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Accumulation of N, P, K, Mg, and Ca in 20 species of herbaceous plants in headwater riparian forest

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ABSTRACT

In this study, results of research related to the bioconcentration of N, P, K, Mg, and Ca in shoots of 20 species of herbaceous plants growing along three headwater streams in northern Poland are presented. Shoots of plants and water samples from streams were taken three times during growing seasons in the period between 2012 and 2014. Concentrations of nutrients in plants represented substantial diversity depending on species, sampling period, and the stream. Based on the results, it was found that many plant species take up nutrients in quantities exceeding their physiological demand, which makes them usable in water purification. Potassium (8,118–42,314), nitrogen (11,716–41,645), and phosphorus (11,607–37,459) had the highest levels of bioconcentration factors (BF). Lower values of BF referred to magnesium (1,161–2,973) and calcium (85–393). Results of Kruskall–Wallis test showed significant statistical differences in values of BF between examined plant species in reference to all analysed nutrients, depending on the stream. The highest value of N, P, K, Mg, and Ca BF were found in shoots of *Filipendula ulmaria, Athyrium filix-femina*, and *Ranunculus acris*; *Veronica beccabunga, Chrysosplenium alternifolium*, and *Epilobium palustre*; *Veronica beccabunga, Ranunculus acris*, and *Chrysosplenium alternifolium*; *Filipendula ulmaria, Stellaria nemorum*, and *Epilobium palustre*; and *Chrysosplenium alternifolium, Geranium robertianum*, and *Cardamine amara,* respectively.

Keywords: Macroelements; Bioconcentration factors; Principal components analysis; Hierarchical cluster analysis; Water purification

1. Introduction

Riparian forests are of high natural and economic value, because they are rich in plant diversity [1,2] and significantly increase water retention in river valleys [3]. The species composition of flora of the woodland ecosystems is closely related to the chemical composition of soils [4] and water [5,6]. Plants of riparian forests growing on banks of streams play an important role in the stabilisation of the bank line and the regulation of the chemical composition of flowing water [7–10]. Seasonal variability of the environment and diverse needs of plants during their lifetime determine dynamic character of the plant–environment relationship. Riparian forest vegetation, through interception, transformation, and accumulation, plays an important role in protecting the river water from eutrophication [11–14]. According to Jansson et al. [15] and Kuglerova et al. [16], plants of riparian forests have easier access to nutrients than in other forest ecosystems, because water transports the mineral materials and organic substances in the form of suspended matter. Depending on plant species, plant nutrient uptake has been shown to account for 3%–47% of nitrogen removal and 3%–60% of phosphorus removal from runoff water in riparian forest [17,18]. In recent years, our attention has been drawn by the species composition of the river bank plants, which substantially modifies physiochemical properties of water in streams [6,19–22] contributing to their purification. Interactions among water, soil, and plants in headwater

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riparian forest are very strict and multidirectional [23,24]. The nutrient dynamics of riparian forest under natural conditions are complicated because of the variety of riparian biotopes, and long-term studies needed to obtain a representative view of the nutrient accumulation dynamics. Despite many studies and results, there are still a number of unresolved issues concerning the role of riparian vegetation in controlling water quality [25–28]. Explanations of relations between physiochemical properties of water in streams and accumulation capacity of various plant species in relation to nutrients in the areas beyond anthropogenic influence contribute to widening the scientific knowledge in order to use plants on a greater scale in the processes in water purification [29,30].

The aim of this paper was to identify the factors that determine nitrogen, phosphorus, potassium, magnesium, and calcium contents in the shoots of 20 species of herbaceous plants growing along three streams in headwater riparian forests and to compare the bioconcentration factors (BF) of particular plant species in relation to nutrients of the highest biological importance (N, P, K, Mg, and Ca).

2. Materials and methods

2.1. Study area

The research was done at the upper flow of the Kamienna Creek, which is a left bank tributary of the Słupia River, situated in the early glacial landscape of Central Pomerania at the territory of Leśny Dwór Forest Inspectorate (54°19′N; 17°10′E) in northern Poland (Fig. 1). The area has an average annual precipitation and ambient temperature of about 770 mm and 7.6°C, respectively. The selection of the research area in the upper course of the river, away from anthropogenic factors, was dictated by the need to investigate the accumulation properties of herbaceous plants in conditions close to natural. The obtained results could be used to compare the accumulation properties of plants originating from contaminated sites. The area of the Kamienna Creek catchment is almost fully covered by spatially diversified forest according to species composition, with domination of beech, pine, and spruce in its plateau part and common alder (*Alnus glutinosa*) at the bottom of the valley [31]. The stream side complex of the riparian ash and alder forest appears at

