OCCURRENCE OF HEAVY METALS IN AQUATIC MACROPHYTES COLONISING SMALL AQUATIC ECOSYSTEMS

WYSTĘPOWANIE METALI CIĘŻKICH W HYDROMAKROFITACH ZASIEDLAJĄCYCH MAŁE EKOSYSTEMY WODNE

Abstract: Concentration of heavy metals in plant tissues results from the total level of contamination of the ecosystem and the degree of development of a species, ecological form and phenophase. Some aquatic plants have a particularly great capacity to accumulate heavy metals and may be classified as hyperaccumulators. The studies of accumulation intensity of heavy metals by macrophytes in five water reservoirs situated in the General Dezydery Chlapowski Landscape Park indicated great variability of Al, Cu, Pb and Zn in both underground and aboveground plant organs. Most plants accumulated elements in surface layers of the rhizomes, mainly in the epidermis and in the cortex mesophyll. The greatest amounts of the analysed elements were found in rhizomes of Oenanthe aquatica (L.).

Keywords: water ecosystems, macrophyte, accumulation, heavy metals

In the recent decades natural element cycles are being influenced to an increasing degree by processes connected with anthropogenic activity. With the development of industry, agriculture, transport or mining a growing number of trace elements is activated. Plants absorb elements and chemical compounds most commonly in the ionic form from the soil solution, bottom deposits, water and air. Some plants (including aquatic macrophytes) together with micro- and macroelements absorb also considerable amounts of toxic ions, which are accumulated in tissues of roots, stems or leaves. Plants absorb ions mainly from the soil solution through roots via active transport at the expense of energy accumulated in ATP or via passive transport by facilitated diffusion [1]. Ion uptake depends on external environmental conditions, mainly on the type of substrate and its reaction and temperature, but also on salinity, which as a consequence may cause acidification or alkalinization of the medium (e.g. the introduction of KCl results in acidification of the substrate, since K\(^+\) ions may be more readily absorbed by plants, in case of the introduction
of NaNO₃, the NO₃⁻ ion is absorbed in greater amounts, while its ion exchanger is OH⁻ or HCO₃⁻, causing alkalization of the substrate).

Hydrophytes, ie aquatic plants, are specific ecological forms. These plants, in order to survive in the aquatic environment, had to adapt by modifying both their anatomical and morphological structure.

In most immersed species we observe an increased sorption surface of submerged leaves, a reduced mesophyll tissue or atrophy of the cuticle. All plants connected with the aquatic environment are characterized by the development of air spaces in all organs, which facilitate gas exchange with the surrounding environment [2].

Evolutionary modifications of organs in aquatic plants to a considerable degree influence uptake of heavy metal ions. Tissues of aquatic macrophytes exhibit a certain tolerance to elevated concentrations of heavy metals in environments exposed to the negative action of these elements [3]. Total concentration of heavy metals in plant tissues results from the total level of contamination of the ecosystem and the degree of development of a species, ecological form and phenophase. Some aquatic plants are characterised by slow growth and limited biomass; however, they have a particularly great capacity to accumulate heavy metals, thanks to which they may be classified as hyperaccumulators.

The aim of the presented study was to determine whether plants of aquatic ecosystems may accumulate ions of toxic metals (Al, Pb), as well as those serving physiologically important functions in plant organs (Zn, Cu). Moreover, investigations aimed at the determination of locations in the cross-sections of plant organs (both underground and aboveground), in which absorbed metal ions are accumulated.

**Experimental**

**Material and methods**

Analyses were conducted in 2009 in the Gen. D. Chlapowski Landscape Park. Concentrations of elements (Al, Cu, Zn and Pb) were determined in organs of macrophytes (*Polygonum hydropiper* (L.), *Glyceria fluitans* (L.) R. Br., *Carex acutiformis* Ehrh., *Phragmites australis* (Cav.) Trin. ex Steud., *Oenanthe aquatica* (L.)) overgrowing aquatic ecosystems differing in terms of their position in the landscape. Moreover, intensity of accumulation was analysed for 4 metals (Al, Cu, Pb and Zn) in cross-sections of leaves or stems and rhizomes of the above-mentioned plants. Plants were selected on the basis of the advantage of a given species in the composition of plants found in the analysed reservoir.

