Nanosilver toxicity thresholds estimative for aquatic and sediment environmental safety

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Abstract

Silver nanoparticles (AgNPs) have been largely utilized. Despite that, comprehensive studies on their ecotoxicological effects and environmental risk are still required. In the present study, the AgNPs effect was assessed to some organisms including algae, plants, microcrustaceans, cnidaria, nematodes, aquatic insect, earthworm, and fish embryos. The no-observed-effect concentration (NOEC) was calculated for each organism using a log-logistic function. AgNPs remained stable in contact with culture media during the analyzed period and conditions employed, presenting dispersion less than 20%, except for *Artemia salina* medium. On this occasion, NPs presented dispersion of approximately 25%, although their size remained unchangeable. The Predicted No Effect Concentration (PNEC) of AgNPs in the aquatic compartment was estimated in the concentration range from 0.13 to 0.66 $\mu\text{g L}^{-1}$. A Risk Quotient (RQ) of 15.1 was derived for the NP tested with the aid of a maximum AgNPs Predicted Environmental Concentration (PEC) estimated value. The RQ value superior to one indicates a risk and the need for its management measures implementation. These data, in addition to the expected increase in AgNPs use, reinforce the importance of the AgNPs safety levels establishment that can contribute to performing their risk assessment studies.

Introduction

Due to their appealing properties for varied applications, nanoparticles (NPs) have been employed in large quantities and inevitably released into different aquatic environmental systems during production, use, discharge, disposal, and recycling. Wastewater effluents are the point source for NPs and discharges of upstream wastewater treatment plants highly affect the water quality downstream (Zhang et al. 2019 a; Bundschuh et al. 2018). Within NPs most used in daily life are AgNPs (Degenkolb et al. 2019, Zhang et al. 2019 b). Commercially, there are many consumer products containing AgNPs, including cosmetics, paints, fabrics, and food containers (He et al. 2019) leading to increased exposure of those materials in the environment. Indeed, Keller and Lazareva (2014) quantified AgNPs released from different industries as 240 metric tons/year emissions to landfill, 45 metric tons/year emissions to soil, 95 metric tons/year emissions to water, and 20 metric tons/year emissions to air, globally.

In relation to food, due to its antimicrobial properties, one possible approach to solve the loss due to spoilage is the silver use viewing the reduction of the microorganism population on the surface of food products (Brito et al. 2020). The use of antimicrobials to the packaging material includes controlled diffusion, reduced antimicrobial contents, and improved cost-effectiveness (Westerbandy and Hicks 2018; Azeredo et al. 2019). Then, AgNPs' food packaging systems promote better food quality and/or stability (Kuuliala et al. 2015). However, the AgNPs' environmental and human health effects are still subject of concern (Ntim et al. 2018). Many studies have found that migration of silver (either as nanoparticle or as ionic form), from food packaging and containers to the food and ultimately to water bodies, can be considered as a matter of environmental and public health concern (Hicks and Temizel-Sekeryan 2019).

It is known that washing or discarding of AgNPs-containing products can lead to silver release into wastewater. Thereafter, silver can reach wastewater treatment facilities and then surface water bodies that may be used as drinking water sources (Tugulea et al. 2014). As a consequence, untargeted aquatic organisms will suffer threats from the discharged NPs, as they can be transferred through the food chain, posing an ecosystem health risk, and ultimately to humans (Zhang et al. 2019 a; Kalantzi et al. 2019). In this way, dietary bioaccumulation of Ag from food can occur in fishes (Clark et al. 2019; Martin et al. 2017).

The behavior of AgNPs is influenced by environmental factors (including pH, dissolved oxygen, sunlight, temperature, and natural organic matter - NOM) which altered their bioaccumulation and toxicity (Deng et al. 2017; He et al. 2019; Lee et al. 2018; Zhang et al. 2018; Zhang et al. 2019 b). Because AgNPs are sensitive to the surrounding environment, transformations of AgNPs, such as aggregation, oxidation, or dissolution, frequently occur (Sharma et al. 2019). Likewise, the complexity of freshwater aquatic systems varies the fate and behavior of AgNPs (Shevlin et al. 2018; Zhou et al. 2017). Besides, AgNPs stability may be influenced by the seasonal variations in the chemistry of the natural water and NP coating (Ellis et al. 2018), and its fate in the environment may be better evaluated taking into account temporal variability in concentrations caused by weather variation (Dumont et al. 2015).

