Biology Honors Thesis

Aluminum Oxide Nanoparticle Impact on Plant Growth and Development

Evan Brinkley

December 2021

APPROVED BY:

Director of Thesis:____

Baohong Zhang

Faculty assessor:____

Peng Xiao and Xiaoping Pan

Director of Undergraduate Studies:___________________________

Jean-Luc Scemama, PhD

On this day,

'I hereby declare I am the sole author of this thesis. It is the result of my own work and is not the outcome of work done in collaboration, nor has any of it been submitted elsewhere for another degree.'

Signed:______________________________ Date: 12/13/2021

Evan Brinkley

Aluminum Oxide Nanoparticle Impact on Plant Growth and Development

Evan L. Brinkley

A thesis submitted to the Department of Biology, East Carolina University, in partial fulfillment of the requirements for Biology Honors Thesis

Advisor: Baohong Zhang, Ph.D

East Carolina University

Department of Biology

Dec 13th, 2021

Aluminum Oxide Nanoparticle Impact on Plant Growth and Development

Evan L. Brinkley

Department of Biology, East Carolina University, Greenville N.C. 27858-4353, U.S.A

ABSTRACT:

Nanoparticles are becoming increasingly more applicable in science as technological barriers are being broken and more broad uses are being discovered. With this ever increasing research and application of nanoparticles, the need for information on their specific impact as pollutants need to be equally researched. This is the cause for the boom of research on individual species of nanoparticles over the past decade in order to better understand the specific effects on plants and animals alike. Aluminum oxide is widely used commercially and because of that it has a presence as a potential pollutant in the environment. In this study, the impact of nanoparticles, aluminum oxide specifically, on plant growth, development, and gene expression was monitored over a period of time. Researching and understanding more about this nanoparticle's impact on plant life is grounds for understanding how dangerous it is as a pollutant. Camelina Sativa is a flowering oilseed plant native to Europe and Central Asia. This study aims to methodically test five varying concentrations of Aluminum Oxide (control, 0.01%, 0.05%, 0.1%, and 1.0%) effect on a large sample of Camelina Sativa seeds as they grow. This protocol acts to minimize any impact of contamination while closely monitoring germination rates, root and plant length, biomass, number of leaves, and gene expression. The experiment itself is compatible with most nanoparticles anytime of year since conditions are constant and agar plates are produced on site. Using Analysis of Variance ANOVA, preliminary findings show a statistical difference in germination rates where the control had higher germination rates on average than the groups with

higher concentrations of Aluminum Oxide. The plant and root weight also showed a large statistical difference in the control's weight in comparison to that of higher concentrations. The average number of roots and plants had moderate statistical difference between groups of higher concentration with a trend downward as concentrations increase. The total plant length and average longest root for individuals in each concentration showed slight correlation between concentrations of Aluminum Oxide.

Acknowledgments

I would sincerely like to thank the people and organizations that made this study possible. My mentors, peers, and fellow lab mates in the Zhang lab who helped me approach this project. My mother, Wendy Brinkley, for her ongoing support and encouragement throughout all of my academic career. The Honors College, the Department of Biology, and East Carolina University for allowing the opportunity. I am especially grateful to my project advisor, Dr. Baohong Zhang for his guidance and knowledge throughout this project and the time that I have known him.

Table Of Contents

Introduction

Background……………………………………………………………10

Significance……………………………………………………………11

Methods

Research Design………………………………………………………13

Agar Development……………………………………………………….13

Sterilization and Seed Transfer……………………………………………..14

Characteristic Recording…………………………………………………14

Results

Germination Rates……………………………………………………..15

Plant Shaft………………………………………………………………...17

Plant Roots……………………………………………………………..20

Averaged Data…………………………………………………………….22

Discussion………………………………………………………………………...27

Literature Cited…………………………………………………………………...29

List of Figures

Figure 1: Germination Rates…………………………….……………………………...16

Figure 2: Average Plant Weight (g) Under Concentrations…………………………….18

Figure 3: Number of Leave Under Concentrations…………………………………….19

Figure 4: Average Plant Length (cm) Under Concentrations…………………………..19

Figure 5: Average Root Weight (g) Under Concentrations…………………………….20

Figure 6: Number of Roots Under Concentrations…………………………………….21

Figure 7: Average Longest Root Length (cm) Under Concentrations…………………21

List of Tables

Table 1: Summary of Germination Rates……………………………………………17

Table 2: ANOVA: Single Factor for Germination Rates…………………………17

Table 3: Averaged Raw Data for Control Group…………………..……………..22

Table 4: Averaged Raw Data for 0.01% Concentration Group…………………..23

Table 5: Averaged Raw Data for 0.05% Concentration Group…………………..24

Table 6: Averaged Raw Data for 0.10% Concentration Group…………………..25

Table 7: Averaged Raw Data for 1.0% Concentration Group……………………26

Introduction

Background:

Nanoparticles are materials that are one dimensional and less than 100 nanometers in diameter. *NNC National Nanotechnology Initiative*. Nanoparticles are becoming more prevalent than ever in industrial fields. This is due to nanoparticles' lightweight, strong materials, and pigment that can be found in sunscreens, scratch-proof glass, cosmetics, and many other commercial uses. *Rittner MN* Aluminum Oxide, otherwise known as Al2O3 can be found as a filler in cosmetics or sunscreen, electrical insulation, sand paper, paint, and many more. Nanoparticles have been used widely for the past twenty years, but only in the past ten years has funding been put forth for research into environmental impacts. In 2011 the funding dedicated to nanoparticle environmental health and safety increased from $35 million in 2005, to $117 million. *NNC National Nanotechnology Initiative.* In the past, studies of nanoparticles have had inhibited seed germination, root elongation, and seedling development. (*Wu YF)* On an even smaller scale, nanoparticles have been known to impact gene expression and can cause variation in protein coding genes and microRNAs which are important regulators to gene expression on their own. (*Gong CC)* Research specific to Al_2O_3 nanoparticles' toxicity impact on microalgae where the growth inhibitory effect of alumina nanoparticles was observed. (*Sadiq*)

With nanoparticles like Aluminum Oxide being heavily relied upon in the near future, there will be an inevitable excess leaked into the environment. Though there has been recently higher funding for nanoparticle environmental health and safety research, there is still need for research on individual plant growth with the added Aluminum Oxide factor. This research hopes to achieve a result that clearly shows a difference in growth or gene expression due to specific amounts of Aluminum Oxide polluted into the agar.

The major guiding question of this study is “What impact does Aluminum Oxide have on plant growth and gene expression in different concentrations.” This question can be answered in the form of noticeable difference, no notable difference, or a difference between agar plates of the varying concentrations of Aluminum Oxide. This brings along potential implications like understanding when Aluminum Oxide becomes harmful, if at all, or if it just slows plant development rather than halting or killing the plant completely. Possible results could imply that seed germination could be impacted separately from potential mutated gene expressions that had occurred after germination.

Significance/Impact

Aluminum Oxide is a widely used nanoparticle and seems to have everlasting new innovations commercially. Part of what makes it so widely useful is its fire resistant, high toughness, insulation, and anti-friction properties.(*Peng*) These structurally helpful properties makes Al_2O_3 the perfect candidate as composite material or in coating/cosmetics. As stated previously, nanoparticle application has been growing exponentially over the past few decades and even more recently there has been research into the safety and potential pollutant effect of the particle. In other Al_2O_3 specific research has supported that Al_2O_3 is dose dependant and can significantly impact root development.*(Yanık)* This was on another species of plant, but additional research adds to the overall sample of specific pollutant knowledge. Camelina Sativa specifically is an oilseed which is commercially sold for both medicinal purposes and as a potential biofuel. (*Eleazer*) Though this specific experiment aims to use the Camelina seeds as an example of general toxicity towards seed development and growth, mutations or specific dependent results have a chance of appearing and would be a significant result. In this project, one of the focuses are the germination rates of the Camelina across the varying concentrations. This can be useful to the biology field in general by being a unique test to determine a level of growth inhibition shown by a new pollutant, Aluminum Oxide. This research is not groundbreaking by any means, but is able to show the degree of impact that specific nanoparticles can have on abundant oilseed and flowering plants as well as adding a large quantity of plant data to ongoing sample sizes for Aluminum Oxide specific research.

Methods:

Research Design:

In this study, the effects of Aluminum Oxide on plant growth and development are tested by observing the germination and growth of Camelina Sativa. This is conducted over five groups of nutritional agar plates with varying concentrations of Al_2O_3 (Control, 0.01%, 0.05%, 0.1%, and 1.0%). Twenty-five Camelina Sativa seeds were distilled, placed in each agar plate, and observed germinating and growing over time. Five plates are created for each of the five concentration groups. The germination rates in the first 14 days were tracked for each plate. Three biological replicates of each concentration group were chosen in order to compare average plant length, plant weight, number of leaves, root weight, longest root length, and number of roots. An single factor analysis of variance (ANOVA) was performed in order on the germination rates in order to create a table displaying the degrees of freedom, sum of squares, mean of squares, R-squared value, F-Ratio, and P-value between the controls and each concentration group.

Agar Development:

The nutritional agar in this experiment was made initially with 50mL of distilled water in a 500 mL beaker. While being mixed by a spinning magnet 0.86g (4.3g/L) of MS medium powder was added to the beaker. Next 0.8g Phytagel (4g/L) and 4g sucrose (20g/L) was added to the solution while it was being mixed. Distilled water is added to the mixture until the volume is 200mL. This solution acts as the control medium for the experiment. Four more solutions were made identical to the control. Each of the solutions were labeled as “control,” “0.01%,” “0.05%,” “0.1%,” and “1.0%.” Each of the solutions has the reciprocating amount of Al_2O_3 added to it containing 0, 1.0, 2.0, and 5.0 g/L Al_2O_3.

Sterilization and Seed Transfer

The solutions were transferred to five different flasks to be autoclaved at 121 ℃ for about fifteen minutes. The produced liquid agar was divided and poured into five petri dishes under a sanitized hood. Five gel agar dishes act as the control group which just has the nutritional agar the plant seeds need to grow. There are also five gel dishes for the four varying concentrations, 0.01%, 0.05%, 0.1%, and 1.0% Aluminum Oxide. Camelina seeds were disinfected and washed with 10% bleach followed by two water rinses. Under a disinfected hood with a lit alcohol lamp, twenty-five individual Camelina seeds were placed on each of the twenty-five total plates in a five by five shape. The plates were placed under a twenty-four hour light and their growth was carefully monitored.

