



Original Research Article

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ANALYSIS OF LIGHT EXPOSURE TIME AND ITS EFFECT ON PHOTOSYNTHETIC PIGMENT CHLOROPHYLL A AND B DEGRADATION

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ABSTRACT: Radiobiology is an area of how living things respond after radiation exposure, specifically ionizing radiation. This is of vital importance to understand how the electromagnetic spectrum affects biomolecules, in this research specifically chlorophyll. During this research, the effect of different light conditions such as complete darkness, natural room light, ultraviolet α (364 nm) and β (305 nm) radiation on the degradation rate of α -chlorophyll and β was evaluated based on quantifying the concentration of these biomolecules using a spectrophotometer and characterized equations for both compounds, through the understanding the relationship between time of exposure, the photon energy, the absorption spectrum, the concentration of this pigment and its degradation velocity in different light environments. After experimentation, it was found that, after one hour of UV- α exposure, α -chlorophyll decreased 20.7%, while β -chlorophyll decreased 11.3%. In the case of UV- β exposure, α -chlorophyll decreased 15.1% and β -chlorophyll decreased 21.8%. It was concluded that α -chlorophyll is approximately 10% more sensitive to UV- α degradation, while β -chlorophyll is 5% more sensitive to UV- β under the experienced conditions.

Keywords: UV Light, Exposure, Chlorophyll, Degradation.

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1. INTRODUCTION

The name "Chlorophyll" was first proposed by Pierre Joseph and Joseph Bienaime in 1818 for the green substance (Greek *chloros*) that could be extracted from leaves (Greek *phyllos*) with the help of alcohol [1-3]. However, in 1912 the chemical composition of what would become chlorophyll α

($C_{55}H_{70}MgN_4O_5$) and chlorophyll β ($C_{55}H_{70}MgN_4O_6$), both major molecules of various tissues of mainly plant origin, became known. The structure of the porphyrin ring was discovered by Discher and co-workers [2-5]. In plants, chlorophyll is mainly found in chloroplasts, organelles located in the center of the leaf (mesophyll layer) [6]. Food and energy production takes place in the chloroplast, transforming light energy into chemical energy [7]. These compounds are complex organic molecules formed by derivatives of porphyrin, a macrocyclic, asymmetric, totally unsaturated structure [3, 8-10]. However, chlorophyll-b has structural differences from chlorophyll-a in that it has the presence of an aldehyde residue instead of a methyl group which is converted to it by the enzyme oxygenase, as seen in figure 1.0 [11-14] Although there are different chlorophylls (α to f), chlorophyll- α accounts for approximately 75% of this pigment found throughout nature [9].

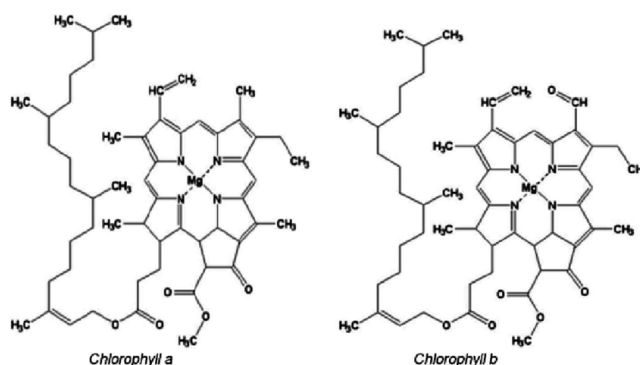


Figure 1.0 Molecular structure of chlorophyll α and β [14.]

Chlorophylls are the molecules that have the function of introducing light into the system. The light is captured by the pigment molecules and used to generate high-energy electrons with high reducing potential [15]. Structural differences generate differences in both the absorption and emission spectra, this is shown in figure 1.1 [16]. Chlorophyll α , d , f absorbs similar intensities in blue, red and, green. This causes α -chlorophyll to appear blue-green, while β -chlorophyll appears bright green [8, 17, 18]. α -Chlorophyll is part of the reactions of photosystem I (PSI) and photosystem II (PSII) [19].

