



Metal Accumulation and Functional Traits of *Maianthemum bifolium* (L.) F. W. Schmidt in Acid Beech Forests Differing with Pollution Level

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Abstract *Maianthemum bifolium* (L.) F. W. Schmidt is a clonal plant with a wide geographical range throughout Europe and Asia. It is also abundant as an understory plant of acid beech forests in southern Poland. The response strategies of this species to heavy metals and their effects on some functional traits (height, specific leaf area (SLA), leaf dry matter content (LDMC), specific rhizome length (SRL) and rhizomes dry matter content (RDMC)) were tested. Selected heavy metal concentrations were measured in leaves, rhizomes and rooting soil of May lily in five beech forests of southern Poland, mainly including an industrialised region of Upper Silesia. The contamination level of these ecosystems was assessed with a single pollution index. The results show significant contamination with Pb even at control sites and moderate with Cd, Zn, Fe and Cu in Upper Silesian forests. May lily accumulated Pb, Zn and Fe mainly in rhizomes, but Ni, Cu and Cd were also translocated to aboveground organs in comparable quantities, which confirms the indicator value of this plant. May lily accumulated up to 21 and 30 mg g⁻¹ Cd, 34 and 90 mg g⁻¹ Pb and 250 and 553 mg g⁻¹ Zn in leaves and rhizomes respectively. Moreover, the accumulation factors show that May lily accumulates Cd in

above- and underground organs in higher amounts than found in soil. Although high concentrations of these toxic elements in tissues, no visible damages on plants were observed, also the measured functional traits show no apparent relationship with the pollution level.

Keywords Bioaccumulation · Heavy metals · Functional traits · *Maianthemum bifolium* · Soil pollution

1 Introduction

One of the most dangerous environmental pollutants are heavy metals. Apart from natural sources such as weathering of rocks or volcanic eruptions, their presence in the environment is related to human activity—industrialisation and urbanisation (Wang et al., 2020; Yuanan et al., 2020). Elevated concentrations of these elements are listed in areas of ore extraction and processing (Demková et al., 2017; Stefanowicz et al., 2014; Yang et al., 2018) and in areas affected by the emissions from various industries (Loska et al., 2004; Zhao et al., 2014), as well as in city centres (Burt et al., 2014; Cai et al., 2013; Zhao & Li, 2013). Such is the situation in Upper Silesia—the southern region of Poland, which has undergone a strong anthropopression since the Middle Ages, which is connected with the presence of metal ores and coal in these areas (Cabała et al.,

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2020). Although this region is strongly industrialised and urbanised, the forests cover about 31.9% of the area, playing an important role in purifying the atmosphere (Jamnická et al., 2013). In the last decades of the twentieth century, industrial pollution and environmental degradation in Poland were highest in Europe (Cole, 1991). Over the past years, the situation has improved significantly; however, the problems of urban pollution, road transport and low emissions are existing (Černíkovský et al., 2016) and still in 2018, Poland was at the forefront of the PM₁₀ and PM_{2.5} annual mean concentrations amongst European countries (Ortiz & Guerreiro, 2020). Studies show that dust emissions are deposited during past decades in the organic level of forest soils, and their acidic reaction contributes to increased metal mobility in the soil and increases metal availability, and thus inclusion in biogeochemical cycles, amongst others, through plant tissue (Jamnická et al., 2007; Magiera et al., 2007).

The influence of heavy metals on living organisms has been studied on a biochemical level (effect on DNA (e.g. Kaur et al., 2014; Siddiqui, 2015) and intracellular enzyme activity (Panda et al., 2011)) and physiological level (such as photosynthetic activity (e.g. Bernardini et al., 2016; Vesley et al., 2012) and transpiration process (e.g. Haag-Kerwer et al., 1999; Singh & Bhati, 2003)). Patterns of metal accumulation in tissues are also widely studied in order to identify indicator, accumulator or excluder plants for phytoremediation, monitoring or ecotoxicity evaluation purposes (e.g. Christou et al., 2017; Dinu et al., 2020; Sert et al., 2019). There are also studies on morphological plant traits; however, they are relatively few and often are performed in laboratory conditions (e.g. Islam et al., 2007; Ouzounidou et al., 1995; Todeschini et al., 2011). The study of plants in natural conditions is most often carried out in areas with very high concentrations of heavy metals in the soil, for example, in areas where metal ores are extracted and processed (e.g. Ashraf et al., 2011; Franiel & Babczyńska, 2011; Wójcik et al., 2013). Nonetheless, such areas occupy a small percentage of the globe's surface (Bradshaw et al., 1989). Heavy metals, however, can affect plants even at low concentrations that do not cause visible damage (Ryser & Sauder, 2006). Despite evidence of heavy metal toxicity to plants under laboratory conditions, their effect is less visible in natural conditions (Bezel' et al., 2001) but can

provide real insight into the functioning of a given organism in the contaminated environment.

Functional plant traits can be defined as the morphological, physiological and phenological properties of plants, measurable from the cellular to the whole organism level, that potentially influence their fitness (Violle, 2007) or the environment they live in (Lavorel & Garnier, 2002). These traits represent critical plant responses to environmental change and the fundamental influence of plants on ecosystem processes. They provide information for interpreting ecological and evolutionary theories and practical solutions for conservation and land management. At the same time, their analysis is relatively easy and cheap (Pérez-Harguindeguy et al., 2013). Universal traits underlying the life cycle of plants include plant size, usually measured by height as well as leaf structure, often represented as specific leaf area (SLA) or leaf dry matter content (LDMC) (Westoby, 1998). SLA of herbaceous plants such as *Plantago lanceolata*, *Plantago major* and *Taraxacum officinale* was used for pollution biomonitoring, presenting higher values in more urbanised, hence polluted, sites (Balasooriya et al., 2009; Datcu et al., 2017; Kardel et al., 2010). For example, SLA and LDMC were determined for plants from landfill waste from Ni smelter to determine their response to harsh environmental conditions (Čierníková et al., 2021).

