01$; Pb: $p < 0.01$; Table 2). Additionally, fewer accumulated elements, including S, Cr, and Rb, were detected in lichens collected at the Hruštinka site during the winter collection period compared to the summer collection period (S: $p < 0.01$; Cr: $p < 0.01$; Rb: $p < 0.05$; Table 2).

The state of pollution state represented by Z-scores calculated from all of the elements in the mosses differed seasonally (Factor 1 - season: $F(1,49) = 9.7$; $p < 0.01$; Factor 2 - location: $p = \text{ns}$; Fig. 2A; Table 3). Here, we demonstrate that the pollution in mosses is more significant during the winter period compared to the summer period at the Pavlova site ($p < 0.01$), while the Hruštinka site did not show a substantial difference in pollution based on seasonality ($p = \text{ns}$; Fig. 2A; Table 3). Correspondingly with the moss pollution, the lichen pollution differed seasonally ($F(1,53) = 7.6$; $p < 0.01$), as opposed to by location ($p = \text{ns}$), though the interaction of both factors represented a robust effect $F(1,53) = 14.3$; $p < 0.001$; Fig. 2B; Table 3). The higher state of pollution in lichens occurred during the winter period, compared to the summer period at the Pavlova site ($p < 0.001$; Fig. 2B; Table 3). The pollution at the Hruštinka site did not differ seasonally, while the pollution during the winter period at Hruštinka was significantly lower compared to the winter period at the Pavlova site ($p < 0.01$; Fig. 2B; Table 3).

Element	Season	N	Mosses Mean (\pm SE)	P value	N	Lichens Mean (\pm SE)	P value
Hg	Summer	15	0.02 (\pm 0.003)	<0.01	15	0.06 (\pm 0.01)	<0.05
	Winter	15	0.22 (\pm 0.06)		15	0.43 (\pm 0.2)	
S	Summer	15	2371.9 (\pm 139.6)	<0.05	15	2012.7 (\pm 138.0)	ns
	Winter	14	3078.1 (\pm 284.3)		15	1985.3 (\pm 199.0)	
Cr	Summer	15	60.4 (\pm 3.9)	<0.05	14	40.7 (\pm 2.5)	ns
	Winter	14	73.0 (\pm 4.1)		15	38.7 (\pm 2.4)	
Fe	Summer	15	1055.9 (\pm 192.1)	ns	14	1123.6 (\pm 92.5)	ns
	Winter	14	839.5 (\pm 112.8)		15	1215.1 (\pm 114.9)	
Rb	Summer	15	11.9 (\pm 0.7)	<0.001	14	7.9 (\pm 0.3)	<0.001
	Winter	14	18.7 (\pm 1.5)		15	10.4 (\pm 0.5)	
Sr	Summer	15	3.1 (\pm 0.4)	<0.01	14	11.0 (\pm 2.0)	<0.01
	Winter	13	6.2 (\pm 1.0)		15	29.0 (\pm 4.6)	
Ba	Summer	15	170.1 (\pm 8.1)	ns	14	152.6 (\pm 9.0)	ns
	Winter	14	170.6 (\pm 9.1)		15	157.3 (\pm 16.2)	
Mn	Summer	15	595.0 (\pm 69.5)	ns	14	246.0 (\pm 47.3)	<0.001
	Winter	14	750.5 (\pm 96.4)		15	805.5 (\pm 122.0)	
Pb	Summer	15	21.1 (\pm 2.3)	ns	14	20.7 (\pm 0.8)	<0.05
	Winter	14	23.7 (\pm 3.8)		15	27.4 (\pm 2.7)	

Table 1. The accumulation of trace elements in mosses and lichens at the Pavlova site during winter and summer. Data are presented as a mean and Standard Error of Mean (SE). Data were statistically evaluated using the Student T-test with 0.95 confidence limits.