As a consequence, the water-soil environment may be exposed to contamination with different Ag species depending on the environmental conditions and the particles' properties (McGillicuddy et al. 2017). Moreover, the trace element dissolved in environmental compartments should be taken into account in toxicity models since it is the most reactive (Wang et al. 2020). The ions release with consequent particles dissolution and oxidative stress are among the most studies AgNPs toxic effects (Zhang et al. 2019 a; Bundschuh et al. 2018; Sayed and Soliman 2017; Mortezaee et al. 2019). These effects can result in cytotoxic and genotoxic damage (Arora et al. 2008; Zhang et al. 2016). Since AgNPs will release Ag⁺ in an aquatic environment, whether dissolved Ag⁺ or AgNPs play the predominant role in the toxicity toward biota is controversial (Zeng et al. 2019). The presence of Ag⁺ near the membrane surface due to NPs oxidation/dissolution seems to be responsible for toxic effects since AgNPs do not diffuse through a lipidic membrane (Guilleux et al. 2018). An increase in AgNPs toxicity is possible due to a potential increase in intracellular silver dissolution (Minghetti et al. 2019).

Viewing this complex scenario and due to the uncertainty surrounding the concentration of AgNPs in the environment, the increase of their use in many different applications has raised concerns over their release into aquatic ecosystems (McGillicuddy et al. 2017). First attempts in considering NP accumulation in environmental sinks have been made by Gottschalk et al. (2009) that used a model to calculate final concentrations in soil and sediment that was recently revised (Gottschalk et al. 2013). The concentrations of total silver in different environmental compartments have been estimated to be in the range of ng L⁻¹ and µL for water (Butz et al. 2019). Maurer-Jones et al. (2013) summarized AgNPs PECs of 0.088-10,000 ng L⁻¹ in surface water. It was reported by Sun et al. (2016) that AgNPs concentrations of 2.2 µg L⁻¹ were found in surface waters. However, considerable uncertainty still remains as to the fate

and behavior of AgNPs entering aquatic environments, resulting in significant variability in exposure estimates (Shevlin et al. 2018).

Comprehending the nature of hazards and defining a value that not causes significant environmental damage requires several studies of the behavior of NPs in different environments (Quik et al. 2011; Amenta et al. 2015). Despite significant advances in analytical methods, it is still not possible to measure the concentrations of NPs in natural systems (Sun et al. 2016). A probabilistic risk assessment of NPs as species sensitivity distributions (SSD) may be used when there is great variability of the observed effects in different ecotoxicological studies and insufficient data (Chen et al. 2018).

Despite these difficulties, it is important to investigate the ecotoxicological potential of AgNPs in aquatic environments to contribute to an ecologically relevant toxicity assessment. In a riverine environment, there is a sediment deposit due to erosion and accumulation. Since NP environmental reworking may occur through different organisms' activity as ingestion and defecation, it can be considered that faunal bioturbation in aquatic environments includes all transport processes carried out by animals that directly or indirectly affect sediment matrices (Kristensen et al. 2012). Therefore, to do more comprehensive studies relating to aquatic contamination effects, it is important not only to include some organisms that could be found in these environments but also those that could have associated processes.

Numerous investigations indicate sediments as the final sink of inorganic nanoparticles, facilitating the exposure of benthic species to them (Lüderwald et al. 2019). Although reports of AgNPs toxicological effects are increasing, its environmental safety levels are not fully known. In this context, knowledge about the risk posed by them to living organisms is primordial to guide management and policies towards sustainable solutions for mitigation of their effects (Gredelj et al. 2018). Then, environmental risk characterization may be based on the PEC in relation to PNEC (Sørensen et al. 2020). Establishing a safe threshold for PNEC is the main goal of ecological risk assessment. Since NOEC is used to estimate a risk concentration that will not cause a significant effect, it is useful to estimate an environmental low-risk concentration for aquatic and sediment ecosystems based on PNEC (Castro et al. 2018). In this context, this study aimed to determine the toxicity of AgNPs on *Raphidocelis subcapitata*, *Lactuca sativa*, *Daphnia magna*, *Artemia salina*, *Hydra attenuata*, *Caenorhabditis elegans*, *Panagrolaimus* sp, *Eisenia fetida*, *Danio rerio*, and *Chironomus sancticarloi* in order to contribute to an index to manage the presence of this NP in the environment.

Materials And Methods

AgNPs synthesis and physicochemical characterization

AgNPs were employed according to the following procedure: 1.84 g of Polyvinyl Alcohol (PVA) was solubilized in 80 mL of deionized water under heating at 60 °C. Next, 720 mg of silver nitrate (AgNO₃) was added and placed in an ice bath under stirring for 30 minutes. Subsequently, 40 mL of sodium borohydride solution (80 mg in 50 mL) was added by dripping (1 drop/second). The final concentration of AgNPs in the solution were 3.85 mg mL⁻¹.

