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Zinc accumulation by two species of *Chara* (Charophyta)

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Abstract — This paper reports the results of experiments on Zn accumulation in two species of green algae: *Chara globularis* and *Chara hispida*. The results of laboratory and field experiments show a rapid accumulation of Zn by charophytes and demonstrate that with the exception of precipitation with calcite, an adsorption phase occurs in Zn accumulation. Both species may be important in Zn circulation in lakes.

***Chara globularis* / *Chara hispida* / Charophytes / freshwater algae / trace metals / Zinc / accumulation / Poland**

Résumé — Accumulation en zinc chez deux espèces de *Chara* (Charophyta). Cette note rend compte des résultats expérimentaux entrepris sur l'accumulation en Zn chez deux espèces d'algues vertes : *Chara globularis* et *Chara hispida.* Les résultats des expériences de laboratoire et de terrain font apparaître une rapide accumulation en Zn par les charophytes et montrent qu'en dehors de la précipitation de la calcite, il existe une phase d'adsorption avec accumulation de Zn. Les deux espèces ont certainement une grande importance dans le flux de circulation du Zn dans les lacs.

***Chara globularis* / *Chara hispida* / Charophytes / algues d'eau douce / métaux trace / Zinc / accumulation / Pologne**

INTRODUCTION

Charophytes (green algae) are one of the submerged macrophyte groups that dominate the vegetation of calcium-rich oligotrophic and moderately eutrophic lakes (Forsberg, 1965; Hutchinson, 1975; Blindow, 1991). In many European lakes, charophytes have a large biomass, forming dense carpets (*Chara* meadows) throughout the year, and are distinguished by their very high calcium content (Hutchinson, 1975; Krause, 1997), which allows them to store large amounts of various elements. In relation to nutrients, they act as a phosphorus sink in lakes in the process of co-precipitation of P with calcite (Blindow, 1991; Hillbricht-Ilkowska, 1989; Kufel & Ozimek, 1994; Murphy *et al.*, 1983). Riemer & Toth (1968) and Lawrence (1971) pointed to the importance of charophytes not only for retaining nutrients but also for the precipitation of trace metals.

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However, in contrast to nutrients, only a few studies have been carried out on the accumulation of trace metals in charophytes, and there are no detailed studies on the rates of accumulation of Zn.

This paper analyses the possible way and time of Zn accumulation in two significantly different species of *Chara*, *Chara hispida* L. and *Chara globularis* Thuillier, based on field and laboratory experiments.

MATERIALS AND METHODS

C. globularis is a small species with a main axis diameter of about 0.5-1 mm, and *C. hispida* is larger (main axis diameter of 1-4 mm) up to 40-50 cm long (Blindow, 1991).

Plant samples were collected from the Lake Blizienko in the northeastern part of Poland, Masurian Lakeland. In all experiments shoot tips (distal 10 cm) were used. Plants were rinsed with lake water and quickly transported to the laboratory. Each assay of 35 g fresh-weight shoot tips (10 cm long) was placed in a glass tank containing 1 litre of lake water plus the precise volume of experimental solution, then incubated in laboratory at 18 ^{o}C for 120 hours under irradiance (144 $\mu mol/m^2/s^{-1}$, day : night 16 : 8 h). Both species of *Chara* were grown at four Zn concentrations in five replicates: 0.3, 0.5, 0.7, 1.0 (mg Zn/l), along with control samples. All solutions were made up on the initial day of the experiment from a 1000 mg/l stock solution.

Field experiments were conducted in the lake in transparent plastic tubes with a diameter of 16 cm for *C. hispida* and of 6 cm for *C. globularis*. Zinc levels and experimental designs were the same as in the laboratory experiment. The water level in tanks was identical to that in the lake. Plants were incubated under natural conditions for 72 hours in the middle of the summer of 2002.

After the experiments (72 and 120 hours), the tested plants were collected, dried at 60 °C and homogenized with 6 ml of a mixture of HNO_3 and H_2O_2 (2:1) using a defined time/program in a CEM microwave. The samples were filtered, and the Zn content was determined using an Avanta Sigma-GBC atomic absorption spectrophotometer. The level of Zn in water was determined by analysis at the start of each experiment and in adequate periods of time after 2, 48, 72 hours in the field experiment, and additionally, after 96 and 120 hours in the laboratory. Water was analyzed after filtration with an AAS graphite furnace. Water samples from Lake Blizienko were collected from the site for physicochemical analysis by standard methods. Temperature, pH and conductivity were determined *in situ*. All analyses were performed in duplicates. Additionally, several samples of the species investigated were taken to determine the "natural" content of Zn. The results were tested by ANOVA and Student t-test ($t_{0.05}$), using the Statistica program, to determine if differences observed through exposure periods were significant.

