

Effects of suspended particles on the toxicity of AgNO_3 and silver nanoparticles to *Daphnia magna*: Importance of adsorption of dissolved Ag and dietary pathway[☆]

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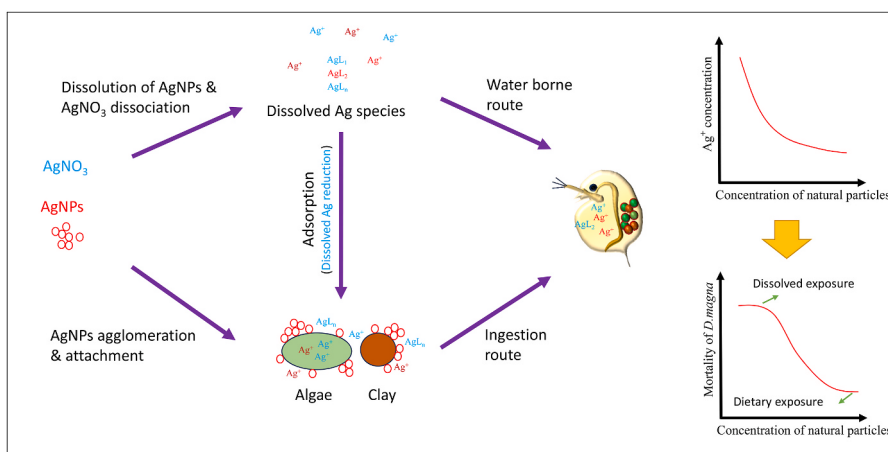
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HIGHLIGHTS

- Suspended particles reduced dissolved Ag by adsorbing Ag to particle surface and incorporating into algal cells.
- The mortality of *D. magna* decreased with suspended particle concentrations, reflecting reduction in dissolved Ag.
- Ingestion of the particle-associated silver provided additional vector for toxicity even when dissolved Ag was low.

GRAPHICAL ABSTRACT



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1. Introduction

Nanoparticles (NPs) are widely utilized across various industries, including manufacturing, commercial products, and medical applications (Jeevanandam et al., 2018; Ahmed et al., 2021; Mabrouk et al., 2021; Wasilewska et al., 2023). The global market for nanomaterials was valued at over USD 7 billion in 2020 and is projected to reach approximately USD 12 billion by 2026 (Ahmed et al., 2022). This substantial increase in the production of engineered nanoparticles (ENPs) has raised considerable concern regarding their potential ecological risks (Baalousha et al., 2016; Jaswal and Gupta, 2023). Once introduced into the environment, NPs may pose unintended ecological risks to both terrestrial and aquatic systems due to their unique physicochemical properties and potential toxic effects (Gupta et al., 2017a,b; Wu et al., 2021).

Among the diverse array of engineered nanoparticles, silver nanoparticles (AgNPs) have garnered significant attention due to their exceptional properties, including antimicrobial efficacy, catalytic activity, and high electrical conductivity (Irene et al., 2020; Xu et al., 2020; Naganthran et al., 2022). AgNPs account for the largest share (>50 %) of the nanomaterials market and represent the fastest-growing segment of nanomaterial production (Inshakova and Inshakov, 2017; Temizel-Sekeryan and Hicks, 2020). Consequently, AgNPs are frequently detected in aquatic environments, where they may exert toxic effects on aquatic organisms (Khoshnamvand et al., 2020). Documented toxicological impacts of AgNPs include developmental abnormalities, reproductive impairments, and histopathological damage in a range of aquatic taxa, including algae, invertebrates, vertebrates, and mammals (Johari et al., 2018; Hartmann et al., 2019; Moon et al., 2019; Liu et al., 2019; Pinheiro et al., 2024).

The acute and chronic toxicity of AgNPs in aquatic organisms is influenced by both their intrinsic physicochemical characteristics and extrinsic environmental factors in the exposure medium (Fabrega et al., 2011; Bondarenko et al., 2013). Well-documented nanoparticle properties that modulate toxicity include size, shape, surface modifications, synthetic methods (Durán et al., 2016; Aguiar et al., 2024; Assis da Silva et al., 2022). In addition, environmental parameters such as ionic strength, redox potential, pH, and dissolved organic carbon (DOC) significantly affect not only the fate and transformation of AgNPs in aquatic systems, but also their bioavailability and toxicity (Liu and Hurt, 2010; Lowry et al., 2012; Maurer-Jones et al., 2013; Almeida et al., 2024). For instance, low ionic strength and acidic conditions enhance the dissolution of AgNPs by promoting their aggregation and oxidation (Zhao and Wang, 2012; Kataoka et al., 2018), leading to increased toxicity due to the release of silver ions (Navarro et al., 2008). Conversely, DOC and other organic constituents have been identified as key modulators of silver speciation, toxicity, and bioavailability (Cáceres-Vélez et al., 2018; Blinova et al., 2013; Kim et al., 2013; Cupi et al., 2015; Nasser and Lynch, 2016). Additionally, ligands such as sulfide and chloride can mitigate toxicity by complexing with silver ions released from AgNPs (Kramer et al., 2002; Choi et al., 2009). Thus, the influence of naturally suspended particles in aquatic environments on AgNPs toxicity remains less well understood (Mackevica et al., 2015; Stevenson et al., 2017; Fernandes et al., 2022).

