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ACCUMULATION OF TRACE METALS BY AQUATIC MACROPHYTES AND THEIR POSSIBLE USE IN PHYTOREMEDIATION TECHNIQUES

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Abstract

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The aim of the performed research was to obtain knowledge on the ability of aquatic plants naturally growing at a site to absorb trace metals contained in bottom sediments and surface water. Furthermore, we compared differences in the accumulation of trace metals by the individual groups of aquatic plants (submerged and emergent) and assessed a possible use of the individual plant species in phytoremediation techniques. Representative samples of water, sediments and aquatic macrophytes were taken from three anthropogenically loaded streams in six monitoring cycles in several collection profiles differing in the distance from a source of contamination. The samples were analysed for the total content of selected trace metals (As, Cd, Pb, Al, Hg, Zn, Fe, Mn, Cr, Ni and Cu). For comparison, one profile at an unloaded site was sampled as well. The obtained results were subjected to multivariate statistical analysis of data. Increased contents of Fe, Al, Mn, Cr and Zn were detected in sediments and plant biomass at loaded sites, namely 2–3× higher than at the comparing site. The contents of metals in surface water samples were altogether below the detection limit of the analytical method. When evaluating the individual plant species, we can state that the lowest contents of metals were detected in shore species (reed canary grass *Phalaroides arundinacea,* wood club-rush *Scirpus silvaticus* and red dock *Rumex aquaticus*); plant species growing in the very water current (water star-wort *Callitriche* sp. and flote-grass *Glyceria fluitans*) exhibited mean contents of metals. In species forming mats (*Fontinalis antipyretica* and *Cladophora* sp.), these contents were several times higher as compared to the previous species. The results of the performed research show that one of important factors, which influence the accumulation of trace metals in plants, is their ecological group (emergent – submerged) affiliation and the species classification within this group. Based on the evaluated data, we can recommend species of moss and algae that form mats eventually species growing in the very water flow for the future use in phytoremediation techniques.

trace metals, freshwater ecosystems, aquatic macrophytes, sediment, contamination

Trace metals belong undoubtedly to the most significant inorganic contaminants of the ecosystem of surface waters. However, as SVOBODOVÁ et al. (1996) state, the concentration of metals detected in waters from different sites of surface waters on the territory of the Czech Republic (CR) in most cases complies with the values valid for water supply courses (note: values determined according to the previously valid Decree of the Government of the CR No. 171/1992 Coll., e.g. for so-called water supply courses: Hg < 0.0005 mg.l^{-1}, Cd < 0.005 mg.l^{-1}, Pb < 0.05 mg.l^{-1}). Even at sites of surface waters heavily loaded by metals for a long time, values valid for water supply courses and for the other surface waters are detected in water samples. Determination of metals only in the liquid part of the ecosystem of the aquatic environment does not convey well the real total pollution of a monitored site. For this reason, it is necessary to pay attention to other components of the aquatic ecosystem as well.

Plants are able in a certain species dependence to absorb metal ions and store them in their tissues. In this regard, special attention is paid to so-called hyperaccumulators, which are such plant species which have an extraordinary ability to accumulate metals in concentrations of up to 1–10 $mg.g^{-1}$ of dry matter. This ability of plants is applied in one of phytodecontamination techniques, which are collectively designated as phytoremediation (SMRČEK, 2002). Phytoremediation techniques can be divided in terms of mechanism into phytodecontamination and phytostabilisation technologies, while phytodecontamination techniques can include phytoextraction, phytodegradation and phytovolatilisation (KAFKA et al., 2002). However, these remediation procedures currently apply above all land (terrestrial) plant species. But higher aquatic plants also accumulate metals very well, especially lead and cadmium (SVOBODOVÁ et al., 1996). In aquatic macrophytes as well, one of main factors influencing the intake of trace metals is a plant species. DRBAL et al. (1995a) states that floating and submerged plants accumulate trace metals more than plants rooting in the bottom.