The beech forest complexes had initially played an essential role in the forest ecosystems of Upper Silesia but are now significantly reduced. The best-preserved beech forests in the area are currently the acidic lowland beech forests (*Luzulo pilosae-Fagetum*) (Cabała et al., 2006; Parusel, 2011). The herb abundant in the herb layer of these forests is *Maianthemum bifolium* (L.) F.W. Schmidt. The species has a wide geographic range and is common throughout Europe and Asia to Japan (Kim & Lee, 2007). It is known to show plastic morphological response to environmental stress but was investigated only in terms of naturally occurring environmental conditions such as different resource availability in contrasting forest habitats (Czarnecka, 1996) or forest sites contrasting with chemistry and light regime (D'Hertefeld & Jonsdottir, 1994).

It was hypothesised that the long-lasting pollution in Silesian forests would affect the populations of May lily, changing the chemical composition of plants and, in consequence, causing changes in functional traits as a way to adapt to environmental

conditions. This study aimed to (i) compare the ability of *M. bifolium* to accumulate heavy metals in leaves and rhizomes, (ii) estimate utility of *M. bifolium* in biomonitoring through accumulation of heavy metal in tissues and/or changes in chosen functional traits and (iii) assess the heavy metal contamination in three acid beech forest complexes in Upper Silesia against relatively unpolluted forests from southern Poland.

2 Materials and Methods

2.1 The Species and the Study Area

Maianthemum bifolium (L.) F. W. Schmidt (May lily) is a monocotyledonous perennial herbaceous plant with underground rhizomes. The plant has vegetative shoots with a single leaf and sexual two-leafed shoots with one inflorescence. The predominant way of spreading is vegetative, through rhizomatous growth (D'Hertefeld & Jonsdottir, 1994).

The study was performed in five *Luzulo-Fagetum* beech forests with *M. bifolium* abundant in the herb layer in southern Poland, differing in the level of pollution. Three of them were located within the Upper Silesian Industrial Region, influenced for many years by heavy industry and urbanisation due to various mineral resources (Kondracki, 2002). These are natural forest fragments, currently subjected to different forms of protection—Segiet (S), Uroczysko Buczyna (UB) and Murcki Forest (M)—formerly transformed through the exploitation of Zn-Pb ores (Segiet) and coal mining (Murcki Forest, Uroczysko Buczyna) (Molenda, 2013). As control sites, forests relatively free from industrial pollution were chosen—Parkowe (P) and Wzgórze Joanny (WJ). Figure 1 presents the localisations of the sampling sites. The beech forests at the study sites are over 120 years old, mostly well-developed and preserved (Cabała et al., 2006). The annual mean temperature in Segiet, Uroczysko Buczyna, Murcki Forest and Parkowe sites is 8.1 °C with annual precipitation ranging from 620 to 890 mm (Woś, 2010), and for Wzgórze Joanny 8.8 °C and 530–570 mm (Polechoński et al., 2018). The forest floor is scarce, beside the *Maianthemum bifolium*; patches of *Vaccinium myrtillus*, *Luzula pilosa*, *Deschampsia flexuosa*, *Oxalis acetosella* and *Convallaria majalis* also occurred.

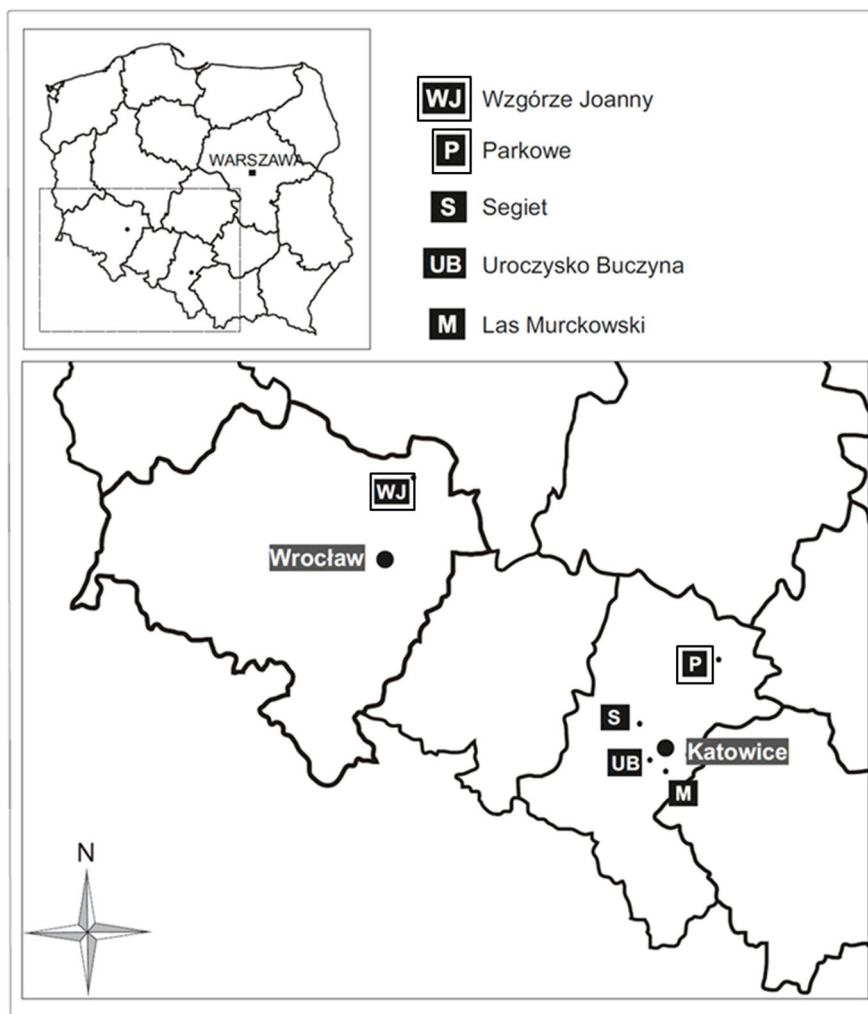
2.2 Field Sampling and Plant Measurements

