



Pellia endiviifolia (Dicks.) Dumort. Liverwort with a Potential for Water Purification

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Abstract

The aim of this study was to assess accumulation ability of the liverwort of *Pellia endiviifolia* Dicks. Dum. in relation with macro- and trace elements uptake from stream water within the mid-forest headwater ecosystem. The research was carried out under conditions of limited impact of anthropogenic factors, which allowed assessing the nutrient needs and providing information on the accumulative possibilities of *P. endiviifolia*. The concentrations of 12 elements (N, P, K, Mg, Ca, Zn, Fe, Cu, Ni, Mn, Al, and Sr) in thalli of liverwort collected from three sites in northern Poland were determined by atomic absorption spectrometry and by microwave plasma atomic emission spectrometry. The thalli of the tested liverwort accumulated, on average, 2502.28 mmol/kg of macro- and trace elements, with macronutrients accounting for 96.22% of this pool. Nitrogen constituted 40.3% of this sum, phosphorus 4.9%, potassium 38.1%, magnesium 5.0%, calcium 12.1%, and trace elements 3.78% of this sum. Among trace elements, the dominant share was represented by iron 56.30%, manganese 24.80%, and aluminum 16.95%, and the share of other elements was lower than 1%. Accumulation in thalli of *P. endiviifolia* high concentrations of iron and manganese confirms the potential use this species in the processes purification of waters contaminated with iron and manganese compounds. Based on the results of the research, it was found that the amount of accumulated Mg, Fe, and Cu was influenced by environmental conditions, whereas N, P, K, Ca, Zn, Ni, Mn, Al, and Sr rather by species-related features than environmental factors.

Keywords Stream · Water · *Pellia endiviifolia* · Accumulation · Macroelements · Trace elements

Introduction

Pellia endiviifolia (Dicks.) Dum. is a representative of thalloid liverworts of the *Pelliidae* subclass, the class of *Jungermanniopsida* (Crandall-Stotler and Stotler 2000; He-Nyngren et al. 2006). *P. endiviifolia* is a species widely distributed in the Northern Hemisphere, and it belongs to the hydrophilous liverworts. It occurs on stones, dead wood, most often in ditches, spring streams, and other damp places. It prefers limestone substrate (Osadowski

2010). In the scientific literature, there is little information about the accumulative properties of this species. Little interest in liverworts is probably due to the difficulty in recognition of species. However, due to the occurrence of *P. endiviifolia* in both clean and polluted waters (Osadowski 2010) and its accumulative properties in relation with some impurities (Satake et al. 1987), this species may be useful in water treatment processes. Cleansing the environment with plants is a promising and future-proof alternative to conventional methods (Marecik et al. 2006; Siwek 2008; Grobelak et al. 2010; Astel et al. 2014; Parzych 2016). Phytoremediation is defined as the use of above-average abilities of some green plants to remove pollutants from the environment (Raskin et al. 1997). Plants used in the phytoremediation process are able to remove harmful substances from waters, soils, and bottom sediments (Hughes et al. 1997; Grobelak et al. 2010; Parzych 2016). Numerous plant species are highly efficient in removing of toxic substances as they have the ability to

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effectively take up or degrade harmful compounds (Parzych 2016; Parzych et al. 2015, 2016). Despite many studies carried out so far, phytoremediation is still at the stage of research and experiments, especially when it comes to the selection of appropriate plant species. Analysis of accumulative properties of *P. endiviifolia* in relation with macro- and trace elements occurring in the forest streams of headwater niches greatly enriches the area of knowledge on the accumulation possibilities and the use of this species in environmental protection.

The aim of the study was to evaluate the accumulation properties of *P. endiviifolia* in relation with N, P, K, Mg, Ca, Zn, Fe, Cu, Ni, Mn, Al, and Sr taken from water and accumulated in the thalli within the forest headwater niches located far from anthropogenic pollution. The results of research carried out in conditions close to natural allow assessing the demand of a given species for nutrients and provide information on its accumulation properties.

