Leaves and roots of *Typha latifolia* L. and *Iris pseudacorus* L. as bioindicators of contamination of bottom sediments by heavy metals

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Abstract: The paper concerns the bioaccumulation of zinc, nickel, iron and manganese in leaves and roots of selected macrophytes from the Krzynia Reservoir (northern Poland). The research was conducted within the area of 10 stations situated in the littoral zone of the reservoir. Samples of surface waters, bottom sediments and plants were taken in summer. Heavy metal content was determined by the atomic absorption spectrometry method (ASA). The concentration of heavy metals in the waters of Krzynia Reservoir was low and no influence of anthropogenic factors was found. Concentration of heavy metals in the examined bottom sediments was low and remained within the limits of the geochemical background for Zn and Fe. In the case of Ni and Mn it sporadically exceeded the level of the geochemical background. The tested plants mainly accumulated heavy metals in roots, with the exception of nickel which appeared in larger quantities in leaves. The relationships among the content of the determined elements in the organs of macrophytes was identical for the tested species and could be arranged into the following sequences: Mn>Fe>Ni>Zn in leaves and Fe>Mn>Zn>Ni in roots. Statistically significant differences were found in the content of Mn in leaves and Zn and Fe in the roots of *Typha latifolia* L. and *Iris pseudacorus* L. By accumulating substantial quantities of heavy metals in their organs, macrophytes constitute an effective protective barrier for the waters and bottom sediments.

Key words: lakes, macrophytes, microelements, bioconcentration factor, translocation factor

Introduction

Due to the environmental pollution of bottom sediments, plants and water it is possible to observe increased concentrations of various substances of anthropogenic origin, including heavy metals (Trojanowski et al. 2005; Parzych 2016; Parzych et al. 2016). To evaluate the quality of reservoirs and water courses, besides bottom sediments, littoral zone plants (macrophytes) are used. They constitute one of the basic elements for the evaluation of the ecological status of the water. At present, the intense exploitation of catchments and the associated inflow of sewage have a substantial impact on the chemical composition of bottom sediments (Salati and Moore 2009). Heavy metals are biologically transformed and can persist in bottom sediments over a long period (Bettinetti et al. 2003; Liu et al. 2009). Bioavailable forms of metals bioaccumulate in the tissues of plants and animals, resulting in an increased risk of poisoning in subsequent links of the trophic chain (Klink et al. 2013; Parzych et al. 2016). Eco-tox-

icological tests prove that plants uptake trace elements selectively from the surrounding environment. Such elements are used for the construction of their own tissues and participate in many metabolic conversions. Uptake of some mineral components from water and bottom sediments by plants is conditioned by a physiological demand for some of them and can also be an effect of the intoxication that results from environmental pollution (Parzych and Jonczak 2013, 2014; Parzych et al. 2016). The heavy metal content in plant tissues depends on the content and the bio-availability of such elements in the surrounding water environment as well as the plant species, the period of vegetation and the morphological differences (Parzych 2014). Plants react differently to increased concentrations of elements in the environment. Intake and bioaccumulation of necessary constituents is part of the natural cycle. The heavy metal content in aquatic plants can exceed their content in the surrounding water environment by many times (Baldantoni et al. 2004), such a wide range of variabil-

ity is related to the biology and ecology of particular species. Some aquatic plants are used as bioindicators (Skorbiłowicz 2003; Rabajczyk and Józwiak 2009; Łojko et al. 2015, Parzych et al. 2015; Parzych 2016). Control of the chemical composition of littoral plants as well as bottom sediments in lakes is necessary, since it allows for specification of the existing and potential hazards that result from the toxic impact of heavy metals on the water environment.

The aim of this study was to compare the accumulation properties of leaves and roots of *Typha latifolia* L. and *Iris pseudacorus* L. for zinc, nickel, iron and manganese. The study takes into account the effect of the water and bottom sediments on the tested macrophytes.

Study area

Krzynia is a retention reservoir situated within the area of the Dolina Landscape Park. It was established in 1925–1926 in conjunction with the construction of a water power station in the River Słupia. It covers an area of 74 ha, with a length of 3.5 km, and its width is 0.4 km. In the surrounding area of the reservoir there are beech forests, and in the direct neighbourhood one can find *Caltha palustris* L., *Chrysosplenium alternifolium* L., *Galium palustre* L., *Cardamine amara* L. and *Myosoton aquaticum* L. while *Phragmites australis* (Cav.) Trin. ex Steud., *Typha latifolia* L., *Glyceria maxima* (Hartm.) Holmb. and *Iris pseudacorus* L. are present in the littoral zone of the lake. The average annual temperature of the air for the examined area is 7.6°C, and annual precipitation is 700 mm. The vegetation period lasts approximately 200 days a year. In summer, the reservoir is used for recreation and tourism.