Element	Season	N	Mosses Mean (\pm SE)	P value	N	Lichens Mean (\pm SE)	P value
Hg	Summer	15	0.04 (\pm 0.003)	<0.001	15	0.04 (\pm 0.003)	<0.001
	Winter	15	0.17 (\pm 0.03)		15	0.2 (\pm 0.01)	
S	Summer	15	3342.7 (\pm 286.1)	<0.05	15	2552.5 (\pm 216.8)	<0.01
	Winter	15	2608.6 (\pm 154.1)		15	1769.2 (\pm 111.0)	
Cr	Summer	15	52.1 (\pm 4.6)	ns	15	45.3 (\pm 3.8)	<0.01
	Winter	15	54.2 (\pm 2.9)		15	29.7 (\pm 2.1)	
Fe	Summer	15	1156.8 (\pm 124.6)	=0.06	15	1154.1 (\pm 138.3)	ns
	Winter	15	1648.3 (\pm 220.0)		15	1070.4 (\pm 114.5)	
Rb	Summer	15	21.5 (\pm 2.5)	<0.05	15	16.7 (\pm 1.7)	<0.05
	Winter	15	27.2 (\pm 0.9)		15	12.5 (\pm 0.8)	
Sr	Summer	10	3.8 (\pm 0.9)	ns	14	5.5 (\pm 1.4)	<0.01
	Winter	15	4.3 (\pm 0.4)		14	11.3 (\pm 1.4)	
Ba	Summer	15	162.0 (\pm 10.7)	ns	15	150.1 (\pm 9.9)	ns
	Winter	15	161.8 (\pm 7.4)		15	130.1 (\pm 12.5)	
Mn	Summer	15	583.9 (\pm 64.2)	=0.06	15	474.5 (\pm 77.7)	ns
	Winter	15	432.8 (\pm 40.1)		15	363.1 (\pm 41.0)	
Pb	Summer	15	17.8 (\pm 1.4)	ns	15	20.5 (\pm 1.8)	<0.01
	Winter	15	19.2 (\pm 1.9)		15	31.3 (\pm 2.6)	

Table 2. The accumulation of trace elements in mosses and lichens in the Hruštinka region during winter and summer. Data are presented as a mean and SE. Data were statistically evaluated using the Student T-test with 0.95 confidence limits.

Discussion

The results of our study show that trace elements accumulated differently in the moss *Sphagnum* sp. and the lichen *Hypogymnia physodes*. Since both species are good indicators of air pollution, one

would expect that the seasonal variations would be similar. However, lichens seem to exhibit better seasonal variations, (especially for lead), and found that seasonal variations also differ between (in the case of sulphur). Two different species may bind elements differently during differing seasons.

