Effects of different calcium salts in growth solutions on the growth of the stems of basil (*Ocimum Basilicum*) plants grown hydroponically

What effects do different calcium salts in growth solutions have on the growth of the stems of basil (*Ocimum basilicum*) plants grown hydroponically?

Biology

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Introduction

Research Question

What effects do different calcium salts in growth solutions have on the growth of the stems of basil (*Ocimum basilicum*) plants grown hydroponically?

Background

Our planet has a population of over 7 billion individuals and we, as a society, must provide the necessary food to ensure the survival of this immense population. Therefore, we're coming up with new and imaginative ways of growing crops to accommodate for the lack of farming space and ever-increasing population. A popular alternative is hydroponics: the growth of plants and crops via soilless media by providing a source of water and soluble nutrients through artificial means (Hydroponics and the Future). Hydroponics has many applications and will be of great significance in the future. As it's estimated that in 2050, the global population will be increasing by 3 billion people, urban hydroponic farming is becoming a conventional alternative to typical rural agriculture (Hydroponics and the Future). In addition to climate change, big cities are aiming to reduce their ecological footprint and one way of achieving this is by incorporating farming into existing architecture as this reduces transport, labor costs, and is a large space-saver as vertical farming is becoming more prevalent (Thomaier et al. 44).

Although the set up is quite expensive, overall output and production increase greatly. The ecological benefits are immense; although these systems require more water initially, the water is recycled and less is needed in the long run. Moreover,

farming of this nature means nations with soil not fertile enough for farming are able to farm regardless of the environment as most hydroponic systems are maintained buildings with controlled environments (Hydroponics and the Future).

Since plants grown in hydroponic systems are not dependent on soil for nutrients, they rely on soluble macronutrients, nutrient needed in large quantities for plant survival, available via irrigation to carry out biological processes (Brown). The most crucial of these nutrients are nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur ("Nutrient Uptake"). The secondary nutrients are not needed in as great a quantity as the primary ones. Therefore, their roles in plant growth could be of more interest because a slight change in the concentration provided could easily disrupt the biological functions that promote growth (Buechel).

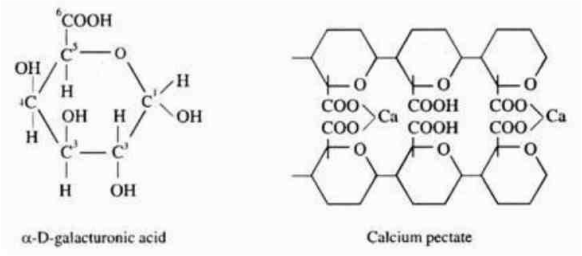


Figure 1: Galacturonic Acid and Calcium Pectate Structure; reprinted from: *Calcium Pectate*. *Biology Discussion*, www.biologydiscussion.com/plant-physiology-2/eukaryotic-plant-cell-with-diagram/226

One secondary macronutrient that's of particular interest is calcium. Calcium has various roles throughout the plant from cell wall formation and strength to assisting in the uptake of other nutrients by changing permeability (Sela). Firstly,

calcium directly affects the formation of the middle lamella during mitosis in plants as it binds with the pectin to form calcium pectate (see Figure 1) that in turn strengthens the lamella ("Calcium: A Central" 1). The middle lamella is the organelle formed during plant cytokinesis and is crucial in connecting adjacent cells and providing strength and sturdiness to plants ("Middle Lamella"). However, the Ca^{2+} cation isn't positively associated with plant cell growth. When the calcium cation concentration is elevated, stem growth can be stunted ("Calcium: A Central" 1). Consequently, lower concentrations of the cation result in increased growth and cell elongation. This may mean a larger crop but can also mean a weaker one, as calcium pectate concentrations will be lower. A so-called weaker crop in agriculture isn't what farmer's want, as these crops are more susceptible to environmental damage such as harsh weather and pests. Calcium is especially critical for cell structure in the roots of plants as they must support and anchor the entire plant (Schwartzkopf 2). Calcium also affects membrane permeability. Ca^{2+} can bind to the phospholipids that make up the cellular membrane of plant cells; resulting in a tighter membrane that's less susceptible to ion leakage ("Calcium: A Central" 2). Conversely, when calcium concentration decreases, plant cells can easily lose other essential ions and aren't able to retain these as well as with the assistance of the Ca^{2+} cation ("Calcium: A Central" 2).

Although calcium does not play as direct a role in plant growth as say other nutrients like nitrogen and phosphorus, it plays a key role in the structure of a plant and its uptake of nutrients. Calcium is an immobile nutrient. meaning that once it has been taken in by the plant, it will remain wherever it's transported forever and its movement relies entirely on water transport in the xylem

("Knowing nutrient") (Morgan). Therefore, new leaves tend to be more calcium deficient than older leaves as the latter already has a supply of calcium. Symptoms of calcium deficiency include leaf tip burn, poor growth, and fruit rotting (White and Broadley 488) (Sela). For this reason, it's imperative to provide constant calcium to plants in order to supply newer leaves with necessary Ca^{2+} for development (Morgan). Transpiration is the movement of water throughout the plant xylem (See Figure 2); this movement is crucial not only for water transport but also mineral transport. If transpiration is halted, so is the movement of essential nutrients, causing deficiency symptoms.

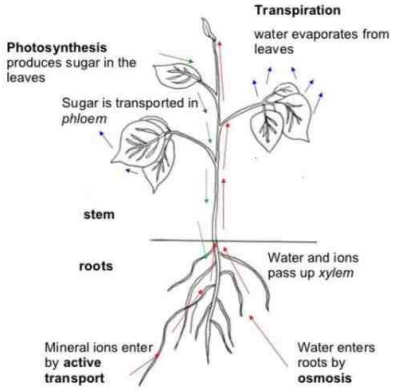


Figure 2: Flow of water and ions through plant assisted by transpiration; reprinted form: *Water Transport in Plants*. *Cheppila*, cheppila.com/?p=194. Accessed 6 Sept.

Calcium salts in hydroponic irrigation are a cost effective source of calcium for farmers (Morgan). Calcium salts are most suitable as they are water-soluble and can provide both calcium and other necessary nutrients such as nitrogen, sulfur, chlorine, and phosphorus. Since calcium relies so greatly on water to reach its target in the plant, it competes with other immobile soluble nutrients in the xylem. Such nutrients include potassium and sodium (Morgan). In addition, calcium can

form insoluble compounds with elements such as phosphorus; neither calcium nor phosphorus will be available to the plant (Sela). In this case, the plant would exhibit both calcium and phosphorus deficiency symptoms: 'tipburn' in newer leaves, inhibited growth, and poor root development (Sela) ("Phosphorous in Soil").

This investigation aims to investigate the effects of different calcium salts on the growth rate of *Ocimum basilicum* grown hydroponically. Salts used were $CaSO_4$, $Ca(H_2PO_4)_2$, $Ca(NO_3)_2$, and $CaCl_2$. In addition, one control group will be supplied solely with tap water for comparison. By changing the calcium salt present in the water source of the plants, one can examine the effects this has on the plant's stem height as well as any observable qualitative changes in the plant. Moreover, one will be able to see the effect different cation and anion combinations have on growth rate.

Using knowledge of calcium in plants one can hypothesize the effects of each salt on the growth rate. If $CaSO_4$ and $Ca(NO_3)_2$ are applied to separate growth solutions, then the basil will have a high growth rate because both salts are soluble and contain primary macronutrients in the form of anions. Sulfur and Nitrogen are both necessary in plans as both are important in protein synthesis and sulfur for chlorophyll synthesis ("Sulfur in Plants"). If $CaCl_2$ is present in the supplied water, then the basil will neither have stunted nor boosted growth as chloride is mainly used for osmotic regulation and photosynthesis ("Chloride - an Essential"). Lastly, the presence of $Ca(H_2PO_4)_2$ in the growth solution will result in stunted growth because $(H_2PO_4)^-$ and Ca^{2+} form insoluble compounds and the basil will be

incapable of using these compounds to get nutrients ("Phosphorous in Soil")(Sela). The basil will manifest calcium and phosphorous deficiency symptoms.

