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Manganese accumulation by two species of *Chara*

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Abstract

This paper reports the results of Mn accumulation in two species of green algae: *Chara globularis* and *Chara hispida*. The results of laboratory and field experiments show a rapid accumulation of Mn by charophytes and demonstrate that deposit formation on the plant surface in the so-called adsorption phase occurs in Mn accumulation. Both species can be an important factor in Mn circulation in lakes.

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INTRODUCTION

Charophytes are one of the submerged macrophyte group of green algae, which dominate submerged calcium-rich vegetation in oligotrophic and moderately eutrophic lakes (Forsberg 1965, Hutchinson 1975, Blindow 1991). They are a common component of the littoral zone in oligo- and moderately eutrophic water bodies. In many lakes, charophytes form dense carpets (chara meadows) present throughout the year and are distinguished by very high calcium content (Hutchinson 1975, Krause 1997). Based on this and on their capacity to develop a high biomass, charophytes can store large amounts of various elements, especially nutrients, and act as a phosphorus sink in lakes in the process of co-precipitation of P with calcite (Murphy et al. 1983, Hillbricht-Ilkowska 1989, Blindow 1991, Kufel and Ozimek 1994). It is also possible that the process of co-precipitation can be important not only for retaining nutrients but for trace metals as well. The trace metals retention has been pointed out by Riemer and Toth (1968) and by Urbaniak (2006), who analyzed the level of zinc and the accumulation ratio in charophytes. However, in contrast to nutrients, only a few studies have been carried out on the accumulation of trace metals in charophytes, and no detailed studies on the rates of accumulation of Mn have been carried out previously.

Through field and laboratory experiments, this paper reports experimental studies on the possible way of Mn accumulation, and tests the time of accumulation in two chara species, which are significantly different in their size and habit: *Chara hispida* and *Chara globularis*.

MATERIALS AND METHODS

Two *Chara* species (*C. globularis* and *C. hispida*) were used in laboratory and field experiments. *C. globularis* is a small species with a shoot diameter of about 0.5-1 mm, and *C. hispida* is a larger species (shoot diameter 1-4 mm) up to 40-50 cm long (Blindow 1991). *C. globularis* contained slight calcium carbonate incrustation on the surface. In the case of *C. hispida* that calcium carbonate deposit was larger and was visible to the naked eye.

Plant samples were collected from Lake Blizienko in the northeastern part of Poland, Mazurian Lakeland. In all the experiments, shoot tips (distal 10 cm) were used. The plants were rinsed with lake water and then quickly transported to the laboratory. Each assay of 35 g fresh-weight of shoot tips (10 cm long) was placed in a glass tank containing 1 liter of lake water, of the appropriate solution, then incubated in laboratory at 18ºC for 120 hours under lighting (144 $\mu m\ m^{-2}\ s^{-1}$, day:night – 16:8 h). Both species of *Chara* were grown at four Mn concentrations in five replicates: 0.2, 0.3, 0.4, 0.5 ($mg\ l^{-1}$ Mn), along with

control samples. At the beginning of all experiments, a precise volume of contaminant solution was added to the medium. All solutions were made up on the initial day of the experiment from a 1000 mg l^{-1} 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*. Manganese levels and experimental procedures 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 2001.

The tested plants were collected after the end of experiments (72 and 120 hours), dried at 60ºC 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 Mn content was determined using an Avanta Sigma-GBC atomic absorption spectrophotometer with an AAS graphite furnace. The level of Mn in the 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. All analyses were performed in duplicate. Additionally, several samples of the plant species under investigation were taken to determine the content of Mn growing in natural conditions.

The results were tested with two-way ANOVA and *T*-Student's test ($t_{0.05}$), using the *Statistica* (2005) program, to determine if differences observed through exposure periods were significant. The concentration of Mn inside the internal wall of internodes was determined by scanning electron microscopy (SEM).

RESULTS

The chemical composition of the lake water used in to the experiments showed that Mn concentrations were below the detection limit. Table 1 shows the changes in Mn concentration after the experiments. The values given are the averages of the experiments, carried out at each of the places of study (laboratory, field). There is a large difference in Mn concentration in water between both species observed during experiments, though in both species the concentration of Mn in the water decreased significantly during the 120- and 72-hour experiments (Table 1).

Mn concentration in the water decreased to 0.04-0.14 mg l^{-1} of the initial concentration, after 120 hours of laboratory tests with *C. globularis* (Table 1). This species accumulated Mn in the range of 66 - 86.7%. During the field experiments on *C. globularis*, aqueous Mn decreased significantly to the range

Table 1

Changes in Mn concentration in water after the experiments concluded.

