WHY SILVER MIGHT NOT BE GOLD IN WATER

An investigation to determine the minimum concentration of nanosilver which causes toxicity to *Daphnia magna*

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PRELIMINARY

Summary

Nanosilver is nanoparticles of silver 1-100 nanometres (nm) in size and is commonly used in commercial products such as textiles and cosmetics due to its strong antimicrobial properties. Nanosilver can enter waterways and aquifers, and despite its widespread use, there is currently no universally accepted threshold level for nanosilver. The objective of this investigation was to determine the minimum concentration of nanosilver which causes toxicity to *Daphnia magna*. This was achieved by conducting a bioassay, in which the population and heart rates of the aquatic organism *Daphnia magna* were studied in different concentrations of nanosilver (0.00 mg/L, 0.25 mg/L, 0.50 mg/L, 0.75 mg/L and 1.00 mg/L) over a period of 60 minutes. The results demonstrated that nanosilver has a significant effect on the *Daphnia magna* because as the concentration of nanosilver increased, the population and heart rates of the *Daphnia magna* decreased. These findings indicate that nanosilver is toxic to *Daphnia magna* at certain concentrations, with the minimum concentration of nanosilver which causes toxicity at 0.26–0.50 milligrams per litre (mg/L). This minimum concentration can then be used as an indicator of water quality, ensuring that healthy aquatic ecosystems can be maintained.

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Abbreviations and Acronyms

Bioassay: a procedure for determining the potency of a substance by measuring its effect on living cells

Bioindicator: a living organism that can be used as a reference indicating toxicity to other organisms

D. magna: abbreviation for *Daphnia magna*

Ecotoxicity: the ability of a chemical or physical agent to have an adverse effect on the environment and the organisms living in it

Lentic: (of organisms or habitats) inhabiting or situated in still fresh water

Suspension: a heterogeneous mixture of a finely distributed solid in a liquid

Threshold level: a value that defines the conditions under which a healthy aquatic organism community is present

Toxicity: the degree to which a chemical substance or mixture of substances can damage an organism

Wastewater: any water that has been contaminated by human activities, stormwater, or sewer inflow

Acknowledgements

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LITERATURE REVIEW

Overview

Nanosilver is nanoparticles of silver 1-100 nm in size and is the most commonly used nanoparticle in consumer goods (Figure 1) such as cosmetics, textiles and disinfectants (Hayes, 2016). Nanosilver is used in these products for its antimicrobial properties which kill bacteria, such as those in clothes to prevent odour. Due to their nanosized particles, nanosilver "poses potential ecotoxicity to ecosystems" (Luo *et al.* 2016, p.1) and to mitigate this damage, a threshold level should be developed for nanosilver.

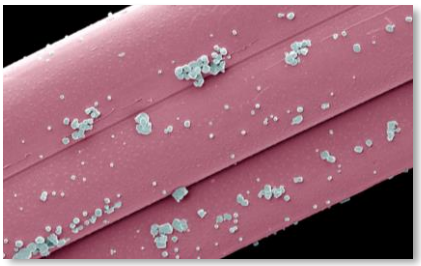


Figure 1: *Coloured scanning electron micrograph of fibres from a nanosilver-impregnated fabric. (Seltenrich, 2013)*

Antimicrobial Properties

Compared to bulk forms of silver, nanosilver particles have a larger surface area to volume ratio, accelerating the release of silver ions, the primary mode of nanosilver toxicity. This makes nanosilver more toxic (Luoma, 2008). Silver ions penetrate cell membranes, leading to cellular compartments leakage, resulting in cellular death (Qing *et al.*, 2018). At the University of Sydney Nano Institute, Associate Professor Wojciech Chrzanowski described how when nanoparticles enter a cell, they damage DNA, lipids and proteins, interfering with intracellular biological functions. This makes nanosilver a powerful microbial agent, a key reason why its use is so widespread.

Environmental Exposure to Nanosilver

As commercial application of nanosilver increases, nanosilver releases into the environment also increase. The largest source of release is clothing, followed by industry, disposal of cosmetics and disinfectants (Hayhurst, 2020). When laundered, fabrics release on average 425 µg Ag/kg of fabric into wastewater (Mitrano *et al.*, 2014) which, once treated (Figure 2), is released into the environment (Nowack, 2010).

Figure 2: *Sources of nanosilver releases into the environment (Nowack, 2010)*

Toxicity to Aquatic Organisms

Cummins (2019) demonstrated the detrimental effects of nanosilver on the population and heart rates of the aquatic organism *Daphnia magna* (Figure 3), establishing the potential for nanosilver to cause environmental toxicity. *Daphnia magna* are an established bioindicator of ecotoxicology and central to the food webs of freshwater lentic habitats (Navarro, 2008). They are an excellent model organism as their body is transparent and the heart is easy to see under the microscope. In addition, Seltenrich (2013) argues that as nanosilver is toxic to aquatic organisms, it follows that nanosilver is also toxic to humans, with the potential for the nanoparticles to cross the blood-brain barrier, posing a threat to human health.