Method

Variables

The independent variable in this investigation was the anion of the calcium salt. I changed this by using different calcium salts ($CaCl_2$, $CaSO_4$, $Ca(H_2PO_4)_2$, and $Ca(NO_3)_2$), the cation remains the same while the anion changes. The anion was changed in order to investigate the effect and relationship of various anions with the Ca^{2+} cation and their uptake by the basil plant. Changing the anion allows the investigation of the relationship of the two ions and how these affect growth rate of the basil.

The dependent variable for the experiment was stem length. Stem length was measured using a ruler. By looking at stem length, one can determine the effect of various calcium salts on the growth rate of basil plants. Stem length is an efficient and direct way of observing growth rate.

The controlled variables go as follows. Firstly, the germination conditions were kept constant for all trials by germinating them for the same duration, growing them in the same growing media, light, and water source. All seeds were germinated with tap water from the same source in order to avoid shocking the seeds and giving them all an equal chance to survive. Similarly, all the plants were grown throughout the course of the experiment in the same rockwool in which they were germinated to avoid differences or damage when transferring the plants.

The nature of the specimen was also kept constant and all seeds came from the same package and were the same brand. In addition, the amount of light exposure, distance from light, and source of light were controlled throughout the experiment as all plants were provided with light originating from a 10W LED plant light that was directly over the nurseries. The duration of the investigation was 42 days and broken down into 12 days of germination where no data collection took place and 30 days where the plants were placed in the solutions and measured every other day. Another variable that was constant was the water added to the containers during the data collection period. When the water in a container was getting low, every container was given the same volume, using a graduated beaker, of water to ensure that the concentration remained the same for every container. Moreover, at the start of the experiment, all containers contained 750ml of solution. The measuring protocol for data collection was another constant in this lab as to reduce random uncertainties (see Figure 3.3). A final controlled variable was the anion concentration in the solutions. This constant was easily controlled since most salts used had a 1:2 Ca^{2+} to anion ratio except for $CaSO_4$, which had a 1:1 cation to anion ratio. Therefore, $CaSO_4$, was added as to make the concentration of that solution 0.1M in order to keep the anion concentration constant for all the containers.

Finally there are the confounding variables. The first is the temperature of the room in which the investigation was carried out. The nurseries containing the plants were kept in the same location, in the same room to keep the surrounding temperature as constant as possible since temperature was not monitored throughout the investigation. The other confounding variable is cation concentration. Although most salts used in the experiment had a 1:2 cation to anion

ratio, $CaSO_4$ had a 1:1 ratio. In this case, twice as much of the salt was added to achieve a constant anion concentration. Therefore, the cation (Ca^{2+}) was twice as much as the other solutions. A last confounding variable, which I discovered later in my investigation, was sample size. Although each solution had ten plants, some solutions had seeds because they had failed to germinate during the germination stage of the procedure. Therefore every solution had at least 9 live basil plants.

Materials

Table 1: Materials and quantities

Equipment/Materials	Use in Experiment	Quantity
VÄXER Nursery and Sprout Box with Lid (IKEA)	Provided a controlled environment for the plants during germination and data collection.	2
VÄXER LED Cultivation Light (IKEA) (10W)	Constant light source for plants throughout investigation.	2
Plastic Container (1.2L)	Held growth solutions as well as the rockwool that housed the plants.	5
Electronic Mass Balance (±1g)	Measured salt added to each solution.	1
1dm^3 Beaker (±25ml)	Measured water needed to make each salt solution and used to mix the solutions.	1
250mL Beaker (±12.5ml)	Used to add water to the containers periodically and accurately throughout the the experiment.	1
Mortar and Pestle	Helped crush calcium salts to facilitate their dissociation.	1
15cm Ruler (±1mm)	Measured stem length	1
Nylon Stockings	Created a surface over the containers so the basil plants were constantly touching the solution.	1
Basil Seeds (Sperli BIO)	Test subject.	50
VÄXER Rockwool Growing Media (IKEA)	Growth medium throughout investigation. Provided constant supply of growth solution.	1 Box (50)

Table 2: Chemicals, formulas, and mass required

Chemicals	Formula	Mass (g)
Calcium Chloride, fused, granular	$CaCl_2$	6g
Calcium Sulfate, dihydride	$CaSO_4$	14g
Monocalcium Phosphate, monohydrate	$Ca(H_2PO_4)_2$	8g
Calcium Nitrate, tetrahydrate	$Ca(NO_3)_2$	12g

Experimental Procedure

Part 1: Germination and Initial Preparation

The seeds were germinated using pieces of rockwool. This ensured that the seeds dried out. I completely saturated the rockwool by soaking the pieces in room temperature water for two minutes. Once saturated, I filled the nursery basin to the maximum line with water. I placed the fifty pieces of rockwool in the 5 by 10 germination tray. I then placed the tray above the basin so the rockwool was in contact with the water below. Then I carefully put one basil seed in each divot of the fifty pieces of rockwool. I placed the nursery lid on top of the basin and turned on the overhead grow light (see Figure 3.1). I left the seeds to germinate and develop for twelve days. I left grow light on for 12 hours daily from 9:00 to 21:00 using an alarm (±1min) to ensure accurate light exposure. After twelve days, the seeds should have opened and have at least two leaves, although three didn't germinate at all.

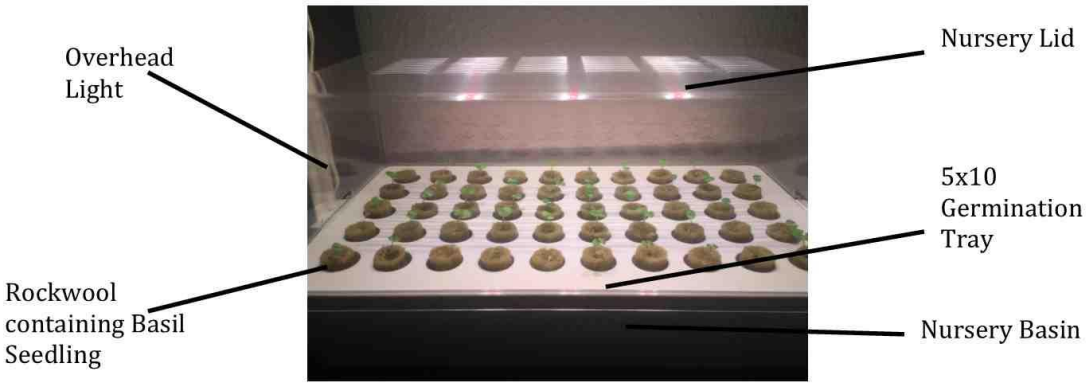


Figure 3.1: Seeds during germination before transferal into solutions

Part 2: Solution Preparation and Final Setup

In order to make the calcium salt solutions for the plants, I measured 1dm^3 of tap water using a beaker (±25ml). Using an electronic mass balance (±1g), I measured out the required mass of calcium salt in order to obtain 0.05M (see Table 2). Then I added said mass of salt to the beaker and stirred the solution using a plastic stirring spoon, to avoid unwanted reactions, until the salt completely dissociated. This was done for every salt that had a 1:2 cation to anion ratio ($Ca(NO_3)_2$, $Ca(H_2PO_4$, and $CaCl_2$). For $CaSO_4$, I added the mass of salt required in order to make a 0.1M solution (see table 2). Once the solutions were mixed, I poured 750ml into the containers. For the tap water group, 750ml was added to a container to control for calcium present in the water. I labeled each container with the name of the solution it contained. I cut the nylon stockings into five cylinders long enough to wrap around the plastic solution-filled containers. I stretched these pieces of nylon over the top of the open containers. I cut ten evenly spaced holes on the surface where the rockwool hosting the basil seedlings could fit with scissors. I took ten pieces of rockwool containing seedlings at random from the germination tray and

