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Toxicity of PVA-stabilized silver nanoparticles to algae and microcrustaceans

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ABSTRACT

Over the years there has been a significant increase in the manufacturing of silver nanoparticles-based products, mainly due to their antimicrobial activity, with application in medicine and textile and food industry. However, the inappropriate use and disposal of these materials can allow the entry of silver nanoparticles (AgNP) into the aquatic environment, with potential toxicological effects. In this study we used *Pseudokirchneriella subcapitata*, *Artemia salina* and *Daphnia similis* as model organisms to investigate the toxicity of PVA-stabilized AgNP at several concentration levels. AgNP were physico-chemically characterized by UV–vis spectroscopy, particle size distribution, zeta potential analyzes and Transmission Electron Microscopy (TEM). AgNP presented a maximum absorption at 400 nm and size range between 2 and 18 nm. Each specific organism was exposed to AgNP concentrations through standardized protocol. For *P. subcapitata* and *A. salina* the EC_{50} value found, 1.09 mg L^{-1} and 5.5 × 10^{-2} mg/L, respectively, were in accordance to previous results reported in the literature. However, for *D. similis*, the EC_{50} 24–48 h values indicate a higher toxicity than other results reported for other daphnids.

1. Introduction

The field of nanomaterials is a fast-growing area and has attracted attention of scientists and engineers because of its multifunctionality and possibility to tailoring the materials properties (Li et al., 2010). The size between 1 and 100 nm (in at least one dimension) of nanomaterials provide unique characteristics that enable novel applications (Vandermoere et al., 2011) in several areas of science including chemistry, physics, biology, engineering and technology (Thakor et al., 2011). Nanotechnology can also be applied to agriculture for improving food nutritional value, and also on the management of supply chain processes associated with food quality (Nair et al., 2010; Scott and Chen, 2013; Singh and Rattanpal, 2014). Several studies have shown the benefits of metal nanoparticles for human health regarding antimicrobial activity (Chen and Schluesener, 2008; Durán et al., 2010). The unique physico-chemical and biological properties of nanoparticles (Abou El-Nour et al., 2010) arises from the larger surface area/volume ratio (compared to microparticles) resulting, for instance, in high chemical reactivity (Wijnhoven et al., 2009). One of the most traditional nanoparticles employed are based on silver (Chen and Schluesener, 2008), which finds application in electronics, toiletries clothing, food industry, paints, sunscreens, cosmetics and medical devices (Ahamed et al., 2010; Ankanna et al., 2010).

The increase of manufacture and consumption of products containing silver nanoparticles (AgNP) can lead to metallic nanoparticles release in the environment if waste is not properly disposed (Hamed Chaman et al., 2012; Ribeiro et al., 2014; Zahir et al., 2012). Silver in natural fresh water can be found in the form of silver chloride (AgCl), silver sulfide (Ag_2S) and the silver ion, the most toxic form (Ribeiro et al., 2014). According to a document of World Health Organization (WHO, 2003), silver is found in natural waters with concentration of 0.2–0.3 μg L^{-1}, while drinking waters can have silver concentrations between "non-detectable" and 5 μg/L. Concentration of these nanoparticles is increasing in aquatic environment and can strongly affect and damage the biota (Angel et al., 2013; Batley et al., 2012). For instance, AgNP concentrations above 5 μg L^{-1} have already been found for groundwater, surface water and drinking water (WHO, 2003). There are many

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Table 1
Concentrations of silver nanoparticles and respective percentages of test-solutions employed in the toxicity experiments.

Concentrations	
%	mg L^{-1}
0.0	0.0
0.001	1.5×10^{-4}
0.01	1.5×10^{-3}
0.1	1.5×10^{-2}
1.0	1.5×10^{-1}
10	1.5

possible reasons for the high toxicity of silver nanoparticles, including its high surface area/volume ratio, which greatly increases its rate of dissolution (Angel et al., 2013). Coating of AgNP with organic materials such as polymer-based stabilizer may also influence its toxicity (Kwok et al., 2012). Another important factor that influences nanoparticles toxicity is the bioavailability related to the aggregation behavior (Angel et al., 2013).

In this context, it becomes of prime importance to investigate the route of AgNP release on the environment and the exposure effects to aquatic organisms (Griffitt et al., 2012). Moreover, studies reporting the influence of AgNP size, concentration and distribution on its toxicity for aquatic microorganisms are still scarce (Ribeiro et al., 2014). In this study we evaluated the toxicity of AgNP for the growth of algal *Pseudokirchneriella subcapitata*, the mobility of the population of *Artemia salina* and the immobilization of *Daphnia similis*. The effect of AgNP in distinct organisms tests was analyzed considering different trophic levels in order to evaluate the toxicity in diverse aquatic ecosystems.

2. Materials and methods

2.1. Preparation of silver nanoparticles (AgNPs)

To obtain highly concentrated stable dispersions of nanosized silver particles, chemical reduction of silver nitrate in aqueous solutions was employed. Deionized water was used to prepare the solutions of polyvinyl alcohol (PVA) (stabilizing agent) with silver nitrate ($AgNO_3$), which were then reduced in the presence of sodium borohydride ($NaBH_4$) (Berni Neto et al., 2008). All reagents were obtained from Sigma-Aldrich and used without further purification. The synthesis can be summarized as follows: 60 mM PVA was added in 10 ml of a 5 mM silver nitrate solution and stirred for 5 min. Next, 5 ml of a freshly prepared sodium borohydride solution was added and stirred for 15 min using a magnetic stirrer. At the end of the synthesis yellow suspensions were obtained confirming the formation of AgNP. The suspensions were then stored in dark bottles and remained stable for more than 2 months. In order to obtain AgNP suspension with concentrations shown in Table 1, aliquots of the original sample were diluted in deionized water, and then used in the experiments with the aquatic organisms.

A borohydride solution of 5 mM (same concentration of silver suspension) was used as control experiment for the aquatic organisms. Such solution was submitted to the same set of experiments of AgNP suspension. PVA was not included in the control solution, once it is considered practically non-toxic for aquatic microorganisms (Wong, 1996).

2.2. Characterization of silver nanoparticles

2.2.1. UV–vis absorption spectroscopy

The optical properties of silver nanoparticles were monitored by UV–vis absorption spectroscopy using a Perkin-Elmer UV–vis Lambda 6. Tree samples were diluted in deionized water and placed in a quartz cell of 2 cm^3 for collecting the absorption spectrum from 250 to 700 nm.

2.2.2. Zeta potential and particle size distribution

The zeta potential of AgNP was evaluated using a Zetasizer Nano ZS (Malvern Instruments Inc., USA), which measures electrophoretic mobility of nanoparticle using phase analysis light scattering. This equipment was also employed to determine the particle size using dynamic light scattering.

