

The Effect of ZnO Nanoparticles on *Arabidopsis* Growth in Elevated CO₂

Independent Research Thesis

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Abstract

The increase in greenhouse gases which causes global warming can also lead to a decrease in the nutrient content of crops, which can impact global malnutrition. Zinc is one of the plant micronutrients most affected by elevated atmospheric carbon dioxide (CO₂). Zinc deficiency is harmful to human health and can stunt growth and impair immune function. In this novel study, zinc oxide (ZnO) nanoparticles were investigated as a potential means of biofortifying plants against the effects of increased CO₂ conditions. Biomass can be a relative indicator of zinc content. ZnO nanoparticle treatments were hypothesized to increase biomass, but only to a certain point, as micronutrients can become harmful at higher concentrations. *Arabidopsis thaliana* plants were grown in ambient and elevated CO₂. Each trial contained ten groups that were treated with various concentrations of ZnO nanoparticles, which were delivered using two different methods. Half of the plants in each trial were soaked as seeds in a solution of ZnO nanoparticles for three hours before being planted, which is a treatment used in other studies, and then watered with 0.5 mL of regular distilled water during growth. The other half of the seeds were watered every three days during growth with 0.5 mL of the solution of ZnO nanoparticles. The concentrations used for each method were 0.0 (control), 0.5, 1.0, 5.0, and 10.0 mg/L of ZnO nanoparticles. After three weeks, the dry biomass above the root was measured. Overall statistical significance was determined by ANOVA tests and post-hoc Tukey tests were used for comparisons between treatments. In ambient CO₂, the 5.0 mg/L soaking treatment yielded the highest mean biomass ($p = 0.001$ compared to control) and the second-highest mean biomass in elevated CO₂ ($p = 0.001$ compared to control). 5.0 mg/L was also more effective than all watering treatments under both ambient and elevated conditions ($p = 0.001$), indicating that *Arabidopsis thaliana* biomass is more affected by three hours of soaking as a seed in the 5.0 mg/L solution than by three weeks of watering with the solutions. 5.0 mg/L was identified as a possible optimal concentration of ZnO nanoparticles to maximize biomass. The presence of ZnO nanoparticles effectively increases crop yield and may be a viable strategy to combat zinc loss due to increased CO₂. As plants are a vital source of zinc for humans around the globe, especially in low-resource areas, measures to stabilize micronutrients in plants must be considered as a response to climate change.

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Introduction

Not only can climate change cause almost irreparable damage to the world's ecosystems, it can also impact the health of humans. Humans intake nutrients either directly from consuming plants or by consuming animals who have eaten those plants. The micronutrient quantities within plants are in a delicate balance, known as homeostasis, much like the human metabolism. Plants depend upon carbon dioxide to perform photosynthesis within their cells, yet as humans continue to burn fossil fuels, carbon dioxide is released into the atmosphere. A major cause of climate change, excess carbon dioxide, can also deplete the micronutrient content of plants. One such micronutrient is zinc, and it is essential to both humans and plants. However, as observed in studies by Myers et al. (2014), this micronutrient is significantly threatened by excess atmospheric carbon dioxide. A leading hypothesis of why nutrients are depleted in elevated carbon dioxide is carbohydrate dilution. As there is more carbon dioxide available, the plant produces more starch, which limits their intake and storage of nutrients. While some nutritional factors can be controlled by humans through dietary modification, when the nutrient content of plants is compromised due to suboptimal environmental conditions, the amount of nutrients available to humans from those sources is limited from the start.

Zinc deficiency, which can arise from consuming foods that contain inadequate zinc, is detrimental to the health of humans and plants. Zinc is a ubiquitous mineral in the body, present in cells throughout, and is utilized in cell growth and repair, along with DNA transcription, the process of copying a strand of DNA to form RNA from DNA. Some of the most serious symptoms of zinc deficiency include stunting of growth, cognitive damage, and even death. In the United States, twelve percent of people are at risk of zinc deficiency, with most in the elderly population (Zinc, 2009). Mathematical models estimate that zinc and iron deficiency, in total, cause a loss of 63 million years of human life annually due to premature death (Myers et al., 2014).

In order to replace the nutrients that the plant lacks, biofortification approaches may be employed to enhance plant nutritional quality through agronomic practices. Not only does biofortification contribute to higher crop yield and biomass, but it also leads to plants that are more resistant to environmental factors such as drought, insects, and diseases (Dimkpa & Bindraban, 2016). An emerging biofortification solution involves nanotechnology, which is an

efficient way to deliver nutrients to the cells of a plant since nanoparticles are more readily absorbed due to their small size.

Specifically, zinc oxide nanoparticles have been known to improve qualities of plants such as biomass, shoot length, and root dry mass, which are key to crop yield, which farmers and seed suppliers seek to increase (Liu & Lal, 2015). However, their efficacy has not yet been studied in environments with elevated atmospheric carbon dioxide, further reinforcing the need for research as the global carbon dioxide levels continue to rise. The purpose of this project was to investigate a potential solution to global malnutrition, which is predicted to worsen with climate. Motivated by the threat of global zinc deficiency, this project aimed to improve the zinc concentration of plants when exposed to the elevated level of atmospheric carbon dioxide that is predicted with climate change.