The stability and dispersion of AgNPs were evaluated in all culture mediums used in the ecotoxicological assays by UV-Vis spectroscopy, Zeta Potential measurements, and Dynamic Light Scattering (DLS). The sample preparation to be analyzed was based on a 10% (w/w) ratio, that is, 1.8 mL of the solution of the silver nanoparticles, which was previously sonicated for 5 minutes, and 16.2 mL of each culture medium. The AgNPs evaluation by UV-Vis spectroscopy was carried out at 0 h, 24 h, 48 h, 72 h, and 96 h, except for Lemna medium evaluation, which was also tested at 120 h and 168 h following the test methodology. Briefly, next are described the procedures employed for UV-Vis absorption spectroscopy, zeta potential, and particle size distribution

1. UV-Vis absorption spectroscopy: The AgNPs were monitored by UV-Vis absorption spectroscopy using a Perkin-Elmer UV-Vis Lambda 6. Three samples were diluted in deionized water and placed in a quartz cell of 2 cm³, which spectra were collected from 250 to 700 nm.
2. Zeta potential and particle size distribution - The zeta potential of NPs was evaluated using a Zetasizer Nano ZS 90 (Malvern Instruments®, UK), which measures electrophoretic mobility of nanoparticles using phase analysis light scattering. The same equipment was also employed to determine the particle size using dynamic light scattering (DLS, Malvern Instruments® Ltd., model ZS Zen 3600, Worcestershire, UK).

Risk assessment measures from the SSD model

Since SSD requires ecotoxicological measures, all ecotoxicity assays were performed using 0 (control); 0,01; 0,1; 1; 10 e 100 mg L⁻¹ of AgNPs. The doses were chosen by a range-finding toxicity test that is useful in the evaluation of unknown toxicity materials. The essays' effective concentrations values were calculated with a 95% confidence interval (Castro et al. 2018). The organisms used, except *Lactuca sativa*, were from Embrapa Environmental cultures. The procedures for each bioassay carried out are summarized below. AgNPs were tested in algae, plants, microcrustaceans, cnidaria, nematodes, aquatic insects, earthworms, and vertebrates (fish embryo) to warrant a comprehensive analysis to determine safe environmental AgNPs concentrations. This analysis took into consideration toxicity testing criteria and requirements (Stubblefield et al. 2020).

Raphidocelis subcapitata. Microalgae were obtained from cultures grown medium prepared according to OECD (1984 a). Plates (96-well) were used to expose de microalgae for 168 hours to 20 ± 2 °C under continuous illumination (~1,300 lux). It was used a total test suspension volume of 300 µL per well containing an initial algae concentration of ~10⁶ cells/mL. A total of 12 replicates were prepared for each test condition. For each test condition, a total of 12 replicates were prepared. Every 24 h for 168 h, algal growth was monitored by absorbance readings at 625 nm (microplate reader, Sunrise Tecan Group Ltd.) of the suspensions. According to OECD (1984 a), the specific growth rate was calculated. The function of the logarithm concentration, according to the fitted regression model, was used to calculate the concentration that inhibited 50% of the specific growth rate (EC₅₀-168 h).

Lactuca sativa. In 12-well polystyrene plates, the trials were carried out in the dark for 96 h at 20 ± 1 °C (Bautista et al. 2013). The lettuce seeds were purchased from ISLA Sementes Ltda, Brazil. A total of 36 seeds were exposed individually in each well. The seeds were placed over the Whatman n° 2 filter paper disk in which was added 400 µL of test solution. Every 24 h, the seed were observed and photographed using an Optika camera 4083B3 coupled to a stereomicroscope (Optika®, Italy). The software Optika View version 7.1.1.5 (Optika®, Italy) was used in order to evaluate the percent of germination and the growth rate of the roots at the end of the exposure period. Linear regression curves of the root size as a function of time were obtained. Angular coefficients were equivalent to the growth rates (Basu and Pal 2011).

Lemna minor. Duckweed seedlings were cultivated in NPK culture medium according to Sipaúba-Tavares and Pereira (2008). The experiments were carried out for 7 d at 24 ± 2 °C, under continuous illumination (~700 lux). Polystyrene plates (12 well) were used to expose two fronds per well containing 5 mL of culture medium. Thus, 24 replicates were assayed for each test condition. The number of fronds was counted daily in order to evaluate the culture growth. At the end of the exposure period, the effect of alteration of the biomass (wet weight) was determined by weighing each seedling. For chlorophyll determination, the substance was extracted by placing the samples in Eppendorf tubes containing 1 mL of ethanol absolute ACS for 24 h at 4 °C (Zhang et al. 2010). The chlorophyll a and b content were determined according to the equations described by Brain and Solomon (2007) in order to calculate the total chlorophyll. The extract absorbance was recorded at 665 and 649 nm by the use of the spectrophotometer Shimadzu (UV-1650). The effective concentration that inhibits the growth rate (fronds production) at 50% after 7days (EC50 – 7d growth) was calculated. The same parameter was calculated for the biomass and chlorophyll reduction, respectively, EC50-7d biomass and EC50-7d chlorophyll.

