International Baccalaureate

Biology Extended Essay

The effect of different light intensities (0 Lux,125 Lux, 175 Lux, 225 Lux, 275 Lux, 325 Lux) on the chlorophyll amount of the *Spinacia oleracea* after a 12-hour lighting period measured by the spectrophotometer absorption rates.

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Table of Contents

Introduction……3

Background Information…….3

Hypothesis……..4

Method Development and Planning……5

Variables……..6

Material List……7

Methodology……8

Ethical, Safety and Environmental Considerations…….9

Resulting Data……10

Evaluation……..16

Conclusion and Further Investigation……17

Bibliography……...18

Appendices

Appendix 1 (Photos from the experiment) …….19

Appendix 2 (Lab permission from Bilkent University)…….21

Introduction

I have always been interested in working with plants as far as I can remember since my grandmother loves gardening and has been growing different fruit trees and vegetables since my grandparents bought their house all the way back in the 1960s. That is why she was very excited when I told her that I was going to be working with spinach plants for my "school project" since spinach was one of the only plants that had never touched her garden and she has been meaning to grow them for a long time. I was first introduced to growing plants back in 4th grade when we were given a home task which was growing a bean sprout and were each given a single bean seed. I was fascinated by the fact that such a large plant could be formed by such a small seed just by leaving it under the sun and occasionally watering it which together with my grandmother's enthusiasm was the reason that made me interested in growing a plant for my extended essay.

Background Information

Chlorophyll is an important pigment for plants that is one of the main components of photosynthesis.[10] Plants are perceived as green since chlorophyll is good at absorbing the red and blue lights of the RGB (Red Green Blue) light spectrum which only leaves the green light to be reflected back to our eyes.

Photosynthesis is the process of creating chemical energy from light using carbon dioxide and water. Chlorophyll is necessary for this process since it is used to absorb light energy from sunlight to be turned into chemical energy in the form of sugars. There are 2 types of chlorophyll in plants called chlorophyll a and chlorophyll b.[3]

The main function of chlorophyll a is to collect sunlight for it to be used in photosynthesis while the function of chlorophyll b is to help chlorophyll a by capturing light

and passing it into chlorophyll a.[5] These pigments work together to capture the required amount of light energy to be used in photosynthesis. They have a certain range of light wavelengths that they can easily absorb between 600nm and 700nm range.

A device called a spectrophotometer can be used to measure the chlorophyll amount in a sample by sending light with different wavelengths into a small cuvette filled with a chlorophyll solution and measuring how much of the light from these wavelengths are reflected and how much of it is absorbed. The peak amount of absorbed light between the 600nm and 700nm range would theoretically give the amount of chlorophyll a and b in the sample. The spectrophotometer graphs these amounts for view and prints a data sheet for closer inspection. I will be using the "MAX" function in excel on the data sheet to find the largest absorption rate and the most suitable light intensity in each of the trials. I will then use the absorption rate of the most suitable wavelength which was always around the 662nm-664nm range to comment on the chlorophyll amount that was in the plant at the end of the lighting period. After I thought of this experiment, I set my research question as **"How does different light intensities (0 Lux, 125 Lux, 175 Lux, 225 Lux, 275 Lux, 325 Lux) affect the amount of chlorophyll in *Spinacia oleracea* (spinach) measured with a spectrophotometer after a 12-hour daylight period?"**

Hypothesis

According to a website I found called "Nagwa", since the plants will only use the light energy to create the required amount of chemical energy for photosynthesis, chlorophyll amount in the higher light intensities will be reduced because the abundance of light will make it easier for the existing chlorophyll to create the required amount of chemical energy.[7] Therefore, reducing the chlorophyll amount in the *Spinacia oleracea.*

Method Development and Planning

I initially wanted use the algae species called *Chlorella vulgaris* for this experiment since I had never grown algae before and I was interested in it. However, I later learned that the algae strain I wanted to use was very expensive for a small amount and it added unnecessary steps into the experiment like setting up photobioreactors and ensuring a constant amount of CO_2 was in the environment, so I decided to use a plant instead of the algae. A Since I wanted to grow the plants myself, I was looking for a plant that I had easy access to and that was already grown in my country so that I would not have a hard time finding seeds for them. The reason for my choice of plant being *Spinacia oleracea* or spinach is that it has a dark green color which is an indication of the chlorophyll amount being higher. It was also very easy and fast to grow, not needing any special equipment or soil. The growth time was an important factor as well since it grows to full maturity in around 2 weeks which meant that it was very convenient and fast for me to grow and would give good results.

