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Influence of heavy metals on fluorescence activity of perspective strains of microalgae and cyanobacteria

Abstract: The article presents the results of a study of the effect of heavy metals on fluorescence activity of microalgae and cyanobacteria. Based on the results of the study determined that the collection of strains of microalgae and cyanobacteria 5 crops resistant to heavy metals, 4 cultures are more sensitive to the studied concentrations of heavy metals. Some strains under the influence of the zinc and copper was observed phenomenon of plasmolysis and deformation of the cells. Influence of heavy metals on cells of microalgae and cyanobacteria have identified a number of surveyed metals toxicity, which is as follows: $Cu^2 + > Zn^2 + > Co^2 + > Ni^{2+}$. From cultures of microalgae and cyanobacteria the following types of selected for further study, as promising strains for bioremediation of contaminated aquatic ecosystems, various heavy metals: *Phormidium autumnale* I-5, *Anabaena variabilis* RI-5, *Synechococcus elongatus* I-4, *Chlorella vulgaris* sp BB-2, *Chlamydomonas reinhardtii* B -4.
Key words: microalgae, cyanobacteria, heavy metals.

Introduction

Environmental pollution by heavy metals is a worldwide phenomenon. Anthropogenic activity is one of the major culprits to the release of heavy metals to the environment. To date, many approaches, e.g. analytical chemistry approaches and biological approaches have been developed to detect the presence of heavy metals. In biological approaches, many cells, e.g. bacteria, algae, cyanobacteria, have been used as the bioindicators or biological reporter groups in biosensors [1]. These cells reflect the real physiochemical toxicity of pollutants to the living organisms. As one of the smallest living entities, these cells can produce distinct responses towards certain analytes, with high sensitivity and rapid responses [2].

From a biological point of view, heavy metals can be divided into two categories: essential and non-essential [3]. However, essential heavy metals have even been reported to be toxic at high concentrations. For example, some heavy metals including copper, zinc, nickel and chromium, are essential for growth at very low concentrations but toxic at slightly levels [4]. The concentration 5 x 10-6 – 10-5 mol.L^{-1} Co^{2+} exerted maximal stimulatory effect on *Chlorella pyrenoidosa* cells at the exponential growth phase in terms of fresh weight (150-160% increase), dry weight (50-60% increase), chlorophylls a and b (45-65% increase), total carotenoids (55-65% increase), water-soluble proteins (19-20% increase) and monosaccharides content (55-60% increase), when compared to the control culture. The effect of Co^{2+} on *Chlamydomonas reinhardtii* observed reduction of growth at 10 ppm Co^{2+} and without change in the morphology of the cells or pH. At 20 ppm Co^{2+}, on the other hand, growth was considerably reduced compared to the control and the colour of the organism became paler and the cells clumped. In addition, the pH value was lower compared to the pH of the control at the end of experimental period. Lu et al. demonstrated that chlorophyll fluorescence analysis could be a useful physiological tool to assess early stages of change in photosynthetic performance of algae in response to heavy metal pollution [5].

In this study, the fluorescence response of microalgae and cyanobacteria to the exposure of heavy metals was determined.

Materials and methods

Isolation and purification of the algae and cyanobacteria: *Chlorella vulgaris sp BB-2* and *Ankistrodesmus sp BI-1* were isolated from the lake Balkhash, *Scenedesmus quadricauda B-1* and *Chlamydomonas reinhardtii B -4* were isolated from the lake Bilikol. Cyanobacteria *Oscillatoria tenuis* RI-4, *Nostoc calcicola* RI-3 and *Anabaena variabilis* RI-5 were isolated from the river Irtysh, *Phormidium autumnale*

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I-5 and *Synechococcus elongatus* I- 4 were isolated from the river Ilek. One single cell from each colony was isolated, transferred to fresh solid medium and subjected to repeated subculturing on fresh solid media before transfer to sterilized liquid nutrient media. Cultures were checked regularly microscopically. These cultures were deemed axenic [6].

Nutrient solution and Culture technique: Tamia medium was used for cultivation of microalgae. Medium BG-1 was used for the cultivation of cyanobacteria. The culture illumination was provided by fluorescent tube lamps giving a light intensity of 120 watts[6].

For water pollution modelling *medium* TM used aqueous solutions of copper sulfate ($CuSO_4 5H_2O \times$), cobalt sulfate ($CoSO_4 \times 7H_2O$), zinc sulfate ($ZnSO_4 \times 7H_2O$), nickel sulfate ($NiSO_4 \times 7H_2O$). The solutions prepared in distilled water, creating concentrations of metal ions: 0.001, 0.01, 0.1 and 1 mg/L.

Fluorescence intensity measurement carried out on spectrofluorimeter Fluorolog -02-panorama (λvozb = 400 nm, λreg = 685 nm) at the end of the experiment (72 hours), the expected relative changes in option (I) in% (Formula 1).

$$I = \frac{I_o}{I_k} \cdot 100\% \qquad (1)$$

where the I_o is the average value of the intensity of fluorescence in the experience, I_k-average value of the intensity of fluorescence in the control [7].

