An investigation to determine the effect of pH on the permeability of beetroot plasma membrane by measuring the leakage of the pigment betalain using a colorimeter.

1: INTRODUCTION.

Beetroot (*Beta Vulgaris*) is a root vegetable that grows as a round bulb with a leafy top poking out above the soil ("Beetroot per 12 Pieces"). They have a high iron content hence, are usually given to anemic people. My elder and younger sisters both have iron deficiency anemia. This is anemia caused by a shortage of iron in the body meaning that the body does not have enough hemoglobin to produce red blood cells. Beetroot's high iron level helps to increase the production of hemoglobin which in turn increases the production of red blood cells and improves oxygen circulation around the body (Userbeets). Hence, because of its benefits, beetroot has been added to my sister's diet. However, in this constantly changing world, where pollution in form of acid rain is increasing, the beneficial abilities that beetroot has for our bodies are at risk of ceasing to exist. Hence, I am interested in studying how pH will affect the growth and nutrient levels of beetroot through effects on its plasma membrane.

2: RESEARCH QUESTION.

How does the pH (1, 2, 3, 4 and 5) of 20 cm^3 of H_2SO_4 solutions affect the permeability of the plasma membrane of a 4 cm long beetroot measured by the concentration of the leaked betalain pigment using a colorimeter?

3: BACKGROUND INFORMATION.

Betalain is a pigment that is found in the vacuole of beetroot. It is well known for its vibrant red-violet colour which is highly noticeable when it leaks out due to any damage to its cell membrane. Under normal conditions, betalain does not pass through the vacuole because the vacuole is surrounded by a membrane known as tonoplast which separates the content of the vacuole from the cytoplasm. The tonoplast is a membrane just as the plasma membrane hence, have a similar structure and can be affected by temperature and pH. The plasma membrane is a very thin layer found on all living cells that controls the exchange of materials such as nutrients and waste products between the cell and its environment because it is selectively permeable. It is made up of a bilayer of phospholipid molecules, cholesterol, protein and carbohydrates ("Components and Structure | Boundless Biology"). The nature and the length of the phospholipid tails determines the plasma membrane fluidity. Fluidity is a parameter describing the freedom of movement phospholipid and proteins constituents within the plasma membrane ("Cell Membrane Fluidity - an Overview | ScienceDirect Topics"). Unsaturated phospholipids and shorter phospholipids tails increases the plasma membrane fluidity. This is because unsaturated phospholipids have kinked hydrocarbon chains that are harder to packed together and the shorter phospholipid tails will increase

fluidity as they are less viscous and more susceptible to changes in kinetic energy ("Membrane Fluidity | BioNinja"). Membrane fluidity and permeability are directly proportional. An increase in plasma membrane fluidity will increase it permeability by increasing the ability of permeant molecules to diffuse through the phospholipid bilayer of the cell membrane (Lande et al.).

Beetroot need a soil pH of 6.0 to 6.8 to grow effectively ("Beet Feeding Instructions - When and How to Apply Beet Plant Fertilizer"). But soil pH is not always constant and can be affected by air pollution. When exhaust gases from car or fuel such as coal or charcoal are burnt, gases containing carbon dioxide (CO_2), nitrogen dioxide (NO_2) and sulphur dioxide (SO_2) are produced. These gases later react with tiny droplets of water from clouds to form carbonic acid (H_2CO_3), nitric acid (HNO_3) and sulphuric acid (H_2SO_4) respectively. The rain from these clouds falls as a weak acid (acid rain) and this is how this acid goes into the soil. When acid rain is present in the soil, it lowers the soil pH which affects the growth of plants by dissolving and washing away the nutrients and minerals in the soil needed for a proper growth of plants and by causing the release of harmful substances such as aluminum into the soil ("What Is Acid Rain?"). This process is known as soil acidification. Also, soil acidification causes harm to the plasma membrane of plants by unfolding and denaturing the proteins that are in the plasma membrane if the pH is too low hence, making it unable to fully cover its functions ("Denaturation - Chemistry Encyclopedia - Structure, Proteins, Metal, Salt").