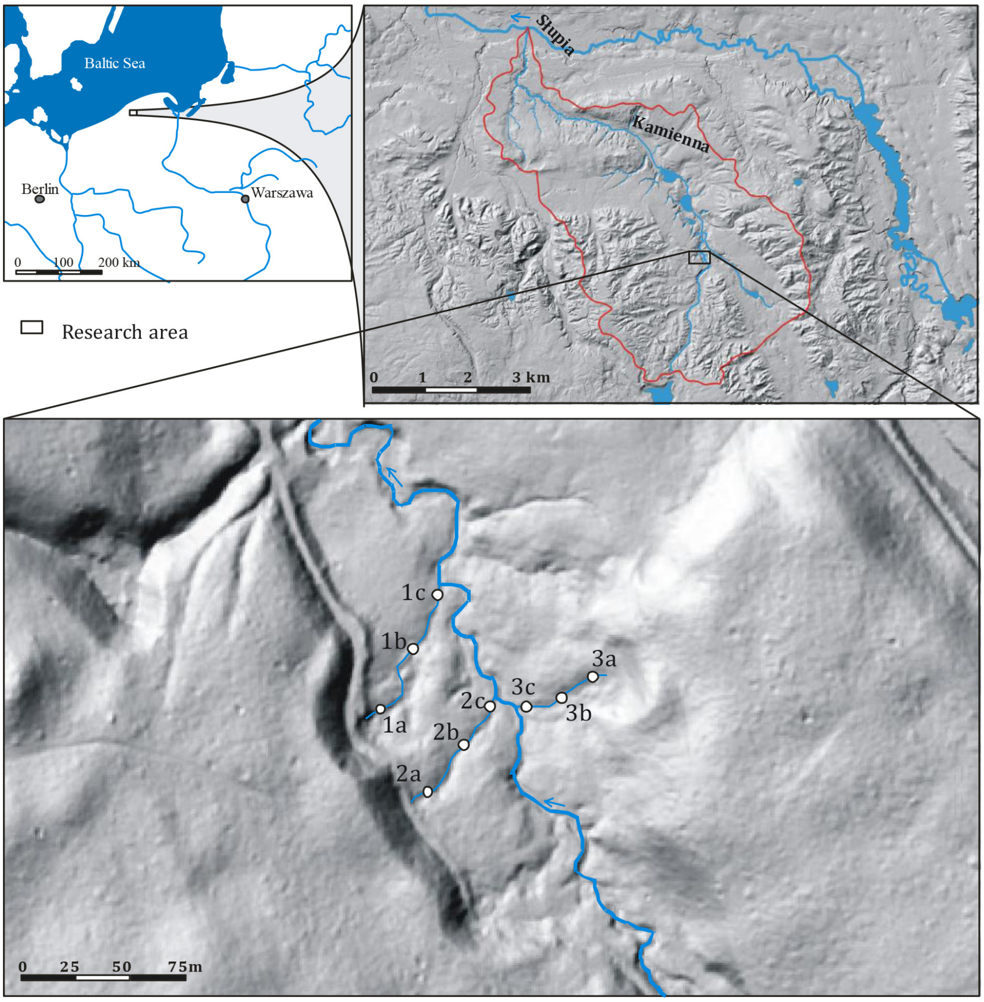

Fig. 1. In hydrographic network, the boundaries of the catchment area of the Kamienna Creek and place of sampling on the background of airborne LIDAR-based digital elevation model. 1, 2, 3 – number of headwater stream, a – initial, b – middle, and c – end part of stream.

the area under consideration in its headwater form, specified as *Fraxino-Alnetum cardaminetosum amarae*, representing simultaneously a local specific characteristic combination of species. Specific attention is drawn by high participation of species generally recognised as outstanding in the complex: *Galium palustre, Lycopus europaeus, Lythrum salicaria, Lysimachia vulgaris*, and *Solanum dulcamara.* There are also locally coexistent species: *Cardamine amara*, *Chrysosplenium alternifolium, Carex paniculata*, and *Scirpus sylvaticus. Alnus glutinosa* with admixture of *Betula pubescens* and *Sorbus aucuparia* constitute the layer of trees along banks of watercourses. Between streams, due to depositions of organic matter, domed peat bogs are made up of forest peat with layers of forest and sedge peat. The peaty sapric histosols were characterised by spatially diverse thickness, not exceeding 1 m. They had a slight acidic and acidic reaction and were rich with nitrogen, potassium, calcium, and magnesium. They were relatively deficient in phosphorus [32].

2.2. Sampling and analysis of water

Water samples for physiochemical analyses were taken in May, July, and September in the years 2012–2014 from three streams (in their initial, middle, and end sector; Fig. 1). The length of the examined streams was 80, 65, and 45 m, respectively. A total of 27 water samples were collected for the study to 0.5 dm^3 polyethylene bottles. Temperature, as well as pH by the potentiometry method (CPI 551 Elmetron, Poland), electrolytic conductivity (CC 315, Elmetron, Poland), and O_2 content by means of the oxygen probe (HI 9146) were measured directly in the field. The content of K^+, NH_4^+, Ca^{2+}, Mg^{2+}, NH_4^+, NO_2^-, NO_3^-, and PO_4^{3-} ions was determined by ion chromatography (881 Compact IC pro, Metrohm, Switzerland). Prior to introduction into a chromatographic column water samples were filtered using filters of porosity of 0.2 mm and diluted by deionised water (Hydrolab HLP-10, Poland) in a proportion of 1:4.

2.3. Sampling and analysis of plants

Among plants covering banks of streams, 20 species of plants characterised by highest frequency and density, which were not under protection, were selected for research (Table 1). The plants were collected along the banks of streams, in the immediate vicinity of flowing water and within 0.3 m from the streams. The samples of aboveground shoots were taken three times during the growing period (May, July, and September) in the years 2012–2014. Each single sample consisted of shoots from several specimens of the given species. A total of 150 plant samples were collected for the study.

After delivering to the laboratory, shoots were washed with distilled water in order to remove mineral parts of the soil. Then, they were dried in temperature of 65°C and homogenised by the use of laboratory grinder (IKA A11, Germany). In order to determine nitrogen and phosphorus, plant samples were digested in a mixture of 98% H_2SO_4 and 30% H_2O_2 to obtain colourless and clear solution. Nitrogen was determined by the Kjeldahl method (Büchi K-350, Switzerland), while phosphorus by the spectrophotometric method with ammonia molybdate (UV-VIS, Hitachi U-5100, Japan). To determine

Table 1
Characteristics of herbaceous plant species studied along headwater streams

Species	Code	Family	Number of headwater stream
Ajuga reptans L.	*Aju_rep*	Lamiaceae	1, 2
Athyrium filix-femina (L.) Roth	*Ath_fem*	Woodsiaceae	1, 3
Berula erecta (Huds.) Coville	*Ber_ere*	Apiaceae	1
Brachythecium rivulare Schimp.	*Bra_riv*	Brachytheciaceae	2
Cardamine amara L.	*Car_ama*	Brassicaceae	2
Carex paniculata L.	*Car_pan*	Cyperaceae	2
Carex remota L.	*Car_rem*	Cyperaceae	3
Chrysosplenium alternifolium L.	*Chr_alt*	Saxifragraceae	1
Epilobium palustre L.	*Epi_pal*	Onagraceae	3
Filipendula ulmaria (L.) Maxim.	*Fil_ulm*	Rosaceae	3
Geranium robertianum L.	*Ger_rob*	Geraniaceae	3
Mentha aquatic L.	*Men_aqu*	Lamiaceae	1, 2, 3
Oxalis acetosella L.	*Oxa_ace*	Oxalidaceae	1
Pellia endiviifolia (Dicks.) Dumort.	*Pel_end*	Peliaceae	2
Ranunculus acris L.	*Ran_acr*	Ranunculaceae	3
Rumex acetosa L.	*Rum_ace*	Polygonaceae	2
Solanum dulcamara L.	*Sol_dul*	Solanaceae	2
Stellaria nemorum L.	*Ste_nem*	Caryophyllaceae	1
Valeriana officinalis L.	*Val_off*	Valerianaceae	2
Veronica beccabunga L.	*Ver_bec*	Scrophulariaceae	1, 2

metallic elements, 0.5 g of the plant sample was digested in a mixture of 65% HNO_3 and 30% H_2O_2 in proportion of 1:1. Then, solutions were filled up to the volume of 50 mL with deionised water, and concentrations of K, Mg, and Ca were measured by atomic spectrometry (Aanalyst 300, PerkinElmer, USA). Analyses were performed in the oxyacetylene flame. The wavelengths at which various metals were detected were as follows: 769.9 nm K, 202.6 nm Mg, and 422.7 nm Ca. All tests were carried out by following the original standards of Merck KGaA (Germany, 1 g/1,000 mL). All analytical measurements were made in triplicate.

