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Polytrichum formosum and *Vaccinium myrtillus* as Phytoindicators of Pollutants from Long-Range Emissions of Environmentally Important Protected Areas (The Tatra National Park, the Central Western Carpathians, Poland)

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Abstract

The study determined the influence of altitude on the content of heavy metals in selected plant species of the Tatra National Park (TNP). The metals (Cd, Cr, Cu, Ni, Pb, and Zn) were identified in two species of plants, i.e., in the moss (*Polytrichum formosum* Hedw.) and in the blueberry leaves (*Vaccinium myrtillus* L.). Plant samples were collected in two test areas every 100 meters of altitude of the area, starting from 1,000 m above sea level in the Lake Morskie Oko test area and from 1,100 m above sea level in the Kasprowy Wierch test area, and ending at 1,400 m above sea level for Lake Morskie Oko and 1,550 m above sea level 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, etc.). The conducted research shows that the content of heavy metals in the studied species of plants increases with the altitude above sea level. Both *P. formosum* Hedw and *V. myrtillus* L. can be good phytoindicators in mountainous areas. In the tested plant species, the contents of heavy metals were also found to be higher than the natural contents, which is most likely related to long-range emission. Long-distance transport of pollutants causes that important natural protected areas, such as the Tatra National Park, are exposed to excessive pollution, including the accumulation of heavy metals in plants.

Keywords: heavy metals, plants, mountains, *P. formosum* moss, *V. myrtillus* blueberry, the Tatras, protected areas

1. Introduction

There are numerous places of natural value in the world. They are often protected areas, such as national parks or nature reserves. However, it should be remembered that such areas are not free from the influence of human activities. In protected areas, humans do not have a direct negative impact on the natural environment, but industrial and automotive emissions reach these areas. These are the so-called long-range emissions, as a result of which fine dust particles are transported over distances of several hundred kilometers [1–3]. Dust particles contain heavy metals, the spreading of which over long distances is related to the long duration of dust pollution in the atmosphere. The length of time during which dust particles remain in the atmosphere depends on the particle size, terrain configuration, and meteorological conditions. Low pressure, strong wind, significant cloud cover, and high precipitation contribute to the spreading of pollutants over long distances [2]. The length of time during which heavy metals remain in the environment differs for individual metals. Lead and cadmium are metals that remain in the atmosphere for a long time and are characterized by the very small diameter of their particles. They are easily transported over long distances and, therefore, contaminate the environment on a global scale [2, 3].

As a result of the transport of pollutants over long distances, areas considered to be of natural value and protected may have a problem with an increased amount of metals in 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 regime of all forms of nature protection in Poland. Together with the Slovak part of the Tatra National Park (Tatranský národný park), it constitutes 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 has continued to this day. The small area of the national park (211 km²) 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 geographic location of the Tatra Mountains in Europe, which is influenced by, among other factors, transitional climate and overlapping ranges of various flora and fauna. Although human pressure on the natural environment of this area concerns the in situ impacts related to tourism, the entire area of the national park is affected by external influences.

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, and chemical, defense, electrical, and electronic industries, as well as by the production of aluminum, nickel, and copper. These industries are a source of heavy metals in the natural environment.

The metals particularly dangerous to living organisms include cadmium, chromium, copper, nickel, lead, and zinc. Currently, the content of these metals in soils and plants is higher than their natural content [4, 5]. The excessive amount of the mentioned heavy metals in the natural environment results from industrial activity and motorisation, and leads to irreversible changes in ecosystems [6, 7].

The phytoindication method is commonly used in environmental monitoring. Technical monitoring is reduced to the direct measurement of pollutants, while biomonitoring supplements technical monitoring and can be carried out within any number of stations. It provides direct information on the level of soil and plant contamination. In this study, the monitoring of contamination with metals was based on two plant species commonly found in the TNP (the European blueberry *V. myrtillus* L. and the moss *P. formosum* Hedw.).

Species recommended as bioindicators (phytoindicators) should meet, inter alia, the following criteria:

- show strong or selective accumulative properties,
- occur in large populations in various habitats of the natural environment [4].

