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Heavy metal accumulation and distribution in *Phragmites australis* seedlings tissues originating from natural and urban catchment

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Abstract

The retention of heavy metal (HM) was studied in root and rhizomes (BLG), stems (ST), and leaves (LF) of *Phragmites australis* (common reed) seedlings collected from different locations, differing in the scale of anthropogenic interference. The analysis includes the reference samples of sediments in uncontaminated lake Garczonki and contaminated roadside ditch in Cieplewo. The concentrations of Zn, Cu, Pb, Cd, Ni, and Cr were analyzed in plant tissues and sediments using the atomic absorption spectrometry and inductively coupled plasma mass spectrometry. The general assessment of sediments collected in the Garczonki lake showed a good environmental status; while in the roadside ditch in Cieplewo, the sediments were considerably polluted with HM. In the first stage of plant growth, all of the analyzed HMs are mainly inhibited by BLG system. The decreasing trend of elements was as follows: BLG > ST > LF. The organs followed different decreasing trends of HM concentration; the trend Zn > Cu > Ni > Cr > Pb > Cd was found in ST and LF for the Garczonki lake seedlings and for BLG and LF for the roadside ditch in Cieplewo seedlings. Zn showed the highest concentration, while Cd the lowest concentration in each of the examined organs. The bioaccumulation factor indicated the higher mobility of HM in seedlings in the Garczonki lake than in the roadside ditch in Cieplewo. The morphological studies suggest the good state and health of seedling from both sites; however, the reduction of root hair surface was observed for the roadside ditch seedlings. The anatomical studies present changes in the size of the nucleus and count of chloroplasts in LF. No reaction on HM contamination sediments in the seedlings from the roadside ditch in Cieplewo in the aerenchyma was noted. Potentially, both types of seedlings can be used to decontaminate environments rich in HM. However, the level of HM absorbed by seedlings (in the first stage of growth) should be considered due to the behavior in the target phytoremediation site.

Keywords

Heavy metals
Phragmites australis
Seedling
Macrophytes
Biomonitoring
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Urban catchment
Natural catchment

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Electronic supplementary material

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Introduction

Heavy metals (HM) belong to the most persistent and toxic contaminants associated with urbanization and industrialization (Adamiec et al. 2016; Alahabadi and Malvandi 2018; Islam et al. 2015). They are not degradable and consequently permanent in the environment (Ali et al. 2013~~)~~; Kulbat and Sokołowska 2019). This critical environmental issue attracts the attention of the government and regulatory authorities to undertake properly planned remediation actions. One of the sustainable approaches which are mostly promoted is phytoremediation. Predominantly, it is economically justified (low cost and low tech) and environmentally friendly, and it gives a high probability of the effectiveness (Sarwar et al. 2016). This technique gains the advantage of the selective uptake abilities of plant underground organs systems (roots and rhizomes), which is dedicated to remove, contain, or render harmless environmental contaminants (Hinchman et al. 1995). Phytoremediation of hazardous compounds relies on several pathways: accumulation inside the plant in roots and rhizomes and transport to aerial organs (phytoextraction and phytovolatilization) or stabilization them into harmless status (phytoimmobilization and phytostabilization) (Liu et al. 2018). For effective phytoremediation effect, HM have to be translocated to the aerial parts of the plant (Sridhar et al. 2005) and removed through harvesting (Vymazal 2016).

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The plants have the abilities to bioaccumulate and translocate the elements through the tissues. The mechanism of HM uptake is the result of several processes such as cation exchange through cell membranes, intracellular transport (in roots and xylem), rhizosphere processes, detoxification, and sequestration of HM across the cell and plant levels (Kabata-Pendias and Pendias 2001; Vymazal and Březinová 2016). HM are incepted actively or passively (Wojciechowska and Waara 2011). Passive uptake occurs by diffusion of ions from external solution into the roots, while active uptake requires metabolic energy (Kabata-Pendias and Pendias 2001). Moreover, plant ability to absorb HM depends on various biotic and abiotic factors such as pH, organic matter content, temperature, salinity, redox conditions, the composition of fine particles, and biogenic elements (Minkina et al. 2019). The degree of plant assimilability of HM from the sediments depends on the chemical form of element. According to Cristaldi et al. (2017) HM in sediments could be found as free metal ions, soluble metal complexes, associated to organic matter, oxides, hydroxides, and carbonates, as well as incorporated into silicate minerals structure. Low bioavailability is a major limiting factor for phytoextraction of contaminants such as Pb (Ali et al. 2013). In particular, the strong binding of HM to sediments particles or precipitation causes a significant fraction of sediment HM to become insoluble. Insoluble HM in sediment/water medium is unavailable for uptake by plants (Sheoran et al. 2011). Three categories of the HM/metalloids bioavailability may be distinguished: readily bioavailable (Cd, Ni, Zn, Cu); moderately bioavailable (Mn, Fe); and least bioavailable (Pb, Cr) (Prasad 2003). Sediments pH is considered as the major factor influencing the availability of elements for plant uptake (Kumar Yadav et al. 2018). A low pH increases the concentration of HM in solution. The secretion of H+ ions by plant root can acidify the rhizosphere and enhance the cation exchange capacity between HM. The result of this process can increase the availability of contaminants for plant uptake by resulting in concentrations of elements at the toxic levels (Kumar Yadav et al. 2018~~)~~; Huerta Buitrago et al. 2013). Kabata-Pendias and Pendias (2001) described the physiologic (normal) and toxic levels of HM in plant leaves with moderate sensitivity and tolerance to their excess (Tab.1).

