## Copper oxide nanoparticles induce oxidative stress, DNA strand breaks and laccase activity in aquatic fungi

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The extensive use of nano metal-based products increases the chance of their release into aquatic environments and may put biota and associated ecological processes at risk. Fungi play a key role in organic matter turnover in freshwaters while they transfer carbon and energy to higher trophic levels. Although aquatic fungi are common in clean and well-aerated freshwaters, they also occur at hyperpolluted sites. We investigated the effects of nano CuO (4 levels,  $\leq$  100 mg  $L^{-1}$ ) on fungal biomass production, extracellular laccase activity, accumulation of reactive oxygen species (ROS), plasma membrane integrity and occurrence of DNA strand breaks, after 5 and 12 days, in 4 fungal isolates: Phoma sp. UHH 5-1-03 (Ph) and Articulospora tetracladia UMB-072.01 (At72) collected at clean sites, and A. tetracladia UMB-061.01 (At61) and Heliscus lugdunensis H-4-2-4 (H4) collected at polluted sites. Fungal biomass was inhibited by the increase in nano CuO concentration and incubation time. After 12 days, the strongest inhibition in biomass production was found in At72 (95.5%) and Ph (81.2%) exposed to 100 mg  $L^{-1}$  nano CuO, while the lowest effects were observed in At61 (59.4%). Laccase activity was induced by all nano CuO concentrations after 12 days, except in At61; maximum activities were found in Ph followed by At72 and H4 (2823, 474 and 166 U  $g^{-1}$ , respectively) when exposed to 100 mg  $L^{-1}$ nano CuO. ROS accumulation increased with increasing nano CuO concentration at both times in all fungi. After 12 days, severe plasma membrane disruption was found in At72 exposed to all nano CuO concentrations and in Ph exposed to 100 mg L<sup>-1</sup> nano CuO. Severe DNA strand breaks were observed in At72 and Ph exposed longer to 100 mg L<sup>-1</sup> of nano CuO. The increase in nano CuO concentration and exposure time led to an increase in DNA strand breaks, which is a signature of an apoptotic process. Overall results suggest that nano CuO is toxic to aquatic fungi, particularly to those isolated from clean streams.

## Keywords: Nano CuO, oxidative stress, DNA strand breaks, laccase

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