ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 05, pp.50147-50152, May, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

STUDIES ON AMBIENT AIR QUALITY AND ITS IMPACT ON MICRO-MORPHOLOGICAL STUCTURE OF LEAVES OF ROAD SIDE PLANT SPECIES, *FICUS BENJAMINA* IN REWA CITY (M.P), INDIA

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ARTICLE INFO

Article History:
Received 17th February, 2017
Received in revised form
25th March, 2017
Accepted 16th April, 2017
Published online 23rd May, 2017

Key words:
Ambient air quality,
SPM, RSPM, SO_2, NO_X,
Epidermal cell, Stomata.

ABSTRACT

This study was undertaken to assess the ambient air quality (seasonal variations of ambient air pollutants SPM, RSPM, SO_2 and NO_X) and its impact on road side plant species *Ficusbenjamina*, in Rewa city with special reference to epidermal characteristics of leaves. The monitored average values of RSPM, SO_2 and NO_X at the sampling sites are well within prescribed limits, whereas average concentrations of SPM in the ambient air of the Rewa city are above the permissible limits as per National Ambient Air Quality Standards (NAAQS) and Central Pollution Control Board (CPCB). The light microscopic studies of this plant indicated marked alternation in epidermal traits, with increased number of stomata and epidermal cells per unit area and decreased length and width of stomata guard cells and epidermal cells on both abaxial and adaxial surface of leaf collected from polluted site than those of control ones. These changes in micro-morphological structures could be an indicator of environmental stress caused due to urban air pollution. These characters could be used in the bio monitoring of urban air quality.

Citation: Riya Shrivastava, 2017. "Studies on ambient air quality and its impact on micro-morphological stucture of leaves of road side plant species, *ficus benjamina* in rewa city (M.P), India", *International Journal of Current Research*, 9, (05), 50147-50152.

INTRODUCTION

Air pollution has become a major environmental problem faced by the people globally in both developing and developed countries in recent times (Nagdeve 2004, Barman *et al.*, 2010, Chaudhary *et al.*2013, Charan and Sahel 2014 and Ahmad and Bano 2015). Rapid urbanization and growing population results serious effect on human life and its environment in recent years resulted in deterioration of ambient air quality. In urban areas and especially metropolitan cities, vehicular exhaust or automobile emissions has been an in escapable reality faced by every individual constituting a major source of environmental pollution in India. In Madhya Pradesh, where the road is most popular way to transportation routes, the running automobiles are in a huge numbers. The major cities of Madhya Pradesh are suffering from damaged road routes and increased number of automobiles like cars, taxies, trucks, buses, three wheelers and two wheelers etc. Most of these vehicles are retired, completed their life and are not in condition to run on the roads by the rule. Thus the problem is much more aggravated due to narrow and congested roads and old poorly maintained vehicles. Petrol and diesel vehicles release variety of pollutants particularly benzene, carbon monoxide , organic compounds, oxides of nitrogen and sulphur and suspended particulate matters like ultra fine primary particles, smoke, metals and dust. Also the ultra fine particles when released quickly coagulate to form larger particles, through reaction with other pollutants like ammonia, sulphur dioxide etc. (Street *et al.*, 1996 and Shrivastava *et al.*, 2013). Various researches have been carried out in India to highlight the effect of air pollution on micro- morphological (Palaniswamy *et al.*, 1995; Morison, 1998; Aggarwal, 2000; Pawar,2013 and Rai and Singh, 2015), anatomical (Salgare and Rawal, 1990; Acharekar and Salgare, 1991; Giri et.al, 2013; Pawar, 2015) and biochemical (Pratibha and Sharma, 2000; Karthiyayini *et al.*, 2005; Ramakrishnaiah and Somashekar, 2003; Gupta *et al.*, 2009) parameters of different plant species at various places. The road side plants play an important role in monitoring and maintaining the ecological balance and also provide enormous leaf area for impingement, absorption and accumulation of air pollutants to reduce the pollution level in the environment acting as efficient interceptors of airborne pollutants. Several studies show that under polluted conditions, plant develop different morphological, physiological and anatomical changes (Inamdar and Chaudhari, 1984; Iqbal, 1985; Gupta and Ghouse, 1988; Gravano *et al.*, 2003; Novak *et al.*, 2003 and Dineva, 2004). Adverse impacts of urban air pollution on leaf structure of plants have been studied by various workers (Kulshreshtha *et al.*, 1994a, 1994b; Sharma and Roy, 1995; Carreras *et al.*, 1996; Pal *et al*,, 2000; Dineva, 2006; Rai and Kulshreshtha, 2006; Sher and Hussain, 2006;

