Nova Biotechnol Chim (2022) 21(2): 954
DOI: 10.36547/nbc.954

Nova Biotechnologica *et* Chimica

The specificity of changes in key performance indicators of green algae of the family Scenedesmaceae under the influence of cerium

Larisa M. Cheban[1, ✉], Alexander B. Shcherbakov[2], Nadiya M. Zholobak[2, 3], Mikhailo M. Marchenko[3]

[1]*Department of Biochemistry and Biotechnology, Chernivtsi National University named after Y. Fedkovych, Kotsiubynsky 2 Str., Chernivtsi 58012, Ukraine*
[2]*Danylo Zabolotny Institute of Microbiology and Virology of the NASU, Acad. Zabolotny 154str., Kyiv 03680, Ukraine*
[3]*Kyiv National University of Technologies and Design, Nemyrovycha-Danchenka 2 str., Kyiv 01011, Ukraine*

✉ Corresponding author: *l.cheban@chnu.edu.ua*

Article info

Article history:
Received: 29th April 2021
Accepted: 7th Januar 2022

Keywords:
Acutodesmus dimorphus
Cerium dioxide nanoparticles
Cerium ions
Desmodesmus armatus
Productivity

Abstract

The effect of various concentrations (1 μM – 100 mM) of citrate-stabilized cerium dioxide nanoparticles or cerium ions (3+) on the biomass production of two species of unicellular green algae *Desmodesmus armatus* (Chod.) Hegew and *Acutodesmus dimorphus* (Turpin) Tsarenko was studied, the amount of chlorophyll, proteins and lipids in the algae biomass was determined. It was shown that at the concentrations of 0.01 M to 0.1 M nanoparticles and cerium salt have pronounced toxic effects on the algal cultures, manifested by a sharp increase in the level of lipids in the biomass combined with the decrease in chlorophyll and protein. At lower concentrations, cerium dioxide nanoparticles stimulate algae biomass accumulation, probably due to a change in key metabolic pathways, accompanied by an increase in the accumulation of carbohydrates in the biomass. For cerium salt, these effects are less pronounced. Thus, depending on the concentration of the objects used, it is possible to obtain an increase in the food biomass production enriched with lipids or carbohydrates as appropriate to the biotechnological objectives.

Introduction

Of interest for the study are the group of rare earth elements, lanthanides, named from the first representative of the group. This group includes cerium, which is characterized by oxygen non-stoichiometry and relatively low toxicity (Ivanov *et al*. 2009; Shcherbakov *et al*. 2020). Cerium (III) salts are used as antineoplastic, antiemetic, antiviral, bacteriostatic and bactericidal agents (Jakupec *et al.* 2005; Zholobak *et al.* 2010; Charbgoo *et al.* 2017). This chemical element has attracted the attention of scientists as an inorganic antioxidant, capable of effectively protecting living systems against oxidative stress.

Despite the low incidence and concentration of lanthanides in soils, these elements can have a stimulating effect on the growth and development of organisms (Chen *et al.* 2001; Goecke *et al.* 2015; Kang *et al.* 2020). Lanthanides are known to significantly improve light energy absorption, energy transfer, electron transfer rate and phosphorylation rate, thus stimulating the accumulation of organic matter in plant cells (Huang *et al.* 2008; Gong *et al.* 2011; Goecke *et al.* 2015). It is also believed that lanthanides can be used as substitutes for macronutrients (Zeng *et al.* 2000; Chen *et al.* 2001; Hong *et al.* 2002).

The ability of lanthanides, including cerium, to partially substitute Ca in plant cells under Ca-deficient conditions is already known (Hong *et al.* 2002; Chao *et al.* 2009). A similar compensation mechanism also works when plant cells are deprived of magnesium or manganese (Yin *et al.* 2009; Yuguan *et al.* 2009a, 2009b; Zhou *et al.* 2011). However, the biological behaviour of these elements is based on the principle of analogy with base metal ions: their properties are similar, but still not identical. Also, the complex mechanism of the correlation between the response of plant organisms to exposure to lanthanides and their amount in the nutrient medium is well established. The effects of even low concentrations of these elements on microalgae cells have also been shown to depend on the food security and the possible stress state of the latter (Laszló *et al.* 2001; Goecke *et al.* 2015; Řezanka *et al.* 2016). On the other hand, there is information on the toxic effects of $CeCl_3$ salt (Chen *et al.* 2001; Tai *et al.* 2010; Kosak *et al.* 2018).

The use of nanoparticles in the form of nanoacquahelates stabilized with carboxylic acids, particularly citric acid, is promising. Carboxylated nanoparticles have the ability to easily penetrate the cytoplasmic membrane and then dissociate from the ligands, which accounts for their high biological activity. The toxicity of nanoacetylates is thought to be much lower than that of the corresponding inorganic salts (Borisevich *et al.* 2012).

Algae, as promising producers of organic raw materials for various needs, are convenient for studying the effects of nanopreparations (Jreije *et al.* 2020). Despite considerable interest in the prospects of using cerium on biological objects of different levels of organization, its effects on microalgae cultures remain understudied.

We have shown the potential of two species of green algae, *Desmodesmus armatus* (Chod.) Hegew and *Acutodesmus dimorphus* (Turpin) Tsarenko, as feed organisms in industrial aquaculture (Cheban and Grynko 2017; Cheban *et al.* 2018). The biomass of genera *Desmodesmus* and *Acutodesmus* microalgae, due to their small size and rather high content of amino acids, proteins, polyunsaturated fatty acids, and carotenoids, is used as an alternative complete feed in aquaculture for feeding zooplankton and fish. At the same time, there is a need for techniques that can increase the productivity of these algal cultures both in overall biomass and in major nutrients. However, the mechanism of factors contributing to the growth of microalgae is not fully studied. Little is known about the mechanism of the positive effect of lanthanides on the cells of higher plants and whether it will be similar in the cells of algae, either. The choice of optimal concentrations that can cause a stimulating effect is also a problem.

