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ORIGINAL RESEARCH ARTICLE

The heavy metal content of soil and shoots of *Vaccinium myrtillus* L. in the Słowiński National Park

Agnieszka Parzych

Pomeranian University in Słupsk, Faculty of Mathematics and Natural Sciences, Institute of Biology and Environmental Protection, Department of Environmental Chemistry, ul. Arciszewskiego 22b, 76-200 Słupsk, Poland.

Tel. +48 59 84 05 347, fax +48 59 84 05 935; e-mail: parzycha1@op.pl

Abstract. The research was carried out in the Słowiński National Park, in an area with 15 research stations in pine coniferous forests situated at locations (1) inaccessible to tourists, (2) most frequently visited by tourists as well as (3) in the vicinity of parking lots. The analysed samples comprised surface generic levels (Ol, Ofh, A), above-ground material (shoots; leaves and stems) and below-ground material (roots) of bilberry (*Vaccinium myrtillus*). The performed analyses showed statistically significant Spearman's correlation coefficients for Zn content in the 'soil – stems:' ($r = -0.44$, $p < 0.05$, $n = 45$) relationship and the 'soil — roots' relationship ($r = -0.52$, $p < 0.05$, $n = 45$). Accordingly, there were significant statistical differences (U Mann-Whitney test) in zinc content in the 'stems – roots' relationship and the 'leaves – roots' relationship. Furthermore, the obtained results reveal an excessive accumulation of Mn in *V. myrtillus*. The content of the investigated heavy metals in *V. myrtillus* shoots decreased in the following order: Mn > Fe > Zn > Cu.

Key words: bilberry, leaves, stems, roots, accumulation of Zn, Fe, Cu, Mn, protected area

1. Introduction

Heavy metals are innate components of the natural environment. The intrinsic content of these elements in the lithosphere shapes the so-called geochemical background and is spatially diversified. In the recent years, the emission due to anthropogenic factors has become a considerable source of heavy metals. These are subject to long-range atmospheric transport as constituents of atmospheric particulate matter (PM10 and PM2.5) or else aerosols, which results in contamination of ecosystems situated quite far from emission sources (Klink et al. 2006; Tainio et al. 2010; Brożek, Zarembski 2011). As natural components of ecosystems, the elements such as zinc (Zn), iron (Fe), manganese (Mn) and copper (Cu) are necessary for appropriate functioning of plants as long as they are available in small amounts. Conversely, excessively high concentrations of these metals are harmful to natural environment. At increased heavy metal contents, ecosystem functioning is disturbed, and this is threatening to plants, animals and humans (Gruca-Królikowska, Wacławek 2006; Malzahn 2009). Heavy metals bioaccumulate in plant and animal tissues, which results in high risk of poisoning with these elements in subsequent links of the trophic chain. Plant uptake of nutrients such as iron, manganese, zinc and copper from soil relies upon plant physiological requirements; nevertheless, it can also be brought about by environmental contamination. The content of metals in plant tissues also depends on their availability in soil as well as on plant species, its growth stage and morphological features. The results of research studies confirm that plants selectively uptake elements from surrounding environment. The nutrients are utilised by plants for own tissue building processes as well as they take part in numerous metabolic pathways. In the world of plants, there exist a distinct tendency to uptake and ac-

