

# Accumulation Characteristics of Some Elements in the Moss *Polytrichum commune* (Bryophytes) Based on XRF Spectrometry

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## ABSTRACT

Bryophytes are broadly used as bioindicators. However, the internal distribution of accumulated elements in the moss tissue is little known. Sampling was carried out in The West Carpathians, Slovakia, in autumn 2012. Seven replicates have been used. The samples were analyzed by XRF Spectrometer Delta Classic. S, Pb, K, Ca, Cr, Mn, Fe, Cu, Rb, Sr, Mo, Ba and Zn were determined. For ordination analysis we used principal component analysis, statistical graphics system STATISTICA have been used for the correlation analysis and for analysis of variance. Results show that sulphur, zinc, chromium, manganese, molybdenum, calcium and copper are preferentially accumulated in the capsula. While lead favors gametophyte, potassium and strontium prefer accumulation in sporophyte. Iron significantly accumulates in the more-year segments, while zinc in the stems. Copper, chromium and sulphur are accumulated preferentially in The Fatra Mts.

**Keywords:** Bioindication; *Polytrichum commune*; The West Carpathians; Elemental Accumulation; XRF Spectrometry

## 1. Introduction

Mosses are suitable in the bioindication of heavy metal deposition because they are perennial without deciduous periods, they have high cation exchange capacity and the cuticle is absent [1,2].

Although the method of bryoinidication in foreign countries has been broadly used, from Slovakia, distinctively from the Tatra Mts are only moderate data present. Šoltés [3] evaluated results of chemical analysis of 267 samples of 26 bryophyte species collected on the territory of the High Tatra Mts, including *Polytrichum commune* Hedw. The peat moss *Sphagnum girgensohnii* Russow used Šoltés [4] for bioindication of imission load of Tatra Mts with special focus to correlation between altitude and heavy metal deposition.

*Polytrichum commune* has been often used as bioindicator. Sidhu [5] found that fluorine accumulation in the moss *Polytrichum commune* was about 10 times greater than in the needles of *Abies balsamea*. Wenstein and Davison [6] reminded that larger fluorine accumulation in the moss *Polytrichum commune* might be interpreted

as reflecting the much greater resistance of *Polytrichum commune* to fluorine. Busoi *et al.* [7] used *Polytrichum commune* for Cr, Pb, Sr, Cu, Zn, Co, Mn and Fe bio-monitoring in Tisa river valley (Romania). Irudayaraj *et al.* [8] used spore germination of *Polytrichum commune* to assess the toxicities of heavy metals Cu, Cd and Zn. Markert and Wtorova [9] used samples of *Polytrichum commune* to evaluate environmental contamination in Forest Biosphere Reserve near Kalinin. Šoltés *et al.* [10] used *Polytrichum commune* to specify the imission influence to non-forest vegetation of the Tatra Mts. *Polytrichum commune* has shown as a highly sensitive indicator. Maňková *et al.* [11] collected 831 samples of six bryophyte species, including *Polytrichum formosum* in order to identify the areas affected by industrial imissions.

Differences in heavy metals accumulation both in gametophyte and sporophyte have been recorded by more authors. Xie and Zhang [12] noticed that gametophytes of *Funaria hygrometrica* accumulated significantly more heavy metals than sporophytes, while concentrations of Zn and Mn in sporophytes were always higher than con-

centrations of other heavy metals. Similar results obtained Basile *et al.* [13], also using *Funaria hygrometrica*.

Water conduction in *Polytrichum commune* is entirely carried out by the central strand [14]. The *Polytrichum* species are well adapted to winter conditions. The lamellar cells of frozen plants are desiccated, the cytosol is shrunken, the ribosomes and mitochondria are aggregated. The thylakoid system of their chloroplasts is irregularly arranged and the grana thylakoids are compressed [15].

Apart from *Polytrichum commune*, other *Polytrichum* species have been used for bioindication also. Grodzińska and Godzik [16] determined Cd, Pb, Ni, Cu, Zn and S in moss species collected in southern Spitzbergen. They found *Polytrichum alpinum* as a poor accumulator of heavy metals.

Even if bryophytes have been extensively used as bioindicators of environmental pollution, still little is known about the internal distribution of heavy metals in the moss tissue.

The following questions are addressed in this paper:

- How accumulated elements are distributed in the tissue of *Polytrichum commune*;
- How accumulated elements are correlated;
- Evaluation of the convenience of the species *Polytrichum commune* for bioindication.

