

Original Research

Differences in Concentrations of Heavy Metals Between Native and Transplanted *Pohlia nutans* (Hedw.) Lindb. – a Case Study from a Dump Exposed to Industrial Emissions in Poland

A. Samecka-Cymerman^{1*}, A.J. Kempers²

¹Department of Ecology and Nature Protection, Wrocław University, ul. Kanonia 6/8, 50-328 Wrocław, Poland

²Department of Environmental Sciences, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands.

Received: May 5, 2006

Accepted: December 12, 2006

Abstract

During a period of 60 days assays were carried out with the moss *Pohlia nutans* transplanted from an uncontaminated control site to a dump consisting of a heavily polluted mine and smelter wastes located near Wałbrzych in southwestern Poland. Within the same period also samples of native *P. nutans* growing on the dump substrate were collected together with the same species from a control site. Concentrations of Al, Fe, Cd, Co, Cr, Cu, Mn, Ni, Pb, V, and Zn, as well as N, P, K, Ca, Mg and S, were determined in *P. nutans*, the dump substrate and the soil of the control site. Atmospheric deposition was the main contribution to the levels of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in the native and transplanted *P. nutans* on the examined dump. The obtained results indicate that the transplanted *P. nutans* accumulated significantly more Al, Cd, Co, Cr, Fe, Ni, Pb and Zn than the native moss.

Keywords: biomonitor, heavy metal, *Pohlia nutans*, transplant

Introduction

Accumulation of heavy metals in the environment may cause chronic damage to living organisms and must be carefully controlled. Bryophytes have been described in the literature as being able to intercept, retain and accumulate pollutants. Their bioconcentration ability is such that they accumulate metals to levels far above their expected physiological needs [1-3] owing to the absence of a cuticle in their tissues and to the abundance of cation exchange sites on their cell walls. The great majority of moss species have been found to have the special advantage that they take the required nutrients almost exclusively from the atmosphere, as they have not developed a

real root system or water-conductive tissues (ectohydrous mosses) [4-5]. Mosses have therefore received increasing attention as a suitable tool for monitoring regional patterns of elemental deposition from the atmosphere, and have been used as such in large-scale studies in various countries, in areas close to industrial installations as well as in areas not expected to be contaminated [6]. Although terrestrial mosses are in general used as biomonitors of atmospheric pollution, interaction of the moss with local litter and soil as element sources should not be underestimated [5, 7, 8]. Therefore in this work we investigated the source of the accumulated metals in *Pohlia nutans*. According to Fernandez et al. [6, 9] and Fernandez and Carballeira [10], mosses have often been used in passive biomonitoring, i.e. where native species are used in the area under study, or for active biomonitoring where trans-

*Corresponding author; e-mail: sameckaa@biol.uni.wroc.pl

plants are used. Fernandez et al. [9] assumed that plants have a capacity to adapt to certain environmental conditions. His study as well as a study of Samecka-Cymerman et al. [11] illustrated that the native mosses accumulated significantly less heavy metals than transplanted mosses of the same species.

In this investigation we compared the bioconcentration of metals in *P. nutans* transplanted from a control site to a dump with the bioconcentration of identical elements in *P. nutans* growing naturally at the contaminated and control sites to evaluate if native moss of this species adapts to pollution on the examined dump.

In the present investigation, the hypotheses to be tested are:

1. Atmospheric deposition is the main contribution to the levels of metals in *P. nutans*
2. Native *P. nutans* exposed to pollution accumulates less metals than the same species transplanted from an uncontaminated site.

The level of nutritional elements in *P. nutans* was investigated as pollution with heavy metals may cause imbalances in the ionic equilibrium within plant tissues and imbalanced reactions between elements may cause chemical stress in plants [12].

No previous investigations were done comparing transplanted and native *P. nutans* growing on a heavy polluted dump. This investigation contributes to the knowledge of the chemical ecology of *P. nutans* growing in extremely contaminated sites, presents the source of metals for this plant and points whether transplanted or native *P. nutans* can be used as the most suitable bioindicator.

Materials and Methods

P. nutans is a terrestrial moss and abundant on acid substrates high in concentration of heavy metals. *P. nutans* is able to accumulate high levels of these elements without visible damage [13]. Therefore, this species is very suitable for studying bioaccumulation of metals.