I looked for online sources on how to extract chlorophyll out of plants[8,9,11] and from my research I was able to come to the conclusion that I needed to use alcohol to get rid of the cell wall and the cell membrane so that the chlorophyll can be collected with the help of some filter paper. The collected chlorophyll can then be put into the spectrophotometer in order to measure the chlorophyll content in the solution gathered from the leaf cells. I chose the light intensities "0 Lux, 125 Lux, 175 Lux, 225 Lux, 325 Lux" since I had a space problem under the fume hood. I wasn't able to fit all the plants under the fume hood by increasing the light intensity by 100 Lux per each trial so I had to lower it to an increase of 50 Lux per trial. I chose the daylight period as 12 hours as 8 to 12 hours is the recommended photoperiod (the amount of time that a plant is exposed to light per day) for spinach plants according to the data I found online.[4]

Variables

Independent Variable: The light intensities shined onto the *Spinacia oleracea* changed by changing the distance between the plants and the light source and using a Lux meter to measure the light intensity. (0 Lux, 125 Lux, 175 Lux, 225 Lux, 275 Lux, 325 Lux)

Dependent Variable: The chlorophyll amount of the *Spinacia oleracea* at the end of a 12-hour daylight period measured with the Spectrophotometer absorption rates.

Controlled Variable	Method of Control	Why Is It Controlled?
The cleanness of the test tubes and the quartz cuvette	The test tubes and the quartz cuvette of the spectrophotometer were washed each time, and the same ones were used.	Any remaining chemicals inside the test tubes or a cuvette for the spectrophotometer could change the experiment results since the remaining chemicals can react with the solutions that are used in the experiment and remaining samples from the other trials can change the spectrophotometer results.
Ambient temperature *(19.0 ± 0.1* °C*)*	I kept the temperature the constant by working in the same lab conditions and using a thermometer.	In case of a temperature difference, the *Spinacia oleracea* may produce different amounts of chlorophyll which would affect the results of the experiment. According to the National Institutes of Health (NIH)'s website chlorophyl production in plants decrease in colder environments as "low temperature suppresses chlorophyll biosynthesis".[2] I kept the *Spinacia oleracea* under the same fume hood which would ensure a constant temperature for all the plants even in slight environmental changes.
Surface Area of the leaf pieces that were grinded	I made sure that all leaf pieces were exactly 1 cm^2 with a ruler.	Differing masses of leaves could change the chlorophyll amount of the solution so there is a certain chlorophyl amount in each leaf cell so using a larger leaf piece would affect the results of the experiment.

Table 1: The Controlled Variables

Material List

Material	Quantity	Unit
Potted *Spinacia oleracea*	5x6=30	-
A Light Source (Fume hood lamps were used)	1	-
Quartz Cuvette	1	10ml ± 0.02ml
Spectrophotometer	1	-
Lux Meter	1	-
Mortar and Pestle	1	-
80% Acetone	500ml	-
Ammonium Hydroxide	0.525milligrams per 150ml of 80% acetone	±0.01 mg
Digital Scale	1	±0.01 mg
Pure Water	500ml	-
Test tubes	30	10ml ± 0.02ml
Filter Paper	30	-
A Ruler	1	30cm ± 0.5 mm
A Pair of Scissors	1	-
Beakers	2	250ml beakers ± 2.5ml
Transparent Dropper	1	5ml ± 0.5

Table 2: Material List

Methodology

1) Buy *Spinacia oleracea* seeds from a local farm.
2) Germinate 30 *Spinacia oleracea* seeds by putting them in a damp towel and leaving them in a dark environment.
3) Grow plants until maturity and put them into individual pots. This step will take around 2 weeks.
4) Put 5 of the plants under the fume hood lights.
5) Adjust the height that they stand on by using a Lux meter and mark the light intensity levels for each plant with a paper (as can be seen in Appendix 1 Figure 9)
6) Put another one of the *Spinacia oleracea* in a dark location to get a control group.
7) Leave the *Spinacia oleracea* under the different light intensities for 12 hours as a daylight period which lets them adjust to the lighting.
8) Cut off a piece of a leaf from a single *Spinacia oleracea* after the daylight period ends.
9) Adjust the cut piece to 1 cm^2 and put it into the mortar.
10) Prepare a solution consisting of 0.525 mg of sodium hydroxide and 150 ml of 80% acetone by adding them into a glass and mixing them thoroughly.
11) Put enough solution to cover the leaf pieces into the mortar.
12) Grind the leaf piece until it has dissolved into the solution with the help of a pestle.
13) Put the solution into a test tube with the help of a filter paper to take out the solid pieces left inside.
14) Add 80% acetone until the solution reaches 3ml.
15) Centrifuge the new solution in 5000rpm for 5 minutes.
16) While the centrifuge is still going, calibrate the spectrophotometer with a sample of pure water.

