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Heavy Metal Content in the Plants (*Pleurozium schreberi* and *Picea abies*) of Environmentally Important Protected Areas of the Tatra National Park (the Central Western Carpathians, Poland)

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Abstract: This work concerns the content of selected heavy metals (Cd, Cr, Cu, Ni, Pb, and Zn), and determines the effect of absolute altitude on the content of metals in the plants of the Tatra National Park (TNP). The metals were determined in two species of plants, i.e., in the moss (*Pleurozium schreberi* (Willd.) Mitten) and in the Norway spruce (*Picea abies* (L.) H. Karst). Plant samples were collected in two test areas every 100 m of the altitude of the area, starting from 1000 m above sea level in the Lake Morskie Oko test area and from 1100 m above sea level in the Kasprowy Wierch test area, and ending at 1400 m above sea level for Lake Morskie Oko, and 1750 m above sea level (the moss) and 1550 m above sea level (the spruce) for Kasprowy Wierch. The two test areas are different from each other in terms of natural and physico-geographical conditions (geological structure, landform, climatic conditions). The conducted research showed that both plant species accumulated greater amounts of heavy metals in the Lake Morskie Oko test area than in the Kasprowy Wierch test area. The moss accumulated higher values of metals compared to the spruce. In both the moss and the spruce, the highest values, exceeding the natural content, were found for Cr, Pb, Cd, and Ni. For these metals, natural values were significantly exceeded: 20 times for Cr; 10 times for Pb; 4 times for Cd; and 3 times for Ni. For both examined areas, an increase in the quantity of accumulated metals in plants was also observed with the increase in altitude. The work focuses on the spreading around of heavy metals and their deposition on plants in protected high mountain (alpine) areas, in connection with altitude. Based on the obtained research results, Spearman's and Kendall's rank correlations were performed, and showed statistically significant relationships between the values for the content of metals and altitude. There are no heavy metal emission sources in the study area, so it is assumed that the metal content in the plants of the TNP is affected by long-range emissions.

Keywords: heavy metals; plants; mountains; *Pleurozium schreberi* moss; *Picea abies* Norway spruce; Tatras

1. Introduction

At present, the natural environment is contaminated with heavy metals, the content of which in soils and plants is significantly higher than their natural content [1–3]. The excessive amount of heavy metals in the natural environment leads to irreversible changes in ecosystems [4]. Increased levels of metals, especially Cd, Cr, Cu, Ni, Pb and Zn, result from industrial and automotive activities. Heavy metals in dust particles are transported over long distances, up to several hundred kilometres [5–7].

As a result of transporting pollutants over long distances, areas considered to be of natural importance and protected areas may have a problem with an increased amount of metals in the soil or vegetation. An example of such an area is the Tatra National Park, where we are dealing with long-range emissions. The Tatra National Park is one of 23 national parks in Poland. It has the highest protection regime of all the forms of nature protection in Poland. Together with the Slovak part of the Tatra National Park (Tatranský národný park), it is a UNESCO biosphere reserve. Its natural value is evidenced by the fact that for many centuries, a large part of this area has not been directly changed by man, and this condition continues to this day. The small area of the national park (211 km^2) contains a wealth of flora and fauna, often endemic and relict species, as well as a variety of landscapes. This is the result of, among other things, the specific geographical location of the Tatra Mountains in Europe, which is influenced by transitional climate or the overlapping ranges of different flora and fauna. Although human pressure on the natural environment of this area concerns in situ impacts related to tourism, the entire area of the national park is affected by external influences. Research on the determination of the content of heavy metals and radionuclides in the soils and plants of the Tatra National Park was carried out by, among others: Kowalska et al. [8]; Kubica et al. [9]; Miechówka et al. [10]; Miechówka and Niemyska-Łukaszuk [11]; Niemyska-Łukaszuk et al. [12]; Paukszto and Mirosławski [13]; Staszewski et al. [14]; Stobiński and Kubica [15]; Świetlik et al. [16]; Wieczorek and Zadrożny [17].

Pollutants from the Czech Republic, Slovakia, and the Silesia region are transported to the TNP area, where they fall and cause increased metal content in soils and plants. In Slovakia and the Czech Republic, the industrial sector is dominated by metallurgy, chemical, defence, electrical, and electronic industries, as well as the production of Ni and Cu. These industries are a source of heavy metals in the natural environment.

Mosses, especially the *Pleurozium schreberi* species, are sensitive indicators of the presence of heavy metals in the environment [18–23]. Mosses have been used as bioindicators owing to the fact that:

- they have a wide range of occurrence, in various habitats;
- they do not have a cuticle or an epidermis, thus, their leaves are easily permeable by metal ions;
- they are devoid of roots and conductive tissue, and they absorb mineral salts as well as heavy metal ions mainly from precipitation and dry deposition;
- they absorb metals mainly through a simple ion exchange process;
- the concentration of heavy metals in mosses is a function of the amount of heavy metal deposition from the air;
- some species have a multi-level structure, with annual increments forming distinct segments [24].

Conifers are also good and frequently used bio-accumulators in environmental research [25–28], e.g., the Norway spruce (*Picea abies*), which is the dominant species in mountain stands. Conifers have the ability to bond air pollutants in their assimilation apparatus for several years. The following factors play a fundamental role in determining the suitability of the Norway spruce (*Picea abies*) for monitoring studies:

- wide geographic range;
- occurrence in various habitats;
- the presence of the annual growth of needles, making the registration of the concentration of chemical elements in various age ranges possible;
- ease of absorption of various components, especially sulphur and heavy metals, from atmospheric emissions.

The aim of this study was to determine the content of heavy metals in two plant species depending on the absolute altitude in mountain areas. This relationship was observed in world studies of various components of the environment (soil, plants) and mountain ranges [29–35]. The mentioned authors observed an increase in the content of metals in plants with increasing altitude. Two plant species representing different taxonomic levels and differing in their ability to absorb heavy metals (Bryophyta, Tracheophyta) were used in the study. Selected plant species were sampled in an important and valuable natural area under protection (the Tatra National Park).

2. Materials and Methods

2.1. Study Area

The study area is located in the Polish part of the Central Western Carpathians, in the northern part of the Tatra Range macroregion [36], which is the highest part of the entire Carpathians (Figure 1).