2.4. Statistical analysis

Distribution of data related to physiochemical parameters of water and the content of N, P, K, Mg, and Ca in the shoots of plants was examined by means of a Shapiro–Wilk test ($p = 0.05$), while the average values of parameters for water were compared and tested by Kruskal–Wallis's test ($p = 0.05$). To identify factors determining the content of macroelements in shoots of plants principal components analysis (PCA) was used. By means of main constituents, two independent factors were separated that explain the variability of N, P, K, Mg, and Ca in shoots of plants growing along the banks of three streams. Basing on concentration of N, P, K, Mg, and Ca in shoots of plants and concentration of NH_4^+, K^+, Mg^{2+}, Ca^{2+}, NO_2^-, NO_3^-, and PO_4^{3-} ions, BF values were calculated. The BF are the ratio of nitrogen, phosphorus, potassium, magnesium, and calcium in the plant shoots to the concentration of respective ions in water. The higher factor the easier absorption of nutrients by plant from the water. Changes of the value of BF of N, P, K, Mg, and Ca in 2012–2014 are presented in figures with results of a Kruskal–Wallis test ($p = 0.05$). Similar analysis was accomplished by the use of hierarchical cluster analysis (HCA) which is well-known and well-described multivariate statistical approach used for pattern recognition purposes. Then, the similarity between herbaceous plant species in the space of BF of N, P, K, Mg, and Ca in shoots was calculated and hence, different groups of similarity were revealed [33]. The distance used for clustering purposes was Euclidean distance while consecutively Ward's method as a linkage method was applied. The correctness of the use of Euclidean distance being the most often measure used to compare profiles of objects across variables was assured, since as is in this case, it is only appropriate for data measured on the same scale. Moreover, the Euclidean distance used together with the Ward's method could be calculated on non-standardised data, which is an advantage [34]. The Ward's method is distinct from all other linkage methods, because it uses an analysis of variance approach to evaluate the distance between groups (clusters). All calculations were performed using the software package STATISTICA 12 (Statsoft Inc., USA).

2.5. Quality assurance/quality control

Quality assurance/quality control of analytical procedures was carried out by analysing the standard certified reference material of aquatic plants (CRM 060) and of water (Multielements Ion Chromatography 89 866-50ML-F, Fluka), adopting the same procedures as for analysed samples. Results of the experimental measurements agreed with reference values. Recoveries were calculated as a ratio of determined value to a certified one and were within the confidence intervals of the certified values. Recoveries were as follows: 99% ± 2% (N), 98% ± 3% (P), 100% ± 1% (K), 96% ± 5% (Mg), and 99% ± 3% (Ca).

3. Results and discussion

3.1. Physicochemical properties of water

Water of examined streams showed diversified physical and chemical properties. Water temperature depended on variable weather conditions and was 7.4°C–7.5°C on average. Temperatures of examined water were slightly lower than average annual temperature of the air in the niche, which was 7.6°C. The minimal variability of the temperature had a positive impact on intensity of processes of mineralisation of organic matter [32]. Water in streams represented a neutral and slightly alkaline reaction (pH = 6.6–8.9; Table 2). The lowest pH was observed in May, which was an effect of the beginning of the growing season and increased demand of plants for alkaline cation [1,6].

Water in streams presented small levels of electrolytic conductivity (180–544 μS cm^{-1}), which show the lack of influence of anthropogenic pressure. Those values indicate low and moderate mineralisation of tested water, which underwent modification due to the contact with diverse plant cover growing along the banks of streams [2]. The concentration of dissolved oxygen was from 8.7 to 9.1 mg dm^{-3} on average, depending on the stream. The oxygen dissolved in water originated almost exclusively from the atmosphere and due to the mixing of water, the contact surface area of the water with the atmosphere increased, along with its gradual oxygenation [35].

Concentration of NH_4^+ ions was on average from 0.0 to 0.2 mg dm^{-3}, and K^+ from 1.4 to 1.6 mg dm^{-3}, depending on the stream. Among the tested cations, ions of Ca^{2+} were the most abundant (Table 2), having range of concentration from 53.7 to 58.0 mg dm^{-3}. Concentration of Mg^{2+} was much lower and varied between 2.6 and 2.9 mg dm^{-3}. Concentration of NO_2^-, NO_3^-, and PO_4^{3-} in water remained at the levels 0.0–0.2, 0.0–5.8, and 0.9–1.4, respectively. The lowest concentrations of ions of NH_4^+, K^+, Mg^{2+}, and Ca^{2+} were observed in July [6]. Nitrogen was assimilated by plants in the form of NH_4^+, or after oxygenation in the form of NO_3^-. Strong diminution of ammonia ions in stream water was a result of intensive processes of nitrification accompanying oxygenation of water in which NO_3^- are formed and uptake of both forms of mineral nitrogen by plants. NO_3^- easily migrates in water and undergo sorption to a limited extent [36]. During a vegetation season connected with dynamic growth and development of plants, nitrates are intensively taken up by plants, which lead to reduction of their concentration in water [37,38]. With a slightly alkaline or neutral reaction of water, many species of plants took up NO_3^- more often than NH_4^+ [39,40]. In July, a lowered concentration of PO_4^{3-} was observed in consequence of increased biological activity of plants connected with the increase of biomass.

Phosphate ions belong to biogenic substances that are indispensable to plants for production of biomass [41,42]. The results of the Kruskal–Wallis test showed statistically

Table 2
Physicochemical properties of headwater streams in years 2012–2014 with Kruskal–Wallis's test results