17) After the centrifuge, put the remaining solution into a test tube and mark it with a marker with the light intensity and the trial number (125-1 for example).
18) Pour the solution into a quartz cuvette with the help of a dropper and put it into the spectrophotometer.
19) Set the spectrophotometer to measure between 0nm and 800nm wavelengths and started it with the chlorophyll solution inside.
20) Record the resulting graph and data sheets.
21) Wash the appliances with acetone and pure water.
22) Repeat the experiment 5 times with each of the 6 light intensities for a total of 30 times for more accurate results.
23) Examine the resulting data sheets to find the highest point on the absorption rate graphs for each of the *Spinacia oleracea* with the different light intensities.

Ethical, Safety and Environmental Considerations

Ethical Considerations

1) None of the chemicals had any direct interactions with the plants themselves. So, after my experiment ended, instead of throwing the plants out, I replanted them onto my grandmother's garden, and they survived for 5 more weeks before withering.
2) Even though some leaf pieces were cut off from the plants, I made sure to not get any excess pieces and make the cuts as small as possible while not taking them from any substantial parts of the plants.

Safety Concerns

Ammonium Hydroxide is a dangerous chemical that should be handled with care.[1]

1) Coming into contact with skin might lead to severe irritation and chemical burns so gloves must always be worn when handling it.

2) Coming into contact with the eyes could lead to eye irritation and permanent eye damage so safety goggles must be worn at all times and the chemical should be used at a lab with safety showers on easy access.
3) Inhaling the chemical could lead to lung irritation and fluid build-up in the lungs which could be lethal.
4) Even though the chemical itself is not combustible, it coming in contact with fire can lead the to the release of ammonia vapors which can be ignited to cause an explosion so fire extinguishers must be kept at close proximity and in case of a fire, it should not be kept close by.

Environmental Considerations

1) None of the chemicals were poured into sewage and was disposed of with chemical containers.
2) The spinach seeds were bought from a local farm and no industrial fertilizers of harmful insecticides were used to grow them.

Resulting Data

For the raw data section, I limited the data I gathered to only include the peak wavelength for each trial since including all the data from the spectrophotometer would give me pages of tables as the spectrophotometer gives the results all the way from 0 nm of light to 800 nm of light which would mean at least 800 lines of data for each trial. That is why I decided to not include it in this extended essay and only show the peak values of each graph. All of the peak absorption rates were gathered between the wavelengths between 662 nm and 664 nm.

Light Intensities shined onto the *Spinacia oleracea* during the lighting period (in Lux)	**Trials**	**The peak absorption rate of the chlorophyll solution obtained from the *Spinacia oleracea* (percentage)**
0 (darkness)	1	0.248780
	2	0.339961
	3	0.476225
	4	0.296219
	5	0.303858
125	1	0.379212
	2	0.645423
	3	0.426225
	4	0.716879
	5	0.303858
175	1	0.351733
	2	0.726847
	3	0.455198
	4	0.898878
	5	0.713478
225	1	0.424244
	2	0.469387
	3	0.753066
	4	0.329841
	5	0.532579
275	1	0.219423
	2	0.514726
	3	0.550594
	4	0.467929
	5	0.510257
325	1	0.392728
	2	0.673650
	3	0.354865
	4	0.431943
	5	0.408139