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cumulate certain elements (Łaszewska et al. 2007). Plants response in different ways to increased heavy metal concentrations in environment. The specific sensitivity of some plant species to the presence of heavy metals in soil allows for the determination of the degree, range and structure of environmental changes. Amongst plant utilised for bioindication purposes, bilberry low-growing shrubs *Vaccinium myrtillus* L. offer practical application for attaining information on a degree of environment contamination (Reimann et al. 2001; Uhlig, Junttila 2001; Salemaa et al. 2004; Białońska et al. 2007; Kukla, Kuklová 2008; Mróz, Demczuk 2010; Kandziora-Ciupa et al. 2013; Remon et al. 2013). Bilberry *V. myrtillus* grows both on contaminated areas and those free of pollution. It is a domineering species in the majority of pine *Pinus silvestris* and mixed forests growing under temperate climate conditions (Białońska et al. 2007). *Vaccinio myrtilli-Pinetum* habitat constitutes the ecological optimum for bilberry plants, which means that the species shows the highest frequency, density, biomass and productivity under these habitat conditions (Moszyńska 1983; Gugnacka-Fiedor 1994; Gerdol 2004; Zvereva, Kozlov 2005; Parzych, Sobisz 2010). Every year, in nearly all forest communities, *V. myrtillus* puts back into the litter high amounts of potassium, calcium, magnesium as well as manganese and iron contained in fallen leaves and adds to important elements for proper functioning of forest ecosystems. Studies on bioindication include bilberry leaves, stems and roots (Mróz, Demczuk 2010; Kozanecka et al. 2002) as well as fruits ((Demczuk, Garbiec 2009; Pająk, Jasik 2012). Bylińska (1992), Reimann et al. (2001) and Boyd (2007) ranked *V. myrtillus* as plant species with superior abilities to accumulate manganese.

The aim of the present study was to: examine Zn, Fe, Mn and Cu contents in the leaves, stems and roots of bilberry *V. myrtillus* L. growing within the protected area, evaluate metal accumulation capacity across plant parts, assess the effect of heavy metal contents in soil on their concentration in bilberry shoots and appraise contamination extent in the Słowiński National Park.

2. Materials and research methods

2.1. Research area

The study comprised forest areas within the Słowiński National Park (SNP) located within the Łebska Spit (protective zones: Rowy, Łeba, Smołdziński Las). The study area was situated at 17°03′–17°33′ east longitude an 54°37′–54°46′ north latitude (Fig. 1). In the park, there prevail pine forests (71.5%), mixed pine forests (21.4%), mixed forests (1.6%) and unclassified forest (5.5%). The groundcover includes mainly bilberry low-growing shrubs, mosses and lichens. The samples of bilberry aboveground parts (leaves and stems) and roots as well as soil samples from organic and humus horizons (Ol, Ofh and A) were collected in September 2011 from 15 research sites placed within SNP area. The sites were positioned either in places with no access for tourists or within most often visited areas or else in the vicinity of parking lots.

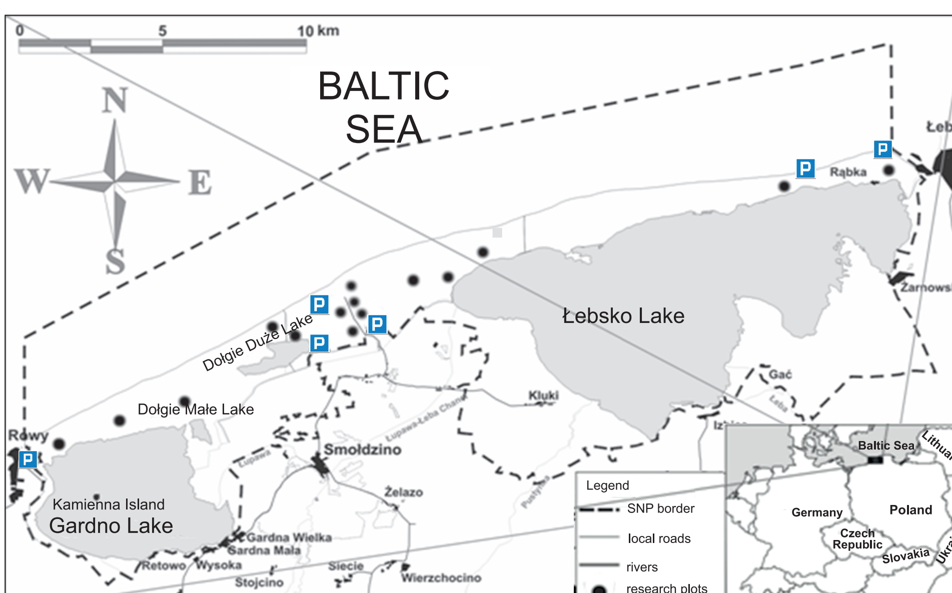

Figure 1. Situation plan of the Słowiński National Park – locations of the study sites and parking