2.2.3. Transmission Electron Microscopy (TEM)

AgNP shape, morphology and size distribution was evaluated by a Transmission Electron Microscope – TEM – (FEI Tecnai G2 F20) using 200 kV accelerating voltage. One drop of AgNP suspension was added on a carbon coated copper grid and the excess was removed with filter paper. The grid remained light protected with the use of a piece of aluminum foil and maintained at environmental temperature. The program ImageJ was used to determine the size of AgNP from TEM images.

2.3. Test organisms exposure to different concentrations of AgNP

2.3.1. P. subcapitata

The unicellular algae *P. subcapitata* (erstwhile *Selenastrum capricornutum*) was used as test organism. The algae was cultured in accordance with the methodology recommended by the Organization for Economic Co-operation and Development (OECD, 1984a), in climatic chambers under controlled temperature at 20 ± 2 °C and luminosity of ~1300 lux. The algal suspension was distributed in Petri dishes (final volume of 15 mL), yielding a concentration of approximately 10^5 cells mL^{-1}. The algae were exposed during 7 days to each of the AgNP suspensions shown in Table 1. Free-AgNP suspension containing only borohydride solution was also evaluated. Periodically, aliquots were taken from the algal suspensions for measuring the absorbance at 750 nm in a Shimadzu spectrophotometer UV-1650 PC, once absorbance is proportional to the cells concentration. The absorbance values (averages of three replicates) were then converted to percentages, which is directly proportional to algae growth. The calculation of the concentration that inhibits algal growth rate by 50% over 7 days (EC_{50}-7d) was based on the relative inhibition of growth rate as a function of the silver nanoparticle concentration (mg L^{-1}).

2.3.2. A. salina

Young individuals of the brine shrimp *A. salina* were used as test organisms. Approximately 24 h before the test, 900 ml of synthetic seawater were placed in a 1 L Erlenmeyer. This water was prepared by adding 30 g of salt "Sera Premium®" (Sera GmbH, Heinsberg) in 1000 mL of water (pH = 7.2; conductivity = 110 μS cm^{-1}) from an artesian well. In this container was added approximately 50 mg of Artemia cysts (INVE Aquaculture Inc., Ogden). The suspension of cysts was kept under intense aeration through a porous stone at a temperature of 25 ± 1 °C and ~6300 lux brightness. The nauplii obtained were exposed to concentrations shown in Table 1 during 48 h at 20 ± 2 °C. Through the use of micropipette, 10 organisms were transferred to beakers containing the test solution (final volume of 30 ml) at different percentages, in duplicate. After 48 h, the number of organisms tested and the concentration that affects mobility in 50% of the population (EC_{50}-48 h) along with its 95% confidence interval were determined (USEPA, 1991).

2.3.3. D. similis

In ecotoxicity testing, usually the filter-feeding freshwater crustacean of the genus *Daphnia* has been used an indicator of the aquatic ecotoxicity tests, anticipating toxicity testing in mammals (Martins et al., 2007). By being a very sensitive organism

toward potential chemical toxicants, such as metal ion species, *Daphnia* has been employed as test organims for the standard protocols of the OECD. Environmental Protection Agency (EPA), and International Standards Organization (ISO) (Li et al., 2010; Baun et al., 2008). Acute toxicity immobilization tests were performed on each of the AgNPs suspension in accordance with OECD Guideline Part I (OECD, 1984b). *D. similis* cultures were maintained in 40 cm × 25 cm × 15 cm glass aquaria containing water from an artesian well, as described in OECD Guideline. The organisms were placed in acclimatized room with controlled temperature at 20 ± 2 °C and with luminosity of approximately 1000 lux. They were fed daily with microalgae (*Chlorella pyrenoidosa* and *P. subcapitata*) suspensions (Prestes et al., 2012).

Acute toxicity tests were performed on *D. similis* neonates that were less than 24 h old, using the AgNP suspension concentrations shown in Table 1. Six or seven neonates were placed in a glass beaker (experimental unit) containing 30 mL of test-solution. A total of 20 organisms were tested for concentrations 1.5, 1.5×10^{-1}, 1.5×10^{-2}, 1.5×10^{-3} and 1.5×10^{-4} mg L^{-1}) divided in three replicates. Immobilization was determined visually after 24 h and 48 h at each concentration, and the respective EC_{50} values were calculated together with the 95% confidence interval. *Daphnias* were examined and photographed after 24 h in order to evaluate their internal content using a stereomicroscope (Model SMZ 2 LED, Optika).

2.3.4. *Statistical analyses for* P. subcapitata, Artemia *and* Daphnia

The calculation of the specific growth rates of *P. subcapitata* was obtained by linear slope values of the respective curves of absorbance increase versus time (log Abs vs. days) (OECD, 1984a). The data were treated by Simple Regression module, contained in Statgraphics Plus (MANUGISTICS, 2001), which allowed calculation of EC_{50}-7d and its 95% confidence interval, and determined the regression model to best fit the data. Specifically, the calculation of the concentration that inhibits algal growth rate by 50% over 7 days (EC_{50}-7d) was based on the relative inhibition of growth rate as a function of the silver nanoparticle concentration (mg L^{-1}). EC_{50} is the effective concentration of toxicant that results in 50% reduction of growth of an organism population, relative to control, at a given duration of time (Chung et al., 2007). For EC_{50}-7d calculation purpose, although the data of the specific growth rate as a function of concentration did not strictly follow a correlated dose–response curve, it was fitted according to the linear regression model "$y = 0.0867 - 0.0411\sqrt{x}$", where "$y$" is a specific growth rate (log $Abs_{750nm} d^{-1}$) and "x" is the silver solution concentration (mg L^{-1}). The EC50-7d values were compared and considered significantly different from each other when their confidence intervals showed no overlap (Yang et al., 2002).

Artemia and *Daphnia* also had the EC_{50} 24–48 h calculated by modulus Probit Analysis of Statgraphics Plus 5.1 software (MANUGISTICS, 2001). Through the same procedures, we also evaluated the toxicity of the borohydride solutions free of AgNP to verify possible interference from other compounds in the test solutions.

