HEAVY METAL ACCUMULATION IN LEAVES OF *HYDROCHARIS MORSUS-RANAE L.* AND BIOMONITORING APPLICATIONS

Ludmiła POLECHOŃSKA[1], Małgorzata DAMBIEC
University of Wrocław, Department of Ecology, Biogeochemistry
and Environmental Protection, Wrocław, Poland

Abstract

In present study the concentrations of Hg, Mn, Zn, Fe and Cu in water, bottom sediments and leaves of *Hydrocharis morsus-ranae* from 11 oxbow lakes of the Odra River were determined by atomic absorption spectrometry. Trace metal concentration in water and bottom sediments were below the geochemical background, indicating no anthropogenic impact in the studied area. On average, the concentrations of metals in leaves of *H. morsus ranae* exceeded natural thresholds. A high bioaccumulation factors for metals were recorded. The significant positive correlations found between the content Zn, Fe and Hg of in water and in the *H. morsus ranae* indicate the potential use of the species in the biomonitoring of environmental contamination with these metals.

Keywords: trace metal, *Hydrocharis morsus ranae*, metal accumulation, biomonitoring

1. INTRODUCTION

Aquatic ecosystems are often the terminal link of pollutant migration. Water bodies of urbanized and agricultural areas are exposed to a strong anthropogenic influence. The increased input of trace metals and biogenic elements into these ecosystems is an urgent problem [13]. Sources of trace metals in water are: industry, agricultural discharge (containing mineral fertilizers and plant

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[1] Corresponding author: University of Wrocław, Department of Ecology, Biogeochemistry and Environmental Protection, ul. Kanonia 6/8, 50-328 Wrocław, Poland,
e-mail: polechonska@gmail.com, tel. +48713752011

protection chemicals), atmospheric pollution such as acid rains and, frequently overlooked, domestic wastewater [10, 27].

Macrophytes play prominent role in biogeochemical cycles of nutrients and heavy metals of many aquatic ecosystems [24]. Some authors indicate aquatic plants as biomonitors for aquatic ecosystems state because of high accumulation ability of a great amount of various substances, among them metals, in plant organs [3, 25]. Many aquatic plants can accumulate considerable amounts of metals dissolved in water and retain them in organs for a long time. In that case analyses of plant tissues provide time-integrated information about quality of the system [3, 13]. Some aquatic plants can accumulate several ten to several thousand times higher concentrations of metals than in their surroundings [18, 24] and at a level correlated with their water or bottom sediment concentration [15]. Because of these abilities, and also high production of biomass, some macrophyte species have been successfully used as biological monitors and remediators of habitats contaminated with heavy metals. The indicator plant species should be: native to the area; easily cultivated and abundant in the field; visible to the naked eye; easy to collect and to handle; easy to identify in the field [6]. In order to obtain accurate indicators of aquatic ecosystem, it is necessary to apply parallel studies on the content of elements in water and sediments [2].

European frog-bit is the only species of the *Hydrocharis* genus native to Poland. It is an annual, herbaceous, free-floating, or rooted, when on mud, aquatic plant [23]. The plant grows best in stagnant water habitats such as swamps, backwaters, quiet areas of bays, low energy shorelines of rivers, streams and lakes [16]. Some authors [2, 5, 10] suggest potential use of this species as a phytoindicator or a phytoremediator in aquatic ecosystems. Moreover, *Hydrocharis morsus-ranae* confirms very well many of the above-mentioned criteria for the good biomonitor organism. Free-floating plants should be used in biomonitoring of water quality in place of rooted species since they draw their nutrients from the water column [6]. Still, there are only a few studies available regarding the capacity of *H. morsus-ranae* to accumulate metal ions from aquatic environment.

The aim of this study was to assess bioaccumualtion of metals in *H. morsus-ranae* in relation to metal concentration in surrounding environment and to evaluate whether this species could be usefully employed in the bioindication by bioaccumulation methods. We evaluated the concentrations of Hg, Mn, Zn, Fe and Cu in water, bottom sediments and leaves of *H. morsus-ranae*.

