Trace metal speciation in sediments and bioaccumulation in *Phragmites australis* as tools in environmental pollution monitoring

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Abstract

In the present study, sequential extraction of metals from bottom sediments, the risk assessment code and *Phragmites australis* bioaccumulation ability were used to assess ecological risk of trace metal pollution on aquatic ecosystems. Surface bottom sediments and leaves of reed collected from agricultural and urban areas were examined for Cu, Fe, Mn, Ni and Pb contents. Results showed that the total metal content in sediment cannot be regarded as a reliable indicator of metal pollution and the risk of environment. High total contents of the metals as well as contents in individual sediments fractions were connected with high organic carbon and domination of the silt–sand, clay–silt or clay particles. However, the percentage of metals bound to each fraction was independent from these properties of sediment. Particular metals characterized by different behavior in the sediments: Mn had the highest percentage bound to the most labile fractions; Cu and Ni had considerable high percentage in potentially bioavailable (reducible or oxidizable) fractions; Fe and Pb had high percentage in the residual fraction. The risk assessment code, sequential analysis and the bioindication method showed generally consistent results: no Cu, Fe and Pb pollution and a high risk of Mn pollution in the study sites, but each one gave different detailed information.

Keywords Aquatic macrophyte · Bioindication · Common reed · Risk assessment code · Sequential extraction

Introduction

In recent decades, the anthropogenic activity has resulted in a substantial increase in trace metals content in the environment and the pollution of many areas worldwide. Trace metals are non-degradable; they accumulate in soil, sediment and living organisms posing serious threat to the environment and humans. Industry, agriculture and urban areas are the source of metals that reach the aquatic environments in which they accumulate in flora and fauna or are incorporated into sediments (Bo et al. 2015; Kabata-Pendias and Pendias 2001; Li et al. 2016). In particular, organisms that have direct contact with sediments, e.g., rooted macrophytes, are under greater influence from metals accumulated in sediments than those dissolved in water (Štrbac

et al. 2014). The mobility of metals, their bioavailability and toxicity to organisms strongly depends on their chemical form. In uncontaminated sediments, metals are usually associated with silicates and primary minerals and therefore their mobility is limited. Metals of anthropogenic origin are weaker bound to the sediment particles (e.g., to the carbonates, oxides, hydroxides and sulfides) which makes them more mobile and bioavailable. Depending on the environmental conditions (e.g., pH, redox potential, organic ligand levels), metals fixed in the sediments can be remobilized (Kabata-Pendias and Pendias 2001; Liu et al. 2009).

To assess the level of pollution and to predict the risk posed by metals in aquatic ecosystems, the analyses of the sediments and living organisms are more reliable than analysis of water which can be affected by accidental discharges of wastes and where pollutants can be easily diluted (Bonanno 2011; Klink et al. 2009; Štrbac et al. 2014). Determination of the total metals content in the sediment is regarded not sufficient to give reliable information on their mobility or the ecological risk (Passos et al. 2010).

Metal pollution in aquatic ecosystems has been frequently studied by the determination of metal content in aquatic macrophytes which are considered good bioindicators. One

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of the most studied wetland macrophytes, characterized by a good chemical elements accumulation ability is common reed *Phragmites australis*. The species is regarded to be a good indicator of the condition of the environment because of a strong positive correlation between the concentrations of metals in the sediment and all reed organs (rhizomes, stems and leaves) (Bonanno 2011; Kabata-Pendias and Pendias 2001; Klink et al. 2009; Štrbac et al. 2014).

Although some studies investigated the metal speciation in bottom sediments and accumulation in leaves of *P. australis* in metal pollution monitoring (Bonanno and Lo Giudice 2010; Bonanno 2013; Klink et al. 2009), to date the studies analyzing the content of metals in plants and their content in various fractions of the bottom sediments simultaneously are lacking. To fill this gap, the present study carried out in September 2017 in west and southwest Poland aimed to: (1) assess trace metal content and sequestration in the sediments characterized by different properties; (2) determine the percent of trace metals binding to the main fractions in bottom sediment; (3) compare bioindication and physical and chemical methods of the metal pollution assessment.

