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ORIGINAL PAPER

ACCUMULATION OF HEAVY METALS IN FOREST DWARF SHRUBS AND DOMINANT MOSSES AS BIOINDICATORS OF ATMOSPHERIC POLLUTION

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Abstract

This article discusses the environmental concerns about heavy metal accumulation in dominant forest mosses and dwarf shrubs. Samples of two different species of mosses, such as glittering wood moss (*Hylocomium splendens*) and feathermoss (*Pleurozium schreberi*), and two species of dwarf shrubs, that is European blueberry (*Vaccinium myrtillus*) and lingonberry (*Vaccinium vitis-idaea*), were collected from specifically chosen areas with evident anthropogenic pollution (vicinity of an airport and close to heavy road traffic) and from contamination-free, secluded forest areas in Lithuania and Sweden. Instrumental analysis of heavy metals, including cadmium, chromium, copper, lead and zinc, revealed that the selected plant species tend to accumulate zinc and copper the most and chromium the least. Forest mosses, used as indicators of airborne pollution, accumulated less heavy metals than dwarf shrubs, indicating more metal accumulation from the soil. The results also revealed that forest dwarf shrub leaves even picked from areas with known anthropogenic pollution sources were safe to use, since concentrations of cadmium and lead remained below the maximum permissible level. A multivariate data analysis model with two principle components explained more than 70% of the data variation. The heavy metal content in soil was the most distinctive factor separating the Lithuanian and Swedish sites. Cadmium and chromium soil contents were more significant in the Lithuanian soils, whereas copper, lead, and zinc were more significant in the Swedish soils.

Keywords: bioaccumulation, contaminated soil, cadmium, feathermoss, *Vaccinium myrtillus, Vaccinium vitis-idaea.*

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INTRODUCTION

Contamination with heavy metals (HM) can originate from both natural and anthropogenic sources. Weathering of the parental material, rock outcropping, volcanoes and wild fires cause natural contamination, whereas agriculture, industry, domestic effluents, waste incineration, landfills and transportation are sources of anthropogenic contamination (Nagajyoti et al. 2010). Heavy metals are released into the environment during these activities in elemental and compound (organic and inorganic) forms. This means that the prevailing contributor is of an anthropogenic source and all terrestrial ecosystems, including snow cover, soil, surface and groundwater and the biota are affected (Thakur et al. 2016). Heavy metals cannot be degraded via microbial or chemical pathways, so they persist in the environment for a very long time. Together with the essential macro- and micronutrients, plants absorb hazardous metals. Nevertheless, HM uptake is not directly related to their concentration in the soil. Factors affecting the uptake of such elements include temperature, soil pH, electrical conductivity, redox potential, moisture content, soil aeration, type of vegetation, fertilization level, etc. (Bolan et al. 2014). Moreover, lower plant species like mosses and lichens can be used to assess airborne heavy metal pollution, on account of lacking a developed root system, which makes them absorb macro- and micronutrients, as well as HM, directly from the atmosphere (Blagnyte, Paliulis 2011, Zhou et al. 2017). Plants provide valuable nutritional elements for humans and animals. They also form the primary link between the soil and air elemental structure and the food chain. Therefore, contamination with HM poses a considerable risk (Gall et al. 2015, Clemens, Ma 2016).

There is high demand for dwarf shrub berries, such as lingonberry and blueberry, not only because of their flavour and use in foodstuffs, but also because of their therapeutic properties. Every year, around 100 000 kg of lingonberries and more than 1 000 000 kg of blueberries are collected and purchased in Lithuania (Lithuanian Department of Statistics 2013). Berries collected in the forest are assumed to be pollutant-free, but atmospheric HM deposition can spoil their quality and affect human and animal health. However, scientific literature on this subject is very limited.

Monitoring HM accumulation in mosses was started in Sweden in 1980, and the general idea of using dominant mosses for this purpose was first mentioned in the late 1960s by Rühling, Tyler (1968). It is assumed that carpet-forming moss species take up most of their nutrients from precipitation (wet, occult or dry). Therefore, they can be used as natural deposition indicators for heavy metals.

