Biol. Mar. Mediterr. (2014), 21 (1): 353-354

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ECOTOXICITY EFFECTS OF NANO-TiO_2 TOWARDS MARINE ORGANISMS

EFFETTI ECOTOSSICOLOGICI DI NANO-TiO_2 SU ORGANISMI MARINI

Abstract - *Engineered nanomaterials (ENMs) are increasing their presence in the worldwide market as display innovative properties that are suitable for various applications in many fields. In particular, TiO_2 nanoparticles ($nTiO_2$) are widely used in products accessible to the mass market. Nevertheless, there is a paucity of general information about their potential effects towards the aquatic species, especially to marine species. In this work the in vivo effects of TiO_2 nanoparticles were checked with a battery of model organisms:* V. fischeri, P. tricornutum, C. gigas *and* M. galloprovincialis. *Moreover, ENMs bioaccumulation was investigated using the ragworm* H. diversicolor. *The comparison of results with literature highlighted some criticisms of toxicity tests with ENMs, mainly due to the difficulty of determining the real exposure scenarios.*

Key-words: *nano-TiO_2, seawater organisms, ecotoxicity, bioaccumulation.*

Introduction - Engineered nanomaterials are at the forefront of ecotoxicologist agendas, due to their increasing use in a broad range of industrial and domestic sectors. In particular, under UV irradiation, $nTiO_2$ shows photocatalytic properties generating Reactive Oxygen Species (ROS). Due to this property it is widely employed in many different applications (Fujishima *et al*., 2008). More specifically, about the 65% of total $nTiO_2$ is estimated to be used for the production of various common cosmetics and sunscreen creams (Clemente *et al*., 2012). As the seawaters are indicated to be the final sink for this kind of emerging pollutants, concerns exist about their effects towards seawater organisms. Anyway, specific data about seawater environment are still scarce (Minetto *et al*., 2014). Our goal was to study the potential toxicity effect of $nTiO_2$ towards a battery of model organisms composed by the bacterium *Vibrio fischeri*, the algae *Phaeodactylum tricornutum* and the mollusc *Crassostrea gigas* and *Mytilus galloprovincialis* (Minetto, 2012; Libralato *et al*., 2013) as well as its bioaccumulation using the ragworm *Hediste diversicolor* (Volpi Ghirardini *et al*., 1999).

Materials and methods - All tests were conducted on the basis of the respective standardized protocol. Some methods were modified to increase sensitivity. *V. fischeri* (ISO, 2007) was exposed to 10^{-9}-10 mg $nTiO_2$ L^{-1} for 5, 15, 30 and 60 minutes. Algae exposure (ISO, 2006) ranged between 0.09-90 mg $nTiO_2$ L^{-1} for 72 h. Mollusc zygotes exposure (ASTM, 2004) ranged between 1-100 mg $nTiO_2$ L^{-1} for 24 h for oysters and between 0.5-64 mg $nTiO_2$ L^{-1} for 48 h for mussels both in total darkness and considering a 12 h photoperiod. Ragworms (USEPA, 1991) were exposed to 5 and 50 mg $nTiO_2$ L^{-1} for 5 weeks. Negative and positive controls were included in all tests.

Results - Results showed embryotoxicity effects in the case of molluscs. Conversely, just a reduced growth inhibition was observed with algae and no bioluminescence inhibition was recorded with bacteria. Anyway, the experimental activities and the comparison with the concerning references evidenced the lack of specific protocols for toxicity tests with ENMs. For these reasons, the control of the variability of experimental conditions is difficult as the knowledge of the real exposure scenario. *C. gigas* showed the maximum embryotoxicity effect (83%) at the minimum concentration (1 mg $nTiO_2$ L^{-1}); conversely, at 10 and 100 mg L^{-1} $nTiO_2$ the toxicity effects were 2% and 4%, respectively. Embryotoxicity effects with *M. galloprovincialis* were evidenced at 4 to 8 mg $nTiO_2$ L^{-1}. Moreover, the presence of light seemed to enhance the ENM effects. All the effects were significantly different from the negative controls ($p<0.05$, ANOVA). *P. tricornutum* growth inhibition was limited (maximum 28% at 36 mg L^{-1}), but significant changes were observed in ENM behavior at 1-10 mg L^{-1} and 16-90 mg L^{-1} ($p<0.05$, ANOVA). This is probably due to a different size and surface area of agglomerates, resulting in simultaneous different exposure scenarios. Probably, according to Savolainen *et al.* (2010), the surface area is a more indicated parameter to describe the effective exposure scenario, respect to the concentration. With *V. fischeri*, no bioluminescence inhibition effects were observed as confirmed by Heinlaan *et al.* (2008). *H. diversicolor* bioaccumulation tests are in progress.

Conclusions - $nTiO_2$ seems to have ecotoxic effects on the basis of the considered model organisms. Decreasing sentitivities were observed: molluscs > algae > bacteria. Criticisms emerged from the experimental activities and the comparison with literature data. The main gap in the knowledge is about the real exposure scenario: it appears as fundamental to have information about the behavior of ENP dispersed in the media and their effective concentration to understand the evolution and the cause of the highlighted adverse effects.

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