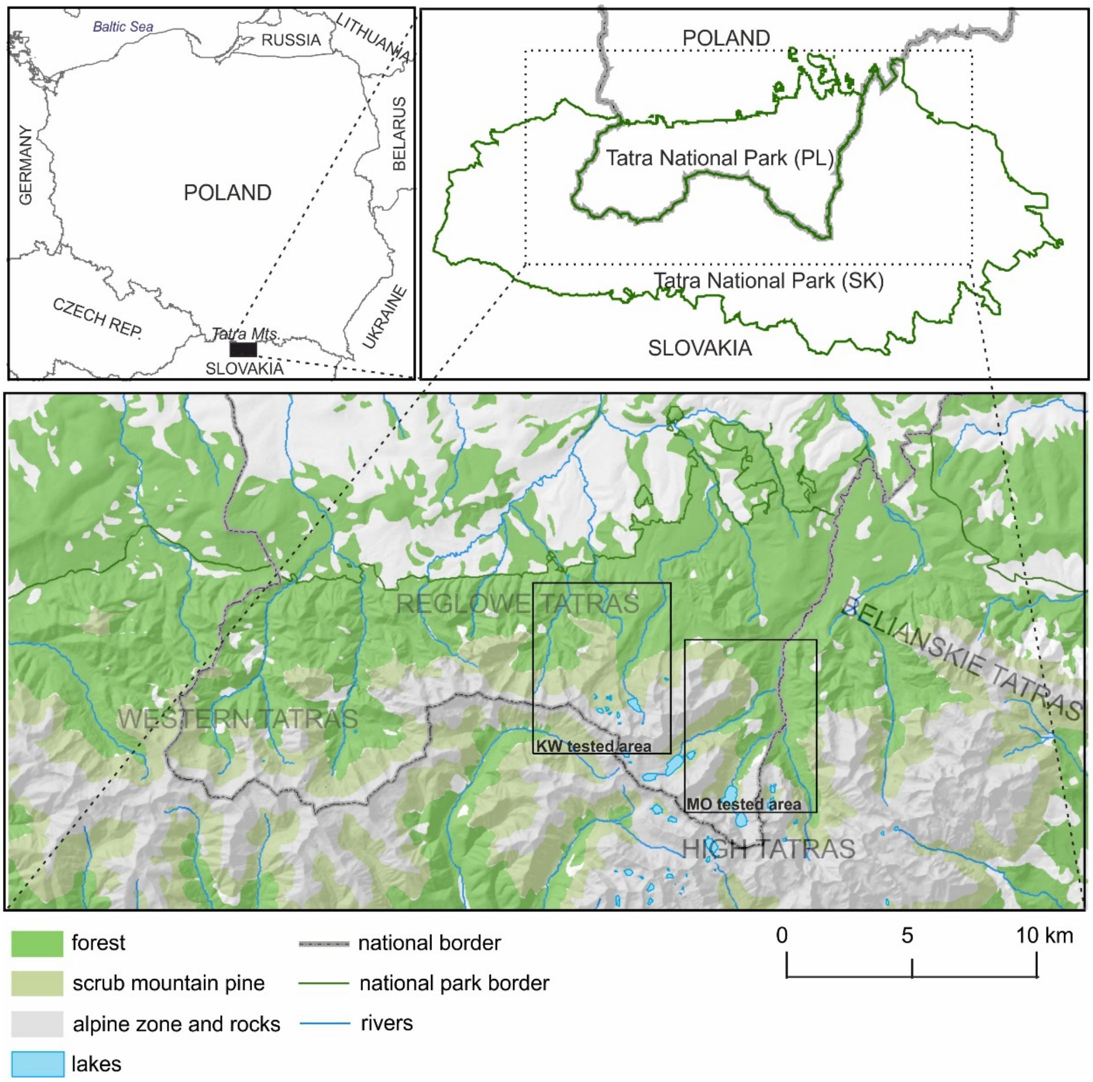

Figure 1. Location of the tested area on the background of the map of Poland and Tatra National Park.

This area is characterized by a complex geological structure [37–39], land relief heterogeneity (fluvial-denudation, karst, and glacial) [40–42], and climatic conditions changing with an increase in altitude (air temperature, total precipitation, etc.). The specific climate conditions of the Tatra Mountains are determined by the frequency of occurrence of various air masses. The largest share in the forming of the weather belongs to the masses of polar maritime air (PPm) (65% of days a year), and to the masses of polar continental air (PPk) (approx. 20% of days a year) [43,44]. The above-mentioned elements determine the features of water circulation (spatially diversified possibility of water retention, the amount of runoff, water chemistry, etc.). The soil cover of the Tatra Mountains is strongly connected with the geological substrate, morphogenetic processes, and climatic conditions, and its characteristic feature is openwork, as well as poorly developed soils (i.e., initial soils) [45]. The specificity of the Tatra Mountains lies in the fully developed physico-geographical zonation characteristic of high mountain areas [46]. Points located in two test areas were selected for study in the Tatra National Park in Poland, and on the northern slope of the Tatra Mountains. These areas were selected owing to the diversity of the natural environment, including physical and geographical location, landscape zone, geological structure, and, in particular, the altered location in relation to the land relief, which affects the deposition of matter. The test areas were given the following working names: Kasprowy Wierch (KW) and Morskie Oko (MO). All points located in the KW test area are located on the slopes near the ridge, with an average slope of approx. approximately 20°. Unlike the previous test area, the other points in the MO test area are located in the relatively wide bottom of the post-glacial valley.

2.1.1. Kasprowy Wierch

The test area covers two physico-geographical mesoregions, i.e., the Reglowe Tatras (sampling points 1–4) and the Western Tatras (sampling points 5–7) [47], and ranges from the forest level to the alpine level (Table 1). The geological structure is strongly diversified in terms of lithology and tectonics. This affects, among other things, the incompatibility of the topographic watershed with the underground watershed. The area belongs to the Bystra catchment (with the sub-catchment of the Potok Jaworzynka) and the Sucha Woda Gąsienicowa catchment, which is part of the Dunajec basin. Depending on the altitude, the mean annual air temperature ranges from 0 °C to 6 °C [48], the annual total of precipitation ranges from 800 mm to 1800 mm, and the length of the snow cover deposition ranges from 100 to 200 days a year [49]. The soil cover is varied and dominated by the following soils: Fluvisols; Rendzic Leptosols; Folic Rendzic Leptosols; Cambic Rendzic Leptosols; Haplic Cambisols (Eutric); Haplic Podzols (Skeletic); Entic Podzols; Leptic Podzols; and Folic Leptosols [50].

Table 1. Characteristics of sampling points in the Kasprowy Wierch test area (KW).

