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Air pollution induced changes in foliar micro-morphology of road side shrub species, *Thevetia peruviana* and *Plumeria alba* in Rewa City, MP, India

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Abstract

This study was undertaken to evaluate the effect of air pollution on micro-morphological characters of two evergreen shrub species viz. Thevetia peruviana and Plumeria alba growing along the road sides in Rewa city. There was marked alternations in the micro-morphological attributes of two shrubs exposed to urban air pollution. The leaf samples of both the species showed increased number of epidermal cells and stomata, stomatal frequency and stomatal index on both dorsal and ventral surface at polluted sites as compared to control sites. The leaf samples collected from polluted sites exhibited decreased length and width of epidermal cell and guard cell as compared to control ones. The results indicate that these two shrubs have developed some adaptations or changes to survive under stressed conditions of air pollution.

Keywords: Air pollution, auto exhaust, epidermal cell, stomata, micro-morphology.

Introduction

The industrialization and vehicular emissions constitutes major sources of environmental pollution in Indian cities. Petrol and diesel engine driven motor vehicles release a wide variety of pollutants particularly benzene, carbon monoxide, organic compounds, oxides of nitrogen, sulphur dioxide and suspended particulate matters like ultra fine primary particles, smoke, metals (Cd, Co, Cu, Pb, etc.) and inert dust. Also the ultra-fine particles when released quickly coagulate to form larger particles, through reaction with other pollutants like ammonia, sulphur dioxide, nitrogen oxides and volatile organic compounds[1]. Plants are sinking for air pollution[2] and air pollution affects plants mainly through the uptake of pollutants into the leaves through stomata. Sulphur dioxide and ozone are the two most important air pollutants which affect plants[3]. The plants growing under the stressed conditions of pollution develop different micro-morphological[4-6], anatomical[7-9] and biochemical[10-13] changes.

Plants growing near the road side plays an important role in accumulation of pollutants and act as efficient interceptors of airborne pollutants. Various researchers have noticed adverse impacts of urban air pollution on leaf structure of plants[14-21]. Vehicular exhausts are the main source of pollution in ambient atmosphere of the Rewa city. This study was undertaken to assess the changes caused by air pollution in epidermal traits of roadside shrub species; *Thevetia peruviana* and *Plumeria alba.*

Materials and methods

Selection of Site: The present study was conducted in Rewa city, which is situated on the north east border of Madhya Pradesh, central part of India.

Selection of plant: For micro-morphological study, road side shrub species, *Thevetia peruviana* and *Plumeria alba* growing at five polluted sites viz; Sirmour Chowk, Saman Chowk, Dhobia Tanki, Transport Nagar and Civil Lines of Rewa city, MP, India and A.P.S. University, Rewa campus, India as control site were selected.

Microscopic studies: Microscopic studies: Leaf surface characteristics were studied with the help of optical microscope. The leaf epidermal peel slides were made by the methods of making lasting impressions. In this method, fresh leaf samples of *Thevetia peruviana* and *Plumeria alba* were collected from control and polluted sites during the four month period from March to June 2016 and were properly washed from distilled water to remove dust and other pollutants from the upper and lower surfaces, wiped out the water from the surfaces Painted a thick patch of clear nail polish on the leaf surface of at least one square centimeter. Nail polish is allowed to dry completely, then a piece of clear cellophane carton sealing tape is taped on the dried nail polish patch. By pulling a corner of the tape gently, peeled out the nail polish patch and the finger nail polish along with the leaf peal. This is the leaf impression which was taped on slides and labeled as abaxial or adaxial surfaces and name of leaf samples etc. Leaf impression was examined under at least 400x magnification 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[22]:

SI = Average number of stomata / (Average number of epidermal cells + Average number of stomata) ×100

Stomatal frequency is calculated by formula:
SF = (Average number of stomata / Average number of epidermal cells) ×100

Results and discussion

The leaves of two evergreen shrub species *Thevetia peruviana* and *Plumeria alba* growing at five polluted sites of Rewa city were observed for number of stomata and epidermal cells, length and width of guard cells and epidermal cells. Similar observations were also made for the respective shrub species growing in the campus of University, a control site.

