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Original Research

The Effect of Zn and Mn on the Toxicity of Cd to the Green Microalga *Desmodesmus armatus* Cultured at Ambient and Elevated (2%) CO_2 Concentrations

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Abstract

A medium modified by 4-fold (Zn) or 2.5-fold (Mn) decreased/increased content of these metals did not influence the growth of the green microalga *Desmodesmus armatus* cultured for 24 h at ambient and elevated (2%) CO_2, as compared with original BBM medium (Zn^{2+} = 2 mg/L, Mn^{2+} = 0.4 mg/L). The 4-fold increased content of Zn in the medium markedly enhanced the toxicity of $CdCl_2$ applied at a concentration (16.8 mg/L) corresponding to $EC_{50/24h}$. The 2.5-fold increase of Mn content reduced the inhibitory effect of Cd on the oxygen evolution rate and F_V/F_M. At low CO_2, Cd did not affect the oxygen evolution but markedly stimulated the activity of carbonic anhydrase (CA). At elevated CO_2, the rate of oxygen evolution was reduced by Cd, whereas CA activity remained unaffected.

Keywords: heavy metals, toxicity, *Desmodesmus*, CO_2

Introduction

Cadmium, widely distributed in the environment, is a 'heavy' metal of well known toxicity. It can induce a wide spectrum of toxic effects on plant physiology, altering enzymatic activities by binding to functional groups (sulfhydryl, carboxyl, imidazole, etc.), or by displacing the metal associated with the enzyme [1]. Zinc and manganese are essential micronutrients for algae and other organisms, but when supplied in excess may become toxic [2, 3]. Both act as important enzyme cofactors. Zn is a component of vial enzymes (e.g. carbonic anhydrase, superoxide dismutase, RNA polymerase), and a structural stabilizer for proteins, membrane and DNA-binding proteins (Zn-fingers) [4]. Mn is an essential nutrient, a component of oxygen-evolving complex, a cofactor in Mn-superoxide dismutase, and an activator of decarboxylase and dehydrogenase [5]. In addition, Mn also plays an important role in detoxification of free radial forms of oxygen [6].

A number of membrane transport protein families are implicated in metal homeostasis in plant cells, and some of them are involved in influx/efflux transport of divalent cations [see rev. by 7]. Thus, competition for a transport system between biologically essential divalent cations such as Zn or Mn and non-essential and toxic heavy metals such as Cd could be documented [8, 9]. In consequence, the antagonistic effect of Zn and Mn on cadmium toxicity was observed in laboratory experiments [10, 11], implying a possible protection of primary phytoplankton producer by nutrient and trace metals against toxic heavy metals present in water bodies.

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Relative contributions of individual dissolved inorganic carbon (DIC) species strongly depends on medium pH. HCO_3^- is a predominant form of DIC in an alkaline environment, and CO_2 is predominant in an acidic one. The cells of algae are able to utilize both forms of DIC. However, photosynthesis in aquatic environments may be limited due to a very low level of free CO_2 or slow diffusion rates in water and into the cells [12]. Therefore, most unicellular algae have evolved a carbon concentrating mechanism (CCM) to compensate for this CO_2 limitation. The crucial role in the CCM in unicellular green algae, played by carbonic anhydrase (CA). Carbonic anhydrase (CA, carbonate-lyase, carbonate dehydratase, EC 4.2.1.1) is a zinc–containg metalloenzyme that catalyzes interconversion of two forms of DIC (CO_2 and HCO_3^-) and provides a substrate (CO_2) for photosynthetic carbon fixation by RuBisCo [13].

Little is known about nutrient-mediated toxicity of Cd to unicellular algae adapted to low and elevated CO_2 concentrations. It is still not clear whether high-CO_2 concentrations reduced the toxicity of Cd or sensitivity of the algae cells to this metal. Therefore, short-term experiments were undertaken to obtain information about the influence of Zn and Mn changed within physiologically relevant concentrations on the toxicity of Cd to the microalgae *Desmodesmus armatus* cultured under CO_2 concentrations at which CCM operates or is switched off.

Materials and Methods

The unicellular green algae *Desmodesmus* (*Scenedesmus*) *armatus* strain (276-4d) used in this study was obtained from Sammlung von Algenkulturen (SAG), Göttingen, Germany.