The concentration of elements inside the internal wall of internodes was determined by scanning electron microscopy (SEM, Hitachi). The accelerating voltage for the analysis was 20 KeV.

RESULTS

Table 1 summarizes the chemical composition of the lake water. Table 2 shows the changes in Zn concentration after the experiments. The values given are the averages of the experiments, carried out in the laboratory or in the field. The concentration of Zn in the water of the experiments showed large differences between the two species, however, the concentration of Zn in the water during the 120- and 72-hour experiments also decreased significantly for both species (Tab. 2).

In the case of *C. globularis,* Zn content in the water decreased to 13.2-29.2% of the initial concentration, after 120 hours of laboratory experimentation (Table 2). This species accumulated Zn in the range of 70.8-86.8%. In the field experiments with *C. globularis*, aqueous Zn decreased significantly to 3.8-6.3% of the initial Zn content in the water and the rates of Zn accumulation were between 93.7 and 96.2%.

For *C. hispida,* the experiment shows a change of Zn of 26.0-34.7% of the initial concentration during the 120-hour run. This species accumulated 65.3-74.0% of initial Zn from water in the laboratory experiments. During the field experiments of *C. hispida*, aqueous Zn decreased significantly from 16.4-26.5% of initial Zn content in water (Zn accumulation was 73.5-83.6%).

Changes in the concentration of Zn in the water by accumulation of both species of *Chara* are shown in Table 2, Figs 1 and 2 (initial concentrations 1.0 mg Zn/l). It is evident (Figs 1, 2) that there is a difference in the rate of accumulation of Zn from water. Accumulation of Zinc by both *Chara* species was more rapid in the field experiments than in the laboratory. In *C. globularis*, aqueous Zn decreased significantly during the first two hours in the field experiment (Fig. 1), but in the laboratory the same level of aqueous Zn was reached only after 48 hours of experiment. Similar processes were observed in *C. hispida* (Fig. 2).

The concentrations of Zn retained in the water at the end of the experiments are shown for *C. globularis* (Fig. 3a, b) and for *C. hispida* (Fig. 3c, d).

Table 1. Lake Blizienko water chemistry data for the period May-September 2002.

Variable	*Mean*	*Range*
Temp. (°C)	19.4	15.0-23.5
pH	8.0	7.7-8.3
Conductivity (μS cm^{-1})	333	280-369
PO_4^{3-} (mg l^{-1})	0.22	0.18-0.28
Ca^{2+} (mg l^{-1})	24.0	7.9-34.2
Na^{+} (mg l^{-1})	3.5	1.7-5.6
NH_4^{+} (mg l^{-1})	0.47	0.26-0.64
NO_3^{-} (mg l^{-1})	0.28	0.17-0.39
NO_2^{-} (mg l^{-1})	0.005	0.001-0.009
K^{+} (mg l^{-1})	1.06	0.43-1.3
Mg^{2+} (mg l^{-1})	7.6	5.7-9.3
Cl^{-} (mg l^{-1})	37.9	32.5-43.7
SO_4^{2-} (mg l^{-1})	33.4	22.7-52.2
Zn^{2+} (μg l^{-1})	<0.1	

Table 2. Changes in Zn concentration in water after the experiments (120 hours of laboratory and 72 of field experiments).

Initial Zn concentration		*Laboratory experiments*				*Field experiments*			
mg/l	*- %*	*Chara globularis* %	*Chara hispida* %	$t_{est.}$	$t_{0.05}$	*Chara globularis* %	*Chara hispida* %	$t_{est.}$	$t_{0.05}$
0.3	100	14.5	30.1	635.2	2.77	5.8	21.8	569.3	2.77
0.5	100	29.2	26.0	523.1		4.8	16.4	226.3	
0.7	100	13.2	34.7	112.1		3.8	26.5	562.1	
1.0	100	23.8	27.9	895.2		6.3	19.1	1012.6	
LSD		0.014	0.074			0.047	0.064		
$F_{est.}$		523411	62354			2335620	56884		
$F_{0.05}$		4.06				4.06			

LSD – the least significant difference
$F_{est.}$ – F estimated
$t_{est.}$ – t estimated