## 2. Methods

### 2.1. Sampling

The samples of *Polytrichum commune* were collected in fairly open stand with a distance of 5 m to the nearest tree. After the return to the laboratory, the plant samples were washed in distilled water and dried at 70°C for two days. After drying, the one-year segments were separated. Growth segments are easy to recognize, they are formed by annually repeated changing patterns of leaf morphology [17]. The geographical coordinates were recorded in the system WGS 84, Garmin eTrex Vista device was used.

Sampling sites:

1) Sub-Tatra Furrow, Tiborova poľana, 933 m a.s.l., coordinates 49°17'44.06"; 20°09'21.94", October 25, 2012.

2) Big Fatra Mts, Turčianska Štiavnička settlement, 532 m a.s.l., coordinates 49°04'27.66"; 19°01'15.24" November 1, 2012.

3) High Tatra Mts, Jakubkova lúka, 1080 m a.s.l., coordinates 49°08'28"; 20°12'44", November 4, 2012.

4) High Tatra Mts, Javorová dolina valley, 1050 m a.s.l., coordinates 49°15'26.82"; 20°08'47.64", October 16, 2012.

5) Small Fatra Lučanská, under Martinské hole hills, 575 m a.s.l., coordinates 49°06'02.12"; 18°52'37.98" October 25, 2012.

6) Sub-Tatra Furrow, Nature Reserve Bor, 957 m a.s.l.,

coordinates 49°16'34.5"; 20°09'40.6", October 25, 2012.

7) Sub-Tatra Furrow, Nature Reserve Bor, 950 m a.s.l., coordinates 49°16'43.02"; 20°09'57.00", October 25, 2012.

### 2.2. Instrumental Analysis

The tissue samples were analyzed by X-ray fluorescence [18], using the hand-held XRF spectrometer DELTA CLASSIC (USA). The following elements were determined: S, Pb, K, Ca, Cr, Mn, Fe, Cu, Rb, Sr, Mo, Ba, Zn. Since some examined elements need different thickness of pellets, e. g. S versus Pb [19] we have decided to use method without pelletization [20-22]. The plant material was crushed in mortar into fine powder. The epoxide frame of 2.0 × 2.4 cm was filled with 1 - 1.2 g of plant powder, up to 1 cm in thickness and analysed directly on protective prolen folium. The accuracy of XRF method is proportional to the mass, to the concentration of elements and to the analysing time. Experimentally we found the optimal analysing time for 30 sec. We have decided for three repetitions. Samples dried at 60°C for 24 hours. Analysis using XRF allows analyze the compact samples without homogenisation. The whole surface of samples was exposed to radiation, the angle of the beam was 90°. The measurement consists of seven replicates.

The samples placed in plastic sleeves are stored in the Institute of High Mountain Biology in Tatranská Javorina, Slovakia.

### 2.3. Statistics

For statistical analysis, the CANOCO 4.5 for Windows package [23] was used. To analyse the relation of environmental variables (element concentrations) and analysed moss segments we had only single data set of variable. Since the length of the first gradient in the log report was < 0.6, we used the linear method of ordination (principal component analysis, the PCA). For ordination analysis we used ordinal elements concentration data without transformation. Statistical graphics system STATISTICA, Release 7 has been used for the correlation analysis and for analysis of variance.

Except for individual differentiation, the samples were sorted into groups according to location (Tatra Mts-Fatra Mts), according to age (One year-More year segments) and according to generation (Gametophyte-Sporophyte). The differences between ecological groups were tested with one-way analysis of variance (ANOVA) of the component scores.

Population of *Polytrichum commune* growing in swampy spruce forest in locality Tiborová (Sub-Tatra Furrow) have been found richly fruitful, the moss is growing in large carpets, herbaceous cover is insignificant. A negative effect of the herbaceous cover and its species rich-

ness on the abundance of *P. commune* was observed [24], we are entitled to assume the optimal ecological conditions in the locality Tiborová. In order to identify the associations among set of variables relating to the population of *Polytrichum commune* growing in optimal condition and other sets of variables we used canonical correlations.

