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The cyanobacterium *Anabaena variabilis* has a significant role in the natural ecosystems, especially by enriching soil with nitrogen. This cyanobacterium, being present in a sufficient quantity in soil, ensures a more intense growth and a higher productivity of the crop plants. The activity of the cyanobacteria is reduced in cases of pollution of their environment. The purpose of this work was to investigate how presence of heavy metals in the environment affects the activity of the cyanobacterium *A. variabilis*.

The tested cyanobacterium *Anabaena variabilis* (locally isolated) was cultivated in the Allen and Arnon's medium [1] (4 times diluted). The cultures were incubated in an air-conditioned culture room (temperature $24 \pm 1^{\circ}\text{C}$) receiving $72 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR (photosynthetically active radiation) for 12 h daily. The batch culture was dosed with different concentrations of copper and zinc from freshly prepared stocks of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ and ZnCl_2 , to obtain the desired metal concentrations (1.0, 2.0 and 4.0 μM for Cu, and 2.5, 5.0 and 10.0 μM for Zn) in the culture medium. At the final day of the experiment (after 15 days of the metal treatment), 15 ml of algal culture suspension were withdrawn from the control as well as from the metal-treated batch and semi-continuous cultures. Different parameters, such as cell number, protein, pigments, pH, phosphate and metal content in the culture, were analyzed by the standard methods.

The experiments resulted in Cu- and Zn-induced reductions of the final yield, protein and chlorophyll *a* of *A. variabilis* in the batch and semi-continuous cultures. The inhibition of protein content and chlorophyll *a* contents of *A. variabilis* was significantly ($P < 0.05$) greater at all the tested concentrations of Cu and Zn in the semi-continuous culture as compared to the batch culture. The control cultures of *A. variabilis* had $183.00 (\pm 6.40)$, $169.30 (\pm 1.20)$ fg cell⁻¹ protein and $77.00 (\pm 2.26)$, $57.00 (\pm 2.90)$ fg cell⁻¹ chl *a* content in the batch and the semi-continuous system, respectively.

The amount of Cu and Zn accumulated in the cells of the test organism was greater in the semi-continuous culture in comparison to the batch culture. The amount Cu and Zn was approximately 2-3 times higher in the semi-continuously grown cells as compared to the batch culture. The percentage of phosphate depletion from their initial concentration (2 mM) was 60, 50, 25 and 62, 50 and 25% from the culture medium dosed with 1, 2 and 4 μM of Cu and 2.5, 5 and 10 μM of Zn, respectively. The control cultures of the batch and semi-continuous systems showed a 70 and 25% depletion of phosphate, respectively.

The carotenoids of the batch grown *A. variabilis* were significantly ($P < 0.05$) higher than in the semi-continuous culture at each tested concentration of metals as well as in the control. The differential toxicity of metals to *A. variabilis* in the batch and semi-continuous cultures might also be due to the different cellular status of the carotenoids. The previous literature documented the role of carotenoids in protecting cells from stress-induced oxidative damage [2]. The mechanism of the metal toxicity amelioration by carotenoids warrants further research.

The present study suggests that the batch culture experiments may underestimate the toxicity of metals to algae, and therefore may be misleading. It recommends the semi-continuous culture (as a better approximation to the natural systems) for predicting the impacts of, and for better understanding of algal responses to metals in the metal-contaminated environments.

Bibliography:

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