Heavy metals and antioxidant responses in the soil ciliate *Cyrtoymena tetracirrata:* a preliminary analysis

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In the last decades, the discharge of anthropogenic heavy metals to the ecosystems has increased worldwide. These heavy metals do not degrade, but get accumulated in the food chain and additionally, some of them produce carcinogenic and toxic effects in human and animals. Ciliated protozoa are cosmopolitan eukaryotic microorganisms which are adapted for life in soil, aquatic, and marine ecosystems. In addition, they are very sensitive to diverse pollutants and toxic agents, and some of them, share a higher degree of functional conservation with human genes than other microbial model microorganisms. In general, bioassays are good tool to assess metal pollution because they have the ability to react and detect only the available fraction of the metal ions. In indirect mechanisms, essential and non-essential metals can generate reactive oxygen species (ROS), e.g., superoxide radical (O^{-2}), hydrogen peroxide (H_2O_2), hydroxyl radical (HO^-), and singlet oxygen ($^1O_2$), in living organisms. Heavy metal induced ROS causes adverse effect by attacking cellular macromolecules, resulting in protein denaturation, lipid peroxidation, DNA damage, and alteration of calcium and redox homeostasis, thus, generating oxidative stress in the organisms. The main aim of our study was to evaluate the cytotoxic effect of the heavy metals Cd, Zn and Cu by means of a combination of ecotoxicological and antioxidant activity assays in the ciliate *Cyrtoymena tetracirrata* isolated from the soil of an agriculture farm located in Marche region (Italy). We have measured the 24-hrs LC_{50} and LC_{20} values for single metal exposures as well as, the effects of binary metal mixtures (Cd + Zn, Cd + Cu, and Cu + Zn) on *C. tetracirrata* viability, applying the Concentration Addition model based on the Toxic Unit approach to assess the type of interaction (i.e. synergistic, antagonistic etc.) between heavy metals. Furthermore, we applied different antioxidant *in vitro* tests, such as total phenol content (TPC), a,adiphenyl- b-picrylhydrazyl (DPPH), Metal chelating assay (MCA) and Hydroxyl Radical Scavenging Assay (HRSA), to evaluate the presence of antioxidant activity in the ciliates exposed to heavy metals. Our preliminary results showed, the LC_{50} values to Cd, Cu, and Zn were 1.16, 0.37 and 32.7 mg L^{-1}, respectively and LC_{20} values were 0.53, 0.22 and 23.0 mg L^{-1}, respectively. The order of toxicity was Cu > Cd >> Zn. In most of the cases, bimetallic (Cd + Zn, Cd + Cu, and Cu + Zn) treatments produced antagonistic effects. The activity of intra- and extra-cellular antioxidant capacity of ciliate cells exposed to different heavy metals conditions was found to be significantly increased with respect to the untreated cells (control). Thus, *C. tetracirrata* seem to have a good potential to be used as a model organism in ecotoxicological studies of soil contaminated with heavy metals. In the long run, our goals will be to analyse the antioxidant enzymes and the expression of potential stress related genes (antioxidant genes) of *C. tetracirrata*.