Algae were transferred from the slants into liquid BBM and incubated for a few days in precultures to adapt them to the conditions of the batch culture. Algae were grown in 200 mL glass test-tubes at 30ºC and illuminated with cool white light from fluorescent tubes (Philips TLD 36W/94) with an irradiance at the surface of the culture vessels of 64 W/m^2 of photosynthetically active radiation (PAR) (380-690 nm). All glass tubes used for the culture were previously cleaned by soaking in 0.1 M HCl for at least 24 h and then rinsed with redistilled water. The initial cell density was 0.4×10^6 cell/mL. The culture vessels were closed with stoppers equipped with glass pipes ending 3 mm above the bottom of the tubes, providing air flow of about 10 L/h (MLW G11, Germany). Cultures were aerated with ambient air (0.05-0.1% CO_2) or with a gas mixture containing 2.0% CO_2, which was passed through a bacteriological filter (Sartorius 2000; 0.2 µm PTFE).

The cells were exposed to cadmium chloride ($CdCl_2 \times H_2O$, Merck) at a concentration of 16.8 mg/L, corresponding to the $EC_{50/24h}$ value determined for algae grown under ambient CO_2. Stock solutions of cadmium salt and medium components were prepared of analytical grade chemicals and bidistilled water. The toxicity of Cd was tested in original BBM medium and in modified BBM media (Table 1). The pH of all media at the start of the experiment was adjusted to 0.1 M HCl or 0.1 M NaOH to 6.9 ± 0.1.

Table 1. Zinc and manganese concentrations in original and modified BBM medium.

Medium	Zn^{2+} (mg/L)	Mn^{2+} (mg/L)
BBM	2.0	0.4
BBM–4×Zn	0.5	0.4
BBM+4×Zn	8.0	0.4
BBM–2.5×Mn	2.0	0.16
BBM+2.5×Mn	2.0	1.0

The cell number was determined in a Bürker chamber under light microscopy using the standard procedure.

The photosynthetic oxygen production was determined in a homemade photosynthetic cylindrical chamber with a 15 mm inside diameter and a capacity of 10 mL, enclosed with an outer jacket for thermostated water, all made of Plexiglas. The chamber was equipped with a Clark-type EO 96 (WTW) oxygen electrode connected to a microprocessor (OXI 96, WTW, Germany).

Chlorophyll *a* fluorescence *in vivo* was measured at room temperature by the pulse amplitude modulation (PAM) method, with an FMS1 fluorometer (Hansatech, Norfolk, UK) run by Modfluor software. The maximum photochemistry yield ($F_V/F_M = (F_M-F_0)/F_M$) was calculated according to Genty et al. [14]. The detailed description of oxygen and fluorescence measurements is given elsewhere [15].

Carbonic anhydrase activity was assayed electrometrically using a modified procedure of Wilbur and Anderson [16]. Cells were harvested by centrifuge (800 × g, 10 min), washed with 60 mM phosphate buffer (pH 8.1) and again centrifuged. Collected cells were resuspended in a 0.5 mL reaction mixture containing 60 mM ice-cold phosphate buffer (pH 8.1), 1 mM EDTA and 0.2 mM DTT (16:1:1), and mixed for 3 min with glass beads (0.4 mm; Merck, England) in conical glass tubes of 10 mL capacity. The homogenate was transferred to an eppendorf tube and the glass beads were washed four times with 0.5 mL of the reaction mixture to a final volume of 2 mL of the extract. After cellular disruption, the suspension was centrifuged (13,800 × g, 30 min, 4ºC) to remove insoluble debris. The supernatant was assayed for total carbonic anhydrase ($CA_{tot.}$) activity that was measured by determining the time necessary for pH to drop from 8.0 to 7.4 at 2ºC in a 2 mL sample. The reaction was started by rapid injection of 2 mL of ice-cold CO_2-saturated distilled water. The enzyme activity units were calculated according to the enzyme unit (EU):

$$10 \times [(t_0 / t_1) - 1],$$

...where t_0 and t_1 are times required for the pH drops in the absence and presence of the enzyme solution, respectively.

Table 2. Cell density (N × 10^6 cells/mL) of *Desmodesmus* cultures exposed for 24 h to cadmium and grown under different medium composition and CO_2 concentration.