Sample Number	Altitude [Meters above Sea Level]	Geographical Coordinates	Dominant Area Exposure	Terrain Slope Grade	Land Cover Features	Geological Structure [38,39]	Physico-Geographical Mesoregion [36]
1	1100	49°15.572′ N 19°59.322′ E	NE	20–30°	Coniferous forest, spruce forest	Boulders, gravel, sand, and silts of stones and river terraces 0.5–3.0 m high, e.g., rivers (Holocene)	The Reglowe Tatras
2	1200	49°15.424′ N 19°59.645′ E	N	30–40°	Coniferous forest, spruce forest	Dolomites, limestones, siltstones, and breccia (Lower Triassic)	The Reglowe Tatras
3	1300	49°15.254′ N 19°59.681′ E	W	20–30°	Glade (area covered with grasses, sedges, herbaceous plants)	Dolomites, limestones, siltstones, and breccia (Lower Triassic)	The Reglowe Tatras
4	1400	49°15.252′ N 19°59.908′ E	NW	20–30°	Rows and groups of the Norway spruce or the Swiss pine in the mountain pine, dense clumps of Norway spruce in the mountain pine	Dolomites and limestones, undivided (Middle Triassic)	The Reglowe Tatras
5	1550	49°14.497′ N 20°00.097′ E	N	0–10°	Glade (area covered with grasses, sedges, herbaceous plants)	Boulders, moraine rock debris, clayey (Pleistocene)	Western Tatras
6	1650	49°14.133′ N 19°59.671′ E	SE	20–30°	The mountain pine, glade (area covered with grasses, sedges, herbaceous plants)	Porphyry granites (Carbon)	Western Tatras
7	1750	49°14.013′ N 19°59.446′ E	E	10–20°	The mountain pine, glade (area covered with grasses, sedges, herbaceous plants)	Boulders and rock debris of rubble cones (screes) (Quaternary)	Western Tatras

2.1.2. Morskie Oko

The area is located within the High Tatras, in the Białka catchment (the Dunajec river basin) drained by the Rybi Potok, the Roztoka, and the Białka (Table 2). With regard to the zonation of the environment, it is entirely located within the forest level. It is part of one of the largest post-glacial grooves in the Tatras (a U-shaped valley). Depending on the altitude, the mean annual air temperature ranges from 2 °C to 4 °C [48], the annual total of precipitation ranges from 1000 mm to 1400 mm, and the length of snow cover deposition ranges from 120 to 160 days a year [49]. The dominant soils in this part are, among others: Haplic Podzols (Skeletic); Haplic Cambisols (Dystric, Skeletic); Lithic Leptosols; and Regosols (Hyperskeletic) [50].

Table 2. Characteristics of sampling points of the Morskie Oko test area (MO).

Sample Number	Altitude [Meters above Sea Level]	Geographical Coordinates	Dominant Area Exposure	Terrain Slope Grade	Land Cover Features	Geological Structure	Physico-Geographical Mesoregion [36]
8	1000	49°15.065′ N 20°05.898′ E	SE	0–10°	Coniferous forest, spruce forest	Boulders, gravel, sand, clayey sands, and silts of cones, of fluvioglacial levels, and terraces 12.0–15.0 m high, e.g., rivers (Pleistocene)	High Tatras
9	1100	49°13.984′ N 20°05.524′ E	NE	20–30°	Coniferous forest, spruce forest	Granodiorites and tonalities, equal grained, grey (Carbon)	High Tatras
10	1200	49°13.270′ N 20°05.647′ E	NE	0–10°	Young Norway spruce stand	Boulders, moraine rock debris, clayey (Pleistocene)	High Tatras
11	1300	49°12.893′ N 20°04.867′ E	NE	10–20°	Coniferous forest, spruce forest	Boulders, rock debris, and silts of dump and alluvial cones (Pleistocene-Holocene)	High Tatras
12	1400	49°12.021′ N 20°04.115′ E	E	10–20°	Coniferous forest, spruce forest	Boulders, rock debris, and silts of dump and alluvial cones (Pleistocene-Holocene)	High Tatras

2.2. Sampling and Analysis

2.2.1. Sampling

Plant samples (two species: the *Pleurozium schreberi* moss (green fragments) and the Norway spruce *Picea abies* (2-year-old needles)) were collected from the area of the Tatra National Park, from the Kasprowy Wierch (KW) and Lake Morskie Oko (MO) test areas. The samples were taken every 100 metres of altitude, starting from the altitude of: 1100 m above sea level for KW, and from 1000 m above sea level for MO. Owing to the limited range of occurrence at higher altitudes, plants (the Norway spruce and the moss) were sampled up to the altitude of 1750 m above sea level for KW. The geographical coordinates of the sampling sites, and the designations adopted are shown in Tables 1 and 2. Ten samples in an amount of ca. 0.1–0.2 kg were taken from each plant species at each location, totalling 240 samples. In the case of the moss, two- or three-year-old green segments of shoots and, in the case of the Norway spruce, two-year-old needles were sampled for the study. Samples were taken according to ICP Vegetation guidelines. The samples were taken at the end of summer/the beginning of autumn. All of the samples were taken in September 2019 under similar weather conditions. The material was placed in polyethylene bags.

2.2.2. Chemical Analysis

According to the suggestions of the following authors: Maňkovska et al. [51] and Sawidis et al. [52], regarding the sample preparation procedure, the plant material was left unwashed. The samples were dried in an electric drier (Model ED 23, Binder, Cracow, Poland) at a temperature of 40 °C for 72 h. Needles were separated from branches. Equal amounts of biomass from primary samples from the same plot were combined. Dry and homogenized samples were pulverized in an electric grinder (Grinder Pulverisette 19, Fritsch, Cracow, Poland). Portions of 1g dry weight material were placed in Teflon vessels, and 5 cm^3 of 65% HNO_3 and 3 cm^3 of 36% H_2O_2 were added to each vessel. The mixture was mineralized in a Berghof Speed Wave microwave in a temperature of 200 °C and at a pressure of 4 MPa. After processing, the samples were diluted with deionized water to a total volume of 50 cm^3, and filtered through a hard paper filter. The final solutions were analysed for heavy metals (Cd, Cr, Cu, Ni, Pb, and Zn) using the inductively coupled plasma mass spectrometry (ICP-MS) method in the Bureau Veritas laboratory. Such standards and reference materials (for plants) were used. The detection limits (µg/g d.m.) were as follows: for Cd, 0.01; Cr, 0.1; Cu, 0.01; Ni, 0.1; Pb, 0.01; and Zn, 0.1. The STD CDV-1 and STD V16 standards were used as reference materials.