Table-1 and Table-2 represents observations on number, length and width of epidermal cells of *Thevetia peruviana* and *Plumeria alba* growing at polluted and control sites of Rewa city, respectively. Results revealed increased number of epidermal cells per unit area on both dorsal and ventral surface of leaves of *Thevetia peruviana* and *Plumeria alba* at polluted sites, as compared to control site (Table-1 and Table-2). This increase in number of epidermal cells at three polluted sites (Samanchowk, Dhobiatanki and Civil lines) was statistically significant on dorsal surfaces but insignificant on ventral surface of the leaves of *Thevetia Peruviana* (Table-3). Whereas in *Plumeria alba* leaves, increase in number of epidermal cells at polluted sites was statistically insignificant on dorsal surface but significant on ventral surface in the leaves (Table-4). Also leaf samples collected from polluted sites showed insignificantly reduced length of epidermal cells on both the surface and significantly reduced width of epidermal cells on ventral surface of *Thevetia peruviana* (Table-3). Whereas leaves of *Plumeria alba* collected from polluted sites showed significantly reduced length and width of epidermal cells on Ventral surface than those of control samples (Table-4). *Thevetia peruviana* has stomata (anisocytic) only on dorsal surface of leaves (hypostomatal), whereas *Plumeria alba* is an amphistomatic species i.e. stomata (paracytic) are found on both the surfaces of the leaves. Number of stomata, length and width of stomatal guard cells, stomatal frequency and stomatal index of leaf samples of Thevetia *peruviana* and *Plumeria alba* collected from polluted and controlled sites are presented in Table-5 and Table-6 respectively. There was significant increase in number of stomata on ventral surface of *Thevetia peruviana* (Table-7) and on both the surfaces of *Plumeria alba* (Table-8) at polluted sites. In leaves of polluted sites the length of guard cells decreased significantly on ventral surface of both the shrub species (Table-7 and Table-8) where as width of guard cells increased significantly on both the surfaces of *Plumeria alba* only (Table-8).

Stomatal shapes were observed distorted on ventral surface of leaves at polluted sites (Figure-1 and Figure-2). The leaves of both the species showed increased values of stomatal frequency and stomatal index at polluted sites, as compared to control ones.

Discussion: Study demonstrated marked alteration in leaf surface characters of *Thevetia Peruviana* and *Plumeria alba* due to auto exhaust urban air pollution in Rewa city. More number of stomata and epidermal cells in leaves of theses shrubs collected from polluted sites supports the findings of other researchers[23,24,16,18,4,19, 25, 26]. The reduction in size of epidermal cells and stomata guard cells in the leaves of two shrubs might have resulted due to inhibited cell elongation, leaf surface area and consequently the increase in frequency of the cell, as suggested by Rai and Kulshreshtha[19]. This reduction in stomata size is considered as an adaptive response of the plant to avoid entry of harmful constituents of exhaust which can otherwise cause adverse effects[27-29].

Distorted shapes of stomata observed in *Thevetia Peruviana* and *Plumeria alba* exposed to auto exhaust pollution (Figure-1 and Figure-2 respectively) might have resulted due to lowering of pH in cytoplasm of the guard cells and also a change in turgour relations of the stomata complex[30] and due to physiological injury inside the leaf[31]. Increase in stomatal frequency and stomatal index at polluted sites in studied plant species is supported by several workers[32,33,18,34].

Table-1: Average number (per mm^2), length (µm) and width (µm) of epidermal cells of *Thevetia peruviana* growing at polluted and controlled sites of Rewa city.

Leaf Surfaces	Epidermal Characters	Polluted Sites					Control Site
		Sirmour Chowk	Saman Chowk	Dhobia Tanki	Transport Nagar	Civil Lines	
Dorsal	LEC	9.0±2.44	8.7±2.62	9.6±2.36	10.2±2.098	9.9±2.84	11.4±7.14
	WEC	6.0±3.16	6.3±2.98	6.6±3.95	6.30±3.302	5.4±2.36	10.5±4.74
	NEC	28.2±11.82	28.9±8.37	30.50±6.80	27.1±11.20	29.5±10.3	21.2±6.74
Ventral	LEC	24.3±9.63	24.6±9.9	25.8±10.78	24.9±8.25	24.6±2.5	27.0±7.34
	WEC	12.6±5.79	12.0±6.16	13.2±5.32	11.4±6.29	12.3±6.40	14.1±5.66
	NEC	36.5±5.56	36.2±5.61	36.7±6.05	38.1±5.40	37.3±4.92	31.9±10.81

LEC= Length of epidermal cells, WE = Width of epidermal cells, NEC= Number of epidermal cells.