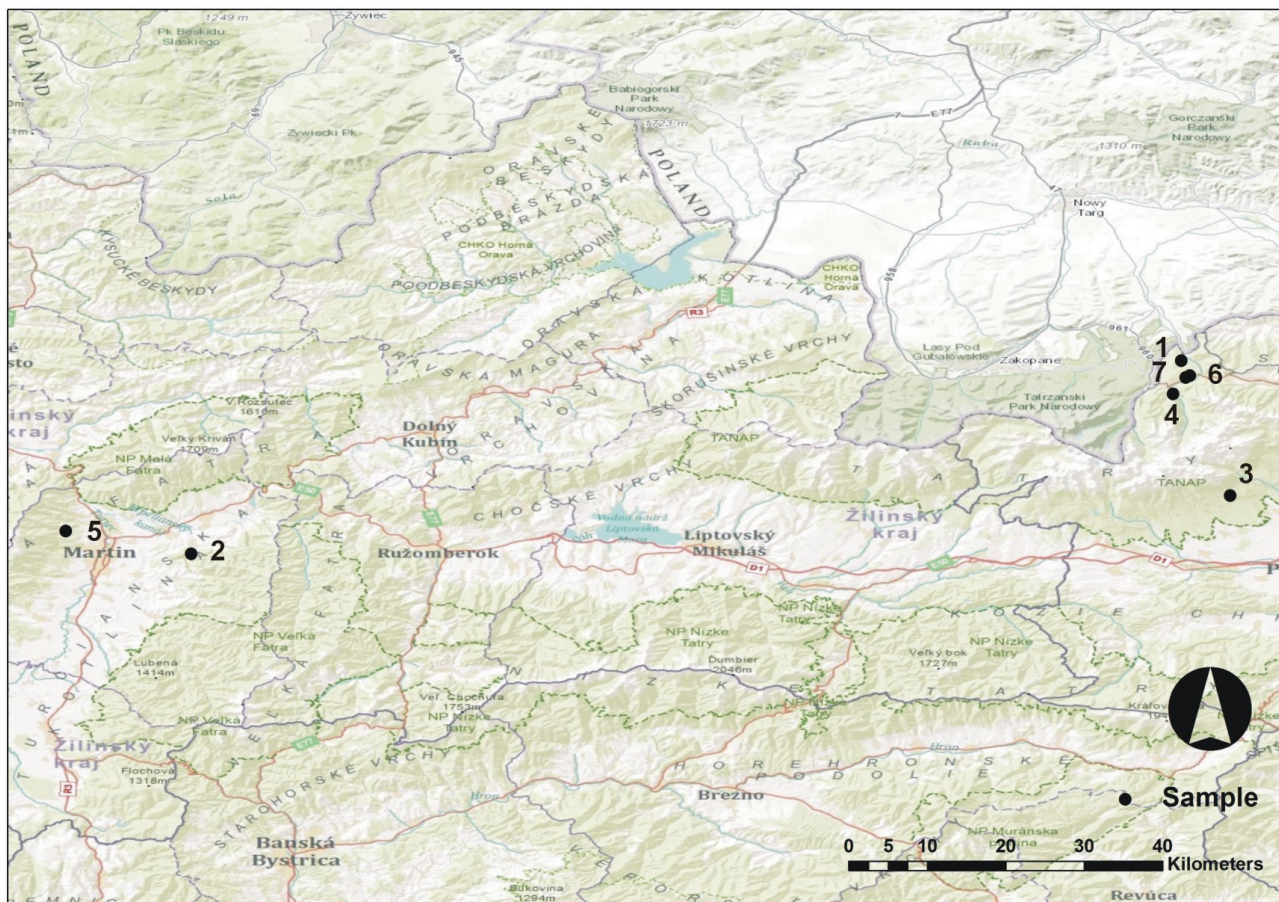
### 3. Results and Discussion

The first axis explains 77.9% of variance, this axis positively correlates with elements concentrating in capsula, *i.e.* zinc, chromium, manganese, copper, calcium, molybdenum and negatively correlates with lead, which has an affinity to gametophyte. The second axis explains 21.7% of variance, this axis positively correlates with elements concentrating in seta, *i.e.* rubidium and in a lesser extent with potassium and strontium.