2.2.3. Statistical Study

Statistical analyses were performed using the Statistica and SAS Studio programs. Owing to the different conditions and differences in the altitude of sampling, the analysis was carried out in two groups depending on the location of the study (MO—Lake Morskie Oko, and KW—Kasprowy Wierch). The Shapiro–Wilk test was used to estimate the normality of distribution. The Mann–Whitney U test and the Kolomogorov–Smirnow test were used to examine the independence of samples between species. The Kruskal–Wallis test was used to examine the equalization of the values for the samples taken at different altitudes. The analyses adopted a confidence level of $p < 0.05$. On the other hand, using the Spearman and Kendall rank correlation, the relationships between the content of metals in plants and altitude were compared, assuming a significance level of $p < 0.05$.

3. Results

On the basis of the average metal content in plants, calculated from all the collected samples, it can be concluded that the *Pleurozium schreberi* moss accumulated higher values of metals (1.9 µg Cd/g, 20.7 µg Cr/g, 16.3 µg Cu/g, 13.2 µg Ni/g, 25.4 µg Pb/g, 58 µg Zn/g dry mass) compared to the *Picea abies* Norway spruce (1.5 µg Cd/g, 16.7 µg Cr/g, 11.7 µg Cu/g, 11.4 µg Ni/g, 14 µg Pb/g, 36.9 µg Zn/g dry mass). Both the *Pleurozium schreberi* moss and the *Picea abies* Norway spruce accumulated Zn in the greatest amounts (58 and 36.9 µg Zn/g d.m. for the moss and the Norway spruce, respectively), and cadmium in the smallest amounts (1.9 and 1.5 µg Cd/g d.m.). The sequences of metal accumulation, in order of the highest metal accumulation in plants, were as follows: Zn > Pb > Cr > Cu > Ni > Cd for the moss, and Zn > Cr > Pb > Cu > Ni > Cd for the Norway spruce. The metal accumulation sequences look similar for both plant species, with the difference in the content of Pb and Cr, where the moss accumulated higher values of Pb than Cr, and in the case of the Norway spruce, it was the opposite (the Norway spruce accumulated higher values of Cr than Pb). For both plant species, the last three metals presented in the sequence were similarly accumulated (higher amounts of Cu compared to Ni and Ca, and Ni compared to Cd).

The average content of heavy metals in plants is presented in the graphs—Figures 2 and 3.

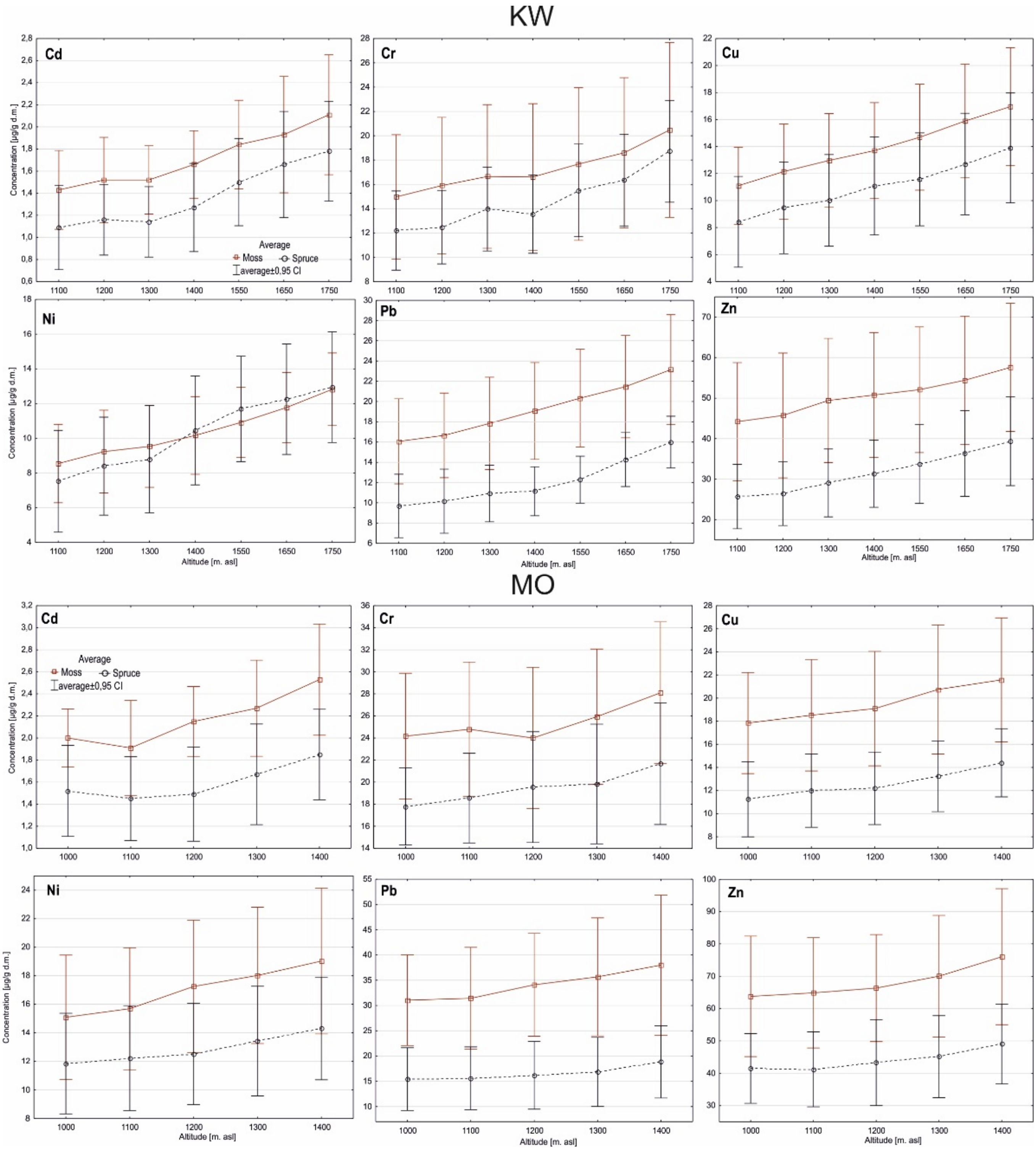

Figure 2. Mean metal content in plants (*Pleurozium schreberi* and *Picea abies*) in Tatra National Park, Poland. Confidence intervals for 95% of observations.

