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CHANGES IN PIGMENTS OF THE LEAVES' DIFFERENT STAGES IN AUTUMN

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Abstract. *The goal of this paper is to research the change in pigments that exist in different species of leaves that were collected before, during and after senescence. The species of leaves that were tested were Cornus Mas, Juglans Regia, Tilia Cordata, Prunus Serrulata and Fraxinus Excelsior with four different samples of each leaf from greenest to brownest. The pigments were extracted by baking the leaves in the oven to prevent bacterial growth and to dry the samples. Next, the leaves were grinded with a mortar and pestle which is then weighed to 60 mg. A solution is made by mixing the leaves with 5 mL of 80% alcohol to extract the pigments more effectively. A spectrophotometer was used to record the wavelength absorbance, then the data was graphed to visualise the absorbance changes throughout the wavelengths. The amount of chlorophyll A and chlorophyll B was calculated using absorbance at 663 and 640 nm, and the data showed that the amount of chlorophyll A in each sample decreases as the 'brownness' of the leaves increases, from 796 to 41,33 microgram/gram. However, this was not the case for chlorophyll B. While most results had shown a decrease of chlorophyll B from 416 to 62.5 microgram/gram dm, some had varied results which did not indicate an increase or decrease. The results have concluded that the content of chlorophyll is inversely proportional to the content of anthocyanins in the leaves as senescence occurs. There were research limitations, for instance each sample of the leaves were not weighed exactly to 60 mg and were off by a few milligrams. This investigation took a few weeks to complete which may also continue the leaves' senescence causing a decrease in chlorophyll. The identification of pigments in autumn leaves is a well established area of study that has yet to be applied in the local conditions of Timisoara because environmental factors also affect leaf colour by influencing anthocyanin and chlorophyll metabolism. It is important to have more local data about plant physiology to assist those in other fields of science for research in this topic.*

Keywords*: Chlorophyll, Spectrophotometer, Pigments, Anthocyanins*

INTRODUCTION

As leaves go through senescence in autumn, their colour changes from green to red to brown. This is because the chlorophyll in leaves degrades to allow other nutrients to be absorbed and adapt to the changing environment which reveals other pigments such as carotenoids and accumulation in anthocyanins (Pei, et all). By using a spectrophotometer, the relationship and correlation of the chlorophyll and anthocyanin content can be seen. Chlorophyll mainly absorbs red and blue light (Vendatu) whilst anthocyanins absorb green light (A H Ahliha, et all). Therefore, the chlorophyll and anthocyanin content can be estimated by analysing the wavelength absorption spectrum of the leaves, where the troughs and peaks are at specific wavelengths. This study investigates the change in pigments in leaves throughout senescence and the relationship between the chlorophyll, carotenoids and anthocyanin content. These findings contribute to the research of plant physiology in Timișoara.

MATERIAL AND METHODS

- Leaf species of *Cornus Mas, Fraxinus Excelsior, Juglans Regia, Prunus Serrulata and Tilia Cordata,* analytical balance, 80% ethanol, *an* ultrasonic bath, a centrifuge and a spectrophotometer (PG instruments),

Five different species of leaves were collected for testing pigments: *Cornus Mas, Juglans Regia, Tilia Cordata, Prunus Serrulata and Fraxinus Excelsior*. The leaves were collected into four types from green to brown with the values of sample 1 being greenest, sample 2 being yellow, sample 3 being orange and sample 4 being brown.

Tilia Cordata

Fraxinus Excelsior

Prunus Serrulata

Juglans Regia

Cornus Mas

Figure 1. Species of leaves study

The leaves were ovenbaked to dry and to prevent bacterial growth. After the leaves had dried, each leaf had been grinded by a mortar and pestle and were placed into test tubes labeled by type, each sample weighing around 60 mg. 5 mL of 80% ethanol was measured and

added into each test tube which were then placed into an ultrasound bath to ensure effective extraction.