Of all the heavy metals, Cd, Cu, Pb and Zn are the ones most often found at increased concentrations in soils and plants. Although Cu, Zn and Cr are essential elements and have several biological functions, an excess of these elements is harmful. Furthermore, Cd and Pb are non-biogenic and do not have any known beneficial purposes in cells (Bolan et al. 2014).

The aim of this research was to evaluate the accumulation of HM in the leaves of dwarf shrubs, such as European blueberry (*Vaccinium myrtillus*) and lingonberry (*Vaccinium vitis-idaea*), and dominant mosses, i.e. glittering wood moss (*Hylocomium splendens*) and feathermoss (*Pleurozium schreberi*) as indicators of anthropogenic airborne pollution.

MATERIAL AND METHODS

Plant samples were collected in summer 2016, from areas subject to anthropogenic pollution and from secluded spots, where anthropogenic influence was minimal. Papiškinė forest in Zapyškis, central Lithuania, and a forest 10 km away from the town Växjö (close to Södre Lake) in southern Sweden were taken as point-source contamination-free sites, i.e., agricultural objects and industrial establishments or infrastructure were absent within the perimeter of at least 10 km. Turžėnai forest near the Kaunas airport in Lithuania and the roadsides of the Växjö-Kalmar arterial road in Sweden were considered to be potentially contaminated territories. Entire moss specimens and leaves of blueberries and lingonberries were picked randomly from 10 ± 2 spots in research subplots (20 x 20 m) in each area (Figure 1).

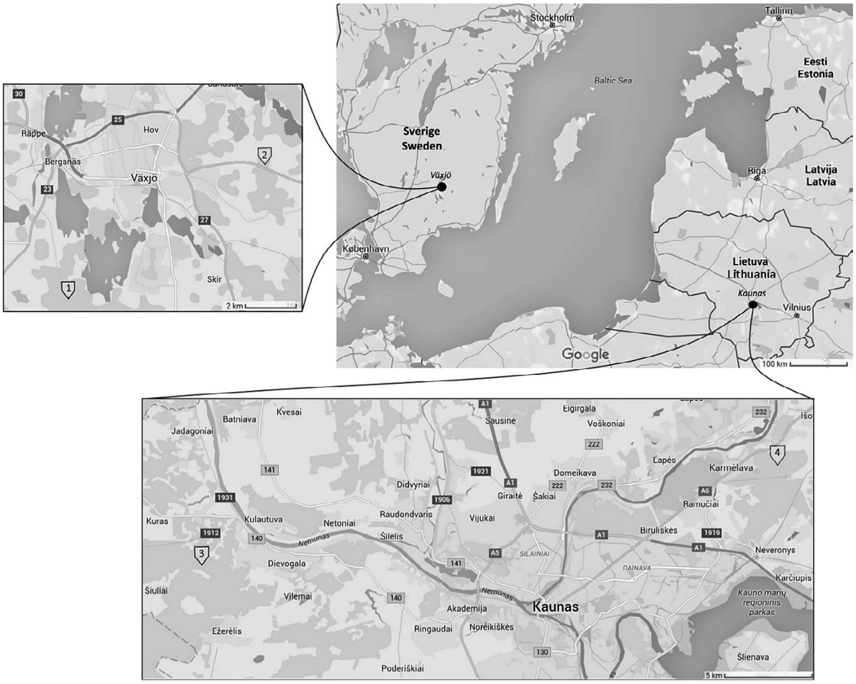

Fig. 1. Locations of subplots in Växjö, Sweden and in Kaunas, Lithuania: 1st tag – forest next to Södre Lake, 10 km away from Växjö city; 2nd tag – sides of the Växjö-Kalmar road; 3rd tag – Papiškinė forest in Zapyškis; 4th tag – Turžėnai forest near Kaunas airport

All selected subplots were fairly open; picking directly under trees was avoided. Joint soil samples were pooled from the same subplots as the dwarf shrubs and mosses. The soil was taken from 0-0.1 m depth, mixed thoroughly, sieved to pass a 2 mm mesh screen, homogenized and brought to the laboratory, where it was air-dried. Soil pH was measured in 1:1 volume to volume suspension of soil and distilled water.

