
INFLUENCE OF WATER CONTAMINATION ON THE ACCUMULATION OF SOME METALS IN *HYDROCHARIS MORSUS-RANAE* L.

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Abstract

European frogbit (*Hydrocharis morsus-ranae*) grows mainly in stagnant, eutrophic and mesotrophic waters. It is found in various water ecosystems, but finds the optimal conditions for development in ecosystems formed with water soldier (*Stratiotes aloides*). The shortage of detailed literature data on the properties of water bodies where European frogbit and water soldier grow has stimulated this study, whose aim has been to determine the values of selected water quality parameters and concentrations of some metals in tissues of both hydrophytes.

The study was conducted in the late spring and summer of 2007 and 2008 and relied on environmental samples of water and plants (9 locations, including a field pond, a draining ditch on Pucka Isle, watercourses in Łwidwie Nature Reserve, an oxbow lake of the Bug River and a flood pool near G³ębokie Lake). The concentrations of N-NO₃, N-NO₂, N-NH₄ and PO₄³⁻ as well as the elements K, Na, Mg, Ca, Cd, Pb, Cu, Zn, Mn and Fe were measured in the water samples. Furthermore, the percentage of oxygen dissolved in water and the pH and electrolytic conductivity of water were determined. In order to assess the accumulation of metals by plants, the content of the same metals was measured in the water.

It was determined that *Hydrocharitetum morsus-ranae* L. associations grow in physiochemically very diverse aquatic environments. The range of differences in the examined water quality indicators was broader for European frogbit than for water soldier. With respect to the concentration of Zn, N and P compounds, pH and the content of dissolved oxygen in water, the ranges were the same for both species. *Hydrocharis morsus-ranae* and *Stratiotes aloides* differed in the accumulation of the metals.

The range of the accumulation of most metals (except of K and Ca) was broader for European frogbit than for water soldier. In respect of Na, the range of accumulation for both plant species was the same. European frogbit spreads in water bodies in which the water is considered contaminated due to the concentrations of Cd, Pb, Cu and P.

Key words: metals, *Hydrocharis morsus-ranae* L., *Stratiotes aloides* L., water.

WPŁYW ZANIECZYSZCZENIA WÓD NA AKUMULACJĘ WYBRANYCH METALI PRZEZ *HYDROCHARIS MORSUS-RANAE* L.

Abstrakt

Żabićciek pływajcy (*Hydrocharis morsus - ranae* L.) występuje w wodach eu- i mezotroficznym. Zasiedla głównie wody stojące. Wchodzi w skład różnych zbiorowisk wodnych, lecz optymalne warunki rozwoju znajduje w obrębie zbiorowiska budowanego wraz z osoką aloesowatą (*Stratiotes aloides*). Ze względu na brak w piśmiennictwie szczegółowych danych dotyczących zawartości wód, w których występują żabićciek pływajcy i osoka aloesowata celem pracy było określenie wartości wybranych wskaźników jakości wód i zawartości niektórych metali w tkankach obu hydrofitów.

W latach 2007-2008, na przełomie wiosny i lata, przeprowadzono badania pobranych ze środowiska próbek wodnych i roślinnych (9 stanowisk obejmujących: oczko śródpolne, rów melioracyjny na Wyspie Puckiej, ciek na obszarze rezerwatu Łwidwie, starorzecze Bugu, rozlewisko przy Jeziorze Głębokim). W próbkach wód zmierzono stężenie: N-NO₃, N-NO₂, N-NH₄ i PO₄³⁻ oraz K, Na, Mg, Ca, Cd, Pb, Cu, Zn, Mn i Fe. Ponadto określono procentową zawartość tlenu rozpuszczonego w wodzie oraz odczyn wody i przewodność elektrolityczną. W celu określenia wielkości akumulacji metali przez rośliny oznaczono zawartość tych samych metali w materiale roślinnym, co w wodzie.