Materials and methods

Study sites and sample collection

Samples for the study were taken from 23 sites designated in west and southwest Poland: the Leszczyńskie Lakeland in Greater Poland (sites numbers 1–4) and the area of the Wrocław agglomeration in Lower Silesia (sites numbers 5–23) (Fig. 1). Designated study sites differ in local sources of pollution and dominant land use. The Leszczyńskie Lakeland is a recreational area dominated by woods, meadows and wetlands. There is no intensive farming or large industrial plants (Rąkowski et al. 2004). Study sites were located at the banks of four glacial lakes: Olejnickie, Górskie, Osłonińskie and Wieleńskie. Study sites around Wrocław were designated in oxbow lakes, backwaters of small rivers and artificial reservoirs. The geological substrate of the region (the Odra Valley) consists of Miocene clays, sands and gravels. Study sites 5–7 were situated in recreational park within the city of Wrocław; sites 8–11 in forested areas near Siechnice and sites 12–14 in Siechnice—a small industrial city, where the main pollution sources are Heat and Power Plant “Czechnica,” a slag heap of a ferrochrome steel plant (closed in 1995) and few small manufacturing plants, e.g., cereals processing plant, metallurgical plants, concrete plant, logistics centers; sites 15–16 near the eastern border of Wrocław in the infiltration fields that are the source of drinking water for the city; sites 17–19 in an oxbow lake “Matunin” in recreational part of Jelcz-Laskowice, a small industrial city; sites 20 and 21 were located in an old gravel

pit near Czernica village; and study sites 22–23 in an anthropogenic lake formed in the former gravel and clay pit at the old Odra riverbed near Kamieniec Wrocławski (Biłyk and Kowal 1993; Fabiszewski 2005a, b; Meinhardt et al. 2014).

In September 2017, at each site three samples of superficial sediment (0–10 cm) were collected randomly within a 5 m × 5 m square using stainless steel collector. Sediment samples were taken at the depth corresponding to the extent of plants roots (0–10 cm) as recommended by Baldantoni et al. (2009) when studying geochemistry of *P. australis*. The same method is usually used for sampling sediments for risk assessment of metals (Bo et al. 2015). Simultaneously, leaves from five randomly selected plants of common reed (*Phragmites australis*) were taken. Ten mature, fully developed, healthy (not affected by insects or injured) leaves were collected from each plant (Karla 1998). Samples were placed in plastic bags to avoid accidental contamination.

Chemical analyses

Plant samples were rinsed with deionized water to eliminate any adhering materials, dried in an oven at 50 °C to mass constancy and homogenized in laboratory mill POLY-MIX PX-MFC 90 D (Kinematica AG, Switzerland). Plant samples (0.5 g) were digested in an open system with nitric acid (65%, for analysis, Merck KGaA) and hydrogen peroxide (30%, for analysis, Chempur) during which temperatures were raised to approximately 95 °C until evolution of nitrous gas had stopped and the digest became clear. The digests were diluted with deionized water to 50 ml and analyzed for Fe and Mn using FAAS (flame atomic absorption spectroscopy) and Cu, Ni and Pb using GFAAS (graphite furnace atomic absorption spectroscopy) (both Avanta PM from GBC).

Field texturing techniques (USDA 2017) were used to determine dominant grain size fractions in the sediment samples. Sediments pH was measured potentiometrically in fresh bottom sediments (sediment/distilled water ratio 1:2.5) (Mikroprozessor-pH-meter HI9107, Hanna Instruments). Air-dried samples of bottom sediments were passed through a 2-mm-diameter sieve and homogenized with a mortar and pestle. Homogenized samples were subjected to organic carbon determination by Tiurin’s method (Mebius 1960) and sequential extraction by modified method proposed by Tessier et al. (1979) with additional determination of the water-soluble fraction according to O’Sullivan et al. (2004). The scheme of the extraction is given in Table 1. The content of metal in the mineral (residual) fraction was calculated by subtracting the sum of the metal contents obtained in fractions 1–5 from the total metal content obtained by the digestion of the sample. Metal contents in the solutions obtained in each step of the sequential extraction were determined using the methods described above for plant samples.

Fig. 1 Location of study sites: 1—Leszczyńskie Lakeland; 2—Wrocław agglomeration

All analyses were done in duplicate. All results for plants and bottom sediments were calculated on a dry weight basis. High quality of analyses had been secured by using Sigma Chemical Co. standards for all elements and blank samples containing the same matrix as the samples. The reproducibility of the elements determination was checked by analysis of the Certified Reference Materials WEPAL-IPE-176 (*Reed/Phragmites communis*, WEPAL, Wageningen, Netherlands). The recovery rates were 98 ± 4 (percent $\pm$ SD).

Calculation of the risk assessment code

The risk assessment code (RAC) was calculated as a percentage of metals present in exchangeable and carbonate phases (first three steps in this study: water-soluble, exchangeable and bound to carbonates) (Perin et al. 1985). Metal pollution in sediments was then evaluated using five-level risk scale of RAC: no risk ($< 1\%$), low risk (1–10%), medium risk (11–30%), high risk (31–50%), and very high risk ($> 50\%$).