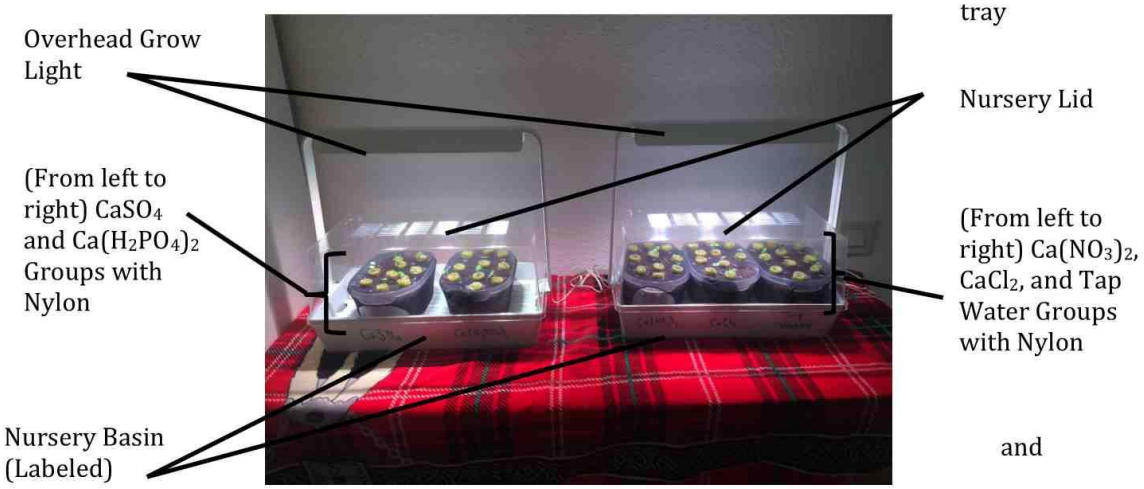


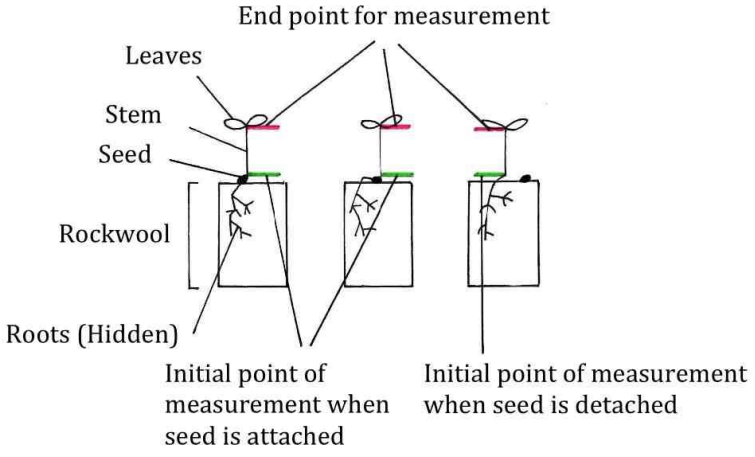
Figure 3.2: Plants in their respective solutions with labeled nurseries and overhead grow lights

placed them in the holes in the

stockings making sure they were in contact with the solutions in the container. Three of the five containers had 9 plants instead of ten as not all seeds germinated. I repeated this with the other solutions. I labeled each plant from 1-10 on the nylon, although not all containers had ten plants. I placed all five containers directly underneath the grow lights in the basins. Three containers fit into one nursery, therefore I placed three in the first nursery and two in the second nursery (see Figure 3.2). I left the grow lights on for 12 hours everyday. Moreover, everyday I checked the water levels in each container and added to every container to ensure constant concentrations.

Part 3: Data Collection Procedure

To measure the stems, I used the same measuring protocol for each plant (refer to Figure 3.3). I measured, with a 15cm ruler (±1mm) and recorded the stem lengths, by holding the ruler parallel to the stem. I recorded data every other day after the seedlings were transferred to their respective solutions. The data collection process went on for 31 days. I disposed of the solutions and basil plants carefully at the end of the investigation.



11 **Figure 3.3:** Specimen Measuring Protocol for Various Stem Orientations

Risk Assessment

The chemicals required for this experiment had the potential to be harmful; therefore it's imperative to handle them with care and to dispose of them responsibly. To avoid health issues, hands were washed immediately after coming into contact with any of the solutions. For calcium salt safety, see appendices 1.1-1.3. The solutions were disposed via the sink, as their solute concentrations were not elevated as to cause environmental damage. Moreover, the plants themselves were discarded along with the rockwool as both are biodegradable and will not cause any damage to the environment.

Data

Raw Data

See Appendices 2.1-2.5

Qualitative Observations

Table 3: Observable quantitative results recorded throughout data collection

Solution	Leaves	Growth	Roots	Other
$CaSO_4$	Leaves were healthy except for slight browning on the edges around day 26; leaves were curved	Constant growth and healthy stem	Healthy roots that penetrated the rockwool	NA
$Ca(H_2PO_4)_2$	Small yellowed leaves that often turned brown and fell	Stunted growth and thin brown stem	Very poor root development; roots were brownish and did not penetrate through rockwool	7/10 plants ended up dying and withering or not growing much
$Ca(NO_3)_2$	Large leaves and some leaves showed brown spots later on	Constant growth and healthy stem; grew quickly from the start	Healthy roots that penetrated the rockwool	One plant died around day 26 of data collection
$CaCl_2$	Healthy leaves that were paler than the other solutions'	Healthy stem with slow constant growth rate	Roots were healthy and relatively longer than the other solutions'	One plant died around day 12
Tap Water	Large, glossy, and unblemished leaves	Quick constant growth from the start; overall tallest plants	Roots were comparable to other solutions but were a little bit paler in color	NA

Processed Data

Table 4.1: Average stem heights for each solution everyday

Average Stem Length (±1mm)					
Time (Days)	**CaSO4**	**Ca(H2PO4)2**	**Ca(NO3)2**	**CaCl2**	**Tap Water**
0	13	12	14	11	11
2	15	13	15	13	13
4	16	13	17	13	15
6	18	13	17	14	16
8	18	13	18	15	17
10	20	13	19	15	18
12	22	13	19	16	21
14	23	13	19	17	23
16	23	14	20	17	23
18	24	14	20	17	24
20	24	14	20	17	25
22	25	14	21	18	26
24	27	14	22	18	28
26	27	14	22	19	29
28	27	14	23	19	30
30	27	14	24	19	31

Table 4.2: Standard deviation for stem heights for each solution everyday

Standard Deviation					
Time (Days)	**$CaSO_4$**	**$Ca(H_2PO_4)_2$**	**$Ca(NO_3)_2$**	**$CaCl_2$**	**Tap Water**
0	3	4	5	3	6
2	3	4	6	3	6
4	2	4	6	3	5
6	3	4	6	3	5
8	3	4	6	3	5
10	3	4	6	3	6
12	3	4	7	4	6
14	4	4	7	4	7
16	4	4	7	4	6
18	4	4	7	5	6
20	4	4	7	5	7
22	4	4	8	5	7
24	5	4	8	5	8
26	5	4	8	5	8
28	5	4	7	5	8
30	5	4	8	6	8