Figure 3: Daphnia magna *under a microscope at 40X magnification. (Kinsman, 2019)*

Threshold Level for Nanosilver

While NSW Environmental Protection Authority sets a threshold level for bulk silver, it does not set a separate level for nanosilver as they have identical molecular identities (Faunce and Watal, 2010). This is despite abundant scientific literature that indicates nanosilver is more toxic. As it is currently unknown which concentration of nanosilver is toxic in ecosystems, the aim of this investigation was: To determine the minimum concentration of nanosilver which causes toxicity to *Daphnia magna*.

To fulfil this aim, a bioassay was used to determine which nanosilver concentration is toxic to *Daphnia magna (D. Magna)* by monitoring their heart rates (proxy for metabolism) – thus establishing if the organism is dying (Amundsen et al., 2015). *D. magna's* heart rates are also elevated by stress, which can occur when moved into a new environment (Cornell University, 2009). By measuring heart rate, it is possible to determine at which nanosilver concentration these organisms die, thus a threshold level can be obtained based on this parameter.

In summary, nanosilver is more toxic than macro forms of silver as its larger surface area to volume ratio releases silver ions more quickly. This underlines the need for an explicit concentration threshold level upon which it is safe to have in the environment.

METHODOLOGY

Hypotheses for Population

H_0: There is no difference in the effect of the tested concentrations of nanosilver on the population of *Daphnia magna.*

H_1: There is a difference in the effect of the tested concentrations of nanosilver on the population of *Daphnia magna.*

Hypotheses for Heart Rates

H_0: There is no difference in the effect of the tested concentrations of nanosilver on the heart rates of *Daphnia magna.*

H_1: There is a difference in the effect of the tested concentrations of nanosilver on the heart rates of *Daphnia magna.*

Independent Variable: The concentration of nanosilver in the spring water

Dependent Variables: Population of the *D. magna* and the heart rates of the *D. magna*

Materials

Colloidal Silver (50 mg/L, 500 mL) (Nature Ultimate brand) (Figure 4), Three *D. magna* culture jars (Southern Biological), two 2 g algae pellets and a Celestron LCD Digital Microscope II (Figure 5) were purchased online. Other equipment used throughout included: permanent markers, digital camera, scissors, digital stopwatch, 15 clear plastic rectangular containers from FPO Packaging, bottled natural spring water (5 L, CS brand) a 10 mL and a 100 mL measuring cylinder, 5 plastic Pasteur pipettes and 3 concavity slides (Carolina Biological Supply).

Figure 4: *Colloidal Silver (500 mL 50 mg/L) purchased online.*

Method A – Nanosilver Suspensions

Using natural spring water, the nanosilver suspensions were prepared by diluting a stock solution of 50 mg/L colloidal silver according to the table in Figure 6. The volume of colloidal silver in mL converts to nanosilver in mg/L and therefore provides the required concentration levels.

Colloidal silver is nanosilver particles suspended in water. A 3 mL Pasteur pipette, 10 mL measuring cylinder and 100 mL measuring cylinder were used. In total, three replicates were completed (Figure 7). Using a stopwatch, each suspension was swirled for 15 seconds with the same amount of force.

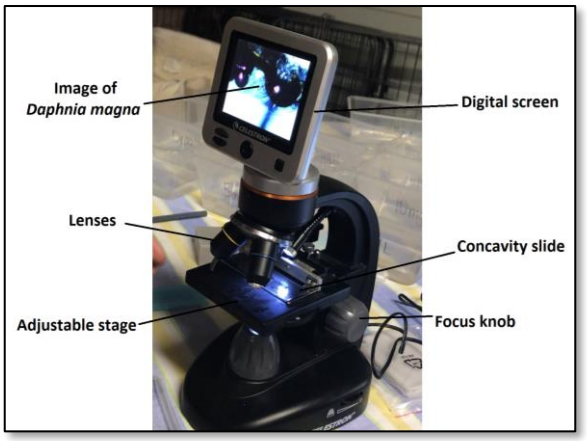


Figure 5: *Celestron LCD Digital Microscope II that was used in the experiment. Purchased online from Australian Geographic.*

Nanosilver concentration (mg/L)	Volume spring water (mL)	Volume colloidal silver (mL)	Total volume of liquid (mL)
0.00	200	0	200
0.25	199	1	200
0.50	198	2	200
0.75	197	3	200
1.00	196	4	200

Figure 6: *Table showing the volume of colloidal silver and spring water required to prepare the nanosilver solutions.*

