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The effect of increased nickel concentrations on *Chlorella sorokiniana* culture

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Microalgae show significant biotechnological potential for remediation of wastewaters polluted with metals, including nickel [1]. Ni enters aquatic ecosystems from fuel combustion, smelting, mining, and electroplating endeavors, and represents a particularly important problem for the access to safe drinking water [2]. It is noteworthy that microalgae utilize Ni as a co-factor of urease. Herein we analyzed the impact of high levels of Ni(II) on freshwater microalga *Chlorella sorokiniana* in the stationary phase of culture growth. In the concentration range 0.5 to 30 mM, Ni induced a drop in cell density and biomass after 7 days of incubation, whereas significant negative impact was present as early as 2 days for 30 mM. High concentrations also induced a decrease in the level of chlorophylls (*a* and *b*) in biomass. In contrast, microalgae that were exposed to moderate stress (1 and 2 mM of Ni) appear to increase photosynthetic activity, indicating the activation of some adaptive mechanism. Scanning electron microscopy showed that *C. sorokiniana* does not release significant amounts of mucilage polymers in response to Ni, in contrast to the response to some other metals [3]. Further, Ni at 1 mM induced an increase in the intracellular production of reactive oxygen species, which appears to reach a plateau after 1 h. The concentration of free thiol groups showed a gradual drop during 24 h of incubation with the same Ni concentration. On the other hand, the decrease in the concentration of reduced glutathione that was observed after 1 h was reversible for 1 mM Ni at 24 h. Ni at 5 mM had a more lasting impact on glutathione. It is important to stress out that the concentrations of reduced glutathione and total glutathione showed very similar trends. This implies that free thiols and reduced glutathione may be 'lost' due to glutathionylation of proteins and synthesis of phytochelatins. Our results demonstrate that the response of *C. sorokiniana* to high Ni levels involves multiple components that may be different than for other metals. Redox regulation and protection of thiol (switches) may represent a crossroad between adaptation and death.

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