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ACCUMULATION OF HEAVY METALS IN MOSS SPECIES *PLEUROZIUM SCHREBERI* (BRID.) MITT. AND *HYLOCOMIUM SPLENDENS* (HEDW.) B.S.G. IN SŁOWIŃSKI NATIONAL PARK

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Abstract

This paper presents the results of research into the heavy metal content in the moss species *Pleurozium schreberi* and *Hylocomium splendens* and in the organic and humus horizons of soil under coniferous pine forests in Słowiński National Park. The test samples were taken in September 2011 from 15 research stations situated in the park. The research stations were set up in places most frequently visited by tourists and in the vicinity of car parks. The examined soil was characterized by strong acid reaction, which had impact on an increased accessibility of the examined heavy metals to plants. Variation coefficients for active acidity and exchangeable acidity were from 5 to 8%. The highest level of organic matter was in the subhorizons Ol, slightly lower in the subhorizons Ofh, and the lowest in the A horizons. It was demonstrated that the active acidity of the soil's surface genetic levels was signnificantly correlated with the manganese and zinc content (in both moss species) and wth zinc (in *H. splendens*). Relationships among the determined heavy metals in both species of moss made the following decreasing series: Fe>Mn>Zn>Cu. The data revealed varied accumulation characteristics of the moss species. The U Mann-Whitney's test on the significance of variation in the content of selected heavy metals in *Pleurozium schreberi* and *Hylocomium splendens* demonstrated statistical differences in the concentration of Cu ($p<0.01$). According to the values of the *Pleurozium*/*Hylocomium* coefficient, *P. schreberi* had higher accumulation potential in relation to Zn, and *H. splendens* in relation to Fe and Cu. Both tested moss species presented similar accumulation characteristics in reference to Mn. The levels of enrichment factors (*EF*) confirmed weak contribution of heavy metals accumulated in soil to the formation of the Fe, Zn, Mn and Cu content in *P. schreberi* and *H. splendens*.

Keywords: *Pleurozium schreberi*, *Hylocomium splendens*, accumulation of Zn, Fe, Cu, Mn.

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AKUMULACJA METALI CIĘŻKICH W MCHACH *PLEUROZIUM SCHREBERI* (BRID.) MITT. I *HYLOCOMIUM SPLENDENS* (HEDW.) B.S.G. W SŁOWIŃSKIM PARKU NARODOWYM

Abstrakt

Badano zawartość metali ciężkich w mchach: *Pleurozium schreberi* i *Hylocomium splendens* oraz w poziomach organicznych i próchnicznych borów sosnowych Słowińskiego Parku Narodowego. Próbki do badań pobierano we wrześniu 2011 r. z 15 stanowisk zlokalizowanych na terenie parku. Stanowiska badawcze zlokalizowano w miejscach najczęściej uczęszczanych przez turystów oraz w sąsiedztwie parkingów samochodowych. Badane gleby charakteryzują się odczynem silnie kwaśnym, co wpływa na zwiększoną dostępność badanych metali ciężkich dla roślin. Współczynniki zmienności dla kwasowości czynnej oraz kwasowości wymiennej wynosiły od 5 do 8%. Największą zawartość materii organicznej stwierdzono w podpoziomach Ol, nieco mniejszą w podpoziomach Ofh, a najmniejszą w poziomach A. Wykazano, że kwasowość czynna wierzchnich poziomów genetycznych gleby pozostaje w istotnym statystycznie związku z zawartością manganu i cynku (w obu gatunkach mchów) oraz cynku (w *H. splendens*). Relacje między oznaczanymi metalami ciężkimi w obu gatunkach mchów układają się w następujący szereg malejący: Fe>Mn>Zn>Cu. Wykazano zróżnicowane właściwości akumulacyjne badanych gatunków mchów. Wyniki testu U Manna-Whitneya istotności zróżnicowania wybranych metali ciężkich w *Pleurozium schreberi* i *Hylocomium splendens* wskazują na istotne statystycznie różnice w koncentracji Cu ($p < 0.01$). Zgodnie z wartościami współczynnika *Pleurozium/Hylocomium* większe właściwości akumulacyjne ma *P. schreberi* w stosunku do Zn, a *H. splendens* w stosunku do Fe i Cu. Oba badane gatunki mchów wykazują zbliżone właściwości akumulacyjne w stosunku do Mn. Wartości współczynników wzbogacenia (*EF*) potwierdzają niewielki udział metali ciężkich zawartych w glebie w kształtowaniu zawartości Fe, Zn, Mn i Cu w *P. schreberi* i *H. splendens*.

