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The Effect of Nanosilver on *Nymphaeaceae* (Water Lily) Germination and Growth

Research Article

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Abstract: Silver nanoparticles, also known as nanosilver, are nanoparticles of silver ranging from 1 to 100 nm in size. Nanosilver is often used in consumer products for their anti-bacterial properties, and stands to be a threat to the environment -especially the aquatic environment- through pollution. Such pollutants constitute a major threat to the interdependent aquatic ecosystems, which is already in need of expeditious attention and treatment from other pollutants. However, due to inconclusive or conflicting findings from prior studies, it is hard to conclude the possible ramifications that nanosilver may have on the aquatic environment. Hence, the purpose of this study was to examine the germination and growth of water lilies when exposed to nanosilver. Two trials were done to test the hypothesis. Concentrations of 0, 5, 10, and 15 ppm of nanosilver were used. Nanosilver's effect on water lilies varied depending on the concentration of nanosilver present. Nanosilver mainly helped water lily growth in small amounts (5 to 10 ppm) in long-term exposure, while higher concentrations (15 ppm) seemed to harm overall growth at any level of exposure.

Keywords: Nanosilver • Water Lilies • Contamination

1. Introduction

1.1. Nanosilver

Silver nanoparticles (AgNP) are parts of silver which are in the range of 1 and 100 nm in size. Over the years, nanosilver has found its way into many consumer products due to its wide range of use (with an estimated 250-312 tons being used worldwide in 2013) [1] Since the beginning of the twenty-first century, nanosilver has gained vast popularity due to its antimicrobial properties and extremely large surface area, which facilitates better contact with microorganisms to kill them. Nanosilver can also be found in hospital equipment and medicine, and

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is most commonly used in diabetes related treatment [1, 2]. It has also gained wide popularity in agriculture, as well as in common daily items such as toothpaste, detergents, plastics, textiles, keyboards, paint, and many more [1]. In addition, The United States Environmental Protection Agency (EPA) has even approved the use of nanosilver in pools and disinfectants. The Food and Drug Administration (FDA) has also approved the usage of "SilvrSTAT", which is an antibacterial wound dressing gel containing nanosilver [1]. Due to its heavy uses in various commodities as well as places, its essence is inevitably found in various polluted areas, especially in bodies of water. The majority of water pollutants originate from waste that is not properly recycled or neutralized. The exact environmental impacts of nanosilver have not been fully explored/investigated yet. With no valid conclusion(s) made within the scientific community, no effective action(s) can be taken for/against it. In addition, if future investigations are done and nanosilver is found to have detrimental effects on the environment, it would be hard for not only the public, but also the government to understand and resolve the issues related to it at a fast rate, prominently due to its vast commercial uses and prior approved uses [3]. Because of these reasons, it will be important to 1) research about the possible environmental impacts of nanosilver 2) raise more awareness among the people about its uses and potential detriments and 3) urge recycling as well as proper waste deposition.

1.2. Water Lilies

Nymphaeaceae is the family name for water lilies, which are aquatic plants that are capable of growing multiple stems and pads, as well as flowers that typically appear one to two seasons after they are planted [4]. Water lilies grow long, sturdy stems and typically grow in ponds and lakes [4, 5]. Water lilies provide food for fish and other wildlife. In addition, birds belonging to the Jacanidae family are known to live on top of the water lily pads, and use them as temporary habitats [6]. Water lilies also hold high cultural importance in many Asian cultures and are ornamental plants. In Bangladesh, water lilies are their national flower, and symbolize love and life and are also used in religious ceremonies [5]. In China, water lilies are known to be used as flowers at weddings and symbolize a long lasting marriage between couples. Furthermore, water lilies are also known to have positive connotations of new wishes and happiness [7]. Water lilies are also used to treat kidney pain, congestion and sore throats. The rhizome of water lilies help alleviate pain and have sedative properties [8]. In addition, water lilies are one of the prominent cut flowers on the international markets.

1.3. Prior studies

The effects of nanosilver have been found to depend on various factors such as its size, concentration, plant species, and ion formation [9, 10]. Prior studies have shown that nanosilver reduces the rate of cell growth, photosynthesis, as well as chlorophyll production in freshwater algae at 0.5 mM, 1.0 mM, 3.0 mM, and 5.0 mM [11]. The nanosilver treated algae also showed signs of cell wall and DNA damage, as the metaphase stage

of the cell seemed to be disturbed during mitosis [11, 12]. On the other hand, when the germination, root growth, and shoot length of castor beans were tested using nanosilver, no effects on those were seen, even at high concentrations of 400 mg/L. But AgNO3 (silver nitrate) treated castor beans at the same concentration displayed slower germination rates. [13] The nanosilver might have accelerated seed coat breakage, which allowed the castor bean seeds to germinate faster by allowing more nutrients to be taken in [9]. Similar results have also been found with the germination of pearl millets, as the group with the higher concentration (50 mg/L) had faster germination rates than those with lower concentration (20mg/L) and control. However, when measuring the growth of the nanosilver treated pearl millets, the shoot and root lengths decreased as the concentration of the nanosilver increased [12]. In contrast to the findings in the pearl millet study, another study that used the common bean and corn plants found an increase in the shoot and root lengths when used at 60 ppm. However, any concentration that was higher than 60 ppm showed a decrease in both shoot and root lengths [14]. Another study examined the prolongation of the vase life of cut lilies when treated with 5, 15, 25 and 30 ppm of nanosilver solution. And according to the data, the nanosilver solution helped to preserve the freshness of the water lilies by eliminating any bacterial contaminants [8]. While this experiment looked at the effects of nanosilver on water lilies, it was limited to only the preservation of the water lilies, not the growth or germination of the plants.