The study area consists of a dump which is situated in the west part of Wałbrzych (South West Poland) in the vicinity of coke and sulphuric acid producing factories. The dump, abandoned in the early eighties and not recultivated, consists of material excavated from a nearby black coal mine and ashes from a nearby coke factory and a municipal thermal-electric power station. The dump is limited by a forest at its West side and a coke factory at its East side. This coke factory produces considerable amounts of dust and gaseous pollution which is considered the highest in industrial Lower Silesia. According to the Polish Provincial Authority for Environmental Protection (WIOS) the soil around this factory contains in mg/kg 0.6-0.7 Cd, 39-44 Cu, 0.32-0.33 Hg, 14-16 Ni, 62-63 Pb, 145-160 Zn, 1340-5100 S (as sulphates), 0.9-1.1 As, and 2.9-3.7 F [14]. The investigated dump forms in some parts green, blue, red, white or brown precipitates caused by atmospheric deposition of exhausts of neighbouring

factories. Daily gas exhausts of yellow-green acrid smoke form in times of mist a persisting smog and create in times of rain acid deposits. A typical composition of these exhausts may consist of 25% CO, 5-8% CO₂, 0.5-15% methane, 45-56% nitrogen and sulphur oxides in amounts dependent on the quality of coal used for the production of coke [14].

A square area of 50 m x 50 m was selected on a dump near Wałbrzych (N 50° 47', E 16° 17'). Within this square, 5 subsquares of 2m x 2m were selected randomly for the transplantation of *P. nutans* from a control site and treated as follows: in each of these 5 subsquares (covered with native *P. nutans*), 5 randomly chosen places were cleaned from vegetation in which patches (20cmx20cm) of mosses (originating from the control site) were transplanted (giving a total of 25 replicates). The remaining part of the subsquare was left covered by native *P. nutans*. The transplanted *P. nutans* originated from a forested control site with a shallow acid soil in the Sowie Mountains (N 50° 45', E 16° 24') at a distance of 15 km E from the dump. According to Vázquez et al. [15], background or reference levels are only applicable to the region for which they are calculated, since they reflect the natural characteristics (especially the climate conditions) of the area where the species occurs. Based on this statement, reference values for the study area were calculated as approximate values for *P. nutans* of the control site. As a control reference *P. nutans* was collected at the same site as where the transplanted *P. nutans* came from at each of five sampling sites in five replicates (giving a total of 25 replicates of soil and plant samples) and replaced again at the control site in order to sort out the effects of transplanting. Both replaced control and transplanted *P. nutans* were transferred together with their soil. The patches of *P. nutans* from the control site were collected remotely from tree canopies so the collected mosses had not been exposed directly to canopy throughfall and placed on the dump also remote from tree canopies and well exposed to wind [16-18]. The experiment lasted 60 days (July and August). Transplant, native and control samples were collected together with soil. Altogether there were 25 native samples taken from the vicinity of the places where the transplants were positioned, 25 transplanted samples and 25 control samples. At the end of the exposure period both native and transplanted mosses were taken to the laboratory for analyses. Only live-green parts of *P. nutans* were collected. Dead material and litter were removed from the samples. Mosses were washed for a few seconds in distilled water [19].

Soil, Dump Substrate and Plant Analysis

Fresh samples from the control soil and dump substrate were used for measurement of the pH_{H2O} and pH_{KCl} (potentiometrically).

All 25 replicates from the control soil and dump substrates were extracted for: PO₄ by means of a solution of

0.3 M sodium citrate and 1 M sodium bicarbonate and for Ca, Mg and K by means of an 1 N ammonium acetate solution [20].

Mosses together with the soil and substrate samples were dried at 50°C and homogenized. Moss samples were homogenized in a laboratory mill. Soil samples were homogenized with mortar and pestle and coarse material was removed using a 2 mm sieve. All replicate soil, substrate and plant samples were analyzed separately. Plant, soil and substrate material (200 mg) were digested in duplicate with nitric acid (pro analyze, 67%) and hydrogen peroxide (pro analyze, 35%), during which temperatures were raised to about 95°C until the evolution of nitrous gas stopped and the digest became clear. After dilution to 10 ml the plant, soil and substrate digests were analyzed for Al, Cr, Fe, Mn, Ni and Zn, the plant digests were additionally analyzed for K, Ca and Mg using ICPEs Spectroflame SIMSEQ and all digests were analyzed for Cd, Co, Cu and Pb using AAS with graphite furnace Philips PU 9200X. P in soil extract and plant digest was measured using a Technicon AutoAnalyser II. Dried and pulverised plant, soil and substrate samples were used for total N and S (Carlo Erba NA-1500 CNS Analyser).

All elements were determined against standards (BDH Chemicals Ltd, pro analyze quality) and blanks prepared in 0.5 M nitric acid. Blanks and standards contained the same matrix as the samples. All results for soil and plants were calculated on a dry weight basis.

The recovery rates, as compared to the results of an interlaboratory study on digesting and analyzing reference material (Wageningen Evaluating Programmes for Analytical Laboratories, WEPAL), were as follows for each of the investigated elements (percentages with SD): Al (98±3), Ca (101±3), Cd (94±5), Co (105±4), Cr (104±4), Cu (99±5), Fe (96±5), K (100±3), Mg (98±3), Mn (105±3), N (103±5), Ni (103±4), P (98±4), Pb (94±5), S (96±6) and Zn (102±4). The reference material consisted of pine needles (IPE 761) and leaves of *Nymphaea alba* (not coded).