Materials and Methods

Research Area

The research was carried out in headwater riparian forests located in the upper course of the Kamienna Creek (54°19'N; 17°10'E). It is an area of the Forest Inspectorate of Leśny Dwór in northern Poland (Fig. 1). Average annual sum of precipitation is about 770 mm and average annual temperatures about 7.6 °C (Kirchenstein and Baranowski 2008).

The area of the Kamienna Creek catchment is nearly in whole covered by forests of a spatially diverse species

composition with domination of beech, pine, and spruce in its plateau part, and common alder (*Alnus glutinosa*) in the valley bottom. The underground water flowing onto the surface form streams in which *P. endiviifolia* is found on the sandy bottom and stones. Soils in the vicinity of streams are rich in nitrogen, potassium, calcium, and magnesium, and relatively low in phosphorus (Jonczak et al. 2015). Plant and water samples were collected from three streams crossing peatbog under headwater riparian forest.

Sampling and Analysis of Water

Water samples were collected at monthly intervals in 2012–2014. Samples were collected using 0.5 dm³ polyethylene bottles. Temperature, pH (CPI 551, Elmetron, Poland), and electrolytic conductivity (CC 315, Elmetron, Poland) were measured in the field. In the laboratory, concentrations of K⁺, NH₄⁺, Mg²⁺, Ca²⁺, NO₃⁻, and PO₄³⁻ using ion chromatography were analyzed (881 Compact IC pro, Metrohm, Switzerland). Water samples were filtered through a 0.20 μm sterile syringe filter and diluted with demineralised water. The accuracy of the output and QA/QC was based on certified reference material (Multi-element Ion Chromatography Anion Standard, Certified 89 866-50ML-F, Fluka, France). The concentration of Fe^{2/3+}, Mn²⁺, Zn²⁺, Cu²⁺, and Ni²⁺ determined by atomic absorption spectrometry (AAS) (Analyst 300, Perkin Elmer, USA) and the concentration of Al and Sr by microwave plasma atomic emission spectrometry (Agilent 4100, MP-AES, Australia). The equipment was calibrated based on the original Merck standard solutions (KGaA, 1 g/1000 ml). The obtained test