Acid rain have a pH range of about 5.0 to 5.5 with the world lowest acid rain level being 1.87 in Scotland ("Acid Rain"). For my investigation, I will be looking at how sulfuric acid (H_2SO_4) solutions of different concentrations and pH but the same volume will affect the permeability of a 4 cm piece of beetroot tap root. Here, H_2SO_4 solutions of different pH will be made by diluting a known concentration solution of H_2SO_4 to the desired concentration. The pH of the solution will be measured by using a digital pH meter. A pH meter is an electric device used to measure hydrogen-ion activity in solution ("PH Meter | Definition, Principle, & Facts | Britannica"). The level of damage of the plasma membrane will be determined by the amount of betalain pigment that leaked out of the beetroot at different pH solutions of H_2SO_4. This will be measured as percentage transmission of light using a colorimeter. It is a light-sensing device that is used to measure the absorbance and transmission of light as it moves through a sample of liquid (Phillips). Here, the larger the readings on the spectrophotometer, the more light is transmitted through the sample solution hence, the less amount of betalain pigment present in that solution.

For this experiment, I have chosen sulfuric acid as a substitute to acid rain. The sample solutions of sulfuric acid containing beetroot pieces will be put in a water bath of 23°C. This is to monitor the temperature so that it does not affect the experiment. The colorimeter is set at wavelength 540 nm to measure the percentage absorbance of betalain in the sample. This is because, 540 nm is the recommended wavelength by the colorimeter brand instructions.

4: HYPOTHESIS.

The lower the pH, the more permeable the plasma membrane will be. This is because at low pH, phospholipids and proteins present in the plasma membrane are deformed and denatured respectively. This damages it and makes it to be more permeable. The increase in permeability will be notice by an increase in the leaking of betalain pigment in the H_2SO_4 solutions.

5: METHODOLOGY.

5.1: Materials and Apparatus.

Materials	5 beetroots (from the school garden), a ruler, a knife, a stopwatch, a 1cm diameter cork borer, a white tile (for cutting), an electronic water bath, a colorimeter set at 540 nm, 2 droppers, a pair of gloves (safety measure), 2 forceps, 10 test tubes, 2 test tube racks, a digital pH meter, a stirrer, tissues.	
Apparatus	Size and uncertainty.	Purpose
1 measuring cylinder	100 cm^3 ±0.5 cm^3	To measure the volume of distilled water needed to make the solutions of different pH.
1 measuring cylinder	50 cm^3 ±0.5 cm^3	To measure the volume of 0.05 mol dm^{-3} H_2SO_4 needed to make the solutions of different pH.
1 measuring cylinder	50 cm^3 ±0.5 cm^3	To measure the 20 cm^3 pH solutions for the experiment.
5 beakers	50 cm^3	To pour the pH solutions containing the leaking betalain pigment from the test tubes.
5 beakers	250 cm^3	To mix both H_2SO_4 and distilled water during dilution to prepare the solutions of different pH.
1 beaker	250 cm^3	To rinse the cuvettes after use with distilled water.

5.2: Chemicals.

- 1 L of 0.05 mol dm^{-3} of sulfuric acid (H_2SO_4).
- 5 L of distilled water.
- Buffer solutions pH 4, 7 and 9.

5.3: Variables.