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Amulya *et al*., 2015; Pawar, 2016). Automobiles are the main source of pollution in urban atmosphere of the Rewa city. This study was undertaken to assess the changes caused by vehicular air pollution on number and size of stomata and epidermal cells in the leaves of a roadside tree species; *Ficusbenjamina.*

MATERIAL AND METHODS

Study area

The present study was conducted in Rewa city, which is situated on the north east border of Madhya Pradesh, central part of India. It is located at 24º18' and 25º12' north latitudes and 81º2' and 62º18' east longitudes and 316 meters above mean sea level (MSL), with a total geographical area 6,314 kilometers having a population about 3.0 lakhs .The climate is humid subtropical with cold, misty winter, a hot summer and a humid monsoon season.

Sampling and selection of plant

Air quality monitoring at eleven selected sites of Rewa city have been carried outviz;Sirmour square, Saman square, Hospital square, Transport nagar, Stadium square, PTS square,Jaistambh square, Nagar nigam, Prakash square and Civil lines along with control site (APS University Campus) of Rewa City for one year (October 2015 to November 2016). Sampling was carried out at the eleven different locations using Respirable Dust sampler (Envirotech model APM 460 BL-411) for 8 hours in a day at an average flow rate of 1.5 LPM as per the standards of Central Pollution Control Board (India). Monitoring is carried out once in a month at sampling sites. Suspended particulate matters (SPM) and respirable suspended particulate matters (RSPM) were collected on the dust cup and glass fabric filter paper respectively.

Table 1. Seasonal average concentrations of air pollutants (µg/m^3) in the ambient air of sampling sites and two way ANOVA showing inter-site and intra-seasonal (P_C-value) with inter site seasonal changes (P_R-value) of Rewa city observed during the year 2015-16

S.No.	Pollutants (µg/m^3)	Seasonal average concentrations			Two way ANOVA			
					F-value		P-value	
		W	S	R	C	R	P_C	P_R
1.	SPM	421.13±150.05	265.98±70.07	180.51±28.97	38.67	04.67	P<0.0001 ***	P=0.0016 **
2.	RSPM	94.04±31.26	81.32±31.60	46.15±16.71	68.47	20.81	P<0.0001 ***	P<0.0001 ***
3.	SO_2	30.89±7.13	23.38±5.48	17.88±3.86	79.69	14.29	P<0.0001 ***	P<0.0001 ***
4.	NO_X	41.60±13.30	30.38±9.35	21.76±5.29	40.89	09.05	P<0.0001 ***	P<0.0001 ***

Samples for determination of gaseous pollutants (SO_2 and NO_x) were collected by bubbling air samples in Sodium tetra chloromercurate and Sodium hydroxide-Sodium arsenate absorbent solutions respectively in impingers at flow rate of 1.5 LPM. These samples were analyzed for SO_2 and NO_X spectrophotometrically. Road side plant species, *Ficusbenjamina* growing at selected polluted sites of Rewa city, M.P. (India) was selected for its micro-morphological study.

