

How does varying concentrations of sulphuric acid (mol/L) affect the percent germination of fenugreek seeds (%) when placed under stable conditions.

## Research Question

How does the concentration of sulphuric acid (mol/L) affect the percent germination of fenugreek seeds (%)?

## Introduction

### Fenugreek Seeds

The scientific name of Fenugreek is *Trigonella Foenum-graecum* it is an endospermic plant meaning that before germination it has endosperm unlike beans. *Trigonella foenum-graecum*, also called Birds Foot, its plant family is Leguminosae or better known Fabaceae. These seeds are found in countries on the eastern shores of the Mediterranean, and they are cultivated in Africa, Morocco and Egypt in India.

A structure of a seed might seem very simple but it is highly complicated therefore not all the parts will be explained. Only the parts pertaining to the experiment will be looked at, the first being the embryo. The Embryo is made up of diploid cells (2n) which are result of fertilization, it is the part that has the cotyledons (baby leafs) that are the first to shoot out during germination. The second part that will be described is the endosperm, which is a triploid (3n) also created during fertilization this is the food storage in a sense because it holds all the nutrients a plant needs to get past germination. These seeds were also used because of the conditions they can survive. Due to the fact that this experiment took place in a Canada and that the seeds would be put under a plant heater, so they have to survive direct plant light for 3 – 4 days straight. Fenugreek seeds are accustomed to frosty weathers, because they should be planted at the end of the winter, beginning of spring when it is still chilly outside for most plants. Also these plants can survive without a lot of water and are accustomed to dry climates.

### Sulphuric Acid

Sulphuric acid is one of the most abundant pollutant's created by man but as it is an acid therefore there come dangers with it when using them in labs. Therefore, in this lab 1 (mol/L) is the highest concentration of sulphuric acid being used. The other reason stands to be that in nature pure sulphuric acid is not what falls down in acid rain it is a highly diluted version of sulphuric acid.

### Acid Rain

Acid rain is an effect of pollution; when car puffs along a street or a factory belches out smoke all those pollutants rise up into the air. The pollutants are, but not restricted to, carbon dioxide, sulphur trioxide, and nitrogen dioxide. All these are found in nature naturally, but due to human dependence on fossil fuels humanity burns and releases more than the natural amount. Out of this sulphur dioxide is one of the most released pollutants, which on due to oxidation becomes sulphur trioxide. When sulphur trioxide reacts with water vapour it creates sulphuric acid:



As if to cleanse the air of pollutants this acid falls down with any form of precipitation and is absorbed by the soil raising its acidity.

### Hypothesis

If the concentration of sulphuric acid (mol/L) is increased, then the percent germination of fenugreek seeds (%) will decrease because the embryo is a major part of the seed that already has mature cells, therefore they have to maintain a pH gradient that suits them. Therefore, by adding acid you are increasing the amount of hydrogen ions thereby exerting the cells energy stored in the endosperm to maintain the pH gradient.

Table 1: The affect of Sulphuric acid on seed germination with control variables related to seed growth and development.

Variables	Relevance
<p>Independent Sulphuric Acid 0.00, 0.25, 0.50, 0.75, 1.00 (mol/L) <b>Explain for how to vary conditions is included</b></p>	<p>Sulphuric acid is one of the most abundant acid's produced by pollution by factories and vehicle exhaust. A third of total acid rain is sulphuric acid, therefore the increase in acidity of the increases the acidity of the soil thereby affecting plants that can only survive in low pH. Therefore, due to the fact that it is commonly used as fertilizer it makes for a perfect variable. The other very important fact is though having varying pH would be the right choice for an independent variable, changing the concentration would work better because of two reasons. Firstly, the instruments in a high school lab are not sophisticated enough to give an accurate pH reading. Secondly changing the concentration is accurate and easier to achieve.</p>
<p>Dependent Percent Germination of Fenugreek seeds (%) <b>Methods for precise measurement is clearly explained</b></p>	<p>Though the dependent variable is percent germination of fenugreek seeds through out the lab this would be calculated near the end. To calculate a percentage there has to be a fraction or a part of a whole. Therefore, for each trial 10 seeds were used so that 10 could be the 100%. The other reason for choosing 10 seeds were from the same species no to seeds would have the same DNA and would all have a different genetic mutation, therefore only a substantial amount of seeds could take that uncertainty into context. The final reason for using many seeds instead of one, lies in the fact that, the seeds have been dormant for a substantial amount of time thus there is no guarantee that that all seeds would germinate.</p>
<p>Control Temperature the seeds were kept under The amount of water the plants received The amount of light the plants received  Reasons Identify all relevant variable</p>	<p>Seeds need the environment to meet a few conditions so that they are able to germinate. Even though the conditions with each seed changes the main conditions which all seeds need are the same: light, water, correct pH level and air. Therefore if the experiment is changing the concentration of sulphuric acid, the temperature, amount of light, air and water would have to be</p>

	kept constant, so not to ruin the experiment.
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### **Materials**

