

Seasonal variation in biochemical parameters of few medicinal plants in industrial area of Gajraula of district Amroha (UP), India

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ABSTRACT

Air is the primary prerequisite for life on the earth. Due to the rapid increase in industrial and automobile emissions, its quality is worsening day by day. The accumulation of air pollutants on the leaves of plants growing along the roadside causes harmful changes in their biochemical parameters. The present investigation deals with the effects of air pollutants on the total chlorophyll and ascorbic acid contents of commonly available medicinal plants in the study area during different seasons. The plants selected for the study were *Azadirachta indica*, *Cassia fistula*, *Ricinus communis*, *Terminalia arjuna*, and *Vachellia nilotica*. It was recorded that the maximum air pollutant level was in the winter season followed by summer and monsoon seasons. The total chlorophyll content decreased and the level of ascorbic acid increased with the increase of air pollutant level. Season-wise, total chlorophyll content was significantly different in all the study plants; also site-wise except for *Cassia fistula*. The ascorbic acid content was significantly different for *Azadirachta indica* and *Cassia fistula* both at site-wise and season-wise. On the flipside, it was non-significant for *Ricinus communis* and *Terminalia arjuna* both at site-wise and season-wise. For *Vachellia nilotica*, season-wise ascorbic acid values were significantly different but site-wise non-significant.

Figure : 00

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KEY WORDS : Air pollution, Ascorbic acid, Total chlorophyll contents

Introduction

Over the past few decades, ambient air pollution caused by human activities has come to be a great problem in developing countries. Industrial cities experience environmental stress in the form of poor air quality due to industrial exhaust and traffic congestion. Air pollution causes health risks to humans as well as plants^{1,27,30}. Due to their large surface area, plant leaves primarily receive air pollutants and respond to changes in the level of ascorbic acid, photosynthetic pigments, proteins, etc. The deposition of dust particles on the leaf surface blocks the sunbeams and alters the photosynthesis and transpiration rate^{4,11,22,28,29}. The sunlight present in polluted air accelerates redox reactions and the bleaching process inside the

chloroplast which results in reactive oxygen species (ROS) formation. These ROS cause a reduction in the amount of chlorophyll under water stress inside the plant leaves and result in a slowdown of plant growth^{5,23,31}. Ascorbic acid present in plant cells, takes part in cell division, cell wall formation, and defence². It also protects the thylakoid membrane lipid content from damages caused by ROS under water scarcity, therefore its concentration increases^{3,31}. The physiological functioning of a plant can be determined by estimation of chlorophyll and ascorbic acid contents⁹.

Though, many studies verify the change in chlorophyll and ascorbic acid contents of the plants growing in industrial areas^{8,24,33} there is a scarcity of reports from plants growing near the roadside in Gajraula

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TABLE-1: Average annual (November 2018-October 2019) values for major air pollutants at site S₁, S₂ and S₃

Study Site	PM _{2.5} (µg/m ³)	PM ₁₀ (µg/m ³)	SO ₂ (µg/m ³)	NO ₂ (µg/m ³)
S ₁	48.16	180.5	15.33	29.5
S ₂	77.58	213.5	22	34.91
S ₃	92.5	243.9	26.83	40

city²⁸. The present study aimed to study the effects of ambient air pollutants in Gajraula city at three sites, on the total chlorophyll and ascorbic acid contents of the five most common medicinal plants *Azadirachta indica* (Neem), *Cassia fistula* (Amaltas), *Ricinus communis* (Arandi), *Terminalia arjuna* (Arjuna) and *Vachellia nilotica* (Babul) growing naturally nearby the road in Gajraula city.

Materials and Methods

Area of Study and Selection of Sampling Sites

This study has been performed at the industrial city Gajraula in the state of Uttar Pradesh, India which is a center of many industries like Insilco Limited, Israeli Pharma Teva API, Jubilant Life Sciences, Navabharath Fertilizers Limited and RACL Geartech, etc. Present

studies were finalized at three different locations of the town i.e. Town Basti, RACL, and Indra Chowk, from November 2018 to October 2019. Factors like population and traffic density, industry location, etc. have been taken into consideration for the selection of the study sites.

S₁ or (Town Basti) Control Site: This site is thinly populated with low traffic density. People are mainly involved in agriculture-related activities. So this site is receiving less pollution as compared to the other two sites. Due to this, the site has been taken as control.