Our experiment tested the growth and germination of the water lilies when treated with nanosilver. To our knowledge, there had not been any papers published about the effect of nanosilver on germination of water lily seeds and plant growth, which was why this experiment.

1.4. Rationale

There have been no prior studies done that test the effects of nanosilver on the growth and germination of water lilies. As a result, in this research, we will be investigating how nanosilver affects *Nymphaeaceae* (water lilies), which are one of the most well-known aquatic plants. Water lilies are also known to be used to treat kidney pain, congestion, and sore throats. The rhizome of water lilies also helps alleviate pain and has sedative properties [8]. In addition, water lilies are one of the prominent cut flowers sold on the international markets. Water lilies can also be used as temporary habitats for birds [6]. Water lilies hold significance in many Asian cultures, and are eaten in many Asian dishes [5, 7].

2. Materials

The water lily seeds for both trial 1 and 2 were bought online from the seller “Golden Autumn Farm” from Amazon.com. The seller did not have any information on the exact species of their water lilies in their description, and stated that they could not provide any further info when contacted. The nanosilver bottles for trial 1 were brought from the seller “Nano Silver” who sells multiple nanosilver related products on Amazon.com. Unfortunately, nanosilver could not be bought from the same seller for trial 2. Instead, extra bottles of nanosilver left from trial 1 were used along with a new bottle of nanosilver from the seller “Holistic Pet Care”. on Amazon.com.

In order to avoid variance from using nanosilver from two different sellers, equal amounts of nanosilver from both bottles of trial 1 were mixed with the nanosilver from the bottles of trial 2 (second brand) and used in trial 2.

3. Methods

3.1. Experimental setup

Table 1. **Nanosilver Concentration Preparation (Trial 1)**

Group Number	Nanosilver Concentration (ppm)	Volume of 20 ppm Nanosilver (mL)	Volume of Water (mL)	Label
Group 1 (Control)	0	0	800	NP-I-01 to NP-I-10
Group 2 (Experimental)	10	400	400	NP-I-11 to NP-I-20

Table 2. **Nanosilver Concentration Preparation (Trial 2)**

Group Number	Nanosilver Concentration (ppm)	Volume of 20 ppm Nanosilver (mL)	Volume of Water (mL)	Label
Group 1 (Control)	0	0	600	NP-II-01 to NP-I-10
Group 2 (Experimental)	5	150	450	NP-II-11 to NP-II-20
Group 3 (Experimental)	15	450	150	NP-II-21 to NP-II-30

Before starting the experiment, all water lily seeds were placed in water to ensure that they would germinate (floating of seeds signal death or infertility of the seeds). Then using sandpaper, all 20 water lily seeds for trial 1 and 30 water lily seeds for trial 2 were scored by filing the hard outer shell of the seeds. The seeds were scored (filed down) from their flat sides (opposite from where the embryonic plants' radical/roots are) to avoid harming the plants in the seed. Scoring the seeds allows the water lilies to grow much faster as it is easier for the embryotic plant to push themselves out (water lily seeds usually have a very hard outer shell). Next, all water lilies were put under running tap water to remove excess debris. All the containers for both control group and experimental group were then labeled for both trials. For the control group in trial 1, a 1000 mL beaker was filled with 800 mL of tap water before it was transferred into a container. This was done for all 10 of the control group containers in trial 1. For the 10 ppm experimental group in trial 1, a 1000 mL beaker was filled with 400 mL of tap water and 400 mL of 20 ppm nanosilver before it was transferred into a container. This was done for all 10 experimental containers. One seed was placed into each the 20 containers (see Table 1).

For trial 2, 5 containers were used for each group and 2 seeds were put into each container with a plastic divider separating the seeds. For the control group of trial 2, 600mL of tap water was measured using a graduated cylinder before it was poured into a control group container. This was done for all 5 containers of the control group. For the 5 ppm experimental group, a 1000mL graduated cylinder was used to measure 450mL of tap water before it was poured into a 5 ppm group container. The same graduated cylinder was then used to measure 75mL of the first brand of nanosilver and 75mL of the second brand of nanosilver. The total 150mL solution of nanosilver was then transferred into the same 5 ppm container. This was done for all containers of the 5 ppm group. For the 15 ppm experimental group, the same 1000mL graduated cylinder was used to measure 150 mL of tap water before it was transferred into a 15 ppm container. The same beaker was then used to measure 225mL of the first brand of nanosilver and then 225mL of the second brand of nanosilver. The total 450mL nanosilver

solution was then transferred into the same 15 ppm container. This was done for all the containers of the 15 ppm group (see Table 2).

For both trials, all containers were placed near the same window and all of the containers were rotated clockwise (Figure 1, obtained from Dr. Blackwell in Francis Lewis High School's Science Research program). All the containers also had their water levels marked from the beginning of the study, so that the water levels could be regulated. All the containers were placed inside a cage to prevent anything from getting into the containers.