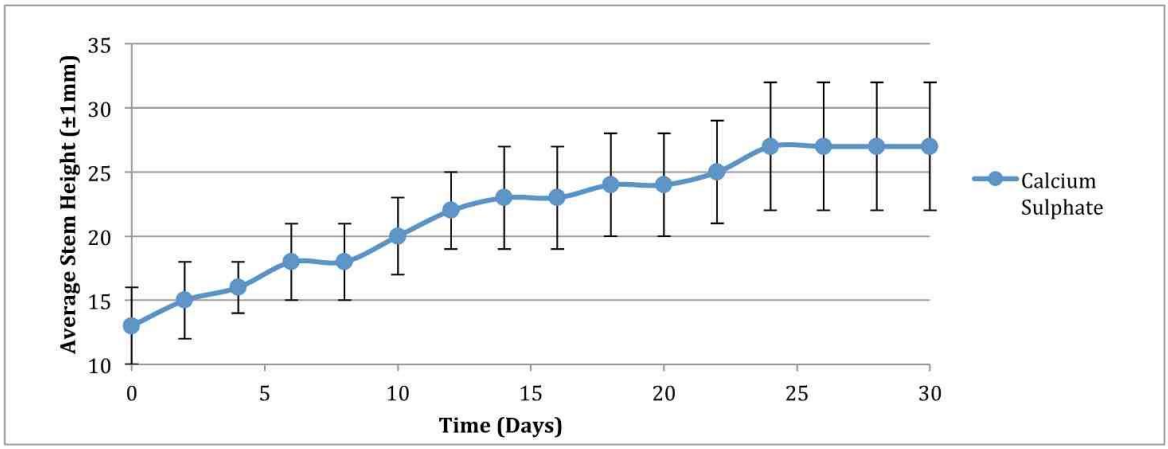


Figure 4.1: Average growth of *Ocimum basilicum* grown in $CaSO_4$ solution over 31 days with standard deviation error bars

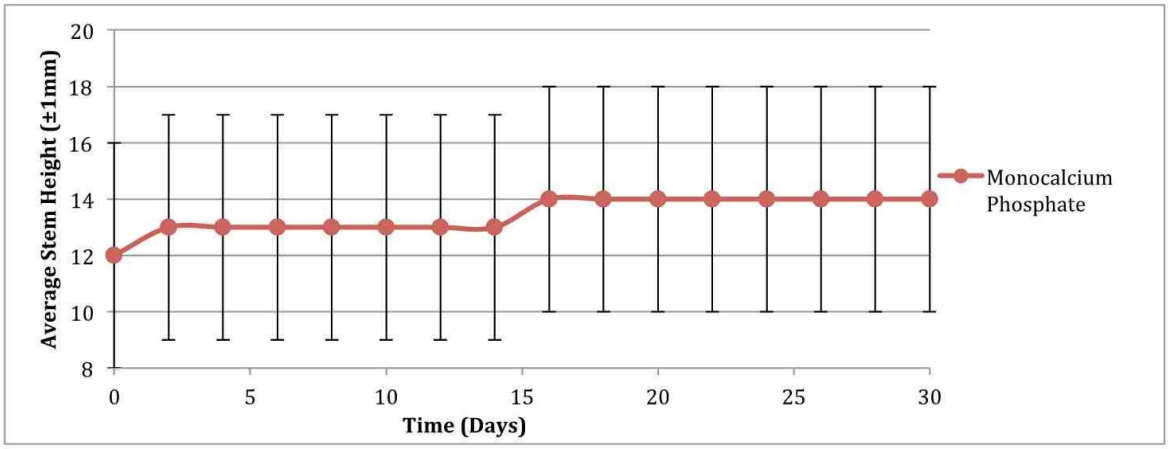


Figure 4.2: Average growth of *Ocimum basilicum* grown in $Ca(H_2PO_4)_2$ solution over 31 days with standard deviation error bars

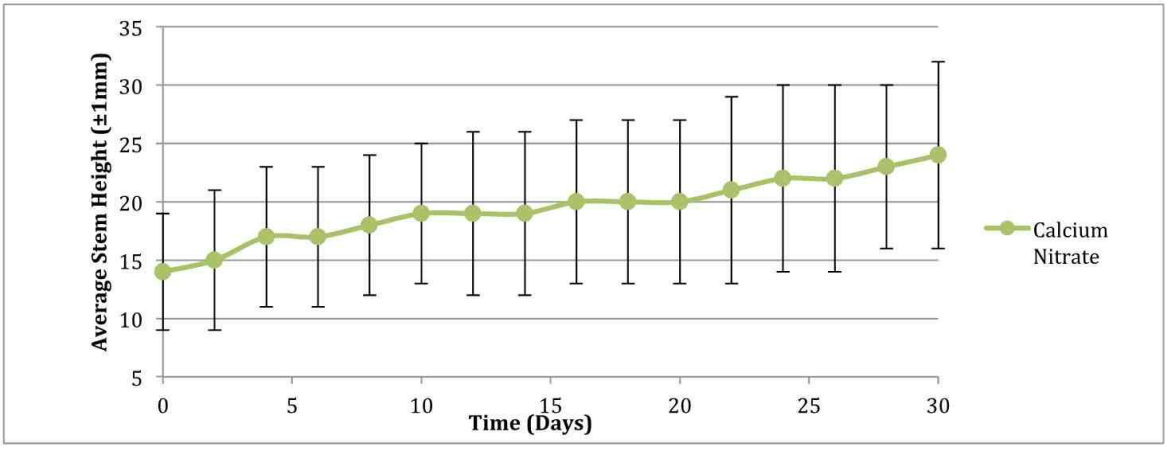


Figure 4.3: Average growth of *Ocimum basilicum* grown in $Ca(NO_3)_2$ solution over 31 days with standard deviation error bars

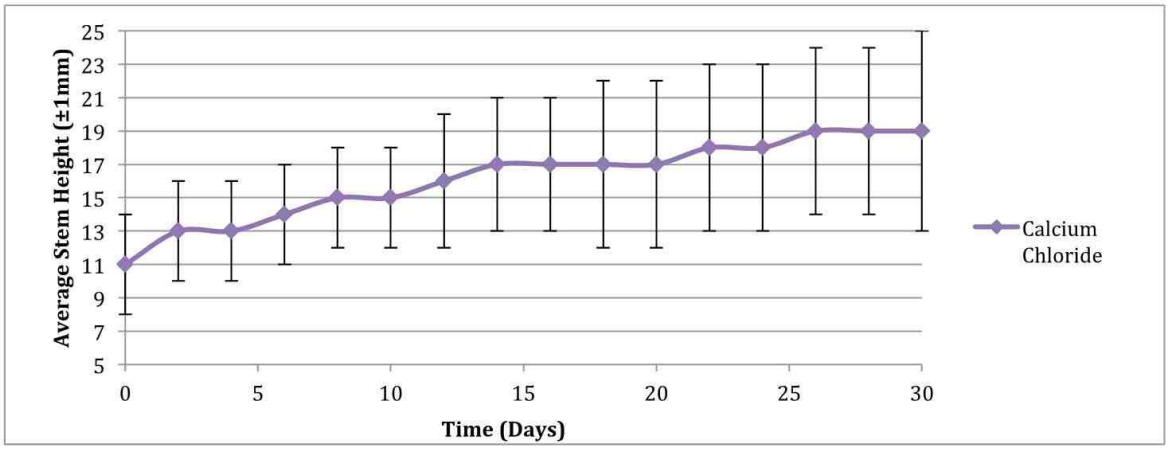


Figure 4.4: Average growth of *Ocimum basilicum* grown in $CaCl_2$ solution over 31 days with standard deviation error bars

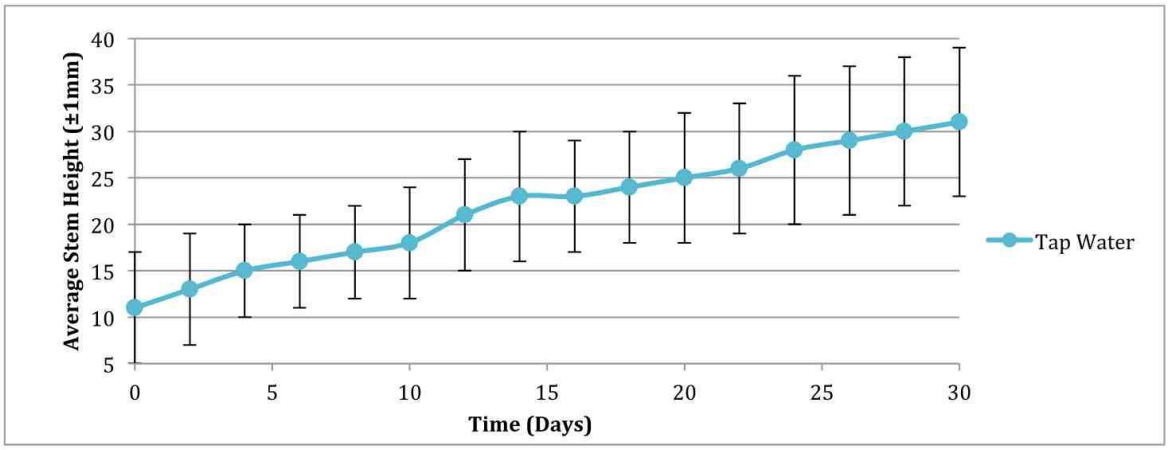


Figure 4.5: Average growth of *Ocimum basilicum* grown in Tap Water over 31 days with standard deviation error bars

