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# Possibilities and limitations of using *Lemna minor*, *Hydrocharis morsus-ranae* and *Ceratophyllum demersum* in removing metals with contaminated water

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## Abstract

The paper presents the assessment of possibilities and limitations of the use of *Lemna minor*, *Hydrocharis morsus-ranae* and *Ceratophyllum demersum* in removing metals from contaminated waters. Synthetically discussed the role of these species in phytotechnology and their importance in the assessment of surface water status. The variability of concentration of selected metals in waters and the content of metals in the organs of the analysed plants are presented. Their advantages and disadvantages in removing metals from waters due to biological features have been characterized. Minimum and maximum efficiency of metal removal depending on the scale of water pollution was determined. It was found that analysed plants can be used for phytoremediation of metals from water, but the limitation of effectiveness of treatments is the toxicity of these metals to plants and the time of exposure. The highest removal efficiency can be obtained thanks to the use of sequences of single-species filtration systems.

**Key words:** phytoremediation, heavy metals, hyperaccumulator, metal removal efficiency, duckweed, European frogbit, hornwort

## INTRODUCTION

Pollution of aquatic ecosystems has a negative effect on organisms occurring therein [GAŁCZYŃSKA, BUŚKO 2016]. Toxic substances (e.g. heavy metals, pesticide residues, polycyclic aromatic hydrocarbons, dioxins or pharmaceuticals) accumulated in tissues of fish, molluscs and crustaceans can constitute a threat to human health when ingested [MENCH *et al.* 2009; OUALI *et al.* 2018]. Currently, there are various technologies used for the purpose of purification of surface waters, urban, domestic and industrial wastewater as well as wastewater sludge. Among these, par-

ticularly noteworthy are solutions based on the use of phytotechnology [BELLO *et al.* 2018; CZYŻYK 2003; OBARSKA-PEMPKOWIAK *et al.* 2015; SKRZYPIEC, GAJEWSKA 2017].

Phytotechnology is based on the application of plants which, using solar energy and their life potential, help to improve the sanitary conditions and environmental protection of aquatic ecosystems. Such plants are characterised by highly efficient biologic systems which uptake and metabolise nutrients and, due to metabolic conversion regulated by photosynthesis, other substances as well [BHUPINDER 2010; WESOŁOWSKI, BRYSEWICZ 2014]. Phytotechnology is considered cost-effective and environmentally

friendly technique of environment decontamination. However, high initial concentration of pollutants present in the growth habitat can be toxic to such plants and, consequently, inhibit or even prevent the growth of the plants. Moreover, out of vegetative season, plant development and the resulting remediation processes may not occur. Prior to removal of any polluted plant material, risk analysis of its further use or processing may be necessary [AKHTAR *et al.* 2017].

Phytoremediation is defined as a method of using higher plants for the purpose of purification of contaminated environment [GHOSH, SINGH 2005] and includes five distinct mechanisms (Tab. 1).

**Table 1.** Types of phytoremediation

Type	Mechanism
Rhizofiltration	plants are used to absorb, concentrate, and precipitate contaminants from polluted aquatic environment by their roots
Phytostabilization	stabilization of contaminated soils by sorption, precipitation, complexation, or metal valence reduction rather than the removal of contaminants
Phytoextraction	phytoaccumulation, process that plants absorb, concentrate, and precipitate the contaminants in the biomass
Phytovolatilization	plants extract certain contaminants in nearby roots and then transpire them into the atmosphere
Phytotransformation	phytodegradation, process that plants remove contaminants from environment by their metabolism

Source: own elaboration.

## SELECTED AQUATIC PLANTS USED FOR ASSESSING WATER STATUS

*Lemna minor*, *Hydrocharis morsus-ranae*, *Ceratophyllum demersum* are widely used in many European countries for the purpose of ecological status assessment of standing and flowing waters (the macrophyte index – MI in Germany, the trophic index of macrophytes – TIM in Germany, the mean trophic rank – MTR in Great Britain, indice biologique macrophytique rivière – IBMR in France, the ecological state macrophyte index – ESMI in Poland, macrophyte river index – MRI in Poland, macrophyte index scheme – MIS in Ireland) [SZOSZKIEWICZ *et al.* 2009]. Only two of them, duckweed and hornwort, prefer water with a high trophic level (the index number of these species in MIR is  $L = 2$ , with  $L$  taking values from 1 for advanced eutrophy to 10 for oligotrophy), and European frogbit level with average trophic level ( $L = 6$ ). These plants differ in the uptake of metals from surface waters.

*Ceratophyllum demersum* is a biological indicator of water pollution with cadmium and lead [DOGAN *et al.* 2015; VAHDATIRAAD, KHARA 2012], *Lemna minor* also serves as an indicator of pollution with lead [PRASAD, FREITAS 2003]. In turn, *Hydrocharis morsus-ranae* is an indicator of an increased concentration of zinc, iron and mercury in water [POLECHOŃSKA, DAMBIEC 2014].

Tables 2–4 present data on metal concentration in waters in which *Lemna minor*, *Hydrocharis morsus-ranae* and *Ceratophyllum demersum* occurred and metal content in organs of the analysed plant species. The data shown present high tolerance of these plants to pollution of water with metals.

**Table 2.** Content of metals in water and tissues of *Lemna minor*

Metal	Water (mg·dm <sup>-3</sup> )	Plant species (mg·kg <sup>-1</sup> d.w.)			Reference
		whole plant	part of plant		
			leaves	roots	
Na	1.5±0.2	–	–	–	SASMAZ <i>et al.</i> [2016]
	–	1500±420	–	–	SIKORA <i>et al.</i> [2018]
Pb	13±0.9	–	–	–	RAI [2009]
	–	–	4.44–7.98	3.80–10.2	KASTRATOVIĆ <i>et al.</i> [2015]
Cu	0.003–0.013	–	7.94–22.3	12.4–33.1	KASTRATOVIĆ <i>et al.</i> [2015]
Zn	0.9±0.1	–	–	–	SASMAZ <i>et al.</i> [2016]
	–	–	41.3–82.5	59.9–115	KASTRATOVIĆ <i>et al.</i> [2015]
Mn	0.008–0.013	–	713–2590	1543–4747	KASTRATOVIĆ <i>et al.</i> [2015]
Co	0.24–0.84	–	–	5.60	KASTRATOVIĆ <i>et al.</i> [2018]
Fe	196±20	–	–	–	SASMAZ <i>et al.</i> [2016]
Ni	0.28–1.08	14.760	0.82	0.82	KASTRATOVIĆ <i>et al.</i> [2018]
Hg	1.8±0.1	–	–	–	RAI [2009]
	–	0.76±0.03	–	–	VARGA <i>et al.</i> [2013]
Cd	<LOD	–	0–0.05	0–0.83	KASTRATOVIĆ <i>et al.</i> [2015]
Cr	<0.002–0.002	–	–	0.79–3.08	KASTRATOVIĆ <i>et al.</i> [2015]
Al	–	0.67±0.047	–	–	RADIĆ <i>et al.</i> [2009]
Tl	–	26.06±1.785	–	–	BABIĆ <i>et al.</i> [2009]

Explanation: LOD = limit of detection.