Suspended particulate matter in both freshwater and marine ecosystems comprises inorganic and organic particles. Organic particles include particulate organic detritus, algal cells, bacteria, viruses, and other microorganisms, while inorganic particles encompass clay minerals, Fe- and Mn-oxyhydroxides, calcite, and silicates, many of which possess high cation exchange capacities and are often coated with negatively charged organic films. These particles can modulate NPs toxicity by adsorbing metal species released from NPs. Moreover, their interactions with NPs can promote aggregation and facilitate sedimentation, thereby reducing NPs bioavailability in the water column (Wang et al., 2016).

Several studies have examined the role of algal (Mackevica et al.,

2015; Stevenson et al., 2017; Sun et al., 2022) and inorganic (Gupta et al., 2016, 2017) suspended particles in modulating NPs toxicity in aquatic organisms. High concentrations of algal particles have been shown to mitigate both acute and chronic NPs toxicity to aquatic biota. For example, Stevenson et al. (2017) demonstrated that increased food availability enhanced the growth, reproduction, and survival of *Daphnia pulicaria* exposed to AgNPs. Similarly, Sun et al. (2022) reported that higher food availability improved survival rates, body length, and fecundity in *Daphnia magna* exposed to ZnO nanoparticles. However, these studies did not explicitly elucidate the underlying mechanisms of toxicity mitigation, but rather alluded to the possibility that the observed reduction in toxicity may be attributed to organic material from food sources (Allen et al., 2010; Naddy et al., 2011; Stevenson et al., 2017), algae-derived dissolved organic carbon (Stevenson et al., 2013), and additional energy allocation for detoxification processes (Sun et al., 2022). Conversely, other studies suggest that organic particles may facilitate dietary uptake of AgNPs, thereby exacerbating toxicity (Lam and Wang, 2006; Zhao and Wang, 2010; Gupta et al., 2016). The heteroagglomeration of AgNPs with natural particles can enhance their internalization via ingestion, increasing nanoparticle bioavailability and toxicity in aquatic organisms (Gupta et al., 2016). Thus, a detailed understanding of the mechanisms by which suspended particles influence the fate, bioavailability, and toxicity of silver nanoparticles and silver ions is critically needed to accurately assess their ecological risks.

To address this gap, the present study systematically examines how natural suspended particles, both organic and inorganic, modulate the toxicity of silver nanoparticles and silver ions in aquatic ecosystems. Specifically, we employed montmorillonite as a model inorganic particle and *Selenastrum capricornutum* as a model organic particle. Montmorillonite, a widely studied clay mineral in environmental research (Gupta et al., 2016; Wang et al., 2021), is known for its pH-dependent surface charge properties (Li et al., 2024). *S. capricornutum* is a unicellular, non-motile freshwater green alga commonly utilized as a bioassay organism for chemical toxicity testing, following standardized protocols established by the U.S. Environmental Protection Agency (EPA), International Standards Organization (ISO), and Organization for Economic Cooperation and Development (OECD). We hypothesize that i) suspended particles will reduce the toxicity of AgNO₃ and AgNPs by adsorbing dissolved silver species, and ii) ingestion of Ag-laden particles, especially organic particles, may partially offset this protective effect. By evaluating toxicity in *Daphnia magna* exposed to two silver forms in the presence of natural particles, this work offers novel insight into the dual role of suspended particles in i) mitigating toxicity by adsorbing dissolved Ag and ii) providing additional exposure route via ingestion of Ag laden particles.

2. Material and methods

2.1. Organisms and culture conditions

Daphnia magna obtained from at the National Institute of environmental Research (NIER, Korea) was cultured in reconstituted hard water (MgSO₄, 9.98 × 10⁻⁴ M; CaSO₄, 6.98 × 10⁻⁴ M; NaHCO₃, 2.28 × 10⁻³ M; KCl, 1.07 × 10⁻⁴ M) following the standardized protocols of the U.S. Environmental Protection Agency (USEPA, 1995). The culture medium had a hardness of 170 ± 5 mg/L, an alkalinity of 110 ± 5 mg/L, and a pH of 7.8 ± 0.2. *D. magna* were maintained under controlled conditions at 20 ± 2 °C with a 16:8 h light-dark photoperiod. The organisms were fed daily with a combination of *Selenastrum capricornutum* (3.5 × 10⁴ cells/mL) and 1.2 mL/L of Yeast-Cerophyll-Trout chow (YCT). The medium in each stock culture vessel was replaced weekly. Neonates (<24 h old) were used in all acute toxicity assays. Overall experimental procedures were summarized in a schematic diagram (Fig. 1).

