Bioaccumulations of heavy metals in submerged macrophytes in the mountain river Biała Łądecka (Poland, Sudety Mts.)

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Keywords: bioaccumulation, heavy metals, submerged plants, Sudety Mts., mountain rivers, Biała Łądecka River.

Abstract: The study was conducted on the Biała Łądecka River which is a mountain river. It is similar to many European mountain rivers in terms of hydromorphology and catchment management. The aim of this study was to determine the bioconcentration factors of heavy metals (Pb, Cd, Hg, Ni, Cr, Cu and Zn) in Ranunculus aquatilis (L.) Dumort., Fontinalis antipyretica (L. ex Hedw.), and Lemanea fluviatilis (L.) C.Ag. The content of metals in water, sediment, and submerged plants was determined. The metal concentrations in plants can be arranged as follows: Hg < Cd < Cr < Ni < Cu < Pb < Zn. The highest concentrations of Hg, Ni, Cr, and Cu were observed in F. antipyretica, but the highest concentrations of Pb, Cd, and Zn were in R. aquatilis. L. fluviatilis always contained the least amounts of heavy metals. Bioconcentration factors (BCFs) were lowest in L. fluviatilis and highest in F. antipyretica. Among the analyzed metals, plants accumulated the highest amount of Zn, and the least of Hg. The BCFs for Zn were from 24111 (in L. fluviatilis) to 97574 (in R. aquatilis), and BCFs for Hg were from 29 (in L. fluviatilis) to 226 (in F. antipyretica).

Introduction

Water pollution is a fact. We are looking for better (cheaper, easier, faster, more accurate) methods for assessing contamination. A particular challenge is to assess the pollution in the fast-flowing waters of mountain rivers. High flow rates result in a change in the content of pollutants in the water.

Macrophytes have proved to be good material for the study since they are easy to obtain and prepare for testing. Among the aquatic plants, submerged plants deserve special attention. Due to the physiology of submerged plants, they may accumulate the largest amounts of pollutants (Senze et al. 2009).

We wanted to make our study as universal as possible. For this reason, we chose for the study the Biała Łądecka river which is a typical European mountain river. The Biała Łądecka River is a right tributary of the Nysa Klodzka River, with a length of 53 km and a basin area of 314.6 km². The Biała Łądecka River begins in the Bialskie Mountains at an altitude of 1090 meters above sea level and turns into the Nysa Klodzka River at its 133.1 km in the village of Krosnowice at 236 meters above sea level. The river basin is dominated by forests, extensive agriculture as well as urban development. Pollution is prominent because of the crystal glass factory, municipal sewage treatment plants and numerous trout farms. The middle-part of the river (16.3 km) is designated as a Special Protected Area “PL.H020035 Biała Łądecka” under the Natura 2000 network.

The study involved macrophytes commonly found in the Biała Łądecka River. There are dominant submerged species of macrophytes in this river, representing different systematic groups of plants.

Ranunculus aquatilis (L.) Dumort. is a species representing a group of Angiosperms Cronquist., of the family Ranunculaceae (Juss.). Ranunculus aquatilis (L.) Dumort. is a perennial plant and hydrophyte. This plant blooms from April to August. It occurs in shallow and slow-flowing water and canals (Cook 1963, Dahlgren 1991). Ranunculus (L.) subgenus Batrachium (DC.) A. Gray – Batrachium was first treated as a subgenus by A. Gray in 1886 but the rank has varied from section to genus (Dahlgren, 1991). Cook (1963) found the subgenus rank to be the most appropriate. The subgenus is mainly located in Atlantic European countries. All species except two occur in Europe. The subgenus Batrachium has 17 species (Dahlgren 1991).

Fontinalis antipyretica (L. ex Hedw.) is a taxon representing the subclass Bryidae. It occurs in Europe, North Asia, northern Africa and North America, in clear-flowing or
standing waters. This moss is common throughout Poland. It grows on underwater rocks, stones, and pieces of wood in shady places. *F. antipyretica* is often found in temperate regions, in flowing freshwater streams and ponds. Of the 20 species of water moss, 18 are native to North America. A brook moss may have shoots 30 to 100 (rarely up to 200) cm long and is usually attached to a stone or a tree root. The most common species is *F. antipyretica*. This species has long, slender branches covered with glossy, yellowish green or dark green phyllids (leaves), 4 to 7 mm long and arranged in three ranks. Male and female reproductive organs are on separate plants (Atherton et al. 2010).

*Lemanea flaviatilis* (L.) C.Ag. is a species representing the division *Rhodophyta*, of the order *Batrachospermales* (Bory de Saint-Vincent 1808). This taxon occurs in Europe (Kučera et al. 2004), in North America (Vis et al. 1992), South America and Australia. The freshwater red algae of the family *Lemaneaeaceae* are characterized by an uniaxial cartilaginous and pseudoparenchymatous gametophyte thallus with internal carposporophytes (Vis et al. 1992). In Poland, the species occurs in several places especially in the southern part of the country (Starmach 1977). There is only a small number of locations with the species *L. flaviatilis* since it needs specific ecological conditions. For this reason, this alga is on the red list, in those countries where this species is defined as vulnerable (Siemińska 2006).

The first aim of this study was to determine the bioconcentration factors (BCFs) for heavy metals (Pb, Cd, Hg, Ni, Cr, Cu and Zn) in *Ranunculus aquatilis* (L.) Dumort., *Fontinalis antipyretica* (L. ex Hedw.), and *Lemanea flaviatilis* (L.) C.Ag. The second aim was to investigate whether the bioconcentration factors depend on basic physicochemical parameters of water as well as the concentration of heavy metals in water and sediment.

