J. Elementol. 2011, 16(1): 31–42

IMPACT OF WATER POLLUTION ON ACCUMULATION OF MAGNESIUM AND CALCIUM BY *STRATIOTES ALOIDES* L.

Małgorzata Gałczyńska, Karolina Żakowiak, Katarzyna Bednarz

Department of General and Ecological Chemistry
West Pomeranian University of Technology in Szczecin

Abstract

Water soldier is a plant growing all across lowlands in Poland, in eutrophic reservoirs of still and slowly flowing water. Because this macrophyte is used as a fertilizer or a component in fodder for cattle or pigs, the purpose of this paper was to determine the content of magnesium and calcium in water soldier depending on pollution of water with selected heavy metals.

The study used water and plant samples collected from the natural environment and a hydroponic experiment (6 variants of water pollution with heavy metals and two terms of plants' exposure). Concentrations of magnesium and calcium in the mineralized samples were measured in three cycles with the atomic absorption spectrometry method on a spectrometer Solaar S AA. Water soldiers (from the hydroponic experiment) contained 5.911 g Mg kg^{-1} d.w. and 16.32 g Ca kg^{-1} d.w. It was determined that both addition of a heavy metal and the exposure time had a statistically significant effect on concentrations of magnesium and calcium in water soldier, as well as on their concentration in water. Except the control and regardless the exposure time, most magnesium remained in those water soldier specimens that had been exposed to iron and cadmium. The smallest amounts of magnesium in a plant were observed when water had been enriched with ions of zinc and cooper. After 6 weeks of exposing the plants to the metals, the content of magnesium was 12% lower than after 3 weeks. On the other hand, the smallest amount of calcium in water soldier was recorded for specimens exposed to iron and cadmium, while the largest amount was found in those grown with an addition of zinc and in the control group. The drop in calcium content measured on the second term, compared to the first one, was 6.119 g kg^{-1} d.w. Pollution of water with heavy metals has a negative effect on development of water soldier and on accumulation of calcium and magnesium. The reduced con-

dr inż. Małgorzata Gałczyńska, Department of General and Ecological Chemistry West Pomeranian University of Technology, Słowackiego 17, 71-434 Szczecin, e-mail: malgorzata.galczynska@zut.edu.pl

tent of calcium and magnesium in plants collected from waters polluted with heavy metals will affect the value of water soldier both as a fertilizer and a fodder additive.

Key words: magnesium, calcium, *Stratiotes aloides*, water.

WPŁYW ZANIECZYSZCZENIA WÓD NA AKUMULACJĘ MAGNEZU I WAPNIA PRZEZ *STRATIOTES ALOIDES* L.

Abstrakt

Osoka aloesowata występuje na całym niżu na terenie Polski w zeutrofizowanych zbiornikach wód stojących i powoli płynących. Ze względu na stosowanie tego makrofitu do użyźniania gleb i jako komponentu pasz dla bydła i świń celem pracy było określenie zawartości magnezu i wapnia w osoce aloesowatej w zależności od zanieczyszczenia wód wybranymi metalami ciężkimi.

W badaniach wykorzystano próbki wód i roślin pobrane ze środowiska naturalnego i z doświadczenia hydroponicznego (6 wariantów skażania wód i 2 czasy ekspozycji roślin na działanie jonów metali ciężkich). Pomiary zawartości magnezu i wapnia w zmineralizowanych próbkach wykonano techniką ASA w 3 powtórzeniach.