2. MATERIAL AND METHODS

2.1. Study area

The investigations were made in the north part of Lower Silesia, near Wołów and Ścinawa (Fig. 1). The study sites were designated in 11 ponds where populations of *H. morsus-ranae* were particularly rich. Study sites were mainly oxbow lakes of the Odra River that are small, permanent water bodies characterized by significant fluctuations in water levels. These ponds are used for a variety of purposes, including recreation, livestock watering and fishing. Some sampling sites (1-7) were located in the wooded region rich in backwaters and swamps which create poor conditions for an intensive development of agriculture and therefore it has a relatively low concentration of farming activity. Other sampling sites (8-11) were located in an agricultural region [26].
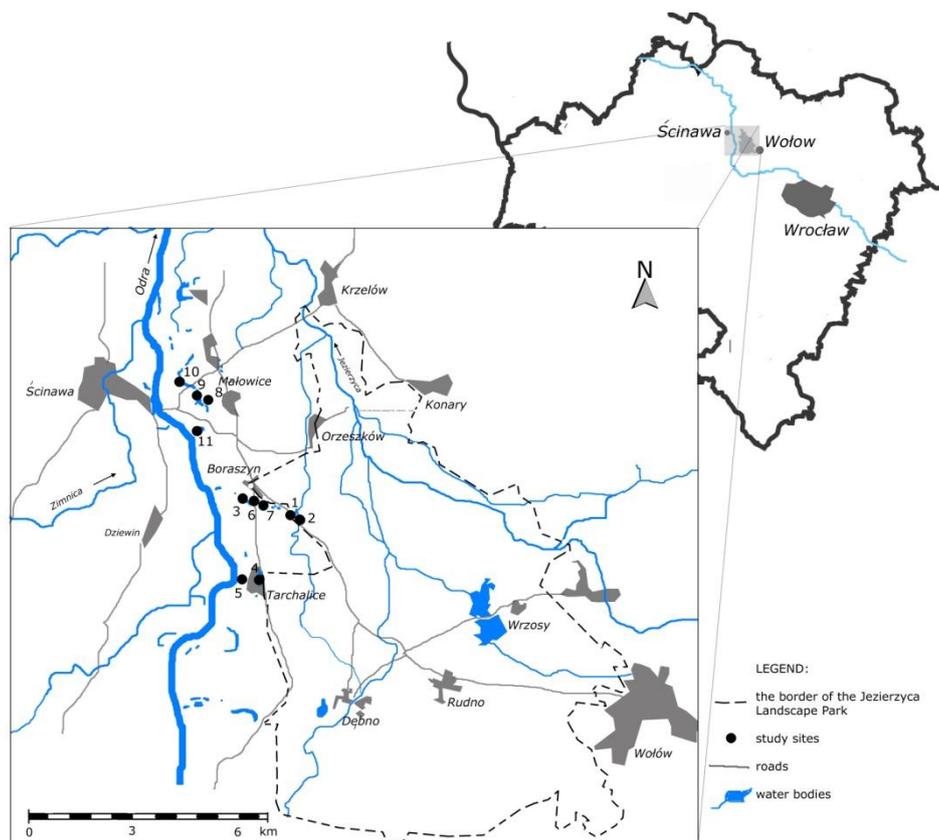

Fig. 1. Location of study sites

2.2. Sample collection

The sampling was conducted in the middle summer of 2012, at the peak of the *H. morsus ranae* growing season. Leaves of *H. morsus ranae* were taken simultaneously with water and bottom sediment samples. Three samples were randomly collected from 3 plots of 1 m^2 located in different parts of each reservoir.

The water samples were taken at depth of 0.5-1 m and they were kept in acid-cleaned polyethylene bottles. The bottom sediment samples were collected using the stainless steel collector at an average soil depth of 0-30 cm. The plant material was washed thoroughly using water from the sampling sites and was then cleaned of any adhering material. After sampling the plant and sediment material was placed in plastic bags, which were sealed to avoid extraneous contamination.