Characteristic Recordings

The germination rate of plants growing was recorded at days seven, fourteen, and twenty-one. Three biological replicates for each concentration were chosen and their characteristics were recorded. The root number, length, and biomass (fresh and dry) was recorded for each seed in the fifthteen plates. The shoot length, biomass (fresh and dry), and number of leaves were recorded for each plate. The fresh biomass was recorded after being removed from the dishes then placed in a dry oven to be recorded again after seven days for the dry biomass. The recorded number of leaves and roots, longest root, plant length, and the root and plant weight, fresh and dry, were averaged and underwent ANOVA statistical analysis. The final germination rate and percentages were also tested for statistically significant variance.

Results

Germination Rate:

After twenty-one days from being seeded into the gel agar plates. The final percentage of seeds that had germinated were recorded and compared. (Figure 1) ANOVA was performed in order to compare the control group to the various concentrations showing a statistically significant drop in germination rates from the control to the higher percentage group. (Table 1) (Table 2)

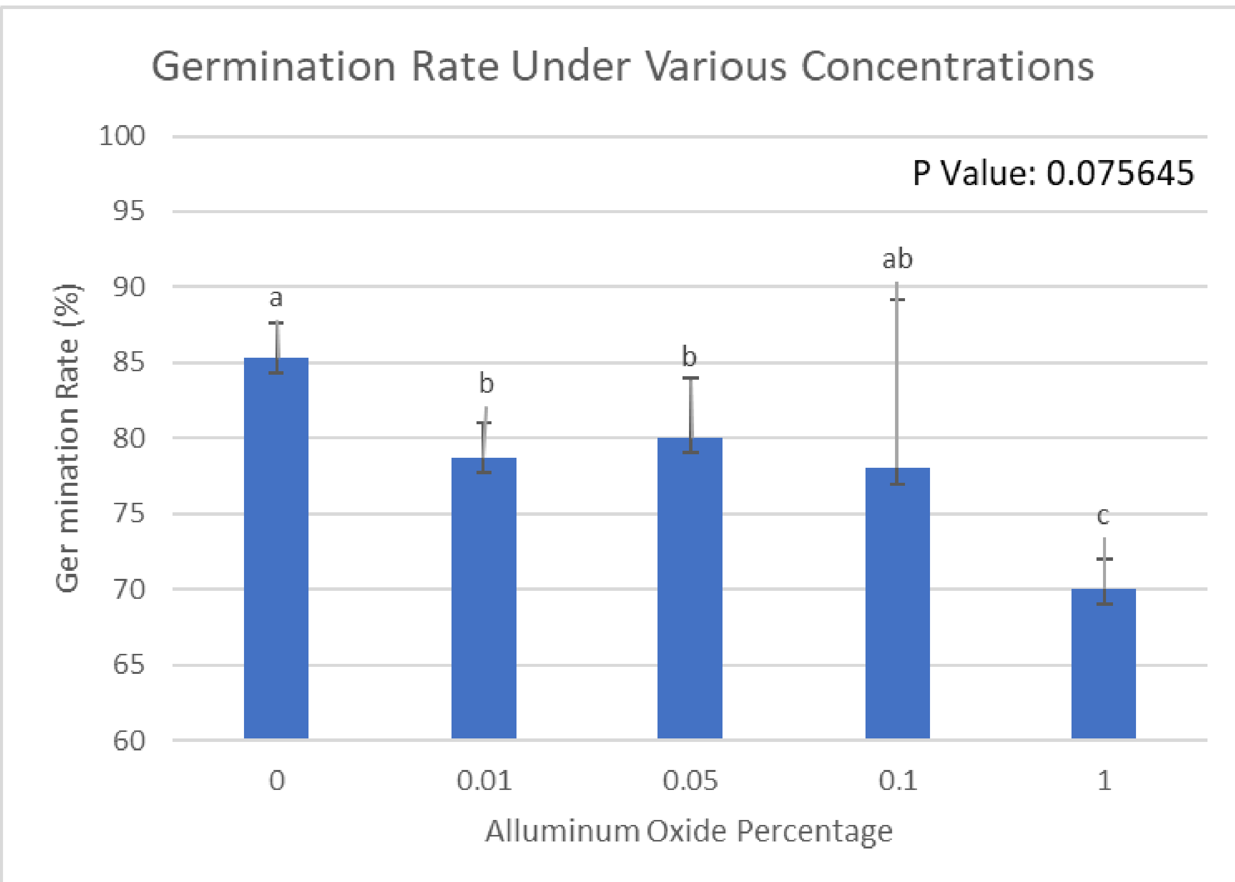

Figure 1: This graph displays the average germination rates across three biological replicate plates from each of the Aluminum Oxide concentration groups.

Groups	Count	Sum	Average	Variance
Control	3	256	85.33333	5.333333
0.01%	3	236	78.66667	5.333333
0.05%	3	240	80	16
0.10%	3	234	78	124
1.00%	3	210	70	4

Table 1: This table displays the statistical summary of germination rates of the twenty-five seeds in each of the concentration groups.

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	364.2667	4	91.06667	2.943966	0.075645	3.47805
Within Groups	309.3333	10	30.93333			
Total	673.6	14				

Table 2: This table displays the single factor Analyzation of Variance (ANOVA) for the germination rates of the twenty-five seeds in each of the trial plates sectioned between groups.