The aim of this work was to comparatively study the effect of nanocerium and cerium salt on the main indicators of growth activity and productivity of two green algae of the family Scenedesmaceae – *D. armatus* (Chod.) Hegew and *A. dimorphus* (Turpin) Tsarenko.

Experimental

The characteristics of the test-objects

As test-objects cerium chloride salt (Sigma, USA) and citrate-stabilized sol contained cerium dioxide nanoparticles (3 – 4 nm particle size, 1 : 1 stabilizer-to-CDN ratios) were used. A citrate solution of equimolar concentration was used as a control.

Citrate-stabilized aqueous sol of CeO_2 was prepared by the method proposed by Ivanov *et al.* (2010). Briefly, 2.0 g of citric acid was mixed with 25 mL of a 4 M aqueous cerium (III) chloride solution). The resulting solution was rapidly poured, under stirring, into 100 mL of a 3 M aqueous ammonia solution, and then exposed for 2 h at ambient conditions and further boiled for 4 h. Then, the solution was cooled to room temperature and purified from precursors and by-products by sedimentation and further re-dispersion. According to TEM data, the average CeO_2 particle size in this sol was about 3 – 4 nm (Fig. 1).

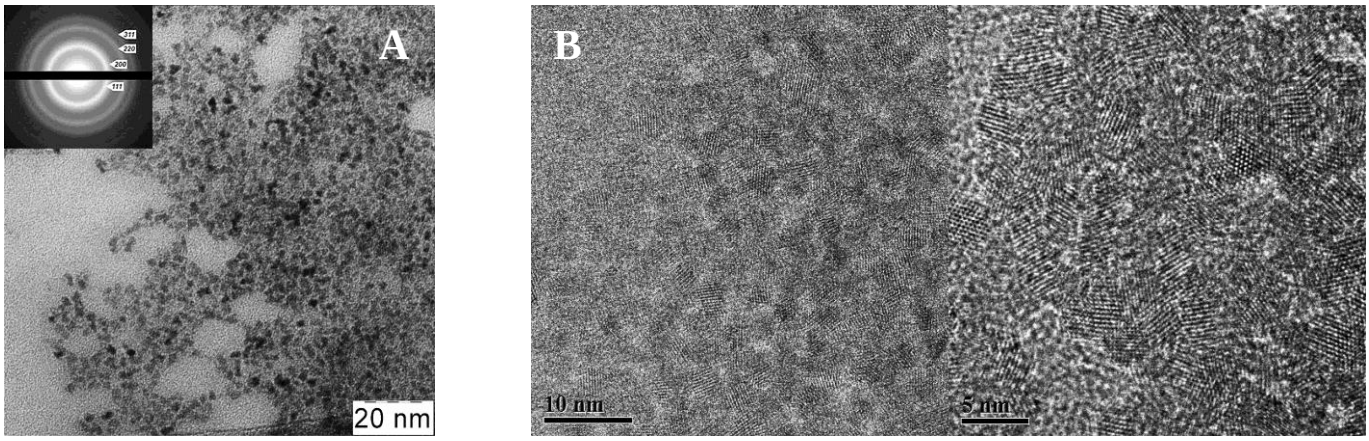


Fig. 1. Photos from (**A**) – TEM microscopy and histogram of size distribution and (**B**) – high-resolution transmission electron microscopy (HRTEM) used cerium nanoparticles.

Biological material and cultivation conditions

The studies were performed on algological pure monocultures of green algae *D. armatus (Chod.) Hegew and A. dimorphus (Turpin) Tsarenko* (IBASH-A), obtained from the collection of the Kholodny Institute of Botany of the National Academy of Sciences of Ukraine, for which we express our gratitude to it.

Algae were pre-cultivated under conditions of cumulative culture to the exponential growth phase in Zender and Gorham's modification of the Fitzgerald's medium N 11 (Zolotareva *et al*. 2008). All manipulations related to the sowing of algae cultures were performed in a laminar box under sterile conditions. The inoculum-to-nutrient medium ratio was 1 : 10. Cultivation was performed in a climatic room with photoperiod of 16 hours provided by fluorescent lamps at a light intensity of 2,500 – 4,000 lux and temperature of 24 ± 2 ºC. To create optimum conditions for studying the effects of cerium salt and cerium nanoparticles, the algal biomass was concentrated and washed of nutrient medium residues in sterile distilled water. Algae cells were isolated from the culture medium by centrifugation at 3,500 g for 15 min. using the *Heraeus Biofuge Stratos benchtop centrifuge (Heraeus Holding GmbH, Hanau, Germany*). The final cell concentration was 2.6×10^5 cells.mL^{-1}. Washed algae cells were incubated in a solution of distilled H_2O with the addition of the appropriate concentration of cerium compounds.

Nano-cerium sol, citrate, or cerium ions (III) were added to the hydrated cells at the concentrations of 0.001 mM, 0.01 mM, 0.1 mM, 1 mM, 10 mM and 100 mM. After 5-day exposure, the amount of algal biomass, cellular chlorophyll *a,* protein and lipid content were assayed.

The amount of biomass was determined from culture density using an optical index at 750 nm at Agilent CaryWin UV 60 (Agilent Technologies, Inc., Santa Clara, USA). The transition from the optical density (OD) units (D750) to the value of absolutely dry biomass (ADB) was carried out through the empirical coefficient k: ADB = k × D750 (Hevorhyz *et al.* 2008). The coefficient k (k = g OD unit per liter) for both cultures was determined experimentally in three independent repeats. Following the cultivation, the entire biomass was separated by centrifugation at 3,500 g and 4 ºC for 15 min and, if necessary, frozen at -20 ºC. Centrifuged microalgae biomass was disintegrated on an USDN-2T. In the hydrated cells, the total proteins were determined by the Lowry (Lowry *et al.* 1951) method and the total lipids were assessed (Knight *et al.* 1972).