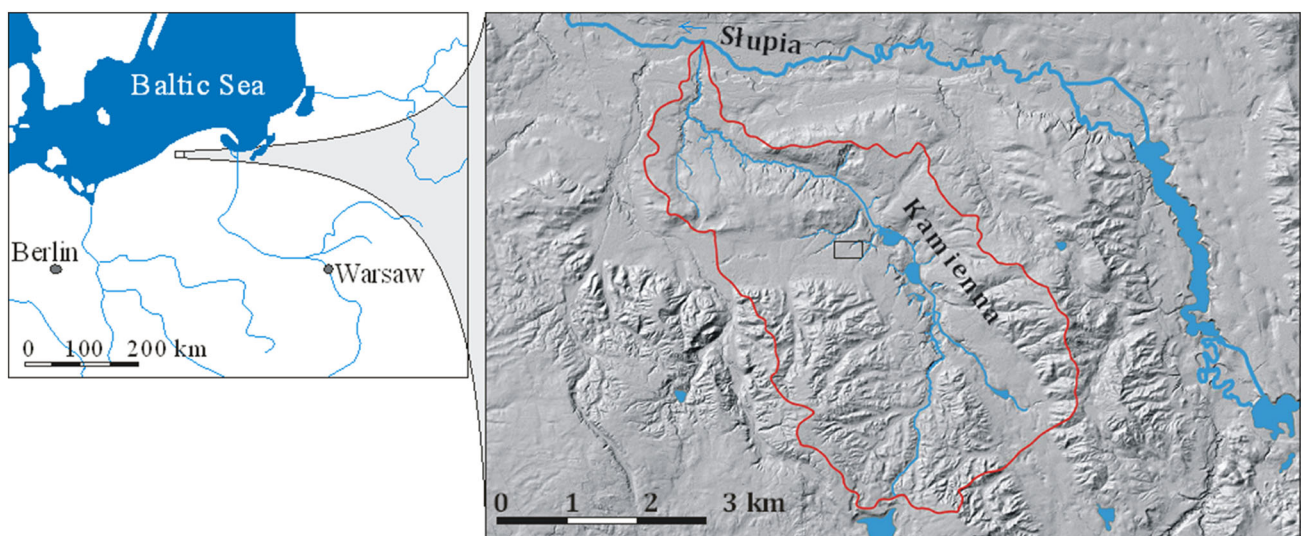


Fig. 1 Hydrographic network, the boundaries of the Kamienna Creek catchment and research area on the background of airborne LIDAR-based digital elevation model (DEM)

results did not exceed the permissible error value of $\pm 3\%$ in relation with the reference material.

Sampling and Analysis of *P. endiviifolia*

The liverwort samples were collected three times during the growing season—in May, July, and September, in the years 2012–2014. *P. endiviifolia* was collected from three streams by hand and washed several times in water at the sites. It was transferred into the laboratory polyethylene bags and washed carefully with deionized water in laboratory to remove attached inorganic and organic particles. Then, it was dried at 65 °C and homogenized using laboratory grinder (IKA 11, Germany). The total nitrogen content was determined by Kjeldahl method (Büchi 350 K, Switzerland) and phosphorus using the molybdate method (spectrophotometer UV–VIS U5100, Hitachi, Japan) after samples digestion in a mixture of 98% H₂SO₄ and 30% H₂O₂ (1:1 v/v). For determination of metallic elements, plant samples were digested in a mixture of 65% HNO₃ and 30% H₂O₂ (1:1 v/v). The concentration of K, Mg, Ca, Zn, Fe, Cu, Ni, and Mn was determined by atomic absorption spectrometry (AAS) (AAnalyst 300, Perkin Elmer, USA), whereas Al and Sr with microwave plasma atomic emission spectrometry (Agilent 4100, MP-AES, Australia). The following wavelengths were used: K 769.9 nm, 202.6 Mg, 422.7 Ca, 213.9 Zn, 248.3 Fe, 324.8 Cu, 232.0 Ni, 279.5 Mn, 396.1 Al, and 407.8 Sr. The tests were carried out following the original standards of Merck (KGaA, 1 g/1000 ml). The quality of the obtained results was controlled based on the analysis of certified samples of aquatic plants (CRM 060).

Statistical Analysis

The data composition was tested by the Shapiro–Wilk test. The physicochemical properties of waters and the chemical composition of *P. endiviifolia* in the tested stands were compared using Kruskal–Wallis test (K–W) at $p < 0.05$. To identify the factors determining the content of macro- and trace elements in thalli of *P. endiviifolia*, the factor analysis was applied (Principal Components Analysis, PCA). By means of the main components, two independent factors were separated, explaining 60% of the variability (variance) of N, P, K, Mg, Ca, Mn, Fe, Zn, Cu, Ni, Sr, and Al concentrations in thalli of *P. endiviifolia*. The demand of *P. endiviifolia* for nutritional components was described by application of ANE method (Accumulation Nutrient Elements) (Ostrowska 1999). The sum of the macro- and trace elements calculated by the ANE method reflects in whole, so called, nutritional factor. The value of the sum of components and its ionic composition determine the circulation of the components between the water and the

plant. The percentage participation of particular components in their sum is determined by alimentary requirements of *P. endiviifolia* in relation with particular components. The sum of components (Y) in mmol_c/kg was calculated based on a formula:

$$Y = \sum_{i=1}^i (Z : z),$$

where Z content of the element in mg/kg, z atomic weight/ion valency.

After the calculation of Y , the percentage (X) of each element in the sum of

$$X = [(Z : z)100]Y^{-1},$$

was calculated. All calculations were performed using the software package Statistica 8.0 (Statsoft Inc., USA).