3. Results

3.1. UV–vis absorption spectroscopy of AgNP

The UV–vis absorption spectrum of precursor silver salt solution and AgNP is displayed in Fig. 1. According to Whelan et al. (2004) and Krklješ et al. (2007) silver nanoparticles exhibit absorption spectra band centered at 400–410 nm. The spectrum shows that AgNP synthesis yielded a single and well-defined peak in the absorbance spectrum, with maximum absorbance at 400 nm, which corresponds to the characteristic surface plasmon resonance of AgNP (Fig. 1). The position of plasmon absorption peak

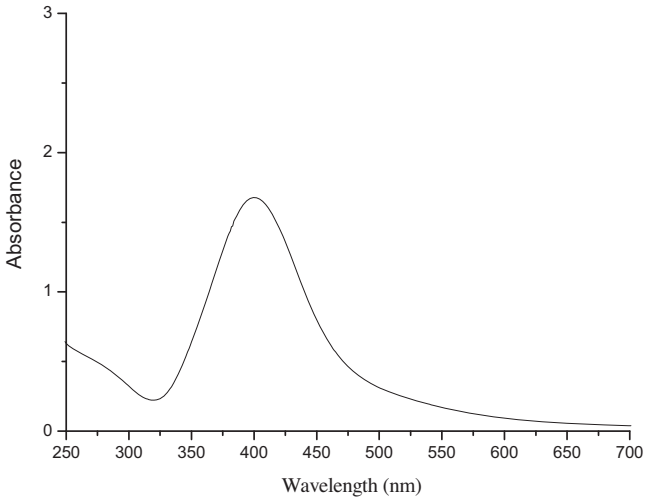

Fig. 1. UV–vis absorption spectrum of AgNP.

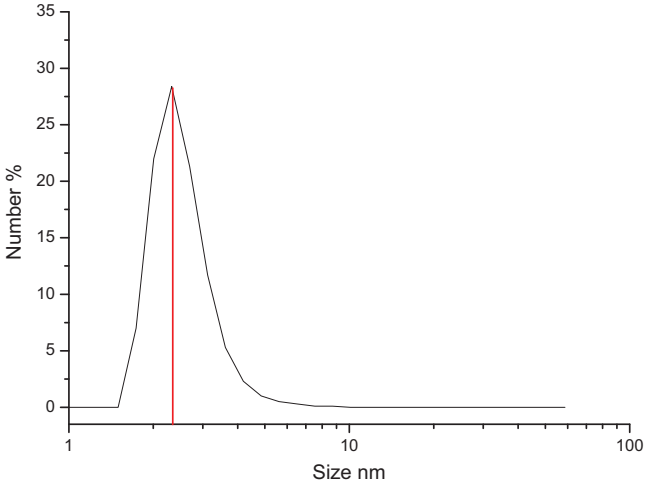

Fig. 2. Particle size distribution of AgNP.

of metal nanoparticles solutions depend on several factors, such as size, shape and polydispersity of the particles (Mock et al., 2002). The relatively narrow band indicates that AgNPs present a narrow size distribution, as further confirmed by TEM analysis. The well-defined peak combined with the yellow color of the colloid solution is an evidence of the non-oxidation of AgNPs (Varkey and Fort, 1993). The silver salt showed no absorbance in this spectral range.

3.2. Zeta potential and particle size distribution

The particle size distribution of AgNP obtained by dynamic light scattering is showed in Fig. 2. AgNP display a polydispersity index (PDI) = 0.179 with an average size of 8.12 nm. The zeta potential obtained for nanoparticles synthesized was around −1 mV at pH of 6.2, which is considered to be low for a permanent stabilization. AgNP suspension presenting low zeta potential values has already been reported in the literature (Loeschner et al., 2011). In the case described by Loeschner et al. (2011), the stability of AgNP suspension was ascribed by electrostatic stabilization, but instead to steric stabilization promoted by the large molecules of polymers PVP. Although in our work we used a distinct stabilizer (PVA), the large molecules of PVA similarly promote steric stabilization of AgNP suspension.

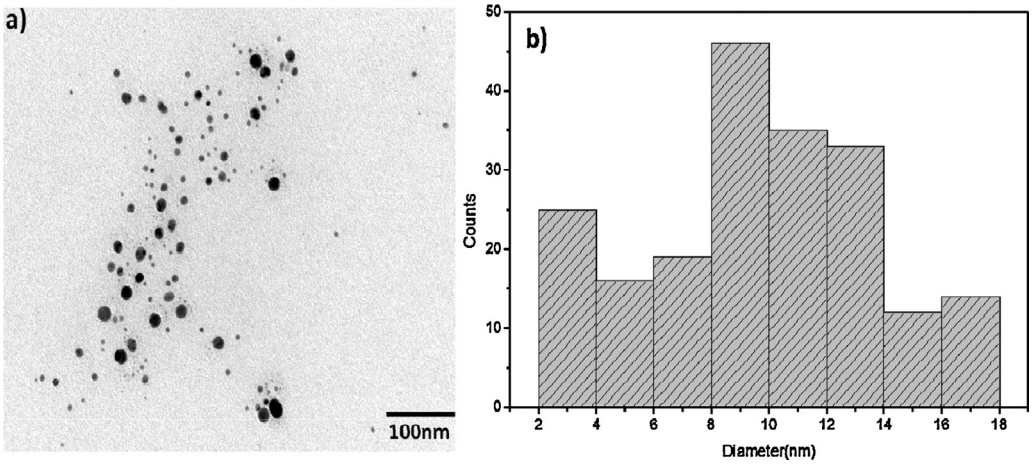

Fig. 3. (a) TEM image of AgNP and (b) histogram of nanoparticles diameter.

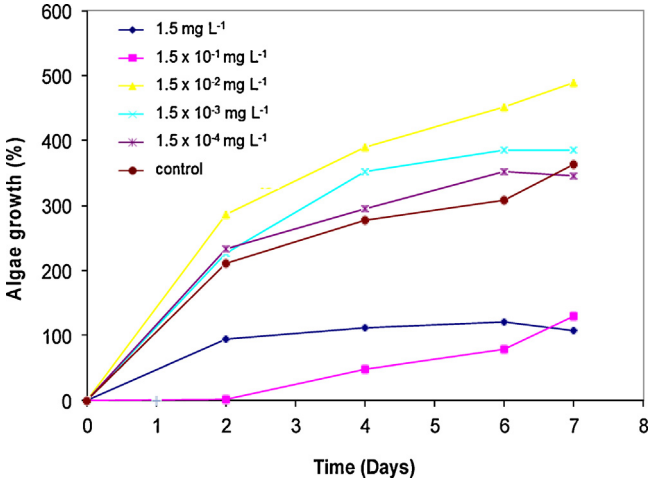

Fig. 4. Algae growth curves of *P. subcapitata* in different concentrations (mg L^{-1}) of silver nanoparticles.