Słowa kluczowe: *Pleurozium schreberi*, *Hylocomium splendens*, akumulacja Zn, Fe, Cu, Mn.

INTRODUCTION

Heavy metals as natural components of ecosystems are necessary in small quantities for sustaining proper functions of plants, although their excess in the environment is harmful. In extremely high concentrations, they disturb whole ecosystems and pose a threat to plants, animals and people (Gruca-Królikowska, Wacławek 2006, Malzahn 2009, Medyńska-Juraszek, Kabała 2012). Heavy metals are bio-accumulated in plant and animal tissues, thus the risk of intoxication grows in subsequent links of the trophic chain. Plants react differently to increased heavy metal concentrations in the environment. The most sensitive ones are used in bio-monitoring to acquire information about the quality of the environment (Migaszewski et al. 2009). Among plant bio-indicators, moss species are useful in monitoring studies. Lacking natural protective barriers such as the epidermis and cuticle, they easily absorb substances, especially metals, which are deposited on their surface (Gjengedal, Steinnes 1990, Berg, Steinnes 1997, Mankovska 1998, Grodzińska, Szarek-Łukaszewska 2001, Reiman et al. 2001, Gerdol et al. 2002, Szczepaniak, Biziuk, 2003, Gałuszka 2006, 2007, Samecka-Cymerman et al., 2006, Dećkowska et al. 2008, Harmens et al. 2010). Moss absorbs

nutritional elements mainly from precipitation and dry deposition. Absorption from the substratum is limited due to the lack of roots (Grodzińska 1980). The influence of soil and soil solutions on the heavy metal content in moss cannot be neglected (Steinnes 1995, Wells, Brown 1996, Økland et al. 1999, Gerdol et al., 2002). *Pleurozium schreberi* and *Hylocomium splendens* found both in pure and polluted habitats are the most popular species, with a widespread geographical distribution, used for evaluation of the air quality. Inter-species comparisons of their chemical content indicate differences in the accumulation of elements. In most cases, higher concentrations were found in *H. splendens*, but this tendency has not been explained in detail. One possible explanation could lie in morphological differences (Halleraker et al. 1998, Grodzińska et al. 1999, Migaszewski et al. 2009).

The objectives of the study were (i) determination of the content of selected heavy metals in *Pleurozium schreberi* and *Hylocomium splendens* growing in a protected area, (ii) comparison of their accumulative characteristics, (iii) evaluation of the influence of heavy metals found in soil on the concentration of heavy metals in moss, and (iv) evaluation of air pollution levels in Słowiński National Park.

SAMPLING SITES AND METHODS

The research was carried out in a forest in Słowiński National Park (SNP), which lies on the Łeba Sandbar. Samples of moss (*Pleurozium schreberi*, *Hylocomium splendens*) and of the organic and humus soil layers were collected in September 2011. They were taken from 15 research stations located in Park's ecosystems of *Empetro nigri-Pinetum*, growing on the Dystric Arenosols and Podzols developed from deep sand dunes (SGP 2011). The research stations were situated in places most frequently visited by tourists and in the vicinity of car parks. The soil samples were submitted to the following determinations: pH in H_2O and 1 M dm^{-3} measured with a potentiometer in a solution of KCl; the organic matter content in a muffle furnace at the temperature of 550°C. The plant material was transported to a laboratory, where it was refined of mineral parts of soil, dried to constant mass at 65°C and homogenized in a grinder (Namieśnik et al., 2000). Moss and soil samples were wet mineralized in a closed system in a mixture of HNO_3 and 30% H_2O_2. In the solutions, the concentrations of Zn, Fe, Mn and Cu were determined by atomic absorption spectrometry (Aanalyst 300, Perkin Elmer) according to Ostrowska et al. (2001). The results were compared to standards (Merck KGaA, 1 g/1000 ml).

