Extended Essay

Biology

Investigation on the effect of two secondary macronutrients – sulphur and magnesium – on the resource allocation in *Pisum sativum* through root, stem and leaf dry weight ratio

Supervisor: My teacher (confidentiality)

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Abstract

There are many useful applications of knowing plant biomass and the value of knowing plant biomass is becoming increasingly important. A way to determine the biomass is by investigating the root shoot ratio non-destructively. Root shoot ratio varies depending on abiotic factors, such as light exposure period, temperature and nutrient availability. The effect of primary macronutrients nitrogen, phosphorous and potassium on root shoot ratio has been better established than the effect of secondary macronutrients magnesium, sulphur and calcium. Therefore, this report examined: **what effect does magnesium and sulphur have on the interrelationship between dry weight of root, stem and leaf for *Pisum sativum*?**

Sulphur was used in form of Na_2SO_4 and magnesium in form of $MgCl_2$. Three concentrations were assigned per element – 0.001M, 0.010M and 0.050M – and one control group with no additional compound supplied. Ten plants per treatment were grown for 40 days and were cultivated in a bottle pot inspired by hydroponics system. The plants were harvested, separated into root, stem and leaf, and dried for one week. They were then measured with an electronic scale. Kruskal-Wallace test was executed to assess the significance of the values.

Results showed that magnesium and sulphur increased allocation of resources to the leaf from the roots, thus decrease Root : Shoot. However, effect of sulphur was not significant while magnesium had a significant effect. *P. sativum* maintained a stable stem dry weight percentage of its total dry weight and a stable Leaf : Stem at approximately 2:1.

Word count: 246

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Introduction

Background information

Ever since industrialization, people are depleting resources and are releasing contaminants into the ecosystem more than ever. A change in dynamics of the natural carbon cycle leads to a change in the ecosystems for plants; therefore affect the plant biomass (Potter, 1999). Plant biomass is the sum of biological material in a living plant or a recently perished plant (Biomass Energy Centre, n.d.). There are many researches that emphasize the importance of measuring plant biomass. Plant biomass may be used as an indication of the condition of the ecosystem in response to climate change (Kardol et al., 2010), biomass as a potential replacement of fossil oil (Henry, 2010) and biomass as an indication of partitioning of contaminants (Calamari et al., 1991). Plant biomass is undoubtedly an important asset for future research.

Despite its importance, the method of measuring plant biomass yet faces limitations (Pieper, 1988). Plant biomasses are either destructively obtained by digging a given sample area or non-destructively obtained by aboveground/shoot sampling using satellite and analysing with equations and conversion factors without terminating the plant (Global Terrestrial Observing System, 2009). Therefore, the data for root biomass lacks compared to shoot biomass and requires more attention to a method that can obtain samples of root biomass without destruction for environmental purposes. Root biomass data collecting can be enhanced by knowing the Root : Shoot ratio – R : S ratio – of the target plant.

Reviews have shown that R : S ratios are dependent on light, nutrients and water (Poorter & Nagel, 2000), (Weaver & Himmel, 1929), (Benjamin et al., 2014). The general trend is that light deficient conditions allocate resources to the shoot to maximize photosynthesis, and nutrient/water deficient conditions allocate resource to the root to maximize nutrient intake. While extensive research on the primary macronutrients for plants – nitrogen, phosphorous and potassium – has been done, comparatively less research has been done on the secondary macronutrients – calcium, sulphur and magnesium. A study by J. Bastow Wilson explained that “the picture is less clear” for minor nutrients, which includes magnesium and sulphur (Wilson, 1988).

This has led to the investigation of the effect of two secondary macronutrients – sulphur and magnesium – on the root, stem and leaf dry weight ratio in *P. sativum*. The choice of this particular species was primarily due to its fast growth rate and tolerance for cool climate, which is suitable for the Swedish cool summer. Other reasons are due to its average maximum height at approximately 50 cm which is feasible for cultivating; *P. sativum* are also found in the wild, mainly in the Mediterranean, thus a potential species for biomass analysis. Sulphur was used as Na_2SO_4 because plants transport sulphur in the form of sulphate and because it is a naturally occurring mineral. Magnesium was used as $MgCl_2$ because it is also a naturally occurring salt and has practical applications for salting roads which may affect its nearby ecosystem. Therefore, these salts are expected to have an abundancy worth of investigation. The concentrations chosen for the investigation are more than what they need for sufficient growth (Hopkins & Hüner, 1995). This is to see how the plant allocates resources when there is an excess supply of a nutrient.

Aim

This has led to investigate **what effect does different concentrations of two secondary macronutrients – sulphur and magnesium – at 0.001M , 0.010M and 0.050M have on the root, stem and leaf dry weight ratio in *P. sativum*?**

This investigation has three purposes. The first purpose is to contribute with the data of Root : Shoot ratio of *P. sativum* by analysing destructively and hopefully lead to a new non-destructive method to determine the biomass. Analysing the nutrient content in soil and the shoot biomass might be more efficient, sustainable and accurate in the future. The second purpose is to serve help for cultivation. Different species provide farmers with different edible compartments. Understanding the effect of different nutrients on growth in stem, root and leaf may be important for the creation of optimal fertilizers in future agriculture. The third purpose is to investigate generally how interdependent root, stem and leafs are to change in nutrient availability.

Prior definitions

Root	Part of plant that is below soil surface.
Stem	Main stalk of the plant
Leaf	Petiole + blade
Shoot	Stem + leaf
Biomass	Root + stem + leaf

Chronological literature review

One of the earliest reports of Root: Shoot ratio was done by Livingston (Livingston, 1906). This report included the result showed by Moeller that dry weight of R : S increases when the solution containing nitrogen is dilute. Livingstons own experiment verified Moeller's result by using a nutrient deficient Takoma soil on wheat. The result also showed that nutrient deficient conditions inhibited lateral root growth. This suggests there is retardation both root and shoot, but retardation in shoot is greater than root in nutrient deficient conditions.

A review by Turner (Turner, 1922) showed that plants treated with diluted potassium phosphate had a decrease in R : S. This is different from Livingston since it was thought that any nutrient deficiency would increase R : S. Therefore, it can be verified that R : S ratios are dependent on the elements and not the concentration value.

Later, more studies on independent elements have been done. A study conducted by J. Bastow Wilson (Wilson, 1988) stated that "…there is some evidence that no S: R response is seen with deficiencies of those minor nutrients that are important in photosynthesis". It has been well established that magnesium and sulphur are both important for chlorophyll. Magnesium is a part of chlorophyll molecule (Peaslee & Moss 1966) and sulphur significantly affects "photosynthesis, stomatal movement…" in form of amino acids and proteins (Mazid, Khan & Mohammad, 2011).