As the May lily spreads mainly by the vegetative growth, it forms distinct patches, and the sampling plots were established in 16 of such patches (9 plots at the sites described as polluted and 7 at control sites), with the average coverage of May lily in the range of 40–60% per patch. The light conditions were similar at all sampling plots. All plots were sampled for topsoil, vegetative shoots and rhizomes. Plant sampling was conducted in July 2014 (at the full development of plants). Only plants with no mechanical damages to the leaf were collected. Rhizomes from 1-m² plots were excavated to be analysed in the laboratory. From 3 to 15 random samplings with 1-m² frame were performed at each sampling patch. After sampling, plants were immediately transported to the laboratory in a cooler bag, then rehydrated with deionised water. The fresh weight of the leaves and rhizomes was determined. The leaf area and rhizome internode length were measured, followed by drying at 70 °C to constant weight. Prepared plant material was subjected to measurements for the following traits: the height of vegetative shoots, specific leaf area (SLA), leaf dry matter content (LDMC), specific rhizome length (SRL) and rhizome dry matter content (RDMC). The measurements of the traits were conducted according to Pérez-Harguindeguy et al. (2013) and Birouste et al. (2014). As a component of SLA, leaf area was measured using the ImageJ software (National Institute of Health, Bethesda, MD, USA). In total, 1863 vegetative shoots and 1494 rhizome internodes were measured.

2.3 Soil and Plant Chemical Analyses

Soil samples were taken from the topsoil (0–10 cm) in five replications per May lily patch and combined into one composite sample per patch (altogether 16 samples). The pH (pH_{KCl}) was determined in each sample, and the organic matter content was measured by the loss-on-ignition (LOI). Soil samples after overnight drying at 105 °C were weighed and placed in a furnace at 550 °C for 8 h. After combustion, the samples were re-weighed, and the percentage of the dry weight loss was calculated (Salehi et al. 2011). Soil total nitrogen was analysed using the Kjeldahl digestion procedure (Rutherford et al., 2007) and available phosphorus and potassium

Fig. 1 Location of the sampling sites (the symbols in frames are the control sites)



contents by the Egner-Rhiem method. The total concentrations of Cd, Pb, Zn, Ni, Fe and Cu were estimated according to Sastre et al. (2002). More precisely, 0.25 g of soil was mineralised in 8 ml of concentrated HNO₃ and 2 ml of 30% H₂O₂ in a microwave oven (Milestone Ethos One, Italy) at 190 °C. After mineralisation, samples were diluted to 25 ml with deionised water and filtered. The potentially bioavailable fraction of heavy metals was extracted with 0.01 M CaCl₂ after shaking 5 g of soil with 50 ml of CaCl₂ for 2 h (Wójcik et al., 2014). The content of metals was determined in the filtered extracts by atomic absorption spectroscopy (Thermo Scientific ICE 3000 Series). All of the analyses included blank samples and certified reference materials (NCS DC 77,302, China National

Analysis Centre for Iron and Steel) to ensure the quality of the analyses. Each measurement was conducted in three replications.

Contamination of the forest soils by heavy metals was assessed using the single factor pollution index (PI) as follows:

$$PI = C_i/S_i, \quad (1)$$

where C_i is the concentration related to the investigated site and S_i is the background value for Upper Silesia soils, for Pb, Zn, Cd and Cu according to Pasieczna et al. (2010); due to lack of literature data for Fe and Ni, the lowest values determined in the control areas were taken as background values. Table 1 presents the classes of the pollution index (PI) according to Hu et al. (2017).

Table 1 Classes of pollution level according to PI values

Class	PI	Class of pollution
1	≤ 1.0	Safety
2	1.0 < PI ≤ 2.0	Slight pollution
3	2.0 < PI ≤ 3.0	Mild pollution
4	3.0 < PI ≤ 5.0	Moderate pollution
5	PI > 5	Severe pollution

Since the studies were conducted in natural ecosystems, and all investigated heavy metals were affecting the plants, the pollution level for each study site was also calculated as integrated pollution index (IPI) (Chen et al., 2005).

$$IPI = \frac{\sum PI_i}{n} \tag{2}$$

where PI_i is the pollution index for each investigated heavy metal and n is the number of investigated heavy metals. The IPI values were used in ordination analysis. IPI values together with PI are presented in Table 2.

Plant material (leaves and rhizomes) was collected at each sampling plot (ca. 50 g f.w) (altogether 16 samples of leaves and 16 samples of rhizomes). Concentrations of Cd, Pb, Zn, Ni, Fe and Cu in above-ground and underground parts of *M. bifolium* were analysed in 0.25-g samples dried at 105 °C, ground and mineralised in concentrated nitric acid and 30% H₂O₂ at 190 °C using a microwave oven (Milestone Ethos One, Italy). After mineralisation, samples were diluted with deionised water to a volume of 25 ml. The concentration of heavy metals was determined by atomic absorption spectroscopy (Thermo Scientific ICE 3000 Series). The reference material (certified reference material CTA-OTL-1 Oriental Tobacco Leaves) was used to evaluate the quality of the

analytic procedure. Each measurement was conducted in three replications.