Source: own elaboration based on literature.

## ANALYSED AQUATIC PLANTS AND THEIR USE IN WATER PURIFICATION

Apart from helophytes (e.g. *Phragmites australis*) also pleustophytes as well as elodeids – represented by the analysed plant species *Lemna minor*, *Hydrocharis morsus-ranae*, and *Ceratophyllum demersum* [CZYŻYK 2003; FOROUGH *et al.* 2010; SCHOLZ, ANDERSON 2003; SZCZERBIŃSKA, GAŁCZYŃSKA 2016] are used for purification of wastewater. In comparison with terrestrial plants, aquatic plants show greater capacity for bioaccumulation of high quantities of toxic metals and biogenic compounds [PRATAS *et al.* 2012]. Such plants, regardless of whether they are free-floating [UYSAL, TANER 2009], submersed [LI *et al.* 2018] or emergent [BELLO *et al.* 2018], are known for their ability to remove heavy metals. Hyperaccumulators of heavy metals can be identified with the use of bioconcentration coefficient (the ratio of the concentration of metal in plant to its concentration in water) or evaluation of the capacity for accumulating these elements in plant tissues in high quantities in relation to the concentration of metals in soil or water [UCER *et al.* 2013]. Currently proposed threshold values in mg·kg<sup>-1</sup> weight for selected elements, above which the plant obtains the hyperaccumulator status, is: 100 mg·kg<sup>-1</sup> Se, Cd and Tl, 300 mg·kg<sup>-1</sup> Cu, Co, Cr; 1000 mg·kg<sup>-1</sup> Ni and Pb; 3000 mg·kg<sup>-1</sup> Zn and 10 000 mg·kg<sup>-1</sup> Mn [VAN DER ENT *et al.* 2013].

**Table 3.** Content of metals in water and tissues of *Hydrocharis morsus-ranae*

Metal	Water (mg·dm <sup>-3</sup> )	Plant species (mg·kg <sup>-1</sup> d.w.)				Reference
		whole plant	part of plant			
			stem	leaves	roots	
Na	1.3–24.8	–	–	–	–	SKWIERAWSKI, SKWIERAWSKA [2013]
	8.3–33.74	1460–11140	–	–	–	GALCZYŃSKA, BEDNARZ [2012]
	–	–	6847–26113	–	11479–32394	POLECHOŃSKA, SAMECKA-CYMERMAN [2015b]
	–	8245	–	–	–	SZCZERBIŃSKA, GALCZYŃSKA [2016]
Ca	12.2–65.2	–	–	–	–	SKWIERAWSKI, SKWIERAWSKA [2013]
	41.89–125.9	1610–30990	–	–	–	GALCZYŃSKA, BEDNARZ [2012]
	–	–	9080–48870	–	13916–64593	POLECHOŃSKA, SAMECKA-CYMERMAN [2015b]
	–	3528	–	–	–	SZCZERBIŃSKA, GALCZYŃSKA [2016]
Pb	0–0.032	–	–	–	–	SKWIERAWSKI, SKWIERAWSKA [2013]
	0–0.211	0–67.18	–	–	–	GALCZYŃSKA, BEDNARZ [2012]
	–	–	0.04–31.9	–	0.42–11.1	POLECHOŃSKA, SAMECKA-CYMERMAN [2015a]
	–	4.23	–	–	–	SZCZERBIŃSKA, GALCZYŃSKA [2016]
	0.60	348.2	–	–	–	GALCZYŃSKA [2012]
Cu	0–0.003	–	–	–	–	SKWIERAWSKI, SKWIERAWSKA [2013]
	0–0.002	–	–	0.01–20.6	–	POLECHOŃSKA, DAMBIEC [2014]
	0–0.190	3.44–29.40	–	–	–	GALCZYŃSKA, BEDNARZ [2012]
	–	–	0.71–61.4	–	0.77–9.49	POLECHOŃSKA, SAMECKA-CYMERMAN [2015a]
	–	35.7	–	–	–	SZCZERBIŃSKA, GALCZYŃSKA [2016]
	0.12	90.9	–	–	–	GALCZYŃSKA [2012]
Zn	0.001–0.020	–	–	–	–	SKWIERAWSKI, SKWIERAWSKA [2013]
	0.0006–0.0042	–	–	9.58–46.8	–	POLECHOŃSKA, DAMBIEC [2014]
	0–0.085	0.48–2219	–	–	–	GALCZYŃSKA, BEDNARZ [2012]
	–	–	9.58–157.1	–	30.4–246	POLECHOŃSKA, SAMECKA-CYMERMAN [2015a]
	–	279	–	–	–	SZCZERBIŃSKA, GALCZYŃSKA [2016]
	2.4	1776	–	–	–	GALCZYŃSKA [2012]
Mn	0.011–0.403	–	–	–	–	SKWIERAWSKI, SKWIERAWSKA [2013]
	0–0.151	–	–	120–6322	–	POLECHOŃSKA, DAMBIEC [2014]
	0.001–3.284	1001–12490	–	–	–	GALCZYŃSKA, BEDNARZ [2012]
	–	–	120–27713	–	2795–42557	POLECHOŃSKA, SAMECKA-CYMERMAN [2015a]
	–	921	–	–	–	SZCZERBIŃSKA, GALCZYŃSKA [2016]
	1.2	823	–	–	–	GALCZYŃSKA [2012]
Fe	0.01–1.49	–	–	–	–	SKWIERAWSKI, SKWIERAWSKA [2013]
	0–0.827	–	–	102–4798	–	POLECHOŃSKA, DAMBIEC [2014]
	0.007–4.032	90–7890	–	–	–	GALCZYŃSKA, BEDNARZ [2012]
	–	–	102–14722	–	1859–14627	POLECHOŃSKA, SAMECKA-CYMERMAN [2015a]
	–	10508	–	–	–	SZCZERBIŃSKA, GALCZYŃSKA [2016]
	2.4	5020	–	–	–	GALCZYŃSKA [2012]
Co	0.0053	155	–	–	–	POLECHOŃSKA, SAMECKA-CYMERMAN [2018]
Ni	0.0571	511	–	–	–	POLECHOŃSKA, SAMECKA-CYMERMAN [2018]

Source: own elaboration based on literature.

Among numerous aquatic plants, *Lemna minor* proves to be a hyperaccumulator of lead, nickel, chromium, copper, cadmium and manganese [AL-KHAFIJI *et al.* 2017; ALVARADO *et al.* 2008; MIRETZKY *et al.* 2006; PRASAD, FREITAS 2003; REZANIA *et al.* 2016; UYSAL, TANER 2010], whereas *Ceratophyllum demersum* serves as a hyperaccumulator of zinc, lead, copper, arsenic, chromium, iron and cobalt [XING *et al.* 2013]. The amount of accumulation of various metals by *Hydrocharis morsus-ranae* does not meet the criteria for identifying the species as hyperaccumulator [UCER *et al.* 2013].