Table 3: Absorption Rates from each trial

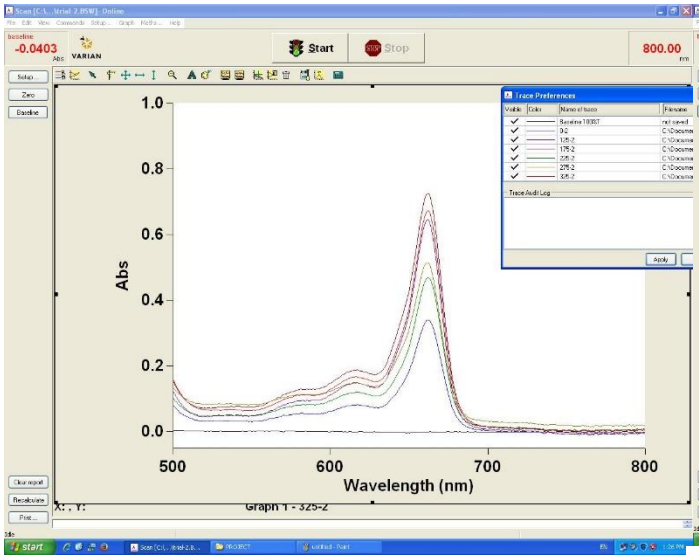


Figure 1: The spectrophotometer graph from the first trial

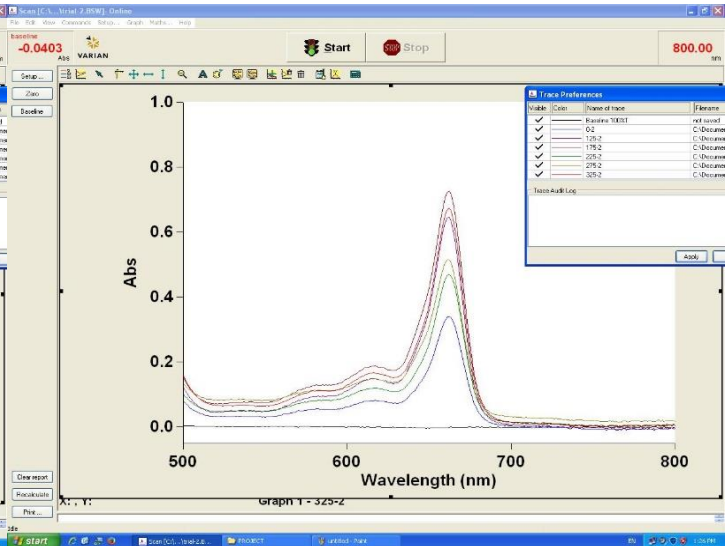


Figure 2: The spectrophotometer graph from the second trial

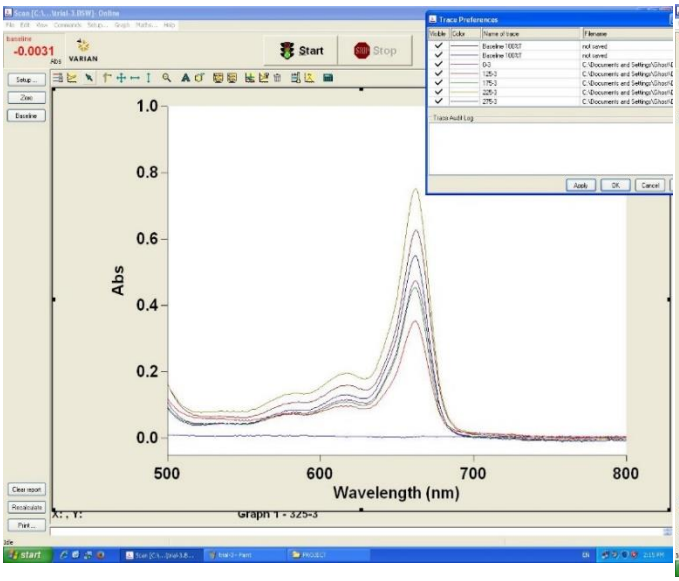


Figure 3: The spectrophotometer graph from the third

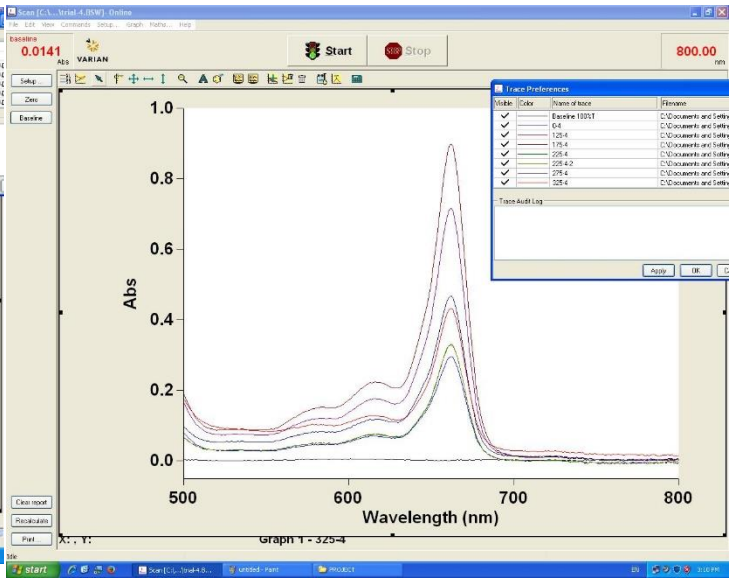


Figure 4: The spectrophotometer graph from the fourth trial

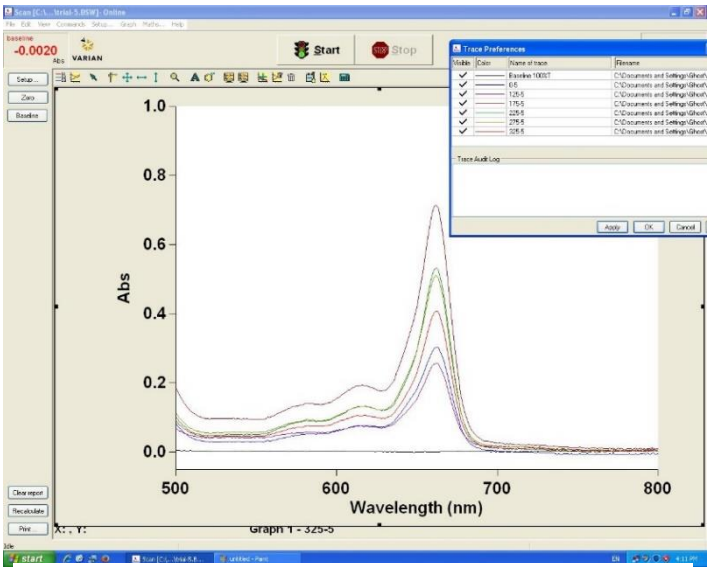


Figure 5: The spectrophotometer graph from the fifth trial

As we can see from the graphs (figures 1-5) and the highest absorption rates (Table 2), the sample with the highest amount of chlorophyll and the lowest