Dry plant material was wet-digested with a mixture of HNO_3 and H_2O_2, whereas the soil was wet-digested in *aqua regia* using a CEM Mars 5 microwave digestion oven. Acid digestions were performed in triplicate for all samples. After cooling and diluting with deionised water, the Cd, Cr, Cu, Zn and Pb concentrations in the digestates were determined on an Analytik Jena AG model AAS-vario 6 flame atomic absorption spectrometer. The results were verified by analysing standard Cd-, Zn-, Cr- Cu- and Pb-nitrate solutions by MERC. The wavelengths and detection limits (DL) used in instrumental analyses were the following: for Cd – 228.8 nm and 0.003 μg l^{-1}, Cr – 359.3 nm and 0.261 μg l^{-1}, Cu – 324.8 nm and 0.392 μg l^{-1}, Pb – 217 nm and 1.103 μg l^{-1}, Zn – 213.9 nm and 4.112 μg l^{-1}, respectively. Glassware and other equipment used for the analyses of metals were thoroughly cleaned, and analytically pure chemicals were used.

Heavy metal uptake by mosses and dwarf shrub leaves was evaluated by calculating the average concentrations and standard deviations. The concentrations of accumulated Cd, Zn, Cu, Cr and Pb in the plant material are presented as mg kg^{-1} in the dry matter (DM). A two-sample t-test was applied to differentiate between the sample means. It was assumed that a two-sample difference was significant when $p < 0.05$. Bioaccumulation factors (BF), as a ratio between the HM concentration in the soil and selected plant organs, were calculated for dwarf shrub leaves (Yoon et al. 2006). Multivariate data analysis was performed using SIMCA-14 (Umetrics). Principle component (PC) analysis was used to identify groups of observations within the data set that indicated distinctive differences between the four analysed areas.

RESULTS AND DISCUSSION

Heavy metal content in the soil

The average HM concentrations in the composite soil samples from the four areas are given in Table 1. Soils obtained from the plots in Sweden showed lower HM content in the soil from the forest and a slightly higher HM content in the soil from the road sides. Surprisingly, soil from Papiškinė forest had slightly higher concentrations of all analysed metals, apart from Cu, than the soil from Turžėnai forest, which was considered a potentially contaminated site. Maximum permissible concentrations (MPC) in the soil, according to the Lithuanian Hygiene Standard HN 60:2015; and limit values

Table 1

Average heavy metal concentrations ± standard deviations (n = 3) in the joint soil samples

Territory	Concentration (mg kg^{-1} DM)				
	Cd	Cr	Cu	Pb	Zn
Turžėnai forest	0.201 ± 0.021	9.004 ± 0.281	10.10 ± 1.134	10.75 ± 0.781	23.05 ± 0.352
Papiškinė forest	0.321 ± 0.011	16.65 ± 1.773	6.217 ± 0.420	15.81 ± 0.850	28.25 ± 1.064
Växjö forest	0.123 ± 0.012	7.150 ± 0.210	11.90 ± 0.422	9.002 ± 0.424	17.45 ± 1.060
Växjö-Kalmar road	0.154 ± 0.014	9.151 ± 0.491	15.45 ± 0.491	20.05 ± 1.483	36.40 ± 0.561
MPC*	1.500	80.00	75.00	80.00	300.0
KM**	0.800	80.00	80.00	50.00	250.0
MKM***	12.00	150.0	200.0	400.0	500.0

* Maximum permissible concentrations according to the Lithuanian Hygiene Standard HN 60:2015.

** Maximum permissible concentrations for the sensitive land use according to the Swedish Environment Quality Standard.

*** Maximum permissible concentrations for the less sensitive land use according to the Swedish Environment Quality Standard.

for sensitive land use (KM) and less sensitive land use (KMK) according to Swedish Environment Quality Standard are also shown in Table 1. None of the analysed soils exceeded the limit values.