<table>
<tr><td>Independent variable</td><td colspan="3">Concentration (in mol dm^{-3}) and pH of sulfuric acid.
The different concentrations and pH are: 0.05 mol dm^{-3}, 0.005 mol dm^{-3}, 0.0005 mol dm^{-3}, 0.00005 mol dm^{-3}, 0.000005 mol dm^{-3} and pH 1, 2, 3, 4 and 5 respectively.</td></tr>
<tr><td>Dependent variable</td><td colspan="3">The amount of betalain pigment that will leak from the beetroot membrane.</td></tr>
<tr><td rowspan="4">Controlled variables.</td><td>Variables</td><td>How</td><td>Explanations</td></tr>
<tr><td>Temperature at which the experiment occurs.</td><td>Make sure that the experiment is carried out under the same temperature. One way to ensure it is by carrying out the experiment in a water bath (the water bath maintains the water temperature at a standard level).</td><td>This is because an increase in temperature will increase the fluidity of the plasma membrane by making the fatty acid tails of phospholipids to become less rigid, allowing more betalain to leak through (“The Effect of Temperature on Cell Membranes”).</td></tr>
<tr><td>Volume (20 cm^{-3}) of sulfuric acid used</td><td>To ensure consistency, use the same volume of H2SO4 for all the five pH concentrations in each of their five trials.</td><td>This is because different volumes of acid will affect the concentration of the solution with the leaked pigment.</td></tr>
<tr><td>Size and length (4cm long and 1 cm diameter) of the pieces of beetroot.</td><td>To ensure consistency, use the same size and length of beetroot for all the five pH concentrations in each of their five trials.</td><td>This is because different size and length of beetroot will have different surface area and diffusion distance hence, affecting the concentration of the solution with the pigment.</td></tr>
</table>

5.4: Safety measures.

1. Sulfuric acid is a highly corrosive chemical that can cause severe skin burns and eyes irritation. Wear eye protection, gloves and handle it gently.
2. The sharp knife can cause wounds. When cutting the ends of beetroots, cut them away from the body.
3. Always add acid to water when preparing the solutions of different pH. This is because heat is released violently, splashing out the acid from the container if acid is added to water.
4. Be careful when using the cork borer, and when making the pieces of beetroots ensure that the beetroot is on the tile and not within the palms of your hand.

5.5: Environmental issues.

After using each solution of H_2SO_4, they are discarded in the sink. Here, the septic tank system will neutralise the acid solutions before they reach the soil. Also, the beetroot samples are thrown after use which is not ecological because it is a non-eatable (non-reusable) waste which is infected by H_2SO_4.

5.6: Procedure.

Part 1: How to prepare solutions of different pH.

a) Preparing a pH 2 solution of H_2SO_4.

I was given 1L of 0.05 mol dm-3 of sulfuric acid (H_2SO_4) which is pH 1. From this concentration, I made the required pH solutions by diluting.

Step 1: Calculate the concentration of H_2SO_4 needed to make a pH 2 solution using the formula: $pH = -log[H+]$

This gives us:

$-log[2x] = 2$

$-log\ 2 - log\ x = 2$

$log\ x = -2 - log\ [2]$

$log\ x = -2.301$

$x = 10^{-2\ .\ 301}$

$x = 0.005\ mol\ dm^{-3}$

Step 2: Calculate the volume of 0.05 mol dm^3 of H_2SO_4 needed to make 250 cm^3 of 0.005 mol dm^3 of H_2SO_4.

Using the formula: $C_1V_1 = C_2V_2$

Where:

$C_1 = 0.05\ mol\ dm^3$ (initial concentration of H_2SO_4)
$C_2 = 0.005\ mol\ dm^3$ (wanted concentration of H_2SO_4)
$V_2 = 250\ cm^3$ (wanted volume of 0.005 mol dm^3 of H_2SO_4)
$V_1 = ?\ cm^3$ (Volume of 0.05 mol dm^3 of H_2SO_4 needed)

$$V_1 = \frac{C_2 V_2}{C_1}$$

$$V_1 = \frac{0.005\ mol\ dm^3 \text{ x } 250\ cm^3}{0.05\ mol\ dm^3}$$

$$V_1 = 25\ cm^3$$

This means that we will need 25 cm^3 of 0.05 mol dm^3 of H_2SO_4 and 225 cm^3 of distilled water mixed together to make 250 cm^3 of 0.005 mol dm^3 of H_2SO_4.