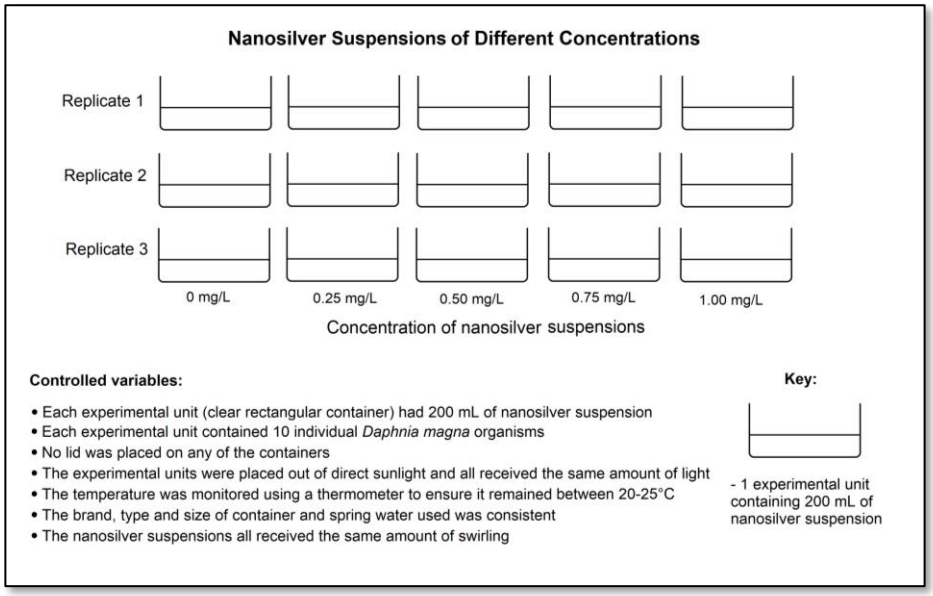


Figure 7 (left): *Diagram showing the experimental set up. There were 3 replicates for each concentration of nanosilver, and all other variables were carefully controlled.*

Method B – *Daphnia magna* cultures

The *D. magna* cultures were removed from the packing material, each culture containing approximately 100 organisms. The culture lids were removed for aeration and the cultures were left for 24 hours out of direct sunlight at room temperature (20-25°C) to allow the organisms to recover from shipping shock. Two 2 g algae pellets were placed into each of the cultures as a food source.

Scissors were used to remove the end of a 3 mL plastic Pasteur pipette at a 45° angle to enable the organisms to be transported without becoming stuck in the pipette tip (Figure 8). Using the pipette, the *D. magna* were placed in a separate container of natural spring water between 20-25°C (Figure 9) before 10 individual organisms were placed into each of the 12 nanosilver suspensions. The *D. magna* were all similar enough to account for an even survival capacity in the treatments.

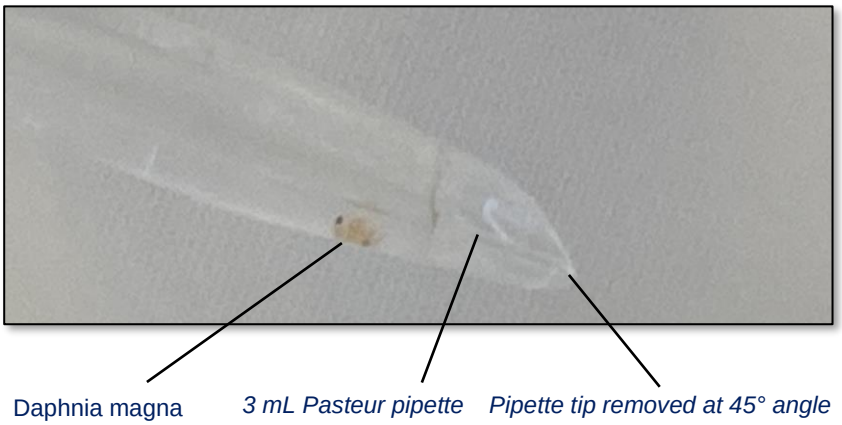


Figure 8: *Close-up image of the end of a 3 mL Pasteur pipette with the tip removed at a 45° angle. This is so the* Daphnia magna *can be transported without becoming stuck in the pipette tip.*

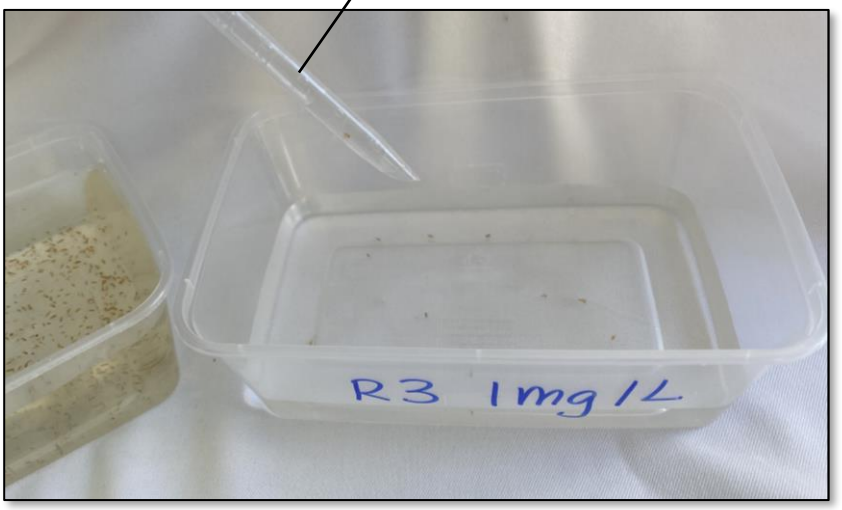


Figure 9: *Labelled image showing the* Daphnia magna *being removed from the separate container and then moved into the 1 mg/L nanosilver container. Note: 'R3' means 'Replicate 3'.*

Method C – Population Number Count

Birds-eye-view videos were taken of the 15 nanosilver suspensions for 5 seconds. From the videos, the number of live *D. magna* was visually determined according to the criteria in Figure 10. The health of the individuals was assessed before placing them into a treatment as healthy organisms were continuously moving. The number of live *D. magna* in the suspensions was counted and recorded once every 15 minutes for 1 hour (Figure 11).