Results

Physicochemical Properties of Water

Waters of the studied streams were characterized by weakly alkaline reaction (pH 7.9–8.1) and the average electrolytic conductivity (EC), which ranged from 106.5 to 279.2 $\mu\text{S}/\text{cm}$ (Table 1). The content of K⁺ was from 0.98 to 1.11 mg/dm³ regardless of the location. NH₄⁺ ions were characterized by very low concentrations throughout the research period, taking average values from 0.05 to 0.08 mg/dm³. The predominant constituents of the stream water were Ca²⁺ ions, occurring in concentrations 56.5–61.2 mg/dm³. Mg²⁺ ions were present in much lower concentrations, on average from 1.83 to 2.65 mg/dm³.

The content of NO₃⁻ in stream waters ranged from 1.20 to 3.77 mg/dm³ depending on the stream, and the concentration of PO₄³⁻ remained at the average level from 0.65 to 0.91 mg/dm³. The concentrations of Fe^{2/3+} and Mn²⁺ ions were on average from 0.23 to 1.45 mg/dm³ and from 0.07 to 0.29 mg/dm³, respectively. The remaining ions (Zn²⁺, Cu²⁺, Ni²⁺, Sr²⁺, and Al³⁺) occurred in less than 0.01 mg/dm³. There were statistically significant differences demonstrated in the concentration of NO₃⁻, Fe^{2/3+}, Mn²⁺, and EC in the waters between the studied streams (Table 1).

Concentration of Macro- and Trace Elements in *P. endiviifolia*

The thalli of *P. endiviifolia* was characterized by varying chemical composition depending on the stream. Slight differences in the physicochemical properties of stream water (Table 1) influenced the amount of accumulated

Table 1 Physicochemical properties of stream water from the studied mid-forest spring niches with Kruskal–Wallis's (K–W) test results

Parameter	Location 1	Location 2	Location 3	K–W, $p < 0.05$
pH	7.9 ± 0.1	7.9 ± 0.3	8.1 ± 0.4	–
EC, $\mu\text{S}/\text{cm}$	279.2 ± 55.5	115.9 ± 152	106.5 ± 143	< 0.01
K^+ , mg/dm^3	1.11 ± 0.7	1.1 ± 0.4	0.98 ± 0.3	–
NH_4^+ , mg/dm^3	0.08 ± 0.1	0.07 ± 0.1	0.05 ± 0.07	–
Mg^{2+} , mg/dm^3	1.83 ± 1.6	2.65 ± 1.7	1.84 ± 1.6	–
Ca^{2+} , mg/dm^3	56.5 ± 10.6	61.2 ± 10.4	57.9 ± 10.3	–
NO_3^- , mg/dm^3	3.77 ± 1.9	3.15 ± 1.9	1.20 ± 1.7	< 0.001
PO_4^{3-} , mg/dm^3	0.65 ± 1.1	0.77 ± 0.4	0.91 ± 0.8	–
$\text{Fe}^{2/3+}$, mg/dm^3	0.23 ± 0.01	1.39 ± 0.7	1.45 ± 0.5	< 0.01
Mn^{2+} , mg/dm^3	0.07 ± 0.001	0.28 ± 0.3	0.29 ± 0.4	< 0.01
Zn^{2+} , mg/dm^3	< 0.01	< 0.01	< 0.01	–
Cu^{2+} , mg/dm^3	< 0.01	< 0.01	< 0.01	–
Ni^{2+} , mg/dm^3	< 0.01	< 0.01	< 0.01	–
Sr^{2+} , mg/dm^3	< 0.01	< 0.01	< 0.01	–
Al^{3+} , mg/dm^3	< 0.01	< 0.01	< 0.01	–

