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Article

Effects of polyethylene microplastics on the growth of *Arabidopsis thaliana* & *Phaseolus vulgaris* and their soil

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SUMMARY

As the amount of manufactured goods in the world increases, their disposal methods also come into question with many plastic products ending up in both the ocean and on land. These plastics can break down, creating microplastics that can persist for long time periods. While extensive research has been done on the effects of microplastics on marine ecosystems, less is known about the effects of different concentrations of microplastics in terrestrial ecosystems. Our study used the plants *Arabidopsis thaliana* and *Phaseolus vulgaris* to explore the effects of microplastics on plant growth and soil quality. We hypothesized that an increase in the concentration of microplastics would result in shorter plant height and root length, as well as a reduced water holding capacity (WHC) of the soil. We found that the majority of the results were not statistically significant, except for the soil's WHC for *P. vulgaris*, where the 0.5 µg/L treatment was lower than the control. These findings can serve as a guide for future studies that can further explore the effect of microplastics on terrestrial ecosystems.

INTRODUCTION

As the world becomes increasingly dependent on manufactured goods, the amount of disposable plastic products is also increasing (1). Plastic production has increased exponentially from 2.3 million tons produced per year in 1950 to 448 million tons in 2015 (1). This number is expected to double by 2050 (1). This exponential growth of plastic production is especially evident in both developing and developed countries where recycling systems are lacking or non-existent (1). Due to the additives that are used in plastics, decomposition takes up to 400 years. Because of this, plastics often end up in landfills or incinerators (1,2). The landfills are susceptible to failures, meaning solid waste and hazardous chemicals can leak through the clay liners and leach into groundwater (2). Burning plastic in incinerators releases acidic gases, carbon monoxide, nitrogen oxides, particulate matter, and various volatile organic compounds which pollute the environment and can cause negative health outcomes such as skin diseases, developmental problems, immune system and liver damage, and even cancer (2). Since plastic takes so long to decompose, considerable amounts of plastic build up both in the ocean and on land, continuously harming the well-being of wildlife and humans.

Since oceans are the main transport pathway for plastic products, they contain the majority of plastic debris. Currently, 8 million tons of plastic end up in our oceans every year (3). With increasing exposure to UV light, plastics start to break down into microplastics; these particles are smaller than 5 nanometers and can have a heavy toll on wildlife (3). Animals are susceptible to being caught in the floating trash or mistaking it for food. Ultimately, these animals die and shift the balance of the ecosystem. Many studies concerning plastic contamination have been on marine life. In 2013, a study showed that in Australia the majority of plastic pollution occurs right off the eastern coast where the main cities are located (4). The plastic contamination levels were similar to the results of studies conducted in the Caribbean Sea and Gulf of Maine (4). These locations are all host to a plethora of wildlife, and negative impacts on megafauna, small fish, and zooplankton have been reported (4). The presence of microplastics in seafood impacts human health as well. Since microplastics are easily mistaken for food by many marine organisms, they accumulate in the gastrointestinal tracts of these organisms since microplastics do not break down easily (5). The ingestion of microplastics by marine organisms negatively impacts humans as well because we can ingest the toxins produced by plastics when eating seafood.

While research on microplastics in marine environments has been conducted since the early 2000s, there is limited literature on the effect of plastic pollution in soils since research on microplastics in terrestrial ecosystems only started in 2016 (6). From this limited research, microplastics disintegrate and insert themselves into soil either directly or through sewage used as fertilizer (7). These microplastics can seep into groundwater and have toxic effects on anyone who uses that water source, which can result in the disruption of the hormone system (7). Microplastics also affect the nutrient cycle in plants (8). Due to their smaller density, microplastics are more likely to be taken up by mechanical transport than nutrients like nitrogen, phosphorus, or potassium (8). This intake of microplastics can then affect the microbial activities in the soil and the nitrogen cycle in particular (8). Increasing concentrations of microplastics in the soil reduces soil enzyme activity, which limits microbial nitrogen transformations. (8). For example, the presence of low-density polyethylene microplastics contributed to a lack of nitrogen in common bean plants (9). Additionally, a recent study found that adding plastic fibers or microplastics like high density polyethylene (HDPE) and polylactic acid (PLA) to soil decreases the germination rate of the *Lolium perenne* plant (10). More specifically, PLA reduced the shoot length of *L. perenne* and HDPE increased its biomass; however, the study found no significant relationship between germination success and root to shoot biomass (10). The presence of microplastics also lowered the pH of soil, the soil bulk density, water holding capacity (WHC), and the relationship between microbial activity and water stable aggregates in soil (10,11).

