Responses of the green alga *Pseudokirchneriella subcapitata* to short- and long-term exposure to heavy metals

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Algal cells can be exposed to toxicants for a short-term due to accidental discharges or, more commonly, for a long term. The green alga *Pseudokirchneriella subcapitata* has been widely used in ecological risk assessment, usually based on the impact of the toxicants in the alga growth. However, the physiological causes that lead algal growth inhibition are not completely understood.

This work pretends to elucidate the main targets of heavy metals toxicity in the alga *Pseudokirchneriella subcapitata* after a short (6 h) or a long (72 h) exposure time. For this purpose, the responses of *P. subcapitata* to three concentrations of Cd(II), Cr(VI), Cu(II) and Zn(II), corresponding approximately to 72h-EC_{10} and 72h-EC_{50} values and a high concentration (above 72h-EC_{90} values) on membrane integrity, esterase activity, mitochondrial function, photosynthetic activity, chlorophyll *a* (Chl *a*) content, intracellular accumulation of reactive oxygen species (ROS) and reduced glutathione (GSH) level were evaluated.

For a short-term exposure (6h), all metals studied (at all concentrations) induced a reduction of esterase activity. A loss of membrane integrity and a decrease of mitochondrial membrane potential in algal cells exposed to 72h-EC_{50} and >72h-EC_{90} concentrations of Cu(II) was also detected. Chl *a* autofluorescence was affected by the presence of Cr(VI) and Cu(II), which suggests the perturbation of photosynthesis.

A long-term (chronic) exposure of algal cells (72h) to Cd(II), Cr(VI) or Cu(II) at >72h-EC_{90} concentrations resulted in a loss of membrane integrity. For all metals tested, an inhibition of esterase activity, in a dose-dependent manner, was observed. Reduction of Chl *a* content, decrease of maximum quantum yield of photosystem II and modification of mitochondrial membrane potential was also verified. Cd(II), Cu(II) and Zn(II), at the highest concentrations tested, induced an increase of intracellular ROS and GSH content. The increase of GSH content might be a form of algal cells to redress the imbalance caused by the oxidative stress. However, the increase of GSH was not enough to protect the algal cells against the long-term exposure to oxidative stress.

In conclusion, the short- and long-term exposure of *P. subcapitata* cells to heavy metals had a negative impact on alga physiology and metabolism. Although a compromising of membrane integrity was observed for >72h-EC_{90} concentrations of Cu(II) and Cd(II), Cr(VI), after long exposure, cell membrane should not be the primary target of the metals action. The main targets of the heavy metals under study have an intracellular localization. The impairment of esterase activity combined with the reduction of Chl*a* content was related with the inhibition of growth caused by a prolonged exposure of Cr(VI) and Cu(II). In the case of Zn (II), in addition to these metabolic parameters, the damage of mitochondrial function was also associated with the growth inhibition. The identification of the targets of the heavy metals studied contributes to the elucidation of the mechanisms of action of these toxicants on the alga *P. subcapitata*.