After that, the test tubes were then placed into a centrifuge to separate and obtain a clear extract to ensure accurate readings for the spectrophotometer. The spectrophotometer was the chlorophyll and carotenoid content. The formulae used to calculate these are the following

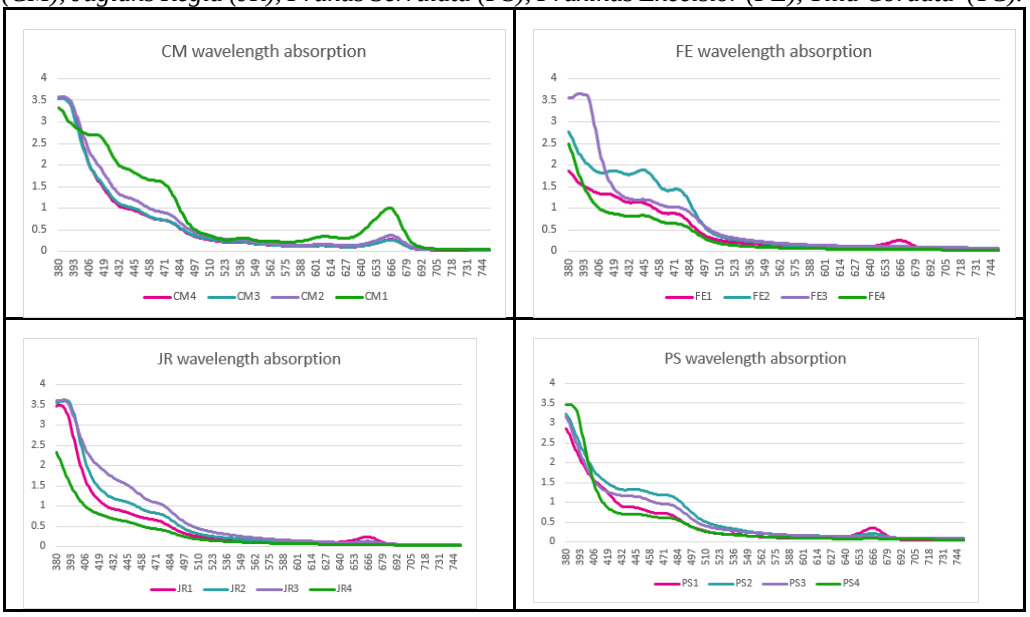
Chlorophyll a (Chl a) = 9.93 × A663 − 0.78 × A640
Chlorophyll b (Chl b) = 17.60 × A 640 − 2.81 × A663
Chlorophyll a + b = 7.12 × A663 + 16.80 × A640
Total carotene = (1000 × A470 − 0.52 × Chl a − 7.25 × Chl b)/226

Where A represents absorption and the number following it is the wavelength in nanometers.
(Mushtaq, Hamid, et al 2025)

The analysis from the spectrophotometer was graphed to indicate the absorption patterns in each sample which can show the relationship between chlorophyll and anthocyanins.

RESULTS AND DISCUSSIONS

In the following results, the leaf species will be referred to by their abbreviations *Cornus Mas (CM), Juglans Regia (JR), Prunus Serrulata (PS), Fraxinus Excelsior (FE), Tilia Cordata (TC).*



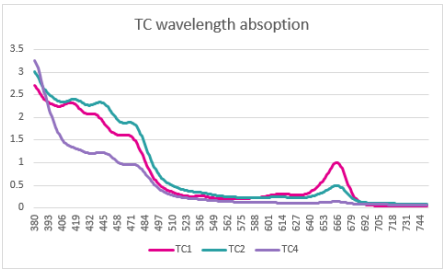


Figure 1. Spectrum of ethanolic extract

Figure 1 above shows the graphs for wavelength absorption for all the species. For all species and samples, the absorption peaks at approximately 640 nm to 680 nm which shows that the chlorophyll content in the leaves is absorbing red light. As the sample gets darker, the peaks get smaller and the amount of red light absorbed decreases which shows that the chlorophyll is degrading. *CM* and *TC* have the largest difference from its greenest and brownest samples. All graphs also peak at approximately 475 nm, where blue light is being absorbed by the chlorophyll and carotenoids. All species show that the darkest sample has the lowest amount of blue light absorbance which further shows how chlorophyll degrades as the leaves go through senescence.