Lead occurs preferably in gametophyte, both in leaves and stems ( $p = 0.057$ , **Figure 1, Tables 1 and 2**), particularly in the more year segments ( $p = 0.187$ ). The oc-

currence in the leaves prefers baryum ( $p = 0.197$ ). Potassium favors sporophyte ( $p = 0.181$ , **Figure 1, Tables 1 and 2**), occurring in seta, also in the capsula. While potassium occurring in the gametophyte, prefers one-year segments ( $p = 0.186$ , **Table 2**). Strontium slightly favors sporofyte also ( $p = 0.290$ , **Figure 1, Tables 1 and 2**), but while occurring in gametophyte, gives priority to more-year segments ( $p = 0.125$ , **Figure 1, Tables 1 and 2**). Molybdenum slightly favors sporophyte ( $p = 0.216$ , **Figure 1, Tables 1 and 2**). A high concentration of some metals in capsula exhibit zinc, chromium, manganese, copper, of the non-metals sulphur (**Figure 1**), but zinc is significantly concentrated in stems ( $p = 0.048$ ). Iron slightly prefers gametophyte ( $p = 0.313$ ), significantly occurs in more year segments ( $p = 0.037$ , **Figure 1, Tables 1 and 2**). Rubidium prefers accumulation in seta, but insignificantly.

Copper and chromium predominate in The Fatra Mts significantly (Cu  $p = 0.035$ ; Cr  $p = 0.020$ ), close to significance predominate in The Fatra Mts sulphur ( $p =$



**Figure 1.** Map of sampling sites: 1: Sub-tatra furrow, Tiborova poľana; 2: Big Fatra Mts, Turčianska Štiavica settlement; 3: High Tatra Mts, Jakubkova lúka meadow; 4: High tatra Mts, Javorová dolina valley; 5: Small Fatra Lučanská, under Martinské hole hills; 6: Sub-tatra furrow, nature reserve Bor; 7: Sub-tatra furrow, nature reserve Bor. (Drawn by J. Solár).

**Table 1. Heavy metals and sulphur concentrations (ppm) in growth segments of *Polytrichum commune*.**

	S	K	Ca	Cr	Mn	Fe	Cu	Zn	Rb	Sr	Mo	Ba	Pb
Site 1													
Capsules	4306	42,336	20,179	109	1339	982	58	532	58	8.7	15	115	23
Seta	nd	42,840	8238	37	925	197	30	132	76	9.9	10.3	53	21
One-year leaves	3154	13,592	5225	72	484	1893	34	nd	27	ND	10	113	44
More-year leaves	4038	14,061	9265	106	794	3277	33	nd	29	ND	13	171	38
One-year stems	2269	11,315	3905	39	392	466	24	65	35	3.3	7.9	55	27
More-year stems	1925	5822	2698	26	278	456	nd	19	32	2.3	6.1	54	33
Site 2													
Capsules	13,378	41,094	116,174	474	1640	3467	87	nd	30	11	19	200	nd
Seta	4404	51,643	23,898	126	627	1340	nd	nd	28	6.4	18	166	26
One-year leaves	5973	23,501	21,493	79	390	2628	50	nd	26	6.2	9	183	36
More-year leaves	3932	17,464	20,280	72	322	3259	46	nd	28	10.5	12	170	39
One-year stems	4417	25,637	14,588	72	303	1601	47	nd	32	8	12	125	29
More-year stems	3224	12,516	10,858	33	206	2763	22	39	30	12.8	10.1	108	30
Site 3													
Capsules	5135	19,661	26,758	147	2760	1948	38	229	42	22	16	150	33
Seta	1906	7197	9567	40	1409	507	23	152	28	26.4	8.7	76	29
One-year leaves	2014	8955	6989	63	875	1703	32	23	27	11.4	12	113	31
More-year leaves	3223	12,519	15,593	166	1771	2516	nd	nd	22	10	14	231	33
One-year stems	2075	8055	4864	31	728	647	16	89	27.6	15.5	9.4	67	25
More-year stems	1220	8674	3032	21	416	320	ND	37	30.1	12.2	9.1	32	22
Site 4													
Capsules	7326	23,628	50,812	95	1417	803	50	87	31	11.2	9	116	19
Seta	nd	51,566	16,805	77	726	553	31	nd	45	8.1	13	112	33
One-year leaves	4962	23,839	15,901	94	643	1639	37	nd	33	5.1	12	170	36
More-year leaves	5415	17,451	21,026	84	947	3155	52	nd	32	12.1	13	206	53
One-year stems	2521	13,822	8405	42	402	806	43	62	31	5.3	9.1	86	28
More-year stems	3066	12,865	9019	37	371	1047	21	21	35	8.5	8.9	92	37
Site 5													
Capsules	9077	38,571	39,711	153	3526	1509	78	nd	30	23	17	222	18
Seta	nd	36,916	16,448	83	1934	2671	nd	nd	42	28	10	187	19
One-year leaves	4206	23,843	11,054	71	1291	4336	33	nd	38	22	8	198	31
More-year leaves	3872	21,152	16,798	98	2529	9880	38	nd	51	37	9	309	35
One-year stems	4557	20,792	8144	47	1072	1561	32	nd	43	21.8	10.3	142	30
More-year stems	3288	22,243	9570	49	965	5184	17	nd	50	36	10.3	182	31
Site 6													
Capsules	nd	33,599	29,084	99	1989	578	39	435	51	6.5	11	78	21
Seta	nd	42,504	7188	37	1492	210	16	52	70	9.1	8.7	51	26
One-year leaves	3692	35,768	6360	81	970	888	29	nd	35	nd	9	109	23
More-year leaves	3816	22,545	9014	93	1154	1082	33	nd	30	nd	10	171	22
One-year stems	1731	20,845	3911	27	758	163	23	64	42	3.1	8.6	39	23
More-year stems	1190	12,238	2069	17	436	160	14	35	39.6	1.8	7.6	32	17
Site 7													
Capsules	4945	16,348	15,028	10	1284	1130	nd	538	29	1	nd	21	10
Seta	nd	36,793	6339	11	831	686	15	146	83	9.2	5.9	20	15
One-year leaves	3004	13,972	2967	6	283	606	nd	36	18	nd	nd	20	10
More-year leaves	2660	8089	4976	20	540	1597	nd	41	13	nd	nd	75	17
One-year stems	1902	10,746	2220	nd	218	277	nd	73	16.6	1.6	nd	nd	6
More-year stems	1732	9605	1714	nd	149	362	nd	47	20.5	1.7	3.1	nd	10