<u>Step 3:</u> Preparing a 250 cm^3 solution of 0.005 mol dm^3 of H_2SO_4.

1. Using a measuring cylinder, measure 225 cm^3 of distilled water. Firstly, put the first 220 cm^3; then, put yourself at eye level to see the bottom of the meniscus then pour the remaining 5.0 cm^3. As you get close to 225 cm^3, use a dropper to add the final few drops.
2. Pour the measured distilled water in a conical flask.
3. Using another measuring cylinder, measure 25.0 cm^3 of 0.05 mol dm^{-3} of H_2SO_4. Firstly, put the first 20.0 cm^3; then, put yourself at eye level to see the bottom of the meniscus then pour the remaining 5.0 cm^3. As you get close to 25.0cm^3, use a dropper (different from the one you used for distilled water) to add the final few drops.
4. Pour the measured H_2SO_4 in the conical flask containing the distilled water.
5. Stir completely using a stirrer to obtain the 250 cm^3 of 0.005 mol dm^{-3} H_2SO_4.

<u>Step 4:</u> Measure the pH of the 0.005 mol dm^{-3} solution of H_2SO_4 using a pH meter to see if its pH is 2.

How to use a pH meter.

a) Operation and pH calibration.

1. Remove the protective cap.
2. Clean the electrode with distilled water and dry up the water attached to the electrode with filter paper.
3. Press the start button to switch power.
4. Dip the pH electrode into pH 4 (buffer solution) and stir lightly.
5. Regulate the pH trimmer with a screwdriver until the buffer solution value corresponds to that of the buffer solution.
6. Clean the electrode by immersing it in distilled water.
7. Repeat step 4 to 6 for pH 9 and 7 respectively.

The aim of pH calibration is to minimize any measurement uncertainty by ensuring the accuracy of test equipment. Hence, by dipping the electrode into buffer solutions (solutions of known pH), and adjusting the reading to the pH number, we are making sure that the pH meter works properly by adjusting the electrode to ensure that the readings are accurate and repeatable.

b) Measuring the pH of the 0.005 mol dm^{-3} solution of H_2SO_4.

1. Rinse and dry the electrode well.
2. Place the electrode in the sample pH solution, press the measure button and wait for about 2 minutes. The reading display on the screen is the pH of the solution.

<u>Step 5:</u> Repeat steps 1, 2, 3 and 4 for pH 3, 4, and 5.

This will give us the following values;

Wanted concentration and volume of H_2SO_4.	**Volume of 0.05 mol dm^3 of H_2SO_4 needed.**	**Volume of distilled water needed.**	**pH of the solutions.**
250 cm^3 of 0.005 mol dm^{-3}	25.0 cm^3	225 cm^3	2
250 cm^3 of 0.0005 mol dm^{-3}	2.50 cm^3	247 cm^3	3
250 cm^3 of 0.00005 mol dm^{-3}	0.250 cm^3	249.75 cm^3	4
250 cm^3 of 0.000005 mol dm^{-3}	0.0250 cm^3	249.975 cm^3	5

<u>Part 2:</u> Experiment.

1. Prepare a water bath at temperature 23°C. Let the water warm.
2. While waiting for the water to warm, cut 25 (5 pieces per pH) pieces of beetroots using a cork borer.
3. Cut the ends of the pieces of beetroots using a knife on a white tile and make sure that all the pieces are 4cm long using a ruler.
4. Rinse the pieces of beetroot in clean water until the water becomes clear to remove the leaked pigment.
5. Measure 20 cm^3 of pH 1 solution using a measuring cylinder and pour it in five test tubes each. The five test tubes are the five trials for one same pH.
6. Add one piece of beetroot in each of the test tubes.
7. Put the five test tubes in the water bath and leave them to stand for 20 minutes.