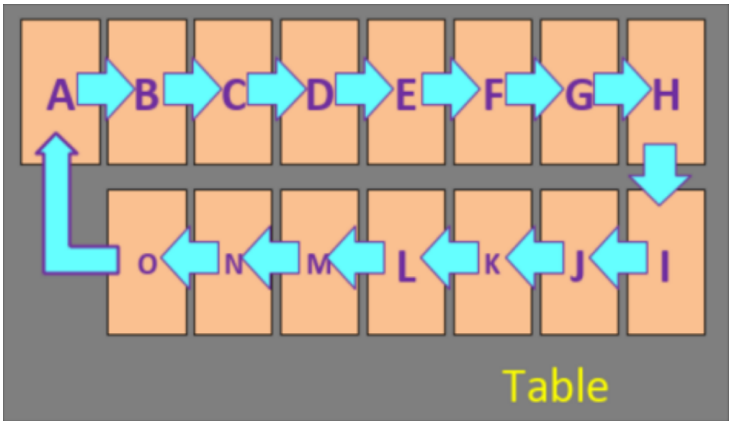

Figure 1. **Example of daily rotation used. Retrieved from Dr. Blackwell in the Francis Lewis High School's Science Research program**

3.2. Experimental Procedure

The plants were measured daily (excluding weekends and other holidays during the experiment) with a ruler or meter stick and data were recorded using Microsoft Excel. Germination was recorded by looking for a sprout in the seed. Stem lengths were measured from the very bottom of the stem that protruded out from the seed to the top the stem, excluding the pads attached at the top. Stems were straightened during measuring to ensure that their full lengths were measured. Pad lengths were measured when they were visible enough to measure. The very bottom to the very top of the pads were measured. When the pads fully unfolded, the horizontal diameter of the pads were measured. The same experimental procedures were followed for both trials.

3.3. Data Analysis

All data for both trials were analyzed/calculated in Microsoft Excel. All graphs (Figures 3-18) were also made on Excel. Averages were found using the average function on Excel. Error bars on graphs were made using the standard deviation function, and were used to show the variance in the data. All data for trial 1 had a two-tailed unpaired, unequal variance (type 3 on Excel) t-test performed to test significance. All data for trial 2 had Analysis of Variance (ANOVA) done, followed by Turkey HSD to test the significance of the data. The three different levels of significance used in this experiment were *$P<0.05$, **$P<0.01$, ***$P<0.001$. The sprouting ratio

was determined using the formula shown below (Equation 1) and graphed using Excel. The same procedures except the level of significance were followed for analyzing the data of both trials. The formula was obtained from the Science Research program at Francis Lewis High School's Plant Lab.

$$S_g = \frac{n_{s,g}}{n_g} \tag{1}$$

In Equation 1, S_g is the sprouting ratio, $n_{s,g}$ is the number of plants that sprouted, and n_g is the total amount of plants.

4. Results

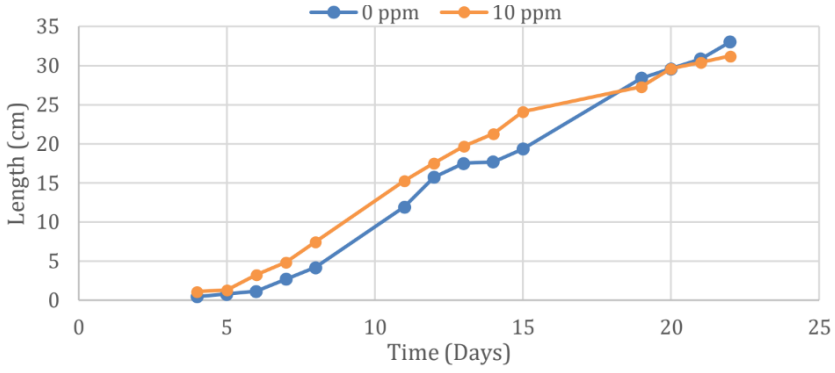

Figure 2. **Average stem growth of stem #1 for both 0 ppm and 10 ppm groups in cm. Both groups had 10 samples and each group was given either 0 ppm or 10 ppm of nanosilver.**

Figure 1 shows that the 10 ppm group began to decrease in growth compared to the control group after day 20, implying that at a there is a negative effect on stem growth with long-term exposure.

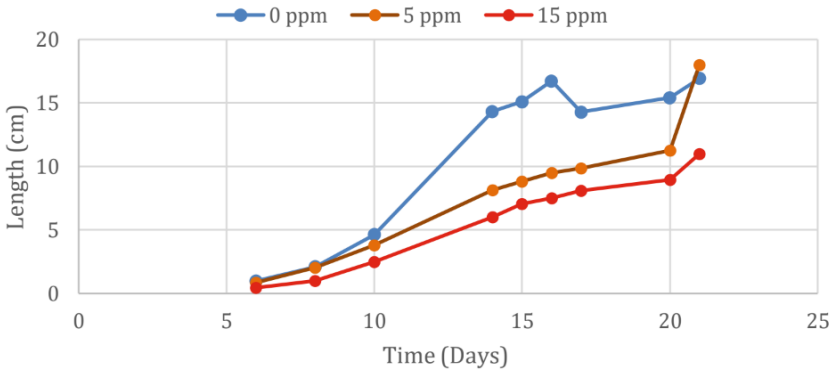

Figure 3. **Average stem growth of stem #1 for the 0 ppm, 5 ppm group, and 15 ppm group, measured in cm. All three groups had 10 samples and were given a 0 ppm, 5 ppm, or 15 ppm concentration of nanosilver.**

The dip in the data for the control group in Figure 3 between days 15 and days 20 is because a very long stem in the control group died. The 5 ppm concentration grew less than the control group did but more than the 15 ppm group did. Its growth did spike during days 20 to 21 and it surpasses the control, implying that a lower concentration of nanosilver may help in long term exposure. 15 ppm grew very little compared to both the 0 ppm and 5 ppm, which implies that 15 ppm may be harmful to the stem growth of water lilies.