The pigments were extracted from the hydrated microalgae cells with chloroform-methanol 2 : 1 and centrifuged at 1,500 g until the discoloration of the extract (Macías-Sánchez *et al*. 2008). Pigment spectra were measured in the combined supernatant. The pigment concentrations were calculated by formulae using ODs at wavelengths corresponding to the absorption maxima (640 – 660 nm) of chlorophyll *a*.

Data analysis

Statistical treatment of data obtained was

performed using a BioStat 2009 Professional 5.8.1 software in accordance with recommendations (Gtantz *et al.* 1998; Gubler and Genkin 1973). All values were measured in six-fold repeatability. Results are presented as the median and the interval between the first and third quartiles. A validity check for the null hypothesis was performed using non-parametric Wilcoxon-Mann-Whitney criteria. The difference between control and experimental groups was judged to be statistically significant at $P < 0.05$.

Results

Biomass accumulation is the first and indicative criterion for assessing the effect of compounds on algal cultures. The Table 1 showed the results of *A. dimorphus* and *D. armatus* biomass accumulation in the presence of $CeCl_3$ solution, citrate or CDN in the cultivation medium. Compared to the intact culture medium, the presence of CDN in the culture medium at a concentration of 0.001 – 10.0 mM resulted in a statistically significant increase of the *A. dimorphus* biomass by 20 – 75 %. Presence of CDN in *D. armatus* culture medium resulted in a 25 – 50 % increase of algae biomass in a significantly narrower concentration range of 0.01 – 1.0 mM. It should be noted that the increase in biomass in concentration 10 – 100 mM of CDN or citrate can be attributed to citrate, a stabilizer in CDN, as an organic substrate, then lower concentrations of 0.001 – 1.0 mM were not caused by the presence of citrate, but rather by CDN. It is interesting to note that the introduction of 0.001 mM citrate into the culture medium statistically significantly inhibited the accumulation of biomass (growth) of the culture compared to the culture grown on a standard medium. The use of CDN stabilized with citrate (M : M) in a similar concentration on the contrary – increased the biomass yield by 20 – 30 %. At the same concentration, cerium salt did not affect accumulation of *A. dimorphus* biomass (Table 1).

Table 1. CDN, citrate, or $CeCl_3$ effect on the biomass accumulation in tested green algae.

The test objects concentration [mM]	***A. dimorphus***			***D. armatus***		
	CDN	**citrate**	**$CeCl_3$**	**CDN**	**citrate**	**$CeCl_3$**
0	9.7 [9.3 - 10.0]			11.4 [10.7 - 11.6]		
0.001	**12.6*** [12.1 - 12.6]	**7.6**** [6.4 - 8.0]	9.5 [9.3 - 9.8]	**12.0**** [11.9 - 12.1]	10.2 [9.6 - 10.7]	10.7 [10.6 - 10.8]
0.01	**14.2*** [14.0 -14.3]	9.0 [8.1 - 10.0]	**11.0*** [10.2 - 11.9]	**14.5*** [14.1 - 14.8]	10.7 [10.6 - 10.8]	10.7 [10.1 - 11.8]
0.1	**15.2**** [14.7 - 15.8]	**10.5*** [10.3 - 11.0]	**12.28**** [11.1 - 12.6]	**15.1*** [14.3 - 15.8]	11.3 [11.0 - 11.8]	12.1 [11.3 - 12.6]
1	**16.9**** [16.5 - 17.8]	**11.0*** [10.3 - 11.6]	**14.5**** [14.0 - 15.1]	**20.0*** [19.5 - 20.7]	11.5 [11.1 - 12.0]	**13.9*** [13.2 - 14.0]
10	**12.0*** [11.9 - 12.1]	**13.6**** [13.4 - 14.1]	**11.5*** [11.2 - 11.8]	**12.8**** [12.7 - 13.2]	**12.9**** [12.9 - 13.0]	11.9 [10.9 - 12.9]
100	**11.1*** [10.5 - 11.2]	**11.0*** [11.0 - 11.2]	9.9 [9.7 - 10.6]	12.0 [11.2 - 12.4]	11.0 [10.4 - 11.7]	11.0 [10.9 - 11.1]

Notes: * – $P < 0.05$; ** – $P < 0.01$, n = 6, results presented as the median and the interval between the first and third quartiles [Q1-Q3].

The maximum quantity of biomass of both cultures was observed when CDN was applied in an amount of 1 mM. Both algocultures showed a similar reactive response to exposure to all factors, some numerical differences being due to the species specifics of the algocultures. Thus, in *A. dimorphus* culture, the amount of biomass increased by 60 % upon CDN exposure, while in *D. armatus* culture it increased by 45 %. The results obtained clearly correlate with the amount of chlorophyll *a* in *A. dimorphus* and *D. armatus* cells (Table 2).

Table 2. CDN, citrate, or $CeCl_3$ effect on chlorophyll *a* concentration in green algae cells.

