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APPLICATION OF MICROALGA *Chlorella sorokiniana* IN WASTEWATER BIOREMEDIATION – CASE OF LAKE ROBULE

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Abstract

Heavy metals remain a major pollutant in waters near mining sites. Water pollution is a current, long-term problem that affects plants and organisms that live in these water systems, and the effect is very harmful not only for individual species and populations, but also for the entire biological community. This study analyzed the potential of the microalga Chlorella sorokiniana in the adsorption of four selected metals: Fe, Cu, Zn and Ni, all present in high amounts in Lake Robule. This pilot study was conducted to evaluate the potential of these microalgae for possible use in future bioremediation treatment of these water. A laboratory study of metal accumulation in lake water samples lasted for 7 days, after which a total decrease in metal concentration was observed, namely Fe ~25%, Cu ~17%, and Zn ~4% on the seventh day. This study confirms the strong potential of microalgae C. sorokiniana to reduce the presence of heavy metals in conditions known for the low pH value of water with a high percentage of Fe and other heavy metals.

Keywords: heavy metals, microalgae, *C. sorokiniana*, Lake Robule.

INTRODUCTION

Pollution of the environment with various compounds, where heavy metals are among the first, is a big problem everywhere. Today, more than before, awareness has been raised about the consequences of water pollution in the vicinity of mines, and monitoring is becoming mandatory, and intensive work is being done on bioremediation projects [1,2]. Microalgae are increasingly attracting the attention of researchers due to their ability to remove heavy metals, inorganic nitrogen, phosphorus and some toxic organic compounds [3–5]. Given the actuality of the mentioned problem and with the aim of maintaining the availability of quality water resources, the biological treatment of wastewater using microalgae stands out as attractive biotechnology. In this field, accelerated development can be observed, which is profitable both economically and ecologically [6–8]. Numerous studies to optimize the growth and productivity of microalgae have focused on the cultivation of microalgae in various wastewater streams, including municipal, industrial and agricultural waste, which, in addition to nutrients, may contain heavy metals, metalloids and various organic pollutants [9]. There is ample evidence that it is possible to use microalgae in wastewater to achieve several goals at the same time: purification of wastewater, synthesis of valuable metabolic products and accumulation of algal biomass that can be used as fertilizer [3,6].

Here, a study was made on real samples of the accumulation of Lake Robule, which was located next to the copper mining excavations in Bor, Serbia. The goal of the work was to evaluate the potential of using the microalgae *Chlorella sorokiniana* for phytoremediation of mining wastewater, ie for the absorption of heavy metals in a highly acidic environment.

MATERIALS AND METHODS

C. sorokiniana cultivation and experimental design

Chlorella sorokiniana strain CCAP 211/8K was obtained from the Culture Collection of Algae and Protozoa (CCAP), UK. Microalgae inoculum was added to 50 ml of 3N-BBM+V medium in 100 ml Erlenmeyer flasks at an initial density of 0.5×10^6 cells ml^{-1}. The initial pH of the medium was ~7.5. Microalgal cultures were grown at 22 °C on orbital shakers (120 rpm) in a growth cabinet with a continuous photon density of 120 µmol m^{-2} s^{-1}. Volume of the samples was corrected for evaporation on day 15 with sterile deionized water. Growth was monitored spectrophotometrically at 750 nm (OD750), which is proportional to cell density. Microalgae were transferred to Robule Lake water on day 20 (early stationary phase). The entire amount of culture was centrifuged at 5000 g for 5 minutes and resuspended in 50 ml of lake water. The resistance of cultures to lake water was examined by measuring the OD750 of control and samples at 1 h, 24 h, 72 h and 168 h after treatment.

At each time point after sampling, 2 ml aliquots of the cultures were centrifuged at 5000 g for 5 min and the supernatant was collected for heavy metal analysis. 4 µl of 65% HNO_3 per 1 ml was added to the supernatant, filtered through a nylon filter with a diameter of 22 µm and diluted 2 times (for Ni) or 20 times for other metals. Control samples remained in 3N-BBM+V medium. All chemicals were purchased from Sigma-Aldrich (St. Louis, MI, USA).

Chemical analysis of heavy metals

The content of Fe, Cu, Zn and Ni in solutions was determined by using the flame atomic emission spectroscopy (AAS Aanalyst 700/Perkin-Elmer). Standard solution (Merck, Darmstadt, Germany) and 18.2 MΩ water were used for preparing calibration standards.