*Polygonum hydropiper* (L.) and *Glyceria fluitans* (L.) R. Br. were found to be predominant in reservoirs located in the Rabin-Blociszewo forest complex (Figs 1 and 2).

*Carex acutiformis* Ehrh. and *Phragmites australis* (Cav.) Trin. ex Steud. were characteristic of the reservoirs located in agricultural areas (Figs 3 and 4). The surroundings of reservoirs comprised mostly arable fields and agriculturally used meadows.

*Oenanthe aquatica* (L.) was found in greatest numbers in the reservoir located in the built-up area (the village of Luszkowo). The reservoir is located in a drainless hollow and it has an area of 432 m² (Fig. 5).
Fig. 1. A forest reservoir (1) with predominant *Polygonum hydropiper* (L.)

Fig. 2. A forest reservoir (2) with predominant *Glyceria fluitans* (L.)

Fig. 3. An agricultural reservoir (3) with predominant *Carex acutiformis* Ehrh.
Preparation of samples for analyses and applied methods

Plant material was collected from 5 reservoirs in the middle of the vegetation season for aquatic plants. Plants were collected from a plot with an area of approx. 3 m², characterised by a uniform substrate structure and the species composition. Plant material was prepared on site. After preliminary rinsing of plants with water from the reservoir in order to remove the bottom deposit fraction, plants were cut into fragments (leaves, stems and rhizomes) and cleaned with blotting paper. Leaves were collected from the middle part of plants, discarding the oldest and youngest organs. Also stems came from the middle part of plants. In case of the underground parts the thickest organs were collected. Plants were
harvested during the vegetation of aquatic plants. The material was transported to the laboratory in coolers at a temperature of 5°C.

In the laboratory the plant material was washed with distilled water. Such prepared samples were analysed whole using Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA ICP-MS) analysis of intensity of occurrence of heavy metals in the tested sample. It is one of the most advanced analytical techniques and has many advantages. It is possible to analyse solid samples thanks to the application of the laser evaporation of a solid sample (a plant organ). This technique consists in surface evaporation of a sample using a laser beam. The analyte in the gas form is transferred to ICP plasma using a stream of argon. Laser evaporation facilitates a detailed analysis of solid samples with no need to mineralize them, which reduces the time of analysis.

In turn, samples to be used in the analyses of concentrations of elements were dried on Petri dishes at room temperature until dry matter was obtained. After drying the plant material was ground in a laboratory jar mill. Such obtained homogenous samples were mineralized in a CEM Star 6 mineralizer by Varian. After incineration the produced solution was filtered on Peel Corner Millipore filters. Next 1 cm³ filtrate was collected and made up to 50 cm³ (dilution of 1:50). Such prepared solution was ready for ICP-MS analyses.

**Results of analyses**

**Concentrations and intensity of accumulation of selected elements in plants of water reservoirs**

In order to verify whether plants of the marsh zone may accumulate metals in their tissues the concentrations of 4 elements were measured both in underground and aboveground parts of plants, characteristic of the reservoirs, in September 2009 (most aquatic and marsh plants complete vegetation and potentially accumulate the highest amounts of ions and compounds in their tissues). Additionally, LA-ICP analyses were simultaneously performed in order to determine precise sites of concentration of selected metals at the cross-section of both underground and aboveground parts of plants (Figs 6, 8, 10, 12 and 14).

**Concentrations of analysed elements in *Polygonum hydropiper* (L.) in reservoir no. 1**

Markedly higher concentrations were found in the underground parts of *Polygonum hydropiper* (L.). This plant accumulated aluminium in highest amounts. In rhizomes its concentration was 1780 mg kg⁻¹ (Table 1), which was a high value, but having no effect of the development of plants within the reservoir. Among the analysed elements, both in rhizomes and in leaves, copper was found in smallest amounts.