8. After 20 minutes, remove the test tubes from the water bath.
9. Pour the water without the beetroots from the five test tubes into five different 50 cm^3 beakers.
10. Make qualitative observations and identify the amount of betalain released from the beetroot, for the pH 1 solution at the constant temperature of 23°C.
11. Use a colorimeter to detect the betalain concentration in water at wavelength 540 nm by putting 1cm^3 of the solution in the cuvettes (see part 3 below).
12. Record data in a table.
13. Wash the five test tubes, beakers and measuring cylinders with distilled water.
14. Repeat steps 5 to 13 for pH 2, 3, 4 and 5.

<u>Part 3:</u> Measuring the amount of leakage of the pigment betalain from beetroot membrane using a colorimeter.

How to use a colorimeter.

1. Switch on the instrument and allow it to warm up for at least 20 minutes before use to allow it to stabilize.
2. Set the desired wavelength with the wavelength knob. In my case, 540 nm.
3. Press the MODE key and select “T” to measure light transmission through the sample.
4. Insert the black block into the first cuvette holder, close the sample compartment cover, set 0,0%T by pressing the /0%T key until display reads 0.0
5. Pull the holder to make the black block not in the light path, set 100%T by pressing the /0Abs/100%T key until the display reads 100.0.
6. Insert the sample solution to be measured into the cuvette holder. Close the sample compartment cover. Then pull the sample to be measured into the light path, wait for the reading to stabilize and read the results directly on the digital display.

<u>Notes.</u>

- When putting the sample solutions in the cuvettes, hold the opaque sides of the cuvettes and make sure that the transparent sides are clean. This is to ensure that the fingers or any dirt do not stain the glass and affect the transmission of light detected by the colorimeter.
- Rinse the cuvettes with distilled water before using them for another sample solution of different pH.

6: ANALYSES.

6.1: Qualitative observations.

I can notice through observation that the concentration of betalain in the solution decreases as pH increases.

pH	Colour description
1	Very reddish
2	Very reddish
3	Reddish
4	Shallow reddish
5	Pale reddish

Table 1.0: Colour description of the acidic solutions containing the betalain pigment.

6.2: Data collection for the percentage transmission of light for pH1, pH2, pH3, pH4 and pH5.

pH	Percentage transmission of light (±0.5 %)				
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
1	5.1	5.7	5.6	5.3	5.2
2	9.6	9.9	9.5	9.0	9.6
3	17.3	17.3	17.0	17.5	17.2
4	24.6	24.0	24.2	24.7	24.3
5	31.2	31.8	31.8	31.7	31.1

Table 1.1: Table of percentage transmission of light in each test tube at the end of 20 minutes measured using a colorimeter.

6.3: Data processing.

Calculating the mean value of the percentage transmission of light for each pH using the formula:

$$\bar{X} = \frac{\sum(x)}{n}$$

Where:
$\bar{X}$ indicates the mean.
$\sum(x)$ indicates the sum of data values.
n indicates the number of data values.

In other words: $\frac{\text{Sum of all the percentage transmission of light for a pH solution sample}}{\text{The number of percentage transmission of light present for that sample}}$