Ustalono, że *Hydrocharitum morsus-ranae* L. rośnie w bardzo różnorodnym fizykochemicznie środowisku wodnym, a zakres zmian wartości badanych wskaźników jakości wody był szerszy w przypadku żabićcieku pływającego niż osoki aloesowatej. W przypadku stężenia Zn, związków N i P, odczynu wody i zawartości tlenu rozpuszczonego w wodzie zakres występowania obu gatunków roślin był taki sam. Rośliny *Hydrocharis morsus-ranae* i *Stratiotes aloides* różniły się zdolnością akumulowania badanych metali. Zakres akumulacji metali, oprócz K i Ca, był szerszy w przypadku żabićcieku pływającego niż osoki aloesowatej. W przypadku zawartości Na zakres akumulacji obu gatunków roślin był taki sam. Żabićciek pływajcy występował m.in. w zbiornikach, gdzie ze względu na stężenie w wodzie: Cd, Pb, Cu i P, wody uznaje się za zanieczyszczone.

Słowa kluczowe: metale, *Hydrocharis morsus-ranae* L., *Stratiotes aloides* L., woda.

INTRODUCTION

Due to human pressure, degradation of aquatic ecosystems is ubiquitous (Koc et al. 2002). Pollutants entering water reservoirs affect the volume and concentration of substances dissolved in water (GRABIŃSKA et al. 2004) as well as their content in the bottom sediments (SKWIERAWSKI 2004, SZYPEREK 2004). The occurrence and distribution of macrophytes in aquatic ecosystems depend on a number of ecological factors, mainly: depth, type of the bottom,

water movement, temperature, transparency and availability of nutrients (BERNATOWICZ, WOLNY 1969, GAMRAT, GAŁCZYŃSKA 2006). The presence or absence of aquatic plants, and their quantitative ratios can define the ecological status of a given ecosystem (SENDER 2007, 2008). Progressing eutrophication affects negatively the species diversity and development of macrophytes (BLINDOW 1992, JEPPESEN et al. 2000). During the natural eutrophic and mesotrophic process of the shrinking of standing waters, plant communities of *Hydrocharitetum morsus-ranae* are the last stage in the development of aquatic vegetation. They supersede communities of plants with floating and submerged leaves, themselves being replaced by plant communities of *Equisetum limosi*, *Phragmitetum*, *Thelypteridi-Phragmitetum*, *Typhetum latifoliae*. By producing huge amounts of phytomass per surface area unit, they cause rapid shallowing of occupied habitats (PODBIELKOWSKI, TOMASZEWICZ 1996). The content of harmful substances in an aquatic environment, such as heavy metals and organic compounds, generates various effects on the growth and development of these plant species (MALEVA et al. 2004, GAŁCZYŃSKA et al. 2011). The shortage of detailed literature data on the properties of water bodies in which European frogbit and water soldier occur has encouraged us to pursue this study in order to determine the values of selected water quality factors and concentrations of some metals in tissues of both hydrophytes. Determination of the impact of water pollution on the accumulation of selected elements by European frogbit can serve as comparative material for studies on other aquatic ecosystems settled by this species.

MATERIAL AND METHODS

Physicochemical studies of plant and water samples were carried out in the late spring and summer of 2007 and 2008. The research material was collected from nine water bodies, including a field pond, a draining ditch on Pucka Isle, watercourses in Łwidwie Nature Reserve, an oxbow lake by the Bug River and a flood pool near Gębokie Lake (Table 1).

In order to determine the metal content in *Hydrocharis morsus-ranae* and *Stratiotes aloides*, all collected plant samples were dried and milled. Afterwards, the samples were mineralized in a mixture of hot acids: HNO₃ and HClO₄. The water samples were mineralized in HNO₃. Determinations of K, Na, Mg, Ca, Cd, Pb, Cu, Zn, Mn and Fe in mineralized plants and water samples in three replications were carried out by the atomic absorption spectrometry technique, using a Solaar S AA spectrometer.

The water samples were also submitted to determinations of nitrogen nitrate(V), ammonium cation, and orthophosphate(V). For determination of N and P in the waters, the colorimetric methods were used in accordance with the Polish Standards. The determinations were performed in a Spekol

Table 1

The occurrence of European frogbit and water soldier in the water bodies

No.	Water and plant samples location	Occurrence	
		European frogbit	water soldier
1.	Draining ditch on Pucka Isle in Szczecin	X	-
2.	Field pond near Myślibórz	X	-
3.	Oxbow lake by the Bug River near Drohiczyn	X	X
4.	Ditch Z2, which is also a canal encompassing Świdwie Lake	X	X
5.	Gunica River flowing from Świdwie Lake	X	X
6.	Bolków-Lęgi canal, which empties into Świdwie Lake	X	X
7.	Struga Żurawia canal, which empties into Świdwie Lake	X	X
8.	Gunica River flowing from Stolsko Lake	X	-
9.	Flood pool by Głębokie Lake in Szczecin	X	-