However, studies on individual species such as *Phaseolus vulgaris* (Cakmak, Hengeler & Marschner, 1994) and *Betula pendula* (Ericsson, 1995), R : S have responded to magnesium and sulphur significantly. In addition, a study on *P. sativum* also showed responses in S : R with Mg deficiency and Sulphur deficiency (Andrews et al., 1999). S : R tended to increase in Mg deficiency while it decreased for S deficiency.

Effects of secondary macronutrients are still unclear. Further research is needed to establish reliable data.

Hypothesis and variables

Hypothesis

Sulphur and magnesium are crucial for photosynthesis and there is some evidence that *P. sativum* responds to sulphur and magnesium changes.

Null hypothesis H_0: There is no change of allocation in root, stem, leaf biomass. Therefore Root : Shoot remains unchanged.

Alternative hypothesis H_1: Plants will allocate its resources to leaf biomass. If this H_1 holds true, there are two scenarios within this hypothesis how the Root : Shoot may change:

1. Leaf biomass is allocated from the stem, meaning that there will be no change in Root : Shoot. It will merely reallocate resources within the shoot.

2. Leaf biomass is allocated from the root, meaning that there will be change in Root : Shoot. More allocation will go from the root to the leafs, therefore reduce Root : Shoot.

Variables

Variable	Description
Dependent	
Dry weight of root, stem and leaves.	The dry weight (in grams) of root, stem and leaf will be measured because they constitute Root : Shoot. Data will be obtained quantitatively after the plants have been cultivated for approximately 2 months.
Independent	
Na_2SO_4 and $MgCl_2$	Plants use different elements for different purposes. These two compounds will be investigated on how it changes the allocation of resources between root, stem and leaf. For each Na_2SO_4 and $MgCl_2$, there will be 3 concentrations at 0.001M, 0.010M and 0.050M.

Variable	Effect on the result	Method of control	Controlled value(s)
Control			
Volume of water	Different volume of solution will mean different abundancy of elements. When plants are exposed to different amounts it may affect the Root : Shoot ratio.	Add same volume of water.	6.5dl.
Type of soil	Components in soil are different when type of soil is different. Type of soil may affect the availability of nutrients and therefore Root : Shoot ratio. In addition, soil with fewest nutrients has been chosen. Plants will show a greater response to change, therefore convenient for testing the effect of elements.	Use same type of soil.	Nutrient content is specified in **Materials and apparatus**.
Same bottle pot design	Different designs may make some plants thrive better than other. Thus it is important to control the initial circumstances.	1 medium/plant. All 1.5L bottles. Same number of ventilation holes, size and position of the holes.	No value.
The depth of planting seed	Pea seeds germinate optimally at 3cm below soil. Therefore different depth may alter development of root and shoot growth.	Plant at the same depth.	3cm below soil.
Abiotic factors	Light exposure, temperature, latitude, weather, components in air, etc. all have a potential effect on Root : Shoot ratio.	Plants were placed near each other on the balcony to expose them to the same conditions.	No value.

Methodology

Design

The design of this experiment was based on bottle pot inspired by hydroponic systems. The choice of this system was to provide individual seeds with their own medium, therefore prevent root tangling.

Materials and apparatus

Bottle pots with nutrient deficient soil

1) 70 1.5 L bottles

2) Soil *ICA plantjord*. Specifications: pH 5.5-6.5, NPK ratio = 11:5:18, NO_3 and NH_4 ≈ 165 mg/L, P ≈ 75 mg/L, K ≈ 270 mg/L, Ca ≈ 1000 mg/L. Other elements are not specified.

3) Porous cloth

Sodium sulphate and magnesium chloride solutions

1) Anhydrous sodium sulphate (Na_2SO_4)

2) Hexahydrous magnesium chloride ($MgCl_2$ x $6H_2O$)

3) Graduated cylinder, 500 ml

4) Beaker, 20 ml

5) 2 Pipette, 5 ml

6) Bucket, ≈20L

Seeds

1) 70+ seeds of *P. sativum* obtained from ICA supermarket. These are produced by the Swedish brand Nelson Garden.

2) Kitchen towel

Method

Bottle pots (refer to picture 2.3 and Pre-investigation in appendix)

1) Bottles were cut 16cm from the bottom.

2) Caps were removed and replaced with porous cloth fixated by rubber bands.

3) Holes were drilled for continuous oxygen supply to the water and soil.

4) Upper part was flipped and inserted on lower part.

Seeds

5) Seeds were immersed in water 1 day prior to planting.

6) Seeds were embedded on kitchen towels to initiate germination. Only the germinated seeds were selected.

7) Soil was filled up to 7cm in the bottles.

8) Seeds were planted and covered with additional 3cm of soil.

Solutions

9) 5dl of solution was poured directly to the bottom of the bottles only once at the beginning of the experiment. Solution should not go through the soil.

10) Additional 1.5dl was poured from the top only once at the beginning of the experiment.

Data recording

11) After 40 days, soil was separated from plant by immersing it in a large bucket of water. Shake it gently to remove excess soil.

12) Leave the sample to dry for a week. Shake gently again to remove excess soil.

13) Leaves, stem and root were separated.

14) Any significant qualitative data were gathered.

15) Leaves, stem and root were dried for one additional week.

16) Dry weights were measured using an analytical scale.

Results

Dataset 1 – Summary of raw data. For the full raw data, refer to appendix.

Table 1 shows the mean dry weight of root, stem and leaf in grams (± 0.0001g); plants survived; and Root : Stem + Leaf (R:S).