100 mL Volumetric  $\pm 0.16$  (mL)  
250 mL Erlenmeyer Flask x 3  
Petri Dish x 5  
Fenugreek Seeds x 250  
Tweezer  
10 mL Pipette  $\pm 0.08$  (mL)  
Small Ziploc Bag x 25  
Paper Towel x 25  
Gloves  
Goggles  
100 mL graduated cylinder  $\pm 0.5$  (mL)  
200 mL Beaker  
Plant Heater

### Methodology

- 1) All the materials were gathered, and goggles and gloves were worn
- 2) Diluted solutions of sulphuric acid were created from 1.00 mol/L of sulphuric acid
- 3) Measured 25 mL of 1 mol/L sulphuric acid in the 100 mL graduated cylinder
- 4) Poured in the 100 mL volumetric
- 5) Measured 100 mL of distilled water in the 200 mL beaker
- 6) Poured water into the 100 mL volumetric till the solution hits the 100 mL mark in the volumetric
- 7) Poured the solution in the first 250 mL Erlenmeyer flask
- 8) Calculated the concentration
- 9) Wrote on a paper: name of the experimenter, concentration of solution,  $H_2SO_4$ , See SDS
- 10) Taped the paper to the first 250 mL Erlenmeyer flask
- 11) Rinsed out the 100 mL volumetric
- 12) Steps 3 – 11 were repeated with 50 mL and 75 mL of sulphuric acid and the second and third 250 mL Erlenmeyer flasks
- 13) Rinsed out the five petri dishes
- 14) Took of their lids
- 15) Used a 10 mL pipette to measure out 20 mL of the first solution in the first Erlenmeyer flask and poured that into each of the petri dishes
- 16) Took five of the same sized paper towel and folded them half short side to short side
- 17) Soaked up the 20 mL of the first solution in each paper towel
- 18) Placed each paper towel in a separate Ziploc bag
- 19) Placed 10 fenugreek seeds in each Ziploc on the top of the paper towel
- 20) Labelled each Ziploc bag with the concentration of the solution being used and labelled each Ziploc bag a different trial from 1 – 5
- 21) Placed the Ziploc bags under the plant heater
- 22) Steps 13 – 21 were repeated with the second solution in the second Erlenmeyer flask and the third solution in the third Erlenmeyer flask
- 23) Rinsed out the Erlenmeyer flasks
- 24) Measured 100 mL of distilled water in one flask and 100mL of 1.00 mol/L concentrated sulphuric acid in the other flask
- 25) Rinsed out the five petri dishes
- 26) Took of their lids

- 27) Used a 10 mL pipette to measure out 20 mL of the distilled water poured that into each of the petri dishes
- 28) Took five of the same sized paper towel and folded them half short side to short side
- 29) Soaked up the 20 mL of distilled in each paper towel
- 30) Placed each paper towel in a separate Ziploc bag
- 31) Placed 10 fenugreek seeds in each Ziploc on the top of the paper towel
- 32) Labelled each Ziploc bag with the concentration of the solution being used and labelled each Ziploc bag a different trial from 1 – 5
- 33) Placed the Ziploc bags under the plant heater
- 34) Repeat with the 1.00 mol/L concentrated sulphuric acid
- 35) Left the Ziploc bags under the plant heater for 12 hours
- 36) Observed the seeds after the 12 hours under the plant heater
- 37) Pulled out the Ziploc bags from under the heater and keep it on the counter with mostly shade and partial light for 12 hours
- 38) Observed the seeds after the 12 hours in the shade
- 39) Placed the Ziploc bags under the plant heater after the 12 hours in the shade
- 40) Observed the seeds after the 12 hours under the plant heater and recorded how many seeds germinated in each Ziploc bag
- 41) Took the average for each test
- 42) Found the percentage germination for each test condition

#### Calculations

How to find concentration?