**Materials and methods**

The research was conducted in 2005, in the section of the river that runs from the mouth (in Krosnowice) to the village of Stary Gierałtòw. Samples of water, sediment, and plants were taken at 9 points (Fig 1), three times a year (in the spring, summer and autumn). Coordinates of sampling sites were determined using GPS map 76CS (GARMIN).

The following physicochemical parameters were determined: temperature (°C), electrolytic conductivity (μS · cm⁻¹), water pH, dissolved oxygen (mgO₂ · dm⁻³), water alkalinity (mgCaCO₃ · dm⁻³), water hardness (mgCaCO₃ · dm⁻³), and the biochemical oxygen demand over 5 days – BOD₅ (mgO₂ · dm⁻³).

The sediment samples and plants were “wet” mineralized in a pressure microwave oven MARS 5 (CEM Corporation, USA). The sediment samples were mineralized with a mixture of HNO₃ and HClO₄ at 3÷1, and the plants with HNO₃.

The amount of metals in mineralize was determined using atomic absorption spectrophotometry (AAS). The flame method (SpectrAA 220 – Varian, Australia) was used to determine Pb, Cd, Ni, Cr, Cu, and Zn. The cold vapor method (TMA 254 – Tesla, the Czech Republic) was used to determine Hg.

The results of the analyses were verified with the certified reference materials: BCR 60 (Commission of the European Communities, Community Bureau of Reference) – aquatic plant *Lagarosiphon major* and CRM 482 (Commission of the European Communities, Community Bureau of Reference)

**Fig. 1. Location of sampling site on Biała Lądecka River**

MASL – meters above sea level
The bioconcentration factors (BCFs) were calculated as the relationship of metal concentration in the plant to the concentration of metal in water.

The statistical analysis of the results (the minima, the maxima, the mean, the standard deviation, differences, correlations) was carried out using Statistica 10.0 (StatSoft).

**Results**

The recorded physical and chemical indicators of water quality are summarized in Table 2. According to the current classification in Poland, the physical and chemical water parameters in the tested section, classified the Biała Łądecka River as having a class I freshwater quality.

The pH of the water of the Biała Łądecka River was slightly alkaline (pH 6.9–8.1). Electrolytic conductivity of the water and its total hardness were relatively low. The content of dissolved O₂ was high, which is typical for the fast-running and stirred-up water of mountain streams and rivers. Average BOD₅ was low, but its maximum value was high. These results may indicate water contamination with organic substances.

The metal contents which were found in the water of the Biała Łądecka River are shown in Table 3. Different levels of metals were found in the water from 0.57 μg · dm⁻³ (Hg) to 8.43 μg · dm⁻³ (Zn). According to Kabata-Pendias et al. (1993), Pb, Ni, Cr, Cu, and Zn concentrations are characteristic for Polish rivers, while concentrations of Hg and Zn indicate contamination.

The contents of five metals – Pb, Hg, Ni, Cu i Zn were examined in sediment (Table 4). The concentration of Hg in the sediment was lowest (0.03 mg · kg⁻¹). Concentrations of Cu and Ni were considered to be at the middle level (13.78 and 14.71 mg · kg⁻¹, respectively), while the concentration of Zn in the sediment was highest (74.24 mg · kg⁻¹).

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**Table 1.** Certified and obtained concentration of Pb, Cd, Hg, Ni, Cr, Cu and Zn in used reference materials (mg · kg⁻¹)

|        | Pb    | Cd    | Hg    | Ni    | Cr    | Cu    | Zn    |
|--------|-------|-------|-------|-------|-------|-------|-------|
| CRM 482 certificated | 40.900 | 0.560 | 0.480 | 2.470 | 4.120 | 7.030 | 100.600 |
| CRM 482 obtained | 42.082 | 0.576 | 0.494 | 2.485 | 4.222 | 7.247 | 102.219 |
| SD     | 0.902 | 0.065 | 0.340 | 0.148 | 0.190 | 0.525 | 2.488  |
| difference (%) | 2.89  | 2.78  | 2.92  | 0.61  | 2.48  | 3.08  | 1.61   |
| BCR 60 certificated | 63.800 | 2.120 | 0.340 | 40.000* | 26.000* | 51.200 | 313.000 |
| BCR 60 obtained | 64.027 | 2.160 | 0.338 | 40.768 | 26.850 | 51.650 | 311.322 |
| SD     | 2.308 | 0.179 | 0.030 | 1.942 | 2.399 | 2.670 | 3.721  |
| difference (%) | 0.36  | 1.89  | -0.53 | 1.92  | 3.27  | 0.87  | -0.54  |
| LGC 6187 certificated | 77.200 | 2.700 | 1.400 | 34.700 | 84.000 | –      | 439.000 |
| LGC 6187 obtained | 78.100 | 2.746 | 1.424 | 34.740 | 84.836 | –      | 438.690 |
| SD     | 1.122 | 0.128 | 0.037 | 1.514 | 1.791 | –      | 1.758  |
| difference (%) | 1.16  | 1.70  | 1.74  | 0.12  | 1.00  | –      | -0.07  |

