Observation of Paraquat Toxicity Lab using Oxidative stress MDA indicator

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Introduction

The herbicide Paraquat, also referred to as 1,1'-dimethyl-4,4'-bipyridylium dichloride and can exhibit profound toxic effects to both plants and primarily lung tissue in mammals in its free radical form after reducing an electron to form a more stable, water-soluble species. (Chia et al, 1982). This radical reacts with Oxygen producing 02- causing lipid peroxidation in the membrane of a cell which is where the degenerative and lethal affects occur with cell contents spilling out (Chia et al, 1982). The purpose of this lab is to investigate the effects that the herbicide Paraquat has on the bean plant species Phaseolus vulgaris through the observation of protein concentration resulting from this spillage from the degraded membranes. This method will be conducted using BSA solution and Malondialdehyde (MDA) which are important indicator molecules to determine the extent of lipid peroxidation stress on the Chloroplast tissue (Hassan et al, 1978).

Materials and Methods

- Please refer to Dr. C. Duxbury, Department of Biology, Spring 2021, Biology 354 Environmental Toxicology, Experiment 4 *TOXICITY OF PARAQUAT*., pp. 1-8, for a full list of the materials and methods conducted in the lab. No deviations were made from the original experiment (Duxbury, C., 2021).

Results

Table 1: Section 1 Class result for Chlorophyl concentrations Obtained from Raw Absorbance in Controlled group.

Control	Weight (g)	700nm	663nm	645nm	[Chl. A] (µg/mL)	[Chl. B] (µg/mL)	Chl. A:B	[Total Chl.] (mg/g)	Dilution
1	30.00	0.011	0.197	0.083	2.169	0.778	2.786:1	2.946	10
2	30.20	0.045	0.323	0.134	3.291	0.737	4.465:1	4.027	10
3	30.20	0.005	0.272	0.102	3.130	0.972	3.221:1	4.101	10
4	30.00	0.026	0.314	0.14	3.351	1.263	2.654:1	4.613	10
5	30.00	0.011	0.422	0.173	4.784	1.786	2.678:1	6.569	50
6	30.00	0.011	0.302	0.123	3.394	1.203	2.822:1	4.596	10
7	30.10	0.006	0.226	0.093	2.560	0.963	2.659:1	3.522	10
8	31.60	0.009	0.435	0.116	5.122	0.457	11.218:1	5.578	10
Avg.	30.26	0.02	0.31	0.12	3.48	1.02	4.06	4.49	15.00
Std Dev.	0.512	0.013	0.079	0.027	0.945	0.378	2.764	1.073	13.229

Calculations for concentration values can be found in sample calculation section in results.
Standard deviation was calculated using excel function for a sample population.

Table 2: Section 1 Class result for Chlorophyl concentrations Obtained from Raw Absorbance in Treated group.

Treated	Weight (g)	700nm	663nm	645nm	[Chl. A] (µg/mL)	[Chl. B] (µg/mL)	Chl. A:B	[Total Chl.] (mg/g)	Dilution
1	30.60	0.003	0.187	0.074	2.146	0.765	2.806:1	2.910	10
2	30.20	0.008	0.182	0.074	2.032	0.697	2.915:1	2.729	50
3	30.00	0.017	0.278	0.122	3.032	1.183	2.563:1	4.214	10
4	29.70	0.029	0.238	0.105	2.450	0.762	3.214:1	3.211	10
5	30.00	0.014	0.296	0.12	3.296	1.108	2.976:1	4.403	10
6	30.20	0.014	0.237	0.1	2.601	0.926	2.809:1	3.526	50
7	30.00	0.014	0.222	0.093	2.429	0.836	2.907:1	3.264	10
8	30.10	0.012	0.286	0.117	3.197	1.122	2.849:1	4.318	10
Avg.	30.10	0.01	0.24	0.10	2.65	0.92	2.88	3.57	20.00
Std Dev.	0.240	0.007	0.041	0.018	0.446	0.177	0.171	0.616	17.321

Table 3: **Raw Absorbance readings at 595nm of Various BSA standard Concentrations**

Tube Number	Volume of standard added (mL)	Absorbance at 595nm	Concentration (mg/mL)
Blank	0	0	0
1	0.20	0.173	0.1

2	0.40	0.194	0.2
3	0.60	0.474	0.3
4	0.50	0.599	0.4
5	1.0	0.918	0.5
6	1.4	1.248	0.7

Concentration of BSA was calculated by taking the volume of 1.0 mg/mL BSA solution and adding a final volume of 2mL of distilled water to achieve final concentration value. The following information is relevant for the next plot, *Figure 1*.