amount of chlorophyll fluctuates as the trials go on. This can be clearly seen by the peak values of the graphs or the values in table-2 which show,

Trial 1: 225 Lux > 325 Lux > 125 Lux > 175 Lux > 0 Lux > 275 Lux

Trial 2: 175 Lux > 325 Lux > 125 Lux > 275 Lux > 225 Lux > 0 Lux

Trial 3: 225 Lux > 275 Lux > 125 Lux > 0 Lux > 175 Lux > 325 Lux

Trial 4: 175 Lux > 125 Lux > 275 Lux > 325 Lux > 225 Lux > 0 Lux

Trial 5: 175 Lux > 225 Lux > 275 Lux > 325 Lux > 0 Lux > 125 Lux

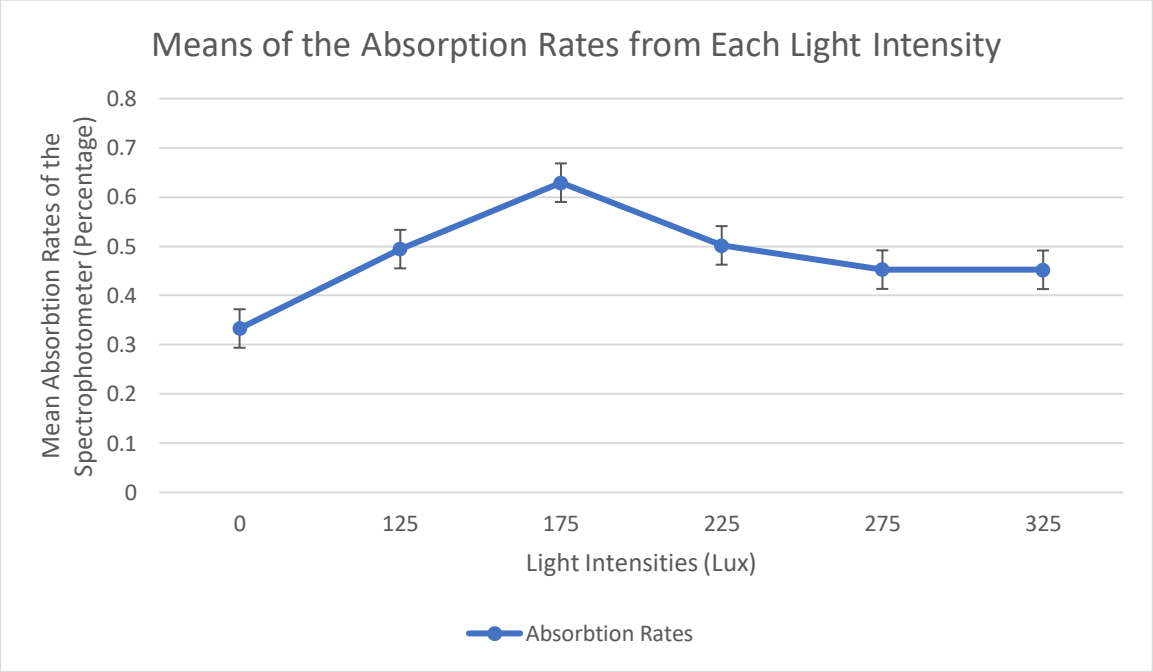
That is why I will be taking the mean values and the standard deviations of the data values in order to comment on the fluctuating data points and graphing them to visualize any correlation.