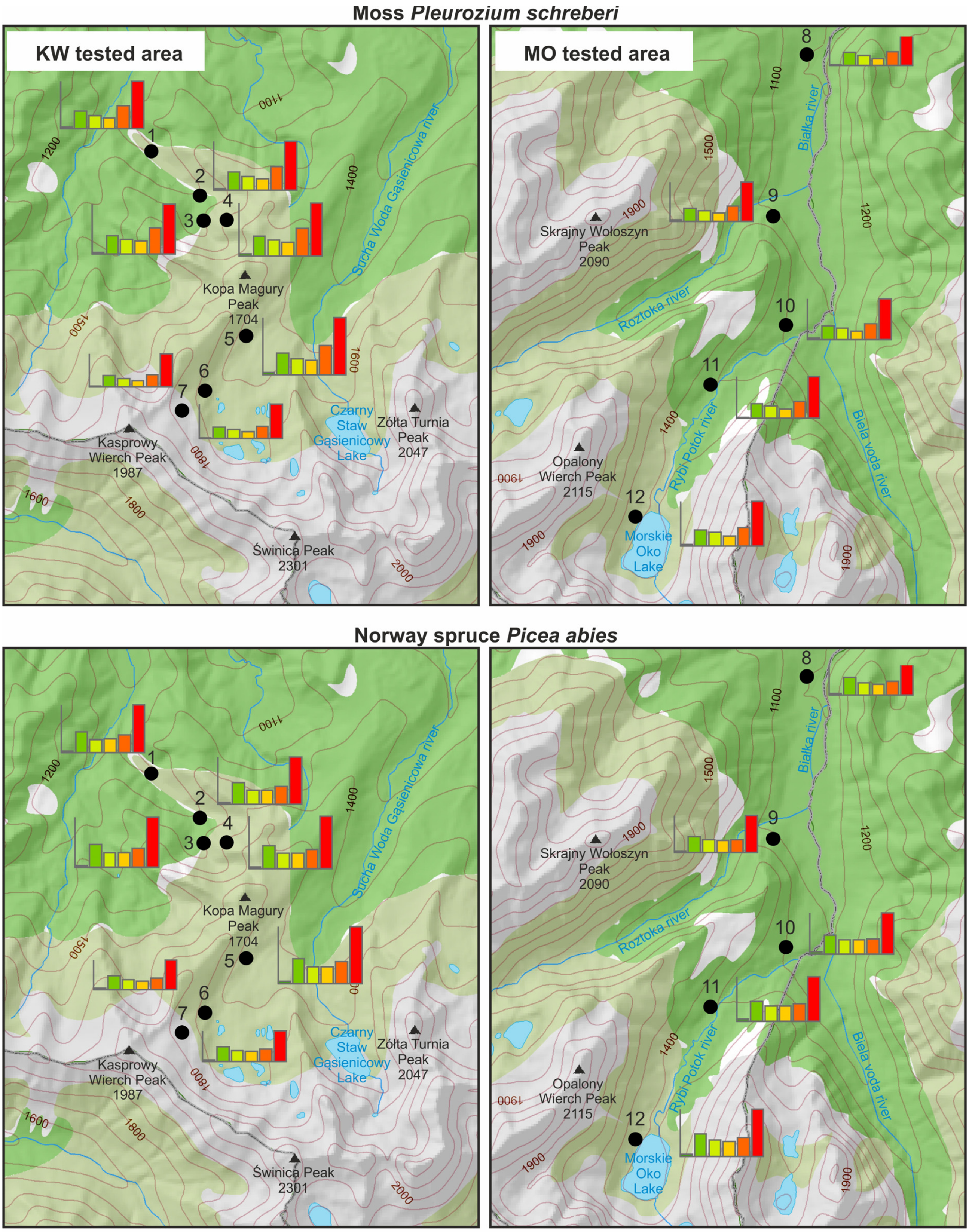

Figure 3. Mean concentrations of heavy metals (µg/g d.m.) in the moss species, *Pleurozium schreberi*, and the Norway spruce, *Picea abies*, in the Tatra National Park, Poland.

Based on the Shapiro–Wilk test, it should be noted that the distribution of metals content is normal only the case of Cd, Cr, and Pb in the Norway spruce, at a confidence interval of 0.05. Therefore, it should be considered that in other cases, the distribution of the analysed values is not normal. Therefore, in order to determine the similarity, non-parametric tests were used in further analysis.

As can be seen in Figure 2, the average metal content for both indicators are different. In order to confirm this fact, and to conduct the analysis separately for both species, the Mann–Whitney U test was carried out. The test results showed statistically significant differences in metal concentrations between the moss and the Norway spruce. Only in the case of Cr (p 0.16) and Ni (p 0.64) in the samples from KW, can the hypothesis that the observed difference in metal content between the species is a coincidence at $p < 0.05$ not be rejected. Complementary tests were performed to confirm this discrepancy in the distribution of metals.

Also, in the Kruskal–Wallis test for the content of Cr and Ni in the moss, and in the Norway spruce in the case of KW, they do not allow the conclusion that the species have a significant influence on the content of metals. As a complement, the non-parametric Kolomogorov–Smirnov test was used to verify that the samples for individual metals were taken from different populations. Only in the case of Cr and Ni content in the samples from KW, are the differences between species statistically significant.

Therefore, it should be concluded that the content of Cr and Ni in the samples from the KW area exhibits characteristics different from those of other metals. The authors only draw readers' attention to this anomaly, but it has not been included in the further analysis. This issue requires further detailed research. On the basis of other cases, it was recognized that both the species diversity and the location of sampling have a significant effect on the content of metals. Therefore, further analysis was made by location and species. The content of heavy metals in both plant species in the two test areas increases together with increasing altitude. The increase in the content of metals with the height of the terrain is presented in Table 3. The presented values were calculated as the ratio of the metal content in a given plant species at an altitude of 1400 m asl for MO and 1750 m asl for KW, to the metal content in plants at 1000 m asl for MO and 1100 m asl for KW.

Table 3. Heavy metal content in plants for extreme altitudes.

Species	Research Site	Cd	Cr	Cu	Ni	Pb	Zn
Moss	MO	1.3	1.2	1.2	1.3	1.2	1.2
	KW	1.5	1.4	1.5	1.5	1.4	1.3
Norway spruce	MO	1.2	1.2	1.3	1.2	1.2	1.2
	KW	1.6	1.5	1.6	1.7	1.7	1.5

The highest values of heavy metals in plants were observed for the Norway spruce near Kasprowy Wierch. The moss, similarly to the Norway spruce, showed higher values in the vicinity of KW compared to the vicinity of MO. The highest value of plants by metals in the higher mountain parts in relation to the lower areas was found for Ni and Pb in the case of the Norway spruce, and for Cd, Cu, and Ni in the case of the moss.