In the samples of organic and humus soil horizons, there were assessed soil active acidity (pH in H_2O), exchangeable soil acidity (1 M·dm^{-3} KCl solution) as well as organic matter content (loss on ignition method using a muffle furnace at 550°C). Plant material was cleaned of soil mineral particles, rinsed in distilled water, divided into above- and underground parts, dried out at 65°C and then homogenised in a blender. The samples of soil and *V. myrtilus* were wet mineralised in a mixture of HNO_3 and 30% H_2O_2 using the closed system. In the obtained solution, the contents of Zn, Fe, Mn and Cu by means of atomic absorption spectroscopy (AAS) with the use of Perkin Elmer Analyst 300 apparatus (Ostrowska et al. 1991) and Merck KGaA standard solutions (1 g/1000 ml) were analysed.

2.2. Analyses of the results

Comparisons of the concentrations of the heavy metals examined in the above- and underground parts of *V. myrtillus* were based on computed mean as well as maximum and minimum values, standard deviations, coefficients of variation (CV) and enrichment factors (*EF*). The normality of distribution of the concentration of Zn, Fe, Mn and Cu in *V. myrtilus* shoots was evaluated using the Shapiro-Wilk test. The non-parametric Mann–Whitney *U*-test was used to compare heavy metal concentrations in the parts of plants examined. All statistical analyses were performed using Statistica 7.1. software.

3. Results and Discussion

The samples of organic and humus soil horizons in the Łebska Spit indicated strongly acidic reaction (Table 1). The organic fermentative humic subhorizon (Ofh) showed the highest active (pH H_2O) and exchangeable acidity (pH KCl) with pH values from 3.1 to 4.1 and from 2.5 to 3.1, respectively. For all 15 research sites, coefficients of variation (CV) of pH (H_2O) and pH (KCl) were from 5% to 8%. Slightly lower acidity, i.e. pH (H_2O) ranging from 3.9 to 5.0, was observed in the humus horizon (A). The largest content of organic matter was found in the litter sub-horizon (Ol), i.e. 91.6–98.5% and then in Ofh (39.0–97.2%), whereas the lowest organic matter content was found in the soil horizon A (0.6–5.9%). With an increasing depth of soil horizon position, organic matter content decreased and varied from 2% to 59%.

The content of the examined heavy metals in upper soil horizons in SNP was differentiated. The largest ammounts of Zn, Fe and Mn were found in the subhorizon Ol, then in Ofh, whereas in A horizon heavy metal contents were the lowest. In case of copper, the highest contents were observed in the subhorizon Ofh. Average concentration of zinc in the soil horizons was: 68.9 mg·kg^{-1} (Ol), 47.0 mg·kg^{-1} (Ofh) and 2.9 mg·kg^{-1} (A), and varied within 15 research sites from 28% to 33% (Table 2). The content of iron showed variation from 35% to 134% within 15 research sites, and on average it was 469.0 mg·kg^{-1} in Ol, 1609.0 mg·kg^{-1} in Ofh and 346.0 mg·kg^{-1} in the horizon A. Average content of manganese was 206.2 mg·kg^{-1} in Ol, 40.0 mg·kg^{-1} in Ofh and 4.2 mg·kg^{-1} in A horizon, whereas variation coefficient for this element took values from 52% to 58%. In the soils tested, there were observed considerably lower contents of copper, i.e. 0.8 mg·kg^{-1} in the subhorizon Ol, 0.9 mg·kg^{-1} in Ofh and 0.09 mg·kg^{-1} in the horizon A. At the same time, Cu concentration in the soil horizons investigated indicated lesser variation as a result of exceptionally low mobility of this element (Kabata-Pendias, Pendias 1999).