Table 1 The scheme of the sequential extraction

Step	Fraction	Procedure
1	Water-soluble	Deionized water 1 h shaking and left overnight, room temp
2	Exchangeable	1 M $\text{CH}_3\text{COONH}_4$ (pH 7) 1 h shaking, room temp
3	Bound to carbonates	1 M CH_3COONa acidified with CH_3COOH to pH 5 5 h shaking, room temp
4	Bound to Fe–Mn oxides	0.04 M $\text{NH}_2\text{OH}\cdot\text{HCl}$ in 25% (v/v) CH_3COOH 5 h shaking, temp. 95 °C
5	Bound to organic matter	0.02 M HNO_3 + 30% H_2O_2 (pH 2) 2 h shaking, temp 85 °C 30% H_2O_2 (pH 2) 3 h shaking, temp 85 °C 3.2 M $\text{CH}_3\text{COONH}_4$ in 20% (v/v) HNO_3 0.5 h shaking, room temp
6	Residual (mineral)	Digestion procedure as described for plant samples

Data analysis

The statistical calculations were performed using Statistica 13 (StatSoft Inc. 2016). After the standardization of the values, the matrix of content of 5 metals (Cu, Fe, Mn, Ni, Pb) in 6 fractions of bottom sediments collected from 23 study sites was used in the numerical classification analysis to identify samples with similar partitioning of metals in the sediment fractions. The Ward clustering method and Manhattan distance similarity method were used.

The normality of features was checked by Shapiro–Wilk's *W* test. Since the variables were not normally distributed, the differences in pH, organic carbon content in sediments and metals content in plants and in sediments between groups distinguished by numerical classification were checked using Mann–Whitney *U* test (Dytham 2011). The relationship between organic carbon content and total metals content in sediments was checked using Spearman correlation coefficient. The probability level was set at 0.05.

For each group, after Box–Cox transformation of the variables, the matrix of the percentage content of five metals in six sediment fractions was analyzed by the principal component and classification analysis (PCCA) to reveal similarities between metals content in specific fractions and to show correlations between the original variables and the first two factors (Legendre and Legendre 1998).

Results and discussion

Results

Metal content in the sediments

Numerical classification helped to identify the groups of study sites with similar pattern of metal content in six sediment fractions. Using a dendrogram of the study sites, two

main clusters were distinguished: A, grouping study sites 5, 6 and 15–20; and B, grouping study sites 1–4, 7–14 and 21–23 (Fig. 2). Total contents of metals studied (Cu, Fe, Mn, Ni, Pb) and metal contents in the individual fractions (with some exceptions) (Table 2) were significantly higher in sediments in group A than B. For all metals except Mn, the content in water-soluble fractions did not differ between sediments in group A and B. Exchangeable fractions of Fe and Pb as well as reducible fractions of Pb were also similar in both groups (Mann–Whitney *U* test, $p > 0.05$). In addition, organic carbon content was significantly higher (Mann–Whitney *U* test, $p < 0.05$) in sediments from group A (range 1.4–12.5%, median 7.2%) than in group B (range 0.15–7.4%, median 0.56%). Significant positive correlations that were found between organic carbon content in sediments and total content of the particular metals: Cu ($r = 0.87$), Fe ($r = 0.81$), Mn ($r = 0.85$), Ni ($r = 0.80$) and Pb ($r = 0.89$), confirmed that higher total metals content was related to higher organic carbon content in the sediments studied. Also, these groups clearly differed in respect of main grain size fractions (Table 3). Group A characterized by domination of the silt–sand, clay–silt or clay fractions and group B characterized by domination of the sand or sand–silt fractions. The pH of sediments did not differ significantly between the groups (Mann–Whitney *U* test, $p > 0.05$) and was in ranges 2.5–6.7 (group A) and 3.4–7.5 (group B).

Different total contents of metals in sediments in group A and B preclude direct comparison of the metal speciation. Thus, the percentage share of each fraction in total metal content was calculated for each metal separately in group A and B and illustrated in Fig. 3. Generally, the greatest percentage of metals was in the residual (mineral) fraction and the lowest in the water-soluble and the exchangeable fractions.