11 spectrophotometer. The percentage of oxygen dissolved in water, water pH and electrolytic conductivity were determined in the water samples using the electroanalytical methods. The following equipment was used for the measurements of the physical parameters: an oxygen meter (HI 9145 microprocessor meter), a laboratory pH meter (CP-411 with a temperature sensor for liquids with sediments and an electrode EPS-1) and a conductometer (inoLab Coud 730 with TetraCon electrode 325).

The results of the physicochemical tests, depending on the presence of European frogbit in a given water body alone or together with water soldier, were statistically elaborated using a single factor analysis of variance. The significance of the differences between the means was determined by Tukey's test, with $p=0.05$. In order to determine the relationship between metal concentration in the tested water and its content in European frogbit, Pearson's linear correlation factor was calculated.

RESULTS AND THEIR DESCRIPTION

Elements leaching from agricultural soils, containing mineral fertilizers and plant protection chemicals, are an important source of water contamination in rural agricultural areas. Particularly high concentrations of PO_4^{3-} in water indicate eutrophication. However, it is worth noticing that the phosphorus concentration in water significantly decreases in summer due to the absorption of phosphorus by plant assemblages of different species of aquatic

plants developing in water reservoirs. The association *Hydrocharitetum mor-sus-ranae* belongs to freshwater macrophyte communities which occur in very shallow water bodies. The field research comprising nine water bodies confirms the literature data (MATUSZKIEWICZ 1984, PODBIELKOWSKI, TOMASZEWICZ 1996) in that this association usually inhabits eutrophic waters with an organic base (lakes or their bays, oxbow lakes, canals), in places which are sunny and sheltered from waves (Table 2). Water bodies number 1, 2 and 3 are located in an agricultural basin. The other analyzed reservoirs have not been exposed to strong human pressure over the last 20 years. In four sampling sites in water bodies nos 1, 2, 8 and 9 (Table 1), European frogbit grew alone, but in the remaining sites (nos 3-7) it occurred with water soldier (Table 1). Rather than competing, European frogbit and water perfectly complement each other in the use of water space. The water depth in which most of the plant communities grew did not exceed 1 meter, and the reaction of water was neutral or slightly alkaline (Table 2). It has been found

Table 2

Physicochemical parameters of water in the water bodies in which European frogbit occurred alone and with water soldier

Water quality indicators		Water bodies with European frogbit				Water bodies with European frogbit and water soldier				LSD _{0.05}
		mean	SD	min.	max.	mean	SD	min.	max.	
Physical parameters	el. conductivity ($\mu\text{S cm}^{-1}$)	555	77	363	703	602	15	562	634	i.d.
	O ₂ (%)	59	18	8	86	71	14	15	90	i.d.
	N-NO ₃ (mg dm ⁻³)	0.455	0.264	0.005	1.086	0.128	0.029	0.075	0.235	i.d.
Chemical parameters	N-NO ₂ ⁻ (mg dm ⁻³)	0.003	0.003	0.0	0.010	0.004	0.003	0.0	0.013	i.d.
	N-NH ₄ ⁺ (mg dm ⁻³)	0.020	0.011	0.0	0.039	0.070	0.041	0.0	0.220	i.d.
	PO ₄ ³⁻ (mg dm ⁻³)	0.254	0.223	0.010	0.919	0.446	0.170	0.010	1.052	i.d.
	N _{min} (mg dm ⁻³)	0.477	0.252	0.054	1.086	0.202	0.039	0.098	0.312	i.d.
	K (mg dm ⁻³)	4.89	1.13	2.16	7.63	4.15	0.42	3.18	5.30	i.d.
	Mg (mg dm ⁻³)	6.59	3.46	0.77	14.58	2.38	1.56	0.56	8.60	i.d.
	Ca (mg dm ⁻³)	81.05	18.69	41.89	125.9	75.63	8.44	43.30	91.97	i.d.
	Na (mg dm ⁻³)	19.40	5.32	10.23	33.74	11.70	1.19	8.30	15.18	i.d.
	Cd (mg dm ⁻³)	0.009	0.006	0.0	0.022	0.001	0.001	0.0	0.007	i.d.
	Pb (mg dm ⁻³)	0.093	0.054	0.0	0.211	0.002	0.002	0.0	0.011	i.d.
	Cu (mg dm ⁻³)	0.050	0.047	0.0	0.190	0.019	0.019	0.0	0.093	i.d.
	Zn (mg dm ⁻³)	0.017	0.005	0.007	0.032	0.025	0.015	0.0	0.085	i.d.
	Mn (mg dm ⁻³)	0.823	0.820	0.001	3.284	0.142	0.097	0.0	0.521	i.d.
Fe (mg dm ⁻³)	1.280	0.926	0.007	4.032	0.327	0.098	0.062	0.558	i.d.	