* – not certificated values; SD – standard deviation

**Table 2.** Physical and chemical parameters of water – temperature (°C), pH, electrolytic conductivity (μS · cm⁻¹), alkalinity (mg CaCO₃ · dm⁻³), total hardness (mg CaCO₃ · dm⁻³), dissolved oxygen (mg O₂ · dm⁻³) and BOD₅ (mg O₂ · dm⁻³)

|        | Temperature | pH | Electrolytic conductivity | Alkalinity | Total hardness | Dissolved oxygen | BOD₅ |
|--------|-------------|----|----------------------------|------------|---------------|-----------------|------|
| Average | 10.12       | –  | 144.35                     | 44.646     | 74.427        | 11.51           | 2.8  |
| Min    | 6.5         | 6.9 | 87                         | 19.99      | 55.34         | 10.2            | 1.1  |
| Max    | 14.0        | 8.1 | 236                        | 99.94      | 110.67        | 12.4            | 7.7  |
| SD     | 2.43        | –  | 42.83                      | 19.05      | 16.93         | 0.60            | 1.22 |
Table 3. Concentrations of Pb, Hg, Cd, Ni, Cr, Cu and Zn in water (µg · dm⁻³)

|       | Pb    | Hg   | Cd    | Ni    | Cr   | Cu   | Zn   |
|-------|-------|------|-------|-------|------|------|------|
| Average | 4.99  | 0.57 | 0.79  | 1.55  | 0.90 | 2.09 | 8.43 |
| Min    | 0.60  | 0.20 | 0.20  | 0.10  | 0.10 | 0.10 | 0.10 |
| Max    | 11.10 | 1.40 | 1.90  | 5.80  | 2.10 | 4.20 | 29.60|
| SD     | 2.86  | 0.31 | 0.42  | 1.36  | 0.55 | 0.86 | 6.32 |

SD – standard deviation

Table 4. Concentrations of Pb, Hg, Ni, Cu and Zn in bottom deposits (mg · kg⁻¹)

|       | Pb    | Hg   | Ni    | Cu   | Zn   |
|-------|-------|------|-------|------|------|
| Average | 56.28 | 0.03 | 14.71 | 13.78| 74.24|
| Min    | 10.39 | 0.01 | 8.94  | 6.66 | 45.35|
| Max    | 134.41| 0.08 | 18.94 | 20.37| 116.58|
| SD     | 40.96 | 0.03 | 14.71 | 13.78| 74.24|

SD – standard deviation

Among the studied metals in plants, Zn was found most frequently, whereas Hg the least (Table 5), and the differences between concentration of these metals were very high. The concentration of Zn in \( F. \) antipyretica was 1,500 times higher than the concentration of Hg in \( F. \) antipyretica. In \( R. \) aquatile the concentration of Zn was 10,300 times higher than the concentration of Hg. The significance of difference between concentrations of metals in different plant species was also calculated (Table 5).

The noted metal content in water and plants allowed for the calculation of the bioconcentration factors (BCFs) . The obtained results are shown in Fig 2 and Table 6. The trends, which are visible on the graph (Fig. 2), have been confirmed by the demonstration of the statistical significance of difference as shown in Table 6 (all metals except Pb).

The correlation between physicochemical parameters of water, metal concentrations in water, sediment, plants and the metal concentration (Tables 7, 8, and 9), and BCFs for metals in plants (Tables 10, 11, and 12) were calculated. Only statistically significant (\( p \leq 0.05 \)) correlations are presented in the Tables.

All other correlations should be considered as strong, \( r \) ranged from -1.0 to -0.5, and from 0.5 to 1.0.

Discussion

Kozubek et al. (2002) conducted research on the Bystrzyca Dusznicka River which is similar to and located near the Biała Łądecka.

These authors found that pH and conductivity of the Bystrzyca Dusznicka River were similar to those of the Biała Łądecka. They also found that BOD₅ was significantly lower (0.82 mg O₂ · dm⁻³), dissolved oxygen levels (9.68 mg O₂ · dm⁻³) were also lower, and total hardness was higher (118.6 mg CaCO₃ · dm⁻³). Simić (2007) gives similar values of the physicochemical water parameters of the Rešava River and the Božička River in Serbia, where there were \( L. \) fluviatilis populations. Say et al. (1983) noted the results of various authors concerning the pH and electrolytic conductivity of water from the habitats of \( F. \) antipyretica and \( R. \) penicilatus. The pH from these sites ranged from 6.5 to 8.1, and the electrolytic conductivity was higher than in the Biała Łądecka River – 70–615 μS · cm⁻¹. During their research Say et al. (1983) determined the parameters of water in the places where \( F. \) antipyretica was found. At these places, the pH was 7.5 and electrolytic conductivity was 296 μS · cm⁻¹.

Metal concentrations in the water of the Biała Łądecka River differed from the concentrations described by Kozubek et al. (2002). They reported higher concentrations of Pb (30.69 μg · dm⁻³), Hg (3.1 μg · dm⁻³), Ni (5.11 μg · dm⁻³), and Zn (28.65 μg · dm⁻³). The values they reported are several times higher than those in the Biała Łądecka River, however, the Cu content was comparable (2.69 μg · dm⁻³), though the Cd content was less comparable (0.3 μg · dm⁻³). In the mountain river Bystrzyca and its tributaries (also in the Sudety Mts), Senze et al. (2007) found higher amounts of Cu, Ni and Zn (for Cu and Zn – 2 times higher), and lower Cd and Pb than we had found in the Biała Łądecka River. On habitats of \( F. \) antipyretica, Say et al. (1983) found similar amounts of Pb (4.2 μg · dm⁻³), smaller amounts of Cd (0.4 μg · dm⁻³), higher amounts of Cu (4.5 μg · dm⁻³) and significantly higher amounts of Zn (83 μg · dm⁻³). Samecka-Cymerman et al. (1999) analyzed bryophytes from mountain rivers in basaltic areas of central France. They found different levels of metals in the water: much higher of Cr (5.1–12.9 μg · dm⁻³), Ni (6.4–29 μg · dm⁻³), and Zn (27–48 μg · dm⁻³), Cu which was comparable (1–4.6 μg · dm⁻³), and significantly lower of Cd (0.003–0.142 μg · dm⁻³) than the metal levels in the Biała Łądecka River.