Daphnia magna. The acute toxicity to this microcrustacean was evaluated according to OECD guidelines (OECD, 2004). *D. magna* was bred in aquaria (40 x 20 x 15 cm) containing water reconstituted with nutrients prepared according to Jonsson and Maia (1999). The temperature and light intensity were maintained at 20 ± 1 °C and 1000 lux, respectively. The microalgae *R. subcapitata* was used to feed the organisms once daily. Neonates less than 24 hours of age were separated from the cultures and used as test organisms. Tests were performed in 12-well polystyrene plates with two *D. magna* neonates per well in a final volume of 5 mL test or control solution. By the use of colony counter equipment, immobilization was recorded visually every 24 h during 48 h. The concentration that immobilized 50% of *D. magna* in 48h was taken as the CE50-48h.

Artemia salina. Viable artemia cysts (Maramar®) were used for the nauplii eclosion of test organisms. As recommended by the manufacturer, the rate of 3 g cysts per liter were used. A saline solution (Sera Premium R - Sera GmbH, Heinsberg, Germany) was prepared in distilled water at 3% (Castro et al. 2018) in order to hatch the cyst that remained 24 h in the constantly aerated solution. Nauplii were exposed to the test material concentrations under controlled temperature (20 ± 2 °C) and light (1000 lux) conditions (USEPA 2002). Twelve-well polystyrene plates were used with two *A. salina* nauplii per well, in a final volume of 5 mL. By the use of a stereomicroscope (Optika, Italy), the immobilization was recorded

visually every 24 h during 48 h. The determination of the concentration that immobilized 50% of *A. salina* in 48h was assessed by the comparison of the test values with the control values.

Hydra attenuata. Hydras were cultured in Hydra medium (1 mM CaCl₂, 1 mM NaCl, 0.1 mM MgSO₄, 0.1 mM KCl, and 1 mM Tris-Cl pH 7.8) and were fed once every two days with freshly hatched *Artemia* nauplii. Non-budding hydras from homogeneous populations were selected to be used in the toxicity assays during 96 h in quadruplicate (n = 12 per group). The organisms were maintained at 20 ± 1 °C with a 12/12 h (light/dark) cycle in 12-well polystyrene plates, each well containing 3 individuals and 4.0 mL of AgNPs in each concentration. The organisms were not fed during the test. The organisms were examined every 24 h using a stereomicroscope (Model SMZ 2 LED, Optika®) and the observed effects were recorded by rating the morphological status and assigning a score from 10 (normal) to 0 (disintegrated), as described by Wilby (1988).

Caenorhabditis elegans. The *C. elegans* wild-type N2 strain was maintained in Petri dishes with agar and feed with *Escherichia coli* OP45 (ISO 10872:2010). At least forty organisms per AgNPs concentration were exposed for 24 h at 20 ± 2 °C in the dark. The organisms were observed every 24 h using an Optika 4083B3 camera coupled to a stereomicroscope (Optika®, Italy). The nematode was considered dead if it failed to respond to a gentle mechanical touch.

Panagrolaimus sp. The tests were performed in the dark for 96 h at 20 ± 1 °C. The nematode cultures were kept in an oatmeal medium (Lara et al. 2007). With the use of overlapping sieves (65, 250, and 400 mesh), the phase J2 organisms were separated using jets of K medium (NaCl, 3.075 g L⁻¹, KCl, 2.42 g L⁻¹). Organisms that were retained in the 400-mesh sieve were removed from a wash bottle using jets of K medium (NaCl, 3.075 g L⁻¹, KCl, 2.42 g L⁻¹). Tests were performed in 24-well polystyrene plates, with 3 repetitions for each test concentration. Ten nematodes were kept per one mL of test solution in each well. A stereomicroscope (Optika®, Italy) coupled to a camera (Optika 408383) was used to record the number of immobile organisms after a period of 96 hours. The concentration that immobilized 50% of *Panagrolaimus sp* in 48h was taken as the CE50-48h.