Table 5: Average absolute and relative change in stem height for each solution

Calculation	Growth Solution				
	$CaSO_4$	$Ca(H_2PO_4)_2$	$Ca(NO_3)_2$	$CaCl_2$	Tap Water
Absolute Change (±2mm)	15	2	10	8	19
Relative Change (%)	124	24	99	72	476
Relative Uncertainty (%)	0.24	1.4	0.33	0.42	0.34
Relative Uncertainty	30	34	33	30	162

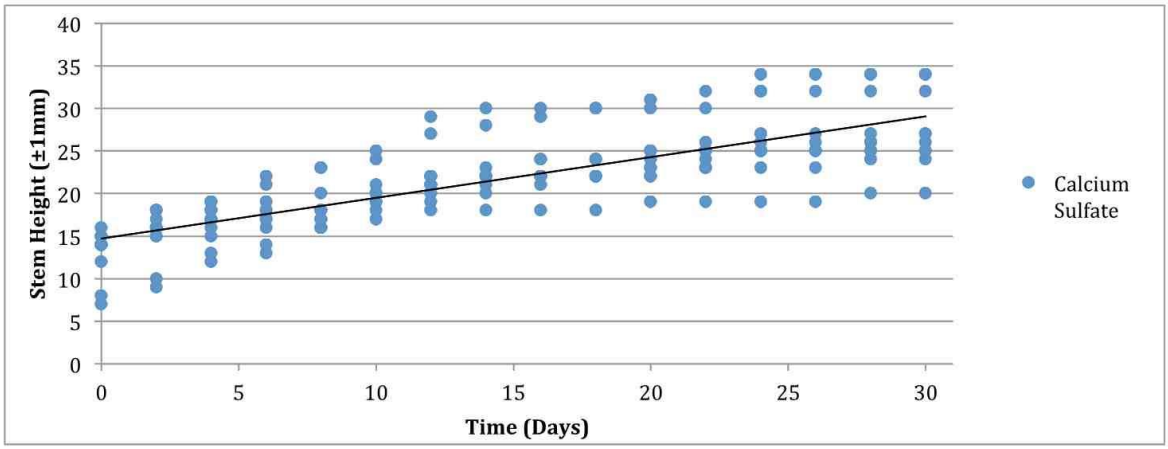


Figure 5.1: Regression Analysis of *Ocimum basilicum* grown in $CaSO_4$ solution over 31 days with linear trend line

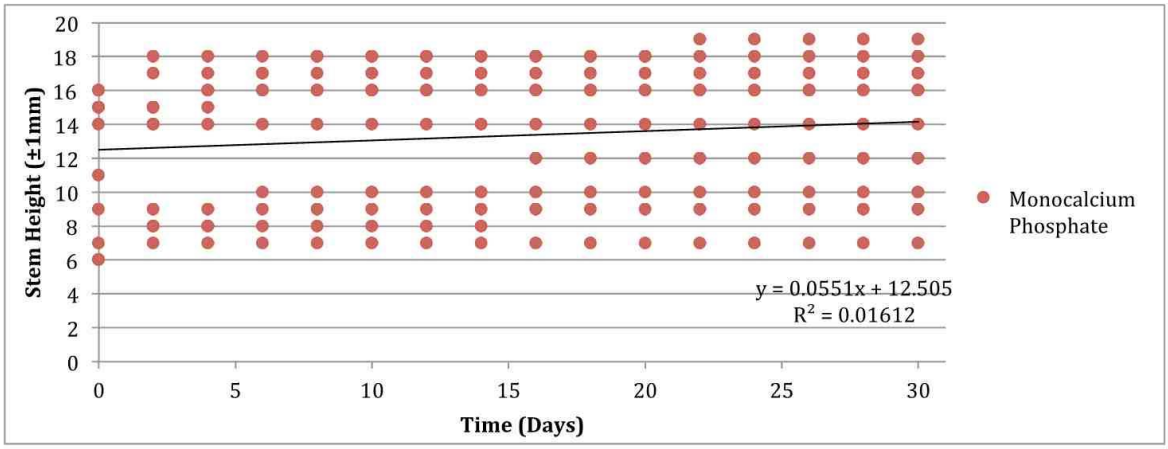


Figure 5.2: Regression Analysis of *Ocimum basilicum* grown in $Ca(H_2PO_4)_2$ solution over 31 days with linear trend line

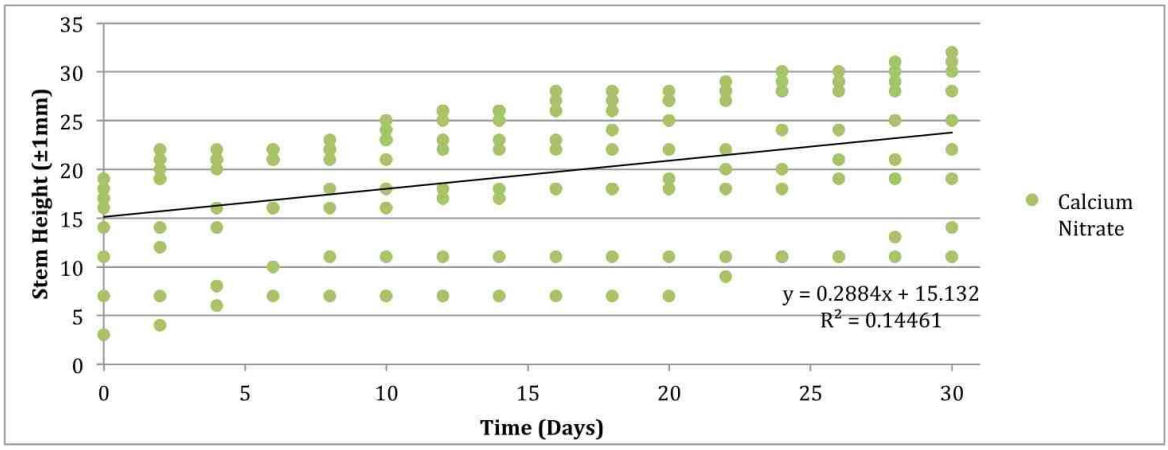


Figure 5.3: Regression Analysis of *Ocimum basilicum* grown in $Ca(NO_3)_2$ solution over 31 days with linear trend line

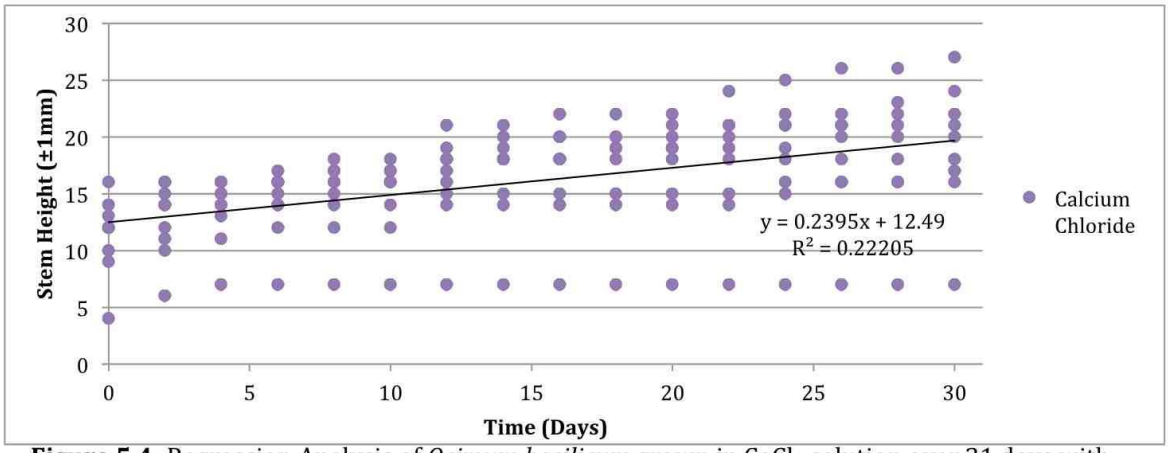


Figure 5.4: Regression Analysis of *Ocimum basilicum* grown in $CaCl_2$ solution over 31 days with linear trend line