### ADVANTAGES AND DISADVANTAGES OF SELECTED AQUATIC PLANTS IN REMOVING METALS FROM AQUATIC ECOSYSTEMS DUE TO BIOLOGIC FEATURES

Plants used as phytoremediators acc. to KOŹMIŃSKA *et al.* [2014] should present the following features:

- 1) fast vegetative growth rates,
- 2) high biomass production,
- 3) well-developed root system (mass, length and the number of root ramifications),
- 4) tolerance to high concentration of heavy metals in soil or low demand for nutrients,

**Table 4.** Content of metals in water and tissues of *Ceratophyllum demersum*

Metal	Water (mg·dm <sup>-3</sup> )	Plant species (mg·kg <sup>-1</sup> d.w.)			Reference
		whole plant	part of plant		
			stem	leaves	
Na	24 ± 1.0	–	–	–	MARKICH <i>et al.</i> [2006]
	–	1478	–	–	SZCZERBIŃSKA, GAŁCZYŃSKA [2016]
Ca	4.8 ± 0.4	–	–	–	MARKICH <i>et al.</i> [2006]
	–	6279	–	–	SZCZERBIŃSKA, GAŁCZYŃSKA [2016]
Pb	<LOD	–	2.74–12.7	3.18–16.8	KASTRATOVIĆ <i>et al.</i> [2014]
	–	44.80	–	–	KREMS <i>et al.</i> [2013]
	–	53.11	55.58	50.64	VAHDATIRAAD, KHARA [2012]
		275	–	–	WANG <i>et al.</i> [2014]
	0.62–1.19	3.9–10.8	–	–	AL-GHANEM [2010]
	–	4.88	–	–	SZCZERBIŃSKA, GAŁCZYŃSKA [2016]
Cu	0.002–0.014	–	6.48–24.5	9.85–34.5	KASTRATOVIĆ <i>et al.</i> [2014]
	–	6.17	–	–	KREMS <i>et al.</i> [2013]
	0.1–1.07	70–128	–	–	MARKICH <i>et al.</i> [2006], AL-GHANEM [2010]
	–	52.3	–	–	SZCZERBIŃSKA, GAŁCZYŃSKA [2016]
Zn	0.002–0.008	–	16.7–75.6	25.6–58.9	KASTRATOVIĆ <i>et al.</i> [2014]
	0.095–0.45	–	–	13.98–160	KREMS <i>et al.</i> [2013]; AL-GHANEM [2010]
	–	255	–	–	SZCZERBIŃSKA, GAŁCZYŃSKA [2016]
	–	114	–	–	WANG <i>et al.</i> [2014]
Mn	0.005–0.014	–	351–1189	539–1984	KASTRATOVIĆ <i>et al.</i> [2014]
	0.022–0.069	2938–4328	–	–	AL-GHANEM [2010]
	–	1785	–	–	SZCZERBIŃSKA, GAŁCZYŃSKA [2016]
	–	8004	–	–	WANG <i>et al.</i> [2014]
Cd	–	0.98	–	–	WANG <i>et al.</i> [2014]
	0.1	41.9	–	–	DOGAN <i>et al.</i> [2015]
	1.0	230.5	–	–	
	10	824.7	–	–	
Ni	–	2662	–	–	WANG <i>et al.</i> [2014]
Fe	0.022–70	4298–5539	–	–	FOROUGH <i>et al.</i> [2010]; MARKICH <i>et al.</i> [2006], AL-GHANEM [2010]
	–	14159	–	–	SZCZERBIŃSKA, GAŁCZYŃSKA [2016]
As	–	16	–	–	WANG <i>et al.</i> [2014]
Co	–	63	–	–	
Cr	–	4242	–	–	

Explanation: LOD = limit of detection.

Source: own elaboration based on literature.

- 5) ability to uptake and metabolise large quantities of toxic organic compounds,
- 6) limited capacity for transporting the accumulated pollutants to shoots,

- 7) ability to immobilise metals through precipitation, reduction or absorption by roots,
- 8) ability to accumulate high concentrations of metals in the aboveground parts.

The use of aquatic plants for the purpose of remediation involves the need to consider the ease of removing the plants in order to remove the accumulated pollutants from the object, as well as the necessity of controlling their population to avoid negative ecological effects (decreased biodiversity of the aquatic ecosystem due to excessive plant expansion).

*Lemna minor* is used mainly in rhizofiltration meeting the conditions listed above: 1, 2, 5, 6, 7. The advantages and disadvantages of this plant in terms of removing metal pollutants from aquatic ecosystems are presented in Table 5. This plant constitutes a very good bio-indicator with high ecological value as regards detecting and monitoring pollution with metals (Tab. 2). Tissue of *Lemna minor* is rich in protein as well as micro- and macro-elements which are a significant dietary ingredient for some animals (also of livestock such as cattle, swine or poultry), water birds (mainly ducks), herbivore fish, and at times even reptiles (e.g. water turtle) and insects such as some beetles [LENG *et al.* 1995].

*Hydrocharis morsus-ranae*. Taking into consideration the optimal features of plants used for purification of aquatic ecosystems from pollution [HANUS-FAJERSKA, KOŹMIŃSKA 2016], *Hydrocharis morsus-ranae* meets several conditions listed above: 1, 3, 4, 6, 7, 8 to be successfully used for removing metals mainly through processes of rhizofiltration and phytoextraction. The advantages and disadvantages of *Hydrocharis morsus-ranae* in removing metals from aquatic ecosystems in terms of physical traits and reproduction of this species are presented in Table 5.

Thick mats of *Hydrocharis morsus-ranae* inhibit light transmission to submersed plants, delay growth of algae as well as limit oxygen diffusion from the atmosphere. *Hydrocharis morsus-ranae* provides shelter and source of food for numerous animals: butterfly larvae, frogs and snails [CATLING *et al.* 2003].

This plant can develop in waters of various metal concentrations and may accumulate significant quantities of such metals (Tab. 5).

*Ceratophyllum demersum* is characterised by features required for species which are to be successfully used in phytoremediation, phytoaccumulation and phytoextraction: 1, 2, 4, 7 [KOŹMIŃSKA *et al.* 2014]. This hydrophyte demonstrated high potential for biomass production and vegetative propagation even in poor dietary conditions [ARAVIND *et al.* 2009] (Tab. 5). Additionally, *Ceratophyllum demersum* provides habitat for small animals (*Stylaria lacustris*, *Caenias macrura*, *Corynoneura*, *Planorbis hortex et al.*) and constitutes food source for some species of fish, snails, crustaceans, insects, water birds (e.g. ducks, rails) and rodents [BERNATOWICZ, WOLNY 1974]. *Ceratophyllum demersum* can be used as a biofilter of pollutants, metals in particular.