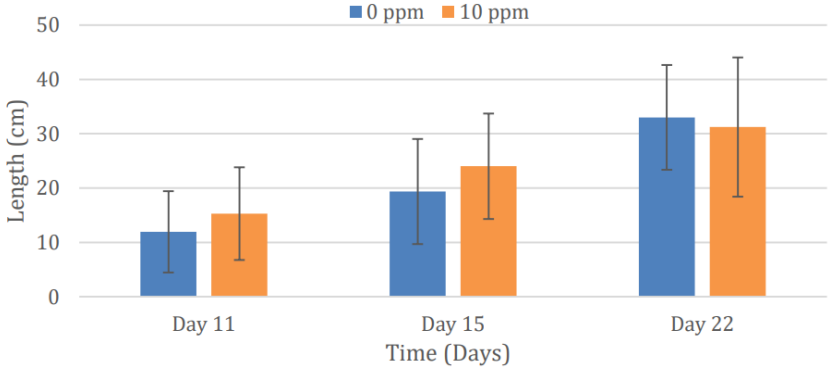

Figure 4. **Average stem growth of stem #1 for both 0 ppm and 10 ppm for days 11, 15, and 22, measured in cm. Both groups had 10 samples and each group was given either 0 ppm or 10 ppm of nanosilver. No significance was found using a two-tailed, unpaired t- test. Error bars represent standard deviation (SD).**

Again, you can see in Figure 4 that days 11 and 15 show a higher stem length in the 10 ppm while day 22 shows a higher stem length for the control, implying a negative long term effect.

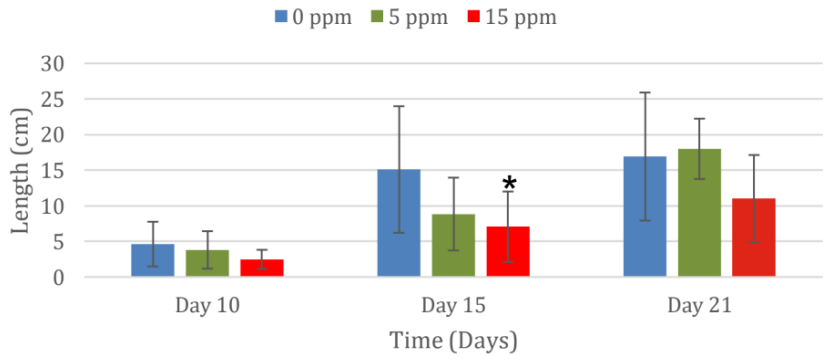

Figure 5. **Average stem growth of stem #1 for 0 ppm, 5 ppm, and 15 ppm during days 10, 15, and 21. Length was measured in cm. All groups had 10 samples each. Error bars represent (SD). ANOVA, followed by Turkey HSD from the website "astatsa.com" was done to find the level of significance between all three groups. Significance was found in day 15 between the 0 ppm group and the 15 ppm group (*p<0.05).**

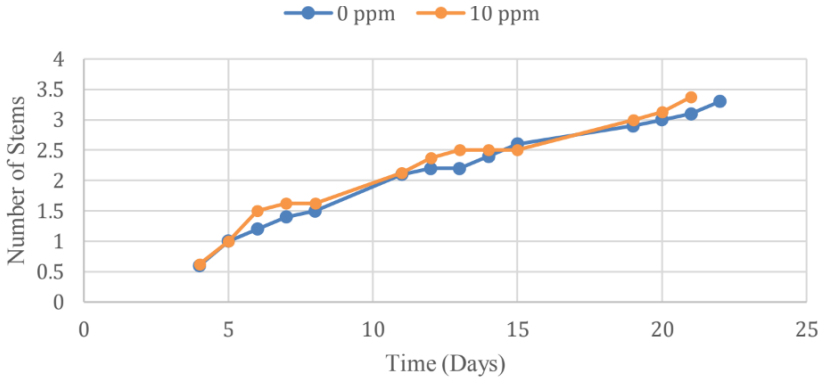

Figure 6. **Average number of stems grown for both groups 0 ppm and 10 ppm. Both groups had 10 samples and were treated with either 0 ppm or 10 ppm of nanosilver. Each group was given either 0 ppm or 10 ppm concentration of nanosilver.**

In Figure 6, days 5 to 8 and 11 to 15, showed an increase in the number of stems, while the control group had a general linear line on the graph. However, when looking towards the end of the experiment, the small spikes in the number of stems did not seem to affect the amount of stems in the end.

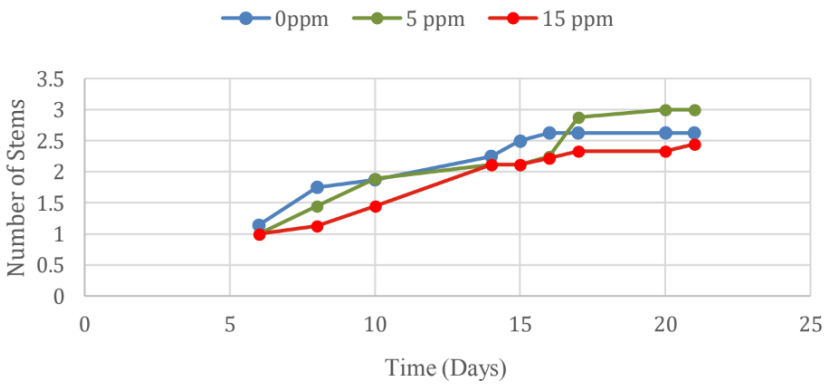

Figure 7. **Average number of stems for 0 ppm, 5 ppm, and 15 ppm. All groups had 10 samples each.**

In Figure 7, the 5 ppm also had another spike in its growth, which surpasses the 0 ppm group. This is a trend seen again throughout trial 2 for the 5 ppm group.