Results and their discussion

Culture exposed to heavy metals, Co2 +, Ni2 +, Zn2 +, Cu2 + at concentrations of 0.001, 0.01, 0.1 and 1 mg/l.,0 the choice of metals due to their the wide spread in natural waters. According to the data content of metals in surface waters far exceeded MAC [8].

Now in ecophysiological studies are widely used indicator of chlorophyll fluorescence. Growth and photosynthetic activity of algae and cyanobacteria that determine the intensity of fluorescence of chlorophyll, are markers of toxicity and the extent of tolerance organisms to this medium. We examined the effect of HM on selected productive strains of 4 cultures of microalgae and cyanobacteria cultures 5. Relative values of chlorophyll fluorescence intensity allocated microalgae with various concentrations of HM are represented on Figure 1. For 100% accepted by the intensity of fluorescence of cells in medium without HM.

The study of the greatest toxicity to selected algae possess the copper ions: fluorescence intensity of all cultures in the presence of copper in the investigated range of concentrations of 0.001-1.0 mg/L decreased to 50-88%. The smallest impact provided nickel and cobalt ions: fluorescence intensity if available microalgae in medium in the same concentrations decreased at 20-60%. Most stable to HM are *Chlorella vulgaris sp* BB-2*, Chlamydomonas reinhardtii* B -4. Most sensitive to the toxic effects of salts of heavy metals are strains of *Scenedesmus quadricauda* B-1 and *Ankistrodesmus sp.* BI-1. Heavy metals in the cells of these strains caused strong plasmolysis, an increase in cell size. This process is clearly observed in the concentration of 1.0 mg/L, Cu^{2+} (Figure 1).

Sensitivity strains to heavy metals may be perspective in bioassay to assess the degree of contamination of water bodies with salts of heavy metals. According to the literature value of fluorescence intensity is closely linked to the absolute number of living cells in culture, then there are HM inhibit microalgae photosynthesis process that affects the viability of cells and their size [9].

Based on the results of the study the effects of these heavy metals on cells of cyanobacteria, it was found that for selected from various sources of cyanobacteria of the four heavy metals studied most toxic are copper and zinc. Least toxic metal turned out to be a nickel. In the initial period of cultivation of cyanobacteria with the introduction of heavy metals resulted in lengthening the lag-phase in all cultures. But in the presence of heavy metals nickel, cobalt linear growth of cyanobacteria quickly restored, and fluorescence intensity crops accounted for 45-55% lower than controls. When making copper and zinc in concentrations of 0.001 mg/L 0.01 mg/L 0.1 mg/L fluorescence intensity declined to 60-80% (Figure 2).

The intensity of the fluorescence *Oscillatoria RI-4* and *Nostoc calcicola RI-3* in all concentrations of copper and zinc declined, and the concentration of 1.0 mg/l fully inhibited the process of photosynthesis. Cultures of cyanobacteria *Oscillatoria RI-4* and *Nostoc calcicola RI-3* proved to be more sensitive to copper and zinc ions. The remaining strains of cyanobacteria extracted from various sources, namely, *Phormidium autumnale I-5, Anabaena variabilis RI-5, Synechococcus elongatus I-4* were found to be more tolerant to the effects of heavy metals.

Based on the results of the study determined that the collection of strains of microalgae and cyanobacteria 5 crops resistant to heavy metals, 4 cultures are more sensitive to the studied concentrations of heavy metals.