The aim of the performed research was to obtain knowledge on the ability of aquatic plants naturally growing at affected sites to absorb trace metals contained in bottom sediments and surface water. Furthermore, we attempted to assess a possible use of these plant species for the detection of chronic load of water streams by metal ions, to compare differences in accumulation of trace metals by the individual groups of aquatic plants (submerged and emergent) and to evaluate the suitability of the individual plant species for a possible use of in phytoremediation techniques.

MATERIAL AND METHODS

Sampling took place in 2005–2006. The selected sites suitable for the project solution were the stream Markovka near the village of Dobrá Voda (below the Pozďátky landfill of hazardous wastes), the stream Svratka just below a landfill of the firm Mars Svratka s. p. (today MARS SVRATKA, a.s.) and the stream Nedvědička near the village of Rožná (below a sludge lagoon of DIAMO, s. p., o. z. GEAM, division ROŽNÁ I). The stream Svratka near the village of Herálec was chosen as a comparing site.

Collected samples

In the course of two years, six monitoring cycles were carried out. Sampling took place in the following terms:

2005: June, August, the turn of September and October

2006: the turn of June and July, September, October.

At each contaminated site, a representative collection of samples of different plant species, bottom sediments and water was conducted in various profiles (differing in the distance from an assumed source of contamination). In the "uncontaminated" comparing site, monitoring was carried out only in one profile. Sediments were taken from a surface layer (of a maximum depth of 15 cm); water samples were re-filtered and fixed with concentrated nitric acid. All samples were placed in sampling bottles stored in ice boxes and transported for laboratory analyses to determine the total amount of the selected trace metals.

Chemical analyses

Collected samples were analysed for the total content of As, Cd, Pb, Al, Hg, Zn, Fe, Mn, Cr, Ni and Cu. Chemical analyses were provided as a service in hydrochemical laboratories of GEOtest Brno, a. s. (accredited by the CAI as a testing laboratory No. 1271). Trace metal concentrations were assessed in accordance with norms ČSN EN ISO 15588 (As), TNV 75 7440 (Hg) and ČSN EN ISO 11885 (Cd, Pb, Al, Zn, Fe, Mn, Cr, Ni and Cu). Decomposition of the samples was realized in accordance with norm ČSN EN ISO 15587-2.

Water

In re-filtered samples fixed with concentrated nitric acid, the total content of trace metals was determined by the method ICP OES, AAS (inductively coupled plasma optical emission spectrometry, atomic absorption spectrometry).

Sediment

Sediment samples were dried, crushed and homogenised. Subsequently, a fraction of grains of < 1 mm in size was taken for decomposition. Such treated samples were mineralised under pressure with nitric acid in a microwave device MWS-2 Berghof. The content of trace metals was determined by the method ICP OES, AAS. The correctness of results of analyses was verified on a certified standard of soil "Loam 7004 Analytica".

Plants

In rooting plants, only their shoot was used for analyses. The material was perfectly rinsed with distilled water, dried, crushed and homogenised. Such treated samples of plant material were mineralised under pressure with a mixture of 65% nitric acid and H_2O_2 in a microwave device MWS-2 Berghof. The content of trace metals was determined by the method ICP OES, AAS. The correctness of results of analyses was verified on a reference plant material BCR No. 61.

Processing of statistics

For data evaluation, a multivariate statistical analysis (method of main components, cluster analysis and dendrograms) was used. A combination of diverse software products was applied: NCSS2000 and SCAN, ADSTAT 2.

RESULTS AND DISCUSSION

As to the results of analyses of surface water for the contents of the selected trace metals, we can state that almost in all cases it has been confirmed that the water samples did not show increased contents of these contaminants (Tab. I). The detected values, in most cases, did not exceed the limits given by Decree of the Government No. 61/2003 Coll. on indicators and values of admissible pollution of surface waters and wastewaters. Only at the site Svratka below the MARS landfill, one time higher contents of Fe, Mn and Zn occurred, namely during ongoing landscaping in the place of the former landfill. But the contents of metals were mostly below the detection limit of the analytical method both at the contaminated sites and at the comparing (reference) one. As stated by SVOBODOVÁ et al. (1996), the concentration of metals in surface waters is not, therefore, the governing indicator of the real long-term contamination of the aquatic environment by these substances. Accordingly DRBAL et al. (1995b), who monitored the migration of trace metals in a pond ecosystem, state that their lowest concentrations are in water, where their content is altogether three orders lower as compared to the other materials (sediments, biological material). Based on these findings, it can thus be clearly stated that the mere analysis of water samples from a site cannot provide any relevant data on the chronic load by contaminants.