Microscopicstudies

Leaf surface characteristics were studied with light microscope. For light microscopic study (LMS), leaf samples of *Ficusbenjamina* were collected from control and polluted sites during October 2015 to November 2016 and were properly washed with tap water and deiodenized water to remove all loose dust particles from their surface. The leaf epidermal peel slides were made by the methods of making lasting impressions. In this method, one square centimeter on surface of leaf was painted by a thick patch of clear or transparent nail polish. Nail polish is allowed to dry completely then a piece of clear cellophane tape is taped to the dried nail polish patch by a carton sealing tape. Gently, peeled out or take out the nail polish patch by pulling a corner of the tape and the finger nail polish along with the leaf peel. This leaf impression was taped on slides and labeled as abaxial and adaxial surface. Leaf impression was examined under 400 x total magnifications by light microscope ("Motic Images plus 2.0 ML" software). Number of stomata and epidermal cells were counted per square millimeter area. Length and width of epidermal cells and stomata guard cells of a leaf were measured in µm with the help of ocular micrometer under high power magnifications by micrometry i.e. "Stage-ocular micrometry".

Stomatal index is calculated by the formula of Salisbury (1927):

$$SI = S / (E+S) \times 100$$

Stomatal frequency is calculated by formula:

$$SF= (S/E) \times 100$$

Where, S= Average number of stomata and E= Average number of epidermal cells.

RESULTS AND DISCUSSION

Seasonal average concentrations of air pollutants in the ambient air of sampling sites have been computed from the basic data (Table 1). There was marked seasonal variation in concentrations of pollutants in the ambient air of Rewa city.

Results showed higher concentrations during winter months to be followed by summer and rainy months. The average concentrations of RSPM, SO_2 and NO_X monitored at sampling sites of Rewa city during three seasons are well within permissible limits as per National Ambient Air Quality Standards (NAAQS) and Central Pollution Control Board (CPCB), New Delhi. Whereas, SPM concentrations exceeded the prescribed permissible limit at all the monitored sites throughout the study period. This trend of seasonal variation in pollutant concentrations under present investigation supports the findings of other workers (Joshi *et al*., 1991, Bhaskar and Mehta 2010, Guttikunda and Jawahar 2011, Nair *et al*., 2014 and Jhamaria and Jadon 2016). The higher concentration of pollutants during winter season can be attributed to low temperature, increasing the density of air and reducing the dispersion of pollutants. Comparatively low concentrations of ambient air pollutants may be attributed to dispersion of pollutants due to high temperature and strong wind speed during summer months, and washing out of pollutants during monsoon months due to rains. The basic data have been computed with suitable statistical approach (ANOVA) to observe the inter as well as intra-sites seasonal differences in concentrations of various pollutants for the year 2015-16.

leaves growing at various sampling sites of the city than those of control sites during the study period. This decrease in length and width of guard cells was statistically significant at most of the sites during winter, summer and rainymonths. The number of stomata increased significantly at all the sites as compared

Table 2. Average number (per mm^2), length (µm) and width (µm) of epidermal cells of *Ficusbenjamina* growing at polluted and controlled sites of Rewa city

L= Length of epidermal cells in µm (micrometer), W= Width of epidermal cell in µm (micrometer), N= Number of epidermal cells per mm^2

Seasons	Sites		Polluted Site	Controlled site	t-test
WINTER	DORSAL	L	21.90±4.01	37.80±3.52	9.419* P <0.0001
		W	10.80±2.09	20.70±4.11	6.783 * P < 0.0001
		NEC	248.70±36.31	208.80±29.79	2.686* P=0.0151
	VENTRAL	L	24.60±3.95	38.40±3.09	8.693* P <0.0001
		W	13.50±2.91	24.60±3.40	7.835 * P <0.0001
		NEC	186.90±18.20	191.60±25.09	4.141 * P=0.0006
SUMMER	DORSAL	L	19.80±4.51	29.40±4.64	4.684 * P=0.0002
		W	11.40±1.89	19.80±3.79	6.261* P <0.0001
		NEC	243.90±45.21	213.30±14.31	2.040* P=0.0562
	VENTRAL	L	22.80±1.54	38.10±3.47	12.70* P <0.0001
		W	14.10±2.02	23.40±4.19	6.313* P <0.0001
		NEC	185.50±11.29	148.60±9.75	7.820* P <0.0001
RAINY	DORSAL	L	20.10±4.01	28.10±3.66	4.657* P=0.0002
		W	7.81±5.41	18.00±3.00	5.214* P<0.0001
		NEC	221.20±33.99	202.90±31.78	1.244 P=0.2296
	VENTRAL	L	21.60±2.75	31.10±3.72	6.482* P <0.0001
		W	12.00±3.16	20.70±2.98	36.329* P <0.0001
		NEC	182.90±7.35	149.60±22.10	4.521* P=0.0003