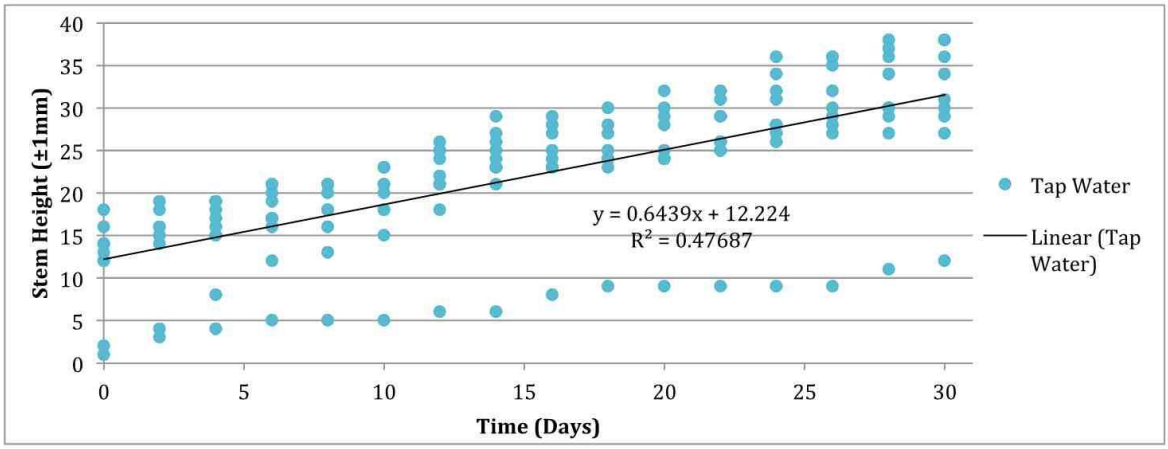


Figure 5.5: Regression Analysis of *Ocimum basilicum* grown in Tap Water over 31 days with linear trend line

Best-fit values	
Slope	0.4784 ± 0.03089
Y-intercept	14.72 ± 0.5438
X-intercept	-30.78
1/Slope	2.090
Goodness of Fit	
R square	0.6029
Sy.x	3.602
Is slope significantly non-zero?	
F	239.9
DFn,DFd	1,158
P Value	< 0.0001
Deviation from horizontal?	Significant

Figure 6.1: Regression Analysis Data for $CaSO_4$

Best-fit values	
Slope	0.05507 ± 0.03423
Y-intercept	12.51 ± 0.6027
X-intercept	-227.1
1/Slope	18.16
Goodness of Fit	
R square	0.01612
Sy.x	3.992
Is slope significantly non-zero?	
F	2.588
DFn,DFd	1,158
P Value	0.1097
Deviation from horizontal?	Not Significant

Figure 6.2: Regression Analysis Data for $Ca(H_2PO_4)_2$

Best-fit values	
Slope	0.2884 ± 0.05886
Y-intercept	15.13 ± 1.036
X-intercept	-52.47
1/Slope	3.467
Goodness of Fit	
R square	0.1446
Sy.x	6.512
Is slope significantly non-zero?	
F	24.01
DFn,DFd	1,142
P Value	< 0.0001
Deviation from horizontal?	Significant

Figure 6.3: Regression Analysis Data for $Ca(NO_3)_2$

Best-fit values	
Slope	0.2395 ± 0.03763
Y-intercept	12.49 ± 0.6625
X-intercept	-52.14
1/Slope	4.175
Goodness of Fit	
R square	0.2220
Sy.x	4.163
Is slope significantly non-zero?	
F	40.53
DFn,DFd	1,142
P Value	< 0.0001
Deviation from horizontal?	Significant

Figure 6.4: Regression Analysis Data for $CaCl_2$

Best-fit values	
Slope	0.6439 ± 0.05659
Y-intercept	12.22 ± 0.9964
X-intercept	-18.98
1/Slope	1.553
Goodness of Fit	
R square	0.4769
Sy.x	6.261
Is slope significantly non-zero?	
F	129.4
DFn,DFd	1,142
P Value	< 0.0001
Deviation from horizontal?	Significant

Figure 6.5: Regression Analysis Data for Tap Water

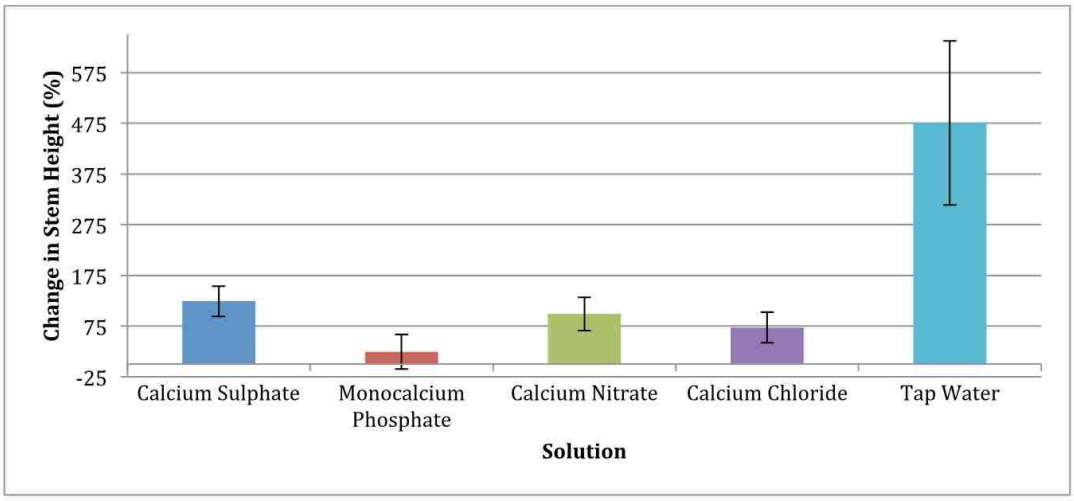


Figure 7: Average relative change in stem height for each growth solution and error bars representing relative uncertainty

Sample Calculations

Sample Calculations	Uncertainties
Average Stem Height for a Solution $\frac{12+16+7+14+8+14+15+14+14+15}{10}$ $= 13\text{mm}$ Excel Formula: AVERAGE (C6:L6)	$\frac{1+1+1+1+1+1+1+1+1+1}{10} = 1$ $\therefore \pm 1\text{mm}$
Average Percentage Change for a Solution $32 - 12 = 20$ $(20 \div 12) \times 100 = 167\%$ $(167+113+243+79+213+143+80+43+86+80) \div 10 = 124\%$ Excel Formula: AVERAGE (C27:L27)	$(2 \div 20) \times 100 = 10\%$ → Relative Uncertainty for absolute change for one trial $(1 \div 12) \times 100 = 8.\bar{3}\%$ → Relative Uncertainty for initial height for one trial $10 + 8.\bar{3} \approx 18\%$ → Relative Uncertainty for relative change for one trial $(18+17+26+25+24+17+23+40+24+23) \div 10 = 24\%$ $0.24 \times 124 = \pm 30$ Excel Formula: AVERAGE (C28:L28)
Standard Deviation for a Solution Excel Formula: STDEVA (C6:L6) $= 3$	NA

Discussion

Conclusion

The results can be summarized in increasing order of average change in stem height:

$$Ca(H_2PO_4)_2 < CaCl_2 < Ca(NO_3)_2 < CaSO_4 < \text{Tap Water}$$

My results show that, excluding the tap water control, $CaSO_4$ resulted in the greatest average percentage change in stem height (see Figure 7) followed by $Ca(NO_3)_2$. This coincides with my hypotheses regarding growth. Sulfur and nitrogen are both primary macronutrients and therefore their presence evidently

affected the overall change in height ("Sulfur in Plants"). Both solutions had significant slopes with $CaSO_4$ having an R^2 value of 0.6029 meaning there was a string connection between growth and the salt used, whereas $Ca(NO_3)_2$ has an R^2 of 0.1446 showing less of a correlation (see Figure 6.1 & 6.3).