By measuring the WHC of the soil, we can determine if the soil has retained enough water for the plants to use. If soils cannot retain water properly, plants will become stressed and not intake the necessary nutrients (12).

The majority of research on microplastics in soil has examined the impact on overall microbial activity, bacterial transport, and the spread of antibiotic resistant genes (ARGs) (13). Polypropylene plastics have shown a positive effect on soil microbial activity, whereas polyacrylic, polyester, and polystyrene plastics show a negative effect (13). However, definite conclusions cannot be drawn since each type of microplastics differed in size and shape (13). It is also possible that polystyrene plastics facilitate the transmission of ARGs in soil environments, but more research is necessary for an accurate conclusion (13).

Of the numerous organisms that live in the soil, only a few have been examined to determine how microplastics affect them (13). These organisms include nematodes, oligochaetes, collembolan, and isopods (13). In nematodes, the 1μm polystyrene resulted in the shortest life span and body length, most likely due to the fact that the 1 μm particles are more easily ingested by the nematodes (13). The effect of microplastics on oligochaetes are dependent on the amount of exposure (13). The oligochaete, *Enchytraeus crypticus,* was not affected until a 10% exposure to the particles (13). Collembolan are more sensitive to microplastic pollution; exposure to 0.1% PVC microplastics for 56 days decreased both growth and reproduction rates (13). The presence of polyethylene microplastics in the soil for 14 days showed little effect on isopods; however, research examining the effect of long-term exposure is necessary (13). Although the effects of microplastics on soil organisms have been explored, there is still limited research in the effects of microplastics on plants, especially since microplastics also pose a threat to the health of humans (14).

Previous research on microplastics in seafood has shown that they accumulate in the tissues of seafood, which could expose humans to pathogenic microbes (14). A past study showed that microplastics have been found in fruits and vegetables (15). They are taken in through the roots of the produce and transported by the xylem and phloem to settle in the plant tissue (15). These microplastics could then enter into people when the fruits and vegetables are consumed. Once microplastics enter into the body, they can potentially cause metabolic disturbances, neurotoxicity, carcinogenic effects, and disrupt the endocrine system (16).

In this project, we investigated how an increase in the concentration of microplastics in soil affects plant growth and soil characteristics. We used *A. thaliana* and *P. vulgaris* due to their quick germination time and simple maintenance and exposed them to polyethylene microspheres, the polymer that is most used in commercial plastics. Previous studies found that 0.02% concentration of polyethylene microplastics negatively impacted germination rate, leaf number, and biomass in garden crest plants, while a 1% concentration of microplastics on wheat plants resulted in negative effects on plant growth (17, 18). Therefore, we exposed the plants to four different microplastics concentrations, 0%, 0.25%, 0.5%, and 1%.

Plant growth is most affected by the amount of water, light, and nutrients they receive, as well as the temperature of their surroundings (19). Since all plants experienced the same temperature, water, and light regimes, we measured plant height, root length, and root to shoot weight ratio to determine if the amount of nutrients the plants intake varied from treatment to treatment. We hypothesized that higher concentrations of microplastics would negatively impact the plant and soil. Specifically, we expected that the treated plants would not grow as much as the controls and would have brittle leaves with a paler color, while the WHC of the soil would decrease with increasing concentration of microplastics (10,11,12). The overall purpose of this study is to examine the effect of microplastics on plant and soil health.