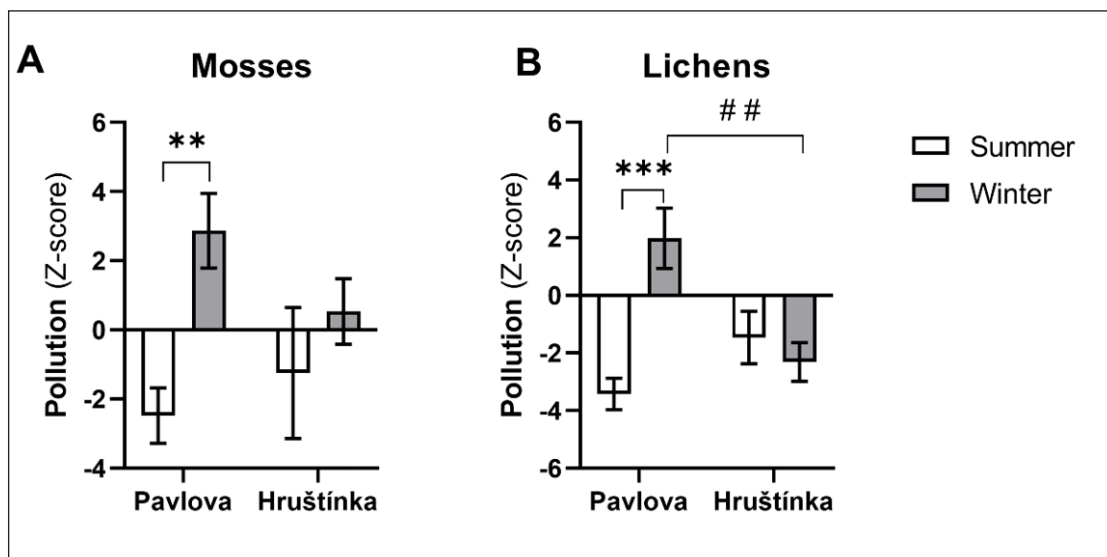


Fig. 2. Pollution state in both areas and seasonal differences. (A) - Seasonal and locational differences in the moss. (B) - Seasonal and locational differences in the lichen. (A) - Pavlova Summer: $n = 15$; Pavlova Winter: $n = 13$; Hruštinka Summer: $n = 10$; Hruštinka Winter: $n = 15$. (B) - Pavlova Summer: $n = 14$; Pavlova Winter: $n = 15$; Hruštinka Summer: $n = 14$; Hruštinka Winter: $n = 14$. *- difference between summer and winter; #- difference between winter in Pavlova and Hruštinka location. The higher Z-score represents more polluted area, while lower less pollution, respectively. ** - $p < 0.01$; ***- $p < 0.001$; # #- $p < 0.01$; 2-way ANOVA, Holm post-hoc test with independent factors season and location.

	Cases	Sum of Squares	DF	Mean Square	F	P value
Mosses	Factor 1 (Season)	163.659	1	163.659	9.709	0.003
	Factor 2 (Location)	3.944	1	3.944	0.234	0.631
	Interaction	41.151	1	41.151	2.441	0.125
	Residuals	825.923	49	16.856		
Lichens	Factor 1 (Season)	73.657	1	73.657	7.554	0.008
	Factor 2 (Location)	19.464	1	19.464	1.996	0.164
	Interaction	139.102	1	139.102	14.266	< 0.001
	Residuals	516.765	53	9.750		

Table 3. Results of two-way ANOVA and statistical determinants the pollution status in the mosses and lichens across seasons (summer vs. winter) and between the two study sites (Pavlova vs. Hruštinka) including their mutual interactions.

This concept has already been addressed in several studies from around the world (Lupšina *et al.* 1992; Evans and Hutchinson 1996; Mikhailova and Sharunova 2008; Caridi *et al.* 2017; Klos *et al.* 2018). For example, at the Pavlova site, we observed significantly higher content of sulphur in mosses than in lichens, especially during the winter season compared to the summer season. On the other hand, at the Hruštinka site, sulphur content was significantly higher for both species during summer than during winter. Pollution load differs from site to site and may be related to local differences, including nearby pollution sources or climate and growth conditions (Bruteig 1993; Küttim *et al.* 2020). Ciężka *et al.* (2022) used the same species of lichen *Hypogymnia physodes* and isotopically identified the sources responsible for the observed contamination (C, S, N and Pb) in Świętokrzyski National Park in Poland. They noticed that residential heating is a major source of contamination in winter, whereas road traffic contributes most to pollution in the summer. When

comparing our two sites, we found significant differences in the pollution in mosses where more trace elements appeared to accumulate in the winter season at the Pavlova site. This may relate to location of the site, as small villages (on Slovak Polish border) nearby primarily use firewood as a heat source. On the other hand, the site is nearby to the main transport route between Poland and Slovakia, and thus, road traffic may play a significant role as a pollutant in the area. With regard to the protected site of Hruštinka, similar sources of pollution are not located in close proximity. As such, regional emissions from the sub-rural areas of lower and upper Orava region are considered to be the most localised sources of pollution, including those from the Ferroalloy Plant in Dolný Kubín (8 km from site). Several studies (Sensen and Richardson 2002; Achotegui-Castells *et al.* 2013; Klos *et al.* 2018) investigating the origin of atmospheric inputs of trace elements show that enrichment factors correlated well with distance of the nearest urbanized and industrial areas. The behavior of some

elements and their higher content in mosses and lichens during winter indicates that these elements may be influenced by the heating season (Klos *et al.* 2018). If we look at the overall pollution of the sites and the studied species (Fig. 2), it is obvious that some trace element values in mosses were higher in winter. Since mosses tend to absorb and exchange substances more quickly, they are therefore able to reflect the current pollution much more effectively and quickly (Reimann *et al.* 1999).