Parameters	Headwater stream 1		Headwater stream 2		Headwater stream 3		Kruskal–Wallis's test
	Mean ± SD (CV, %)	Minimum–maximum	Mean ± SD (CV, %)	Minimum–maximum	Mean ± SD (CV, %)	Minimum–maximum	*p*
Temp, °C	7.4 ± 2.6 (36)	2.0–12.0	7.5 ± 3.1 (43)	0.5–13.0	7.5 ± 3.2 (42)	0.5–13.0	0.9629
pH	7.9 ± 0.4 (5)	6.7–8.8	7.9 ± 0.4 (5)	6.6–8.9	7.9 ± 0.4 (6)	6.6–8.9	0.7472
EC, µS cm^{-1}	291 ± 72 (21)	200–544	275 ± 66 (24)	191–435	254 ± 60 (24)	180–393	**0.0085**
O_2, mg dm^{-3}	9.1 ± 1.2 (11)	4.4–12.2	8.8 ± 1.2 (11)	3.4–12.4	8.7 ± 1.1 (11)	4.0–11.7	0.9193
NH_4^+, mg dm^{-3}	0.2 ± 0.3 (152)	0.0–1.4	0.1 ± 0.2 (160)	0.0–1.1	0.1 ± 0.2 (121)	0.0–0.7	0.8757
K^+, mg dm^{-3}	1.1 ± 0.8 (44)	0.1–3.1	1.2 ± 0.5 (42)	0.2–2.8	1.2 ± 0.4 (48)	0.4–3.1	0.3508
Mg^{2+}, mg dm^{-3}	1.9 ± 1.5 (75)	0.1–5.5	1.9 ± 1.6 (78)	0.1–4.9	1.9 ± 1.5 (80)	0.0–5.5	0.6712
Ca^{2+}, mg dm^{-3}	58.0 ± 18.8 (26)	28.8–191.8	57.7 ± 17.4 (32)	26.0–143.8	53.7 ± 15.5 (27)	26.0–111.2	**0.0451**
NO_2^-, mg dm^{-3}	0.2 ± 0.3 (158)	0.0–1.2	0.2 ± 0.4 (199)	0.0–1.6	0.2 ± 0.3 (220)	0.0–1.6	0.5827
NO_3^-, mg dm^{-3}	2.3 ± 1.2 (50)	0.0–7.5	2.4 ± 1.3 (48)	0.0–8.9	0.9 ± 1.2 (125)	0.0–7.4	**<0.001**
PO_4^{3-}, mg dm^{-3}	0.4 ± 0.6 (129)	0.0–2.6	0.5 ± 0.8 (121)	0.0–3.1	0.4 ± 0.5 (149)	0.0–2.6	**0.0015**

Note: Mean pH value calculated as follows pH = $-\log_{10}[H^+]$, EC – electrical conductivity, SD – standard deviation, CV – coefficient of variation, and *p* – level of significance. Statistically significant correlation are in bold.

significant differences in the concentration of Ca^{2+}, NO_3^-, and PO_4^{3-} and electrolytic conductivity between water of the examined streams (Table 2).

In general, the water of the studied streams were characterised by low concentration of dissolved substances dominated by Ca^{2+} and HCO_3^- ions, which confirms the lack of influence of anthropogenic factors. Due to the mid-forest location of the research facility and the lack of use of the catchment for industrial and commercial purposes, stream water can be included in the first class of purity [6,42].

3.2. Nutrients concentrations in plants

Uptake of nutritional components by plants is, to a large extent, determined by plant-dependent factors, such as species, age, developmental stage, and ion reactions of a synergic and antagonistic character [43]. Concentrations of nutrients in the shoots of the examined plants showed great diversity depending on the species and the stream. The lowest average nitrogen content (7,967 mg kg^{-1}) was in the case of *Brachythecium rivulare*, while the largest (20,676 mg kg^{-1}) in the shoots of *Chrysosplenium alternifolium* (Table 3). According to Ostrowska and Porębska [44], the natural nitrogen content in the green parts of plants is usually between 13,000 and 31,000 mg kg^{-1}. According to Jansson et al. [15] and Kuglerova et al. [16], due to high level of the water table, plants in the riparian forests have easier access to many nutrients than in other forest ecosystems. In the examined riparian forest, in spite of the high level of the water table and efficient processes of mineralisation of organic matter supplying additional nutrients [4], the plant species under consideration showed relatively little nitrogen content. The reason was the low concentration of NH_4^+ and NO_3^- in water of the streams [6] and soils [4] and intensive outflow of mineral forms of nitrogen along with water of streams outside the range of the roots [45].

The highest concentration of phosphorus was found in the shoots of *Veronica beccabunga* (4,416 mg kg^{-1}) while the lowest in *Ajuga reptans* (1,857 mg kg^{-1}; Table 3). According to Ostrowska and Porębska [44], obtained results were within the natural contents of phosphorus in the shoots of plants (1,000–4,000 mg kg^{-1}). Concentration of P showed variability within growing season resulting from the changeable demand of various species of plants for phosphorus and dynamics of PO_4^{3-} in the water of the streams (CV = 121%–149%; Table 2). Variation coefficients (CV) were from 6% in *Berula erecta* to 42% in *Athyrium filix-femina.* Strong moisture of the area characteristic for riparian forest had a positive impact on the uptake of phosphorus by plants [46]. Water of streams contained very small quantities of phosphate ions [6], and the soils within the area of one examined headwater were relatively poor in phosphorus [32]; therefore, one phosphorus may be taken up by plants in the form of $H_2PO_4^-$ and HPO_4^{2-} [47], originating mainly from the decomposition of organic matter [32,48].

The results presented by Samecka-Cymerman and Kempers [50] indicate that in the case of higher concentration of phosphates in water (1.9 mg kg^{-1}), *Veronica beccabunga* accumulates much more phosphorus content in its shoots (18,048–27,937 mg kg^{-1}).

In the case of potassium, the highest average concentration was also found in the shoots of *Veronica*

Table 3
Nutrients concentration (average, mg·kg^{-1} ± SD) in shoots of herbaceous plants in riparian forest in years 2012–2014