Wykorzystana w doświadczeniu hydroponicznym osoka aloesowata zawierała 5,911 g Mg kg^{-1} s.m. i 16,32 g Ca kg^{-1} s.m. Na podstawie analizy statystycznej ustalono, że zarówno dodatek metalu ciężkiego, jak i czas ekspozycji miały istotny statystycznie wpływ na zawartość Mg i Ca w osoce aloesowatej i stężenie tych pierwiastków w wodzie. Najwięcej Mg, poza kontrolą, niezależnie od czasu ekspozycji, pozostało w osobnikach osoki aloesowatej poddanych działaniu Fe i Cd. Najmniejsze ilości Mg w roślinie stwierdzono po dodatku do wody jonów Zn i Cu. Po 6 tygodniach ekspozycji roślin na działanie metali zawartość Mg była o 12% mniejsza niż po 3 tygodniach. Najmniejszą zawartość Ca w osoce aloesowatej zanotowano u osobników poddanych działaniu Fe i Cd, a największą po dodatku Zn i w kontroli. Spadek zawartości Ca w II terminie w stosunku do I wyniósł 6,119 g kg^{-1} s.m. Zanieczyszczenie wód metalami ciężkimi ma niekorzystny wpływ na rozwój osoki aloesowatej oraz kumulację Ca i Mg. Zmniejszona zawartość Ca i Mg w roślinach pobranych z zanieczyszczonych metalami ciężkimi wód obniży wartość zarówno uzyskanego nawozu, jak i dodatku paszowego.

Słowa kluczowe: magnez, wapń, *Stratiotes aloides,* woda.

INTRODUCTION

Pollution of surface waters with compounds of nitrogen, phosphorus and heavy metals affects the presence and development of different kinds of hydrophytes (SMOLDERS et al. 1996, 2000, 2003, MALEVA et al. 2004). Water soldier colonizes both eutrophic and mesotrophic reservoirs of still and slowly flowing water. Apart from Poland, where it occurs over its entire lowlands (SZMEJA 2006, TARKOWSKA-KUKURYK 2006), it is found in continental waters on the whole European territory of Russia, in the Caucasus, in Scandinavia, within the basins of the Baltic and Mediterranean Seas and the European part of the Atlantic Ocean basin, in Western Siberia and Northern Kazakhstan (ROELOFS 1991, EFREMOV, SVIRIDENKO 2008). In some European countries, water soldier is protected by law (SCULTHORPE 1967, BRAMMER 1979), while in the countries where this species covers large areas of water reservoirs (SUUTARI et al. 2009) it is used as a fertilizer and a component in fodder for cattle or pigs (COOK, URMI-KÖNIG 1983)

The purpose of this paper was to determine the content of magnesium and calcium in water soldier, depending on pollution of water with selected heavy metals.

MATERIAL AND METHODS

The samples of water and plants, used for chemical determinations, were collected from the natural environment and from a hydroponic experiment. In 2008, in the Drawskie Lake District Protected Landscape Area and in Świdwie Nature Reserve, nine habitats of water soldier were established, from which samples of water and plants were collected on 11 August. In the same year, a hydroponic experiment was run to grow water soldier in solutions of NPK with addition of heavy metals. On 20 June, water soldier specimens used for that experiment were collected from a ditch encircling Lake Świdwie and transported to a laboratory, where they underwent cleaning and selection procedures. Next, they were grouped into pairs *Stratiotes aloides* (a mature and a young specimen), which did not differ in their masses and represented similar development stages, and placed in 14 vases containing 8 liters of distilled water each. In order to satisfy nutritional needs of the plants, the vases were fed with solutions of biogenic compounds, namely: NH_4NO_3, K_2HPO_4, KNO_3. Concentration of NPK compounds was 0.92 mg NH_4^+ dm^{-3}, 2.2 mg PO_4^{3-} dm^{-3}, 2.76 mg NO_3^- dm^{-3} and 2.56 mg K^+ dm^{-3}. After one week, the water in the vases where the water soldier specimens were growing was supplemented with solutions of such heavy metals as: $CuCl_2 \cdot 2H_2O$, $ZnSO_4 \cdot 7H_2O$, $Pb(CH_3COO)_2 \cdot 3H_2O$, $MnSO_4 \cdot H_2O$, $FeCl_3$, $3CdSO_4 \cdot 8H_2O$. In the vases, the concentration of the heavy metals was: 12.3 mg Fe dm^{-3}, 6.08 mg Mn dm^{-3}, 12.1 mg Zn dm^{-3}, 0.6 mg Cu dm^{-3}, 3.04 mg Pb dm^{-3} and 0.05 mg Cd dm^{-3}. In the experiment, the control sample was a vase with two specimens of *Stratiotes aloides*, growing in a solution of biogenic compounds without any addition of heavy metals. The plants were exposed to the solution of heavy metals for 3 and 6 weeks (terms 1 and 2). For further tests, water and plant samples were collected from the vases on two dates, i.e. on 21 July and 11 August, and from the natural environment – on 11 August 2008. All the collected plant samples were dried up and ground. The water samples were subjected to mineralization in HNO_3, while the plant samples were mineralized in heat, in a mixture of the acids HNO_3 and $HClO_4$. The content of magnesium and calcium in the mineralized samples was measured with the atomic absorption spectrometry method on a spectrometer Solaar S AA in three cycles.