S₂ or (RACL) Industrial Site: RACL is an automotive industry and this site is located near NH-24. The traffic movement is very frequent near this site.

S₃ or (Indra Chowk) Commercial Site: It is a residential area with moderate commercial activities. Many shops and workshops are situated near this site. Throughout

TABLE-2 : Site-wise and season-wise variation of total chlorophyll and ascorbic acid content in *Azadirachta indica*

<i>Azadirachta indica</i> (Neem)						
Site	Total Chlorophyll Content (mg/g)			Ascorbic Acid content (mg/g)		
	Winter	Summer	Monsoon	Winter	Summer	Monsoon
S ₁	2.93	3.64	4.31	8.01	6.65	5.81
S ₂	2.38	3.07	3.86	8.32	7.59	6.29
S ₃	2.61	3.17	3.81	8.53	7.88	6.31
ANOVA	Within Sites, F = 42.41, Fcrit = 6.94, α = 0.05, p = 0.002*			Within Sites, F = 9.3, Fcrit = 6.94, α = 0.05, p = 0.03*		
	Within season, F = 249, Fcrit = 6.94, α = 0.05, p = 0.000*			Within season, F = 70.24, Fcrit = 6.94, α = 0.05, p = 0.000*		

TABLE-3 : Site-wise and season-wise variation of total chlorophyll and ascorbic acid content in *Cassia fistula*

<i>Cassia fistula</i> (Amaltas)						
Site	Total Chlorophyll Content (mg/g)			Ascorbic Acid content (mg/g)		
	Winter	Summer	Monsoon	Winter	Summer	Monsoon
S ₁	6.81	7.88	9.12	10.1	9.55	8.21
S ₂	5.96	7.3	8.91	11.23	9.89	9.03
S ₃	5.87	8.31	8.79	11.89	10.52	9.75
ANOVA	Within Sites, F = 1.36, Fcrit = 6.94, α = 0.05, p = 0.35			Within Sites, F = 27.45, Fcrit = 6.94, α = 0.05, p = 0.004*		
	Within season, F = 34.21, Fcrit = 6.94, α = 0.05, p = 0.003*			Within season, F = 57.59, Fcrit = 6.94, α = 0.05, p = 0.001*		

the year, more pollution was recorded at this site due to the emissions from the industries located nearby. Traffic movement is slow and highly congested due to traffic jams and encroachment on both sides of the road. More vehicles gather on the road near the railway line crossing.

Average annual (November 2018-October 2019) values for major air pollutants recorded from all three sites at Gajraula city have been presented in Table 1, which indicates the pollution level in the order of site S₁ < site S₂ < site S₃. The values of PM_{2.5}, PM₁₀, SO₂, and NO₂ were measured by Respirable Dust Sampler APM-460 NL (Envirotech, New Delhi), Fine Particulate Sampler (Envirotech, New Delhi, Model: APM-550), West-Geake method³² and modified method¹³, respectively.

Selection of Plant species

Five medicinal plant species have been selected which are abundantly available all over the Gajraula city. The sampling was done in three stages. The first stage of sampling has been done in January 2019 (winter season). The second stage of sampling was done in May 2019 (summer season) while the third stage of sampling was done in July 2019 (monsoon season). The leaf samples were collected in the morning between 7:30-9:30 AM to avoid any variation in biochemical parameters. The samples were kept in polyethylene bags and sent to the laboratory for analysis. The samples were processed immediately within the 3-4 hours of collection.

Biochemical parameters analysis

Total chlorophyll contents

The amount of chlorophyll-a and chlorophyll-b was estimated by the formula²⁰. The amount of total chlorophyll was calculated by summation of chlorophyll-a and chlorophyll-b. The amount of chlorophyll content was expressed in unit of 'mg pigment per g of fresh leaves'.

$$\text{Chlorophyll-a} = \frac{12.3 \text{ O.D.}_{663} - 0.86 \text{ O.D.}_{645}}{d \times 1000 \times W} \times V$$

$$\text{Chlorophyll-b} = \frac{19.3 \text{ O.D.}_{645} - 3.6 \text{ O.D.}_{663}}{d \times 1000 \times W} \times V$$

$$\text{Total Chlorophyll} = \text{Chlorophyll-a} + \text{chlorophyll-b}$$

Where V is the volume of the chlorophyll supernatant solution, d is the length of the light path in centimeters, W is the fresh weight of leaves and O.D. is the absorbance at respective wavelengths.