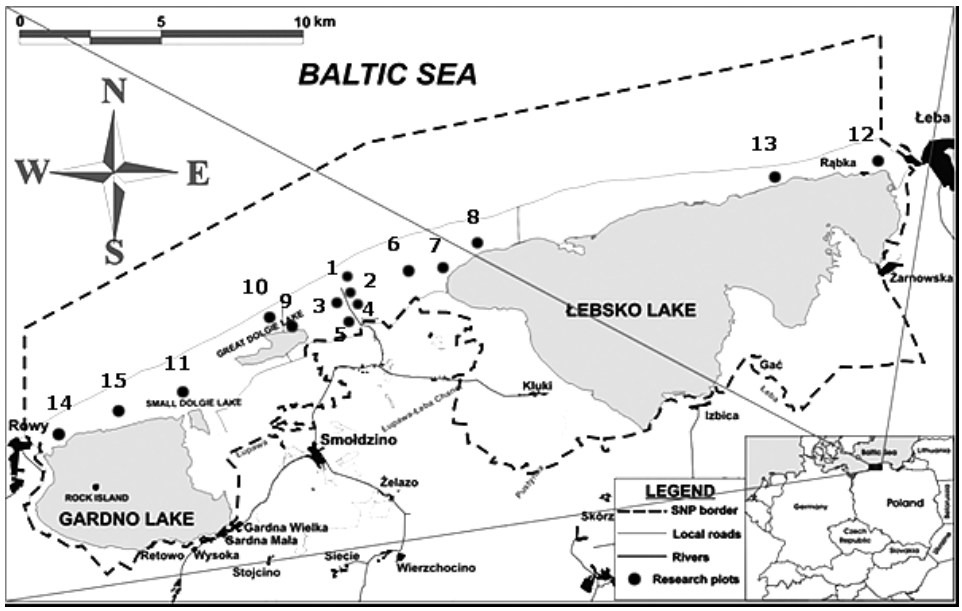

Fig. 1. Situation plan of the Słowiński National Park – locations of the study sites

STATISTICAL ANALYSIS

In order to characterize and compare concentrations of the selected heavy metals in the examined moss species, average, minimum, maximum, medians, standard deviations, variation coefficients (CV), Spearman's correlation coefficients, *Pleurozium/Hylocomium* coefficients and enrichment factors (*EF*) were calculated. The validity of statistical variation between the moss species and the heavy metal content in the soil was checked by the non-parametric U Mann-Whitney's test. Statistica software was used for calculations (7.1).

RESULTS AND DISCUSSION

The samples of organic and humus horizons taken from 15 research stations within the area of the Łeba Sandbar had strong acid reaction (Table 1). The variation coefficients for active acidity (pH, H_2O) and exchangeable acidity (pH, KCl) were from 5 to 8%. The highest clevel of organic matter was in the subhorizons Ol, slightly lower in the subhorizons Ofh, and the lowest in the A horizons. The organic matter content decreased with the depth, conversely to the diversity of this parameter, which increased (CV 2 59%).