The ability to accumulate heavy metals in the leaves and rhizomes of *M. bifolium* was assessed by calculating the bioconcentration (BCF) and the bioaccumulation (BAC) factors and the mobility of elements in plants by the translocation factor (TF) (Ari-fin et al., 2012; Rezvani & Zaefarian, 2011).

$$BCF = \frac{[traceelement]_{rhizome}}{[traceelement]_{soil}} \tag{3}$$

$$BAC = \frac{[traceelement]_{leaves}}{[traceelement]_{soil}} \tag{4}$$

$$TF = \frac{[traceelement]_{leaves}}{[traceelement]_{rhizomes}} \tag{5}$$

A value of BAC > 1 indicates the ability to accumulate the element, whilst BAC < 1 indicates exclusion of the element by the plant. A value of TF > 1 indicates translocation of the element from underground to aboveground organs.

2.4 Statistical Analyses

To obtain the normal distribution of data, the Box-Cox transformation was used (mean values of each trait presented in tables and figures are non-transformed). The differences between the heavy metal content in soils, plant organs and plant functional traits were tested using ANOVA, and when the significant differences were identified, a post hoc test (Tukey HSD) was performed. Relationships amongst soil and plant metal concentrations were analysed using Pearson’s correlation analysis. The analyses were conducted with Statistica v.12.

The ordination analyses were performed to evaluate the influence of chemical and biological properties of the investigated habitat on the chosen *M. bifolium* functional traits. The functional traits of plants from the sampling plots were used as separate

Table 2 Pollution indices of investigated metals in beech forest soils

	PI						IPI
	Cd	Pb	Zn	Fe	Cu	Ni	
S	4.4	40.9	7.4	2.9	4.4	2.0	10.8
UB	2.9	17.4	3.8	3.3	3.4	2.1	6.9
M	2.0	22.7	3.1	2.8	3.8	2.2	6.4
P	0.4	4.8	0.7	1.0	1.0	1.0	1.7
WJ	0.4	3.6	0.8	1.3	2.8	1.2	2.0

variables with log transformation $Y = \log(Ay + C)$. The detrended canonical analysis (DCA) revealed the gradient length lower than 3SD (0.187), which indicates the linear structure of the data and therefore allows to use the redundancy analysis (RDA) in further analyses. As the environmental variables in the redundancy analysis, the chemical properties of the soil, including the integrated pollution index, were used. RDA was followed by the forward stepwise selection of environmental variables and the unrestricted Monte Carlo test with 499 permutations. The ordination analyses were performed with the Canoco v 4.5 package (Ter Braak & Smilauer, 2002).

3 Results and Discussion

3.1 Basic Soil Properties

The physicochemical properties of sampling sites are presented in Table 3. All of the sites were characterised with acidic soil pH (from 2.95 to 3.59). Soils at the UB and M sites can be classified as organic (over 30% organic matter) whilst S, P and WJ as mineral (from 4.5 to 7.9) (Bednarek et al., 2004). The content of the macroelements (N, P, K) was higher in organic soils of UB and M sites.

3.2 Heavy Metal Content in Investigated Soils

The studies of heavy metal pollution of the forest ecosystems soils show that pollutants entering the soil are accumulated mainly in the surface layer of organic soil (Jamnická et al., 2007; Magiera et al., 2007). The total fraction of the investigated heavy metals in soils showed significant differences between the polluted and the unpolluted sites in the case of almost all elements. Only Cu concentration at the WJ control site did not differ from UB and M sites. Amongst

the investigated soils, the highest concentrations concerned Fe and Pb even at the control sites. Ni and Cd occurred in the lowest concentrations.

The concentrations of Cd, Pb and Zn at the polluted sites have multiple times exceeded the values obtained for the control sites, which are reflected by very high PI values (Table 2). The Upper Silesian forests are severely polluted with Pb, also the Cd, Zn and Cu pollution is considerable. However, the concentrations of Pb at the control sites also indicate moderate pollution with this element. According to the PI index, the WJ control site is mildly polluted with Cu, and the Fe and Ni contamination at all polluted sites is mild.

Amongst the investigated communities in the upper soil layer, significantly elevated Cd, Pb and Zn concentrations were found in the S site, which exceeded the concentrations recorded in soils in relatively free of pollution sampling sites, 11, 9.5 and 8.5 times, respectively. At this location, considerable heavy metal pollution is connected with the proximity (about 15 km) of the zinc smelter in Miasteczko Śląskie and centuries of mining and processing of Zn and Pb ores in this area (Ullrich et al., 1999). At the S site, the forest is growing on the old Zn and Pb ore excavation area, which is considered as a source of environmental pollution—soils developed at such locations might be highly polluted with As, Cd, Pb and Zn (Stefanowicz et al., 2014, 2016).

Pb concentrations in the soil, potentially toxic to plants, are considered values above 500 mg kg^{-1} (Kabata-Pendias & Pendias, 1999). These values were exceeded at the S and M sites. Also, a significant incensement in Cd concentration in the Upper Silesian forest ecosystems was observed. The obtained results of Cd, Pb, Zn and Ni concentrations in investigated forests are similar to values obtained in nearby Silesian forests (Magiera et al., 2007). Generally, the soils of Upper Silesia are in varying

Table 3 Physicochemical properties of the soil (mean values \pm SD)