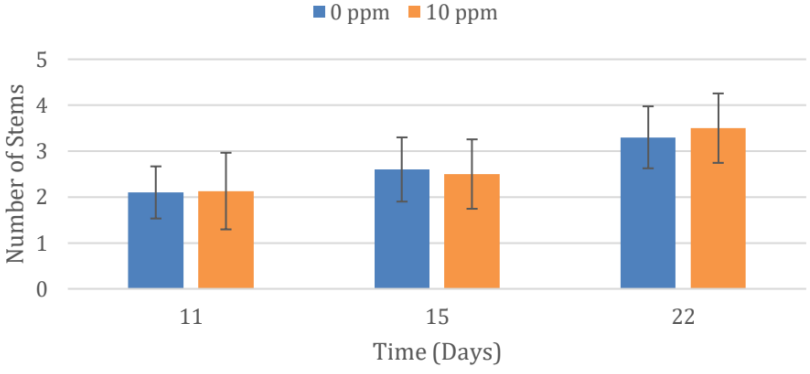

Figure 8. **Average number of stems grown for both groups 0 ppm and 10 ppm on days 11, 15, and 22. Both groups had 10 samples. Each group was given either 0 ppm or 10 ppm concentration of nanosilver. Error bars represent SD. Level of significance was found using a two-tailed, unpaired t-test. There was no significance found**

There was no major difference in the total number of stems for both groups, implying no major effect.

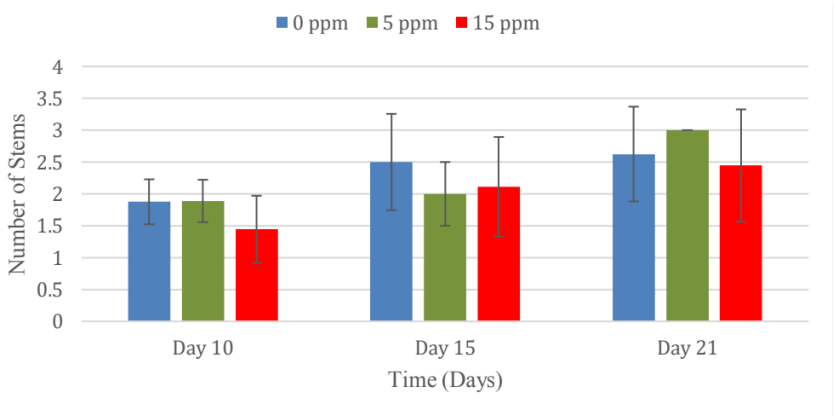

Figure 9. **Average number of stems for 0 ppm, 5 ppm, and 15 ppm groups on day 10, 15, and 21. Both groups had 10 samples each. Error bars represent SD. ANOVA, followed by Turkey HSD from the website "astatsa.com" was done to find the level of significance between all three groups. No significance was found for all days between all three groups. All seeds in the 5 ppm group had 3 stems by day 1, which is why there are no error bars. The 5 ppm group had a higher average number of stems than the control group did by day 21.**

Again, the 5 ppm group had a higher average number of stems than the control group as trial 2 went out, a trend also seen in Figure 7.

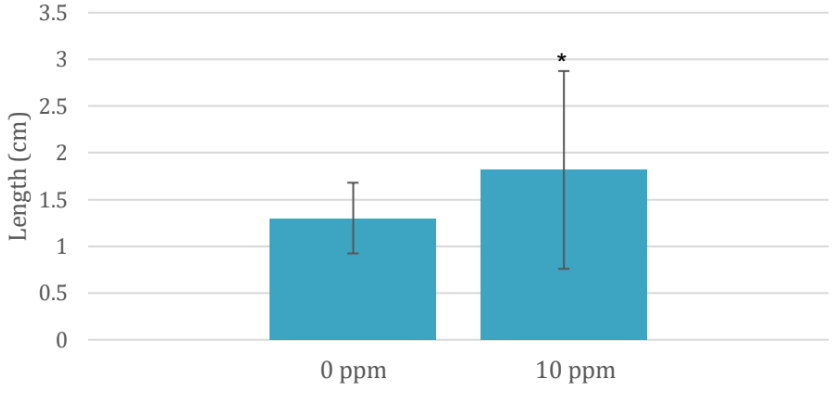

Figure 10. **Average pad length for both groups 0 ppm and 10 ppm. Measured in cm. Both groups had 10 samples each. A significance of *p<0.05 between both groups was found through an unpaired t-test. Error bars represent SD.**