Statistical Analysis

Differentiation between sampling sites with respect to concentration of elements in soil, dump substrate and *P. nutans* were evaluated by a one way ANOVA on log transformed data. The normality of the analyzed features was checked by the Shapiro-Wilk's W test and homogeneity of variances was checked by Bartlett's test [21, 22]. *Post hoc* LSD test was applied for evaluation differences between transplanted, native and control *P. nutans* [22].

t-test was applied on log transformed data to compare the concentration of soil elements.

In order to remove fluctuations of absolute values and to give a clue sources of metals found in the moss, raw data of concentrations were normalized to the soil crustal

abundance pattern by calculating the Enrichment factor (Ef) defined as

$$Ef = \frac{\left(\frac{X}{Al}\right)_{Moss}}{\left(\frac{X}{Al}\right)_{Soil}}$$

[23, 24].

All calculations were done with the program CSS Statistica 7.1 [25].

Results and Discussion

Mean concentrations of elements in the dump substrate, control soil and *P. nutans* from the dump and control sites are presented in Table 1, Figs. 1a-1d. The *t*-test indicated that control soil and dump substrate differ significantly in respect to the contents of the examined elements. Analysis of variance indicated that mosses of all sampling sites differ significantly in respect to the contents of the examined elements.

The concentration of heavy metals in *P. nutans* from the control site was lower than the concentration of these elements in *Pleurozium schreberi* from the non-polluted Puszcza Biała Forest [26], indicating that the selected control site may be recognized as relatively free from influence of anthropogenic pollution.

Compared to the control values concentrations of P, K, Ca, Mg were significantly lower and concentrations of N, S and all investigated heavy metals were significantly higher in substrate and mosses of the dump site.

The mean pH_{KCl} of the dump substrate was 2.9 and that of the control soil was 4.2.

High concentration of metals in native and transplanted *P. nutans* from the dump is in agreement with Huttunen [27], who states that this species commonly colonizes polluted areas in cities and in the surroundings of industrial plants. The low amount of Ca was probably caused by high levels of toxins present in the substrate of the dump. According to Markert and Wtorova [28], Vázquez et al. [29] and Figueira and Ribeiro [30], the presence of high concentrations of heavy metals seems to be directly associated with an exclusion of Ca and sometimes also other nutritional elements, which may be indicated by a concentration of Ca and K below the critical growing limit in both species [31].

According to Dongarra and Varrica [32] and Fernández and Carballeira [17], elements were considered to be enriched when the average Enrichment factor (Ef) was greater than 3 and for a higher degree of certainty, at least 30% of the samples should have an Ef > 3%. Tyler [13], Bargagli [23] and Fernandez and Carballeira [17] have proposed that this way of calculation the enrichment could serve as a method to demonstrate that the presence of metals in mosses comes from another source than soil. Table 2 shows some descriptive statistics of the Ef for *P. nutans* as well as the percentage of samples with Ef > 3%.

Table 1. Range, mean and SD of pH, soil fraction (sf) >2 mm (% weight) and concentration (mg/kg on a dry weight basis) of elements in samples from the soils of dump and control sites. $t_{0.05(48)} \text{ tabular} = 2.01$, t-test results are all significant at $p < 0.05$.

	Dump soil range	mean	SD	Control soil range	mean	SD	t-test
pH H ₂ O	3.0-5.2	3.9	0.5	5.1-5.3	5.2	0.1	-7.8
pH KCl	2.2-4.8	2.9	0.4	4.1-4.3	4.2	0.1	-4.5
sf	21-61	45	7	24-28	25.9	1.5	6.4
N	2130-2960	2651	140	1570-1599	1582	7.2	11.0
P	2.3-3.8	2.9	0.5	231-247	241	5.2	-462
K	0.65-8.7	4.2	1.1	10.6-11.8	11.1	0.4	-2.8
Ca	21-43	33	3.2	55-58	56	0.8	-14.5
Mg	5.0-12.3	8.9	2.4	18-21	20	0.7	-19.3
S	21400-38900	27736	929	1150-1170	1161	7.5	15.7
Al	9026-10139	9610	344	331-439	365	32	7.54
Cd	0.76-0.85	0.81	0.03	0.05-0.08	0.07	0.01	-14.2
Co	19.8-24.8	21.3	1.3	2.0-2.9	2.5	0.3	7.86
Cr	37-49	42	3.1	13-19	16	1.2	14.5
Cu	50-62	54	3.4	14-cze	10	1.4	10.5
Fe	2284-2380	2336	31	618-639	627	8.0	12.1
Mn	472-531	498	17	137-184	157	8.2	13.3
Ni	18-35	25	1.3	0.39-0.47	0.4	0.03	3.7
Pb	140-189	159	12	19-31	27	0.6	11.1
Zn	328-338	332	3.2	21-33	28	3.4	12.8

The results suggest that for Cd, Co, Cr, Cu, Fe, Mn, Ni and Pb in cases of native and transplanted moss, there were other sources of contamination apart from the soil.