In sediment samples, effects of the long-term load of the sites have already been manifested by anthropogenic pollution. The increased contents of Fe, Al, Mn, Cr and Zn were detected at all the contaminated sites. These values were several times higher than at the reference site. However, after the comparison with literary sources, these increased values are by no means extreme. For instance, ŠVEHLA et al. (1999) monitored the content of selected trace metals in sediments of twelve České Budějovice ponds. The average content of Zn and Cr amounted to 66.6 and 47.2 $mg.kg^{-1}$ of dry matter respectively. Accordingly, SPURNÝ et al. (2002) detected, among others, the concentration of Zn and Cr at 3 sites of the upper course of the Jihlava River, while collecting samples of water, sediments and biological material. Increased contents of the monitored metals were recorded at all monitored sites in different components of the aquatic ecosystem. The highest concentrations were identified in sediments (e.g. at site 2 the following values were detected (in $mg.kg^{-1}$): Zn 80.290 and Cr 8.713). When observing the movement of trace metals in the pond Naděje by Lomnice nad Lužnicí, the content of trace metals was also determined in its sediments (DRBAL et al., 1995b). The authors detected the following values (in $mg.kg^{-1}$ of dry matter): Mn 288; Fe 20.350; Zn 125; and Cr 64.1. Even though the concentrations detected by us do not reach markedly high values, they can pose a relatively serious threat to the river ecosystem. As given by KUBAL et al. (2002), they can suddenly be washed out by a mere change in the surrounding conditions (for instance, by lowering pH). The same problem is pointed out by SUSCHKA (1993), who addressed the occurrence of trace metals in surface waters of Upper Silesia, where ores containing Zn, Pb and Fe are mined to a large extent. In particular, he deals with the River Biala Przemsza, which is the main recipient of wastewaters from the premises of the so-called "Bukowno Komplex" (operation with the greatest production of zinc in the world). The author states that trace metals contained in the wastewaters are deposited to a considerable extent in sediments of the recipient and can, even after the end of ore mining and processing, contaminate the river water for centuries.