Table 1

The physiological and phytotoxic level of heavy metals in plant leaves with moderate sensitivity and tolerance to their ~~excel~~excess (Kabata-Pendias and Pendias 2001)

Contamination level	Heavy metal concentrations [mg/kg d.w.] in plant leaves					
	Cu	**Zn**	**Pb**	**Cd**	**Ni**	**Cr**
Physiological level	5–30	20–150	5–10	0.05–0.2	0.1–5	0.1–0.5
Phytotoxic level	20–100	100–400	30–300	5–30	10–100	5–20

The harmfulness or deficiency of elements may be a secondary phenomenon that results from interactions (antagonistic and/or synergistic) between them in plant organisms. The combination of Pb and Cd leads to additive toxic effects, while for Cd-Cu, Cd-Zn, and Zn-Cu~~,~~ the antagonistic effect is observed. Element pairs among which both antagonistic and synergistic effects can be noted are Ni-Cu, Ni-Zn, and Ni-Cd (Kabata-Pendias and Pendias 2001).

Several studies indicate the sediments as an important sensor of assessment of the urban environmental quality (Chabukdhara and Nema 2013; Wang et al. 2017). The surface layer of sediments is commonly considered as the most exposed to HM part of the environment; the highest content of HM occurs particularly in the top layer (Acosta et al. 2015). On the other hand, the macrophytes (e.g., *Phragmites australis*) are frequently treated as the indicator of contaminated sites (Bonanno, 2011; Bonanno and Lo Giudice 2010; Minkina et al. 2018; Vymazal and Březinová 2016). Recently Elshamy et al. (2019) proved the phytoremediation efficiency of *Portulaca oleracea* L. However, *P. australis* is the species most frequently studied and used to phytoremediation diverse sites (Vymazal and Březinová 2016). *P. australis* (common reed) is a large perennial rhizomatous grass that grows in the natural environment to 1.5–

3 m tall. It absorbs HM from sediments and is planted widely in constructed wetlands for the treatment of metal-supplemented wastewater and effluents. In urban areas, it is applied to create green zones, due to the increasing knowledge about sustainable techniques (Cristaldi et al. 2017). This species demonstrates high plasticity and the ability to adapt to prevailing environmental conditions (in contamination context) (Bonanno and Lo Giudice 2010; Lee and Scholz 2006; Southichak et al. 2006). The uptake of HM matters the most at the first phase of the vegetation period (Kabata-Pendias and Pendias 2001; Obarska-Pempkowiak et al. 2015). However, the differences in the use of diverse seedlings and its properties with metal uptake at the first stage of growth are often omitted.

The main goal of our study was an investigation of HM (Zn, Cu, Pb, Cd, Ni, Cr) concentration in the seedlings of *P. australis* collected from natural (lake) and man-affected (roadside ditch) stands (Kaszuby District, northern Poland) in order to ascertain the capability of these two types of seedlings of HM extraction from originating sites. We checked the HM content in *P. australis* in the initial phase of growth, which could be important for the selection of the different type of seedling in phytoremediation sites. Moreover, the anatomical and morphological reactions were considered to assess the ecological plasticity of *P. australis* seedlings under potential anthropogenic stress caused by HM.

Materials and methods

Study area

Plant species were collected at two locations in Kaszuby District (northern Poland), subjected to different levels of human impact. The first stand was Garczonki lake located 40 km from Gdansk (54° 03′ 33.8″ N 18° 15′ 01.9″ E). It belongs to standing water type, covering the area of 15 ha, the average depth is 8 m. In this location, human impact (in accordance with HM distribution) is negligible. The second site was a roadside ditch (80 m long) located near the busy trunk road (DK91) in Cieplewo (54° 13′ 58.3″ N 18° 37′ 59.5″ E), which is located about 15 km distance from Gdansk. This ditch is mainly affected by surface runoff (from road and farmland). The average temperature during the year is 8.7 °C (0 °C—January, 18 °C—July, August), the total rainfall during the year is 397.1 mm. The reed vegetative season in Poland lasts from the 2nd half of April even up to November.

Sample collection

The samples of sediments and *P. australis* seedlings were collected in the 2nd half of April 2018 (at 15th, 21th, and 30th of April). Five sampling points at the lake edge (100 m distance) and five along the roadside ditch (80 m distance) were randomly chosen. Sampling consisted of plant seedling (about 20–30 cm height). In each sampling point, 8–10 samples of *P. australis* seedlings and sediments associated were collected within a 5 × 2 m^2 plot. The top layers of sediments (approx. 5 cm) were collected using a plastic sampler. Collected samples of sediments were placed in PE bags, while seedlings were transported in a bucket filled with tap water. All samples were transported in cooling conditions (4 ± 1 °C) and taken to the laboratory within 1 h after extraction.