**Table 5.** The advantages and disadvantages of *Lemna minor*, *Hydrocharis morsus-ranae* and *Ceratophyllum demersum* in removing metals from aquatic ecosystems due to their construction and reproduction characteristics

Selected biological features of the plant	Advantages	Disputable	Disadvantages
<b><i>Lemna minor</i></b>			
Free-floating freshwater aquatic plant	<ul style="list-style-type: none"> <li>– the ability to take mineral substances from the water, has a high content of nitrogen and phosphorus</li> <li>– pH of water from 3.5 to 10</li> <li>– a wide range of temperature tolerances</li> </ul>	–	generally does not participate in the removal of metals and biogenic compounds from bottom sediments
Plant with one, two, three or four leaves each having a single root hanging in the water	<ul style="list-style-type: none"> <li>– accumulates more metals through completely immersed in water</li> <li>– removes shading</li> </ul>	–	small biomass of single plant
It reproduces mainly vegetatively by division	<ul style="list-style-type: none"> <li>– short breeding time – double number increase takes place within 1–4 days</li> <li>– trouble-free gathering from the water surface</li> </ul>	the length of the growing season determines the period of use as a biofilter	<ul style="list-style-type: none"> <li>– in the development of large biomass causes the cutting off of light, causing deterioration of growth conditions for other vascular plants and phytoplankton, which even leads to their disappearance</li> <li>– dense floating mats limit diffusion of oxygen from the atmosphere to water</li> </ul>
<b><i>Hydrocharis morsus-ranae</i></b>			
Free floating plant	participates in the removal of metals and biogenic compounds from the euphotic layer of surface waters	–	in most cases does not participate in the removal of metals and biogenic compounds from bottom sediments
Cold-resistant up to –20°C, perennial water plant	there is no need to obtain new plants every year	will the next year's new rosettes be more resistant or more sensitive to contaminants?	–
Densely leafed rhizome	the ability to accumulate metal in leaves	–	short lifespan of leaves
Relatively short water roots with long root hairs	the ability to accumulate metals in roots	–	–
Grows vegetatively throughout the summer season	biomass growth in the vegetative season leading to the creation of a dense cluster of plants covering the surface of small water reservoirs	the length of the vegetative season determines the time of biofiltration	dense plant mats limit the diffusion of oxygen from the atmosphere
<b><i>Ceratophyllum demersum</i></b>			
Free floating plant	<ul style="list-style-type: none"> <li>– plant submerged</li> <li>– the vulgar species produces winter shoots lying on the bottom of the tank</li> <li>– present in area aquatic plants and rush</li> </ul>	light intensity, expansive species	sensitive to reduction water level and drying, does not freeze into the ice
No roots	<ul style="list-style-type: none"> <li>– easy to obtain from the environment</li> <li>– resistant to phytotoxicity of metals, e.g. Pb</li> </ul>	–	no possibility of collecting nutrients and impurities from bottom sediments
Stalk densely foliage	the possibility of metal accumulation in leaves	–	–
Produces winter buds	there is no need to obtain new plants	–	–
Vegetative reproduction	<ul style="list-style-type: none"> <li>– the possibility of pollination in water</li> <li>– fast biomass growth – creates compact phytocoenoses</li> <li>– the reproduction possible even with limited availability of nutrients</li> </ul>	the length of the growing season determines the period of use as a biofilter	–

Explanations: selected biological features of the plant for *Lemna minor* acc. to BERNATOWICZ and WOLNY [1974], and KŁOSOWSKI and KŁOSOWSKI [2001], for *Hydrocharis morsus-ranae* acc. to BUCZACKI [1997] and PODBIELKOWSKI and TOMASZEWCZ [1996] and for *Ceratophyllum demersum* acc. to BERNATOWICZ and WOLNY [1974] and PARNIAN *et al.* [2016].

Source: own elaboration.

## EFFICIENCY OF REMOVAL OF METALS FROM THE WATER ENVIRONMENT DEPENDING ON THE TIME OF PLANT EXPOSURE ON METAL CONTAMINATION AND METAL TOXICITY

### EFFICIENCY OF METAL REMOVAL FROM AQUATIC ENVIRONMENT

Analyses of aquatic plants with respect to determining the potential for removing metals from aquatic ecosystems were conducted for *Lemna minor* [AL-KHAFIJI *et al.* 2017; BASILE *et al.* 2012; BOKHARI *et al.* 2016; GOSWAMI *et al.*

2014; MIRANDA *et al.* 2014; SEKOMO *et al.* 2012; SZMIT *et al.* 2017; UYSAL 2013], *Hydrocharis morsus-ranae* [GAŁCZYŃSKA 2012; POLECHOŃSKA, SAMECKA-CYMERMAN 2018] and *Ceratophyllum demersum* [CHEN *et al.* 2015; DUMAN, KOCA 2014; KARA 2005; MATACHE 2013; REZANIA *et al.* 2016; UMEBESE, MOTAJO 2008].

***Lemna minor.*** In biomonitoring of surface waters, *Lemna minor* is considered to be one of the main bioindicators of the level of pollution with selected metals. The conducted analyses of concentrations of, among others, heavy metals in biota, may provide much valuable information on pollutants introduced to aquatic ecosystems. The results of analyses confirmed usability of *Lemna minor* as a biomoni-

tor of point sources of aquatic ecosystem pollution with, among others, lead and nickel [SZMIT *et al.* 2017].

GOSWAMI *et al.* [2014] showed that Common duckweed indeed has the potential for removing arsenic from aquatic ecosystems. The maximum removal of more than 70% of arsenic was reached with initial concentration of arsenic on the  $0.5 \text{ mg}\cdot\text{dm}^{-3}$  15 day of the experimental period lasting 22 days.

In a hydroponic experiment, BOKHARI *et al.* [2016] assessed the phytoremediation potential of Common duckweed in relation to elements Cd, Cu, Pb and Ni. Concentration of heavy metals in water and soil samples was analysed following 3, 10, 17, 24 and 31 day. The comparison of results in terms of efficiency of removal, metal uptake and bioconcentration coefficient confirmed accumulation of heavy metals in the plant and subsequent decrease in concentration determined in wastewater. For all metals, determined efficiency of removal was greater than 80%, and the maximum removal for nickel was 99% for industrial wastewater.

In an experiment, AL-KHAFAJI *et al.* [2017] showed that mean efficiency of Cd, Cr, Ni and Pb removal for *Lemna minor* was 44.93%, 32.26%, 74.48% and 79.10%, respectively, whereas SEKOMO *et al.* [2012] evidenced that regardless of metal accumulation rate, Cr removal coefficient in a pond overgrown with Common duckweed was 94%. The elements such as Pb, Cd and Cu were removed with the following efficiency: 36%, 33% and 27%.