Zn was the only element in the native *P. nutans* with an $E_f < 3\%$. Also 30% of the transplanted *P. nutans* has an $E_f < 3$. Čeburnis and Valiulis [33] state that some metals such as Zn enter the moss also from non atmospheric origin. Also, Szczepaniak and Biziuk [24] and Zechmeister et al. [5] are of the opinion that *P. nutans* appears to be an excellent deposition bioindicator for monitoring elements such as Cd, Co, Cr, Cu, Fe, Ni, Pb and only partly Zn. Chiarenzelli et al. [34] suggested that Zn is accumulated by mosses and retained by other way than via deposition of airborne particulates. The values of E_f s for Cd, Co, Cr, Cu, Fe, Mn, Ni, and Pb reflected the emissions produced by the coke factory and the municipal thermal-electric power station [12]. Similar values of E_f s were also reported by Fernandez and Carballeira [17] for *Scleropodium purum* collected in the vicinity of a power station in Spain.

All results taken together show that transplants of *P. nutans* are convenient for biomonitoring air pollution with metals.

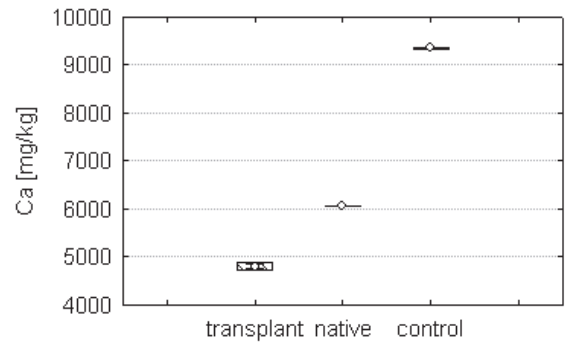
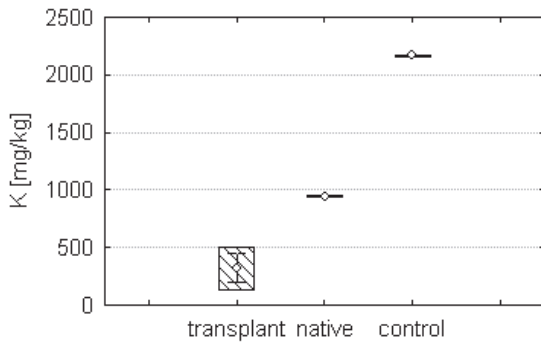
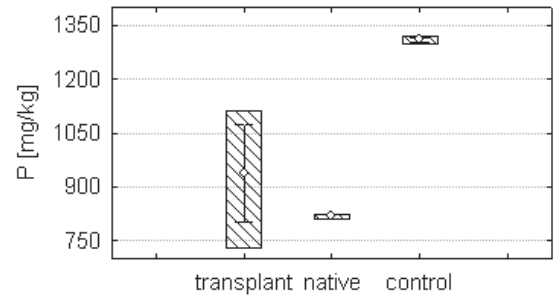
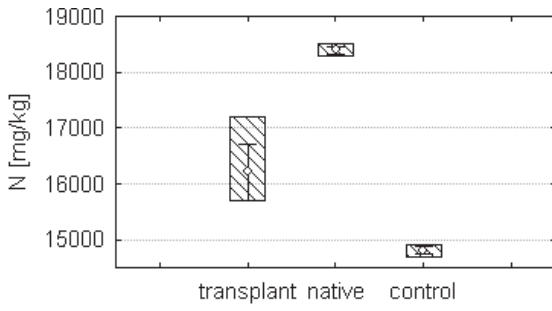
Comparison of concentrations of elements in transplants and native *P. nutans* with *P. nutans* growing on the control soil (Fig.1a.1b.1c.1d) revealed that there was a

significant difference between these mosses in concentration of all elements. Concentrations of P, K, Ca and Mg were significantly higher and all other elements significantly lower in mosses growing at the control site.