Sample analysis

Seedlings of common reed were preliminary cleaned using Milli-Q water and dissected in roots and rhizomes (belowground parts—BLG), stems (ST), and leaves (LF). Ordered plant tissues and sediments were placed in Petri dishes and lyophilized to a constant weight. The BLG were not separated for analysis due to the low proportion of roots to significant rhizomes (approx. 10 to 90% of the biomass, respectively). For further mineralization, plant organs were grounded in the mill (Millmix 20), and sediments were grounded into fine powder in a mortar. Homogenized plant material (0.5 g of subsample—0.001 g accuracy) was mineralized with 65% HNO_3 (Suprapur) at 60 °C during 12 h. After that, nitric acid was evaporated at 130 °C. When the samples cooled down the 70% $HClO_4$ (Suprapur) was added to each sample and heated to 220 °C. The mineralized subsamples were diluted with 10 ml of 0.1 M HNO_3 (Suprapur) and placed in PP test tubes. The sediment subsample of 0.5 g was weighted into Teflon bombs. Extra pure chemical reagents—$HClO_4$, HF, and HCl (3:2:1; Suprapur)—were added. Subsequently, the Teflon test tubes were placed in an oven (140 °C) for 4 h. Then the solution was evaporated to dryness and 5 ml of concentrated HNO_3 (Suprapur) was added and evaporated. The dried residue was dissolved in 10 ml of 0.1 M HNO_3 (Suprapur) and transferred to PP test tubes. Sediment samples were analyzed using atomic absorption spectrometry (Shimadzu 6800), while plant samples were analyzed using inductively coupled plasma mass spectrometry (Perkin Elmer). All analyses were performed in three replicates, and the results for *P. australis* and sediments were calculated on a dry weight basis. Blank samples were digested according to the same procedure. Analytical accuracy was determined using certified reference sediments (IAEA-433) and strawberry leaves (LGC716). Recoveries were within 10% of the certified values.

To describe the internal morphology of leaves and rhizomes, we used the light microscopy (× 100, × 400, × 1000). The cross-sections of rhizome and surface fragments of leaf were cut by hand applying the technique proposed by Thomas and Dudley (1894). Microscopic measurements were performed on freshly cut seedlings. One root and leaf from each site (L and RD) was analyzed. To distinguish cell walls and nucleus from the background, the material was stained with Lugol's fluid. The diameters of root and leaf cells were measured using a micrometric ocular.

Statistical analysis

The results of chemical analysis correspond to the mean of three replicates. Standard deviation (SD) was used as a measure of variance. The data analysis was performed using STATISTICA software. Plant ability to accumulation a metal into its tissues from the surrounding environment (sediments) was evaluated by the bioconcentration factor (BCF) (Ali et al. 2013), while the efficiency of the plant in translocating the accumulated metal from its BLG parts (root and rhizome) to stem and leaf and between aboveground parts (stem-ST and leaf-LF) was calculated using translocation factor (TF) (Bonanno and Vymazal 2017). The described factors are expressed by the following ratios: BCF = $[\text{trace element}]_{BLG}/[\text{trace element}]_{sediment}$, TF = $[\text{trace element}]_{ST}/[\text{trace element}]_{BLG}$, TF = $[\text{trace element}]_{LF}/[\text{trace element}]_{BLG}$, TF = $[\text{trace element}]_{LF}/[\text{trace element}]_{ST}$. The assessment of the status of sediments contamination by heavy metals in accordance with up-to-date contamination indices: contamination factor (CF) and the degree of contamination (C_{deg}) (Hakanson 1980; Tomlinson et al. 2013; Likuku et al. 2013) was performed. The described indices are expressed by the following formulas: CF = $[\text{trace element}]_{Sediments}/[\text{trace element}]_{Background}$, $C_{deg} = \sum CFi$, where i is the trace metal studied. The values of $[\text{trace element}]_{Background}$ were established using GEOLOG Software of Polish Geological National Research Institute (GeoLOG) for the surface soil layer in the analyzed area (L: Cu, 6.85; Zn, 45.0; Pb, 17.5; Cd, 0.500; Ni, 5.65; Cr, 5.60; RD: Cu, 11.6; Zn, 69.0; Pb, 17.5; Cd, 0.600; Ni, 14.9; Cr, 15.9 [mg/kg d.w.]). The general description of contamination is as follows: $CF < 1$, low; $1 \leq CF < 3$, moderate; $3 \leq CF < 6$, considerable; $CF \geq 6$, very high; $C_{deg} < 8$, low degree of contamination; $8 \leq C_{deg} \leq 16$, moderate degree of contamination; $16 \leq C_{deg} \leq 32$, considerable degree of contamination; $32 \leq C_{deg}$, very high degree of contamination.

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Results

Chemical analysis of sediments and *P. australis* seedlings

Heavy metal concentrations in sediments from the source areas from which the seedlings were taken are presented in Table 2. The element concentrations were significantly different at both source sites with definitely higher values noted for the sediments collected from the roadside ditch in Cieplewo. The average concentration level was 6 to 18 times higher for copper and lead respectively. Trace metal concentrations followed the same decreasing trend according to the type of sediment sites, Zn > Cu > Pb > Cr > Ni > Cd. The pH value for sediments from the lake was in the range 7.0–7.1, while for the roadside ditch in Cieplewo 6.9–7.4.