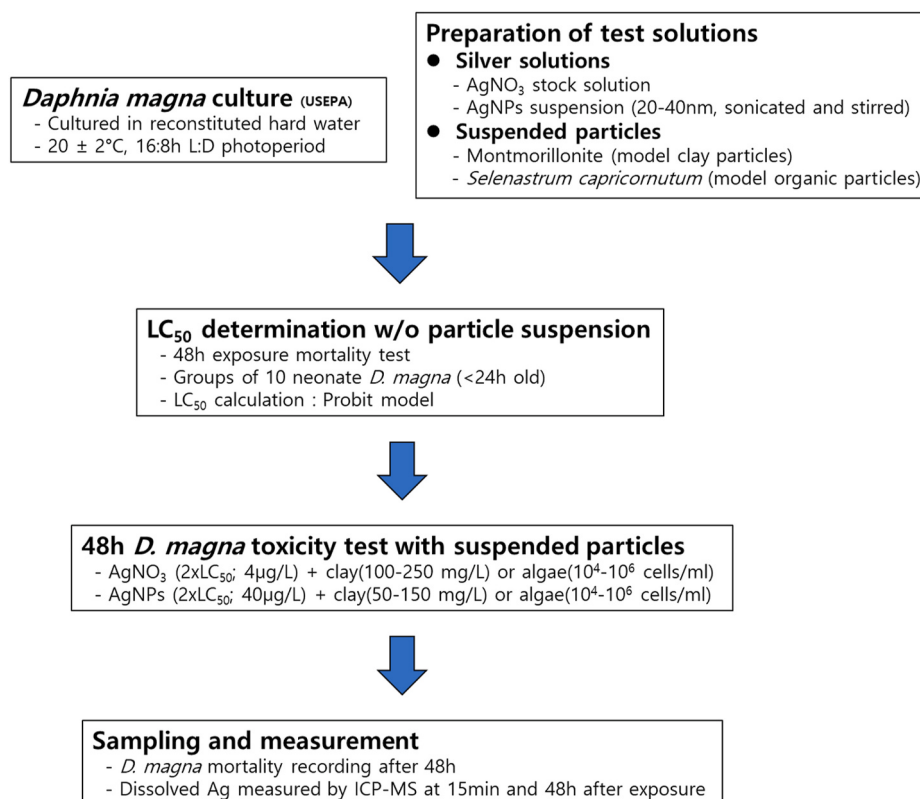


Fig. 1. Schematic diagram of experimental design.

2.2. AgNPs and suspended particles

Silver nanoparticles (AgNPs) were procured from Alfa Aesar (MA, USA), with a nominal particle size of 20–40 nm. The morphology and size distribution of AgNPs were characterized using transmission electron microscopy (TEM) (JEOL JEM-2000FX II, Tokyo, Japan). TEM samples were prepared by depositing a drop of AgNPs suspension in deionized water onto carbon-coated copper grids, followed by air-drying at 25 °C overnight to facilitate solvent evaporation.

For the inorganic particle suspension experiments, commercially available montmorillonite (Al₂O₃·4SiO₂·xH₂O; Sigma-Aldrich, MO, USA) was used as the clay component. According to the manufacturer, the specific surface area and particle size of montmorillonite were 220–270 m²/g and 18.3 µm, respectively.

The freshwater phytoplankton species *Selenastrum capricornutum* was cultured in the laboratory following USEPA guidelines (USEPA, 1978). The algal cultures were continuously shaken at 100 rpm and incubated at 24 °C on a rotary shaker under an irradiance of 255 µmol photons m⁻² s⁻¹. The hydrodynamic diameter of AgNPs were additionally characterized using dynamic light scattering (DLS) with a Zetasizer Nano ZS (Malvern Instruments, UK).

2.3. Preparation of AgNPs stock and AgNO₃ solution

The AgNPs stock solution was prepared by dispersing 0.02 g of AgNPs powder in 1 L of deionized water (18.2 MΩ cm, Millipore) and subjecting the suspension to ultrasonication (80 W, 45 kHz) (Transsonic 570/H; Elma Co., Singen, Germany) for 15 min. The suspension was subsequently stirred using a magnetic stir bar for 48 h at 25 °C to maintain dispersion. Prior to experimental use, additional ultrasonication was applied for 15 min to further disperse any aggregated nanoparticles. The desired experimental concentrations were prepared by diluting the stock solution in reconstituted hard water. For AgNO₃ treatments, a stock solution was prepared and serially diluted in

reconstituted hard water to achieve the required exposure concentrations.

2.4. Median lethal concentration (LC₅₀) of AgNPs and AgNO₃

The acute toxicity of AgNO₃ and AgNPs to *Daphnia magna* was assessed following the Organization for Economic Co-operation and Development (OECD) Guideline 202: *Daphnia* sp. *Acute Immobilization Test* (OECD, 2004). To determine the 48 h median lethal concentration (LC₅₀), groups of ten neonates (<24 h old) were exposed to a series of AgNO₃ or AgNPs concentrations. The nominal exposure concentrations for dissolved Ag (as AgNO₃) were 0, 1, 2, 3, and 4 µg/L, whereas those for AgNPs were 0, 10, 20, 30, and 40 µg/L. These concentrations were chosen from several preliminary range-finding experiments. All toxicity assays were conducted in triplicate under identical experimental conditions. The 48-h LC₅₀ values were calculated using the Probit model implemented in the EPA Probit Analysis Program v1.5 (USEPA, 1999). The estimated LC₅₀ values were used in the experiments for evaluating the effects of suspended particles in the following section.

2.5. Toxicity test with suspended particles

To evaluate the influence of suspended particles on the toxicity of AgNO₃ and AgNPs, *Daphnia magna* neonates were exposed to 4 µg/L of dissolved Ag (as AgNO₃) and 40 µg/L of AgNPs in media containing various concentrations of suspended particles. These exposure levels were set at twice the respective LC₅₀ values to account for potential toxicity mitigation by suspended particles.