that the association *Hydrocharitetum morsus-ranae* grows in aquatic environments that possess highly different physiochemical properties (Table 2). Despite the generally higher average values of the determined water quality parameters in the water bodies with European frogbit growing alone, the performed statistical analysis indicates that there are no significant differences between the average values of these parameters depending on the classification of the examined water reservoirs (Table 2). The range of values of the analyzed examined water quality indicators was broader for the habitats occupied exclusively by European frogbit than for the ones in which it cooccurred with water soldier (Table 2). Previous studies by PINDEL and WO•NIAK (1998), OBOLEWSKI et al. (2009) and GAŁCZYŃSKA et al. (2011) suggest that water soldier can grow in waters with higher conductivity values than European frogbit. This study has revealed that the range of occurrence of both plant species was the same with respect to the concentrations of zinc, nitrogen and phosphorus compounds, water pH and the content of dissolved oxygen in water (Table 2).

The references seem to contain no information on concentrations of nitrogen and phosphorus compounds in waters occupied by European frogbit. The data given by TARKOWSKA-KUKURYK (2006) on concentrations of nitrate nitrogen(V) and ammonia nitrogen in waters inhabited by water soldier are consistent with our results for the water bodies with European frogbit and water soldier (Table 2). The analysis of the results from OBOLEWSKI et al. (2009) shows that *Stratiotes aloides* inhabits waters which are characterized by even higher concentrations of orthophosphate(V) ($0.63\text{-}1.31\text{ mg dm}^{-3}$) than shown in this work (Table 2). In respect of pH, the literature data indicate better adaptation of water soldier to fluctuations in water pH. PINDEL, WO•NIAK (1998) reported that *Hydrocharis morsus-ranae* grew in waters where pH ranged from 7.0 to 7.9. On the other hand, the research carried out by RENMAN (1989) shows that water soldier develops in waters with pH of 5.64 to 7.50. and OBOLEWSKI et al. (2009) report that in oxbow lakes adjacent to the Łyna River water soldier colonized waters with pH of 7.6 to 8.49.

Among the analyzed water bodies (Table 2), the highest concentration of PO_4^{3-} was measured in the water of the canal surrounding Œwidwie Lake (1.052 mg dm^{-3}), whereas the highest N_{min} was found in the field pond near MyŒlibórz (1.086 mg dm^{-3}). The lowest concentration of these elements ($0.010\text{ mg PO}_4^{3-}\text{ dm}^{-3}$ and $0.054\text{ mg N}_{\text{min}}\text{ dm}^{-3}$) was measured in the Gunica River flowing from Stolsko Lake. The highest concentration of the above metals was measured in three sampling points of reservoirs nos 1-3 located in the agricultural basin. The highest concentrations of potassium, calcium, magnesium, lead and copper were measured in the waters of the field pond near MyŒlibórz, and the highest concentrations of sodium, cadmium, manganese and iron were determined in the water of the draining ditch on Pucka Isle in Szczecin. The highest concentration of zinc in water was assayed in the oxbow lake of the Bug River (Table 2). According to the Regulation of the

Minister for Environment of 20th August 2008 on classification of surface waters, it has been concluded that European frogbit occurs in water bodies in which the water is considered to be contaminated due to the concentrations of cadmium, lead, copper and phosphorus.