Table 2

Heavy metal concentrations [mg/kg d.w.] in bottom sediments of Garczonki lake and roadside ditch in Cieplewo at the seedlings collection sites

Type of sediments	Value	Heavy metal concentrations [mg/kg d.w.] in source sites of *P. australis*					
		Cu	Zn	Pb	Cd	Ni	Cr
Garczonki lake	Min	14.7	40.8	4.31	0.036	2.31	3.92
	Max	15.5	43.8	4.87	0.044	2.66	4.03
	Mean	15.1	42.4	4.57	0.040	2.55	3.97
	SD	0.3	1.2	0.21	0.003	0.12	0.04
Roadside ditch Cieplewo	Min	86.1	621	84.0	0.622	23.8	48.8
	Max	88.9	656	85.1	0.674	26.1	56.6
	Mean	87.9	642	84.5	0.654	25.4	54.2
	SD	1.0	14	0.4	0.020	0.8	2.8

The calculated values of CF and C_{deg} are presented in Table 3. The sediments from the lake were considered as slightly contaminated with low impact of human activities in reference to CF and analyzed HMs. The opposite assessment applies to sediments collected from the roadside ditch. These sediments in reference to CF index were recognized as strongly polluted by Zn. The considerable and/or moderate contamination was noted for Pb, Cr, and Ni. The complex pollution index (C_{deg}) gave an unambiguous assessment for RD sediments as considerably contaminated.

Table 3

The assessment of the heavy metals contamination status with contamination factor (CF) and contamination degree (C_{deg}) of sediments collected with the *P. australis* seedlings from Garczonki lake and roadside ditch in Cieplewo

Site	Heavy metal

Site	Heavy metal					
	Cu	Zn	Pb	Cd	Ni	Cr
CF						
Garczonki lake	2.2	0.9	0.3	0.1	0.5	0.7
Roadside ditch in Cieplewo	7.6	9.3	4.8	1.1	1.7	3.4
Cdeg						
Garczonki lake	5					
Roadside ditch in Cieplewo	28					

The results of HM contents in plants are presented in Table 4. The most abundant concentrations were detected in BLG parts, while the lowest—in ST for Garczonki lake (L) seedlings and LF for roadside ditch (RD) seedlings. Concentrations of elements in the Garczonki lake decreased in the order of BLG > LF > ST; only in the case of Cr the concentration decrease was as follows: BLG > ST > LF. In the case of plant tissues collected from the roadside ditch, the decrease followed the order BLG > ST > LF for all metals except for Ni (BLG > LF > ST). The element concentrations in the roadside ditch in Cieplewo (BLG) were from 1.6 (Zn) to almost 30 (Pb) times higher than those registered in the Garczonki lake. Heavy metals accumulated in *P. australis* seedlings were mainly sequestrated in root tissues. The highest concentrations of trace metals in the Garczonki lake (BLG) were noted for Zn; the order of elements drop was as follows: Zn > Cu > Cr > Pb > Ni > Cd. In the roadside ditch in Cieplewo (BLG), the highest concentration was observed also for Zn; however, the decrease of remaining elements was dissimilar: Zn > Cu > Ni > Cr > Pb > Cd. The decreasing trend of metal concentrations was, in ST (Garczonki lake): Zn > Cu > Ni > Cr > Pb > Cd, in ST (roadside ditch in Cieplewo): Zn > Cu > Cr > Ni > Pb > Cd, in LF (Garczonki lake): Zn > Cu > Ni > Cr > Pb > Cd, and in LF (roadside ditch in Cieplewo): Zn > Cu > Ni > Cr > Pb > Cd. In accordance with the toxicology and normal (physiological) level of heavy metals in LF, we observed for the Garczonki lake the proper status for plants requirements (except for Cu, Pb, and Cd—with the lower values than expected as physiological level). The opposite situation was noted for seedlings LF collected from the roadside ditch in Cieplewo. The phytotoxic level was detected in the case of Zn, while Cr was above the physiological value.

Table 4

Heavy metal concentrations [mg/kg d.w.] in organs of the seedlings of *P. australis* collected from Garczonki lake and roadside ditch in Cieplewo

Site	Part of plant	Heavy metal concentrations [mg/kg d.w.] in plants tissues					
		Cu	Zn	Pb	Cd	Ni	Cr
Garczonki lake	BLG	3.19 ± 0.11	23.1 ± 1.1	2.88 ± 0.16	0.107 ± 0.005	1.69 ± 0.07	3.81 ± 0.16
	ST	0.827±0.029	11.7 ± 0.5	0.153 ± 0.008	0.007 ± 0.000	0.264 ± 0.012	0.241 ± 0.010
	LF	1.92 ± 0.07	24.5 ± 1.1	0.212 ± 0.012	0.015 ± 0.001	0.845 ± 0.037	0.219 ± 0.009
Roadside ditch Cieplewo	BLG	66.2 ± 2.3	691 ± 32	4.43 ± 0.24	0.833 ± 0.038	12.4 ± 0.55	6.84 ± 0.29
	ST	14.4 ± 0.5	473 ± 22	0.109 ± 0.006	0.055 ± 0.003	0.594 ± 0.026	0.901 ± 0.038
	LF	11.3 ± 0.4	340 ± 16	0.039 ± 0.002	0.021 ± 0.001	1.06 ± 0.05	0.868 ± 0.037

BLG roots and rhizomes, *ST* stems, *LF* leaves

The BCF and TF were below unity 1 (Table 5). If the relationship is reversed (BCF > 1 and TF > 1), and at the same time, the plants give large amounts of biomass, they are referred to be a hyper-accumulators (Kumar Yadav et al. 2018). Generally, mobility of HM was higher from sediment to plant than within organs (except LF/ST). In addition, the BCF was higher in the Garczonki lake than in the roadside ditch *P. australis* samples. The highest BCF was observed for Cd at both sites. The lowest BCF in the Garczonki lake was observed for Cu, while in the roadside ditch for Pb. The TF in Garczonki lake was generally higher for LF/BLG and LF/ST than for ST/BLG ratio. In the roadside ditch in Cieplewo, the transport from BLG to ST was more important than transport to LF. To sum up, the average ST/BLG ratio was in the same level for both sites (approx. 0.2); LF/BLG was higher in seedlings in Garczonki lake (0.41), than in roadside ditch site (0.15), while the mean LF/ST ratio was 1.9 for the Garczonki lake and 0.83 for the roadside ditch in Cieplewo.