Medium	low-CO_2 cells		high-CO_2 cells	
	N	%	N	%
	cadmium			
BBM	3.36 (0.08)	100	4.29 (0.19)	100
BBM+Cd	1.60 (0.15)**	47.6	2.26 (0.24)**	52.7
	cadmium ± zinc			
BBM–4×Zn	3.36 (0.14)	100	4.36 (0.36)	100
BBM–4×Zn+Cd	2.21 (0.15)**	65.8	1.74 (0.02)**	39.9
BBM+4×Zn	3.45 (0.33)	100	4.55 (0.54)	100
BBM+4×Zn+Cd	0.58 (0.03)**	16.8	1.04 (0.03)**	22.8
	cadmium ± manganese			
BBM–2.5×Mn	3.47 (0.16)	100	4.10 (0.40)	100
BBM–2.5×Mn+Cd	1.49 (0.14)**	42.9	1.21 (0.01)**	29.5
BBM+2.5×Mn	3.39 (0.19)	100	4.24 (0.004)	100
BBM+2.5×Mn+Cd	1.32 (0.10)**	38.9	1.88 (0.01)**	44.3

Values are means of 3-9 experiments ± SE (in parentheses). Asterisks stand for values statistically different from the controls at **P<0.01. The initial density was 0.4 × 10^6 cells/mL.

Statistical analysis was performed using an Origin 7.5 program (OriginLab Corporation, USA). Student's t-test was applied to compare results obtained for control and treated cultures (*P<0.05 and **P<0.01), as well as for comparison of the controls performed in original and modified BBM media ($^{+}$P<0.05 and $^{++}$P<0.01).

Results and Discussion

The growth of algae in the control cultures performed for 24 h under conditions of ambient CO_2 (in laboratory 0.05-0.1%) level was markedly lower in comparison to that observed at elevated (2%) CO_2 concentration, but it was not affected by the medium modification with Zn and Mn (Table 2). Cadmium reduced the growth of algae to about 50% at both CO_2 levels, when algae were grown in original BBM medium. The reduced content of Mn in the medium enhanced the Cd toxicity to high-CO_2 cells. In contrast, this enhancement was distinct in both low- and high-CO_2 cells grown with increased Zn content (Table 2). It is well documented that Zn is an essential nutrient for algae, but at higher concentrations reduces the cell division, photosynthetic pigments and total amino acid content, or phosphatase activity [3, 17]. However, there are reports that high concentrations of Zn in the medium lessened the uptake of Cd by algae [9], resulting in the protection of cells against Cd toxicity [10]. In our experiment, to exclude the toxic effect of Zn and Mn to *D. armatus*, we changed the metal content in the medium in the range that did not modify the growth of cells, as compared with cells cultured in original BBM medium. Our findings indicate that Cd-induced toxicity was not alleviated by Zn supplementation. Synergistic effects of Cd and Zn on cell growth inhibition were observed.

The ratio F_V/F_M, often used as a stress indicator, expresses the potential yield of the photochemical reaction [18]. We have found that F_V/F_M was significantly lower in low-CO_2 than in high-CO_2 cells, and it was valid for original and modified media. Cadmium lowered the values of F_V/F_M (Table 3). Literature data indicate quite different effects of Cd on the values of F_V/F_M, observed also in such a related organism as species of the same genus [19, 15]. The increased content of Zn in the medium enhanced Cd-induced drop in F_V/F_M, especially in high-CO_2 cells. Ilangovan et al. [11] observed that Cd (0.5-1.0 mg/L) diminished F_V/F_M in *Scenedesmus* cells, while Cd-Zn (Zn = 50 mg/L) restored it up to the control value when cultured in suspension but not in immobilized cultures. On the other hand, Zn alone (8.0 mg/L) markedly reduced the pigment content in *Scenedesmus* cells [3], while at a concentration applied in the experiment reported herein in the Zn-supplemented medium (8.0 mg/L) it had no effect on the growth of *Desmodesmus* cells. It suggests that the overcoming of Cd toxicity to photosynthetic apparatus by Zn [20] depends rather on a subtle Zn/Cd balance within the chloroplast than on the concentration of metals in the medium, on the organism, culture condition or time of exposure.

Table 3. The maximum photochemistry yield (F_V/F_M) of *Desmodesmus* cells exposed for 24 h to cadmium and grown under different medium composition and CO_2 concentration.