All four analysed soils showed slightly acidic pH values. pH in the soil from Turžėnai forest was 6.0 ± 0.11, Papiškinė forest – 6.2 ± 0.14, Växjö road – 5.9 ± 0.20, and Växjö forest – 6.4 ± 0.05. Soil pH is often regarded to have a significant influence on the mobility of HM and their bioavailability to plants. As stated in Parzych (2014), acidity of the upper soil layer can have influence on the HM uptake by mosses as well. Both soils from the potentially contaminated sites had a slightly lower pH as compared with the point-source contamination-free sites. However, the differences between samples were insignificant and had no clear pattern with regards to the bioaccumulation of HM.

Heavy metal accumulation in mosses

It was expected that mosses collected from potentially contaminated sites would have higher HM concentrations as an indication of some anthropogenic impact, especially airborne pollution. However, the results did not provide a clear pattern (Figure 2).

Glittering wood moss from Papiškinė forest, which was considered to be a site free from anthropogenic influence, accumulated less HM than its counterpart from Turžėnai forest, which is located close to the Kaunas airport. Moreover, the soil from Papiškinė forest had a higher HM content, except for Cu. Heavy metal accumulation in glittering wood moss was very similar

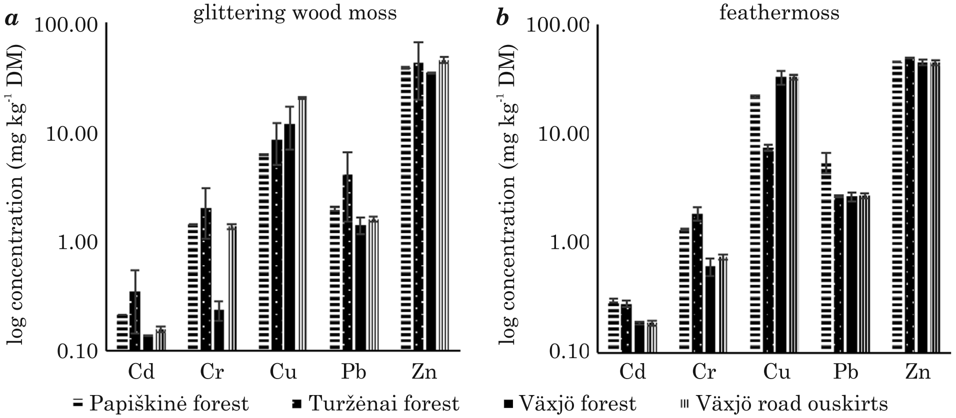

Fig. 2. Average heavy metal concentrations ± standard deviations (n = 3) in glittering wood moss (a) and feathermoss (b)

at both sites in Sweden. Only Cr accumulation was significantly higher (by 1.7 times) in glittering wood moss from the Växjö-Kalmar roadsides. Therefore, these results are in line with other studies (e.g. Bargagli 2002), stating that HM enter mosses from the atmosphere rather than from the soil.

Feathermoss from Papiškinė forest accumulated more Cu and Pb (21.80 and 5.611 mg kg^{-1} DM, respectively) than the feathermoss from Turžėnai forest (7.441 and 2.721 mg kg^{-1} DM, respectively). It can be speculated that the higher Pb content in the feathermoss from Papiškinė forest is related to the higher Pb content in the soil, but it is not clear why Cu accumulation was also higher when the soil-Cu content was low. The feathermoss samples from the Swedish sites had very similar HM concentrations despite having been collected from two different places.