Table 5 Please merge columns in the TF row above ST/BLG, LF/BGL, and LF/ST.

The bioconcentration factor (BCF) and translocation factor (TF) for lake and roadside ditch s

Element	BCF		TF			
			ST/BLG		LF/BLG	
	Garczonki lake	roadside ditch in Cieplewo	Garczonki lake	Roadside ditch in Cieplewo	Garczonki lake	Roadside ditch in Cieplewo
Cu	0.21	0.75	0.26	0.22	0.60	0.17
Zn	0.54	1.08	0.51	0.68	1.06	0.49
Pb	0.63	0.05	0.05	0.02	0.07	0.01
Cd	2.68	1.27	0.07	0.07	0.14	0.03
Ni	0.66	0.49	0.16	0.05	0.50	0.09
Cr	0.96	0.13	0.06	0.13	0.06	0.13
Mean	0.95	0.63	0.18	0.20	0.41	0.15

BCF - Bioconcentration factor; TF- Translocation factor; BLG- roots and rhizomes; ST-

Morphological studies of *P. australis* seedlings

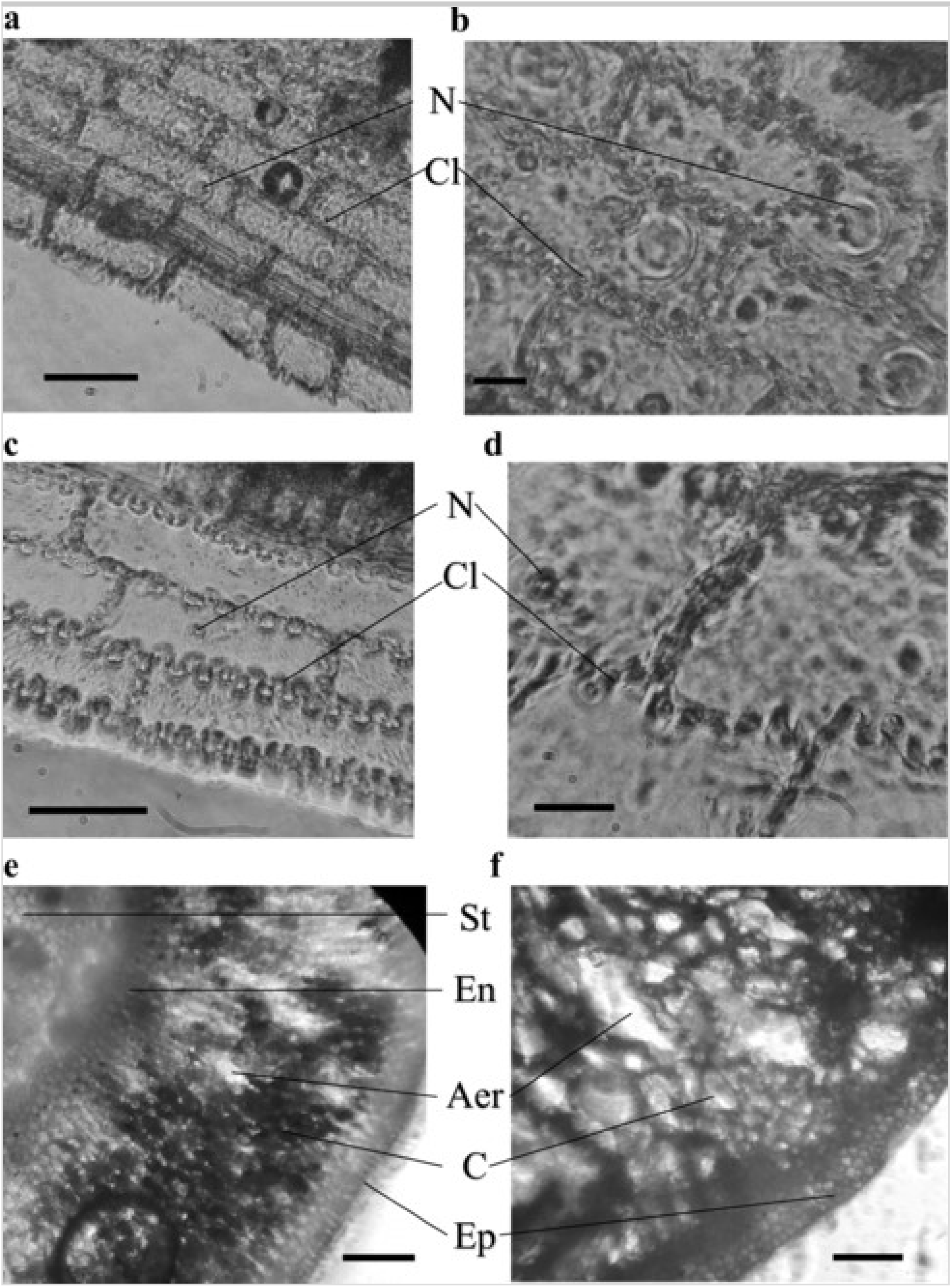
The total length of *P. australis* seedlings collected from the Garczonki lake and roadside ditch in Cieplewo were in the range between 20 and 30 cm. The ST and LF length of seedlings were as follows: 13–22 cm (ST) and 5–16 cm (LF) for Garczonki lake and 10–21 cm (ST) and 6–15 cm (LF) for the roadside ditch in Cieplewo. Leaves were green, glossy with good vigor. The morphometric changes between seedlings of *P. australis* extracted from different sites were observed in BLG parts. On rhizomes between internode (separated by the node with bud scales), the presence and size of roots were smaller in seedlings from the roadside ditch than from Garczonki lake (Fig. S1). The roots of the plants growing on sediments with high metals content had smaller dimensions with relatively thinner barks. The root hair surface was reduced in the roots of the Garczonki lake seedlings.