The test objects concentration [mM]	*A. dimorphus*			*D. armatus*		
	CDN	**citrate**	**$CeCl_3$**	**CDN**	**citrate**	**$CeCl_3$**
0	10.5 [10.2 - 11.0]			10.3 [10.0 - 10.6]		
0.001	11.0 [10.9 - 11.7]	10.1 [8.8 - 11.2]	10.1 [9.7 - 10.6]	9.9 [9.7 - 10.1]	10.0 [9.7 - 10.4]	9.9 [9.3 - 10.5]
0.01	10.4 [10.0 - 11.0]	10.3 [9.6 - 11.1]	10.4 [10.1 - 10.9]	10.3 [10.0 - 10.6]	9.9 [9.7 - 10.1]	10.6 [10.0 - 11.0]
0.1	10.0 [9.5 - 11.5]	11.1 [10.7 - 11.4]	10.6 [10.3 - 10.9]	10.7 [10.1 - 10.9]	9.9 [9.2 - 10.5]	9.9 [9.4 - 10.0]
1	10.2 [9.8 - 10.6]	10.9 [10.1 - 11.6]	10.5 [10.4 - 11.1]	9.7 [9.5 - 10.4]	9.4 [9.2 - 10.6]	10.0 [9.7 - 10.6]
10	11.0 [10.7 - 11.2]	10.6 [10.4 - 10.9]	**6.7**** [6.5 - 7.0]	9.9 [9.2 - 10.1]	**9.0*** [8.8 - 9.8]	**8.7**** [8.1 - 8.9]
100	10.6 [10.3 - 11.3]	10.8 [10.4 - 11.0]	**6.1**** [5.7 - 6.8]	10.4 [10.0 - 10.9]	**9.5*** [8.7 - 9.9]	**7.9**** [7.3 - 8.0]

Notes: * – $P < 0.05$; ** – $P < 0.01$, n = 6, results presented as the median and the interval between the first and third quartiles [Q1-Q3].

Its amount does not significantly differ from the control values, except for the concentration variants in which the growth activity of both algocultures is inhibited (10 – 100 mM). But even under these conditions, a decrease in the amount of chlorophyll *a* was noted when exposed to $CeCl_3$, while in the presence of CDN, on the contrary, the negative effect of high concentrations of cerium ions on algocultures is compensated. Despite the positive effect of cerium both in ionic form and in the form of nanoparticles on the growth activity of *A. dimorphus* and *D. armatus*, a biochemical analysis showed some negative effect of $CeCl_3$ on the process of protein accumulation in the algal biomass (Table 3).

Table 3. The effect of CDN, citrate or $CeCl_3$ on the protein content (% of biomass) in the biomass of tested green algae.

The test objects concentration [mM]	*A. dimorphus*			*D. armatus*		
	CDN	**citrate**	**$CeCl_3$**	**CDN**	**citrate**	**$CeCl_3$**
0	**49.9 [48.6 - 50.9]**			**56.7 [56.0 - 57.5]**		
0.001	50.0 [46.1 - 51.2]	50.9 [49.5 - 51.5]	**33.9*** [33.3 - 34.8]	**53.5*** [53.3 - 54.6]	**52.4*** [51.9 - 53.6]	**46.0**** [45.5 - 46.8]
0.01	50.9 [49.3 - 51.4]	50.7 [49.6 - 51.8]	**34.1*** [33.1 - 35.0]	**54.5*** [53.4 - 55.7]	**54.6*** [53.5 - 55.2]	**46.5**** [46.0 - 47.5]
0.1	49.6 [49.2 - 50.7]	50.0 [48.3 - 51.0]	**33.5*** [32.8 - 34.7]	**58.0*** [57.8 - 58.1]	**54.4*** [53.5 - 55.3]	**44.3**** [43.9 - 44.8]
1	**44.5**** [42.7 - 45.7]	47.3 [47.0 - 48.6]	**32.5*** [31.3 - 33.5]	**54.5*** [52.5 - 55.3]	**53.6*** [52.4 - 54.7]	**43.5**** [42.3 - 44.0]
10	**37.0**** [35.1 - 38.0]	**45.0*** [44.1 - 45.8]	**25.3*** [24.1 - 26.0]	**52.8**** **[52.2 - 53.1]**	**50.5**** **[50.0 - 51.6]**	[39.9 - 42.1]
100	**28.5*** [27.9 - 30.5]	**43.6*** [43.1 - 44.7]	**18.1*** [17.5 - 22.1]	**50.5**** **[49.6 - 51.7]**	**50.9**** **[50.3 - 51.1]**	**40.0**** [38.9 - 41.6]

Notes: * – $P < 0.05$; ** – $P < 0.01$, n = 6, results presented as the median and the interval between the first and third quartiles [Q1-Q3].

Thus, a rather low content of total protein in cells was noted in the presence of CDN ≥ 1 mM. The use of nanocerium in a smaller amount does not reliably reduce the protein content in algal cells. The use of $CeCl_3$ in all cases is accompanied by a decrease in the amount of protein in *A. dimorphus* cells by 25 – 55 %, and in *D. armatus* cells by 10 – 25 %. While the amount of protein tended to

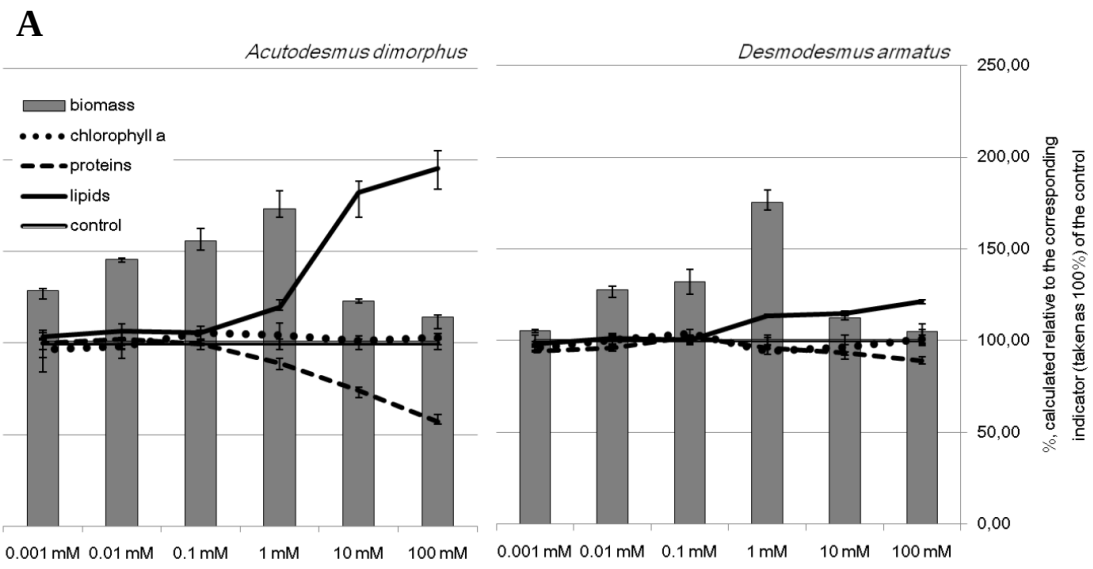
decline, there was an increase in the accumulation of lipids in the biomass of both cultures (Table 4).