Mosses are not consumed or used for medical purposes, so the HM content in their tissues is not legally regulated. Because a moss survey is included into the International Cooperative Programs (ICP) for integrated monitoring of long-range transboundary air pollution, and because this survey includes large areas in Europe and many elements to be analysed, it is possible to use moss data as an indication of the atmospheric input of pollutants into various catchment basins (Harmens et al. 2010, Harmens et al. 2013, Schröder et al. 2016). Dominant mosses were collected from 28 countries in Europe by Harments et al. (2010). Feathermoss and glittering wood moss covered more than 60% of all collected plant samples. The Lithuanian and Swedish data showing average HM concentrations in mosses are collated in Table 2. Heavy metal concentrations detected in the Lithuanian moss samples during our study were higher in all cases except for Pb. The HM content in moss tissues from the Swedish sites were higher for all analysed metals except Cr. In both cases, the Cu and Zn concentrations were considerably higher than those reported by Harmens et al. (2010). Although

Table 2

Average heavy metal concentration ± standard deviation in mosses collected in Lithuania and Sweden during the moss survey (Harmens et al. 2010)

Concentration (mg kg^{-1} DM)	Country	
	Lithuania	Sweden
Cd	0.130 ± 0.031	0.140 ± 0.067
Cr	1.012 ± 0.381	3.971 ± 0.612
Cu	5.193 ± 1.612	3.564 ± 1.523
Pb	4.643 ± 1.077	2.154 ± 2.106
Zn	17.78 ± 4.925	30.64 ± 10.69
Number of sites	146.0	538.0

differences may be due to a low number of samples in our study and/or different extraction techniques, it is clear that plants even from sites free from anthropogenic influence are also exposed to atmospheric HM deposition.

Heavy metal accumulation in dwarf shrubs

Figure 3 shows that blueberry (a) and lingonberry (b) leaves in most cases accumulated similar ($p > 0.05$) HM concentrations, regardless of the site from which they were collected. Only Cd was significantly higher in the Swedish sites. This could be due to a larger mobile fraction. As reported in the scientific literature, approximately sufficient or normal HM concentrations in mature leaf tissues for various species are: 0.05-0.2 mg kg^{-1} DM for Cd, 0.1-0.5 mg kg^{-1} DM for Cr, 5-30 mg kg^{-1} DM for Cu, 5-10 mg kg^{-1} DM for Pb and 27-150 mg kg^{-1} DM for Zn (Intawongse, Dean 2005, Magahud et al. 2015, Arif et al. 2016). None of the analysed HM exceeded these values. Furthermore, Cr, Cu and Zn are essential to living organisms, so there are

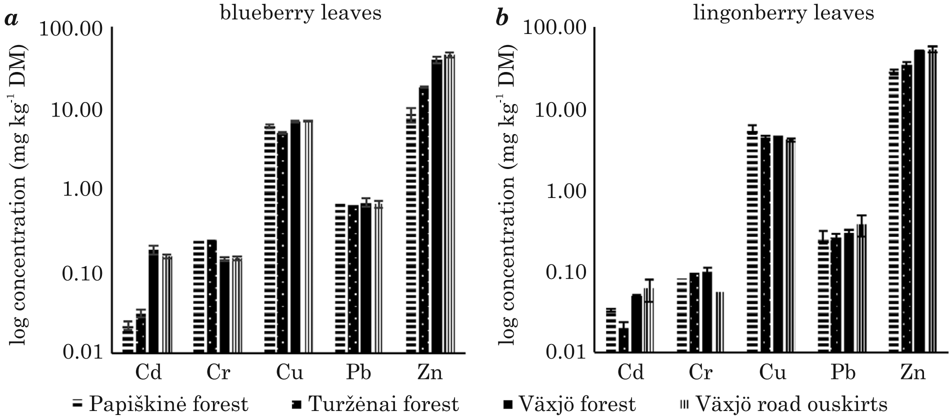

Fig. 3. Average heavy metal concentrations ± standard deviations ($n = 3$) in blueberry (*a*) and lingonberry (*b*) leaves

no MPC limits in foodstuff for these elements. However, Cd and Pb are toxic even at low concentrations. Commission Regulation 1881/2006 sets MPCs for certain contaminants in foodstuffs. The maximum permissible concentration is 0.2 mg kg^{-1} for Cd and 0.1 mg kg^{-1} for Pb in fresh herbs (berries excluded). Assuming that fresh leaves of dwarf shrub contain up to 70-80% water, the Cd concentration should not exceed 1 mg kg^{-1} DM and the Pb concentration should not exceed 1.5 mg kg^{-1} DM. Both Cd and Pb were below the MPC values, meaning that leaves collected even from areas with known anthropogenic pollution sources were safe to use. The Cd concentrations detected in the lingonberry and blueberry leaves from the Papiškinė and Turžėnai forests were very similar (0.030 and 0.023 mg kg^{-1} DM, respectively). The blueberry and lingonberry leaves from Växjö contained more Cd than those from the Lithuanian sites. The highest Cd concentration, 0.181 ± 0.021 mg kg^{-1} DM, was detected in blueberry leaves which were picked from plants growing along the Växjö-Kalmar road.