The concentration of Zn in the above- and underground *V. myrtillus* shoots showed considerable variation depending on the research site and the plant section analysed. The content of zinc was from 14.9 to 69.4 mg·kg^{-1} in bilberry leaves, from 38.0 to 108.0 mg·kg^{-1} in the stems and from 9.7 to 51.4 mg·kg^{1} in the roots (Fig. 2). The highest Zn

Table 1. pH and organic matter in organic and humus horizons in SNP

Statistical measures	pH (H_2O)			pH (KCl)			Organic matter [%]		
	Ol	Ofh	A	Ol	Ofh	A	Ol	Ofh	A
Average	4.4±0.2	3.8±0.2	4.4±0.3	3.5±0.2	2.8±0.2	3.3±0.2	96.6±0.02	76.9±0.2	3.1±0.01
Minimum	4.1	3.1	3.9	3.0	2.5	2.9	91.6	39.0	0.6
Maximum	4.7	4.1	5.0	3.8	3.1	3.6	98.5	97.2	5.9
Median	4.4	3.9	4.4	3.6	2.8	3.4	97.5	73.8	2.6
CV [%]	5	7	7	8	6	8	2	23	59

CV – coefficient of variation

Table 2. Heavy metals content (mg·kg^{-1}) in organic and humus horizons in SNP

Statistical measures	Zn			Fe			Mn			Cu		
	Ol	Ofh	A	Ol	Ofh	A	Ol	Ofh	A	Ol	Ofh	A
Average	68.9±19.0	47.0±15.4	2.9±1.1	469±505	1609±2161	346±120	206.2±113	40.0±23	4.2±2.1	0.80±0.14	0.90±0.29	0.09±0.03
Minimum	37.1	24.2	1.3	118	478	124	48.7	12.2	1.2	0.50	0.60	0.06
Maximum	101.0	82.0	5.7	2071	8517	568	415.0	86.2	8.3	1.10	1.70	0.19
Median	65.5	42.7	2.7	279	907	381.1	198.9	37.4	3.7	0.8	0.9	0.08
CV [%]	28	33	38	108	134	35	55	58	52	18	31	35