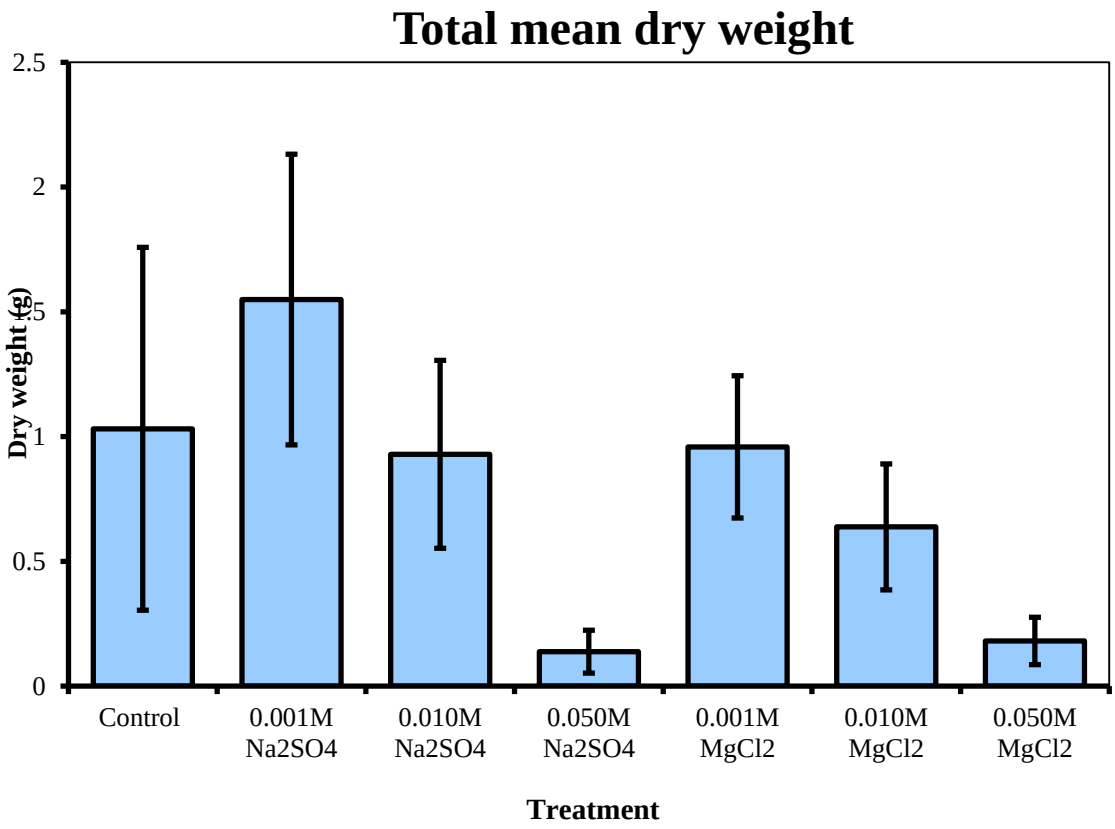
Control	**Root (g)**	**Stem (g)**	**Leaf (g)**	**R:S**
Mean	0.5015	0.1808	0.3489	0.9468
Range (min-max)	0.2141-0.8563	0.0221-0.4406	0.0208-0.7816	
Plants survived	6			
0.001M Na_2SO_4	**Root (g)**	**Stem (g)**	**Leaf (g)**	**R:S**
Mean	0.6892	0.2884	0.5717	0.8013
Range (min-max)	0.0313-1.4718	0.1085-0.4640	0.0543-1.0105	
Plants survived	10			
0.010M Na_2SO_4	**Root (g)**	**Stem (g)**	**Leaf (g)**	**R:S**
Mean	0.3795	0.1882	0.3613	0.6906
Range (min-max)	0.0819-0.8922	0.0173-0.3258	0.0541-0.6494	
Plants survived	10			
0.050M Na_2SO_4	**Root (g)**	**Stem (g)**	**Leaf (g)**	**R:S**
Mean	0.0440	0.0315	0.0627	0.4671
Range (min-max)	0.0055-0.1330	0.0085-0.0523	0.0092-0.1637	
Plants survived	8			
0.001M $MgCl_2$	**Root weight (g)**	**Stem (g)**	**Leaf (g)**	**R:S**
Mean	0.2951	0.2147	0.4490	0.4446
Range (min-max)	0.1179-0.5379	0.0417-0.3742	0.7230-0.2173	
Plants survived	10			
0.010M $MgCl_2$	**Root (g)**	**Stem (g)**	**Leaf (g)**	**R:S**
Mean	0.1769	0.1516	0.3098	0.3834
Range (min-max)	0.0427-0.4031	0.0285-0.3374	0.0715-0.5232	
Plants survived	10			
0.050M $MgCl_2$	**Root (g)**	**Stem (g)**	**Leaf (g)**	**R:S**
Mean	0.0374	0.0419	0.1019	0.2601
Range (min-max)	0.0163-0.0638	0.0032-0.0719	0.0056-0.1786	
Plants survived	7			

Dataset 2 – Total mean biomass

Table 2 shows the total mean dry weight for each treatment.

	Control	0.001M Na2SO4	0.010M Na2SO4	0.050M Na2SO4	0.001M MgCl2	0.010M MgCl2	0.050M MgCl2
Total mean dry weight (g)	1.0312	1.5493	0.9290	0.1382	0.9588	0.6382	0.1812

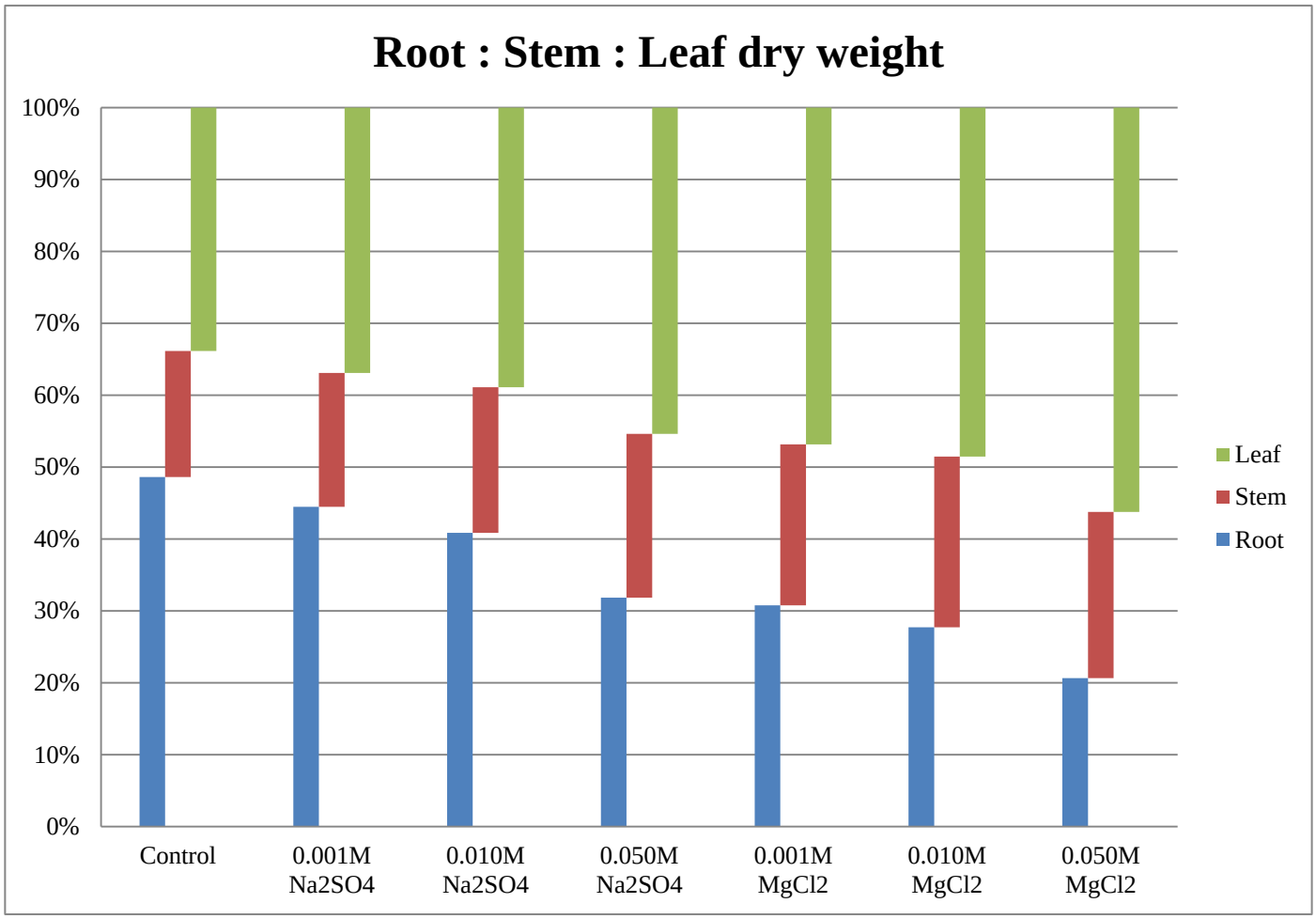
Figure 2 shows the total mean dry weight of the 7 different treatments with 95% CI as error bar. The error bar shows the range in which the true value lies with 95% certainty.



