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Article

The effect of nanosilver particles on the lifespan of *Daphnia magna* in pond water

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SUMMARY

Nanosilver particles are currently used in over 1,000 consumer products because of their ability to kill fungi and bacteria by attaching to and penetrating their cell wall. Nanosilver particles are utilized in the production of consumer goods; however, a deficiency in research exists when it comes to potential harmful effects on the environment. The purpose of our research was to determine the effect of silver nanoparticles on *Daphnia magna* in pond water. *D. magna* are a species of water flea that have previously been used as an indicator species for the health of pond ecosystems. We hypothesized that the *D. magna* exposed to the highest concentration nanosilver particle (25 µg/L) would reach 100% mortality the quickest and exhibit a shorter lethal 50 time (LT50) compared to the control and the lower concentrations of nanosilver particles (5 µg/L and 10 µg/L). We saw that *D. magna* exposed to 25 µg/L silver nanoparticles reached 100% mortality the fastest (9.6 hours) compared to *D. magna* exposed to the 5 µg/L and 10 µg/L concentrations. Our findings provide evidence that a means of preventing nanosilver particle runoff into the environment is necessary in order to prevent ecological damage.

INTRODUCTION

Particles of silver between 1 to 100 nanometers in size are defined as nanosilver particles. The use of nanosilver particles in the production of consumer goods has increased exponentially over the last two decades (1). The antifungal and antibacterial properties of nanosilver particles are the primary reason for their increased use in the production of a myriad of consumer products, such as washing machines, clothing, cosmetics, food containers, and wound dressings (2). Through runoff, the disposal of wastewater treatment, or directly through industrial discharges, nanosilver particles enter freshwater systems (3). This is concerning since silver is ranked second as the most toxic metal to aquatic organisms, and nanosilver particle runoff could disrupt the aquatic ecosystem (4).

Daphnia magna, a specific breed of water flea, is a keystone species in ponds and other freshwater ecosystems. They have previously been used a model organism for assessing the health of a pond (5). Additionally, *D. magna* are one of the most commonly studied model organisms to date (6). This is because of its even distribution throughout the world and its ability to thrive in simple aquatic environments with minimal care. These qualities make the species useful in experiments pertaining to ecotoxicology (6). While prior research has shown that nanosilver particles can be toxic to other aquatic organisms, research specifically focusing on how these particles affect *D. magna* remains limited (7, 8). Most studies that have been conducted involving nanosilver particles and *D. magna* have been done in deionized water or a M4 medium, specifically formulated water that contains buffers to maintain a neutral pH, and a gap in current research exists when it comes to how it affects these organisms in pond water specifically (9, 10).

Therefore, we utilized *D. magna* to determine the toxicity of nanosilver particles in pond water. Specifically, we investigated the effects of nanosilver particles on *D. magna* by exposing them to varying concentrations (5 µg/L, 10 µg/L, 25 µg/L) of nanosilver particles in pond water. We hypothesized that the *D. magna* exposed to the highest concentration of nanosilver particles would reach 100% mortality the quickest and have a shorter LT50 (median lethal time). Our results supported our hypothesis as the *D. magna* exposed to 25 ug/L silver nanoparticles reached 100% mortality rate the fastest (9.6 hours) compared to the two weaker solutions (5 µg/L and 10 µg/L) and the control. These results reflect the importance of obtaining a better understanding of the effects of nanosilver particles on living organisms in pond water.

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RESULTS

We tested the toxicity of nanosilver particles on *D. magna* by exposing them to three different concentrations of nanosilver particles (5, 10, and 25 µg/L) and measuring the mortality every two hours over a 24-hour time period. From the mortality data we also calculated the LT50 for each concentration. In total, we conducted five trials in the same controlled environment, at the same time, and in the same manner.

We saw that the *D. magna* exposed to 25 µg/L of nanosilver particles reached 100% mortality an average of 4 hours earlier than the 10 µg/L group, an average of 10 hours earlier than 5 µg/L group, and an average of 12 hours earlier than the control group (**Figure 1**). Additionally, the 25 µg/L group on average reached LT50 in the least amount of time (4 hours) (**Figure 2**).The 10 µg/L solution reached LT50 between hours 8 and 10 and the 5 µg/L solution reached LT50 at hour 10.

DISCUSSION

Our data supported the hypothesis that 100% mortality in *D. magna* is reached faster at higher concentrations of nanosilver particles. Additionally, our study addresses a gap in current research by testing the effects of different concentrations of nanosilver particles on *D. magna* in pond water samples. From our results, it can be inferred that nanosilver particles can pose a threat to ecosystems given that *D. magna* are an indicator species of overall ecosystem health. Furthermore, the results of this experiment provide a foundation for further research to take place to determine the short- and long-term effects of nanosilver particles on organisms within freshwater ecosystems and the lasting effects on the environment.