The PCCA ordination showed different patterns of the percentage metal content in specific fractions in group

Fig. 2 A diagram for 23 study sites based on concentrations of Cu, Fe, Mn, Ni and Pb in six sediment fractions, with Ward clustering method and Manhattan distance similarity method

A (Fig. 4) and in group B (Fig. 5). In group A, the first principal factor positively correlated with water-soluble, exchangeable and bound to carbonates fractions and negatively correlated with mineral fraction and, to a lower extent, to organic matter fraction. The second principal component correlated positively with the fraction bound to the Fe–Mn oxides and negatively with the fraction bound to organic matter. The first principal component discriminated between Cu, Pb and Fe (negative scores) and Ni and Mn (positive scores). The second principal component discriminates between Cu and Pb (negative scores) and Fe, Ni and Mn (positive scores). The plot showed that Fe, Cu and Pb were the metals with high percentage content in mineral fraction and low in the most labile fractions (water soluble, exchangeable and bound to carbonates). At the same time, the second principal factor discriminated between these metals: Fe had high percentage found in the fraction bound to Fe–Mn oxides and Cu and Pb were bound to organic matter. Nickel and Mn had a relatively high share in labile fractions (water soluble, exchangeable and bound to carbonates) and the fraction bound to Fe–Mn oxides, while lower in mineral fraction and the fraction bound to organic matter. In group B, the first principal factor positively correlated with water soluble, exchangeable and bound to carbonates fractions and negatively correlated with mineral fraction. The second principal component correlated positively with mineral fraction and negatively with the fractions bound to Fe–Mn oxides and to organic matter. The first principal factor discriminated between Pb and Fe (negative scores, metals with

high percentage in mineral fraction) and Mn (positive scores, metal with high percentage in labile fractions). The second principal factor discriminated between Pb and Mn (mostly positive scores, metals with relatively low percentage in the fractions bound to Fe–Mn oxides and to organic matter) and Fe, Ni and Cu (mostly negative scores), which characterized by high percentage bound to organic matter or to Fe–Mn oxides fractions.

The percentage content in the most labile fractions (steps 1–3; sum of metal concentrations in water soluble, exchangeable and bound to carbonates fractions) for the metals studied was similar in both groups (Mann–Whitney *U* test, $p > 0.05$) and followed the order: Mn (41%) > Ni (16%) > Cu (10%) > Pb (9%) > Fe (2%) in group A and Mn (43%) > Ni (24%) > Pb (14%) > Cu (11%) > Fe (3%) in group B. The percentage content of the metals in fractions 1–5 (sum of metal concentrations in water soluble, exchangeable, bound to carbonates, bound to Fe–Mn oxides and bound to organic matter fractions), which are potentially bioavailable, can be ordered as follows: Mn (73%) > Ni (66%) > Cu (54%) > Pb (49%) > Fe (39%) in group A and Cu (70%) > Mn (66%) > Ni (64%) > Fe (40%) > Pb (25%) in group B. Only for Cu and Pb, the percentage content of potentially bioavailable fractions differed significantly between group A and B (Mann–Whitney *U* test, $p < 0.05$).

Table 2 Median, minimum, maximum and average deviation (AD) of content [mg kg^{-1} DW] of metals in bottom sediment fractions in groups of study sites and the results of Mann–Whitney U test (Z and p values)