When analysing the distribution of metals in marsh pepper smartweed it may be stated that in leaves such elements as zinc or lead were accumulated with the highest intensity, as it is evidenced by values of concentrations (Table 1). The location of the concentration for these metals was the main vein (high peaks in Figs 7b and 7d). The distribution of concentrations of all elements for rhizomes was similar (Figs 7 e-h); however, Al and Zn were absorbed with highest intensity and they were retained mainly in the outer areas of rhizomes.
Table 1

Concentrations of selected elements in organs of *Polygonum hydropiper* (L.)

<table>
<thead>
<tr>
<th><em>Polygonum hydropiper</em> (L.)</th>
<th>Al [mg kg(^{-1})]</th>
<th>Zn [mg kg(^{-1})]</th>
<th>Cu [mg kg(^{-1})]</th>
<th>Pb [mg kg(^{-1})]</th>
</tr>
</thead>
<tbody>
<tr>
<td>leaves</td>
<td>95.100</td>
<td>8.910</td>
<td>0.766</td>
<td>2.810</td>
</tr>
<tr>
<td>rhizomes</td>
<td>1780.000</td>
<td>31.000</td>
<td>6.900</td>
<td>14.800</td>
</tr>
</tbody>
</table>

Fig. 6. A cross-section of: A) a leaf, B) rhizomes of *Polygonum hydropiper* (L.)
Concentrations of analysed elements in *Glyceria fluitans* (L.) R. Br. in reservoir no. 2

*Glyceria fluitans* (L.) R. Br. was the plant found in greatest numbers in reservoir no. 2, observed in the area of the entire water body in considerable scattering. Analyses showed that this plant accumulated aluminium in highest amounts, particularly in its underground parts (Table 2). In rhizomes of floating manna grass 10-fold higher concentrations of zinc and copper were recorded in comparison with the concentrations found in leaves. In case of lead greater amounts were observed in leaves, which could have been caused by precipitation containing elevated amounts of this element.

Using laser ablation and ICP cross-sections of plant organs were performed (Fig. 8) and graphs of intensity of metal accumulation were prepared. As it results from graphs given below (Fig. 9), markedly higher concentrations were found in rhizomes, in which the distributions of zinc and copper were very similar (Figs 9f and 9g). In turn, aluminium was absorbed by rhizomes and accumulated in external sections of underground organs of plants, as evidenced by the distribution of intensity in time (Fig. 9e).

Table 2

<table>
<thead>
<tr>
<th><em>Glyceria fluitans</em> (L.) R. Br.</th>
<th>Al [mg kg⁻¹]</th>
<th>Zn [mg kg⁻¹]</th>
<th>Cu [mg kg⁻¹]</th>
<th>Pb [mg kg⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>leaves</td>
<td>34.400</td>
<td>5.310</td>
<td>0.724</td>
<td>9.790</td>
</tr>
<tr>
<td>rhizomes</td>
<td>1110.000</td>
<td>50.400</td>
<td>7.430</td>
<td>2.190</td>
</tr>
</tbody>
</table>
Fig. 8. A cross-section of: A) a leaf, B) rhizomes of *Glyceria fluitans* (L.) R. Br.
Concentrations of analysed elements in *Carex acutiformis* Ehrh. in reservoir no. 3

*Carex acutiformis* Ehrh., found within the littoral zone, formed compact patches, which covered 35% of the reservoir. Aboveground parts, i.e. leaves, and underground parts, i.e. rhizomes, were collected for analyses.

Lead was found at high concentrations in leaves of marsh sedge; the value of 30 mg kg\(^{-1}\) (Table 3) may have a toxic action on plants, depending on the species, variety and conditions in the habitat. In this case this value did not cause visible changes in plants, which may indicate an increased tolerance of marsh sedge to this element. In rhizomes of the analysed plants aluminium was found in highest amounts, with its concentration at 190 mg kg\(^{-1}\).

