

Research Article

Effects of Air Pollution on Chlorophyll Content and Ascorbic Acid Concentration and Defence Mechanism of Different Plant Samples

Preeti R. Dwivedi*, Aashutosh O. Singh and Vikram C. Singh

Vikas Degree College of Arts, Science and Commerce, Kannamwar Nagar, Vikhroli East, Mumbai-83

Abstract

The present study tries to relate the variation in ascorbic acid content with the tolerance and sensitivity of four selected plant species viz., *Saraca asoca*, *Mangifera indica*, *Syzygium cumini* and *Arthocarpus heterophyllus* by calculating their Air Pollution Tolerance Index (APTI) during summer season from March to April in the urban city, Mumbai. In the present paper, comparative studies have also been done to find effect of pollutants on total chlorophyll content of same plant species. Photosynthetic pigments chlorophyll A and chlorophyll B were quantified. For both experiments, leaf samples of the above plant species were collected from roadside of Kannamwar police station Vikhroli east Mumbai and Vikas College Vikhroli. Reduction in chlorophyll content of plant leaves growing in roadside of Kannamwar police station Vikhroli east (polluted zone) as compared to Vikas college Vikhroli (control zone), was due to degradation of chlorophyll into phaeophytin by the loss of magnesium ions.

Keywords: Chlorophyll, Air Pollution, Photosynthetic Pigments, Total Chlorophyll content, APTI and biochemical parameters

*Correspondence

Author: Preeti R. Dwivedi
Email: dwipreeti@gmail.com

Introduction

The most important resource for sustenance of life [1] is air, but today this air has become highly polluted due to industrialization [2] and urbanisation [3] as industrial pollution takes a back seat and vehicular emissions is the major cause of urban air pollution [4]. From industries and automobile, oxides of nitrogen and sulphur and fly-ash [5] are the major proportions for the gaseous and particulate emissions. The exposure of these pollutants to the leaves cause a reduction in the concentration of their photosynthetic pigments viz., chlorophyll and carotenoids. Chlorophyll is the principal photoreceptor in photosynthesis, while carotenoid is a class of natural fat soluble pigment found principally in plants, algae and photosynthetic bacteria. With the help of air pollution tolerance index (APTI) [6], the inherent ability of plant to encounter stress arising from pollution can be analysed as it provides a reliable method for screening large number of plants with respect to their susceptibility to air pollutants. APTI is calculated by using four biochemical parameters such as ascorbic acid, chlorophyll, leaf extract pH and relative water content in leaf samples. Species having higher APTI value are more tolerant to air pollution than those having lower APTI value which may act as bio-indicators of pollution.

The main function of ascorbic acid is to work as a cofactor for enzymes involved in regulating photosynthesis, hormone biosynthesis and regenerating other antioxidants. Simultaneously, it regulates cell division and growth and is involved in signal transduction.

Materials and Methods

Sample Collection

The leaves sample of four plant species viz., *Saraca asoca*, *Syzygium cumini*, *Mangifera indica*, *Arthocarpus heterophyllus* were collected from roadside of Kannamwar nagar police station, Vikhroli East, Mumbai and Botanical garden of Vikas College campus for the study. i.e. some polluted leaves are collected from more polluted zone and some polluted leaves are collected from less polluted zone. Leaf samples were taken during the summer season (March) for analysis during the early morning and at a height of 2.5 to 3.0 meters. They were stored in a polythene bag and transferred to the laboratory for biochemical analysis.

All chemicals and reagents viz., Standard ascorbic acid, Oxalic acid, EDTA, Orthophosphoric acid, Sulphuric acid and acetone used were of the analytical grade (AR) and were purchased from Sigma Aldrich. Double distilled water was used throughout the experiment.

By spectrophotometric method, absorbance of ascorbic acid in the leaves four different plant species viz., *Saraca*

asoca, *Syzygium cumini*, *Mangifera indica*, *Artocarpus heterophyllus* of more polluted zone and less polluted zone has been measured. From the calibration curve method, concentration of ascorbic acid in the same leaves species has been calculated.