I had also hypothesized that the basil in the $CaCl_2$ solution would neither have a particularly low nor high growth rate. The data shows that indeed, out of all the solutions, $CaCl_2$ was in the middle. As seen in Figure 4.4, the basil in this solution has a smaller growth than $Ca(NO_3)_2$, but a larger growth than $Ca(H_2PO_4)_2$. Along the same lines, its average percent change in stem height (see Figure 7) was greater than that of $Ca(H_2PO_4)_2$, and smaller than that of $Ca(NO_3)_2$. These results are explained by the role of the chloride ion in plants: to assist photosynthesis and osmotic regulation("Chloride - an Essential"). Therefore, Chloride has no particular effect on growth rate because it's used in other physiological plant processes. This is further supported by the R^2 that was a mere 0.222; although it's still significant in terms of growth (see Figure 6.4).

Figure 8: Withered basil plant grown in $Ca(H_2PO_4)_2$ solution

The last of my hypotheses stated that the $Ca(H_2PO_4)_2$ solution would have the lowest overall growth and the plants would manifest symptoms of calcium and phosphorus deficiency; the results supported these predictions. For average change in height and average growth rate (see Figures 4.2 and 7), basil grown in $Ca(H_2PO_4)_2$ was lowest in both categories. This is also seen from the regression results as there is no significant

slope, or a significant R^2 value: 0.01612 (see Figure 6.2). All the plants grown in the solution grew small and had yellowish brown leaves with wrinkled and dark brown stems, most of which died (see Figure 8). These are amalgamations of calcium and phosphorus deficiency symptoms. Since the two ions form insoluble compounds, the basil cannot absorb these nutrients ("Phosphorous in Soil")(Sela).

Quantitatively, my hypotheses were, mostly correct. However, quantitatively, I hadn't considered the plants in these solutions would experience toxicity. Plants in the $CaSO_4$ solution showed browning on leaf edges, which is a symptom of sulfur toxicity ("Symptoms of Deficiencies"). Additionally, I hadn't taken chlorine toxicity came to my attention after researching the reasons for the yellowing leaves and lower growth rate in the $CaCl_2$ solution basil ("Symptoms of Deficiencies").

The cases of toxicity made me question whether or not the concentrations of salts used were too elevated. Prior to the investigation, I had done research on salinity resistance and ideal salinity for crops; however, there is agreement that there isn't much literature on salinity requirements for basil (Shannon and Grieve 32). I settled for 0.05M for all solutions except $CaSO_4$ (0.1M), as I wanted to control anion concentration. In hindsight, I think that since the sources revolved mainly on soil based salt, the salt present in my water solutions was too elevated as it was more readily available to the basil. This point is further backed up by the growth of the tap water group (see Figure 4.5) . Overall, *Ocimum basilicum* grown in tap water outgrew the other plants, as its average change is greater than that of $CaSO_4$ by 352% (see Table 5).

Evaluation

This investigation was successful and produced promising results as well as giving me deeper insights on nutrient uptake in plants. The reason that *Ocimum basilicum* was used in this experiment was because of its relatively quick growth rate and its use as a crop. I used basil over other crops, as I didn't have time to grow crops such as tomatoes, as fruit take a longer time to grow. In addition, the reason for which I chose to measure stem height rather than plant mass for growth rate had to do with the fact that I grew my plants hydroponically. The wet rockwool would have been difficult to remove from the mass when measuring. With height, it was simple to measure and had little error. Consequently, I chose to grow the basil hydroponically for two reasons. Firstly, growing them in water meant it was easier to control which nutrients were present as well as having a easily locatable initial point for measurement. Secondly, hydroponics is very relevant to today's world as we have an ever-increasing population and farmers are getting more creative with their cultivation and growth methods. All in all this method worked quite well for this investigation. Lastly, I chose to investigate calcium due to the fact that not much research has been done on its effect on growth rate compared to nitrogen and other primary macronutrients. Therefore, I wanted to explore its effect on growth rate of a plant, which also doesn't have much research done on it.

Limitations of this project mainly revolve around the materials used. Firstly, the balance used to measure the salts had no decimal places, only whole numbers. Therefore, the concentrations of the solutions could have been slightly inaccurate. Moreover, the ruler I used to measure the basil plants had as a smallest unit 1mm. Furthermore, the fact that temperature was not monitored is a slight limitation as

it can affect growth. However, the specimens were all in the same room, therefore, if temperature had an effect, it would have affected them equally. Finally there was the slight difference in sample size for each solution. This occurred because not all fifty seeds germinated during the initial part of the experiment and therefore, some solutions had one unopened seed, slightly skewing the data. Lastly, the number of trials for each solution also varied as not all seeds germinated and therefore, some solutions only has 9 plants compared to ten. However, this would not have changed the averages by much and is almost negligible.

If time allowed it, I would have used better equipment with smaller units for measurements. Moreover, I would set up more than fifty seeds to germinate rather than exactly fifty in the likely event that not all of them sprout. I could have measured the mass of the plants with a better hydroponic system. Since more literature is available on tomatoes, I would use these instead to calculate percentage error. Calcium deficiency in tomatoes has been readily researched as the symptoms manifest themselves in rotting fruit, having a more direct connection to agriculture and business. Lastly, since cell wall strength and calcium concentration are correlated, measuring cell wall sturdiness would be interesting. Although studies have been done studying this strength, it isn't an investigation that would be easily replicable in a high school laboratory.

Appendices

Signal: Danger
GHS Hazard Statements
H302: Harmful if swallowed [Warning Acute toxicity, oral]
H318: Causes serious eye damage [Danger Serious eye damage/eye irritation]
H335: May cause respiratory irritation [Warning Specific target organ toxicity, single exposure; Respiratory tract irritation]
H373: Causes damage to organs through prolonged or repeated exposure [Warning Specific target organ toxicity, repeated exposure]

Precautionary Statement Codes
P260, P261, P264, P270, P271, P280, P301+P312, P304+P340, P305+P351+P338, P310, P312, P314, P330, P403+P233, P405, and P501
(The corresponding statement to each P-code can be found here.)

Inhalation causes irritation of nose and throat. Ingestion causes irritation of mouth and stomach. Contact with eyes (particularly by dust) causes irritation and possible transient corneal injury. Contact of solid with dry skin causes mild irritation; strong solutions can cause marked irritation, even a superficial burn. (USCG, 1999)

INHALATION: move to fresh air; if discomfort persists, get medical attention. INGESTION: give large amounts of water. EYES: promptly flood with water and continue washing for at least 15 min.; consult an ophthalmologist. SKIN: flush with water. (USCG, 1999)

Appendix 1.1: Safety Hazards and Health Protocols for $CaCl_2$; reprinted from: "Calcium Chloride." *PubChem*, pubchem.ncbi.nlm.nih.gov/compound/Calcium_dichloride#section=Safety-and-Hazards. Accessed 27 Aug. 2017

Signal: Danger
GHS Hazard Statements
Aggregated GHS information from 22 notifications provided by 776 companies to the ECHA C&L Inventory. Each notification may be associated with multiple companies.