Table 4. The effect of CDN, citrate or $CeCl_3$ on the lipid content (% of biomass) in the biomass of tested green algae.

The test objects concentration, mM	*A. dimorphus*			*D. armatus*		
	CDN	**citrate**	**$CeCl_3$**	**CDN**	**citrate**	**$CeCl_3$**
0	**15.2 [14.6 - 15.6]**			**16.1 [15.8-16.7]**		
0.001	15.8 [15.5 - 16.1]	15.3 [14.7 - 15.9]	**16.6*** [16.0 - 17.4]	**19.5**** [18.7 - 20.1]	**19.6**** [19.1 - 20.7]	**29.0**** [28.1 - 29.7]
0.01	16.2 [14.8 - 16.8]	**16.3*** [15.9 - 17.2]	**19.0**** [18.9 - 19.5]	**18.4*** [18.0 - 18.9]	**17.9*** [17.2 - 18.6]	**29.8**** [29.0 - 30.0]
0.1	16.1 [15.2 - 16.3]	**16.0*** [15.8 - 16.6]	**22.9**** [22.8 - 23.6]	**18.2*** [17.9 - 18.7]	17.8 [16.5 - 18.7]	**21.5**** [21.0 - 22.0]
1	**18.2*** [18.0 - 18.9]	**17.1*** [16.8 - 17.3]	**28.4**** [27.7 - 29.5]	16.1 [15.2 - 16.3]	16.9 [16.3 - 17.3]	**18.9**** [18.2 - 20.0]
10	**27.8**** [25.7 - 28.7]	**16.4*** [15.9 - 17.0]	**31.1**** [30.8 - 31.6]	16.2 [14.8 - 16.8]	16.0 [15.7 - 16.6]	**16.9*** [16.8 - 17.3]
100	**29.8**** [28.1 - 31.3]	**17.0*** [16.7 - 17.6]	**31.3**** [30.2 - 32.5]	15.8 [15.5 - 16.1]	15.6 [15.1 - 16.0]	16.4 [16.0 - 16.9]

Notes: * – $P < 0.05$; ** – $P < 0.01$, $n = 6$, results presented as the median and the interval between the first and third quartiles [Q1-Q3].

There is a clear correlation between the amount of $CeCl_3$ in the medium and the increase in the number of total lipids in *A. dimorphus* and *D. armatus* cells. It should be noted that judging by changes in the number of total lipids in algal biomass in response to the presence of cerium ions. *A. dimorphus* culture is more sensitive than that of *D. armatus*. Thus, in the presence of 0.01 mM $CeCl_3$ in the culture medium the amount of total lipids in *A. dimorphus* cells increases by 20 % while in the presence of ≥1 mM of $CeCl_3$ this indicator increases by almost 100 %. CDN does not have such a strong impact on lipid accumulation by algae cells, which can be considered as an indirect sign of its lower toxicity. In the presence of 10 – 100 mM of CDN in the culture medium an increase of 80 – 90 % in the number of lipids in *A. dimorphus* biomass compared to control samples biomass was recorded. Thus, the results obtained allowed us to estimate the effect of CDN on cells of two green algae. *A. dimorphus* and *D. armatus.* Having compared all indicators. We determined the optimal CDN concentrations which when applied to bring an increase of the biomass in both algocultures and optimize this biomass by productivity outputs (Fig. 2).