Plants treated with Na_2SO_4 have higher total mean biomass than plants treated with $MgCl_2$ for all concentrations except for 0.050M. The only treatment that has higher biomass than the control group is Na_2SO_4 at 0.001M. For both Na_2SO_4 and $MgCl_2$, the biomass is decreasing as concentration gets higher, therefore showing a negative correlation with increasing concentration.

Dataset 3 - Ratio of Root : Stem : Leaf in percentage

Figure 3 is a visual representation of the average dry weight ratio of Root : Stem : Leaf. Values are given by percentage occupied of the total dry weight.



All plants treated with sulphur or magnesium shifted the allocation of resources to the leaf. The ratio with the lowest leaf dry weight is by the control group at approximately 33.8%. The ratio with the highest leaf dry weight is by the 0.050M $MgCl_2$ group at approximately 56.2%. The range is therefore 22.4%. However, the range between lowest and highest stem ratio is only 6.3%. In addition, plants treated with $MgCl_2$ have a higher allocation to leaf in all concentrations than plants treated with Na_2SO_4.

Statistical analysis

Dataset 4 – Root : Shoot

Table 4a shows the median of Root : Shoot.

	Control	0.001M Na2SO4	0.010M Na2SO4	0.050M Na2SO4	0.001M MgCl2	0.010M MgCl2	0.050M MgCl2
Median	1.1675	0.8044	0.5990	0.2816	0.4556	0.3508	0.2722

Figure 4 shows the Root : Shoot ratio with upper and lower whiskers as error bars.

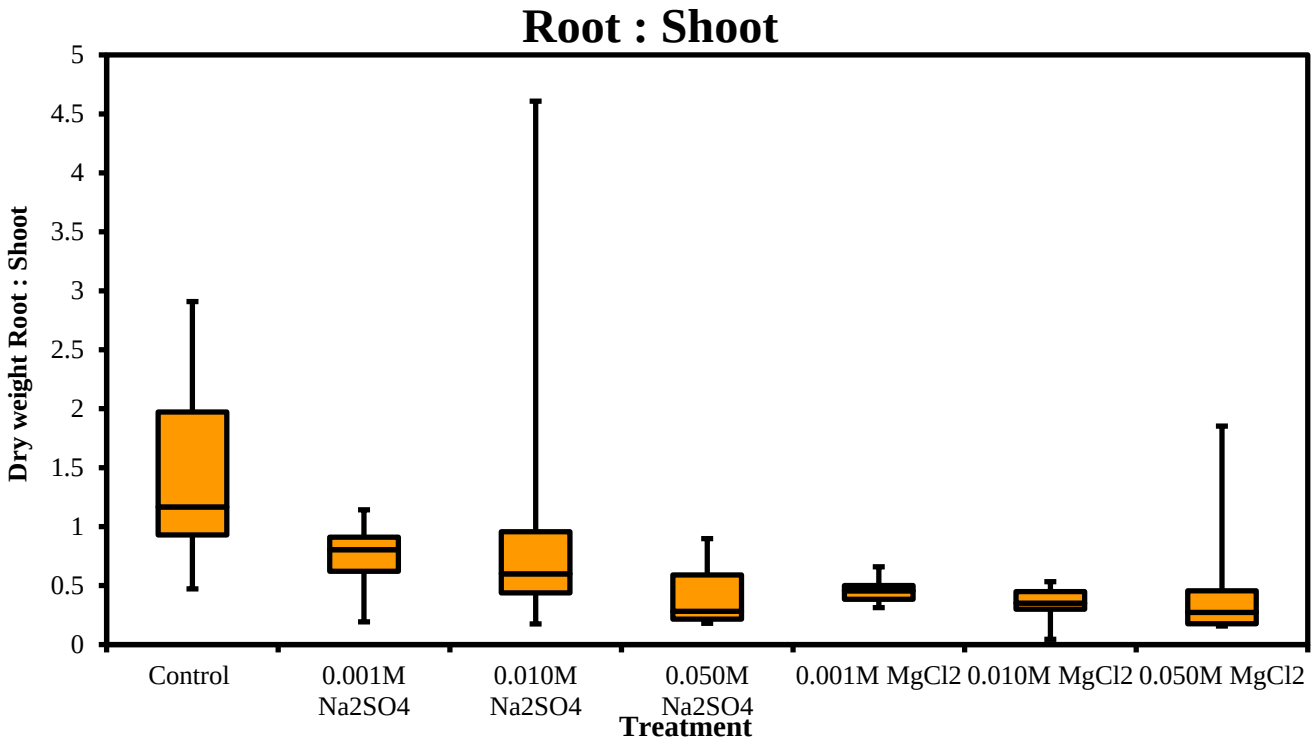


Table 4b shows the *P* values for different solution groups.

Solution groups	*P* value
0.001M, 0.010M and 0.050M Na_2SO_4	7.60%
0.001M, 0.010M and 0.050M $MgCl_2$	11.93%
0.001M Na_2SO_4 with Control	6.52%
0.001M $MgCl_2$ with Control	0.17%

Dataset 5 – Leaf : Stem

Table 5a shows the median of Leaf : Stem.

	Control	0.001M Na2SO4	0.010M Na2SO4	0.050M Na2SO4	0.001M MgCl2	0.010M MgCl2	0.050M MgCl2
Median	1.9226	1.9492	1.9448	1.5901	2.0184	2.0441	2.2268

Figure 5 shows the Leaf : Stem ratio in form of box plot.