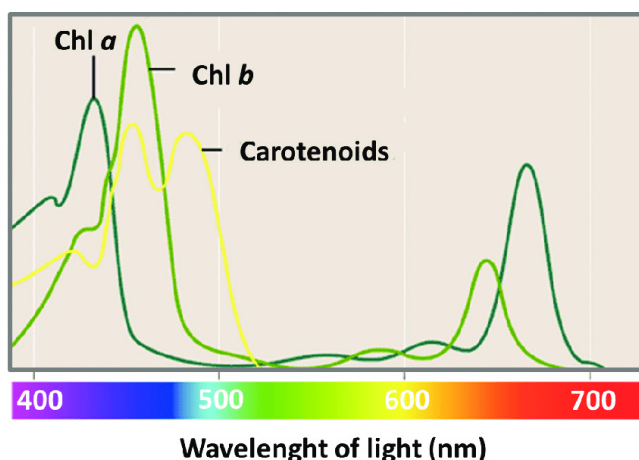


Figure 1.1 Absorption spectra of chlorophyll α , β and, carotenoids [16].

These compounds absorb mainly the visible part of the spectrum and when exposed to UV rays their structure and functionality appear altered. This may be due to different factors such as enzymes, temperature and pollutants. Chlorophyll degradation may be due to light exposure in which the energetic imputation of photons catabolizes this molecule creating photoproducts with it [20]. The use of chlorofluorocarbons has removed some of the ozone layers in the atmosphere, and thus more ultraviolet radiation (including UV- α and UV- β) is likely to enter the earth. Ultraviolet radiation can generate cell deterioration, including plant tissue that is more capable of absorbing it [21-23]. The damage caused by radiation is not only in the degradation of chlorophyll, but also in morphological changes, increase of phenolic compounds, inhibition of photosynthesis, reduction of biomass and rubisco activity, among others [24-27].

2. MATERIALS AND METHODS

The materials and reagents to be used for the extraction and measurement for the concentration of the different photosynthetic pigments are:

- 1 Mortar
- 10 ml graduated Pyrex cylinder
- 1 paper punch
- 1 Centrifuge
- 1 Visual light spectrophotometer.
- 10 2 mL Spectrophotometer cuvettes

Reactants:

- 100 mL 95% ethanol
- 10 Filter paper
- 50ml Corning tubes
- 100 2 mL microcentrifuge tubes

The methods used during this experimentation can be described in three separate parts, the extraction of the pigments, the lighting exposure and, the measurement of their absorbance.

The method used for pigment extraction is:

1. cut several circles with the whole paper punch and place them in the mortar.
2. Place 5ml of 95% ethanol.
3. Crush the leaves with the pestle and mortar for approximately 3 minutes.
4. Pass the extract through a coffee filter paper.
5. Put the extract in Corning tubes and centrifuge at 4°C with a speed of 5000xg for 15 min.
6. Place 2 ml of the supernatant in 25 microcentrifuge tubes.

Exposure time-lapses: All groups will be exposed equally to their corresponding light conditions, and the absorbance will be measured in similar time lapses (every 10 minutes, from 0 to 60 min).

The method to measure the absorbance of the extraction after exposing the extract to the corresponding light condition is:

1. Calibrate the spectrophotometer using 95% ethanol as blank.
2. Using a micropipette place the 2 ml of the extract in a cuvette of the spectrophotometer.