Metal	Fraction	GROUP A, $n=8$				GROUP B, $n=15$				Z	p
		Median	Min	Max	AD	Median	Min	Max	AD		
Cu	Total	17.4*	11.3	53.9	4.9	4.0*	2.3	7.5	0.42	−3.84	<0.001
	Water soluble	0.07	0.04	3.0	0.36	0.05	0.01	0.17	0.01	−1.84	>0.05
	Exchangeable	0.30*	0.19	0.64	0.05	0.10*	0.03	0.26	0.02	−3.52	<0.001
	Carbonate	1.2*	0.40	3.8	0.39	0.17*	0.06	0.52	0.04	−3.65	<0.001
	Reducible	1.2*	0.75	3.0	0.25	0.66*	0.46	0.93	0.04	−3.39	<0.001
	Oxidizable	5.7*	3.2	17.9	1.9	1.6*	0.77	2.2	0.13	−3.84	<0.001
	Residual	7.0*	5.0	28.4	2.7	1.3*	0.07	4.4	0.29	−3.84	<0.001
Fe	Total	24,425*	8522	50,036	4522	1849*	826	9480	572	−3.78	<0.001
	Water soluble	30.6	5.6	624	74.9	15.5	1.1	120	9.1	−1.13	>0.05
	Exchangeable	3.1	0.60	358	44.5	1.6	0.39	7.0	0.51	−0.87	>0.05
	Carbonate	209*	88.1	2048	233	30.7*	2.6	206	14.4	−3.45	<0.001
	Reducible	5417*	4275	6937	289	552*	211	4209	263	−3.84	<0.001
	Oxidizable	2501*	837	8268	919	156*	41.2	509	37.1	−3.84	<0.001
	Residual	16,176*	3312	31,801	3081	1072*	471	4634	298	−3.78	<0.001
Mn	Total	541*	238	1357	154	31.6*	6.4	226	14.9	−3.84	<0.001
	Water soluble	9.6*	6.7	37.0	3.5	1.4*	0.48	24.7	1.6	−3.26	<0.001
	Exchangeable	69.9*	23.0	500	57.1	5.9*	0.82	45.8	3.4	−3.58	<0.001
	Carbonate	73.5*	41.2	224	23.9	2.7*	0.44	51.3	3.6	−3.78	<0.001
	Reducible	207*	59.8	387	49.6	3.9*	2.5	46.8	3.2	−3.84	<0.001
	Oxidizable	16.4*	4.4	41.9	4.4	0.60*	0.22	2.1	0.15	−3.84	<0.001
	Residual	143*	35.5	470	65.2	12.7*	0.24	82.3	5.5	−3.58	<0.001
Ni	Total	21.9*	12.8	40.9	3.4	5.1*	0.60	8.9	0.71	−3.84	<0.001
	Water soluble	0.09	0.06	5.9	0.72	0.04	0.01	0.68	0.06	−1.65	>0.05
	Exchangeable	0.42*	0.15	1.6	0.19	0.14*	0.02	1.8	0.12	−2.10	<0.05
	Carbonate	2.3*	1.7	3.1	0.18	0.49*	0.09	1.6	0.12	−3.84	<0.001
	Reducible	6.6*	5.5	15.4	1.2	0.97*	0.20	2.1	0.17	−3.84	<0.001
	Oxidizable	3.6*	2.0	8.0	0.77	0.33*	0.05	2.7	0.17	−3.65	<0.001
	Residual	9.1*	1.2	16.0	1.9	1.3*	0.07	4.4	0.39	−3.07	<0.01
Pb	Total	26.1*	16.1	135	15.6	3.4*	1.6	10.4	0.78	−3.84	<0.001
	Water soluble	0.05	0.01	0.15	0.02	0.05	0.002	0.23	0.02	0.16	>0.05
	Exchangeable	0.09	0.01	1.8	0.22	0.08	0.01	1.9	0.12	−0.16	>0.05
	Carbonate	2.4*	0.51	6.4	0.79	0.22*	0.03	2.1	0.14	−3.20	<0.001
	Reducible	0.37	0.00	1.9	0.21	0.14	0.08	0.72	0.04	−1.45	>0.05
	Oxidizable	14.5*	4.4	33.9	3.4	0.14*	0.08	1.4	0.09	−3.84	<0.001
	Residual	10.1*	1.3	95.4	11.9	2.5*	1.1	7.7	0.54	−2.94	<0.01

*The values with the asterisk differ significantly between groups (Mann–Whitney U test, $p < 0.05$)

Metal content in *P. australis*

The contents of metals in leaves of *P. australis* are summarized in Table 4. No significant differences were found between groups A and B distinguished by numerical classification based on the metal content in the sediments (Mann–Whitney U test, $p > 0.05$). In both groups, the most abundant element in plant's leaves was Mn followed by Fe and Cu, while contents of Ni and Pb were the lowest.

The risk assessment code

The risk assessment code (RAC) calculated for the sediments in group A and group B is given in Table 5. In group A, RAC was lower than 10% and the pollution risk was evaluated as low for Cu, Fe and Pb, and for Ni RAC was slightly higher and the risk assessed as medium for this metal. In group B, RAC was lower than 10% for Fe indicating low pollution risk and higher than 10% for Cu, Ni and

Table 3 The properties of the bottom sediments in the study sites

Study site	Group	Organic carbon %	pH	Dominant grain size fractions
1	B	0.29	7.09	SAND
2	B	0.95	7.28	SAND–SILT
3	B	0.89	5.64	SAND–SILT
4	B	0.23	7.50	SAND–SILT
5	A	4.47	6.73	SILT–SAND
6	A	8.34	6.09	SILT–SAND
7	B	0.22	5.21	SAND
8	B	6.33	6.19	SAND–SILT
9	B	7.38	3.42	SAND–SILT
10	B	0.68	4.30	SAND–SILT
11	B	0.56	6.78	SAND
12	B	0.15	5.68	SAND
13	B	0.98	6.87	SAND–SILT
14	B	1.77	5.63	ORGANIC
15	A	9.90	4.12	CLAY
16	A	1.41	4.60	CLAY
17	A	7.47	5.31	CLAY
18	A	2.30	5.67	CLAY
19	A	12.45	2.51	ORGANIC
20	A	6.90	6.01	CLAY–SILT
21	B	0.39	6.81	SAND
22	B	0.20	7.43	SAND
23	B	0.27	6.81	SAND

Pb, signaling medium pollution risk. The pollution risk of Mn was evaluated as very high ($RAC > 31\%$) in both groups.