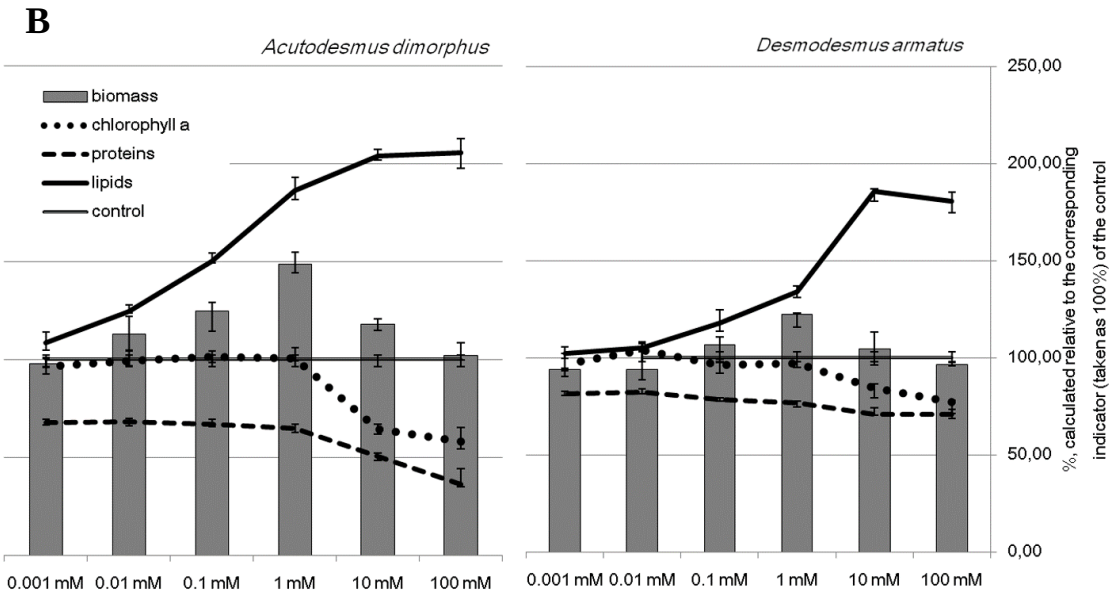


Fig. 2. The effect of CDN (**A**) or $CeCl_3$ (**B**) on key indicators of the green algae viability. The data on the content of biomass, chlorophyll *a*, proteins, and lipids in percent are given, calculated relative to their amount in the control samples of biomass grown on a standard medium.
Notes: on the abscissa – the concentration of cerium; on the y-axis – the percentage of content of biomass, chlorophyll *a*, proteins, and lipids in percent, calculated relative to their amount in the control samples of biomass grown on a standard medium (taken as 100 %), n = 6, error bars indicate [Q1-Q3] of the mediane.

It was determined that 0.1 mM of CDN is the optimal amount of the preparation that causes positive changes in cells *A. dimorphus* and *D. armatus*. Under such conditions, it is possible not only to increase the yield of biomass of algal crops, but also to achieve balanced levels of chlorophyll *a*, proteins, and lipids. The concentration of 1 mM of CDN can be considered as suboptimal, recommended for use in cases of an urgent need for rapid mass growth of cell mass of green algae *A. dimorphus* and *D. armatus*. The use of $CeCl_3$ in all analyzed concentrations leads to increased growth activity of algocultures, but also in all cases there is negatively affects the processes of proteins and lipids accumulation. The generalized results are presented in the form of a summary table, which allows to clearly determine, depending on the goal, the dose of CDN or $CeCl_3$ (Table 5).

Table 5. The nontoxic (CC_0. mM) concentration of CDN and $CeCl_3$.

Criteria	*Acutodesmus dimorphus*			*Desmodesmus armatus*		
	CDN	**citrate**	**$CeCl_3$**	**CDN**	**citrate**	**$CeCl_3$**
Chlorophyll *a*	**100.0**	100.0	**1.0**	**100.0**	1.0	**1.0**
Total proteins	**0.1**	0.5	**<0.001**	**<0.001**	<0.001	**<0.001**
Total lipids	**0.1**	0.001	**<0.001**	**<0.001**	<0.001	**<0.001**
Biomass increasing (> 20 %)	**<0.001 – 1.0**	–	**0.1 – 1.0**	**0.01 – 1.0**	–	**1.0**

Discussion

Like most trace elements, Ce depending on the concentration can have both positive and negative effects on the growth and development of algae. It is known that in the aqueous medium NP CeO_2 undergo redox modifications, form polydisperse aggregates, which lead to a change in their catalytic activity (Santschi *et al.* 2017; Gagnon *et al.* 2018; Rozhin *et al.* 2021).

First of all, it should be noted that the sharp increase in the amount of lipids found in *D. armatus* and *A. dimorphus* cells is a well-known response of algae cells to stress, in our case to the presence of high concentrations of cerium in the growth medium. A similar increase in the amount

of lipids in response to the presence of alkaline earth metals has been described for *Chlorella vulgar*is and *Scenedesmus obliquus* (Gorain *et al*. 2013). *Dunaliella tertiolecta,* and *Tetraselmis suecica* (Ghafari *et al*. 2016). Stress activation of metabolic pathways of lipid synthesis by Ce^{3+} ions may be due to inhibition of further conversion of the intermediate product of glycolysis – glyceraldehyde-3-phosphate. In this case, instead of the synthesis of carbohydrates, the synthesis of triacylglycerols (Wang *et al*. 2009; Li *et al*. 2010) will take place, which will be accompanied by an increase in the accumulation of lipids in the cells of microalgae. As a consequence of such processes, the redistribution of the relation between the major macromolecules in the cells of algae, which we found.

It is known that high concentrations ($\approx 30\ \mu M.L^{-1}$) of lanthanides lead to a sharp slowdown in the growth processes of unicellular algae (Tai *et al*. 2010), while low concentrations stimulate these processes (Laszló *et al*. 2001; Goecke *et al*. 2015; Řezanka *et al*. 2016). In our studies it was shown that in the presence of ≤ 1 mM cerium concentration in the medium, there is an intensification of growth processes in cultures *D. armatus* and *A. dimorphus* and, as a consequence an increase in the amount of biomass by more than 25 %. However, the effect of any chemical element depends also on the form of its introduction into the incubation medium (Chen *et al*. 2000; Tai *et al*. 2010). $CeCl_3$ has a more pronounced toxic effect on algae cultures than CDN. A similar effect was shown by zirconium on the cells of the alga *Chlorella pyrenoidosa* (Laszló *et al*. 2001).