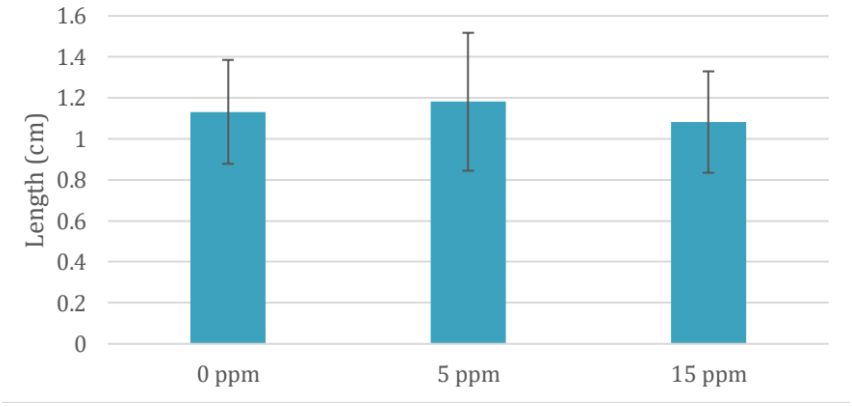

Figure 11. **Average pad length for 0 ppm, 5 ppm, and 15 ppm groups, measured in cm. Each group had 10 samples each. Error bars represent SD. ANOVA, followed by Turkey HSD from the website"astatsa.com" was conducted and no significance was found.**

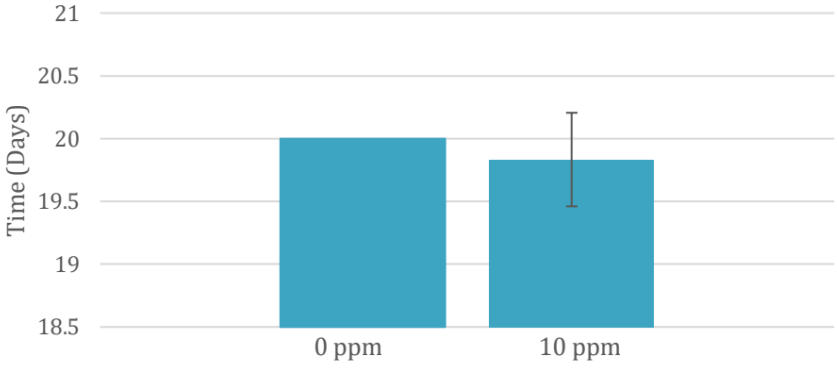

Figure 12. **Average pad unfolding time for both the 0 ppm and 10 ppm groups measured in days. Both groups had 10 samples. Pads first came out their stems folded half way vertically before eventually unfolding at some point during the experiment. The control group only have sample of data, so a t-test was unable to be conducted to test significance. SD could also not be done for the same reason, hence the lack of an error bar in the 0 ppm group.**

It is to be noted in Figure 12 that 10 ppm group had more (6 total) pads unfold during the experiment than the control did, which implies that nanosilver may affect the pads of water lilies more than their stems.

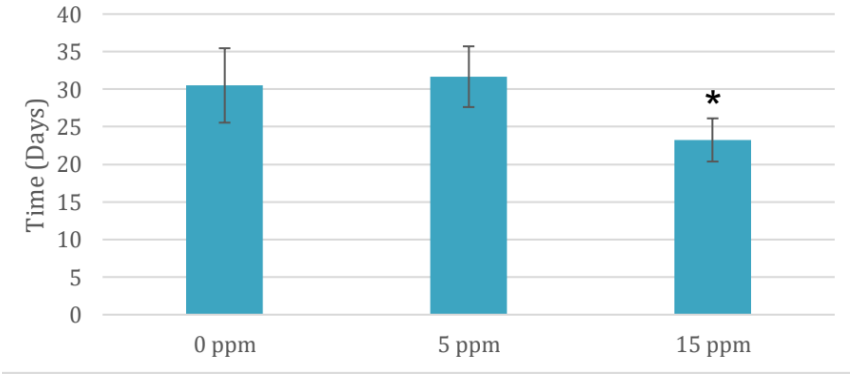

Figure 13. **Average pad unfolding time for the 0 ppm, 5 ppm and 15 ppm groups measured in days. Each group had 10 samples. ANOVA, followed by Turkey HSD from the website "astatsa.com" was done to find the level of significance between all three groups. A significant difference was found between the 0 ppm and 15 ppm group (*$p<0.05$). The 0 ppm had 2 pads unfolded while the 5 ppm had 3 pads unfolded and the 15 ppm had 4 pads unfolded.**

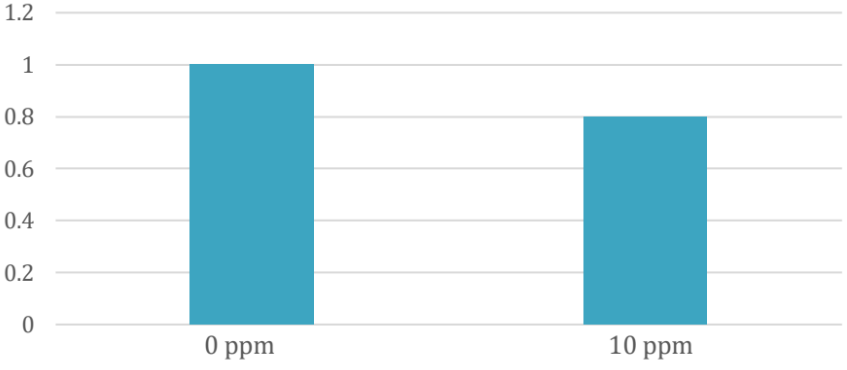

Figure 14. **The sprouting ratio of both the 0 ppm and 10 ppm groups. The sprouting ratio was done with a 0-1 scale, with 0 meaning no germination and 1 indicating that there was germination. Both groups had 10 samples. Each group was given either 0 ppm or 10 ppm concentration of nanosilver. Error bars represent SD. The entire control group germinated, which is why there are no error bars. A t-test was not performed because the sprouting ratio data only provided the numbers 1 and 0.**