nd: below detection limit.

**Table 2. Results of ANOVA, distribution of elements with respect to generations (gametophyte-sporophyte), segments age (one year-more year) and The West Carpathians mountain ranges (Tatra Mts, including).**

	Gametophyte-sporophyte		One year-more year segments		Tatra Mts-Fatra Mts	
	F	P	F	p	F	p
Pb	175.5	0.057	15.62	0.187	1.512	0.547
S	6.780	0.280	0.379	0.820	170.2	0.058
K	16.79	0.181	13.20	0.186	2.966	0.413
Ca	0.948	0.683	0.134	0.948	3.813	0.370
Cr	2.694	0.425	0.948	0.637	1432.2	0.020
Mn	27.70	0.142	10.54	0.227	2.417	0.445
Fe	5.310	0.313	368.1	0.037	6.836	0.282
Cu	4.538	0.337	0.99	0.627	387.8	0.035
Rb	1.294	0.571	12.46	0.209	82.74	0.083
Sr	7.029	0.290	31.25	0.125	10.57	0.226
Mo	10.55	0.226	0.08	0.975	49.40	0.108
Ba	1.659	0.519	7.61	0.265	2.957	0.414
Zn	29.55	0.137	0.90	0.648	1.614	0.534

0.058), insignificantly predominates in The Fatra Mts rubidium ( $p = 0.083$ ) (Table 2). Except for the effects of the Silesian and Polish metalurgy, an important source of polymetallic deposition impacting The Fatra Mts is domestic Ferrous-Alloy Mills Istebné. The key components are Mn, Cr the other risk elements are Ti, Zr, Cu, Pb [25]. With a regard to the distribution of chromium and copper (Tatra Mts-Fatra Mts, Table 2), our results confirmed this conclusions [25].

Differences in heavy metals accumulation in both gametophyte and sporophyte have been recorded by more authors. Xie and Zhang [12] noticed that gametophytes of *Funaria hygrometrica* accumulated significantly more heavy metals than sporophytes, while concentrations of Zn and Mn in sporophytes were always higher than concentrations of other heavy metals. Similar results noticed Basile *et al.* [13], also using *Funaria hygrometrica*.

We have recorded higher concentrations of Zn and Mn in the sporophyte of *Polytrichum commune* also (Tables 1 and 2, Figure 1). Brown and Buck [26] found, that degeneration of the gametophyte of *Funaria hygrometrica* was accompanied by loss of K and a gain in Ca.