The selection of indicator plants was based primarily on the prevalence of given species in the Tatra National Park, followed by the criterion of their ability to accumulate heavy metals. Literature data [8–11] as well as our own observations [12, 13] have provided some indication of the heavy metal accumulation capacity of selected plant species. The moss species *Pleurozium schreberi*, recommended in the European monitoring programme, has been abandoned owing to its wide geographical range. Despite the fact that it is a very good indicator of environmental pollution, it nevertheless occurred within a too small number of sites in the studied mountainous area. In particular, it rarely appeared within dense spruce stands. On the other hand, samples of the moss *P. formosum* Hedw. as one of the most widespread species in the forest areas of the Tatra National Park were collected. The samples of the plant material were collected at the end of September, that is at the end of the growing season in the mountains. Markert and Weckert [14] provide some clue as to the date of moss sampling. They state that the heavy metal content in the *P. formosum* Hedw. is subject to significant seasonal fluctuations, and this variation is often more important than the variation between sites. Therefore, in order to obtain comparable results, it is recommended that moss is sampled for biomonitoring purposes in the same period of time, preferably at the end of the plant growing season, i.e. at the turn of September and October.

The main objective of the study is to determine the content of heavy metals in two plant species depending on the absolute altitude in protected mountain areas.

2. Materials and methods

2.1 Study area

The research area is located in the Tatra National Park (**Figure 1**). The park covers an area of 211.64 km², of which 149.84 km² is under strict protection, 34.69 km² is under active protection, and 27.14 km² under landscape protection. In 2019, the TNP was visited by 3.9 million people. The Tatras are the only alpine mountains in Poland, where mainly alpine relief is protected, as well as valuable species of plants and animals (including endemics and relics). The research area is located in the Polish part of the Central Western Carpathians, in the northern part of the Tatra Range macroregion [15] and it is the highest part of the entire Carpathians. The specificity of this area is the complex geological structure [16–18], land relief heterogeneity (fluvial-denudation, karst, and glacial) [19–21], climatic conditions changing with the increase in altitude above sea level (air temperature, total precipitation, etc.). The specificity of the climate of the Tatra Range is determined by the incidence of different air masses. Arctic maritime air masses (PPm) have the largest share in the formation of weather, i.e., 65% of days a year, while continental polar air masses (PPk) approximately 20% of days a year [22, 23]. The above elements determine the specificity of water circulation (spatially diversified possibility of water retention, the volume of runoff, water chemistry, etc.). The soil cover of the Tatra Mountains is strongly related to, among other features, their geological substrate, morphogenetic processes, and climatic conditions, and its

Figure 1.
Location of the study area on the background of the map of Poland and Tatra National Parks.

characteristic feature is openwork, as well as poorly developed soils (i.e., initial soils) [24]. All the physico-geographical zones, characteristic of high mountain areas, have developed in the Tatra Mountains [25]. Two test areas in the Tatra National Park in Poland, on the northern slope of the Tatra Mountains, were selected for the study. These areas were selected owing to the diversity of the natural environment, including the physico-geographical location, landscape zone, and geological structure. The test areas were given working names—Kasprowy Wierch (KW) and Morskie Oko (MO).

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 point 5) [26], 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 0–6°C [27], the annual total of precipitation ranges from 800 mm to 1,800 mm, and the length of the snow cover deposition ranges from 100 to 200 days a year [28]. The soil cover is varied and dominated by the following soils:

Sample no.	Altitude [m asl]	Geographical coordinates	Dominant area exposure	Terrain slope grade	Land cover features	Geological structure [17, 18]	Physico-geographical mesoregion [15]
1	1100	N49°15.572' E19°59.322'	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	N49°15.424' E19°59.645'	N	30°-40°	Coniferous forest, spruce forest	Dolomites, limestones, siltstones, and breccia (Lower Triassic)	The Reglowe Tatras
3	1300	N49°15.254' E19°59.681'	W	20°-30°	Glade (area covered with grasses, sedges, herbaceous plants)	Dolomites, limestones, siltstones, and breccia (Lower Triassic)	The Reglowe Tatras
4	1400	N49°15.252' E19°59.908'	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	N49°14.497' E20°00.097'	N	0°-10°	Glade (area covered with grasses, sedges, herbaceous plants)	Boulders, moraine rock debris, clayey (Pleistocene)	Western Tatras
6	1000	N49°15.065' E20°05.898'	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
7	1100	N49°13.984' E20°05.524'	NE	20°-30°	Coniferous forest, spruce forest	Granodiorites and tonalities, equal grained, gray (Carbon)	High Tatras
8	1200	N49°13.270' E20°05.647'	NE	0°-10°	Young Norway spruce stand	Boulders, moraine rock debris, clayey (Pleistocene)	High Tatras
9	1300	N49°12.893' E20°04.867'	NE	10°-20°	Coniferous forest, spruce forest	Boulders, rock debris and silts of dump and alluvial cones (Pleistocene–Holocene)	High Tatras
10	1400	N49°12.021' E20°04.115'	E	10°-20°	Coniferous forest, spruce forest	Boulders, rock debris and silts of dump and alluvial cones (Pleistocene–Holocene)	High Tatras

Table 1.
Characteristics of sampling points in the Kasprowy Wierch (KW) test area - samples No. 1–5, and the Lake Morskie Oko (MO) test area - samples No. 6–10.

Fluvisols, Rendzic Leptosols, Folic Rendzic Leptosols, Cambic Rendzic Leptosols, Haplic Cambisols (Eutric), Haplic Podzols (Skeletal), Entic Podzols, Leptic Podzols, and Folic Leptosols [29].

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 1**). 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 2–4°C [27], the annual total of precipitation ranges from 1,000 mm to 1,400 mm, and the length of snow cover deposition ranges from 120 to 160 days a year [28]. The dominant soils in this part are, among others: Haplic Podzols (Skeletal), Haplic Cambisols (Dystric, Skeletal), Lithic Leptosols, and Regosols (Hyperskeletal) [29].

2.2 Sampling and analysis

2.2.1 Sampling

Plant samples (two species: the moss *Polytrichum formosum* Hedw. (green parts) and the European blueberry *Vaccinium myrtillus* L. (leaves) were sampled in the area of the Tatra National Park, from the Kasprowy Wierch (KW) test area and the Lake Morskie Oko (MO) test area. The samples were taken every 100 meters of altitude, starting from an altitude of: 1,100 m above sea level for KW and from 1,000 m above sea level for MO. Owing to the limited range of occurrence at higher altitudes, the plants were sampled up to 1,550 m above sea level for KW. The geographical coordinates of the sampling sites and the designations adopted are presented in **Table 1**.

2.2.2 Chemical analysis

According to the suggestions of the following authors: Maňkowska et al. [30] and Sawidis et al. [31] regarding the sample preparation procedure, the plant material was left unwashed. The samples were dried in an electric drier at a temperature of 400°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. Portions of 1 g dry weight material were placed in Teflon vessels. 5 cm³ of 65% HNO₃ and 3 cm³ of 36% H₂O₂ were added to each vessel. The mixture was mineralized in a Berghof Speed Wave microwave at 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 cm³ and filtered through a hard paper filter. The final solutions were analyzed 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 dm) were as follows: for Cd: 0.01, Cr: 0.1, Cu: 0.01, Ni: 0.1, Pb: 0.01 and Zn: 0.1.

2.2.3 Statistical study

Statistical analyses were performed using the IBM SPSS program. 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 tests (MO Lake Morskie

Oko and KW Kasprowy Wierch). In order to assess the compliance of the distributions with the normal distribution, the Kolmogorov-Smirnow tests were performed. As the distributions differed from the normal distribution, non-parametric methods were used for further analyses. Spearman's coefficients were used to assess the relationship between the variables. In order to assess the significance of differences between the two groups, descriptive statistics were calculated and Mann-Whitney tests were performed. $P = 0.05$ was assumed as the limit of statistical significance below which the results were considered significant.

3. Results

The mean content of heavy metals in plants is presented in **Table 2**.

On the basis of the mean metal content in plants, calculated from all the collected samples, the following series of heavy metal concentrations were obtained:

$Zn > Pb > Cr > Cu > Ni > Cd$ for the moss *P. formosum* Hedw.

$Zn > Cr > Pb > Cu > Ni > Cd$ for the European blueberry *V. myrtillus* L.