Table 1

pH and organic matter in organic and humic horizons in SNP

Specification	pH (H_2O)			pH (KCl)			Organic matter (%)		
	Ol	Ofh	A/AC	Ol	Ofh	A/AC	Ol	Ofh	A/AC
Average	4.4	3.8	4.4	3.5	2.8	3.3	96.6	76.9	3.1
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
Standard deviation	0.2	0.2	0.3	0.2	0.2	0.2	0.02	0.2	0.01
CV (%)	5	7	7	8	6	8	2	23	59

The content of the analyzed metals in the soil was varied. The largest quantities of Zn, Fe and Mn were found in the Ol subhorizons, slightly smaller ain Ofh, and the smallest in the A horizons. The highest concentrations of Cu and Fe were found in the Ofh subhorizons, and Zn and Mn were most abundant in the Ol subhorizons. The variation coefficients for the zinc content were from 28 to 38 %, for iron – from 35 to 134 %, manganese – from 52 to 55 %, and copper – from 18 to 35 %, depending on the genetic level of soil (Table 2).

Table 2

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

Specification	Zn			Fe			Mn			Cu		
	Ol	Ofh	A/AC	Ol	Ofh	A/AC	Ol	Ofh	A/AC	Ol	Ofh	A/AC
Average	68.9	47.0	2.89	469	1609	346	206.2	40.0	4.17	0.80	0.90	0.09
Minimum	37.1	24.2	1.28	118	478	124	48.7	12.2	1.15	0.50	0.60	0.06
Maximum	101.0	82.0	5.68	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
Standard deviation	19.0	15.4	1.08	505	2161	120	112.6	23.1	2.15	0.14	0.29	0.03
CV (%)	28	33	38	108	134	35	55	58	52	18	31	35

The moss samples collected from the Łeba Sandbar in Słowiński National Park were determined to contain different levels of Zn, Fe, Mn and Cu. The zinc content varied from 47.9 to 95.2 (mg kg^{-1}) in *Pleurozium schreberi* and from 38.7 to 84.9 mg kg^{-1} in *Hylocomium splendens* (Figure 2, Table 3). The Zn concentration in the tested moss revealed was highly varied. The mean variability coefficients (CV) of the Zn content were 21.0% for *P. schreberi* and 24.0% for *H. splendens*. At most research stations, higher accumulation of zinc in *P. schreberi* than in w *H. splendens* was discovered. The Zn content in *P. schreberi* and *H. splendens* moss in the examined coniferous forests is comparable to the results obtained by other, e.g. Gałuszka (2006) 33-54 mg kg^{-1} in Wigierski National Park, Kozanecka et al. (2002)