This experiment examined zinc oxide nanoparticles, which could effectively deliver zinc in plant transportation systems and enhance the overall zinc concentration. To biofortify the zinc in *Arabidopsis thaliana* plants, they were treated with varying concentrations of zinc oxide nanoparticles and grown in environments with ambient and elevated carbon dioxide. The plants' height and biomass were measured, while temperature, light exposure, and administered water volume were controlled. The plant *Arabidopsis thaliana* was employed as a model organism due to its quick growth rate and the need to maintain a delicate homeostasis with the minerals in its cells. *Arabidopsis thaliana* is also a C3 plant, meaning that a three-carbon compound is formed in photosynthesis, and it is more susceptible to changes in atmospheric carbon dioxide. Its nutrient content is more likely to be depleted, as seen in other C3 plants, such as wheat and rice (Myers et al., 2014). In this experiment, it was crucial to test multiple concentrations of zinc oxide nanoparticles because plants and humans need small amounts of zinc, and an excess can also be harmful. The results of the experiment could potentially influence global public health efforts in terms of agronomic approaches to prevent further zinc deficiency.

Methods and Materials

The design of this procedure was based upon components from past scientific literature. The articles included Dimkpa & Bindraban (2016), Handling (n.d.), Liu & Lal (2015), and Myers et. al. (2014). The procedure was conducted in ambient CO₂ and then repeated in elevated CO₂. The entire experiment was then repeated with expanded treatment groups and three times as many plants, which will be referred to as the second experiment. The Methods and Materials section of this paper describe the original experiment, with some additional information about the second experiment. The duration of each experimentation phase of the project was seven weeks. In summary, *Arabidopsis thaliana* seeds were incubated in the refrigerator, treated with solutions of varying concentrations of zinc oxide nanoparticles, and planted in soil. They then germinated and grew for three weeks in a growth chamber. The height was measured throughout the growing process and the dry biomass was measured at the conclusion of the growing process.

Phase 1: Seed Incubation

For each trial, 100 *Arabidopsis thaliana* seeds were counted and placed into a container with 25 milliliters of cool tap water. The container was placed in the refrigerator, which was set to a temperature of 4 degrees Celsius. The seeds were incubated in the refrigerator for 2 days, or 48 hours. This procedure breaks the dormancy of seeds and improves synchrony, leading to more precise results (Handling, n.d.).

Phase 2: Zinc Oxide Nanoparticle Solutions Preparation

A digital balance was utilized to measure 200 milligrams, or 0.200 grams, of zinc oxide nanoparticles, in order to prepare the stock solution. The balance was placed inside a contained laboratory environment that had a clear barrier to protect the user from the chemicals that they may be handling inside. The final reading of the balance was 0.2005 grams. Gloves and goggles were worn when handling the nanoparticles.

The measured nanoparticles were added to a small beaker with 20 milliliters of distilled water. A magnetic stirrer was placed within, and the solution was mixed above a spinning magnet, on high speed for 30 minutes. By the end, the zinc oxide nanoparticles had been evenly dispersed throughout. After the mixing period had elapsed, the beaker was removed from the stirring machine and the stock solution was stored in two vials, labeled accordingly.

To create the solutions, distilled water was first poured into a small beaker. Solutions of three different concentrations of zinc oxide nanoparticles were created: 10.0 mg/L, 1.0 mg/L, and 0.5 mg/L. The solvent of distilled water was added to each vial in the correct amount to form a solution of that concentration. Before the stock solution was extracted, the vial was moved back and forth in multiple directions at least ten times, or until it appeared to be thoroughly mixed. To prepare the final solutions that would be used to soak the seeds, the stock solution was measured with a micropipette and expelled into the vials to create solutions of different concentrations. The solutions were thoroughly mixed. They had the desired final concentrations of 10.0 mg/L, 1.0 mg/L, and 0.5 mg/L. These solutions are notably dilute because zinc, being a micronutrient, is needed by small plants such as *Arabidopsis thaliana* in especially small concentrations.

Phase 3: Seed Treatment

After the seeds were refrigerated, using a micropipette set for 100 μ L, the seeds were extracted from the container and transferred onto a paper towel. The paper towel absorbed the liquid from the seeds. Then, the seeds were lifted by the micropipette again and placed into their respective containers. This process transferred the seeds one-by-one, or in groups of a counted quantity.

Twenty seeds were placed into each of the three vials, and the remaining forty were stored in the original container, with only tap water. When all of the necessary seeds had been transferred to the proper containers and the tops had been tightly closed, the seeds were left to soak in the solutions for three hours.