H272 (75%): May intensify fire; oxidizer [Danger Oxidizing liquids; Oxidizing solids]
H302 (36.08%): Harmful if swallowed [Warning Acute toxicity, oral]
H315 (61.08%): Causes skin irritation [Warning Skin corrosion/irritation]
H318 (27.19%): Causes serious eye damage [Danger Serious eye damage/eye irritation]
H319 (60.82%): Causes serious eye irritation [Warning Serious eye damage/eye irritation]

Information may vary between notifications depending on impurities, additives, and other factors. The percentage value in parenthesis indicates the notified classification ratio from all companies. Only Hazard Codes with percentage values above 10% are shown.

Precautionary Statement Codes
P210, P220, P221, P264, P270, P280, P301+P312, P302+P352, P305+P351+P338, P310, P321, P330, P332+P313, P337+P313, P362, P370+P378, and P501

Dust causes mild irritation of eyes. (USCG, 1999)
EYES or SKIN: flush with water and seek medical assistance. (USCG, 1999)

Appendix 1.2: Safety Hazards and Health Protocols for $Ca(NO_3)_2$; reprinted from: "Calcium Nitrate." *PubChem*, pubchem.ncbi.nlm.nih.gov/compound/calcium_nitrate#section=Safety-and-Hazards. Accessed 27 Aug. 2017.

Signal: Danger
GHS Hazard Statements
Aggregated GHS information from 9 notifications provided by 58 companies to the ECHA C&L Inventory. Each notification may be associated with multiple companies.

H315 (46.55%): Causes skin irritation [Warning Skin corrosion/irritation]
H318 (43.1%): Causes serious eye damage [Danger Serious eye damage/eye irritation]
H319 (48.28%): Causes serious eye irritation [Warning Serious eye damage/eye irritation]

Information may vary between notifications depending on impurities, additives, and other factors. The percentage value in parenthesis indicates the notified classification ratio from all companies. Only Hazard Codes with percentage values above 10% are shown.

Precautionary Statement Codes
P264, P280, P302+P352, P305+P351+P338, P310, P321, P332+P313, P337+P313, and P362

Exposure Routes: inhalation, skin and/or eye contact Symptoms: Irritation eyes, skin, upper respiratory system; conjunctivitis; rhinitis, epistaxis (nosebleed) Target Organs: Eyes, skin, respiratory system (NIOSH, 2016)

Eye: If this chemical contacts the eyes, immediately wash the eyes with large amounts of water, occasionally lifting the lower and upper lids. Get medical attention immediately. Contact lenses should not be worn when working with this chemical. Skin: If this chemical contacts the skin, wash the contaminated skin with soap and water. Breathing: If a person breathes large amounts of this chemical, move the exposed person to fresh air at once. Other measures are usually unnecessary. (NIOSH, 2016)

Appendix 1.2: Safety Hazards and Health Protocols for $CaSO_4$; reprinted from: "Calcium Sulphate." *PubChem*, pubchem.ncbi.nlm.nih.gov/compound/calcium_sulfate#section=Hazards-Identification. Accessed 27 Aug. 2017.

Appendix 2.1: Raw Data Collection for $CaSO_4$ Solution

$CaSO_4$ Solution										
[redacted]	**Stem Length (± 1mm)**									
Time (Days)	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8	Trial 9	Trial 10
0	12	16	7	14	8	14	15	14	14	15
2	16	18	9	15	10	16	18	16	15	17
4	17	19	12	15	13	18	19	16	17	18
6	17	22	13	16	14	21	19	17	18	18
8	17	23	16	16	16	23	20	17	18	18
10	19	24	19	18	20	25	20	17	21	19
12	21	27	21	20	22	29	22	18	22	19
14	23	28	22	21	22	30	22	18	22	20
16	24	29	22	21	24	30	22	18	22	22
18	24	30	22	22	24	30	24	18	22	22
20	25	31	23	22	25	30	24	19	23	22
22	26	32	23	23	25	30	24	19	25	23
24	32	34	23	25	25	32	25	19	26	27
26	32	34	23	25	25	34	25	19	26	27
28	32	34	24	25	25	34	26	20	26	27
30	32	34	24	25	25	34	27	20	26	27

Appendix 2.2: Raw Data Collection for $Ca(H_2PO_4)_2$ Solution

$Ca(H_2PO_4)_2$ Solution										
	Stem Length (±1mm)									
Time (Days)	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8	Trial 9	Trial 10
0	7	15	15	14	9	11	6	16	6	16
2	8	15	15	14	9	14	8	17	7	18
4	8	15	16	16	9	14	8	17	7	18
6	8	16	16	17	10	14	9	17	7	18
8	8	16	16	17	10	14	9	18	7	18
10	8	16	16	17	10	14	9	18	7	18
12	8	16	16	17	10	14	9	18	7	18
14	8	16	16	17	10	14	9	18	7	18
16	12	16	16	17	10	14	9	18	7	18
18	12	16	16	17	10	14	9	18	7	18
20	12	16	16	17	10	14	9	18	7	18
22	12	16	16	17	10	14	9	19	7	18
24	12	16	16	17	10	14	9	19	7	18
26	12	16	16	17	10	14	9	19	7	18
28	12	16	16	17	10	14	9	19	7	18
30	12	16	16	17	10	14	9	19	7	18

Appendix 2.3: Raw Data Collection for $Ca(NO_3)_2$ Solution

$Ca(NO_3)_2$ Solution									
	Stem Length (±1mm)								
Time (Days)	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8	Trial 9
0	17	11	18	19	17	14	3	16	7
2	20	12	21	22	19	14	4	19	7
4	21	14	21	22	21	16	6	20	8
6	22	16	21	22	21	16	7	21	10
8	22	16	22	22	23	18	7	21	11
10	24	16	23	23	25	18	7	21	11
12	26	17	23	26	25	18	7	22	11
14	26	17	23	26	25	18	7	22	11
16	26	18	23	27	28	18	7	22	11
18	26	18	24	27	28	18	7	22	11
20	27	18	25	27	28	19	7	22	11
22	28	18	27	29	28	20	9	22	11
24	30	18	28	29	28	20	11	24	11
26	30	19	29	29	28	21	11	24	11
28	30	19	29	31	28	21	13	25	11
30	30	19	31	32	28	22	14	25	11

Appendix 2.4: Raw Data Collection for CaCl2 Solution

$CaCl_2$ Solution									
	Stem Length (±1mm)								
Time (Days)	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8	Trial 9
0	10	13	16	12	12	14	9	12	4
2	10	15	16	12	14	14	11	16	6
4	11	15	16	14	14	15	13	16	7
6	12	16	17	14	15	16	14	16	7
8	12	16	18	14	15	17	16	17	7
10	12	16	18	14	16	17	16	17	7
12	14	16	19	15	18	18	17	21	7
14	14	18	20	15	19	18	18	21	7
16	14	18	20	15	20	18	18	22	7
18	14	18	20	15	20	19	19	22	7
20	14	18	21	15	20	19	19	22	7
22	14	18	21	15	20	19	21	24	7
24	16	18	22	15	21	19	21	25	7
26	16	18	22	16	21	20	21	26	7
28	16	18	23	16	21	20	22	26	7
30	16	18	24	17	21	20	22	27	7

Appendix 2.5: Raw Data Collection for Tap Water

Tap Water									
[redacted]	Stem Length (±1mm)								
Time (Days)	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8	Trial 9
0	1	2	12	14	14	16	18	13	12
2	4	3	16	15	15	19	18	16	14
4	8	4	18	16	15	19	19	17	15
6	12	5	20	16	17	21	21	19	17
8	13	5	21	16	18	21	21	20	18
10	15	5	23	18	20	21	23	21	18
12	18	6	25	22	21	26	25	24	21
14	21	6	26	23	24	27	29	25	23
16	23	8	27	23	24	28	29	25	23
18	24	9	27	24	24	28	30	25	23
20	25	9	29	24	24	30	32	28	24
22	25	9	29	26	25	31	32	29	26
24	26	9	32	28	27	34	36	31	28
26	29	9	35	30	27	36	36	32	28
28	29	11	38	30	27	36	37	34	30
30	29	12	38	30	27	36	38	34	31

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