Medium	low-CO_2 cells	high-CO_2 cells
	cadmium	
BBM	0.751 (0.009)	0.814 (0.008)
BBM+Cd	0.714 (0.023)	0.763 (0.038)
	cadmium ± zinc	
BBM–4×Zn	0.751 (0.009)	0.807 (0.010)
BBM–4×Zn+Cd	0.733 (0.008)	0.759 (0.029)
BBM+4×Zn	0.744 (0.012)	0.785 (0.009)$^{+}$
BBM+4×Zn+Cd	0.712 (0.013)*	0.666 (0.012)**
	cadmium ± manganese	
BBM–2.5×Mn	0.754 (0.008)	0.807 (0.009)
BBM–2.5×Mn+Cd	0.711 (0.010)**	0.784 (0.002)*
BBM+2.5×Mn	0.747 (0.009)	0.799 (0.010)
BBM+2.5×Mn+Cd	0.727 (0.017)	0.782 (0.008)

Values are means of 4-6 experiments ± SE (in parentheses). Asterisks stand for values statistically different from the controls at **P<0.01 and *P<0.05, cross means significant difference between original and modified BBM medium at $^{+}$P<0.05.

Table 4. The influence of cadmium on photosynthesis rate [$\mu molO_2/h/1\times10^6$ cell] of *Desmodesmus* cells grown for 24 h under different medium composition and CO_2 concentration.

Medium	low-CO_2 cells	high-CO_2 cells
	cadmium	
BBM	0.178 (0.029)	0.261 (0.017)
BBM+Cd	0.147 (0.008)	0.244 (0.021)
	cadmium ± zinc	
BBM–4×Zn	0.182 (0.023)	0.284 (0.014)
BBM–4×Zn+Cd	0.147 (0.010)	0.170 (0.015)**
BBM+4×Zn	0.136 (0.012)	0.117 (0.002)$^{++}$
BBM+4×Zn+Cd	0.123 (0.011)	0.091 (0.003)**
	cadmium ± manganese	
BBM–2.5×Mn	0.234 (0.031)	0.307 (0.027)
BBM–2.5×Mn+Cd	0.091 (0.024)**	0.164 (0.038)**
BBM+2.5×Mn	0.205 (0.028)	0.344 (0.012)$^{++}$
BBM+2.5×Mn+Cd	0.160 (0.051)	0.259 (0.021)**

Values are means of 3-11 experiments ± SE (in parentheses). Asterisks stand for values statistically different from the controls at **P<0.01, crosses indicate significant difference between original and modified BBM medium at $^{++}$P<0.01.

In contrast to Zn, the enhanced inhibitory effect of Cd on F_V/F_M was observed in algae grown in a medium with the reduced content of Mn. This was observed particularly in low-CO_2 cells (Table 3). Mn is an important component of the oxygen-evolving complex in the donor site of photosystem II (PSII) [21], but binding of Cd to the essential Ca site during photoactivation is proposed to be an important mode in the action *in vivo* for inhibiting photosynthesis in unicellular algae such as *Chlamydomonas* [22]. Our results indicate that 2.5-fold increased content of Mn in the medium was sufficient to protect the photosynthetic apparatus of low-CO_2 cells against Cd toxicity, but not the growth of these cells. It suggests that nucleocytoplasmic processes such as DNA replication, mitosis or protoplast fission were less protected than the chloroplast ones.

The inhibitory influence of Cd on oxygen evolution was more pronounced in high-CO_2 than in low-CO_2 cells (Table 4). The effect of Cd on the activity of carbonic anhydrase (CA) also strongly depended on CO_2 concentration, but it was not affected by Zn and Mn content in the medium (Table 5). In low-CO_2 cells, the activity of CA was stimulated within the range from 160 to 300% of the control (the exception is the lack of effect in BBM+4×Zn medium), whereas in high-CO_2 cells Cd had generally no statistically significant influence on CA activity. The exception is 270% stimulation noted in BBM-2.5×Mn medium. It seems that different sensitivity of *D. armatus* cells to Cd at low and high CO_2 concentrations may result from differently functioning CCM. One component of this mechanism is CA, a Zn-containing enzyme that is produced in large amounts when algae are grown on limiting CO_2. CA activity increased with an increase of medium pH [23]. In the control cultures, the low-CO_2 cells alkalize the medium (7.99-9.15), whereas in high-CO_2 cells the pH of media remains unchanged (6.82-6.96). For this reason, we observed 2-3-fold higher activity of CA in cells grown at low CO_2 than at elevated CO_2 (Table 5). However, we noticed increased activity of CA in Cd-treated cells (pH of the medium 7.25-7.66) as compared to control low-CO_2 cells, regardless of Zn and Mn medium content. Therefore, we conclude that the stimulation of CA activity in cells after exposure to Cd did not result from pH changes in the medium. There are only a few data about stimulation activity of CA in algae exposed to Cd [24, 25]. Unfortunately, the reason for this stimulation remains unclear.