Additionally, in the water solutions where the *Stratiotes aloides* specimens had grown, both in the natural environment and the hydroponic experiment, the concentrations of N-NO_3, N-NH_4 and orthophosphates(V) were determined. To determine compounds of nitrogen and phosphorus in water, colorimetric methods were applied in compliance with the Polish standards. The measurements were made in a spectrophotometer Spekol 11. In the water samples, by means of electro-analytical methods, percentage of waterborne oxygen, pH and electrolytic conductivity were determined. For measuring physical qualities, an oxygen meter (a micro-processor unit HI 9145), a laboratory pH-meter (CP-411 with a temperature probe, for liquids, with an electrode EPS-1) and a conductometer (inoLab Coud 730 with an electrode TetraCon 325) were used.

The test results were processed statistically, based on two-factor ANOVA. Significance of differences between the mean values was determined with Tukey's test, at α=0.05.

RESULTS AND DISCUSSION

In polluted aquatic environments, water plants can absorb nutrients and heavy metals both from water and ground sediments. Most of the metals affect the microelements critical for maintenance of life of aquatic organisms and display toxic effects only when their concentration available to an organism exceeds the value necessary to satisfy its nutritional requirements. Copper, iron, zinc, manganese, cobalt and selenium are all very important for metabolism. On the other hand, exposure of aquatic organisms to high concentrations of the same elements may strongly affect their development. Other metals such as lead, cadmium and mercury, do not play any significant role in life cycles of organisms; nevertheless, they can cause damage to an organism's tissues if they are present in the environment in toxic concentrations. Reactions of plants to heavy metals depend on the individual sensitivity of each plant, intensity of the stress (duration, concentration) and the form the metal is available in. The toxic effect of absorption of the heavy metals results from their high concentration in cells. By making bonds with functional groups of proteins and polynucleotides, heavy metals upset functions of membranes in the photosynthetic and mitochondrial transport of electrons and inactivate many enzymes involved in regulating the basic cellular metabolism. As a result, these phenomena lead to reduction of the cell energy balance, disturb absorption of minerals, impair growth and development of plants, or even cause their death (GRUCA-KRÓLIKOWSKA, WACŁAWEK 2006).

In our hydroponic experiment, we observed that water soldier absorbed heavy metals. After three weeks of water soldier plants' exposure to heavy metals, a decrease in masses of the specimens in question, as a result of their negative influence on the plant development, was recorded. After another three weeks, decomposition of older leaves of the plants was seen analogously in the control group (Table 1).

Table 1

Percentage loss of mass of water soldier (%)

Addition of heavy metal	Loss mass (%)						
	control	Pb	Cd	Fe	Zn	Cu	Mn
I term	6.25	28.4	23.5	50.5	11.3	45.5	62.7
II term	54.2	40.6	52.2	49.9	59.1	68.3	68.9

In order to determine whether the decomposition of external leaves of water soldier had to be attributed to the heavy metals alone, comparative analysis was performed for natural habitat conditions of *Stratiotes aloides* and the conditions in the hydroponic experiment. The analysis was based on physical and chemical parameters of the water reservoirs where water soldier had grown (Table 2).