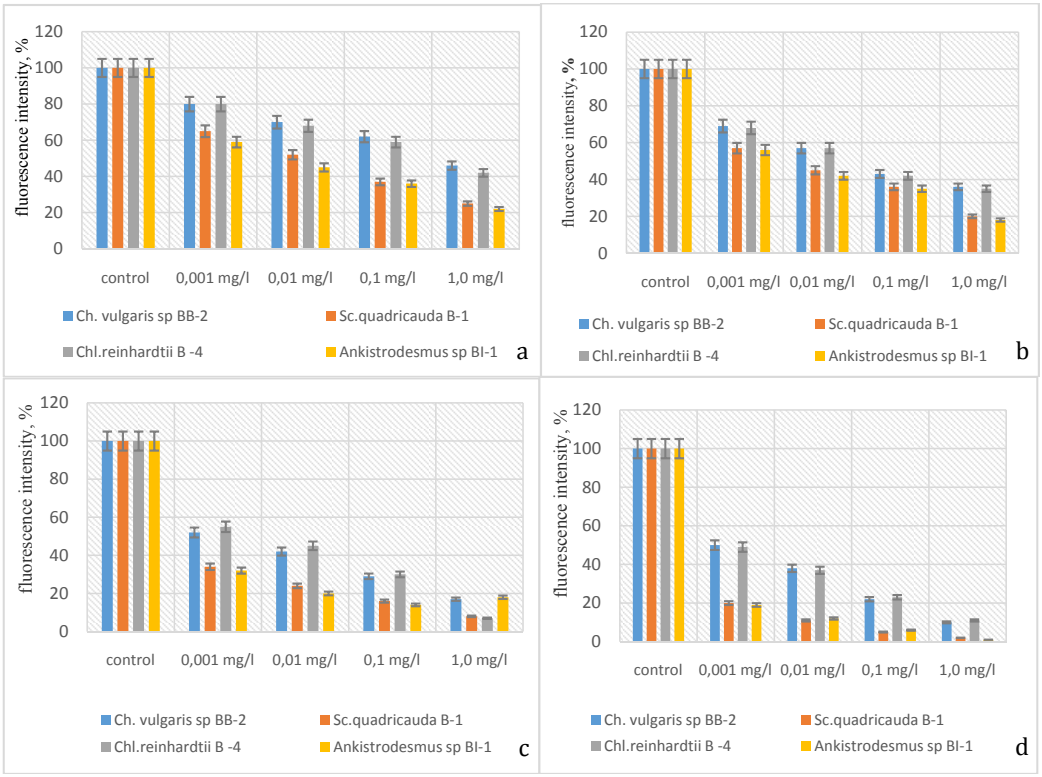

Figure 1 – Relative intensity values of chlorophyll fluorescence of microalgae, subjected to the effects of heavy metals in different concentrations, a – Co^{2+}, b – Ni^{2+}, c – Zn^{2+}, d – Cu^{2+}

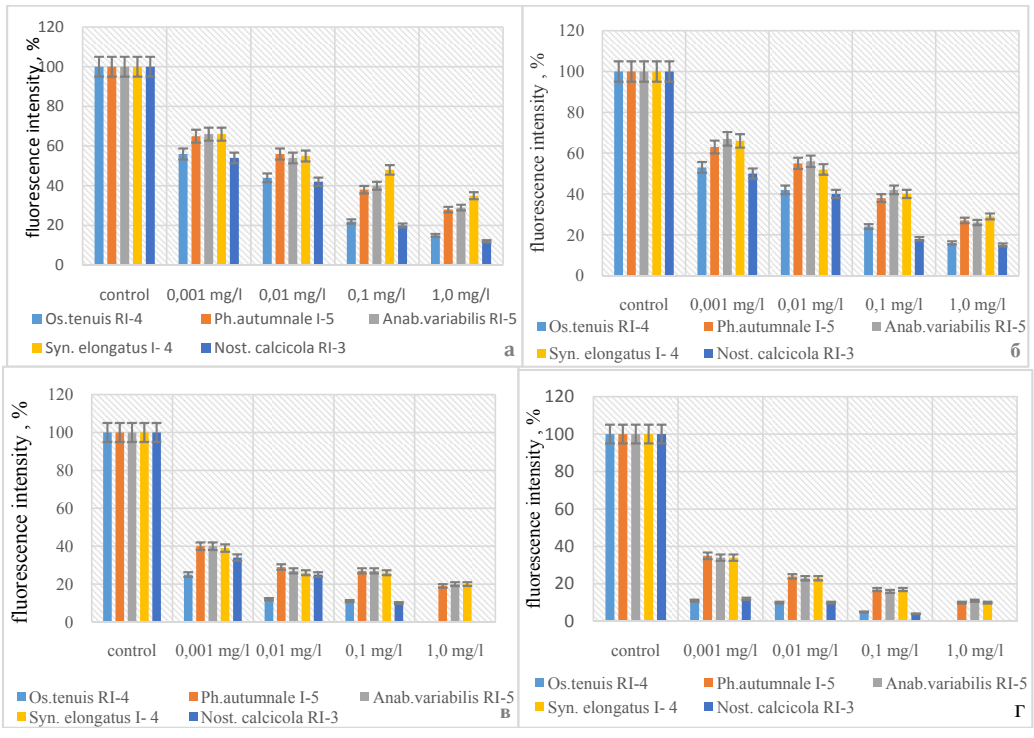

Figure 2 – Relative intensity values of chlorophyll fluorescence of cyanobacteria, subjected to the effects of heavy metals in different concentrations, а – Co^{2+}, б – Ni^{2+}, в – Zn^{2+}, г – Cu^{2+}

Some strains under the influence of the zinc and copper was observed phenomenon of plasmolysis and deformation of the cells. Influence of heavy metals on cells of microalgae and cyanobacteria have identified a number of surveyed metals toxicity, which is as follows: Cu_2 + > Zn_2 + > Co_2 + > Ni_2 +. From cultures of microalgae and cyanobacteria the following types of selected for further study, as promising strains for bioremediation of contaminated aquatic ecosystems, various heavy metals: *Phormidium autumnale I-5, Anabaena variabilis RI-5, Synechococcus elongatus I-4, Chlorella vulgaris sp BB-2, Chlamydomonas reinhardtii B -4.*

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