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RESEARCH PAPER

Plant leaf as pollution monitoring device

D. SARALA THAMBAVANI* AND V. PRATHIPA
Department of Chemistry, Sri Meenakshi Government Arts College for Women (Autonomous), MADURAI (T.N.) INDIA

ABSTRACT

Biological effect monitoring of urban industrial pollutants was carried out using an air pollution tolerance index (APTI) of plants. For this purpose, the four leaf parameters namely, ascorbic acid, chlorophyll, relative water content and leaf extract pH were combined together in a formulation signifying the APTI of plants. The index indicated the plant response at the cell membrane and chloroplast levels. Sampling sites P_1 and P_2 are situated in western parts of Dindigul near the junction of national highways and industries. The control site was selected as Lakshmanapuram to categorize plants as sensitive or resistance. Air pollution tolerance index was calculated. The APTI showed a marked gradation as the pollutant load decreased from zone P_1 and P_2 to the control site (P_3). The APTI can be used as a good indicator to find the impact of pollution on plants.

Key Words : Urban industrial pollutant, APTI, Total chlorophyll, Ascorbic acid, Leaf extract pH, Relative water content (RWC)

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Rapid industrialization, urbanization and traffic density cause atmospheric pollution all over the country. Vegetation plays the role of major sink of atmospheric dust containing a fair amount of highly toxic heavy metal particles. Air pollution is one of the severe problems worlds facing today. It deteriorates ecological condition and can be defined as the fluctuation in any atmospheric constituent from the value that would have existed without human activity. Various efforts have been done for environmental restoration in India but still it seems to be a formidable task. Dindigul town is no exception. Its environment has undergone irreparable damage due to the population growth and its subsequent requirements in terms of housing and traffic density continuously increasing road traffic is a primary culprit. The changed ambient environment due to the air pollutants in urban area of Dindigul which has exerted a profound influence on the morphological, biochemical and physiological status of plants, and therefore its responses.

The responses of plants to pollutants provide a simple and low cost method of monitoring gaseous pollutants (Posthumus, 1985). However, to use plants as bio indicators, a proper selection of plant characteristics is of vital importance. A number of plant parameters has been used individually for the purpose, *viz.*, visible foliar injury (Davis and Wilhour, 1976), membrane permeability (Farooq and Beg,1980), ascorbic acid (Keller and Schwager, 1977), relative water content (Rao, 1979), chlorophyll content (Bell and Mudd,1976) , leaf extract pH (Chaudhary and Rao, 1977) and peroxides activity (Eckert and Houston,1982). Usually for biological effect monitoring, pollution induced changes in individual parameters of plants are quantified and correlated with level of pollutants. However, evaluation of plant response based on single criterion alone may not be feasible in the complex circumstances of an urban industrial environment where a variety of unspecified pollutants present.

Work have been done in the direction to study the sensitivity of plants based on the selected parameters such as ascorbic acid, relative water content (RWC) chlorophyll

*** Author for correspondence**
V. Prathipa, Department of Chemistry, P.S.N.A. College of Engineering and Technology, DINDIGUL (T.N.) INDIA

content, leaf extract pH. Only one particular character recognizing sensitivity of a plant is not justifiable. Therefore, incorporated the above four characters together to calculate the air pollution tolerance index (APTI) to categorize a plant as sensitive to the air pollutants. APTI of some plants have been calculated by Karthiyayini *et al.* (2005) using the APTI formula. Several contributors agree that air pollutants effect plant growth adversely (Sarala *et al.*, 2009).

Ascorbic acid along with leaf pH plays a significant role in determining pollutant response of plants (Chaudhary and Rao, 1977). Its reducing power is low at lower pH and at lower concentrations. A decreased reducing power of this ascorbic acid will render the enzymes of the CO_2 fixation cycle and total chlorophyll liable to inactivation by the accumulation of SO_2 induced free radical (S) (Shimazaki *et al.*, 1980; Tanaka *et al.*, 1982). Ascorbic acid possibly protects chloroplast functions and chlorophyll degradation through its pH dependent reducing power. On exposure to pollutant the relative water content of leaf tissue represents the plant response at the cell membrane level (Singh *et al.*, 1991). The main focus of this work is to provide an assessment of the use of biochemical parameters of plants as indicators of air pollution. So that, these biochemical indicators can be used for air quality monitoring. The proposed study will provide a technical support to the air quality management in the Dindigul town. The data generated will help us to find out the exact position and also the corrective measures which are required to take up to bring the system to its normal or pristine stage.

