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# Effect of silver nanoparticles on marine organisms belonging to different trophic levels

Chiara Gambardella [a, *], Elisa Costa [b], Veronica Piazza [a], Adele Fabbrocini [c], Emanuele Magi [d], Marco Faimali [a], Francesca Garaventa [b]

[a] CNR – ISMAR, Via De Marini 6, 16149 Genova, Italy
[b] CNR – ISMAR, Arsenale – Tesa 104, Castello 2737/F, 30122 Venezia, Italy
[c] CNR – ISMAR, Via Pola 4, Lesina, 71010 Foggia, Italy
[d] DCCI, University of Genova, Via Dodecaneso 31, 16146 Genova, Italy

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ABSTRACT

Silver nanoparticles (Ag-NPs) are increasingly used in a wide range of consumer products and such an extensive use raises questions about their safety and environmental toxicity. We investigated the potential toxicity of Ag-NPs in the marine ecosystem by analyzing the effects on several organisms belonging to different trophic levels. Algae (*Dunaliella tertiolecta*, *Skeletonema costatum*), cnidaria (*Aurelia aurita* jellyfish), crustaceans (*Amphibalanus amphitrite* and *Artemia salina*) and echinoderms (*Paracentrotus lividus*) were exposed to Ag-NPs and different end-points were evaluated: algal growth, ephyra jellyfish immobilization and frequency of pulsations, crustaceans mortality and swimming behavior, and sea urchin sperm motility. Results showed that all the end-points were able to underline a dose-dependent effect. Jellyfish were the most sensitive species, followed by barnacles, sea urchins, green algae, diatoms and brine shrimps. In conclusion, Ag-NPs exposure can influence different trophic levels within the marine ecosystem.



## 1. Introduction

Nanotechnology is rapidly expanding with applications in different fields, from electronics to medicine, from remediation to engineering and food industry (Oberdörster et al., 2007; Das et al., 2013; Massarky et al., 2013). Nowadays the products containing silver nanoparticles (Ag-NPs) are increasing, as well as their worldwide diffusion for industrial processes and treatments (Myrzakhanova et al., 2013), due to their importance as antimicrobial agents (Mohan et al., 2007; Zheng et al., 2008) and their particular magnetic, optical, electronic and catalytic properties, that make Ag-NPs suitable for applications in a wide range of fields (Johari et al., 2013). The Woodrow Wilson Database (2011) has listed about 1317 NP-based consumer products currently on the market, 311 of which contain Ag-NPs. Nanotechnology enables the incorporation of these NPs into many daily personal care products, wound dressings, kitchen-ware, children toys, washing machine coatings, wall paints, food packaging and many more (Kim et al., 2007; Sotiriou and Pratsinis, 2010). Moreover 53% of the EPA (Environmental Protection Agency) -registered biocidal silver products likely contain Ag-NPs (Nowack et al., 2011). Such an extensive use and growing production raises questions about Ag-NP safety and environmental toxicity. To date the predicted environmental concentrations (PECs) for Ag-NPs in the environment are at the range of ng $L^{-1}$ to mg $kg^{-1}$ (Fabrega et al., 2011a; Reidy et al., 2013) and this value is estimated to be 0.03–0.08 μg $L^{-1}$ in the water compartment, representing a high potential risk induced by Ag-NPs in the aquatic ecosystem (Mueller and Nowack, 2008). The investigation of Ag-NPs effects in the aquatic ecosystem is very important, since the wide variety of the applications containing Ag-NPs can potentially end up in the aquatic environment and reach the sea during waste disposal (Asharani et al., 2008) as most of NPs do. Ag-NPs may aggregate and/or dissolve in the aquatic environment (Baun et al., 2008), so these processes may alter the fate, transport and toxicity of such NPs (Lowry et al., 2012).

Most of the currently available ecotoxicological data regarding

* Corresponding author.
*E-mail address:* chiara.gambardella@ge.ismar.cnr.it (C. Gambardella).