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

Leaf Surfaces	Epidermal Characters	Polluted Sites					Control Site
		Sirmour Chowk	Saman Chowk	Dhobia Tanki	Transport Nagar	Civil Lines	
Dorsal	LEC	31.20±4.73	30.60±5.25	30.90±3.47	31.20±2.53	31.50±4.74	33.6±5.79
	WEC	17.10±3.47	16.50±3.80	16.20±3.22	15.30±3.59	17.40±2.75	18.0±3.16
	NEC	99.20±10.50	99.60±10.10	98.60±8.20	100.20±9.53	95.0±8.51	93.3±8.67
Ventral	LEC	26.40±3.09	25.80±2.89	24.90±3.17	23.70±2.62	26.4±3.09	47.7±4.11
	WEC	14.40±2.757	14.10±2.47	14.40±3.09	13.80±2.53	14.4±3.09	22.5±3.24
	NEC	66.30±5.51	67.10±4.88	68.0±4.92	69.30±5.39	65.3±5.55	139.1±7.56

LEC= Length of epidermal cells, WE = Width of epidermal cells, NEC= Number of epidermal cells.

Table-3: Values of 't' test between number of epidermal cells and size of epidermal cells of *Thevetia peruviana* leaves of polluted and controlled sites of Rewa city.

Leaf Surfaces	Epidermal Characters	Polluted Sites				
		Sirmour Chowk	Saman Chowk	Dhobia Tanki	Transport Nagar	Civil Lines
Dorsal	LEC	1.006 P=0.3278	1.123 P=0.2763	0.7569 P=0.4589	0.5099 P=0.6163	0.613 P=0.5448
	WEC	2.497** P=0.0224	2.371** P=0.0291	1.999* P=0.0610	2.299** P=0.0337	3.046*** P=0.0070
	NEC	1.626 P=0.1212	2.265** P=0.0361	3.070*** P=0.0066	1.427 P=0.1707	2.125* P=0.0477
Ventral	LEC	0.7048 P=0.4900	0.6126 P=0.5478	0.2908 P=0.7745	0.6013 P=0.551	0.9788 P=0.3407
	WEC	0.5855 P=0.5655	0.7936 P=0.4378	0.3661 P=0.7186	1.009 P=0.3265	0.6662 P=0.5137
	NEC	1.196 P=0.2472	1.116 P=0.2791	1.225 P=0.2365	1.622 P=0.1223	1.437 P=0.1678

LEC= Length of epidermal cells, WE=Width of epidermal cells, NEC=Number of epidermal cells. * Significant; 't' value at 18 d.f. on 0.05% level is 1.734.

Table-4: Values of 't' test between number of epidermal cells and size of epidermal cells of *Plumeria alba* leaves of polluted and controlled sites of Rewa city.

Leaf Surfaces	Epidermal Characters	Polluted Sites				
		Sirmour Chowk	Saman Chowk	Dhobia Tanki	Transport Nagar	Civil Lines
Dorsal	LEC	1.015 P=0.3236	1.213 P=0.2407	1.264 P=0.2224	1.201 P=0.2453	0.8875 P=0.3865
	WEC	0.6064 P=0.5518	0.9586 P=0.3505	1.262 P=0.2232	1.785* P=0.0912	0.4529 P=0.6560
	NEC	1.370 P=0.1876	1.497 P=0.1518	1.404 P=0.1774	1.693 P=0.1076	0.4425 P=0.6634
Ventral	LEC	13.09**** P<0.0001	13.77**** P<0.0001	13.88**** P<0.0001	15.56**** P<0.0001	13.10**** P<0.0001
	WEC	6.021**** P<0.0001	6.520**** P<0.0001	5.714**** P<0.001	6.693**** P<0.0001	5.721**** P<0.0001
	NEC	24.60**** P<0.0001	25.29**** P<0.0001	24.92**** P<0.0001	23.55**** P<0.0001	24.88**** P<0.0001

LEC= Length of epidermal cells, WE = Width of epidermal cells, NEC= Number of epidermal cells. *Significant; 't' value at 18 d.f. on 0.05% level is 1.734

Table-5: Average length (µm) and width (µm) of guard cells, number of stomata (per mm^2), stomatal frequency and stomatal index of *Thevetia peruviana* growing at polluted and controlled sites of Rewa city.