Table 2

Physicochemical parameters of water

Parameters		Natural reservoirs		Hydroponic experiment	
		mean	SD	mean	SD
Physical	pH	7.05	0.24	7.23	0.42
	electrolytic conductivity (μS cm^{-1})	503	208	258	106
	O_2 (%)	72.7	13.6	49.1	21.1
Chemical	N-NO_3 (mg dm^{-3})	0.099	0.014	0.216	0.084
	N-NH_4 (mg dm^{-3})	0.079	0.078	0.129	0.165
	PO_4^{3-} (mg dm^{-3})	0.466	0.631	0.106	0.065

The natural water pH was close to neutral, whereas a slightly higher pH value was determined in the hydroponic experiment (Table 3). According to the research conducted by RENMAN (1989), water soldier grows in waters with pH between 5.64 do 7.50. However, a study conducted by OBOLEWSKI et al. (2009) in the area of the River Łyna oxbow lakes showed that water soldier populated waters with pH from 7.6 to 8.49.

In their work, PINDEL and WOŹNIAK (1998) proved that *Stratiotes aloides* grew in waters where electrolytic conductivity equaled 240 µS cm^{-1} and was similar to the conditions in the hydroponic experiment. On the other hand, in the tested waters collected from the natural environment, the value of this parameter was more than double. Nevertheless, OBOLEWSKI et al. (2009) inform that in the area of the River Łyna oxbow lakes water soldier occurred in waters with electrolytic conductivity within the range from 364 to 471 µS cm^{-1}.

In the analyzed waters, collected from the natural environment, the content of water-borne oxygen was much higher than in the water from the hydroponic experiment. The drop in the value of this parameter proves consumption of oxygen in bio-chemical processes resulting from decomposition of dead leaves of water soldier.

In respect of its presence in plant tissues, nitrogen is the fourth most abundant element, after carbon, oxygen and hydrogen. Nitrogen participates in almost all bio-chemical reactions taking place in cells, and its shortage frequently hampers formation of new tissues. The main forms of N available to plants include the ions NO_3^- i NH_4^+ (SAKAKIBARA et al. 2006). According to TARKOWSKA-KUKURYK (2006), water soldier grows in waters where concentration of N-NO_3 remained within the range from 0.03 to 0.24 mg dm^{-3}, and N-NH_4 from 0.19 to 0.27 mg dm^{-3}. Similar concentrations of N-NO_3 and N-NH_4 were observed in waters collected from the natural environment, as well as from the hydroponic experiment.

Phosphorus is absorbed by plants generally in an oxidized form: $H_2PO_4^-$ or HPO_4^{2-}, and is a macronutrient playing a critical role in both catabolism and anabolism. It is also involved in the mechanism of transporting organic compounds and inorganic ions by cellular membranes. When there is a deficit of phosphorus, the intensity of various metabolic transformations, especially anabolic ones, slows down, leading to a slower growth of plants (SCHACHTMAN et al. 1998). According to the results obtained by OBOLEWSKI et al. (2009), *Stratiotes aloides* dwells in waters with even higher concentration of orthophosphates(V) (0.63-1.31 mg dm^{-3}) than we found in the water samples collected from natural reservoirs or particularly from the hydroponic experiment. The decreased concentration of orthophosphates(V) in the hydroponic experiment from 2.20 to 0.1 mg dm^{-3} may indicate that water soldier absorbs large quantities of phosphorus from the aquatic environment.