Preparation of Ascorbic acid solution

A stock solution of 1000 ppm ascorbic acid was prepared by dissolving 100mg of ascorbic acid in 100ml of double distilled water using a volumetric flask. Standard solution for calibration was prepared according to the **Table 1**. After 15 minutes, the absorbance was taken at 760 nm and with the help of $C_1V_1=C_2V_2$, the final concentration of oxalic acid was calculated (**Table 2**). Then, the standard calibration curve was plotted between absorbance versus concentration of standard ascorbic acid solution (**Figure 1**).

Table 1: Volume of solution added in cm³

1000 ppm stock solution	Oxalic acid-EDTA extract solution	Ortho-phosphoric acid	5% H ₂ SO ₄	5% Ammonium Molybdate	D/W	Total
0.1	4	1	1	2	3	11.1
0.2	4	1	1	2	3	11.2
0.3	4	1	1	2	3	11.3
0.4	4	1	1	2	3	11.4
0.5	4	1	1	2	3	11.5
0.6	4	1	1	2	3	11.6
0.7	4	1	1	2	3	11.7
0.8	4	1	1	2	3	11.8
0.9	4	1	1	2	3	11.9

Table 2: absorbance of oxalic acid at different concentration

Concentration of solution in ppm	Absorbance at 760 nm
9.01	0.068
17.86	0.204
26.54	0.326
35.09	0.487
43.48	0.662
51.72	0.826
59.82	1.034
67.79	1.228
75.63	1.428

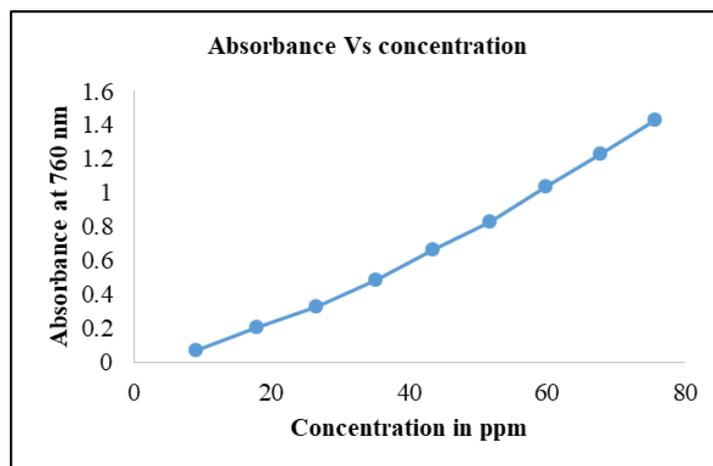


Figure 1: Standard calibration curve

Sample analysis of plant leaves

Fresh leaves of all the four plants, individually, were washed thoroughly, and blot them to dry. A weighed quantity of leaves was grinded in a mortar pestle. 1g of each of the sample were taken individually in a test tube and then add 4

ml of oxalic acid- EDTA extracting solution (0.5g of oxalic acid + 0.075g of EDTA solution in 100 ml standard measuring flask and dilute up to the mark) in each test tubes. Add 1ml of orthophosphoric acid, 1ml of 5% H₂SO₄, 2.0 ml of 5% ammonium molybdate and then add 3.0 ml of distilled water in each of the sample. Allow them to stand for 15 minutes (**Figure 2**) and take the absorbance of each of the sample at 760 nm (**Table 3 and 4**).

With the help of above graphs for all four different plant species, concentration of ascorbic acid, plotted against absorbance and concentration of unknown sample for more and less polluted areas, can be calculated via calibration curve method. The concentration of ascorbic acid in plant species are given in **Table 5**. Variations in physiological characteristics of selected plant species exposed to vehicular smoke, dust pollutants are given below.