Species		N	P	K	Mg	Ca
Headwater stream 1	*Ajuga reptans*	13,603 ± 1,813	2,137 ± 737	20,743 ± 3,186	3,065 ± 833	15,499 ± 5,524
	Athyrium filix-femina	13,969 ± 2,064	2,266 ± 435	16,902 ± 3,013	4,068 ± 1,477	4,907 ± 2,933
	Berula erecta	11,305 ± 2,581	2,513 ± 160	28,019 ± 7,861	4,470 ±1,072	7,342 ± 5,945
	Chrysosplenium alternifolium	20,676 ± 2,458	3,827 ± 504	35,343 ± 5,936	4,351 ± 659	22,781 ± 9,084
	Mentha aquatica	13,125 ± 4,597	2,377 ± 342	26,184 ± 3,780	3,179 ± 726	14,737 ± 3,004
	Oxalis acetosella	13,759 ± 1,678	2,184 ± 420	17,975 ± 3,275	3,569 ± 1,261	7,502 ± 2,849
	Stellaria nemorum	15,190 ± 2,953	2,116 ± 737	34,792 ± 8,219	5,716 ± 746	10,493 ± 3,492
	Veronica beccabunga	16,509 ± 1,171	4,416 ± 687	45,699 ± 3,918	4,900 ± 1,073	16,662 ± 6,064
Headwater stream 2	*Ajuga reptans*	12,711 ± 2,435	1,857 ± 382	24,743 ± 3,411	2,205 ± 666	16,530 ± 6,022
	Brachythecium rivulare	7,967 ± 1,752	2,178 ± 745	9,742 ± 6,176	2,376 ± 808	13,315 ± 4,158
	Cardamine amara	16,744 ± 2,027	4,346 ± 864	33,697 ± 8,279	3,808 ± 646	19,149 ± 9,677
	Carex paniculata	11,676 ± 1,862	1,896 ± 492	21,263 ± 4,155	2,676 ± 635	5,618 ± 2,952
	Mentha aquatica	14,062 ± 2,187	2,656 ± 716	21,990 ± 4,633	2,816 ± 388	13,902 ± 4,037
	Pellia endiviifolia	12,666 ± 1,734	3,447 ± 503	33,254 ± 8,279	2,802 ± 1,042	9,792 ± 4,203
	Rumex acetosa	18,291 ± 3,467	2,679 ± 525	31,961 ± 5,004	4,131 ± 383	5,857 ± 1,373
	Solanum dulcamara	15,858 ± 3,530	2,298 ± 463	24,678 ± 4,580	3,247 ± 709	10,493 ± 3,021
	Valeriana officinalis	12,740 ± 1,884	2,298 ± 463	29,533 ± 8,863	2,616 ± 352	9,075 ± 5,094
	Veronica beccabunga	15,037 ± 4,385	4,017 ± 908	30,897 ± 15,511	4,500 ± 1,086	15,851 ± 5,043
Headwater stream 3	*Athyrium filix-femina*	14,140 ± 2,937	2,177 ± 916	21,809 ± 4,869	3,781 ± 561	6,879 ± 1,333
	Carex remota	10,053 ± 4,446	2,200 ± 535	25,087 ± 4,445	2,588 ± 932	4,771 ± 1,213
	Epilobium palustre	11,694 ± 1,056	3,692 ± 754	24,594 ± 3,315	5,361 ± 2,151	15,443 ± 10,861
	Filipendula ulmaria	14,159 ± 2,179	2,743 ± 484	22,597 ± 4,171	5,650 ± 722	11,903 ± 4,925
	Geranium robertianum	10,943 ± 2,114	3,096 ± 320	26,748 ± 5,231	2,887 ± 680	19,775 ± 5,303
	Mentha aquatica	11,574 ± 1,923	2,220 ± 535	23,209 ± 3,229	3,145 ± 603	17,705 ± 6,788
	Ranunculus acris	11,841 ± 865	3,287 ± 363	39,414 ± 4,832	3,284 ± 944	16,769 ± 4,176
Minimum		7,967	1,857	9,742	2,205	4,771
Maximum		20,676	4,416	45,699	5,716	22,781
Natural content in plants		13,000–31,000[a]	1,000–4,000[a]	2,000–18,000[a]	1,000–3,000[b]	1,000–33,000[a]

[a]Kabata-Pendias and Pendias [43].
[b]Markert [49].

beccabunga (45,699 mg kg^{-1}) while the lowest in *Brachythecium rivulare* (9,742 mg kg^{-1}; Table 3). In most species, the obtained results exceeded the natural contents of potassium in plants (2,000–18,000 mg kg^{-1}) [44]. The contents of K in shoots showed changeability within the growing seasons. Variability coefficients of the concentration of phosphorus were from 9% in shoots of *Veronica beccabunga* to 74% in the case of *Berula erecta*. Potassium uptake by plants was strongly connected with dynamics of K^+ in the water of streams (CV = 42%–48%; Table 2). This confirms strong interactions between water and plants in ecosystems of headwaters [24,51]. Neutral and slightly alkaline reaction of water in the streams had positive impact on potassium intake by plants (Table 2). Potassium is a nutrient that is often taken up by plants in excess, exceeding their nutritional demand [46], especially in forest ecosystems with a high level of groundwater table [31]. At the same time, potassium limits uptake of other nutrients, especially magnesium. High content of K in shoots of various species of plants that are found at highly moisturised areas is confirmed by Parzych et al. [52], especially in the shoots of *Veronica beccabunga* [50,51].

The highest concentration of magnesium was found in shoots of *Stellaria nemorum* (5,716 mg kg^{-1}) while the lowest in the case of *Ajuga reptans* (2,205 mg kg^{-1}; Table 3). According to Markert [49], obtained results exceeded in most examined species the natural content of Mg in shoots of plants (1,000–3,000 mg kg^{-1}), which indicates their good supply with this macroelement. For proper growth and development of plants, the presence of Mg in the minimum quantity of 1,000 to 1,300 mg kg^{-1} is usually necessary [53]. Mg in shoots of the plants was characterised by substantial dynamics of concentration. CV were from 9% in the shoots of *Rumex acetosa* to 40% in *Epilobium palustre* and were the result of changeable concentration of Mg^{2+} in water of the streams (Table 2) and in the soil [31]. Comparable results in Mg content in the shoots of *Veronica beccabunga* and *Berula erecta* were obtained at headwater areas of Poland by Horska-Schwarz and Spałek [51] and Samecka-Cymerman and Kempers [50]. The presence of magnesium in plants is necessary for correct metabolism at the cellular level and in the whole organism [43]. Magnesium is a mobile element and can be easily washed out of soil, especially peaty soils and peaty and half-bog soils, enriching the water of the streams. The intensity of assimilation of magnesium by plants depends, among other things, on the content of other ions. The presence of NO_3^- has a positive impact on magnesium uptake, whereas NH_4^+ and the excess of potassium have a negative impact on it. In addition, magnesium uptake may limit too acidic reaction of the soil solution [46].

In the case of calcium, the highest concentrations were found in the shoots of *Chrysosplenium alternifolium* (22,781 mg kg^{-1}) while the lowest in *Carex remota* (4,471 mg kg^{-1}). Obtained results were within the limits of the values typical for most species (Table 3). Plants need a relatively small quantity of calcium for their growth. The optimum content of Ca in shoots of most plants is within the limit of 1,000–33,000 mg kg^{-1} [44]. In the years 2012–2014, the CV of Ca in the shoots of the examined plants remained at the level of 19% in the case of *Athyrium filix-femina* and as much as 81% in *Berula erecta*. Changes were due to in the chemistry of water in the streams during the growing season [6]. Concentration dynamics of Ca^{2+} in water changed in the range between 26% and 32% (Table 2), depending on the stream. High moisture of the soils characteristic for headwater mid-forest niches and a slightly alkaline reaction of water had a positive impact on the uptake of calcium by plants [31], mostly in the leaves [53]. At the end of the vegetative season, increased Ca content in shoots was observed in most species, which indicates that plants were getting older [44]. Comparatively, the content of Ca in the riparian forest was found among the species of the plants growing on domed peat bogs [31].

3.3. Factors determining the share of nutrients in plants

In order to identify the factors determining the participation of macroelements in the shoots of the examined plant species PCA was applied. In calculations, 3 years' means were used (for May, July, and September) of macroelements content (N, P, K, Mg, and Ca) in shoots of herbaceous plants (Table 4). By application of the method of main components, two independent factors explaining 63%–75% of the variance of the data concerning chemical composition of plants growing along three streams were separated. Only the values of factor loading exceeding 0.7 were used for interpretation.