When comparing the *Stratiotes aloides* habitat conditions in its natural environment to those in the hydroponic experiment, one may conclude that a decrease in the mass and decomposition of older leaves of water soldier specimens resulted from the negative influence of heavy metals on the development of plants and, probably, from the shortage of phosphorus. Those factors led also to the accumulation of magnesium and calcium in the plants concerned (Table 3, Figure 1). Many researchers point to the fact that water soldier dwells in waters with high concentration of calcium. It has been

Table 3

Mean content of magnesium and calcium in water soldier and concentration of these elements in water depending on the addition of a heavy metal and the exposure time

Treatment		Content of elements in lant ($g\ kg^{-1}$ d.w.)		Concentration of elements in water ($mg\ dm^{-3}$)	
		Ca	Mg	Ca	Mg
Addition of heavy metal (D)	control	11.21	5.293	6.161	5.976
	Pb	3.50	4.915	4.781	3.634
	Cd	9.404	5.074	1.694	4.063
	Fe	1.831	5.628	0.114	1.367
	Zn	12.450	2.773	6.604	1.522
	Cu	8.271	3.050	7.088	1.402
	Mn	1.921	4.344	0.045	0.976
Exposure time (C)	I	10.00	4.712	4.039	3.319
	II	3.881	4.167	3.528	2.092
$LSD_{0.05}$	D	0.074	0.052	0.047	0.029
	C	0.214	0.152	0.137	0.083
	DC	0.349	0.248	0.224	0.135

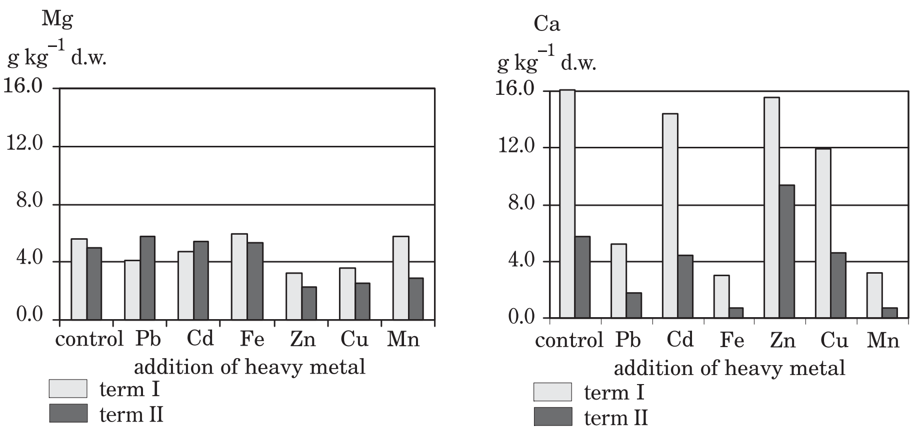

Fig. 1. Content of magnesium and calcium in water soldier from the hydroponic experiment

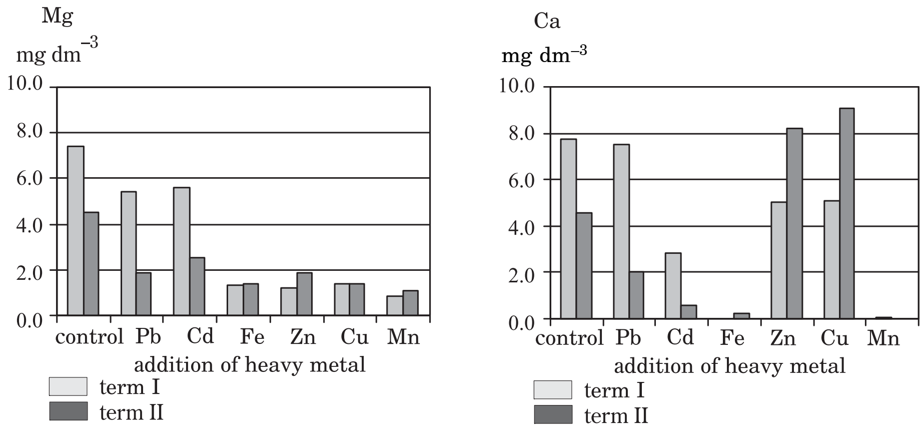

Fig. 2. Concentration of magnesium and calcium in water from hydroponic experiment