UYVAL [2013] demonstrated that *Lemna minor* has the ability to remove Cr(VI) ions from wastewater in a hydrophyte system. The following factors were identified: growth rate, chlorophyll content and ratio of dry or fresh weight of the plant to measurement of chromium toxicity. It was also found that chromium was successfully removed from water, and the greatest decrease was observed during the first 24 hours of the experiment (approx. 65–99% of chromium added to medium with *Lemna minor*). Chromium removal efficiency was within the range of 41–66% in the first 7 days. During the study (21 days) *Lemna minor* demonstrated removal efficiencies of 40.8%, 48.5%, 29.0%, 26.0% and 21.8% of added chromium ions, respectively in concentrations of 0.5, 1.0, 2.0, 4.0 and  $5.0 \text{ mg}\cdot\text{dm}^{-3}$ .

MIRANDA *et al.* [2014] proved that among the analysed aquatic plants, Common duckweed is characterised by the highest efficiency of capturing most of heavy metals in-

cluding almost complete i.e. 99% removal of Cu and 87% uptake of Co. Concentrations Ni, Zn, Fe and Cd were reduced with lower efficiency (61%, 58%, 43.4% and 57%, respectively).

BASILE *et al.* [2012] observed that the highest efficiency of Cd removal was reached as early as after 7 hours of incubation and amounted to 95%, whereas removal efficiency of Pb reached its maximum following 8 hours, i.e. 93%. For Cu the maximum removal efficiency was recorded after 24 hours, i.e. 86.5%, and in the case of Zn the efficiency was 63.5%. KHELLAF and ZERDAOUI [2009] observed that metals such as Cu and Cd may cause visible damage to Common duckweed. Chlorosis was observed as well as separation of leaves from the root. With low concentration ( $0.5 \text{ mg}\cdot\text{dm}^{-3}$ ) of Cu, after 24 hours necrosis was observed. Cd inhibited the growth of Common duckweed at all concentrations selected for this analysis,  $0.5 \text{ mg}\cdot\text{dm}^{-3}$  concentration in medium resulted in visible damage a day after treatment, whereas at concentrations to  $0.4 \text{ mg}\cdot\text{dm}^{-3}$ , there was a decrease in growth rate, colony separation and change in the colour of the leaves. Therefore, Zn was clearly less toxic element for Common duckweed as the manifestation of toxicity (slight discolouration and leaves separation) was observed only with  $18 \text{ mg}\cdot\text{dm}^{-3}$  Zn and at higher concentrations.

HOU *et al.* [2007] noticed that Cd, even at low concentrations –  $0.05 \text{ mg}\cdot\text{dm}^{-3}$ , demonstrates toxicity through a decrease in photosynthetic pigment content in *Lemna minor*. The minimum and maximum removal efficiency of selected metals at various starting concentrations of this metal from the aquatic environment by *Lemna minor* were collected and shown in Figure 1a.

**Hydrocharis morsus-ranae.** GAŁCZYŃSKA [2012] conducted studies in the period of 3 and 6 weeks on the reaction of *H. morsus-ranae* to five metal solutions: Pb – 0.6, Zn – 2.4, Cu – 0.12, Mn – 1.2 and Fe –  $2.4 \text{ mg}\cdot\text{dm}^{-3}$ . The highest content of the discussed metals in frogbit was determined in aquatic environment without sludge and amounted to  $348.2 \text{ mg}\cdot\text{kg}^{-1}$  d.w., for lead,  $90.9 \text{ mg}\cdot\text{kg}^{-1}$  d.w., for copper,  $1776 \text{ mg}\cdot\text{kg}^{-1}$  d.w., for zinc and  $802 \text{ mg}\cdot\text{kg}^{-1}$  d.w., for manganese. In the case of iron, the highest content of this metal, which amounted to  $5020 \text{ mg}\cdot\text{kg}^{-1}$  d.w., was found in the plant grown in a system with sludge. Pollution of water with salts of zinc or manganese in the amount of 2.4 and  $1.2 \text{ mg}\cdot\text{kg}^{-1}$  d.w., respectively, resulted

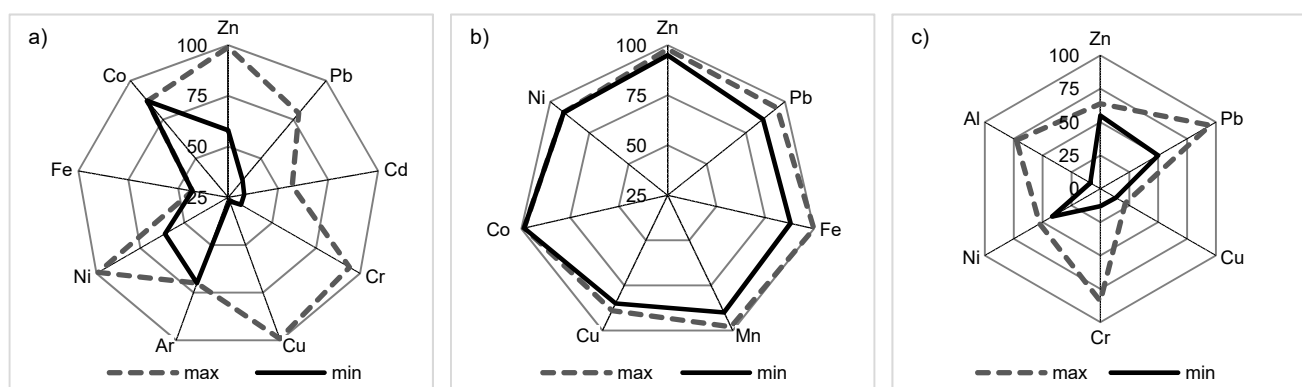


Fig. 1. Effectiveness of metal removal from the aquatic environment by: a) *Lemna minor*, b) *Hydrocharis morsus-ranae*, c) *Ceratophyllum demersum*; source: own study

in a decrease of frogbit biomass production. In aquatic ecosystem with sludge, mean value of uptake efficiency coefficient –  $Eu$  (difference between metal content in dry weight of polluted plants and the content of metal in dry weight of plants in a control specimen divided by the introduced dosage of metal) for five metals was: 95% ( $Eu_{Fe} = 105\%$ ,  $Eu_{Zn} = 98\%$ ,  $Eu_{Pb} = 95\%$ ,  $Eu_{Mn} = 90\%$  and  $Eu_{Cu} = 89\%$ ), and in environment without sludge the mean value was lower by only 6% ( $Eu_{Mn} = 98\%$ ,  $Eu_{Zn} = 95\%$ ,  $Eu_{Fe} = 88\%$ ,  $Eu_{Pb} = 86\%$  and  $Eu_{Cu} = 85\%$ ).