The metal concentrations found in the water of the Biała Łądecka River are similar to the concentrations found in other clean European rivers (Neal et al. 1998, Vázquez et al. 2000, Samecka-Cymerman et al. 2005, Gecheva et al. 2011).

The metal content in sediment was significantly higher than that found in the water of the Biała Łądecka River. The metal concentration in sediment to metal concentration in
Volatility can be expressed as a variability coefficient, respectively, and a higher variability coefficient for Pb and Hg (73% and 100%, respectively). The metal content of the sediment had different letters indicate statistically significant differences, ab – p ≤ 0.05, AB – p ≤ 0.01; SD – standard deviation.

| Table 5. Concentrations of Pb, Hg, Cd, Ni, Cr, Cu and Zn in *R. aquatile*, *F. antipyretica* and *L. fluviatilis* (mg · kg⁻¹ in dry weight) |
|---------------------------------------------------------------|
| **Ranunculus aquatile**                                        |
| Average            | Pb  | Hg  | Cd  | Ni  | Cr  | Cu  | Zn  |
|                   | 77.44<sup>a</sup> | 0.068<sup>a</sup> | 2.560<sup>a</sup> | 10.25<sup>a</sup> | 5.289<sup>a</sup> | 23.46<sup>a</sup> | 701.1<sup>a</sup> |
| Min                | 8.63 | 0.27  | 0.680 | 5.59  | 2.453 | 16.86 | 148.9  |
| Max                | 245.65 | 0.126 | 4.460 | 18.61 | 9.385 | 34.11 | 209.34  |
| SD                 | 74.12 | 0.024 | 1.093 | 4.22  | 2.381 | 5.59  | 604.3  |

| Fontinalis antipyretica                                        |
| Average            | Pb  | Hg  | Cd  | Ni  | Cr  | Cu  | Zn  |
|                   | 35.66<sup>b</sup> | 0.104<sup>b</sup> | 0.689<sup>b</sup> | 11.95<sup>b</sup> | 6.446<sup>a</sup> | 23.76<sup>a</sup> | 160.3<sup>b</sup> |
| Min                | 9.10  | 0.045 | 0.243 | 6.78  | 2.077 | 9.34  | 71.8   |
| Max                | 76.95 | 0.149 | 1.146 | 17.68 | 10.042 | 41.90 | 257.4  |
| SD                 | 20.68 | 0.035 | 0.283 | 3.89  | 2.319 | 12.23 | 74.8   |

| Lemanea fluviatilis                                          |
| Average            | Pb  | Hg  | Cd  | Ni  | Cr  | Cu  | Zn  |
|                   | 25.07<sup>b</sup> | 0.017<sup>c</sup> | 0.289<sup>b</sup> | 4.98<sup>b</sup>  | 1.735<sup>b</sup> | 11.29<sup>b</sup> | 113.6<sup>b</sup> |
| Min                | 8.99  | 0.010 | 0.190 | 2.91  | 0.830 | 6.67  | 79.7   |
| Max                | 51.20 | 0.025 | 0.428 | 8.20  | 2.732 | 15.94 | 145.4  |
| SD                 | 12.43 | 0.005 | 0.085 | 1.72  | 0.531 | 2.64  | 25.0   |

different letters indicate statistically significant differences, ab – p ≤ 0.05, AB – p ≤ 0.01; SD – standard deviation.

| Table 6. Bioconcentration factors (BCF) for Pb, Hg, Cd, Ni, Cr, Cu and Zn in *R. aquatile*, *F. antipyretica* and *L. fluviatilis* |
|---------------------------------------------------------------|
| **Ranunculus aquatile**                                        |
| Average            | Pb  | Hg  | Cd  | Ni  | Cr  | Cu  | Zn  |
|                   | 17851<sup>a</sup> | 149<sup>a</sup>  | 4496<sup>a</sup> | 9084<sup>a</sup>  | 5523<sup>a</sup> | 13224<sup>a</sup> | 97574<sup>a</sup> |
| Min                | 1952  | 39   | 755  | 3004  | 1950  | 34108  | 11134  |
| Max                | 58197 | 319  | 12038 | 37538 | 11731 | 309482  |         |
| SD                 | 17483 | 92   | 3746 | 8461  | 3688  | 6624   | 91435  |

| Fontinalis antipyretica                                        |
| Average            | Pb  | Hg  | Cd  | Ni  | Cr  | Cu  | Zn  |
|                   | 8047<sup>a</sup> | 226<sup>a</sup>  | 920<sup>a</sup>  | 26404<sup>ab</sup> | 12258<sup>a</sup> | 13789<sup>a</sup> | 33978<sup>b</sup> |
| Min                | 3838  | 65   | 280  | 3601  | 4096  | 32404  | 95321  |
| Max                | 21066 | 410  | 1432 | 67776 | 20766 | 39202   |         |
| SD                 | 5218  | 123  | 456  | 23676 | 5774  | 10144  | 29803  |

| Lemanea fluviatilis                                          |
| Average            | Pb  | Hg  | Cd  | Ni  | Cr  | Cu  | Zn  |
|                   | 7036<sup>a</sup> | 29<sup>b</sup>  | 396<sup>b</sup>  | 12046<sup>ab</sup> | 3029<sup>a</sup> | 5857<sup>b</sup> | 24111<sup>b</sup> |
| Min                | 2362  | 10   | 128  | 1020  | 838  | 2996   | 11892  |
| Max                | 13371 | 43   | 681  | 33234 | 4914  | 8951   | 53007  |
| SD                 | 4187  | 13   | 198  | 11580 | 1616  | 2000   | 14809  |

different letters indicate statistically significant differences, ab – p ≤ 0.05, AB – p ≤ 0.01; SD – standard deviation.