macro- and trace elements in the liverworts. The average nitrogen content in *P. endiviifolia* was maintained at the level from 12,666 to 14,230 mg/kg, phosphorus from 3447 to 3941 mg/kg, potassium from 33,254 to 39,516 mg/kg, magnesium from 2443 to 3382 mg/kg, and calcium from 9792 to 12,708 mg/kg (Fig. 2). The average content of zinc was from 33 to 46 mg/kg, iron from 1743 to 4019 mg/kg, copper from 11 to 24 mg/kg, nickel from 16 to 20 mg/kg, manganese from 393 to 2866 mg/kg, aluminum from 312 up to 518 mg/kg, and strontium from 43 to 60 mg/kg (Fig. 2).

To identify the determinants of the chemical composition of the thalli of *P. endiviifolia*, principal component analysis (PCA) was used. In the calculations, the concentrations of N, P, K, Mg, Ca, Zn, Fe, Mn, Cu, Ni, Sr, and Al in the thalli of liverworts in 2012–2014 were used (Table 2). By means of the principal components, two independent factors explaining 60% variability (variance) of the chemical composition were distinguished. The values of factor loading, greater than 0.7, were used to interpret the data. Factor 1 explained 39% of variance and grouped P, K, Mg, and Mn characterized by very high positive factor loadings. Factor 2 explained 21% variability of chemical composition and was created by N and Zn, characterized by high positive and negative factor loadings (Table 2), respectively.

Accumulation of Macro- and Trace Elements in *P. endiviifolia*

The thalli of the liverwort tested accumulated on average 2502.28 mmol_c/kg of the analyzed components, with macronutrients accounting for 96.22% of this pool (Table 3).

The share of nitrogen accounted for 40.3% of this sum, phosphorus 4.9%, potassium 38.1%, magnesium 5.0%, calcium 12.1%, and trace elements accounted for 3.78% of the total amount. In total amount of micronutrients, iron had a dominant share with 56.30%, manganese had 24.80% and manganese 16.95% (Table 3), while the remaining components accounted for less than 1%.

Discussion

Physicochemical Properties of Water

Waters in the streams showed weakly alkaline reaction (Table 1), which is typical for young-glacial areas of northern Poland due to the prevalence of CaCO_3 in the outflows of groundwater (Mazurek 2008; Parzych et al. 2016). Electrolytic conductivity of waters depends on the number of positive and negative ions, and its value in the case of the studied waters indicates for low mineralization (Parzych et al. 2018a, b). The studied streams flowed through the forest, which meant that an important role in modifying the chemistry of waters (Parzych et al. 2016) was played by organic matter falling down from the trees (Jonczak et al. 2016). The streams of water were regularly supplied with nutrients released from the decaying plant fall (Jonczak et al. 2016), which due to favourable conditions prevailing in the niches was almost completely decomposed during the first year (Jonczak et al. 2015). The concentration of calcium in stream waters was largely dependent on the carbonate balance (Bukowska-Jania 2003), and during growing seasons additionally on the vegetation demand for Ca^{2+} ions (Parzych et al. 2017, 2018a, b). The results of Osadowski's (2010)