Lanthanides can also replace certain metals in some physiological processes, as has been demonstrated, in particular, in plants with Ca^{2+} or Mg^{2+} deficiency (Huang *et al*. 2008a; Chao *et al*. 2008; Gong *et al*. 2011) The primary cause of this phenomenon may be the property of lanthanides, whose combinatorial ability is much higher than that of other divalent cations, to interact with a large number of biological macromolecules to form stable complexes (Goecke *et al*. 2015). It is believed that the ability of lanthanides, including cerium, to mimic some biological functions of Ca^{2+}, primarily affects the processes of photosynthesis and ion transport in plant cells (Wei *and* Zhou 2000; Gong *et al*. 2011). Obviously, this property of cerium was decisive in the manifestation of the stimulating effect on the growth processes of green algae cultures *D. armatus* and *A. dimorphus*, studied by us. The stimulating effect of Ce on photosynthetic organisms is associated with the ability of the latter to accumulate in chloroplasts as well at low concentrations of Mg^{2+} to replace it in the chlorophyll molecule (Zhou *et al*. 2011). The effects we found are most likely related to an increase in carbohydrate production. The mechanism of this process is due to the influence of Ce ions on intracellular anabolic pathways in particular, transmebranic mitochondrial transport of pyruvate and oxaloacetate production. It is known that rare earth metals in general, and cerium in particular, significantly improve the process of light energy absorption, energy transfer, electron transfer rate and phosphorylation rate, thus stimulating the accumulation of organic matter in cells (Zeng *et al*. 2000; Gong *et al*. 2011).

Conclusion

Compared to CDN, $CeCl_3$ solution is more toxic to green algae cells. CDN is 100 – 1,000 times less toxic than $CeCl_3$ solution. It is shown that the use of high concentrations of Ce is accompanied by a well-known response of algae cells to the action of a stress factor – an increase in total lipids and a decrease in the synthesis of proteins and chlorophyll *a*. The introduction of CDN into the culture of algae in non-toxic concentrations (CC_0 and below) provides a significant increase (25 – 75 %) in the total amount of biomass, whereas with the use of $CeCl_3$ solution such an effect is absent. The obtained results allow assessing the mechanism of realization of biological effects of cerium dioxide nanoparticles.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

Borisevich VB, Kaplunenko VG, Kosinov NV, Borisevich

BV, Sukhonos VP, Khomin NM, Teliatnikov AV, Voloshina NA, Tkachenko SM, Doroshchuk VA, Korzh AV, Litvinenko DI, Kulida MA, Kulinich SL, Borosevich VB Jr, Borisevich IB, Dimchev VA (2012) Nanomaterials and nanotechnologies in the veterinary practice. Kyiv: VD «Avitsena». 512 p.

Chao L, Bofu P, Weiqian C, Hao H, Liang C, Xiaoqing L, Xiao W, Faishu H (2008) Influences of calcium deficiency and cerium on growth of spinach plants. Biol. Trace Elem. Res. 121: 266-275.

Chao L, Weiqian C, Yun L, Hao H, Liang C, Xiaoqing L, Fashui H (2009) Cerium under calcium deficiency – influence on the antioxidative defense system in spinach plants. Plant Soil. 323: 285-294.

Charbgoo F, Ahmad MB, Darroudi M (2017) Cerium oxide nanoparticles: green synthesis and biological applications. Int. J. Nanomed. 20: 1401-1413.

Cheban L, Grynko O (2017) Use of *Acutodesmus dimorphus* (Turpin) Tsarenko as a fodder organism for *Daphnia* growing. Acta Biol. Univ. Daugavp. 17: 141-148.

Cheban L, Grynko O, Dorosh I (2018) Co-cultivation of *Daphnia magna* (Straus) and *Desmodesmus armatus* (Chod.) Hegew. in recirculating aquaculture system wastewater. Fish. Aquat. Life 26: 57-64.

Chen WJ, Gu YH, Zhao GW. Tao Y, Luo JP, Hu TD (2000) Effects of rare earth ions on activity of RuBPcase in tobacco. Plant Sci. 152: 145-151.

Chen WJ, Tao Y, Gu YH, Zha GW (2001) Effect of lanthanide chloride on photosynthesis and dry matter accumulation in tobacco seedlings. Biol. Trace Elem. Res. 79: 169-176.

Gagnon C, Bruneau A, Turcotte P, Pilote M, Gagné F (2018) Fate of cerium oxide nanoparticles in natural waters and immunotoxicity in exposed rainbow trout. J. Nanomed. Nanotechnol. 9: 2.

Ghafari M, Rashidi B. Haznedaroglu BZ (2016) Effects of macro and micronutrients on neutral lipid accumulation in oleaginous microalgae. Biofuels. 9: 147-156.

Goecke F, Jerez C, Zachleder V, Figueroa FL, Bišová K, Řezanka T, Vítová M (2015) Use of lanthanides to alleviate the effects of metal ion-deficiency in *Desmodesmus quadricauda* (*Sphaeropleales*, *Chlorophyta*). Front. Microbiol. 6: 1-12.

Gong X, Hong M, Wang Y, Zhou M, Cai J, Liu C, Gong S, Hong F (2011) Cerium relieves the inhibition of photosynthesis of maize caused by manganese deficiency. Biol. Trace Elem. Res. 141: 305-316.

Gorain PC, Bagchi SK, Mallick N (2013) Effects of calcium magnesium and sodium chloride in enhancing lipid accumulation in two green microalgae. Environ. Technol. 3: 1887-1894.

Gtantz S (1998) Medical-biological Statistics. Praktika, Moscow.

Gubler EV, Genkin AA (1973) Application of non-parametric statistic criteria in medical-biological research. Medicine, Moscow, 142 p.

Hevorhyz RH, Shchepachyov SH (2008) Metodyka yzmerenyia plotnosty suspenzyy nyzshykh fototrofov na dlyne volny sveta 750 nm. Sevastopol: Otdel byotekhnolohyy y fytoresursov YnBIuM NAN Ukraynu. (In Russian).

Hong F, Wei Z, Zhao G (2002) Mechanism of lanthanum effect on chlorophyll of spinach. Sci. China C. Life Sci. 45: 166-76.

Huang H, Liu XQ, Qu CX, Liu C, Chen L, Hong FS (2008) Influences of calcium deficiency and cerium on the conversion efficiency of light energy of spinach. Biometals 21: 553-561.

