Title: Effect of Sulphur Spring Water on the Biosynthesis of Chlorophyll and overall growth in Cabomba *caroliniana*

Research Question: "How does Sulphur Spring Water have an effect on the Biosynthesis of chlorophyll a, b & total chlorophyll content along with the overall growth in Cabomba *caroliniana*?"

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INTRODUCTION

1: Introduction

Indonesia, a vast archipelago of more than 13,000 islands, is one of the most complex jewels of South East Asia. Its remarkable and diverse scenery includes countless contrasts; from idyllic white sandy beaches to blazing volcanoes, and from harsh barren landscapes to thick jungles populated by wild animals and exotic plantations.
Moreover, Indonesia could be considered a nation with many massive active volcanoes. It's the foremost volcanoes compared to any country within the world, with 76 volcanoes that have erupted a minimum of 1100 plus times until now. The Smithsonian establishment has 141 Indonesian records in its Volcano Index. Indonesia has over a hundred thirty active volcanoes that are a part of the Pacific Ring of Fire, and has faced the biggest range of eruptions leading to deaths, destruction to productive land, wreckage, tsunamis, volcanic rock domes, and pyroclastic flows.

Due to these damages to arable land, only a handful of selected plants grow around on the lands that are, or have been affected by the volcanic eruptions or structures as a whole. I visited one of the Volcanic Craters myself for my Extended Essay project. The crater is known as Kawah Putih, which is located about 50 kilometers south of Bandung in West Java in Indonesia. I observed that the types of vegetation around this attraction was nothing but dead tree branches, sediment rocks and sand.

Interestingly, there was one more plant that grew around, which was something that surprised me even more. Coffee and Tea had been growing at a short distance away from the crater. Lets dig further into the science behind this. Basically the bulk of volcanic deposits are made up of "tephra." Tephra is a mixture of volcanic rocks (ash) and rock fragments which can be excreted from a volcano after an eruption. Tephra melts down through the years to create what we call “volcanic soil”. Most volcanic soils are referred to as Andisols , derived from the Japanese phrases which mean "darkish colored soil." Andisols are quite necessary for plant rooting for loads of reasons. Firstly, they have a low density but a porous structure, which facilitates the soil to preserve water correctly and makes it especially ready against drought. Since they're exwtraordinarily permeable, the roots of various floras also can develop deep and drain quickly, which prevents the roots from getting too moist and rotten. Coffee plantations want need more than few nutrients to develop, which can be produced via way of means of simplicity of this unique soil. Volcanic soils are in part fertile due to the fact they're comparatively "young"; they maintain a good deal of the nutrients that had been graved with inside the preliminary rock.

After carrying out some more extensive research about the conditions around the volcanoes, while it varies from volcano to volcano, I found out an article[1], that states that due to the presence of heavy metals around the volcanoes here in Java, the Andisols usually contain copper, phosphorus, potassium, calcium, magnesium, zinc, iron and boron, thus affecting growth of plants around the crater. This is why there are very less plantations that you can see around the volcanic crater.

[1] https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/volcanic-activity

In contrast, Cabomba a national importance herb, is known to be one of the worst weeds due to its invasiveness, spread capacity and its impacts on the economy and environment. It tends to block watercourses alongside the coasts of water bodies. Cabomba grows rapidly and provides a substantial quantity of plant material. Water storage space and drinking water supply can be drastically decreased. Water treatment rates can be raised by up. Heavy invasions also increases water levels causing overflows and serious sedation losses. It is highly tenacious and can take over a whole water body. It may also have an effect on native species. Cabomba's thick figure of submerged leaf and stem pose a challenge to aquatic water. As this vegetation dies, the decay causes drastic decreases of oxygen and water with bad odour. This plant can also adjust to any such harsh environments and continue to grow at a rapid pace even then.

Thus, I look forward to perform an experiment to note how changes in the concentrations of sulphur spring water affect the biosynthesis of chlorophyll in a particular species of plant. Taking both extremes of characteristics, as one Sulphur Water contain important chemicals that may affect growth, and the other which is the plant that grows rapidly. I will look to figure out the concentration that has the least and most negative effects on the structure and growth of the plant as a whole. I had also carry out a supporting experiment to prove the claim I had made earlier on the basis of an article I had found, mentioned earlier. I will be working with a Copper Sulphate solution, where Copper comes under one of the many heavy metals found in the area.

I made a nutrient solution known as the Knops Solution, which was a medium for the plants to grow in along with the different concentrations of Sulphur Spring Water. I carried out a test experiment to see whether everything was going to work out. The process is same as the controlled experiment and is included in the method section. I selected the *Cabomba caroliniana* as it was a plant that is used to being submerged in water, and as a result it may deliver faster results than those who are not that exposed to a large quantity of water during growth, making it easier to observe changes during experiments

RESEARCH QUESTION

- "How does sulphur spring water have an effect on the biosynthesis of chlorophyll a,b & total chlorophyll along with the overall growth in *Cabomba caroliniana*

HYPOTHESIS

If the concentration of the Sulphur Spring Water in the nutrient solution is increased, then the growth and biosynthesis of chlorophyll will decrease because at higher concentrations contents of the solution are exposed to the plant much more which hampers the overall growth of the plant.

LITERATURE REVIEW

Chlorophyll

Chlorophyll[1], comes under one of the most crucial pigments involved in photosynthesis; a mechanism where light energy from the sun is transformed to chemical energy via way of means of the synthesis of natural compounds. Chlorophyll is present in nearly all of the photosynthetic species, which includes inexperienced plants, cyanobacteria and algae. It absorbs power from mild; this power is then used to transform carbon dioxide to carbohydrate. In photosynthesis, the energy of the sun is converted to chemical energy by species classified as photosynthetic species. However not all wavelengths of sunlight are used equally in photosynthesis. Instead, organisms involved in photosynthesis contain light-absorbing molecules called pigments, which help absorb only particular wavelengths of visible light while they tend to reflect others that are not needed.

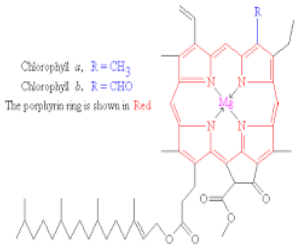


Figure 1. Chlorophyll Structure

Most photosynthetic organisms have a number of 'one-of-a-kind' pigments, that allows them to absorb a huge variety of wavelengths from the absorption spectrum, however maximum of those molecules grasp blue and red. We can hence say that chlorophyll is very important for photosynthesis, as they are the main component to help plants absorb energy from sunlight and help make their own food. This just shows that it is an integral component of a plant and is something that contributes majorly to its day-to-day life. Learning how such a small structure in a plant can be so important to them can only get interesting.

Chlorophyll occurs in a variety of discrete forms: chlorophyll a and b are the primary types found in higher plants and algae mostly in ; chlorophylls c and d are found, often with a, in different algaes. Chlorophyll occurs in green plants in membranous disk-like structures called thylakoids which are present in organelles known as chloroplasts. The chlorophyll molecule is a hydro phobic molecule and it comprises of a central magnesium atoms surrounded by a structure called a *porphyrin ring* that contain nitrogen. It is a long chain that contains carbon-hydrogen side chain, called the *phytol chain.* The changes are due to minor alterations in certain side groups. Chlorophyll is similar in structure to haemoglobin; a pigment found in the red blood cells of mammals and other vertebrates, for the purpose of carrying oxygen.

The range of wavelengths absorbed by the pigment is known as an absorption spectrum. The diagram below shows the absorption spectra of three primary pigments chlorophyll a, chlorophyll b, and β-carotene. The collection of wavelengths that the pigment does not absorb are reflected, which is what we see as color. For example, plants have a green appearance because they contain variosus of chlorophyll a and b molecules that reflect light colored green.

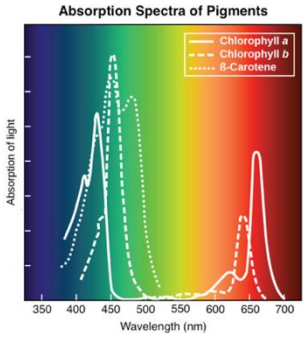


Figure 2. Absorption Spectra of Pigments

Carotenoids

Carotenoids[2] also come under the main category of pigments that absorb light. It grasps the colors violet and blue-green, that can be seen spectrum figure above. Bight colored carotenoids are present in fruits like red tomatoes yellow corn seeds and peels of oranges. It also helps attract insects.

Carotenoids aids in absorbing light during photosynthesis, but they also have an important part to play in reducing extra light energy. During this process, leaves are exposed to full light and as a result they absorb a tremendous amount of energy; if that energy is not properly treated, it may destroy photosynthetic machines. Carotenoids in chloroplasts help to absorb excess energy and dissipate it as heat.

When any sort of pigment absorbs a photon of light, it tends to be in an excited state, which means that it has extra energy is no longer in its natural state. In a subatomic language, it is due to the electron being bumped into an orbital of greater energy, which lies farther away from the nucleus. This is actually one of the main reasons as to why different pigments absorb varied ranges of wavelengths of light. The so called "energy gaps" that lie in the middle of the orbitals are different for each pigment, meaning that photons containing varied wavelengths are required in each of te case to successfully provide a so called energy boost that fits the difference.

An 'excited' pigment is not stable and usually looks out for a number of options available to make it more stable than it currently is. It can either transfer its extra energy or its excited electron to a nearby molecule. These are what is classified as a light-dependent reaction.

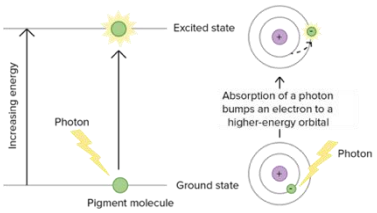


Figure 3. Energy states of pigment

[2] https://www.britannica.com/science/carotenoid

Biosynthesis of Chlorophyll

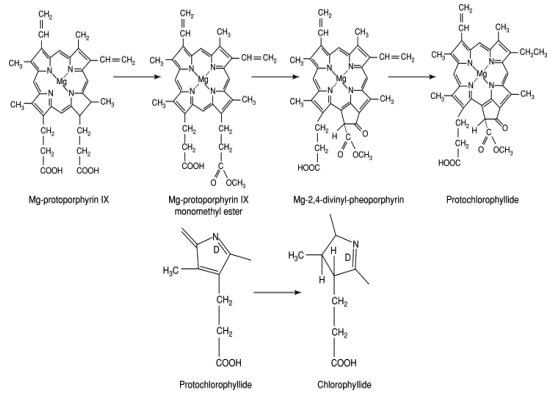


Figure 4. Steps of Biosynthesis of Chlorophyll in plants

Chlorophyll in plant foods are synthesized from δ-aminolevulinic acid (ALA), the perform of that is incontestible within the biogenesis of the nucleus of tetrapyrrole. Once ALA is formed, 2 molecules are condensed to create porphobilinogen also known as PBG , they do this by turning associate organic compound into an aromatic one. The head-to-tail condensation of 4 PBG molecules leads to the creation of the primary intermediate tetrapyrrole and what is known as linear hydroxymethylbilane porphyrin. This linear molecule is enzymatically closed order to form the first cyclic tetrapyrrole, and uroporphyrinogen III

With the chemical change of the carboxylic acid groupsof decarboxylase-catalyzed on the rings of A, B, C and D. Uroporphyrinogen III is also then converted to Coprophorphyrinogen III. The oxidization of the carboxylic acid cluster and the aromatization additionally alters formation of protoporphyrin IX that is shown on the last step of the figure.