RESULTS

In this experiment, we exposed *A. thaliana* and *P.* to four different concentrations of polyethylene microplastics (0%, 0.25%, 0.5%, 1%) for a three-week period. We chose the type and concentration of microplastics based on the results of previous studies examining the effects of microplastics on other plants (10,11,12). We measured the WHC of the soil of both plants to determine if they were able to properly intake nutrients (12). We also measured the plant height and root length as indicators of plant growth (19).

Over the three-week period, the number of *P. vulgaris* sprouts remained relatively constant across all treatments. Five seeds were put into each trial for *P. vulgaris*. At the end of three weeks, an average of 1.8 sprouts grew across all trials (p = 0.3580, **Table 1**). No more than three seeds sprouted for any trial and only two of the twelve trials did not have any sprouts (p = 0.3580, **Table 1**). The number of *A. thaliana* sprouts were not as consistent. We planted seven to nine *A. thaliana* seeds for each condition and an average of 2.6 sprouts grew across all trials (p = 0.4112, **Table 2**). No more than eight seeds sprouted for any trial and four of the twelve trials had zero sprouts (p = 0.4112, **Table 2**).

The height of *P. vulgaris* sprouts did not differ between the control and treatments (p = 0.2027, **Figure 1A-C**) (7). Average height across all *P. vulgaris* sprouts on day 14 was 21.04 cm +/- 10.71. Additionally, as the concentration of microplastics increased, the leaves of the *P. vulgaris* plants were darker and the stems started to turn purple (**Figure 2**). When observing the *P. vulgaris* plants, we found brown spots on the leaves of the treated plants. These leaves also had a more brittle texture than the leaves of the plants with no treatment. These

Treatment	Trial Number	14 days since original planting	18 days since original planting	22 days since original planting
0%	1	5	8	8
0%	2	1	2	2
0%	3	1	2	2
0.25%	1	3	3	3
0.25%	2	3	3	3
0.25%	3	0	0	0
0.50%	1	0	0	0
0.50%	2	2	2	2
0.50%	3	0	0	0
1%	1	0	0	0
1%	2	3	5	5
1%	3	5	6	6

Table 1. The number of sprouts for each treatment for *P. vulgaris*. Conducting a single factor ANOVA test showed that the number of seeds sprouted did not differ between the control and treatments (p = 0.3580).

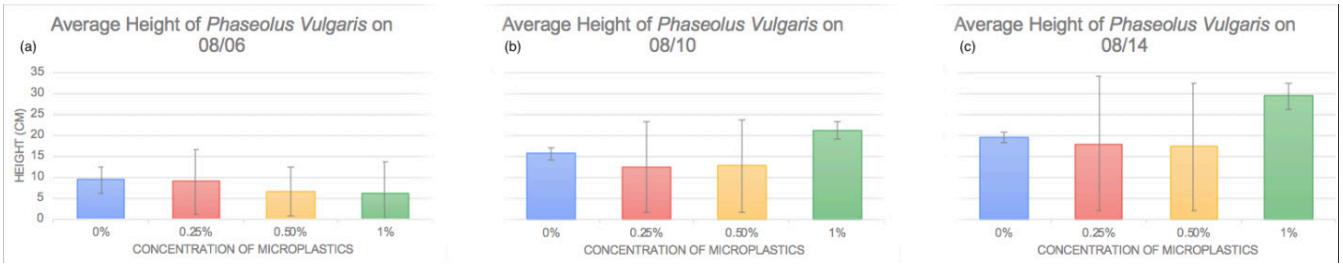

Figure 1: Plant height over time. (A) The average height of *P. Vulgaris* on the first day of measurement, August 6th, 2021. The bar represents the mean height and the error bars are the standard deviation. **(B)** The average height of *Phaseolus Vulgaris* on the second day of measurement, August 10th, 2021. The bar represents the mean height and the error bars are the standard deviation. **(C)** The average height of *Phaseolus Vulgaris* on the last day of measurement, August 14th, 2021. The bar represents the mean height and the error bars are the standard deviation. Conducting a double factor ANOVA test without replacement showed that the average height of the *Phaseolus vulgaris* plant did not differ between the control and the treatments (p = 0.2027).

data were not quantified. We did not measure the height of *A. thaliana* sprouts since only the top of the sprouts were visible above the soil (**Figure 3**).