Initial Mn concentration (mg l^{-1})	Mn concentration in water after 120 hours laboratory experiments (mg l^{-1})				Mn concentration in water after 72 hours field experiments (mg l^{-1})			
	Chara globularis	*Chara hispida*	$t_{est.}$	$t_{0.05}$	*Chara globularis*	*Chara hispida*	$t_{est.}$	$t_{0.05}$
0.2	0.05	0.07	55.3	2.77	0.02	0.04	214.3	2.77
0.3	0.04	0.06	31.6		0.04	0.07	69.7	
0.4	0.08	0.12	56.9		0.05	0.09	112.3	
0.5	0.14	0.18	41.1		0.05	0.08	856.4	
LSD	0.09	0.08			0.13	0.06		
$F_{est.}$	1165254	2236354			756542	775869		
$F_{0.05}$	4.06				4.06			

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

of 0.02-0.05 mg l^{-1} of initial Mn concentration in the water and rates of Mn accumulation were between 64 and 80%.

Initial concentrations for *C. hispida* during the 120-hour run changed to the level of 0.06-0.18 mg l^{-1}. This species accumulated 87.5 – 90% of initial Mn from water in the laboratory experiments. During the field experiments of *C. hispida*, aqueous Mn decreased significantly to the level of 0.04-0.09 mg l^{-1} of initial Mn concentration in water (rates of Mn accumulation were 76.5 – 80%).

Changes of Mn concentrations in water by both species of chara are shown in Table 1, Fig. 1 a-b and Fig. 2 a-b (initial concentrations 0.3 mg Mn l^{-1}. It is evident (Fig. 1-2) that there is a difference in the rate of accumulation of aqueous Mn from water. In the field experiments, Mn accumulation by both chara species was more rapid than in the laboratory. During the study of *C. globularis*, aqueous Mn decreased significantly during the first two hours (Fig. 1 a-b). In contrast, in the laboratory the same level of aqueous Mn was reached after 48 hours. Similar processes were observed in *C. hispida* (Fig. 2 a-b). Accumulation of aqueous Mn was different depending on experimental conditions – field and laboratory experiments. This is probably as a result of fresh plants being used in the experiment, which in the case of field studies absorbed Mn much faster, than the plants in the laboratory, which were often observed in various experiments.

In all, 50 samples of the whole plant were analyzed upon completion of the experiment, and the results are presented in Fig. 3. The concentrations of Mn retained from water by plants at the end of the experiments are shown for *C. globularis* (Fig. 3 a-b) and for *C. hispida* (Fig. 3 c-d). *C. globularis* has the