In this case the concentration is being figured out through dilution therefore the equation

$$C_1V_1 = C_2V_2$$

$$C_2 = C_1V_1/V_2$$

Concentration of solution = Concentration of 1.00 (mol/L) concentrated sulphuric acid \* Volume of 1.00 (mol/L) concentrated sulphuric acid / Volume of total solution

\*Volume in this equation is always in Litres

#### Example

$$C_1 = 1.00 \text{ (mol/L)}$$

$$V_1 = 25 \text{ mL} = 0.025 \text{ L}$$

$$V_2 = 100 \text{ mL} = 0.1 \text{ L}$$

$$C_1V_1 = C_2V_2$$

$$C_2 = C_1V_1/V_2$$

$$C_2 = 1.00 * 0.025 / 0.1$$

$$C_2 = 0.25 \text{ mol/L}$$

Table 2: Portraying the percent death of the fenugreek seeds after being treated with varying concentration of H<sub>2</sub>SO<sub>4</sub>. For there to be less uncertainty in the results five trials for each concentration take place.

Concentration of H <sub>2</sub> SO <sub>4</sub> (mol/L)	Percent Germination of Fenugreek Seeds (%)					
	Test	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
0.0 ± 0.16 (mL)		90	100	90	90	100
0.25 ± 0.82 (mL)		80	70	70	80	80
0.50 ± 0.82 (mL)		60	60	60	70	60
0.75 ± 0.82 (mL)		50	50	40	50	30
1.0 ± 0.16 (mL)		20	10	30	40	20

#### Method for calculating uncertainty

To calculate uncertainty of the solution in this case would be to add the uncertainties up.

#### Example

Test 0.00 (mol/L)

To measure 20 (mL) of this solution a 10 (mL) pipette was used:

Uncertainty of a 10 (mL) pipette is ± 0.08 (mL), therefore if used twice the uncertainties would add up.

$$0.08 + 0.08 = 0.16$$

Therefore, the uncertainty for the first solution is ± 0.16 (mL)

#### Method for calculating percentage

To calculate the number of seeds that survived count the number of seeds that sprouted in each trial containing 10 seeds. Once the number is known divide by total number of seeds which are 10 in each trial and multiply by 100 to find the percentage

#### Example

Test 0.00 (mol/L)

Trial 1

Number of Seeds that survived = 9 (seeds)

Percent of seeds that survived = number of seeds survived/total number of seeds \* 100

$$P = 9/10 * 100$$

$$P = 90 (\%)$$

Table 3: The observations taken from the moment the fenugreek seed touches water to the point when the first cotyledons pop out of the seed. This is all due to the idea of having the seeds survive with only 20 (mL) through the 3 – 4 days it takes Fenugreek seeds to germinate.

Day 1	<p>Fenugreek Seeds</p> <ul style="list-style-type: none"> <li>• They are small in size the biggest seeds are about 0.25 (cm) <math>\pm</math> 0.05 (cm)</li> <li>• Their shell colour is brown</li> <li>• They have a musky odour that overpowers other smells</li> <li>• They weigh about 0.03 (g) <math>\pm</math> 0.01 (g)</li> <li>• The all have a small dent separating the point where the cotyledons sprout out of</li> <li>• Have a hard shell</li> </ul> <p>Sulphuric Acid</p> <ul style="list-style-type: none"> <li>• Is a clear liquid</li> <li>• Has no strong identifiable smell</li> <li>• It has the same consistency as water</li> <li>• Sometimes you can see sliver of clear liquid separate from the solution, but disappears when the solution is shaken</li> </ul>
Day 2	<p>Fenugreek Seeds</p> <ul style="list-style-type: none"> <li>• Their size's have increased</li> <li>• They look ready to burst</li> <li>• The musky odour has almost disappeared</li> </ul>
Day 3	<p>Fenugreek Seeds</p> <ul style="list-style-type: none"> <li>• Some of the seeds have sprouted</li> <li>• The cotyledons are white in colour</li> <li>• The seeds which have cotyledons seem smaller and a bit shrivelled</li> </ul>
Day 4	<p>Fenugreek Seeds</p> <ul style="list-style-type: none"> <li>• More seeds have sprouted</li> <li>• The ones that sprouted a day before are very shrivelled</li> <li>• The seeds which just sprouted their cotyledons seem smaller and a bit shrivelled than Day 2</li> </ul>

Table 4: Average percent germination of fenugreek seeds in different concentrations of sulphuric acid ranging from 0.0 (mol/L) to 1.0 (mol/L). This average was found by finding the mean of the five trials of each test condition.