Every set of variables highly correlates with the set of independent variables, except for location Nr. 3, nevertheless, the correlation is close to significance ( $p = 0.057$ , Table 3). From the ecological point of view, site 3 is a slightly different location. This is the highest localized sampling site (1080 m a.s.l.) placed in the south facing slope of The High Tatra Mts. The others are located on the northern slopes of the The Tatra Mountains, or have their origin in The Fatra Mts.

Vázquez *et al.* [27] found that metals are mostly taken up to the extracellular compartment than to the intracellular compartment, nevertheless zinc prefers intracellular

compartment [28]. *Polytrichales* have highly developed conducting strands [29]. An internal water-conducting system consists of highly elongate cells lacking cytoplasmic contents at maturity. Traditionally these cells are called hydroids and form a central strand which is sometimes referred to as hydrom [29] and outer leptom. Hydroids are specialized for conduction of water. Leptom of *Polytrichum* stem conduct assimilates and ionic solutes such as sulphate and lead [30]. High enzymatic activity is indirect evidence for the role of leptoides in conduction of organic compounds [30], ions are accumulated in the leptom cells [31]. The accumulated elements are during vegetation period transferred to their growing tips. We have found, that most rapidly is increasing K and Ca content of developing sporophyte, this was observed by Brown and Buck [26] also.

It seems that some bryophytes thrives in polluted environments. Species commonly growing in such environments, like *Pohlia nutans*, produce protonemata and gametophytes much faster [32]. *Bryum argenteum* frequently occurs in urban environments and therefore appears to have the ability to tolerate high levels of such atmospheric pollutants as lead. Plants from different populations contained extremely different concentrations of lead and other metals. Panda and Choudhury [33] investigated the effect of chromium, copper and zinc on nitrate reductase activity and on total chlorophyll content in the moss *Polytrichum commune*. The response of *Polytrichum commune* to toxic concentrations of Cr, Cu and Zn appears to induce oxidative damage as observed by increase in malondialdehyde content.

Klavina *et al.* [34] recorded following concentration in *Polytrichum commune* (ppm) K 6210, Ca 2561, Fe 779, Mn 126, Cu 8.1, Zn 42, Pb 3.0. These are usually the



**Table 3. Canonical correlation, sums of partial elements concentrations (ppm) in growth segments.**

	Capsule	Seta	One y-leaves	More-y-leaves	One-y-stems	More-y-stems	Eigen values	Canonical correlation	p
Independent variables									
1	70,061	52,569	24,648	31,825	18,603	11,351			
Dependent variables									
2	176,574	82,282	54,374	45,635	46,871	29,852	0.8498	0.9219	0.0100
3	56,939	20,969	20,848	36,098	16,649	13,826	0.6447	0.8029	0.0570
4	84,404	69,969	47,371	48,446	26,262	26,628	0.9560	0.9778	0.0009
5	92,935	58,338	45,131	54,808	36,452	41,625	0.8674	0.9313	0.0072
6	65,990	51,664	44,272	34,154	25,907	15,067	0.8737	0.9347	0.0071
7	39,344	44,954	20,922	18,028	15,460	13,644	0.8180	0.9044	0.0146

concentrations that correspond with our measured values, except for Mn, Zn and Pb, where our values are higher. Busuioac *et al.* [7] recorded extreme high chromium concentration in *Polytrichum commune*, of 2040 ppm.

*Polytrichum commune* sensitively responds to the nutrient supply. When ecological conditions are changed, the moss quickly occupy appropriate habitats [35]. At the sub-arctic Abisco site the mosses showed individualistic response to environmental changes (temperature, nutrients). While *Hylocomium splendens* showed reduced growth due to increased temperature or increased nutrient supply, *Polytrichum commune* increased its growth when nutrients were added [36].

#### 4. Conclusions

- Zinc, chromium, manganese, molybdenum, calcium, copper and of the non-metals sulphur are preferentially accumulated in the capsule.
- If *Polytrichum commune* is used as bioindicator, the structure of samples collected must be unified in the whole monitored territory (only gametophyte or only gametophyte + sporophyte), because some elements are particularly accumulated in sporophytes.
- Lead prefers accumulation in gametophyte, potassium, strontium slightly prefers accumulation in sporophyte.
- While zinc accumulates in the stems, iron significantly accumulates in the more-year segments.
- Increased accumulation of copper, chromium and sulphur in The Fatra Mts indicate increased immision deposition in this area.

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