Table 3. Comparison of Zn content in *Chara globularis* and *Chara hispida* (mg/kg d. w.) after laboratory and field experiments. $t_{est.}$ = t estimated

Initial Zn concentration mg/l	*Laboratory experiments*				*Field experiments*			
	C. globularis	*C. hispida*	$t_{est.}$	$t_{0.05}$	*C. globularis*	*C. hispida*	$t_{est.}$	$t_{0.05}$
0.0	0.12	0.13			0.12	0.13		
0.3	2.7	2.2	10.0	2.22	2.2	1.9	2.0	2.22
0.5	3.0	3.7	13.6		3.7	2.0	10.7	
0.7	4.1	4.0	21.7		4.0	2.6	9.2	
1.0	4.6	4.4	18.3		4.4	2.8	9.5	

C. globularis has accumulated twice as much Zn from water (laboratory experiments) than *C. hispida*. Similar results were obtained in field experiments.

Fifty samples of whole plants were analyzed and the results are presented in Table 3. Generally the whole plant had higher metal content than control samples, and almost all differences between the species used in the experiments were significant.

Analyses of the Zn content from extant plants under natural conditions (as collected from the water-body), contained 0.1-0.3 mg Zn/kg for *C. globularis* and 0.1-0.2 mg Zn/kg for *C. hispida*.

DISCUSSION

The results of these experiments show that the charophytes *C. globularis* and *C. hispida* can rapidly respond to inputs of Zn in the environment. However, the accumulation of aqueous Zn is different depending if the experiment is carried on in the field or in the laboratory (Figs 1, 2). These differences could be a