3. Place the cuvette inside the machine and measure the absorption of the extraction at wavelengths 649nm and, 664nm.

3. RESULTS AND DISCUSSION

The raw data for this investigation will be absorbance values of the extracts exposed to different light conditions at different time-lapses. The data will fluctuate from 0 to 1 depending on the amount of light absorbed compared to 95% ethanol, these values can be found in Table 1.1. The concentrations of the different photosynthetic pigments will be calculated using the equations proposed by Sumanta, N. et al. in the article "Spectrophotometric Analysis of Chlorophylls and Carotenoids from Commonly Grown Fern Species by Using Various Extracting Solvents" [28]. There are many equations for determining chlorophyll concentration, but the efficiency depends on the solvent used to obtain it, which in this case is ethanol. The equations in Table 1.0 are used to obtain the concentration in micrograms per milliliter ($\mu\text{gr}\cdot\text{ml}^{-1}$) of the different pigments.

Table 1.0 Equations to obtain the concentration of α and β chlorophyll.

Chlorophyl α Concentration $C\alpha = 13.36 \cdot A_{664} - 5.19 \cdot A_{649}$	Chlorophyl β Concentration $C\beta = 27.43 \cdot A_{649} - 8.12 \cdot A_{664}$
Where:	
<ul style="list-style-type: none"> - "$C\alpha$" represents the concentration ($\mu\text{gr}\cdot\text{ml}^{-1}$) of α-chlorophyll. - "$C\beta$" represents the concentration ($\mu\text{gr}\cdot\text{ml}^{-1}$) of β-chlorophyll. - "A_{649}" represents the absorbance at 649 nm. - "A_{664}" represents the absorbance at 664 nm. 	

Table 1.1 Light absorption at 664 and 649 nm lengths of α and β chlorophyll exposed to different light conditions ± 0.001 and repetition (1 to 5).

Time (Min)	Rep.	UV- β (305 nm)		UV- α (364 nm)		Natural Room Light		No Light	
		664 nm	649 nm	664 nm	649 nm	664 nm	649 nm	664 nm	649 nm
0	1	0.854	0.625	0.674	0.544	0.940	0.721	0.902	0.689
	2	0.849	0.625	0.674	0.544	0.931	0.712	0.899	0.688
	3	0.849	0.624	0.740	0.549	0.934	0.719	0.898	0.685
	4	0.861	0.632	0.674	0.549	0.936	0.723	0.900	0.687
	5	0.861	0.633	0.674	0.550	0.924	0.716	0.900	0.685
10	1	0.852	0.618	0.660	0.524	0.910	0.704	0.881	0.682
	2	0.853	0.616	0.667	0.523	0.909	0.709	0.886	0.689
	3	0.850	0.617	0.667	0.524	0.916	0.710	0.882	0.697
	4	0.850	0.617	0.656	0.524	0.916	0.711	0.877	0.681

	5	0.854	0.619	0.673	0.525	0.909	0.701	0.883	0.681
20	1	0.818	0.605	0.656	0.540	0.912	0.706	0.892	0.676
	2	0.818	0.605	0.655	0.521	0.914	0.706	0.887	0.676
	3	0.818	0.605	0.655	0.543	0.914	0.700	0.892	0.676
	4	0.820	0.607	0.648	0.543	0.914	0.710	0.893	0.674
	5	0.823	0.608	0.662	0.525	0.911	0.696	0.898	0.678
30	1	0.785	0.573	0.631	0.514	0.902	0.691	0.885	0.672
	2	0.774	0.570	0.637	0.514	0.907	0.692	0.884	0.669
	3	0.789	0.583	0.647	0.521	0.886	0.693	0.886	0.670
	4	0.788	0.581	0.619	0.507	0.903	0.692	0.880	0.674
	5	0.767	0.568	0.645	0.527	0.896	0.691	0.881	0.676
40	1	0.755	0.549	0.516	0.505	0.883	0.697	0.868	0.666
	2	0.753	0.547	0.603	0.494	0.896	0.680	0.882	0.684
	3	0.754	0.556	0.568	0.497	0.889	0.688	0.882	0.683
	4	0.752	0.553	0.667	0.486	0.877	0.697	0.868	0.695
	5	0.754	0.554	0.656	0.493	0.894	0.685	0.868	0.673
50	1	0.718	0.506	0.583	0.487	0.880	0.684	0.870	0.676
	2	0.712	0.510	0.609	0.514	0.882	0.686	0.864	0.682
	3	0.727	0.513	0.574	0.476	0.890	0.682	0.865	0.679
	4	0.720	0.506	0.574	0.488	0.885	0.680	0.872	0.684
	5	0.714	0.510	0.574	0.471	0.880	0.685	0.870	0.670
60	1	0.700	0.504	0.560	0.470	0.875	0.678	0.860	0.670
	2	0.721	0.508	0.562	0.473	0.870	0.680	0.864	0.673
	3	0.705	0.501	0.561	0.468	0.880	0.682	0.868	0.668
	4	0.728	0.506	0.559	0.472	0.883	0.676	0.859	0.675
	5	0.718	0.503	0.554	0.469	0.880	0.680	0.861	0.665