The average root length of the *A. thaliana* plant did not differ between the control and the treatments (p = 0.4507, **Figure 4A**). The average root length of the *P. vulgaris* plant also did not differ between the control and the treatments (p = 0.87, **Figure 4B**). There was no difference in the average root to shoot weight ratio between the control and the treatments for *P. vulgaris* (p = 0.7592, **Figure 5**).

The WHC of the *A. thaliana* soil did not differ between the control and the treatments (p = 0.1573, **Figure 6A**). However, the WHC for the *P. vulgaris* soil treated with 0.5 µg/L microplastics was significantly lower than the control (p = 0.0306, **Figure 6B**). There was no difference in WHC for *P. vulgaris* between any of the microplastics concentrations and the control. When conducting the WHC test for both types of plants, after three hours there was more white material floating at the top for treatments with a higher concentration of microplastics. Finally, the control and treatment 0.25 µg/L had a soil pH of 7 and while treatments 0.5 µg/L and 1 µg/L had a soil pH of 6.5 (**Table 3**).

DISCUSSION

Our study showed that increasing the concentration of microplastics did not have a statistically significant effect on the growth of *A. thaliana* and *P. vulgaris* plants as measured by plant height and root length. Our results of no microplastic effect on plant growth suggest that more studies with a

Treatment	Trial Number	14 days since original planting	18 days since original planting	22 days since original planting
0%	1	2	2	2
0%	2	1	1	1
0%	3	2	3	3
0.25%	1	1	1	1
0.25%	2	1	2	2
0.25%	3	0	0	0
0.50%	1	2	2	2
0.50%	2	2	3	3
0.50%	3	0	0	0
1%	1	1	3	3
1%	2	1	3	3
1%	3	2	2	2

Table 2. The number of sprouts for each treatment for *A. thaliana*. Conducting a single factor ANOVA test showed that the number of seeds sprouted did not differ between the control and treatments (p = 0.4112).

Figure 2: Photo of *P. vulgaris* at the end of three weeks. The treatments with higher concentrations are slightly more colored in the stems. From left to the right, the first column had 0% microplastics, the second 0.25%, the third 0.5%, and the last column 1%. From top to bottom, the first row is trial 1, the second trial 2, and the third trial 3.

Figure 3: Photo of *A. thaliana* at the end of three weeks. The plants did not grow enough for their heights to be measured. From left to the right, the first column had 0% microplastics, the second 0.25%, the third 0.5%, and the last column 1%. From top to bottom, the first row is trial 1, the second trial 2, and the third trial 3.