POLECHONSKA and SAMECKA-CYMERMAN [2018] described the reaction of *H. morsus-ranae* to water pollution with nickel and cobalt in the form of separate solutions and solutions of both metals with the following concentration of nickel 10.7, 18.7, 32.7, 57.1, 100 ( $\mu\text{g}\cdot\text{dm}^{-3}$ ), cobalt: 5.33, 9.32, 16.3, 28.6, 50.0 ( $\mu\text{g}\cdot\text{dm}^{-3}$ ), and 10.7 Ni + 5.33 Co, 18.7 Ni + 9.32 Co, 32.7 Ni + 16.3 Co, 57.1 Ni + 28.6 Co, 100 Ni + 50.0 Co ( $\mu\text{g}\cdot\text{dm}^{-3}$ ). The content of Co and Ni in the plant increased with an increase in concentration of metals in the medium. In a solution of both metals, the presence of Ni disrupted accumulation of Co which resulted in lower Co content than that determined in plants grown in Co solutions. Notably high content of Co (till 155  $\text{mg}\cdot\text{kg}^{-1}$  d.w.) and high efficiency of Ni uptake makes this species a very good accumulator of these metals. High content of both of these trace metals in plant tissue (till 511  $\text{mg}\cdot\text{kg}^{-1}$  d.w. Ni and 155  $\text{mg}\cdot\text{kg}^{-1}$  d.w. Co) had no effect on plant growth which demonstrates tolerance of the plant to these toxic substances. The plant also showed an exquisite ability to remove Co (till 98.6% in solution with 5.33  $\mu\text{g}\cdot\text{dm}^{-3}$  Co) and Ni (91.4% in solution 57.1  $\mu\text{g}\cdot\text{dm}^{-3}$  Ni and 28.6  $\mu\text{g}\cdot\text{dm}^{-3}$  Co) from the medium.

The minimum and maximum removal efficiency of selected metals at various starting concentrations of this metal from the aquatic environment by *Hydrocharis morsus-ranae* were collected and shown in Figure 1b.

***Ceratophyllum demersum*.** KARA [2005] observed that submerged species accumulate relatively high amount of heavy metals, as compared with emergent species. This is due to the physical traits of the species as because of its very thin epithelium the plants can remove heavy metals from water using its whole surface [REZANIA *et al.* 2016]. The analyses of biosorption conducted by, among others, KESHINKAN *et al.* [2004] and KREMS *et al.* [2013] showed that *Ceratophyllum demersum* can be used in Pb, Cu and Zn biosorption and bioaccumulation. XING *et al.* [2013] indicate that *Ceratophyllum demersum* demonstrates a positive adaptive strategy in response to exposure to heavy metals in *in situ* studies. EL-KHATIB *et al.* [2014] described strong Pb accumulation potential of *Ceratophyllum demersum* in 7-day long study of this plant in hydroponic systems supplemented with various concentrations of Pb (25, 50, 75  $\text{mg}\cdot\text{dm}^{-3}$ ). The highest accumulation values for *Ceratophyllum demersum* were observed for Pb (164.26  $\text{mg}\cdot\text{kg}^{-1}$  f.w.), the largest quantity of metal was accumulated following 1 day (91.72  $\text{mg}\cdot\text{kg}^{-1}$ ). After 7 days, with 75  $\text{mg}\cdot\text{dm}^{-3}$ , there was a significant decrease in the amount of photosynthetic pigment and occurrence of morphological symptoms such as chlorosis and leaves fragmentation. EL-KHATIB *et al.* [2014], DUMAN and KOCA [2014], EL-

-KHATIB *et al.* [2014], and MISHRA *et al.* [2006] showed that this plant is characterised by strong tolerance to lead and chromium following 7 days of exposition.

DUMAN and KOCA [2014] conducted an experiment to determine the way in which Cr(III) and Cr(VI) may affect various physiological and biochemical parameters of a plant. *Ceratophyllum demersum* was exposed to Cr(III) and Cr(VI) at various concentrations (0.05; 0.1; 0.3 and 0.5  $\text{mg}\cdot\text{dm}^{-3}$ ) and different exposition time (1, 2, 4 and 7 days), wherein the following was analysed: Cr accumulation, relative growth rate (RGR), malondialdehyde content (MDA), photosynthetic pigmentation, proline content and activity of antioxidant enzymes. In the case of application of Cr(III) and Cr(VI) it was found that concentration has a significant effect on all of the analysed parameters. However, exposition time showed no statistically significant effect on proline content with application of Cr(III), nor on MDA and protein with application of Cr(VI). It was found that the factor of Cr concentration had a synergistic effect on the changes in the content of RGR, electrical conductivity, protein and antioxidant enzymes both with application of Cr(III) and well as Cr(VI).

CHEN *et al.* [2015] conducted a hydroponic experiment to identify the features of lead bioaccumulation and tolerance of *Ceratophyllum demersum* to exposition to various concentrations of this metal (1.04–16.58  $\text{mg}\cdot\text{dm}^{-3}$ ) lasting 7, 14 and 21 days. Pb accumulation increased with an increase in concentration of this metal in the solution, to a maximum accumulation 4016.4  $\text{mg}\cdot\text{kg}^{-1}$  f.w., which shows hiperaccumulation of this element by *Ceratophyllum demersum*. At Pb concentration 1.04  $\text{mg}\cdot\text{dm}^{-3}$ , accumulation showed an increase throughout the whole experiment, with a rapid increase on day 7 and 14 when the plants accumulated 50% and 92% Pb, respectively. With concentration of this metal 2.07  $\text{mg}\cdot\text{dm}^{-3}$ , the plants accumulated the maximum quantity of lead on 14 day, however, on day 21 there was a significant decrease in Pb content. With an initial concentration of lead in water was 1.04  $\text{mg}\cdot\text{dm}^{-3}$ , *Ceratophyllum demersum* removed 60% on day 21, reaching its highest efficiency. Additionally, a reaction of the plant to the stress caused by lead concentration was observed, i.e. a change in protein content in plants and change in biomass increase. Levels of malondialdehyde showed a considerable increase at lead concentration below 4.14  $\text{mg}\cdot\text{dm}^{-3}$ , which additionally proves that this metal is toxic for plants. The results suggest that *Ceratophyllum demersum* has strong tolerance to a specific range of Pb concentration and it is speculated that 8.29  $\text{mg}\cdot\text{dm}^{-3}$  is the tolerance threshold of this plant to Pb in water.

REZANIA *et al.* [2016] indicated that efficiency of heavy metals removal by *Ceratophyllum demersum* with respect to Cr amounted to 13.0–84.3%, and for Pb from 92.0 to 95.0%. KESHINKAN *et al.* [2004] conducted laboratory analyses on Cu, Zn and Pb sorption by *Ceratophyllum demersum* using approx. 2 g of fresh weight of the plant and concentrations 2, 4, 8, 16, 32 and 64  $\text{mg}\cdot\text{dm}^{-3}$  of these metals in a time period of 120 min. It was found that the maximum absorption capacity ( $q_{\text{max}}$ ) for *Ceratophyllum demersum* was 6.17  $\text{mg}\cdot\text{g}^{-1}$  for Cu (II), 13.98  $\text{mg}\cdot\text{g}^{-1}$  for Zn (II), and 44.8  $\text{mg}\cdot\text{g}^{-1}$  for Pb.