Leaf Surfaces	Stomata Characteristics	Polluted sites					Control site
		Sirmour Chowk	Saman Chowk	Dhobia Tanki	Transport Nagar	Civil Lines	
Ventral	LGC	12.9±5.66	11.70±5.376	12.0±5.65	11.10±5.48	12.0±5.47	15.90±3.47
	WGC	8.10±4.48	9.30±3.59	9.6±2.75	9.0±3.16	9.0±3.74	10.80±2.09
	NS	20.7±7.212	21.20±6.52	21.80±6.61	21.0±7.39	21.1±6.64	14.40±4.00
	SF	56.71	58.56	59.40	55.11	56.56	45.14
	SI	36.18	36.93	37.26	35.53	36.13	31.10

LGC=Length of guard cells, WGC=Width of guard cells, NS=Number of stomata, SF=Stomatal frequency, SI=Stomatal index

Table-6: Average length (µm) and width (µm) of guard cells, number of stomata (per mm^2), stomatal frequency and stomatal index of *Plumeria alba* growing at polluted and controlled sites of Rewa city.

Leaf Surfaces	Stomata Characteristics	Polluted sites					Control site
		Sirmour Chowk	Saman Chowk	Dhobia Tanki	Transport Nagar	Civil Lines	
Dorsal	LGC	14.10±12.33	13.50±11.93	12.30±10.81	11.10±11.83	14.9±13.42	6.6±8.80
	WGC	12.90±10.05	12.60±9.77	12.60±9.57	11.70±10.33	12.3±11.52	3.9±5.10
	NS	1.10±0.87	1.50±0.850	1.40±0.96	1.60±1.07	1.30±0.823	0.6±0.84
	SF	1.10	1.50	1.41	1.50	1.36	0.64
	SI	1.09	1.48	1.40	1.48	1.34	0.63
Ventral	LGC	25.20±4.29	24.60±4.85	24.90±4.70	23.40±5.44	25.5±3.77	31.2±6.03
	WGC	24.60±3.95	24.0±4.24	24.30±4.11	21.90±2.47	25.5±4.06	37.5±4.74
	NS	13.80±2.04	14.0±2.00	14.0±1.054	14.30±0.823	13.80±1.03	12.3±2.25
	SF	20.81	20.86	20.5	20.63	21.13	8.84
	SI	17.22	17.26	17.07	17.10	17.44	8.12

LGC=Length of guard cells, WGC=Width of guard cells, NS=Number of stomata, SF=Stomatal frequency, SI=Stomatal index.

Table-7: Values of 't' test between average number of stomata and size of guard cells of *Thevetia peruviana* leaves of polluted and controlled sites of Rewa city.