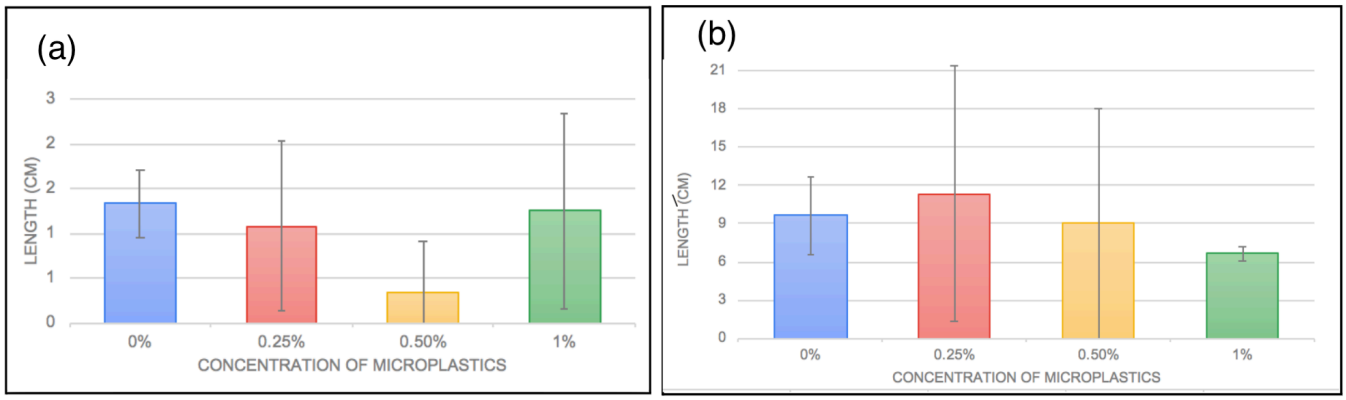

Figure 4: Average root length of *A. thaliana* and *P. vulgaris* measured three weeks after planting. (A) The bar represents the mean root length and the error bars are the standard deviation. Conducting a single factor ANOVA test showed that the average root length of the *A. thaliana* plant did not differ between the control and the treatments (p = 0.4507). **(B)** The bar represents the mean root length and the error bars are the standard deviation. Conducting a single factor ANOVA test showed that the average root length of the *P. vulgaris* plant also did not differ between the control and the treatments (p = 0.87).

greater range of microplastic sizes and plant types need to be conducted to further understand the effect of microplastics on soil-plant interactions. The concentrations of microplastics used in this study were very low, so it is possible that such a small concentration did not impact the overall growth of these plants. Additionally, the actual experiment took place over the course of three weeks, which limited how tall the plants grew, especially the *A. thaliana*.

We also found that microplastics led to plant discoloration, possibly due to impaired nutrient uptake. In the plants we treated with higher concentrations of microplastics, we observed brown spots on the leaves. Brown spots often indicate a lack of phosphorus (20). Though we were unable to quantify this pattern, we first observed the difference in coloration on the second day of observation and continued for the duration of the experiment.

Additionally, we found that the WHC of the soil for *P. vulgaris* was lower in the 0.5 µg/L treatment compared to the control while the WHC of the soil for *A. thaliana* did not differ by treatment. Previous studies indicated that soil properties such as structure, function, or microbial diversity, are influenced by microplastics, but that these specific impacts differ by type of microplastic (21). Polyester fibers have been found to increase the WHC of soil, whereas polyethylene and poly acrylic acid have no clear impact on WHC (21). In contrast, the polyethylene microplastics that we used did impact the WHC of the soil in the *P. vulgaris,* suggesting that the effect of microplastics on soil WHC is complex

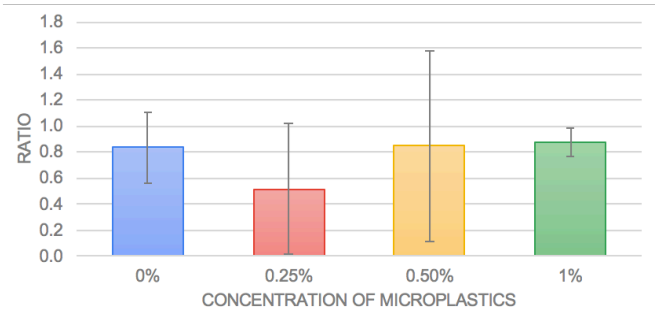

Figure 5: Average root to shoot weight ratio for *P. vulgaris* taken three weeks after planting. The bar represents the mean root to shoot ratio and the error bars are the standard deviation. Conducting a single factor ANOVA test showed that there was no difference in the average root to shoot weight ratio between the control and the treatments for *P. vulgaris* (p = 0.7592).

The soil used for this experiment contained lime, and after letting the soil rest for three hours in 40 mL of water, more lime was present at the tops of the cups which contained a higher concentration of microplastics in the soil . Liming soil helps restore acidic soil to its natural pH and increases the amount of nutrients available (22). Without the application of lime and the presence of fertilizer, the pH of soil decreases (23).