The pattern of green light absorption shows that all the species have an increase as the samples get darker. This shows the increasing level of anthocyanin content as the leaves go through senescence as more green light is being absorbed. However, it is interesting to note that the final samples will have a large decrease in green light absorption. For example, Table 2 below shows the absorption for *FE* at 525 nm which shows the decrease numerically.

Table 1

Wavelength (nm)	FE 1	FE 2	FE 3	FE 4
525	0.204	0.269	0.299	0.134

Cornus Mas

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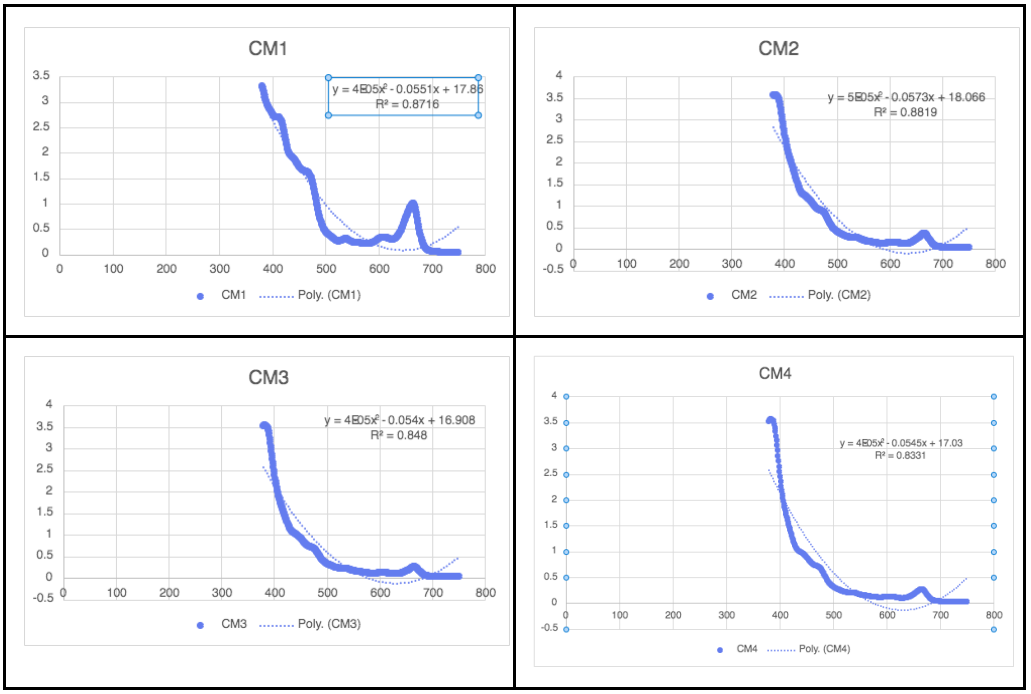
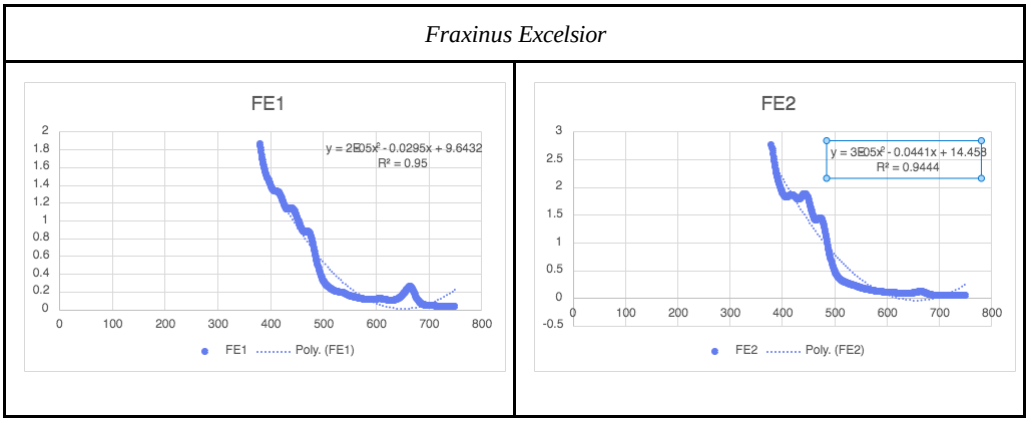