Light Intensities (Lux)	0	125	175	225	275	325
Mean Values (Percentage)	0.333009	0.494319	0.629227	0.501823	0.452586	0.452265
Standard Deviation of the Values	0.08639	0.17785	0.22171	0.15868	0.13359	0.12688

Table 4: Means and Standard Deviations of the Absorption Rates from Each Light Intensity

As we can see from the mean values, the *Spinacia oleracea* with the 175 Lux light intensity had the highest chlorophyll content out of all the trials since it has the highest absorbance rate in the spectrophotometer result. Since it is one of the lower light intensities, we can say that there is no direct correlation between the chlorophyll amount and the light intensity. The 325 Lux *Spinacia oleracea* having the second lowest intensity is another point that can be made for these means. The 175 Lux light intensity also had the highest standard deviation which means that some data deviated from the mean of the data at a high rate which could point to it being inconsistent.

I will graph these means to see if an equation can be drawn from the data given from the means of the absorption rates.



Graph 1: The Mean absorption rates from the different light intensities

The graph (Graph 1) shows that no equation could be drawn from the data given by the means of the absorption rates. The error bars on the graph represent the standard error of the data. I will now use the ANOVA data analysis technique to see the differences between the means of the data. The following graph shows the results of the ANOVA calculations created by using Excel. I will form two hypotheses for the result of my p-value from the ANOVA graph and differentiate between them by seeing if my p-value is under or over 0.05. H_0 meaning the p-value is over 0.05 and there is not a direct correlation between the data. This would mean that that the different light intensities were not able to cause a sizable difference in the chlorophyll amount in the *Spinacia oleracea* and H_1 meaning the p-value is under 0.05 and there is a direct correlation between the data which would mean that the different light intensities have caused a sizable difference in the chlorophyll amount in the *Spinacia oleracea*.

H_0: There is not a significant difference between mean absorption rates when the light intensities are changed.

H_1: There is a significant difference between mean absorption rates when the light intensities are changed.

The table below shows the results of the ANOVA test that I used on my data.

SUMMARY

Groups	*Count*	*Sum*	*Mean*	*Variance*
0 Lux	5	1,665043	0,333009	0,007464
125 Lux	5	2,471597	0,494319	0,031632
175 Lux	5	3,146134	0,629227	0,049157
225 Lux	5	2,509117	0,501823	0,025179
275 Lux	5	2,262929	0,452586	0,017848
325 Lux	5	2,261325	0,452265	0,016101

ANOVA

Source of Variance	*Sum of Squares*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Group	0,230152	5	0,04603	1,873924	0,136513	2,620654
Within Groups	0,589526	24	0,024564			
Total	0,819678	29				

Table 5: Results of my ANOVA test

The H_0 hypothesis seems to be correct since the p-value is over 0.05, around 0.1 which means that the independent variable does not have a significant effect on the dependent variable. This means that the dependent variable which is the chlorophyll amount isn't heavily affected by the light intensity. We can see this as there is no steady increase or decrease in the chlorophyll amount as the light intensity goes up. We can see this since the 325 Lux *Spinacia oleracea* never had the highest chlorophyll amount throughout the 5 trials.