The following step within the method pigment synthesis begins with the chelation of protoporphyrin IX regulated by Mg chelatase. this is often among methylation of 1 of the residues of propanoic acid to make Mg-protoporphyrin-n-monomethyl ester

And finally the ultimate step is distinguished by the changing Mg-protoporpyrin-Me to protochlorophyllide and protochlorophyllide to chlorophyllide, with reduction of the vinyl replacement in the side chain of the B ring, that is additionally accompanied by a process called oxidization of photoreduction of the D ring together with the esterification process of the propionate substituent to pyrrole D ring with geranyl geranium, which is then followed by a reduction method of phytyl.

Sulphur Spring Water

Figure 5. Sulphur Spring Water Reserve[3]

Sulphur Acid (or sulfuric water) is a condition in which water is detected as hydrogen sulfide gas, resulting in a distinct odor of rotten eggs. Hydrogen sulfide can be a chemical compound with the H_2S equation. It could be a colorless cacogenic hydride gas with an incomparable foul odor like a spoiled egg. It is harmless, destructive and flammable. Hydrogen sulfide is further formed by microbial decomposition of natural substances in the absence of aerated oxygen, such as in swamps and sewers; This tool is commonly referred to as anaerobic absorption by sulfate-reducing microorganisms.

H2S is also found in volcanic emissions, natural gas and some well water supplies. The human body produces small amounts of H2S and uses it as a signaling molecule. Swedish chemist Karl Wilhelm Scheil is credited with inventing the chemical structure of hydrogen sulfide in 1777. Sulphur water is made up of minerals dissolved in water that contain sulfate. These include barite epsomite and gypsum. It has been reported that large differences in taste of water differ from the form of sulphate acting as water.

Figure 6. Composition of Sulphate Minerals[4]

Hydroxide and Hydrous Sulfate Minerals:	
Mineral	Composition
Gypsum	$CaSO_4 \cdot 2H_2O$
Chalcanthite	$CuSO_4 \cdot 5H_2O$
Kieserite	$MgSO_4 \cdot H_2O$
Starkeyite	$MgSO_4 \cdot 4H_2O$
Hexahydrite	$MgSO_4 \cdot 6H_2O$
Epsomite	$MgSO_4 \cdot 7H_2O$
Meridianiite	$MgSO_4 \cdot 11H_2O$
Melanterite	$FeSO_4 \cdot 7H_2O$
Antlerite	$Cu_3SO_4(OH)_4$
Brochantite	$Cu_4SO_4(OH)_6$
Alunite	$KAl_3(SO_4)_2(OH)_6$
Jarosite	$KFe_3(SO_4)_2(OH)_6$

[3] https://en.wikipedia.org/wiki/Mineral_spring

[4] https://geology.com/minerals/sulfur.shtml

Cabomba *caroliniana*

C.*caroliniana* is a herbaceous, submerged, rooted aquatic species that can grow up to 0.4-1.2 meters and 6 meters deep in water. Plants have both underwater and floating leaves.
The sunken leaves are placed on the opposite side and are 4 centimeters long. Extends to along and extended version with 3 to 200 terminal components.

The floating leaves are either marked at the base or have a hollow margin. The diameter of the flowers is about 6-15 millimeters and the color of the flowers is white to purple or purple, petals. The fruit length of the plant is about 4-7 millimeters and the seed size is 1-3 millimeters.

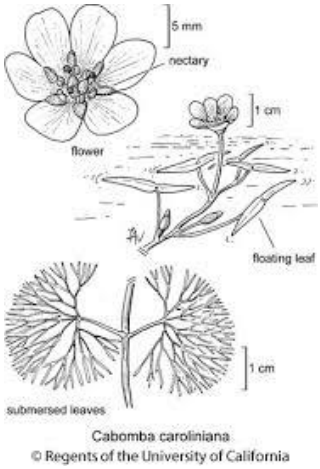


Figure 7. Dimensions of a Cobomba plant

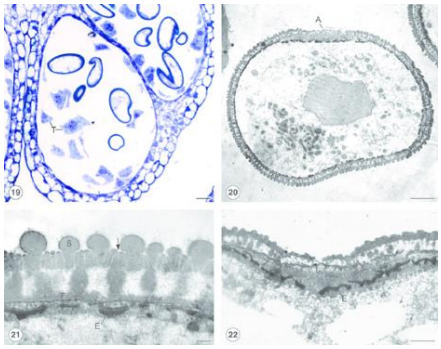


Figure 8. Cobomba Plant under a microscope

The plant is completely underwater and sometimes develops floating leaves and flowers. It grows roots in stagnant soil to slow flowing water, including rivers and streams. It grows in lakes, ponds, reservoirs, slopes, pits and canals, but can survive for six to eight weeks. The plant grows delicate roots, the green shoots of the straight shoots are green and mostly reddish brown, and the horizontal rhizomes are only long.

C. *caroliniana* flowers from the month of May all the way to the month of September and is often used as an aquarium plant due to its delicate appearance. It is also made commercially in Asia and sold in Europe and other regions of the world. In some countries there is small-scale territorial agriculture. In its natural ecosystem, C. *caroliniana* is eaten by water birds and other trout and provides habitat for some small fish and plankton.
The plant grows well in high nitrogen, low pH conditions, but loses its leaves in more brackish water. High calcium levels inhibit growth and, unlike other aquatic weeds, it can thrive in cloudy water. It prefers a humid, humid environment with temperatures ranging from 13 all the way to 27 degrees Celsius, but it can survive if the body surface of the water freezes.

Table 1. Taxonomy of species Cambomba *caroliniana*

Kingdom	Plantae
Phylum	Viridiplantae
Class	Magnoliopsida
Order	Nymphaeales
Family	Cabombaceae
Genus	*Cabomba*

EXPERIMENT

2: Investigation
Variables and manipulation

Aim: To estimate the amount of chlorophyll concentration in Cabomba *caroliniana* before and after it is treated with different concentrations of sulphur spring water enriched with equal volume of Knops nutrient solution.

Table 2. Investigation Variables and Manipulation

Variables	
2.1: Independent Variables	**Type of Sulphur Spring Water** – The Sulphur Spring Water was collected from the same source, keeping the water contents same. **Concentration of Growth Medium** – mixture of the sulphur spring water and nutrient solution was made for a concentration of 0 (controlled), 2,4,8,16 and 32% were made using serial dilution
2.2: Dependent Variables	**Changes in Colour of the Plant** – Plants kept in different growth medium react differently as the concentration increases, thus resulting in a more prominent colour change as the intensity of growth medium increases. **Absorbance rate** - Absorbance (A), also known as optical density (OD), is the volume of light consumed by the solution. Transmittance is the sum of light that travels into the solution. Absorbance and percent propagation are also used in spectrophotometry. The chlorophyll content along with Acetone of 8% is the solution in this case.
	Temperature-, the plants and nutrient medium were kept in a conical flask and then onto a basket kept near the window to maintain moist and suitable conditions for growth and development of the plant. Somewhere around 25-30 degrees were a suitable temperature range for the plants.

2.3: Controlled Variables	**Nutrient medium** – • A modified version of the Knops Solution • A Copper Sulphate Solution of different concentrations 0 (controlled), 50, 100, 200, 400, 800 ppm for supporting experiment **pH** – the same types of nutrient medium was used for all the samples resulting in a constant pH of 7 **Time taken** – 7-8 day's time was given to the plants to undergo growth and development in the different concentrations of mediums. This provided enough time to observe any and all changes that had occurred to the sample. **Amount of solution used** – Same sulphur spring water enriched with the same volume of Knops nutrient medium were put in the conical flask along with the plant. **Type of Plant** – Cabomba *caroliniana* were bought from aquarium resellers and kept in a water medium for less than a day before the experiment was conducted to prevent any changes in the in the overall structure of the plant Length and Mass of Plant – Each strand of Cabomba plant was accurately measured to 7 cm(s) in length to be used to be submerged in the nutrient solution. A mass of 0.1g of the plant was used during the extraction process of the chlorophyll of the plant, to determine the initial and final (after a week) effect of nutrient solution on the chlorophyll concentration of the C.*caroliniana* treated with different concentration of sulphur spring water. **Concentration of Chlorophyll**

3: Procedure

3.1: For experiment,

Table 3. Materials & Apparatus

Apparatus	Details
Sulphur Spring Water	5 Litres (x2) jelly cans of sulphur spring water from the source
Electronic balance (±0.001)	to measure components of the nutrient solution and the accurate weight of the plants
pipette	6 – one pipette used for each concentration of nutrient solution to avoid mixture
1000 ± 5 cm^3 Beaker	1
500 ± 5 cm^3 Beaker	2
250 ± 5 cm^3 Conical Flasks	30
Distilled Water	6 Litres (6 Bottles)
Acetone	80% Concentration 400 m^3
50 ± 5 cm^3 Measuring cylinder	1
Funnels	30
Mortar and Pestle	10 (Repeatedly used again)
Test Tubes	30
Ruler	1 (30 cm Ruler)
Filter Paper	30
Stirring rod	To stir the nutrient medium completely
Sticky Labels	Used to label the flasks, to avoid confusion
Light microscope with eyepiece graticule	1 To observe changes that had occurred to the inner components of the plant, after the experiment
Labels	To label the different concentration mixtures
Metal Tweezers	1, To handle the plants gently
Filter Paper	Used to collect the extra residue in the chlorophyll extract or solution
Aluminium Foil	Used to cover the acetone, to prevent it from evaporating
Spectrophotometer	To measure the absorbance of the solutions.
Knops Solution Chemicals	Calcium nitrate, $Ca(NO_3)^2$, 3 g Magnesium sulfate, $MgSO_4$, 1 g Potassium nitrate, KNO_3, 1g Potassium phosphate, dibasic, K_2HPO_4 **(±0.001cm)**

3.2: Organisms

20 mini pots of Cabomba plants obtained from the aquarium reseller market. (Note that each mini-pot consists of 7 to 9 strands of the Cabomba plant)

Safety Procedures

Safety measures experiment I took some measures to ensure my safety, due to the use of toxic chemicals:

1. I maintained social distance by wearing a mask during the pandemic
2. Wear glasses to avoid contact with my eyes
3. Wash all equipment and equipment with a detergent solution
4. Clean the area with water and tissues after the experiment
5. Wash your hands with hand soap and use the surface from experiment
6. Low density CuSO4 (400, 200, 100, 50, 25, 0 (controlled) ppm) and high concentrations of nutrient solution can increase safety risks and be toxic to plants
7. Wear eye protection and work in a well-ventilated room

3.3: Experimental Method

Firstly, the Knops Solution was mixed with the different concentrations of sulphur solution, and then poured back into the 1 Liters (L) distilled water bottles and labeled for each of the concentrations. Then, 200 mL of the nutrient solution were poured into 30 conical flasks, using a 250 cm^3 (± 0.5 cm^3) beaker, (Where 5 flasks were provided for each concentration of the nutrient solution). Thirdly, for each concentration, the Cabomba plant was placed on an electronic mass balance which helped measure the accurate mass of the plant and also another set was weighed to 0.1g. A 30cm ruler was also used to cut the plant to a length of 7cm(s) and placed into the nutrient solution, where it would be monitored for a period of one week, so that the after results could be obtained. This was done to keep each trial uniform. After that, 30 test tubes were prepared with filter paper on them to start the extraction process.