Plant Shaft:

The weight of the plant shaft, number of leaves, and length of the plant shaft were recorded and composited together. The average plant weight seemed to drop the most with exposure to a higher Aluminum Oxide concentration. (Figure 2) The average number of leaves stayed very similar throughout the concentration with slight statistical differences amongst the various concentrations. (Figure 3) The average plant length amount the group also did not have a statistical difference in plants. (Figure 4)

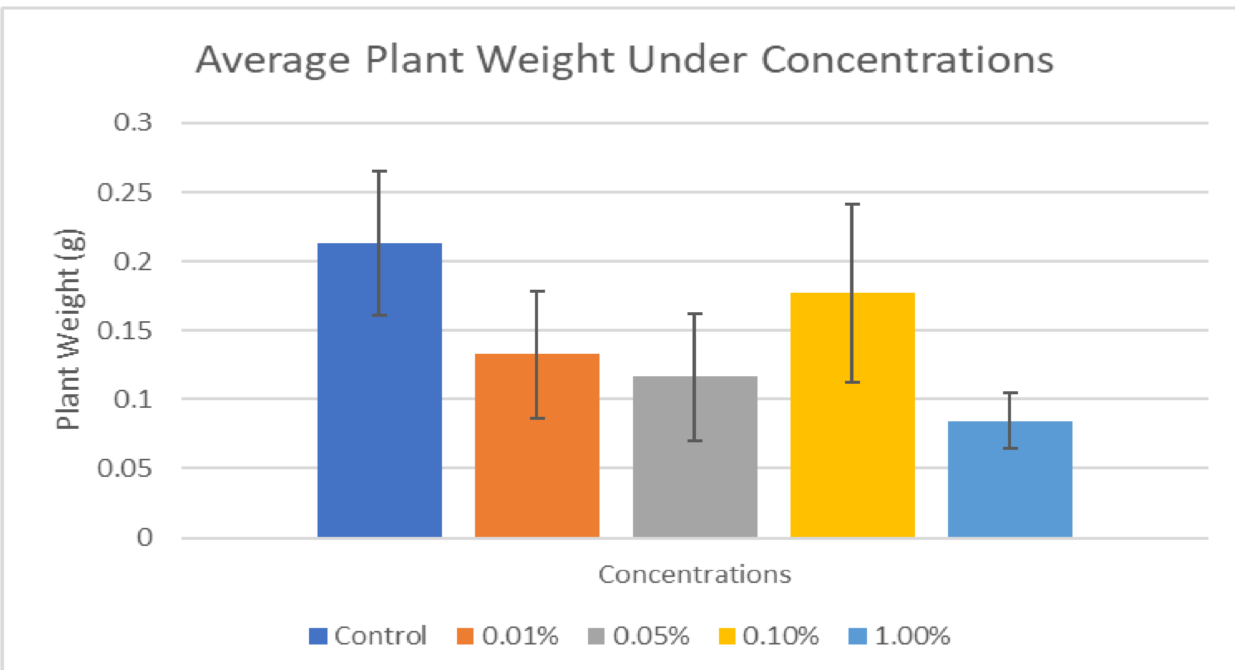

Figure 1: This graph shows the average plant shaft weight in grams of the three replicants for each concentration with standard deviation within the groups.

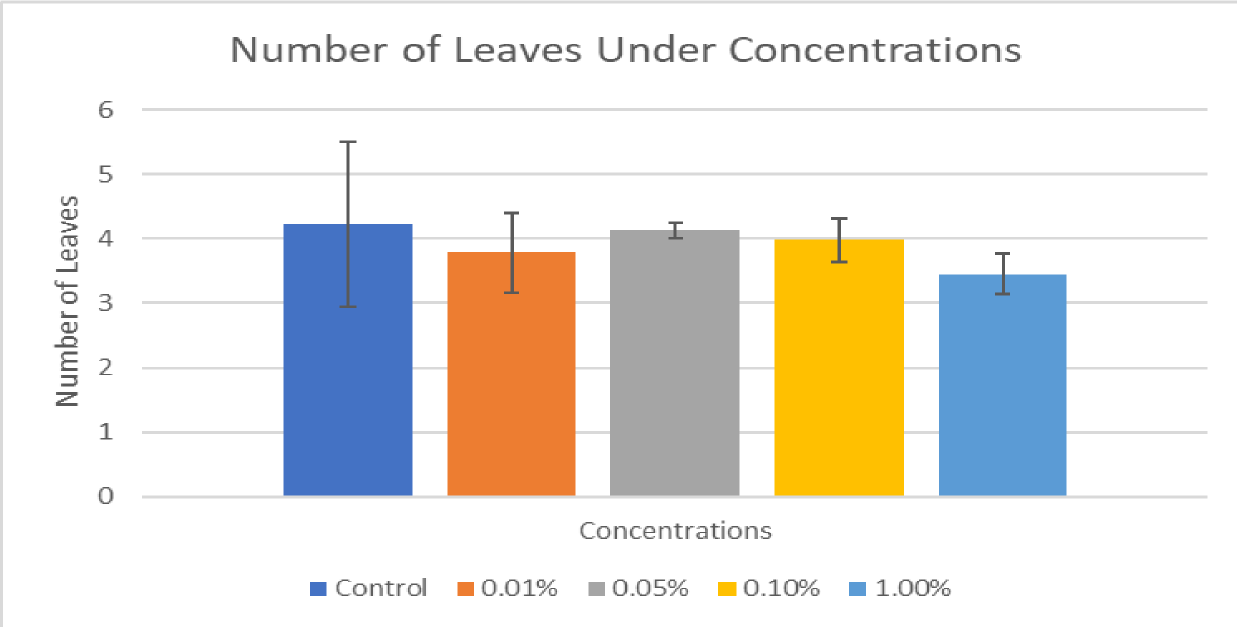

Figure 2: This graph shows the average number of leaves for each plant in the three replicants for each concentration with standard deviation within the groups.

