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## **THE INFLUENCE OF HEAVY METAL IONS ON ABSORPTION SPECTRA OF CHLOROPHYLL FROM *EUGLENA GRACILIS* (IN VITRO)**

Heavy metal environmental pollution is one of the most important ecological problems. Photosynthetic organisms are frequently used as test systems, among which the preference is given to microorganisms such as *E. gracilis*. The changes of physiological characteristics of cells are used as criteria for evaluation of aquatic environmental pollution. At the same time, to understand the essence of physiologic response on the influence of heavy metal ions it is necessary to understand the mechanisms of interactions of pollutant with biochemical systems of a cell.

In this research the influence of  $\text{Fe}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Cu}^{2+}$  on spectral properties of chlorophyll isolated from *E. gracilis* culture is studied. The results showed that the mentioned above ions replace  $\text{Mg}^{2+}$  in chlorophyll molecule. This causes the decrease of absorption maxima at 666 nm. The most significant spectral changes were observed when chlorophyll interacted with  $\text{Fe}^{2+}$ , while  $\text{Cu}^{2+}$  had the least impact. The assumption is given that the values of effective ionic radii and redox potential of pair of  $\text{Me}^{2+}/\text{Me}^0$  influence the efficiency of interaction of heavy metal ions with molecules of chlorophyll. This interaction can be shown by the changes in absorption spectra of chlorophyll.

**Key words:** electronic absorption spectra, chlorophyll, *Euglena gracilis*, ions of heavy metals, effective ionic radii, redox potential.

**Introduction.** Nowadays remediation of environment is one of the most actual challenges. High plants as well as aquatic photosynthetic organisms like algae and cyanobacteria can be used for such purposes. Ions of heavy metals such as  $\text{Cr}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Mn}^{2+}$  are essential microelements for photosynthetic microorganisms. High concentrations of these ions are toxic and significantly influence metabolism of aquatic photosynthetic microorganisms [1]. The studies with chloroplasts showed that ions of heavy metals cause damages in photosystem I (PS I) and photosystem II (PS II) [2, 3]. These damages can be observed in electronic absorption spectra of chlorophyll (Chl).

According to *in vitro* experiments, majority of  $\text{Cu}^{2+}$  interact with the component of PS II, and this system is more sensitive to toxicity of these ions in comparison to PS I. It was detected that  $\text{Cu}^{2+}$  inhibits electronic transport [4, 5]. In *in vivo* experiments it was expressed that  $\text{Zn}^{2+}$  cause activation of chlorophyllase which leads to the decrease of Chl concentration in cells of *E. gracilis* [6].  $\text{Fe}^{2+}$  has negative impact on photosynthetic organisms only at very high concentrations [7]. The content of Chl in cells is a very important indicator for the estimation of metabolic activity of photosynthetic aquatic microorganisms and it is used for the determination of photosynthetic and respiratory rates. In order to assert the influence of heavy metal ions

on photosynthetic apparatus of aquatic microorganisms, it is necessary to know which spectral changes occur during interaction of Chl with heavy metal ions. The study of the spectral changes of these interactions was the aim of this research.

**Main part.** In this study the strain of *E. gracilis* was used from the collection of Belarusian State University.

The algae were grown in the medium with the composition:  $(\text{NH}_4)_2\text{SO}_4$ ,  $\text{K}_2\text{HPO}_4$  and  $\text{MgSO}_4$  each for 0.1 g, 1 ml of soil extract and 3 drops of 1% aquatic solution of  $\text{FeSO}_4$  for 100 ml of media at pH 6.0. We added 50 ml of *E. gracilis* culture to 300 ml of such media and incubated at  $(25 \pm 2)^\circ\text{C}$  with day light during 10 d.

For the extraction of Chl the cells were separated by centrifuge at 6,000 rpm, the supernatant was deleted, and the sediment resuspended in  $\times 20$  volume of 70% aquatic acetone solution. After it was incubated for 10 min at room temperature and again centrifuged for 10 min at 6,000 rpm. Then the supernatant was separated and used in spectrophotometric experiments.

The tests were done in cuvettes with 1 cm width at spectrophotometer Specord 200 Plus at the range of wave length 450–800 nm at room temperature. We placed 2.5 ml of Chl solution into cuvette and then successively added to it the equal volumes of 50  $\mu\text{l}$  of heavy metal salt solu-

tion with concentration  $10^{-4}$  M. Each portion of heavy metal salt solution was added 20 min after the previous addition. The result concentrations of heavy metal ions were  $1.96 \cdot 10^{-6}$ ,  $3.85 \cdot 10^{-6}$  and  $5.65 \cdot 10^{-6}$  M after the first, the second and the third additions, respectively.