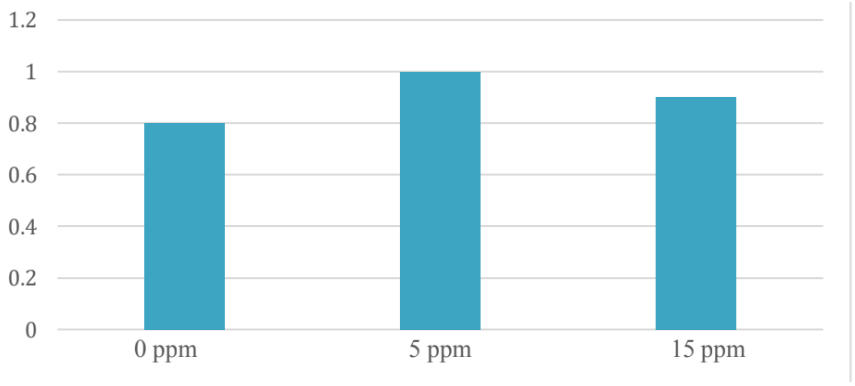

Figure 15. **The sprouting ratio of 0 ppm, 5 ppm, and 15 ppm groups. There were 10 samples in each group. The ratio was done on a 0-1 scale, with 0 meaning that the plant did not germinate and 1 meaning that the plant germinated. ANOVA and Turkey HSD were not performed because the sprouting ratio only provided the numbers 1 and 0.**

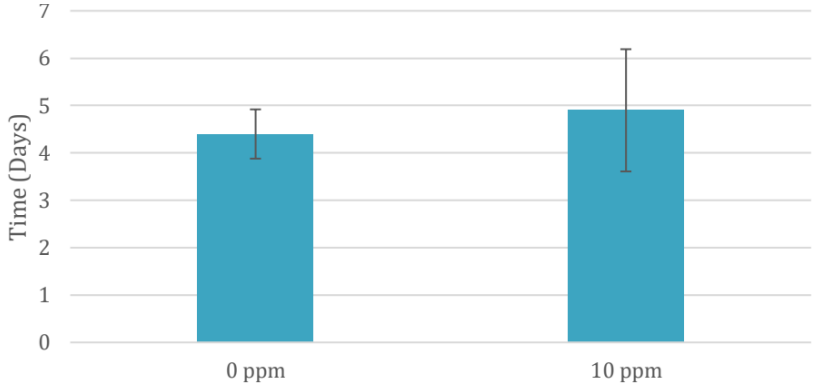

Figure 16. **Average sprouting time for both the 0 ppm and 10 ppm groups measured in days. Both groups had 10 samples. Error bars represent SD. A two-tailed, unpaired t-test was conducted but no significance was found. Error bars represent SD. The 0 ppm group germinated faster by around half a day.**

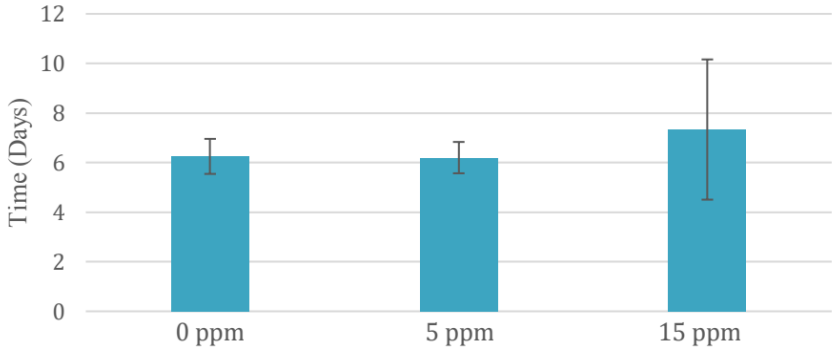

Figure 17. **Average sprouting time for the 0 ppm, 5 ppm, and 15 ppm groups. Time was measured in days Error bars represent SD. ANOVA, followed by Turkey HSD was conducted and no significance was found. The 15 ppm group germinated the latest while the 0 and 5 ppm groups germinated around the same time.**

5. Conclusion

The hypothesis for our experiment was that the nanosilver treated water lilies would show signs of decline in their germination time and stem and pad growth. Based on the data collected, the effects of nanosilver on the growth of water lilies, which includes if it's beneficial, detrimental or non-effective, depends on the concentration being used. Nanosilver was seen to decline the germination time of the water lily seeds at all the different concentrations used for this study (5 ppm, 10 ppm and 15 ppm) (Figures 16 & 17), which does not support past studies that concluded that nanosilver helps seed germination by quickening the seed coat breakage [9, 12]. For the sprouting ratio of the first trial, while all of seeds of the control group germinated, two seeds from the 10 ppm group did not germinate. And for trial 2, while 1 seed did not germinate from the 15 ppm group, two seeds did not germinate from the control group. The seeds from the 5 ppm group in trial 2 had all germinated, further implying that nanosilver may not have any effect (Figures 14 & 15).

The average stem length of the water lilies from the 10 ppm group was also slightly smaller (2 cm) than that of the control, despite the fact that for majority of the experiment, the nanosilver treated groups had higher stem lengths (Figures 2 & 4). In contrast, water lilies from the 5 ppm and 15 ppm groups both showed smaller stem length growth during the beginning of the second trial. But around day 20, the water lilies from the 5 ppm group showed a large spike in their stem length which surpassed the control group's stem lengths (Figures 3 & 5). However the stem length of water lilies from the 15 ppm group grew consistently smaller than that of the control group and 5 ppm group during trial 2. This trend implies that nanosilver might only have a negative effect on the plants' growth after a prolonged period of exposure, at a concentration of 10 ppm or higher and only helps the plant in short term exposure, with a higher concentration of 15 ppm harms the growth at any level of exposure. However, long term exposure of a concentration of 5 ppm, is helpful to the plant's growth.