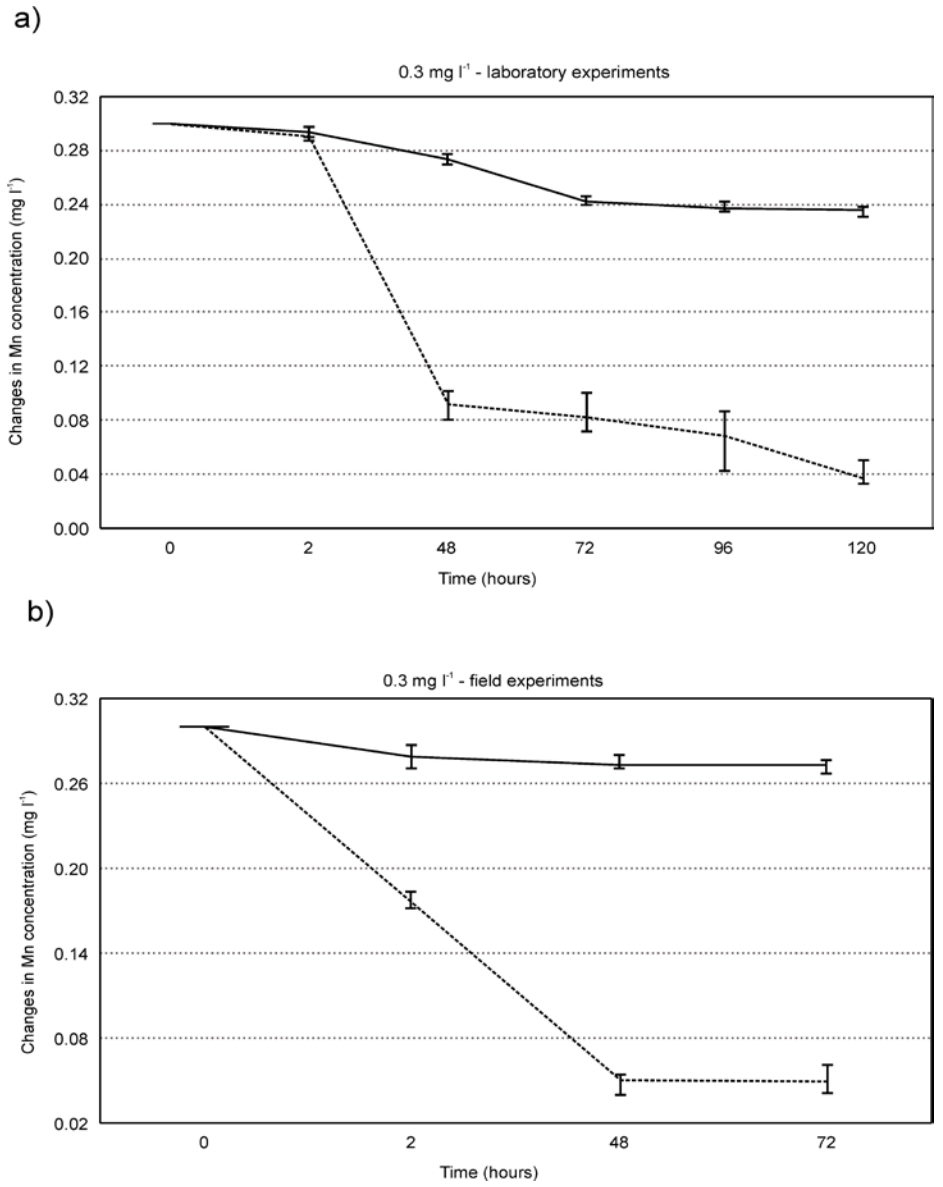

Fig. 1. Changes in Mn concentration in water (mg l^{-1}) - *Chara globularis* straight line - control, dotted line – experiments.