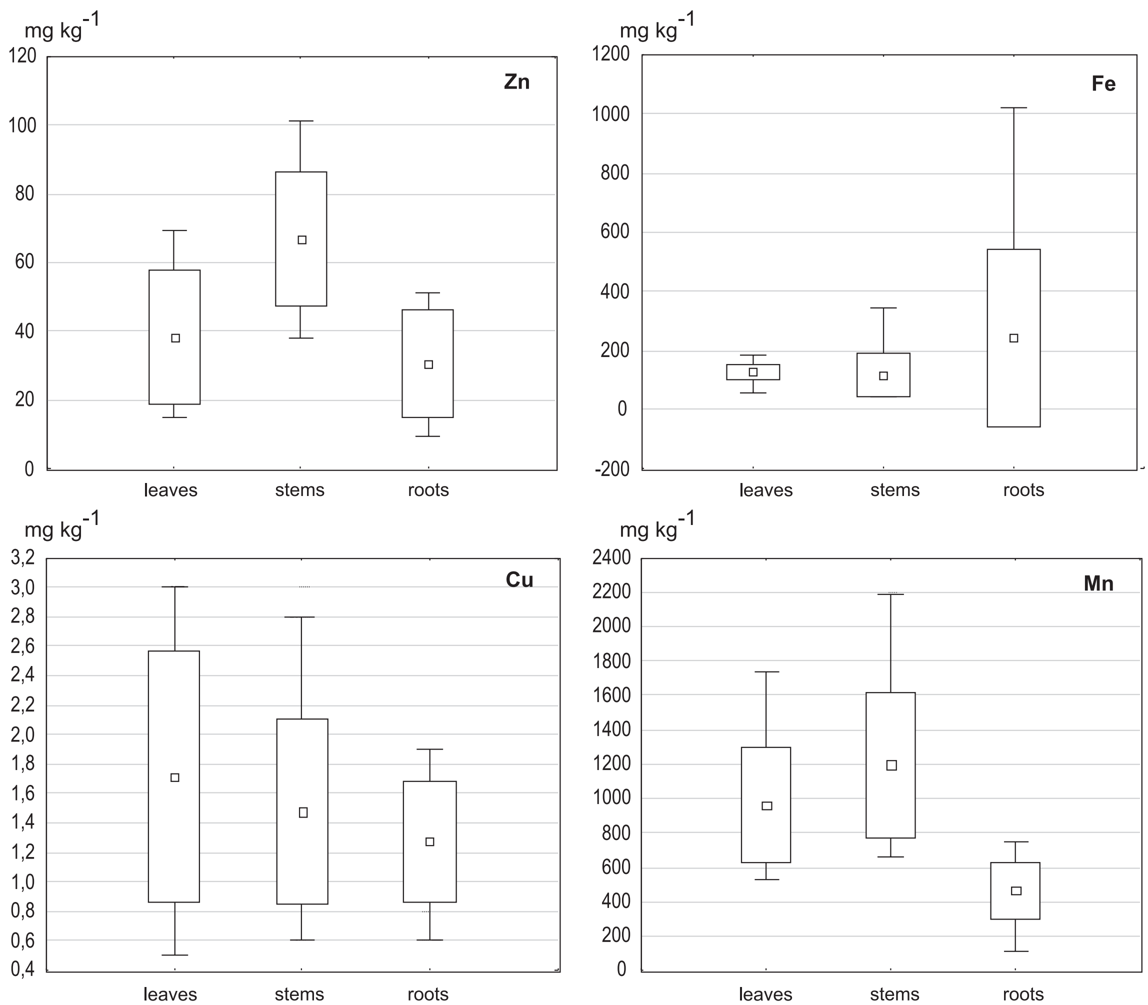

Figure 2. The heavy metal content in the leaves, stems and roots of *Vaccinium myrtillus* in SPN, point (mean), rectangle (standard deviation), whiskers (minimum – maximum)

amounts were observed in the shoots of bilberry growing within the research sites located in the central areas of the Park – those in parking lot vicinity. The concentration of zinc showed the highest values of CV for bilberry roots (51.3%), and the lowest for the stems (29.3 %). Plants uptake Zn in the amounts proportional to its concentration in soil. Several studies showed that the content of zinc in *V. myrtillus* growing on non-contaminated areas regularly amounted to approximately 16.8 mg·kg^{-1} (Mróz, Demczuk 2010) or else 20.0 mg·kg^{-1} (Kozanecka et al. 2002). The content of zinc in the shoots of bilberry growing on contaminated areas is usually higher and amounts to 24.4 mg·kg^{-1} (Pająk, Jasik 2012) or even to 107.8 mg·kg^{-1} (Gworek, Degórski 2000). Leaf Zn concentration ranging from 15 to 30 mg·kg^{-1} usually assures the fulfilment of physiological requirements of the majority of plants. The present study carried out within the territory of the Słowiński National Park shows that Zn is accumulated in higher amounts in bilberry stems when compared with the leaves, which is in line with the results obtained by Kozanecka et al. (2002). Similar to deciduous trees loosing leaves in the fall, bilberry low-growing shrubs accumulate larger amounts of nutrients in their stems and roots when compared with the leaves (Moszyńska 1983; Gugnacka-Fiedor 1994). Low Zn concentration in bilberry shoots reflects low amounts of this element in podzolic soils of the Park, which is derived from poor dune sands (Tobolski et al. 1997) as well as the fact that the plants grow under conditions of relatively clean atmosphere (Brożek, Zarembski 2011)