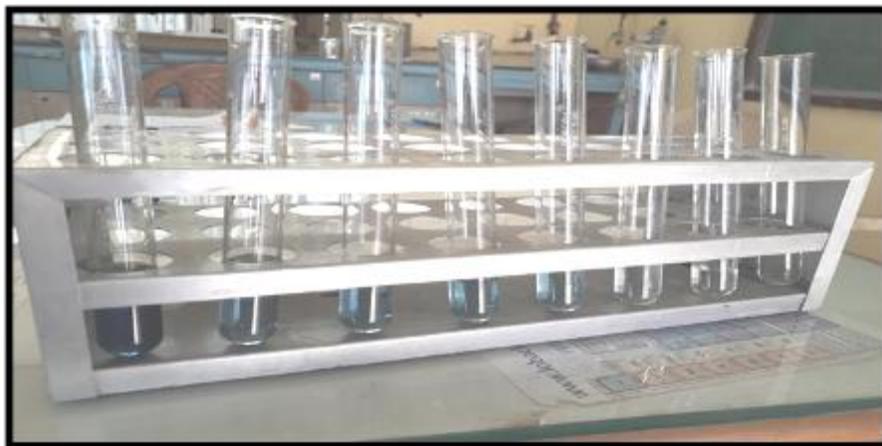


Figure 2: Different concentration of ascorbic acid solution

Table 3: Absorbance of oxalic acid for more polluted leaves.

Species of leaves	Absorbance at 760 nm
Saraca asoca (Ashoka)	0.452
Mangifera indica (Mango)	1.164
Syzygium cumini (Blueberry)	0.220
Artocarpus heterophyllus (Jackfruit)	0.692

Table 4: Absorbance of oxalic acid for less polluted leaves

Species of leaves	Absorbance at 760 nm
Saraca asoca (Ashoka)	0.085
Mangifera indica (Mango)	0.204
Syzygium cumini (Blueberry)	0.048
Artocarpus heterophyllus (Jackfruit)	0.074

Method for estimation of total chlorophyll content: Preparation of sample

1 gm of fresh leaves of each four plants was mined with 10 ml of distilled water and crushed using mortar and pestle. 0.5 ml of aliquot of each sample were mixed with 4.5 ml of 80% acetone and left for 15 minutes for extraction. The liquid portion was decanted into another test tube and centrifuged. The supernatant was collected to measure the absorbance of leaf extract of each plant sample individually at 645 nm and 663 nm for chlorophyll A and chlorophyll B using U.V-Visible spectrophotometer. The total chlorophyll content ($\mu\text{g/ml}$) was calculated by using Arnon equation for 80% acetone from chlorophyll A and chlorophyll B:

$$\text{Chlorophyll A } (\mu\text{g/ml}) = 12.7 (A_{663}) - 2.69 (A_{645})$$

$$\text{Chlorophyll B } (\mu\text{g/ml}) = 22.9 (A_{645}) - 4.68 (A_{663})$$

$$\text{Total chlorophyll content } (\mu\text{g/ml}) = 20.2 (A_{645}) + 8.02 (A_{663})$$

The data obtained for all four plants species at polluted and non-polluted zone and at absorbance 645 nm and 663 nm were summarised in the following **Tables 6 and 7**.