Concentration of H <sub>2</sub> SO <sub>4</sub> (mol/L)	Average Percent Germination of Fenugreek Seeds (%)
0.0	94
0.25	76
0.50	62
0.75	44
1.0	24

#### Overview and Sample Calculations

#### Finding the Average/Mean Percent Germination of Fenugreek Seeds (%)

$$\text{Mean} = \text{Trial 1} + \text{Trial 2} + \text{Trial 3} + \text{Trial 4} + \text{Trial 5} / 5$$

Example

Test1: 0.00 (mol/L)

$$\text{Mean} = \text{Trial 1} + \text{Trial 2} + \text{Trial 3} + \text{Trial 4} + \text{Trial 5} / 5$$

$$\text{Mean} = 90 + 100 + 90 + 90 + 100 / 5$$

$$\text{Mean} = 94 (\%)$$

Why this calculation?

This calculation is one of the ways of obtaining average the two other methods are called medium and mode. Both of these do not take outliers into context:

Mode

Finding the Average Percent Germination of Fenugreek Seeds (%)

Mode = Find the most reoccurring number in the data

Mode = x, x, y, x, y

Mode = x

Example

Test1: 0.00 (mol/L)

Mode = Find the most reoccurring number in the data

Mode = 90, 100, 90, 90, 100

Mode = 90 (%)

Medium

Finding the Average Percent Germination of Fenugreek Seeds (%)

Medium = Place data in numerical order from least to greatest. Then cross of the first and last number in the number line till only two numbers are left or one number is left. If two numbers are left in the center add them and divide them by two. If there is one number left then that is the medium.

Medium = x, x, x, y, y

Medium = x, x, y

Medium = x

Example

Test1: 0.00 (mol/L)

Medium = Place data in numerical order from least to greatest. Then cross of the first and last number in the number line till only two numbers are left or one number is left. If two numbers are left in the center add them and divide them by two. If there is one number left, then that is the medium.

Medium = 90, 90, 90, 100, 100

Medium = 90, 90, 100

Medium = 90 (%)

Both of these methods do not take the less occurring numbers or outliers into context whereas mean takes every piece of data in context. Therefore, mean is the most accurate method of calculating average.

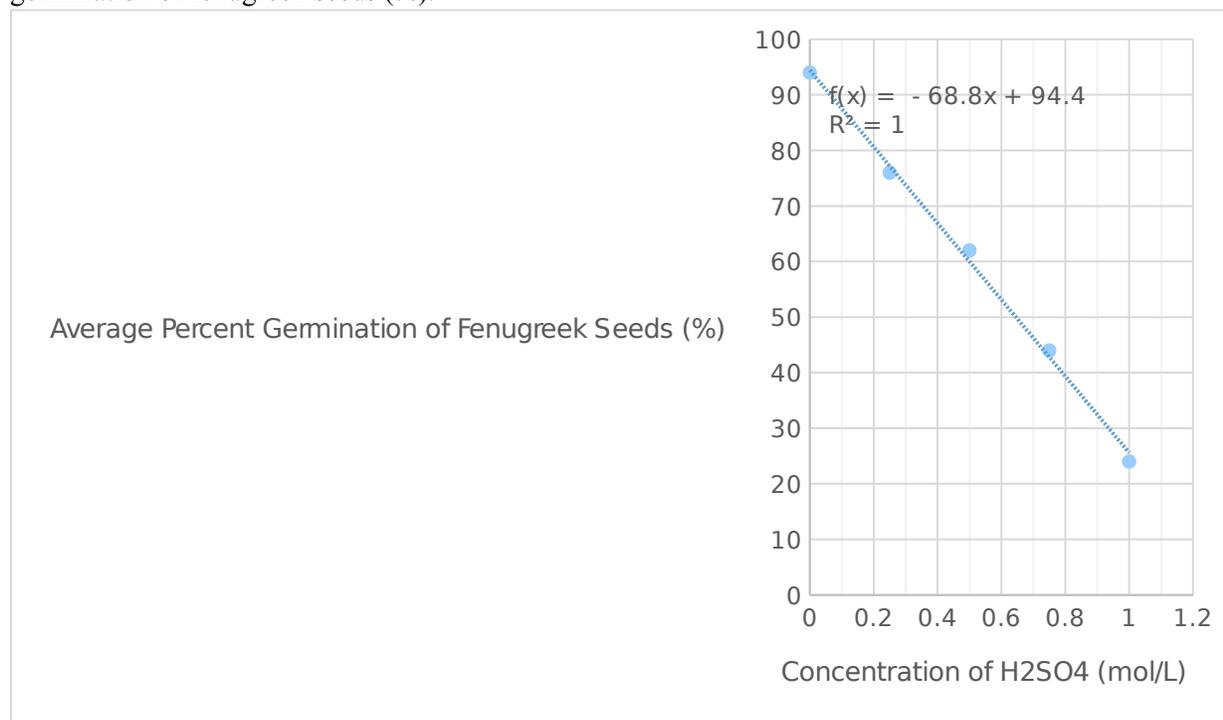
Table 5: The errors in this lab due to conventional ideas failing from paper to reality.