State	Conditions
Live	• Movement of legs, antennae, body • Heart beating
Dead	• Absence of movement • No heartbeat

Figure 10: *When the conditions indicating the* D. magna *was live were observed, the organism was recorded as being live. When the conditions including an absence of movement and no heartbeat was observed, the organism was recorded as being dead.*

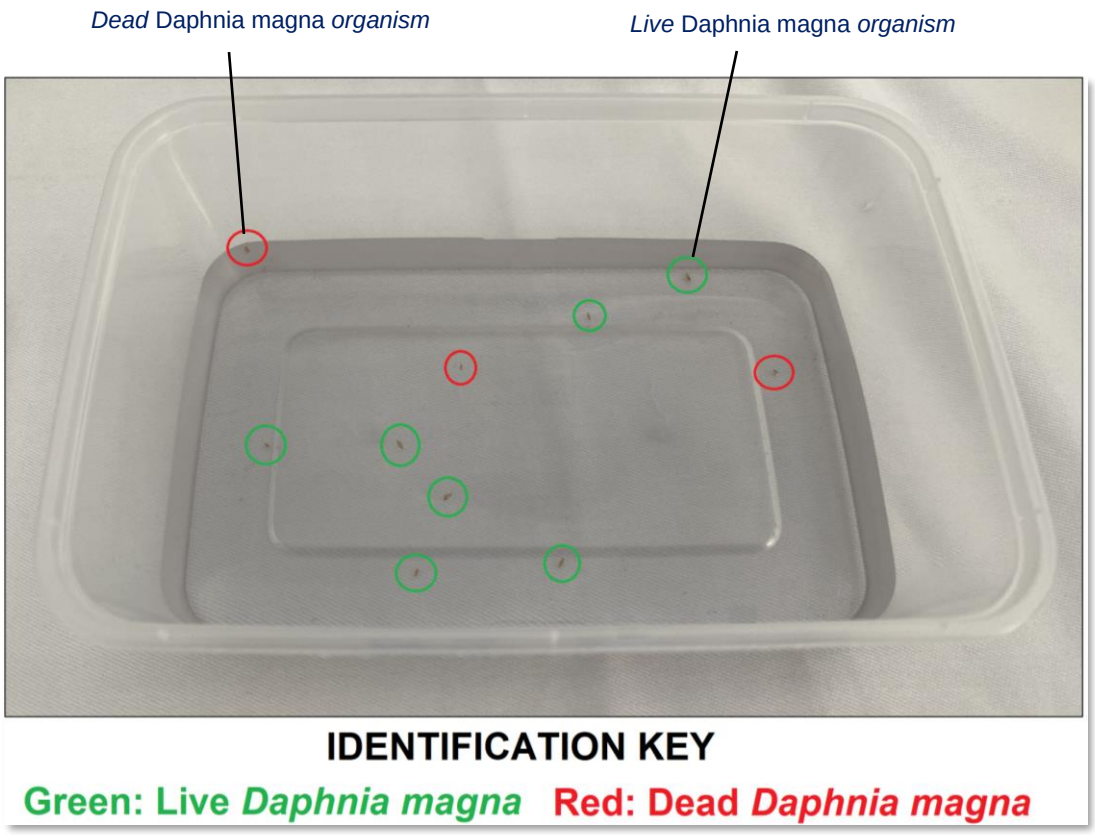


Figure 11: *A birds-eye-view of the 1 mg/L nanosilver suspension after 15 minutes. As seen in the image, there are 7 live* Daphnia magna *and 3 dead.*

Method D – Heart Rates

A single *D. magna* was randomly removed from the 0 mg/L suspension using the 3 mL Pasteur pipette, placed in the well on a concavity slide and placed under the microscope. Excess water was removed using the pipette.

As shown in Figure 13, the heart is located behind the eye spot. Using the microscope's recording function, the *D. magna's* heartbeat was recorded for 5 seconds (Figure 12). Two more *D. magna* were randomly removed from the 0 mg/L suspension and their heartbeats recorded. Once completed, all three *D. magna* were returned to the suspension. This was completed for each of the 15 nanosilver suspensions every 15 minutes for 1 hour.

Figure 12: *Image showing a* Daphnia magna *on the microscope's digital screen under 10X magnification.*

After the experiment was completed, the videos of the heartbeat were watched in slow mode, and the number of heart beats in 5 seconds was multiplied by 12 and recorded using the unit of beats per minute (bpm).