Methods

Samples of water, bottom sediments and leaves and roots of *Typha latifolia* L. and *Iris pseudacorus* L. were collected from 10 stations located in the littoral zone of the Krzynia Reservoir (Northern Poland, 54°20'47"N 17°12'16"E). Water pH was measured directly in the field using the potentiometer method (CPI 551, Elmetron, Poland), and electrical conductivity with the conductometric method (CC 315, Elmetron, Poland). Surface water samples were taken in polyethylene bottles of 1.5 dm³ in volume. Bottom sediments were collected with an Eckman sampler from the depth of 0–15 cm. Following transport to the laboratory, the samples were dried at a temperature of 65°C after which they were ground in a mortar and passed through a 1mm sieve. The bottom sediments were examined for active acidity (pH, H₂O), exchange acidity (pH, KCl), and organic

matter content using the heat loss method in a muffle furnace at a temperature of 550°C. Macrophyte samples taken from within the area of each station were tested from several sprouts by preparation of mixed samples, separately made of leaves and separately of roots. After the transport to the laboratory the plant material was cleaned of mineral parts of the soil, flushed in distilled water, dried at a temperature of 65°C and then homogenized in a laboratory grinder (IKA A11, Germany).

All samples (water, bottom sediments, plants) were digested wet with concentrated nitric acid (65%) and hydrogen peroxide (30%). The concentration of Zn, Ni, Fe and Mn was determined by atomic absorption spectrometry (AAnalyst 300, Perkin Elmer, USA). The analyses were performed in an oxyacetylene flame. The wavelengths at which the various metals were detected are as follows 213.9 nm Zn, 232.0 Ni, 279.5 Mn and 248.3 Fe. All the analyses were performed in three replicates. The tests were carried out following the original standards (Merck KGaA, 1g per 1000 ml, Germany).

The analytical quality of the results were checked against the following reference materials: CRM 060 (aquatic plant), provided by the European Commission Institute for Reference Materials and Measurements. The results of the experimental measurements agreed with the recommended reference values of the material.

Statistical analysis

The distribution of the content of the analysed elements of water, of bottom sediments and leaves and roots of *T. latifolia* and *I. pseudacorus* was tested by the Shapiro-Wilk test. In order to identify the factors determining the heavy metal content in the bottom sediments, the factor analysis was applied (*Principal Components Analysis, PCA*). By application of the method of main components, 2 independent factors were separated explaining 67% of variance of the physicochemical composition of bottom sediments. The significance of the differences in the heavy metals in the leaves and roots in the content of *T. latifolia* and *I. pseudacorus* was verified by the non-parametric Mann Whitney U test and compared to the natural content of Zn, Ni, Fe and Mn in plants (Tab. 4). The concentration of heavy metals in the sprouts of water plants, in the water and in the bottom sediments was used for calculation of bioconcentration factors (BCF). The bioconcentration factor was calculated as the ratio: concentration of elements in leaves/concentration of element in bottom sediments (or in water) (Salem et al. 2014). The relationship between the content of heavy metals in leaves and in roots of *T. latifolia* and *I. pseudacorus* was determined by translocation factors (TF). TF was calculated as the

ratio: concentration of element in leaves/concentration of element in roots according Bose et al. (2008).

Results and discussion

Physicochemical properties of water and bottom sediments

Surface waters as well as bottom sediments of the Krzynia Reservoir were characterized by a neutral and slightly alkaline reaction (Table 1). The levels of electrolytic conductivity revealed low mineralization of the waters (EC; 0.26–0.27 mS cm⁻¹). The organic matter content in the bottom sediments remained on average at the level of 45.8% and was substantially diversified among the stations. The heavy metal content in the surface waters of the Krzynia Reservoir was low and did not exceed the level of 0.647 mg dm⁻³ (Table 2), which indicated no anthropogenic pollution. Manganese was characterized by the highest variability of concentration within the research area (67.6%). Slightly lower variability was found in the nickel (32.2%) and zinc (28.9%). The natural origin of heavy metals in the case of water reservoirs depends on the geological structure and chemical composition of the rocks of the catchment (Czamara and Czamara 2008). Concentration of heavy metals in the examined bottom sediments was low and remained within the limits of the geochemical background for Zn and Fe. In the case of Ni and Mn it sporadically exceeded the level of the geochemical background (Table 2). The highest diversity among the stations was found for nickel (45.4%).