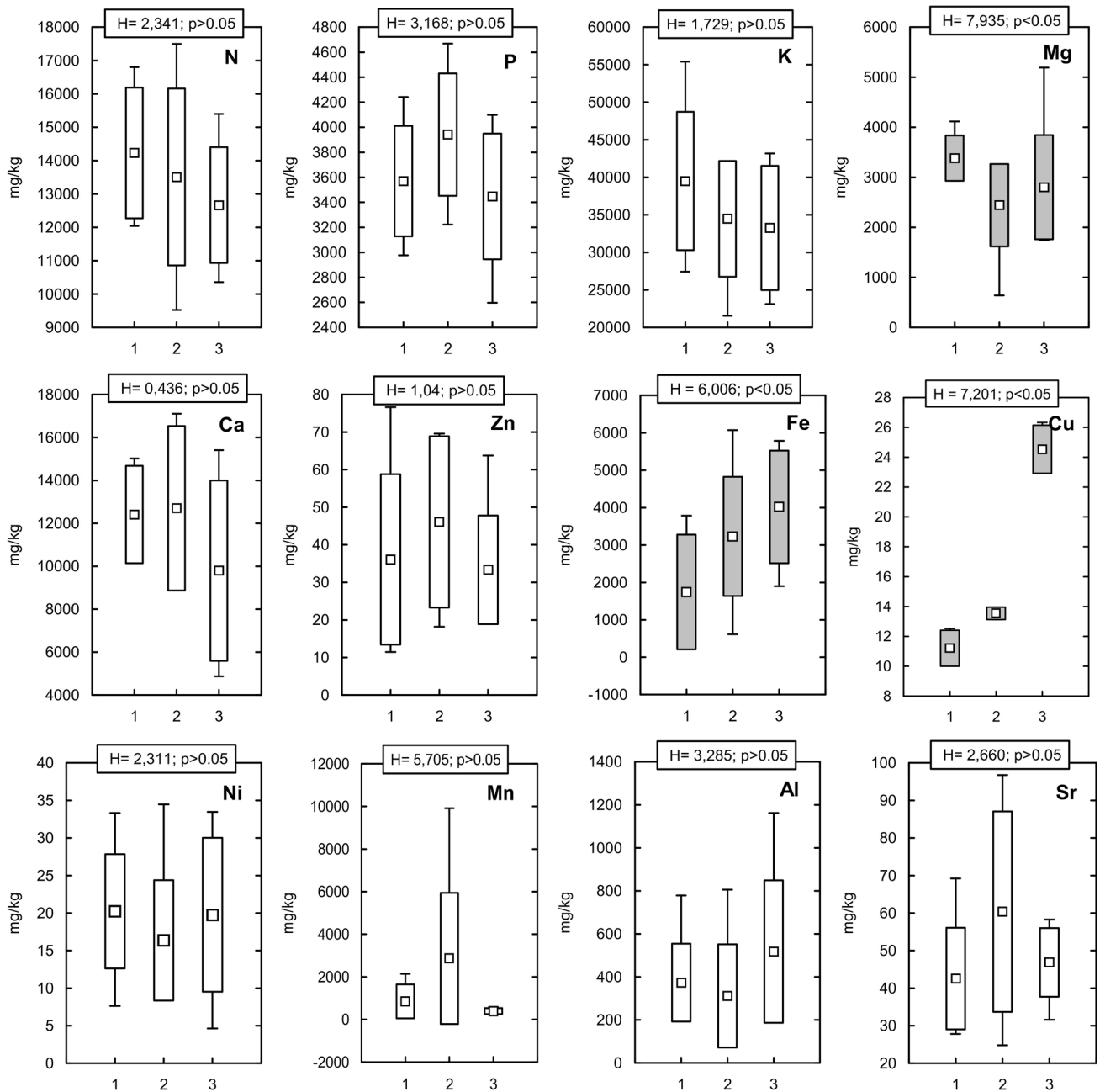


Fig. 2 Mean, minimum, maximum, and standard deviation of elements concentration in liverworts of *Pellia endiviifolia* in locations 1, 2, and 3 with Kruskal–Wallis test results. Colorless rectangles—no statistical significance, gray rectangles—statistically significant ($p < 0.05$)

research indicate that the communities of *P. endiviifolia* are eager to occur in streams the waters of which are characterized by an increased content of PO_4^{3-} ions. Although the soils in the vicinity of the streams contained a low phosphorus, their waters were fed with phosphorus compounds mainly from decomposition of organic matter (Jonczak et al. 2016), lying in the bottom of the streams. Both PO_4^{3-} and NO_3^- ions during vegetation seasons are intensively absorbed by plants (Parzych et al. 2016). In addition, NO_3^- ions easily migrate in water and are subject

to sorption to a small extent (Parzych 2011; Parzych et al. 2018a, b).