For AgNO₃ treatments, montmorillonite clay concentrations of 100, 120, 150, 200, and 250 mg/L and algal densities of 1 × 10⁴, 1 × 10⁵, and 1 × 10⁶ cells/mL were used. In AgNPs treatments, montmorillonite concentrations of 50, 70, 100, and 150 mg/L and algal concentrations of 1 × 10⁴, 1 × 10⁵, 3.68 × 10⁵, and 1 × 10⁶ cells/mL were employed. Algal density was quantified using a Neubauer hemocytometer under light

microscopy.

Prior to introducing the test organisms, the particle suspensions were gently stirred for 15 min to ensure homogeneous distribution. Groups of ten *D. magna* neonates were then introduced into 1 L of AgNO₃- or AgNPs-containing media with suspended particles. Following a 48 h exposure period, immobilized individuals were recorded.

To quantify dissolved silver concentrations, 10 mL aliquots of each treatment solution were collected at 15 min and 48 h post-exposure. Samples were filtered through an Amicon centrifugal ultrafilter (3 kDa, Millipore) via centrifugation at 3243×g for 1 h (FLETA40; Hanil Co., Daejeon, South Korea). The 3-kDa filter had a nominal retention range of 1–20 nm, effectively removing particulate silver while retaining dissolved species. The concentration of dissolved silver in the filtrates was subsequently analyzed using inductively coupled plasma mass spectrometry (ICP-MS; Agilent Technologies 7700 series, CA, USA).

2.6. Statistical analysis

To evaluate the effects of suspended particle concentration on silver toxicity, one-way analysis of variance (ANOVA) was performed separately for each treatment condition (AgNO₃ or AgNPs) and particle type (clay or algae). The dependent variables analyzed included *Daphnia magna* mortality, as well as dissolved silver concentrations measured at 15 min and 48 h post-exposure. Statistical analyses were conducted using IBM SPSS Statistics (version 30, IBM Corp., Armonk, NY, USA), and a significance level of $p < 0.05$ was applied throughout. A summary of F-values and corresponding p -values for each treatment condition is presented in Table 1.

3. Results

3.1. AgNPs characterization

Transmission electron microscopy (TEM) revealed that the AgNPs had an average diameter exceeding 50 nm, with some nanoparticles exhibiting aggregation due to their high affinity (Fig. 2a). The dynamic light scattering (DLS) analysis indicated an average particle size distribution of 45.7 ± 30.8 nm, which was slightly larger than the nominal particle size (20–40 nm), likely due to agglomeration (Fig. 2b). The zeta potential of the AgNPs, a key indicator of colloidal stability and inter-particle repulsive forces, was measured at -18.57 mV, suggesting moderate stability in suspension.

Table 1
Summary of one-way ANOVA results.

Treatment	Particle type	Source	df	F-value	p-value
AgNO ₃ treatment	Clay	Mortality	5	55.622	<0.001
		Ag ion conc. (15 min)	3	105.880	<0.001
		Ag ion conc. (48 h)	3	61.541	<0.001
	Algae	Mortality	3	19804.250	<0.001
		Ag ion conc. (15 min)	3	21.934	<0.001
		Ag ion conc. (48 h)	3	80.755	<0.001
AgNPs Treatment	Clay	Mortality	4	35.461	<0.001
		Ag ion conc. (15 min)	3	180.172	<0.001
		Ag ion conc. (48 h)	3	364.378	<0.001
	Algae	Mortality	4	19952.300	<0.001
		Ag ion conc. (15 min)	3	77.749	<0.001
		Ag ion conc. (48 h)	3	617.331	<0.001

3.2. LC₅₀ for AgNO₃ and AgNPs

The 48 h median lethal concentrations (LC₅₀) for AgNO₃ and AgNPs in the absence of natural suspended particles were determined to be 1.79 µg/L and 17.05 µg/L, respectively (Fig. 3). The calculated LC₅₀ value for AgNPs was approximately ten times higher than that for AgNO₃, indicating a comparatively lower acute toxicity of AgNPs to *Daphnia magna*.

3.3. Influence of suspended particles on toxicity and dissolved Ag

The presence of suspended natural particles significantly attenuated the toxicity of both AgNO₃ and AgNPs to *Daphnia magna*, with the extent of mitigation dependent on particle concentration (Fig. 4, $p < 0.001$). In AgNO₃-exposed treatments, toxicity reduction was observed at montmorillonite concentrations exceeding 100 mg/L and algal cell densities exceeding 10⁴ cells/mL (Fig. 4). Similarly, in AgNPs-exposed treatments, toxicity was significantly reduced at montmorillonite concentrations above 50 mg/L and algal densities above 10⁵ cells/mL (Fig. 4, $p < 0.001$).

At 15 min of exposure in AgNO₃-containing media, the dissolved Ag concentration decreased from 3.2 µg/L in the control (without suspended particles) to 1.5 µg/L with increasing montmorillonite concentration and to below the detection limit with increasing algal cell density (Fig. 4). After 48 h of exposure, dissolved Ag concentrations declined further, from 2.4 µg/L in the control treatment to below the detection threshold at higher concentrations of both particle types.

In AgNPs-exposed treatments, the dissolved Ag concentration in the control (without natural particles) was initially 1.8 µg/L at 15 min, corresponding to approximately 5 % dissolution of the AgNPs suspension (Fig. 4). In the presence of montmorillonite, dissolved Ag levels decreased from 1.8 µg/L to 0.8 µg/L at the highest clay concentration, whereas they fell below the detection limit as algal concentration increased. After 48 h of exposure, the dissolved Ag concentration in AgNPs treatments further decreased from 1.0 µg/L to below the detection threshold in the presence of both particle types.