RESEARCH METHODOLOGY

Study area:

The study area is located in the southern part of India, close to Kodaganar river basin, mainly in hard rock terrain. The area is known for its leather industries. It lies between 10^0 13'44"–$10^0$26'47" N latitude and $77^0$55'08"–$78^0$01'24" E longitude and falls in survey of India Top sheet No. 58 F/15 and J/3, in the state of Tamil Nadu, India. The selected area is located in the central part of Dindigul town and along Madurai, Batlagundu and Ponmandurai roads.

Monitoring town, Dindigul :

Dindigul is the interior region of Tamil Nadu. It lies on the banks of Kudavanar river. The total landscape of Dindigul is 6058 sq. km. The urban population is 3,76,445. Inspite of its geographical location there are about 110 tanneries both registered and non-registered in and around Dindigul.

Sampling stations:

Monitoring was carried out at three locations in the town of Dindigul broadly classified into three main categories, tanneries, commercial cum traffic and residential. All these locations are prominent places in the Dindigul town and are typical representatives of their respective categories. The frequency of monitoring for SPM was 24 hourly and 8 hourly with respect to SO_2 and NO_X. The details of monitoring locations are given in the Table A.

Table A : Sampling sites

Site No.	Site	Location description	Category
1.	Thomaiyarpuram (P_1)	Tanneries, Small scale industries	Industrial Category (Tannery area)
2.	Dindigul Bus stand (P_2)	Traffic, hotels, shopping complex, theatre, commercial complex, market	Commercial-cum-Traffic
3.	Lakshmanapuram (P_3)	Residential areas of lower and middle classes, small shops	Residential

Collection of samples and analysis:

The samples were collected bimonthly during winter and summer seasons. The parameters monitored for ambient air are suspended particulate matter (SPM), sulphur dioxide (SO_2) and oxides of nitrogen (NO_X). The ambient air monitoring was carried out in each station and samples were collected with the help of high volume sampler, model APM-415 of envirotech with provisions for gaseous sampling. A brief description of the sampling and analytical procedure for ambient air quality monitoring is given below.

PM sampling:

SPM were collected using standard high volume sampler fitted with respirable dust sampler. The sampler was calibrated using orifice control root meter. The particle size of the samples collected by RDS is smaller than 10μm. The samples were collected on glass fibre filter sheets (GF/A from Whatman) continuously for 24 hour every week at three different locations at 8 hourly intervals (Singh *et al.*, 2008). These samples were gravimetrically analysed according to analysis procedures recommended by TNPCB. The methodologies are given in Table B.

Sulphur dioxide (SO_2) and oxides of nitrogen (NO_X):

Sulphur dioxide (SO_2) and oxides of nitrogen (NO_X) were collected on 8 hourly basis for 24 hour by drawing air flow at 1 L/min through potassium terachloro mercurate and sodium hydroxide as an absorbing solutions, respectively. Sulphur dioxide was determined by West – Gaeke spectrophotometric method (standard method for air sampling and analysis) and oxides of nitrogen was determined by Jacob – Hochhieiser spectrophotometric method (Katz, 1977).

Table B: Monitored pollutants and the methodologies employed for ambient air quality study			
Methodology	SPM	SO_2	NOx
Indian standard No.	1S5182	1S5182	1S5182
	P: 4-2005	P: 2-2001	P: 6-1998
Sampling equipment	High volume sampler	High volume sampler with impinger	High volume sampler with impinger
Duration sampling	8 hourly for 24 hour	8 hourly for 24 hour	8 hourly for 24 hour
Collection media	GF/A Filter paper	Tetrachloro mercurate	NaOH

Table C: Seasonal variations in air quality in Dindigul area in $\mu g/m^3$				
Parameters	Seasons	Tannery site	Commercial-cum-traffic site	Residential site
SPM	Winter	111.2	158.4	98.0
	Summer	99.4	147.6	65.5
SO_2	Winter	11.4	15.0	14.2
	Summer	9.5	18.7	9.9
NO_x	Winter	32.0	25.6	20.0
	Summer	33.3	29.7	14.7

The observations made during the sampling period are listed in Table C.