Phase 4: Seed Planting

Soil was extracted from a supply, using the tool provided by the manufacturer, and placed into each of the individual cells. Soil was filled at a depth of approximately 4 inches. The cells were moved thoroughly back and forth in order to level out the surface of the soil. More soil was removed or added until all of the cells contained close to the same amount, approximately three-quarters full. This step was repeated for each of the trays and for the number of cells required for the seeds.

Using a micropipette, the seeds were extracted from their respective containers and expelled into a container with some cool tap water. They were then transferred from the container to a cell in their respective trays. The seeds were placed on top of the soil, in order to

allow light and air for germination. It was crucial that the seeds did not become covered in soil, which would inhibit their germination.

This procedure was repeated until at least one seed had been planted in all 100 cells. Up to 4 seeds can successfully grow in the same location. Afterward, the bottoms of the trays were filled with 300 mL of cool tap water to provide the seeds with moisture. The trays were covered by their clear plastic covers, which were slightly propped up on one side, to allow for air flow to the seeds. The light in the room was kept on throughout the night and the next day in order to facilitate germination.

Phase 5: Routine Monitoring

The next day, the trays were placed into the growth chamber. The growth chamber was plugged in and set to 16 hours of light each day. During the first trial, it contained ambient carbon dioxide, so no changes were made to the carbon dioxide levels originally in the laboratory room. The temperature was approximately room temperature. The plastic cover contained sealing zippers. The image below displays the setup of the plant trays and the growth chamber.

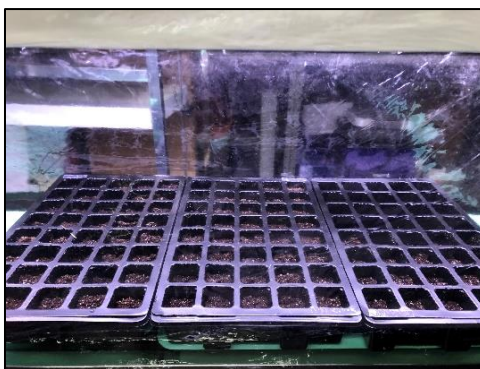


Figure 2: This figure displays the plant tray arrangement in the growth chamber. The seeds were recently planted, so they had not yet sprouted.

For the first week, the plants were provided water through the bottom of the trays, 300 mL each. This was to ensure that the water did not cause any of the seeds to slip below the soil or become overwatered before they had been able to grow roots. After the plants had been growing for one week, they were watered from the top, instead. Each cell was watered with 0.5 mL of liquid every 3 to 4 days. The seeds that were soaked at the beginning received tap water, and the group that received the zinc oxide nanoparticle solution throughout the growing process was watered with a 10.0 mg/L solution of zinc oxide nanoparticle and distilled water. The four other groups of plants were watered with cool tap water.

The plants were first monitored for their height after they had been sprouting for a week. To measure the plants, the ruler was placed as close to the plant as possible. The height measurement was taken, in centimeters, at the point where the topmost leaf of the plant reached. The plants were measured under a large light for accurate viewing.

Phase 6: Measuring Biomass

After three weeks of growing time, which is typically half the full growth period of *Arabidopsis thaliana*, each plant was gently removed from the soil. The final height was measured according to the procedure outlined above. This time, the roots were also gently pulled away from the stem, because they were not to be included in the biomass measurement. The plants were dehydrated to ensure that the biomass measurements reflected their tissues, and not the liquid within them. This was to control the accuracy of the biomass measurement, so that it did not take the water within the plants or soil on the roots into account.

The temperature for the dehydrator, which is similar to an oven that heats and evaporates the moisture from the plants with a fan, was set to 120 degrees Celsius. It dehydrated the plants for 24 hours. The racks of the dehydrator were wrapped in tinfoil. This measure was to contain the small plants on a flat surface and prevent them from falling through the wire openings. A second piece of tinfoil was attached loosely to one end of the rack, near the fan, and was used to cover the plants so that the gentle fan in the dehydrator did not alter their position.

After 24 hours of dehydration, the biomass of each individual plant was measured on the balance. The weigh boat was brushed off in between each plant in order to eliminate any remaining plant matter. The image below displays a biomass recording.

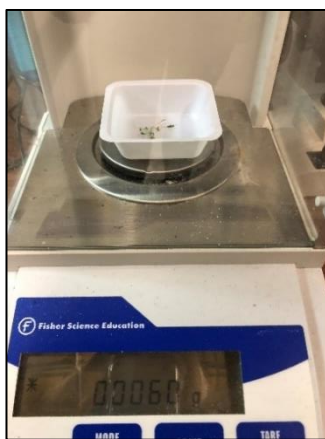


Figure 3: This figure displays the mass of a dehydrated plant being measured on the balance. Here, 0.0060 grams of biomass was recorded.