The distribution of concentrations of the elements in plants are most evident in Figure 11f, showing the intensity of zinc occurrence in rhizomes, with this element being accumulated at two locations at the cross-section. This distribution of copper in rhizomes is analogous to that in this cross-section; however, it is at much lower intensity (Fig. 11g). The distribution of aluminium, both in leaves and in rhizomes, is more uniform (Figs 11a and 11e), while for Zn, Cu and Pb in leaves and Pb in rhizomes it is difficult to determine the distribution of the analysed elements (Figs 11b-d and 11h).
Fig. 11. Intensity of accumulation: a)-d) Al, Zn, Cu and Pb in leaves; e)-h) Al, Zn, Cu and Pb in rhizomes of Carex acutiformis Ehrh.
Concentrations of selected elements in organs of *Carex acutiformis* Ehrh.

<table>
<thead>
<tr>
<th><em>Carex acutiformis</em> Ehrh.</th>
<th>Al [mg kg(^{-1})]</th>
<th>Zn [mg kg(^{-1})]</th>
<th>Cu [mg kg(^{-1})]</th>
<th>Pb [mg kg(^{-1})]</th>
</tr>
</thead>
<tbody>
<tr>
<td>leaves</td>
<td>13.500</td>
<td>9.560</td>
<td>1.160</td>
<td>31.200</td>
</tr>
<tr>
<td>rhizomes</td>
<td>190.000</td>
<td>15.900</td>
<td>2.450</td>
<td>0.598</td>
</tr>
</tbody>
</table>

**Concentrations of analysed elements in *Phragmites australis* (Cav.) Trin. ex Steud. in reservoir no. 4**

*Phragmites australis* (Cav.) Trin. ex Steud. was collected for chemical analyses from reservoir no. 4; this plant was found in the littoral zone as a predominant species and covered 82% area of the entire reservoir.

Similarly as marsh sedge, common reed accumulated lead in leaves in amounts of more than 30 mg kg\(^{-1}\), while in rhizomes only 0.18 mg kg\(^{-1}\) Pb were detected (Table 4). Much higher concentrations of the other elements were recorded in rhizomes.

In graphs showing the intensity of occurrence for all the analysed elements in leaves we may clearly see that their higher concentration was found in living tissues (darker colour - Fig. 12A), which is manifested in graphs given below (Figs 13a-d). Graphs of distributions for the elements in rhizomes show in detail in which locations aluminium, zinc and copper were accumulated (high peaks in Figs 13e-g), while slight concentrations of lead in rhizomes hinder its analysis (intensity of accumulation was very low, below 500 units).

**Table 4**

Concentrations of selected elements in organs of *Phragmites australis* (Cav.) Trin. ex Steud.

<table>
<thead>
<tr>
<th><em>Phragmites australis</em> (Cav.) Trin. ex Steud.</th>
<th>Al [mg kg(^{-1})]</th>
<th>Zn [mg kg(^{-1})]</th>
<th>Cu [mg kg(^{-1})]</th>
<th>Pb [mg kg(^{-1})]</th>
</tr>
</thead>
<tbody>
<tr>
<td>leaves</td>
<td>8.980</td>
<td>5.320</td>
<td>0.783</td>
<td>30.500</td>
</tr>
<tr>
<td>rhizomes</td>
<td>54.400</td>
<td>19.500</td>
<td>2.6900</td>
<td>0.180</td>
</tr>
</tbody>
</table>

Fig. 12. A cross-section of: A) a leaf, B) rhizomes of *Phragmites australis* (Cav.) Trin. ex Steud.
Concentrations of analysed elements in *Oenanthe aquatica* (L.) in reservoir no. 5

*Oenanthe aquatica* (L.) is a plant, which appeared in reservoir no. 5. In the structure of fine-leaved water dropwort the rhizome and the lower section of the stem are predominant,
characterised by a large diameter at the cross-section in comparison with the other organs of the plant. Due to the complex structure of leaves the stem was collected for analyses as the aboveground part, while the rhizome was collected as the underground part.