proven that calcium is accumulated by plants until September and during intensive photosynthesis the surplus of this element precipitates in the form of carbonates on surfaces of submerged leaves (COOK, URMI-KÖNIG 1983, BRAMMER, WETZEL 1984, RENMAN 1989, KRÓLIKOWSKA 1997, SZMEJA 2006). On average, the water soldier collected from the natural reservoirs accumulated 6.837 g Mg kg^{-1} d.w. and 23.28 g Ca kg^{-1} d.w. at a concentration of magnesium in water 1.756 mg dm^{-3} and calcium 52.42 mg dm^{-3}. According to the results obtained by OBOLEWSKI et al. (2009), water soldier grows in waters with a much higher concentration of ions of this element (8.90-12.90 mg Mg dm^{-3}). In plants, magnesium occurs in large quantities in chloroplasts and ribosomes. It is a very important element, which participates in activating enzymes involved in processes of photosynthetic and oxidative phosphorylation, glycolysis, the citric acid cycle, synthesis of multi-molecular building and spare materials and in transformation of nitric compounds. With the transpiration current, calcium is transported to the top of the plant, and because of its low mobility it is accumulated mainly in older leaves. Any surplus of calcium, which can have a negative effect, is excluded from metabolic transformations and bound with oxalic acid in the form of crystals. As a potassium antagonist, calcium causes lower elasticity of cellular walls and membranes, particularly in ageing cells (PASTERNAK et al. 2010).

The water soldiers grown in the hydroponic experiment contained 5.911 g Mg kg^{-1} d.w. and 16.32 g Ca kg^{-1} d.w. In the course of the tests, it was determined that both an addition of a heavy metal and the exposure time had a statistically significant effect on concentrations of magnesium and calcium in water soldier, as well as on their concentration in water (Table 3). Except the control and regardless the exposure time, most magnesium remained in those water soldier specimens that had been exposed to iron and cadmium. The smallest amounts of magnesium in a plant were observed where water had been enriched with ions of zinc and copper. After 6 weeks of exposing the plants to the metals, the content of magnesium was 12% lower than after 3 weeks. On the other hand, the smallest amount of calcium in water soldier was recorded in specimens exposed to iron and cadmium, while the largest amount was found in those with an addition of zinc and in the control group. The drop in calcium content on the second term, compared to the first term, was 6.119 g kg^{-1} d.w.

Apart from the control group, the highest content of calcium in the plant material was observed on the 1^{st} term in the variant enriched with zinc and cadmium, whereas the lowest was seen in the one supplemented with iron and manganese. On the 2^{nd} term, apart from the control group, the highest content was recorded for the copper and cadmium variant, while the lowest content of that element was observed in the variant with the addition of iron and manganese (0.667 g Ca kg^{-1} d.w. and 0.685 g Ca kg^{-1} d.w.). The values of the results obtained from the hydroponic experiment are much lower than those obtained from the analysis of the plant material collected from the natural environment. Accelerated by the presence of heavy metals, decomposition of older leaves of water soldier resulted in the fact that our determinations of calcium accumulation concerned generally younger leaves, which contained smaller quantities of this element.

As a result of the accelerated atrophy and decomposition of the oldest water soldier leaves, ions of calcium and magnesium appeared in the water solutions (Figure 2).

On the 2^{nd} term, a slight growth in the magnesium content was observed in the water supplemented with manganese and zinc, while a drop was seen in the control group and the lead and cadmium variant. On the 1^{st} term, concentrations of magnesium in water remained within the range from 0.860 to 7.432 mg Mg dm^{-3}, whereas on the 2^{nd} term, this range was from 1.097 to 4.513 mg Mg dm^{-3}. The results obtained from the hydroponic experiment exceed the limits stated by Obolewski et al. (2009), as well as those achieved in our own tests in the natural environment.