Figure 2. Spectra of Cornus Mas

Fraxinus Excelsior



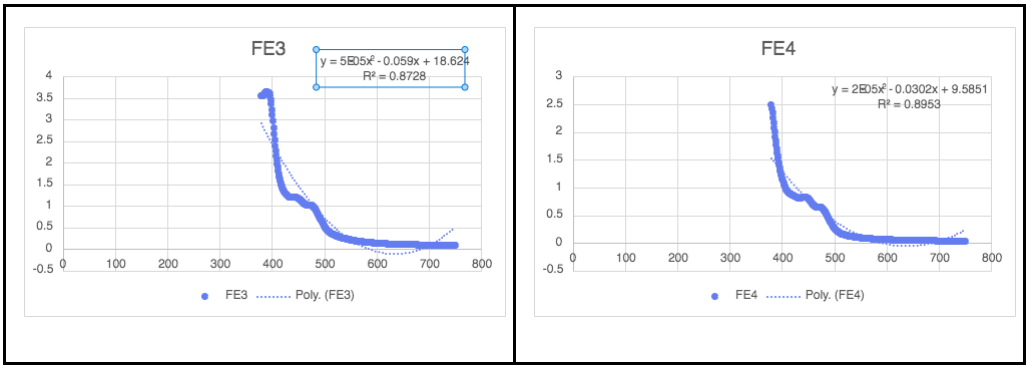


Figure 3. Spectra of Fraxinus Excelsior

Juglans Regia