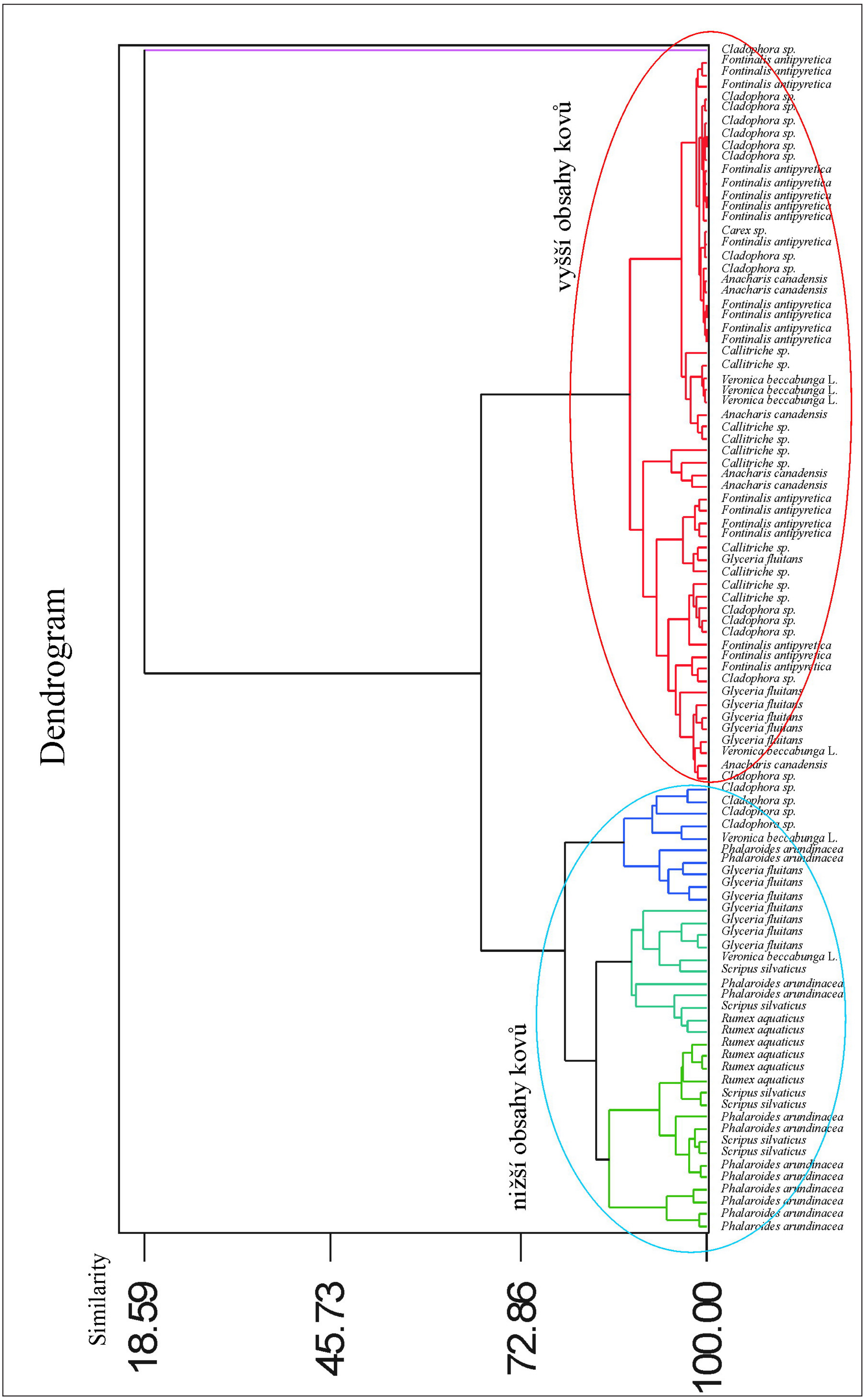
The content of trace metals detected in the sediments was reflected in their amounts identified in the plant organisms. In them, the highest values for Fe, Al, Mn, Cr and Zn were detected as well. It is, however, very important to emphasise the striking preference of the informative value of analyses of plant samples to sediments. Plants permanently growing at a site really show the conditions of the site, whereas sediments can, especially after rain, be washed from the upper parts of streams and cover original sediments. When comparing these values with the values from the unloaded site, it was shown that the contents of metals in the tissues of the aquatic macrophytes in the anthropogenically affected sites were 2–3× higher (Tab. I). KLUMPP et al. (2002) state that the long-term use of fungicides containing copper in cocoa plantations of southern Brazil caused the contamination of regional freshwater ecosystems. As bioindicators of this pollution, the authors chose the floating aquatic macrophytes water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes*), in which the contents of Al, Cr and Cu were determined. Higher contents of metals in plants were detected in lower sections of streams; the authors recommend using aquatic macrophytes as bioindicators of the load of the aquatic ecosystem by trace metals. The aforementioned facts show that aquatic plants could be used in the evaluation of the real ecological state of the ecosystem of surface waters in terms of their contamination by trace metals.

The distinct difference in the content of trace metals can be traced between the individual plant species (Fig. 1). The highest contents were detected in species forming mats (*Fontinalis antipyretica*, *Cladophora* sp.), whereas these contents were several times lower in shore species (reed canary grass *Phalaroides arundinacea,* wood club-rush *Scirpus silvaticus,* red dock *Rumex aquaticus*). Plant species occurring just in the water flow (water star-wort *Callitriche* sp., flotegrass *Glyceria fluitans*) showed mean contents of metals. DRBAL et al. (1995a) also state that plants are able to selectively take in trace metals. The authors monitored the content of trace metals in macrophytes taken from three pools lying in the floodplain of the River Lužnice. According to the authors, the difference in the intake of trace metals between floating (duckweed, water-weed) and emergent plants was confirmed. According to SVOBODOVÁ et al. (1996), I: *Values of selected trace metals (dm = dry matter)*