Overall difference is based on two-way ANOVA. Test was performed for comparison of all the parameters at their respective locations, where F values in ANOVA are significant. Significant difference at $\rho<0.001$ by multiple comparison tests.

Site P_1, is Thomaiyarpuram which is crowded with tannery industries. It is junction of national highways. It receives at the emissions from automobile exhaust and leather tanneries. The major pollutants SO_2, NO_X, SPM and ammonia (NH_3). The other pollutants are particulate matter, hydrocarbons and heavy metals.

Site P_2 is Dindigul bus stand which is along the National highways and receives emission from automobile exhaust. The other sources of pollutants are pollutant from diesel and petrol engines.

Site P_3 is Lakshmanapuram in Dindigul town was taken as a control site P_3 and two polluted sites were selected in the western part of Dindigul.

A Total of six plants species growing at polluted and control site were selected. For this study *Azadiracta indica*, *Delonix elata, Moringa tinctoria*, *Calotrophis*, *Thyme/ Rosemary* and *Cyanadon dactylon* were investigated with respect to total chlorophyll, ascorbic acid and relative water contents and leaf extract pH. Fully expanded leaves, devoid of any leaf injury symptoms were collected between 8.00 and 9.00 am. For trees three individuals were identified along the pollution sources and the control site selected randomly in a belt transect measuring 50 x 75 m and foliar. Samples in triplicate were taken from each of them. The samples were taken three times at an interval of 20 days.

The mean values of different parameters were used to calculate the per cent of control values of each parameter of plants growing at the three polluted sites. In another sampling protocol, the same plants were sampled in the pollution zones I and II. Plants were sampled (as described earlier) along the lower, middle and upper regions of each pollution zone. The APTI of plants growing in the lower, middle and upper region of a pollution zone were estimated separately.

The ascorbic acid content (mg g^{-1} dry weight) was determined using the modified colorimetric 2, 6-dichlorophenol indophenol method described by Keller and Schwager (1977). Total chlorophyll (mg g^{-1} dry weight) was estimated following the method of Maclachlan and Zalik (1963). Leaf samples (0.59) was crushed and homogenized in some deionised water, the mixture was centrifuged and supernatant was collected for detection of pH using pH meters. The per cent relative water content (RWC) was calculated by using the initial weight and dry weight of leaf material. Ascorbic acid, leaf extract pH, total chlorophyll content and relative water content (RWC) were taken in the form of mathematical expression to obtain an empirical value, signifying the air pollution tolerance index (APTI) (Raza *et al.*, 1985).

$$\mathbf{APTI = A(T+P)+R/10}$$

where,

A = Ascorbic acid content (mg g^{-1} fresh wt)

P = leaf extract pH

T = Total chlorophyll (mg g^{-1} dry wt) and

R = Per cent relative water content of leaf.

Based on the development and evaluation of APTI values among the samples they were categorized into three groups as given in Table D.

Table D: Categories of tree species based on APTI	
APTI value	Remarks
1-10	Sensitive
10-16	Intermediate
More than 17	Tolerant

RESEARCH AND REMONSTRATION FINDINGS

Different plants species showed significant variation in the response to air pollutant. The level of total chlorophyll, leaf pH, Ascorbic acid and relative water content in plants reduced to various extent depending upon the pollution load and its impact on the different sampling sites (Table A and Table 1, 2, 3 and 4).

However, plants maintained a certain level of parameters due to a balance between the injury caused by air pollutant and the homeo static processes governing repair.

Azadiracta indica:

The level of pH in *Azadiracta indica* at the control site was 5.85. It showed 5.12 per cent decline at the traffic area (site P_2) and 1.19 per cent decline at the tannery area (site P_3). There was decrease in pH in site P_2 and P_3 but the significant fall was found at the traffic area. The relative water content of *Azadiracta indica* at the sampling sites at P_1, P_2 and P_3 were 75.0, 65.0 and 62.8, respectively. The total chlorophyll content of *Azadiracta Indica* at the selected sampling sites at P_1, P_2 and P_3 were 0.69 mg/g, 0.478 mg/g and 0.85 mg /g, respectively. The tannery industrial effluent and the traffic pollutant had the impact on total chlorophyll. There was 43.76 per cent and

Table 1 : Total chlorolphyll (TC)) (mg/g) of the selected plant species along traffic and tannery area