Scanning electron microscopy (SEM) analysis of *Selenastrum capricornutum* exposed to AgNPs revealed substantial nanoparticle aggregation and the formation of clusters on the algal cell surface (Fig. 5), suggesting that physical association with algal surfaces may influence AgNPs fate and bioavailability in aquatic environments.

4. Discussion

Most previous studies on AgNPs toxicity in aquatic organisms have been conducted without accounting for the influence of naturally suspended particles. In this study, we demonstrated that natural particles can modulate the toxicity of AgNPs to aquatic organisms through not only the adsorption of dissolved silver onto particle surfaces, which reduces their overall bioavailability and toxicity, but also the ingestion of Ag-associated particulates, which provides an alternative pathway for internal silver exposure. These processes can significantly alter the fate, transport, and biological uptake of silver species, ultimately influencing toxicity outcomes. To our knowledge, this is the first experimental investigation to systematically compare the modifying effects of both algal and clay particles on the toxicity of dissolved and nanoparticulate silver and provide mechanistic explanation.

The dissolution behavior of silver nanoparticles (AgNPs) plays a pivotal role in their overall toxicity, particularly in aquatic environments. In the control treatment, where no suspended particles were present, dissolved silver (Ag) reached 1.8 µg/L after 15 min of exposure, corresponding to approximately 5 % of the initial AgNPs concentration. This dissolved Ag was likely released during the 48-h stock preparation period. These results align with previous studies, which have reported AgNPs dissolution rates ranging from 4 % to 7 % under varying conditions. For instance, research on PVP-coated AgNPs demonstrated a

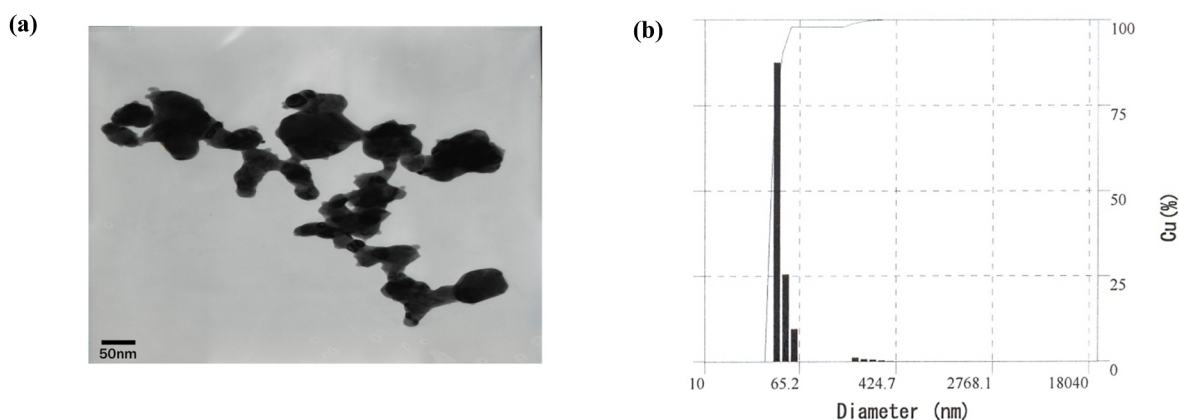


Fig. 2. Transmission electron microscopy micrograph of silver nanoparticles (AgNPs) in deionized water (a) and size distribution of silver nanoparticles analyzed by dynamic light scattering (b). Cu: cumulative percentage (%).

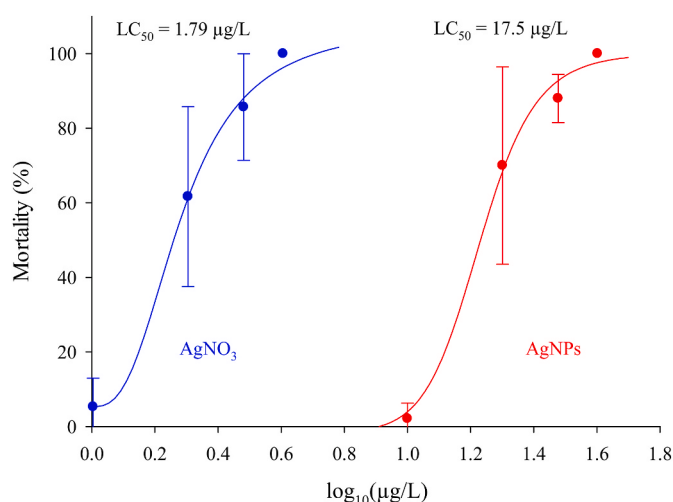


Fig. 3. Percent mortality (mean $\pm$ SD, $n = 3$) of the *Daphnia magna* exposed for 48 h to AgNO_3 (blue line) and AgNPs (red line) without suspension of natural particles.

dissolution rate of approximately 5.3 % within one week in biological media, with some of the dissolved silver re-precipitating as silver chloride (Loza and Epple, 2018). Similarly, single-particle ICP-MS analysis indicated that AgNPs dissolution in natural and laboratory waters was approximately 5 % in the initial stages (Mitrano et al., 2014). The dissolution of AgNPs is a key driver of their toxicity, particularly to aquatic organisms such as *Daphnia magna* (Radwan et al., 2019). This process is further influenced by environmental parameters such as temperature, ionic strength, and the presence of organic matter, all of which can modulate the dissolution and subsequent bioavailability of AgNPs (Radwan et al., 2019). Our results confirm that AgNPs dissolution occurs rapidly during early exposure, but the concentration of this dissolved Ag is significantly changed in the presence of suspended particles.