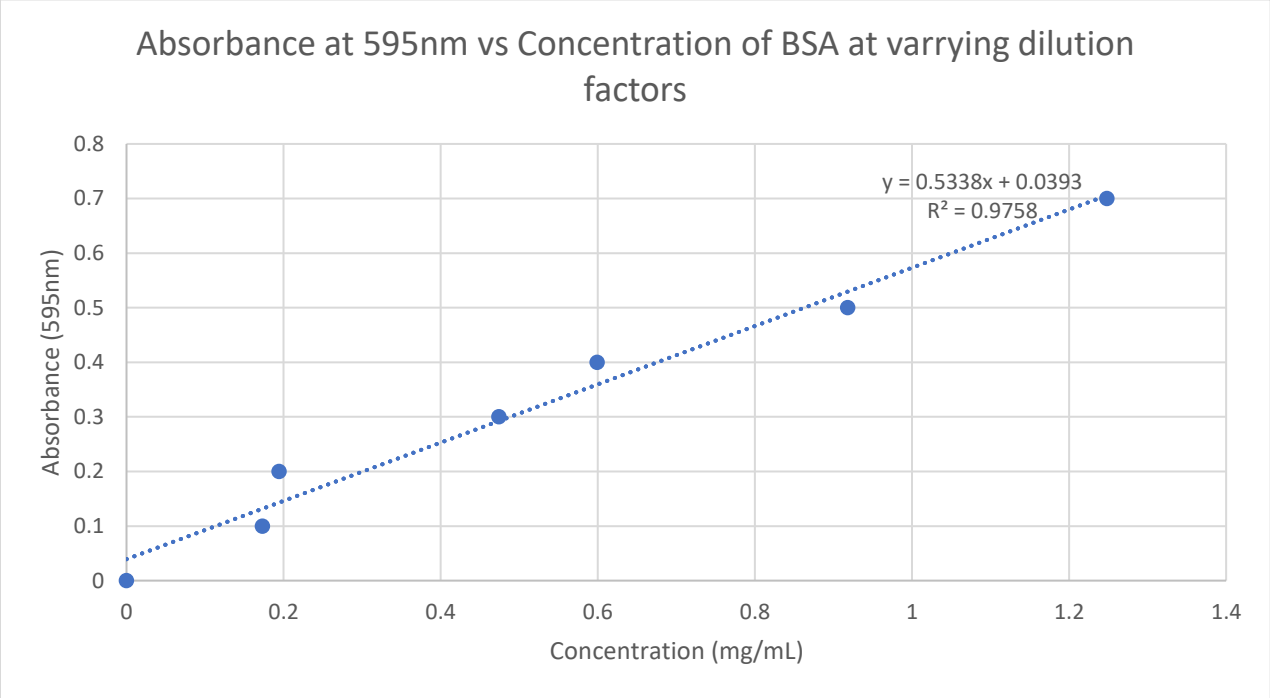

Figure 1: Standard Curve of BSA concentration. Absorbance is plotted on the Y axis and Concentration is plotted on the X axis in mg/mL with a positive correlation of increasing concentration and absorbance.

Table 4: Protein Concentration of Control and Treated sample

Sample	Control	[Protein] (mg/ml)	Treated	[Protein] (mg/ml)
1	0.117	2.93	0.32	6.73
2	0.459	9.33	0.892	17.45
3	0.423	8.66	0.273	5.85
4	0.37	7.67	0.276	5.91
5	0.708	14.00	0.338	7.07
6	0.183	4.16	0.192	4.33
7	0.157	3.68	0.201	4.50
8	0.329	6.90	0.508	10.25

Avg.	0.343	7.167	0.375	7.761
Std Dev.	0.182	3.411	0.216	4.047

Table 5: Table of control and treated MDA samples

Sample	**Control MDA Abs**	**[Control MDA] (nMol/mg)**	**Treated MDA Abs**	**[Treated MDA] (nMol/mg)**
1	0.235	4.39	0.505	4.11
2	0.395	2.32	0.359	1.13
3	0.513	3.25	0.437	4.09
4	0.631	4.51	0.572	5.30
5	0.63	2.47	0.465	3.60
6	0.399	5.25	0.633	8.01
7	0.385	5.73	0.748	9.11
8	0.471	3.74	0.645	3.45
Avg.	0.457	3.957	0.546	4.850
Std Dev.	0.125	1.161	0.119	2.419
T-test	-	0.1475	-	0.1475