In order to identify factors that determine the participation of physicochemical components in bot-

Table 3. Factor loading obtained from the principal component analysis (PCA) method on the basis of the metal content in the bottom sediments of Krzynia Reservoir

Parameter	F1	F2
Organic matter	-0.77	0.12
pH (KCl)	-0.46	-0.70
pH (H ₂ O)	-0.64	-0.01
Zn	-0.77	0.38
Ni	0.13	0.91
Fe	-0.72	0.12
Mn	-0.93	-0.03
Eigen values	3.19	1.49
Explained variance [%]	0.46	0.21
	67	

Factor loadings higher than 0.7 are presented in boldface.

tom sediments, factor analysis was applied (PCA). In calculation, organic matter content, active acidity (pH, H₂O), exchange acidity (pH, KCl) and Zn, Ni, Fe and Mn content were taken into account (Table 3). By means of application of the method of main constituents, 2 independent factors were separated, explaining 67% of variability of the physicochemical composition of bottom sediments. For interpretation of data, only such factor loading values were used which exceeded 0.7. Factor 1 explained 46 % of variability and grouped organic matter, manganese, iron and zinc characterized by high, negative factor loads. The composition of 1 factor indicates that Mn, Fe and Zn are strongly associated with decomposing organic matter which is a valuable source of metals (Jonczak et al. 2015). Factor 2 explained 27 % of variability of the physicochemical

Table 1. Physicochemical properties of water and bottom sediments of Krzynia Reservoir

Value	Water		Bottom sediments		
	pH*	EC [mS cm ⁻¹]	pH* (H ₂ O)	pH* (KCl)	Organic matter [%]
Mean (median*)±SD	7.50 ± 0.16	0.26	6.90 ± 0.19	5.74 ± 0.17	45.8 ± 6.4
Minimum	7.13	0.26	6.56	5.31	36.4
Maximum	7.67	0.27	7.17	5.92	56.3

Table 2. Heavy metal content in water (mg dm⁻³) and in bottom sediments (mg kg⁻¹) of Krzynia Reservoir

	Value	Zn	Ni	Fe	Mn
Water	Mean ±SD	0.032 0.001	0.023 0.007	0.521 ±0.06	0.060 ±0.04
	Minimum	0.020	0.014	0.444	0.008
	Maximum	0.051	0.035	0.647	0.139
	CV [%]	28.9	32.2	12.1	67.6
Bottom sediments	Mean ±SD	32.5 ±5.9	11.4 ±5.2	10361.8 ±2521.8	599.3 ±83.3
	Minimum	26.7	4.8	7246.7	467.3
	Maximum	47.2	21.0	14770.0	699.0
	CV [%]	18.3	45.4	24.3	13.9
	Geochemical background	48.0 ¹	5.0 ¹	15 000.0 ²	500.0 ¹

¹Woitke et al. (2003); ²Bojakowska and Sokołowska (1998).

composition of bottom sediments and was created by Ni and exchange acidity characterized by high, opposite factor loads.

Heavy metals content in plants

The heavy metal content in the organs of aquatic plants was substantially diversified. The highest levels of average zinc quantity were discovered in roots of *T. latifolia* (61.7 mg kg^{-1}) and the lowest, in leaves of *I. pseudacorus* (22.6 mg kg^{-1}), (Table 4). Zn content in the organs of plants usually takes values from 10 to 70 mg/kg (Kabata-Pendias and Pendias 1999) excluding the roots, which often exceed 70 mg kg^{-1} (Fig. 1, Table 4). Zinc is an essential micronutrient for plants (Deng et al. 2004). The sufficient concentration in leaves falls within the limit of $15\text{--}30 \text{ mg kg}^{-1}$ which covers the physiological needs of most plants (Kabata-Pendias and Pendias 1999). Zn is a metal commonly introduced to surface waters along with surface flows. Similar relations in zinc distribution in aboveground and underground sprouts of various aquatic plants have been described in the works of Vardanyan and Ingole (2006), Parzych et al. (2015) and Parzych et al. (2016).