The absorption spectra were registered 20 min after the addition of each new portion.

Chl concentration was determined by the measurement of absorption of extract at absorption

maxima 666 nm using extinction coefficient  $\varepsilon = 82.04 \text{ L} \cdot \text{g}^{-1} \cdot \text{cm}^{-1}$  [8].

According to the results, heavy metal ions caused the decrease of absorption intensity at 666 nm. However,  $\text{Fe}^{2+}$  caused more significant changes in different regions of spectra, what we think can be due to the light scattering because of formation of Fe (III) hydroxide.

In Fig. 1 electronic absorption spectra of Chl in presence of  $\text{Cu}^{2+}$  is illustrated.

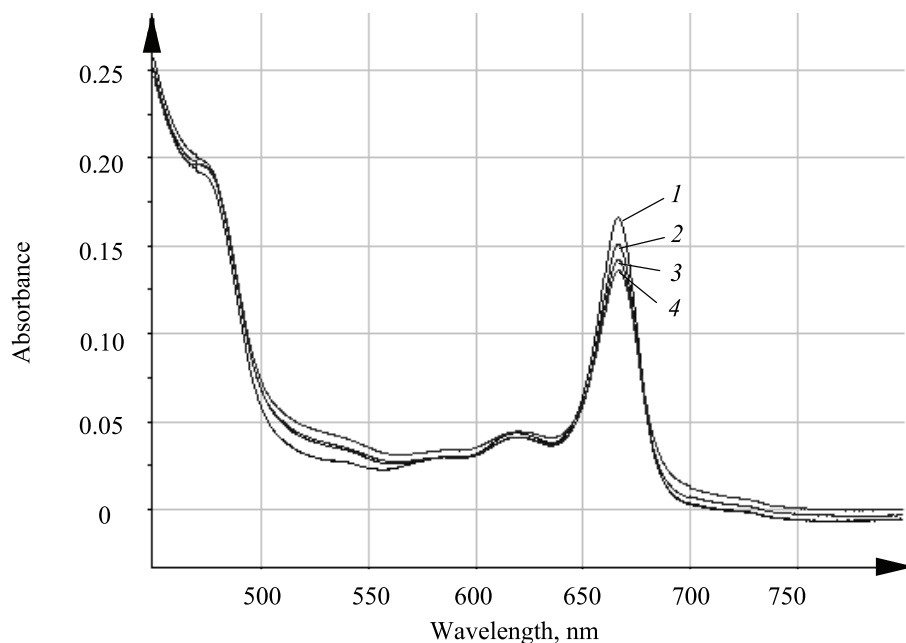


Fig. 1. Electronic absorption spectra of Chl in presence of  $\text{Cu}^{2+}$ :  
1 – control; 2 –  $1.96 \cdot 10^{-6}$  M  $\text{CuSO}_4$ ; 3 –  $3.85 \cdot 10^{-6}$  M  $\text{CuSO}_4$ ; 4 –  $5.65 \cdot 10^{-6}$  M  $\text{CuSO}_4$