Table 3 shows the BF values for blueberry and lingonberry leaves. A bioaccumulation factor was calculated only for dwarf shrub plants because they have roots and can uptake nutrients and other elements from the soil solution. The transport of HM from the roots to other organs depends on electrochemical differences between the elements. Cadmium and zinc are moderately mobile elements, whereas Cu, Cr and Pb are strongly bound in roots cells (Schützendübel, Polle 2002). This study confirms the above tendency, and based on their potential to be transferred from the soil to leaves heavy metals can be ranked in the following order: Zn > Cd ≥ Cu > Cr > Pb. Most medicinal herbs can accumulate rather high levels of HM, if they are present in the habitat. Furthermore, older plants contain higher metal content. Usually, their curative properties come from organic compounds, such as glycosides or alkaloids (Annan et al. 2013). Therefore, HM can have additional impacts on plant quality and food safety. Plant cell structure also plays a role in element bioaccumulation. Table 3 shows that in most cases

Table 3

Bioaccumulation factors for blueberry and lingonberry leaves

Site and plant	Element				
	Cd	Cr	Cu	Pb	Zn
Turžėnai forest, blueberry	0.114	0.037	0.598	0.061	0.370
Turžėnai forest, lingonberry	0.162	0.015	0.543	0.037	1.186
Papiškinė forest, blueberry	0.100	0.012	0.836	0.047	0.631
Papiškinė forest, lingonberry	0.061	0.014	0.712	0.029	1.183
Växjö forest, blueberry	1.533	0.024	0.588	0.087	2.233
Växjö forest, lingonberry	0.416	0.017	0.372	0.034	2.878
Växjö-Kalmar road, blueberry	1.023	0.027	0.455	0.031	1.242
Växjö-Kalmar road, lingonberry	0.405	0.018	0.262	0.021	1.407

Cd, Cr, Cu and Pb were transported more actively from the soil to blueberry leaves than to lingonberry leaves. It is difficult to explain why the bioaccumulation of Cd is so different between the two analysed dwarf shrubs. Zinc bioaccumulation showed the opposite trend, and was always significantly higher in lingonberries.

Finally, it should be noted that mosses like glittering wood moss usually consist of at least 3 years' growth, building one floor each year. Blueberry is deciduous and sheds leaves every year and lingonberry is evergreen. It has to be acknowledged that the accumulation of HM in leaves from dwarf shrubs may depend on the HM soil concentration, air pollution and the age of leaves. The older the leaf, the more soil and air metal it should potentially contain. Therefore, it is difficult to compare the accumulation patterns of different plants.

Principle component analysis

Most of the results in the multivariate data analysis model are described by two principle components: PC1 and PC2, and together they explain 74% of the data variation (Figure 4). The score scatter plot (Figure 4*a*) shows how observations from the four investigated sites are located in the score space with respect to each other. The plot shows groupings of two because the third and most outlying value for each triplicate was discarded. The loading scatter plot (Figure 4*b*) is used to explain which variables cause the grouping because the averages for each variable were loaded at the same position and correlate with their scores. The most distinctive factor separating the Lithuanian and Swedish sites was the HM content in the soil. Both the Cd and Cr concentrations in the moss species and Cr in the dwarf shrub leaves were grouped close to the soil Cr and Cd (located left from 0; 0), which indicated a positive correlation between these observations and the Lithuanian sites. On the other hand, both Swedish territories correlated with the soil Cu content, whereas the soil Pb and Zn contents were more distinctive for the site next to the Växjö-Kalmar road. The Cu contents in dwarf shrub leaves and