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Ag-NPs are limited to freshwater species used in regulatory testing (i.e. OECD, ISO), that represent key environmental organisms, such as algae, crustaceans and fish (Miao et al., 2010; Hoheisel et al., 2012; Kashiwada et al., 2012; Wu and Zhou, 2013). The toxicity of Ag-NPs measured in freshwater depends on the test species (Blinova et al., 2013). For example, Ag-NPs are reported to be toxic for crustaceans at very low concentration ($EC_{50}$ < 0.1 mg $L^{-1}$), followed by algae ($EC_{50}$ = 0.23 mg $L^{-1}$), but the toxicity to fish is relatively low ($EC_{50}$ = 7.1 mg $L^{-1}$, Kahru and Dubourguier, 2010; Ashgari et al., 2012).

On the contrary, the current knowledge on the fate, behavior and ecotoxicity of Ag-NPs in the marine ecosystem is scarce. Recent findings indicate that salinity influences the stability and aggregation of Ag-NPs (Wang et al., 2014), therefore the fate of such NPs is primary to aggregate in the water column, precipitate and accumulate in sediments following release into the marine environment (Keller et al., 2010; Buffet et al., 2013). To date only sparse data on the potential toxicity of Ag-NPs to marine species (e.g. their effects on sea urchin and oyster development, fish and oyster physiology and blue mussel accumulation, Chae et al., 2009; Ringwood et al., 2010; Zuykov et al., 2011; Gambardella et al., 2013; McCarthy et al., 2013) are available.

Ag-NPs cause a significant decrease in marine biofilm volume and biomass (Fabrega et al., 2011b), inhibit the photosynthetic performance of green algae (Oukarroum et al., 2012) and induce mortality and a cyst hatching decrease in brine shrimp (Arulvasu et al., 2014). As a contribution to this field, the effects of Ag-NPs on environmental relevant marine test species belonging to different trophic levels have been examined in the present paper. In order to obtain a comprehensive assessment of Ag-NP effects on seawater column organisms, toxicity testing was carried out across a battery of six species belonging to different trophic levels (primary producers and consumers), including algae (*Skeletonema costatum* and *Dunaliella tertiolecta*), cnidaria (*Aurelia aurita*), crustaceans (*Artemia salina* and *Amphibaanus amphitrite*) and echinoderms (*Paracentrotus lividus*). The diatom *S. costatum*, the green alga *D. tertiolecta*, the sea urchin *P. lividus*, the brine shrimp *A. salina* and the barnacle *A. amphitrite* were selected because they are established model species in standardized toxicity tests, ecotoxicological studies and in ecological risk assessment (Wong et al., 1995; UNI EN ISO, 2000; ASTM, 2004; Faimali et al., 2006; Losso et al., 2007; Pane et al., 2008; Dineshrama et al., 2009; Pétinay et al., 2009; Garaventa et al., 2010; Piazza et al., 2012).

In addition, the jellyfish *A. aurita* was used in this work since it has been recently proposed as a very new, sensitive and innovative model organism in ecotoxicological studies. Besides occupying a key evolutionary position as basal metazoan (Faimali et al., 2014; Costa et al., 2015), cnidarians are important components of marine food webs both as major consumers of zooplankton (Riisgård et al., 2007) and preys (Cardona et al., 2012; Titelman et al., 2006). Moreover, increasing evidence has shown that jellyfish have an influence on microbial food webs, through direct and indirect effects, and are important regulators of marine biogeochemical fluxes (Turk et al., 2008).

Therefore, the aim of this study was to expand knowledge on the effects of Ag-NPs on the marine ecosystem, by analyzing different end-points, such as algal growth, jellyfish immobility and frequency of pulsation, crustacean mortality and swimming behavior, and sea urchin sperm motility.

