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Mechanisms of detoxification of high manganese concentrations by the microalga *Chlorella sorokiniana*

Snežana Vojvodić[1*], Marina Stanić[1], Bernd Zechmann[2], Milena Dimitrijević[1], Miloš Opačić[1], Jelena Danilović Luković[1,3], Arian Morina[1], Jon K. Pittman[4], Ivan Spasojević[1]

[1] *Life Sciences Department, Institute for Multidisciplinary Research, University of Belgrade, Belgrade, Serbia*
[2] *Center for Microscopy and Imaging, Baylor University, Waco, Texas, USA*
[3] *Institute for Application of Nuclear Energy, University of Belgrade, Belgrade, Serbia*
[4] *Department of Earth and Environmental Sciences, School of Natural Sciences, University of Manchester, Manchester, UK*

Many neutrophilic and acidophilic microalgal species tolerate high metal concentrations and can survive or colonize metal-polluted waters. They show significant biotechnological potential for the remediation and wastewaters processing. On the other hand, negative effects of metal pollution on microalgae may affect the function of aquatic ecosystems because these photosynthetic microorganisms represent the primary producers of O_2 and biomass. However, adaptive mechanisms that microalgae employ to detoxify metal excess are largely unknown. Herein we analyzed the response of the freshwater microalga *Chlorella sorokiniana* to high but non-toxic levels of Mn^{2+}. Manganese is a key metal pollutant, with five possible oxidation forms that can bind to a variety of different ligands. At pH below 7, it is predominantly present in Mn^{2+} form. Scanning electron microscopy showed that in response to 1 mM Mn^{2+}, *C. sorokiniana* released mucilage polymers within 1 h. Electron paramagnetic resonance spectroscopy (EPR) showed that the early response involved loose Mn^{2+} binding to mucilage and/or the cell wall. The amount of loosely bound Mn^{2+} was significantly decreased after 24 h, whereas biomass showed significant accumulation of Mn, O and P, as determined by energy dispersive X-ray spectrometry, indicating the production of polyphosphates, which may sequester Mn. Further, it was found that the exposure to Mn^{2+} resulted in rapid and transient decrease of total free glutathione concentration; the drop was observed after 1 h, and the concentration returned to initial values after 24 h. EPR measurements showed a similar trend in the level of reduced thiols. The observed changes can be explained either by the synthesis of phytochelatins – sulfur-rich short-chain peptides that sequester metals, or by glutathionylation of proteins. Reduced thiols could not be detected in the extracellular space, indicating that C. sorokiniana did not release thiols in response to high Mn. These results demonstrate that the adaptive response of C. sorokiniana to high Mn levels involves multiple components and time phases. The early phase involves mucilage release, phytochelatins and/or protection of protein thiols, whereas the successive phase involves Mn coordination by polyphosphates and other mechanisms that remain to be resolved.

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E-mail address: snezanavojvodic@gmail.com