Site	C [%]	N [%]	C/N	P ₂ O ₅ [mg/100 g]	K ₂ O [mg/100 g]	Ca [g kg ⁻¹]	Mg [g kg ⁻¹]	pH _{KCl}
S	7.45 \pm 2.6	1.01 \pm 0.1	7.21	23.33 \pm 3.3	21.97 \pm 2.9	2.27 \pm 0.34	1.26 \pm 0.04	3.51
UB	36.35 \pm 2.6	1.01 \pm 0.1	36.64	62.44 \pm 8.5	55.07 \pm 5.0	1.27 \pm 0.06	1.18 \pm 0.06	3.59
M	30.97 \pm 11.9	1.99 \pm 0.6	15.30	21.21 \pm 15.0	42.18 \pm 9.9	1.29 \pm 0.41	0.90 \pm 0.19	3.07
P	7.92 \pm 0.7	0.76 \pm 0.2	11.52	19.17 \pm 5.6	18.88 \pm 3.2	1.53 \pm 0.16	0.67 \pm 0.06	2.95
WJ	4.52 \pm 1.8	0.77 \pm 0.3	6.02	1.88 \pm 1.0	17.15 \pm 1.9	1.28 \pm 0.3	1.27 \pm 0.4	3.33

degrees contaminated with heavy metals, which results firstly from the geological construction of the region, namely the occurrence of zinc-lead ore deposits (Lis & Pasieczna, 1997) and the resulting industrial activities. Concentrations of heavy metals in the surface soils of the control sites were only a fraction of heavy metal concentration levels in Upper Silesia soils, demonstrating a relatively low exposure to metalliferous pollution.

The concentrations of the bioavailable fraction of heavy metals varied depending on the element. Cd had the highest level of bioavailability (up to 26% of the total content of the element in the soil), followed by Zn (up to 14% of the total fraction) and Ni (3.8%). The availability of Pb, Cu and Fe was low and ranged from 1.5, 1.1 to 0.1% of the total content of these elements, respectively (Table 4). The bioavailability of the investigated heavy metals was in the typical ranges estimated for forest soils (Kim et al., 2015). In the case of all elements besides Fe, it correlated mainly with the total metal concentration, and in the case of Cd, Zn and Fe with soil pH. The highest bioavailability of Cd, Zn, Pb and Cu was observed at the control P site, presumably related to the lowest pH and relatively low organic matter content at this location. These soil properties enhance metal availability (Shahid et al., 2016).

3.3 Heavy Metals in Leaves and Rhizomes of *Maianthemum bifolium*

The concentrations of heavy metals in the rhizomes and leaves of the May lily plants collected from the polluted (S, UB, M) and control sites (P, WJ) are presented in Table 5. The highest levels concerned Fe and Zn, both in aboveground and underground plant parts. The greatest concentration of investigated elements, except for Cu at the control sites, was found in the rhizomes. Ni was found in the lowest concentrations in leaves as well as rhizomes. The concentrations in leaves and rhizomes were significantly higher for the polluted sites than the control sites. The concentrations assessed for the unpolluted regions did not differ from the ranges reported in other pollution-free regions of Poland (Kozanecka et al., 2002). The general pattern of metal concentration in *M. bifolium* organs was similar to that found in wetland species (Deng et al., 2004) and in plants growing near highways (Huseyinova et al., 2009), as well as in plants

Table 4 Total and bioavailable concentrations of the selected metals (mg kg⁻¹) in soil samples (mean values ± SD) (n=48)

Site	Cd		Pb		Zn		Ni		Fe		Cu	
	Total	Bioavailable	Total	Bioavailable	Total	Bioavailable	Total	Bioavailable	Total	Bioavailable	Total	Bioavailable
S	7.00 ± 0.71a	1.32 ± 0.28a	1155.66 ± 139.19a	5.00 ± 0.84a	850.87 ± 18.04a	37.60 ± 7.46a	2.41 ± 0.25 a	0.03 ± 0.01b	24,325.18 ± 3101.85a	2.40 ± 0.99b	71.38 ± 27.72a	0.33 ± 0.24a
UB	4.85 ± 1.07b	0.96 ± 0.17b	493.49 ± 83.79b	3.33 ± 0.96b	462.02 ± 56.15b	34.09 ± 7.34a	2.64 ± 0.29a	0.07 ± 0.00a	27,197.90 ± 3842.50a	2.65 ± 0.78b	54.68 ± 10.59ab	0.19 ± 0.07a
M	3.51 ± 1.13c	0.74 ± 0.23c	646.27 ± 167.34b	4.99 ± 2.03a	390.31 ± 48.94b	32.19 ± 10.02a	2.88 ± 0.81a	0.11 ± 0.07a	23,521.38 ± 6236.58a	27.89 ± 19.49a	61.57 ± 13.61ab	0.28 ± 0.08a
P	0.89 ± 0.22d	0.24 ± 0.03d	138.73 ± 23.93c	2.12 ± 0.31b	107.07 ± 11.81c	15.09 ± 5.55b	1.23 ± 0.40b	0.02 ± 0.01bc	8282.66 ± 707.50b	6.24 ± 2.62ab	16.68 ± 3.63c	0.19 ± 0.04a
WJ	0.42 ± 0.18e	0.03 ± 0.01e	103.07 ± 27.85d	0.73 ± 0.19c	91.60 ± 23.67c	1.92 ± 0.63c	1.44 ± 0.32b	0.01 ± 0.01c	10,999.08 ± 4088.72b	5.57 ± 6.34b	44.53 ± 12.54b	0.21 ± 0.08a
Geochemical background	1*		28*		105*		1.2**		8264**		16*	

Different letters in the same column indicate a significant difference at the 0.05 level. Geochemical background values according to * Pasieczna et al., 2010, ** lowest value of the control site in this study

Table 5 Concentrations of heavy metals (mg kg⁻¹ d. w.) in *Maianthemum bifolium* leaves (n = 86) and rhizomes (n = 76) (mean values ± SD)