The metal concentration series look similar for both plant species with the difference in lead and chromium content, where the moss *P. formosum* Hedw. accumulated more lead than chromium, while in the case of the European blueberry

Species	Study area	Altitude [m asl]	Heavy metals [µg/g d.m.]					
			Cd	Cr	Cu	Ni	Pb	Zn
Moss <i>P. formosum</i> Hedw.	MO	1000	2.1	24.9	18.7	16.7	30.2	70.8
		1100	2.1	24.8	18.8	17.0	30.8	70.6
		1200	2.3	25.1	19.5	17.7	32.3	71.9
		1300	2.4	26.6	20.3	18.1	33.7	74.2
		1400	2.5	27.9	20.9	18.8	35.5	76.9
	KW	1100	1.6	15.9	12.6	10.2	17.6	45.6
		1200	1.8	16.4	12.7	10.3	18.0	46.4
		1300	1.8	17.4	13.1	10.8	18.6	47.7
		1400	2.0	17.6	13.6	11.2	19.3	49.4
		1550	2.2	18.3	14.2	11.7	19.9	50.7
European blueberry <i>V. myrtillus</i> L.	MO	1000	1.5	17.0	11.8	12.2	15.9	42.4
		1100	1.6	17.3	12.2	12.3	15.9	43.8
		1200	1.6	17.7	12.5	12.7	16.6	44.5
		1300	1.7	18.7	13.2	13.2	17.3	45.6
		1400	1.8	19.7	14.1	14.3	18.2	48.1
	KW	1100	1.1	11.1	9.3	8.7	10.0	26.1
		1200	1.2	11.6	9.8	9.3	10.6	26.2
		1300	1.3	12.2	10.2	9.5	11.0	27.6
		1400	1.4	12.2	10.9	10.1	11.5	28.0
		1550	1.5	13.2	11.4	11.0	12.1	28.6

Table 2.
Mean concentrations of heavy metals in the moss species *Polytrichum formosum* Hedw. And in the European blueberry *Vaccinium myrtillus* L.

V. myrtillus L. the opposite was true (the European blueberry accumulated more chromium than lead). For both plant species, the last three metals presented in the series were similarly accumulated (higher amounts of copper compared to nickel and cadmium, and nickel compared to cadmium).

Comparing the mean metal contents in both plant species, it can be seen that the moss *P. formosum* Hedw. was characterized by a greater accumulation of metals (2.1 µg Cd/g dm, 21.5 µg Cr/g dm, 16.4 µg Cu/g dm, 14.2 µg Ni/g dm, 25.6 µg Pb/g dm, 60.4 µg Zn/g dm) compared to the European blueberry *V. myrtillus* L. (1.5 µg Cd/g dm, 15.1 µg Cr/g dm, 11.5 µg Cu/g dm, 11.3 µg Ni/g dm, 13.9 µg Pb/g dm, 36.1 µg Zn/g dm). Both the moss *P. formosum* Hedw. and the European blueberry *V. myrtillus* L. accumulated zinc in the highest amounts (60.4 and 36.1 µg Zn/g dm for the moss and the European blueberry, respectively), and cadmium in the smallest amounts (2.1 and 1.5 µg Cd/g dm).

Analyzing the data in **Table 2**, it can also be concluded that both plant species accumulated greater amounts of metals in the Morskie Oko test area than in the Kasprowy Wierch test area. For the same absolute altitudes, the content of heavy metals in plants, in particular of lead (1.8), nickel (1.7) and zinc (1.6) for the moss and of zinc (1.7) and lead (1.6) for the European blueberry, were almost twice as high in the Morskie Oko test area (**Table 3**). Smaller differences in the metal content in plants between the test areas were observed for cadmium, copper, and chromium.

The absolute altitude coefficient was calculated as the quotient of the heavy metal content in plants in the test areas for a given altitude (e.g. for Cd 1,100 m asl, the coefficient is the quotient of the Cd content in the MO test area to the Cd content in the KW test area for an altitude of 1,100 m asl).