In the case of species growing along the headwater stream 1, factor 1 explained 54% of variance and grouped K, N, P, and Ca, characterised by high and negative factor loadings (Table 4). Factor 2 explained 21% of the variability of the chemical composition of selected species of plants and was contributed by Mg, characterised by a high, positive value of the factor loading. In the case of plants growing on the banks of headwater stream 2, factor 1 explained 47% of variances and grouped K, N, and P, characterised by high and negative factor loadings. Factor 2 explained 20% of the chemical composition of the examined species and was contributed by Ca, characterised by a high factor value. Some other factors made up the nutrient content in the shoots of the plants growing along banks of headwater stream 3. Factor 1 explaining 38% of variance comprised high negative factor loadings of P and Ca (Table 4). Factor 2 explained 25% of the changeability of the chemical composition of the examined plants and was contributed by Mg.

Differences in the composition of factors developing the content of nutrients in shoots of herbaceous plants growing along the banks of the three streams result from the differences in chemical composition of water in streams and depend on the type of substratum in the streams [51]. In streams 1 and 2, numerous organic remains were deposited, which enriched water of the streams with additional nutrients due to efficient processes of mineralisation [32]. In the case of stream 3, the substratum was stony, which, in turn, impacted the washing out of ions of Ca^{2+} and the insignificant participation of N and P in the creation of these factors [6].

3.4. Bioconcentration factors of nutrients in plants

BF reflect diverse accumulative properties of the examined species of herbaceous plants in relation to the macroelements included in the water of the streams. The BF of N, P, K, Mg, and Ca reflect the plant's ability to accumulate the nutrients from its surrounding environment. BF values in Figs. 2 and 3 show that different species of herbaceous plants in mid-forest spring had different abilities to absorb nutrients. Potassium (8,118–42,314), nitrogen (11,716–41,645), and phosphorus (11,607–37,459) had the highest levels of BF.

Table 4
Factor loading obtained from the principal components analysis (PCA) method on the basis of the nutrients concentrations in the herbaceous plants (in bold factor loading higher than 0.70 are highlighted)

Nutrients	Headwater stream 1		Headwater stream 2		Headwater stream 3	
	PC 1	PC 2	PC 1	PC 2	PC 1	PC 2
K	**–0.86**	0.11	**–0.79**	–0.39	–0.63	0.55
N	**–0.78**	0.09	**–0.73**	–0.16	–0.37	–0.35
P	**–0.81**	–0.29	**–0.85**	0.14	**–0.87**	–0.13
Mg	–0.41	**0.87**	–0.57	–0.11	–0.02	**–0.90**
Ca	**–0.71**	–0.41	–0.41	**0.89**	**–0.80**	–0.11
Eigenvalues	2.68	1.04	2.37	1.01	1.92	1.26
Explained variance (%)	0.54	0.21	0.47	0.20	0.38	0.25
	75		67		63	

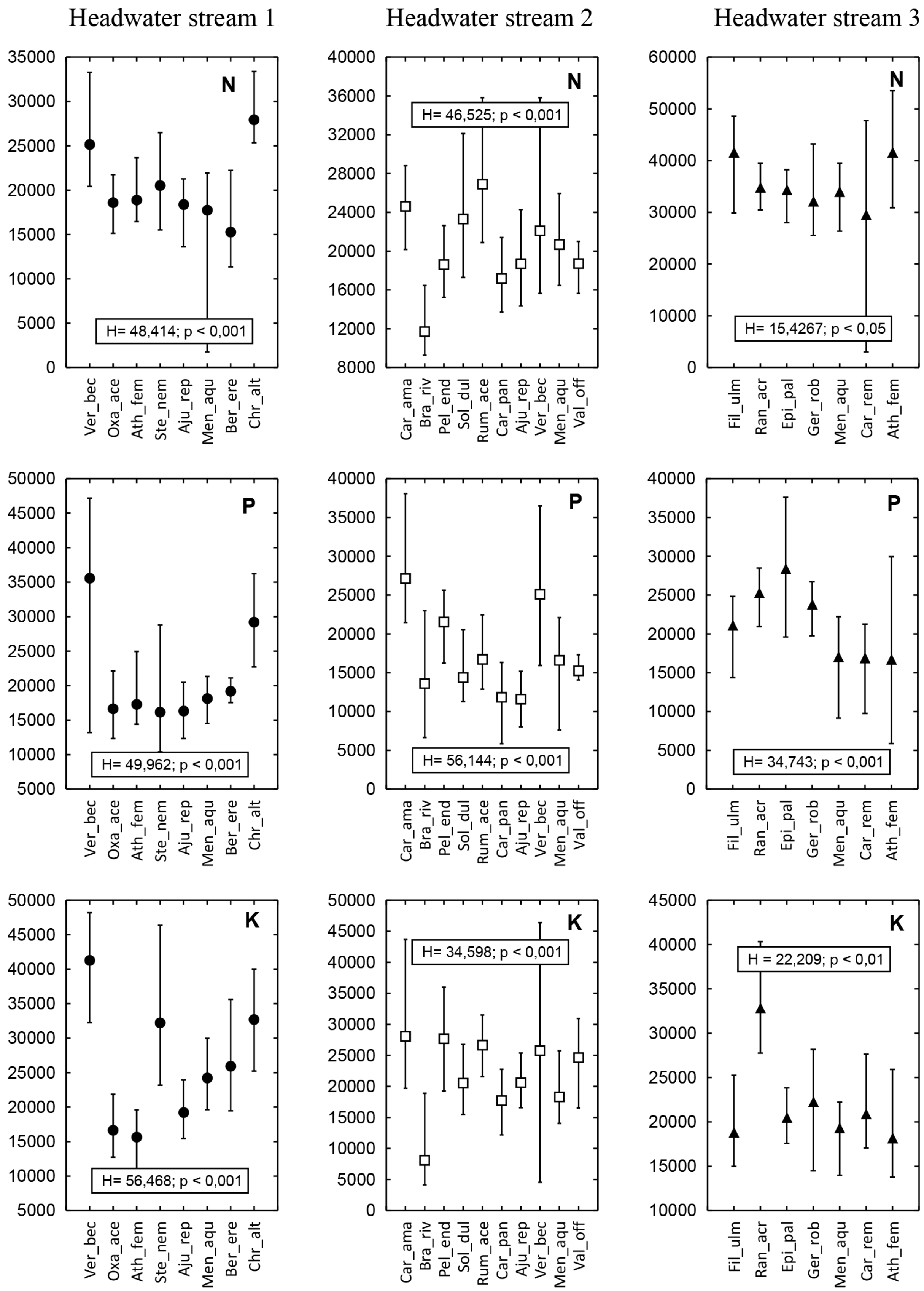

Fig. 2. Changes of bioconcentration factors of N, P, and K in shoots of herbaceous plants in 2012–2014 years with Kruskal–Wallis's test results.