Anatomical studies of *P. australis* seedlings

Palisade layer of seedling LF is presented in the Fig. 1a–d. The palisade layers of Garczonki lake and roadside ditch seedlings are composed of cylindrical cells with small intercellular spaces. Cytoplasm containing the nucleus (N) and chloroplast (Cl) adhere to the cell walls. Other organelles are visible in Fig. 1b (probably plastids, mitochondria, ribosomes, etc.—the size < 4 μm). The nucleus is concentrated in the cells (Fig. 1b, c). It seems to be smaller in LF cells in the roadside ditch in Cieplewo seedling than in the Garczonki lake seedlings. The number of organelles is lower in the roadside ditch LF, and their shape is more irregular therein. The light optical examination of *P. australis* seedlings showed that reed rhizomes from Garczonki lake and roadside ditch in Cieplewo (Fig. 1e, f) consist of the stele (St), an internal layer formed by endodermis (En), an exterior layer formed by epidermis (Ep) and cortical parenchyma (C) between them. The air spaces (aerenchyma AER) are larger in the roadside ditch seedlings rhizomes. In the external part of Ep, the cells are multisided and closely packed. The En in the Garczonki lake seedling rhizome consists of closely packed cells around the axis cylinder (St). The important changes of the irregular shape are observed for the roadside ditch seedling rhizomes in the cortical parenchyma (C) between En and Ed layers.

Fig. 1 I have attached the Figure 1 in the 500 DPI resolution - maybe it will be clearer and transparent.

Optical microscopy image of **a**–**d** leaves and **e**, **f** rhizomes of *P. australis* collected from **a**, **b**, **e** Garczonki lake and **c**, **d**, **f** roadside ditch in Cieplewo. The scale bar is (μm) **a** 50, **b** 10, **c** 50, **d** 10, **e**, **f** 100 (mark: N nucleus, Cl chloroplast, Aer aerenchyma, Ep epidermis, En endodermis, St stele, C cortical parenchyma)



Discussion

The results of our study showed the various distribution of HM in the first phase of the vegetation period of *P. australis* seedlings collected from different sites. Bearing in mind that there is the long-term need of decontamination of HM affected sites to avert the negative effects on human health (Kumar Yadav et al. 2018), the phytoremediation method shows a promising and environmentally friendly approach for HM removal (Cristaldi et al. 2017). Seedlings of *P. australis* have the ability to accumulate great quantity of more than single metal. In general, underground organs are the main pathway of trace elements to plants (Bonanno 2011; Vymazal et al. 2007). However, other tissues (rhizomes and leaves) have the abilities to readily translocate HM (Bonanno 2011). Transport of toxic elements can occur through passive mechanisms such as the flow of transpiration, or through active mechanisms (Cristaldi et al. 2017). Our investigations confirmed the greater accumulation of HM in underground organs. This is consistent with the research of other authors in the context of macrophytes (Bonanno and Vymazal 2017; Obarska-Pempkowiak et al. 2005; Obarska-Pempkowiak et al. 2015~~)~~; Wojciechowska and Obarska-Pempkowiak 2008). In response to high concentrations of Cu, Zn, Pb, and Cr (87.9, 642, 84.5, and 54.2 mg/kg d.w., respectively) in sediments from the roadside ditch in Cieplewo, the high concentrations of these metals were found in root and rhizome tissues (66.2, 691, 4,43, 6.84 mg/kg d.w., respectively). The sediments from the Garczonki lake were characterized by very low HM contamination. Analyzing both locations in terms of potential HM sources, it seems obvious that the main impact on sediments contamination in RD site had the vehicle emission: burning of fuels (gasoline and diesel), corrosion products of car bodies, tire abrasion, etc. The elevated occurrence of HM elements in urban and motorway road dust in Poland was reported by Adamiec et al. (2016). The similar level of heavy metal content as in RD sediments was noted for urban river sediments in Yangtze River Delta in Ningbo (Zuo et al. 2018), Nakivubo channelized stream sediments (Sekabira et al., 2010), or in stormwater treatment plant and retention tanks located in the urbanized catchment in Poland (Sałata and Dąbek 2017; Wojciechowska et al. 2019).