In rhizomes of this plant the highest concentrations of the elements were recorded from among all the plants found in the reservoirs. Aluminium concentration in this part of the plant exceeded 2.5 g kg$^{-1}$, also concentrations of zinc, copper and lead were high, amounting to 0.31, 0.098 and 0.091 g kg$^{-1}$ d.m., respectively (Table 5). Concentrations of metals in the stem of this plant were much lower, which shows that this plant accumulates mainly ions of the elements in the rhizome and transports them to the aboveground parts in slight amounts.

The distribution of metals, both in stems and rhizomes, is clearly manifested for Al, Zn and Cu. Distributions of these elements show in which locations in the stem and rhizomes the analysed elements are accumulated (Figs 15a-c and Figs 15e-g). We need to stress here similar distributions of copper and zinc, which may indicate similar mechanisms of uptake for these elements.

<table>
<thead>
<tr>
<th>$Oenanthe$ aquatica (L.)</th>
<th>Al [mg kg$^{-1}$]</th>
<th>Zn [mg kg$^{-1}$]</th>
<th>Cu [mg kg$^{-1}$]</th>
<th>Pb [mg kg$^{-1}$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>stems</td>
<td>4.480</td>
<td>3.950</td>
<td>0.479</td>
<td>7.000</td>
</tr>
<tr>
<td>rhizomes</td>
<td>2570.000</td>
<td>310.000</td>
<td>98.700</td>
<td>90.900</td>
</tr>
</tbody>
</table>

Fig. 14. A cross-section of: A) a stem, B) rhizomes of $Oenanthe$ aquatica (L.)
Analyses conducted in Poland and abroad confirm the accumulation capacity of hydrophytes. It results from analyses conducted on plants in the reservoirs of the city of Poznan that macrophytes, such as *Typha latifolia* (L.) and *Phragmites australis* Cav. Trin. ex Steud., may accumulate heavy metals (Cu, Pb, Cd, Zn), while Zn and Cu, as less mobile elements, were accumulated in greater amounts by the underground organs. An opposite situation was observed in case of Cd and Pb, where higher concentrations were recorded in aboveground parts of the analysed plants [4, 5]. Analyses conducted in the Leszno Lake District showed that leaves of *Phragmites australis* Cav. Trin. ex Steud. may accumulate Mn and Cr at concentrations exceeding physiological levels for most plants [6].

Analyses concerning bioaccumulation of heavy metals by aquatic plants are conducted in many parts of the world. In China eg concentrations of Cd, Pb, Cu, Zn and Mn were...
analysed in two species of pondweed (*Potamogeton* sp.) [7]. Water hyacinth (*Eichhornia crassipes*) was the object of investigations in India [8]. *Pistia stratiotes* (L.) was examined under hydroponic conditions in Nigeria [9], while plants from genera *Typha* sp. and *Persicaria* sp. were studied in Australia [10].

Analyses concerning concentrations of metal ions in aquatic macrophytes are conducted on a broad scale in Poland, focusing on the most common species, predominant in the littoral zone. In literature most frequently studies have investigated two macrophytes considered the best hyperaccumulators, ie *Phragmites australis* Cav. Trin. ex Steud. and *Typha* sp.

Very interesting results were presented by Cardwell et al [10], who analysed 15 species of aquatic and marsh plants overgrowing watercourses in Brisbane. They recorded much higher concentrations of heavy metals in rhizomes of plants in comparison with concentrations found in leaves mainly in case of *Typha domingensis* Pers. and *Schoenoplectus validus* (Vahl) A.&D. Löve. The concentration in rhizomes was so high that it exceeded values recorded in the adjacent bottom deposits. Such a situation was explained by the higher accumulation of ions of these elements by plants and the contamination of urban areas with these metals mainly by sewage discharges and leaching of metal ions from urban and industrial areas. The authors stated that the elements are accumulated in the epidermis and mesophyll of the cortex in greater amounts than in the pith of the mesophyll (a section of the central cylinder).