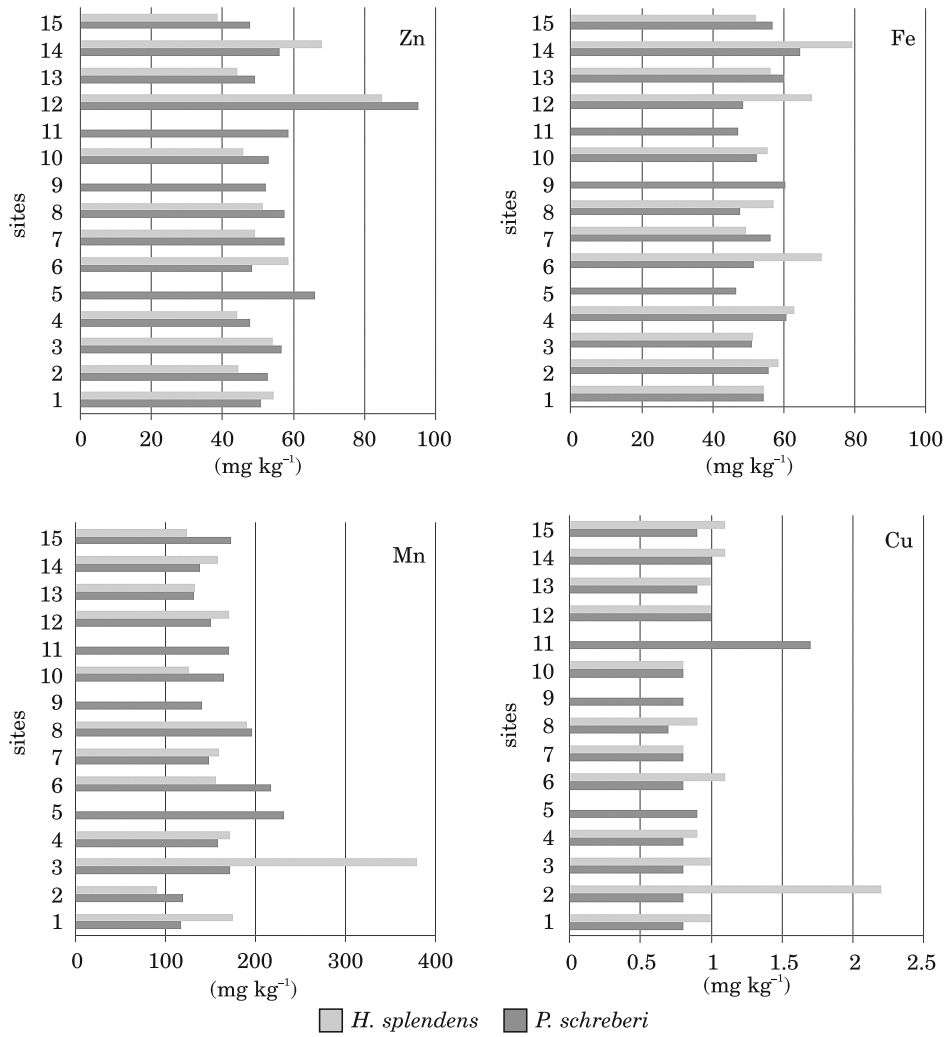

Fig. 2. Content of selected elements (mg kg^{-1}) in *Pleurozium schreberi* and *Hylocomium splendens*

33-43 mg kg^{-1} in Biała Primeval Forest, or MALZAHN (2009) 46 mg kg^{-1} in Białowieska Primeval Forest.

The iron content in moss was also diverse. In *P. schreberi* the Fe level ranged from 233.0 to 323.0 (mg kg^{-1}), and in *H. splendens* it varied from 247.0 to 396.0 (mg kg^{-1}) (Figure 2, Table 3). Variability coefficients for the Fe concentration were from 10.4% (*P. schreberi*) to 15.0% (*H. splendens*). These values of the Fe content in moss in SNP are substantially lower than determined earlier by GRODZIŃSKA (1980). The current results indicate that the pollution in the park had decreased. Similar iron concentrations in *P.*

schreberi moss were obtained by: GAŁUSZKA (2006), 194-795 mg kg^{-1}, and KOZANECKA et al. (2002), from 326 to 650 mg kg^{-1}.

The manganese content in the tested moss was from 116.3 to 231.4 mg kg^{-1} (*P. schreberi*) and from 90.5 to 379.0 mg kg^{-1} (*H. splendens*) – Figure 2, Table 3. The Mn concentration in *H. splendens* was twice as varied as in *P. schreberi* (CV = 42.3% and CV = 20.7%, respectively). According to KABATA-PENDIAS, PENDIAS (1999), the plant's demand for manganese is diverse but in most cases 10-25 mg kg^{-1} is sufficient. A concentration of about 500 mg kg^{-1} can be toxic for most plants. The average manganese content in *P. schreberi* from Biała Primeval Forest is about 383 mg kg^{-1} (KOZANECKA et al. (2002). The Mn content in both moss species originating from Lithuania was from 50.0 to 744.0 mg kg^{-1} (ČEBURNIS, STEINNES 2000).

The copper content in moss was from 0.7 to 1.7 mg kg^{-1} in *P. schreberi* and from 0.8 to 2.2 mg kg^{-1} in *H. splendens*, showing much higher variation among the stations. The mean variation coefficients of the Cu concentration in moss were 26.2% (*P. schreberi*) and 34.4% (*H. splendens*) – Figure 2, Table 3. Some earlier tests of the chemical composition of *P. schreberi* by GRODZIŃSKA et al. (1999) in SNP demonstrated the concentration of Cu equal 7 mg kg^{-1}. The average Cu content in *P. schreberi* in Poland, according to DEĆKOWSKA et al. (2008), is 10.7 mg kg^{-1}. The Cu content in moss from SNP determined in our study is very low and sufficient just to cover the plant's physiological needs.