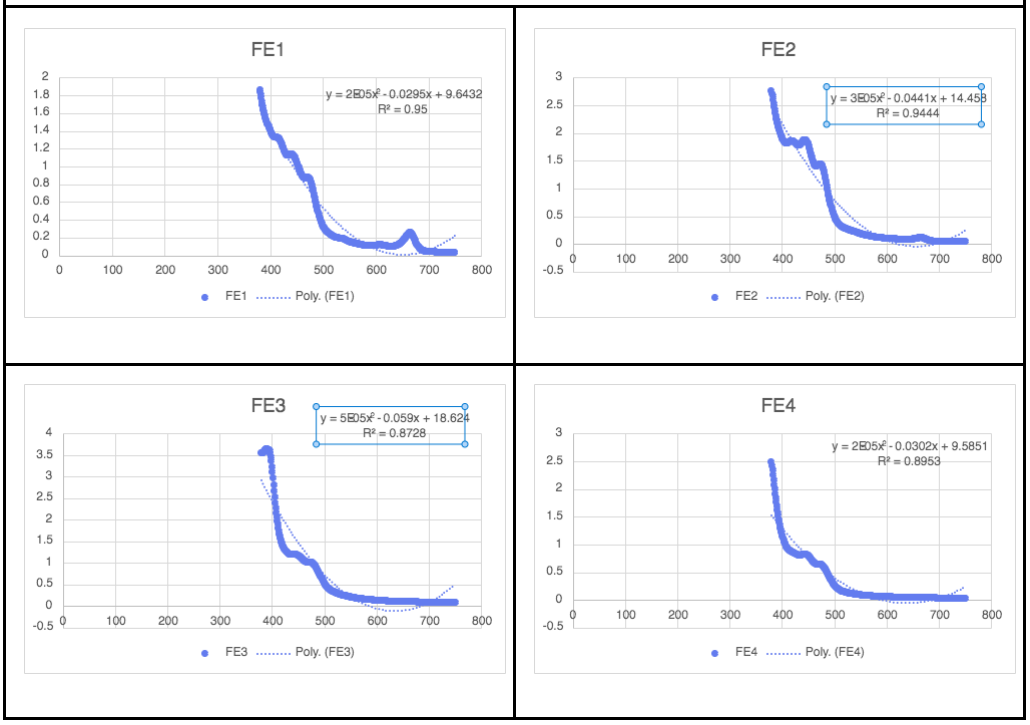


Figure 4. Spectra of Juglans Regia

Prunus Serrulata

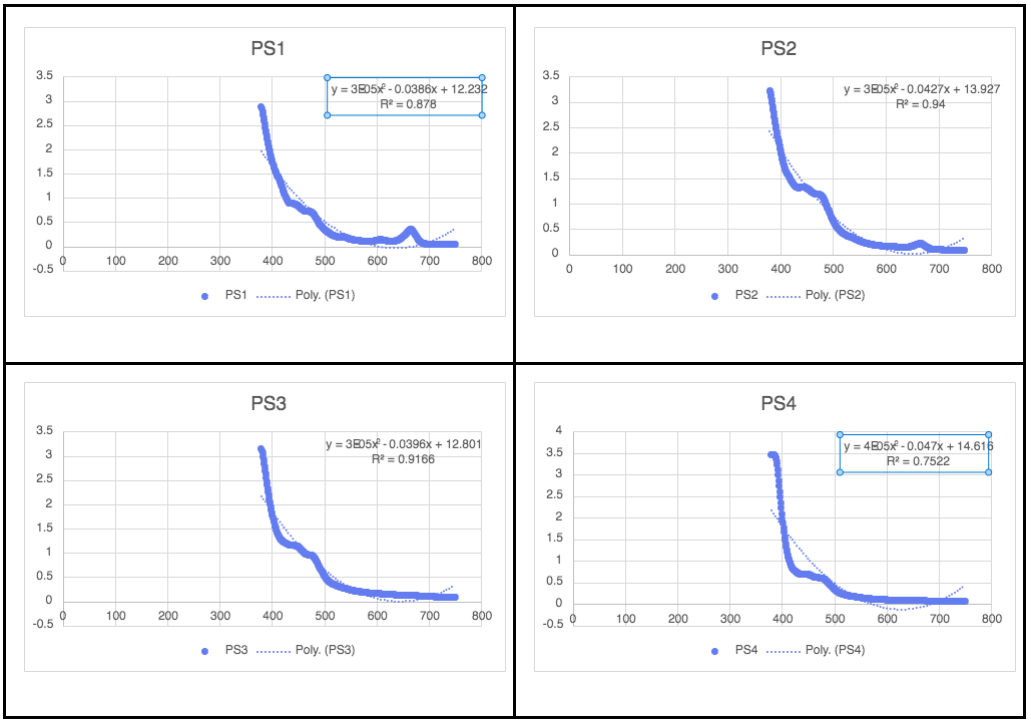
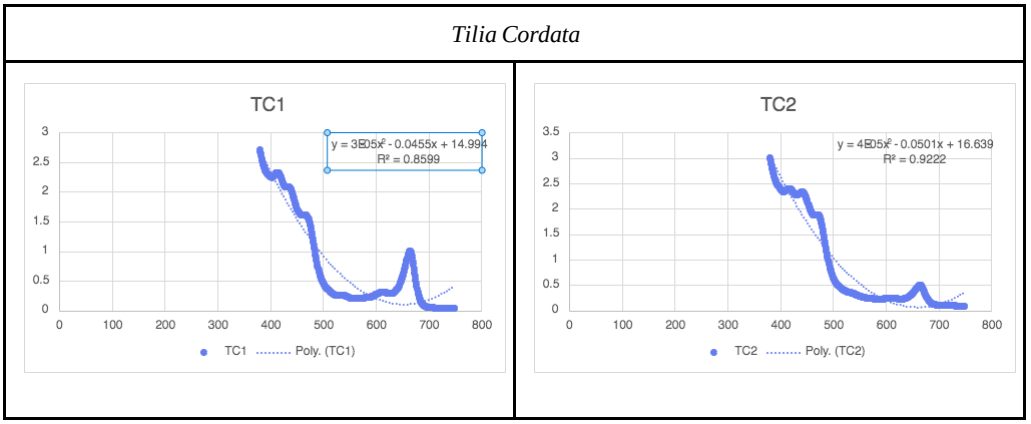