Chironomus sancticaroli. *C. sancticaroli* culture was maintained according to OECD (2004, 2011). The organisms were maintained at 23-26 °C in a 16/8 light/dark cycle with daily feeding. After seven days of separation from the reproducers (approximately 2nd instar larvae) (Lee et al. 2016; Waissi et al. 2017), organisms were selected to the acute and subchronic tests based on.

Acute test lasted 48 h with mild aeration in 50 mL glass Becker's. The organisms were not fed and were observed at every 24 h to determine immobility (lack of movement for 15 seconds after mechanical stimulation) and the development of pupae. The immobile larvae were considered to be dead as well as individuals not found. At the end of the assay, the mortality percentage was calculated for each AgNPs concentration after 24 and 48 hours with probit analysis using the Statgraphics Plus software version 5.1.

Subchronic test was performed for 8 days of exposure (Pinto et al. 2021). For that, four larvae were used in each of the four replicates of all dilutions - control and AgNP concentrations, totalizing 20 larvae per treatment and 96 in total. Only in the first day, 0.5 ml of feed was provided (Tetramin® 20 g / L) to larvae. The solution exchange was carried out on alternate days, with the objective of renewing the culture medium, to avoid the sharp drop of dissolved oxygen and the proliferation of fungi by the remainder of uneaten feed. The organisms were observed daily 24 h till the end of test. The immobility (% immobility/h) was calculated for each exposure concentration in order to determine the EC10-192 h values.

Eisenia fetida. Adult earthworms (350 and 500 mg) with well-developed clitella were cultured at 25 °C ($\pm$ 1) in an artificial soil test substrate at 35% humidity containing charcoal, vermiculite, and humic acids. The culture was fed with decomposed manure and bran oats. Contact filter paper test was performed in a 9 cm Petri dish according to the OECD (1984 b). It was used ten replicates for AgNPs concentration. Before the dose-response test start, earthworms were maintained on filter paper moistened without test-substance, in a dark environment at 21 $\pm$ 1 °C for 24h viewing expel the intestinal contents. After that, the paper was substituted by another soaked with 2 mL AgNPs solution. Then, the dish was incubated at 21 $\pm$ 1 °C for 48 h in order to assess mortality. Earthworms that failed to respond to a gentle mechanical touch were considered dead. The mortality in the controls should not exceed 10 percent during the test.

Danio rerio. Wild-type adults were housed in a rack system. After mating, eggs were collected, washed, and selected for their viability to assay by microscopic observation. It was used 10 eggs per group, keep in fish embryo medium in microplates with pH 7.5 $\pm$ 0.5 according to USEPA (2002). Facilities were maintained in a 14/10 h light/dark cycle at 27 $\pm$ 1 °C. Embryos were evaluated daily for mortality or malformation appearance till 96 h. The software Optika View Version 7.1.1.5, Optika®, previously calibrated was used for verifying embryos hatching. It was also used for larvae total length measurement with 2-fold magnification. Animal handling procedures were approved by Embrapa Environment's Ethics Commission for Animal Use (Embrapa Environment, protocol 010/2018).

Determination of low-risk environmental concentrations

The effective concentration causing an effect of the parameters evaluated in 50% of the organisms during the test period (EC50) was determined using the "Probit Analysis" and "Simple Regression" modules of the Statgraphics Centurion XVII program version 1.17.04 (Stat Point Technologies, 2014). NOEC was determined using the one-way ANOVA module of the same program and was considered as the tested concentration that had no statistically significant effect when compared with the control within the test exposure time. NOEC was estimated based on the EC50/10 ratio (OECD, 1995). Alternatively, NOEC was also estimated by the effective inhibitory concentration at 10% test-organism growth or mortality (EC10) (OECD, 2002) when NOEC was considered as the EC10 upper limit of the 95% confidence interval (Hoekstra and Van Ewijk 1993). Parameters tested in each organism were based on their ecological relevance (Warne et al. 2015) and were shown in Table 1.

The hypothetical environmental concentration at which only 5% of the species (5th percentile of the fitted distribution) of an aquatic environment would be affected (HC5) can be derived from the species sensitivity distribution (SSD). SSD is used to derive PNECs for chemicals since it correlates the concentration of a chemical to the proportion of species being affected. These data represent a random sample from a statistical distribution representative of a given ecosystem (Sørensen et al. 2020). HC5 is commonly used as the basis for the environmental risk assessment of chemicals. It was calculated with a 50% confidence level (HC5-50) (Traas and van Leeuwen 2007; Aldenberg and Slob 1993). Then, HC5 is assumed to be the concentration that is sufficient to protect 95 % of the species of given ecosystems following the addition of an extra safety factor that ranges between 1 and 5 (ECHA 2008). Thenceforth, PNEC was calculated by applying the risk factor evaluation (factors values = 1 and 5) to the HC5-50, as described by Liu et al. (2016). HC5 was determined by the log-logistic distribution of the NOEC values (OECD 1995; Castro et al. 2018), using the simple regression module contained in the Statgraphics Centurion XVII program version 1.17.04 (Zolezzi et al. 2005). The normality distribution of the NOEC values was analyzed by the Kolmogorov-Smirnov test using the same program.