If my p value was under 0.05, I would have used another data analysis technique called TUKEY. The TUKEY technique can be used when the p-value of the ANOVA test is lower than 0.05 and it is used to determine which of the data have a significant difference between them instead of giving a general value for all the data.

Evaluation

As we can see from these values, The *Spinacia oleracea* with the highest amount of chlorophyll and the lowest amount of chlorophyll constantly fluctuates between the trials. Because of this fact I was not able to deduce a correlation between the light intensity and the chlorophyll amount. In some of the trials some of the *Spinacia oleracea* even went below the 0 Lux *Spinacia oleracea* that I kept in darkness for the 12-hour daylight period. This might emphasize that even though photosynthesis halts with a lack of sunlight, chlorophyll production continues to some extent. The 175 Lux *Spinacia oleracea* was the most consistent one to have the highest amount of chlorophyll. This might be the effect of *Spinacia oleracea* health and genetic make-up rather than the effect of the light intensity. Some limitations of my experiment were the number of leaves that each plant had, the human errors in the watering of the plants, and the plants getting slightly different amounts of sunlight in their growth phase. Some strengths of my experiment were that I used a spectrophotometer which is a highly calibrated device that leaves no room for human errors while taking measurements and that I grew the plants in the same conditions which could eliminate other variables that might affect the chlorophyll amount in the plants, thus, giving better results. The test tubes filled with the chlorophyll solution having different colors depending on their chlorophyll content (as can be seen on Appendix 1 figure 6) gives a visual representation to the numerical data that was gathered.

Conclusion and Further Investigation

My research question was **"How does different light intensities (0 Lux, 125 Lux, 175 Lux, 225 Lux, 275 Lux, 325 Lux) affect the amount of chlorophyll in *Spinacia oleracea* (spinach) measured with a spectrophotometer?"** However, the results do not seem to support my hypothesis that the chlorophyll amount will decrease as the light intensity is increased. According to a paper released by the science journal called "HortScience", my hypothesis was true since in their tests, chlorophyll production in a plant reduced when the light intensity was increased as explained with this quote from the paper, "The chlorophyll concentration of plants was significantly influenced by the different light intensities. Among all treatments, the chlorophyll concentration was greatest in the L_{10} treatment and then decreased consistently as the light intensified, lowest in the L_{90} treatment".[6] L_{10} and L_{90} being the Lux values that were used in the experiment. However, I wasn't able to get the same result from this experiment. This might have been because I used a different plant species than the *Anoectochilus blume* that HortScience used for their experiment. Since I could not find a solid correlation between the *Spinacia oleracea* with the different light intensities, I have a few ways to improve the experiment to investigate this matter further. Firstly, seeds from the same parent plant could be used to recreate the experiment which would eliminate the genetic factors that could affect the chlorophyll production. Secondly, the plant leaf sizes could affect the results of the experiment so leaf sizes could be changed by pruning the leaf ends so they would have the same size. Thirdly, dried and withering leaves could be cut so that they stop using the plant's resources which could get rid of the unwanted energy use of the plants. Fourthly, the plants can be grown in the desired light intensities to eliminate the need to give them a daylight cycle to adapt into the light intensities since they were already grown in that environment. Lastly, the plants can be kept under the different light intensities for a longer period which would give them more time to adapt to the conditions, thus giving more accurate results.