Plastic usage and waste is rapidly increasing across the world with many counties lacking proper disposal methods, so understanding the effect of microplastics in our ecosystem has become more important than ever (1). Research in aquatic systems has shown the negative effect of the accumulation of microplastics in aquatic organisms, such as crustaceans and fishes (24). While less research has been conducted on the effect of microplastics in terrestrial ecosystems, it appears to have similar effects to microplastics in water (24, 25). Microplastics can easily be ingested by species that are important for ecosystem processes such as nutrient cycling and decomposition (25). If these species, which are often keystone species, are negatively impacted, then the entire ecosystem could be at risk.

Currently, there are few studies that examine the effect of microplastic concentration on terrestrial plant growth. Therefore, the results from this study are important in understanding the impact of microplastics on plant and soil health. Since the majority of the results from this study were not statistically significant, future studies should investigate these effects either over a longer period of time or use a higher concentration of microplastics to understand how terrestrial plants are impacted by microplastics. The roots and stems of these plants can also be examined to determine if microplastics can enter into the plants and affect its intake of nutrients and well as using different plants for the study.

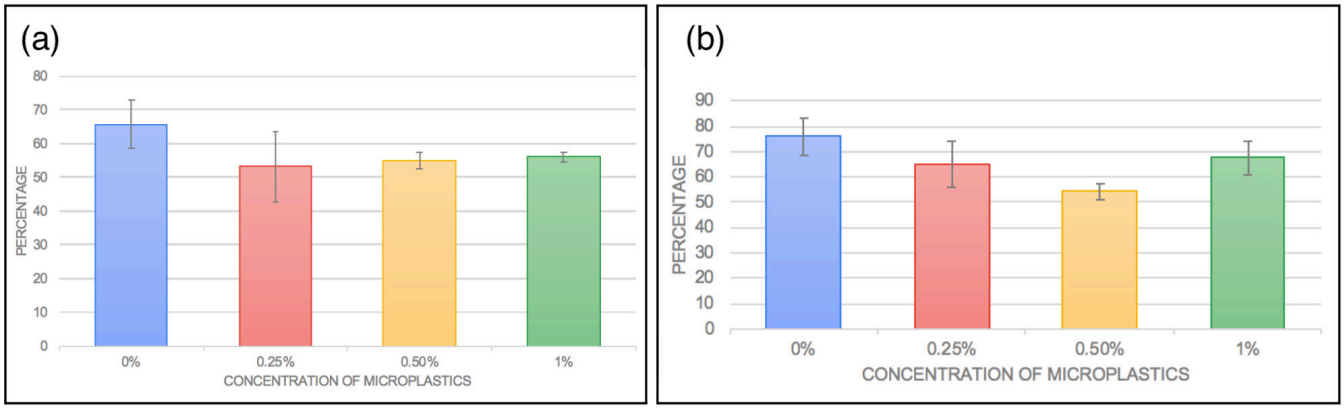

Figure 6: Average WHC for *A. thaliana* and *P. vulgaris* taken three weeks after planting. (A) The bar represents the mean WHC and the error bars are the standard deviation. Conducting a single factor ANOVA test showed that the WHC of the *A. thaliana* soil did not differ between the control and the treatments (p = 0.1573). **(B)** The bar represents the mean WHC and the error bars are the standard deviation. Conducting a single factor ANOVA test showed that the WHC for the *P. vulgaris* soil differed by treatment (p = 0.0306).

MATERIALS AND METHODS

Growth Conditions

To examine the effects of microplastics on plant and soil health, we chose *A. thaliana* and *P. vulgaris* because they have short germination periods and are simple to grow (26,27). This project took place in Cupertino, CA from July 23, 2021 to August 15, 2021. The average temperature during this time period was 24ºC during the day and 15ºC during the night (28). About 10 g of air cleaning indoor soil (containing a mixture of peat, perlite, lime, and worm castings) was added to fill three-fourths of a 1.5 X 1.5 X 2 in pot for all 24 trials. We added "clear polyethylene microspheres 0.96 g/cc – 1 µm to 1700 µm" (Cospheric) in concentrations of 0%, 0.25%, 0.5%, and 1% to the soil of both *A. thaliana* and *P. vulgaris*. The microplastics were added on top of the soil, and a small layer of soil was added on top of the microplastics. For *P. vulgaris,* five seeds were added to each pot and placed indoors on the ground under a well-lit floor-to-ceiling window. The *A. thaliana* seeds were purchased in a pack of 100 seeds (Arabidopsis Biological Resource Center). Due to their small nature, 100 *A. thaliana* seeds were put into a bowl and mixed with water. A small pipette was used to pick up six to nine seeds that were spread evenly through the pots. The *A. thaliana* plants were kept in the freezer at 4ºC for three days to stratify them and then put under a strong light during the night, since they needed to consistently be under a strong light source at all times (26,29). They were placed in a well-sunlit area during the day, alongside the black bean plants. Each condition was performed in triplicate (**Figure 7**).