Conclusion

The obtained results indicate that the 4-fold increased content of Zn in the medium did not reduce toxicity effect of Cd on the growth and photosynthesis of algae, regardless of CO_2 concentrations. The 2.5-fold increase of Mn content did not change the inhibitory effect of Cd on the growth of cells but clearly protect the photosynthetic apparatus of low-CO_2 cells against toxicity of cadmium. The increased activity of CA in low-CO_2 cells after exposure to Cd may

Table 5. Total carbonic anhydrase ($CA_{tot.}$) activity of *Desmodesmus* cells exposed for 24 h to cadmium and grown under different medium composition and CO_2 concentration.

Medium	low-CO_2 cells		high-CO_2 cells	
	$CA_{tot.}$	pH	$CA_{tot.}$	pH
	cadmium			
BBM	15.87 (2.59)	8.65	7.68 (1.64)	6.96
BBM+Cd	28.80 (0.20)**	7.52	11.58 (2.18)	6.78
	cadmium ± zinc			
BBM–4×Zn	21.53 (4.16)	9.15	9.10 (6.37)	6.93
BBM–4×Zn+Cd	43.05 (7.25)*	7.31	8.43 (3.49)	6.74
BBM+4×Zn	19.93 (7.38)	7.99	8.50 (4.69)	6.84
BBM+4×Zn+Cd	37.80 (7.50)	7.66	11.70 (1.79)	6.14
	cadmium ± manganese			
BBM–2.5×Mn	18.35 (4.77)	8.33	8.43 (3.16)	6.82
BBM–2.5×Mn+Cd	55.25 (17.05)*	7.38	31.30 (2.30)**	6.44
BBM+2.5×Mn	23.0 (4.96)	8.40	6.90 (3.27)	6.96
BBM+2.5×Mn+Cd	36.97 (2.24)*	7.25	16.30 (1.13)	6.72

Values are means of 3-6 experiments ± SE (in parentheses). Asterisk stands for values statistically different from the controls at *P<0.05 and **P<0.01. Activity of $CA_{tot.}$: $EU \times 10^{-2}$ per 1 mln cells.

suggest that the amount of the substrate (CO_2) provided for carbon fixation by RuBisCo was not a limiting factor of the photosynthesis. It could explain the lack of the influence of Cd on the photosynthetic activity observed in low-CO_2 cells.