Ascorbic acid

The ascorbic acid content of the leaf sample was calculated by the formula¹⁶ with the help of a spectrophotometer.

Ascorbic acid was estimated by the following formula:

$$\text{Ascorbic acid (mg/g fresh weight)} = \frac{(E_o - E_s - E_t) V}{W \times 100} \times 100$$

TABLE-4 : Site-wise and season-wise variation of total chlorophyll and ascorbic acid content in *Ricinus communis*

<i>Ricinus communis</i> (Arandi)						
Site	Total Chlorophyll Content (mg/g)			Ascorbic Acid content (mg/g)		
	Winter	Summer	Monsoon	Winter	Summer	Monsoon
S₁	4.23	8.17	10.78	4.12	3.89	3.17
S₂	3.12	7.12	9.6	5.28	5.11	4.13
S₃	3.08	5.37	9.13	5.46	7.23	4.74
ANOVA	Within Sites, F = 10.5, Fcrit = 6.94, $\alpha = 0.05$, $p = 0.02^*$			Within Sites, F = 8.67, Fcrit = 6.94, $\alpha = 0.05$, $p = 0.35$		
	Within season, F = 120.65, Fcrit = 6.94, $\alpha = 0.05$, $p = 0.000^*$			Within season, F = 4.04, Fcrit = 6.94, $\alpha = 0.05$, $p = 0.10$		

Where V is the volume of the extract, W is the weight of the leaf sample (g) and E₀, E_s, and E_t are optical densities of blank, plant sample, and sample with one drop of 1% ascorbic acid respectively.

Statistical Analysis

For statistical analysis, we used Analysis Tool Pak in Microsoft Office Excel 2007. A two-way ANOVA at $\alpha = 0.05$ was performed to know the season-wise and site-wise significant ($p < 0.05$) variations in biochemical parameters.

Results and Discussion

Total chlorophyll contents

The site-wise and season-wise variation of total chlorophyll contents of five selected plant species has been summarized in Tables 2-6. The amount of total chlorophyll content was recorded highest in the monsoon season followed by winter and summer seasons. Without consideration of study sites, the mean value for total chlorophyll had been, 4.03, 5.66, and 7.29 mg/g, respectively for all the study plants in all three different seasons *i.e.* winter, summer, and monsoon. The highest chlorophyll content had been recorded 10.78 mg/g in *Ricinus communis* at site S₁, during monsoon season. The lowest value was reported at 1.81 mg/g in *Vachellia nilotica* at site S₃ during winter. The site-wise and season-wise significant difference ($p < 0.05$) in chlorophyll values was analyzed by two-way ANOVA at $\alpha = 0.05$ for every plant species. Season-wise, the level

of total chlorophyll content was significantly different for all the study plant species. On the other hand, site-wise, it was significantly different for all the study plants except *Cassia fistula*.

The amount of chlorophyll pigments in a plant leaf represents its photosynthetic performance and this photosynthesis process is responsible for the growth and increase in biomass of plants. Many studies indicated that the amount of chlorophyll is changed with the age of the leaf and in response to the abiotic stress caused by surrounding air pollutants^{6,10,14,25}, therefore its level is used to estimate the impact of air pollution on plants. A study from Lucknow recorded a decline in total chlorophyll contents with the dust settling on the surface of leaves in a polluted environment⁷.

Our study recorded a decline in the total chlorophyll contents from site S₁ to S₃ and from the monsoon season followed by winter and summer seasons. Studies suggested that air pollutant levels and dust settle were maximum in the winter season followed by summer and monsoon seasons^{24,28}. This increase in the level of pollutants and maximum dust settling could be a cause of retardation in chlorophyll production because air pollutants have many hazardous metals and polycyclic aromatic compounds, which slow down the enzyme synthesis required for chlorophyll formation. Apart from this, they retard the gaseous diffusion by obstructing stomata and decline in photosynthesis by hindering incident sunlight^{12,15,18,21,24}.