3.3. Transmission Electron Microscopy (TEM)

TEM image of AgNP is showed in Fig. 3a, revealing that the primary morphology of the nanoparticles is spherical. It can be seen that the nanoparticles are well dispersed, without aggregation and have small particle size in the range 2–18 nm. Although TEM images are greatly subjected to sub-sampling, the results obtained (in terms of size range) agree with those obtained by dynamic light scattering technique. Very small nanoparticles can be observed in TEM micrographs, indicating good stabilization by the PVA, which was also inferred by the Zeta potential analysis. The corresponding particle size distribution histogram of AgNP obtained by TEM images is given in Fig. 3b.

3.4. Toxicity assays

3.4.1. P. subcapitata

Fig. 4 presents the curves of Algae growth as a function of exposure time (up to 7 days) of *P. subcapitata* in different concentrations (mg L^{-1}) of silver nanoparticles. The comparison of the curves suggest that the solution concentration of 1.5×10^{-1} mg L^{-1} was the one that most affected *P. subcapitata*, inhibiting the growth of algae substantially when compared with the other concentrations. The data show that two days of exposure led to a decrease in absorbance and onwards the absorbance (and consequently the growth) of *P. subcapitata* had a slight increase, but without exceeding 0.05. However, when we calculated the specific growth rate obtained from the slope of the regression of log Abs vs. time (OECD, 1984a), the lowest specific growth rate were observed for the 1.5 mg L^{-1} and 1.5×10^{-1} mg L^{-1} concentrations. Specifically, 1.5×10^{-1} mg L^{-1} solution yielded a growth rate of 0.054 ($R^2 = 0.524$, provided by the mathematical fitting), while the solution of 1.5 mg L^{-1} yielded a growth rate of 0.039 ($R^2 = 0.589$, provided by the mathematical fitting). The 1.5×10^{-2}, 1.5×10^{-3} and 1.5×10^{-4} mg L^{-1} solution concentrations of AgNP shows behavior similar to control, not interfering in the specific growth rate, which were in the order of 0.08–0.09. Such behavior probably arises because of the relative low concentration of silver, which was not sufficient to induce algal toxicity. The calculation of EC_{50}-7d for this AgNP suspension, according to the model employed (see experimental section for details), was 1.09 mg L^{-1} (0.59–3.15 mg L^{-1} at 95% confidence interval).

Similarly, the EC_{50} was determined for the sodium borohydride solution in terms of % of solution, which yielded EC_{50}-7d = 61.56%. Such value indicates that sodium borohydride solution is much less toxic than the AgNP suspension (EC_{50}-7d = 7.29%), presenting EC_{50}-7d value 8.5 times higher than AgNP.

The AgNP used in this work showed lower toxicity to *P. subcapitata* compared to other investigations reported in the literature. For instance, $EC_{50} = 3.24 \times 10^{-2}$ mg L^{-1} was obtained for alkane-coated AgNP of 7.5 nm (Ribeiro et al., 2014), while $EC_{50} = 1.95 \times 10^{-2}$ mg L^{-1} was obtained for PVP-coated AgNP with dimensions from 9.9 to 20 nm (Angel et al., 2013). The higher toxicity found for AgNP in Ribeiro et al. (2014) and Angel et al. (2013) can be ascribed to the nature of their respective coatings, indicating that PVA may present lower toxicity as stabilizing agent.

The EC_{50} obtained in our study, however, was similar to that reported by Mc Laughlin and Bonzongo (2012), who determined an $EC_{50-96\,h}$ for silver nanoparticles equivalent to 1.6 mg L^{-1} for *P. subcapitata* grown in natural water. Little is known about the action mechanism of AgNP on *P. subcapitata*. Navarro et al. (2008) indicate that there are several factors that influence the toxicity of AgNP to *P. subcapitata*, including size and surface area of nanoparticles, biotic interactions between organisms and chemical conditions of the environment that also affect the dissolution of silver ions from AgNP.

The precise mechanism by which distinct coating can affect aquatic toxicity is still a matter for debate. For instance, the toxicity found against *P. subcapitata* and other tests organisms can be influenced by the coating of the AgNP. The AgNP used in this investigation was coated by poly(vinyl alcohol) (PVA). PVA and AgNP

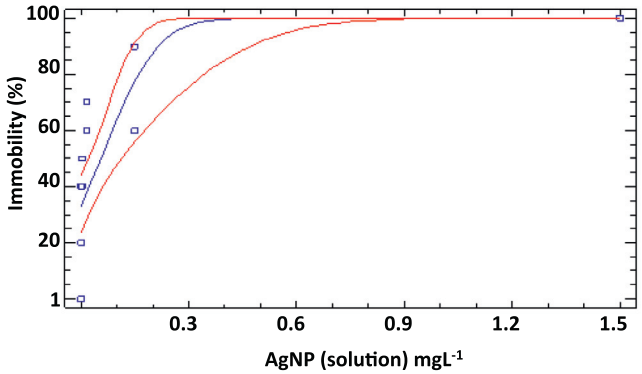

Fig. 5. Dose–response (in blue) and 95% confidence interval (in red) to *A. salina* under different concentrations of silver nanoparticles present in the solution.

present high interfacial compatibility, given by non-covalent bonds (Lin et al., 2012). The hydroxyl groups (OH^-) of PVA strongly interact with water molecules and OH^- neighbors of the environment, thereby increasing the molecular mobility (González-Campos et al., 2012). This molecular mobility of PVA causes a mobility of AgNP allowing release of these nanoparticles to the environment that could be toxic (DeMerlis and Schoneker, 2003).

According to Kwok et al. (2012) silver nanoparticles with different coating of organic materials (capable of enhancing their dispersion in water), present different degrees of toxicity to Japanese rice fish. In addition, AgNP coated by layers of polymers can increase the electrostatic repulsion and increase stability in suspension. The same authors attributed PVP-coated AgNP toxicity to a more positive surface charge found in the polymer surface, resulting in greater interaction with aquatic organism. Besides, the chemical interactions between the polymer surface coverage with aquatic environment can influence the release of AgNP.

3.4.2. A. salina

The results regarding exposure of *A. salina* at different concentrations of the test solution is expressed in Fig. 5. The value EC_{50}-48 h is 5.5×10^{-2} ($2.2–11.2 \times 10^{-2}$ mg L^{-1} at 95% confidence interval, and percentage of deviance = 60, 56) indicates the very high toxicity to this test-organism (<0.1 mg L^{-1}) (USEPA, 1985). This value appears to be higher than that found by Falugi et al. (2013) when assessing the toxicity of AgNP for brine shrimp using with 1–10 nm in serial dilutions (from 1.0×10^{-1} mg L^{-1} up to 100 mg L^{-1}). Falugi et al. (2013) obtained LC_{50}-48 h (lethal concentration) value of 7.3×10^{-3} mg L^{-1}. Solutions containing only borohydride showed no toxicity, since the mobility pattern of the organisms was similar to the control.