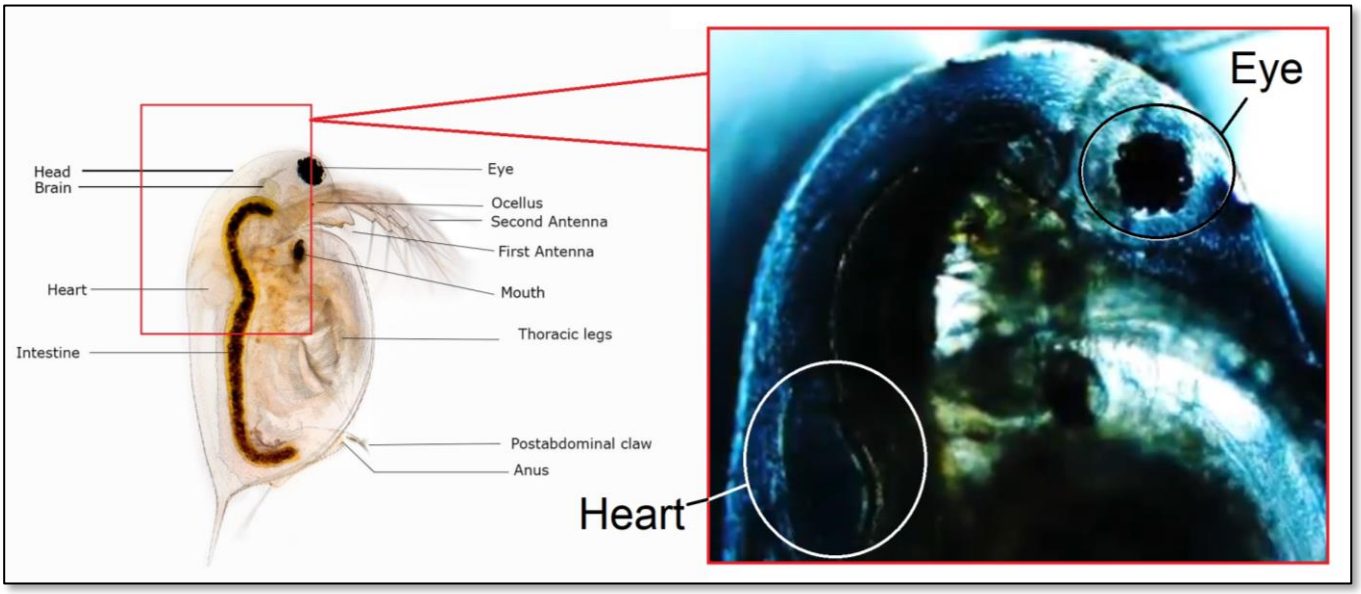


Figure 13: *Using the microscope's 10X magnification, the* Daphnia magna *was located by adjusting the stage. On the digital screen, the heart was located behind the eye, denoted by the labelled white circle.*

Assessment of Relevant Ethical Issues

This project adhered to the RSPCA code for the care and use of animals for scientific purposes as it implemented the 3Rs: replacement, reduction and refinement (Miller, 2021).

Firstly, while the method was unable to avoid or replace animal use, the animals were suited to the purpose of the project, fulfilling the condition of replacement. *D. magna* were appropriate to use in this project because they can address the research question as they are sensitive to changes in water chemistry. *D. magna* are invertebrates and lack a central nervous system, and as such, cannot feel pain, minimising their suffering. As a result of this, *D. magna* are regularly used in ecotoxicology studies and are bred as live fish food. Hence, *D. magna* were suited to use in this experiment.

Secondly, the condition of reduction was satisfied as the minimum number of animals were used to answer the scientific research question. In total, 150 organisms were used across 5 treatments, with each replicate containing 10 individuals. Extensive background research was carried out before the start of the project to avoid the wastage of animals, and effective experimental design and statistical analysis optimised the number of organisms.

Lastly, respect for the *D. magna* underpinned every decision and action involving their care and use, fulfilling the condition of refinement as their suffering was reduced. After delivery, the lid of the culture was loosened to allow for gas exchange. The culture was also kept at room temperature (20-25°C) and out of direct sunlight. The organisms may have experienced shipping shock, so were given 24 hours to recover and resume normal movement. Once daily during the experiment, the *D. magna* were fed 2 g algae pellets, maintaining their food supply. Natural spring water was used as opposed to demineralized water as it most closely simulates *D. magna's* natural environment of freshwater lentic habitats. The pipette tip was removed at a 45° angle so that the organisms were not harmed as they were transported between containers during the experiment. Lastly, after the experiment was completed, the surviving *D. magna* were returned to a freshwater tank in the aquarium they were purchased from.

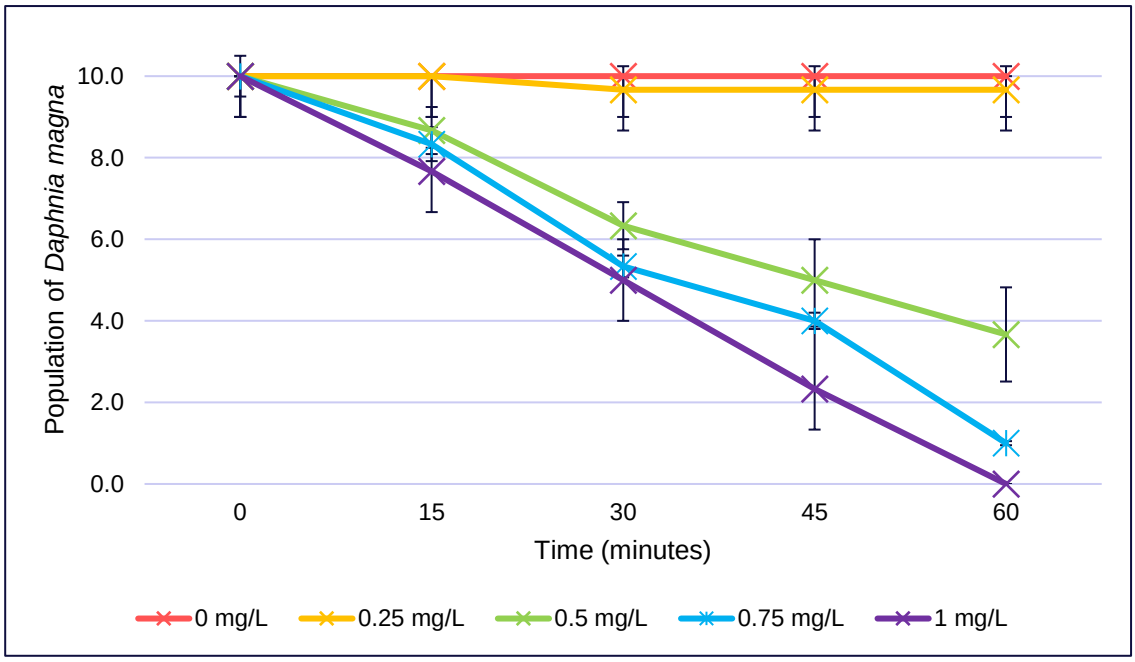
In conclusion, *D. magna* were appropriate to use in this project and during and after the research, the organisms' well-being was supported in several ways. Hence, for these reasons, it was ethical to use the animal *D. magna* in this project.