When analyzing calcium concentration in water samples, we found that it distinctly decreased on the 2^{nd} term of measuring this element both in the control group and in the variant enriched with zinc, copper and manganese, which can be a sign that calcium, freed from older leaves, is very well absorbed by younger ones. The results obtained from the vase experiment regarding the calcium content in water are much lower than those obtained from the water samples collected from the natural environment.

Because of the high content of biogenic elements, water soldier is sometimes used as a fertilizer or compost. In some countries, e.g. Finland, the plant is used as fodder for cattle and pigs. Calcium and magnesium accumulated by the plant are exceptionally valuable elements. Fertilizers enriched with these elements can contribute to reduction of soil acidification and, when introduced as an additive to the fodder, they can lower the risk of occurrence of such diseases as rickets, hypocalcaemia, hypomagnesaemia and allergies in cattle and pigs. Considering the increasing pollution of waters with heavy metals, one must admit that introduction of water soldiers to the nutritional chain of plants (soil fertilization) and animals (fodder additives) will not only contribute to economic utilization of *Stratiotes aloides*, but also to dispersion of heavy metals. However, a lower content of calcium and magnesium in the plants collected from waters polluted with heavy metals depresses the value of water soldier as a fertilizer or a fodder additive.

CONCLUSIONS

1. Pollution of water with heavy metals has a negative effect on development of water soldier and on accumulation of calcium and magnesium.

2. The reduced content of calcium and magnesium in plants collected from waters polluted with heavy metals will affect the value of water soldier both as a fertilizer and a fodder additive.

REFERENCES

BRAMMER E.S. 1979. *The littoral role of the water-soldier (Stratiotes aloides* L. Acta Univ. Ups., 540: 1-22.

BRAMMER E.S., WETZEL R.G. 1984. *Uptake and release of K^+, Na^+ and Ca^{2+} by the water soldier, Stratiotes aloides* L. Aquat. Bot., 19(1-2): 119-130.

COOK C.D.K., URMI-KÖNIG K. 1983. *A revision of genus Stratiotes (Hydrocharitaceae)*. Aquat. Bot., 16: 213-249.

EFREMOV A.N., SVIRIDENKO B.F. 2008. *The Ecobiomorph of water soldier Stratiotes aloides L. (Hydrocharitaceae) in the West Siberian part of its range*. Inl. Wat. Biol., 1(3): 225-230.

GRUCA-KRÓLIKOWSKA S., WACŁAWEK W. 2006. *Metale ciężkie w środowisku [Heavy metals in the environment]*. Zesz. Nauk., 1-2: 41-55. (in Polish)

HALL J.L. 2002. *Cellular mechanisms for heavy metal detoxification and tolerance*. J. Exp. Bot., 53:1-11.

KRÓLIKOWSKA J. 1997. *Eutrophication processes in shallow, macrophyte-dominated lake- species differentiation, biomass and contribution of submerged macrophytes in Lake Łuknajno (Poland)*. Hydrobiologia, 342: 411-416.

MALEVA M.G., NEKRASOWA G.F., BEZEL V.S. 2004. *The response of hydrophytes to environmental pollution with heavy metals.* Rus. J. Ecol., 35(4): 230-235.