In the literature there are few reports on the ecotoxicity of silver nanoparticles against *A. salina*. Kumar et al. (2012) obtained LC_{50} value of 10 nM ($\sim 10^{-3}$ mg L^{-1}) by evaluating the toxicity of AgNP with average size of 33–44 nm against *A. salina*. Kowalska-Góralska et al. (2011) reported that Brine Shrimp specimens exposed by 6 h in silver solution at a concentration of 10 mg L^{-1} showed average survivability near to 93%. More recently, investigations on the toxicity of inorganic nanoparticles for *A. salina* have also been reported. For instance, Ates et al. (2013) described the impregnation of the gut of *A. salina* with TiO_2 nanoparticles (including adults and nauplii). These facts suggest that in nanometric, inorganic compounds can agglomerated in gut of aquatic organisms and prevent the nutrients absorptions, once mostly of these organisms are filter feeder.

3.5. D. similis

The AgNP suspension interfered on the survival of *Daphnia*. The calculation for 24 h EC_{50} for this AgNP suspension was 3.42×10^{-4} mg L^{-1} ($4.86–2.52 \times 10^{-4}$ mg L^{-1} at 95% confidence interval). For the 48 h the exposure, the EC_{50} calculation was 2.62×10^{-4} mg L^{-1} ($1.80–4.03 \times 10^{-4}$ mg L^{-1} at 95% confidence interval). The toxicity of the control solution of borohydride was determined, which proved the very low contribution (0.92%) on the overall toxicity of the solution containing AgNP.

The low EC_{50} 48 h value equivalent to 2.62×10^{-4} mg L^{-1} for *D. similis* is somehow consistent with findings in the literature in which the EC_{50}-48 h values for metals nanoparticles are lower than 1×10^{-1} mg L^{-1}. Because of this fact, we can categorize it as "very highly toxic" to the test-organism (UN United Nation, 2009). For example, Li et al. (2010) found the value of 2×10^{-3} mg L^{-1} for the silver ion, when testing the effects in *D. magna*. Asghari et al. (2012) obtained EC_{50} of the same value on *D. magna* for spherical silver nanoparticles of average size of 16.6 nm. The lower EC_{50} value for *D. similis* found in this work comparatively to other values reported in the literature can be explained by the higher sensitivity of the former to toxicant materials (Jardim et al., 2008; Romanholo Ferreira et al., 2011).

Ag^+ inhibits the flow of sodium ions (Na^+), by blocking the action of enzymes, Na^+, K^+ and ATPase, causing a disturbance in ion regulation of these aquatic organisms (Allen et al., 2010; Bianchini and Wood, 2003; Zhao and Wang, 2010; Stensberg et al., 2014). At the moment that silver nanoparticles are exposed to the aquatic environment they undergo surface oxidation by releasing the ionic silver (Ag^+) in water, which presents the highest toxicity for aquatic organisms (Angel et al., 2013; Allen et al., 2010; Lee et al., 2005). Jo et al. (2012) also obtained high toxicity for some of their formulations (24 h-EC_{50} $9.0–14.3 \times 10^{-3}$ mg L^{-1}) to *Daphnia*, assigning the toxic effect of the silver ions released from the surface of silver nanoparticles.

Besides the sensitivity to pollutants, *Daphnia* compose the bottom of the food chain in aquatic freshwater ecosystems. A subtle change in the quality and quantity of *Daphnia* population affects other populations of aquatic organisms, resulting in major environmental impacts (Martins et al., 2007). Fig. 6 shows changes suffered by *Daphnia* adults and neonates exposed for 24 h to a solution of AgNP with concentration of 1.5 mg L^{-1}. The dark coloration observed in the lines of the intestine indicates that these organisms ingested the solution of silver nanoparticles. It can be observed that, for both adult and neonate, the AgNP suspension modified the morphology of the eyes and that there are AgNP agglomerates impregnated in the carapace, antennules and other parts of body. Ingestion of AgNP may interfere on food availability for these organisms and also impair intestine cleaning (Lee et al., 2005). Zhao and Wang (2011) also showed brown color in their gut lines when exposed at high AgNP concentrations (200 and 500 mg L^{-1}). Similar results were found (Ates et al., 2013) for other nanoparticles, which showed that *Daphnias* had difficulty to clean up the gut after TiO_2 nanoparticles ingestion.

3.5.1. Final remarks

The physical and biological conditions of aquatic systems are complex and composed of many variables, and therefore, it is difficult to reproduce in vitro conditions. Thus, the toxicity of metals, especially metallic nanoparticles, is largely affected by water quality parameters such as pH, temperature and organic composition.

Generally, toxicity of Ag salts are higher compared to AgNP, nevertheless AgNP can also have toxic effects depending on concentration of dissolved ionic silver, probably due to additional effects of particles and agglomerations on cell membranes. Such behavior depends on various factors like media, organic molecules,