Our analysis of the average levels of metals in European frogbit depending on its occurrence alone or together with water soldier showed no statistically significant differences between the means except for the concentration of zinc (Table 3). Considering the two species in the *Hydrocharitetum morsus-ranae* association, it was noticed that European frogbit and water soldier differed in the accumulation of the analysed metal ions (Tables 3 and 4). The scope of accumulation of these elements was broader for European frogbit than for water soldier, with the exception of potassium and calcium ions. In the case of sodium ions, the scope of their accumulation was the same for both plant species. With its shallow root system, European frogbit uses only the upper water layers, while water soldier's roots reach deep and take up nutrients from the lower parts of a reservoir (PODBIELKOWSKI, TOMASZEWICZ 1996).

Potassium and nitrogen dominate among the macronutrients in plants, but there are quite large amounts of calcium and much less of sulphur, phosphorus and magnesium. It was determined that the average content of potassium was 56.84 mg K g⁻¹ d.w. of water soldier and 13.59 mg K g⁻¹ d.w. of European frogbit. No effect of the potassium level in water on its content in dry matter of European frogbit was found. Many researchers stress that *Hydrocharitetum morsus-ranae* associations occur in waters rich in calcium

Table 3

Concentrations of metals in European frogbit either growing alone or in associations with water soldier

Metal	Water bodies with European frogbit				Water bodies with European frogbit and water soldier				LSD _{0.05}
	mean	SD	min.	max.	mean	SD	min.	max.	
K (mg g ⁻¹ d.w.)	16.34	10.53	8.59	47.80	10.84	2.26	7.79	19.79	i.d.
Mg (mg g ⁻¹ d.w.)	3.86	2.04	1.23	9.94	3.50	1.96	1.10	11.32	i.d.
Ca (mg g ⁻¹ d.w.)	12.48	6.44	1.61	27.55	16.42	4.03	7.93	30.99	i.d.
Na (mg g ⁻¹ d.w.)	5.83	1.84	2.90	11.14	4.28	1.57	1.46	10.38	i.d.
Cd (µg g ⁻¹ d.w.)	0.0	0.0	0.0	0.0	0.20	0.20	0.0	1.01	i.d.
Pb (µg g ⁻¹ d.w.)	16.32	7.11	0.0	32.33	21.26	11.79	0.47	67.18	i.d.
Cu (µg g ⁻¹ d.w.)	16.56	1.37	14.47	20.49	16.63	4.25	3.44	29.40	i.d.
Zn (µg g ⁻¹ d.w.)	888.4	510.9	70.96	2219	34.99	9.17	0.48	52.98	850.1
Mn (mg g ⁻¹ d.w.)	4.70	2.69	0.69	12.49	5.03	1.70	0.01	9.71	i.d.
Fe (mg g ⁻¹ d.w.)	2.60	1.77	0.67	7.89	0.66	0.24	0.09	1.43	i.d.

Table 4

Concentrations of the selected metals in dry matter of water soldier sampled from the water bodies where it co-occurred with European frogbit

Metal	Water bodies with European frogbit			
	mean	SD	min.	max.
K (mg g ⁻¹ d.w.)	56.84	21.53	22.20	78.65
Mg (mg g ⁻¹ d.w.)	6.98	2.78	3.41	10.45
Ca (mg g ⁻¹ d.w.)	31.46	15.82	5.40	42.46
Na (mg g ⁻¹ d.w.)	7.89	3.88	4.43	14.52
Cd (µg g ⁻¹ d.w.)	0.001	0.002	0.0	0.004
Pb (µg g ⁻¹ d.w.)	6.61	3.89	2.12	11.52
Cu (µg g ⁻¹ d.w.)	10.02	5.04	1.59	15.10
Zn (µg g ⁻¹ d.w.)	32.21	22.26	0.16	61.36
Mn (mg g ⁻¹ d.w.)	3.46	2.18	0.033	5.54
Fe (mg g ⁻¹ d.w.)	0.577	0.975	0.015	2.309

(ROSTAFIŃSKI 1956, RENMAN 1989). Water soldier accumulates calcium until September and its surplus precipitates in the form of carbonates on the surface of submerged leaves (KRÓLIKOWSKA 1997). In this study, water soldier accumulated an average of 31.46 mg Ca g⁻¹ d.w. (Table 4) and European frogbit - just 14.45 mg Ca g⁻¹ d.w. (Table 3). In all the water bodies with elevated concentrations of calcium in the water, the content of this element in the dry matter of European frogbit decreased significantly (Table 5). On the other hand, in the water bodies where European frogbit grew alone, the correlation was negative (Table 5). In the water bodies where European frogbit co-occurred with water soldier, an increase in the concentration of magnesium in water led to its significantly higher content in the dry matter of European frogbit (Table 5). Additionally, in the same water reservoirs, a statistically significant, albeit negative, linear relationship between the sodium concentration in water and its content in the dry matter of European frogbit was found (Table 5).