More Polluted leaves

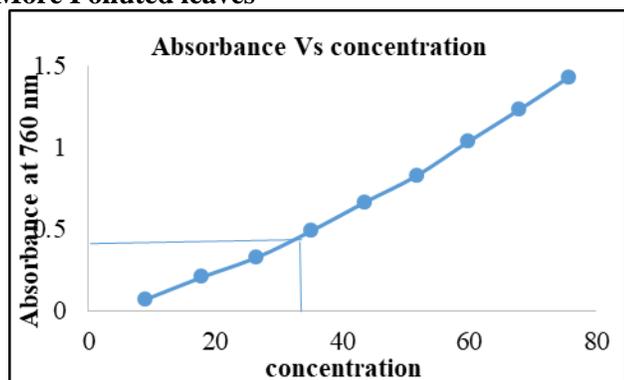


Figure3: Concentration of ascorbic acid in *Saraca asoca*

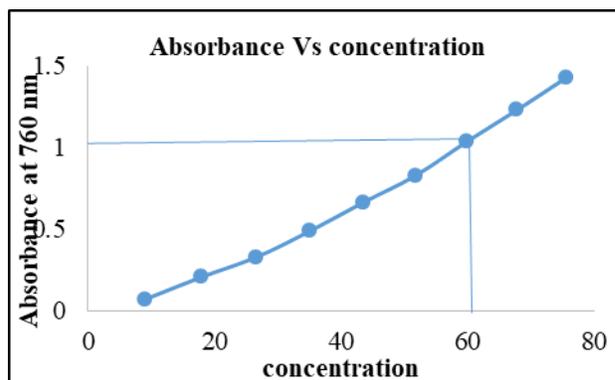


Figure 4: Concentration of ascorbic acid in *Mangifera indica*

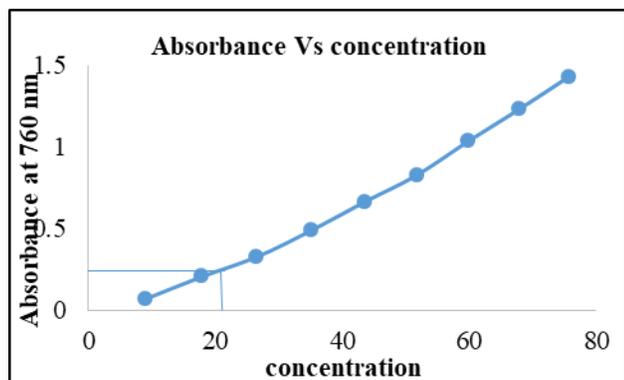


Figure5: Concentration of ascorbic acid in *Syzygium cumini*

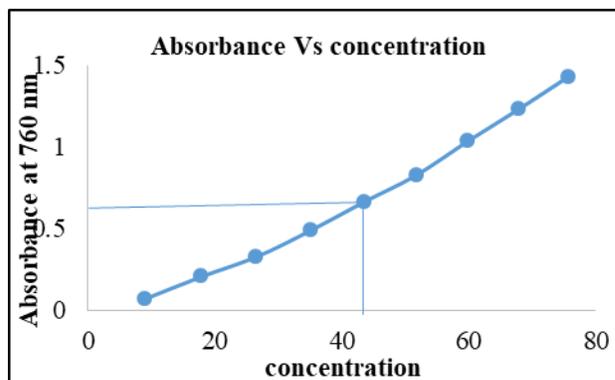


Figure6: Concentration of ascorbic acid in *Arthocarpus heterophyllus*

Less Polluted Area

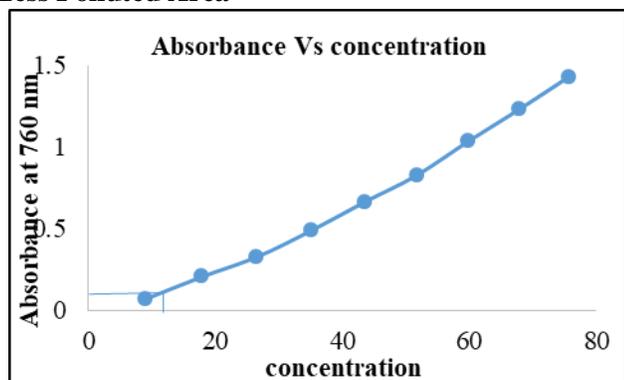


Figure7: Concentration of ascorbic acid in *Saraca asoca*

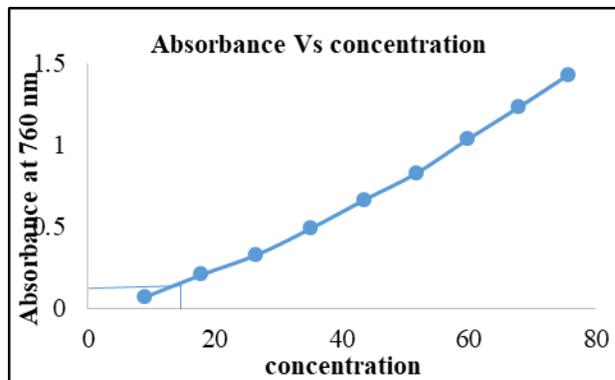


Figure8: Concentration of ascorbic acid in *Syzygium cumini*

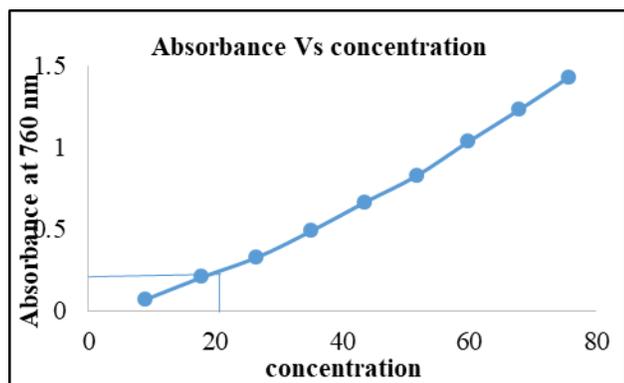


Figure9: Concentration of ascorbic acid in *Mangifera indica*

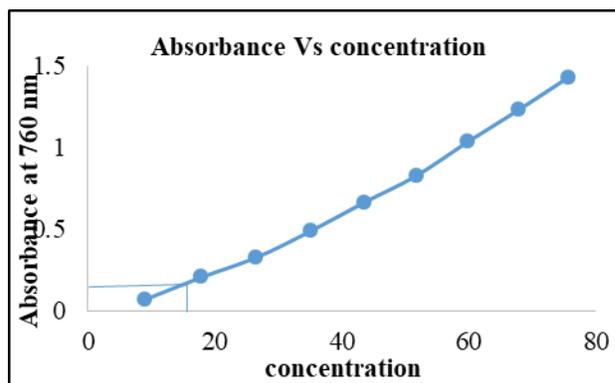


Figure10: Concentration of ascorbic acid in *Arthocarpus heterophyllus*

Table 5: Concentration of ascorbic acid against absorbance at more and less polluted zone

Species of leaves	Concentration at more polluted zone (760nm)	Concentration at less polluted zone (760nm)
Saraca asoca (Asoka)	31.223	13.5112
Mangifera indica (Mango)	65.5848	19.2543
Syzygium cumini (Blueberry)	20.0265	11.7256
Artocarpus heterophyllus (Jackfruit)	42.8056	12.9804

Table 6: Absorbance of plants at polluted zone

Plant species name	Absorbance at 645 nm	Absorbance at 663 nm
Mangifera indica (Mango)	0.342	0.351
Syzygium cumini (Blueberry)	0.127	0.190
Artocarpus heterophyllus (Jackfruit)	0.138	0.196
Saraca asoca (Ashoka)	0.082	0.134