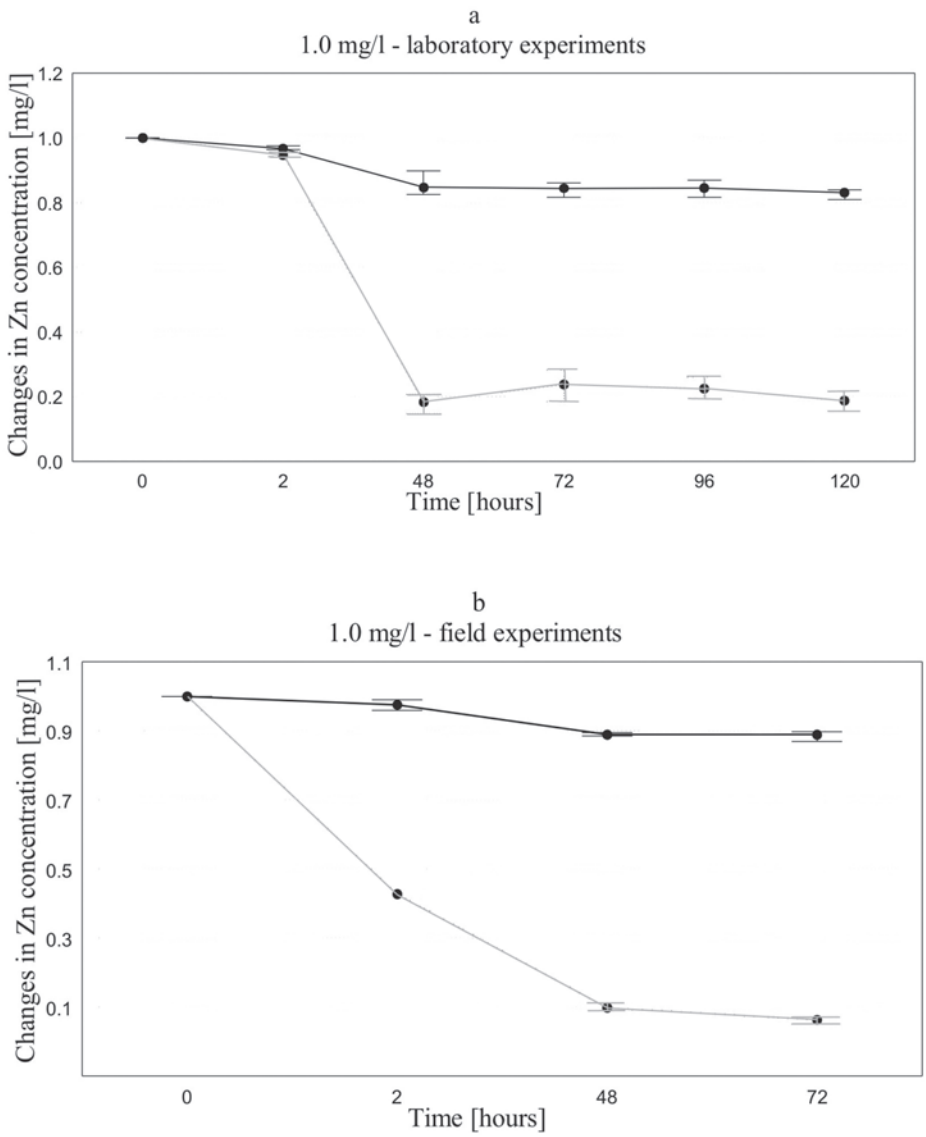

Fig. 1. Changes in Zn concentration in water (mg/l) – *Chara globularis* – in the field (a) and laboratory (b). Straight line = control, gray line = experiments.

response to the use of intact fresh plants for the field studies, instead of the cut shoot tips used in the laboratory.

These results seems to confirm Riemer & Toth's (1968) observation of high amounts of zinc in *Chara* sp. with very high calcium content, namely 95 and 125 mg Zn/kg. However, other *Chara* species investigated by the authors had only 25 and 28.5 mg Zn/kg.

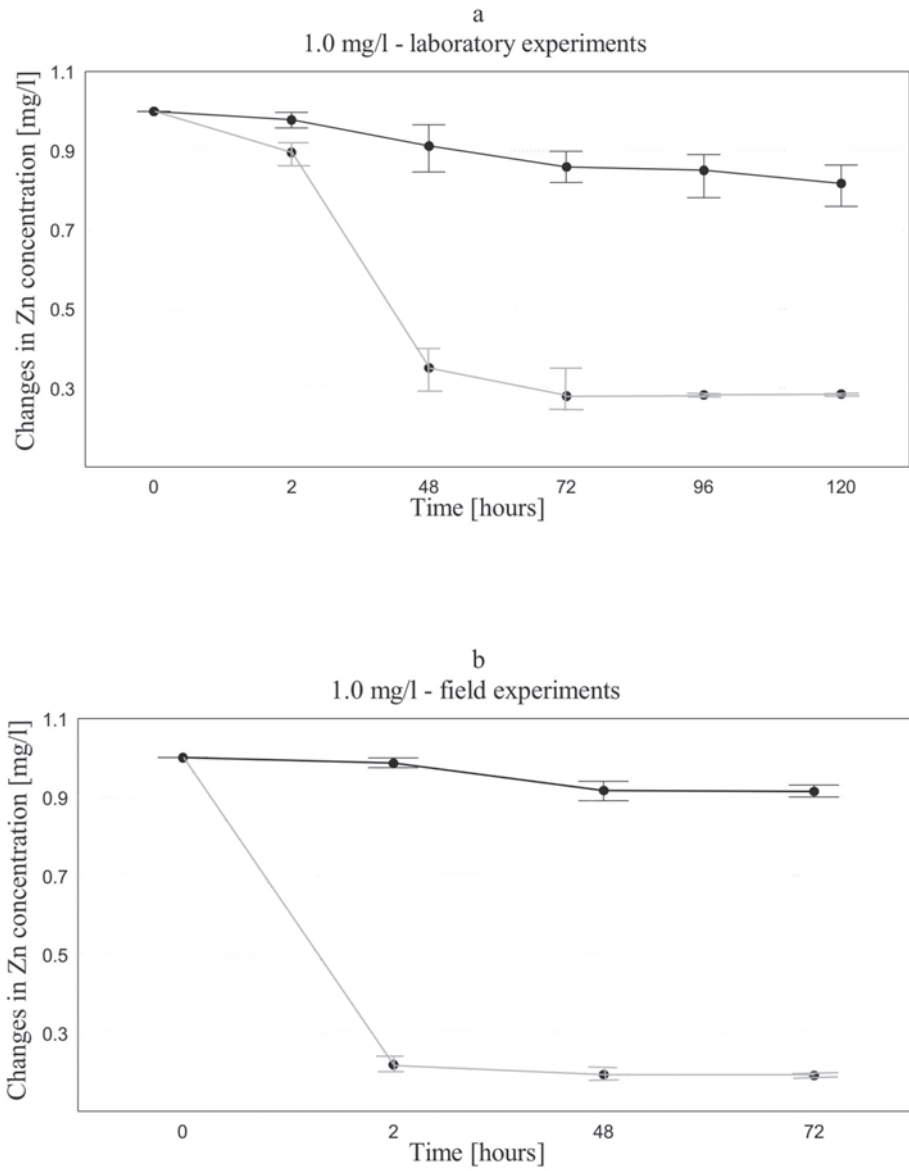

Fig. 2. Changes in Zn concentration in water (mg/l) – *Chara hispida* – in the field (a) and laboratory (b). Straight line = control, gray line = experiments.