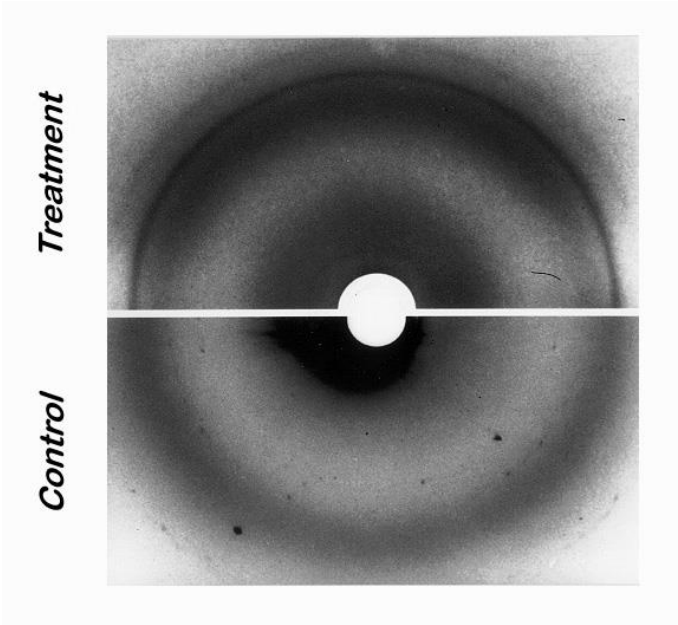

Figure 2: X-Ray Diffraction of Control and Treated Chloroplast Membrane Samples. Greater darker banding patterns on the outer membrane surface of the treated sample.

Figure 3: Control (untreated) P. vulgaris. Plant looks to be in healthy condition with bright green leaf indicating a healthy plant.

Figure 4: Paraquat treated P. vulgaris. Plant condition looks unwell with white discoloration and wilted leaves, less green pigmentation on the leaves compared to untreated sample. This damage does appear to be indicative of the mode of action of paraquat.

Sample Calculations:

Table 1: Control

A_{645}= Absorbance at 645nm – Absorbance at 700nm

A645=0.083 -0.011 = 0.072

A_{663}= Absorbance at 663nm – Absorbance at 700nm

A663=0.197 – 0.011 = 0.186

[Chlorophyl A] (in ug/mL) = (12.7 x A663) – (2.69 * A645)

= (12.7 * 0.186) – (2.69 * 0.072)

= 2.16852 ug/mL

[Chlorophyl B] (in ug/mL) = (22.9 * A645) – (4.68 * A663)

= (22.9 * 0.72) – (4.68 * 0.186)

= 0.77832 ug/mL

Control Ratio A:B = 2.169/0.778 = 2.786 : 1 ratio

[Total Chl.] in (ug/mL) = (20.2 *A645) – (8.02 *A663)

= (20.2 * .072) – (8.02 *0.186)

= 2.946 ug/mL

Average = 30.60+30.20+30.00+29.70+30.00+30.20+30.00+30.10 / 8 = 30.26

Std Dev. = $\sigma = \sqrt{\frac{\sum(x_i - \mu)^2}{N}}$ = 4.49 (total [Chl.])

Table 2: Treated

A_{645}= Absorbance at 645nm – Absorbance at 700nm

A645=0.074 -0.003 = 0.071

A_{663}= Absorbance at 663nm – Absorbance at 700nm

A663=0.187 – 0.003 = 0.184

[Chlorophyl A] (in ug/mL) = (12.7 x A663) – (2.69 * A645)

= (12.7 * 0.071) – (2.69 * 0.184)

= 2.146 ug/mL

[Chlorophyl B] (in ug/mL) = (22.9 * A645) – (4.68 * A663)

= (22.9 * 0.184) – (4.68 * 0.071)

= 0.765 ug/mL

Control Ratio A:B = 2.146/0.765 = 2.806 : 1 ratio

[Total Chl.] in (ug/mL) = (20.2 *A645) – (8.02 *A663)

= (20.2 * 0.184) – (8.02 *0.003)

= 2.910 ug/mL

Table 3: BSA concentrations

C1V1=C2V2

(1.0mg/mL)(0.2mL)= C2 (2mL)

C2= .1 mg/mL

Table 4: Protein Concentration of Control and Treated sample

Y= 0.5338x + 0.0393

C = Absorbance at 595nm + 0.0393 / 0.5338 = 0.235

Table 5: MDA calculation

0.235 / 1.56x10^{5}M-1cm-1)/1.17cm X (3.5/.5) = 1.29x10-5mol/L → Convert to nMol/L
10^9/1000→ 12.87 nMol/L → Convert to nMol/mg /2.93 → = 4.39 nMol/mg