Sr. No	Sampling location	*Azadiracta indica*		*Delonix elata*		*Moringa tinctoria*		*Calotrophis*		*Thyme/Rosemary*		*Cyanadon dactylon*	
		TC	%R	TC	%R	TC	%R	TC	%R	TC	%R	TC	%R
1.	Residential area (P_3)	0.85		0.531		0.948		0.674		0.80		0.66	
2.	Traffic area (P_2)	0.478	43.76	0.384	27.68	0.636	32.91	0.47	30.26	0.458	42.75	0.42	36.36
3.	Tannery area (P_1)	0.69	18.82	0.76	-43.12	0.48	49.36	0.43	36.20	0.56	30	0.37	43.93

where %R = Percentage reduction. Over all difference is based on two way ANOVA. Test was performed for comparison of pH of all plant species at their respective locations, where F values in ANOVA are significant difference at $\rho<0.001$ by multiple comparison tests.

Table 2: Leaf extract pH of the selected plant species along traffic and tannery area

Sr. No	Sampling location	*Azadiracta Indica*		*Delonixelata*		*Moringa tinctoria*		*Calotrophis*		*Thyme/ Rosemary*		*Cyanadon dactylon*	
		pH	%R	pH	%R	pH	%R	pH	%R	pH	%R	pH	%R
1.	Residential area (P_3)	5.85		7.53		6.75		7.60		5.24		6.35	
2.	Traffic area (P_2)	5.55	5.128	9.56	-26.95	5.5	18.51	4.56	40	4.85	7.44	3.65	42.51
3.	Tannery area (P_1)	5.78	1.196	6.92	8.10	7.2	-6.66	8.52	-12.10	5.20	0.763	6.60	-3.95

where %R = Percentage reduction. Over all difference is based on two way ANOVA. Test was performed for comparison of pH of all plant species at their respective locations, where F values in ANOVA are significant difference at $\rho<0.001$ by multiple comparison tests.

Table 3: Ascorbic acid (AA) (mg/g) of the selected plant species along traffic and tannery area

Sr. No.	Sampling location	*Azadiracta indica*		*Delonixelata*		*Moringa tinctoria*		*Calotrophis*		*Thyme/ Rosemary*		*Cyanadon dactylon*	
		AA	%R	AA	%R	AA	%R	AA	%R	AA	%R	AA	%R
1.	Residential area (P_3)	2.08		2.65		1.65		2.56		1.96		3.88	
2.	Traffic area (P_2)	4.05	-94.71	3.58	-35.09	3.55	-115.15	3.65	-42.57	2.35	-19.89	4.56	-17.5
3.	Tannery area (P_1)	2.62	-25.96	3.15	-18.86	3.68	123.0	1.89	26.17	1.08	44.89	2.34	39.69

where %R = Percentage reduction. Over all difference is based on two way ANOVA. Test was performed for comparison of pH of all plant species at their respective locations, where F values in ANOVA are significant difference at $\rho<0.001$ by multiple comparison tests.

Table 4 : Relative water content (RWC) of the selected plant species along traffic and tannery area

Sr. No.	Sampling Location	*Azadiracta indica*		*Delonixelata*		*Moringa tinctoria*		*Calotrophis*		*Thyme/ Rosemary*		*Cyanadon dactylon*	
		RWC	%R	RWC	%R	RWC	%R	RWC	%R	RWC	%R	RWC	%R
1.	Residential area (P_3)	62.8		65.3		72.05		70.5		72.3		75.6	
2.	Traffic area (P_2)	65	-3.503	55.3	15.31	62.5	13.25	58.5	17.02	62.5	13.55	56.5	25.26
3.	Tannery area (P_1)	75	-19.42	79	-20.98	82	-13.80	86.5	-22.69	72.8	-0.69	77.5	-2.51

Where %R = Percentage reduction. Over all difference is based on two way ANOVA. Test was performed for comparison of pH of all plant species at their respective locations, where F values in ANOVA are significant difference at $r<0.001$ by multiple comparison tests.

18.82 per cent decrease at site at P_2 and P_3, respectively. The ascorbic acid content at the control site, traffic site and tannery site were 2.08 mg/g, 4.05 mg/g and 2.62 mg/g, respectively. Reduction of chlorophyll in plants could unfavorably affect the photosynthesis, growth and productivity of plants (Lauenroth and Dodd, 1981).