The values of the heavy metals plant enrichment factor metals depending on the absolute height were determined as the second factor. This coefficient was calculated as the quotient of the heavy metal content for the lowest altitude to the highest metal content in the plants for a given test area, e.g. Cd 1,400 m asl to Cd 1,000 m asl for MO). The values of the enrichment coefficient are presented in **Table 4**.

The enrichment factors, calculated for all absolute heights, reach a value greater than or equal to 1.0. This indicates the presence of heavy metals accumulation in plants that is lower or similar to the highest altitude tested for a given test area (1,400 m asl for MO and 1,550 m asl for KW). The increase in the accumulation of metals in plants along with altitude was observed for the two plant species and for

Species	Study area	Altitude [m a.s.l.]	Absolute altitude coefficient					
			Cd	Cr	Cu	Ni	Pb	Zn
Moss <i>Polytrichum formosum</i> Hedw.	MO/KW	1100	1.3	1.6	1.5	1.7	1.8	1.5
		1200	1.3	1.5	1.5	1.7	1.8	1.6
		1300	1.3	1.5	1.5	1.7	1.8	1.6
		1400	1.3	1.6	1.5	1.7	1.8	1.6
European blueberry <i>Vaccinium myrtillus</i> L.	MO / KW	1100	1.4	1.6	1.3	1.4	1.6	1.7
		1200	1.4	1.5	1.3	1.4	1.6	1.7
		1300	1.3	1.5	1.3	1.4	1.6	1.7
		1400	1.3	1.6	1.3	1.4	1.6	1.7

Table 3.
Absolute altitude coefficients.

Species	Study area	Altitude [m a.s.l.]	Enrichment factor					
			Cd	Cr	Cu	Ni	Pb	Zn
Moss <i>Polytrichum formosum</i> Hedw.	MO	1400/1000	1.2	1.1	1.1	1.1	1.2	1.1
		1400/1100	1.2	1.1	1.1	1.1	1.2	1.1
		1400/1200	1.1	1.1	1.1	1.1	1.1	1.1
		1400/1300	1.0	1.0	1.0	1.0	1.1	1.0
	KW	1550/1100	1.3	1.2	1.1	1.1	1.1	1.1
		1550/1200	1.2	1.1	1.1	1.1	1.1	1.1
		1550/1300	1.2	1.0	1.1	1.1	1.1	1.1
		1550/1400	1.1	1.0	1.0	1.0	1.0	1.0
European blueberry <i>Vaccinium myrtillus</i> L.	MO	1400/1000	1.2	1.2	1.2	1.2	1.1	1.1
		1400/1100	1.1	1.1	1.2	1.2	1.1	1.1
		1400/1200	1.1	1.1	1.1	1.1	1.1	1.1
		1400/1300	1.1	1.1	1.1	1.1	1.1	1.1
	KW	1550/1100	1.3	1.2	1.2	1.3	1.2	1.1
		1550/1200	1.3	1.1	1.2	1.2	1.1	1.1
		1550/1300	1.2	1.1	1.1	1.2	1.1	1.0
		1550/1400	1.1	1.1	1.0	1.1	1.1	1.0

Table 4.
Heavy metals plant enrichment factors.

Species	Study area	Altitude [m asl]	Plant accumulation coefficient					
			Cd	Cr	Cu	Ni	Pb	Zn
Moss <i>Polytrichum formosum</i> Hedw./ European blueberry <i>Vaccinium myrtillus</i> L.	MO	1000	1.4	1.5	1.6	1.4	1.9	1.7
		1100	1.3	1.4	1.5	1.4	1.9	1.6
		1200	1.4	1.4	1.6	1.4	1.9	1.6
		1300	1.4	1.4	1.5	1.4	1.9	1.6
		1400	1.4	1.4	1.5	1.3	1.9	1.6
	KW	1100	1.5	1.4	1.4	1.2	1.8	1.7
		1200	1.5	1.4	1.3	1.1	1.7	1.8
		1300	1.4	1.4	1.3	1.1	1.7	1.7
		1400	1.4	1.4	1.3	1.1	1.7	1.8
		1550	1.5	1.4	1.2	1.1	1.6	1.8

Table 5.
Heavy metals plant accumulation coefficients.

each tested element. The increase in the content of all metals in plants occurs for two test areas.