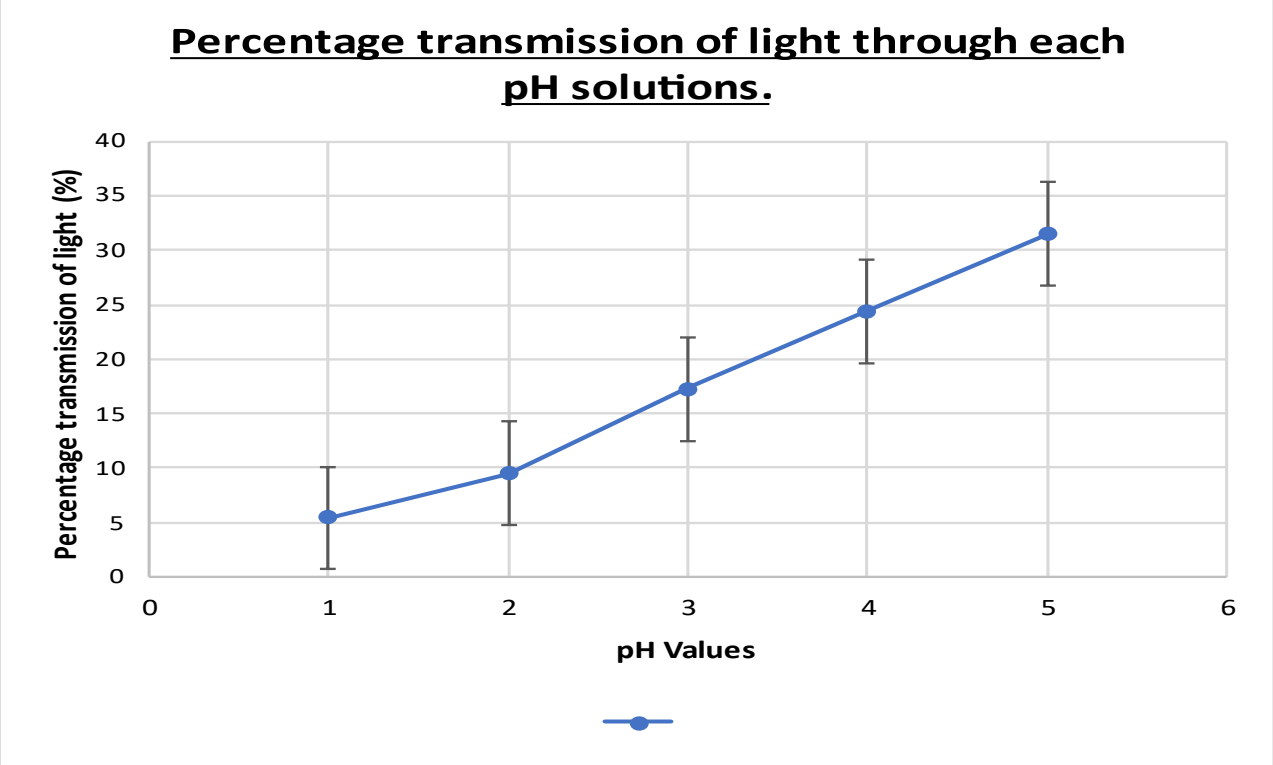
This gives us:

pH	Percentage transmission of light (±0.5 %)
1	5.4
2	9.5
3	17.3
4	24.4
5	31.5

Table 1.2: Table of the mean percentage transmission of light for each pH solutions at the end of 20 minutes.

6.4: Graph and standard deviation.

The graph below shows the relationship between pH level of a solution and the percentage transmission of light through the sample solutions due to the leakage of betalain pigment through the plasma membrane of beetroots. The standard deviation represented as error bars is a measure that will show how the data collected is spread from the mean values of the data set.



Graph 1.0: Percentage transmission of light through the sample solutions of pH 1, 2, 3, 4 and 5 containing betalain pigment.

6.5: t-Test.

A t-Test (one-tailed hypothesis with a 0.05 significance level) is conducted on the mean percentage transmission of light in table 1.2 above in order to determine if the transmission of light through each of the pH solutions are significantly different.

t-Test Results		
pH	**t-value**	**p-value**
1 and 2	- 22.19273	0.00001
2 and 3	- 46.25535	0.00001
3 and 4	- 46.61378	0.00001
4 and 5	- 35.80000	0.00001

Table 1.3: t-Test results comparing samples of pH solutions in pair.

7: CONCLUSION.

In conclusion, it was hypothesis that at lower pH, the plasma membrane will be more permeable. This because at low pH, the phospholipids and proteins present in the plasma membrane will be deformed and denatured respectively causing damages to the plasma membrane which initially being a selectively permeable membrane, will not be able to control the exchanges of substances between the cell and it surrounding.