By correlating the content of individual metals with the altitude, an analysis was performed for individual species and locations (Figure 4). The analysis was performed using two approaches. First, non-parametric correlations were made taking into account all the observations. However, it should be noted that the number of samples was very limited, owing to the research area (samples for the tests were collected in a protected natural area). Also, the technical conditions of sampling, including the lack of given species on a specific measurement site, and the morphological diversity of plants (sampling of 2-year-old needles (the Norway spruce) and 3-year-old green parts (the moss)) resulted in outliers. Therefore, in the second approach, rank correlations of mean values were used, with outliers being rejected.

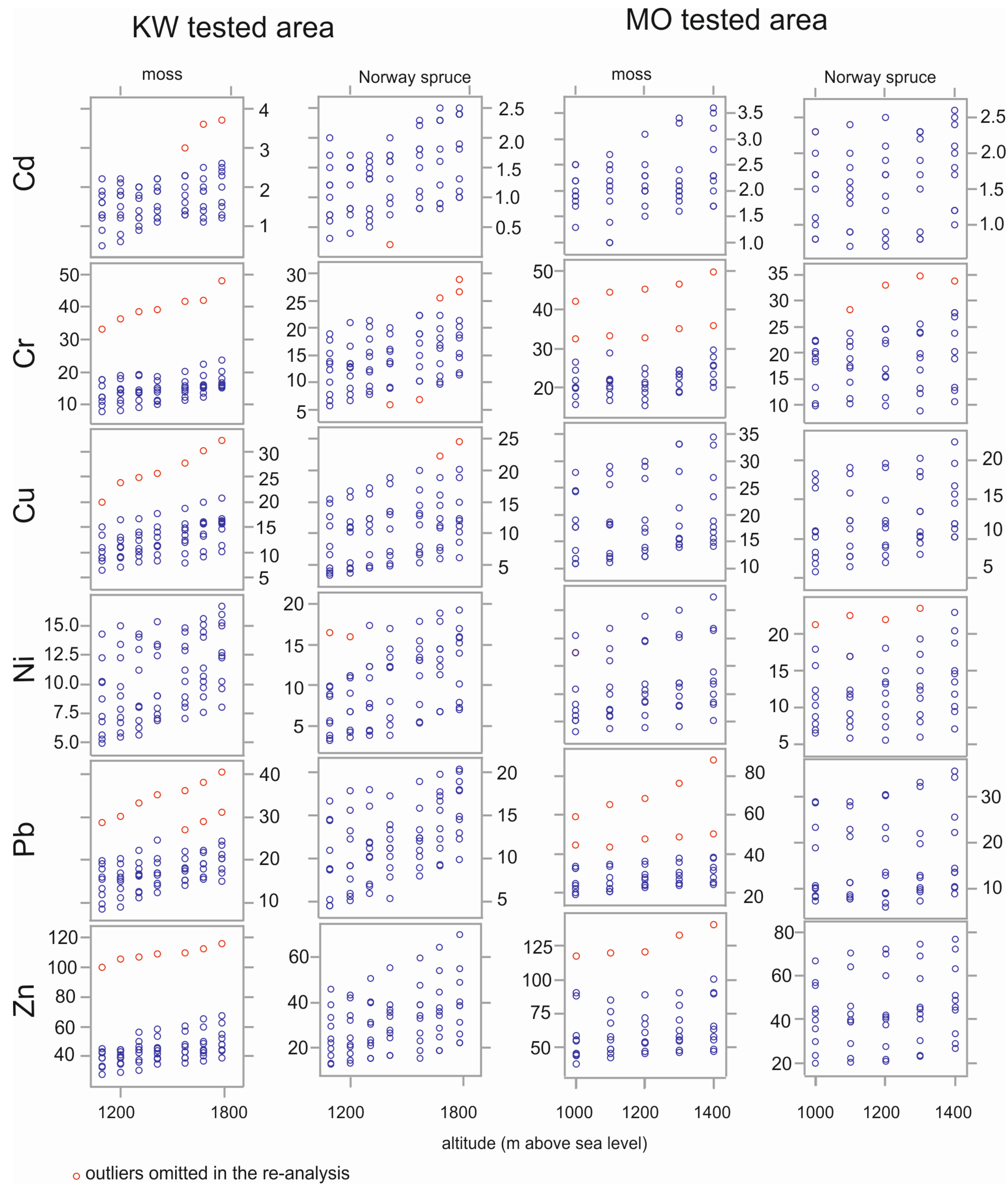

Figure 4. Dependence of heavy metal content (µg/g d.m.) in plants (*Pleurozium schreberi* and *Picea abies*) on the altitude, broken down into test areas (Kasprowy Wierch-KW and Morskie Oko-MO) in the Tatra National Park, Poland.

Two Spearman and Kendall correlations were used in the analysis. In the normal case, the Kendall correlation is more robust and efficient than the Spearman correlation. This means that the Kendall correlation is preferred when there are small samples or some outliers. The Kendall correlation has an O(nˆ2) computation complexity, comparing with O(n logn) for the Spearman correlation, where n is the sample size. Spearman's Rho is usually larger than Kendall's Tau. The interpretation of Kendall's Tau, in terms of the probabilities of observing the agreeable (concordant) and non-agreeable (discordant) pairs, is very direct. The selection of non-parametric dependence measures was determined by altitude measures, which are not continuous measures.

Spearman's and Kendall's rank correlations showed statistically significant relationships at the level of p <0.05 only between the values for the content of metals and altitude, and only for the samples from KW. In the case of samples from MO, statistically significant correlations appeared (Table 4) in the case of NI (the moss) and Cu (the Norway spruce), however, these values were small, and amounted to about 0.22. In the vicinity of KW, statistically significant correlations were found for all metals in the case of the moss and the Norway spruce. For mosses, the strongest statistically significant positive correlation was found in the case of Cu and Ni. In the case of the Norway spruce, the strongest relationships with altitude were observed in the case of Pb and Ni.

Table 4. Spearman's and Kendall's rank correlations for plants in the test areas (Kasprowy Wierch-KW and Morskie Oko-MO) in the Tatra National Park, Poland.