Table 5 summarizes the plant accumulation coefficients calculated as the ratio of the content of a given metal in the moss to the content of the same metal in the European blueberry for the same absolute height and the same test area.

On the basis of the calculated accumulation coefficients it is clearly visible that the moss *P. formosum* Hedw. has a greater ability to absorb and accumulate heavy metals than the European blueberry *V. myrtillus* L. For each of the heavy metals determined, it was the moss that accumulated greater amounts. The largest differences in the accumulation of metals between the tested plants were observed for lead (a coefficient of 1.6–1.9) and zinc (a coefficient of 1.6–1.8), and the lowest for nickel (a coefficient of 1.1–1.4). The coefficients obtained for cadmium, chromium, and copper were similar and fell within the range of: 1.2–1.6.

Based on the Kolmogorov–Smirnov test (**Table 6**), distributions close to normal were recorded only for the content of Cd in the moss and Cu in the European blueberry. The other variables had distributions deviating from the normal distribution. Therefore, in order to determine the similarity, non-parametric tests were used in further analysis.

A statistically significant positive relationship was found between the Cd content and altitude in the moss ($\rho = 0.227$; $p = 0.023$). There was no statistically significant relationship between the altitude and the content of other elements (**Table 7**).

Species		Altitude	Cd	Cr	Cu	Ni	Pb	Zn
Moss	N	100	100	100	100	100	100	100
	Test statistics	0.135	0.085	0.110	0.136	0.096	0.174	0.219
	p	0.000	0.070	0.005	0.000	0.025	0.000	0.000
European blueberry	N	100	100	100	100	100	100	100
	Test statistics	0.135	0.142	0.135	0.069	0.096	0.212	0.109
	p	0.000	0.000	0.000	0.200	0.024	0.000	0.005

Table 6.
Assessment of compliance with the normal distribution - Kolmogorov–Smirnov test for one sample.

Species	Spearman's rho	Cd	Cr	Cu	Ni	Pb	Zn
Moss	rho	0.227	−0.081	−0.064	−0.047	−0.069	−0.057
	p	0.023	0.422	0.525	0.642	0.493	0.573
European blueberry	rho	0.132	−0.002	0.085	0.088	0.065	−0.076
	p	0.190	0.988	0.403	0.386	0.521	0.455

Table 7.
Assessment of the relationship between the variables (metal-altitude) - Spearman's coefficients.

Species		Cd	Cr	Cu	Ni	Pb	Zn
Moss	Z	−4.798	−6.571	−5.726	−5.330	−6.132	−6.250
	p	0.000	0.000	0.000	0.000	0.000	0.000
European blueberry	Z	−4.962	−5.057	−2.479	−3.200	−2.775	−5.291
	p	0.000	0.000	0.013	0.001	0.006	0.000

Table 8.
Mann–Whitney test results for species.

There were statistically significant differences between the locations for both the moss and the European blueberry. Each element had a lower concentration in the measurements at Kasprowy Wierch than at Lake Morskie Oko (**Table 8** and **Figure 2**).

Figure 2.
Assessment of the relationships between variables (metal-altitude) - scatter dot plots for Cd, Cr, Cu, Ni, Pb and Zn.

Location		Cd	Cr	Cu	Ni	Pb	Zn
Morskie Oko	Z	−7.036	−5.106	−5.954	−3.709	−5.512	−5.653
	p	0.000	0.000	0.000	0.000	0.000	0.000
Kasprowy Wierch	Z	−6.390	−4.472	−2.096	−1.759	−5.937	−6.067
	p	0.000	0.000	0.036	0.079	0.000	0.000

Table 9.
Mann–Whitney test results for location.

Considering the differences in the accumulation of metals by the moss and the European blueberry, statistically significant differences were obtained for each location. Moss accumulated more of all the tested metals compared to the European blueberry. The only exception was the Ni content in the Kasprowy Wierch test area, for which no statistically significant differences were found in accumulation by the moss and the European blueberry (**Table 9**).