Copper and zinc uptake is mostly metabolically controlled. Both metals belong to the essential elements absorbed by BLG system passively (the flow of transpiration), or actively (transport proteins associated with the cell membrane) (Obarska-Pempkowiak et al. 2015; Wojciechowska and Waara 2011; Cristaldi et al. 2017; Kumar Yadav et al. 2018). Copper concentrations in sediments from the Garczonki lake correspond to the moderate, while in the roadside ditch in Cieplewo to very high contamination. The Cu uptake in BLG parts was low for the Garczonki lake seedlings (3.19 mg/kg d.w.) and much higher for the roadside ditch seedlings (66.2 mg/kg d.w.). These values correspond to the BCF: 0.21 and 0.75 for Garczonki lake and roadside ditch in Cieplewo, respectively. The concentration of Cu in LF was below the physiological level for lake seedlings and in the range of physiological level for the roadside ditch seedlings. The BLG systems have the capability to inhibit Cu transport to shoots in the situation of both Cu deficiency and Cu excess (Kabata-Pendias and Pendias 2001). The copper TF for ST/LF (TF = 2.32) shows the high demand of Cu supply to leaves. The zinc BCF for the Garczonki lake seedlings was below 1, while for the roadside ditch in Cieplewo greater than unity 1. The concentration of Zn in sediments from the roadside ditch in Cieplewo gave the “plentiful” supplies of this element, which has a bearing on the BCF value. In addition, the highest Zn content of plant LF occurs always during the phase of intensive growth (Yang et al. 2017). The concentration of Cu and Zn vary in wide ranges, showing that these HM occur in a mobile (bioavailable) form which is a key process in the nutrition of the plant. The concentration of Cu and Zn in ripe common reed growing in constructed wetlands in Czech Republic (Vymazal et al. 2007) was as follows for Cu: BLG-28, ST-10.1, LF-19.1 mg/kg d.w. and for Zn: BLG-61.3, ST-11.6, LF-20.3 mg/kg d.w. In the ex-mining region in Malaysia (Ashraf et al. 2011), the Cu concentration was from 10 times for BLG to 40 times for LF higher than observed in our study for the roadside ditch seedlings. In the case of Zn, in our study, the concentration was 2 times higher for BLG and approx. 1.5 times higher for ST and LF than obtained in the ex-mining region in Malaysia (356, 211, and 228 mg/kg d.w. for root, stem, and leaf, respectively). It confirms the high ability of *P. australis* seedlings to adapt to prevailing conditions; however, these HMs could become toxic when they are present in too high concentrations. If it happens, the plants utilize various mechanisms to retain or stabilize them, for example, they reduce in the size of intercellular air spaces and increase in the size the endodermal cells (Minkina et al. 2018).

Lead and cadmium are reported to be commonly found in plants, but they are referred to as non-essential and passively uptaken elements in tissues (Bonanno and Lo Giudice 2010; Divan et al. 2009). Despite the considerably various Pb concentrations in sediments, the concentrations in BLG systems of both analyzed seedlings were on a similar level (2.88 and 4.43 mg/kg d.w. for the Garczonki lake and the roadside ditch in Cieplewo, respectively). The concentrations in the leaves also did not reach the physiological level (0.212 and 0.039 mg/kg d.w. for lake and roadside ditch, respectively). However, the properties of two types of sites vary in BCF, which is higher for the Garczonki lake seedlings (BCF = 0.63) and low for the roadside ditch seedlings (BCF = 0.05). The TF between tissues is negligible (except TF = 0.75 for ST/LF in Garczonki lake seedlings). Possibly, the mechanism of Pb uptake in *P. australis* seedlings is not so intensive as Zn uptake. Results of our study revealed that Pb was absorbed mainly by BLG system and was stored to a considerable degree in cell walls (Kabata-Pendias and Pendias 2001). Ashraf et al. (2011) reported up to 129 mg/kg d.w. of Pb in LF of *P. australis* in the ex-mining region; Bestari Jaya, Peninsular Malaysia. This proves that common reed has the property of collecting toxic doses of Pb, which is probably associated with passive uptake of Pb. The Cd content in analyzed sediments corresponded to the low (Garczonki lake) and moderate (roadside ditch in Cieplewo) contamination status. The highest concentration of Cd occurred in BLG parts. At the same time, the BCF reached a high value—2.68 for the Garczonki lake seedlings and 1.27 for the roadside ditch seedlings. In general, origin sites rich in Cd cause damages in plants—oxidative stress by releasing free radicals and reactive oxygen species. This leads to the death of plants by damaging membrane lipids, proteins, pigments, and nucleic acids (Bonanno 2011; Bonanno and Lo Giudice 2010). It was reported by Ederli et al. (2004) that the roots of *P. australis* were considered to be Cd accumulators and should be used for Cd detoxification. *P. australis* has a high tolerance to a high concentration of Cd. The defense strategy of a common reed is based on increased antioxidant enzyme activities (Bonanno and Lo Giudice 2010; Ederli et al. 2004). In our study Cd concentration in the Garczonki lake seedlings was similar for BLG, ST, and LF obtained for constructed wetlands by Vymazal et al. (2007). Even for the worst samples (BLG parts in the roadside ditch seedlings), Cd concentration was below the physiological level for plant LF. In accordance with Bonanno and Lo Giudice (2010), Cd content in plants from unpolluted sediments is in the range from 0.01 to 0.3 mg/kg d.w. This proves that Cd content in *P. australis* roots could be helpful to establish the level of origin site pollution.