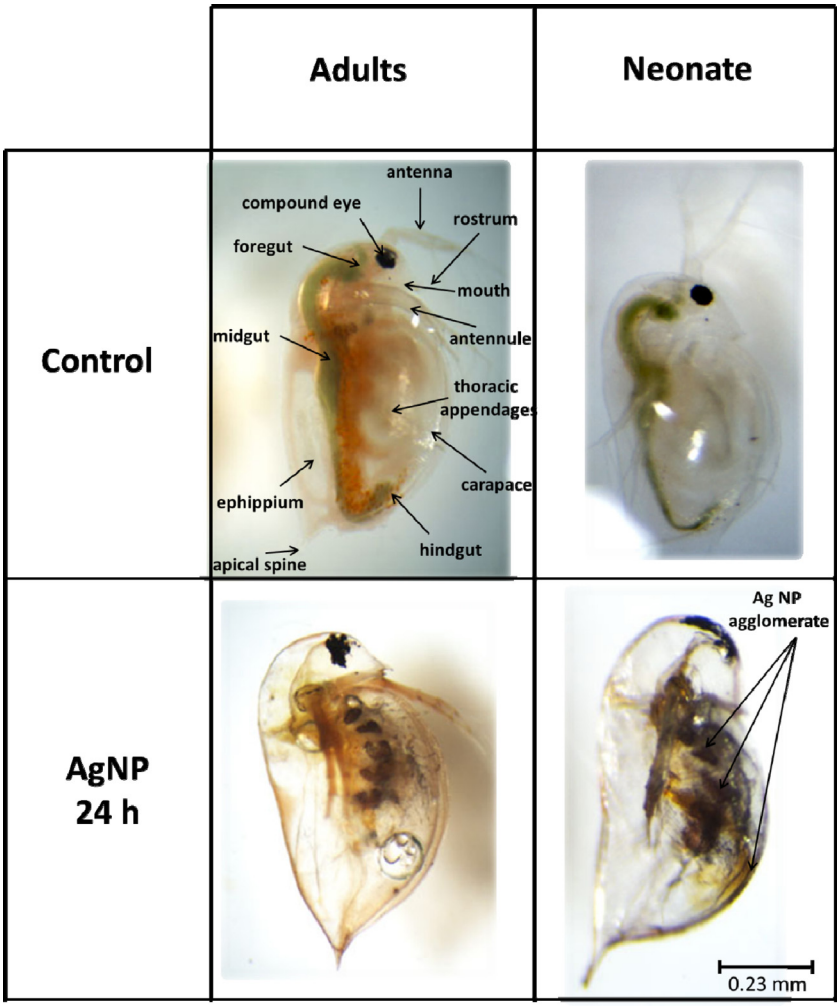

Fig. 6. Typical alteration observed in *Daphnia* adults and neonates after 24 h of silver nanoparticles exposure.

light conditions and particle size or NP coating (Boenigk et al., 2014). In this context, Vannini et al. (2013) compared the effects of $AgNO_3$ and AgNPs on *Eruca sativa* and observed that only the AgNP exposure causes the alteration of some proteins related to the endoplasmic reticulum and vacuole indicating these two organelles as targets of the AgNPs action. According to the authors, these data add further evidences that the effects of AgNPs are not simply due to the release of Ag ions.

Specifically in Brazil, the concentration limit of Ag in the aquatic compartment is ruled by the CONAMA 357/05 decreet (BRASIL, 2005), which establishes 0.01 mg L^{-1} for freshwaters and 0.005 mg L^{-1} for saline waters. According to our results, the effective concentration (EC_{50}-48 h) for the most sensitive organism is about 20 times lower than the limit established by CONAMA decreet. Considering the acute toxicity (EC_{50}) for the most sensible organism and an application factor of 100 in order to prevent the chronic adverse effects in such species and protect other species (Gherardi-Goldstein et al., 1990), a concentration of 2.62×10^{-6} mg L^{-1} was calculated (2.62×10^{-4} mg L^{-1}/100) for this purpose. For achieving this concentration, as reported by Zagatto (2006) and Crane et al. (2003) in order to assess the safety of materials, it would be necessary a direct application of approximately 7.86 mg of AgNP over an area equivalent to 1 ha, with a water column depth of 30 cm. Thus, according to the considerations above, AgNP concentration limits previously established should be carefully reviewed for correct handling and disposing of AgNP, avoiding accumulation in aquatic compartments.

4. Conclusions

This study investigated toxic effects of silver nanoparticles stabilized with PVA for aquatic organisms, such as *P. subcapitata* algae, *A. salina* and *D. similis*, representing different trophics levels of the food chain. According to dynamic light scattering measurements, the AgNPs in solution are well dispersed, with size range 2–18 nm. Among the organisms studied, AgNP showed lower toxicity to *A. salina* and *P. subcapitata* organisms compared with data found in the literature, with EC_{50} 5.5×10^{-2} mg L^{-1} and 1.09 mg L^{-1} respectively. The AgNP showed high toxicity to *D. similis* with EC_{50} (48 h) 2.62×10^{-4} mg L^{-1}. According to the EC_{50} values, the order of toxicity for the test-organisms is *D. similis* > *A. salina* > *P. subcapitata*. This difference is statistically significative ($p < 0.05$) since the 95% confidence intervals of the EC_{50s} do not overlap to each other (Yang et al., 2002). These nanoparticles were impregnated along the *Daphnia* gut and shell as well as in the appendices, including altered morphology eye. Considering the technological applications of AgNP, investigation on its effects for aquatic organisms are crucial to help to establish protocols for the use and disposal of AgNP on the environment, minimizing toxic effects.

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References

Abou El-Nour, K.M., Eftaiha, A., Al-Warthan, A., Ammar, R.A., 2010. Synthesis and applications of silver nanoparticles. Arab. J. Chem. 3, 135–140.

Ahamed, M., AlSalhi, M.S., Siddiqui, M., 2010. Silver nanoparticle applications and human health. Clin. Chim. Acta 411, 1841–1848.

Allen, H.J., Impellitteri, C.A., Macke, D.A., Heckman, J.L., Poynton, H.C., Lazorchak, J.M., Govindaswamy, S., Roose, D.L., Nadagouda, M.N., 2010. Effects from filtration, capping agents, and presence/absence of food on the toxicity of silver nanoparticles to *Daphnia magna*. Environ. Toxicol. Chem. 29, 2742–2750.

Angel, B.M., Batley, G.E., Jarolimek, C.V., Rogers, N.J., 2013. The impact of size on the fate and toxicity of nanoparticulate silver in aquatic systems. Chemosphere 93, 359–365.

Ankanna, S., Prasad, T., Elumalai, E., Savithramma, N., 2010. Production of biogenic silver nanoparticles using *Boswellia ovalifoliolata* stem bark. Dig. J. Nanomater. Biostruct. 5.

Asghari, S., Johari, S.A., Lee, J.H., Kim, Y.S., Jeon, Y.B., Choi, H.J., Chaul Moon, M., Je Yu, I., 2012. Toxicity of various silver nanoparticles compared to silver ions in *Daphnia magna*. J. Nanobiotechnol. 10, 1–14.

Ates, M., Daniels, J., Arslan, Z., Farah, I.O., 2013. Effects of aqueous suspensions of titanium dioxide nanoparticles on *Artemia salina*: assessment of nanoparticle aggregation, accumulation, and toxicity. Environ. Monit. Assess. 185, 3339–3348.

Batley, G.E., Kirby, J.K., McLaughlin, M.J., 2012. Fate and risks of nanomaterials in aquatic and terrestrial environments. Acc. Chem. Res. 46, 854–862.

Baun, A., Hartmann, N., Grieger, K., Kusk, K.O., 2008. Ecotoxicity of engineered nanoparticles to aquatic invertebrates: a brief review and recommendations for future toxicity testing. Ecotoxicology 17, 387–395.

Berni Neto, E., Ribeiro, C., Zucolotto, V., 2008. Síntese de nanopartículas de prata para aplicação na sanitização de embalagens. Embrapa Instrumentação Agropecuária. Comunicado Técnico.

