Effects of nano CuO on aquatic decomposers: from community to cellular responses

<u>Arunava Pradhan</u>¹, Seena Sahadevan¹, Stefan Helm², Katharina Gerth², Gerd-Joachim Krauss², Dirk Wesenberg², Dietmar Schlosser³, Cláudia Pascoal¹, Fernanda Cássio¹

¹Centre of Molecular and Environmental Biology, Department of Biology, University of Minho, 4710-057 Braga, Portugal

²Institute of Biochemistry and Biotechnology, Martin-Luther-University, Halle-Wittenberg, Germany ³Helmholtz Centre for Environmental Research, Leipzig, Germany E-mail contact: <u>arunava2006molbio@gmail.com</u>

Intensive use of metal nanoparticles increases the chance of their release into freshwaters that may pose risk to biota and associated ecological processes. In streams, microbes play a key role in detritus foodwebs transferring carbon and energy from plant litter to invertebrate shredders. Here, we investigated the effects of nano CuO (<50 nm, nanopowder, Sigma) on aquatic detritus foodwebs by examining i) leaf-litter decomposition by bacterial and fungal communities, ii) cellular damage and physiological responses of fungal populations collected from non-polluted and metal-polluted streams, and iii) survival, growth and leaf consumption by an invertebrate shredder. Results were compared with those obtained with ionic copper.

Stream-dwelling microbial communities were obtained by immersion of leaves in a nonpolluted stream (Portugal). Microbial communities were exposed in microcosms to nano CuO (\leq 500 mg L⁻¹) and Cu²⁺ (\leq 30 mg L⁻¹). Leaf decomposition decreased with increasing concentrations of nano and ionic copper. Both copper forms reduced biomass of bacteria and fungi, and fungal reproduction. Cu²⁺ had stronger effects than nano CuO. Exposure to Cu²⁺ and nano CuO led to a decrease in fungal diversity and to shifts in species dominance.

Increased concentrations of nano CuO (\leq 100 mg L⁻¹) stimulated extracellular laccase activity by fungi. Populations from non-polluted streams were more affected by nano CuO than those from polluted streams, as shown by a stronger inhibition of biomass production, higher Cu adsorption, higher levels of reactive oxygen species and DNA strand breaks.

Acute lethality tests suggested low toxicity of nano CuO to the shredder *Allogamus ligonifer*. However, sublethal concentrations of nano CuO ($\leq 75 \text{ mg L}^{-1}$) strongly reduced leaf consumption and invertebrate growth under aqueous and dietary exposure. Concentration of leached Cu²⁺ in the stream water increased with increasing nano CuO concentration. Exposure to 75 mg L⁻¹ of nano CuO via water or food led to higher Cu adsorption and accumulation in larvae. Moreover, leached Cu²⁺ appeared to have a role in inducing toxicity of nano CuO.

Keywords: Nano CuO, aquatic microbial communities, fungal isolates, invertebrate shredder.

Acknowledgement: FEDER-POFC-COMPETE, DAAD and FCT supported this work (PEst-C/BIA/UI4050/2011, FCT-DAAD-2010-2011, NANOECOTOX-PTDC/AAC-AMB/121650/2010) and A. Pradhan (SFRH/BD/45614/2008).