RESULTS

Summary

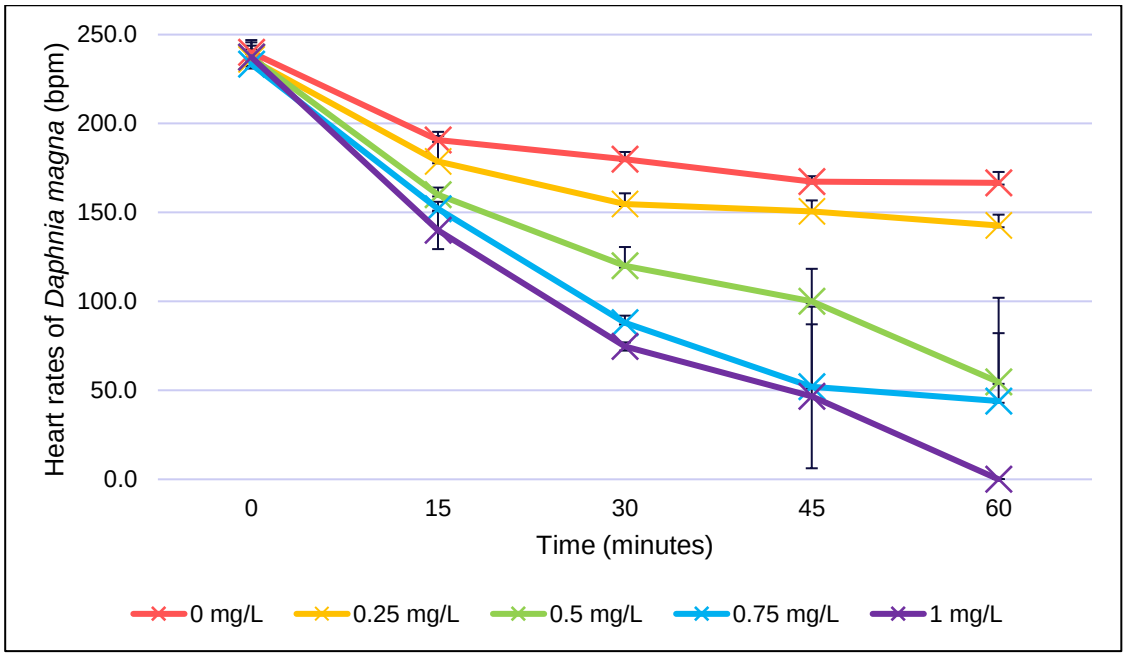
The results obtained in this investigation demonstrated that there was a difference between the effect of different concentrations of nanosilver on the population numbers (survival) and heart rates (metabolic integrity) of *D. magna*. As the concentration of nanosilver increased, the population of *D. magna* decreased (Graph A.1) and their heart rates decreased (Graph B.1). A two-step procedure was used to conduct statistical analyses of the data: a one-way analysis of variance (ANOVA), followed by post-hoc analysis using the Tukey HSD Test (a Multiple Comparison Test). The results indicated that the minimum concentration of nanosilver which causes toxicity for *D. magna* is in the range of 0.26–0.50 mg/L of nanosilver.

Graph A.1 – Average Population of *Daphnia magna* over one hour at different nanosilver concentrations



Note: the error bars on the graph represent the standard deviation

Graph B.1 – Average Heart Rate of *Daphnia magna* over one hour at different nanosilver concentrations



Note: the error bars on the graph represent the standard deviation

Table A.2 – Results of ANOVA Test on *Daphnia magna* Population after 60 Minutes of Exposure to Different Nanosilver Concentrations

Value	Result
P-value	1.66E-08
Significance level (α)	0.05
F value	126
F critical value	3.48

Table B.2 – Results of ANOVA Test on *Daphnia magna* Heart Rates after 60 Minutes of Exposure to Different Nanosilver Concentrations

Value	Result
P-value	0.0001
Significance level (α)	0.05
F value	19.6
F critical value	3.48

Table A.3 – Results of Tukey Test on *Daphnia magna* Population after 60 Minutes of exposure to nanosilver concentrations