Ivanov VK, Polezhaeva OS, Shaporev AS, Baranchikov AE, Shcherbakov AB, Usatenko AV, (2010) Synthesis and thermal stability of nanocrystalline ceria sols stabilized by citric and polyacrylic acids. Russ. J. Inorg. Chem. 55: 328-332.

Ivanov VK, Usatenko AV, Shcherbakov AB (2009) Antioxidant activity of nanocrystalline cerium dioxide against anthocyanins. J. Inorg. Chem. 54. 10: 1596-1601.

Jakupec MA, Unfried P, Keppler BK (2005) Pharmacological properties of cerium compounds. Rev. Physiol. Biochem. Pharmacol. 153: 101-111.

Jreije I, Azimzada A, Hadioui M, Wilkinson K J (2020) Measurement of CeO_2 nanoparticles in natural waters using a high sensitivity, single particle ICP-MS. Molecules 25: 5516.

Kang T, Kim Y G, Kim D, Hyeon T (2020) Inorganic nanoparticles with enzyme-mimetic activities for biomedical applications. Coord. Chem. Rev. 403: 1-21.

Knight JA, Anderson S, Rawle JM (1972) Chemical basis of the sulfo-phospho-vanillin reaction for estimating total serum lipids. Clin. Chem. 18: 199-202.

Kosak N, Röhder LA, Brandt T, Sigg L, Behra R (2018) Uptake and effects of cerium (III) and cerium oxide nanoparticles to *Chlamydomonas reinhardtii*. Aquat. Toxicol. 197: 41-46.

Laszló S, Fodor M, Pais I (2001) Effects of zirconium on the growth and photosynthetic pigment composition of *Chlorella pyrenoidosa* green algae. J. Plant Nutr. 24: 159-174.

Li YT, Han DX, Hu GR, Sommerfeld M, Hu QA (2010) Inhibition of starch synthesis results in overproduction of lipids in *Chlamydomonas reinhardtii*. Biotechnol. Bioeng. 107. 2: 258-268.

Lowry OH, Rosebrough NJ, Farr AL. Randall RJ (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275.

Macías-Sánchez MD, Mantell Serrano C, Rodrígues Rodrígues M, Martínez de la Ossa E, Lubián LM, Montero O (2008) Extraction of carotenoids and chlorophyll from microalgae with supercritical carbon dioxide and ethanos as cosolvent. J. Separ. Sci. 31: 1352-1362.

Řezanka T, Kaineder K, Mezricky D, Řezanka M, Bišová K, Zachleder V, Vítová M (2016) The effect of lanthanides on photosynthesis. growth. and chlorophyll profile of the green alga *Desmodesmus quadricauda*. Photosynth. Res. 130: 335-346.

Rozhin P, Melchionna M, Fornasiero P, Marchesan S (2021) Nanostructured Ceria: Biomolecular tmeplates and (Bio)applications. Nanomater. 11: 2-34.

Santschi C, Von Moos N, Koman VB, Slaveykova VI, Bowen P, Martin OJ (2017) Non-invasive continuous monitoring of pro-oxidant effects of engineered nanoparticles on aquatic microorganisms. J. Nanobiotechnol. 7. 15: 19.

Shcherbakov AB, Zholobak NM, Ivanov VK (2020) Biological. biomedical and pharmaceutical applications of cerium oxide. *In* Scirè S, Palmisano L (Eds.), Cerium Oxide (CeO_2): Synthesis. Properties and Applications, Elsevier, pp. 279-358.

Tai P, Zhao Q, Su D, Li P Stagnitti F (2010) Biological toxicity of lanthanide elements on algae. Chemosphere 80: 1031-1035.

Wang ZT, Ullrich N, Joo S Waffenschmidt S. Goodenough U (2009) Algal lipid bodies: stress induction, purification, and biochemical characterization in wild-type and starchless *Chlamydomonas reinhardtii*. Eukaryot. Cell. 12: 1856-1868.

Wei YZ, Zhou XB (2000) Effect of neodymium on physiological activities in oilseed rape during calcium starvation. J. Rare Earths 18: 57-61.

Yin S, Ze Y, Liu C, Li N, Zhou M, Duan Y, Fashui H (2009) Cerium relieves the inhibition of nitrogen metabolism of spinach caused by magnesium deficiency. Biol. Trace Elem. Res. 132: 247-258.

Yuguan Y, Sitao Y, Zhe J, Luyang L, Chao L, Fashui H (2009a) Influences of magnesium deficiency and cerium on antioxidant system of spinach chloroplasts. Biometals 22: 941-949.

Yuguan Z, Min Z, Luyang L, Chao L, Sitao Y, Yanmei D, Na L, Fashiu H (2009b) Effects of cerium on key enzymes of carbon assimilation of spinach under magnesium deficiency. Biol. Trace Elem. Res. 131: 154-164.

Zeng FL, An Y, Ren L, Deng RW, Zhang MF (2000) Effects of lanthanum and calcium on photoelectron transport activity and the related protein complexes in chloroplast of cucumber leaves. Biol. Trace Elem. Res. 77: 83-91.

Zholobak NM, Olevynskaia ZM, Spivak NYa, Shcherbakov AB, Ivanov VK, Usatenko AV (2010) Antiviral action of cerium oxide nanoparticles stabilized with low molecular weight polyacrylic acid. Microbiol. J. 72: 42-47.

Zhou M, Gong X, Wang Y, Liu C, Hong M, Wang L, Fashui H (2011) Cerium relieves the inhibition of chlorophyll biosynthesis of maize caused by magnesium deficiency. Biol. Trace Elem. Res. 143: 468-477.

Zolotareva O, Shnyukova E, Sivash O, Mihaylenko N (2008) Prospects of use of microalgae in biotechnology. Alterpres, Kyiv, 234 p.