Site	Cd		Pb		Zn		Ni		Fe		Cu	
	Leaves	Rhizomes	Leaves	Rhizomes	Leaves	Rhizomes	Leaves	Rhizomes	Leaves	Rhizomes	Leaves	Rhizomes
S	20.65 ± 7.85ab	27.07 ± 20.18ab	23.98 ± 9.14b	64.90 ± 33.11a	122.48 ± 60.46b	487.56 ± 293.35ab	0.78 ± 0.26b	0.83 ± 0.14b	178.91 ± 64.87b	244.39 ± 132.35b	11.27 ± 1.80bc	15.28 ± 9.29 bc
UB	21.49 ± 6.07a	30.90 ± 4.08a	34.85 ± 2.61a	90.18 ± 11.41a	249.90 ± 23.08a	553.16 ± 47.26a	1.27 ± 0.22a	1.73 ± 0.07a	303.86 ± 63.09a	644.00 ± 65.15a	13.84 ± 1.19ab	40.66 ± 9.74a
M	12.66 ± 3.51bc	15.11 ± 2.16bc	22.84 ± 7.63b	60.40 ± 33.21ab	118.85 ± 16.35b	263.56 ± 86.05bc	1.14 ± 0.10a	1.69 ± 0.32a	175.52 ± 19.98bc	288.35 ± 55.52b	17.31 ± 12.34a	22.91 ± 9.44b
P	8.81 ± 2.71c	8.47 ± 2.08c	11.58 ± 1.74c	34.37 ± 10.87c	35.63 ± 9.48c	188.06 ± 47.55c	0.76 ± 0.13b	0.88 ± 0.09c	123.22 ± 26.93 cd	211.02 ± 78.25b	10.11 ± 1.14c	10.10 ± 3.42c
WJ	1.41 ± 0.86d	1.57 ± 0.33d	9.81 ± 2.17c	19.48 ± 7.50c	29.67 ± 9.25 c	58.39 ± 13.09d	0.73 ± 0.16b	0.96 ± 0.18c	106.36 ± 38.54d	213.85 ± 64.37b	12.03 ± 1.27ac	11.30 ± 2.35c

Different letters in the same column indicate a significant difference at the 0.05 level

from the understory of beech forests on former Zn-Pn mines (Luwe, 1995; Stefanowicz et al., 2016). However, the element’s accumulation in the leaves differed between the polluted and control sites and can be presented in different orders. For the control sites, the highest concentrations were of the essential elements Fe > Zn > Cu > Pb > Cd > Ni, whilst for the polluted ones, the concentrations were as follows: Fe > Zn > Pb > Cd > Cu > Ni, despite the high bioavailability of Cd and the low bioavailability of Pb. In rhizomes of plants growing at the polluted sites, Zn prevailed over Fe, whilst the rest of the element’s content decreased as follows: Pb, Cu, Cd and Ni.

The concentration of investigated elements in plant tissues depended mainly on their soil concentration. The positive and statistically significant correlation between heavy metal content in soil and leaves and the May lily rhizomes was obtained in all elements studied besides Cu in above- and underground organs and Pb in rhizomes. A strong correlation was found for Cd and Fe content in soil and leaves (Tables 6 and 7).

Cd phytotoxic concentrations are reported at 5–10 mg kg⁻¹ for susceptible plants, whilst 10–30 mg kg⁻¹ for resistant plants (Kabata-Pendias & Pendias, 1999). This study determined cadmium concentration values above 10 mg kg⁻¹ even in plants from control plots, and values exceeding 30 mg kg⁻¹ characterised plants from the S and UB sites. These results suggest that *M. bifolium* is relatively resistant to the elements mentioned above, as no visible traces of damage to leaves or underground organs were observed. Moreover, high bioaccumulation factors (BAC, BCF) indicate increased cadmium uptake, which concentrations in the plant exceeded those in the soil. On average, the Cd concentration in the rhizomes was almost six times as high, and in above-ground organs, and 4.2 times as high as in the soil, which can describe *M. bifolium* as an accumulator of this element (Mehes-Smith et al., 2013). These findings are consistent with the research conducted near Finnish steelworks (Mukherjee & Nuorteva, 1994), in which the ability of *M. bifolium* to accumulate Cd was also reported. Concentrations of Zn above the value of 100 mg kg⁻¹, especially at the UB site reaching 600 mg kg⁻¹ in underground parts, are considered toxic (Kabata-Pendias & Pendias, 1999). Comparing the accumulation coefficients of the other elements, apart from Cd, only Zn was characterised by

Table 6 Pearson's correlation coefficients between heavy metals in leaves, rhizomes and soils of *Maianthemum bifolium*

	Cd	Pb	Zn	Fe	Cu	Ni
Leaves – soil ($N=18$)	0.793***	0.562*	0.586*	0.749***	0.377	0.671**
Rhizomes – soil ($N=17$)	0.561*	0.351	0.589*	0.497*	0.467	0.626**
Leaves – rhizomes ($N=70$)	0.874***	0.597***	0.663***	0.743***	0.159	0.711***

*** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$

Table 7 Heavy metal translocation factor (TF), bioconcentration factor (BCF) and bioaccumulation factor (BAC) in *Maianthemum bifolium*

	Cd	Pb	Zn	Ni	Fe	Cu
TF	0.74	0.40	0.37	0.76	0.58	0.76
BCF	5.68	0.12	0.96	0.60	0.02	0.44
BAC	4.24	0.05	0.33	0.45	0.01	0.31

the values of bioaccumulation coefficients higher than 1 (at UB and P sites, data not shown), signalling the ability of May lily to accumulate this element as well. Despite higher concentrations of Pb, Ni, Fe and Cu in plants from the polluted area, phytotoxic concentrations of these elements were not exceeded. However, the content of Cd, Pb and Zn was many times higher than in plants collected from the P and WJ sites, reflecting soil pollution in the studied areas. The ability to accumulate toxic metals was also reported in other understory plants such as *Mycelis muralis*, *Melica nutans* and *Mercurialis perennis* in beech forests growing on former mining areas and no visible negative effect on plant growth (no necrosis, chlorosis) was observed (Luwe, 1995; Stefanowicz et al., 2016; Woch et al., 2017). Nevertheless, these findings confirm that some heavy metals might be transferred to higher trophic levels and potentially cause adverse effects for the herbivores.