Water ratio was almost 10,000 for Pb, Ni and Zn. For Cu, this ratio was 6600. For Hg, the ratio was quite different; only 53.

In terms of metal content, the bottom sediment of the Biała Łądecka River (Table 4) belongs to class I classification, only the Pb content was typical of the class II classification of bottom sediment (Bojakowska 2001). These values do not differ from the values reported by other researchers for other rivers (Say et al. 1983, Ciszewski 2001, Ibragimow et al. 2013, Pokorny et al. 2013, Jabłońska-Czapla et al. 2014).

The metal content in the water and sediment of the Biała Łądecka River was characterized by high volatility. The volatility can be expressed as a variability coefficient calculated from the formula: Standard Deviation/Average · 100%. For metals in water, the variability coefficient ranged from 41% (for Cu) to 88% (for Ni). The metal content of the sediment had a lower variability coefficient for Ni, Cu, and Zn (23%, 32%, and 29%, respectively), and a higher variability coefficient for Pb and Hg (73% and 100%, respectively).

The variability coefficient for the metal content in plants was lower than the variability coefficient for the metal content in water and sediment. However, the variation coefficient differed depending on the plant species. The highest was for *R. aquatile* (from 24% to 96% for Cu and Pb, respectively) and the lowest was for *L. fluviatilis* (from 22% to 50% for Zn and Pb, respectively).

The metal concentrations in all the species of plants from the Biała Łądecka River study, can be arranged as follows: Hg < Cd < Cr < Ni < Cu < Pb < Zn, and arrangement is the same for the water. Among the studied plants, the lowest metal concentrations were found in *L. fluviatilis*. Of all species of plants, *F. antipyretica* contained the highest amount of Hg, Ni, Cr, Cu, and *R. aquatile* contained the highest amount of Pb, Cd and Zn. We found statistically significant differences (Pb – p ≤ 0.05, all other – p ≤ 0.01) between the content of each metal in *L. fluviatilis*, and in *R. aquatile*.

The contents of all metals in *F. antipyretica* were higher than the metals content in *L. fluviatilis*. The differences for Hg, Ni, Cr, and Cu were statistically significant (p ≤ 0.01).

As indicated by available literature, the metal levels in plants from the present Biała Łądecka River study were similar to the results of other authors. In *R. aquatile*, Martinez...
Table 7. Statistically significant (p ≤ 0.05) correlations between physicochemical parameters of water and content of the investigated metals in water, sediment and the metal concentration in *R. aquatilis*

| Relations between (x and y) | Regression equation | Correlation coefficient (r) | Significant level (p) |
|-----------------------------|---------------------|----------------------------|---------------------|
| Cond : Zn in R. aq.         | y = -268.6133 + 6.7003 x | 0.5084                     | 0.0312              |
| Cond : Cd in R. aq.         | y = 1539.251 + 100.213 x | 0.6955                     | 0.0009              |
| Alk : Ni in R. aq.          | y = 5.4408 + 0.1053 x   | 0.5198                     | 0.0226              |
| Th : Zn in R. aq.           | y = 716.4021 + 18.7345 x| 0.5313                     | 0.0233              |
| BOD₅ : Pb in R. aq.         | y = -26.6462 + 36.4196 x| 0.6582                     | 0.0022              |
| Pb in water : Pb in R. aq.  | y = 0.7298 + 14574.4153 x| 0.5512                     | 0.0144              |
| Zn in water : Cd in R. aq.  | y = 1.2915 + 231.4913 x | 0.8333                     | 0.0053              |
| Ni in water : Ni in R. aq.  | y = 6.9403 + 1855.1593 x| 0.6035                     | 0.0062              |
| Cr in water : Zn in R. aq.  | y = 1209.9989 + 5.1465E5 x| -0.4948                    | 0.0369              |
| Pb in sed. : Pb in R. aq.   | y = 21.8663 + 1.5595 x  | 0.8688                     | 0.0000              |
| Zn in sed. : Zn in R. aq.   | y = 1859.2892 – 14.8605 x| -0.5531                    | 0.0173              |
| Cu in sed. : Zn in R. aq.   | y = 2031.2182 – 92.7053 x| -0.7159                    | 0.0008              |
| Ni in sed. : Zn in R. aq.   | y = 2251.2317 – 102.9232 x| -0.5887                    | 0.0102              |
| Hg in sed. : Zn in R. aq.   | y = 1163.0296 – 11629.7886 x | -0.5988                   | 0.0086              |

