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 bryoindication 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 immission 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 than larger fluorine accumulation in the moss *Polytrichum commune* might be interpreted as reflecting the much greater resistence of *Polytrichum commune* to fluorine. Busoic 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 immission influence to non-forest vegetation of the Tatra Mts. *Polytrichum commune* has shown as a highly sensitive indicator. Maňkovská 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 hygrometica.

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 convenciency 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 polana, 933 m a.s.l., coordinates 49°17'44.06"; 20°09'21.94", October 25, 2012.
2) Big Fatra Mts, Turčianska Štvavnička settlement, 532 m a.s.l., coordinates 49°04'27.66"; 19°01'15.24" November 1, 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 x 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 stroncium.

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 occurrence 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 sporophyte 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* = 0.048).
Table 1. Heavy metals and sulphur concentrations (ppm) in growth segments of *Polytrichum commune*.

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<td>831</td>
<td>686</td>
<td>15</td>
<td>146</td>
<td>83</td>
<td>9.2</td>
<td>5.9</td>
<td>20</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>One-year leaves</td>
<td>3904</td>
<td>13,972</td>
<td>2967</td>
<td>6</td>
<td>283</td>
<td>606</td>
<td>nd</td>
<td>36</td>
<td>18</td>
<td>nd</td>
<td>20</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>More-year leaves</td>
<td>2660</td>
<td>8089</td>
<td>4976</td>
<td>20</td>
<td>540</td>
<td>1597</td>
<td>nd</td>
<td>41</td>
<td>13</td>
<td>nd</td>
<td>75</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>One-year stems</td>
<td>1902</td>
<td>10,746</td>
<td>2220</td>
<td>nd</td>
<td>218</td>
<td>277</td>
<td>nd</td>
<td>73</td>
<td>16.6</td>
<td>1.6</td>
<td>nd</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>More-year stems</td>
<td>1732</td>
<td>9605</td>
<td>1714</td>
<td>nd</td>
<td>149</td>
<td>362</td>
<td>nd</td>
<td>47</td>
<td>20.5</td>
<td>1.7</td>
<td>3.1</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

*nd*: below detection limit.
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 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 [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 thrive 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
accumulation characteristics of some elements in the moss Polytrichum commune (Bryophytes) based on XRF spectrometry

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

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

concentrations that correspond with our measured values, except for Mn, Zn and Pb, where our values are higher. Busuoic 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, nutrient supply). When ecological conditions are changed, Polytrichum commune increased its growth when 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 a 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.

5. Acknowledgements

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REFERENCES