According to Riemer & Toth (1968), Zn can be easily precipitated along with calcium forming the calcareous encrustation on the plant surface, as shown by Otsuki & Wetzel (1972). This seems to be true, particularly in waters with a high content of calcium. Zinc can be found as $ZnCO_3$ and $ZnSO_4$ in water rich in $CaCO_3$ or SO_4, these compounds depending on water pH value. When the pH is

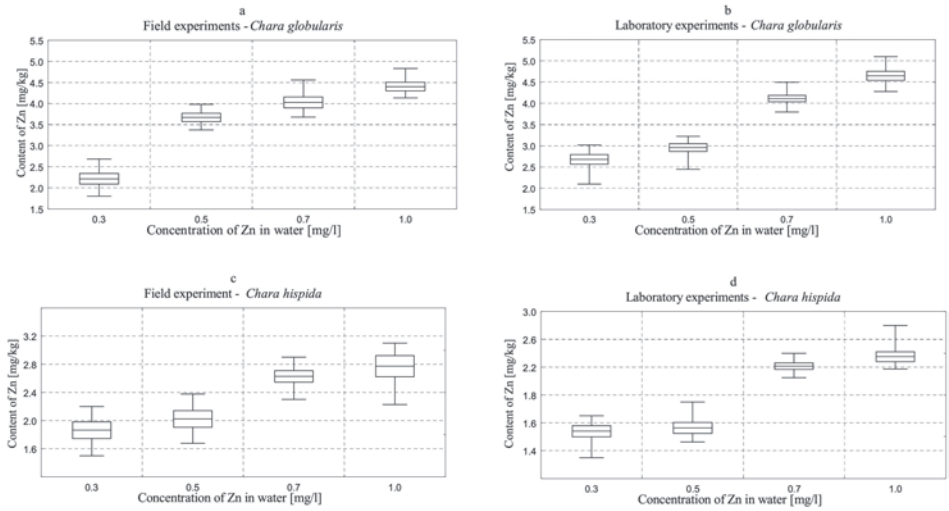

Fig. 3. Comparison of Zn content in *C. globularis* (a, b) and *C. hispida* (c, d) (mg/kg d. w.) at the end of laboratory and field experiments.

between 7.0 and 7.5, zinc salts are hydrolyzed and when the pH rises to 8.0, $Zn(OH)_2$ can be easily deposited on the plant surface (Dojlido, 1995). However, the mechanism of precipitation cannot be the only way of trace metals accumulation by charophytes.

According to Beaugelin-Seiller *et al.* (1995), there exists the possibility that two successive phases can occur in the accumulation of trace metals by all aquatic plants. They are: surface adsorption on the calcium carbonate deposit and biological absorption. In other words, the whole accumulation process can be summarized as a fast initial adsorption phase (first phase) followed by a slower biological absorption phase (second phase) which is generally longer and slower. The importance and mechanism of the adsorption phase for algae such as biological absorption was described in detail for ^{65}Zn, ^{60}Co and ^{110}Ag by Baudin (1974) and Garnier & Baudin (1989).

Surface adsorption

The experiments showed that the concentration curve reached a constant value in the adsorption phase (Figs 1, 2), which is expected if surface adsorption occurs, in agreement with previous authors who indicated adsorption as one of the mechanisms responsible for trace metals accumulation. This seems to be contradicted by the results of experiments, especially those made *in situ,* during which in the first 2 hours a short intense Zn accumulation was observed on *C. hispida*. In the laboratory, the proposed adsorption phase was much longer and slower. After 2 days of exposure, the Zn accumulation in the adsorption phase was estimated at 65-80% (laboratory experiments) with both species, and in comparisons after 2 hours approximately 80% in the field experiments with *C. hispida*.