Discussion

Total metals content in the sediments

Differences in total metal contents in sediments in groups of the study sites (A and B) distinguished by numerical classification (Fig. 2) were connected with differences in sediment properties, i.e., dominant grain size fraction and organic carbon content (Table 3). According to Kabata-Pendias and Pendias (2001), Conrad and Chisholm-Brause (2004) as well as Neto et al. (2006), metals are largely sorbed to the organic matter and the surfaces of particles, so the sediments containing a higher percentage of high surface area particles (e.g., clay) and higher concentration of organic carbon are expected to have higher metals content than sandy sediments and sediments with low organic matter content. In the present study, total contents of all metals in sediments from group B were lower than geochemical background (GB) for freshwater bottom sediments (Cu 30, Fe 15,000, Mn 500, Ni 20 and Pb 20 mg kg^{-1}) (Woitke et al. 2003), while in group A, median contents for Fe, Mn, Ni and Pb were higher than these values, and Cu concentrations were higher in some study sites only. The observation confirmed statement that the total metal content in sediments did not give reliable information on anthropogenic pollution because is strongly affected by sediments properties (Passos et al. 2010).

Fig. 3 Fractionation of Cu, Fe, Mn, Ni and Pb in sediments in groups A and B distinguished by numerical classification

Fig. 4 Ordination plot of five metals studied based on their percentage content in six sediment fractions and projection of the content in these fractions on the component plane in group A

Fig. 5 Ordination plot of five metals studied based on their percentage content in six sediment fractions and projection of the content in these fractions on the component plane in group B

Metal speciation in the sediments

Individual metals in this study characterized by different behavior in the sediments (Table 2 and Fig. 3). Metals associate with the sediment by different mechanisms which is

reflected by the fractions they are bound to. Different behavior of metals is also regarded to give an information of the origin of the metals and anthropogenic pollution of the sediments (Passos et al. 2010). The metals extracted in the first stages of sequential analysis (water soluble, exchangeable and bound to carbonates) are the most labile and bioavailable fractions which can be directly absorbed by the biota. Simultaneously, they are considered to give an information on the pollution potential and the recent contamination (Li et al. 2016; Naji et al. 2010; Passos et al. 2010). In this study, among the metals studied, Mn had the highest percentage bound to the most labile fractions (Fig. 3). Cooper and Ni were the metals with considerable high percentage in reducible (bound to Fe–Mn oxides) or oxidizable (bound to organic matter) fractions which are known to be potentially bioavailable depending on physical and chemical conditions, e.g., oxygen content, redox potential changes or bacterial activity, which affect their solubility. Strong associations between trace metals and the reducible or oxidizable fractions are considered to indicate pollution that occurred in an earlier period of time than that in more labile fractions, usually being related to the industry emissions. The residual (mineral) fraction represents metals of the natural origin, largely embedded in the crystal lattice of the sediment and usually not available. High percentage content of metals in the residual fraction (Fe and Pb in this study) indicates the relatively unpolluted environment (Li et al. 2016; Naji et al. 2010; Passos et al. 2010).

The high affinity of Cu to organic matter found in this study (36.4% of total content in group A and 40.5% in group B) (Fig. 3) was also reported by other authors. High percentage of Cu (51%) bound to organic matter and in the residual fraction (43%) was found by Sobczykński and Siepak (2001) in bottom sediments from relatively unpolluted lakes in Wielkopolski National Park in Poland. On the contrary to present results, authors reported no detectable Cu in exchangeable forms nor bound to carbonates and only 5.8% bound to Fe–Mn oxides. Similarly, organic matter was the major scavenger for Cu among the non-lithogenous in the sediments of polluted Mahanadi river in India (Sundaray et al. 2011).

Iron was another metal characterized by the high percentage content in the residual fraction (Fig. 3.). Relatively high percent (25% in group A and 28% in group B) of the total Fe content was also bound to Fe–Mn oxides. Simultaneously, Fe had the lowest percentage content in the three most labile fractions of the sediments. Similar behavior of Fe was reported by Sobczykński and Siepak (2001) and Sundaray et al. (2011): The highest percent of the total content was bound to mineral fraction, the second highest was bound to Fe–Mn oxides and only the marginal percent was bound to organic matter, carbonates and the exchangeable fraction. These findings correspond to Kabata-Pendias and

Table 4 Median, minimum, maximum and average deviation (AD) of content [mg kg^{-1} DW] of metals in leaves of *Phragmites australis* in groups of study sites and the results of Mann–Whitney *U* test (*Z* and *p* values)

Metal	GROUP A, <i>n</i> = 8				GROUP B, <i>n</i> = 15				<i>Z</i>	<i>p</i>
	Median	Min	Max	AD	Median	Min	Max	AD		
Cu	5.97	5.32	7.49	0.30	5.16	2.75	8.89	0.41	−1.84	>0.05
Fe	152	110	223	12.3	141	104	235	7.78	−1.32	>0.05
Mn	652	83.3	1791	183	457	102	1094	90.0	−0.94	>0.05
Ni	0.89	0.44	1.62	0.12	0.87	0.47	1.45	0.07	0.03	>0.05
Pb	1.11	0.64	2.27	0.19	0.79	0.37	3.31	0.24	−0.74	>0.05