Delonixelata **:**

The leaf extract pH of *Delonixelata* at the control site was 7.53 but it showed increase of 26.95 per cent at the traffic site (P_2) and a loss of 8.10 per cent at the tannery site (P_1). The relative water content of *Delonixelata* was found to be 65.3, 55.3 and 79.0 at sites P_3, P_2 and P_1, respectively. The total chlorophyll content at the control site was 0.531 mg/g while it exhibited significant reduction (27.68%) at the traffic site and an increase trend at the tannery site (43.12%). The ascorbic acid content of *Delonixelata* at the control site (P_3) was 2.65 mg/g while it showed maximum stimulation 35.09 per cent at the traffic site (P_2) and 18.86 per cent at the tannery site (P_1).

Moringa tinctoria **:**

Moringa tinctoria showed the variation of all the biochemical parameters significantly. The leaf extract pH of *Moringa tinctoria* at control site (P_3) was 6.75 while it showed decreasing trend (18.51%) at the traffic site (P_2) and increasing trend (6.66%) at the tannery site (P_1). The relative water content of *Moringa tinctoria* was observed as 82.0, 62.5 and 72.05 at different sampling sites P_1, P_2 and P_3, respectively. The total chlorophyll content at the control site (P_3) was found to be 0.948 mg/g, while the maximum reduction was found at the traffic site (P_2) and the tannery site ((P_1)(32.91% and 49.36%), respectively. Ascorbic acid was found to be increasing at the pollutated site (P_1 and P_2) as compared to control site. Maximum increase of > 100% was evident at the traffic site (P_2) and the tannery site (P_1), respectively.

Calatrophis:

The level of pH in *Calotrophis* at the control site was 7.60. It showed 40 per cent decease at the traffic area (site P_2) and increased (12.10%) at the tannery area (site P_3). The relative water content of *Calotrophis* at the sampling sites at P_1, P_2 and P_3 were 86.50, 58.5 and 70.50, respectively. The total chlorophyll content of *Calotrophis* at the selected sampling sites at P_1, P_2 and P_3 were 0.43 mg/g, 0.47 mg/g, and 0.674 mg/g, respectively. The tannery industrial effluent and the traffic pollutant had the impact on total chlorophyll. There was 30.26 and 36.20 per cent decrease at site at P_2 and P_3, respectively. The ascorbic acid content at the control site, traffic site and tannery site were 2.56 mg/g, 3.65 mg/g and 1.89 mg/g, respectively. Reduction of chlorophyll in plants could unfavorably affect the photosynthesis, growth and productivity of plants.

Thyme/Rosemary:

The leaf extract pH of *Thyme/Rosemary* at the control site was 5.24 but it showed the decease of 7.44 per cent at the traffic site (P_2) and 0.763 per cent at the tannery site (P_1). The relative water content of *Thyme/Rosemary* was found to be 72.8, 62.5 and 72.3 at sites P_3, P_2 and P_1, respectively. The total chlorophyll content at the control, site was 0.8 mg /g while it exhibited significant reduction (42.75 %) at the traffic site and 30 per cent at the tannery site (P_3). The ascorbic acid content of *Thyme/Rosemary* at the control site (P_3) was 1.96 mg /g while it showed stimulation 19.89 per cent at the traffic site (P_2) and 44.89 per cent reduction at the tannery site (P_1).

Cyandon dactylon:

Cyandon dactylon showed the variation of all the biochemical parameters significantly. The leaf extract pH of *Cyandon dactylon* at control site (P_3) was 6.35 while it showed the decreasing trend (42.51%) at the traffic site (P_2) and increasing trend (3.95 %) at the tannery site (P_1). The relative water content of *Cyandon dactylon* was observed as 77.5, 56.5 and 75.6 at different sampling sites P_1, P_2 and P_3 respectively. The total chlorophyll content at the control site (P_3) was found to be 0.66 mg/g, while the maximum reduction was found at the traffic site (P_2) and the tannery site (P_1) (36.36 % and 43.93 %), respectively. Ascorbic acid was found to be increasing at the pollutated site (P_1 and P_2) as compared to control site. Maximum increase of > 100 % was evident at the traffic site (P_2) and tannery site (P_1), respectively.

The reduction in all the four parameters was highest in plants at site P_1 than at sites P_2 and P_3 (Table 5) indicating there by that the pollutant impact on plants was maximum at site P_1 followed by sites P_2 and P_3 which was obvious as pollutant concentration was more at site P_1 followed by sites P_2 and P_3 (Table 1). But if any one parameter is to be singled out for bio indication purpose, the situation becomes complex, since none of the four parameters responds consistently in all the species.