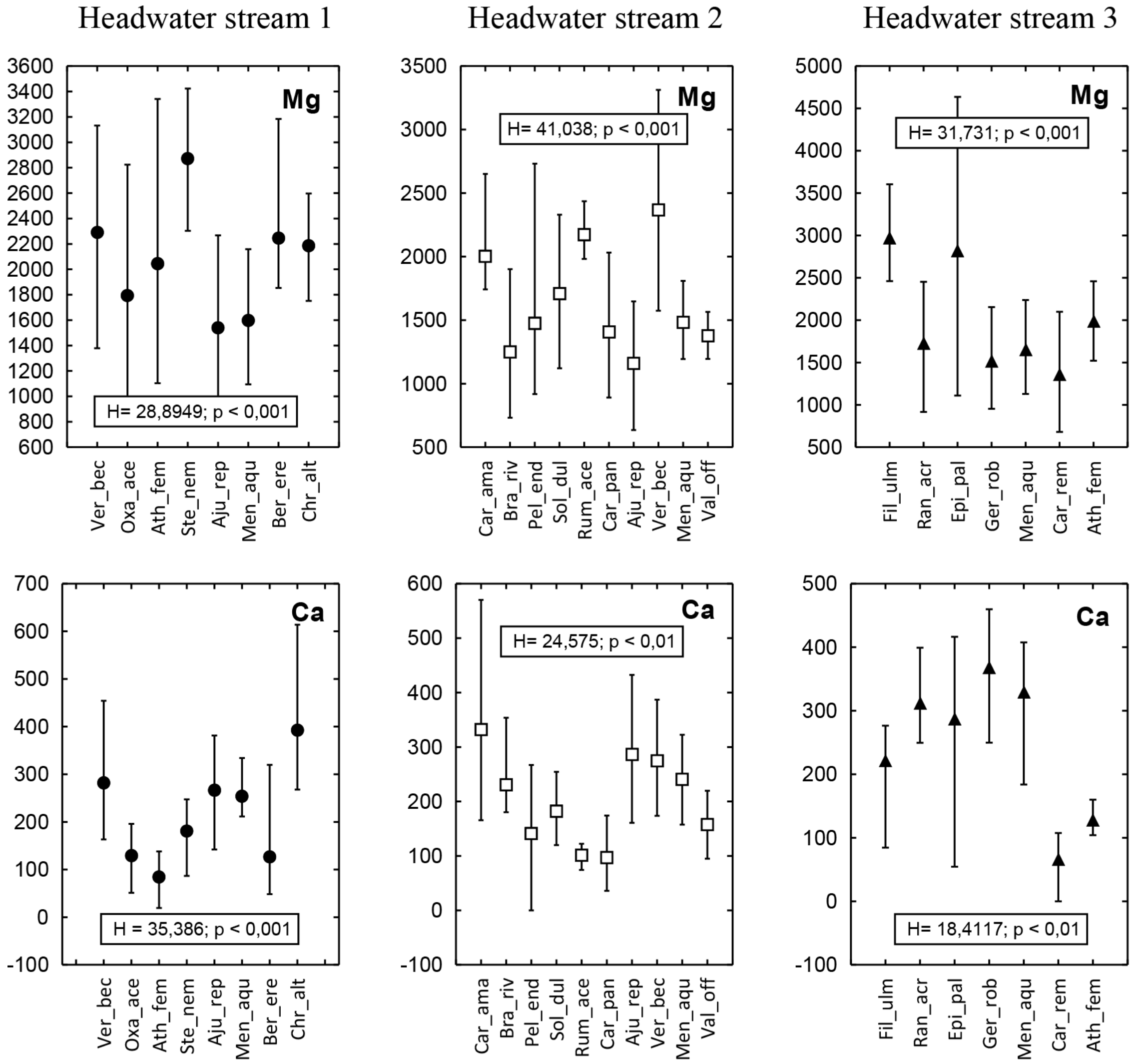

Fig. 3. Changes of bioconcentration factors of Mg and Ca in shoots of herbaceous plants in 2012–2014 years with Kruskal–Wallis's test results.

Lower values of BF referred to magnesium (1,161–2,973) and calcium (85–393).

The highest levels of BF of nutrients were in the following species:

N: *Fil_ulm* > *Ath_fem* > *Ran_acr* > *Epi_pal* > *Men-aqu*
P: *Ver_bec* > *Chr_alt* > *Epi_pal* > *Car_ama* > *Ran_acr*
K: *Ver_bec* > *Ran_acr* > *Chr_alt* > *Ste_nem* > *Car_ama*
Mg: *Fil_ulm* > *Ste_nem* > *Epi_pal* > *Ver_bec* > *Ber_ere*
Ca: *Chr_alt* > *Ger_rob* > *Car_ama* > *Men_aqu* > *Ran_acr*

The results of Kruskall–Wallis test showed significant statistical differences in values of BF between the examined plant species in reference to all analysed nutrients, depending on the stream (Figs. 2 and 3). Results show that the highest levels of BF of nitrogen, phosphorus, potassium, magnesium, and calcium were found in shoots of *Veronica beccabunga, Filipendula ulmaria,* and *Chrysosplenium alternifolium*. High BF values for N, P, K, Mg, and Ca are the result of high demand of plants for macroelements [44,46] and low concentrations of these components in the water of the studied streams (Table 2). Strong accumulation properties of some plant species growing on the banks of rivers and streams have been described by Zang et al. [33] and Parzych et al. [52].

The relatively high potential for bioaccumulation of nutrients in *Veronica beccabunga* shoots was demonstrated by Samecka-Cymerman and Kempers [50] who examined rivers polluted by urban, agricultural, and textile industry sewages, as well as Horska-Schwarz and Spałek [51] who examined selected springs of Opole Silesia region. Equally high BF values for N, P, K, Mg, and Ca in herbaceous plants of the source areas were described by Parzych et al. [31] and Parzych and Jonczak [54].