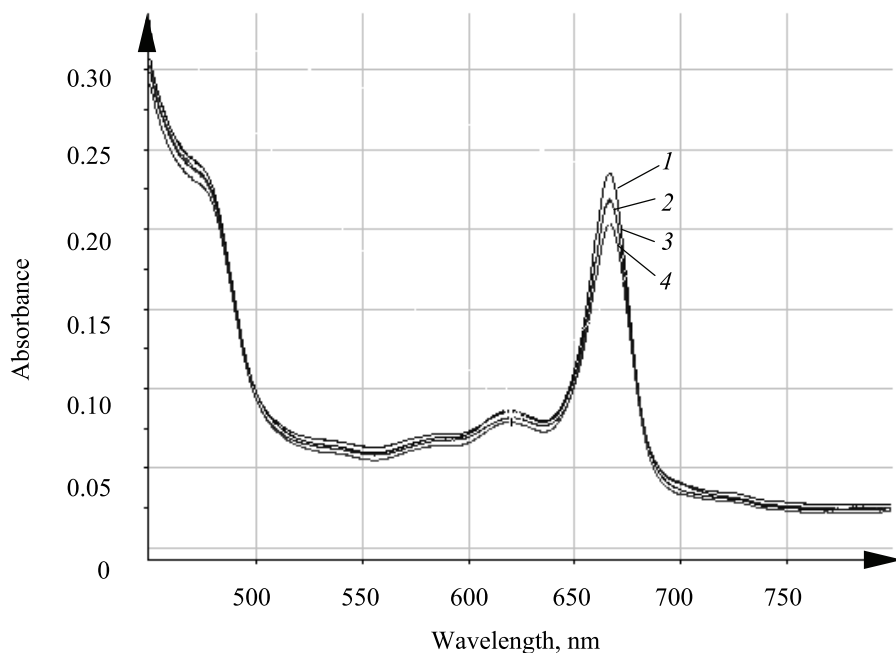


Fig. 2. Electronic absorption spectra of Chl in presence of  $\text{Zn}^{2+}$ :  
1 – control; 2 –  $1.96 \cdot 10^{-6}$  M  $\text{ZnSO}_4$ ; 3 –  $3.85 \cdot 10^{-6}$  M  $\text{ZnSO}_4$ ; 4 –  $5.65 \cdot 10^{-6}$  M  $\text{ZnSO}_4$

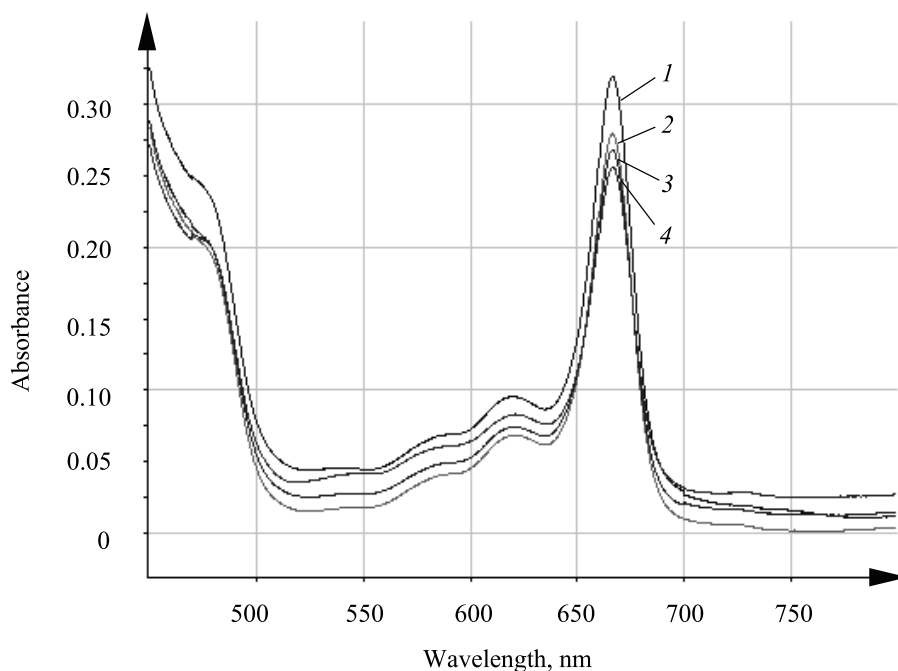


Fig. 3. Electronic absorption spectra of Chl in presence of  $\text{Fe}^{2+}$ :  
 1 – control; 2 –  $1.96 \cdot 10^{-6}$  M  $\text{FeSO}_4$ ; 3 –  $3.85 \cdot 10^{-6}$  M  $\text{FeSO}_4$ ; 4 –  $5.65 \cdot 10^{-6}$  M  $\text{FeSO}_4$

It is clear from Fig. 1 that intensity of absorption at 666 nm decreases with increase in concentration of  $\text{Cu}^{2+}$ .

De Filippis showed that copper is able to replace the magnesium in the Chl molecule [9]. He observed it by the decrease of absorption intensity as well as by the shift of absorption spectra to the shorter wave length region. However, in our experiments such shift was not noticed.

Fig. 2 and 3 show the results of experiments with interaction between Chl and  $\text{Zn}^{2+}$ , Chl and  $\text{Fe}^{2+}$ , respectively.

In Chl structure  $\text{Mg}^{2+}$  can be replaced by heavy metal ions, namely by  $\text{Zn}^{2+}$ . Toxic concentrations of  $\text{Zn}^{2+}$  also decrease the levels of Chl *a* and *b* as well as the Chl *a/b* ratio [10].