## 2. Materials and methods

### 2.1. Ag NPs characterization

Ag NPs were obtained from Polytech Inc. (Germany) as a 1000 ppm suspension of metallic silver in deionized water, with a nominal particle size provided by the producer in the range of 1–10 nm. Ag-NPs were suspended in 0.22 μm filtered natural seawater (FNSW, supplied from the Aquarium of Genova (Italy, pH 8.27; Salinity 36.9‰) and sampled at few miles from the Ligurian Sea coast) to obtain a concentration of 1 mg $mL^{-1}$ according to Gambardella et al. (2013), before bringing them to the different concentrations used in the tests (Table 1). After NP suspension preparation the toxicity tests were immediately performed. The testing concentrations were chosen on the basis of the results of a preliminary screening test using an order-of-magnitude dilution series (0-0.1-1-10-100 mg $L^{-1}$), with which we assessed the ecotoxicological end-points.

Ag-NPs were diluted 1:100 in deionized water in order to determine Ag concentration by Plasma Emission Spectrometry (ICP-OES). The ICP-OES instrument was an axially-viewed Varian (Springvale, Australia) Vista PRO with the following main operating conditions: RF Power: 1100 W; Plasma gas flow rate: 15.0 L $min^{-1}$. Sample uptake rate: 0.8 mL $min^{-1}$. To compensate for non-spectral interferences, the on-line internal standardization (4 g $mL^{-1}$ Lutetium standard solution) was applied. Ag NP size (determined by Dynamic Light Scattering) and effective surface charge (ζ-potential) characterization, available in Gambardella et al. (2015), were 990 nm (diameter) and −3 ± 2 mV, respectively.

### 2.2. Toxicity tests

#### 2.2.1. Algae

The potential of Ag-NPs to inhibit algal growth was evaluated using the green alga *D. tertiolecta* and the diatom *S. costatum*. Algae were obtained from culture collection of CNR ISMAR (Genova, Italy). Algal cells were cultured in artificial seawater Instant Ocean® with complete F2 culture medium (Guillard and Ryther, 1962) at 20 ± 0.5 °C with a 12–12 h light dark period and light intensity of 6000–10,000 lux (Sbrilli et al., 1998). Toxicity tests were performed according to the method ISO 10253, 2006. Three replicates for each Ag-NP suspension, including the control, were prepared. After 72 h, culture growth was stopped by using Lugol's solution (Throndsen, 1978; ICRAM, 2001) and the algal growth inhibition was evaluated by counting cells with a haemocytometer Thoma, using a Leitz Diavert inverted microscope (Leitz, Germany). The reliability of the test was verified using cadmium as reference toxicant, according to UNI EN ISO 10253 method.

#### 2.2.2. Cnidarians

Colonies of *A. aurita* polyps attached on PVC tubes were supplied by the "Acquario di Genova, Costa Edutainment S.p.A."; once in the CNR − ISMAR laboratories, they were kept at 20 ± 0.5 °C in 1.5 L dark plastic tanks, covered with a lid in order to keep polyps in dark conditions. Tanks were filled with FNSW (37‰) and gently aerated. *A. aurita* ephyra were obtained from polyps as described by Faimali et al. (2014). Once released by strobilation, ephyrae were transferred into a beaker and immediately used for the toxicity tests.

Ephyrae were placed into multi-well plates, one individual for each well containing 2 mL of Ag-NP solution (Table 1). For each NP suspension, three replicate plates were prepared, each replicate consisting of 8 wells containing one ephyra in order to avoid interactions among organisms. Plates were then sealed and kept at 20 ± 0.5 °C in dark conditions. A toxicity test using cadmium nitrate as reference toxic compound was also performed, according to Faimali et al. (2014).

After 24 and 48 h, the acute (immobility) and sub-lethal endpoint (frequency of pulsations) were evaluated. The acute endpoint consisted in organism inability to perform any kind of movement (without changing their own barycentre position) for