Iron and manganese in groundwater outflows are quite common, sometimes in high concentrations, and their presence is associated with the weathering of igneous and sedimentary rocks. The redox and pH conditions are the main factor determining their migration potential (Osadowski and Strzelczak 2009). In the studied area, there is the accumulation of organic matter in the form of peat (Jonczak and Parzych 2016; Parzych and Jonczak 2018), which

Table 2 Results of principal component analysis (PCA) of macro- and trace elements concentrations in thalli of *Pellia endiviifolia*

Elements	FC 1	FC 2
N	– 0.43	0.71
P	0.86	– 0.31
K	0.80	– 0.01
Mg	0.79	0.15
Ca	– 0.60	– 0.43
Zn	0.23	– 0.71
Fe	– 0.24	– 0.00
Cu	– 0.46	– 0.18
Ni	0.56	0.64
Mn	0.82	– 0.18
Al	– 0.68	0.49
Sr	– 0.60	– 0.67
Eigen values	4.66	2.48
Explained variance (%)	39	21
	60	

Factor loading higher than 0.7 are in bold

Table 3 Average accumulation (\pm standard deviation, SD) of components^a in thalli of *Pellia endiviifolia*

Elements	Average \pm SD	Participation (%)
\sum macro (mmol _c /kg)	2407.3 \pm 183	96.22
% N in \sum	40.3	
% P in \sum	4.9	
% K in \sum	38.1	
% Mg in \sum	5.0	
% Ca in \sum	12.1	
\sum trace elements (mmol _c /kg)	95.03 \pm 31	3.78
% Sr in \sum	0.63	
% Zn in \sum	0.66	
% Cu in \sum	0.28	
% Ni in \sum	0.38	
% Fe in \sum	56.30	
% Mn in \sum	24.80	
% Al in \sum	16.95	
\sum macro + \sum trace elements	2502.28	100

^aExpressed as the sum of the components and their share in the total

is a potential source of Fe^{2/3+} and Mn²⁺ ion supply to stream waters. Low concentrations (< 0.01 mg/dm³) of Zn²⁺, Cu²⁺, Ni²⁺, Sr²⁺ and Al³⁺ ions in stream waters unambiguously indicate the lack of influence of anthropogenic factors on the studied headwater niches.

Concentration of Macro- and Trace Elements in Thalli of *P. endiviifolia*

Concentrations of macroelements in *P. endiviifolia* are in the range of average values resulting from the physiological needs of most plants (Markert 1992; Ostrowska and Porębska 2002). The obtained results indicate also that the studied thalli accumulated increased amounts of nickel (> 10 mg/g) and large amounts of iron (> 1000 mg/kg) and manganese (> 500 mg/kg) (Kabata-Pendias and Pendias 1999). The physiological demand of plants for nickel is 0.1–5.0 mg/kg, for manganese most often from 10 to 25 mg/kg, and in the case of iron, it is usually below 375 mg/kg. Samecka-Cymerman et al. (1997) indicate that thalli of *Pellia epiphylla* also accumulated large quantities of Fe (900–7500 mg kg⁻¹). For most species of plants, high concentrations of trace elements in their organs have negative impact on their growth and development, which is most often visible in morphological changes (Szatanik-Kloc 2004). Basile et al. (2017) suggest that in the case of *Pellia neesiana*, severe alterations were observed in chloroplasts from samples exposed in the most polluted (Cd, Pb) site of Sarno River in Italy. According to the Basile et al. (2012), at the ultrastructural level, sub-lethal concentrations of the heavy metals caused induced cell plasmolysis and alterations of the chloroplast arrangement in *Lemna minor*, *Elodea Canadensis*, and moss of *Leptodictyum riparium*. Esposito et al. (2012) showed that sub-lethal concentrations of cadmium caused the most severe modifications in the *Leptodictype riparium*, while other heavy metals caused only slight changes. The results of research by Satake et al. (1987) confirm strong accumulative properties of *P. endiviifolia* in relation with iron and manganese. In the thalli of the liverwort, they found from 44.7 to 2900 mg/kg Mn and from 286 to 15,100 mg/kg Fe at the concentration of Mn²⁺ < 0.02 mg/dm³ and Fe^{2/3+} < 0.03 mg/dm³ in waters in Japan. Accumulation of high levels of iron and manganese in *Pellia endiviifolia* confirms the potential use this species in the processes purification of waters contaminated with iron and manganese compounds. Both our and Satake et al. (1987) studies have been conducted under conditions of low Fe and Mn contents in water. It is difficult to predict changes in liverwort thallus and what amounts of these metals would accumulate in *Pellia endiviifolia* under their higher concentrations. This issue still requires further research to clarify accumulative properties of *Pellia endiviifolia* under various contamination conditions.