One technical limitation encountered during the experiment was the challenge of assessing the health of the *D. magna* specimens prior to experimentation. When the *D. magna* arrived in a refrigerated container, there was no recommended process for assessing their initial health. This limitation was partially overcome by allowing for a 12-hour adjustment period. This adjustment period allowed time for behavioral and physiological adaptation for the specimens. The *D. magna* that were unable to adapt most likely died within the 12-hour period and were then disposed of prior to experimentation. Implementing this adjustment period allowed for reproducible experimental results in the future. However, even with the adjustment period, there was still room for error and the potential for unhealthy *D. magna* to be used for the experiment. Although it is difficult to assess the impact of unhealthy specimens on the experiment, the trends in the data were consistent and reproducible across multiple trials suggesting that the key findings of the experiment remained constant. In future experiments, this could be addressed by viewing each specimen under a microscope prior to experimentation. Under the microscope one could look for any abnormal swimming patterns or physical deformities. This could help prevent unhealthy specimens from being used in the experiment.

Nanosilver from consumer products such as textiles and cosmetics can be released into wastewater during their use and disposal, ultimately entering aquatic ecosystems through wastewater runoff. As the production of consumer products containing nanosilver increases, it is vital that research be conducted on the effects this pollution has on living organisms in the environment (1). *D. magna* are essential to the ecosystem and food webs in lakes and ponds and are the predominant food source for a multitude of species (6). If ecosystems containing *D. magna* continue to be polluted with nanosilver particles, then entire ecosystems risk collapse (6). It is imperative that case studies be performed regarding other organisms and the effects that nanosilver has on their physiology as well as their lifespan. Additional experiments need to be conducted that test varying concentrations of nanosilver particles and how they affect other freshwater organisms, such as zebrafish or freshwater snails. Long-term ecological monitoring as well as *in situ* experiments testing varying concentrations of nanosilver particles should

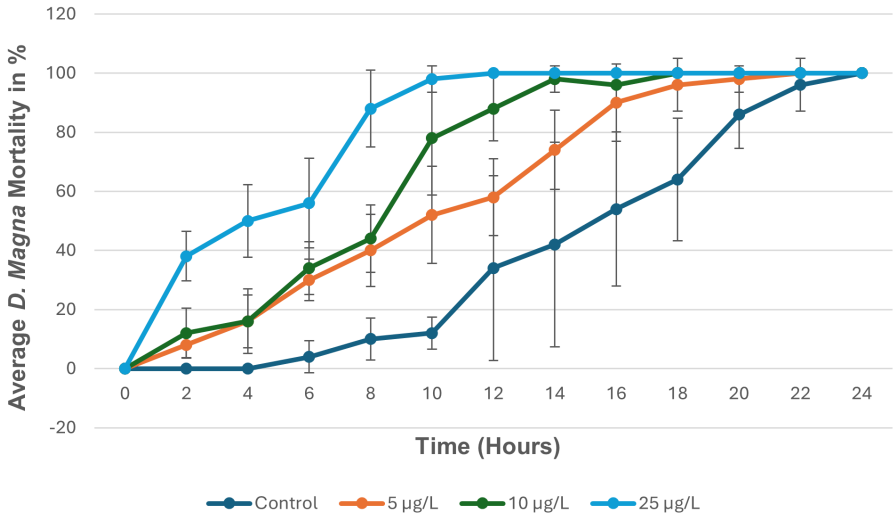

Figure 1. Average mortality of *D. magna* over 24 hours. Average mortality rate of *D. magna* at 0 µg/L, 5 µg/L, 10 µg/L, and 25 µg/L. *D. magna* exposed to the 25 µg/L solution reached 100% mortality the quickest at hour 12 on average. The 10 µg/L solution reached 100% mortality at hour 18 on average. The 5 µg/L solution reached 100% mortality at hour 20 on average. The control group reached 100% mortality at hour 24 on average. Data shown as mean ± SD (n = 5 replicates with 10 *D. magna* each for each concentration).

