Assessing toxicity of citrate-gold nanoparticles at different marine trophic levels (microalgae, copepods and bivalve mollusks)

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Abstract

Engineered nanoparticles (ENPs) may offer benefits to society in general, although they sometimes inherently have unintended effects on ecosystems. As a consequence, assessment of the environmental safety of ENPs has become a major issue worldwide [1]. Within the metallic ENPs, gold nanoparticles have been used extensively in drug delivery, gene therapy, biosensing and contrast agent for imaging [2]. However, studies about the effects of gold nanoparticles are limited and they are specially focused on “in vitro” experiments rather than “in vivo” systems. Additionally, estuaries and coastal ecosystems are the final receptors of substances dumped in the environment wherefore the effects of these substances should be tested in representative site specific organisms. In order to assess the effect of gold-citrate nanoparticles on aquatic ecosystems, toxicity tests were carried out in three groups of model organisms belonging to different trophic levels: the marine microalgae species *Cylindrotheca closterium, Chlorella autotrophyca, Phaeodactylum tricornutum, Pleurochrysis pseudoroscoffensis* and *Rhodomonas salina* (Fig. 1), the copepod, *Tisbe battagliai* (Fig. 2) and the clam *Ruditapes philippinarum* (Fig. 3). The gold-citrate NPs employed were citrate reduced AuNPs in the range of 20 – 30 nm, or soluble gold, H(AuCl4) as positive control.

For the toxicity test with microalgae, the selected endpoint was population growth after 72 hours of exposure. The cells were incubated in batch cultures of 50 mL in artificial seawater enriched with simple medium (nitrate, phosphate, silica) and exposed under continuous light conditions at 20±1ºC to different dissolved Au or NPs concentrations. Growths of experimental populations were compared with controls, and concentrations which imply an inhibition of 50% respect the controls (EC50%) are calculated (Fig. 4). Dissolved Au toxicity ranged from 0.052 ± 0.001 mg•L^{-1} for *Rhodomonas salina* to 0.50 ± 0.15 mg•L^{-1} for *Chlorella autotrophyca*. Concentrations at ecologically significant values for NPs (up to 0.3 mg•L^{-1}) did not imply growth inhibitions over 50%. For copepods, nauplii (< 24 h-old) were exposed (48 h) to increasing concentrations of Au-NPs in 12-well plates (5 ml/well, 4 nauplii/well and 5 replicates/concentration) under the above described laboratory conditions [3]. The results are shown in Figure 5. The clam, *Ruditapes philippinarum* was exposed for 28 days to two Au-NPs concentrations: 6 and 30 µg·L^{-1}. Clams were collected different at sampling points and target tissues (gills, digestive gland and mantel) were dissected and stored at -80ºC until their analysis. No significant mortality was recorded during the experiment and bioaccumulation in the digestive gland along the experiment was measured (Figure 6).

In summary, no acute toxicity was recorded at ecological relevant concentrations for assayed Au-NPs. Nevertheless, further research should be necessary to know the effect of chronic exposure to these NPs and to improve the knowledge about their environmental risk assessment.

References

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[2] O. Bar-Ilan, R. M. Albrecht, V. E. Fako, D. Y. Furgeson. Small, **5** (2009) 1897-1910
[3] Diz F.R., Araujo C.V.M., Moreno-Garrido I., Hampel M., Blasco J. Ecotoxicol. Environ. Safety **72** (2009) 1881-1886

Figures

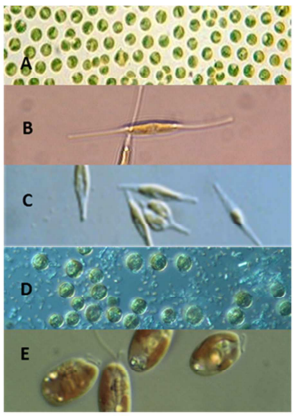

Figure 1. Phytoplankton species (A. *Chlorella autotrophyca* (CHLOROPHYCEAE); B. *Cylindrotheca closterium* (BACILLARIOPHYCEAE); C. *Phaeodactylum tricornutum* (BACILLARIOPHYCEAE); D. *Pleurocrysis pseudoroscoffesis* (PRIMNESIOPHYCEAE) ; E. *Rhodomonas salina* (CRYPTOPHYCEAE))

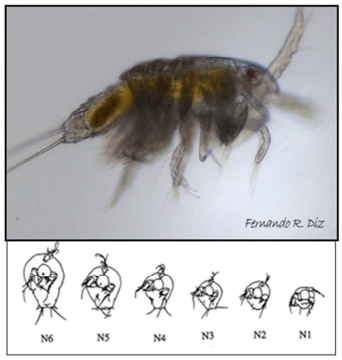

Figure 2. Second stage copepodid and naupliae stage of Tisbe battagliai (Crustacea: Copepoda).

Figure 3. Specimens of the clam *Ruditapes philippinarum*

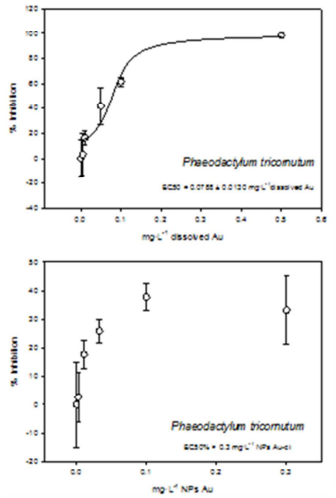

Figure 4. Effect of Au-NPs and Au(III) on *P. tricornutum*

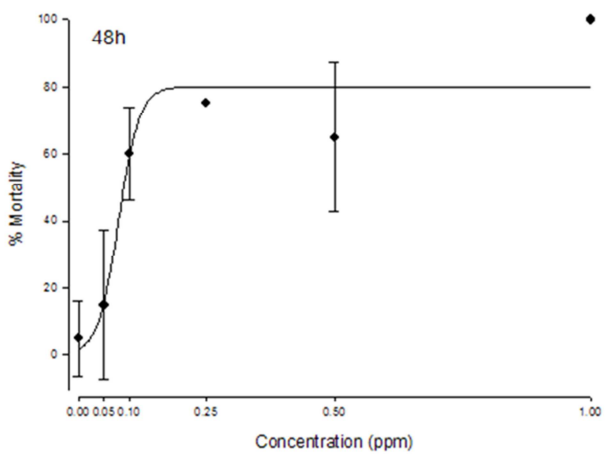

Figure 5. *Tisbe battagliai* mortality (%) vs Au-NPs concentration

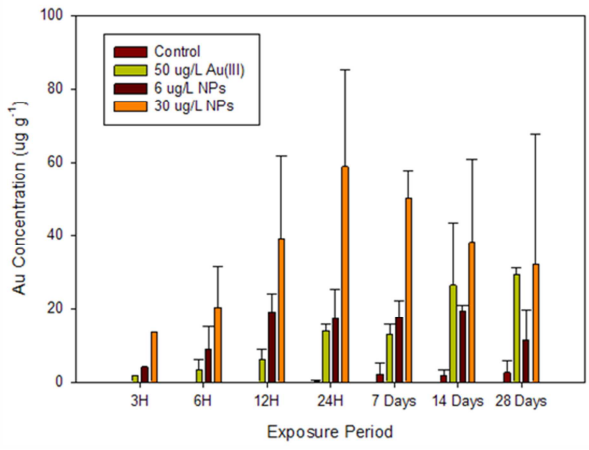

Figure 6. Gold concentration in digestive gland of *R. philippinarum* collected at several exposure times