Relationships among the determined heavy metals in both species of moss made the following decreasing series: Fe>Mn>Zn>Cu. Identical relationships among the tested elements were discovered by KOZANECKA et al. (2002) and MALZAHN (2009).

Table 3

Mean, standard deviation (SD), coefficient of variation (CV), minimum (min), maximum (max), median concentrations of different elements (mg kg^{-1}) in *Pleurozium schreberi* and *Hylocomium splendens* and correlation coefficient

Plant		Zn	Fe	Mn	Cu
Pleurozium schreberi	mean (mg kg^{-1})	56.7	271.1	161.5	0.9
	SD	11.8	28.2	33.4	0.24
	CV (%)	21.0	10.4	20.7	26.2
	min	47.9	233.0	116.3	0.7
	max	95.2	323.0	231.4	1.7
	median	53.0	271.0	158.2	0.8
Hylocomium splendens	mean (mg kg^{-1})	53.2	298.1	169.1	1.1
	SD	12.7	44.8	71.2	0.37
	CV (%)	24.0	15.0	42.3	34.4
	min	38.7	247.0	90.5	0.8
	max	84.9	396.0	379.0	2.2
	median	50.3	283.5	158.2	1.0
Correlation *P. schreberi-H. splendens*		**0.81**[a]	0.26[b]	0.21[b]	0.27[b]

[a] statistically significant for the $p < 0.01$, [b] – not statistically significant

Comparing the accumulation of the tested metals in *P. schreberi* and *H. splendens*, a statistically significant, positive correlation was demonstrated only for the zinc content ($r = 0.81$, $n = 36$, $p < 0.05$) – Table 3. Correlations between Fe, Mn and Cu in both moss species were statistically unimportant.

The results of the U Mann-Whitney's test on the significance of variation in the content of the heavy metals in the moss species *Pleurozium schreberi* and *Hylocomium splendens* sampled in SNP demonstrate statistically significant differences only for the Cu concentration ($p < 0.01$).

The *Pleurozium/Hylocomium* coefficients calculated for moss from SNP demonstrate higher accumulation potential of *P. schreberi* in relation to Zn, and *H. splendens* in relation to Fe and Cu (Table 4). Similar results were obtained by REIMAN et al. (2001). In the case of iron and copper, a higher accumulation potential of *H. splendens* than *P. schreberi* was proven (0.96 and 0.80 respectively). Similar relationships between the content of these metals in moss were discovered by GAŁUSZKA (2007) or BERG, STEINNES (1997). As for manganese, similar accumulative values were discovered for *P. schreberi* and in *H. splendens.*

Table 4

Pleurozium/Hylocomium concentration (median) ratios of 4 elements in *H. splendens* and *P. schreberi* derived from SNP

Zn	Fe	Mn	Cu	References
1.05	0.96	1.00	0.80	Results of their own, SNP - 2011
0.97	0.95	0.81	0.89	GAŁUSZKA (2007)
1.03	0.89	0.95	0.80	REIMAN et al. (2001)
-	0.63	1.30	0.76	BERG and STEINNES (1997)