Treatments pair		Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inference
A vs B	0.00 mg/L vs 0.25 mg/L	0.7906	0.8999947	insignificant
A vs C	0.00 mg/L vs 0.50 mg/L	15.0208	0.0010053	** p<0.01
A vs D	0.00 mg/L vs 0.75 mg/L	21.3454	0.0010053	** p<0.01
A vs E	0.00 mg/L vs 1.00 mg/L	23.7171	0.0010053	** p<0.01
B vs C	0.25 mg/L vs 0.50 mg/L	14.2302	0.0010053	** p<0.01
B vs D	0.25 mg/L vs 0.75 mg/L	20.5548	0.0010053	** p<0.01
B vs E	0.25 mg/L vs 1.00 mg/L	22.9265	0.0010053	** p<0.01
C vs D	0.50 mg/L vs 0.75 mg/L	6.3246	0.0082052	** p<0.01
C vs E	0.50 mg/L vs 1.00 mg/L	8.6963	0.0010053	** p<0.01
D vs E	0.75 mg/L vs 1.00 mg/L	2.3717	0.4876193	insignificant

Table B.3 – Results of Tukey Test on *Daphnia magna* Heart Rates after 60 Minutes exposure to different concentrations of nanosilver

Treatments pair		Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inference
A vs B	0.00 mg/L vs 0.25 mg/L	1.5127	0.7996027	insignificant
A vs C	0.00 mg/L vs 0.50 mg/L	7.0591	0.0038482	** p<0.01
A vs D	0.00 mg/L vs 0.75 mg/L	7.7314	0.0019779	** p<0.01
A vs E	0.00 mg/L vs 1.00 mg/L	10.5046	0.0010053	** p<0.01
B vs C	0.25 mg/L vs 0.50 mg/L	5.5464	0.0188532	* p<0.05
B vs D	0.25 mg/L vs 0.75 mg/L	6.2187	0.0091746	** p<0.01
B vs E	0.25 mg/L vs 1.00 mg/L	8.9919	0.0010053	** p<0.01
C vs D	0.50 mg/L vs 0.75 mg/L	0.6723	0.8999947	insignificant
C vs E	0.50 mg/L vs 1.00 mg/L	3.4455	0.1825712	insignificant
D vs E	0.75 mg/L vs 1.00 mg/L	2.7732	0.3489148	insignificant

DISCUSSION

Population Count

A relationship between nanosilver concentrations and population of *D. magna* was observed. In the 0.00 mg/L nanosilver treatment, the population remained constant at 10 *D. magna* individuals for the experiment's duration. In contrast, there were nearly 3 times less individuals in the 0.50 mg/L treatment than the control after 1 hour and 5 times less individuals in the 0.75 mg/L treatment (Graph A.1). Thus, all concentrations above 0.25 mg/L had a detrimental effect on the survival of *D. magna* after only 1 hour of exposure. This indicates the treatments had different effects on the population count, supported by an ANOVA test that found a statistical difference between the treatments at 60 minutes as the p-value is less than the 0.05 significance level (Table A.2).

These results are supported by scientific literature which shows nanosilver has detrimental effects on populations of *D. magna* as it putatively interrupts biological processes, resulting in cellular death (Qing et al., 2018). As higher concentrations of nanosilver contain greater numbers of silver ions, the key mode of nanosilver toxicity, it follows that these higher concentrations have a greater impact on *D. magna* populations (Luoma, 2008).

The greatest decrease in population was observed in the 1.00 mg/L nanosilver treatment as all of the organisms died, followed by the 0.75 mg/L and 0.50 mg/L concentrations (Graph A.1). Post-hoc analysis found there was no significant difference between 0.00 mg/L and 0.25 mg/L, and 0.75 mg/L and 1.00 mg/L suspensions as $p>0.05$ (Table A.3). This indicates that there is no difference in the effect of nanosilver on the metabolism of *D. magna* at 0.000 mg/L and 0.25 mg/L concentrations. However, there was a difference between 0.25 mg/L and 0.50 mg/L suspensions ($p\leq0.05$) (Table A.3), indicating that *D. magna* at these concentrations were detrimentally affected in treatments greater than 0.25 mg/L of nanosilver.

Thus, this provides evidence against the null hypothesis that there is no difference between the tested concentrations of nanosilver.

Heart Rates

A relationship between nanosilver concentrations and heart rates of *D. magna* was observed. Initially, the *D. magna's* heart rates were high at 224–244 bpm (Graph B.1). This was consistent with the scientific literature that heart rates are increased by stress, which can occur when moved into a new environment (Cornell University, 2009). By 30 minutes, heart rates had decreased from the initially high level. In the control treatment, heart rates stabilised around 160–180 bpm, inside the homeostasis range for *D. magna*, and for the experiment remained consistently in this range (Graph B.1). In the 0.25 mg/L treatment, heart rates were slightly lower at 150–160 bpm. The nanosilver concentration of 1.00 mg/L had the most detrimental effect on the heart rates of *D. magna*, followed by the 0.75 mg/L and 0.50 mg/L treatments, as in all cases there was a significant drop ($p<0.05$) in bpm compared to the control (Graph B.1). This implied that *D. magna's* metabolic rate lowered, indicating the organisms were dying (*Amundsen et al*., 2015). The treatments had different effects on heart rates, supported by the ANOVA test that found a statistical difference between the treatments at 60 minutes as the p-value was 0.0001 ($p<0.05$) (Table B.2).