For the extraction process of the chlorophyll the following process mentioned below was followed:

Steps for Chlorophyll Extraction Process

1. 0.1 g of fresh leaves were taken and grinded with 10 cm^3 of 80 % acetone
2. It was then filtered with filter paper
3. the filtrate was transferred to a cuvette. The absorbency of the solution was read at 645 and 663 nanometres against the blank solution which is the 80% acetone.
4. Recalibrate the cuvette before checking the next sample

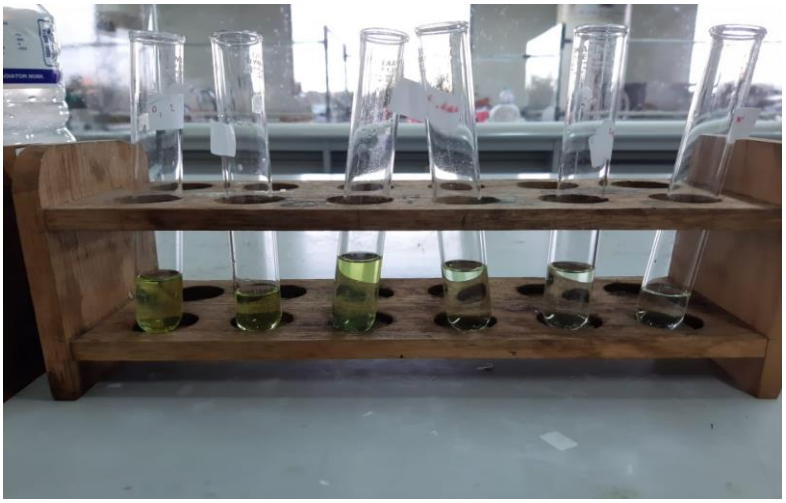


Figure 9. Shows appearance of solution after chlorophyll has been extracted

To determine the absorbency of the solution, the following steps mentioned below were followed

Steps for Calibration of Spectrophotometer

1. The instrument must be warmed up for at least 15 minutes, prior to use.
2. Use the wavelength knob to set the desired wavelength of 645 nm
3. Wipe the cuvette containing blank solutions and place it into the sample. Close the cover and set the meter reading '0' on the absorbance scale
4. Remove the blank, wipe off the sample and filled the cuvette with chlorophyll extract. Insert it and close the cover.
5. Read and record the absorbance

The above processes were carried out, so that the chlorophyll content could be extracted from the specimen and the rest of the plant was kept aside for further evaluation. The same steps mentioned above were followed for both the wavelengths of 645nm and 663nm for chlorophyll a and chlorophyll b correspondingly. The chlorophyll content was then calculated using these formulas.

Equation for Estimation of Chlorophyll Content

- **Sample calculation for Chlorophyll a (0% Concentration, Trial 1) [After]**

  Formula to calculate Chlorophyll a (µg/ml) = 12.7 (A_{663}) - 2.69 (A_{645})

  = 12.7 x (0.401) – 2.69 x (0.242)

  = 5.0927 – 0.6509

  = **4.442 µg/ml**

- **Sample calculation for Chlorophyll b (0% Concentration, Trial 1) [After]**

  Formula to Calculate Chlorophyll b (µg/ml) = 22.9 (A_{645}) - 4.68 (A_{663})

  = 22.9 x (0.242) – 4.68 x (0.401)

  = 5.5418 – 1.8766

  = **3.665 µg/ml**

- Sample calculation for Total Chlorophyll (0 % Concentration, Trial 1) [After]

  Formula to Calculate Total Chlorophyll **(µg/ml) = 20.2 (A_{645}) + 8.02 (A_{663})**

  = 20.2 x (0.242) + 8.02 x (0.401)

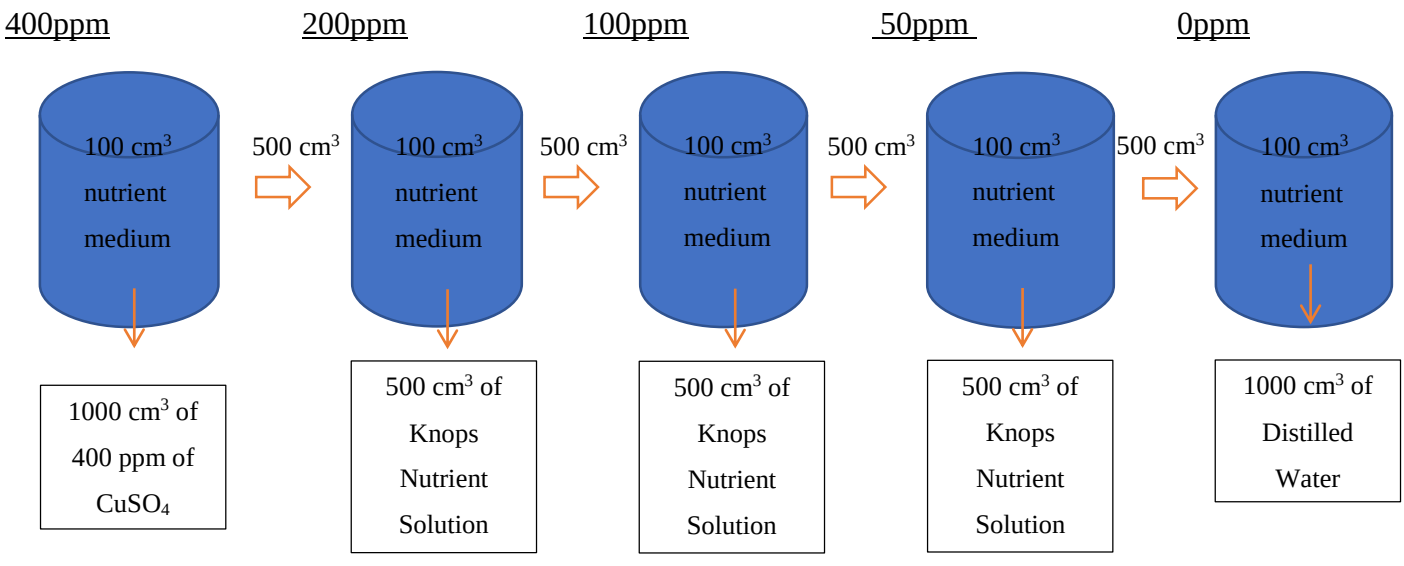
  = 8.48 + 3.21

  = 11.696 µg/ml

Further on, for the detailed evaluation of the specimens, one plant from each of the concentrated nutrient solution were taken and put on a glass slide with a drop of water, so that the inner structures of the cells could be distinguished and ready to be examined under the light microscope. Each specimen was analyzed under the light microscope at a (x40) magnification and the difference in structure was noted and captured using a camera. This sampling and analysis of was repeated for 5 of the specimens from each concentration

Serial Dilution Procedure

A process by which the concentration of the solution is reduced through a serial dilution. This reduces the previous concentration to half. For this reason, only a small amount of nutrient media was used to prepare the 400 ppm solution. The following visual representation shows the process used for serial dilution.



Preparing the dilution series of sulphur spring water enriched with Knop's nutrient solution

- Label six 1000 cm^3 beaker of (controlled) 0, 2, 4,8,16 and 32 % concentration of sulphur spring water
- Use original sulphur spring water collected from the source and Knop's nutrient solution and distilled water to make 1000 cm^3 of spring water of the following concentrations 2,4,6,8,16 and 32%

The table below shows the volumes of original spring water, and the Knop's solution and distilled water to make up 1000 cm^3 of each concentration

Table 4: Quantity of Sulphur Spring Water and Nutrient solution taken during Serial Dilution

Concentration of Sulphur Spring Water	Volume of Sulphur Spring Water from the Spring	Volume of Nutrient Solution (±5cm)	Volume of Distilled Water (±5cm)	Final Volume different Concentration of Sulphur Spring Water
0% (controlled)	0	500	500	1000
2%	20	500	480	1000
4%	40	500	460	1000
8%	80	500	420	1000
16%	160	500	340	1000
32%	320	500	180	1000

3.4: Limitations

This investigation is limited to how different concentrations of sulfur water affect the overall structures of the Cabomba plant. Another supporting experiment was conducted to verify and prove the claims made during further detailed research.

4.0: Processed Results/ Data

* Data processing was conducted using the raw data in the appendix These show the equations used in processing the data, along with sample calculations.

Table 5: Effect of different concentrations of sulphur spring water on absorbance of chlorophyll extract (645 nm and 663 nm)

Concentrations	Trials	Absorbance (Before) (645 nm)	Mean Absorbance (Before) (645 nm)	Absorbance (Before) (663 nm)	Mean Absorbance (Before) (663 nm)	Absorbance (After) (645 nm)	Mean Absorbance (After) (645 nm)	Absorbance (After) (663 nm)	Mean Absorbance (After) (663 nm)
0 % (controlled)	1	0.358	0.354	0.678	0.678	0.242	0.242	0.400	0.401
		0.349		0.678		0.242		0.401	
	2	0.284		0.562	0.558	0.170	0.172	0.339	0.339
		0.289	0.287	0.553		0.174		0.338	
	3	0.258	0.259	0.517	0.512	0.303	0.301	0.554	0.554
		0.259		0.507		0.299		0.553	
	4	0.254	0.257	0.488	0.492	0.228	0.228	0.438	0.438
		0.259		0.495		0.227		0.437	
	5	0.179	0.178	0.346	0.346	0.176	0.178	0.314	0.315
		0.176		0.345		0.179		0.315	
2%	1	0.370	0.370	0.693	0.693	0.234	0.234	0.435	0.436
		0.369		0.693		0.233		0.436	
	2	0.291	0.292	0.566	0.563	0.212	0.214	0.435	0.435
		0.292		0.560		0.216		0.434	
	3	0.243	0.243	0.457	0.455	0.278	0.278	0.503	0.504
		0.242		0.452		0.277		0.504	
	4	0.237	0.235	0.450	0.449	0.179	0.179	0.357	0.355
		0.233		0.447		0.178		0.353	
	5	0.175	0.178	0.345	0.344	0.206	0.207	0.416	0.416
		0.181		0.342		0.208		0.415	
4 %	1	0.302	0.302	0.572	0.571	0.540	0.550	0.127	0.127
		0.301		0.570		0.560		0.126	
	2	0.254	0.253	0.481	0.481	0.141	0.140	0.330	0.331
		0.251		0.480		0.138		0.332	
	3	0.306							
		0.302	0.304	0.558	0.555	0.740	0.735	0.148	0.149
				0.552		0.730		0.149	
	4	0.245	0.246	0.494	0.489	0.800	0.825	0.197	0.198
		0.246		0.484		0.850		0.198	
	5	0.252	0.252	0.466	0.463	0.810	0.820	0.193	0.193
		0.251		0.459		0.830		0.192	