2.3. Sample analysis

Prior to analysis, the water samples were filtered using a Whatman glass microfiber filter (GF/C) and acidified with concentrated nitric acid (65%, pro analysis). The concentrations of Zn, Mn, Fe, Cu were determined by atomic absorption spectrometry with electro thermal atomization (AVANTA PM by GBC Scientific Equipment). Mercury concentrations were determined using Advanced Mercury Analyser (AMA 254). The bottom sediment samples were air dried, passed through a 2 mm diameter sieve and pulverized with a mortar and a pestle. The plant material was washed thoroughly in distilled water, dried at 50ºC to a constant weight and ground into a fine powder in a POLYMIX PX-MFC 90 D laboratory mill. Homogenized plant and sediment material (0.5 g) was subsequently digested in an open system with concentrated nitric acid (65%, pro analysis) and hydrogen peroxide (30%, pro analysis). The concentrations of Zn, Mn, Fe and Cu were determined by atomic absorption spectrometry with flame atomization (AVANTA PM by GBC Scientific Equipment). Mercury concentrations were determined using Advanced Mercury Analyser (AMA 254). The bottom sediments and water pH were determined with the pH meter.

The precision of the measurements was determined by comparing the results of trace metal content in the solutions made from two separate weighted portions of each sample, which had been analysed using identical methods. All elements were measured against standards (Atomic Absorption Standard Solution from Sigma Chemical Co.). Results for sediments and plants were calculated on a dry weight basis. Blank samples were digested and analysed in the same manner. The reproducibility of the methods was compared to the results of an inter-laboratory study through digesting and analysing reference materials INCT-

OBTL-5 (Oriental Basma Tobacco Leaves, Institute of Nuclear Chemistry and Technology Warszawa Poland) and GBW 07402 Anthropogenic Soil (Institute of Geophysical and Geochemical Exploration Lanfang China). Values were found to be 98±4 (percent ± standard deviation).

2.4. Statistical analysis

Statistical differences between study sites in terms of concentrations of elements in bottom sediments, water and plants were evaluated by one-way ANOVA on log-transformed data to obtain a normal distribution of features [29]. Pearson regression and correlation coefficients (n=33) were calculated to examine relationships between the concentrations of the elements in water and plant tissues [19]. The t test was used to determine differences of element concentrations between the group of study sites located in agricultural areas (8-11) and the group of study sites with low farming activity (1-7) [22]. Statistical confidence was set at p=0.05. To evaluate metal transfer from environment to plants, a bioaccumulation factor (BF) was calculated as a ratio of metal concentration in plant tissues to that dissolved in the surrounding water, as European frog-bit is free-floating plant. The value was calculated for each study site separately and then averaged [1, 25]. All statistical calculations were carried out using the CSS-StatisticaStatsoft[©] [21].

3. RESULTS AND DISCUSSION

The ranges of total metal contents in water, bottom sediment samples and leaves of *H. morsus ranae* examined in present study are summarized in Tab. 1 and 2. The mean concentrations of all elements, except of Hg, in water, bottom sediments and plants differed significantly between study sites (ANOVA, p=0.05).

The trace metal concentrations found in water and bottom sediments of examined ponds decreased in the order: Fe > Mn > Zn > Cu > Hg. The concentrations of metals studied in the water and the bottom sediments were mostly within the ranges typical of European background values [8, 12, 28, 30] (Tab. 1) which shows that oxbow lakes studied can be regarded as relatively free from the influence of anthropogenic pollution.

Table 1. Minimum/maximum values, mean±standard deviation (SD) of pH
and concentrations of metals in water and bottom sediments in the study sites

	Element	Min	Max	Mean±SD	Geochemical background
Water	pH	7.09	9.46	7.99	-
	Zn [µg dm^{-3}]	0.56	4.18	1.92±0.82	0.5-1.5[b]
	Mn [µg dm^{-3}]	BDL	151	25.9±33.7	20-60[a]
	Fe [µg dm^{-3}]	BDL	827	126±218	10-1400[a]
	Cu [µg dm^{-3}]	0.45	2.22	1.37±0.43	2[b]
	Hg [µg dm^{-3}]	BDL	0.4	0.1±0.1	0.2-1.2[a]
Bottom sediments	pH	1.72	6.92	3.70	-
	Zn [mg kg^{-1} d.w.]	2.67	320	25.4±57.1	35[d]
	Mn [mg kg^{-1} d.w.]	3.24	440	73.0±89.9	500[c]
	Fe [mg kg^{-1} d.w.]	138	7625	1847±2285	15 000[c]
	Cu [mg kg^{-1} d.w.]	BDL	59.6	10.1±13.8	9[d]
	Hg [mg kg^{-1} d.w.]	0.003	0.338	0.064±0.146	0.2[a]