The average nickel content remained at the level of 29.0 mg kg^{-1} in leaves and 31.6 mg kg^{-1} in roots of *T. latifolia* and 90.8 mg kg^{-1} in leaves and 40.2 mg kg^{-1} in roots of *I. pseudacorus* (Table 4). At all stations higher Ni content in the organs of the examined plants was found. The natural nickel content in the leaves is usually $0.1\text{--}5.0 \text{ mg kg}^{-1}$ (Kabata-Pendias and Pendias 1999), but these levels can be higher since nickel easily undergoes bioaccumulation in water plants (Sarosiek and Woźakowska-Natkaniec 1993).

In the case of iron, very high levels of this element were found in the roots of *T. latifolia* ($6986.9 \text{ mg kg}^{-1}$) and *I. pseudacorus* ($2375.2 \text{ mg kg}^{-1}$) (Fig. 1, Table 4). Iron concentration in leaves was low and remained at $145.0\text{--}198.6 \text{ mg kg}^{-1}$. Iron is among the low mobility elements in a plant and is mostly gathered in the un-

derground parts (Kabata-Pendias and Pendias 1999). A high content of iron in the roots of plants can indicate accumulative properties of that species in respect to Fe. At the same time, minor concentrations in the leaves (Fig. 1, Table 4) indicate the existence of a protective barrier, enabling the transfer of the iron compounds from the roots to leaves (Hozina et al. 2001).

The highest content of manganese was found in the roots of *I. pseudacorus* (774.0 mg kg^{-1}) (Fig. 1, Table 4). Mn concentration in leaves was lower at 759.1 mg kg^{-1} (*T. latifolia*) and 382.8 mg kg^{-1} (*I. pseudacorus*). Plant demand for manganese is diverse, in most cases a sufficient level is $10\text{--}25 \text{ mg kg}^{-1}$ (Kabata-Pendias and Pendias 1999). The increased Mn content in organs of aquatic plants indicates a positive influence of these macrophytes on purification of waters and bottom sediments from manganese compounds in relation to physiological demand and can be a genetic feature. Manganese concentrations are generally high in most plants as a consequence of its easily moving within plants to the above-ground plant organs (Salem et al. 2014).

The relationships regarding the content of the determined heavy metals in the organs of macrophytes was identical for the tested species and could be arranged into the following sequences: $\text{Mn} > \text{Fe} > \text{Ni} > \text{Zn}$ in leaves and $\text{Fe} > \text{Mn} > \text{Zn} > \text{Ni}$ in roots. The non-parametrical Mann-Whitney U test revealed a series of statistically salient differences in the content of Mn in the leaves and Zn and Fe in the roots of *Typha latifolia* L. and *Iris pseudacorus* L (Table 4). Through the accumulation of substantial quantities of heavy metals in their organs macrophytes created an effective protective barrier for the waters and bottom sediments.

Bioaccumulation and translocation factors of heavy metals

The examined water plants showed diversified accumulation properties in relation to zinc, nickel, iron and manganese present in water and bottom sediments. Levels of BF_w indices ranged from 278.3 to 12 900. The

Table 4. The heavy metal content (mg kg^{-1}) $\pm$ SD (standard deviation) in leaves and roots of *Typha latifolia* and *Iris pseudacorus* and statistical differences in their concentration

		<i>Typha latifolia</i>		<i>Iris pseudacorus</i>		U Mann Whitney test p level	Natural content in plant ¹	Toxity content in plant ¹
		mean	range	mean	range			
Zn	Leaves	28.6 ± 5.7	21.1–38.9	22.6 ± 7.2	11.6–33.4	0.180194	10–70	>100
	Roots	61.7 ± 14.3	44.9–90.5	40.3 ± 11.2	18.2–60.5	0.004429		
Ni	Leaves	29.0 ± 14.1	14.5–60.6	90.8 ± 158.4	11.2–533.3	0.140237	0.5–5	>10
	Roots	31.6 ± 18.9	10.5–68.9	40.2 ± 77.5	4.6–258.9	0.122911		
Fe	Leaves	145.0 ± 30.7	111.5–215.0	198.6 ± 121.8	109.2–537.1	0.069151	5–375	toxic not know
	Roots	6986.9 ± 2553.1	3198.0–11913.0	2375.2 ± 1721.7	200.2–4793.7	0.000300		
Mn	Leaves	759.1 ± 206.7	470.6–1241.7	382.8 ± 191.4	106.8–789.5	0.004429	10–25	>500
	Roots	770.7 ± 230.9	348.9–1118.3	774.0 ± 396.8	346.1–1681.7	0.871760		

¹Kabata-Pendias and Pendias (1999).