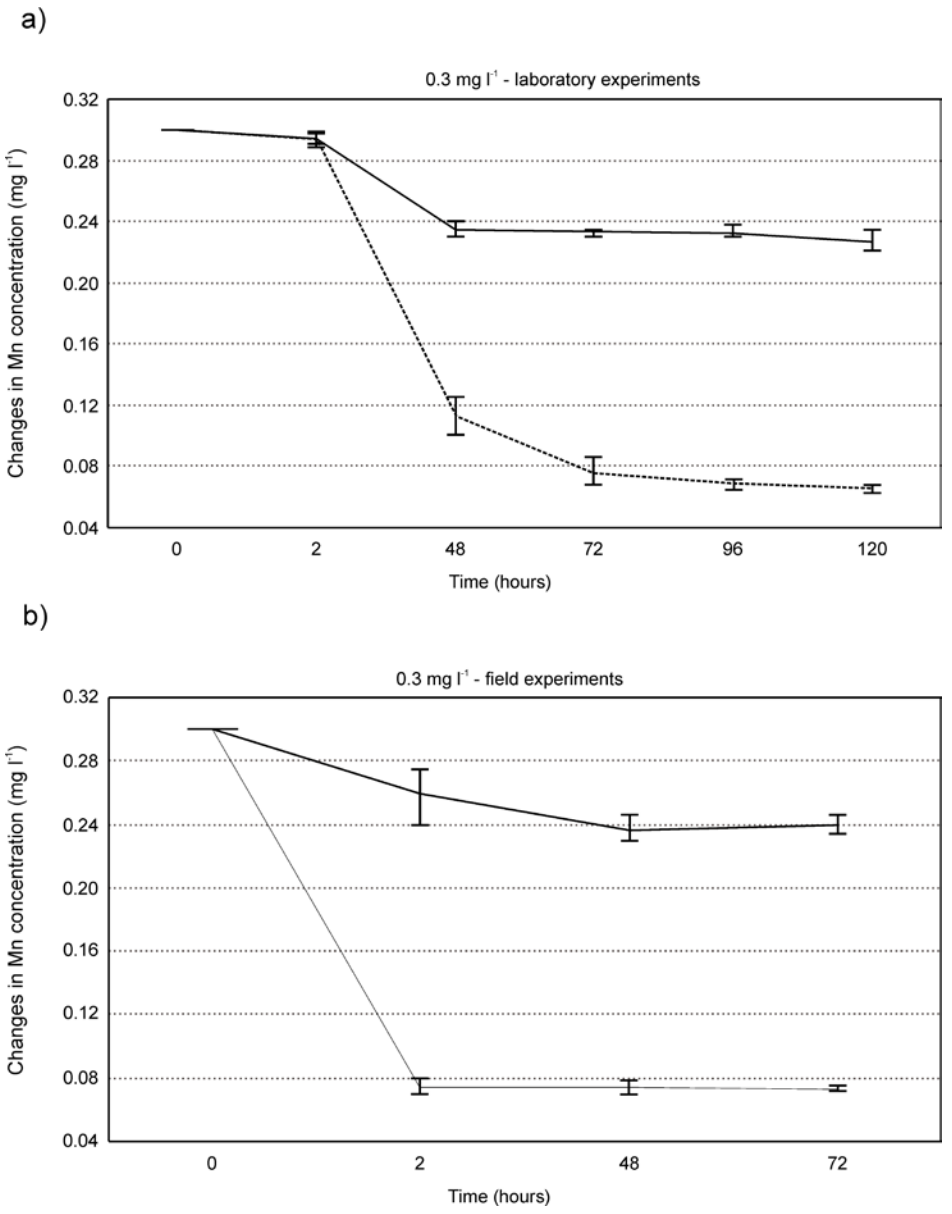

Fig. 2. Changes in Mn concentration in water (mg l^{-1}) - *Chara hispida* straight line - control, dotted line – experiments.

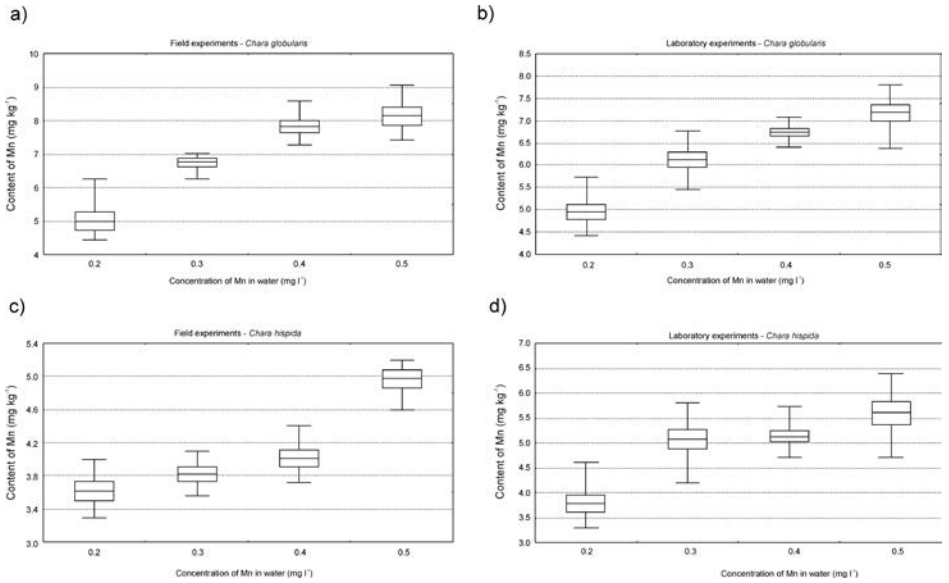

Fig. 3. Comparison of Mn content in *Chara globularis* and *Chara hispida* (mg kg $^{-1}$ d.w.) after finished laboratory and field experiments.

potential to accumulate twice as much Mn from the water (laboratory experiments) as *C. hispida*. Similar results were obtained during field experiments. All the differences between the two species used in the experiments were significant. Plants taken directly from the environment contained 0.1 mg kg^{-1} Mn for *C. globularis* and *C. hispida*.

DISCUSSION

The results of these experiments show that charophytes: *C. globularis* and *C. hispida* can rapidly compensate for environmental inputs of Mn. These results seems to confirm the observation of Riemer and Toth (1968), who found considerable amounts of manganese in *Chara* sp. with very high calcium content, namely 100 and 6500 mg kg^{-1} Mn.

According to Beaugelin-Seiller et al. (1995), it is possible 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 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 has been described in detail for ^{65}Mn, ^{60}Co, and ^{110}Ag by Baudin (1974) and Garnier and Baudin (1989).