In order to examine the influence of the soil's chemical composition on the content of heavy metals in moss, the Spearman's correlation coefficients were calculated (Table 5). It was demonstrated that the active acidity (pH, H_2O) had a significant statistical correlation with Mn ($r = -0.33$ *P. schreberi* and $r = -0{,}32$ *H. splendens*, where $n = 36$, $p < 0.05$) ($r = 0.32$, $n = 45$, $p < 0.05$, *P. schreberi*). Exchangeable acidity (pH, KCl) was statistically significantly and positively correlated with iron contained in both moss species ($r = 0.41$, $n = 45$ and $r = 0.41$, $n = 36$, $p < 0.05$) and with manganese ($r = -0.33$, $n = 36$, $p < 0.05$) in the case of *H. splendens*. The organic matter accumulated in the surface layers of soil had strong impact on the Zn content in *P. schreberi* ($r = -0.37$, $n = 45$, $p < 0.05$) and on the Mn content in *H. splendens* ($r = 0.48$, $n = 36$, $p < 0.05$). The content of heavy metals in the soil demonstrated a very weak impact on the content of these metals in moss. A positive correlation was demonstrated in the case of zinc in the soil and in *P. schreberi* ($r = 0.38$, $n = 45$, $p < 0.05$). As the iron content in the soil increased, the Cu concetration in moss ($r = -0.31$, *P. schreberi* and

Table 5

The correlation coefficients Spearmann selected soil properties and heavy metals in mosses

Plant	Elements	pH, H_2O	pH, KCl	Organic mater	Zn	Fe	Mn	Cu
Pleurozium schreberi (n = 45, p < 0.05, $r_{crit.}$ = 0.30)	Zn	**0.32**	-0.01	**-0.37**	**0.38**	-0.16	0.17	0.01
	Fe	-0.05	**0.41**	0.17	0.02	-0.09	0.29	0.08
	Mn	**-0.33**	**-0.33**	0.22	0.19	0.22	-0.28	0.08
	Cu	0.08	-0.06	-0.05	0.09	**-0.31**	0.09	0.05
Hylocomium splendens (n = 36, p < 0.05, $r_{crit.}$ = 0.30)	Zn	-0.08	0.15	0.16	-0.16	**0.41**	**-0.42**	0.56
	Fe	-0.23	**0.40**	0.01	0.16	0.04	-0.03	0.45
	Mn	**-0.32**	-0.25	0.48	0.13	0.13	**-0.39**	-0.15
	Cu	-0.15	0.16	0.14	0.17	-0.43	0.08	-0.22

r = -0.43, *H. splendens*) decreased while the Zn content rose (r = 0.41, n =36, p < 0.05). Moreover, manganese included in the soil had some impact on decreasing the content of Zn (r = -0.42) and Mn (r = -0.39), while soil copper resulted in an increase in Zn (r = 0.56) and Fe (r = 0.45) in *H. splendens* (Table 5).

The heavy metal content in the soil had a weak influence on the Zn, Fe, Mn and Cu content in moss. Similar relationships were obtained by Gerdol et al. (2002). A small content of the metals in soils and moss implies relatively clean air (Brożek, Zarembski 2011) and translates into small levels of the enrichment factor (*EF*) – Figure 3. The lowest enrichment factor was discovered in the case of iron (average 0.3 for *P. schreberi* and *H. splendens*), copper (averge 1.5 for *P. schreberi* and 1.7 for *H. splendens*) and zinc (average 1.5 for *P. schreberi* and 1.4 for *H. splendens)*. The highest *EF* was found only in the case of manganese (average 2.8 for *P. schreberi* and *H. splendens*).

Little quantities of heavy metals in the soil suggested an alluvial form of pollution, e.g. brought by dry and wet precipitation. However, in the examined case, quantities of heavy metals originating from such sources were minimal. This was supported by the results of tests on suspended particulate matter PM10 (a potential source of heavy metals) held in 2010 in Słowiński National Park – 17 μg m^{-3} (Brożek, Zarembski 2011). In 1995, the amount of emitted dust containing heavy metals (529 t) decreased substantially in comparison to 1990 (1165 t) (GUS 1991, 1995). The area of Słowiński National Park has been recognized by many scholars as one of the cleanest nature reserves in Poland (Grodzińska 1980, Grodzińska et al. 1990). The enrichment factor (*EF*) values confirm small contribution of heavy metals in the soil to the formation of the Fe, Zn, Mn and Cu content in *P. schreberi* and *H. splendens* (Figure 3). Similar levels of the EF coefficients for the *EF* were achieved in *H. splendens* by Bargagli et al. (1995).