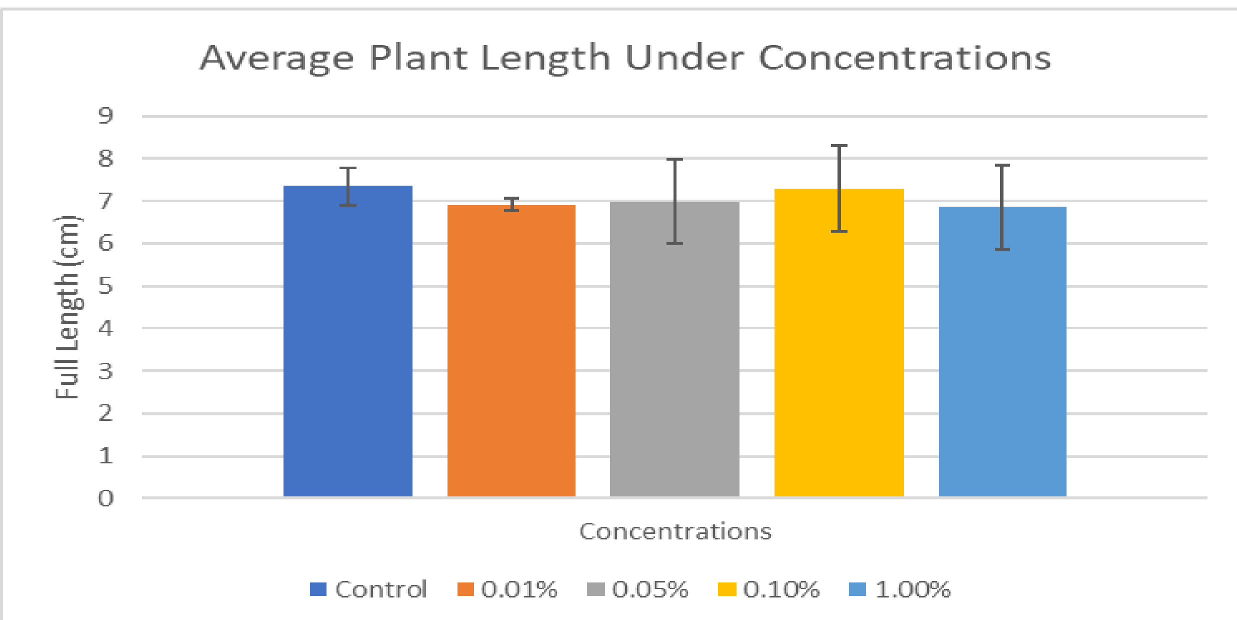

Figure 3: This graph shows the average plant shaft length in centimeters of the three replicants for each concentration with standard deviation within the groups.

Plant Roots:

The final weight of the isolated plant roots, number of plant roots, and the average longest individual root from each plant were recorded and compared together. The average root weight had the largest difference where a higher concentration seemed to suggest a lower average root weight, (Figure 4) The average number of roots each plant had seemed to be stagnant and have slight statistical difference by slightly dropping with higher concentrations. (Figure 5) The average longest root had much variability with some individual plants having a few uniquely long roots. This comparison had the least correlation.