Heavy metals such as copper, zinc, iron and manganese are involved in many key metabolic processes. The exact role of lead and cadmium in the lifecycle of organisms is not known. The response of plants to metals, including heavy ones, depends on the sensitivity of individual plants, the severity of the stress and the form in which metal is available. The toxic effect of heavy metal absorption is related to their very high concentration in plant cells, which leads to impaired function of the membranes in photosynthetic and mitochondrial electron transport and inactivation of many en-

Table 5

Relationship between concentrations of the elements in water
and in the dry matter of European frogbit

Statistical parameters	Water bodies with European frogbit and with European frogbit and water soldier									
	metal									
	K	Mg	Ca	Na	Cd	Pb	Cu	Zn	Mn	Fe
<i>c</i>	0.029	0.083	i.d. 0.717	i.d. 0.202	0.096	i.d. 0.406	i.d. 0.445	i.d. 0.202	0.719	0.990
<i>s</i>	0.940	0.832	0.030	0.603	0.806	0.278	0.230	0.602	0.029	0.001
Water bodies with European frogbit										
<i>c</i>	i.d. 0.107	i.d. 0.592	0.940	i.d. 0.323	i.d.	i.d. 0.957	i.d. 0.534	i.d. 0.426	i.d. 0.428	0.994
<i>s</i>	0.893	0.408	0.050	0.671	i.d.	0.043	0.466	0.574	0.572	0.006
Water bodies with European frogbit and water soldier										
<i>c</i>	0.601	0.998	i.d. 0.259	i.d. 0.853	i.d.	i.d. 0.164	i.d. 0.534	i.d. 0.904	0.400	0.706
<i>s</i>	0.283	0.001	0.675	0.046	i.d.	0.792	0.466	0.035	0.505	0.136

c - correlation coefficient, *s* – significance level,

i.d. – insufficient data; statistically significant correlations in bold

zymes involved in the regulation of the basic cell metabolism. These events cause disorders of in the growth and development of plants (GRUCA-KRÓLIKOWSKA, WACŁAWEK 2006, GAECZYŃSKA et al. 2011).

MALEVA et al. (2004) reported that when water was contaminated with cadmium or with copper at a concentration of 0.025 g dm³, the bioaccumulation factor for European frogbit was 597 and 618, respectively, and when the contamination reached 0.25 g dm³, it equalled 276 and 336. In the analyzed water bodies, the influence of the content of cadmium and copper in water on the concentration of these metals in the dry matter of European frogbit was not detected. With regard to lead, a statistically significant negative correlation was observed only in the water bodies where European frogbit grew alone. In turn, as the zinc concentration in water increased, the amount of this metal content in the dry matter of European frogbit significantly decreased, but this relationship was verified only in the water bodies overgrown with European frogbit and water soldier (Table 5). In respect of manganese and iron in all the water reservoirs, a significant and positive linear correlation between the concentration of these elements in water and their content in the dry matter of European frogbit was detected. In the water bodies where this species occurred independently, this relationship was identified only for iron.

CONCLUSIONS

1. European frogbit was able to live in water bodies in which the water was deemed contaminated due to excessive concentrations of cadmium, lead, copper and phosphorus.

2. The ecological range of occurrence of European frogbit, in terms of most chemical parameters of a habitat, is wider than that of water soldier. In respect of the concentrations of zinc, N and P compounds in water, the range of occurrence of both plant species is the same.

3. The range of accumulation of metals, except for potassium and calcium, was wider for European frogbit than for water soldier. By accumulating manganese and iron, European frogbit reflects concentrations of these metals in water. The occurrence of European frogbit along with water soldier affects the size of accumulation of zinc by European frogbit.

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