Metal	Moss				Norway Spruce			
	MO		KW		MO		KW	
	Spearman's	Kendall's	Spearman's	Kendall's	Spearman's	Kendall's	Spearman's	Kendall's
Cd	0.26259	0.19887	0.33778	0.25776	0.22449	0.16848	0.40877	0.32051
p	0.0654	0.0661	0.0042	0.0037	0.1170	0.1185	0.0004	0.0003
Cr	0.23334	0.18348	0.36764	0.29170	0.18775	0.13722	0.37305	0.27619
p	0.1029	0.0838	0.0017	0.0008	0.1917	0.1963	0.0015	0.0015
Cu	0.21225	0.17629	0.43813	0.33968	0.27554	0.21572	0.33454	0.25811
p	0.1389	0.0969	0.0001	0.0001	0.0528	0.0425	0.0046	0.0030
Ni	0.28137	0.22182	0.43869	0.32976	0.19064	0.14316	0.43630	0.33837
p	0.0478	0.0368	0.0001	0.0002	0.1848	0.1764	0.0002	0.0001
Pb	0.24461	0.19711	0.40418	0.30308	0.19067	0.14885	0.48218	0.36990
p	0.0869	0.0633	0.0005	0.0005	0.1847	0.1605	0.0001	0.0001
Zn	0.24894	0.19186	0.41102	0.30760	0.18769	0.14395	0.33803	0.25094
p	0.0813	0.0697	0.0004	0.0004	0.1918	0.1737	0.0042	0.0039

Subsequently, an analysis of the relationship between the mean values of metal content and altitude was performed. Owing to the limitation of the outliers, they were omitted in the analysis. In the analysis of the mean values of metal content and altitude, higher values of the relationship can also be observed in the case of KW, although the differences are not large. Only in the case of Cr in the moss and Cd in the Norway spruce in MO, do the relationships not meet the assumed significance at the level of $p < 0.05$ (Table 5).

Table 5. Spearman's and Kendall's rank correlations without outliers for plants in the test areas (Kasprowy Wierch-KW and Morskie Oko-MO) in the Tatra National Park, Poland.

Metal	Moss				Norway Spruce			
	KW		MO		KW		MO	
	Spearman's	Kendall's	Spearman's	Kendall's	Spearman's	Kendall's	Spearman's	Kendall's
Cd	0.99103	0.97590	0.90000	0.80000	0.96429	0.90476	0.70000	0.60000
p	0.0001	0.0024	0.0374	0.0500	0.0005	0.0043	0.1881	0.1416
Cr	0.96429	0.90476	0.70000	0.60000	0.96429	0.90476	1.00000	1.00000
p	0.0005	0.0043	0.1881	0.1416	0.0005	0.0043	0.0001	0.0143
Cu	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000
p	0.0001	0.0016	0.0001	0.0143	0.0001	0.0016	0.0001	0.0143
Ni	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000
p	0.0001	0.0016	0.0001	0.0143	0.0001	0.0016	0.0001	0.0143
Pb	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000
p	0.0001	0.0016	0.0001	0.0143	0.0001	0.0016	0.0001	0.0143
Zn	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	0.90000	0.80000
p	0.0001	0.0016	0.0001	0.0143	0.0001	0.0016	0.0374	0.0500

The presented research results showed that there is a relationship between the content of selected metals in plants and altitude. The determined content of Cd, Cr, Cu, Ni, Pb, and Zn was also affected by long-range emissions in the TNP area.

In order to determine the accumulation capacity of the studied plant species, the bioconcentration factor (BCF) was determined. The BCF was calculated as the quotient of the metal concentration in the plant to the metal concentration in the soil (Table 6). Data for the soils sampled at the same measurement points as plants, included in the article by Korzeniowska and Krąż [53], were used.

Table 6. The bioconcentration factor (BCF) for plants in the test areas (Kasprowy Wierch-KW and Morskie Oko-MO) in the Tatra National Park, Poland.

Tested Area	Sample Number	Species of Plants	The Bioconcentration Factor (BCF)					
			Cd	Cr	Cu	Ni	Pb	Zn
KW	1	Moss	1.1	0.3	0.9	0.5	0.1	0.4
		Norway spruce	0.8	0.2	0.7	0.5	0.1	0.2
	2	Moss	1.3	0.4	1.0	0.6	0.1	0.4
		Norway spruce	1.0	0.3	0.8	0.6	0.1	0.2
	3	Moss	1.4	0.4	1.2	0.8	0.2	0.5
		Norway spruce	1.0	0.3	0.9	0.7	0.1	0.3
	4	Moss	1.9	0.5	2.1	1.3	0.2	0.6
		Norway spruce	1.4	0.4	1.7	1.4	0.1	0.4
	5	Moss	2.6	0.6	2.6	1.8	0.2	0.7
		Norway spruce	2.1	0.5	2.1	1.9	0.1	0.4
	6	Moss	2.7	0.6	3.8	1.8	0.3	0.7
		Norway spruce	2.4	0.5	3.0	1.9	0.2	0.5
MO	7	Moss	4.2	0.8	4.3	2.3	0.4	0.8
		Norway spruce	3.6	0.7	3.5	2.3	0.3	0.5
	8	Moss	1.5	0.7	1.2	2.6	0.2	0.5
		Norway spruce	1.2	0.5	0.8	2.0	0.1	0.3
	9	Moss	1.9	0.8	1.5	3.1	0.2	0.6
		Norway spruce	1.5	0.6	1.0	2.4	0.1	0.4
	10	Moss	2.4	0.8	1.6	3.4	0.3	0.8
		Norway spruce	1.7	0.6	1.1	2.5	0.1	0.5
	11	Moss	3.8	0.9	1.9	5.8	0.3	1.0
		Norway spruce	2.8	0.7	1.2	4.3	0.1	0.7
	12	Moss	5.0	1.0	2.3	7.0	0.3	1.5
		Norway spruce	3.8	0.8	1.6	5.3	0.2	0.9

The bioconcentration factor (BCF) in the test areas reaches the highest values for Cd, Cu, and Ni for both the moss and the Norway spruce. For both plant species, the factor values increase with the altitude of the terrain. In the case of Cd and Ni for the moss and the Norway spruce, the values of the BCF are higher in the Morskie Oko test area compared to the Kasprowy Wierch test area.

4. Discussion

The literature provides ranges of the natural content of metals in plants [4], and they are as follows: 0.05–0.2 µg Cd/g; 0.1–0.5 µg Cr/g; 5–30 µg Cu/g; 0.1–5.0 µg Ni/g; 5–10 µg Pb/g; 27–150 µg Zn/g d.m. However, metal concentrations in the following ranges are considered to be values which are toxic and unfavourable for plant development: 5–30 µg Cd/g; 5–30 µg Cr/g; 20–100 µg Cu/g; 10–100 µg Ni/g; 30–300 µg Pb/g; 100–400 µg Zn/g d.m. On the basis of the conducted research, it was found that the content of Cu and Zn in plants is within the natural ranges. On the other hand, the content of Cd, Cr, Ni, and Pb in both plant species exceeded the natural values, and indicated anthropogenic pollution in selected research areas.