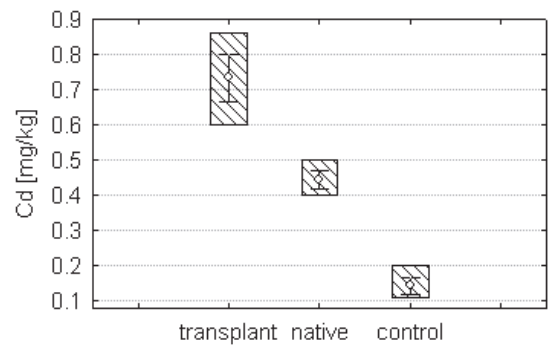
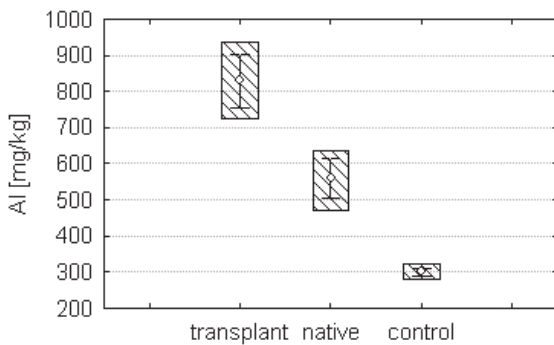
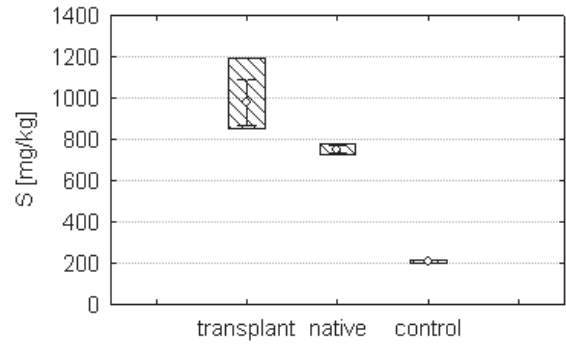
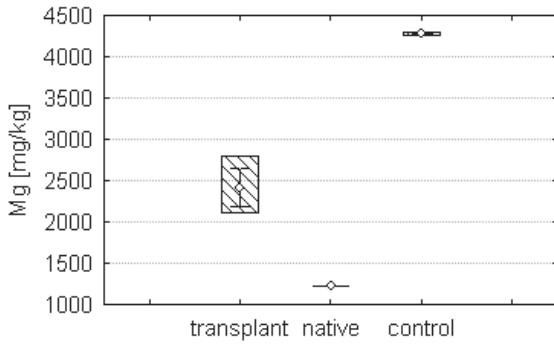
In soils and plants chemical elements are not independent of each other [35, 36]. The dump consists of wastes containing high levels of heavy metals and is additionally regularly polluted with exhausts containing, among other things, high amounts of sulphur oxides and heavy metals. These pollutants probably modified the accumulation of elements in the transplanted mosses so that their concentration was different from that of the mosses growing naturally at the control site having a higher pH than that of the dump substrate, which was extremely acidic. Bryophytes transplanted by Vázquez et al. [29] to severely contaminated sites showed loss of intracellular K (indicating an altered membrane permeability), loss of extracellular Mg and loss of both extra- and intracellular Ca (implying damage to the cell wall). The transplanted moss contained significantly more (Fig.1a.1b) N and S (forming the main components of industrial exhausts deposited on the dump site), heavy metals, and significantly less P, K, Ca and Mg compared to the mosses growing at the control site.

An LSD test for comparison between transplanted *P. nutans* with native *P. nutans* growing on the dump revealed

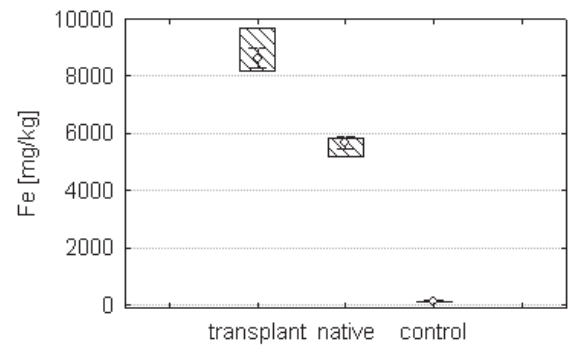
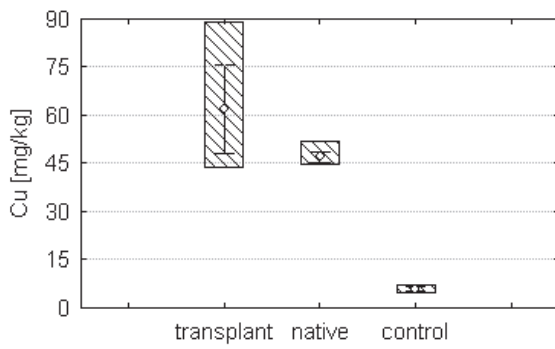
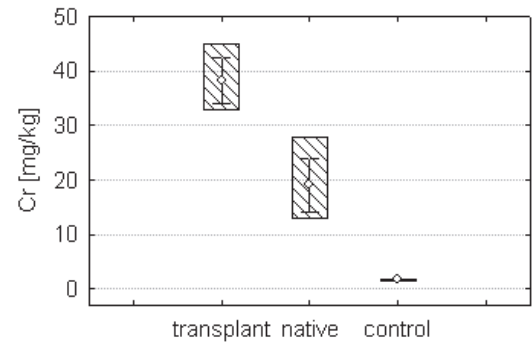
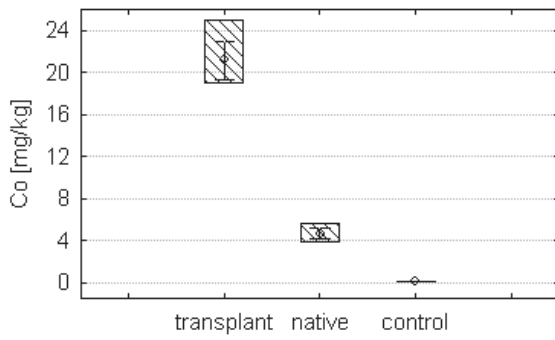
a