Phase 7: Elevated Carbon Dioxide

After the first trial in ambient carbon dioxide, the experiment was repeated in elevated carbon dioxide. Since the carbon dioxide was in pressurized form, it was vital that it be stabilized during the process. The carbon dioxide tank was firmly attached to a support pole in the laboratory room. A regulator was attached to the tubing for the carbon dioxide tank. A carbon dioxide controller was placed inside the growth chamber, approximately in the center. The plastic covering was sealed as tightly as possible so as to prevent gas leakage. The target high CO₂ level on the carbon dioxide controller was set to 550 parts per million, and the target low CO₂ level was set to 450 parts per million, as in Myers et. al. (2014).

The statistical tests chosen for data analysis were the ANOVA test and the post-hoc Tukey test. The ANOVA test indicated whether there was a statistically significant difference somewhere in all of the data collected as a whole. If the result indicates significance, a post-hoc Tukey test can be performed to identify between which two groups a difference lies. A Tukey test was selected because it is conservative in assigning significance to small sample sizes, which is needed for this project, due to a sample size of approximately 25 seeds.

In the replication of the experiment, the same procedures were used, except that both watering and soaking treatments were expanded to test the control, 0.5 mg/L, 1.0 mg/L, 5.0 mg/L, and 10.0 mg/L.

Results

To analyze the data gathered through the first round of experimentation, the mean and standard deviation of plant height and biomass were calculated. The values are displayed in the tables below.

The majority of the values were within one standard deviation from the mean, but not the control or the 10.0 mg/L soaking treatment, indicating that the data was not normally distributed. In ambient carbon dioxide, the group that was soaked in 1.0 mg/L yielded the highest mean biomass and final height. 10.0 mg/L during growth produced the second highest mean biomass and final height. 0.5 mg/L produced the lowest mean biomass, while 0.0 mg/L, the control group, produced the lowest mean height.

Table A: Mean and Std Dev of <i>A. thaliana</i> in Ambient CO ₂					
Treatment	(mg/L)	Biomass		Height	
		Mean	Std Dev	Mean	Std Dev
0.0		0.0048	0.0011	3.0	1.3
0.5		0.0041	0.0004	3.8	0.1
1.0		0.0060	0.0008	4.0	0.7
10.0		0.0043	0.0013	3.5	1.1
10.0 During		0.0053	0.0007	4.0	0.7

The biomass values for ambient carbon dioxide are displayed in the bar graph below, with error bars that represent standard deviation.

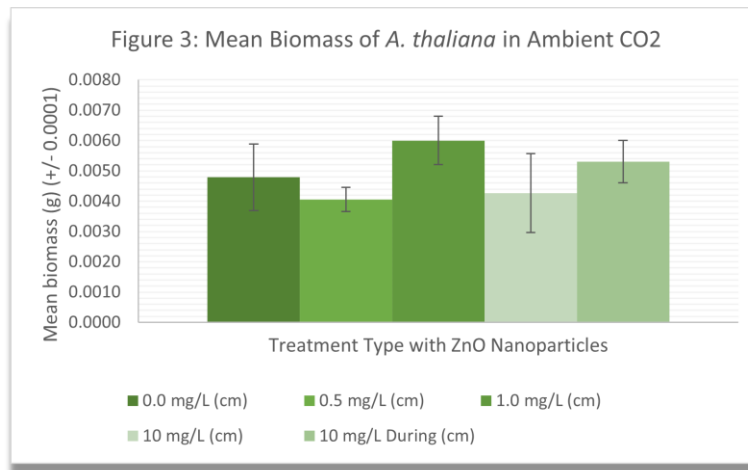


Figure 3: This figure is a graphical representation of the mean biomass of *Arabidopsis thaliana* plants in ambient carbon dioxide. Error bars = standard deviation

In the figure above, there is clear, visual variation between the means of the experimental groups. The group with the largest standard deviation is the group treated with 10.0 mg/L of zinc oxide nanoparticles, and the group with the smallest standard deviation is the group treated with 0.5 mg/L. The rightmost column represents the data for the plants that were grown with 10.0 mg/L during the process, and the column adjacent to it represents the data for the plants that were soaked, per the traditional treatment, with 10.0 mg/L at the start. The biomass data does not seem to follow a linear relationship with respect to zinc oxide nanoparticle concentration, suggesting there could be little variation or a point of diminishing return.

The height values for ambient carbon dioxide are displayed in the bar graph below, with error bars that represent standard deviation.