TABLE-5 : Site-wise and season-wise variation of total chlorophyll and ascorbic acid contents in *Terminalia arjuna*

<i>Terminalia arjuna</i> (Arjuna)						
Site	Total Chlorophyll Content (mg/g)			Ascorbic Acid content (mg/g)		
	Winter	Summer	Monsoon	Winter	Summer	Monsoon
S₁	6.17	7.82	9.42	9.1	7.84	6.93
S₂	5.35	7.19	9.23	8.23	8.78	7.67
S₃	5.28	7.03	9.2	7.9	8.91	7.93
ANOVA	Within Sites, F = 8.86, Fcrit = 6.94, $\alpha = 0.05$, $p = 0.03^*$			Within Sites, F = 0.17, Fcrit = 6.94, $\alpha = 0.05$, $p = 0.84$		
	Within season, F = 255.1, Fcrit = 6.94, $\alpha = 0.05$, $p = 0.000^*$			Within season, F = 1.98, Fcrit = 6.94, $\alpha = 0.05$, $p = 0.25$		

Ascorbic Acid

The site-wise and season-wise variation of ascorbic acid contents of five selected plant species has been outlined in Tables 2-6. The value of ascorbic acid had been recorded maximum during winters followed

by summer and was lowest in monsoon. Irrespective of the study sites, the mean value for ascorbic acid had been reported as 9.24, 8.57, and 7.23 mg/g for all the plant species for winter, summer, and monsoon seasons, respectively. The highest ascorbic acid value of 15.87

TABLE-6 : Site-wise and season-wise variation of total chlorophyll and ascorbic acid content in *Vachellia nilotica*

<i>Vachellia nilotica</i> (Babul)						
Site	Total Chlorophyll Content (mg/g)			Ascorbic Acid content (mg/g)		
	Winter	Summer	Monsoon	Winter	Summer	Monsoon
S₁	2.96	3.32	4.81	10.3	9.63	8.89
S₂	1.88	2.82	4.23	14.28	12.31	9.68
S₃	1.81	2.67	4.19	15.87	12.84	9.84
ANOVA	Within Sites, F = 18.51, Fcrit = 6.94, $\alpha = 0.05$, $p = 0.009^*$			Within Sites, F = 6.12, Fcrit = 6.94, $\alpha = 0.05$, $p = 0.06$		
	Within season, F = 118, Fcrit = 6.94, $\alpha = 0.05$, $p = 0.000^*$			Within season, F = 8.57, Fcrit = 6.94, $\alpha = 0.05$, $p = 0.03^*$		

mg/g had been shown by *Vachellia nilotica* at site S₃ during winter while the lowest value of 3.17 mg/g by *Ricinus communis* at site S₁ during monsoon season. In two-way ANOVA analysis at $\alpha = 0.05$, the level of ascorbic acid content was significantly ($p < 0.05$) different for *Azadirachta indica* and *Cassia fistula* (Tables 2-3) both site-wise and season-wise. On the flip side, variation was non-significant for *Ricinus communis* and *Terminalia arjuna* (Tables 4-5) both site-wise and season-wise. But for *Vachellia nilotica* site-wise variation was non-significant but seasonal variation was significant (Table- 6).

Our results are similar to previous studies^{10,25,33}. However, a previous finding showed contradictory trends²⁶. They reported elevated ascorbic acid at the control site and while lower amount at polluted sites²⁶. In our study, the same pattern was shown by *Terminalia arjuna* (Table 5) during the winter season means a more ascorbic acid level at the control site (S₁), and a lower level at polluted sites (S₂ and S₃).

Ascorbic acid functions as an antioxidant and gives resistance to the plants against environmental stresses, by nullifying the free oxygen radicals formed inside the cytoplasm¹⁹. Its amount in cytoplasm increases with the elevation in pH of cytoplasm. At higher pH, the hexose sugar conversion to ascorbic acid is increased which provides more tolerance against environmental stresses¹⁷.

Conclusion

Pollution levels in different seasons affect leaf biochemical parameters by a decline in the synthesis of enzymes required for chlorophyll synthesis. Air pollutants along with the dust settling, slow down gases (CO₂ and O₂) exchange by obstructing stomata and decline in photosynthesis by hindering incident sunlight. In contrast, the ascorbic acid content of leaves increases to overcome these environmental stresses. All these variations exert an effect on the normal physiological functioning of plants.

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