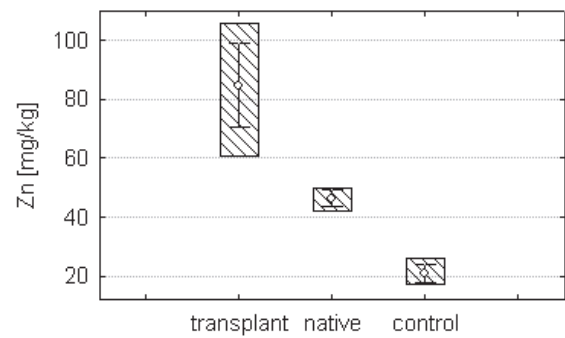
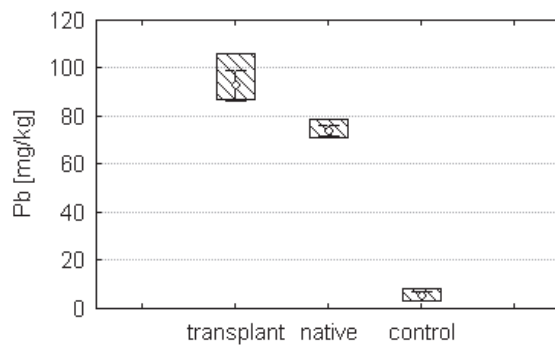
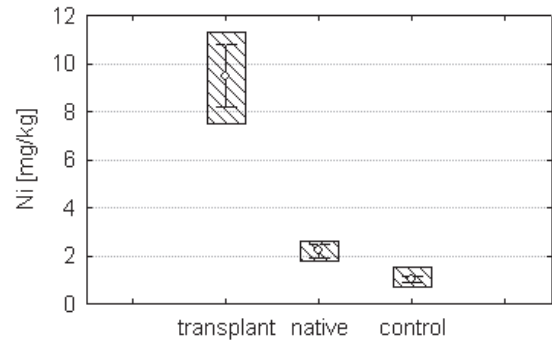
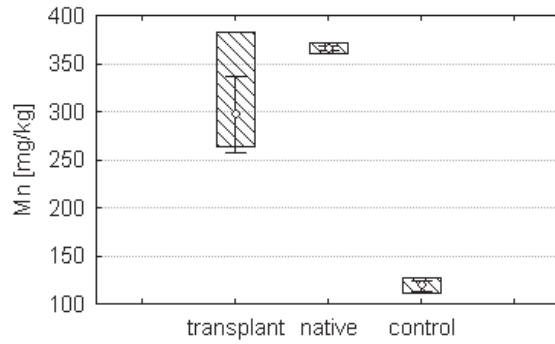
b



c



d



Figs. 1a, b, c, d. Minimum/maximum values (vertical bars), mean (°) and SD (I) of concentrations of elements in the transplanted, native and control *Pohlia nutans*.

Table 2. Assessment of mean values, standard deviation (SD) and range of enrichment factors (Efs) of different metals in *P. nutans* transplants, native and control relative to the total metal content of the soils and the percentage of samples of each metal with Ef >3% (see text).

Element	Mean Ef	Minimum	Maximum	SD	%
Transplant					
Cd	10	8.3	12	1.0	100
Co	4.6	3.6	5.8	0.8	100
Cr	11	8.5	13.3	1.3	100
Cu	13	9.9	18	2.4	100
Fe	44	38	53	3.9	100
Mn	6.9	5.8	8.8	0.6	100
Ni	4.5	2.7	5.6	0.8	100
Pb	6.8	5.4	8.3	0.7	100
Zn	2.9	2.3	3.6	0.3	30
Native					
Cd	14	11	25	3.4	100
Co	4.9	3.9	6.2	0.3	100
Cr	7.8	5.0	11	1.1	100
Cu	19	14	26	2.3	100
Fe	43	37	52	1.5	100
Mn	13	11	15	0.9	100
Ni	5.5	3.6	8.9	0.7	100
Pb	3.7	2.9	4.6	1.2	96
Zn	2.4	2.2	2.7	0.1	0

($p < 0.001$) that the transplants contained significantly more Al, Cd, Co, Cr, Fe, Ni, Pb and Zn and native mosses significantly more N, K and Ca. There was no statistical significant difference between native and transplanted mosses in concentrations of P, Mg, S, Cu and Mn.