Table 7: Absorbance of plants at non-polluted zone

Plant species name	Absorbance at 645 nm	Absorbance at 663 nm
Mangifera indica (Mango)	0.375	0.469
Syzygium cumini (Blueberry)	0.168	0.278
Artocarpus heterophyllus (Jackfruit)	0.205	0.311
Saraca asoca (Ashoka)	0.117	0.270

The values obtained with polluted and non-polluted zone of *Saraca asoca*, *Mangifera indica*, *Artocarpus heterophyllus*, and *Syzygium cumini* were compared and given in **Tables 8-11**. In general, plants showed a decrease in photosynthetic pigments due to air pollution. All the four plant species showed a significant reduction in total chlorophyll content, chlorophyll A and chlorophyll B in the study period. According to the data in the given tables, it is clear that *Mangifera indica* has more affinity to tolerate air pollution, while, *Artocarpus heterophyllus* can withstand under extreme polluted zone.

Table 8: Concentration of different Photosynthetic Pigments ($\mu\text{g/ml}$) in the Leaves of *Saraca a.* Collected from Polluted and Control Sites

Parameter	Polluted	Non polluted	Percentage reduction
Chlorophyll A	1.48	3.16	53.16
Chlorophyll B	1.25	1.41	12.00
Total chlorophyll content	2.73	4.20	35.00

Table 9: Concentration of Different Photosynthetic Pigments ($\mu\text{g/ml}$) in the Leaves of *Mangifera i.* Collected from Polluted and Control Sites

Parameter	Polluted	Non polluted	Percentage reduction
Chlorophyll A	3.54	4.94	28.34
Chlorophyll B	6.19	6.40	3.28
Total chlorophyll content	9.72	11.33	14.21