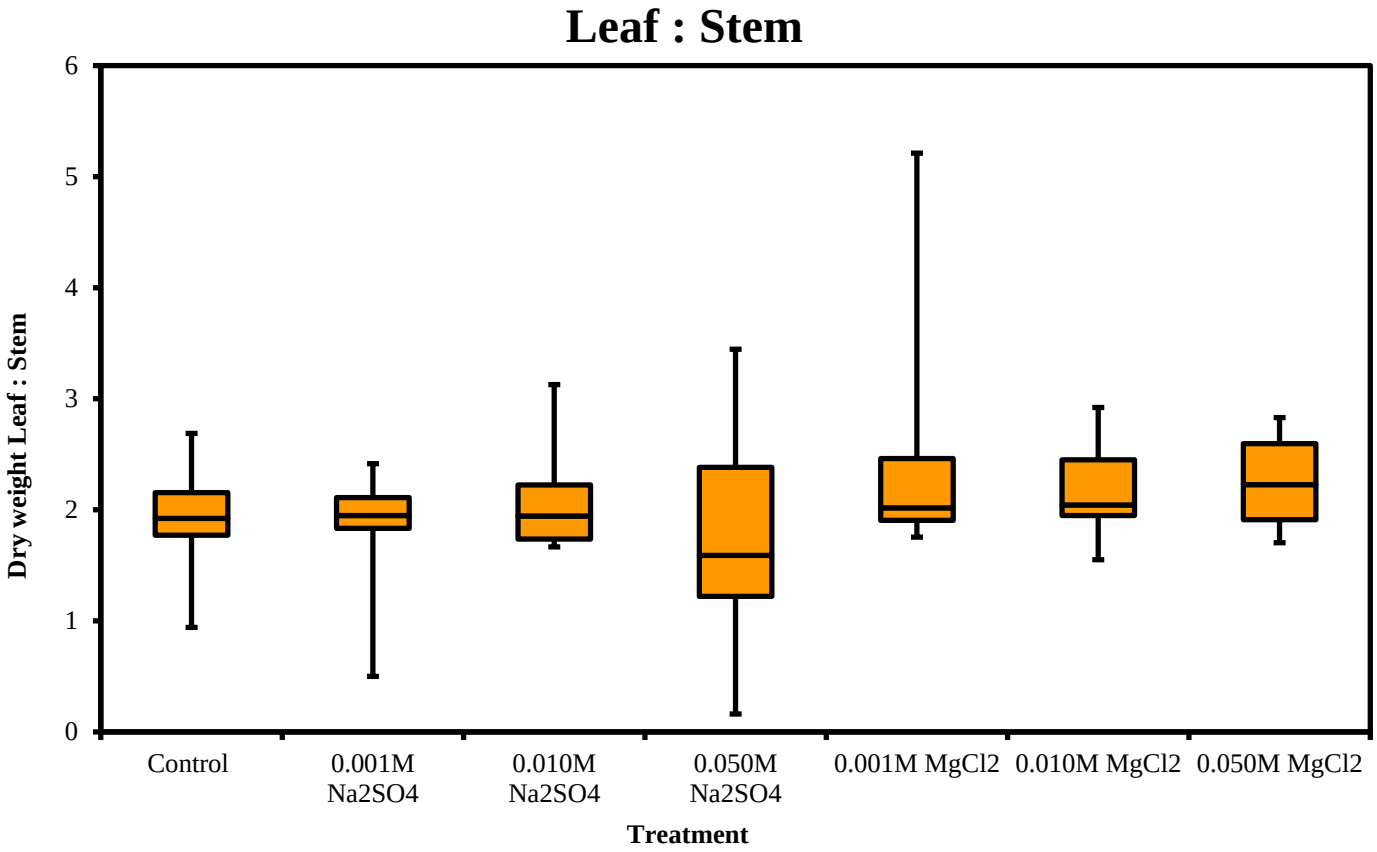


Table 5b shows the *P* value of Leaf : Stem ratio for all treatments.

Solution groups	*P* value
All solutions	68.58%

Dataset 6 – Leaf : Root

Table 6a shows the median of Leaf : Root.

	Control	**0.001M Na2SO4**	**0.010M Na2SO4**	**0.050M Na2SO4**	**0.001M MgCl2**	**0.010M MgCl2**	**0.050M MgCl2**
Median	0.6143	0.8119	0.9083	1.0664	1.4530	1.7909	2.7147

Figure 6 shows the Leaf : Root ratio in form of box plot.

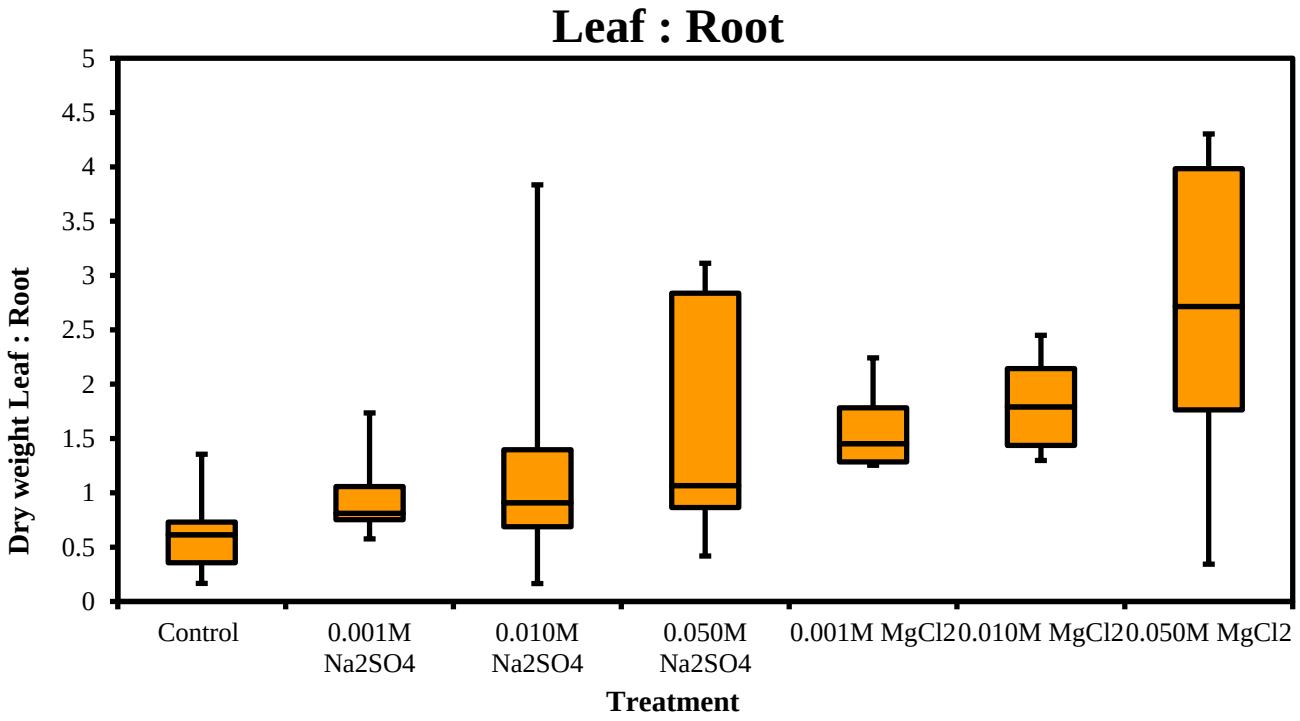


Table 6b Figure 4c shows the *P* values for different solution groups.