Total Chl. Fresh Weight= [Total Chl .] * V acetone V sample

Total Chl. Fresh Weight= 1.005 μg mL * 6mL * 1mg 1000 μg 0.05mL* 30g FW/30mL

Total Chl. Fresh Weight=0.125mg/g

Discussion:

The goal of this lab was achieved in observing the toxic effects of paraquat on the bean plant species through different means of stress testing. The first part of the lab was observing Chlorophyl A and B production through the use of a spec 20 device, as Chlorophyl A absorbs maximally at 663 nm and Chlorophyll B absorbing maximally around 645nm (Chia et al, 1981). In the untreated control group, the average Chlorophyll A and B formation were 3.48 mg/ml and 1.02 mg/ml respectively while the total Chlorophyll Concentration is 4.49 mg/ml. And in the treated group, the average Chlorophyll A and B formation were 2.65 mg/ml and 0.92 mg/ml and the total Chlorophyll Concentration is 3.57 mg/ml. This trend is similar across all aspects of the data in Tables 1 and 2 as the paraquat toxicity does indeed confirm its site and mode of action in targeting the chloroplast of the cell and affecting the photosynthetic membrane, Photosystem I (Fuerst et al, 1990).

Lipid Peroxidation was analyzed in table 3 using varying concentrations of BSA, the result of MDA formation will be indicative of the catalytic effects of paraquat on the chloroplast membrane oxidation (Chia et al, 1981). As seen in figures 3 and 4, significant discoloration has occurred on the Phaseolus vulgaris plant leaf surface and is a result of the free radical formation

in the paraquat causing this damage (Chia et al, 1982). In the experiment the control had an average MDA concentration of 3.957 nMol/mg and 4.850 nMol/mg, this increase in MDA concentration means there is greater oxidative stress on the treated groups vs the untreated control group, further proving Paraquats toxic effects.

This also proves the data in table 4 observing the protein concentration production makes sense as with greater oxidative stress on the phospholipid membrane of the chloroplast cell, more cellular contents containing protein are able to leak out and escape into solution which should yield a greater protein concentration. This is exactly what happened as the control groups average protein concentration was 7.167 mg/ml while the treated groups protein concentration was slightly higher at 7.761 mg/ml.

Separate Questions:

1. Expressing the MDA values per g fresh weight would alter how the data is interpreted as it would provide a value of MDA that would be very small to work with that would make it difficult to asses oxidative stress so it would be difficult to meaningfully make sense of the data. Another way to express MDA would be to measure it as a means of chromatography using thiobarbituric acid which fluoresce a pink hue under low light (Moselhy, et., al., 2013).
2. Some mechanisms that plants use to gather a resistance to paraquat toxicity is by reducing the amount of the free radical form of paraquat to be up taken by the plant and into its cytoplasm or with the expression of transgenes that can co-express enzymes that can enhance the plants tolerance (Hawkes, 2014).

Work Cited:

Duxbury, C., 2021. Biol 354 Environmental Toxicology 1 Laboratory Manual. University of Waterloo, Biology Department: pp 1-8.

Chia, L.S., D.G. McRae and J.E. Thompson. 1982. Light-dependence of paraquat-initiated membrane deterioration in bean plants. Evidence for the involvement of superoxide. Physiol. Plant. 56: 492-499.

Chia, L.S., J.E. Thompson and E.B. Dumbroff. 1981. *Simulation of the effects of leaf enescence on membranes by treatment with paraquat*. Plant. Physiol. 67: 415-420.

Hassan, H.M., and I. Fridovich. 1978. *Superoxide radical and the oxygen enhancement of the toxicity of paraquat in E. coli*. J. Biol. Chem. 253: 8l43.

Hawkes T. R. (2014). *Mechanisms of resistance to paraquat in plants*. *Pest management science*, *70*(9), 1316–1323. https://doi.org/10.1002/ps.3699

Fuerst, E. Patrick, and Kevin C. Vaughn. "*Mechanisms of Paraquat Resistance*." *Weed Technology* 4, no. 1 (1990): 150–56. doi:10.1017/S0890037X0002515X.

Moselhy, F., H., Reid, G., R., Yousef, S., Boyle, P., S. 2013. A specific, accurate, and sensitive measure of total plasma malondialdehyde by HPLC. J. Lipid Res., 54(3), 852 – 858.