OBOLEWSKI K., GLIŃSKA-LEWCZUK K., KOBUS SZ. 2009. *Próba oceny wpływu jakości wód na strukturę jakościowo-ilościową epifauny zasiedlającej Stratiotes aliodes* L. *na przykładzie starorzecza Łyny [An attempt at assessing the effect of water quality on the qualitative and quantitative structure of epifauna colonizing Stratiotes aliodes* L., *a case study of oxbow lakes in the Łyna River channel].* J. Elementol., 19(1): 119-134. (in Polish)

PASTERNAK K., KOCOT J., HORECKA A. 2010. *Biochemia magnezu [Biochemistry of magnesium].* J. Elementol., 15(3): 601-616. (in Polish)

PINDEL Z., WOŹNIAK Ł. 1998. *Naturalne warunki występowania wybranych ozdobnych gatunków roślin wodnych i bagiennych [Natural conditions for presence of some ornamental water and peatbog plants].* Fol. Univ. Agric. Stetin. 187, Agricultura, 70: 83-87. (in Polish)

PN-82/C-04576/08. *Woda i ścieki. Badania zawartości związków azotu. Oznaczanie azotu azotanowego metodą kolorymetryczną salicylanem sodowym [Water and wastewater. Determination of content of nitrogen compounds. Determination of nitrate nitrogen by colorimetry with sodium salicylate].* (in Polish)

PN-89/C-04537/02. *Woda i ścieki. Oznaczenie rozpuszczonych ortofosforanów kolorymetryczną metodą molibdenianową z chlorkiem cynowym jako reduktorem [Water and wastewater. Determination of dissolved orthophopshates with the molybdenum colorimetric method using tin chloride as a reducing agent].* (in Polish).

PN-ISO 7150-1/2002. *Oznaczenie azotu amonowego. Manualna metoda spektrometryczna [Determination of ammonia nitrogen. The manual spectrophotometric method].* (in Polish)

RENMAN G. 1989. *Life histories of two clonal populations of Stratiotes aloides* L. Hydrobiologia, 185: 211-222.

ROELOFS J.G.M. 1991. *Inlet of alkaline river water into peaty lowlands: effects on water quality and Stratiotes aloides* L. *stands*. Aquat. Bot., 39: 267-293.

SAKAKIBARA H, TAKEI K., HIROSE N. 2006. *Interactions between nitrogen and cytokinin in the regulation of metabolism and development.* Trends Plant Sci., 11(9): 440-448.

SCHACHTMAN D.P., REID R. J., AYLING S.M. 1998. *Phosphorus uptake by plants: from soil to cell.* Plant Physiol., 116: 447-453.

SCULTHORPE C.D. 1967. *The biology of aquatic vascular plants*. Edward Arnold Ltd, London.

SMOLDERS A.J.P., LAMERS L.P.M., DEN HARTOG C., ROELOFS J.G. M. 2003. *Mechanisms involved in the decline of Stratiotes aloides* L. *in the Netherlands. sulphate as a key variable*. Hydrobiologia, 506(1): 603-610.

SMOLDERS A.J.P., ROELOFS J.G.M., DEN HARTOG C. 1996. *Possible causes for the decline of the water soldier (Stratiotes aloides* L.*) in the Netherlands*, Arch. Hydrobiol., 136: 327-342.

SMOLDERS A.J.P., VAN RIEL M.C., ROELOFS J.G.M. 2000. *Accumulation of free amino acids as an early indication for physiological stress (nitrogen overload) due to elevated ammonium levels in vital Stratiotes aloides* L. stands. Arch. Hydrobiol., 150: 169-175.

SUUTARI E., SALMELA J., PAASIVIRTA L., RANTALA M.J., TYNKKYNEN K., LUOJUMKI M., SUHONEN J. 2009. *Macroarthropod species richness and conservation priorities in Stratiotes aloides* (L.) *lakes*. J. Insect. Conserv., 13: 413-419.

SZMEJA J. 2006. *Przewodnik do badań roślinności wodnej [A guidebook for studying aquatic plants].* Wyd. UG, Gdańsk. (in Polish)

TARKOWSKA-KUKURYK M. 2006. *Water soldier Stratiotes aloides* L. *(Hydrocharitaceae) as a substratum for macroinvertebrates in a shallow eutrophic lake*. Pol. J. Ecol., 54: 441-451.