Nickel is an element regarded as toxic for plants if its concentration exceeds the physiological level, while chromium is nonessential and also can be toxic (Hu et al. 2014). Ni concentrations in plants are harmful over 5 mg/kg d.w. (Kabata-Pendias and Pendias 2001). The LF Ni content for both sites seedlings was in the range referred to as a natural level. The values of bioaccumulation in the roadside ditch seedling organs agree with results presented by Vymazal et al. (2007) in constructed wetlands. The BCF noted in our study was 0.66 and 0.49 for the Garczonki lake and the roadside ditch in Cieplewo, respectively, and showed moderate transport from sediments to BLG organs. In our study, Cr content in LF of the Garczonki lake seedlings presented the physiological level, while in the roadside ditch seedlings the LF concentration was above this level. Bonanno and Lo Giudice (2010) suggest that Cr concentrations higher than 0.5 mg/kg d.w. are toxic to plants. In reference to this principle, all tissues of common reed seedlings collected from the roadside ditch in Cieplewo were contaminated by Cr at the toxic level. The Cr concentration in BLG for RD seedling was 2 times lower in comparison to constructed wetland described by Vymazal et al. (2007). For aerial parts of the roadside ditch seedlings, Cr concentration was 3 and 4 times higher (for LF and ST, respectively) than the mean value reported for constructed wetlands. The Cr BCF calculated for seedlings in our study was close to unity 1 for L and significantly lower for the roadside ditch seedlings (0.13). The TF was generally low, except for LF/ST ratio (0.91 for the lake and 0.96 for the roadside ditch seedlings). In the senescent part of *P. australis*, Bragato et al. (2006) observed the plant ability to translocate Cr and Ni during the growing season—the accumulation of these HM may increase sharply at the end of the vegetation. It is probably connected with the way of elimination of some part of the metal burden.

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It is clear that urban plants are capable of reducing environmental contamination through bioaccumulation in their bodies. In both sites—pristine and contaminate—the uptake of HM was noted. Incorporating the green infrastructure, like phytoremediation techniques, in planning the cities development should become one of significant and complementary elements woven into everyday practices of creating city infrastructure.

The high concentration of HM in seedlings of *P. australis* suggests that this species, when grown in environments susceptible to high pollution loads, tends to adapt a tolerance strategy relying on BLG systems as principal accumulator organs. The morphological analysis confirms the adequate status of seedling collected at both sites. It shows the high tolerance to HM contamination or good ability to ability to adapt to the stressful environmental conditions. It was widely reported that plants growing at sites with higher metal concentrations, exhibit characteristic unhealthy growth (Ashraf et al. 2011; Rout and Das 2003). In the case of *P. australis*, the changes involve yellow color, less glossy, more fragile leaves, and smaller roots. However, in case of *P. australi*s seedlings, there were no changes observed in LF and ST. Only in case of BLG system, the seedlings presented some differences in size and roots evolvement. Rucin (2016) reported that abnormalities in metal-stressed plants frequently include the decreased elongation of the primary root, impaired secondary growth, increased root dieback and reduced root hair surface (due to the elevated concentrations of Cd, Cr, Ni, Pb, Cu, Zn). Some changes observed at the cell level in reference to chloroplasts could result from a decrease in the level of metabolic processes that ensure plant growth. The disturbance of chloroplast ultrastructure in the presence of HM is an important and active influence. This is due to the decrease in the content of pigments in macrophytes and the general reduction in the photosynthesis rate (Minkina et al. 2018). In our study, no reaction on the disturbance on Ep cells was noted, and there was no response of *P. australis* seedlings rhizomes in the reduction in the size of intercellular air spaces, which usually occurs in contaminated sites.

Conclusions

This study shows the differences of HM absorbed by *P. australis* seedlings at the initial stage of growth in reference to the originating sites. The contents of HM in the sediments from both investigation sites varied significantly, showing low human impact on the lake sediments and considerable pollution in the roadside ditch sediments. Mobility was rather variable according to the bioavailability of studied HM and was generally higher from sediment to plant than through plants tissues. In the first stage of plant growth, all of analyzed HM are mainly retained by BLG system, which can store the bulk of trace elements in order to protect the plants themselves against the harmful effects of toxic concentrations on photosynthetic organs. Concentration of elements in the Garczonki lake seedlings decreased in the order of BLG > LF > ST (except Cr: BLG > ST > LF); while for plant tissues collected from the roadside ditch in Cieplewo, the decrease was BLG > ST > LF (except Ni: BLG > LF > ST). The BCF was generally greater than 1 for Cd and Zn. Our investigations confirm by far that *P. australis* can be used for decontamination of sediments contaminated with heavy metals due to the high ecological amplitude and phytoremediation characteristics. However, the differences between the origin of seedlings should be considered, due to their possible influence on the efficiency of phytoextraction. The analysis of *P. australis* seedling morphological properties demonstrated the tendency to adapt a tolerance strategy on high HM concentration—the seedling present good vigor and healthy growth. However, the changes in the reduction of hair root surfaces were found in the roadside ditch seedlings. The anatomical properties included changes in the size of the nucleus and count of chloroplasts in LF. No reaction on HM contamination sediments in the seedlings from the roadside ditch in Cieplewo in the aerenchyma was noted. Potentially, both types of seedlings can be used to decontaminate environments rich in HM, but the level of HM absorbed by seedlings (in the first stage of growth) may affect the plant behavior in the target phytoremediation site. The possible response of seedlings may consist of reed adaptation or loss of vigor, absorption of more metals, or even their release into the environment. Further research should be focused on the analysis of the different types of seedlings in the high contaminated sediments to show the described possible responses.

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Electronic supplementary material

ESM 1

(DOCX 631 kb)

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