Table 1.2 α -Chlorophyll and β -chlorophyll concentration as a function of light conditions $\pm 0.02 \mu\text{g}\cdot\text{ml}^{-1}$.

Time (Min)	Rep.	UV- β (305 nm)		UV- α (364 nm)		Natural Room Light		No light	
		C α	C β	C α	C β	C α	C β	C α	C β
0	1	8.17	10.2	6.18	9.45	8.82	12.1	8.47	11.6
	2	8.10	10.2	6.18	9.45	8.74	12.0	8.44	11.6

	3	8.10	10.2	7.04	9.05	8.75	12.1	8.44	11.5
	4	8.22	10.3	6.16	9.59	8.75	12.2	8.46	11.5
	5	8.22	10.4	6.15	9.61	8.63	12.1	8.47	11.5
10	1	8.18	10.0	6.10	9.01	8.50	11.9	8.23	11.6
	2	8.20	10.0	6.20	8.93	8.46	12.1	8.26	11.7
	3	8.15	10.0	6.19	8.96	8.55	12.0	8.17	12.0
	4	8.15	10.0	6.04	9.05	8.55	12.1	8.18	11.6
	5	8.20	10.0	6.27	8.94	8.51	11.8	8.26	11.5
20	1	7.79	9.95	5.96	9.49	8.52	12.0	8.41	11.3
	2	7.79	9.95	6.05	8.97	8.55	11.9	8.34	11.3
	3	7.79	9.95	5.93	9.58	8.58	11.8	8.41	11.3
	4	7.80	9.99	5.84	9.63	8.53	12.1	8.43	11.2
	5	7.84	9.99	6.12	9.03	8.56	11.7	8.48	11.3
30	1	7.51	9.34	5.76	8.98	8.46	11.6	8.34	11.2
	2	7.38	9.35	5.84	8.93	8.53	11.6	8.34	11.2
	3	7.52	9.59	5.94	9.04	8.24	11.8	8.36	11.2
	4	7.51	9.54	5.64	8.88	8.47	11.6	8.26	11.3
	5	7.30	9.35	5.88	9.22	8.38	11.7	8.26	11.4
40	1	7.24	8.93	4.27	9.66	8.18	11.9	8.14	11.2
	2	7.22	8.89	5.49	8.65	8.44	11.4	8.23	11.6
	3	7.19	9.13	5.01	9.02	8.31	11.7	8.24	11.6
	4	7.18	9.06	6.39	7.91	8.10	12.0	7.99	12.0
	5	7.20	9.07	6.21	8.20	8.39	11.5	8.10	11.4
50	1	6.97	8.05	5.26	8.62	8.21	11.6	8.11	11.5
	2	6.87	8.21	5.47	9.15	8.22	11.7	8.00	11.7
	3	7.05	8.17	5.20	8.40	8.35	11.5	8.03	11.6
	4	6.99	8.03	5.14	8.72	8.29	11.5	8.10	11.7
	5	6.89	8.19	5.22	8.26	8.20	11.6	8.15	11.3
60	1	6.74	8.14	5.04	8.34	8.17	11.5	8.01	11.4
	2	7.00	8.08	5.05	8.41	8.09	11.6	8.05	11.4
	3	6.82	8.02	5.07	8.28	8.22	11.6	8.13	11.3
	4	7.10	7.97	5.02	8.41	8.29	11.4	7.97	11.5
	5	6.98	7.97	4.97	8.37	8.23	11.5	8.05	11.2