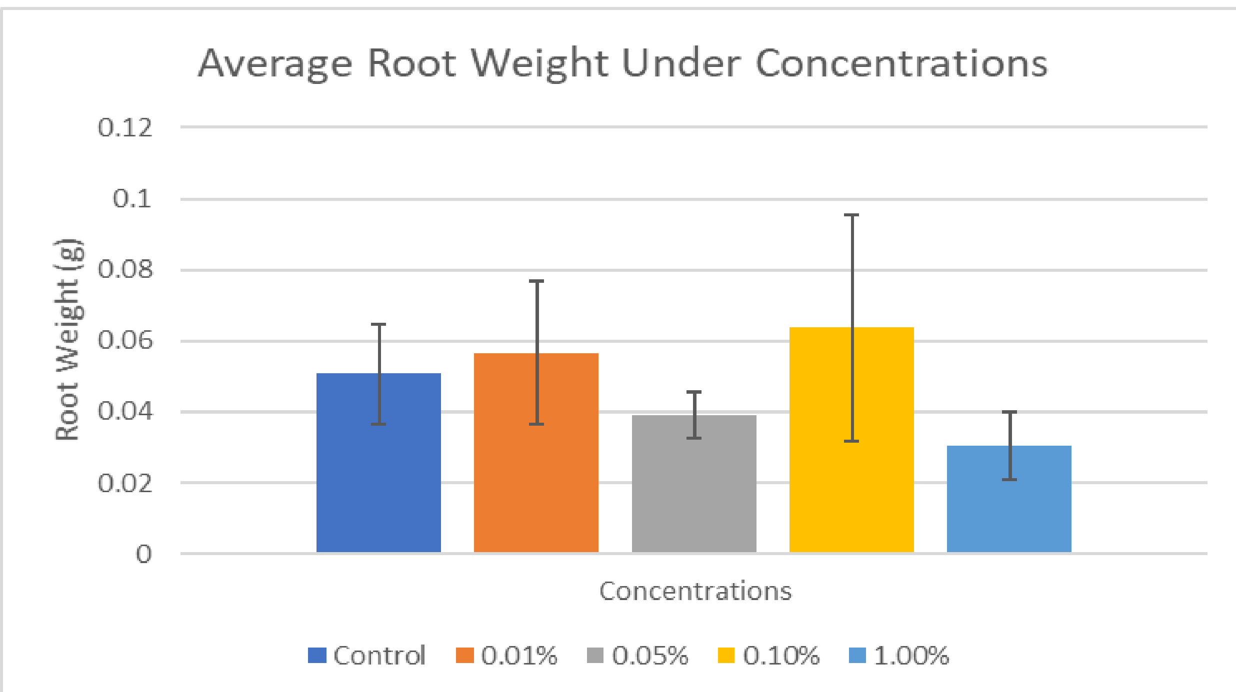

Figure 4: This graph shows the average root weight in grams for each plant in the three replicants for each concentration.

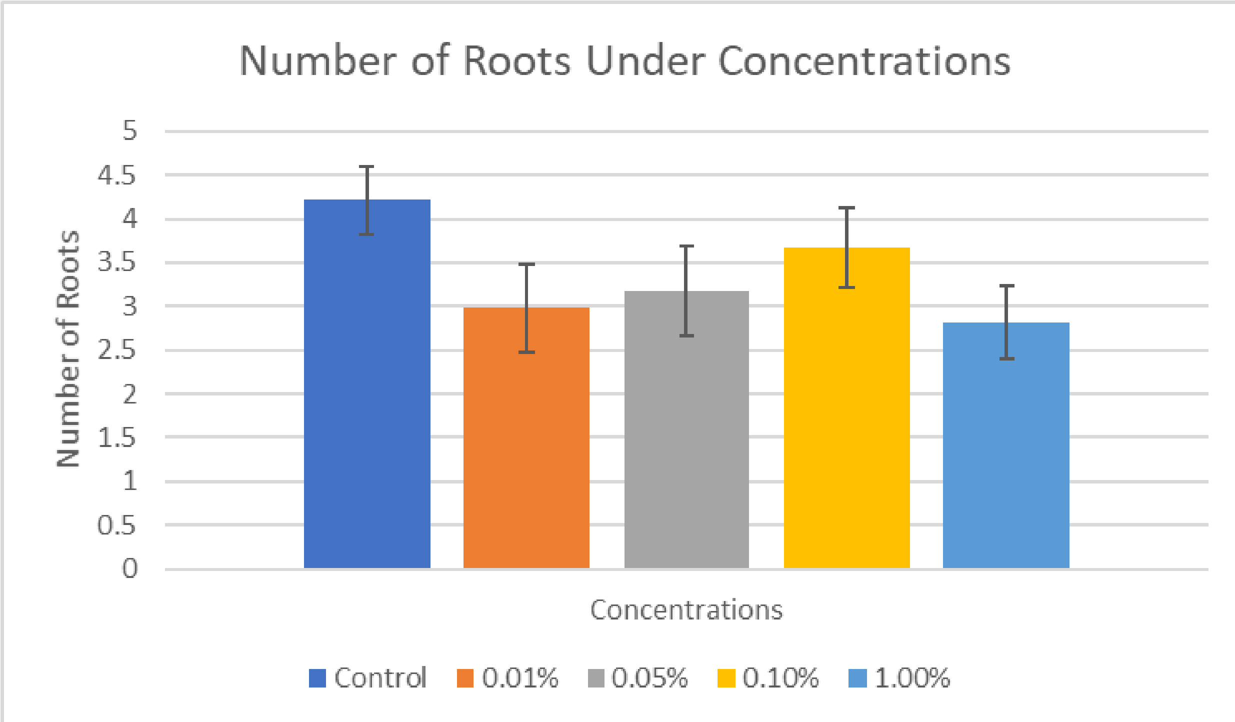

Figure 5: This graph shows the average number of roots for each plant in the three replicants for each concentration.