Results

AgNPs Physicochemical characterization

Before performing the toxicity tests, it should be ensured that the AgNPs can be well dispersed in the culture medium. Therefore, it is recommended to characterize the test nanomaterial, including the degree of aggregation/agglomeration or change of particle size distribution (Kennedy et al. 2017; Rasmussen et al. 2018). This variation ideally may not be more than $\pm 20\%$ (Petersen et al. 2015).

In order to verify the stability of the AgNPs together with the culture media, DLS and Zeta Potential analysis were performed. UV-Vis Spectroscopy was used to verify changes in morphology of the AgNP (Fig. 1). With these tests, it was possible to observe that the nanoparticles remained stable when in contact with culture media during the analyzed period and conditions used similar to the test's protocols. The AgNPs presented dispersion less than 20% except for *Artemia salina* medium that presented approximately 75% of the initial absorbance at the end of the observational period. However, the NPs size remained unchangeable that was considered acceptable.

Toxicity assays and PNEC estimative

Different criteria were used to calculate the NOEC values, which were transformed into logarithms and plotted as the log-logistic function. A concentration-response ratio was established for all the tested organisms. Table 1 shows the EC50 values for each organism and the parameter evaluated in ascending order. The cumulative distribution function generated by SSD is shown in Fig. 2.

The data showed the variability in susceptibility of the test-organisms to AgNPs. AgNPs were toxic in a decreasing severity to *Daphnia magna*; *Danio rerio*; *Caenorhabditis elegans* and *Hydra attenuata*; *Lemna minor*; *Raphidocelis subcapitata*, and *Panagrolaimus sp.*; *Lactuca sativa* and *Eisenia fetida*; *Artemia*

salina and *Chironomus sancticaroli*. As an example, Fig. 3 shows the *Lemna minor* test with different concentrations of AgNPs.

Derived from the fitted curve, a value of $p > 0.1$ was obtained with the Kolmogorov-Smirnov test, which means that the data correspond to a logistic function. The HC5-50 (confidence limits in parentheses) value calculated from such a distribution was 0.00066 (0.00014 – 0.0027) mg L^{-1} for AgNPs. The safest values associated with the PNEC were calculated by dividing these values by a risk assessment factor (5 and 1). Thus, the PNEC of AgNPs in the aquatic compartment, below which adverse effects will most likely not occur during long or short-term exposure, was estimated as 0.13 to 0.66 $\mu\text{g L}^{-1}$.

According to Maurer-Jones et al. (2013), the highest AgNPs PEC reported for surface waters is 10,000 ng L^{-1} . From this value we can derive an $\text{RQ} = 15.1$ ($\text{RQ} = \text{PEC/PNEC} = 10 \mu\text{g L}^{-1} / 0.66 \mu\text{g L}^{-1}$) for our test substance.

Discussion

It is known that AgNPs can exert different toxic effects depending on the environment and their surface properties (Zhang et al. 2019 b). From an environmental risk perspective, AgNPs' characteristics can predict which ecological niches of an ecosystem would be more impacted (Auffan et al. 2020). Also, speciation and transformation of AgNPs (as coating materials) rather than their concentrations drive the biological responses and could influence their bioactivity and toxicity (Abbas et al. 2020). The toxicity of AgNPs is enhanced by PVP- and sodium citrate- coatings (Chen et al. 2018). However, risk evaluation can be difficult due to the several AgNPs dissolution mechanisms (Liu et al. 2020).

Due to these facts, a more extensive evaluation of the AgNPs' environmental risk assessment is needed. To estimate the AgNPs environmental risk, it can be used the species sensitivity distribution (SSD) that infers the environmental toxicity using some organisms that pertain to different levels of an ecosystem as bioindicators (Lu et al. 2020). Because of that, AgNPs' toxic effects were studied on algae, plants, microcrustaceans, cnidaria, nematodes, aquatic insects, earthworms, and vertebrates (fish embryo) viewing obtain AgNPs concentration limits.