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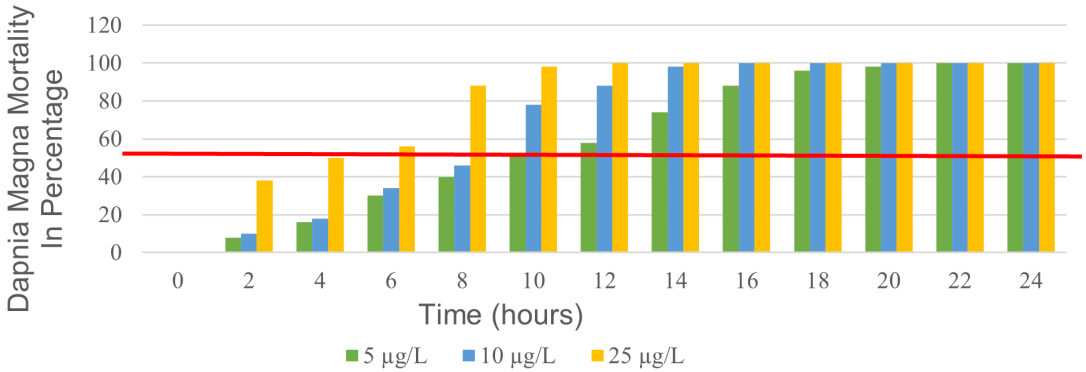

Figure 2. Average median lethal time (LT50) for nanosilver particle-treated *D. magna*. The red bar marks the point at which 50% mortality was reached (LT50). LT50 was reached at the fastest rate in the 25µg/L solution at hour 4. The 10µg/L solution reached LT50 between hours 8 and 10 and the 5µg/L solution, the weakest solution, reached LT50 at hour 10 which was the slowest. (n = 5 trials with 10 *D. magna* each for each concentration).

be implemented in order to assess the long-term damage of nanosilver exposure on ecosystems. Future research like this would prove to be invaluable in providing a framework for establishing regulatory standards for the levels of nanosilver particles sustainable in freshwater ecosystems.

MATERIALS AND METHODS

Preparation of nanosilver solutions

Pond water was obtained from Carolina Biological. Nanosilver particles were obtained from Silver Wings Nanosilver through Amazon. We prepared three concentrations of nanosilver particles using pond water as the solvent: 5 µg/L, 10 µg/L, and 25 µg/L. Five independent replicates of each concentration of nanosilver particles was used.

Preparation of *D. magna*

The pond water, nanosilver particles, and 10 *D. magna* (Carolina Biological) were placed in clear sterile 0.59 L plastic cups. The sterile experiment cups containing the nanosilver particles and pond water solutions were placed in a controlled environment which included a closed room with no direct sunlight and a temperature that stayed between the recommended range of 19– 21°C (11). *D. magna* used were from the same order and all came from the same container. Upon arrival, they were visually examined with a hand lens, and the lid of the container was opened to allow for air exchange. Any suboptimal specimens, those that were not actively swimming, were assumed dead and were disposed prior to the adjustment period. The *D. magna* was placed in the previously mentioned controlled environment for 12 hours for an adjustment period prior to the start of the experiment. After this period, the containers were examined again and any other suboptimal organisms were discarded. Following the adjustment period, 10 healthy *D. magna* were placed in each cup using a 1 mL transfer pipette. The tip of the transfer pipette was cut at an angle to widen the opening so the *D. magna* would not be harmed during the transport.

Nanosilver concentrations

On average, the lifespan of *D. magna* is 40 days and the need to change the pond water by hour 72 is recommended to sustain viability (12). The three concentrations of nanosilver particle solutions used were 5 µg/L, 10 µg/L, and 25 µg/L. The three concentrations of nanosilver particles that were used were representative to that of real-world waterways where concentrations are estimated to be approximately 10 µg/L in surface waters (13). A higher concentration (25 µg/L) was used so that polluted water near large industries could also be represented. The 5 µg/L concentration was selected in order to represent a lower, environmentally relevant exposure level, as nanosilver concentrations in surface waters have been reported to reach or exceed this level in areas impacted by this form of pollution. These concentrations were additionally selected to ensure that mortality occurred within 72 hours allowing for a manageable timeline for data collection. In addition, by choosing these three concentrations, we limited the need to change the water and thus avoided addition of extra variables to the experiment.

Mortality quantification

Percent mortality was selected as the dependent variable for assessment of toxicity. The cups containing the *D. magna* and varying concentrations of nanosilver particles were observed with a hand lens on a continual basis every 2 hours over a 24-hour time period until all of the *D. magna* had reached 100% mortality. Mortality was assessed visually by counting the number of immobile organisms that failed to respond to gentle agitation with a pipette tip. If there were any uncertainties in the viability of the *D. magna*, then a 50x magnification power microscope (Binocular Compound microscope, Poothoh) was used to verify the existence of a heartbeat. At the end of the 24 hour time period, all of the specimens were determined to be dead.