8%	1	0.433 0.426	0.430	0.803 0.842	0.823	0.610 0.600	0.605	0.391 0.392	0.392
	2	0.399 0.400	0.400	0.581 0.582	0.582	0.750 0.740	0.745	0.194 0.195	0.195
	3	0.340 0.346	0.343	0.575 0.576	0.576	0.920 0.900	0.910	0.211 0.214	0.213
	4	0.291 0.282	0.287	0.541 0.537	0.539	0.137 0.136	0.137	0.322 0.324	0.323
	5	0.269 0.270	0.270	0.517 0.522	0.520	0.162 0.161	0.162	0.372 0.373	0.373
16%	1	0.468 0.454	0.461	0.842 0.841	0.842	0.111 0.110	0.111		0.266
	2	0.355 0.361	0.358	0.674 0.675	0.675	0.107 0.108	0.108	0.247 0.246	0.247
	3	0.347 0.354	0.351	0.694 0.695	0.695	0.185 0.184	0.185	0.42 0.421	0.421
	4	0.275 0.278	0.277	0.508 0.509	0.509	0.135 0.134	0.135	0.321 0.320	0.321
	5	0.321 0.326	0.324	0.635 0.634	0.635	0.146 0.145	0.146	0.344 0.345	0.345
32 %	1	0.346 0.347	0.347	0.676 0.678	0.677	0.219 0.218	0.219	0.545 0.544	0.545
	2	0.331 0.332	0.332	0.611 0.628	0.620	0.860 0.840	0.850	0.173 0.172	0.173
	3	0.325 0.313	0.319	0.614 0.613	0.614	0.950 0.940	0.945	0.196 0.195	0.196
	4	0.265 0.254	0.260	0.497 0.494	0.496	0.730 0.720	0.725	0.158 0.157	0.158
	5	0.261 0.263	0.262	0.516	0.512	0.119 0.121	0.120	0.288 0.287	0.288
Average			0.301		0.565		0.380		0.321

Table 6: Effect of different concentrations of Sulphur spring water on the concentration of chlorophyll a, chlorophyll b and Total chlorophyll

Concentrations	Trials	Chlorophyll a (before)	Chlorophyll b (before)	Total Chlorophyll (before)	Chlorophyll a (after)	Chlorophyll b (after)	Total Chlorophyll (after)	Total change in Chlorophyll a	Total change in Chlorophyll b	Total change in Chlorophyll
0 % (controlled)	1	7.658	4.934	12.588	4.442	3.665	8.104	**3.216**	**1.269**	**4.484**
	2	6.315	3.961	10.272	3.843	2.352	6.193	**2.472**	**1.609**	**4.079**
	3	5.806	3.535	9.338	6.226	4.300	10.523	**-0.420**	**-0.765**	**-1.185**
	4	5.557	3.583	9.137	4.949	3.171	8.118	**0.608**	**0.412**	**1.019**
	5	3.915	2.457	6.370	3.522	2.602	6.122	**0.363**	**-0.145**	**0.248**
2%	1	7.806	5.230	13.031	4.908	3.318	8.224	**2.898**	**1.912**	**4.807**
	2	6.365	4.052	10.413	4.949	2.865	7.812	**1.416**	**1.187**	**2.601**
	3	5.125	3.435	8.557	5.653	4.007	9.658	**-0.528**	**-0.572**	**-1.101**
	4	5.070	3.280	8.347	4.027	2.438	6.463	**1.043**	**0.842**	**1.884**
	5	3.890	2.466	6.354	4.726	2.793	7.518	**-0.836**	**-0.327**	**-1.164**
4 %	1	6.439	4.244	10.679	0.133	12.001	12.129	**6.306**	**-7.777**	**-1.450**
	2	5.428	3.543	8.968	3.827	1.657	5.483	**1.601**	**1.886**	**3.485**
	3	6.231	4.364	10.591	-0.085	16.134	16.042	**6.316**	**-11.770**	**-5.451**
	4	5.549	3.345	8.890	0.295	17.966	18.253	**5.254**	**-14.530**	**-9.363**
	5	5.202	3.604	8.803	0.245	17.875	18.112	**4.957**	**-14.271**	**-9.309**

8%	**1**	9.295	5.995	15.286	3.351	12.020	15.365	**5.944**	**-6.025**	**-0.079**
	2	6.315	6.436	12.747	0.472	16.148	16.613	**5.843**	**-9.712**	**-3.866**
	3	6.393	5.159	11.548	0.257	19.842	20.09	**6.136**	**-14.683**	**-8.542**
	4	6.073	4.050	10.120	3.734	1.626	5.358	**2.339**	**2.424**	**4.762**
	5	5.878	3.749	9.624	4.301	1.964	6.264	**1.577**	**1.785**	**3.360**
16%	**1**	9.453	6.616	16.065	3.080	1.297	4.376	**6.373**	**5.319**	**11.689**
	2	7.609	5.039	12.645	2.846	1.317	4.163	**4.763**	**3.722**	**8.482**
	3	7.882	4.785	12.664	4.849	2.266	7.113	**3.033**	**2.519**	**5.551**
	4	5.719	3.961	9.677	3.714	1.589	5.301	**2.005**	**2.372**	**4.376**
	5	7.193	4.448	11.637	3.989	1.729	5.716	**3.204**	**2.719**	**5.921**
32 %	**1**	7.664	4.778	12.438	6.332	2.465	8.795	**1.332**	**2.313**	**3.643**
	2	6.981	4.701	11.678	-0.089	18.655	18.557	**7.070**	**-13.954**	**-6.879**
	3	6.940	4.432	11.368	-0.053	20.723	20.661	**6.993**	**-16.291**	**-9.293**
	4	5.600	3.633	9.229	0.056	15.863	15.912	**5.544**	**-12.230**	**-6.683**
	5	5.798	3.604	9.398	3.335	1.400	4.734	**2.463**	**2.404**	**4.664**
Average		6.327	4.223	10.547	3.013	7.323	10.333	3.312	-3.090	0.214

Table 7 : Weight of Cabomba Plant Before/After/ Difference of weights				
Concentration	**Initial Mass (±0.001g)**	**Final Mass (±0.001g)**	**Change in Mass (±0.001g)**	**Percentage Change (%)**
0 % (controlled)	0.586	0.478	0.108	10.8%
	0.485	0.387	0.098	9..8%
	0.641	0.532	0.109	10.9%
	0.518	0.489	0.029	2.9%
	0.674	0.548	0.126	12.6%
2%	1.880	0.370	1.510	15.10%
	0.789	0.399	0.390	39.0%
	0.415	0.172	0.243	24.3%
	0.408	0.072	0.336	33.6%
	0.507	0.156	0.351	35.1%
4%	0.945	0.53	0.415	41.5%
	0.828	0.788	0.040	4.0%
	0.608	0.35	0.258	25.8%
	0.588	0.238	0.350	35.0%
	1.360	0.758	0.602	60.2%
8%	0.342	0.26	0.082	8.2%
	0.465	0.447	0.018	1.8%
	0.795	0.662	0.133	13.3%
	0.418	0.304	0.114	11.4%
	0.527	0.26	0.267	26.7%
16%	0.425	0.361	0.064	6.4%
	0.342	0.26	0.082	8.2%
	0.795	0.662	0.133	13.3%
	0.465	0.447	0.018	1.8%
	0.483	0.304	0.179	17.9%
32%	0.505	0.374	0.131	13.1%
	0.861	0.728	0.133	13.3%
	0.35	0.243	0.107	10.7%
	1.045	0.743	0.302	30.2%
	0.372	0.174	0.198	19.8%

- **Calculation for Average Absorption Value for 0% Concentration Trial 1 (645nm) [Before]**

$$\text{Mean} = \frac{\frac{(\text{Trial } 0{,}1+0{,}2)}{2}}{\frac{0.358 + 0.349}{2}} = 0.354$$

- **Calculation for Average Absorption Value for 0% Concentration Trial 1 (645nm) [After]**

$$\text{Mean} = \frac{\frac{(\text{Trial } 0{,}1+0{,}2)}{2}}{\frac{0.242 + 0.242}{2}} = 0.242$$

- **Calculation for Average Absorption Value for 0% Concentration Trial 1 (663nm) [Before]**

$$\text{Mean} = \frac{\frac{(\text{Trial } 0{,}1+0{,}2)}{2}}{\frac{0.678 + 0.678}{2}} = 0.678$$

- **Calculation for Average Absorption Value for 0% Concentration Trial 1 (663nm) [After]**

$$\text{Mean} = \frac{\frac{(\text{Trial } 0{,}1+0{,}2)}{2}}{\frac{0.400 + 0.401}{2}} = 0.400$$

*** All values have been taken to 3 significant decimal places**

- **Calculation for Average Absorption Value for 0% Concentration Trial (645nm) [After]**

$$\text{Mean} = \frac{\frac{(\text{Trial 1})}{2} + \frac{(\text{Trial 2})}{2} + \frac{(\text{Trial 3})}{2} + \frac{(\text{Trial 4})}{2} + \frac{(\text{Trial 5})}{2}}{5}$$

$$= \frac{\frac{(0.242 + 0.242)}{2} + \frac{(0.170 + 0.174)}{2} + \frac{(0.303 + 0.299)}{2} + \frac{(0.228 + 0.227)}{2} + \frac{(0.176 + 0.179)}{2}}{5}$$

$$\text{Mean} = \frac{0.354 + 0.287 + 0.259 + 0.257 + 0.178}{5} = 0.267$$

$\therefore$ Mean $[\bar{x}]$ **= 0.267**

- **Calculation for Average Absorption Value for 0% Concentration Trial (663nm) [Before]**

$$\text{Mean} = \frac{\frac{(\text{Trial 1})}{2} + \frac{(\text{Trial 2})}{2} + \frac{(\text{Trial 3})}{2} + \frac{(\text{Trial 4})}{2} + \frac{(\text{Trial 5})}{2}}{5}$$

$$= \frac{\frac{(0.678 + 0.678)}{2} + \frac{(0.562 + 0.553)}{2} + \frac{(0.517 + 0.507)}{2} + \frac{(0.488 + 0.495)}{2} + \frac{(0.346 + 0.345)}{2}}{5}$$

$$\text{Mean} = \frac{0.618 + 0.558 + 0.512 + 0.492 + 0.346}{5} = 0.517$$

$\therefore$ Mean $[\bar{x}]$ **= 0.517**

- **Calculation for 0% concentration for the standard deviation of 5 trials, with 2 sub- trials each (645nm) [After]**

$$\textbf{Standard Deviation} = \frac{\sqrt{(\Sigma X_{trials} - \bar{x})}}{5}$$

$$\frac{\sqrt{\Sigma\,(0.242 - 0.267)^2 + (0.172 - 0.267)^2 + (0.301 - 0.267)^2 + (0.228 - 0.267)^2 + (0.178 - 0.267)^2}}{5}$$

$$= \frac{\sqrt{0.000625 + 0.009025 + 0.001156 + 0.001521 + 0.007921}}{5} = 0.0636$$