The laboratory experiment conducted by CHOROM *et al.* [2012] lasted 15 days and used concentrations of Ni 6, 4, 2, and 1 mg·dm<sup>-3</sup>. Efficiency of metal removal from water was 41.7%, 50.0%, 52.5% and 46%, respectively. With lengthening of exposition time, there was an increase in Ni removal efficiency from the medium. With a slow rate of Ni removal from the medium, *Ceratophyllum demersum* showed biomass increase and possibility of greater Ni accumulation.

PARNIAN *et al.* [2016] cultivated *Ceratophyllum demersum* for 8 days in greenhouse conditions using medium modified with an increasing Cd and Ni (0, 1, 2, 4 and 6 mg·dm<sup>-3</sup>) dosage. The obtained results showed that an increase in heavy metals concentration in medium resulted in a decrease in the plant biomass. Efficiency of metal removal by *Ceratophyllum demersum* was 82.0% for Cd and 52.5% for Ni. UMEBESE and MOTAJO [2008] exposed *Ceratophyllum demersum* to toxic concentrations of Al (3 and 9 mg·dm<sup>-3</sup>), Zn (3 and 9 mg·dm<sup>-3</sup>) and Cu (2.5 and 7 mg·dm<sup>-3</sup>) in a solution for 15 days. It was found that *Ceratophyllum demersum* has much higher tolerance to aluminium ( $Eu_3 = 72.8\%$  and  $Eu_9 = 68.8\%$ ) than to zinc ( $Eu_3 = 63.7\%$  and  $Eu_9 = 54.9\%$ ), whereas its tolerance to copper ( $Eu_{2.5} = 21.6\%$  and  $Eu_7 = 13.43\%$ ) was very low. Greater dose of aluminium brought about a significant increase in chlorophyll content in the first 6 days of the experiment, whereas other metals caused a considerable decrease in chlorophyll content.

The minimum and maximum removal efficiency of selected metals at various starting concentrations of this metal from the aquatic environment by *Ceratophyllum demersum* were collected and shown in Figure 1c.

#### USE OF THE CAPABILITY OF REMOVAL OF METALS FROM WEDNESDAY WATER BY ANALYZED WATER PLANTS

Plants differ in their potential for metal uptake depending on the concentration values of a given metal in water, exposition time and availability of biogenic compounds [CHEN *et al.* 2015; DUMAN, KOCA 2014; GAŁCZYŃSKA 2012; UYSAL 2013]. The amount of biomass as well as efficiency of metal uptake from water affects the usability of plant for the purpose of phytoremediation of water. High concentration of metals in water may result in limited plant development and growth [GAŁCZYŃSKA 2012; PARNIAN *et al.* 2016]. This is an unfavourable phenomenon due to a decrease in biomass and temporal decline of efficiency of metal uptake. Many researchers stress that finding an optimum exposition time of a plant to pollution has a significant effect on reaching the greatest effect on removing metals from aquatic environment [CHEN *et al.* 2015; GAŁCZYŃSKA 2012; GOSWAMI *et al.* 2014; UYSAL 2013]. Most often, short-term exposure to high concentrations of metals allows for obtaining high level of purification. Such a relationship for *Lemna minor* was determined by UYSAL [2013]. It was found that in a period of 7 days, the efficiency of chromium removal was within the range of 41–66%, and following 21 days, regardless of metal concentration levels, the efficiency decreased (22–49%). Additionally, GAŁCZYŃSKA [2012] observed that due to

short time of leaves mortality in *Hydrocharis morsus-ranae* rosettes and high efficiency of metal uptake in the period of 3 weeks of plant exposure to metal both at lower (aquatic environment without sludge – 90%, aquatic environment with sludge – 93%) as well as higher (aquatic environment – 91%, soil-water environment – 98%) concentrations of nitrogen, phosphorus and potassium compounds, there is no need to lengthen the exposition time of plant to pollutants present in the environment. The same trend was observed by CHEN *et al.* [2015] with respect to lead concentration equal to 2.07 mg·dm<sup>-3</sup>, where *Ceratophyllum demersum* accumulated the maximum lead quantity on the 14 day, however with a considerable decrease in Pb on the 21 day of the experiment. In turn, according to CHOROM *et al.* [2012], following application of 2 mg dm<sup>-3</sup> Ni, after 15 days, *Ceratophyllum demersum* showed the highest metal removal efficiency (52.5%) which was additionally accompanied by an increase in biomass.

Taking into consideration the possibility for development of the analysed plants in environment polluted with metals, the rank of their applicability due to demonstrated tolerance to metal concentration and uptake efficiency was determined (Fig. 2).

The use of *Lemna minor*, *Hydrocharis morsus-ranae* and *Ceratophyllum demersum* for the purpose of purification of water polluted with metals can be achieved by immersing the plants located in special containers in water – the containers would enable easy removal of the plants from the aquatic ecosystem under purification. When designing the containers, the need for protection of *Lemna minor* and *Hydrocharis morsus-ranae* from wind and water waves must be taken into account. The most favourable time for plant use is the vegetative season. For species such as *Hydrocharis morsus-ranae* and *Lemna minor*, this period lasts from mid-May to mid-September, and for *Ceratophyllum demersum* this period is slightly longer and lasts till mid-October. Following the period of no more than 2 weeks, the plants should be removed from water, dried and the resulting residue should be further utilised.

#### SUMMARY

On the grounds of the above-mentioned research by numerous authors, it was found that *Lemna minor* in the period of 21 days can remove from 22 to 41% chromium ions in concentration 0.5–5.0 mg·dm<sup>-3</sup> from the aquatic environment. In the case of arsenic, removal efficiency reached values of more than 70% at the concentration of 0.5 mg·dm<sup>-3</sup> following 15 days. In turn, mean Cd, Cu and Pb removal efficiency from aquatic solutions (0.038–0.054 mg·dm<sup>-3</sup> Cd; 0.0024–0.032 mg·dm<sup>-3</sup> Cu; 5.0–10.0 mg·dm<sup>-3</sup> Pb) in the period of 31 days reached values over 81%, and for Ni (2.5–5.0 mg·dm<sup>-3</sup>) 99%. *Hydrocharis morsus-ranae* shows 95% potential for removing Pb (0.6 mg·dm<sup>-3</sup>), Cu (0.12 mg·dm<sup>-3</sup>), Zn (2.4 mg·dm<sup>-3</sup>), Mn (1.2 mg·dm<sup>-3</sup>), Fe (2.4 mg·dm<sup>-3</sup>) from aquatic environment in the period of 14 days, and Co (0.005 – 0.050 mg·dm<sup>-3</sup>) and Ni (0.011–0.100 mg·dm<sup>-3</sup>) in the period of 7 days. *Ceratophyllum demersum* demonstrated the potential for metal removal in the period of 15 days from 73% to 95% Pb (1 mg·dm<sup>-3</sup>), Cr