SSD distribution ranks the species based on their sensitivity to the NP (Garner et al. 2015) and derives hazardous concentrations that protect 95% of species from chemical contaminants (HC5) following the addition of an extra safety factor that ranges in between 1 and 5 (Chen et al. 2018). PEC is estimated for exposure and the PNEC for chemical hazards. PEC/PNEC ratio derives the risk characterization (Mueller and Nowack 2008). No environmental risk is to be expected when RQ is lower than 1 whereas an RQ superior than 1 means that the PEC is capable to cause adverse effects on the organisms. However, the evaluation of the PEC value is influenced by several basic settings of the model being applied. PECs values depend on the chemical production volume, released quantity in a given region and environmental transformation, suitable geographical region, and life cycle stage considered (Wigger et al. 2020).

In the present study, the PNEC of AgNPs in the aquatic compartment was estimated in the concentration range of 0.13 to 0.66 $\mu\text{g L}^{-1}$. This last value calculated by HC5/1 is the same as the HC5 described by Kwak et al. (2016). It is also so close to the HC5 described by Chen et al. (2018) for PVP coated AgNPs (HC5 = 1.1 $\mu\text{g L}^{-1}$). Using a maximum PEC estimated value for AgNPs, an RQ = 15.1 was derived for tested AgNPs. These data reinforce the importance of performing risk assessment studies with AgNPs.

Literature data are in the same direction as the present results, since it can be noticed that AgNP can produce some damage in various organisms. AgNPs can enter plants inducing toxicological effects on them. The accumulation of AgNPs in *Lactuca sativa* is influenced by size and concentration, but not by nanoparticle coating when it was exposed during 9 days to different coated and sized AgNPs. On the other hand, AgNPs' translocation to shoots was more pronounced for neutral charged and large-sized NPs at higher NP concentrations (Torrent et al. 2020). In *Lemna minor*, Ag⁺ entered the plant and provoked serious activation of the defense system indicating that it can be a good bioindicator of water quality fluctuation. Excessive application of Ag⁺ would affect biomass and morphology of *L. minor* (Li et al. 2020). Also, Hasan et al. (2021) observed that AgNPs have a stimulatory seedling growth effect at moderate concentrations, but at higher concentrations, the release of Ag⁺ ions caused inhibitory effects.

For invertebrates, sediment served as the main reservoir and as an important exposure source of AgNPs and different NP uptake mechanisms take place in these organisms as oysters (Shao and Wang, 2020). Moreover, the potential for great bioaccumulation and biomagnification of AgNPs in benthic invertebrates (e.g., shrimp, shellfish) and fish highlights the risk of aquatic food products consumption to the human health risks (Xiao et al. 2019). Also, chironomid larvae are closely associated with benthic substrates and link primary producers and secondary consumers. Given their trophic position and their life habits, these larvae can be considered the entry point for the transference of Ag to the higher food web trophic levels (Williams et al. 2018). In this respect, Lee et al. (2016) observed that the toxicity of AgNPs for *Chironomus riparius* is strongly affected by specific natural conditions as sulfidation and dissolved organic matters. Also, these NPs can lead to pronounced induction of genes related to oxidative stress and detoxification (Nair et al. 2013). Regarding AgNPs presence in the terrestrial environment, some invertebrates like *E. fetida* can also bioaccumulate Ag in a limited way suggesting a link between Ag and metallothioneins, which are key proteins in the sequestration and detoxification of metals (Courtois et al. 2021).

In vertebrates, like rainbow trout, ions released as a result of NPs dissolution are internalized in the cell through transporter proteins or ion channels (Abbas et al. 2020). In common carp, even exposure to sublethal concentrations of AgNPs LC50 (96 h) for 21 days can induce some toxicity (Vali et al. 2020). Also, Liu et al. (2019), studying the contribution of particle size and surface coating to toxicity, observed that AgNP with small size and citrate coating showed greater toxicity to zebrafish gills and intestines. These findings imply that already a battery of various bioassays is required for a reliable evaluation of the ecological risk of NPs as Nam et al. (2015) conducted for gold nanoparticles.

In summary, the bioassays used in the present study illustrate the biological responses to AgNPs exposure at various trophic levels. Our study showed a high susceptibility variability of the organisms to the nanomaterial tested. This difference was approximately 126,000 times between the more sensitive and the less sensitive species. This fact, together with the extremely low NOEC value for *D. magna*, given a very low PNEC value for the nanoparticle. A similar occurrence was also observed in Kwak et al (2016) who reported *Daphnia magna* as the most sensitive organism for AgNPs with a NOEC value of 0.001 mg L⁻¹. Also, Butz et al. (2019) observed the effects of AgNPs concentration and surface coating on toxicity to *Prorocentrum minimum* when calculated NOEC values were 24.0, 19.0, 0.2 µg L⁻¹ for citrate (cit-AgNPs), polyvinylpyrrolidone (PVP-AgNPs), and AgNO₃ respectively.