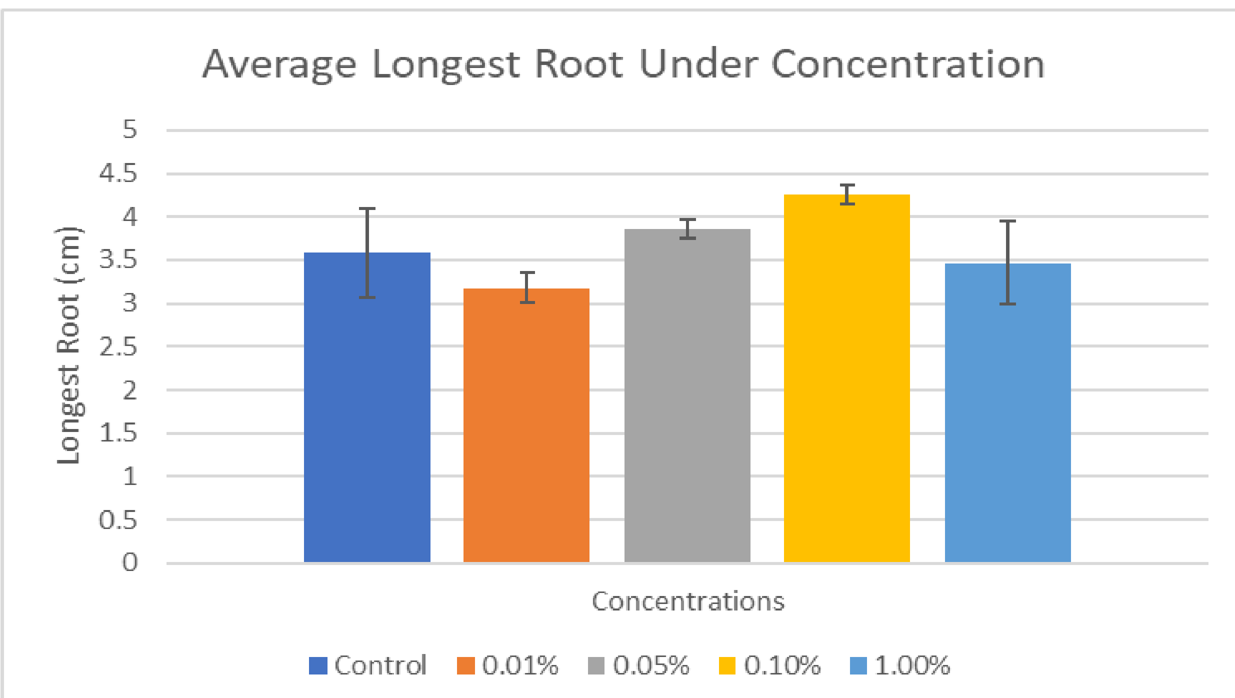

Figure 6: This graph shows the average longest root for each plant in the three replicants for each concentration with standard deviation within the groups.

Averaged Data:

The averaged number of leaves, number of roots, full length (cm), plant length (cm), root weight (g), and plant shoot weight (g) were tabled together along with their own variation amongst the three biological replicates of the control, (Table 3) 0.01% group, (Table 4) 0.05% group, (Table 5) 0.1% group, (Table 6) and 1.0% group. (Table 7)

Control	# of leaves	# of roots	full length (cm)	Root length (longest)(cm)	root weight (g)	shoot weight (g)
Replicant 1	3.22727273	4.45	7.2	3.709091	0.056273	0.188409
Replicant 2	3.76190476	3.761904	7.857143	3.019048	0.034667	0.273095
Replicant 3	5.66667	4.428571	7.009524	4.02381	0.061143	0.178667
Average	4.21861583	4.213492	7.355556	3.583983	0.050694	0.21339
Std	1.282226	0.391232	0.444705	0.513932	0.014092	0.051935

Table 3: Above shows a tabular version of the already averaged number of leaves, roots, full length (cm) , root length (cm) , root weight (g), and shoot weight (g) from the twenty-five seeds in each of the replicants in the control group. Further the data from each of the replicants were averaged together to form a new standard deviation.

0.01%	# of leaves	# of roots	full length (cm)	Root length (longest)(cm)	root weight (g)	shoot weight (g)
Replicant 1	3.2631579	2.789474	6.978947	3.278947	0.036474	0.159211
Replicant 2	3.6	2.6	6.74	2.98	0.05695	0.12765
Replicant 3	4.47619048	3.55	7.01	3.285	0.0765	0.11095
Average	3.77978279	2.979825	6.909649	3.181316	0.056641	0.132604
Std	0.6261816	0.502792	0.147739	0.174371	0.020015	0.024509

Table 4: Above shows a tabular version of the already averaged number of leaves, roots, full length (cm) , root length (cm) , root weight (g), and shoot weight (g) from the twenty-five seeds in each of the replicants in the 0.01% group. Further the data from each of the replicants were averaged together to form a new standard deviation.

0.05%	# of leaves	# of roots	full length (cm)	Root length (longest)(cm)	root weight (g)	shoot weight (g)
Replicant 1	4.15789474	2.631579	7.184211	3.752632	0.046158	0.163737
Replicant 2	4	3.65	6.865	3.88	0.0336	0.07145
Replicant 3	4.23809524	3.238095	6.914286	3.957143	0.036952	0.113143
Average	4.13199666	3.173225	6.987832	3.863258	0.038903	0.11611
Std	0.12114194	0.5123	0.171845	0.103278	0.006502	0.046215

Table 5: Above shows a tabular version of the already averaged number of leaves, roots, full length (cm) , root length (cm) , root weight (g), and shoot weight (g) from the twenty-five seeds in each of the replicants in the 0.05% group. Further the data from each of the replicants were averaged together to form a new standard deviation.

0.10%	# of leaves	# of roots	full length (cm)	Root length (longest)(cm)	root weight (g)	shoot weight (g)
Replicant 1	3.89473684	3.157895	6.842105	3.426316	0.036474	0.241211
Replicant 2	3.7	3.85	6.535	3.675	0.0987	0.1767
Replicant 3	4.35294118	4	8.511765	5.664706	0.056059	0.112882
Average	3.98255934	3.669298	7.29629	4.255341	0.063744	0.176931
Std	0.33521283	0.449194	1.063773	1.226863	0.031817	0.064164

Table 6: Above shows a tabular version of the already averaged number of leaves, roots, full length (cm) , root length (cm) , root weight (g), and shoot weight (g) from the twenty-five seeds in each of the replicants in the 0.01% group. Further the data from each of the replicants were averaged together to form a new standard deviation.