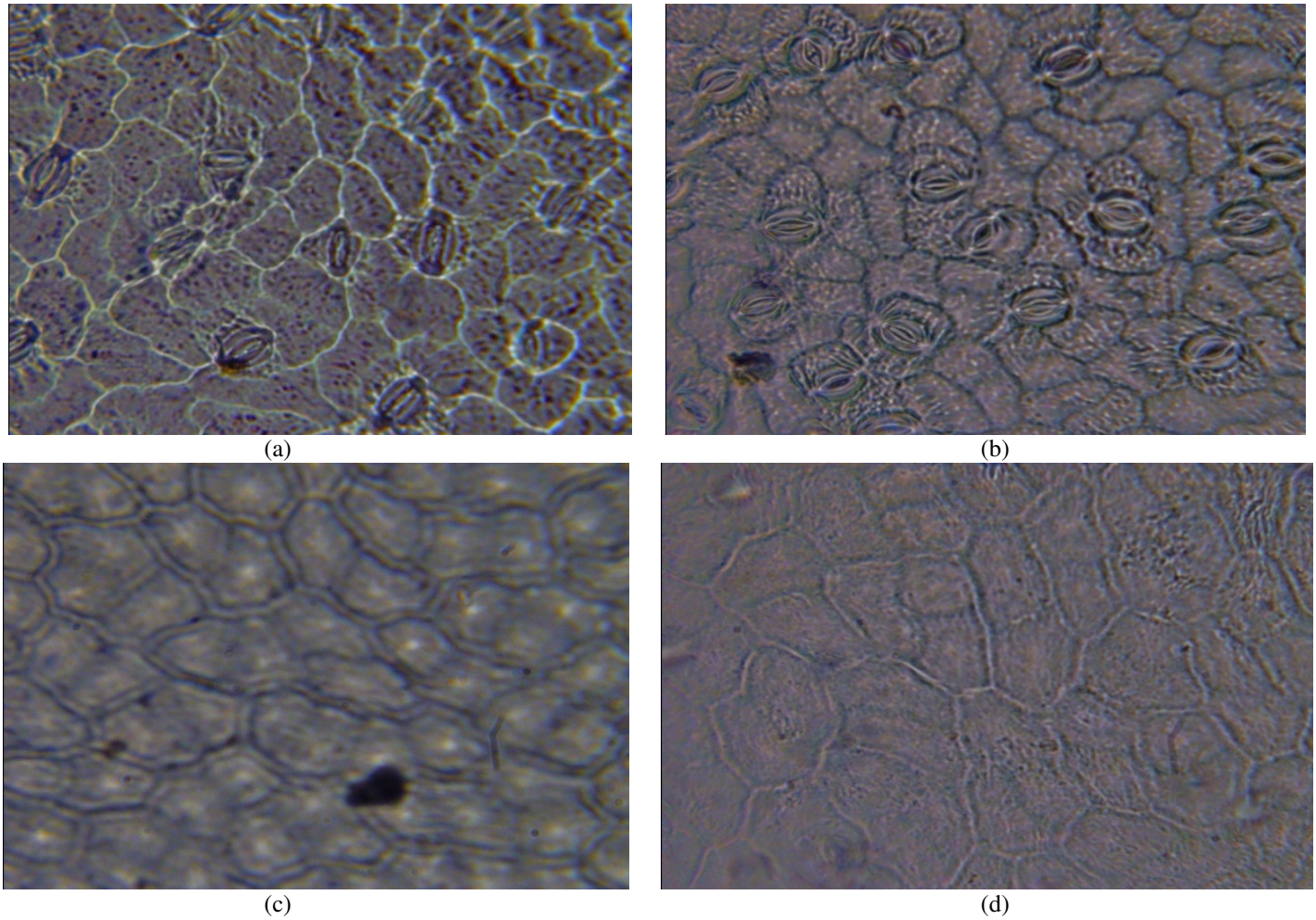
Leaf Surfaces	Stomata characteristics	Polluted sites				
		Sirmour Chowk	Saman Chowk	Dhobia Tanki	Transport Nagar	Civil lines
Ventral	LGC	1.428 P=0.1704	2.074* P=0.0527	1.859* P=0.0795	2.338** P=0.0311	1.902* P=0.0732
	WGC	1.726 P=0.1015	1.141 P=0.2688	1.097 P=0.2871	1.500 P=0.1509	1.327 P=0.2010
	NS	2.415** P=0.0266	2.810** P=0.0116	3.026*** P=0.0073	2.483** P=0.0231	2.732** P=0.0137

LGC = Length of guard cells, WGC = Width of guard cells, NS = Number of stomata, SF = Stomatal frequency, SI = Stomatal index. *Significant ;'t' value at 18 d.f. on 0.05% level is 1.734.

Table-8: Values of't' test between average number of stomata and size of guard cells of *Plumeria alba* leaves of polluted and controlled sites of Rewa city.

Leaf Surfaces	Stomata characteristics	Polluted sites				
		Sirmour Chowk	Saman Chowk	Dhobia Tanki	Transport Nagar	Civil lines
Dorsal	LGC	1.566 P=0.1348	1.472 P=0.1583	1.293 P=0.2123	0.9648 P=0.3474	1.636 P=0.1193
	WGC	2.535** P=0.0207	2.496** P=0.0225	2.537** P=0.0207	2.141** P=0.0462	2.108** P=0.0493
	NS	1.307 P=0.2075	2.382** P=0.0285	1.983* P=0.0628	2.318* P=0.0324	1.886* P=0.0756
Ventral	LGC	2.564** P=0.0195	2.697** P=0.0147	2.600** P=0.0179	3.037*** P=0.0071	2.535** P=0.020
	WGC	6.611**** P<0.0001	6.713**** P<0.0001	6.653**** P<0.0001	9.230**** P<0.001	6.080**** P<0.001
	NS	1.560 P=0.1361	1.786* P=0.0910	2.164* P=0.0442	2.640* P=0.0166	1.916* P=0.0714

LGC=Length of guard cells, WGC=Width of guard cells, NS=Number of stomata, SF=Stomatal frequency, SI=Stomatal index.
* Significant; 't' value at 18 d.f. on 0.05% level is 1.73.