Table 10: Concentration of Different Photosynthetic Pigments ($\mu\text{g/ml}$) in the Leaves of *Artocarpus h.* Collected from Polluted and Control Sites

Parameter	Polluted	Non polluted	Percentage reduction
Chlorophyll A	2.18	3.40	35.88
Chlorophyll B	2.24	3.24	30.86
Total chlorophyll content	4.36	6.64	34.33

Table 11: Concentration of Different Photosynthetic Pigments ($\mu\text{g/ml}$) in the Leaves of *Syzygium c.* Collected from Polluted and Control Sites

Parameter	Polluted	Non polluted	Percentage reduction
Chlorophyll A	2.07	3.08	32.7
Chlorophyll B	2.02	2.48	18.5
Total chlorophyll content	4.09	5.62	27.2

Result and Discussions

The results obtained with polluted and non-polluted areas for *Saraca asoca*, *Artocarpus heterophyllus*, *Mangifera indica* and *Syzygium cumini* were compared. In general, plants showed a decrease in photosynthetic pigments due to air pollution. *Saraca asoca*, *Artocarpus heterophyllus*, *Mangifera indica* and *Syzygium cumini* showed a significant reduction in total ascorbic acid content in the study period. From the **Figure 13**, we concluded that the concentration of ascorbic acid of leaves at more polluted zone is greater than less polluted zone.

From **Figure 14**, samples were collected under non polluted zone for comparison with polluted zone to observe change in chlorophyll content under both conditions. From **Figure 15**, high concentration of total chlorophyll content in plant species have been studied. From the graph, it is concluded that some plants species like *Mangifera indica* and *Artocarpus heterophyllus* has resistant against air pollution and also have higher chlorophyll content in both species of plant leaves in polluted zone it is due to no loss of Magnesium ions from chlorophyll skeleton.

The order of highest obtained chlorophyll content under polluted conditions:

Mangifera indica > *Artocarpus indica* > *Syzygium cumini* > *Saraca asoca*

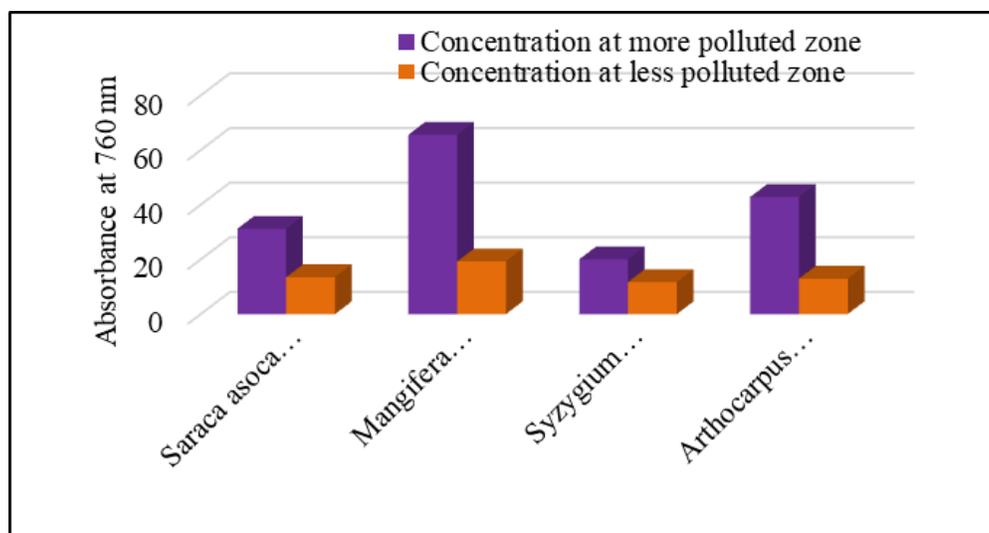


Figure 13: Concentration of ascorbic acid of leaves at more polluted zone and less polluted zone