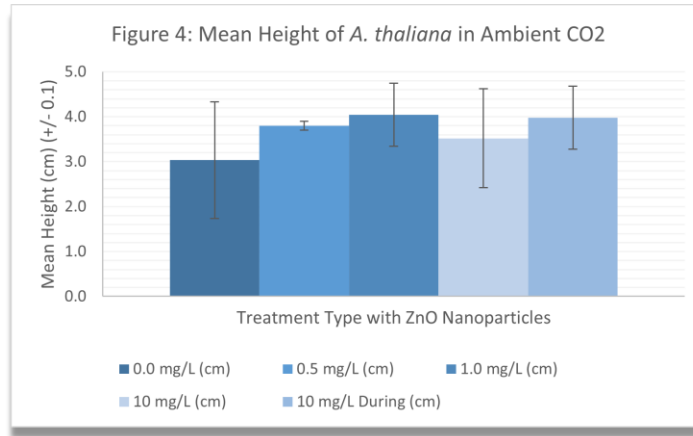


Figure 4: This figure is a graphical representation of the mean height of *Arabidopsis thaliana* plants in ambient carbon dioxide. Error bars = standard deviation

The group with the largest standard deviation in height is the control group, with 0.0 mg/L of zinc oxide nanoparticles, and the group with the smallest standard deviation is the group treated with 0.5 mg/L. The heights follow a similar pattern as the biomasses in ambient carbon dioxide. The height data also does not seem to follow a linear relationship with respect to zinc oxide nanoparticle concentration.

The mean and standard deviation of plant height and biomass in elevated carbon dioxide are displayed in the table below. The majority of the values were within one standard deviation from the mean, so the data was more normally distributed than that of the plants grown in ambient carbon dioxide. The groups that were soaked in 0.5 mg/L and those treated with 10.0 mg/L during the growth process yielded the highest mean final height of 3.1 centimeters. In elevated carbon dioxide, 1.0 mg/L and 10.0 mg/L during growth produced plants with the highest biomass, of 0.0013 grams. The values for the plants grown in elevated carbon dioxide are markedly lower than those grown in ambient carbon dioxide.

Treatment	(mg/L)	Biomass		Height	
		Mean	Std Dev	Mean	Std Dev
0.0		0.0007	0.0004	2.7	0.9
0.5		0.0008	0.0007	3.1	0.9
1.0		0.0013	0.0007	2.7	1.1
10.0		0.0009	0.0005	2.6	1.0
10.0 During		0.0013	0.0006	3.1	0.8

The biomass values for elevated carbon dioxide are displayed in the bar graph below, with error bars that represent standard deviation.

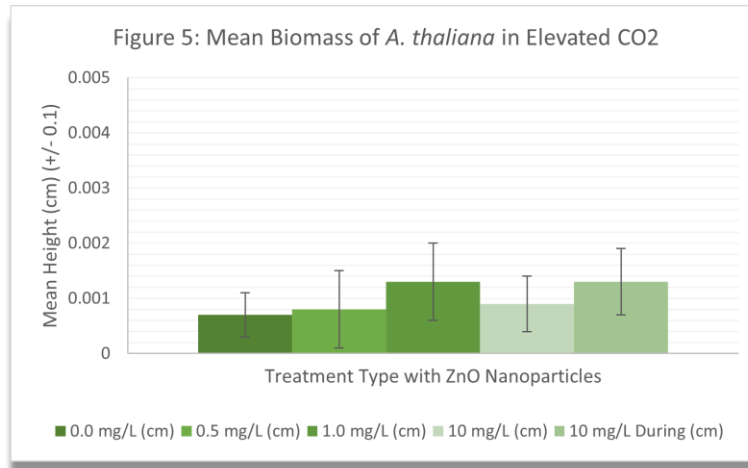


Figure 5: This figure is a graphical representation of the mean biomass of *Arabidopsis thaliana* plants in elevated carbon dioxide. Error bars = standard deviation

The group with the largest standard deviation in biomass in elevated carbon dioxide is the group treated with 10.0 mg/L of zinc oxide nanoparticles, and the group with the smallest standard deviation is the group treated with 0.5 mg/L. A similar trend as in ambient carbon dioxide, 1.0 mg/L and 10.0 mg/L during produced the highest biomasses of the trial.

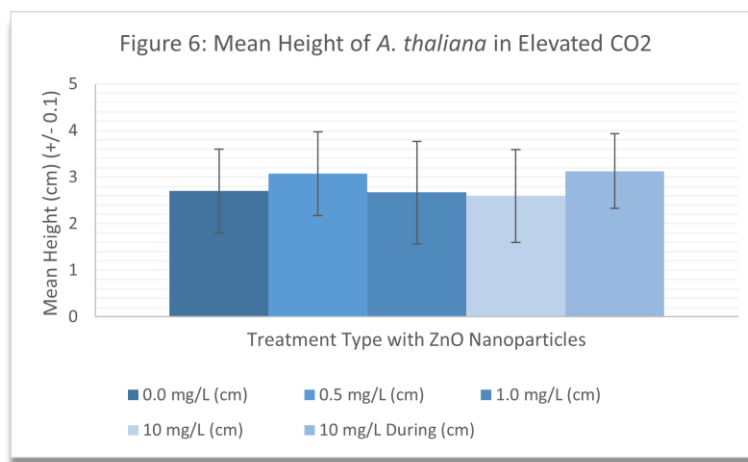


Figure 6: This figure is a graphical representation of the mean height of *Arabidopsis thaliana* plants in elevated carbon dioxide. Error bars = standard deviation