(a) (b) (c) (d)

Figure-1: Lower foliar surface of *Thevetia peruviana* showing anisocytic stomata at control (a) and polluted (b) site; upper foliar surface showing waxy-walled epidermal cells at control(c) and polluted (d) site.

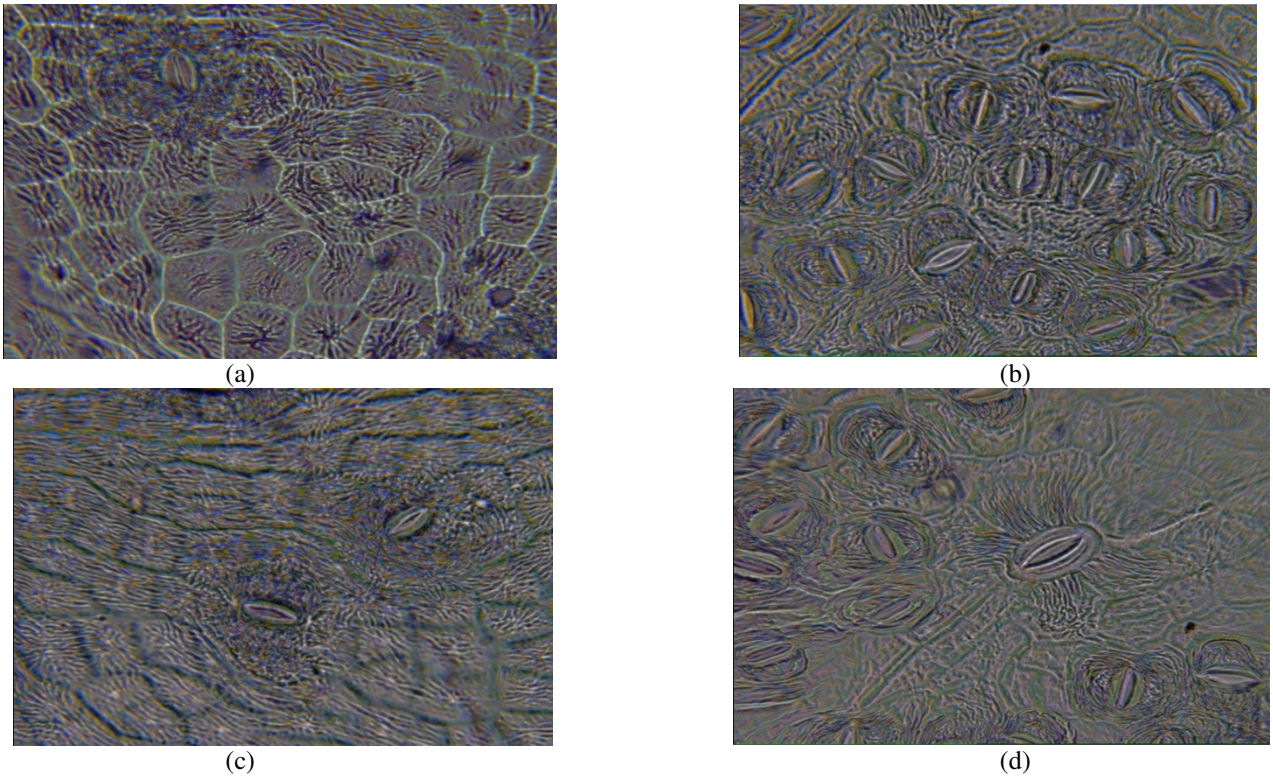


Figure-2: Lower foliar surface of *Plumeria alba* showing paracytic stomata at control (a) and polluted (b) site; upper foliar surface showing paracytic stomata and epidermal cells at control (c) and polluted (d) site.

Conclusion

This study illustrates significant alternation in micro-morphological attributes of *Thevetia peruviana* and *Plumeria alba* leaves exposed to urban air pollution in Rewa city, with increased number of stomata and epidermal cells and decreased length and width of stomata guard cells and epidermal cells on both abaxial and adaxial surface at polluted sites, 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 auto exhaust pollution.

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