The concentration of iron in *V. myrtillus* also showed variation depending on the research site and plant part. Leaf Fe concentration was from 57.0 to 182.0 mg.kg^{1}, whereas in the stems it was from 47.0 to 344.0 mg kg^{-1}, and in the roots from 50.0 to 1019.0 mg kg^{-1} (Fig. 2). The highest Fe content was observed at the research sites situated in vicinity of parking lots. Coefficients of variation of Fe concentration were from 22.4% for bilberry leaves to 120.2% for the roots. The highest Fe concentrations were observed in the underground shoots. Similar Fe content in bilberry shoots was found by Mróz and Demczuk (2010) – 120.0–217.0 mg kg^{-1}, and Gworek and Degórski (2000) – 95.0–104.0 mg kg^{-1}. In some bilberry samples collected in SNP, there was observed an increased content of Fe, which can indicate advanced ability to accumulate iron in this plant species. Strongly acidic soil environment in the Park (Table 1) enhances the availability of heavy metals to plants. According to Bylińska (1992), *V. myrtillus* has superior capability to accumulate iron. Within the areas free of contamination, Fe content is usually 74 mg kg^{-1} in bilberry leaves and 62 mg kg^{-1} in the stems (Kozanecka et al. 2002).

The content of copper in bilberry shoots indicated considerable differentiation among the research sites and was from 0.5 to 3.0 mg·kg^{-1} in the leaves, from 0.6 to 2.8 mg·kg^{-1} in the stems and from 0.6 to 1.9 mg·kg^{-1} in the roots. Coefficients of variation of Cu concentration in *V. myrtillus* were on average from 32.0% in the roots to 49.5% in the leaves (Fig. 2). Plant copper shows low mobility and the amount of approximately 2 mg·kg^{-1} is sufficient to cover physiological needs of the majority of plants. The content of copper in plants is usually below 4–5 mg·kg^{-1} and considerably varies depending on plant part, developmental stage as well as plant species and variety. Average Cu content in the aboveground parts of plants is 5–20 mg·kg^{-1} (Kabata-Pendias, Pendias 1999). The results of chemical analyses of *V. myrtillus* shoots carried out by Gworek and Degórski (2000) in several locations in Poland indicate that depending on contamination degree, the concentration of Cu can be from 0.5 to 8.1 mg·kg^{-1}. Earlier chemical studies on *V. myrtillus* aboveground shoots carried out in the fresh pine forests within the Słowinski National Park showed Cu concentration 3.1 mg·kg^{-1} (Parzych et al. 2012). The content of Cu in bilberry plants from the Park is low and not threatening – in contrast, it is sufficient for covering *V. myrtilus* physiological needs.

Fairly different status was observed for manganese contents. These were from 529.2 to 1736.0 mg·kg^{-1} in bilberry leaves, from 658.0 to 2182.0 mg·kg^{-1} in the stems and from 105.9 to 746.3 mg·kg^{-1} in the roots (Fig. 2). The concentration of Mn indicated related variation – from 34.9 to 35.3%, regardless of the part of plant analysed. According to Kabata-Pendias and Pendias (1999), plant requirements for manganese are much differentiated, but in most cases the amount of 10–25 mg·kg^{-1} is sufficient. The concentration of about 500 mg·kg^{-1} can be toxic for the majority of plants. The results obtained in this study indicate excessive accumulation of manganese in *V. myrtillus* shoots. Reimann et al. (2001) as well as Bylińska (1992) noted that bilberry plants were distinctive of high contents of Mn, apart from those observed in soil. Boyd (2007) described this low-growing shrub as manganese accumulator in view of the fact that the concentration of manganese in bilberry tissues can be even above 2000 mg·kg^{-1}. The results of research reported by Gworek and Degórski (2000) indicated that *V. myrtillus* could accumulate in its tissues even 2489.0 mg·kg^{-1}, which proves the large capability of this species to accumulate manganese.

The relationships among heavy metals determined both in the aboveground and underground parts of *V. myrtillus* showed the following decreasing order: Mn > Fe > Zn > Cu.

The analyses carried out revealed statistically significant Spearman's correlation coefficients for bilberry zinc content in the relationship soil – stems (r = -0.44, p < 0.05, n = 45) as well as soil – roots (r = -0.52, p < 0.05, n = 45). The decrease of zinc content in soil was accompanied by the increase of Zn content in *V. myrtillus* stems and roots, which is confirmed by the uptake of high amounts of this element from the soil solution

(Table 3). Other heavy metals tested (Fe, Cu, Mn) did not show statistically significant relationships between their concentrations in soil and those in billberry shoots, which can indicate leaf uptake of these elements from either precipitation or atmospheric particulate matter settling to the ground (Łaszewska et al. 2007).