Another method of processing the data is to average the different trails for the same light conditions and different time lapses. This will be used to compare between the different groups with values that

best represent the different paths taken. This formula can be seen in the formulas below. The error bars of the group mean calculations will be calculated using the standard deviation; this can show the variation between the mean of the values and each value. By this analysis it is easier to understand how the values are spread between the same groups, the formula is seen below.

Table 1.3 Mean chlorophyll concentration as a function of exposure time to different lighting conditions.

Light Type	UV- β (305 nm)		UV- α (364 nm)		Natural Room Light		No light	
	C α	C β	C α	C β	C α	C β	C α	C β
Exposure Time (Min)								
0	8.16	10.3	6.34	9.43	8.74	12.1	8.46	11.5
10	8.18	10.0	6.16	8.98	8.51	12.0	8.22	11.7
20	7.80	9.97	5.98	9.34	8.55	11.9	8.41	11.3
30	7.44	9.43	5.81	9.01	8.42	11.7	8.31	11.3
40	7.20	9.02	5.47	8.69	8.28	11.7	8.14	11.6
50	6.95	8.13	5.26	8.63	8.26	11.6	8.08	11.6
60	6.93	8.03	5.03	8.36	8.20	11.5	8.04	11.4

Table 1.4 Mean percentage difference of α and β chlorophyll concentration as a function of exposure time to different illumination conditions (%).

Light Type	UV- β (305 nm)		UV- α (364 nm)		Natural Room Light		No light	
	C α	C β	C α	C β	C α	C β	C α	C β
Expo time. (Min)								
0 a 10	0.17	-2.54	-2.86	-4.80	-2.55	-1.13	-2.79	1.08
10 a 20	-4.41	-3.02	-5.69	-0.97	-2.19	-1.96	-0.51	-2.05
20 a 30	-8.79	-8.23	-8.32	-4.48	-3.66	-3.68	-1.73	-2.30
30 a 40	-11.7	-12.3	-13.7	-7.85	-5.20	-3.49	-3.73	0.27
40 a 50	-14.8	-20.9	-17.1	-8.46	-5.52	-4.55	-4.46	0.18
50 a 60	-15.1	-21.8	-20.7	-11.3	-6.15	-5.11	-4.89	-1.31

With the formulas already shown, the values of chlorophyll concentration and mean chlorophyll concentration as a function of illumination conditions and exposure time can be calculated. These values are shown in Figures 1.2 and 1.3. As the different samples had different initial concentrations, it is impossible to directly compare the amount of chlorophyll decrease would only be related to the initial amount, instead, comparing the % change seems a better way to observe how the illumination conditions and time affect the concentration.