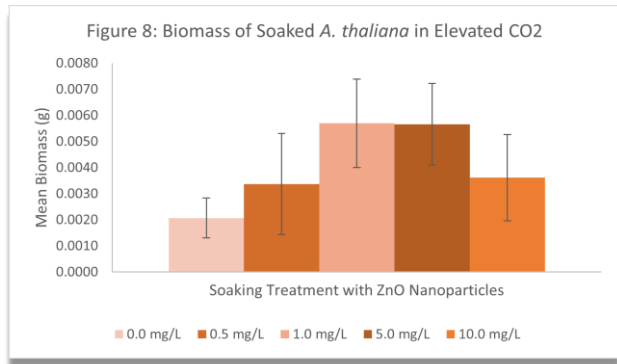
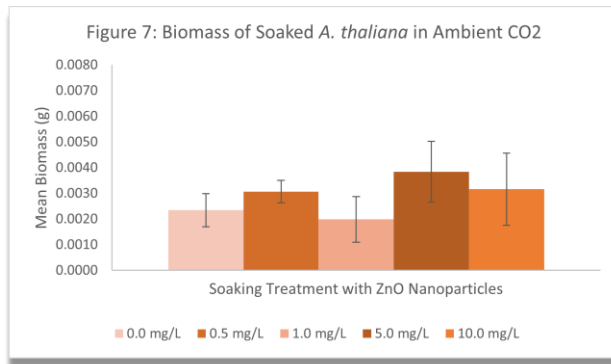
In the figure above, the variation between experimental groups is less evident. The group with the largest standard deviation is the group treated with 1.0 mg/L of zinc oxide nanoparticles, and the group with the smallest standard deviation is the group treated with 10.0 mg/L during the growth process.

Statistical tests were employed to determine whether the differences between means were significant, and could be extrapolated to the population at large. The null hypothesis was that zinc oxide nanoparticles had no effect upon the biomass or height of *Arabidopsis thaliana* plants, in either environment. The alternate hypothesis was that zinc oxide nanoparticles were responsible for statistically significant variation in the data, meaning that they did have a pronounced effect.

The data was not parametric, as the standard variations were not equal and the data did not display a normal distribution when in a histogram format. Thus, a single-factor ANOVA test was performed on the heights and biomasses, which can function with non-parametric data. For the plant heights in ambient and elevated carbon dioxide, the *F statistic* value was smaller than the *F critical* value, meaning that there was not statistically significant variation between the groups. However, the ANOVA test determined that there was statistically significant variation between the biomasses of the treatment groups in both ambient and elevated carbon dioxide, because the *F statistic* value was greater than the *F critical* value. Therefore, it was investigated as to which groups are statistically different.

The experimental groups were not of the same sample size, and they were relatively small, under 30, so a Tukey test was performed to identify between which groups the statistic differences lay. Each of the groups were tested against the control group, which was 0.0 mg/L, and all of the other groups. The p-value produced must be less than the alpha value, 0.05, in order for the data to be considered statistically significant. In ambient carbon dioxide, it was found that the plants treated with 1.0 mg/L achieved significantly higher biomass than the 10.0 mg/L group, producing a p-value of 0.013. In elevated carbon dioxide, the plants treated with 1.0 mg/L achieved significantly higher biomass than the control, producing a p-value of 0.005. The plants watered with 10.0 mg/L during growth displayed significantly higher biomass than the control and the soaking treatment of 10.0 mg/L, producing p-values of 0.001 and 0.043, respectively.

In the expanded replication of the experiment, both watering and soaking treatments were expanded to test the control, 0.5 mg/L, 1.0 mg/L, 5.0 mg/L, and 10.0 mg/L. It was found that the intermediate treatment of 5.0 mg/L led to the highest biomass.



Figures 7 & 8: These figures are a graphical representation of the mean height of soaked *Arabidopsis thaliana* plants in ambient and elevated carbon dioxide, respectively with expanded treatment groups. Error bars = standard deviation

In terms of the results for soaked seeds, in both ambient and elevated carbon dioxide, the 5.0 mg/L treatment was the most effective, producing the highest biomass. A post-hoc Tukey test revealed that 5.0 mg/L led to statistically greater biomass than the control in both conditions, with p-values of 0.001. In elevated carbon dioxide specifically, the 5.0 mg/L treatment was more effective than all but the 1.0 mg/L group.

The results for watered seeds, in contrast, did not have notable variation. There were no statistically significant differences between all concentrations of watering treatments. Watering is not as effective as soaking in this experiment since all mean biomasses for watering treatment were lower than the most effective soaking treatment, 5.0 mg/L, with a p-value of 0.001. More information about p-values can be found in Appendix D.