Table 3. Spearman correlation coefficients (r) of heavy metals in the shoots *V. myrtillus* relative to 'soil – leaves', 'soil – stems', 'soil – roots' ($n = 45$, $p < 0.05$, $r_{cryt.} = 0.30$)

r in relation	Fe	Mn	Zn	Cu
soil – leaves	0.17	0.25	-0.28	0.20
soil – stems	0.01	0.27	-0.44	-0.21
soil – roots	0. 25	-0.06	-0.52	-0.01

Low contents of Zn, Fe and Cu in the above- and underground *V. myrtillus* shoots as well as in surface soil layers are well reflected by the low values of enrichment factors (*EF*). The highest *EF* values were obtained for Mn (*EF* > 20), which confirms its tendency to elevated accumulation in plants (Fig. 3), and the lowest for copper (Mn > Fe > Zn > Cu). The results obtained in the National Słowiński Park indicate that zinc and manganese are accumulated to the largest extent in *V. myrtillus* stems, whereas copper in the leaves and iron in the roots. According to Kłos (2009), *EF* values >10 can indicate the influx of exterior pollution, for example, with dry and wet precipitation. However, heavy metal influx is in general relatively small. The results of the study on particulate matter carried out in 2010 within the area of the Słowiński National Park

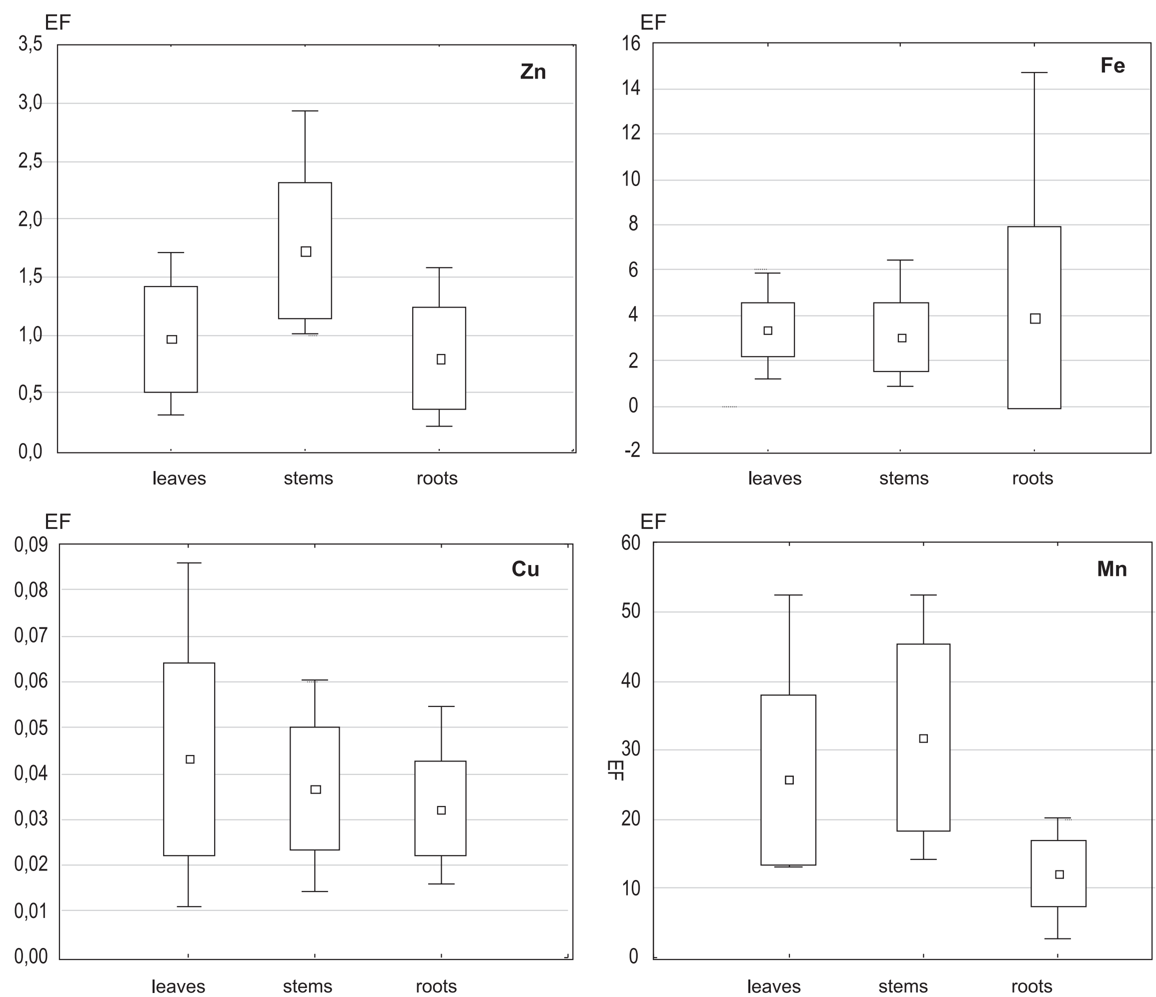

Figure 3. Enrichment factors (*EF*) investigated metals in the leaves, stems and roots of *V. myrtillus*, point (mean), rectangle (standard deviation), whiskers (minimum – maximum)

showed PM10 content 17 μg/m^3 (Brożek, Zarembski 2011), and this did not exceed the threshold value. SNP area has been considered by many researchers as one of the cleanest in Poland. Among others, this is confirmed by chemical studies on forest bioindicative flora (Grodzińska et al. 1990, 1999; Bykowszczenko et al. 2006; Parzych et al. 2012; Parzych, Sobisz 2012).

Comparative analyses of heavy metal concentrations in the above- and underground shoots of bilberry *V. myrtillus* were performed using the Mann–Whitney *U*-test (Table 4). The results obtained showed statistically significant differences in zinc content in the relationship stems – root and leaves – stems as well as manganese content in the relationship stems – roots and leaves – roots No significant differences were found for Fe and Cu concentrations in bilberry above- and underground shoots.