Based on the BF of nutrients in plants, the tested species were clustered into two distinct groups (A and B) by HCA for headwater streams (Fig. 4). The distinction reflects slight differences in headwater niche ecosystems. The first group (A) comprises two subgroups (A1 and A2), with nine species of plants (*Fil_ulm_3, Ath_fem_3, Men_aqu_3, Car_3* and *Ran_acr_3, Chr_alt_1, Epi_pal_3, Ger_rob_3, Ver_bec_1*). The plant species of A1 subgroup exhibited a relatively high values of BF for

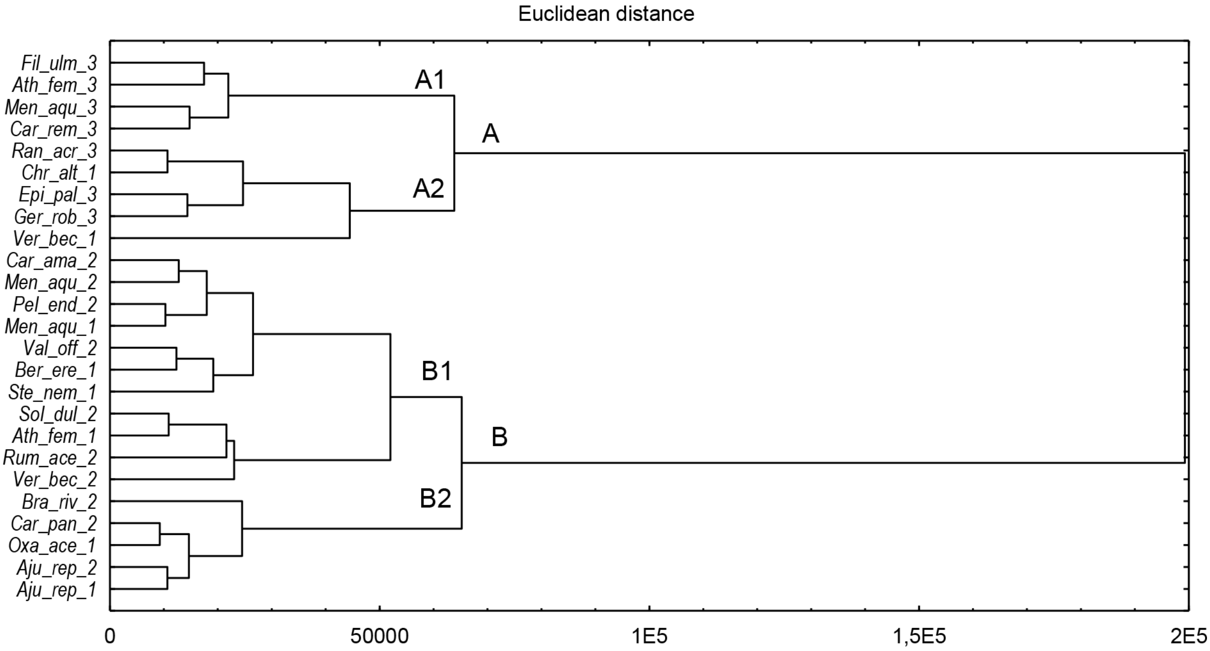

Fig. 4. Variability of the investigated plant species of headwater streams (1, 2, and 3) in relation to the bioconcentration factors of N, P, K, Mg, and Ca (Euclidean distance and Ward's clustering method).

N and P and low values for K and Mg. The plant species of A2 subgroup were characterised by the highest values of BF for N, P, K, and Ca. Such linkage proves that plants growing in headwater stream 3 bioconcentrated the most popular biogens (N and P) due to favourable local conditions. Moreover, an abundance of phosphorus facilitates an uptake of potassium and calcium. The second group (B) included two subgroups (B1 and B2) with 15 species (*Car_ama_2, Men_aqu_2, Pel_end_2, Men_aqu_1, Val_off_2, Ber_ere_1, Ste_ere_1, Sol_dul_1, Ath_fem_1, Rum_ace_2, Ver_bec_2* and *Bra_riv_2, Car_pan_2, Oxa_ace_1, Aju_rep_2, Aju_rep_1*) with lack of samples collected in the area of headwater stream 3. The plant species of B1 subgroup exhibited a relatively high values of BF for N and K and average values for P and Ca. B2 subgroup included species, which had relatively low values of BF for P, K, and Mg. The obtained research results confirm very close interactions between the main components of the headwater niche – water and vegetation, and their character varies depending on the place of sampling and the plant species. It clearly indicates that headwater streams of 1 and 2 differ from stream 3 according to availability of phosphorus. In the case of species growing on the edges of different streams, it was demonstrated that in the shoots of *Athyrium filix-femina* and *Veronica beccabunga* the amount of accumulated macronutrients was predominantly influenced by the environmental conditions resulting from the sampling site while in the case of *Ajuga reptans* the characteristics of the species dominated (Fig. 4).

Simultaneously, it was observed that in the case of *Mentha aquatica*, the amount of accumulated nutrients was strongly influenced by the species characteristics modified by environmental conditions. A relatively high ability for bioaccumulation of nutrients by shoots of *Veronica beccabunga* was shown by Samecka-Cymerman and Kempers [50] and Horska-Schwarz and Spałek [51], and in the shoots of *Athyrium filix-femina* by Parzych [40] and Samecka-Cymerman et al. [55].

4. Conclusion

Plants usually take up macroelements in proportion to their concentration in water and soil. However, in the case of the headwater riparian forest under consideration, it was discovered that concentrations of all nutrients were much higher in the dry matter of plants than in their concentrations in water. It was found that many plant species take up nutrients in quantities exceeding their physiological demand, which makes it possible to be used in water purification. The demonstrated differences in composition of conditions that shape macronutrient content in shoots of herbaceous plants growing on the edges of streams explain from 63% to 75% of variance.

High levels of BF confirm very strict interactions between basic components of headwater niches — water and plants — and their character is diverse, depending on the element and the species of the plant. Potassium (8,118–42,314), nitrogen (11,716–41,645), and phosphorus (11,607–37,459) had the highest levels of BF. Lower values of BF referred to magnesium (1,161–2,973) and calcium (85–393). The highest values of BF K were found in shoots of *Veronica beccabunga, Ranunculus acris*, and *Chrysosplenium alternifolium*; N in shoots of *Filipendula ulmaria, Athyrium filix-femina*, and *Ranunculus acris*; P in shoots of *Veronica beccabunga, Chrysosplenium alternifolium*, and *Epilobium palustre*; Mg in *Filipendula ulmaria, Stellaria nemorum*, and *Epilobium palustre*; and Ca in shoots of *Chrysosplenium alternifolium, Geranium robertianum*, and *Cardamine amara.* The results of a non-parametric Kruskall–Wallis test showed significant statistical differences in the values of BF between the examined plant species in reference to all analysed nutrients, depending on the stream. In the case of species growing on the edges of different streams, it was demonstrated that in the shoots of *Athyrium filix-femina* and *Veronica beccabunga* the amount of accumulated macronutrients was predominantly influenced by the environmental conditions resulting from the sampling site while in the case of *Ajuga reptans* the characteristics of the species dominated. Simultaneously, it was observed that in the case of *Mentha aquatica*, the amount of accumulated nutrients was strongly influenced by the species characteristics modified by environmental conditions.

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