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References

1. SANITÁ DI TOPPI L., GABBRIELLI R. Response to cadmium in higher plants. Environ. Exp. Bot. **41**, 105, **1999**.
2. ROUSCH J. M., SOMMERFELD M. R. Effect of manganese and nickel on growth of selected algae in pH buffered medium. Water Res. **33**, (10), 2448, **1999**.
3. OMAR H. H. Adsorption of zinc ions by *Scenedesmus obliquus* and *S. quadricauda* and its effect on growth and metabolism. Biol. Plant **45**, (2), 261, **2002**.
4. VALLEE B. L., AULD D. S. Zinc coordination, function and structure of zinc enzymes and other proteins. Biochemistry **29**, 5647, **1990**.
5. MARSCHNER H. Mineral nutrition of higher plants. Academic Press, London **1995**.
6. SHENKER M., PLESSNER O. E., TEL-OR E. Manganese nutrition effects on tomato growth, chlorophyll concentration, and superoxide dismutase activity. J. Plant Physiol. **161**, 197, **2004**.
7. HANIKENNE M., KRÄMER U., DEMOULIN V., BAURAIN D. A comparative inventory of metal transporters in the green alga *Chlamydomonas reinhardtii* and the red alga *Cyanidioschizon merdae*. Plant Physiol. **137**, 428, **2005**.
8. KOLA H., WILKINSON K. J. Cadmium uptake by a green alga can be predicted by equilibrium modeling. Environ. Sci. Technol. **39**, 3040, **2005**.
9. TÖPPERWIEN S., BEHRA R., SIGG L. Competition among zinc, manganese, and cadmium uptake in the freshwater alga *Scenedesmus vacuolatus*. Environ. Toxicol. Chem. **26**, (3), 483, **2007**.
10. SUNDA W. G, HUNTSMAN S. A. Antagonisms between cadmium and zinc toxicity and manganese limitation in a coastal diatom. Limnol. Oceanogr. **41**, (3), 373, **1996**.
11. ILANGOVAN K., CAÑIZARES-VILLANUEVA R.O., GONZÁLEZ-MORENO S., VOLTOLINA D. Effect of cadmium and zinc on respiration and photosynthesis in suspended and immobilized cultures of *Chlorella vulgaris* and *Scenedesmus acutus*. Bull. Environ. Contam. Toxicol. **60**, 936, **1998**.
12. GHOSHAL D., GOYAL A. Carbon concentration mechanism(s) in unicellular green algae and cyanobacteria. J. Plant Biochem. Biotech. **10**, 83, **2001**.
13. KAPLAN A., REINHOLD L. CO_2-mechanisms in photosynthetic microorganisms. Annu. Rev. Plant Physiol. Plant Mol. Biol. **50**, 539, **1999**.
14. GENTY B., BRIANTAIS J. M., BAKER N. R. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophys. Acta **990**, 87, **1989**.

15. TUKAJ Z., BAŚCIK-REMISIEWICZ A., SKOWROŃSKI T., TUKAJ C. Cadmium effect on the growth, photosynthesis, ultrastructure and phytochelatin content of green microalga *Scenedesmus armatus*: A study at low and elevated CO_2 concentration. Environ. Exp. Bot. **60**, 291, **2007**.
16. WILBUR K. M., ANDERSON N. G. Electrometric and colorimetric determination of carbonic anhydrase. J. Biol. Chem. **176**, 147, **1948**.
17. TRIPATHI B. N., GAUR J. P. Physiological behavior of *Scenedesmus* sp. during exposure to elevated levels of Cu and Zn and after withdrawal of metal stress. Protoplasma **229**, 1, **2006**.
18. BJÖRKMAN O., DEMMIG B. Proton yield of O_2 evolution and chlorophyll fluorescence at 77K among vascular plants of diverse origins. Planta **170**, 489, **1987**.
19. MALLICK N., MOHN F. H. Use of chlorophyll fluorescence in metal - stress research: a case study with the green microalga *Scenedesmus*. Ecotoxicol. Environ. Saf. **55**, 64, **2003**.
20. ARAVIND P., PRASAD M. N. V. Zinc protects chloroplasts and associated photochemical functions in cadmium exposed *Ceratophyllum demersum* L., a freshwater macrophyte. Plant Sci. **166**, 132, **2004**.
21. FERREIRA K. N., IVERSON T. M., MAGHLAOUI K., BARBER J., IWATA S. Architecture of the photosynthetic oxygen-evolving center. Science **303**, 1831, **2004**.
22. FALLER P., KIENZLER K., KRIEGER-LISZKAY A. Mechanism of Cd^{2+} toxicity: Cd^{2+} inhibits photoactivation of photosystem II by competitive binding to the essential Ca^{2+} site. Biochim. Biophys. Acta **1706**, 158, **2005**.
23. DUDOLADOVA M. V., KUPRIYANOVA E. V., MARKELOVA A. G., SINETOVA M. P., ALLAKHVERDIEV S. I., PRONINA N. A. The thylakoid carbonic anhydrase associated with photosystem II is the component of inorganic carbon accumulating system in cells of halo- and alkaliphilic cyanobacterium *Rhabdoderma lineare*. Biochim. Biophys. Acta **1767**, 616, **2007**.
24. WANG B., LIU C-Q., WU Y. Effect of heavy metals on the activity of external carbonic anhydrase of microalga *Chlamydomonas reinhardtii* and microalge from Karst Lakes. Bull. Environ. Contam. Toxicol. **74**, 227, **2005**.
25. LANE T. W., MOREL F. M. M. A biological function for cadmium in marine diatoms. Proc. Natl. Acad. Sci. **97**, (9), 4627, **2000**.