Bibliography

1) *Hazardous Substance Fact Sheet*. NJ Health. (2011, July). Retrieved December 22, 2022, from https://www.nj.gov/health/eoh/rtkweb/documents/fs/0103.pdf
2) F., S., Z., L., Z., Z., & C., Y. (2020, February 19). *Effect of Low Temperature on Chlorophyll Biosynthesis and Chloroplast Biogenesis of Rice Seedlings during Greening*. International journal of molecular sciences. Retrieved November 13, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7073065/#:~:text=Chlorophyll%20during%20the%20greening%20process,C%20(Figure%202C).
3) Khan Academy. (2016). *Intro to photosynthesis (article)*. Khan Academy. Retrieved November 13, 2022, from https://www.khanacademy.org/science/ap-biology/cellular-energetics/photosynthesis/a/intro-to-photosynthesis#:~:text=Photosynthesis%20is%20the%20process%20in,is%20released%20as%20a%20byproduct.
4) K., H., C., C., & K., T. (2000, February). The Potential Use of Photoperiod during Transplant Production under Artificial Lighting Conditions on Floral Development and Bolting, Using Spinach as a Model. Retrieved January 15, 2023, from https://journals.ashs.org/hortsci/view/journals/hortsci/35/1/article-p43.pdf
5) Lakna Panawala. (n.d.). *(PDF) home » Science » Biology » Cell Biology » difference between ...* ResearchGate. Retrieved November 12, 2022, from https://www.researchgate.net/publication/316584030_Home_Science_Biology_Cell_Biology_Difference_Between_Chlorophyll_A_and_B_Difference_Between_Chlorophyll_A_and_B_Main_Difference_-_Chlorophyll_A_vs_Chlorophyll_B.
6) Ma, Z., Li, S., Zhang, M., Jiang, S., & Xiao, Y. (2010, June 1). *Light intensity affects growth, photosynthetic capability, and total flavonoid accumulation of Anoectochilus Plants*. HortScience. Retrieved November 12, 2023, from https://journals.ashs.org/hortsci/abstract/journals/hortsci/45/6/article-p863.xml
7) *Describing the relationship between light intensity and synthesis of chlorophyll*. Retrieved November 15, 2023, from Nagwa. (n.d.). https://www.nagwa.com/en/videos/627178468285/
8) *Selective Chlorophyll Removal Method to "Degreen" Botanical Extracts*. Journal of natural products. (2020, June 26). Retrieved January 27, 2023, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7398693/#:~:text=Chlorophyll%20pigments%20are%20readily%20extracted,such%20as%20MeOH%20and%20EtOH.
9) Team, B. J. (2019, April 15). *Chlorophyll fluorescence*. BIOLOGY JUNCTION. Retrieved January 13, 2024, from https://biologyjunction.com/chlorophyll-fluorescence/.
10) Wikimedia Foundation. (2024, January 8). *Chlorophyll*. Wikipedia. Retrieved January 19, 2024, from https://en.wikipedia.org/wiki/Chlorophyll#:~:text=Chlorophyll%20is%20any%20of%20several,Chlorophyll%20at%20different%20scales.
11) wildlandschoolvideo. (2011). *Chlorophyll Extraction and Analysis*. *YouTube*. YouTube. Retrieved January 27, 2023, from https://www.youtube.com/watch?v=rXGDg_g-8Ik.

Appendix 1

Photos from the experiment

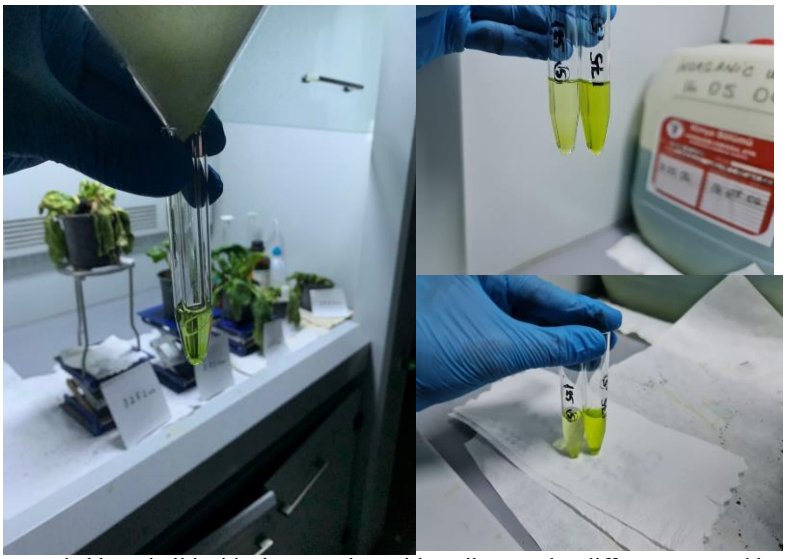


Figure 6: The extracted chlorophyll inside the test tubes with a vibrant color difference caused by the difference in chlorophyll amount

Figure 7: The leaf piece after it was crushed with a mortar.

Figure 8: My experimentation setup in the laboratory

Figure 9: The Spinacia oleracea that I used for the experiment.

Figure 10: The Luxmeter that I used to measure the light intensity on the plants.

Appendix 2

Bilkent University
Department of Chemistry

[redacted] has done his IB diploma program extended essay experiment in Bilkent University Chemistry Department Laboratory by himself with the supervision of our staff.

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