Fig. 1. Median, minimum and maximum content of heavy metals in the organs of aquatic plants in Krzynia Reservoir

highest bioaccumulation was found in the case of manganese and iron and slightly lower with respect to zinc and nickel. The reaction of the examined water was a positive factor for the accumulation of Mn and Fe (Table 1). The main factor influencing the availability of heavy metals for the examined plants is the reaction of water and bottom sediments. Solubility of metals is low to neutral and alkaline reactions increase along with a lowering of the value of pH. Increased mobility of Zn and Mn is most effective with pH = 6, Ni at pH = 5.5, while for Fe at pH = 4. Manganese, however, is also characterized by increased solubility in an alkaline

environment (Alloway 1995; Smal and Salomons 1995). Much higher quantities of heavy metals were accumulated in the roots than in the leaves of the examined species with the exception of *I. pseudacorus*, in which case larger quantities of Ni were found in leaves. The highest levels of BF_{bs} were found for Ni (2.54–7.96), slightly lower for Zn (0.69–1.89) and Mn (0.64–1.29). Bioaccumulation factors for Fe had the lowest values (0.01–0.67), both in the case of leaves and roots of *T. latifolia* and *I. pseudacorus* (Table 5). The high levels of BF_{bs} for nickel confirm the high mobility and ease of accumulation of this element in the plant tissues. The

Table 5. Bioaccumulation factors (BF) of heavy metals in plants from Krzynia Reservoir

Species		Zn		Ni		Fe		Mn	
		BF_{bs}	BF_{w}	BF_{bs}	BF_{w}	BF_{bs}	BF_{w}	BF_{bs}	BF_{w}
<i>Typha latifolia</i>	Leaves	0.88	893.7	2.54	537.0	0.01	278.3	1.27	12651.7
	Roots	1.89	1228.1	2.77	585.2	0.67	13410.5	1.29	12845.0
<i>Iris pseudacorus</i>	Leaves	0.69	706.2	7.96	1681.5	0.02	381.2	0.64	6380.0
	Roots	1.89	1259.4	3.53	744.4	0.23	4558.9	1.29	12900.0

Note: BF_{bs} – calculated on the basis of heavy metals concentration in bottom sediments, BF_{w} – calculated on the basis of heavy metals concentration in water.

Table 6. Translocation factor (TF) of heavy metals in organs (Leaves/Roots) of *Typha latifolia* and *Iris pseudacorus* from Krzynia Reservoir

Species	Zn	Ni	Fe	Mn
<i>Typha latifolia</i>	0.46	0.91	0.02	0.98
<i>Iris pseudacorus</i>	0.56	2.26	0.08	0.49

strong cumulative property of some macrophytes in reference to nickel has been confirmed in the research of Salt and Kramer (2000), Mays and Edwards (2001), Parzych (2014) and Parzych et al. (2016). The values of BF_w and BF_{bs} confirm the very close interactions between water, bottom sediments and plants.

By means of translocation factors (TF) mobility of zinc, nickel, iron and manganese in relation leaves-roots was represented. From among the examined heavy metals, nickel showed the highest mobility in the organs of *I. pseudacorus* (TF = 2.26). The levels of translocation factors in the case of the remaining metals were $TF < 1.0$ (Table 6). Nickel is easily accumulated by plants and transported to their aboveground parts, and when in excess, it is accumulated in the roots (Kabata-Pendias and Pendias 1999).

Conclusion

The analysis of the physicochemical and chemical analysis of the waters, bottom sediments and organs of macrophytes revealed substantial variations between the stations and species. The concentration of heavy metals in the waters and bottom sediments of the Krzynia Reservoir was low and excluded any impact of anthropogenic factors. Two independent main factors, determined by PCA, explain 67% of the total variability of the physicochemical composition of the bottom sediments. Factor 1 explained 46% of variants and its composition confirmed the strong relationship between Mn, Fe and Zn with organic matter. Factor 2 explained 27% of variability of the physical and chemical composition of the examined bottom sediments and was made up of Ni and pH (KCl), thus confirming the increasing mobility of nickel along with acidification of the environment. The tested plants mainly accumulated heavy metals in roots, with the exception of nickel which appeared in larger quantities in the leaves. The relationships respecting the content of the determined elements in the macrophyte organs was identical for the tested species and could be arranged into the following sequences: $Mn > Fe > Ni > Zn$ in leaves and $Fe > Mn > Zn > Ni$ in roots. Statistically significant differences were found in the content of Mn in the leaves and Zn and Fe in the roots of *Typha latifolia* and *Iris pseudacorus*. By accumulating substantial quantities of heavy metals in their organs, macrophytes constitute an

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