Solution groups	*P* value
0.001M, 0.010M and 0.050M Na_2SO_4	47.20 %
0.001M, 0.010M and 0.050M $MgCl_2$	15.08 %
0.001M Na_2SO_4 with Control	13.32 %
0.001M $MgCl_2$ with Control	0.39%

Conclusion

Discussion

The data from Table 1 shows that the Root : Shoot ratio decreases in the order of Control, 0.001M Na_2SO_4, 0.010M Na_2SO_4, 0.050M Na_2SO_4, 0.001M $MgCl_2$, 0.010M $MgCl_2$ and 0.050M $MgCl_2$. This implies that sulphur and magnesium does tend to have an effect on Root : Shoot ratio for *P. sativum*. It also shows that plants for Control, 0.050M $NaSO_4$ and 0.050M $MgCl_2$ did not survive as well as the plants treated with 0.001M and 0.010M. The perished plants did not either sprout, grew at most 50mm with thin white dotted leaves or dried out despite sufficient water access. Thus it suggests that magnesium and sulphur are vital but at the same time toxic in excessive amounts. However, the phenomenon of over-fertilization causing negative results is not unusual as many species have shown in various studies (Weinbaum, Johnson, & DeJong, 1992), (Fernández-Escobar et al., 2006).

The data from Figure 2 indicates that plants did not only change their Root : Shoot depending on the treatment, but they also thrived and grew differently in different conditions. Plants that thrive well will likely to have faster increase in biomass due to increased metabolism and therefore higher total dry weight. It can be deduced that plants treated with Na_2SO_4 and $MgCl_2$ affected the growth of the plant. For *P. sativum*, a concentration of Na_2SO_4 over 0.010M and concentration of $MgCl_2$ over 0.001M inhibited the growth. There is an indication that plants were short in sulphur since plants treated with Na_2SO_4 0.001M had a higher dry weight than the controlled plants. Thus *P. sativum* seems to need sulphate at a concentration around 0.001M. Nevertheless, 0.001M does not necessarily mean that it is the optimum concentration and there may have even been significant amount of sulphur in the soil that the manufacturer did not inform about. Magnesium does not seem to give a positive dry weight from 0.001M and upwards. Thus it cannot be concluded with confidence that the decrease in Root : Shoot causes decrease in total dry weight and vice versa.

The data from Figure 3 shows which compartments are interdependent. There is a positive correlation between concentration and leaf biomass for both elements, while there is a negative correlation between concentration and root biomass for both elements. This suggests that there is an inverse

relationship between leaves and roots, and that the increase in shoot is primarily due to the increase in leaf and not the stem. Thus it can be implied that *P. sativum* is striving for a stable stem biomass over its total biomass regardless of treatment. This finding may prove to be very valuable when it comes to underground root estimation. By knowing the stem dry weight, the total dry weight may be extrapolated. That value may then be subtracted by the stem and leaf dry weight to determine root dry weight non-destructively.

The data from Figure 4 was to statistically determine whether the treatments had significant effect on Root : Shoot. The result indicated that plants treated with Na_2SO_4 did not significant effect on Root : Shoot since the *P* value was 7.60%, which is above 5%. Likewise, the *P* value for $MgCl_2$ was 11.9%, which is also above 5%. Thus the null hypothesis is accepted. This means that the increments of concentration does not affect the Root : Shoot dramatically enough to reject the null hypothesis. Further, the effect on Root : Shoot was compared with the Control group and a 0.001M group. It showed that *P* value for 0.001M and Control was 6.52%. Although very close to 5%, the null hypothesis is accepted. However, the *P* value for 0.001M and Control was 0.17%. Thus null hypothesis is rejected, meaning that a 0.001M addition of magnesium increased the Root : Shoot significantly. Thus it can be expected that soil with high magnesium content will have a significantly high Root : Shoot ratio for *P. sativum.*

The data from Figure 5 compared the Leaf : Stem for all treatments. The *P* value with all treatments considered was as high as 68.6%. This means that null hypothesis can be accepted by a large marginal. This suggests that sulphur and magnesium does not affect the Leaf : Stem. This in turn means that *P. sativum* is striving for a constant biomass ratio between leaf and stem, and that leaf and stem are highly interdependent on each other. The ratio seems to be close to 2:1, indicating that *P. sativum* allocates resources to leaf around twice as much than the stem. There might be a way for plants to communicate between leaf and stem to regulate the allocation of resources to give them constant proportions regardless of nutrient level. This is truly an interesting finding.

The data from Figure 6 compared the Leaf : Root for all treatments. *P* values for treatments within Na_2SO_4 and treatments within $MgCl_2$ were higher than 5%. This means that the increment within the added element has no significant effect on the Leaf : Root. *P* value for 0.001M with Control was still higher than 5% meaning that additions of sulphur does not significantly affect Leaf : Root. Nevertheless, *P* value for 0.001M $MgCl_2$ with Control was very low at 0.39%. This means that addition of magnesium does indeed significantly increase Leaf : Root. These results are similar to Figure 4, which is understandable. Root : Shoot took into account of both stem and leaf, and since stem and leaf were deduced to maintain a constant ratio, it is logical that Leaf : Root show similar trends in *P* values like Root : Shoot.

Revisiting the research question

The focus of this experiment was to determine the effect of sulphate and magnesium on the allocation of resources between root, stem and leaf.

From the evidence in **Results** on and deductions in **Discussion**, H_1 is accepted. Within H_1, first hypothesis is rejected because the resource allocation goes from the stems to the leaves. Therefore the second hypothesis of H_1 is accepted because the second hypothesis stated that resource allocation will go from the roots to the leaf. This was primarily supported from Figure 3 and Figure 5.

Evaluation

Limitations and improvements

A major limitation was that sulphur and magnesium content in soil were unknown. Although it is thought to have very small amounts, it may still be significant for root and shoot development. Thus the result gathered may have the risk of unknown uncertainty. In order to solve this problem, it is recommended to cultivate the plants in an aerated hydroponics system without any nutrient containing soil. The nutrients may be then controlled and varied in a confident way that significantly reduces uncertainty in nutrient that plant is receiving.

Not all bottles were identical. Some systems were different because bottles were gathered by asking people and not all had the same type of bottles. Since the systems were all not identical, the volume of initial soil might have differed. The range is estimated to be around ±10g. This means that the initial N-P-K availability may have been different. This may cause different rate of growth or even different root, stem, leaf ratio. It would therefore be ideal to use identical bottles with the same shape and volume.

There was loss of root during the process of extracting. Roots of *P. sativum* were extremely thin and there was undeniably loss of root although the roots were washed with extreme caution. When parts of root are lost, the uncertainty reflects the root, stem and leaf ratio. It would then provide a lower Root : Shoot ratio than what it would have been if the extraction was perfect. A suggestion would be to grow the species in fine soil so they are easily removed in water. The soil used in this experiment contained coconut coir which made root extraction difficult.