In the control treatment without suspended particles, dissolved Ag concentrations declined after 48 h of incubation. This reduction can be attributed to the adsorption of Ag onto the walls of the test containers, as well as onto the external surfaces of organisms and excreted materials (see Fig. 4). The pronounced decline in dissolved Ag concentrations with increasing particle concentrations can be primarily explained by the adsorption of dissolved Ag onto suspended particle surfaces and the incorporation of silver ions into algal cells. These processes effectively reduce the bioavailability of Ag in both AgNO_3 and AgNPs exposure media, underscoring the critical role of adsorption and biotic uptake in

mitigating silver toxicity in aquatic systems. The rapid decline of dissolved Ag to below the detection limit in algal suspensions highlights the superior capacity of algal particles, compared to clay particles, in sequestering dissolved metals (Lucaci et al., 2020; Ordóñez et al., 2023). Previous studies have demonstrated that algae can effectively accumulate metals by adsorbing them onto cell surfaces and incorporating them into cytoplasmic components (Gu and Lan, 2021; Mahana et al., 2021). Similarly, clay minerals, the most ubiquitous inorganic suspended particles, exhibit high adsorption capacities for heavy metals and other chemical species (Gupta et al., 2016). The surfaces of these natural suspended particles in aquatic environments are typically negatively charged and coated with organic films, which facilitate the adsorption of positively charged metal species (Forero López et al., 2021). Our results clearly demonstrate that both types of particles reduced dissolved Ag concentrations in AgNO_3 and AgNPs exposure media and this reduction was particle concentration dependent.

We are confident that the reduction in mortality with increasing particle concentrations for both particle types was primarily due to the reduction of dissolved Ag concentrations in AgNO_3 and AgNPs exposure media. Allen et al. (2010) demonstrated that food-derived organic material binds dissolved silver ions, reducing their bioavailability and minimizing toxic effects on *Daphnia magna*. Similarly, Naddy et al. (2011) emphasized that food not only directly binds Ag^+ , but also enhances physiological detoxification capacity, thereby improving organismal resilience. Other studies have also reported that high concentrations of algal particles can mitigate both acute and chronic nanoparticle toxicity in aquatic organisms. For example, Stevenson et al. (2017) observed that the growth, reproduction, and survival rates of *Daphnia pulicaria* exposed to 75 and 200 $\mu\text{g/L}$ of AgNPs significantly increased when food availability was enhanced up to 400 times the natural environmental levels. This study suggested that food-derived dissolved organic carbon (DOC) mitigates AgNPs toxicity via silver ion complexation, whereas insufficient food exacerbates nanoparticle toxicity due to limited energy availability for detoxification mechanisms. Similarly, Sun et al. (2022) found that increased food availability improved survival, body length, and fecundity in *Daphnia magna* exposed to ZnO nanoparticles, further supporting the notion that energy-rich environments enhance detoxification capacity and mitigate nanoparticle toxicity. However, previous studies have not explicitly elucidated the mechanistic basis by which suspended particles reduce nanoparticle toxicity. In this regard, our study provides novel mechanistic insights, demonstrating that adsorption of dissolved silver onto particle surfaces largely contribute to the mitigation of AgNO_3 and AgNPs toxicity.

The mortality of organisms exposed to AgNO_3 with montmorillonite suspensions was generally correlated with dissolved Ag concentrations,