Conducted analyses of heavy metal concentrations in *M. bifolium* tissues also showed differences in underground and aboveground organs. The value of the translocation factor ($TF < 1$) indicates that *M. bifolium*, like many other plant species, accumulates heavy metals in underground parts (Jamnická et al., 2013; Stefanowicz et al., 2016; Wójcik & Tukiendorf, 2005) as an “exclusion” strategy, protecting plants from the harmful effects of excess of these elements. The lowest translocation factor values referred to Zn and Pb, followed by Fe. Pb is not very mobile in the soil and the plant and is usually accumulated in the

roots (Ociepa-Kubicka & Ociepa, 2012). Fe behaves similarly to the Pb in the plant (Kabata-Pendias & Pendias, 1999). Also, Zn and Cd are usually accumulated in underground organs (Jamnická et al., 2013; Klink et al., 2013; Yoon et al., 2006). Cd, Ni and Cu are almost equally distributed in the aboveground and underground organs, and therefore the TF is relatively high (> 0.7) and confirms the indicator value of May lily (Oliva & Espinosa, 2007). The TF of Cu in plant tissues from control sites reached values > 1 (data not shown), indicating the accumulation of this element in aboveground parts. This strategy also applies to other forest floor plants such as *Mercurialis perennis* and *Polygonatum multiflorum* (Luwe, 1995), *Carex pilosa*, *Galium odoratum* or *Dentaria bulbifera* (Jamnická et al., 2013). Studies show that copper is sometimes accumulated in similar concentrations in aboveground and underground parts (Chakroun et al., 2010). An increase in its accumulation in underground organs is associated with an increase in its concentration in the soil (Madejón et al., 2003). Such is the situation observed for the studied species in areas with elevated heavy metal concentrations. The translocation of Cu to aboveground organs may be due to the essential functions of this element in the plant body, being a structural element of proteins and involved in electron transport during photosynthesis, mitochondrial respiration, response to oxidative stress, cell wall metabolism or hormonal signalling (Yruela, 2005). Relatively high translocation rates associated with Ni may result from its high

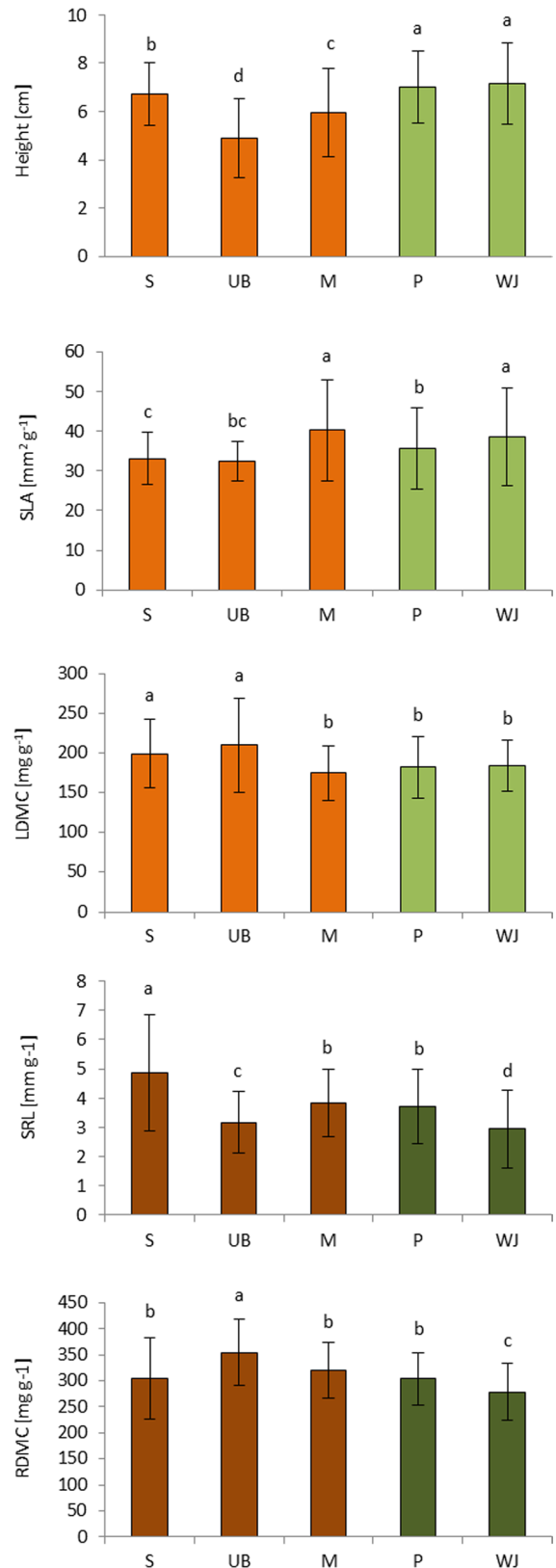
Fig. 2 Mean values of investigated functional traits of *Maianthemum bifolium*. Different letters in one figure indicated a significant difference at $p < 0.05$ (orange – polluted sites, green – control sites)

mobility in plants (Kabata-Pendias & Pendias, 1999). Concentrations of this element determined in organs of herbaceous plants from southern areas of Poland showed similar concentrations in leaves and roots, or concentrations slightly higher in underground organs (Łaszewska et al., 2007).

3.4 Functional Traits

Figure 2 presents the mean values of measured functional traits of May lily across the investigated populations. The height of plants ranged from 4.8 cm at the M site to 7 cm at the P and WJ sites. The SLA index reached its highest value in the M and WJ population (40.3 and 38.5 $\text{mm}^2 \text{mg}^{-1}$ respectively). The lowest value of the index was characteristic for the leaves of the UB and S populations (32.5 and 33.15 $\text{mm}^2 \text{mg}^{-1}$ respectively), whilst plants from the same populations had the highest content of dry matter in tissues (LDMC)—at UB site 210 and S site 199 mg g^{-1} . In the rhizome traits, the highest SRL value was obtained for the S population (4.9 mm g^{-1}), the lowest for the WJ population (2.9 mm g^{-1}). Similar to the dry matter content in the aboveground tissues, the highest RDMC was calculated for the UB population (354.4 mg g^{-1}). The lowest values were found in the WJ population (279 mg g^{-1}).