It results from the analyses conducted in the Gen. D. Chlapowski Landscape Park that most plants accumulate elements in surface layers of the rhizomes, mainly in the epidermis and in the cortex mesophyll. In leaves of *Phragmites australis* (Cav.) Trin. ex Steud the physiological level was exceeded for lead. Values in leaves were higher than those recorded for rhizomes, which may indicate that lead to a considerable degree was absorbed by leaves from dry or wet depositions. Definitely highest concentrations of zinc, copper and lead were found for rhizomes of *Oenanthe aquatica* (L.). Recorded concentrations of lead and copper fluctuated around the toxic content level, while zinc concentration exceeded the physiological level. This suggests that plants may accumulate in their underground parts considerable amounts of noxious elements, with no simultaneous inhibition of the basic physiological processes. Macrophytes may accumulate considerable amounts of toxic elements in their tissues (mainly in stolons and rhizomes) - with the exception of lead, which was accumulated primarily in leaves. As it was shown by the analyses conducted in different areas, aquatic and marsh plants may not only be good bioindicators of water cleanliness, but first of all be effective in the purification of surface waters and serve the function of the biogeochemical barrier.

**Conclusions**

1. Analysed plants turned out to be good accumulators of micropollutants (Al, Zn, Cu and Pb). The greatest amounts of the analysed elements were found in rhizomes of *Oenanthe aquatica* (L.).
2. Higher concentrations of Al, Zn and Cu were recorded in rhizomes of the analysed plants, while Pb predominated in aboveground parts of analysed macrophytes.
3. Marsh plants accumulate toxic ions of the elements mainly in the epidermal layer and the cortex mesophyll, as it is indicated by analyses conducted using one of the most
advanced analytical analyses, i.e, Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA ICP-MS).

4. Some aquatic macrophytes may accumulate in their organs considerable amounts of noxious elements, with no simultaneous inhibition of the basic physiological processes.

5. Aquatic and marsh plants, particularly *Oenanthe aquatica* (L.), may be used not only as bioaccumulators of pollutants, but first of all as an effective element in hydrophytic treatment plants.

Acknowledgements

This work was financially supported by grant N N 305 085635.

References


**WYSTĘPOWANIE METALI CIĘŻKICH W ORGANACH HYDROMAKROFITÓW ZASIEDLAJĄCYCH MAŁE EKOSYSTEMY WODNE NA TERENIE PARKU KRAJOBRAZOWEGO IM. GEN. D. CHŁAPOWSKIEGO**

Wydział Ogrodnictwa i Architektury Krajobrazu, Uniwersytet Przyrodniczy w Poznaniu

**Abstrakt:** Koncentracja metali ciężkich w tkankach roślin jest wypadkową ogólnego poziomu zanieczyszczenia ekosystemu i stopnia rozwoju gatunku, formy ekologicznej oraz fazy fenologicznej. Niewielkie ilości metali ciężkich, pełniący funkcję hiperakumulatorów. Badania nad intensywnością akumulacji metali ciężkich przez makrofity, zasiedlające pięć zbiorników wodnych na terenie Parku Krajobrazowego im. gen. Dezyderego Chłapowskiego, wykazały duże zróżnicowanie stężeń Al, Cu, Pb i Zn w częściach nadziemnych i podziemnych roślin. Większość roślin gromadziła pierwiastki w wierzchnich warstwach kłączka, głównie skórki, oraz w mięśniu kory pierwotnej. Największe ilości analizowanych pierwiastków stwierdzono w kłączu Oenanthe aquatica (L.).

**Słowa kluczowe:** ekosystemy wodne, makrofity, akumulacja, metale ciężkie