Beyond that, an RQ increase in the future is expected due to the increase in AgNPs use. The present study fills a data gap about these NPs thresholds that can affect non-target aquatic and sediment organisms. In addition, the obtained values can subsidize the risk assessment of AgNPs. For the future, since several factors are involved in AgNPs ecotoxicity (Chen et al. 2018), more focused investigations of these NPs environmental risk need to be performed.

Conclusion

In light of the present findings, AgNPs were more toxic to *Daphnia magna* and were less toxic to *Artemia salina* and *Chironomus sancticaroli*. The PNEC of AgNPs in the aquatic compartment was estimated in the concentration range from 0.13 to 0.66 µg L⁻¹. Based on the PNEC calculation and the maximum PEC value estimated for AgNPs, we found an RQ = 15.1 for the tested AgNPs. The results showed that environmental concentrations of AgNPs may have ecological implications for aquatic populations and enhance potential risks to them. These data reinforce the importance of performing risk assessment studies with AgNPs and contribute to the establishment of its governance. However, it is important to note that the available data should be analyzed according to specific environmental conditions since abiotic factors can also influence the observed effects caused by NPs. In view of that, it is important to look for the most accurate estimative of the potential benefits and limitations that may be due to the presence of these NPs in the environment.

Declarations

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Ethics approval - The experimental procedures with *Danio rerio* used in this study were previously approved by the Embrapa Environment Ethics Commission (protocol 010/2018).

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Table 1

Table 1. Acute toxicity of AgNPs and hypothetic values of NOEC (* = not determined)

Test-organism/ parameter	EC50 and confidence limit 95% (mg L ⁻¹)	Exposure time (h)	Toxicity classification (USEPA, 2020)	Estimated CENO (mg L ⁻¹)
<i>Daphnia magna</i> (Microcrustacean) immobilization	0.002 (0.002-0.003)	48	Very highly toxic	0.0002
<i>Danio rerio</i> (Fish embryo) mortality	0.06 (0.05-0.08)	96	Very highly toxic	0.01
<i>Caenorhabditis elegans</i> (Nematoda) mortality	0.12 (0.11-0.14)	24	Highly toxic	0.01
<i>Danio rerio</i> growth	0.19 (0.16-0.22)	96	Highly toxic	0.04
<i>Hydra attenuata</i> (cnidaria) mortality	0.41 (0.29 a 0.54)	96	Highly toxic	0.04
<i>Raphidocelis subcapitata</i> (microalgae) growth inhibition	3.67 (2.46-5.31)	168	Moderately toxic	0.58
<i>Panagrolaimus sp.</i> (Nematoda) immobilization	8.45 (5.33-19.17)	96	Moderately toxic	0.84
<i>Lemna minor</i> (Plant) growth inhibition	11.44 (4.71 – 32.88)	168	Slightly toxic	0.33
<i>Lemna minor</i> wet weight	4.01 (2.50 – 9.05)	168	Moderately Toxic	0.26
<i>Lemna minor</i> total chlorophyll	53.58 (19.18 – 254.27)	168	Slightly toxic	0.37
<i>Lactuca sativa</i> (Plant) growth rate	35.31 (10.75-18.50)	96	Slightly toxic	1.45
<i>Eisenia fetida</i> (Annelida) mortality	>100	48	Practically nontoxic	10.00
<i>Chironomus</i>	143.50	192	Practically	29.43

<i>sancticaroli</i> (Aquatic insect) sub chronic test immobilization	(63.06 – n.d.*)		nontoxic	
<i>Chironomus sancticaroli</i> (acute test) mortality	186.02 (114.39-921.65)	48	Practically nontoxic	(n.d.*)
<i>Artemia salina</i> (Microcrustacean) immobilization	265.48 (192.97-349.17)	48	Practically nontoxic	26.00

Figures

Figure 1

Absorbance spectrum of AgNPs

Figure 2

Log-logistic function of the cumulative sensitivity according to NOEC values of AgNPs for the test organisms: a – *Daphnia magna*; b – *Danio rerio* (embryo); c – *Caenorhabditis elegans*; d – *Hydra attenuata*; e – *Lemna minor*; f – *Raphidocelis subcapitata*; g – *Panagrolaimus* sp.; h – *Lactuca sativa*; i – *Eisenia fetida*; j – *Artemia salina*; k – *Chironomus sancticaroli*. Dotted lines correspond to the 50% confidence interval of the curve. HC5-50 = 0.00066 (0.00014 – 0.0027) mg L⁻¹

Figure 3

Example of test with various organisms (in this case *Lemna minor*) with different concentrations of AgNPs