The percentage transmission of light through the pH solutions in table 1.2 tells us about the amount of betalain that leaked from the beetroot vacuole into the solution due to damages on the plasma membrane because of change in pH level. If the amount of betalain pigment in the solution is high, there will ne a low percentage transmission of light through the solution but if the amount of betalain pigment in the solution is low, there will be a high percentage transmission of light through the solution.

The t-Test conducted in table 1.3 has for null hypothesis $p \geq 0.05$. This means that a p-value equal or greater than 0.05 will imply that the difference in the results of two sample is not significant. The p-values in table 1.3 above are all less than 0.05. This suggest that there is a true effect of pH on beetroot plasma membrane permeability because there is a significant difference in the amount of betalain that is leaking in the different pH solutions. From this, it can be stated that graph 1.0 shows that the percentage transmission of light in the solutions is directly proportional to increasing pH level. As pH increase from 1 to 5, the percentage transmission of light through the solution also increase from 5.4% to 31.5%. A decrease in percentage transmission of light implies an increase in the amount of betalain leaking into the solution therfore, lower pH damages the beetroot plasma membrane leading to a greater leakage.

To conclude, low pH affects the structure of the plasma membrane of beetroots which in turn affects it permeability by making it more permeable. If the beetroot plasma membrane is unable to control the exchange of substances between its cells and the environment, it will have difficulty controlling water uptake into its cells. Water contain dissolved iron which is crucial for the growth of beetroot ("Function of Iron - Learn about the Role of Iron in Plants"). The uptake of water contributes to the iron content of beetroot. Water uptake in cells is done through osmosis. Osmosis is the net movement of water molecules across a semi-permeable membrane from a region of low solute concentration to a region of high solute concentration high-water potential to a region of low water potential through a selectively permeable membrane ("Osmosis | BioNinja"). A damaged plasma membrane will not efficiently uptake water from the soil into the cells. This might lead to less or no iron in the cell. Without iron, the beetroot will not be able to produce chlorophyll which is used in the manufacture of food nutrients. Iron deficiency in plant is known as iron chlorosis and can be seen on plant leaves having a silky yellowish colour due to lack of chlorophyll ("Why Do Plants Need Iron? - Green as It Gets"). Failure of beetroot to uptake water by osmosis through its plasma membrane will hence, affect the nutritional content of beetroot.

8: LIMITATIONS.

In the process of the investigation, the following random and systematic errors were present.

1. **Time since harvest of beetroots:** A beetroot that has been harvested way before another might contain more degraded proteins as compared to the proteins in beetroots which have been harvested recently. In this case, the leaking rate of betalain in the older beetroots will be faster than that in the recent beetroots. This is because it will be easier for the acid to damage the degrading proteins as compared to those that are intact. Hence, this contributes to the variation of data and affects the reliability of the results.
2. **Rinsing the beetroots in clean tap water:** Rinsing beetroot with clean tap water after cutting it to remove the leaking betalain does not ensure that betalain leakage has stopped. Hence, a source of limitation to the investigation because failure to wash off all the leaking pigment will affect the readings of the transmission of light through the sample will not only be due to the acidic solutions.

9: EXTENSIONS.

Air pollution can also lead to global warming. Global warming is the slow increase in the average temperature of the earth's atmosphere ("Climate Change, What Is It? Understanding the Basic Facts about Global Warming"). This affects the world and lead to extreme temperatures. Extreme temperature can affect beetroot germination as it grows better under cool temperature *(Southern States Cooperative).* The extreme temperature can affect germination by increasing evaporation which will decrease the amount of moisture present in soil. Also, high temperature might denature enzymes in beetroot hence, slowing doing it metabolism. Investigating the effect of temperature as being one of the effects of pollution on beetroot can add value to this investigation as a further comparism could be done to see the extent to which pollution can affect beetroot through high temperature and pH levels.

10: WORK CITED.

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