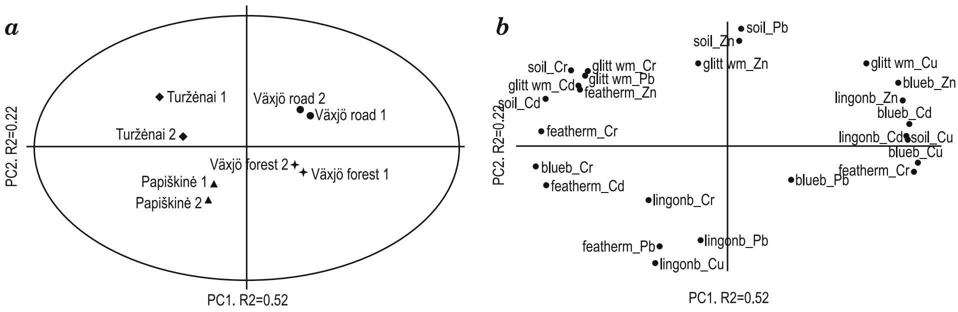

Fig. 4. Principle component analysis of HM content in the soil, dwarf shrub leaves and mosses: *a* – score scatter plot, *b* – load scatter plot. PC1 and PC2 – principal components 1 and 2, respectively, blueb – blueberry leaves, lingonb – lingonberry leaves, featherm – feathermoss, glitt wm – glittering wood moss

glittering wood moss were grouped next to the soil Cu content. There was a strong negative correlation between soil Pb content and the Pb concentration in dwarf shrub leaves and feathermoss, as these points are located far away from each other. This coincides with a very low BF for both dwarf shrub species.

It was expected initially that both dominant mosses and dwarf shrub leaves would accumulate increased amounts of HM when growing in environments with a heavy anthropogenic impact, such as airport and road traffic, compared with areas located away from urban transportation and industrial activities. In most cases, this assumption was correct. Our findings coincide with those of GRIGALAVIČIENĖ et al. (2005), who investigated the accumulation of Pb, Cu and Cd in roadside forest soil. They concluded that the highest HM concentrations were found 5 m from the highway, and they tended to decrease as deeper into the forest. However, in this study the accumulated HM concentrations in plants taken from secluded places seldom differed significantly, and in some cases the concentrations in certain species were even higher in the contamination-free areas than in the ones with anthropogenic impact. Such findings may indicate that HM compounds are actively dispersed by air transport, and that ecosystems considered as contamination-free are affected as well. But lacking values considered as alerting HM concentrations in mosses and having MPC values only for fresh herbs and only for Cd and Pb, further analysis of our results is difficult.

CONCLUSIONS

Instrumental analysis was used to detect the Cd, Cr, Cu, Pb and Zn concentrations in forest plant samples. The sequence Zn > Cu > Pb > Cr > Cd for total heavy metal content was the same for both blueberry and lingonberry. Both Cd and Pb concentrations remained below the maximum permissible limits, meaning that dwarf shrub leaves collected even from areas with known anthropogenic pollution sources were safe to use.

Two dominant moss species were used for comparison as indicators of anthropogenic airborne pollution. The results revealed that mosses collected from the sites free from anthropogenic influence contained similar heavy metal concentrations as their counterparts from the sites with evident anthropogenic pollution. This means that plants growing in secluded places can be exposed to atmospheric heavy metal deposition as well. Furthermore, use of feathermoss and glittering wood moss is a conventional method for rapid identification of atmospheric pollution owing to a large set of data available from the previous and ongoing surveys.

The multivariate data analysis model with two principle components explained 74% of the data variation. The heavy metal content in the soil was the most distinctive factor separating the Lithuanian and Swedish sites. Cadmium and Cr soil contents were more significant in the Lithuanian soils, whereas Cu, Pb and Zn were more significant in the Swedish soils.

Sequential extraction or a bioavailability assay should be included in future studies in order to clarify tendencies in heavy metal bioaccumulation.

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