The content of metals in the moss and in the Norway spruce in the test area of Kasprowy Wierch (KW) and Morskie Oko (MO) was compared to the content of metals in plants in mountain areas. It turned out that they were higher than the content of Cd, Cu, Cr, Ni, Pb, and Zn in the *Pleurozium schreberi* moss in the Alps [31], and for Cd, Cu, Pb, and Zn concentrations in spruce needles in the Tatra National Park, as stated by Staszewski et al. [14]. The contents of all determined metals in both plant species collected in the TNP compared to the content of metals in the moss, *Pleurozium schreberi*, in the states of Eastern Europe were among the highest values [54]. The contents of Cr, Cu, Ni, and Pb in the moss and the Norway spruce were the highest among the values found in Europe, and comparable with the values recorded in the moss, *Pleurozium schreberi*, in Ukraine, Bulgaria, Romania, Hungary, the Czech Republic, and Slovakia. The content of Cd in the tested TNP plants was higher than the content in the *Pleurozium schreberi* moss found in Lithuania, Croatia, Germany, Austria, and Hungary, and similar to the content of Cd for Russia, Romania, Belarus, the Czech Republic, and Slovakia. Comparing the Zn content in the moss and the Norway spruce in the tested areas of the TNP with the content of this metal in other European countries, it can be seen that the concentrations found were average and similar to those obtained in Germany, Austria, Belgium, the Netherlands, and Switzerland [54]. The content of Cd and Cr in the moss and the Norway spruce found in the study is approximately four times higher than the content of these metals in the *Pleurozium schreberi* in other Polish national parks. The content of Cu is about twice as high in the Tatra National Park than in other national parks in Poland. Only the content of Pb and Zn in the Norway spruce in the Tatra National Park was similar to the concentration of these metals in other Polish national parks. In the case of the content of Pb and Zn in moss in the Tatra National Park, the concentrations were approximately twice as high as in other Polish national parks [55]. The content of Cu in the tested plants was similar to the content of this metal in the *Pleurozium schreberi* and *Hylocomium splendens* mosses in the Słowiński National Park (Poland) [56], however, the content of Cu in the tested samples in the TNP was over ten times higher than the content of this metal in the mosses of the Słowiński National Park. This may indicate the negative impact of human activity on the natural environment. In mountainous areas, the content of heavy metal in plants is affected by long-range emissions. Traffic and industrial pollutants from areas with increased emissions are transported over long distances, up to several hundred kilometres [31,57]. The transport of pollutants is consistent with the prevailing wind directions. For the TNP area, the dominant wind direction is south-west. Dusts containing heavy metals are transported from this direction, i.e., from industrial areas (Silesia, Poland), as well as from the Czech Republic and Slovakia.

Long-range emissions contribute to the high concentrations of metals in the plants of the protected area. Moreover, the high metal content in the plants in the higher parts of the mountains is also affected by high wind velocity and a large amount of rainfall [58]. By determining the metal content in plants in the test areas, the authors found an increase in the presence of metals with an increase in altitude. The increase in the content of metals concerned all the metals tested, but in the case of the Kasprowy Wierch area, it was higher than in the Lake Morskie Oko area. Comparing both tested plant species, it turned out that the difference in the metal content between the sampling points located at the lowest and highest altitudes was the greatest in the case of the Norway spruce. In the case of the moss, there was also a difference in the content of metals, but not as significant as for the Norway spruce.

Similar to the authors of the present study, an increase in the content of metals in plants along with an increase in altitude was found in the research by: Shetekauri et al. [29] in the western Caucasus Mountains for As, Cd, Ti, W in mosses; Zechmeister [31] in the Alps for As, Pb, Zn, and V in the *Pleurozium schreberi* and *Hylocomium splendens*; and Šoltés [32] for the content of Pb in the *Sphagnum girgensohnii* in the Tatra Mountains in Slovakia.

Comparing the accumulation of metals in the two studied species of plants, it can be seen that the *Pleurozium schreberi* moss accumulates greater amounts of metals compared to the *Picea abies* Norway spruce. The higher accumulation of metals in the case of the moss results from its morphological structure and the ability to accumulate pollutants. Moreover, the moss accumulated pollutants longer than the Norway spruce, because in the case of the moss, it was the green parts of the plant (stem and leaves), estimated to be about 3 years old, that were sampled for the analysis, and in the case of the Norway spruce, it was 2-year-old needles. This gives a longer accumulation time of pollutants. The analysed plant species occur in specific areas with characteristic natural conditions. Their habitat requirements are strictly defined. The moss, *Pleurozium schreberi,* is a species widespread in Poland and Europe. However, the Norway spruce, *Picea abies,* is found mainly in mountainous areas up to a certain altitude (i.e., up to about 1600–1700 m above sea level). The structure of the moss and the Norway spruce is completely different, which definitely affects the accumulation capacity of both these plant species. Moreover, it is considered that the metal content in the moss is directly proportional to the emissions of pollutants in the air. The Norway spruce, as a vascular plant, has a very well-developed root system, and some of the metals accumulated in the plant are certainly also the result of absorbing them from the soil.

On the basis of the conducted research, the authors showed that in the protected area with special natural values, which is the TNP, we are dealing with long-range emissions. These emissions contribute to the increased content of Cd, Cr, Cu, Ni, Pb, and Zn in the tested plants in the TNP. In addition, the research carried out showed that the areas located higher (at higher altitudes) may be characterized by a greater content of metals in plants than the areas located lower (at lower altitudes).

The obtained research results can be extrapolated to other mountain areas with similar natural conditions (relief, air condition, climate, vegetation) and geographic conditions (location, vicinity of urbanized areas, course of communication routes), such as: Mt. Mala Fatra; Mt. Wielka Fatra; the Nizke Tatry Mountains (Slovakia); the Făgăraș Mountains (Romania); and selected fragments of the Alps (Europe), the Pyrenees (Europe), and the Rocky Mountains (North America).

5. Conclusions

The conducted research shows that the content of heavy metals in plants changes with altitude, and that *Pleurozium schreberi* and *Picea abies* can be good bio-monitors in mountainous areas. Both the moss and the Norway spruce at the highest sampling points showed higher metal content than at points located lower (lower altitude). The bioconcentration factors for the two plant species tested, in particular for Cd, Cu, and Ni, indicated the accumulation capacity of the moss and the Norway spruce. Mountainous areas are extremely exposed to the impact of long-range emissions and the influx of pollutants in line with the prevailing wind direction. It is worth mentioning that the phenomenon of transporting dusts over long distances results from the fact that important natural protected areas, such as the Tatra National Park, are exposed to excessive environmental pollution, including the accumulation of heavy metals in plants.

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