Table 5 The risk assessment code (RAC) in groups of study sites distinguished by numerical classification

GROUP A			GROUP B	
RAC value		Metal pollution in sediments	RAC value	Metal pollution in sediments
Cu	9.7	Low risk	10.9	Medium risk
Fe	1.9	Low risk	3.0	Low risk
Mn	40.6	High risk	42.9	High risk
Ni	15.9	Medium risk	23.7	Medium risk
Pb	8.7	Low risk	14.2	Medium risk

Pendias (2001) who stated that Fe content in easily soluble and exchangeable species is usually low.

Manganese was mainly bound to Fe–Mn oxides, mineral and exchangeable fraction (Fig. 3.). This metal is rather rarely reported in the studies on the metals speciation in sediments. Sobczyński and Siepak (2001) found that in relatively unpolluted sediments, Mn was mainly bound to Fe–Mn oxides (37%). On the contrary to the present study, abovementioned authors found higher content (25%) of Mn bound to carbonates and organic matter (11%) but only 17% bound to the mineral fraction and 9.5% in exchangeable fraction. Similarly as in this study, the sediments in the polluted river studied by Sundaray et al. (2011), contained high percentage of Mn bound to Fe–Mn oxides and low-bound to the organic matter and carbonates. However, in the study of Sundaray et al. (2011), Mn content in the residual fraction was considerably higher (59%), and in the exchangeable fraction (4.6%), definitely lower than in the present study. The differences may be connected with different sediments physical and chemical properties that caused leaching of Mn or with recent Mn emissions in areas covered by the present study (Kabata-Pendias and Pendias 2001).

According to Kabata-Pendias and Pendias (2001), Ni is mainly bound in the mineral fraction but significant percentage of this metal is also found in organic and Fe–Mn oxides fractions, the latter being easily available to plants. Sobczyński and Siepak (2001) revealed that in the relatively unpolluted lakes of Wielkopolski National Park, Ni was bound mostly to the mineral fraction (52%). In this study,

Ni characterized by relatively low percentage content in the mineral fraction (about 35% in both groups A and B) which can suggest an anthropogenic pollution. Nickel contents in the fractions bound to Fe–Mn oxides (25%) and bound to the organic matter (18%) reported by Sobczyński and Siepak (2001) were similar as in this study but percent bound to the most labile fractions (up to 5%) was substantially lower. Passos et al. (2010) found 21% of the total Ni content bound to Fe–Mn oxides and high percentage (> 30%) in the organic matter fraction which was connected with the significant human activity in the region.

According to Kabata-Pendias and Pendias (2001), Pb is usually associated with clay minerals, Fe–Mn oxides and organic matter. In this study, Pb was also mainly bound to less mobile phases. Similar findings were reported by Sundaray et al. (2011) who noted that 67% of Pb was bound in the residual fraction in the sediments of polluted Mahanadi river in India. On the other hand, Passos et al. (2010) stated that Pb was mainly bound to organic matter (over 30%) and to Fe–Mn oxides (22% of the total) which was related to the discharges of industrial effluents and indicated potential bioavailability of this metal.

Metal content in *P. australis*

Copper, Fe, Ni and Pb contents in leaves of *P. australis* in the present study were found to be within the ranges of natural metal levels in plants given by Kabata-Pendias and Pendias (2001) and Markert (1992) and were not higher than median contents of these metals reported by Brook and Robinson (1998) for vascular plants from uncontaminated freshwaters (Table 4). Manganese content in leaves of the studied plants was significantly higher than natural levels of this metal given by Kabata-Pendias and Pendias (2001) and Markert (1992) (20–500 mg kg^{-1}) as well as the value given by Brook and Robinson (1998) for uncontaminated aquatic plants (370 mg kg^{-1}). Extraordinary accumulation of Mn (196–775 mg kg^{-1}) in the reed growing in the relatively unpolluted area was previously reported by Klink et al. (2009) suggesting that *P. australis* is a Mn accumulator. On the contrary, Štrbac et al. (2014) and Bonanno and Lo Giudice (2010) founded low (up to 109 and 308 mg kg^{-1} , respectively) Mn content in leaves of *P. australis* from

polluted Tisza River in Serbia and Imera Meridionale River in Italy. Thus, the problem of high Mn content in reed should be further investigated to clarify if it can be related to natural properties of the species to accumulate Mn or be the result of the affinity of Mn to bioavailable fractions of the sediments or some specific conditions favoring the uptake of this metal by plants.