$\therefore$ **Standard Deviation = $\pm$ 0.06**

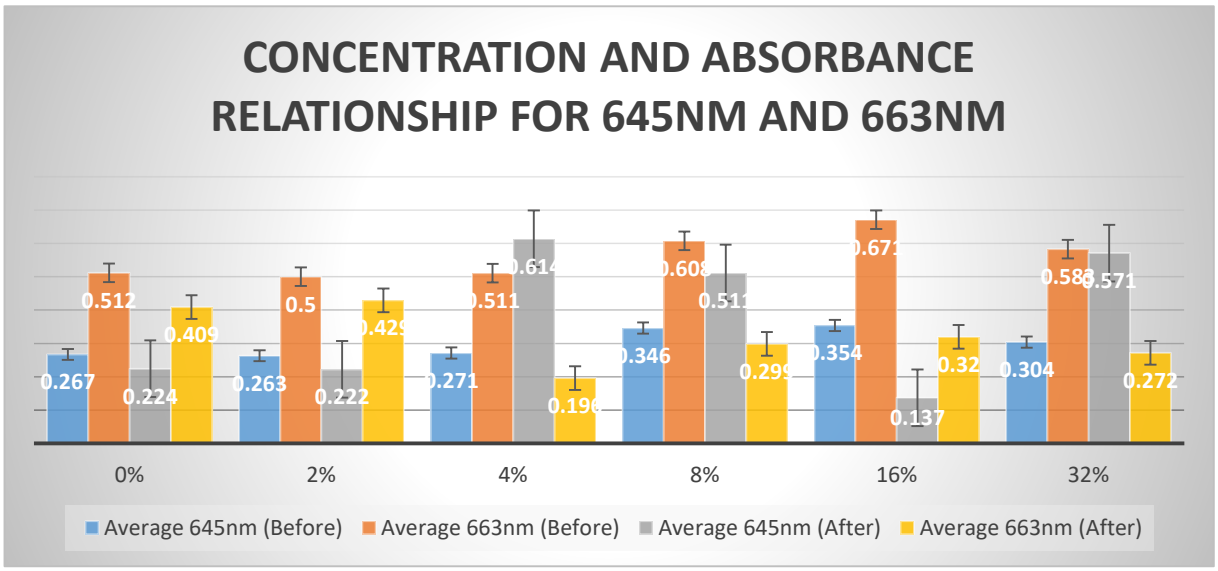
Averaged Values for All Trials

Replicates	• **Table 8: SD for % concentration for the standard deviation of average value for 5 trials, (645nm) [Before]**						
Concentration	1	2	3	4	5	Average	SD
0%	0.354	0.287	0.259	0.257	0.178	0.267	± 0.06
2%	0.370	0.292	0.243	0.235	0.178	0.263	± 0.07
4%	0.302	0.253	0.304	0.246	0.252	0.271	± 0.02
8%	0.430	0.400	0.343	0.287	0.270	0.346	± 0.06
16%	0.461	0.358	0.351	0.277	0.324	0.354	± 0.07
32%	0.347	0.332	0.319	0.260	0.262	0.304	± 0.04

Replicates	• **Table 9: SD for % concentration for the standard deviation of average value for 5 trials, (663 nm) [Before]**						
Concentration	1	2	3	4	5	Average	SD
0%	0.678	0.558	0.512	0.492	0.346	0.512	± 0.11
2%	0.693	0.563	0.455	0.449	0.344	0.500	± 0.13
4%	0.571	0.481	0.555	0.489	0.463	0.511	± 0.04
8%	0.823	0.582	0.576	0.539	0.520	0.608	± 0.12
16%	0.842	0.675	0.695	0.509	0.635	0.671	± 0.11
32%	0.677	0.620	0.614	0.496	0.512	0.583	± 0.07

Replicates	• **Table 10: SD for % concentration for the standard deviation of average value for 5 trials, (645nm) [After]**						
Concentration	1	2	3	4	5	Average	SD
0%	0.242	0.172	0.301	0.228	0.178	0.224	± 0.05
2%	0.234	0.214	0.278	0.179	0.207	0.222	± 0.05
4%	0.550	0.140	0.735	0.825	0.820	0.614	± 0.20
8%	0.605	0.745	0.910	0.137	0.162	0.511	± 0.30
16%	0.111	0.108	0.185	0.135	0.146	0.137	± 0.03
32%	0.219	0.850	0.945	0.725	0.120	0.571	± 0.30

Replicates	• **Table 11: SD for % concentration for the standard deviation of average value for 5 trials, (663nm) [After]**						
Concentration	1	2	3	4	5	Average	SD
0%	0.401	0.339	0.554	0.438	0.315	0.409	± 0.09
2%	0.436	0.435	0.504	0.355	0.416	0.429	± 0.05
4%	0.127	0.331	0.149	0.198	0.193	0.196	± 0.07
8%	0.392	0.195	0.213	0.323	0.373	0.299	± 0.09
16%	0.266	0.247	0.421	0.321	0.345	0.320	± 0.06
32%	0.545	0.173	0.196	0.158	0.120	0.272	± 0.16



Graph showing relationship between absorbance and concentration at wavelengths 645 and 663 nm

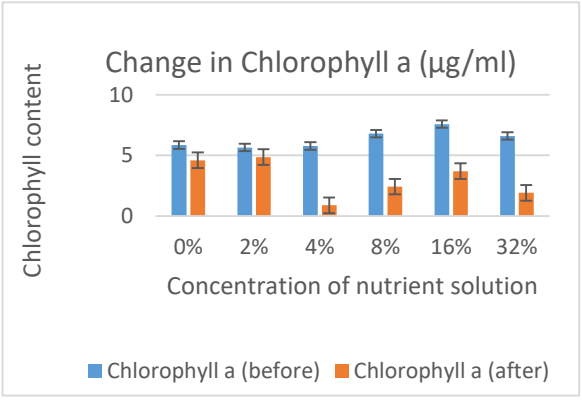
Note* - The Error bars are plotted for Standard Error (SE)

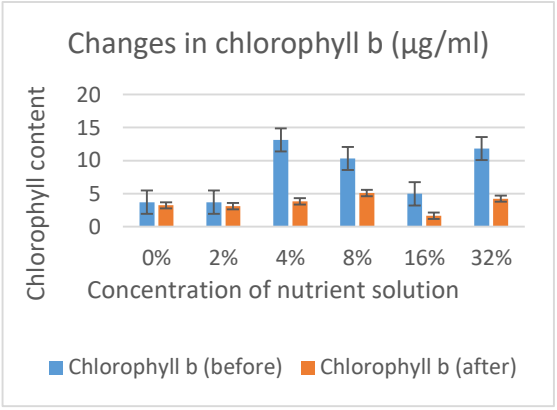
Replicates	**Table 12: Difference in Chlorophyll A (Before)** µg/ml							
Concentration	1	2	3	4	5	Average	SD	Variance
0%	7.658	6.315	5.806	5.557	3.915	5.850	± 1.3	1.69
2%	7.806	6.365	5.125	5.070	3.890	5.651	± 1.4	1.96
4%	6.439	5.428	6.231	5.549	5.202	5.769	± 0.5	0.25
8%	9.295	6.315	6.393	6.073	5.878	6.790	± 1.4	1.96
16%	9.453	7.609	7.882	5.719	7.193	7.571	± 1.3	1.69
32%	7.664	6.981	6.940	5.600	5.798	6.596	± 0.8	0.64

Replicates	**Table 13 : Difference in Chlorophyll A (After)** µg/ml							
Concentration	1	2	3	4	5	Average	SD	Variance
0%	4.442	3.843	6.226	4.949	3.522	4.596	± 1.0	1.0
2%	4.908	4.949	5.653	4.027	4.726	4.852	± 0.5	0.25
4%	0.133	3.827	-0.085	0.295	0.245	0.883	± 1.6	2.56
8%	3.351	0.472	0.257	3.734	4.301	2.423	± 1.9	3.61
16%	3.080	0.108	0.185	0.135	0.146	3.695	± 0.8	0.64
32%	6.332	-0.089	-0.053	0.056	3.335	1.916	± 2.8	7.84

Replicates	**Table 14 : Difference in Chlorophyll B (Before)** µg/ml							
Concentration	1	2	3	4	5	Average	SD	Variance
0%	4.934	3.961	3.535	3.583	2.457	3.694	± 0.8	0.64
2%	5.230	4.052	3.435	3.280	2.466	3.692	± 0.4	0.16
4%	12.001	1.657	16.134	17.966	17.875	13.126	± 6.8	46.24
8%	12.020	16.148	19.842	1.626	1.964	10.320	± 8.2	67.24
16%	6.616	5.039	4.785	3.961	4.448	4.969	± 1.0	1.0
32%	2.465	18.655	20.723	15.863	3.604	11.821	± 9.1	82.81

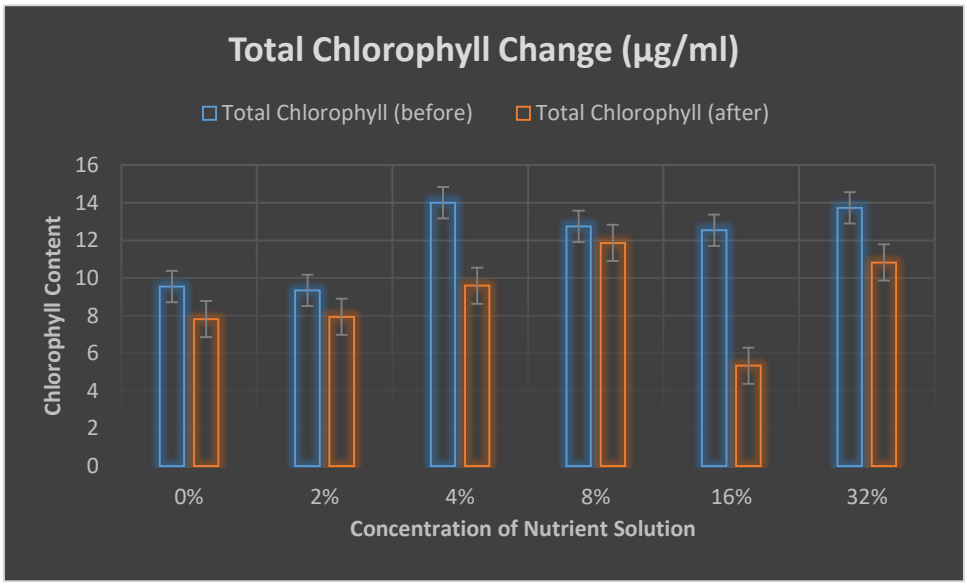
Replicates	**Table 15: Difference in Chlorophyll B (After)** µg/ml							
Concentration	1	2	3	4	5	Average	SD	Variance
0%	3.665	2.352	4.300	3.171	2.602	3.218	± 0.7	0.49
2%	3.318	2.865	4.007	2.438	2.793	3.084	± 0.6	0.36
4%	4.224	3.543	4.364	3.345	3.604	3.820	± 0.2	0.04
8%	5.995	6.436	5.159	4.050	3.749	5.077	± 1.7	2.89
16%	1.297	1.317	2.266	1.589	1.729	1.639	± 0.3	0.09
32%	4.778	4.701	4.432	3.663	3.604	4.229	± 0.5	0.25