Discussion & Conclusion

The claim was that if the concentration of zinc oxide nanoparticles added to the soil increases, then the biomass and height of *Arabidopsis thaliana* plants will increase to a certain point of maximum yield because this substance heightens the bioavailability of the zinc micronutrient but becomes toxic at higher concentrations. The results of the experiment supported the hypothesis. The data aligned with the findings reported in Liu & Lal (2015), in that soaking the seeds in 10.0 mg/L of zinc oxide nanoparticles was too concentrated of a treatment to have a positive effect. In the extended repetition, the 5.0 mg/L soaking treatment produced the highest biomass, which was a consistent finding in both elevated and ambient carbon dioxide. 5.0 mg/L could have been the optimal concentration with which to soak the seeds of the plants, delivering the zinc mineral in a concentration that was manageable to the seed. In the original experiment, 1.0 mg/L soaking and 10.0 mg/L watering treatments were the most effective. The second round of experimentation revealed that in between these concentrations, 5.0 mg/L led to the highest biomass. Also, since 10.0 mg/L soaking treatment was less effective than the 5.0 mg/L soaking treatment, there may be a point of diminishing return, and zinc oxide nanoparticles could be harmful at higher concentrations, as hypothesized.

In the first experiment, plant height and biomass in elevated carbon dioxide was notably lower than in ambient carbon dioxide. Prior research suggested that this trend was due to zinc deficiency causing stunting of growth. Since the environment contained excess carbon dioxide, the gas that the plant took in was significantly altered from normal levels, affecting photosynthesis and nutrient absorption. Elevated carbon dioxide causes zinc deficiency and stunting in plants, which may have contributed to the visible height and biomass difference between the two trials. Although these results indicate that zinc oxide nanoparticles could be a potential solution to enhancing the zinc content of crops, it is valuable to note the implications of the biomass of plants in elevated carbon dioxide not being as large as those in ambient carbon dioxide. When extrapolated to the real world, this could indicate that zinc oxide nanoparticles are a viable compound to influence zinc content, but that they are not yet a solution enough within themselves to completely replenish the zinc lost due to climate change.

A potential source of error in this project could have sourced from the carbon dioxide controller. The monitor was set to the correct level of 550 parts per million, at which it was supposed to halt carbon dioxide flow, but the actual concentration that it reported was notably

higher than that level, such as 800 parts per million. The level was consistently elevated beyond 550 parts per million, even though the carbon dioxide was not flowing out of the valve during those times. This could have contributed to the effects on biomass becoming more pronounced than they would have at the predicted elevated level in 2050, 550 parts per million. Another source of error could have been the balance for measuring plant biomass. The plants are of extremely low biomass, so the balance needed to detect values less than 0.01 grams, which could have impaired the accuracy of the machine, since it was not designed for such small masses. The balance needed to be recalibrated several times in order to detect the mass placed on it, and it sometimes read a weight of 0.0000 grams, even though this was not the case. Appendix B contains more information about potential limitations and assumptions of this experiment.

Ultimately, this experiment indicated that treating plants with zinc oxide nanoparticles was an effective method by which to increase the biomass of plants, which can be extended to improving the zinc content of crops. As climate change becomes an increasingly pressing issue for the global population, the micronutrients within plants are threatened to be significantly depleted. Zinc oxide nanoparticles were selected as a chemical compound that could be applied to the plants and contribute to higher levels of zinc in the tissues, thereby providing more zinc-fortified food to humans around the world. Zinc oxide nanoparticles were needed in small amounts for this approach, and were simple to prepare and administer, yet with significant positive effects upon plant growth and nutrition. This indicated that they may be an affordable, efficient, and effective technique to be incorporated into agronomic practices, especially in the low resource areas that are most affected. Malnutrition, especially zinc deficiency, is predicted to be an even larger issue for public health in the coming years, affecting populations around the globe, as the nutrients in plants will be limited under climate change. Therefore, any solution that has the potential to positively impact the zinc concentration of plants is worth further investigation. Human capabilities in other realms, the economy, and survival all heavily depend upon nutrition, and zinc oxide nanoparticles have been shown to enhance the nutrients in humans' original source of nourishment, plants, potentially leading to widespread positive affects throughout the world, sustaining through climate change.

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Appendices

Appendix A: Acknowledgements

I would like to thank Dr. Crowthers for the valuable guidance, enthusiasm, and dedication that he provided throughout the course of my project. In the beginning, he inspired me to become excited about the possibilities for research this year, and he informed me of all of the options available. He assisted me in developing methodology, especially on the first day that I came into school, teaching me how to handle a micropipette and perform calculations about solution concentrations. It was a significant time commitment to be at school every three days for me to come and record data. Many thanks to Dr. Crowthers also for negotiating with representatives and acquiring a gas tank and regulator for my project. I am endlessly grateful for his encouragement and expertise.

I would also like to thank Mr. Bower at Airgas and Dr. Rulfs from the Biology department at Worcester Polytechnic Institute. They kindly donated a carbon dioxide tank and regulator for use in my project. Without their generosity, I would not have been able to continue my experiment with climate change conditions.

Additionally, I am grateful for the assistance and encouragement from my colleagues at Mass Academy. They were instrumental in peer reviewing my writing and offering suggestions for my presentation.