Roots were not cleaned completely. Although some roots were very successfully removed, there were always some chunks of soil that are sticking to the root hair. This would add on the root mass, hence affect the ratio values. The loss is estimated to be up to 0.05g which is definitely a value that can have a large impact on root shoot ratios. The suggestion would be the same as above.

There might have been difference in light received due to position of plants because some plants were

positioned behind and some were in front of each other. As mentioned in introduction, light exposure affects the allocation of resources in leaves. This might partially explain why the plants treated with 0.001M Na_2SO_4 and 0.001M $MgCl_2$ had highest total biomass because 0.001M Na_2SO_4 and 0.001M $MgCl_2$ were positioned in the front. A suggestion is to distribute the plants and change the position regularly. Exposing the plants directly under the sun is not an option since Sweden is a country with high frequency of precipitation. Precipitation may dilute the treatment concentrations and cause random errors.

Plants had different biomass. When plants have grown to a certain point, the ratio of root, stem and leaf changes (Troughton, 1956), (Wilson, 1988). Therefore, the ideal scenario would be that the biomass of all treatments are all equal with only the dry weight of root, stem and leaf ratio differing. However, the difference in biomass may be due to the treatments itself and therefore inevitable. A way to minimize the difference would be to artificially control the abiotic factors, in particular the light exposure. In addition, segregating the plants might reduce the chance that some become more dominant than the other and create shadow upon less developed plants.

Not all plants survived. Not all plants in Control group, 0.050M Na_2SO_4 and 0.050M $MgCl_2$ survived, thus affects reliability of the roots, stem and leaf ratio. Since this is an issue of reliability, more trials would alleviate the effect of plant's death.

There were wide error bars on Figures 4, 5, and 6, on some treatments. This might be due to some outliers or the difficulties in root extraction as mentioned above. In either case, the error bars may be reduced by having more plants per treatment.

For future research, it is recommended to reproduce the experiment with these improvements mentioned above and delve into the relationship between the plant's stem height and its stem : leaf : root ratio. This could cast light on how the plant's allocation of resources is changing as it ages which can further help to understand the mechanism of how plants regulate its growth in different nutrient available conditions.

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Appendix

Pre-investigation

A pilot test was executed to verify the function of bottle pot.

The picture below shows the successful root growth after the shoot has been removed.

Calculations

The solutions of Na_2SO_4 and $MgCl_2$ were calculated as follows.

Solution	Volume of water/plant
Water added on the bottom of bottle pots	5.5dl
Water added from the top of bottle pots	1.0dl
Total	6.5dl

Each concentration has 10 trials, thus needs 6.5L per concentration.

	Na_2SO_4 solution (anhydrous) m = 142.04 g/mol	$MgCl_2$ solution (hexahydrous) m = 203.31 g/mol
0.001M (6.5L = 6.5dm^3)	Mole required = 0.001mol Mass required = 0.92326g	Mole required = 0.001mol Mass required = 1.321515g
0.010M (6.5L = 6.5dm^3)	Mole required = 0.010mol Mass required = 9.2326g	Mole required = 0.010mol Mass required = 13.21515g
0.050M (6.5L = 6.5dm^3)	Mole required = 0.050mol Mass required = 46.163g	Mole required = 0.050mol Mass required = 66.07575g
Total mass needed	56.31886g	80.612415g

Required mass for each concentration of each compound were calculated in school using a digital scale. These are the weighed masses.

	Na_2SO_4 solution (anhydrous) m = 142.04 g/mol	$MgCl_2$ solution (hexahydrous) m = 203.31 g/mol
0.001M (6.5L = 6.5dm^3)	0.9252g	1.3672g
0.010M (6.5L = 6.5dm^3)	9.2561g	13.2050g

0.050M (6.5L = 6.5dm^3)	46.1578g	66.0433g

Formulae

$$Shoot = Stem\ dry\ weight + Leaf\ dry\ weight$$

$$R : S = Root : Shoot = \frac{Root\ dry\ weight}{Stem\ dry\ weight + Leaf\ dry\ weight}$$

$$Leaf : Stem = \frac{Leaf\ dry\ weight}{Stem\ dry\ weight}$$

$$Leaf : Root = \frac{Leaf\ dry\ weight}{Root\ dry\ weight}$$

Raw data

Table 1.1 shows the dry weight values of shoot, root and leaves for plants treated with no sulphur or magnesium in grams (± 0.0001g).

Added compound: Not added **Concentration: Control**										
	1	2	3	4	5	6	7	8	9	10
Stem	0.1305	0.3091	0.1383	0.0221	-	-	0.0439	-	-	0.4406
Root	0.7914	0.8563	0.4457	0.1248	-	-	0.2141	-	-	0.5765
Leaves	0.2311	0.6402	0.3019	0.0208	-	-	0.1180	-	-	0.7816

Table 1.2 shows the dry weight values of shoot, root and leaves for plants treated with sulphur in three different concentrations in grams (± 0.0001g).

Added compound: Na_2SO_4 **Concentration: 0.001M**										
	1	2	3	4	5	6	7	8	9	10
Stem	0.4184	0.4370	0.1413	0.2144	0.2860	0.3700	0.1911	0.2530	0.4640	0.1085
Root	1.4718	0.9088	0.2395	0.6653	0.9548	0.4547	0.5146	0.6451	1.0061	0.0313
Leaves	1.0105	0.7711	0.2587	0.5128	0.5495	0.6813	0.3840	0.5002	0.9945	0.0543
Added compound: Na_2SO_4 **Concentration: 0.010M**										
	1	2	3	4	5	6	7	8	9	10
Stem	0.3084	0.3237	0.0422	0.3258	0.1949	0.0173	0.1084	0.3040	0.1546	0.1022
Root	0.3661	0.3482	0.1162	0.5765	0.5217	0.3290	0.3814	0.8922	0.0819	0.1819
Leaves	0.5270	0.5785	0.0784	0.5601	0.4409	0.0541	0.1806	0.6494	0.3141	0.2302
Added compound: Na_2SO_4 **Concentration: 0.050M**										
	1	2	3	4	5	6	7	8	9	10
Stem	-	0.0523	0.0164	0.0475	0.0441	0.0143	0.0364	0.0327	-	0.0085
Root	-	0.0344	0.0619	0.1330	0.0681	0.0055	0.0258	0.0133	-	0.0102
Leaves	-	0.0960	0.0525	0.1637	0.0593	0.0023	0.0768	0.0414	-	0.0092

Table 1.3 shows the dry weight values of shoot, root and leaves for plants treated with magnesium in three different concentrations in grams (± 0.0001g).