Figure 5. Spectra of Prunus Serrulata

Tilia Cordata



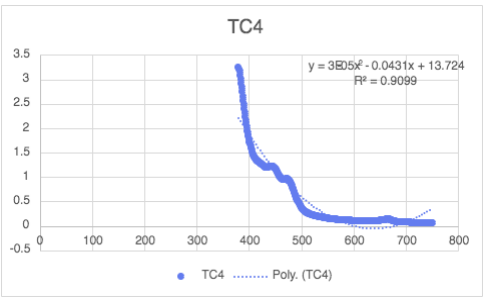


Figure 6. Spectra of Tilia Cordata

The Figures 2 to 6 show the individual samples' wavelength absorption with a regression line, the line equation and the R^2 value. In almost all the graphs, the R^2 is above 0.8 which shows that the polynomial line explains more than 80% of the wavelength absorption. The peaks and troughs of the wavelength absorptions are due to the chlorophyll and anthocyanins absorbing the blue,red and green light respectively. These graphs also show the precision of the samples that follow the same pattern consistently.

Table 2

Content of chlorophyll and carotenoids

Species	Chlorophyll A	Chlorophyll B	Chlorophyll A + B	Total Carotenoids
CM 1	9.56772	5.00982	14.48096	0.5186
CM 2	3.45993	1.83359	5.25832	0.3328
CM 3	2.51721	1.41477	3.90536	0.2709
CM4	2.5875	1.3773	3.9384	0.2698
FE 1	2.40273	1.34169	3.71912	0.3399
FE 2	1.09551	1.18423	2.26104	0.5922
FE 3	1.0431	1.70886	2.72688	0.3954
FE 4	0.49644	0.75606	1.24128	0.2609
JR 1	2.24775	1.29765	3.5212	0.2448
JR 2	2.24775	1.30509	2.51192	0.3230

JR 3	1.06083	1.52805	2.566	0.4322
JR4	0.54297	0.81321	1.34408	0.1665
PS 1	3.297	1.29958	4.56864	0.2702
PS 2	1.96539	1.92251	3.85688	0.4590
PS 3	1.22532	2.02646	3.22208	0.3578
PS 4	0.81066	1.19452	1.98736	0.2315
TC 1	9.42939	3.46683	12.8194	0.5720
TC 2	4.64247	3.04593	7.63384	0.7270
TC 4	1.30752	1.4934	2.7776	0.3761