Bianchini, A., Wood, C.M., 2003. Mechanism of acute silver toxicity in *Daphnia magna*. Environ. Toxicol. Chem. 22, 1361–1367.

Boenigk, J., Beisser, D., Zimmermann, S., Bock, C., Jakobi, J., Grabner, D., Sures, B., 2014. Effects of silver nitrate and silver nanoparticles on a planktonic community: general trends after short-term exposure. PLOS ONE 9.

BRASIL, 2005. Ministério do Meio Ambiente. Conselho Nacional do Meio Ambiente CONAMA. Resolução n0 357, de 17 de março de 2005. Diário Oficial da União, Brasília.

Chen, X., Schluesener, H., 2008. Nanosilver: a nanoproduct in medical application. Toxicol. Lett. 176, 1–12.

Chung, M., Hu, R., Wong, M., Cheung, K., 2007. Comparative toxicity of hydrophobic contaminants to microalgae and higher plants. Ecotoxicology 16, 393–402.

Crane, M., Whitehouse, P., Comber, S., Watts, C., Giddings, J., Moore, D.R.J., Grist, E., 2003. Evaluation of probabilistic risk assessment of pesticides in the UK: chlorpyrifos use on top fruit. Pest. Manag. Sci. 59, 512–526.

DeMerlis, C., Schoneker, D., 2003. Review of the oral toxicity of polyvinyl alcohol (PVA). Food Chem. Toxicol. 41, 319–326.

Durán, N., Marcato, P.D., Conti, R.D., Alves, O.L., Costa, F., Brocchi, M., 2010. Potential use of silver nanoparticles on pathogenic bacteria, their toxicity and possible mechanisms of action. J. Braz. Chem. Soc. 21, 949–959.

Falugi, C., Aluigi, M.G.A., Faimali, M., Ferrando, S., Gambardella, C., Gatti, A.M., Ramoino, P., 2013. Dose dependent effects of silver nanoparticles on reproduction and development of different biological models. EQA – Int. J. Environ. Qual. 8, 61–65.

Gherardi-Goldstein, E., Bertoletti, E., Zagatto, P.A., Araujo, R.P.A., Ramos, M.L.L.C., Série Manuais/Secretaria do Meio Ambiente 1990. Procedimentos para a utilização de testes de toxicidade no controle de efluentes líquidos. CETESB, São Paulo, pp. 17 p.

González-Campos, J.B., Prokhorov, E., Sanchez, I.C., Luna-Bárcenas, J.G., Manzano-Ramírez, A., González-Hernández, J., Lopez-Castro, Y., del Rıo, R.E., 2012. Molecular dynamics analysis of PVA-AgnP composites by dielectric spectroscopy. J. Nanomater. 2012, 1–11.

Griffitt, R.J., Brown-Peterson, N.J., Savin, D.A., Manning, C.S., Boube, I., Ryan, R., Brouwer, M., 2012. Effects of chronic nanoparticulate silver exposure to adult and juvenile sheepshead minnows (*Cyprinodon variegatus*). Environ. Toxicol. Chem. 31, 160–217.

Hamed Chaman, S., Arab, M., Roozban, M., Ahmadi, N., 2012. Postharvest longevity and quality of cut carnations 'pax' and 'tabor', as affected by silver nanoparticles, vol. 1012. In: VII International Postharvest Symposium, pp. 527–532.

Jardim, G.M., Armas, E.D., Monteiro, R.T.R., 2008. Ecotoxicological assessment of water and sediment of the Corumbataí River, SP, Brazil. Braz. J. Biol. 68, 51–59.

Jo, H.J., Choi, J.W., Lee, S.H., Hong, S.W., 2012. Acute toxicity of Ag and CuO nanoparticle suspensions against *Daphnia magna*: the importance of their dissolved fraction varying with preparation methods. J. Hazard. Mater. 227, 301–308.

Kowalska-Góralska, M., Lawa, P., Senze, M., 2011. Impact of silver contained in the nanosilver preparation on the survival of brine shrimp (*Artemia salina* leach 1819) larvae. Ecol. Chem. Eng. A 18, 371–375.

Krklješ, A.N., Marinović-Cincović, M.T., Kacarevic-Popovic, Z.M., Nedeljković, J.M., 2007. Radiolytic synthesis and characterization of Ag-PVA nanocomposites. Eur. Polym. J. 43, 2171–2176.

Kumar, P., Senthamil Selvi, S., Lakshmi Prabha, A., Selvaraj, M., Macklin Rani, L., Suganthi, P., Sarojini Devi, B., Govindaraju, M., 2012. Antibacterial activity and in-vitro cytotoxicity assay against brine shrimp using silver nanoparticles synthesized from *Sargassum ilicifolium*. Dig. J. Nanomater. Biostruct. 7.

Kwok, K.W., Auffan, M., Badireddy, A.R., Nelson, C.M., Wiesner, M.R., Chilkoti, A., Liu, J., Marinakosc, S.M., Hinton, D.E., 2012. Uptake of silver nanoparticles and toxicity to early life stages of Japanese medaka (*Oryzias latipes*): effect of coating materials. Aquat. Toxicol. 120, 59–66.

Lee, D., Cohen, R.E., Rubner, M.F., 2005. Antibacterial properties of Ag nanoparticle loaded multilayers and formation of magnetically directed antibacterial microparticles. Langmuir 21, 9651–9659.

Li, T., Albee, B., Alemayehu, M., Diaz, R., Ingham, L., Kamal, S., Rodriguez, M., Bishnoi, S.W., 2010. Comparative toxicity study of Ag, Au, and Ag–Au bimetallic nanoparticles on *Daphnia magna*. Anal. Bioanal. Chem. 398, 689–700.

Lin, J.J., Lin, W.C., Dong, R.X., Hsu, S.H., 2012. The cellular responses and antibacterial activities of silver nanoparticles stabilized by different polymers. Nanotechnology 23, 12.

Loeschner, K., Hadrup, N., Qvortrup, K., Larsen, A., Gao, X., Vogel, U., Mortensen, A., Lam, H.R., Larsen, E.H., 2011. Distribution of silver in rats following 28 days of repeated oral exposure to silver nanoparticles or silver acetate. Part. Fibre Toxicol. 8, 18.

MANUGISTICS, 2001. In: Group RM (Ed.), Rockville: Manugistics Group.

Martins, J., Oliva Teles, L., Vasconcelos, V., 2007. Assays with *Daphnia magna* and *Danio rerio* as alert systems in aquatic toxicology. Environ. Int. 33, 414–425.

Mc Laughlin, J., Bonzongo, J.C., 2012. Effects of natural water chemistry on nanosilver behavior and toxicity to *Ceriodaphnia dubia* and *Pseudokirchneriella subcapitata*. Environ. Toxicol. Chem. 31, 168–175.