The results of the investigations as reported in this paper on the bioaccumulation of elements were clearly different for native mosses than for the transplanted mosses. Comparison of bioconcentrated elements in the native *P. nutans* with those in *P. nutans* transplanted from uncontaminated sites indicated that the latter accumulated significantly more Al, Cd, Co, Cr, Fe, Ni, Pb and Zn. Higher levels of K and Ca in the native *P. nutans* indicate a possible mechanism preventing the loss of these elements as observed in bryophytes transplanted to severely contaminated sites [29].

Conclusions

1. Atmospheric deposition is the main contribution (according to the Enrichment factor Ef) to the levels of Cd, Co, Cr, Cu, Fe, Mn, Ni and Pb in the native and transplanted *P. nutans*.

2. *P. nutans* transplanted from uncontaminated to polluted dump sites accumulated significantly more Al, Cd, Co, Cr, Fe, Ni, Pb and Zn than the native *P. nutans* growing naturally on the dump. Therefore, the transplanted species reflects a better level of contamination of the metals in the examined area.

References

1. GALSOMIES L., LETROUIT M.A., DESCHAMPS C., SAVANNE D., AVNAIM M. Atmospheric metal deposition in France: initial results on moss calibration from the 1996 biomonitoring. *Sci. Total Environ.* **232**, 39, **1999**.
2. CARBALLEIRA A., FERNANDEZ J.A. Bioconcentration of metals in the moss *Scleropodium purum* in the area surrounding a power plant. A geotopographical predictive model for mercury. *Chemosphere* **47**, 1041, **2002**.
3. GALUSZKAA. Geochemical Background of Selected Trace Elements in Mosses *Pleurozium schreberi* (Brid.) Mitt. And *Hylocomium splendens* (Hedw.)B.S.G. from Wigierski National Park. *Pol. J Environ Stud.* **15**, 72, **2006**.
4. GERDOL R., BRAGAZZA L., MARCHESINI R., ALBER R., BONETTI L., LORENZONI G., ACHILLI M., BUF-