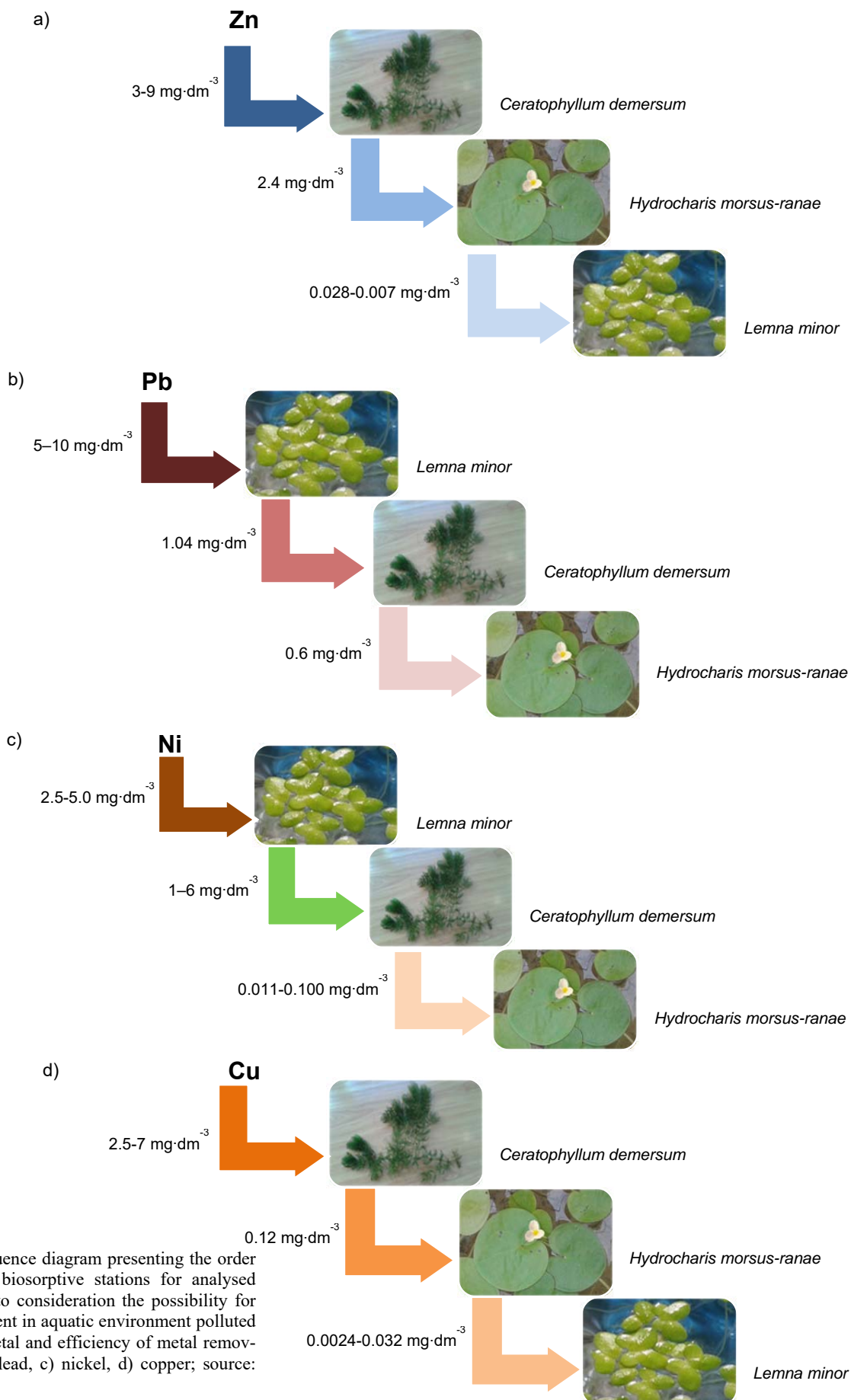


Fig. 2. The sequence diagram presenting the order of one-species biosorptive stations for analysed metal taking into consideration the possibility for plant development in aquatic environment polluted with a given metal and efficiency of metal removal: a) zinc, b), lead, c) nickel, d) copper; source: own study

(2 mg·dm<sup>-3</sup>), Cd (2 mg·dm<sup>-3</sup>) and Al (3 mg·dm<sup>-3</sup>) or from 21% to 63% Zn (3 mg·dm<sup>-3</sup>), Ni (2 mg·dm<sup>-3</sup>) and Cu (3 mg·dm<sup>-3</sup>), with a decrease in metal removal efficiency following 21 days. *Lemna minor*, *Ceratophyllum demersum* and *Hydrocharis morsus-ranae* can be used for phytoremediation of toxic metals from aquatic ecosystems, however, toxicity of these metals and exposition time poses some limitation to efficacy of the plants' use. Geoclimatic conditions influence limitation of biomass increase, therefore there is a lack of sufficient amount of biomass of heavy metals hyperaccumulators to reach 100% efficiency of metal removal. The greatest efficiency of metal removal can be achieved using sequences of one-species filtration systems. Providing that excess plants are removed from hydrophyte system (e.g. *Lemna minor* is removed several times in a year, *Ceratophyllum demersum* and *Hydrocharis morsus-ranae* 3 times), a decrease of metal pool in the system is permanent.

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### Możliwości i ograniczenia stosowania *Lemna minor*, *Hydrocharis morsus-ranae* i *Ceratophyllum demersum* w usuwaniu metali z wód zanieczyszczonych

#### STRESZCZENIE

W pracy przedstawiono ocenę możliwości i ograniczeń stosowania rzęsy drobnej, żabiścieku pływającego i rogatka sztywnego w usuwaniu metali z wód zanieczyszczonych. Syntetycznie omówiono rolę tych gatunków w fitotechnologii i ich znaczenie w ocenie stanu wód powierzchniowych. Przedstawiono zmienność stężenia wybranych metali w środowisku występowania roślin i zawartość metali w organach analizowanych roślin. Scharakteryzowano ich wady i zalety w usuwaniu metali z wód ze względu na cechy biologiczne. Określono minimalną i maksymalną efektywność usuwania metali w zależności od skali zanieczyszczenia wód. Ustalono, że wszystkie analizowane rośliny mogą być wykorzystywane do fitoremediacji metali toksycznych z ekosystemów wodnych, ale ograniczeniem skuteczności zabiegów jest toksyczność tych metali dla roślin i czas ekspozycji. Największą efektywność w usuwaniu metali można uzyskać dzięki zastosowaniu sekwencji jednogatunkowych układów filtracyjnych.

**Słowa kluczowe:** fitoremediacja, hiperakumulatory, metale ciężkie, rogatek sztywny, rzęsa drobna, skuteczność usuwania metali, żabiściek pływający