These results are in line with the scientific literature that states that silver ions cause toxicity in cells as they putatively disrupt biological processes (Qing *et al.*, 2018). Hence, as the concentration of nanosilver increased, so too did its detrimental impact on heart rates due to the greater number of silver ions that cause toxicity.

Post-hoc analysis of the heart rates of *D. magna* using the Tukey Test found most treatments were statistically different from the control. There was no difference between the control and the 0.25 mg/L treatment, 0.50 mg/L and 0.75 mg/L, 0.50 mg/L and 1.00 mg/L, and 0.75 mg/L and 1.00 mg/L suspensions. This was supported by a stringent post hoc test ($p<0.01$) (Table B.3). These heart rate values are concordant with those for population as they demonstrate there was no statistical difference between 0.00 mg/L and 0.25 mg/L suspensions, but there was a significant difference between 0.25 mg/L and 0.50 mg/L. While some results show a large standard deviation (as shown by error bars in Graph B.1), this is because the 0 bpm heart rates were included.

This provides evidence against the null hypothesis that there is no difference between the tested concentrations of nanosilver.

Identification of Threshold Level

Based on the statistical analysis for both population count and heart rates, there is no difference between the control and the 0.25 mg/L treatments (Table A.3, Table B.3). Yet, there is a significant difference between the 0.25 mg/L and 0.50 mg/L treatments ($p<0.05$), indicating that the threshold level for nanosilver is greater than 0.25 mg/L but less than 0.50 mg/L. Hence, the research question has been answered to the extent that is possible within the experimental limitations as the minimum concentration of nanosilver which causes toxicity to *D. magna* lies within the range of 0.26–0.50 mg/L.

The results demonstrate that at a certain concentration above 0.25 mg/L, nanosilver is toxic to *D. magna* and potentially other freshwater organisms. As nanosilver releases into the environment continue from clothing, industry, cosmetics and disinfectants, it is important that nanosilver is regulated based on a threshold level developed specifically for it as current regulatory thresholds are only applicable for macro forms of silver. Thus, to prevent potential toxicity occurring in living organisms, nanosilver concentrations in the environment must be monitored so that they do not exceed their threshold level of 0.26–0.50 mg/L.

Sources of Error

D. magna heart rates were measured sequentially, not in parallel, as only one microscope was available at the time of experimentation. This meant that measurements may not be indicative of true heart rates at the time recorded, compromising accuracy.

Secondly, the health of the *D. magna* prior to the experiment was unable to be determined, so some organisms included in the experiment may have already been weak. In 0.25 mg/L suspensions, only 1 organism died across the three replicates, indicating it was possible the organism was unwell before the experiment. To attempt to address this, 10 *D. magna* were used in each replicate, the greatest number that could be used due to limitations on time and resources.

Thirdly, preliminary experiments (Cummins, 2019) showed nanosilver concentrations of 0.00 mg/L, 1.00 mg/L, 2.00 mg/L, 3.00 mg/L, 4.00 mg/L and 5.00 mg/L were statistically insignificant as within 60 minutes, all *D. magna* died (Graph A.1). Lower nanosilver concentrations were used in the main study, producing results addressing the aim and making the experiment valid. However, due to limitations on time and resources, the experiment with lower concentrations could only be carried out once with 3 replicates, rather than 5 or more times which would have been preferable.

Fourthly, it was difficult to keep *D. magna* from moving on the slides to accurately determine their heart rates. To address this, a pipette was used to remove excess water to limit movement. This may have temporarily placed stress on the organisms and contributed to changes in their heart rates.

Fifthly, an assumption was made that the 50 mg/L concentration as labelled was the true concentration of the colloidal solution and that the size of nanoparticles was uniform. Also, while the solution was swirled before use, it was unable to be ascertained if the nanoparticles were evenly distributed.

Finally, the heart rates were too fast to count in real time under a microscope. To address this, heart rates were recorded digitally and then carefully calculated later in slow motion. This improved the accuracy of results, reducing the risk of error and improving validity of the method.

Applications of Results

In a scientific context, the results provide a minimum concentration of nanosilver which is not harmful to aquatic organisms. This minimum concentration level can be used as an indicator of water quality in terms of nanosilver pollution, thus ensuring that healthy aquatic ecosystems can be maintained. This threshold level can be used both in environmental monitoring by local and national government agencies, as well by industries to ensure that their waste output will not cause nanosilver levels to exceed their safe threshold level of 0.26–0.50 mg/L.

Furthermore, the NSW Environmental Protection Authority can use this threshold level to propose that a new level for nanosilver, separate to bulk silver, is required in order to regulate nanosilver levels in the environment.

Hence, this project contributes to improving water quality in the environment as it defines the threshold level for nanosilver as 0.26–0.50 mg/L, underlining how a healthy aquatic ecosystem can exist when nanosilver levels are below this threshold level.

CONCLUSIONS

Conclusion 1: This study has found that as the concentration of nanosilver in freshwater increased, the population and heart rates of *D. magna* significantly decreased over a short period of one hour.

Conclusion 2: The minimum concentration of nanosilver which causes toxicity to *D. magna* is within the range of 0.26–0.50 mg/L. This has important environmental implications as currently the regulatory toxicity threshold for nanosilver is well above this concentration range.

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