[a] [12], [b] [8], [c] [28], [d] [30]
BDL- below detection level, (limits of detection: Cu 0.001 mg kg^{-1}; Mn 0.45 µg dm^{-3};
Fe 0.3 µg dm^{-3}; Hg 0.003 ng kg^{-1})

Tab. 2. Minimum/maximum values, mean±standard deviation (SD) of concentrations
of metals [mg kg^{-1} d.w.] in leaves of *Hydrocharis morsus-ranae* and bioaccumulation
factors (BF)

Element	Min	Max	Mean±SD	Geochemical Background	BF (mean±SD)
Zn	9.58	46.8	21.5±8.38	10-17[a]	12 984±6 706
Mn	120	6322	1979±1735	20-500[a]	248 597±337 309
Fe	102	4798	997±1216	50-200[b]	55 610±88 352
Cu	0.01	20.6	4.09±3.39	5-30[a]	3 499±3 495
Hg	0.0056	0.0305	0.011±0.005	0.02-0.5[a]	141±164

[a] [12], [b] [14]

The plant leaves differed from water and bottom sediments in respect of
examined elements concentration sequence, which was as follows: Mn > Fe >
Zn > Cu > Hg. The mean concentrations of Zn, Mn and Fe were found to be
relatively high and exceeded the physiological thresholds [12, 14]. The
concentrations of Mn and Fe in leaves from some study sites were found to be
within the toxic ranges for most plant species proposed by above-mentioned
authors (400-1000 mg kg^{-1} and 10-200 mg kg^{-1}, respectively). Deviations from

the natural contents in plant may indicate species that could be suitable for biomonitoring [17]. Compared to element concentrations in *H. morsus ranae* collected by other authors [2, 20, 30] in polluted regions, plants from area studied showed lower concentrations of Cu, Fe and Hg. However, concentrations of Mn and Zn were higher than noted in plants growing in contaminated water that were on average 816.78 ± 36.43 mg kg^{-1} for Mn and ranged from 0.48 to 22.19 mg kg^{-1} for Zn [2, 10].

The concentrations of Fe, Cu and Mn in leaves of *H. morsus ranae* were higher than the ranges published for some other floating macrophyte species. Iron concentrations in plants from some study sites exceeded the concentrations found in shoots of *Eichornia crassipes* collected from metal-contaminated water bodies [1, 7], and also in *Lemna* sp. [24]. Concentrations of Mn in leaves from most study sites were higher than reported for water hyacinth [1, 9] (148 and 681 mg kg^{-1}, respectively) but lower than in *Lemna* sp. and *Salvinia* sp. (2717 and 3000 mg kg^{-1}, respectively) [5, 24]. Whereas Cu concentrations were lower than in *Eichornia crassipes* [1, 9, 11] but higher than in *Salvinia* sp. collected from the area under intensive agricultural influence (3.83 mg kg^{-1}) [5].

Study sites surrounded by fields (8-11) had significantly higher Mn concentrations in water than study sites with low farming activity (t test, p=0.05). Fertilizers and plant protection products are considered to be a significant source Mn [12]. Whereas the plants collected from study sites 1-7, located in non-agricultural regions, showed significantly greater accumulation of Zn and Cu (t test, p=0.05). This may be related to the fact that some of these study sites (1, 2, 4, 6 and 7) are situated near roads with high vehicle traffic [26]. These results are in agreement with statement that Cu and Zn comes principally from motor oil additives, tires, brake liners, metal corrosion, pavement and motorway material [7].