Surface adsorption

If surface adsorption by the investigated charophytes is one of the mechanisms responsible for trace metals accumulation, which is often postulated by the authors cited above, the concentration curve would reach a constant value in the adsorption contamination phase (Fig. 1 a-b and 2 a-b). This seems to be contradicted by the results of experiments, especially those made *in situ*, during which a short intense Mn accumulation was observed in the first two hours. In the laboratory, the proposed adsorption phase was much longer and slower. After two days of exposure, the Mn accumulation in the adsorption phase was estimated at 33-44% (laboratory experiments) for both species, and for comparison after two hours approximately 23-46% in the field experiments with *C. hispida*.

Biological absorption

If biological absorption by the investigated charophytes was the mechanism responsible for trace metal accumulation, which is also postulated by Beaugelin-Seiller et al. (1995), the decrease of Mn concentration in the water should be longer and slower than in the first phase. However, this stage of accumulation is not clearly displayed in the experiments presented here and probably does not exist. In the case of both investigated species, on the second day of exposure of *C. hispida* and *C. globularis*, when the proposed biological absorption should start, the concentration of aqueous Mn was very low in laboratory experiments (Fig. 1 a-b and 2 a-b). Similarly, in the field experiments with *C. globularis*, the duration of the second phase was also not clear. The process seems to have started after 48 hours, and similarly low Mn content was detected. The possible explanation is that during the proposed first phase, almost all Mn has been accumulated on the plant surface by both chara species. This seems to be confirmed by the analysis of the internal wall of internodes by SEM microanalysis (Fig. 4 a-b, Fig. 5). It showed no presence of Mn content inside the investigated plants.

CONCLUSION

The proposed accumulation phase, or so-called “biological absorption”, has not been observed as yet during the experiments. It is very probable that charophytes accumulated Mn by adsorption on the plant surface, which seems to be the most likely manner of Mn accumulation. It is widely known that the

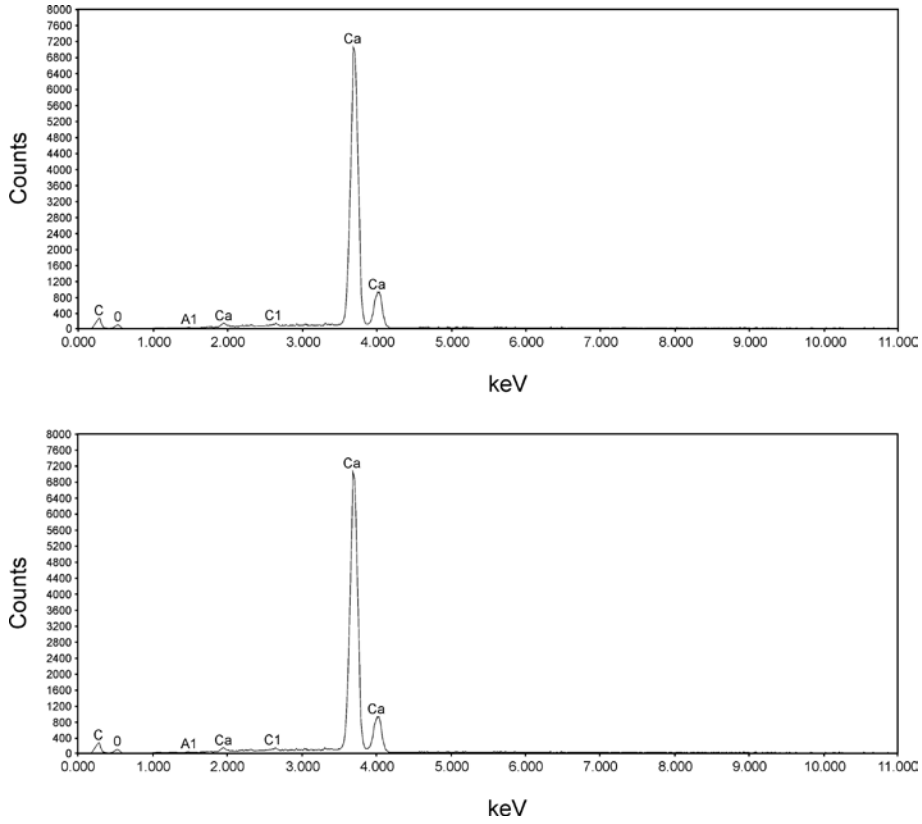

Fig. 4. SEM microanalysis spectra. Analysis of element content in the internal wall of internodes; a) *Chara globularis*, b) *Chara hispida.*

external cell wall in the numerous species of *Chara* is covered by calcium carbonate (Fig. 5), and these huge carbonate deposits can play a key role in the accumulation of elements by water plants and especially charophytes. Mn and the other elements dissolved in water can be easily deposited on the external cell surface. This process is characteristic for all the aquatic plants, which have heavy calcium deposit on the surface, and more especially for charophytes.