Statistical analysis

To test for significant effects, Tukey's post hoc test was used to test for significant differences in metal absorbance at different time points among different sample groups. Tukey's post hoc tests were performed using IBM SPSS statistical software (version 20.0, SPSS Inc., Chicago, IL, USA). The significance threshold value was set at 0.05.

RESULTS AND DISCUSSION

The initial metal concentration in the water sample is Cu 48.125 ± 0.006 mg/L: Zn 19.272 ± 0.003 mg/L; Fe 296.550 ± 0.006 mg/L and Ni 0.687 ± 0.033 mg/L. The adsorbed amount of metals shown by time points and for target metals (Table 1) shows that there is a reduction in water pollution with metals in the presence of microalgae *C. sorokiniana*. The best results were obtained with Fe, where after 7 days (168 h) there was a reduction of more than 25% of the initially present amount of metal and for Cu ~17%, and Zn ~4% in the same, 7 day.

Table 1 *The decrease in the amount of Fe, Cu, Zn and Ni in the Lake Robule water after treatment with microalgal culture. The amount of aforementioned metals was measured after 1 h, 24 h, 72 h and 168 h (7 days)*

Amount of adsorbed metals in [mg/L]	**1 h**	**24 h**	**72 h**	**168 h**
Cu	0.480 ± 0.009	7.610 ± 1.320	4.229 ± 0.105	8.453±0.050
Zn	0.423 ± 0.005	4.050 ± 0.053	1.939 ± 0.021	8.453±0.050
Fe	16.870 ± 3.150	68.635 ± 7.245	48.580 ± 2.940	73.350±1.320
Ni	/	/	/	/

The percentage values shown in Figures 1–3 (decrease of each metal in water of Lake Robule after treatment with microalgal culture at different time points) are percentages calculated in relation to the measured values of metals in the water before treatment and the values measured at a given time point. The decrease is expressed as the percentage of the metal amount in untreated water and can be attributed to adsorption and/or absorption of metal by microalgal cells.

The Figures below (Figure 1 and Figure 3) show that Cu and Fe are absorbed in the same way; the greatest effect of absorption is in the first 24 hours, while it then decreases to return in seven days to the same value as in 24 hours. In the case of zinc (Figure 2), the absorption dynamics are different. It reaches a maximum of 24 hours, while after that it only reaches a decrease in the value of the absorbed amount of metal.

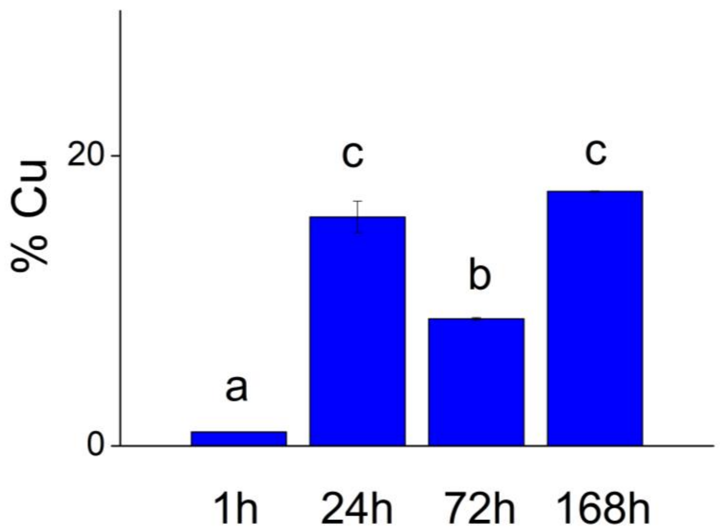

Figure 1 *Amount of absorbed Cu and/or absorbed expressed as a percentage by time points. Different letters indicate significant differences based on Tukey's test*

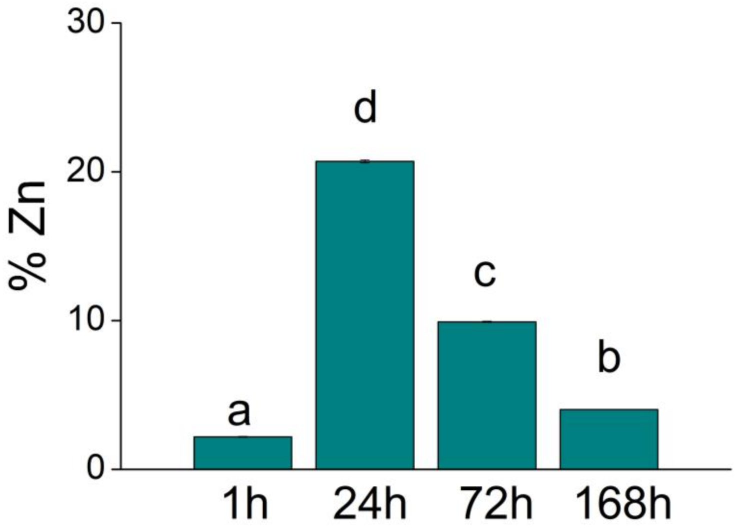

Figure 2 *Amount of absorbed and/or absorbed Zn expressed as a percentage by time points. Different letters indicate significant differences based on Tukey's test*