When looking at the average number of stems, the 10 ppm group had their stems appear in waves at a

much faster rate. Through days 5-8 and 11-15, the 10 ppm group showed spikes/increase in their overall average number of stems while the control group had a generally linear line. Though, the 10 ppm group had no major difference in average number of stems by the end of the experiment. (Figures 6 & 8). In the 5 ppm, there was only one big spike in their average number of stems near the end of experiment (Figure 7), before it surpassed both the control group's average and the 15 ppm group's average (Figure 9). This again indicates that a smaller concentration of nanosilver may help the growth of the water lilies in long term exposure. The 15 ppm treated water lilies showed no spikes in their number of stems like the group with 5 ppm or 10 ppm experienced, and instead showed a consistently lower number of stems than the 5 ppm and control group in trial 2 (Figure 7 & 9). This signifies that much like the stem lengths of the plants, a short term exposure to 10 ppm helps the plants' growth, a long term exposure to 5 ppm helps the plants' growth, and 15 ppm harms the plants' growth at any level of exposure.

For the pad lengths, when compared to the control group, the average length of the pads for the 10 ppm group were bigger by nearly 1 cm (Figure 10). However, the water lilies from 5 ppm and 15 ppm group did not show any big difference in their pad lengths when compared to the control group (Figure 11).

The water lilies from the 10 ppm group also had more pads unfold during the experiment (6 unfolded pads in the 10 ppm group and 1 unfolded pad in the control). This was seen again in trial 2, with 3 pads unfolding in 5ppm, 4 pads unfolding in 15 ppm, and 2 pads unfolding in the control.

In addition to the average pad lengths, average pad unfolding time was also recorded. The pads from the 10 ppm group took slightly faster to unfold than the control group did (though, significance was not able to tested because the control group only had one unfolded pad) (Figure 12). For the 5 ppm group, the pads surprisingly took nearly the same amount of time as the control group did, which does not align with the stem length and number data seen throughout the experiment. However, in the 15 ppm group, the pads took significantly ($*p<0.05$) less time than the control to unfold (Figure 13).

The differences in the pad data (Length, pad amount, and unfolding time) for both trials 1 and 2 are interesting when contrasting them with data of the stems. This bigger difference in the pads of the water lilies imply that while nanosilver may not have any major significant effect on water lily stems, they affect the pads' unfolding frequency, the pads' pace of unfolding, and the pads' lengths.

As aforementioned, the t-test performed on the data in trial 1 showed that the average stem length and average number of stem were not significant at any level. Data that was found to be significant was the pad lengths of both groups. In addition, in trial 2, all data except the pad unfolding time and stem lengths between the control group and the 15 ppm group were found to also be insignificant by ANOVA and Turkey HSD. The difference between the time taken for the 0 ppm group to unfold and the 15 pp group to unfold had a p-value of $*p<0.05$, and the stem lengths of the 0 ppm group and 15 ppm group on day 15 had a p-value of $*p<0.05$. So, while our data show some difference in certain areas (number of stems, stem length, etc.), it is not statistically significant. The lack of significance in the data may be because of the errors made while conducting the experiment.

In addition, the sample size was small, which might have affected the p-value as well.

6. Discussion

The experiment had some sources of error. There were days when the experimenters could not check on the plants to measure them or rotate them to ensure the uniform distribution of sunlight among the water lilies. In such cases, some water lilies might have been in a more favorable position to grow/germinate than others as they received more sun light than the other plants. Constant and frequent visits should be done for future trials to regulate the rotating process and maintain the correct water level marked on the containers. Another error was that multiple stems and pads of water lilies from both the control and experimental group dried up, rotted, or ripped during the experiment, and because of that, our data was more susceptible to outliers. In addition, in trial 1, 2 seeds from the 10 ppm sample did not germinate and for trial 2, 2 seeds from the control group and 1 seed from the 15 ppm group did not germinate. Upon terminating the experiments and examining the seeds, it was seen that most of the seeds had rotted (they had a black cotyledon), while others most likely experienced seed dormancy (a condition where a plant seems its conditions unfavorable for growth), as they did not show any signs of irregularities. Some plants also grew multiple stems of the same or similar length, which made it difficult for the experimenters to keep track of the correct measurement of a certain stem (for example, the experimenter might measure stem 2 as stem 1). The data also had only 10 seeds in each group for each trial, which made it more susceptible to errors and bias. Some of the water lily sprouts were growing out of their containers and interacting with the outside environment more than others, which may have also affected their growth. To prevent this from occurring again, future trials should have a larger sample size as well as larger containers. Future research includes comparing the effects of nanosilver on Nymphaeaceae with more varieties of concentrations, with all the errors made in this experiment taken into consideration to minimalize any chance of additional errors in the experiment and data. In addition, for more authentic conclusions, the effects of nanosilver should also be tested with other aquatic plant species.

Conflict of Interest

There are no conflicts of interest for this study.

Human Studies/Informed Consent

This study does not involve human participants. No human studies were conducted for this article.

Animal Studies

This study does not involve animals. No animal studies were conducted for this article.

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