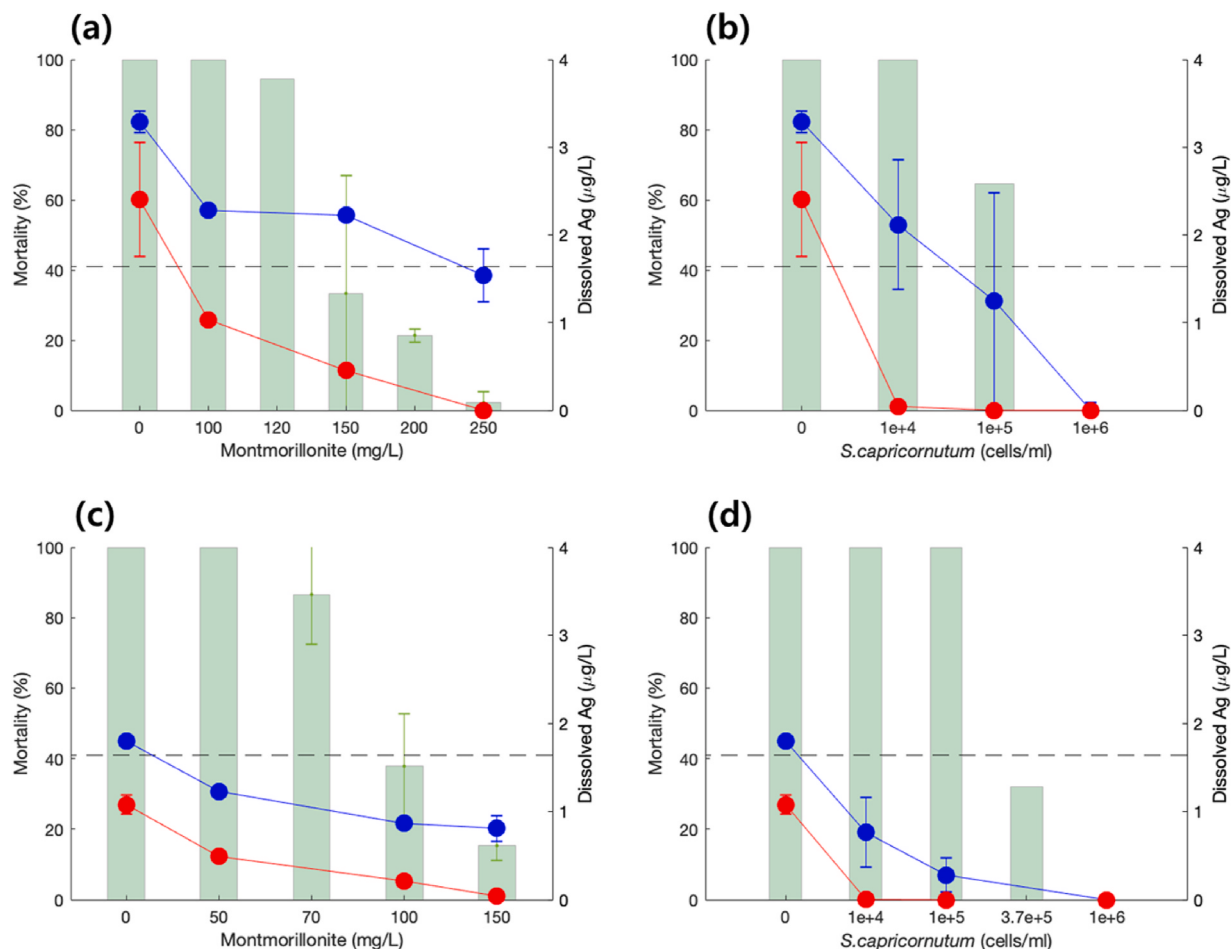


Fig. 4. Effects of montmorillonite (a, c) and *Selenastrum capricornutum* (b, d) on the *Daphnia magna* mortality and dissolved Ag concentration after 48 h exposure to 4 $\mu\text{g/L}$ AgNO_3 (a, b) and 40 $\mu\text{g/L}$ AgNPs (c, d). Bars indicate percent mortality (mean $\pm$ SD, n = 3) and lines show dissolved Ag concentrations measured at 15 min (blue circles) and 48 h (red circles). The dotted line represents the 48 h- LC_{50} determined in the independent experiment exposing the animals to AgNO_3 without particle suspension (see Fig. 3). Dissolved Ag at 70, 120 and 200 mg montmorillonite/L and 3.7×10^5 cells/L were not determined.

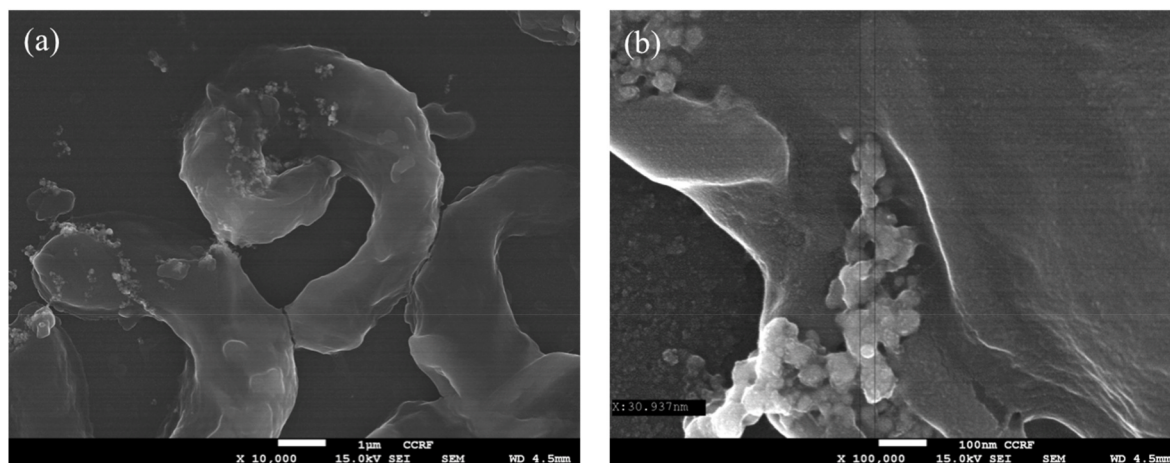


Fig. 5. Scanning electron microscope image of AgNPs agglomerated on the *Selenastrum capricornutum* cell (a) (scale bar = 1 μm) or (b) (scale bar = 100 nm).