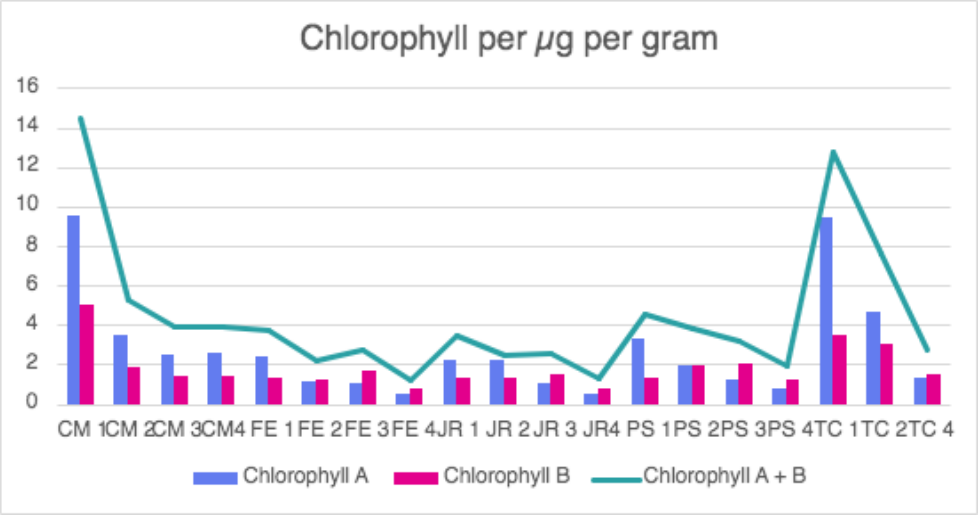


Figure 7. Chlorophyll content of leaves

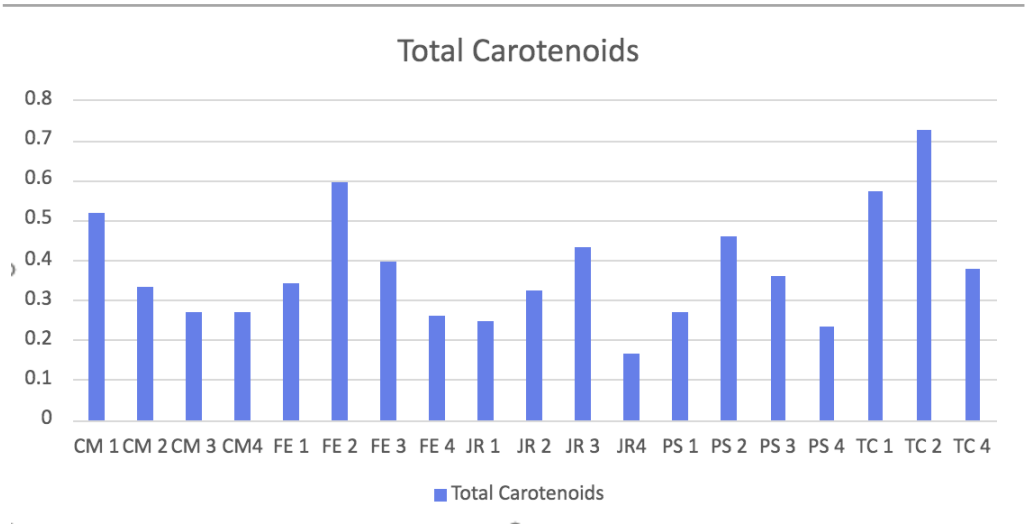


Figure 8. Total carotenoids in leaves

Figure 7 shows that the content of chlorophyll generally shows a peak at the greenest sample and decreases as the samples get browner and the chlorophyll degrades. It is interesting to see that *JR* had the smallest amount of initial chlorophyll and *TC* had the highest initial amount.

The total amount of carotenoids can show how healthy or stressed a plant is where the higher the number is the healthier the plant is. Typically, the total number of carotenoids should decrease as the leaf nears its end as it is shown in Figure 8, this only applied to CM whilst the rest of the species had its carotenoid peak at the second or third sample. This could be that the chlorophyll content hides the carotenoids in the beginning stages of the leaf's life and as the chlorophyll degrades, the carotenoids are no longer hidden. All species and samples have the carotenoid value of <1 which indicates that they have low pigment values or are older leaves because the leaves were collected on the ground after they fell.

CONCLUSIONS

The results have concluded that the content of chlorophyll is negatively correlated to the content of anthocyanins in the leaves as senescence occurs. The analysis from the spectrophoto meter shows that the blue light absorption and the green light absorption have a negative correlation as the sample gets darker. This proves that while the leaves go through senescence, the chlorophyll content decreases as the anthocyanin content increases with a negative correlation.

There were research limitations, for instance each sample of the leaves were not weighed exactly to 60 mg and were off by a few milligrams. This investigation took a few weeks to complete which may also continue the leaves' senescence causing a decrease in chlorophyll.

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