The mean values of bioaccumulation factor in leaves of *H. morsus ranae* are presented in Tab. 2. The decreasing trend of BF was the same as trend of metals concentrations in leaves of species studied. Obtained values are higher than reported for freshwater vascular plants in uncontaminated water, which were: 52 857 for Mn, 2600 for Zn and 1128 for Cu [6]. Compared to *Eichhornia crassipes*, a floating macrophyte that has been shown to hyper accumulate trace elements [6], *H. morsus ranae* was characterized by higher factor of Cu, Mn and Zn accumulation. In water hyacinth growing in the drain from industrial wastes and agricultural runoff in Australia, BF amounted up to 3879.6 for Zn and 595 for Cu [11]. Also bioaccumulation factors for *Eichornia crassipes* growing in metal-contaminated coastal water, which were: 68 for Zn, 43 for Cu and 82 for Mn [1], were lower than for *H.morsus ranae* in our study. A high bioaccumulation factor for metals at low concentrations in surrounding

environment is important for phytoremediation [2, 11]. So species studied have a significant promise in the field.

Regarding trace metals correlation between plants and their environment, *H. morsus ranae,* as it is free-floating plant, is expected to show a relationship with the concentrations in water [6]. In our study, positive correlation expressed by Pearson's correlation ($p < 0.05$) was found between Zn (0.39), Fe (0.68) and Hg (0.57) concentrations in water and its accumulation in the leaves of species studied. Plants can be useful for ecological control when they show direct response to the environmental status [4].

4. CONCLUSIONS

Iron, Zn, Mn, Cu and Hg concentrations in water and bottom sediments at all studied locations were below the geochemical background, indicating no anthropogenic impact with respect to these metals.

Generally, the concentrations of Zn, Mn, Fe and Cu in leaves of *H. morsus ranae* exceeded the normal ranges stated in plants and reported for other free-floating macrophytes. Common frog-bit showed a high bioaccumulation factor for metals at low concentrations in surrounding environment. So species studied may be considered an accumulator of these metals. Plants growing near roads with high vehicle traffic show significantly greater accumulation of Zn and Cu than plants in agricultural regions.

The positive correlations between the content of metals in water and the level of these elements in the *H. morsus ranae* indicate that the plant is potentially useful for biomonitoring in general and for Zn, Fe and Hg in particular.

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AKUMULACJA METALI CIĘŻKICH W LIŚCIACH *HYDROCHARIS MORSUS-RANAE* L. I MOŻLIWOŚCI JEGO ZASTOSOWANIA W BIOMONITORINGU

S t r e s z c z e n i e

Rośliny wodne od lat znajdują zastosowanie w bioindykacji skażenia środowiska wodnego metalami śladowymi, ze względu na istniejącą zależność między zawartością pierwiastków w ich tkankach i w otaczającym je środowisku. Stopień kumulacji metalu zależy zarówno od rodzaju metalu, jak i od gatunku rośliny. Gatunek wskaźnikowy powinien być reprezentatywny dla obszaru badań, wszechobecny oraz łatwy w identyfikacji i zbieraniu, a także charakteryzować się wysoką tolerancją na metale i wysokimi współczynnikami ich kumulacji.

Celem badań była ocena zdolności do akumulacji metali ciężkich przez *Hydrocharis morsus-ranae* oraz określenie jego przydatności w bioindykacji. Pobrano próbki wody, osadów dennych oraz liści żabiścieku pływającego z 11 zbiorników wodnych okolic Ścinawy. W próbkach oznaczono zawartości Hg, Mn, Zn, Fe i Cu. Zawartości metali w wodzie, osadach i roślinach wykazały istotne statystycznie różnice pomiędzy stanowiskami. Stężenia metali w wodzie i osadach dennych były niższe niż tło biogeochemiczne, co wskazuje na brak antropogenicznego zanieczyszczenia tymi metalami. Wysokie stężenia metali oraz wysokie współczynniki akumulacji w roślinach, a także dodatnie korelacje między zawartościami Zn, Fe i Hg w liściach *H. morsus-ranae* i wodzie wskazują na możliwość wykorzystania tego gatunku w biomonitoringu. Rośliny rosnące w zbiorniach wodnych zlokalizowanych przy drogach o silnym natężeniu ruchu charakteryzują się znacznie wyższą zawartością Cu i Zn od roślin rosnących w rejonach rolniczych.

Słowa kluczowe: metale ciężkie, *Hydrocharis morsus ranae*, akumulacja
                   biomonitoring