Pollution level assessment

Results of the metal speciation showed that the content of Cu in most labile fractions were low indicating no severe recent Cu pollution in the area studied (Passos et al. 2010). Taking into account high natural affinity of Cu to organic matter (Kabata-Pendias and Pendias 2001), we can assume also no severe pollution in the past. Only in group B, Cu content bound to Fe–Mn oxides was considerably high (18.5%) which can make this metal potentially bioavailable in favorable physical and chemical conditions. Similarly, high percentages of Fe and Pb were immobilized (in the mineral fraction) and not available for biota. However, about one-quarter of the total Fe content bound to Fe–Mn oxides made this metal potentially bioavailable under reducing conditions and suggested industrial pollution (Passos et al. 2010). The results obtained in this study suggested relatively high bioavailability of Ni compared to other metals studied and possible contribution of human activity to Ni content in the sediments. Similarly, low Mn content in the mineral fraction and high in the most labile fractions, especially in the exchangeable fraction, suggested that the bioavailability of this metal could be considerable high and, similarly to Ni, the anthropogenic pollution may have significant contribution to its content in the sediments studied (Passos et al. 2010).

Analyzes of the metals contents in leaves of good plant bioindicator *P. australis* (Bonanno and Lo Giudice 2010; Klink et al. 2009; Štrbac et al. 2014; Vymazal and Brezinová 2016), similarly as RAC values, indicated no Cu, Fe and Pb pollution and confirmed high risk of Mn pollution in the study sites. Assuming that Mn was present in the most labile fractions, in the sediments, considerably high content of this metal in reed leaves was also the result of high bioavailability not only the potential ability of reed to accumulate high amounts of this metal. Although considerably high percent of total Fe content was bound to Fe–Mn oxides which made this metal potentially bioavailable under reducing conditions, its content in reed leaves did not exceed natural level in plants which can suggest that the conditions in the sediments did not favor the release of Fe. For Ni pollution assessment, the conclusions drawn from metal speciation analyses, the calculations of RAC and the use of the bioindicator were not consistent. Both methods using Ni content in sediments indicated some pollution risk but content of

this metal in the leaves of reed did not exceed natural content in plants (Brook and Robinson 1998; Kabata-Pendias and Pendias 2001; Markert 1992). Bonanno and Lo Giudice (2010), Duman et al. (2007) and Vymazal et al. (2007) noted that reed accumulated significantly more Ni in roots than in leaves. Thus, it can be assumed that in this study the sediments could be polluted with Ni easily available for plants, but it was retained in roots and not relocated to the aboveground organs.

An important observation is that significantly higher total metal contents in the sediments in group A compared to group B generally did not result into significantly higher RAC values or higher metal content in reed's leaves. Thus, the pollution risk of the sediments in both groups was similar and not directly related to the total metal content. This can be explained by the differences in the properties of the sediments, especially in the dominant grain fractions and organic matter content (Table 3).

Conclusion

Total metal content in the sediment cannot be regarded as a reliable indicator of the metal pollution and the risk of the environment because the properties of the sediments (e.g., the dominant grain fractions, clay content and organic matter content) strongly influence total content and the bioavailability of the metals in sediments. Total contents of the metals studied (Cu, Fe, Mn, Ni and Pb) as well as content in individual sediments fractions were higher in sediments with high organic carbon and characterized by domination of the silt–sand, clay–silt or clay particles than in sediments with low carbon level and characterized by domination of the sand or sand–silt particles. Although the different total contents of metals in sediments differing in properties preclude direct comparison of the metal speciation, the percentage of metals bound to each fraction show similar trends. Generally, the greatest percentage of all metals was in the residual (mineral) fraction and the lowest in the water-soluble and the exchangeable fractions. Great shares of Fe, Mn and Ni were also bound to Fe–Mn oxides, while Cu and Pb associated with organic matter.

The methods of the metal pollution assessment tested in this study (the risk assessment code RAC, the sequential analysis and the bioindication) generally showed consistent results but each one gave different detailed information. All methods indicated that there was no risk of Cu, Fe and Pb pollution and a high risk of Mn pollution in the study sites; however, the methods were not consistent for Ni pollution risk assessment. The risk assessment code (RAC) allowed to estimate the potential threat from mobile forms of metals. Sequential analysis gave similar information and additionally allowed to estimate the anthropogenic contribution to

the metal content in the sediment and approximate time of the pollution, but it was much more labor-intensive and time-consuming. However, none of methods based on sediment analysis did show the actual environmental consequences of pollution, while common reed used as a bioindicator gave additional information on the reaction of living organisms.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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