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Salinity-Based Toxicity of CuO Nanoparticles, CuO-Bulk and Cu Ion to *Vibrio anguillarum*

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Bacteria are used in ecotoxicology for their important role in marine ecosystems and their quick, reproducible responses. Here we applied a recently proposed method to assess the ecotoxicity of nanomaterials on the ubiquitous marine bacterium *Vibrio anguillarum*, as representative of brackish and marine ecosystems. The test allows the determination of 6-h EC_{50} in a wide range of salinity, by assessing the reduction of bacteria actively replicating and forming colonies. The toxicity of copper oxide nanoparticles (CuO NPs) at different salinities (5-20-35 ‰) was evaluated. $CuSO_4$ $5H_2O$ and CuO bulk were used as reference toxicants (solubility and size control, respectively). Aggregation and stability of CuO NP in final testing dispersions were characterized; Cu^{2+} dissolution and the physical interactions between *Vibrio* and CuO NPs were also investigated. All the chemical forms of copper showed a clear dose-response relationship, although their toxicity was different. The order of decreasing toxicity was: $CuSO_4$ $5H_2O$ > CuO NP > CuO bulk. As expected, the size of CuO NP aggregates increased with salinity and, concurrently, their toxicity decreased. Results confirmed the intrinsic toxicity of CuO NPs, showing modest Cu^{2+} dissolution and no evidence of CuO NP internalization or induction of bacterial morphological alterations. This study showed the *V. anguillarum* bioassay as an effective tool for the risk assessment of nanomaterials in marine and brackish environments.

Keywords: metal oxide, marine bacteria, bioassay, nanoparticle behavior, copper dissolution, salinity influence

INTRODUCTION

The metal nanoparticles, including metal oxides, represent one of the major classes of commercial nanomaterials, which are manufactured on a large scale for both industrial and household applications (Chang et al., 2012). Copper (II) oxide nanoparticles (CuO NPs) are increasingly used in several products (Huang et al., 2010; Chang et al., 2012; Rossetto et al., 2014). The wide variety of applications entails the risk of environmental contamination, as a consequence of the environmental release of CuO NP during their production, use and disposal (Weinberg et al., 2011; Sanchís et al., 2013; Fan et al., 2014). This kind of contamination could bias both organisms and