RDA was performed to identify whether physiochemical soil properties influenced the May lily traits (Fig. 3). The first axis, which represents the fertility of soils, explains 30.4% of the total variation in the measured traits. The second axis represents the heavy metal pollution gradient and explains 20.6% of the total variation. Amongst the six variables taken into consideration in the analysis, only the concentration of available K significantly influenced the investigated May lily functional traits ($\text{Lambda A} = 0.26$, $p = 0.006$), and its effect explains 26% of the general variance. According to the analysis, the investigated functional traits of May lily were in a slight degree associated with metal pollution. Although the height of plants measured at the control sites was greater than at the polluted sites, there was no significant correlation with the integrated pollution index (neither



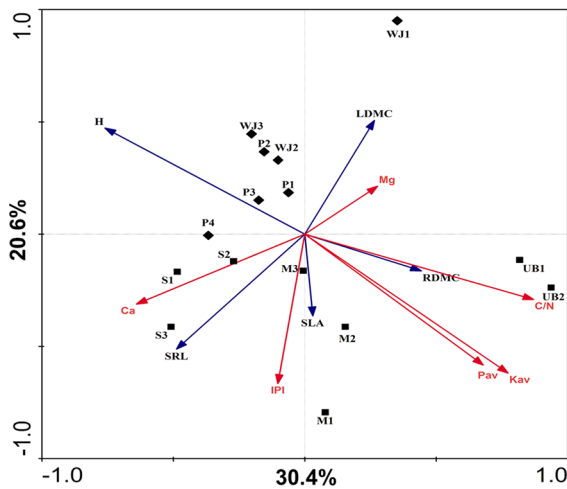


Fig. 3 The relationships between environmental variables (red arrows) at sampling points (diamonds represent the control sites P and WJ; squares, the polluted sites S, UB and M) and the functional traits (blue arrows). LDMC – leaf dry matter content, RDMC – rhizome dry matter content, SLA – specific leaf area, SRL – specific rhizome length, H – height, Pav – available phosphorus, Kav – available potassium, IPI – integrated pollution index

with investigated elements, data not shown). In experimental conditions, Cd and Zn were found to reduce plant height (Chaves et al., 2011), although many species do not show any alterations in their morphological traits (e.g. Papazoglou et al., 2005; Jiménez-Ambriz et al., 2007). Meta-analysis of growth characteristics of plants growing near point polluters showed that growth reduction concerned only annual herbaceous plants (Zvereva et al., 2010). The higher pollution level might influence SRL and LDMC; at the polluted stands, the rhizomes were longer and thinner (higher SRL), and leaves had a higher level of hydration (lower LDMC). Higher SRL and SLA are connected with fast growth, usually at resource-rich sites (Shovon et al., 2020). Elongation of rhizomes might also be a strategy of avoiding unfavourable conditions (Oborny, 1994; Skálová et al., 1997), but it can only speculate whether heavy metals were related to this process. Studies on *Alternaria philoxeroides* and *A. sesillis* showed that Cd treatment negatively impacted plant traits, including internode length (Wang et al., 2021). Pb also had a negative effect on rhizome internode elongation in *Phragmites australis*; however, the reductions were reduced after a more extended period of the experiment, which was explained

compensation to loss caused by disturbances in early stages of growth (Zhang et al., 2015). Under Ni and Cu treatment, length of stolon internodes of *Potentilla anserina* was not affected with heavy metals; however, they were thinner in relation to controls (Koi-vunen et al., 2004). Nonetheless, it should be stressed that the studies cited were carried out under experimental conditions, whilst data collected under natural conditions are very scarce. Higher RDMC indicates a resource-conservative strategy at resource-poor sites (Birouste et al., 2014) observed at nitrogen-poor UB site (C/N over 30 indicates low availability of N). Presumably, biotic factors such as soil fertility and light availability predominantly affect plant traits at sites with a long contamination history. As the beech forests naturally colonise such areas in southern Poland in the natural succession, Woch et al. (2017) hypothesised that the accompanying plants might have some opportunities to form local metal-tolerant populations, which might also occur in the presented study.

4 Conclusions

The studies were conducted in natural forest habitats that had arisen in areas formerly entirely transformed by the exploitation of mineral deposits and which have returned to the biocenoses that are characteristic of these areas after human activity ceased. Therefore, levels of soil heavy metals are relatively high. Although clean technologies nowadays replace the heavy industry, their effects remain in the ecosystems, reflected by severe pollution with Pb, moderate pollution with Cd, Zn and Cu, even at forests relatively distant from the polluters. Hence, the concentrations of heavy metals recorded in the tissues of May lily from polluted populations were elevated. Generally, the investigated elements were accumulated in the underground parts, but Cd, Cu and Ni were translocated to the aboveground parts. Therefore this species can be proposed as an indicator plant in forest ecosystems. May lily has also shown a distinct capacity to accumulate cadmium at concentrations much higher than those found in soils. There was no clear evidence that heavy metal pollution has adverse effects on the functional traits of May lily. In natural conditions, the biotic factors probably play the primary role in displaying the morphological traits, and plants have adapted to the long-lasting pollution.

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Author Contribution Conceptualisation, methodology, analysis and investigation and writing the manuscript – Karolina Bierza.

Data Availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Code Availability Not applicable.

Declarations

Conflict of Interest The author declares no competing interests.

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