Addition of  $\text{FeSO}_4$  solution showed the decrease in absorption of Chl. Also, the transport of  $\text{Fe}^{2+}$  to the chloroplasts reduces the Chl content [11]. As well as the results of *in vivo* experiments with cyanobacteria *Spirulina platensis*, Babu et al. have reported the concentration dependent decrease of intensity absorption and fluorescence of Chl at 666 and 679 nm, respectively, when the cells were exposed to the action of  $\text{Ag}^+$  and  $\text{Cr}^{3+}$  [12].

The decrease in absorption intensity at 666 nm under the action of  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Fe}^{2+}$  can be due to the replacement of central atom in Chl molecule by these ions. The similar effect was observed by Petrovic [13]. Probably, the extent of Chl was damaged by metals correlates to the value

of standard redox potential of pair  $\text{Me}^{2+}/\text{Me}^0$ . The action of heavy metal ions in Chl is a kind of exchange between  $\text{Mg}^{2+}$  and  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Fe}^{2+}$  which results in a decrease of intensity of absorption at 666 nm.

This decrease is proportional concentration of metal ions which is shown in Fig. 4.

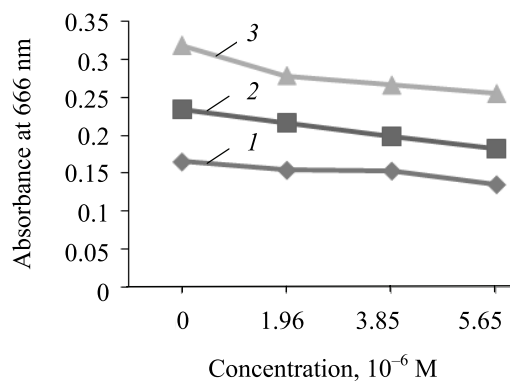


Fig. 4. Dependence of absorption on the concentration of salts:  
 1 –  $\text{CuSO}_4$ ; 2 –  $\text{ZnSO}_4$ ; 3 –  $\text{FeSO}_4$

The extent of the impact of these ions can be placed in the following order:  $\text{Cu}^{2+} < \text{Zn}^{2+} < \text{Fe}^{2+}$ . The extent of  $\text{Mg}^{2+}$  replacement in Chl molecule can be connected to the value of effective ionic radii ( $r_{\text{ion}}$ ) of divalent cation or redox potential ( $E^0$ ) of pair  $\text{Me}^{2+}/\text{Me}^0$  [14, 15]. These values are given in the Table.

**The effective ionic radii and redox potential**

| Me <sup>2+</sup>                  | Zn <sup>2+</sup>                  | Cu <sup>2+</sup>                  | Fe <sup>2+</sup>                  |
|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| r <sub>ion</sub> , pm             | 74                                | 73                                | 61                                |
| Me <sup>2+</sup> /Me <sup>0</sup> | Zn <sup>2+</sup> /Zn <sup>0</sup> | Cu <sup>2+</sup> /Cu <sup>0</sup> | Fe <sup>2+</sup> /Fe <sup>0</sup> |
| E <sup>o</sup> , V                | -0.76                             | +0.34                             | -0.44                             |

The substitution efficiency of Mg<sup>2+</sup> can be connected to the value of effective ionic radii of corresponding cation. According to Fig. 4, the highest and the lowest influences of heavy metal ions on the decrease of absorption intensity at 666 nm belong to Fe<sup>2+</sup> and Cu<sup>2+</sup>, respectively.

Also, it is expressed in the table that the value of redox potential does not correlate with changes of absorption intensity which means that there is

no direct dependency between redox potential and measured parameters.

**Conclusion.** We assume that Fe<sup>2+</sup> will be the most effective for the replacement of Mg<sup>2+</sup> in Chl molecule because of the fact that Fe<sup>2+</sup> has the least effective ionic radii. Both Zn<sup>2+</sup> and Cu<sup>2+</sup> have very close values of ionic radii, however Cu<sup>2+</sup> replaces the central atom in Chl molecule weaker than Zn<sup>2+</sup>. But among the investigating ions only the pair of Cu<sup>2+</sup>/Cu<sup>0</sup> has a positive redox value.

Thus, the tests have shown that the efficiency of the central atom replacement in Chl molecule by divalent cations depends on the effective ionic radii and redox potential of the pair Me<sup>2+</sup>/Me<sup>0</sup>. As further research, we are going to study such interactions in *in vivo* system with *E. gracilis*.

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Received 10.02.2015