It was demonstrated that there were statistically significant differences in concentration of magnesium, iron, and copper in the thalli of the liverwort (Fig. 2). The amount of Mg, Fe, and Cu accumulated was determined by

environmental conditions, and in the case of N, P, K, Ca, Zn, Ni, Mn, Al, and Sr, the species-related features had stronger influence than environmental impacts.

Principal components analysis (PCA) showed that two factors played an important role in shaping chemical composition of *P. endiviifolia* (Table 2). The first one was created by macroelements (P, K, and Mg) necessary for proper functioning and Mn collected in excessive quantities from waters of an alkaline reaction (Table 1). These results are confirmed by Alloway (1995) oraz Kabata-Pendias and Pendias (1999), who show that manganese compounds show the highest bioavailability for plants at pH 6 and 8. The second factor created by N and Zn indicates that in periods of maximum demand for nutrients, zinc is less available for plants in alkaline water (Table 1). According to Kabata-Pendias and Pendias (1999), the increase in zinc mobility is the most effective at pH 6.

Accumulation of Macro- and Trace Elements in Thalli of *P. endiviifolia*

The uptake of nutrients by *P. endiviifolia* is to a large extent dependent on the species traits and physicochemical properties of stream water (Parzych et al. 2017). According to Ostrowska and Porębska (2002), the average values of the sum of components accumulated in plants usually range from 1200 to 2500 mmol/kg, and the share of micro-elements usually fluctuates within 1%. *P. endiviifolia* accumulates slightly larger amounts of nutrients than other aquatic plants (Parzych and Cymer 2014; Parzych et al. 2015) and comparable to the amount of ingredients accumulated by herbaceous plants in peatlands (Parzych et al. 2018a, b).

The percentage of Fe and Mn in the sum of the components accumulated in the thalli of *P. endiviifolia* indicates a high demand for these micronutrients and shows the strong accumulation properties of this species in relation with iron and manganese. The results of the research presented in our work and data obtained by Satake et al. (1987) unequivocally indicate significant accumulation potential of the tested liverwort in relation to iron and manganese compounds that can be used in water treatment processes.

Conclusions

Analysis of accumulative properties of *P. endiviifolia* in relation with macro- and trace elements occurring in the forest headwater streams not affected significantly by anthropogenic factors, allowed to assess the nutrient needs and provided information on the accumulation capacities of *P. endiviifolia*. The thalli of the tested hepatic accumulated,

on average, 2502.28 mmol/kg of macro- and trace elements, with macronutrients accounting for 96.22% of this pool. The predominant share among trace elements was represented by Fe, Mn, and Al and the share of other elements was lower than 1%. Differences statistically significant between stations indicate that the environmental conditions were decisive for the accumulated amount of Mg, Fe, and Cu, while for N, P, K, Ca, Zn, Ni, Mn, Al, and Sr (no statistically significant differences), the influence of species characteristics was stronger than in case of environmental features. Accumulation in thalli of *P. endiviifolia* high concentrations of iron and manganese confirms the potential use this species in the processes purification of waters contaminated with iron and manganese compounds.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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