R. aq. – *Ranunculus aquatilis*; Cond – electrolytic conductivity; Alk – alkalinity; Th – total hardness; sed. – sediment
### Table 8. Statistically significant (p ≤ 0.05) correlations between physicochemical parameters of water and content of the investigated metals in water, sediment and the metal concentration in *F. antipyretica*

| Relations between (x and y) | Regression equation | Correlation coefficient (r) | Significant level (p) |
|-----------------------------|---------------------|-----------------------------|-----------------------|
| Alk : Cr in Font.           | y = 14.0267 – 0.2254 x | -0.9581                     | 0.00005               |
| Th : Cu in Font.            | y = -45.5121 + 1.0276 x | 0.7118                      | 0.0315                |
| O$_2$: Cu in Font.          | y = 219.207 – 16.8977 x | -0.6875                     | 0.0407                |
| Pb in water : Pb in Font.   | y = 8.4316 + 5117.0033 x | 0.7504                      | 0.0198                |
| Pb in water : Ni in Font.   | y = 6.6759 + 991.1837 x | 0.7730                      | 0.0146                |
| Zn in water : Pb in Font.   | y = 73.4723 – 6065.2731 x | -0.7263                    | 0.0267                |
| Zn in water : Ni in Font.   | y = 18.5588 – 1060.3333 x | -0.6751                    | 0.0460                |
| Ni in water : Pb in Font.   | y = 20.4559 + 13036.7567 x | 0.8171                      | 0.0072                |
| Hg in water : Cr in Font.   | y = 3.7307 + 4610.7845 x | 0.6847                      | 0.0419                |
| Pb in sed. : Pb in Font.    | y = 14.8068 + 0.3328 x | 0.8211                      | 0.0067                |
| Pb in sed. : Cu in Font.    | y = 12.0882 + 0.1862 x | 0.7767                      | 0.0138                |
| Pb in sed. : Ni in Font.    | y = 7.6636 + 0.0684 x  | 0.8976                      | 0.0010                |
| Zn in sed. : Pb in Font.    | y = -11.9592 + 0.6015 x | 0.7434                      | 0.0217                |
| Hg in sed. : Pb in Font.    | y = 15.5155 + 479.7585 x | 0.7457                      | 0.0211                |

Font – *Fontinalis antipyretica*; Alk – alkalinity; Th – total hardness; O$_2$ – dissolved oxygen; sed. – sediment

### Table 9. Statistically significant (p ≤ 0.05) correlations between physicochemical parameters of water and content of the investigated metals in water, sediment and the metal concentration in *L. fluviatilis*

| Relations between (x and y) | Regression equation | Correlation coefficient (r) | Significant level (p) |
|-----------------------------|---------------------|-----------------------------|-----------------------|
| Cond : Hg in Lem.           | y = -0.0029 + 0.0002 x | 0.8831                      | 0.0016                |
| Th : Ni in Lem.             | y = -7.4287 + 0.1938 x | 0.6706                      | 0.0481                |
| Th : Cd in Lem.             | y = -0.4263 + 0.0112 x | 0.7812                      | 0.0129                |
| Th : Cr in Lem.             | y = -2.6022 + 0.0677 x | 0.7597                      | 0.0175                |
| Zn in water : Cr in Lem.    | y = 2.8998 + 207.1143 x | -0.7709                    | 0.0150                |
| Pb in sed. : Pb in Lem.     | y = 15.0003 + 0.1627 x | 0.6733                      | 0.0468                |
| Cu in sed. : Hg in Lem.     | y = 0.0258 – 0.0006 x  | -0.6933                     | 0.0384                |
| Ni in sed. : Hg in Lem.     | y = 0.0304 – 0.0009 x  | -0.7854                    | 0.0121                |

Lem. – *Lemanea fluviatilis*; sed. – sediment; Cond – electrolytic conductivity; Th – total hardness;

### Table 10. Statistically significant (p ≤ 0.05) correlations between physicochemical parameters of water and content of the investigated metals in water, sediment and the BCF for metal in *R. aquatile*

| Relations between (x and y) | Regression equation | Correlation coefficient (r) | Significant level (p) |
|-----------------------------|---------------------|-----------------------------|-----------------------|
| Zn in water : BCF Pb in R. aq. | y = 3456.1036 + 1.6003E6 x | 0.62                        | 0.004                 |
| Pb in water : BCF Cd in R. aq. | y = 1137.7484 – 1.186E6 x | -0.75                       | 0.020                 |
| Zn in water : BCF Cd in R. aq. | y = 936.8764 + 6.4978E5 x | 0.68                        | 0.043                 |
| Cd in R. aq. : BCF Ni in R. aq. | y = 18054.0579 – 3598.0412 x | -0.67                     | 0.047                 |
| Cond : BCF Cu in R. aq.     | y = 1539.251 + 100.213 x  | 0.70                        | 0.001                 |
| Alk : BCF Cu in R. aq.      | y = 2733.5963 + 229.571 x | 0.72                        | 0.001                 |
| Th : BCF Cu in R. aq.       | y = -7840.0059 + 274.0981 x | 0.72                      | 0.001                 |
| Ni in water : BCF Cu in R. aq. | y = 9001.8029 + 2.3636E6 x | 0.49                        | 0.033                 |
| Cr in water : BCF Cu in R. aq. | y = 18950.3289 – 5.7569E6 x | -0.49                     | 0.033                 |
| Hg in sed. : BCF Cu in R. aq. | y = 17272.3509 – 1.0657E5 x | -0.50                     | 0.029                 |