1.00%	# of leaves	# of roots	full length (cm)	Root length (longest)(cm)	root weight (g)	shoot weight (g)
Replicant 1	3.16666667	2.388889	6.355556	2.911111	0.019889	0.061333
Replicant 2	3.77777778	3.222222	7.5	3.75	0.033111	0.095389
Replicant 3	3.41176471	2.823529	6.723529	3.752941	0.038235	0.096353
Average	3.45206972	2.811547	6.859695	3.471351	0.030412	0.084358
Std	0.30754279	0.416796	0.584247	0.485184	0.009466	0.019946

Table 7: Above shows a tabular version of the already averaged number of leaves, roots, full length (cm) , root length (cm) , root weight (g), and shoot weight (g) from the twenty-five seeds in each of the replicants in the 1.0% group. Further the data from each of the replicants were averaged together to form a new standard deviation.

Discussion

Analysis of Variance ANOVA, findings show a statistical difference in germination rates where the control had higher germination rates on average than the groups with higher concentrations of Aluminum Oxide. As seen in Table 1, Table 2, and Figure 1 there is a downward trend as concentration increases with the control group having an average germination rate of 85.33% ± 5.33 where the highest concentration group showed a 70% ± 4 germination rate. There is high variance in the 0.1% group due to one specific plate producing a high germination rate and higher than expected overall plant growth. Without this outlier the rest of the 0.1% replicants followed the trend of lower germination rates as concentration increases.

The plant weight also showed a large statistical difference in the control's weight in comparison to that of higher concentrations. As seen in figure 3 there is a downward trend as concentrations increase. Again the 0.1% group had an outlying high growth plate that did not outgrow the control, but significantly raised the average weight of the concentration group overall. As seen in Tables 3 and Table 7, the control group's average weight was 0.213 g ± 0.05g in comparison to the 1.0% group being almost one third of this weight at 0.084 g ± 0.02 g average.

Root weight showed a large statistical difference in the control's weight in comparison to that of higher concentrations.As seen in figure 4 there is a downward trend as concentrations increase. Again the 0.1% group had an outlying high growth plate that outgrow the control, and significantly raised the average weight of the concentration group overall. Since root weight is generally a relatively small number, being 0.050g ± 0.014 in the control and 0.03g ± 0.009 in the 1.0% group. It is easier for variance to occur with the 0.1% group having an average root weight of 0.063g ± 0.031g.

The average number of leaves had moderate statistical difference between groups of higher concentration with a trend downward as concentrations increase. As seen in Figure 2, there is a slight trend downward in the average number of plant leaves with the average number of leaves always staying within 3-4 leaves.

The average number of roots had moderate statistical difference between groups of higher concentration with a trend downward as concentrations increase. As seen in Figure 5 there is a downward trend in the number of roots with the lowest being the 1.0% concentration group averaging less than 3 roots per plant while the control group averaged over 4.

The total plant length and average longest root for individuals in each concentration showed slight correlation between concentrations of Aluminum Oxide. The average total plant length stayed roughly similar throughout concentrations while slightly trending downward the total average difference from the control to the 1.0% group was only 0.5 cm on average. The longest root for each of the plants showed low correlation where the 0.05% and 0.1% group had the highest average longest root. At 3.86 cm $\pm$ 0.10 cm and 4.25 cm $\pm$ 1.22 cm respectively in comparison to the control that was 3.58 $\pm$ 0.51.

Literature Cited

1. Eleazer P. *Resurreccion*, Javad Roostaei, Mason J. Martin, Randy L. Maglinao, Yongli Zhang, Sandeep Kumar, The case for camelina-derived aviation biofuel: Sustainability underpinnings from a holistic assessment approach, Industrial Crops and Products, 10.1016/j.indcrop.2021.113777, 170, (113777), (2021).
2. Gong CC, Wang LH, Li XL, Wang HS, Jiang YX, Wang WX (2019) Responses of seed germination and shoot metabolic profiles of maize (Zea mays L.) to Y2O3 nanoparticle stress. RSC 580 Adv 9:27720–27731 Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR)
3. NNC National Nanotechnology Initiative. Washington, D.C: National Nanotechnology Coordination Office. National Nanotechnology Initiative (U.S.), Office.
4. Rittner MN, Abraham T. Nanostructured materials: an overview and commercial analysis. *Journal of the Minerals, Metals, and Materials Society.* 1998;50:37–38.
5. Sadiq, I. Mohammed, et al. "Studies on Toxicity of Aluminum Oxide (al2o3) Nanoparticles to Microalgae Species: Scenedesmus Sp. and Chlorella Sp.." *Journal of Nanoparticle Research*, Springer Netherlands, 4 Feb. 2011, from https://link.springer.com/article/10.1007/s11051-011-0243-0.
6. Wu YF, Peng WMH, Dong ZD, Jiang QQ, Yu XR, Chen G, Xiong F (2020) Exogenous application of NaBiF4 nanoparticle affects wheat root development. BMC Plant Biol 20:140
7. Yanık, Fatma, and Filiz Vardar. "Toxic Effects of Aluminum Oxide (al2o3) Nanoparticles on Root Growth and Development in Triticum Aestivum." *Water, Air, & Soil Pollution*,

Springer International Publishing, 13 Aug. 2015, from
https://link.springer.com/article/10.1007/s11270-015-2566-4.