* Significant

➢ ‘t’ value at 18 d.f. on 0.5% level is 1.734

Results revealed that there was significant difference in SPM, RSPM, SO_2 and NO_X concentrations in ambient air of Rewa city between three seasons. Similarly, the observed pollutants concentrations varied significantly between different studied sites as well as within sites with respect to three seasons. Observations on number, length and width of epidermal cells of *Ficusbenjamina* growing at polluted sites and control site of Rewa city are presented in Table 2. Results revealed significant decrease in length and width of epidermal cells on both dorsal and ventral surfaces at all polluted sites, as compared to control site plants. The leaf samples from different sites collected during three seasons registered mixed results for number of epidermal cells on both the surfaces, as compared to control samples. Table 3 shows number of stomata, length and width of stomatal guard cells, stomatal frequency and stomatal index of leaf samples collected from polluted and controlled sites. Studies were conducted only on ventral surface of leaves of this species for guard cells because stomata were absent on dorsal surface due to hypostomatous nature of the plant. Results indicated greater decrease in length and width of guard cells on ventral surface of *Ficusbenjamina*

to control sites. The values of stomatal frequency and stomatal index were observed to be increased at all the sites during winter and rainy months. The summer leaf samples have shown lesser values of two parameters at all the sampling sites than those of control samples. This study demonstrated marked alteration in leaf surface characters of *Ficusbenjamina* due to urban or vehicular air pollution in Rewa city. More number of stomata and epidermal cells in leaves of this plant collected from polluted sites supports the findings of other researchers. Similar increase in the number of stomata and epidermal cells has been observed in various other plant species like *Jasminumsambac* (Kulshreshtha *et al*., 1980), *Calotropis gigantean* (Ramanathan *et al*., 1989), *Azadirachtaindica and Dalbergiasissoo*(Sharma and Roy, 1995), *Polyalthialongifolia* (Pal *et al*., 2000), *Cassia siamea* (Aggarwal, 2000), *Nyctanthesearbortristis, Quisqualisindica* and *Terminaliaarjuna* (Rai and Kulshreshtha, 2006), *Calotropisgigantia and Ipomoea Fistulosa* (Tiwari, 2012) and *Pongamia Pinnata* (Rai and Mishra, 2013). The reduction in the size of epidermal cells and stomata guard cells resulted due to inhibited cell elongation, leaf surface area and consequently the increase in frequency of the cell, as suggested by Rai and Kulshreshtha (2006). This reduction in stomata size is considered as an adaptive response of this plant to avoid entry of harmful constituents of exhaust which can otherwise cause adverse effects (Satyanarayana *et al*., 1990; Salgare, 1990 and Zarinkamar *et al*., 2013).

number of stomata and epidermal cells and decreased length and width of stomata guard cells and epidermal cells at polluted, as compared to control site. Reduction in the size of cells of stomata and epidermal cells was probably because of the adaptation in the plants in order to struggle with stressed condition of vehicular pollution.

Table 3. Average length (µm) and width (µm) of guard cells, number of stomata (per mm^2), stomatal frequency and stomatal index of *Ficusbenjamina* growing at polluted and controlled sites of Rewa city

LGC= Length of guard cells in µm (micrometer), WGC= Width of guard cells in µm (micrometer), NOS= Number of stomata per mm^2, SF= Stomatalfrequency, SI= Stomatal index