Mortality was graphed and then the LT50 for each of the three nanosilver solutions was calculated. The LT50 provides a clear benchmark for the toxicity of nanosilver particles and marks the amount of time it took for 50% of the *D. magna* specimens to die (14).Taking into consideration that *D. magna* can survive on average 36 hours without eating and they came in containers with pond water containing microorganisms, no additional food was provided throughout the duration of the experiment (15). This was done to limit the variables being tested that could potentially skew the results. *D. magna* were disposed of according to Carolina Biological safety recommendations which involved adding 2mL of bleach to the specimen cups and flushing the contents down the drain with a surplus of hot water (15).

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REFERENCES

1. Potter, Phillip M, et al. "Transformation of Silver Nanoparticle Consumer Products during Simulated Usage and Disposal." *Environmental Science. Nano*, 2019. PMCID: PMC6854907.
2. "Nanosilver." Beyond Pesticides, 2022, "Nanosilver." *Beyond Pesticides*, www.beyondpesticides.org/resources/antibacterials/nanosilver. Accessed 01 May 2025.
3. Hayhurst, Lauren. "Silver Uncontrolled: How Nanosilver Gets into Our Freshwater and What We Need to Do About It." *IISD Experimental Lakes Area*, 17 Apr. 2020, https://sdg.iisd.org/commentary/guest-articles/how-nanosilver-gets-into-our-freshwater-and-what-we-need-to-do-about-it/. Accessed 13 Nov. 2023.
4. Ihtisham, Muhammad, et al. "Silver Nanoparticle's Toxicological Effects and Phytoremediation." *MDPI*24 Aug. 2021, https://doi.org/10.3390/nano11092164.
5. Javier Esteban Mussin, and Gustavo Giusiano. "Biogenic Silver Nanoparticles as Antifungal Agents." *Frontiers in Chemistry*, vol. 10, 6 Oct. 2022, https://doi.org/10.3389/fchem.2022.1023542.
6. Ebert, Dieter. "Daphnia Magna as a Versatile Model System in Ecology and Evolution." *EvoDevo*, vol. 13, no. 16, 2022. https://doi.org/10.1186/s13227-022-00199-0.
7. Zhang, Jing, et al. "Nano Silver-Induced Toxicity and Associated Mechanisms." *International Journal of Nanomedicine*, vol. Volume 17, Apr. 2022, pp. 1851–1864, https://doi.org/10.2147/ijn.s355131.
8. Akter, Mahmuda, et al. "A Systematic Review on Silver Nanoparticles-Induced Cytotoxicity: Physicochemical Properties and Perspectives." *Journal of Advanced Research*, vol. 9, Jan. 2018, pp. 1–16, www.ncbi.nlm.nih.gov/pmc/articles/PMC6057238/, https://doi.org/10.1016/j.jare.2017.10.008.
9. Asghari, Saba, et al. "Toxicity of Various Silver Nanoparticles Compared to Silver Ions in Daphnia Magna." *Journal of Nanobiotechnology*, vol. 10, no. 1, 2012, p. 14, https://doi.org/10.1186/1477-3155-10-14.
10. Hu, Yi, et al. "Distinct Toxicity of Silver Nanoparticles and Silver Nitrate to Daphnia Magna in M4 Medium and Surface Water." *Science of the Total Environment*, vol. 618, Mar. 2018, pp. 838–846, https://doi.org/10.1016/j.scitotenv.2017.08.222.
11. Müller, Mara F., et al. "Temperature-Driven Response Reversibility and Short-Term Quasi-Acclimation of Daphnia Magna." *PLOS ONE*, vol. 13, no. 12, 21 Dec. 2018, https://doi.org/10.1371/journal.pone.0209705.
12. Elenbaas, Molly. "Daphnia Magna." *Animal Diversity Web*, 2023, https://animaldiversity.org/accounts/Daphnia_magna/. Accessed 04 Jan. 2025
13. Ramirez, Rosenberg et al. "Environmental Risk Assessment of Silver Nanoparticles in Aquatic Ecosystems Using Fuzzy Logic." *MDPI*, vol. 14, no. 12, 2022. https://doi.org/10.3390/w14121885
14. "LT50." *ScienceDirect Topics*, www.sciencedirect.com/topics/agricultural-and-biological-sciences/lt50. Accessed 02 Nov. 2024.
15. Wardsci, www.wardsci.com/www.wardsci.com/images/Wards_Daphnia_LMCS.pdf. Accessed 01 Nov. 2024