For example in *Azadiracta indica* at highly pollutated sites P_1, the percentage of reduction in pH was 1.196, total chlorophyll 18.82 and ascorbic acid 25.96. In *Delonixelata* at the same site, the percentage of reduction in pH was 8.10, total chlorophyll 43.12 and ascorbic acid 18.86.In *Moringa tinctoria* at the same site, the reduction of total chlorophyll was found to be 49.36 per cent.

Calotrophis showed 40 per cent of reduction in pH at traffic area while 30.26 per cent and 36.27 per cent reduction in total chlorophyll in traffic and tannery area, respectively. It also showed significant reduction in ascorbic acid and relative water content at pollution site. *Thyme/Rosemary* showed significant reduction (42.75%, 30%), total chlorophyll 7.44 per cent and 0.786 per cent reduction in leaf extract pH at polluted sites and also showed reduction in ascorbic acid and relative water content (RWC) in the traffic and tannery area.

Cyandon dactlyon showed 36.36 per cent and 43.93 per cent reduction in chlorophyll at the polluted site. It showed 42.51 per cent reduction in leaf extract pH at the traffic area and the significant reduction in ascorbic acid and relative water content (RWC) in the traffic and tannery area.

No single parameters uniformly indicated the same degree of impact of pollutants on plants. In few plants chlorophyll was the most sensitive parameters. It showed a gradual decrease as the pollution load increased. For example *Moringa tinctoria* showed significant reduction in total chlorophyll at the pollutated site P_1 and P_2.

The APTI of plants determined in two pollution zones P_1 and P_2 (marked on the basis of pollution concentration) showed that as the pollution load decreases from zone P_1, P_2 to control site (P_3), the tolerance index of the plant decreased. For example in *Azadiracta indica* in zone P_1 ranged from 9 -10, in zone P_2 8 – 9 and at control site it was 7.7. In *Delonix elata* the APTI ranged from 10 -11 in zone P_1 and from 9 – 10 in zone p_2 and at control site (P_3) it was 8.7. In *Moringa tinctoria* the APTI ranged from 11–12 in zone P_1, from 8 – 9 in zone P_2 and it was 8.5 at control site (P_3). In *Calotrophis* the APTI ranged from 10-11 in zone P_1, from 7-8 in zone P_2 and it was 9.168 at control site (P_3). In *Thyme/Rosemary* the APTI ranged from 10-11 in zone P_1 from 7-8 in zone P_2 and it was 8.41 at control site (P_3). In *Cyandon dactylon* the APTI ranged from 9-10 in zone P_1 from 7-8 in zone P_2 and it was 10.27 at control site (P_3) (Table 9). Thus, it seems that there was marked changes in the APTI of plants.

Table 5 : Air pollution tolerance index of plants growing in different pollution zones and at control site

Plant species	Pollution zone		Control site
	P_1	P_2	P_3
AzadiractaIndica	9 - 10	8 - 9	7.7
Delonix elata	10 -11	9 -10	8.7
Moringatinctoria	11 - 12	8 - 9	8.5
Calotrophis	10-11	7-8	9.168
Thyme/Rosemary	10-11	7-8	8.41
Cyanadon dactylon	9-10	7-8	10.27

The observed variation in plant distribution was contributed due to the air pollutants and sensitivity of the plant. Reports by Dwivedi *et al.* (2000) and Waugh *et al.* (2006), where abnormality in plant distribution of plant, response, of fossil fuel pollution supports our finding using APTI. Pollution impact zones could be marked by determining the range of index of a suitable indicator species for a particular pollution zone. It can be said that the air pollution tolerance index (APTI) values estimated using the four biochemical parameters in plant leaves *viz.*, relative water content (RWC), chlorophyll, pH and ascorbic acid can be used as predictor of air quality. These parameters are significant in studies in plant environment interactions and used for development of bioindicator groups.

The present study suggests that plants have the potential to serve as excellent morphometric, quantitative and qualitative indices of pollution level. Biomonitoring of plants is an important tool to evaluate the impact of air pollution on plants. It is evident that the plants can act as pollution indicator and it shows how the plants tolerate against the industrial and automobile emission pollution.

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