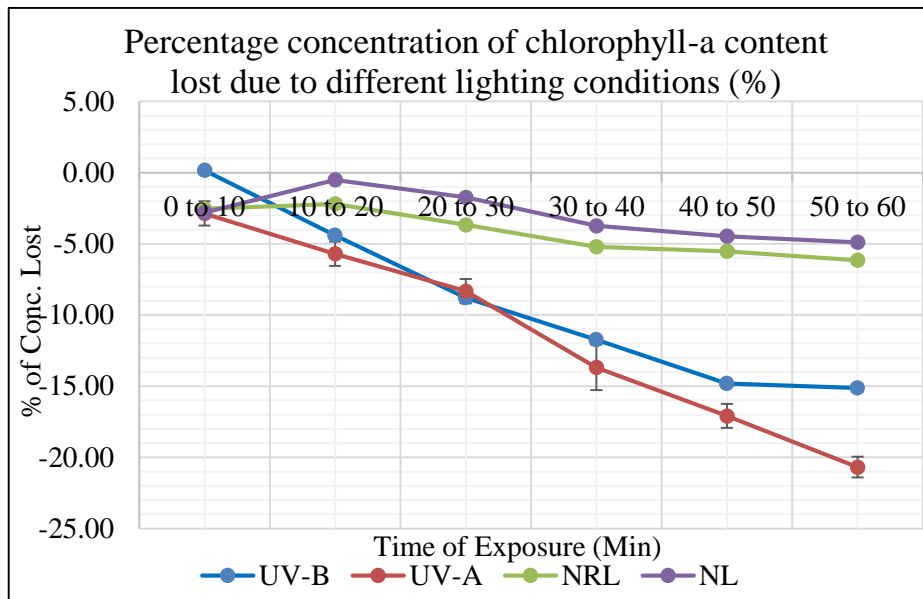


Figure 1.1 Percentage concentration lost under different illumination conditions (%) – α -Chlorophyll.

Following the analysis of Table 1.3 and Figure 1.1, a decrease in the concentration of photosynthetic content is observed after exposure to different illumination conditions, and it is observed that UV- α light degrades chlorophyll- α more than any of the other conditions. After 60 min of exposure, UV- α light decreased pigment concentration by 20.7%, while UV- β light decreased it by 15.1% (5.6% less); natural illumination decreased total content by 6.15% and no illumination by 4.89%. The two UV lights (305nm & 364nm) were the lighting conditions with the highest degraded α -chlorophyll content. After comparing the results obtained from the experiment with the literature it was found that they were similarities as Petrović, S. mentioned that chlorophyll underwent a 50% decrease in 30 min (when exposed to UV light) and in 390 min (in white light) [20]. The idea that UVR lights are more destructive to chlorophyll- α is supported by the literature. UV- α light showed a greater decrease in chlorophyll- α content than UV- β light, being 5.6% slower in inducing catabolism of this pigment. This difference between the degradation rate is observed after making the linear correlation between degradation and time; This is supported by Table 1.5, having a higher constant in the UV- α condition (3.675) compared to its degradation in UV- β (3.1618). The data suggest a greater influence of this type of light on the degradation of chlorophyll α . After looking at the literature, it was found that the greater decrease in chlorophyll- α content by UV- α light is a similar result that Zvezdanović, J. had when scalded pigments, she noted that UV- α light reduced the initial amount of chlorophyll more even when compared to UV- β [29].