Seasons	Sites		Polluted Site	Controlled Site	t-test
WINTER	VENTRAL	LGC	30.30±2.62	35.40±5.44	2.669* P=0.0156
		WGC	22.20±1.54	28.50±5.52	3.473* P=0.0027
		NOS	41.71±12.90	31.20±3.79	2.472* P=0.0237
		SF	21.55	20.40	-
		SI	17.73	16.94	-
SUMMER	VENTRAL	LGC	32.40±3.95	35.40±4.85	1.517 P=0.1467
		WGC	22.20±3.22	25.20±4.73	1.657 P=0.1148
		NOS	35.30±2.40	30.40±6.57	2.215* P=0.0399
		SF	19.02	20.45	-
		SI	15.98	16.98	-
RAINY	VENTRAL	LGC	31.80±7.77	37.50±4.74	1.980* P=0.0632
		WGC	19.20±4.94	28.80±8.02	3.221* P=0.0047
		NOS	25.40±3.16	12.30±2.25	10.66* P<0.0001
		SF	13.98	8.22	-
		SI	12.19	7.59	-

* Significant

- ‘t’ value at 18 d.f. on 0.5% level is 1.734

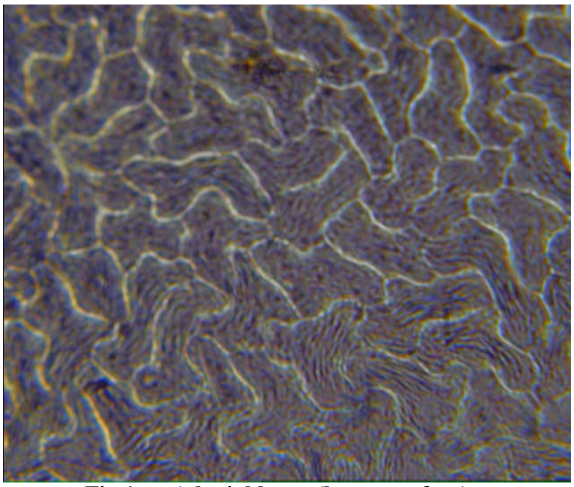

Fig 1. a. Adaxial layer (lower surface) of leaf of *Ficusbenjamina*

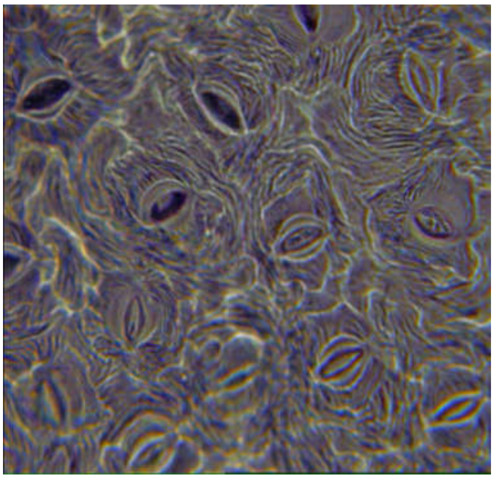

Fig. 1. b. Abaxial layer (upper surface) of leaf of *Ficusbenjamina*

Decline in stomatal frequency and stomatal index under present investigation supports the findings of Samal *et al*. (2002), Raina and Aggrawal (2004), Raina and Sharma (2006) and Raina and Bala (2011). Distorted shapes of stomata observed in *Ficusbenjamina* populations exposed to exhaust pollution (Fig.1) might have resulted due to lowering of pH in cytoplasm of the guard cells and also a change in turgor relations of the stomata complex (Kondo *et al*. 1980) and due to physiological injury inside the leaf (Ashenden and Mansfield 1978). This study illustrates significant alternation in micro-morphological attributes of *Ficusbenjamina* leaves exposed to urban air pollution in Rewa city, with increased

Conclusion

Plants play an important role in monitoring and maintaining the ecological balance and also provide enormous leaf area for impingement, absorption and accumulation of air pollutants to reduce the pollution level in the environment.

The present finding indicate that the exhaust released from automobiles and diesel engine vehicles causes abnormal changes in the micro morphological characters of leaf of *Ficusbenjamina* and these changes could be considered as indicator of environmental stress.

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