4. Discussion

The contents of heavy metals in the tested plants were compared to the natural and toxic contents of metals in plants provided by Kabata-Pendias and Pendias [4], which amount to, respectively: 0.05–0.2 and 5–30 µg Cd/g, 0.1–0.5 and 5–30 µg Cr/g, 5–30 and 20–100 µg Cu/g, 0.1–5.0 and 10–100 µg Ni/g, 5–10 and 30–300 µg Pb/g, 27–150 and 100–400 µg Zn/g dm. It was found that the contents of copper and zinc in plants are within the natural ranges. However, the contents of cadmium, chromium, nickel, and lead in both plant species for both test areas exceeded the natural values. They were exceeded by several, or several dozen times: more than 3 times for lead and nickel, several times for cadmium, and the highest - several dozen times for chromium. Such high exceedances in the content of heavy metals in the tested plant species in relation to the natural value of these metals in plants indicate the anthropogenic pollution of the selected research areas. Owing to their location (TNP) and great natural value, the research areas should be free from anthropogenic pollutants, however, long-range emission in this case has a large impact on the quality of the natural environment. It should be noted that in mountain areas, the content in plants of heavy metals is influenced by long-range emissions. Communication and industrial pollutants from areas with increased emissions are transported over long distances, even several dozen to several hundred kilometers. The transport of pollutants is related to the prevailing wind directions. For the TNP area, the dominant wind direction is to the south-west. Dusts containing heavy metal are transported from this direction, i.e., from industrial areas in Poland (Silesia) as well as from the Czech Republic and Slovakia. Long-range emissions result in such a high accumulation of metals in plants of the protected area [32–34]. Additionally, the high accumulation of metals in the higher parts of the mountains is also influenced by high wind velocity and a large amount of precipitation [34].

Determining the content of heavy metals in the plants in the tested test areas, an increase in metals was found with increasing altitude. The increase in the metal content concerned all of the tested metals and two plant species. However, this increase was slight and similar for the Kasprowy Wierch and Morskie Oko test areas. Similarly to the author of the present study, an increase in the content of metals in plants along with an increase in altitude was found in their research by Shetekauri et al. [35] in the western Caucasus Mountains for As, Cd, Ti, W in mosses, Sahin et al. [36] in the Kumalar Mountains for Cu, Zn in herbaceous plants, Zechmeister [32] in the Alps for As, Pb, Zn, and V in the *P. schreberi* and *Hylocomium splendens*, Šoltés [37] for the content of Pb in the *Sphagnum girgensohnii* in the Tatra Mountains in Slovakia, Samecka-Cymerman et al. [38] in the Tatra National Park for the content of Cd, Ni, and Zn in the *Athyrium distentifoliu*, Panek [39] in the Poland's Carpathian region for Pb in *P. formosum* and Kuklová et al. [40]. Kuklová et al. [40] found an increase in the content of Cu and Zn in three plant species (the *Dryopteris filix-mas*, *Rubus idaeus*, and *V. myrtillus*) with an increase in altitude. They observed an increase in the Cd content for two plant species: the *D. filix-mas* and the *V. myrtillus*. The re-search was carried out in the Slovak Paradise National Park (Slovenský Raj National Park), Eastern Slovakia, collecting samples

of plants growing at an altitude of 750, 760, 950, 960, 1,000, and 1,110 m above sea level.

Comparing the accumulation of metals in the two tested plant species, it can be seen that the moss *P. formosum* Hedw. has higher amounts of metals compared to the European blueberry *V. myrtillus* L. The higher accumulation of metals in the moss results from its morphological structure and the ability to accumulate pollutants. In addition, the moss accumulated pollutants for longer than the European blueberry, 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 analysis, while in the case of the European blueberry, it was the leaves (about six months old) that were sampled for analysis. This gives several times longer accumulation time of pollutants.

5. Conclusions

In order to determine the content of heavy metals in the natural environment, indicator plants are used, the so-called phytoindicators. These include plant species that can absorb and accumulate toxic substances, such as heavy metals. The conducted research makes it possible to make the conclusion that both plant species showed a high ability to absorb air pollutants, which directly translated into high concentrations of heavy metals in plants. Both the moss *P. formosum* Hedw. as well as the European blueberry *V. myrtillus* L. can be good phytoindicators in mountainous areas. The conducted research showed that both plant species accumulated greater amounts of heavy metals in the Morskie Oko test area compared to the Kasprowy Wierch area.

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