Additionally, the co-precipitation of trace elements white calcite and especially Mn, proposed by Riemer and Toth (1968), seems not to be observed. Mn in water cannot be so easily precipitated with calcium carbonate as can Zn, for example (Dojlido 1995).

In light of the foregoing discussion it seems that rapid accumulation of Mn in the plant tissue can be an important factor in Mn circulation in lakes. Blindow (1992) and Scheffer et al. (1993) described similar processes for

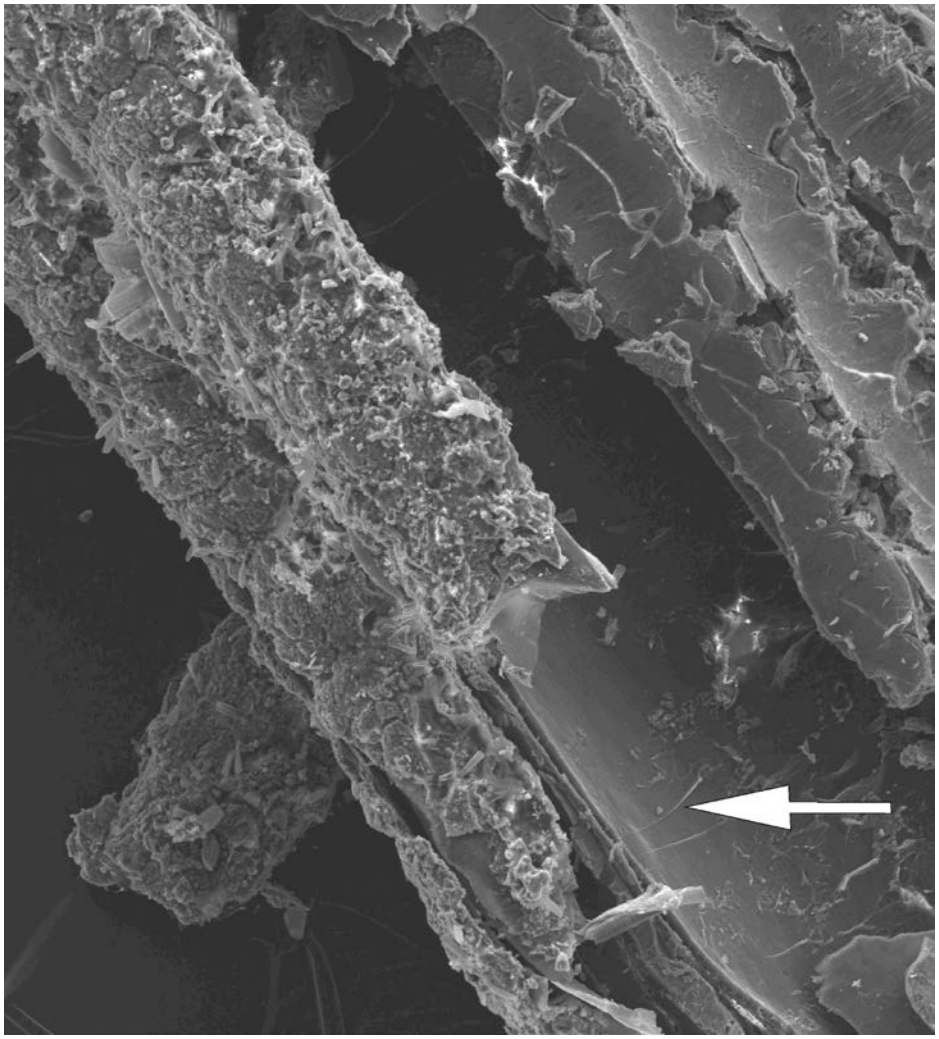

Fig. 5. Part of internodal cell of *Chara hispida* covered by heavy calceorus incrustation. An arrow is showing an investigated area of the plant.

nutrients. Owing to high biomass, charophytes could store large amounts of Mn. Extensive and dense carpets of charophytes found in lakes are able to store aqueous Mn in greater amounts per lake area than other algae species, such as *Mougeotia* or *Cladophora* or vascular plants. However, more studies are needed for a better understanding of this process of accumulation.

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