As previously mentioned, the compared sites had the same character of habitat conditions, including species composition of trees, age of stand, and degree of protection. However, these two strictly protected areas are 64 km apart, and the potential local and regional sources of pollution are different. Additionally, the local climate differs, including precipitation conditions which can be associated with total precipitation deposition of trace elements from the air. The highest differences were recorded in the case of sulfur, chromium and manganese. These differences may have various causes. In the case of sulfur, significant differences were observed not only between locations, where sulfur values were significantly higher in Hruštinka than in Pavlová, but differences were also observed between seasons. The seasonal influence on the content of trace elements is also confirmed in a study by Mikhailova and Sharunova (2008). Authors compared seasonal changes and concluded that the input of emitted particles onto lichens surface in summer may be limited by deciduous tree crowns and the herb-dwarf shrub layer. In addition, Van Herk *et al.* (2003) showed that the lichen *H. physodes* is able to store sulfur from historical deposition. This could explain these increased values at the Hruštinka site, where pollution may come from historical deposition. Though the Ferroalloy Plants have operated here for many years, the results also indicate significant differences in sulfur content during the seasons, where lower values were found in Pavlova in the summer months than in Hruštinka. Significant differences in the content of trace elements can also be caused by seasonal changes and the associated differences in precipitation. Their influence is pointed out by Čeburnis *et al.* (1999) and Staines (1995), where they proved through both direct and experimental research that the longer the rain acts on moss or lichen, the greater the leaching of trace elements. This fact may also be the reason for such significant differences in the summer period at the studied localities, where there were significant differences in the total precipitation. Table 4 are presents precipitation rates (provided by Slovak Hydrometeorological Institute - SHMÚ)

for individual months of sample collection, where we can see that site Hruštinka had higher precipitation rate during winter and less precipitation rate during summer than Pavlova. This situation of high precipitation therefore might be affected content of sulphur in mosses and lichens.

Each species of moss or lichen can bind different amounts of trace elements, even within the species (Abulude *et al.* 2021). Each individual plant may have different surface characteristics connected to collecting abilities (Kansanen and Venetvaara 1991). Nevertheless, for some elements (Cr, Hg, Pb, S), mosses and lichens showed some similarity in the accumulation of trace elements. On the other hand, differences in some trace elements, like Fe and Mn, may be caused by the different impact of dry and wet deposition in individual periods (Berg *et al.* 1995). For example, Pavlova lichens had higher values of Fe in winter due to higher dry deposition. Conversely, values of Mn were higher at Hruštinka than at Pavlova, likely as a result of higher wet deposition. Harmful elements such as Hg and Pb exhibited higher values for both species during the winter period, but lichens had slightly higher values than mosses. This may be due to the lichen's increased ability to bind mercury from dry deposition (Kansanen and Venetvaara 1991). Also, Evans and Hutchinson (1996) demonstrated that lichens accumulate mercury from the atmosphere more readily than mosses. Conversely, for mosses, the lower mercury and lead content may be due to the layer of snow that protects mosses from pollution in winter (Reimann *et al.* 1999).

Conclusion

In conclusion, it should be noted that the study contributes to the general knowledge about the seasonal accumulation of trace elements in one of the most protected localities in Slovakia. Our selected bioindicators proved to be very suitable for this kind of work because of their accumulation capacity.

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Year	2023	2023	2024	Winter	2024	2024	2024	Summer
Month	11	12	1	Σ 11-1	6	7	8	Σ 6-8
Hruštinka	110.0	104.8	92.7	307.5	132.0	72.2	67.7	271.9
Pavlová	121.6	55.0	79.3	255.9	159.9	135.7	202.1	497.7

Table 4. Monthly precipitation (sum) during sample collection in study site Pavlova (station Tatranská Javorina) and Hruštinka (Station Oravský Podzámok) data provided by SHMÚ.

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