Treatment	Trial Number	pH
0%	1	7
0%	2	7
0%	3	7
0.25%	1	7
0.25%	2	7
0.25%	3	7
0.5%	1	6.5
0.5%	2	6.5
0.5%	3	6.5
1%	1	6.5
1%	2	6.5
1%	3	6.5

Table 3: The pH of the soil after mixing with 40 mL of water for three hours.

Growth Measurements

Once the *P. vulgaris* seeds started to sprout after nine days, the number of sprouts in each condition was recorded, as well as observations about plant height, texture and color of the leaves and stem. The height of the plants was measured using a 30 cm ruler and the texture was evaluated through touch. If few bumps were felt throughout a leaf, it was qualified as smooth; otherwise, it was qualified as brittle. These measurements were taken every four days until the end of the experiment. On the final day, to record the color of the leaves, a printed green color scale showing 20 different shades was used to determine how the leaves from each treatment compared to each other (30). Because the *A. thaliana* seeds did not grow as quickly, the number of sprouts and height of the plants were measured on days 18 and 22.

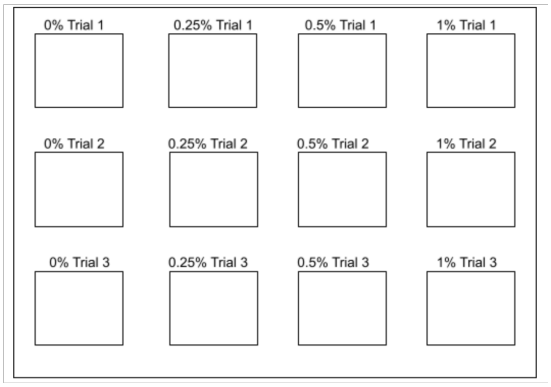

Figure 7: The experimental setup for both *A. thaliana* and *P. vulgaris*.

Root Length

After three weeks of growth, all plants were excavated from the soil and the tallest sprout's roots were measured and recorded. The weight of the roots and shoots of all the *P. vulgaris* plants were measured using a regular kitchen scale in grams. The root to shoot weight ratio of the *P. vulgaris* was then calculated. The root to shoot ratio for *A. thaliana* was not calculated since they did not grow beyond a small sprout that extended slightly above the soil line (**Figure 2**).

pH and Water Holding Capacity

The WHC of all plants were measured after three weeks of growth. All the soil was dug out of the pots and placed in a 100mL beaker with 40mL of water. The mixture was stirred with a glass rod and left to sit for three hours. After this time, pH was used to record the pH of the soil. To measure the WHC, filter paper was placed into a funnel over a large cup and the soil mixture was poured into the funnel. The mixture was left to sit for 10 minutes and the water that fell into the cup was poured into a 50mL graduated cylinder, and the amount of water was recorded for all 24 trials (31).

Data Analysis

The data were analyzed using Excel. First, we calculated the mean and standard deviation for each measurement (plant height, soil WHC, and root length). Next, using the mean values from each condition, the p-value between each treatment was calculated by using a single factor ANOVA test for all categories except the height of the plants which used a double factor ANOVA test without replacement because the data was collected over three different days. Significance was evaluated at $p < 0.05$. After the ANOVA tests, when there was a statistically significant difference, a Tukey Kramer post hoc test was used. Each trial was compared to each other by finding a q value and comparing it to the studentized range Q Table ($p < 0.05$).

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