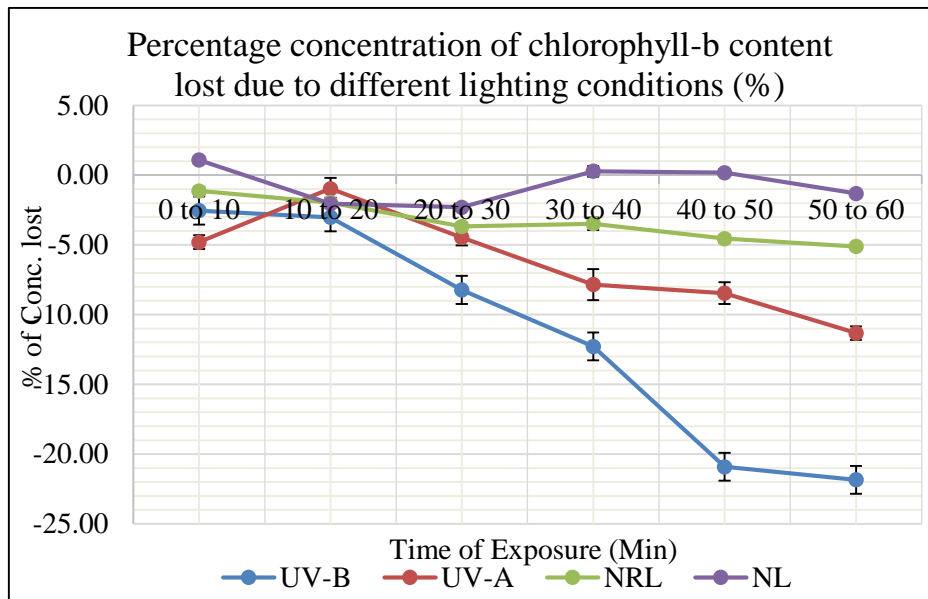


Figure 1.2 Percentage concentration lost in different illumination conditions (%) – β -chlorophyll.

Another process to be performed is linear regression to obtain the relationship and the intensity of this relationship between the different variables.

After studying the Table 1.3 and Figure 1.2 of the degradation of β -chlorophyll, it was observed that UV- β light was the illumination condition that decreased the concentration of this pigment the fastest, decreasing the original concentration by 21.8%. The next illumination condition with the greatest decrease in the concentration of this pigment was UV- α light with 11.3% (10.5% less); ambient illumination decreased the concentration by 5.11% and no illumination by 1.31%. Comparing temporal continuity and degradation by visible light it can be said that β -chlorophyll seems less affected by these factors than β -chlorophyll. The two UV lighting conditions showed the greatest decrease in pigment concentration, this can be compared with the results reported by Hediya M.H. Salama in which all plants exposed to UV lights showed lower chlorophyll concentration [30]. In contrast to the degradation of α -chlorophyll, the concentration of β -chlorophyll was reduced to a greater extent in UV- β exposure, with 10.5% being the difference between these two. Another comparison of this degradation can be made by studying the correlation in which the slope of the linear regression of the different lights showed that UV- β has a higher rate, being approximately 2.6 times higher reduction by exposure than that caused by UV- α .

Table 1.5 Regression equation and regression coefficient of the mean percent concentration functions lost during exposure under different light conditions.

Type	Regression Equation		Regression Coef.	
	C α	C β	C α	C β
UV- β	$y = -3.1618x + 1.9497$	$y = -4.4064x + 3.9522$	0.951	0.9466
UV- α	$y = -3.675x + 1.4744$	$y = -1.6696x - 0.4687$	0.9907	0.7362
NRL	$y = -0.8446x - 1.2555$	$y = -0.7856x - 0.5713$	0.9167	0.9362
NL	$y = -0.6959x - 0.5834$	$y = -0.0771x - 0.4192$	0.5996	0.0108

For all lighting conditions and chlorophyll type (α and β), a decrease in concentration was observed with increasing exposure time. This was identified by observing the graphs in which a constant decrease is presented in the different groups. The rate of decrease varied as a function of time and illumination conditions, as UV- α and β showed greater changes in concentration. After looking at the literature, this result was supported, since it was found that, as reported by Hollósy in 2002, UV- β radiation resulted in a greater reduction in the amount of β -Chlorophyll compared to α -Chlorophyll, suggesting that Chlorophyll- β might have a more selective destruction [31].

4. CONCLUSION

After the analysis performed in the discussion, it was found that there was a moderate relationship between wavelength and pigment degradation, as UV- α and β lights showed higher reduction than normal and no lighting conditions. This relationship is not strong since, UV- α light showed greater effects on chlorophyll- α even that has a longer wavelength than UV- β . In chlorophyll- β , the relationship between wavelength and degradation seems stronger as UV- β light shows a greater decrease in concentration. The degradation of these two molecules is going to depend on several factors, the molecule itself and especially the photon energy. A general decrease in degradation was observed when light intensity decreases. A general growth of degradation was observed in all groups when the exposure time was increased, this is due to the interaction of photons and pigments. The increased interaction caused a greater decomposition of the molecules, decreasing the concentration of the molecules.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The author confirms that the data supporting the findings of this research are available within the

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CONFLICT OF INTEREST

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