- FONI A., DE MARCO N., FRANCHI M., PISON S., GIAQUINTA S., PALMIERI F., SPEZZANTO P. Monitoring of heavy metal deposition in Northern Italy by moss analysis. *Environ. Pollut.* **108**, 201, **2000**.
5. ZECHMEISTER H.G., HOHENWALLNER D., RISS A., HANUS-ILLNAR A. Variations in heavy metal concentrations in the moss species *Abietinella abietina* (Hedw.) Fleisch. According to sampling time, within site variability and increase in biomass. *Sci. Total Environ.* **301**, 55, **2003**.
 6. FERNANDEZ J.A., ABOAL J.R., CARBALLEIRA A. Use native and transplanted mosses as complementary techniques for biomonitoring mercury around an industrial facility. *Sci. Total Environ.* **256**, 151, **2000**.
 7. BROWN D.H., BATES J.W. Bryophytes and nutrient cycling. *Bot. J. Linn. Soc.* **104**, 129, **1990**.
 8. REIMANN C., NISKAVAARA H., KASHULINA G., FILZMOSER P., BOYD R., VOLDEN T., TOMILINA O., BOGATYREV I. Critical remarks on the use of terrestrial moss (*Hylocomium splendens* and *Pleurozium schreberi*) for monitoring of airborne pollution. *Environ. Pollut.* **113**, 41, **2001**.
 9. FERNANDEZ J.A., REY A., CARBALLEIRA A. Differences in the responses of native and transplanted mosses to atmospheric pollution: a possible role of selenium. *Environ. Pollut.* **10**, 73, **2000**.
 10. FERNANDEZ J.A., CARBALLEIRA A. Differences in the response of native and transplanted mosses to atmospheric pollution: a possible role of selenium. *Environ. Pollut.* **110**, 73, **2000**.
 11. SAMECKA-CYMERMAN A., KOLON K., KEMBERS A.J. Comparison of Native and Transplanted *Fontinalis antipyretica* Hedw. as Biomonitors of Water Polluted with Heavy Metals. *Sci. Total Environ.* **341**, 97, **2005**.
 12. KABATA-PENDIAS A. Trace Elements in Soils and Plants, CRC Press, Boca Raton, London, New York, Washington D.C., pp. 413, **2001**.
 13. TYLER G. Bryophytes and heavy metals: a literature review. *Bot. J. Linn. Soc.* **104**, 231, **1990**.
 14. Wojewódzki Inspektorat Ochrony Środowiska WIOS [Polish Provincial Authority for Environmental Protection]. Raport o stanie środowiska w województwie dolnośląskim [Report about the quality of the environment in the Lower Silesia province]. Biblioteka Monitoringu Środowiska: Wrocław, pp. 237, **2001**.
 15. VÁZQUEZ M.D., WAPPELHORST O., MARKERT B. Determination of 28 Elements in Aquatic Moss *Fontinalis antipyretica* Hedw. And Water from the Upper Reaches of the River Nysa (Cz, D), by ICP-MS, ICP-OES and AAS. *Water Air Soil Poll.* **152**, 153, **2004**.
 16. RÜHLING A. Atmospheric heavy metal deposition in Europe-estimations based on moss analysis. *Nordic Council of Ministries Copenhagen Nord* **9**, 44, **1994**.
 17. FERNANDEZ J.A., CARBALLEIRA A. A comparison of indigenous mosses and topsoils for use in monitoring atmospheric heavy metal deposition in Galicia (northwest Spain). *Environ. Pollut.* **114**, 431, **2001**.
 18. UNECE. Heavy metals in European mosses 2000/2001 survey. UNECE ICP Vegetation. Compiled by A. Buse and D. Norris and H. Harmens and P. Buker and T. Ashenden and G. Mills, Center for Ecology and Hydrology, Bangor University of Wales Bangor, UK. pp. 45, **2003**.
 19. FERNANDEZ J.A., REY A., CARBALLEIRA A. An extended study of heavy metals deposition in Galicia (NW Spain) based on mosses analysis. *Sci. Total Environ.* **254**, 31, **2000**.
 20. SCHLICHTING E., BLUME, H.P., STAHR, K. *Bodenkundliches Praktikum*. Blackwell Wissenschafts Verlag, Berlin, **1995**.
 21. ZAR H. *Biostatistical analysis*. Prentice Hall: Upper Saddle River, New Jersey, pp. 931, **1999**.
 22. SOKAL R.R., ROHLF F.J. *Biometry. The Principles and Practice of Statistics in Biological Research*. Freeman and Company: New York, pp. 859, **2003**.
 23. BARGAGLI R. Trace elements in terrestrial plants. *An Ecophysiological Approach to Biomonitoring and Biorecovery*, Springer Verlag: Berlin, pp. 324, **1998**.
 24. SZCZEPANIAK K., BIZIUK M. Aspects of the biomonitoring studies using mosses and lichens as indicators of metal pollution. *Environ. Res.* **93**, 221, **2003**.
 25. STATSOFT, STATISTICA (data analysis software system), version 7.1, **2005**, www.statsoft.com.
 26. KOZANECKA T., CHOJNICKI J., KWASOWSKI W. Content of heavy metals in Plant from Pollution-Free Regions. *Pol. J Environ Stud.* **11**, (4), 395, **2002**.
 27. HUTTUNEN S. Reproduction of the moss *Pleurozium schreberi* and *Pohlia nutans* in the surroundings of copper smelters at Harjavalta, SW Finland. *J. Bryol.* **25**, 41, **2003**.
 28. MARKERT B., WTOROVA W. Inorganic chemical investigations in the Forest Biosphere Reserve near Kalinin. Part 3. Comparison of the multielement budget with a forest ecosystem in Germany – aspects of rejection indication and accumulation of chemical elements, *Vegetatio* **98**, 43, **1992**.
 29. VÁZQUEZ M.D., FERNANDEZ J.A., LOPEZ J., CARBALLEIRA A. Effects of water acidity and metals concentration on accumulation and within-plant distribution of metals in the aquatic bryophyte *Fontinalis antipyretica*. *Water Air Soil Poll.* **120**, 1, **2000**.
 30. FIGUEIRA R., RIBEIRO T. Transplants of aquatic mosses as biomonitors of metals released by a mine effluent. *Environ. Pollut.* **136**, 293, **2005**.
 31. MARKERT B. Multi-element Analysis in Plant Materials – Analytical Tools and Biological Questions in: D.C. Adriano (ed). *Biogeochemistry of Trace Metals*, Lewis Publishers, Boca Raton, pp. 402-428, **1992**.
 32. DONGARRA G., VARRICA D. The presence of heavy metals in air particulate at Volcano Island (Italy). *Sci. Tot. Environ.* **212**, 1, **1998**.
 33. ČEBURNIS D., VALIULIS D. Investigation on absolute metal uptake efficiency from precipitation in moss. *Sci. Total Environ.* **226**, 247, **1999**.
 34. CHIARENZELLI J., ASPLER L., DUNN C., COUSENS B., OZARKO D., POWIS K. Multi-element and rare earth element composition of lichens, mosses, and vascular plants from the central Barrenlands. Nunavut, Canada. *Appl. Geochem.* **16**, 245, **2001**.
 35. GENONI P., PARCO V., SANTAGOSTINO A.S. Metal biomonitoring with mosses in the surroundings of an oil-fired power plant in Italy. *Chemosphere* **41**, 729, **2000**.
 36. SCHLEPPI P., TOBLER P., BUCHER J.B., WYTTEBACHA. Multivariate interpretation of the foliar chemical composition of Norway Spruce (*Picea abies*). *Plant Soil* **219**, 251, **2000**.