Errors	
1) Seed Dormancy Due To Lack Of Water	Seeds wait for the suitable conditions to present themselves so that they can germinate. This being an experiment the amount of solution used for each ten seed was estimated to be 20 (mL). This seems to have harmed the possibility for the seeds that remained dormant to germinate.
2) Movement of seeds might have caused a disturbance	Fenugreek seeds are part of the legume family which are known to not sprout if they are disturbed therefore when they are planted they have to be kept in biodegradable pots that can be kept into the soil with out taking out the seed. This procedure required the transfer of the seeds from light to the shade this could have upset some seeds and killed them.

Table 6: The improvements that will guarantee a successful lab no matter who performs it

Improvements	
1) Use soil to contain water	Though fenugreek seeds need easily drainable soil to grow in, it would be better to get soil that would hold at least moisture to help the seeds that were dormant to get ample supply of water if they need it.
2) Turning Of the Heater instead of moving seeds	Due to the fact that there were other experiment under the heater with this one, closing the heater would become a problem for others. Thus if this experiment was being performed at home close of the heater rather than moving the legumes for better results.

Fig 1: The affect of a changing the concentration of sulphuric acid (mol/L) has to the average percent germination of fenugreek seeds (%).



## CONCLUSION

In conclusion the experiment portrayed by thinking perfectly, that by increasing the concentration of sulphuric acid (mol/L) there would be a decrease in percentage fenugreek seed germination (%). This is shown in Fig 1 as a downwards linear equation with an  $r^2$  value of 0.9. The downwards trend line indicates a decrease in percentage germination and the  $r^2$  value tells that there is an almost perfect correlation between the points. Also the error bars are not very large indicting the fact that the trials had numbers that were very close to each other. The final two data points have error bars that coincide, which means that those two data points could be the same therefore they can be treated as one data point. This tells that as the concentration of sulphuric acid increases the average percentage of seeds that will germinate becomes distorted and there is no clear percentage. The most interesting part of this experiment was the first data point in Fig 1, because even though those seeds had been germinated in distilled water the average was not 100% it was 94%. This was a very peculiar thing considering that all the seeds were placed under perfect conditions to germinate, thus there can only be two reasons for this mishap. The first reason is that the seed was dead, meaning that the embryo was damaged to such a point it could not grow no matter the conditions. The second reason, though the most unlikely reason it could be that for a couple of seeds even the conditions that were set out in the first test were not enough to wake up the dormant seed. This is because seeds can remain dormant for many years, it is almost like a hibernation in which they wait for the perfect temperature, water content and soil acidity so they can sprout. There was one other experiment conducted by another person similar to this one, in their experiment they used cress seeds, but used the same acid as the one in this lab. There results where surprisingly close to the one this

experiment received. The other lab was calculating growth of the seed rather than germination itself. The specific point is that due to the fact that fenugreek seeds usually survive 6.5 pH increasing the concentration would definitely harm the seed. Therefore, all in all this experiment was a success due to the extraordinary results acquired from the lab.

#### Bibliography

B. (n.d.). The Chemistry of Acid Rain. Retrieved February 12, 2016, from <http://www.usetute.com.au/acidrain.html>

M., L., & S. (n.d.). MAINTAINING CELLULAR CONDITIONS: PH AND BUFFERS. Retrieved February 12, 2016, from <http://www.tiem.utk.edu/~gross/bioed/webmodules/phbuffers.html>

Reid, J. S. G. (1971). Reserve Carbohydrate Metabolism in Germinating Seeds of *Trigonella foenum-graecum* L. (Leguminosae). *Planta*, 100(2), 131–142. Retrieved from <http://www.jstor.org/stable/23369370>

U. (2005, Spring). The Seed Biology Place - Seed Structure and Anatomy. Retrieved February 12, 2016, from <http://www.seedbiology.de/structure.asp>