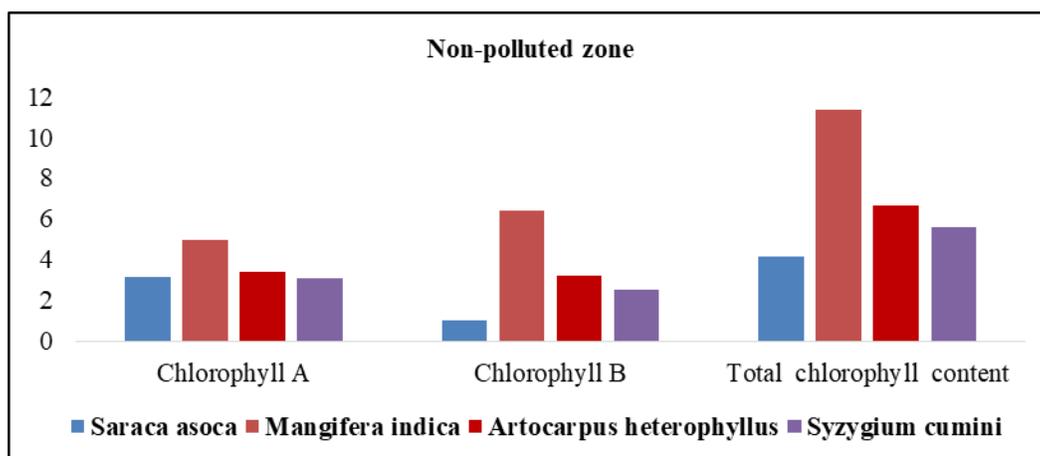


Figure 14: Graph of photosynthetic pigment against plant species for non-polluted zone

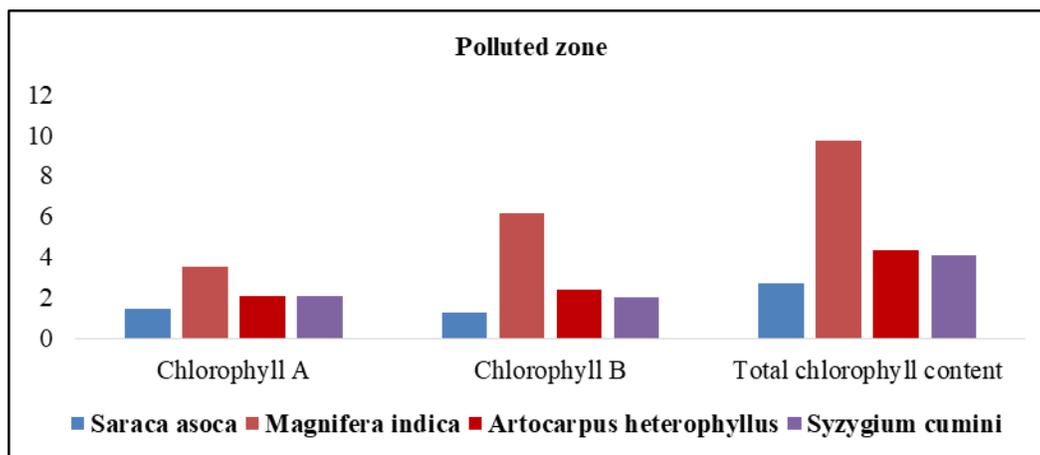


Figure 15: Graph of photosynthetic pigment against plant species for polluted zone

Conclusions

The results of this study indicated a decline in ascorbic acid content in trees growing in polluted area. According to the results, highest ascorbic acid was observed in case of *Mangifera indica* species followed by *Artocarpus heterophyllus* whereas the least was observed in *Syzygium cumini*. Ascorbic acid was significantly affected by distance from the roadside. Similarly, there was a decline in chlorophyll content in trees growing in polluted area. Highest TCH was observed in case of *Mangifera indica* species followed by *Artocarpus heterophyllus* whereas the least was observed in *Saraca asoca*. Chlorophyll content is important for healthiness of plants canopy and the rate of photosynthesis and can also be used to study medicinal and natural therapeutic properties.

From the study, we recommend to grow *Mangifera indica* (Mango) and *Artocarpus heterophyllus* (Jackfruit) in more polluted zone. As the concentration of ascorbic acid in all plant samples is more at more polluted zone as compare to less polluted zone, we should plant more trees which have high concentration of ascorbic acid to decrease the pollution, whose defense mechanism is good against pollution.

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