Replicates	**Table 16: Difference in Total Chlorophyll (Before)** µg/ml							
Concentration	1	2	3	4	5	Average	SD	Variance
0%	12.588	10.272	9.338	9.137	6.370	9.541	± 2.2	4.84
2%	13.031	10.413	8.557	8.347	6.354	9.341	± 2.5	6.25
4%	12.129	5.483	16.042	18.253	18.112	14.003	± 5.3	28.09
8%	15.365	16.613	20.090	5.358	6.264	12.738	± 6.5	42.25
16%	16.065	12.645	12.664	9.677	11.637	12.537	± 2.3	5.29
32%	8.795	18.557	20.661	15.912	4.734	13.731	± 6.7	44.89

Replicates	**Table 17: Difference in Total Chlorophyll (After)** µg/ml							
Concentration	1	2	3	4	5	Average	SD	Variance
0%	8.104	6.193	10.523	8.118	6.122	7.812	± 1.8	3.24
2%	8.224	7.812	9.658	6.463	7.518	7.935	± 1.1	1.21
4%	10.679	8.968	10.591	8.890	8.803	9.586	± 0.9	0.81
8%	15.286	12.747	11.548	10.120	9.624	11.865	± 2.2	4.84
16%	4.376	4.163	7.113	5.301	5.716	5.333	± 1.1	1.21
32%	12.438	11.678	11.368	9.229	9.398	10.822	± 1.4	1.96



This is a Graphical Representation of changes in total chlorophyll content as concentration of nutrient solution increases

***Note-** The Error Bar plotted for changes in chlorophyll a, b, and total chlorophyll on the graph are **Standard Error (SE)**

Statistical Analysis

T - Test Introduction

The t test helps to compare two averages of similar data to find out how important the difference between the variables is. It uses t values, distribution values and degree of freedom to calculate the probability of difference between the data. The formula for calculating the t value for independent samples and unequal variables is:

$$t = \frac{|\mu^{\circ} - \mu|}{\sqrt{\frac{V^{\circ}}{n^{\circ}} + \frac{V}{n}}}$$

To find the degree of freedom to compare the calculated t-value to find if there is a significant difference:

DF = (n - 1) + (n - 1)
DF = (5 - 1) + (5 -1)

The critical value for the DF = 8,

the t test is based on a table that corresponds to a **degree of freedom of 8** and a **confidence level of 5%** (the most used) is usually the critical value

If the t value is more than significant The null value hypothesis is rejected and an alternative hypothesis is accepted, but in this case the t-value obtained is less than the critical value, which suggests that the null hypothesis is true and there is no statistically significant difference between the samples.

Null Hypothesis (H_O) – *C. caroliniana* is not affected by the nutrient solution

Alternate Hypothesis (H_A) – *C. caroliniana* is affected by the nutrient solution
Now I will perform the test, taking 0ppm of nutrient solutions as control.

Variables

μ° = to the mean of the control
μ = mean of the sample
V° = variance of the control
V = variance of the sample
n°= number of measurements of the control
n = number of measurements of the sample

Replicates	**Table 18: t-test for Difference in Total Chlorophyll (After) µg/ml**					
Concentration	Average	SD	Variance	*Diff* $(X - M)$	*Sq. Diff* $(X - M)^2$	T test
0% (CONTROLLED)	7.81	± 1.8	3.24	-2.45	5.99	-
2%	7.93	± 1.1	1.21	-2.32	5.40	0.13
4%	14.00	± 5.3	28.09	3.74	14.02	0.04
8%	12.73	± 6.5	42.25	2.48	6.15	1.67
16%	5.33	± 1.1	1.21	-4.93	24.26	2.10
32%	13.73	± 6.7	44.89	3.47	12.06	1.09
Mean				**M: 10.26**	**SS: 67.87**	

T-value Calculation

$s^2_p = ((df_1/(df_1 + df_2)) * s^2_1) + ((df_2/(df_2 + df_2)) * s^2_2) = ((5/10) * 1.83) + ((5/10) * 13.57) = 7.7$

$s^2_{M1} = s^2_p/N_1 = 7.7/6 = 1.28$

$s^2_{M2} = s^2_p/N_2 = 7.7/6 = 1.28$

$t = (M_1 - M_2)/\sqrt{(s^2_{M1} + s^2_{M2})} = 0.36/\sqrt{2.57} = \mathbf{0.22}$

Treatment 2

N_2: 6
$df_2 = N - 1 = 6 - 1 = 5$
M_2: 10.26
SS_2: 67.87
$s^2_2 = SS_2/(N - 1) = 67.87/(6-1) = 13.57$

The *t*-value is 0.22.

The *p*-value is 0.41. The result is **significant** at ***p* < .05**.

DISCUSSION

The first set of experiments using the sulphur nutrient solution resulted in decrease of amount of chlorophyll level extracted and very high disintegration along with a lot of deformations in the inner organelles and structures of the plant. Upon further analysis I found out that the plants had different number of leaves per structure or even the fact that the water they have been grown in could defer. The exposure to excessive moisture must have in a way affected the inner structures as well. This data was not used and this experience proved valuable as I became more careful about controlling external factors.

The results of chemical analysis of fluorine, lead and other specific elements are not very difficult. Contaminated fluorine and lead are usually found in low concentrations in plant tissues, and increased concentrations usually indicate an increase in the emission of contaminants from rapid and accumulation. However, sulfur is a common component of plants, and its concentration depends on the species, location and many other conditions of the plant. Therefore, when interpreting sulfur concentrations in plant leaves, it was important for me to consider comparisons with specific plant species, geographic location, seasons and plant growth stages.

EFFECT OF THE SULFUR ON THE CHLROROPHYL AND INNER STRUCTURES OF THE PLANT

Under the microscope, all the inner structures looked circular with a thick silhouette. But there were several instances of disintegration where the inner structures started to resemble like black decayed particles particularly at higher concentrations. In some cases, all the inner structures of the plant had disintegrated while most cases had both instance of clearly visible inner structures as well as disintegrated ones.

Below are pictures of the results observed by me during my experiment:

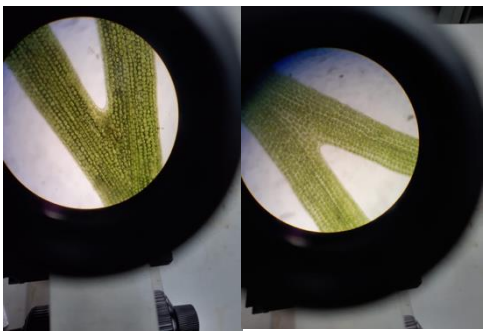


Figure 10. Clear visible inner structure

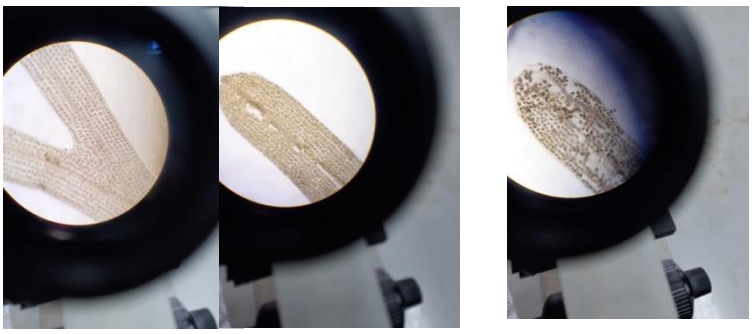


Figure 11& 12. Viable inner structure visible / Disintegrated Inner Structure

GRAPHICAL ANALYSIS

The Cabomba plants treated with the sulphur spring water enriched nutrient medium showcased that there was in fact a decrease in the plant's chlorophyll amount and therefore the rate of absorbency of the solution as well, when the concentration of the sulphur nutrient medium was increased. The decrease was not disproportional and almost all samples tested showed a percentage decrease when the concentration was decreased.

The plants that had been kept in the solution of the highest concentration, 32% had a strong odour showed a lot of disintegration. The disintegration rate increased with concentration. The growth ate decreased as the concentration decreased similar to a decreasing divergent graph.
In comparison to the initial concentrations like 0%(controlled), 2%, 4%, small decrease in growth rate as well as visible structures were observed at lower concentrations. There was a general decrease in chlorophyll percentage over increasing concentration. The plant as a whole in this solution looked much darker in green colour under the microscope compared to the other solutions and showed little to no disintegration at lower concentrations. There is a sudden and steep decrease in the chlorophyll percentage between the last few concentrations as all the structures as well as the plant itself had completely disintegrated

CONCLUSION

The t-test through the results clearly suggests that at most concentrations the nutrient solution does have a substantial effect on the chlorophyll and growth of the plant. This is important to note as it shows how damaging the concentration of sulphur spring water enriched with nutrient solution can be on plants. Along with the t results, the standard deviation results also show how concentrations affect the plant as a whole and make them more volatile and distributed on both ends of the spectrum. The Qualitative analysis also showed damaged changes in structures of the plant at high concentrations of the sulphur concentrations.

The results clearly state that though at some concentrations of some of the nutrient sulphur solution, the plant's growth is definitely affected, there is an overall decrease in the general characteristics of the plant such as the amount of chlorophyll present afterwards, the absorbency rate of the solution and the images under the microscope tend to suggest that the the nutrient solution are definitely toxic to the plants. Especially high concentrations of the sulphur spring water should be avoided as they completely disintegrate the plant as a whole. Therefore, to prevent harm to to the plants and somewhat provide them enough nutrients and maximize their growth, a low to moderate concentration should be used.

My supporting experiment that I carried out also provides proofs regarding the claim. The claim made was that the plants around the volcanic areas are also affected by the heavy metals present in the soil. After working and testing the plants with a Copper Sulphate solution, where copper comes under one of the many heavy metals found in the area. The results of my supporting experiment (present in the appendix) does infact prove that the plants are affected by the heavy metals in the area as well, and not only the main source of toxicity which was the sulphate in this case.

In an agricultural perspective, Sulfur (S) is an essential element in the formation of proteins, enzymes, vitamins and chlorophylls in plants. It is crucial for efficient nitrogen fixation in the development of knots and legumes. Protein synthesis requires large amounts of sulfur, especially in the formation of oils within the seed, and sulfur is a component of several amino acids and vitamins found in plants and animals. Therefore, sulfur is an important factor in determining the nutritional quality of foods. Sulfur is also important in photosynthesis and contributes to the winter hardiness of the crop. Adequate supply of sulfur is important not only for crops that require more sulfur, but also for crops that require more nitrogen, which cannot optimize nitrogen use.

If used in excessive amounts, Sulfur and Sulfur dioxide inhibits photosynthesis by disrupting the photosynthetic mechanism. The opening of the stomata is promoted by sulfur dioxide, resulting in an excessive loss of water. This may affect the environment as well because when sulfur dioxide combines with water and air, it forms sulfuric acid, which is the main component of acid rain. Acid rain can cause deforestation. acidify waterways to the detriment of aquatic life. Therefore, moderate use is recommended and necessary

All my findings and data supports the hypothesis constructed earlier in this report. As the concentration of the sulphur spring water enriched with nutrient solution increased, the growth and chlorophyll content percentage decreased, while growth was supported for a few to some extent. At higher concentrations as well, my hypothesis was accepted, as the plant structures was deformed and the length of plant did not rise.