Site	Type of sample		Al	Zn	Mn	Fe	Cr
Pozďátky	Water [µg.l^{-1}]	Average min-max	149	58	335	148	<10
			<50-1000	<20-362	41-1 860	60-491	
	Sediment [mg.kg^{-1} of dm]		10 273	60	530	15 013	154
			6 544-19 820	37-116	231-779	9 460-29 400	90-282
	Macrophytes [mg.kg^{-1} of dm]		5 671	230	5 222	8 923	54
			80-26 600	26-2 920	90-49 8000	150-61 400	<5-310
Svratka	Water [µg.l^{-1}]		139	214	96	1 173	15
			<50-426	<20-2 290	42-580	340-7 070	<10-45
	Sediment [mg.kg^{-1} of dm]		7 369	3 976	150	11 240	235
			2 920-27 800	35-32 800	51-481	3 080-62 800	5-2 093
	Macrophytes [mg.kg^{-1} of dm]		5 760	759	2 488	12 176	23
			149-40 470	52-5 860	49-15 280	328-34 400	<2-287
Rožná	Water [µg.l^{-1}]		51	<20	27	142	<10
			<50-135		<25-56	68-360	
	Sediment [mg.kg^{-1} of dm]		7 875	61	462	11 987	20
			378-21 280	25-118	239-955	7 060-30 900	9-76
	Macrophytes [mg.kg^{-1} of dm]		9 676	266	8 237	12 813	29
			224-25 000	25-710	91-35 000	266-26 400	6-49
Herálec (comparing site)	Water [µg.l^{-1}]		140	<20	25	294	<10
			84-191		<25-60	119-411	
	Sediment [mg.kg^{-1} of dm]		3 812	39	125	4 326	7
			1 154-6 560	8-74	31-238	1 180-7 500	5-9
	Macrophytes [mg.kg^{-1} of dm]		2 737	103	657	3 357	23
			270-6 260	35-475	120-3 100	376-14 200	<5-235

floating plants have the highest contents of metals, reeds the lowest ones. SANCHEZ et al. (1994) identified the content of trace metals (Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn) in samples of sediments, bryophyte *Brachythecium rivulare*, vascular plants (soft rush *Juncus effusus* and common catttail *Typha latifolia*) and fish (brown trout *Salmo trutta* m. *fario* and European eel *Anguilla anguilla*), which were taken from different sites of a river flowing through an area of former lead and zinc mining. High concentrations of both the trace metals were detected, especially in sediments and in bryophyte, in which the content of Pb reached up to 1.690 mg.kg^{-1} and that of Zn up to 11.800 mg.kg^{-1} (in sediments: 1.300 mg.kg^{-1} and 8.710 mg.kg^{-1}, respectively in soft rush 5.2 mg.kg^{-1} and 620 mg.kg^{-1}, respectively). FÖRSTNER and WITTMANN (1983) report that the most suitable algae are filamentous green ones in terms of monitoring the content of trace metals because they can easily be separated from the other algae due to their size. Different results were presented by HELEŠIC and SCHEIBOVÁ (2000), who identified the content of trace metals at 5 sites – surface streams within the territory of the CR, witch each of the sites characterising a certain hydrobiological zone. Besides samples of zoobenthos, sediments and macrophytes, also samples of algae were taken, particularly it was *Cladophora* sp. The content of As, Cd, Cu, Ni, Pb, Hg and Zn was determined in the samples. The values detected in the alga *Cladophora* sp. were lower as compared to the content of metals both in the moss representative *Fontinalis antipyretica*, and in the macrophyte representatives *Batrachium fluitans* and *Myriophyllum* sp.