4. Conclusions

In the above- and underground shoots of bilberry *V. myrtillus* growing on the territory of the Słowinski National Park located within the Łebska Spit, there were found varied contents of Zn, Fe, Mn and Cu depending on research site localisation and the part of plant. The highest amounts of the heavy metals tested were found in the sites in vicinity of parking lots, which tells on the role of road traffic in point contamination of the Park. The metals investigated were accumulated in *V. myrtillus* shoots to different extents. The highest amounts of copper were found in bilberry leaves, those of iron – in the roots, and those of zinc and manganese were observed in the stems. The relationships among heavy metals determined both in the aboveground and underground parts of *V. myrtillus* showed the following decreasing order: Mn > Fe > Zn > Cu.

Small contents of Zn, Fe and Cu in bilberry shoots as well as in the surface horizons of soil translate into the low enrichment factor (*EF*) values obtained in this study. Strongly acidic soil environment enhanced the bioavailability of the heavy metals tested to bilberry shoots. The highest *EF* values were obtained for Mn and Fe, which is a sign of greater accumulation ability of *V. myrtillus* as for these elements, despite their low contents in soil.

The analyses carried out showed statistically significant differences in Zn content in the relationships leaves – stems and stems – roots as well as in Mn content in the relationship stems – roots and leaves – roots. No significant differences were found for Fe and Cu concentration in the above- and underground shoots of bilberry *V. Myrtillus.*

Table 4. The significance of variation of heavy metals concentration in the tested shoots *V. myrtillus* (Mann–Whitney *U*-test)

Metal	Relations		
	leaves – stems	stems – roots	leaves – roots
Zn	**	***	ns
Fe	ns	ns	ns
Cu	ns	ns	ns
Mn	ns	***	***

The significance level: ***$p < 0.001$, *$p < 0.05$, ns – no differences

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