EVALUATION

Biggest sources of errors:

Each experiment tries to prove its hypothesis in a very positive way; However, there can be many problems that can change the experiment negatively. There can also be many problems that can change the results and make the experiment inaccurate. Here are few problems I faced:

- When testing the cuttings, there may have been problems within the plant that could not be determined. The cutout plant may nothave been healthy. This has a significant impact on the experiment as it affects growth patterns.
- Once the cuttings have been placed, errors may have occurred due to the amount of water supplied to the plants. The cuttings may have been overwatered or overwatered.
- The measurements of the cuttings may not be accurate. The stem may not be unequal and the ruler may not allow correct answers
- Procedural error occurs when different techniques are used to answer the same question and give slightly different answers. One round up or one round down is a procedural error
- Conducting an experiment in a natural environment can improve the accuracy of results. Experiments carried out on a large number of plants can better reflect the agricultural effects of sulfate.
- Light energy is converted into chemical energy and is used by photosynthesis to make organic compounds. Therefore, photosynthesis is a very important measure for the growth rate of plants. As a result, plant growth is also affected by factors that affect photosynthesis. The number of photons in light-accelerating chlorophyll oils increases with increasing light intensity. It also increases the rate of

photosynthesis. More light means more energy. In one experiment, the perspectives of sunlight photons may be slightly different for groups. Patterns can be exposed to light rays from different angles. Thus, the energy they receive may vary, so it affects photosynthesis and growth rates.

- Interference with any microorganisms is also a major source of error. Although all conditions have been tried to be the same for all plants, it is also possible that the microorganisms were formed in one of the plant pots placed in water or nutrient solution. These organisms may inhibit plant growth
- Another source of error is genetic variation, as genes and environmental conditions determine plant growth, photosynthesis rate, hormone production and maintenance. All these factors affect the growth of the plant.

Problems in design:

- Filter Paper – The filter paper had to be made into perfect sized cones to put on the flask, so that the solution could be poured, while extracting the chlorophyll. This was a hard task because it kept on sinking in and not fitting into the flask. This process was ineffective and quite a few trials had to be executed again.
- Human error while measuring the lengths and taking a picture through the microscope – Most plants were not straight which resulted in an approximate length data recorded rather than the exact length, as well as it was extremely difficult to focus the phone camera onto the microscope.
- Droppers and Pouring of solution – Somewhere, a very negligible amount of extra solution might have been dropped too.

Improvements for the experiment:

- The experiment should be conducted maybe using the water the plants are actually used to, rather than laboratory distilled water
- Use a higher resolution microscope to get a more accurate images of the plants so that better results could be observed and decided upon whether the experiment was successful or not
- Use a wider range of plant concentrations to get more data
- Use smaller differences between each concentration to find the exact concentration that produces optimal growth and where it starts to actually affect the plant
- Use different types of heavy metals solutions to see which ones affect more than others (for the supporting experiment)
- I should have used plants that grow in a different climatic condition but same type to evaluate whether the nutrient solutions and the metallic ones have similar effects throughout those types or not.

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APPENDIX A

Preparation of the Knops Solution

Materials

Calcium nitrate, $Ca(NO_3)_2$, 3 g	Sucrose, 50 g (optional)
Magnesium sulfate, $MgSO_4$, 1 g	Water, distilled or deionized (DI)
Potassium nitrate, KNO_3, 1 g	Balance, 1-g
Potassium phosphate, dibasic, K_2HPO_4, 1 g	Beaker, 1-L

Safety Precautions

Calcium nitrate is a strong oxidizer; a potential fire risk when in contact with organic material; and may explode when shocked or heated. Potassium nitrate is a strong oxidant; fire and explosion risk when heated or when in contact with organic material as well as a skin irritant. Wear chemical splash goggles, chemical-resistant gloves and a chemical-resistant apron whenever working with chemicals, heat or glassware. Wash hands thoroughly with soap and water before leaving the laboratory. Follow all laboratory safety guidelines. Please review current Material Safety Data Sheets for additional safety, handling and disposal information.

Procedure

1. Measure 1-L of distilled or deionized water into a 1-L beaker.
2. Mass 3 g of calcium nitrate. Add the calcium nitrate to the water.
3. Mass 1 g of each of the following chemicals—magnesium sulfate, potassium nitrate and potassium phosphate and add to the solution.
4. For immediate use add 5-L of DI water to the original stock solution. *Note:* This 1% solution may need to be shaken before use to mix undissolved salts.
5. Pour solution into desired containers and autoclave.
6. (Optional) Add 50 g of sucrose to 500 mL of Knop's solution to stimulate the formation of zoospores.

Serial Dilution Steps

- Serial dilution involves the process of taking a sample and diluting it through a series of standard volumes of sterile diluent, which can either be distilled water or 0.9 % saline.
- Then, a small measured volume of each dilution is used to make a series of plates.
- Depending on the estimated concentration of cells/organisms in a sample, the extent of dilution is determined. For e.g., if a water sample is taken from an extremely polluted environment, the dilution factor is increased. In contrast, for a less contaminated sample, a low dilution factor might be sufficient.
- Serial two-fold and ten-fold dilutions are commonly used to titer antibodies or prepare diluted analytes in the laboratory.
- The dilution factor in a serial dilution can be determined either for an individual test tube or can be calculated as a total dilution factor in the entire series.
- The dilution factor of each tube in a set:

$$\frac{volume\ of\ sample}{volume\ of\ sample + volume\ of\ diluent}$$

- For a ten-fold dilution, 1 ml of sample is added to 9 ml of diluent. In this case, the dilution factor for that test tube will be:

$$\text{Dilution factor} = \frac{1\ ml}{1\ ml + 9\ ml} = \frac{1}{10} = 10^{-1}$$

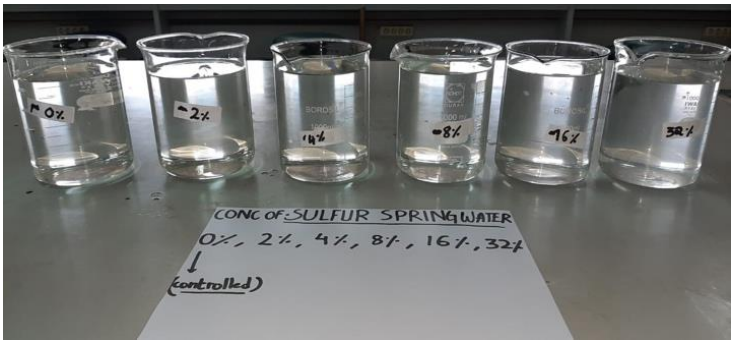
- After the first tube, each tube is the dilution of the previous dilution tube.

Now, for total dilution factor,

- Total dilution factor for the second tube = dilution of first tube × dilution of the second tube.

Example:
For the first tube, dilution factor = 10-1 (1 ml added to 9 ml)
For the second tube, dilution factor = 10-1 (1ml added to 9 ml)
Total dilution factor = previous dilution × dilution of next tube
= total dilution of 10-1 × 10-1 = 10-2

Result of Serial Dilution



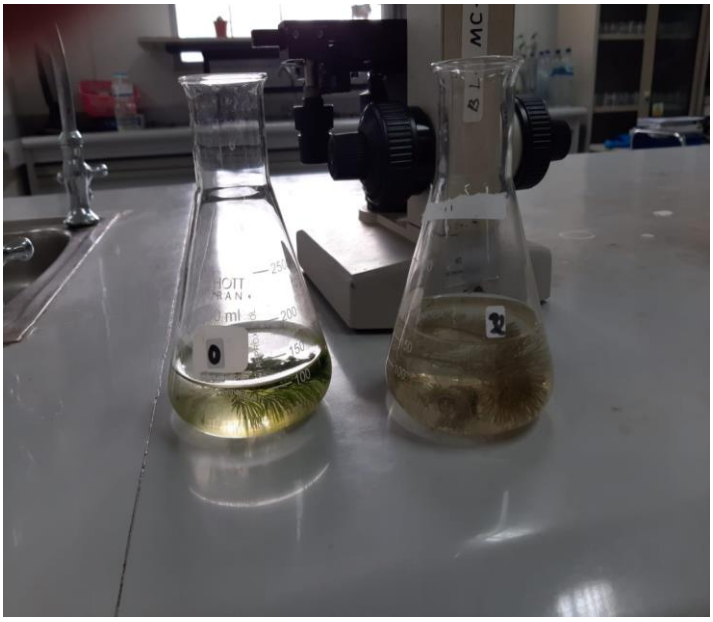
APPENDIX B

Pictures taken for and during experiment:

The images show Nutrient solution of different concentration

Cabomba Plant kept in the different concentrations of nutrient solution

Chlorophyll extract of the plants kept in different concentration



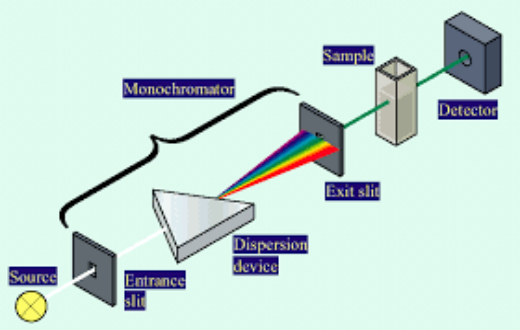
Difference in color between plants kept in 0% concentration and 32% concentration over a 7-day period

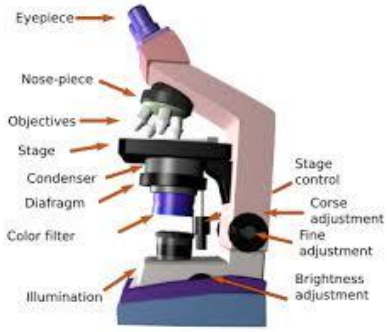
Plants kept in nutrient solution enriched with sulphur spring water facing the window to provide optimum environment for plant development.