Added compound: $MgCl_2$ **Concentration: 0.001M**										
	1	2	3	4	5	6	7	8	9	10
Stem	0.1030	0.3230	0.1846	0.1804	0.0417	0.1322	0.3742	0.3144	0.2858	0.2073
Root	0.1179	0.5379	0.2884	0.2585	0.1708	0.2028	0.3431	0.4872	0.3064	0.2378
Leaves	0.2643	0.6745	0.3970	0.3420	0.2173	0.3646	0.7230	0.6126	0.5314	0.3637
Added compound: $MgCl_2$ **Concentration: 0.010M**										
	1	2	3	4	5	6	7	8	9	10
Stem	0.0542	0.1403	0.0285	0.0590	0.3374	0.1405	0.1796	0.2005	0.1320	0.2438
Root	0.0427	0.2104	0.0443	0.1185	0.4031	0.1342	0.2202	0.1943	0.1654	0.2355
Leaves	0.1046	0.4099	0.0715	0.1631	0.5232	0.2896	0.3597	0.4562	0.2255	0.4942
Added compound: $MgCl_2$ **Concentration: 0.050M**										
	1	2	3	4	5	6	7	8	9	10
Stem	0.0612	0.0378	0.0032	-	-	0.0719	0.0513	-	0.0236	0.0441
Root	0.0638	0.0182	0.0163	-	-	0.0422	0.0553	-	0.0396	0.0263
Leaves	0.1732	0.0783	0.0056	-	-	0.1786	0.1389	-	0.0402	0.0982

Table 1.4 shows morphology of roots, stem and leaves for plants treated with no sulphur or magnesium.

Added compound: Not added **Concentration: Control**
Not all sprouted, but out of those who did, everyone grew very healthy with robust stem and even entered the phase of flowering. There were also sigs of reproductive allocation with onset of small seeds. Root grew very long and thick out of the bottle. Leaf had many white dots.

Table 1.5 shows morphology of roots, stem and leaves for plants treated with sulphur in three different concentrations.

Added compound: Na_2SO_4 **Concentration: 0.001M**
Virtually all plants were very robust and healthy. Their stem seemed thick and very bright green colour. There were no signs of wilting and even signs of reproductive allocation. 3 plants had signs of reproductive allocation. All plants had bundles of root growing out from the bottle, although not all were in same thickness. Leaves were as largest 5cm x 3 cm.
Added compound: Na_2SO_4 **Concentration: 0.010M**
All of the plants were healthy as well. Although they did not grow as much in length as 0.001M Na_2SO_4. Some stem were thin while some where thick as plants treated with 0.001M. Nevertheless, the majority were thick and showed strong green colour. The sizes of leaves were relatively small but there were more of them than 0.001M.

There were 2 plants with signs of reproduction allocation.
Added compound: Na_2SO_4 **Concentration: 0.050M**
Stem were generally short. Not all sprouted, and some died after sprouting or not grown above 3 cm.

Table 1.6 shows morphology of roots, stem and leaves for plants treated with magnesium in three different concentrations.

Added compound: $MgCl_2$ **Concentration: 0.001M**
Majority of plants were very healthy and stems were rigid and intact. One plant had signs of wilting. There were some varieties of root growth. Some roots were very thin, but long and some roots were very thick, dense and had many lateral growth. There were few plants with white dots on the leaves. White dots seemed to start from the centre and expand out. Only one plant showed signs of reproductive allocation.
Added compound: $MgCl_2$ **Concentration: 0.010M**
Only few grew very well, two did not sprout and the rest of the plants grew shorter than plants treated with 0.001M $MgCl_2$. The numbers of leaves were similar as plants treated with 0.001M, ranging from 20-40 leaves.
Added compound: $MgCl_2$ **Concentration: 0.050M**
Shoot on all are generally thin. Not many have sprouted or it is short. There was almost no root growth and no lateral growth. There were a few signs of wilting.

Explanation of statistical analysis

Choice of statistical test:

Kruskal-Wallace tests were carried out to determine the association between the concentrations in plants treated with Na_2SO_4 and $MgCl_2$. There are four reasons for choosing this statistical test. Firstly, the concentrations are independent samples, i.e. they are not dependent on each other. Secondly, there are more than two concentrations for each Na_2SO_4 and $MgCl_2$ and this test enable one to test more than two groups at once. Thirdly, the data is not assumed to have a normal distribution. Thus a non-parametric test that is more forgiving to outliers is suitable. Fourthly, Kruskal-Wallace test enables one to test more than two different concentrations at the same time, which is very suitable for this experiment with three concentrations per Na_2SO_4 and $MgCl_2$.

Meaning of *P* values:

H_0 is the null hypothesis. It assumes that the median values of all the concentrations are identical. This means that when the calculated *P* value is $P < 5\%$, the null hypothesis may be rejected. In other words, there is more than 95% certainty that there is a significant difference in at least one of the categories.

H_1 is the alternative hypothesis. When $P > 5\%$, this hypothesis is accepted. This means that there is not a significant difference between the medians and therefore the samples treated with different solutions do not have an effect on allocation of resources.

Diagram explanation:

The suitable diagram for Kruskal-Wallace test is a box plot. A box plot includes the median, first quartile, third quartile and upper and lower whiskers as error bars. *P* values for Kruskal-Wallace test was determined using an add-on to excel called *Merlin*.

Pictures

Picture 2.1 shows the process and the end-product of bottle pots.

Picture 2.2 shows preparation of seeds by soaking them overnight.

Picture 2.3 shows the seed and soil level in the bottle pots.

Picture 2.4 shows plants after 14 days.

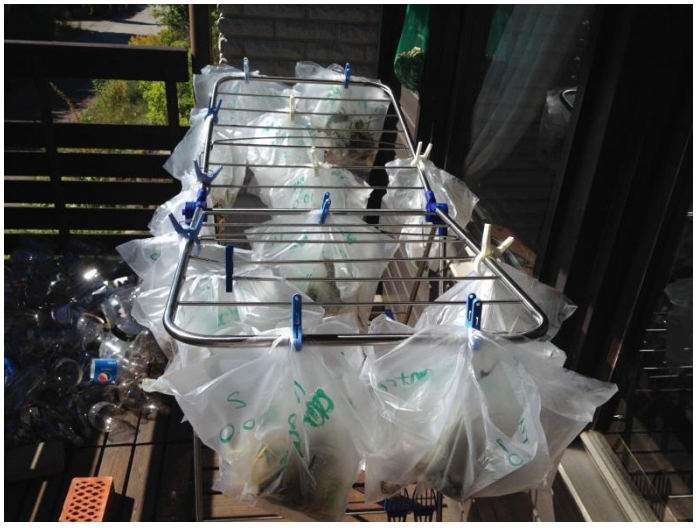
Picture 2.5 shows plants after 22 days.

Picture 2.6 shows plants after 40 days.

Picture 2.7 shows the removal of soil from plant.

Picture 2.8 shows the process of separating the root, leaf and stem and letting them dry.

Note that the plastic bags were well ventilated with holes in the bottom and top so water could condense down or evaporate.



Picture 2.9 shows how dry weight was measured.