R. aq. – *Ranunculus aquatile*; Cond – electrolytic conductivity; Alk – alkalinity; Th – total hardness; sed. – sediment
Table 11. Statistically significant ($p \leq 0.05$) correlations between physicochemical parameters of water and content of the investigated metals in water, sediment, and plant and the BCF for metal in *F. antipyretica*

| Relations between (x and y) | Regression equation | Correlation coefficient (r) | Significant level (p) |
|-----------------------------|---------------------|-----------------------------|-----------------------|
| Hg in water : BCF Pb in Font. | $y = 814.1135 + 1.2283E7 \times x$ | 0.81 | 0.008 |
| Th : BCF Hg in Font. | $y = -468.5711 + 10.2205 \times x$ | 0.68 | 0.030 |
| $O_2$ : BCF Hg in Font. | $y = 2602.9746 - 205.9489 \times x$ | -0.80 | 0.006 |
| Hg in sed. : BCF Ni in Font. | $y = 47120.207 - 4.9324E5 \times x$ | -0.67 | 0.048 |
| Th : BCF Cu in Font. | $y = -52677.9782 + 985.9972 \times x$ | 0.82 | 0.006 |
| $O_2$ : BCF Cu in Font. | $y = 2.1071E5 - 17024.9992 \times x$ | -0.84 | 0.005 |
| Pb in sed. : BCF Zn in Font. | $y = 3880.3069 + 480.2388 \times x$ | 0.82 | 0.007 |
| Zn in sed. : BCF Zn in Font. | $y = -31942.6097 + 832.6085 \times x$ | 0.71 | 0.031 |

Font – *Fontinalis antipyretica*; Th – total hardness; $O_2$ – dissolved oxygen; sed. – sediment

Table 12. Statistically significant ($p \leq 0.05$) correlations between physicochemical parameters of water and content of the investigated metals in water, sediment, and plant and the BCF for metal in *L. fluvialis*

| Relations between (x and y) | Regression equation | Correlation coefficient (r) | Significant level (p) |
|-----------------------------|---------------------|-----------------------------|-----------------------|
| Cd in water : BCF Pb in Lem. | $y = 13153.7168 - 6.7147E6 \times x$ | -0.80 | 0.009 |
| Alk : BCF Cd in Lem. | $y = 1010.384 - 18.8766 \times x$ | -0.71 | 0.033 |
| Cu in water : BCF Cd in Lem. | $y = 697.097 - 1.4022E5 \times x$ | -0.68 | 0.045 |
| Hg in sed. : BCF Ni in Lem. | $y = 22830.9852 - 2.5995E5 \times x$ | -0.73 | 0.025 |
| BOD$_5$ : BCF Cu in Lem. | $y = 12016.3846 - 2399.7702 \times x$ | -0.77 | 0.015 |
| Cd in water : BCF Cu in Lem. | $y = 8658.223 - 3.0745E6 \times x$ | -0.77 | 0.015 |
| Pb in sed. : BCF Zn in Lem. | $y = 10519.1522 + 219.5411 \times x$ | 0.76 | 0.017 |
| Zn in sed. : BCF Zn in Lem. | $y = -6254.2987 + 388.7518 \times x$ | 0.71 | 0.034 |

Lem. – *Lemanea fluitans*; Alk – alkalinity; sed. – sediment

et al. (2011) found higher amounts of Hg and Cu (respectively, 1.045 mg · kg$^{-1}$ and 53.15 mg · kg$^{-1}$) but smaller amounts of Cd and Zn (respectively, 0.555 mg · kg$^{-1}$ and 61.25 mg · kg$^{-1}$). Samecka-Cymerman et al. (1996) found in *R. aquatile* (from contaminated river) higher amounts of Hg and Ni (0.16 mg · kg$^{-1}$ and 9.5 mg · kg$^{-1}$) but smaller Pb, Cd, Cr, Cu and Zn (1.6 mg · kg$^{-1}$, 0.63 mg · kg$^{-1}$, 2.4 mg · kg$^{-1}$, 7.1 mg · kg$^{-1}$ and 101 mg · kg$^{-1}$).

There is much more information in the literature concerning *F. antipyretica*. In our study, the indicated concentrations of Pb, Cd, Ni, Cr, and Cu in this moss were in the range reported by the following authors (Say et al. 1983, Vazquez et al. 2004, Gapeeva et al. 2010, Gecheva et al. 2011, Martinez et al. 2011). However, in the case of Hg and Zn, the maximum values given by the authors are much higher than those obtained in our study. In *F. antipyretica*, Vazquez et al. (2004) even found 0.76 mg · kg$^{-1}$ (5 times more than the maximum value found in our study) and 1107 mg · kg$^{-1}$ Zn (4 times more). In *F. antipyretica*, Say et al. (1983) found 2825 mg · kg$^{-1}$ Zn (11 times more).

Correlation coefficients between the analyzed factors (physical and chemical parameters of water, metal concentration in water, sediment, and plants) were calculated. Among the statistically proven correlations, some can be distinguished. What would seem to be the obvious relationship of the metal content in water and the content of the same metal in plant, was actually only statistically confirmed in three cases. The three cases were: 1) Pb in water and Pb in *R. aquatile*, and 2) Pb in water vs. Pb and *F. antipyretica*, and 3) Ni in water and Ni in *R. aquatile*. All these correlations are positive and strong. Statistically significant correlations between a specific metal content in sediment and that specific metal content in plant were equally rare. The correlations were statistically confirmed only for Pb in sediment and Pb in all of plant species, and the correlations were statistically confirmed for Zn in sediment and Zn in *R. aquatile*. Interestingly, with an increase of Zn content in sediment, there was a decrease in the concentration of Zn in *R. aquabile* ($r = -0.55$). Samecka-Cymerman et al. (1999) have confirmed other correlations: Cu in water and Ni in bryophytes, Cr in water and Pb in bryophytes, Pb in water and Cd in bryophytes. But their research was carried out in different water conditions, where, above all, there was a low pH (5.7–6.3).