which decreased over time with increasing particle concentrations (Fig. 4). However, 100 % mortality was observed at 10^4 algal cells/mL and approximately 70 % mortality at 10^5 cells/mL in the AgNO_3 treatment, despite initial dissolved Ag concentrations being near the 48 h LC_{50} value and final concentrations dropping below detection limits. The mortality in algal suspensions cannot be solely explained by

dissolved Ag concentrations. Instead, additional toxicity mechanisms must be considered. As discussed earlier, algal cells can effectively sequester dissolved Ag, and subsequent ingestion of Ag-laden algal cells by *Daphnia magna* likely contributed to increased toxicity. The absence of this mechanism in the montmorillonite treatments probably was due to the selective feeding behavior of *Daphnia magna*, which preferentially

ingests organic particles over inorganic particles based on size and nutritional value (Hadas et al., 1982; Ger et al., 2019). Additionally, aquatic organisms often exhibit higher assimilation efficiency when ingesting metals associated with organic particles than those associated with inorganic particles (Lee and Lee, 2005; Lee et al., 1998). Therefore, the ingestion of Ag-laden algal cells may act as a secondary pathway for metal uptake, providing additional toxic vector to dissolved-phase exposure.

On the other hands, the organisms exposed to AgNPs in both particle suspensions exhibited 100 % mortality, even when initial and final dissolved Ag concentrations were below the 48 h LC₅₀ (Fig. 4). The impact of dissolved Ag on mortality was lower in the algal suspension treatment than in the clay treatment, particularly at 10⁵ cells/mL, where dissolved Ag concentrations were significantly lower than the LC₅₀ value. Similar to AgNO₃ exposure, the toxicity of AgNPs cannot be fully explained by dissolved Ag alone. The fact that 100 % mortality was observed in control treatments without natural particle suspensions, despite dissolved Ag concentrations remaining below LC₅₀ values, indicates that additional mechanisms, such as direct contact with AgNPs or ingestion of AgNPs aggregates, contributed to toxicity. Our results underscore the complex interplay between particle-mediated processes and nanoparticle behavior, where agglomeration, surface adsorption, and ingestion collectively shape toxic outcomes.

In treatments with suspended particles, some of the dissolved Ag released from AgNPs was adsorbed onto clay particles or absorbed/incorporated into algal cells, explaining the observed decrease in dissolved Ag concentrations with increasing particle concentrations. Vincent et al. (2017) demonstrated that silver ions released from AgNPs are rapidly taken up by phytoplankton, further reducing free Ag⁺ concentrations in the surrounding medium. Further, we observed that AgNPs were aggregated on the surfaces of algal cells (Fig. 4) and this agglomeration of nanoparticles is common phenomena when they exposed together with algal cells (Ketter et al., 2018). Similarly, nanoparticles such as ZnO particles reported to agglomerate on montmorillonite via electrostatic interactions (Gupta et al., 2017a,b). Therefore, if *Daphnia magna* is able to ingest these Ag-associated particles, this ingestion pathway can account for the residual toxicity not explained by dissolved Ag exposure alone. In fact, many studies have demonstrating that *Daphnia magna* is able to ingest AgNPs adhered to algae and toxicity resulted from this dietary exposure (Vincent et al., 2017; Zhao and Wang, 2011; Lekamge et al., 2019). Taken together, these mechanisms highlight the need to consider both dissolved and particle-bound pathways in evaluating nanoparticle risk in realistic aquatic settings.

The dissolved silver concentrations reported for surface waters and wastewater effluents ranges from several ng/L to over 1 µg/L, with higher values particularly in regions receiving urban and industrial discharges (Gottschalk et al., 2009; Kaegi et al., 2011). Although some of these values approaches the LC₅₀ values determined for *Daphnia magna* in this study, most of the values are lower than the LC₅₀ values used in the present study. Similarly, the predicted and measured Ag concentrations in the environments from use of AgNPs are much lower than the values used in this study (Wimmer et al., 2019). In the present study, use of the high concentrations of AgNO₃ and AgNPs were necessary to explicitly demonstrate the effects on suspended particles on acute toxicity of aquatic organisms. Thus, while the study conditions may not fully replicate environmental silver concentrations, the findings provide critical insights into the mechanistic role of suspended particles in modulating nanoparticle toxicity. In the future studies in the evaluating role of suspended particles in modulating toxicity of dissolved metal or nanoparticles could use environmentally relevant metal concentrations to determine effects at the chronic to the cellular and molecular levels.

5. Conclusions

Our findings highlight the critical interactive role of natural organic and inorganic suspended particles in modulating the toxicity of

anthropogenic silver compounds to *Daphnia magna*, a freshwater cladoceran. Irrespective of particle type, these suspended particulates effectively attenuated the toxicity of both AgNO₃ and AgNPs by reducing the bioavailable dissolved Ag concentration in the exposure medium. Moreover, our results underscore that particle-bound silver, particularly when associated with ingestible organic matter such as algae, may represent a distinct and underrecognized pathway of toxicity, complementing the effects of dissolved silver species. These findings contribute fresh mechanistic insight to the current body of knowledge on AgNPs behavior and highlight the importance of including environmentally relevant particle types in future ecotoxicological risk assessments.

CRedit authorship contribution statement

Jihyuk Kim: Writing – original draft, Project administration, Methodology, Data curation. **Hee Yoon Kang:** Writing – review & editing, Methodology, Conceptualization. **Chang-Keun Kang:** Writing – review & editing, Conceptualization. **Byeong-Gweon Lee:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Hee Yoon Kang reports financial support was provided by National Research Foundation of Korea. Jihyuk Kim reports financial support was provided by National Forensic Service of Korea. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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