Lastly, I wholeheartedly thank my family for their support. I could not have done this project without their encouragement and selflessness in donating their time to drive me to school, staying there during many long hours of data collection, looking over my writing, and being a practice audience for my presentations.

Appendix B: Limitations and Assumptions

1. Limitation: Especially in the ambient carbon dioxide trial, some of the sample sizes were limited, such as less than 20 plants. The smallest sample size was in the 0.05 mg/L group, so the standard deviation and averages may be misleading.
2. Limitation: Although it was set for the correct level, the carbon dioxide controller consistently displayed values that were a few hundred parts per million higher than it was

set for. This could have contributed to the drastic height and biomass differences between plants in ambient and elevated carbon dioxide.

3. Limitation: The plants were not grown under free-air CO₂ enrichment technology, which requires a large field for planting and a higher budget. Free-air CO₂ enrichment technology would be more representative of the environmental conditions of crops growing outside, rather than in cells in a growth chamber.
4. Assumption: Biomass was measured as a relative indicator because zinc is key for cell growth. In studies such as that by Dimkpa & Bindraban (2016), zinc concentration was directly correlated with biomass. However, in order to conclusively confirm the efficacy of zinc oxide nanoparticles under elevated carbon dioxide, the plants must be tested for the zinc concentration in their tissues. This impacts the influence of the results of this experiment.
5. Assumption: It was assumed that the trends observed in *Arabidopsis thaliana* plants would also be present after a longer growth period. It was also assumed that the same effects would be observed in crops, such as wheat and rice. *Arabidopsis thaliana* is not a plant that is consumed by humans, and therefore does not contribute to human nutrition as directly as crops.

Appendix C: Tukey Test p-Value Tables – First Experiment

The figures below display the p-values generated from Tukey tests of the biomass data for ambient and elevated carbon dioxide in the first experiment. Each group was compared to all of the other groups. A p-value less than the alpha value of 0.05 indicated the statistical significance of the difference of those groups from each other.

Table C: Tukey Test of Biomass in Ambient CO ₂ (D = During)					
Zinc Oxide Nanoparticle Concentration (mg/L)					
	0.0	0.5	1.0	10.0	10.0 D
0.0	x	x	x	x	x
0.5	0.900	x	x	x	x
1.0	0.223	0.182	x	x	x
10.0	0.842	0.900	0.013	x	x
10.0 D	0.860	0.573	0.642	0.214	x

Table D: Tukey Test of Biomass in Elevated CO ₂ (D = During)					
Zinc Oxide Nanoparticle Concentration (mg/L)					
	0.0	0.5	1.0	10.0	10.0 D
0.0	x	x	x	x	x
0.5	0.900	x	x	x	x
1.0	0.005	0.086	x	x	x
10.0	0.780	0.900	0.284	x	x
10.0 D	0.001	0.043	0.900	0.195	x

Appendix D: Tukey Test p-Value Tables – Second Experiment

The figures below display the p-values generated from Tukey tests of the biomass data for ambient and elevated carbon dioxide in the second experiment. Each treatment group (soaked or watering) was compared to all of the other groups.-

Table F: Tukey Test of Soaked Biomass in Ambient CO ₂					
Zinc Oxide Nanoparticle Concentration (mg/L)					
	0.0	0.5	1.0	5.0	10.0
0.0	x	x	x	x	x
0.5	0.235	x	x	x	x
1.0	0.900	0.033	x	x	x
5.0	0.001	0.359	0.001	x	x
10.0	0.134	0.900	0.017	0.560	x

Table G: Tukey Test of Soaked Biomass in Elevated CO ₂					
Zinc Oxide Nanoparticle Concentration (mg/L)					
	0.0	0.5	1.0	5.0	10.0
0.0	x	x	x	x	x
0.5	0.210	x	x	x	x
1.0	0.001	0.002	x	x	x
5.0	0.001	0.006	0.900	x	x
10.0	0.087	0.900	0.015	0.034	x

Table H: Tukey Test of Watered Biomass in Ambient CO ₂						
Zinc Oxide Nanoparticle Concentration (mg/L)						
	0.0	0.5	1.0	5.0	10.0	15.0
0.0	x	x	x	x	x	x
0.5	0.720	x	x	x	x	x
1.0	0.498	0.900	x	x	x	x
5.0	0.900	0.900	0.900	x	x	x
10.0	0.900	0.900	0.739	0.900	x	x
15.0	0.144	0.900	0.900	0.500	0.366	x

Table I: Tukey Test of Watered Biomass in Elevated CO ₂						
Zinc Oxide Nanoparticle Concentration (mg/L)						
	0.0	0.5	1.0	5.0	10.0	15.0
0.0	x	x	x	x	x	x
0.5	0.900	x	x	x	x	x
1.0	0.900	0.900	x	x	x	x
5.0	0.757	0.900	0.900	x	x	x
10.0	0.075	0.789	0.589	0.900	x	x
15.0	0.394	0.900	0.900	0.900	0.900	x