Say et al. (1983) demonstrated a correlation between Cu and Zn in water and in *F. antipyretica*. They also showed a correlation between the contents of Cd, Pb, Cu, and Zn in sediment and in *F. antipyretica*.

The occurrence of a positive correlation between the content of the metal in water and its concentration in plant may indicate that the metal is absorbed by green part of plant, which is washed by water. The lack of such correlation does not necessarily imply a different way of metal absorption. It
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may be due to the fact that metal is present in water in less bioavailable forms. Positive correlations between the metal content in sediment and metal concentrations in plant could indicate metal absorption by the roots or rhizoids.

Bioconcentration factors vary significantly depending on the metal and the plant species. The calculated BCFs for metals, arranged in ascending order, are as follows:

\[
\begin{align*}
\text{Ranunculus aquatilis} & \quad \text{Hg} < \text{Cd} < \text{Cr} < \text{Ni} < \text{Cu} < \text{Pb} < \text{Zn} \\
\text{Fontinalis antipyretica} & \quad \text{Hg} < \text{Cd} < \text{Pb} < \text{Cr} < \text{Cu} < \text{Ni} < \text{Zn} \\
\text{Lemanea fluviatilis} & \quad \text{Hg} < \text{Cd} < \text{Cr} < \text{Cu} < \text{Pb} < \text{Ni} < \text{Zn}
\end{align*}
\]

Among the analyzed metals, plants accumulated the highest amount of Zn, and the least of Cd and Hg. For nickel, the lowest BCF was calculated in \textit{R. aquatilis}, for all other metals the lowest BCF was calculated in \textit{L. fluviatilis}.

In conclusion, we can say that the lowest bioaccumulation of metals was characterized by \textit{L. fluviatilis}, and the highest by \textit{F. antipyretica}.

Physicochemical parameters may have statistically significant influence ($p \leq 0.05$) on the BCFs for metals in plants. The following statistically significant relationships were confirmed: BCF for Cu in \textit{R. aquatilis} was correlated with the alkalinity, electronitological conductivity, and total hardness; BCF for Cu in \textit{F. antipyretica} was correlated with total hardness and dissolved oxygen; and BCF for Cu in \textit{L. fluviatilis} was correlated with BOD$_5$. The BCF for Hg in \textit{F. antipyretica} was correlated with total hardness and dissolved oxygen. The BCF for Cd in \textit{L. fluviatilis} was correlated with alkalinity.

We managed to see some significant trends. The bioconcentration factor for Zn in \textit{F. antipyretica} and \textit{L. fluviatilis} was positively ($r > 0.7$) correlated with the contents of Pb and Zn in river sediments. The bioconcentration factor for Ni was inversely proportional to concentrations of Hg in the sediment, for \textit{F. antipyretica}, and \textit{L. fluviatilis}. Whereas, in the case of \textit{R. aquatilis}, Hg in sediment correlated (also negatively) with BCF for Cu.

Conclusions
1. The lowest concentrations of Hg and the highest concentrations of Zn were determined in the environmental elements of the Biała Łądecka River.
2. In ascending order, the metal concentrations were: water < sediments < plants.
3. The concentrations of metals in the water and in the plants can be arranged as follows: Hg < Cd < Cr < Ni < Cu < Pb < Zn.
4. Out of three plants species, the lowest concentrations of all metals were determined in \textit{L. fluviatilis}.
5. In all plants species, the lowest BCFs were calculated for Hg, and the highest for Zn.

Acknowledgments
We wish to thank Dr. Pavel Kučera from the Department of Botany, Masaryk University Brno, the Czech Republic, and Dr. Petr Marvan from the Academy of Science of the Czech Republic, Institute of Botany Brno, for their help in the determination of \textit{Lemanea fluviatilis} (L.) C.Ag.

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**Bioakumulacja metali ciężkich w makrofitach submersyjnych górskiej rzeki Białej Łądeckiej (Polska, Sudety)**

**Streszczenie:** Badania przeprowadzono na górskiej rzce Białej Łądeckiej. Pod względem hydromorfologicznym i zagospodarowania zlewni jest ona porównywalna z wieloma europejskimi rzekami górnymi. Celem pracy było określenie współczynników biokoncentracji metali ciężkich (Pb, Cd, Hg, Ni, Cr, Cu i Zn) w *Ranunculus aquatilis* (L.) Dumort., *Fontinalis antipyretica* (L. ex Hedw.) i *Lemanea fluviatilis* (L.) C.Ag. Określono zawartość metali w wodzie, osadzie, i roślinach zanurzonych. Stężenia metali w roślina można uszeregować w następujący sposób: Hg <Cd <Cr <Ni <Cu <Pb <Zn. Najwyższe stężenia Hg, Ni, Cr i Cu stwierdzono w *F. antipyretica*, a najwyższe stężenie Pb, Cd i Zn w *R. aquatilis*. Stężenia metali w *L. fluviatilis* zawsze były najniższe. Na podstawie zawartości metali w wodzie i w roślinach obliczono współczynniki biokoncentracji (BCFs). Współczynniki BCFs były najniższe dla *L. fluviatilis* a najwyższe dla *F. antipyretica*. Spośród analizowanych metali, rośliny kumulowały najwięcej Zn a najmniej Hg. BCFs dla Zn wynosiło od 24111 (w *L. fluviatilis*) do 97574 (w *R. aquatilis*), a BCFs dla Hg wynosiło od 29 (w *R. fluviatilis*) do 226 (w *F. antipyretica*).