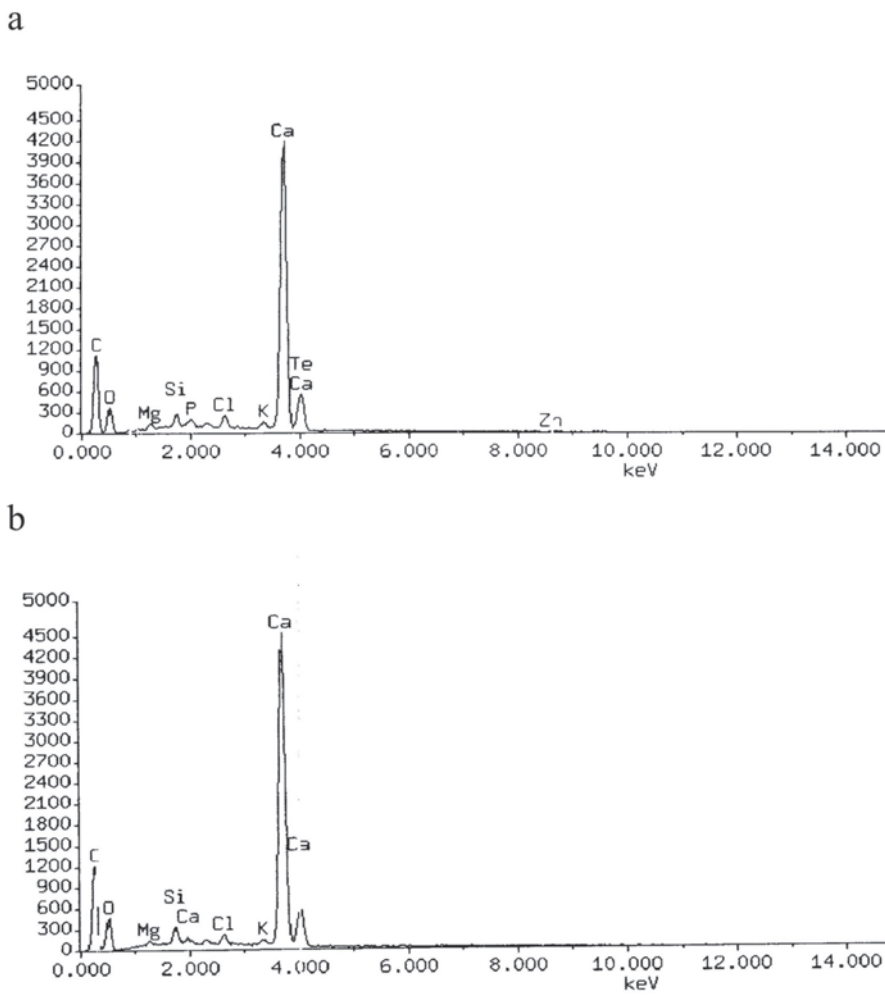

Fig. 4. SEM microanalysis spectra. Analysis of elements content in the internal wall of internodes of *Chara globularis* (a) and *Chara hispida* (b).

Biological absorption

Beaugelin-Seiller *et al.* (1995), postulated biological absorption as the mechanism responsible for trace metal accumulation in charophytes, in which case, a decrease in Zn content in the water should take place longer and slower than the in the first phase, but this part of accumulation is not clear in the laboratory experiments. In the case of *C. globularis* and *C. hispida*, the concentration of aqueous Zn was very low in laboratory experiments during the second day (Figs 1, 2). Similarly, in the field experiments with *C. globularis,* the duration of the second phase is not clear. The process seems to have started after the 48 hours, and similarly the Zn content was low. The possible explanation is that during the proposed first phase and during precipitation, almost all Zn has

been accumulated. It seems to be confirmed by the analysis of the internal wall of the internodes by SEM microanalysis (Fig. 4). It showed almost no presence of Zn content inside the investigated plants.

The proposed accumulation phase — the so called "biological absorption" — has not been observed during the experiments. Therefore, it can be concluded that charophytes accumulated Zn by two ways: precipitation and adsorption on the plant surface. Both processes can proceed simultaneously, but more studies are needed for a better understanding of the process of accumulation.

In light of the foregoing discussion, it seems that rapid accumulation of Zn on the plants can be an important factor in Zn circulation in lakes. Similar processes were described by Blindow (1992) and Scheffer *et al.* (1993) for nutrients. Owing to their high biomass, charophytes could store larger amounts of Zn. Large dense carpets of charophytes found in lakes are able to store aqueous Zn in greater amounts per lake area than other algae species e. g. *Mougeotia* or *Cladophora* or higher plants.

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