Mock, J., Barbic, M., Smith, D., Schultz, D., Schultz, S., 2002. Shape effects in plasmon resonance of individual colloidal silver nanoparticles. J. Chem. Phys. 116, 6755–6759.

Nair, R., Varghese, S.H., Nair, B.G., Maekawa, T., Yoshida, Y., Kumarm, D.S., 2010. Nanoparticulate material delivery to plants. Plant Sci. 179, 154–163.

Navarro, E., Piccapietra, F., Wagner, B., Marconi, F., Kaegi, R., Odzak, N., SIGG, L., RENATA, B., 2008. Toxicity of silver nanoparticles to *Chlamydomonas reinhardtii*. Environ. Sci. Technol. 42, 8959–8964.

OECD, 1984a. Organization for Economic Co-operation and Development. Guidelines for the Testing of Chemicals: 201 – Alga, Growth Inhibition Test. Paris.

OECD, 1984b. Guidelines for testing of chemicals. *Daphnia* sp., acute immobilisation test and reproduction test, n. 202. Organization for Economic Co-operation and Development, Paris.

Prestes, E.B., Jonsson, C.M., Castro, V.L.S., 2012. Avaliação da toxicidade aguda de piraclostrobin, epoxiconazol e sua mistura em *Daphnia similis*. Pesticidas (UFPR) 22, 43–50.

Ribeiro, F., Gallego-Urrea, J.A., Jurkschat, K., Crossley, A., Hassellöv, M., Taylor, C., Soares, A., Loureiro, M.V.M.S., 2014. Silver nanoparticles and silver nitrate induce high toxicity to *Pseudokirchneriella subcapitata*, *Daphnia magna* and *Danio rerio*. Sci. Total Environ. 466, 232–241.

Romanholo Ferreira, L.F., Mario, M.A., Tâmara, G.M., Geórgia, B.P., Ana, l.P.S.D., Regina, T.M., 2011. Evaluation of sugar-canevinasse treated with *Pleurotussajor-caju* utilizing aquatic organisms as toxicological indicators. Ecotoxicol. Environ. Saf. 74, 132–137.

Scott, N., Chen, H., 2013. Nanoscale science and engineering for agriculture and food systems. Ind. Biotech. 9, 17–18.

Singh, G., Rattanpal, H., 2014. Use of nanotechnology in horticulture: a review. Int. J. Agric. Sci. Vet. Med. 2, 34–42.

Stensberg, M.C., Madangopal, R., Yale, G., Wei, Q., Ochoa-Acuña, H., Wei, A., McLamore, E.S., Rickus, J., Porterfield, D.M., Sepúlveda, M.S., 2014. Silver nanoparticle-specific mitotoxicity in *Daphnia magna*. Nanotoxicology 8, 833–842.

Thakor, A.S., Jokerst, J., Zavaletam, C., Massoud, T.F., Gambhir, S.S., 2011. Gold nanoparticles: a revival in precious metal administration to patients. Nano Lett. 11, 4029–4036.

UN United Nation, 2009. Globally Harmonized System of Classification and Labelling of Chemicals (GHS). New York and Geneva.

USEPA, 1985. United States Environmental Protection Agency. Hazard Evaluation Division. Standard Evaluation Procedure: Acute Toxicity Test for Freshwater Invertebrates. EPA-540/9-85-005. Washington, DC., pp. 12.

USEPA, 1991. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Fourth edition. EPA/600-/4-90/027. United States Environmental Protection Agency, Washington, pp. 293.

Vandermoere, F., Blanchemanche, S., Bieberstein, A., Marette, S., Roosen, J., 2011. The public understanding of nanotechnology in the food domain: the hidden role of views on science, technology and nature. Public Underst. Sci. 20, 195–206.

Vannini, C., Domingo, G., Onelli, E., Prinsi, B., Marsoni, M., Espen, L., Bracale, M., 2013. Morphological and proteomic responses of *Eruca sativa* exposed to silver nanoparticles or silver nitrate. PLOS ONE 8.

Varkey, A.J., Fort, A.F., 1993. Some optical properties of silver peroxide (AgO) and silver oxide (Ag_2O) films produced by chemical-bath deposition. Solar Energy Mater. Solar Cells 29, 253–259.

Whelan, A.M., Brennan, M.E., Blau, W.J., Kelly, J.M., 2004. Enhanced third-order optical nonlinearity of silver nanoparticles with a tunable surface plasmon resonance. J. Nanosci. Nanotechnol. 4, 66–68.

WHO, 2003. Silver in Drinking-water, Background Document for Development of WHO Guidelines for Drinking-water Quality, vol. 2.

Wijnhoven, S.W., Peijnenburg, W.J., Herberts, C.A., Hagens, W.I., Oomen, A.G., Heugens, E.H., Roszek, B., Bisschops, J., Gosens, I., Van De Meent, D., Dekkers, S., Jong, W.H., Van Zijverden, M., Sips, A.J.A.M., Robert, E., Geertsma, M.S., 2009. Nano-silver – a review of available data and knowledge gaps in human and environmental risk assessment. Nanotoxicology 3, 109–138.

Wong, D., Parasrampuria, J., 1996. Polyvinyl alcohol. In: Brittain, H.G. (Ed.), Analytical Profiles of Drug Substances and Excipients. Academic Press Inc., Redwood, pp. 1–619.
Yang, X., Buschman, L.L., Zhu, K.Y., Margolies, D.C., 2002. Susceptibility and detoxifying enzyme activity in two spider mite species (Acari: Tetranychidae) after selection with three insecticides. J. Econ. Entomol. 95, 399–406.
Zagatto, P.A., 2006. Avaliação de risco e do potencial de periculosidade ambiental de agentes químicos para o ambiente aquático. In: Zagatto, P.A., Bertoletti, E. (Eds.), Ecotoxicologia Aquática – Princípios e Aplicações. São Carlos (2006). Editora Rima, p. 478.
Zahir, A.A., Bagavan, A., Kamaraj, C., Elango, G., Rahuman, A.A., 2012. Efficacy of plant-mediated synthesized silver nanoparticles against *Sitophilus oryzae*. J. Biopest.
Zhao, C.M., Wang, W.X., 2010. Biokinetic uptake and efflux of silver nanoparticles in Daphnia magna. Environ. Sci. Technol. 44, 7699–7704.
Zhao, C.M., Wang, W.X., 2011. Comparison of acute and chronic toxicity of silver nanoparticles and silver nitrate to *Daphnia magna*. Environ. Toxicol. Chem. 30, 885–892.