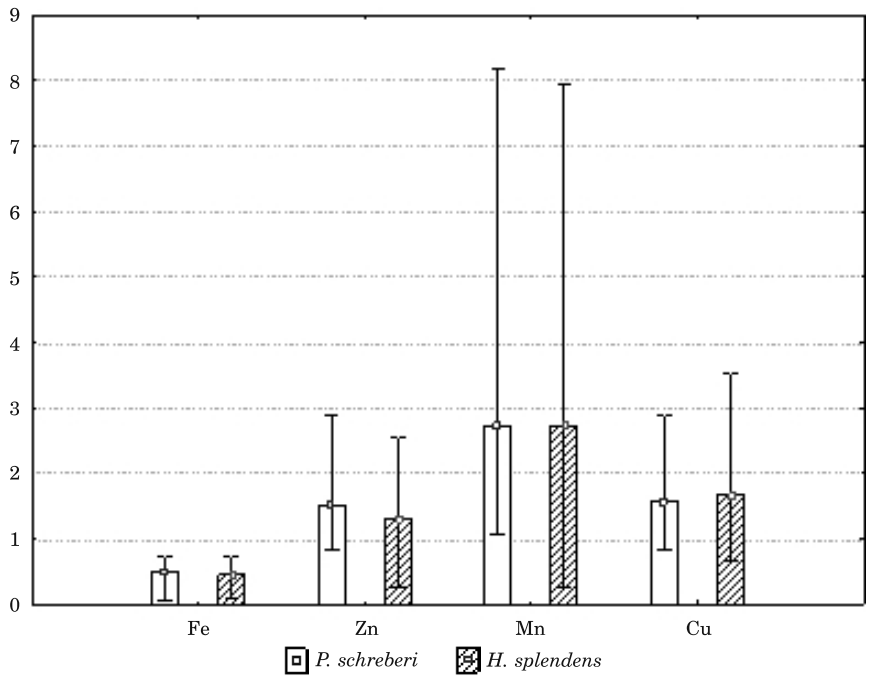

Fig. 3. Enrichment factor of *P. schreberi* and *H. splendens* in heavy metals, point (mean), rectangle (standard deviation), whiskers (minimum-maximum)

CONCLUSIONS

The moss samples from Słowiński National Park had varied concentrations of Zn, Fe, Mn and Cu. The examined soil was characterized by strong acid reaction, which had impact on an increased accessibility of the examined heavy metals to plants. It was demonstrated that the active acidity (pH, H_2O) of the soil's surface genetic levels had a significant statistical correlation with the manganese and zinc content (in both moss species) and wih zinc (in *H. splendens*). The Mn content in the tested moss was from 116.3 to 231.4 mg kg^{-1} in *P. schreberi* and from 90.5 to 379.0 mg kg^{-1} in (*H. splendens*) (CV = 42.3% and CV = 20.7% respectively*)*. The relationships between the determined heavy metals in both moss species made an identical decreasing series: Fe>Mn>Zn>Cu.

Comparing the accumulation of the examined heavy metals in *P. schreberi* and *H. splendens*, a statistically significant and positive correlation was demonstrated for zinc ($r = 0.81$, $n = 36$, $p < 0.05$). Correlations between Fe, Mn and Cu in both species of moss proved to be statistically unimportant. The results of the U Mann-Whitney's test concerning the significance

of variations of selected heavy metals in the moss *Pleurozium schreberi* and *Hylocomium splendens* demonstrates substantial differences in the Cu content ($p < 0.01$).

The *Pleurozium*/*Hylocomium* coefficients demonstrate higher accumulation potential of *P. schreberi* in relation to zinc and *H. splendens* in relation to iron and copper. Both examined moss species demonstrated similar accumulation capabilities regarding manganese. The values of enrichment factors (*EF*) confirm small contribution of heavy metals in soil to the formation of the Fe, Zn, Mn and Cu content in *P. schreberi* and *H. splendens.*

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