Based on the obtained results, we can state that the aquatic macrophytes are able to accumulate trace metals in their tissues, but this depends on the species. These facts are also reflected in the evaluation of the suitability of their use in phytoremediation techniques. In our monitoring, the lowest contents of metals were detected in the shore species of plants. In addition, these plant species are imbedded in sediments by a relatively spreading root system. No data of the content of trace metals in the root system are available to us; only the shoot of the plants was analysed. It would be appropriate here to establish



1: Contents of metals in individual plant species more precise data by other analyses, e.g. in laboratory conditions. However, based on the data given by other authors, it can be assumed that a certain portion of metals is absorbed in roots. STUCHLÍKOVÁ (2003), e.g., reports that the largest accumulation of trace metals occurs in roots. Also CARDWELL et al. (2002), who identified the content of trace metals in macrophytes growing in recipients of municipal wastewaters in Australia, divided collected plants prior to analysis into individual morphological parts and confirmed that metals accumulated differently in individual parts of plants. It was found out that roots of plants contained higher concentrations of metals than stems or leaves. But no marked difference between stems and leaves was identified. The highest content of metals was detected in the submerged species parrot's feather *Myriophyllum aquaticum* and also emergent species contained relatively a plenty of metals in their roots. The leaves of submerged and floating species contained higher concentrations of trace metals as compared to the leaves of emergent plants. Based on the given facts, we can state that when removing only the shoot, the root system can decompose in sediments and subsequently the trace metals which have already been absorbed may be released back into the surrounding environment. For this reason, the use of these plant species may be limited in phytoremediation processes. The contents of trace metals detected in species of mat-forming algae and moss were several times higher as compared to the aforementioned species. Under suitable conditions, these species create a large amount of relatively easily removable biomass.

SOUHRN

Akumulace toxických kovů vodními makrofyty a možnost jejich využití ve fytoremediačních postupech

Cílem prováděného výzkumu bylo získat poznatky o schopnosti vodních rostlin přirozeně rostoucích na lokalitě absorbovat toxické kovy obsažené v sedimentu dna a povrchové vodě. Dále jsme porovnávali rozdíly v akumulaci toxických kovů jednotlivými skupinami vodních rostlin (submerzní a emerzní) a posuzovali možnost využití jednotlivých rostlinných druhů ve fytoremediačních postupech. Na třech antropogenně zatížených tocích byly v šesti monitorovacích cyklech na několika odběrných profilech, lišících se vzdáleností od zdroje kontaminace, odebrány reprezentativní vzorky vody, sedimentu a vodních makrofyt. Vzorky byly analyzovány na celkový obsah vybraných toxických kovů (As, Cd, Pb, Al, Hg, Zn, Fe, Mn, Cr, Ni a Cu). Pro srovnání byl rovněž proveden odběr z jednoho profilu na nezatížené lokalitě. Získané výsledky jsme podrobili vícerozměrné statistické analýze dat. V sedimentu a rostlinné hmotě byly na zatížených lokalitách zjištěny zvýšené obsahy Fe, Al, Mn, Cr a Zn a to 2–3× vyšší ve srovnání s lokalitou srovnávací. Ve vzorcích povrchové vody byly obsahy kovů vesměs pod mezí detekce analytické metody. Při hodnocení jednotlivých rostlinných druhů můžeme konstatovat, že nejnižší obsahy kovů byly zjištěny u pobřežních druhů (chrastice rákosovitá *Phalaroides arundinacea,* skřípina lesní *Scirpus silvaticus,* šťovík *Rumex aquaticus*), rostlinné druhy rostoucí přímo ve vodním proudu (hvězdoš *Callitriche* sp., zblochan vzplývavý *Glyceria fluitans*) vykazovaly střední obsahy kovů. U druhů tvořících nárosty (*Fontinalis antipyretica*, *Cladophora* sp.) byly tyto obsahy několikanásobně vyšší oproti předchozím druhům. Z výsledků provedeného výzkumu je zřejmé, že jedním z důležitých faktorů, které ovlivňují akumulaci toxických kovů v rostlinách, je jejich druhová příslušnost. Na základě vyhodnocených dat můžeme pro budoucí využití ve fytoremediačních postupech doporučit druhy mechů a řas, které tvoří nárosty, popř. druhy rostoucí přímo v proudu vody.

toxické kovy, sladkovodní ekosystémy, vodní makrofyta, sediment, kontaminace

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