Instruments Used

1.Spectrophotometer

2. Light Microscope





3.Digital Weighing Balance

APPENDIX C

All Raw Data Tables

Table 1: Raw Data Table [Chlorophyll Absorbance 645nm and 663nm] (Before Reading)

Concentration	Wave Length 645nm (Before Reading #1)	Wave Length 645nm (Before Reading #2)	Wave Length 663 nm (Before Reading #1)	Wave Length 663 nm (Before Reading #2)
0,1	0.358	0.349	0.678	0.678
0,2	0.284	0.289	0.562	0.553
0,3	0.258	0.259	0.517	0.507
0,4	0.254	0.259	0.488	0.495
0,5	0.179	0.176	0.346	0.345
2,1	0.370	0.369	0.693	0.693
2,2	0.291	0.292	0.566	0.560
2,3	0.243	0.242	0.457	0.452
2,4	0.237	0.233	0.450	0.447

2,5	0.175	0.181	0.345	0.342
4,1	0.302	0.301	0.572	0.570
4,2	0.254	0.251	0.481	0.480
4,3	0.306	0.302	0.558	0.552
4,4	0.245	0.246	0.494	0.484
4,5	0.252	0.251	0.466	0.459
8,1	0.433	0.426	0.803	0.842
8,2	0.399	0.400	0.581	0.582
8,3	0.340	0.346	0.575	0.576
8,4	0.291	0.282	0.541	0.537
8,5	0.269	0.270	0.517	0.522
16,1	0.468	0.454	0.842	0.841
16,2	0.355	0.361	0.674	0.675
16,3	0.347	0.354	0.694	0.695
16,4	0.275	0.278	0.508	0.509
16,5	0.321	0.326	0.635	0.634
32,1	0.346	0.347	0.676	0.678
32,2	0.331	0.332	0.611	0.628
32,3	0.325	0.313	0.614	0.613
32,4	0.265	0.254	0.497	0.494
32,5	0.261	0.263	0.516	0.507

Raw Data Table 2: Weight of Cabomba Plant Before/After/ Difference of weights

Concentration	Weight (Before)	Weight (After)	Weight Difference
0,1	0.586	0.478	0.108
0,2	0.485	0.387	0.098
0,3	0.641	0.532	0.109
0,4	0.518	0.489	0.029
0,5	0.674	0.548	0.126
2,1	1.880	0.370	1.510
2,2	0.789	0.399	0.390
2,3	0.415	0.172	0.243

2,4	0.408	0.072	0.336
2,5	0.507	0.156	0.351
4,1	0.945	0.530	0.415
4,2	0.828	0.788	0.040
4,3	0.608	0.350	0.258
4,4	0.588	0.238	0.350
4,5	1.360	0.758	0.602
8,1	0.342	0.260	0.082
8,2	0.465	0.447	0.018
8,3	0.795	0.662	0.133
8,4	0.418	0.304	0.114
8,5	0.527	0.260	0.267
16,1	0.425	0.361	0.064
16,2	0.342	0.260	0.082
16,3	0.795	0.662	0.133
16,4	0.465	0.447	0.018
16,5	0.483	0.304	0.179
32,1	0.505	0.374	0.131
32,2	0.861	0.728	0.133
32,3	0.350	0.243	0.107
32,4	1.045	0.743	0.302
32,5	0.372	0.174	0.198

Table 3: Raw Data Table [Chlorophyll Absorbance 645nm and 663nm] (After Reading)

Concentration	Wave Length 645nm (After Reading #1)	Wave Length 645nm (After Reading #2)	Wave Length 663 nm (After Reading #1)	Wave Length 663 nm (After Reading #2)
0,1	0.242	0.242	0.400	0.401
0,2	0.170	0.174	0.339	0.338
0,3	0.303	0.299	0.554	0.553
0,4	0.228	0.227	0.438	0.437
0,5	0.176	0.179	0.314	0.315
2,1	0.234	0.233	0.435	0.436

2,2	0.212	0.216	0.435	0.434
2,3	0.278	0.277	0.503	0.504
2,4	0.179	0.178	0.357	0.353
2,5	0.206	0.208	0.416	0.415
4,1	0.540	0.560	0.127	0.126
4,2	0.141	0.138	0.330	0.332
4,3	0.740	0.730	0.148	0.149
4,4	0.800	0.850	0.197	0.198
4,5	0.810	0.830	0.193	0.192
8,1	0.610	0.600	0.391	0.392
8,2	0.750	0.740	0.194	0.195
8,3	0.920	0.900	0.211	0.214
8,4	0.137	0.136	0.322	0.324
8,5	0.162	0.161	0.372	0.373
16,1	0.111	0.110	0.243	0.245
16,2	0.107	0.108	0.247	0.246
16,3	0.185	0.184	0.42	0.421
16,4	0.135	0.134	0.321	0.320
16,5	0.146	0.145	0.344	0.345
32,1	0.219	0.218	0.545	0.544
32,2	0.860	0.840	0.173	0.172
32,3	0.950	0.940	0.196	0.195
32,4	0.730	0.720	0.158	0.157
32,5	0.119	0.121	0.288	0.287

Table 4 : Supporting Experiment Data Table (Before Results)

Concentration	Chlorophyll content **before** putting copper solution **(645 nm)**	Chlorophyll content **before** putting copper solution **(645 nm)**	Chlorophyll content **before** putting copper solution **(663 nm)**	Chlorophyll content **before** putting copper solution **(663 nm)**
0,1	0.168	0.167	0.349	0.350
0,2	0.210	0.210	0.454	0.447
0,3	0.116	0.122	0.245	0.246

50,1	0.241	0.248	0.522	0.523
50,2	0.255	0.256	0.537	0.547
50,3	0.284	0.278	0.580	0.581
100,1	0.114	0.116	0.234	0.232
100,2	0.245	0.246	0.492	0.495
100,3	0.255	0.252	0.526	0.528
200,1	0.210	0.200	0.416	0.414
200,2	0.349	0.348	0.695	0.693
200,3	0.205	0.203	0.410	0.411
400,1	0.245	0.242	0.491	0.494
400,2	0.234	0.229	0.495	0.493
400,3	0.233	0.234	0.460	0.456
800,1	0.155	0.156	0.284	0.286
800,2	0.223	0.224	0.493	0.501
800,3	0.260	0.258	0.545	0.550

Table 5 : Supporting Experiment Data Table [Copper Sulfate Solution] (After Results)

Concentration	Chlorophyll content **after** putting copper solution **(645 nm)**	Chlorophyll content **after** putting copper solution **(645 nm)**	Chlorophyll content **after** putting copper solution **(663 nm)**	Chlorophyll content **after** putting copper solution **(663 nm)**
0,1	0.138	0.153	0.244	0.251
0,2	0.162	0.16	0.326	0.319
0,3	0.198	0.182	0.372	0.359
50,1	0.128	0.120	0.202	0.228
50,2	0.147	0.148	0.405	0.395
50,3	0.208	0.203	0.203	0.204
100,1	0.034	0.033	0.039	0.038
100,2	0.049	0.033	0.047	0.048
100,3	0.083	0.084	0.327	0.321
200,1	0.052	0.042	0.044	0.052
200,2	0.074	0.079	0.082	0.074
200,3	0.052	0.056	0.040	0.052
400,1	0.065	0.039	0.041	0.065
400,2	0.046	0.038	0.037	0.046

400,3	0.047	0.036	0.037	0.047
800,1	0.029	0.094	0.096	0.029
800,2	0.034	0.007	0.003	0.034
800,3	0.029	0.010	0.012	0.029

Table 6: Averaged Values of Data Table 1 for 645nm and 663nm (Before Results)		
Concentration	**Average Wave Length 645nm (Before Reading)**	**Average Wave Length 663 nm (Before Reading)**
0,1	0.354	0.678
0,2	0.287	0.558
0,3	0.259	0.512
0,4	0.257	0.492
0,5	0.178	0.346
2,1	0.370	0.693
2,2	0.292	0.563
2,3	0.243	0.455
2,4	0.235	0.449
2,5	0.178	0.344
4,1	0.302	0.571
4,2	0.253	0.481
4,3	0.304	0.555
4,4	0.246	0.489
4,5	0.252	0.463
8,1	0.430	0.823
8,2	0.400	0.582
8,3	0.343	0.576
8,4	0.287	0.539
8,5	0.270	0.520
16,1	0.461	0.842
16,2	0.358	0.675
16,3	0.351	0.695
16,4	0.277	0.509
16,5	0.324	0.635
32,1	0.347	0.677
32,2	0.332	0.620
32,3	0.319	0.614
32,4	0.260	0.496
32,5	0.262	0.512

Table 7: Averaged Values of Data Table 1 for 645nm and 663nm (After Results)		
Concentration	**Average Wave Length 645nm (After Reading)**	**Average Wave Length 663 nm (After Reading)**
0,1	0.242	0.401
0,2	0.172	0.339
0,3	0.301	0.554
0,4	0.228	0.438
0,5	0.178	0.315
2,1	0.234	0.436
2,2	0.214	0.435
2,3	0.278	0.504
2,4	0.179	0.355
2,5	0.207	0.416
4,1	0.550	0.127
4,2	0.140	0.331
4,3	0.735	0.149
4,4	0.825	0.198
4,5	0.820	0.193
8,1	0.605	0.392
8,2	0.745	0.195
8,3	0.910	0.213
8,4	0.137	0.323
8,5	0.162	0.373
16,1	0.111	0.266
16,2	0.108	0.247
16,3	0.185	0.421
16,4	0.135	0.321
16,5	0.146	0.345
32,1	0.219	0.545
32,2	0.850	0.173
32,3	0.945	0.196
32,4	0.725	0.158
32,5	0.120	0.288

Table 8: Calculated Chlorophyll 'a' & 'b' values, Total Chlorophyll present for Before Experimentation Results			
Concentration	**Chlorophyll a (Before)**	**Chlorophyll b (Before)**	**Total (Before)**
0,1	7.658	4.934	12.588
0,2	6.315	3.961	10.272
0,3	5.806	3.535	9.338
0,4	5.557	3.583	9.137
0,5	3.915	2.457	6.370
2,1	7.806	5.230	13.031
2,2	6.365	4.052	10.413
2,3	5.125	3.438	8.557
2,4	5.070	3.280	8.347
2,5	3.890	2.466	6.354
4,1	6.439	4.244	10.679
4,2	5.428	3.543	8.968
4,3	6.231	4.364	10.591
4,4	5.549	3.345	8.890
4,5	5.202	3.604	8.803
8,1	9.295	12.020	15.365
8,2	6.315	16.148	16.613
8,3	6.393	19.842	20.090
8,4	6.073	1.626	5.358
8,5	5.878	1.964	6.264
16,1	9.453	6.616	16.065
16,2	7.609	5.039	12.645
16,3	7.882	4.785	12.664
16,4	5.719	3.961	9.677
16,5	7.193	4.448	11.637
32,1	6.332	2.465	8.795
32,2	-0.089	18.655	18.557
32,3	-0.053	20.723	20.661
32,4	0.056	15.863	15.912
32,5	3.335	1.400	4.734

Table 9: Calculated Chlorophyll 'a' & 'b' values, Total Chlorophyll present for After Experimentation Results			
Concentration	**Chlorophyll a (After)**	**Chlorophyll b (After)**	**Total (After)**
0,1	4.442	3.665	8.104
0,2	3.843	2.352	6.193
0,3	6.226	4.300	10.523
0,4	4.949	3.171	8.118
0,5	3.522	2.602	6.122
2,1	4.908	3.318	8.224
2,2	4.949	2.865	7.812
2,3	5.653	4.007	9.658
2,4	4.027	2.438	6.463
2,5	4.726	2.793	7.518
4,1	0.133	12.001	12.129
4,2	3.827	1.657	5.483
4,3	-0.085	16.134	16.042
4,4	0.295	17.966	18.253
4,5	0.245	17.875	18.112
8,1	3.351	5.995	15.286
8,2	0.472	6.436	12.747
8,3	0.257	5.159	11.548
8,4	3.734	4.050	10.120
8,5	4.301	3.749	9.624
116,1	3.080	1.297	4.376
16,2	2.846	1.317	4.163
16,3	4.849	2.266	7.113
16,4	3.714	1.589	5.301

16,5	3.989	1.729	5.716
32,1	7.664	4.778	12.438
32,2	6.981	4.701	11.678
32,3	6.940	4.432	11.368
32,4	5.600	3.633	9.229
32,5	5.798	3.604	9.398