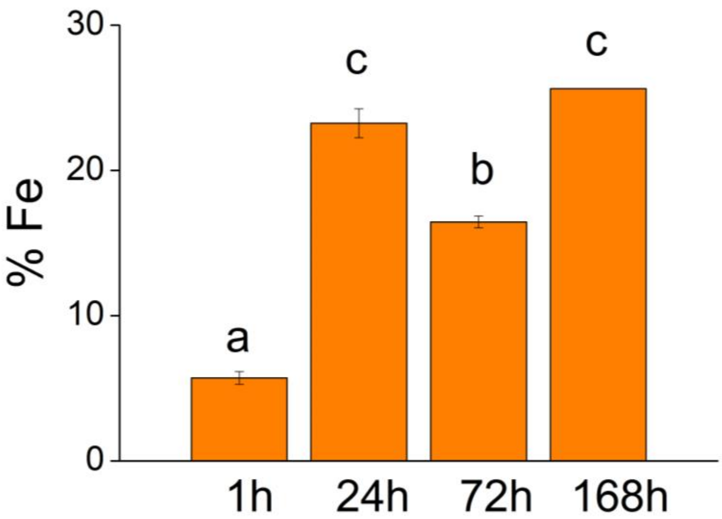

Figure 3 *Amount of absorbed and/or absorbed Fe expressed as a percentage by time points. Different letters indicate significant differences based on Tukey's test*

Figure 4 shows the percentage share of target metals Fe, Cu, Zn and Ni based on the percentage presence of those four metals, which are absorbed by phytoremediation with algae *C. sorokiniana* followed in the actual sample of Lake Robule.

Based on the presented data, that is, the different presence of metals, it can be seen that there is also competition in the adoption of metals, which is also a promising result. Although nickel is present in the smallest amount, it is not removed from the water for seven days.

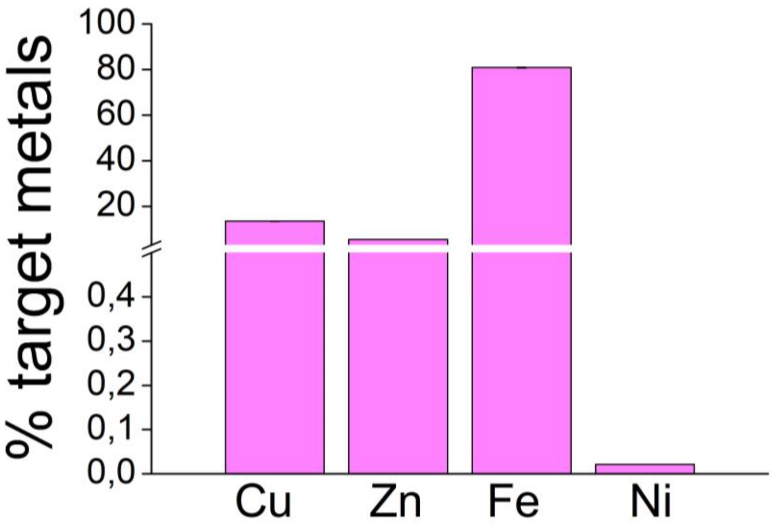

Figure 4 *Percentage of each target metal adsorbed to and/or absorbed in the biomass. Iron dominates, which is expected, as it is the metal most represented in the lake water. It is followed by copper and zinc, and lastly nickel, which is the least abundant in the lake water itself*

CONCLUSION

The decrease in the amount of metals in the water of Lake Robule shows that the microalgae *C. sorokiniana* is a good candidate for natural bioremediation of acidic wastewater. For Cu and Fe, 24 h was the optimal time for maximum removal, because on the seventh day there is a slight additional metal removal. Although Zn had different removal dynamics, but reached the maximum in 24 h. These results show the need to examine and optimize the treatment of each polluted site depending on the composition of polluting metals.

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