EFFECT OF DUST POLLUTION ON THE ANATOMICAL STRUCTURE OF LEAVES OF *ANNONA SQUAMOSA* AND *DALBERGIA SISSOO* GROWING ALONG THE ROAD SIDE OF REWA CITY (M. P.)

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Abstract: *Plants growing near the roadsides absorb the pollutants at their foliar surface. In this paper, anatomical changes induced by air pollutants in Annona squamosaand Dalbergia sissoo leaves were seen. Some plant species able to absorb, detoxify and tolerate high levels of pollution. Number of stomata and epidermal cells, length and width of epidermal cells and stomatal guard cells, stomatal frequency and stomatal index of these species from highly polluted sites and low polluted sites were investigated by light microscopy. Results revealed increase in stomatal number, epidermal cells number and stomatal index and stomatal frequency in leaves with decrease in stomatal size of leaves from polluted sites as compared to control ones. These alternations in anatomical structure of leaves could be an indicator of pollution stress.*

Keywords: Foliar Surface, Anatomical, Epidermal Cells, Stomatal index and Stomatal frequency.

Introduction:Air pollution is a major environmental problem faced by all developed and developing countries of the world. Dust particles form a major part of air pollutants arising due to industrialization, unplanned urbanization or expansion of cities, alarming increase in vehicle fleet, increase in traffic, population explosion rapid economic development and higher level of energy consumption that pose serious threat to the ecosystem. In India approximately 30-35% of air pollutants comprises of dust particles only. Air pollution in urban area is mainly due to emissions of industrial gases, traffic related particulates and activities such as coal mining, quarrying, stone crushing, thermal power plants, cement industries etc., adds huge quantities of dust to the environment which undergo dispersion, transport and chemical reaction in the atmosphere and are deposited as gaseous ions, solid and liquid particles. Rapid urbanization and growing population results serious effect on human life and its environment in recent years resulted in deterioration of ambient air quality.Vehicular exhaustis a major source of environmental pollution in India.

Pollutants commonly found in dust present on the roads are harmful not only to roadside vegetation but also to wildlife and the neighboring human settlements. Plants are used for monitoring air pollutants as different plant species respond to different types of air pollutants because leaf is the most sensitive part to be affected by air pollutants instead of all other plant parts such as stem and roots as it provides enormous area for the absorption and accumulation of air pollutants and have been regarded as bio-filters that reduce the pollution level in the ambient air environment. The sensitivity rests on the fact that the major portions of the important physiological processes are concerned with leaf because after deposition dust particles have a tendency to stick to the leaf surfaces for a definite period of time till they get washed off with rain or shredding of the leaf itself.

Researchers also reported that plants which are sensitive to air pollutants had showed changes in their morphology, anatomy, physiology and biochemistry[1-2] (Reig-Armiñana et al., 2004 and Silva et al., 2005). Various authors investigated the effects of pollution on different plant species. Various researches carried out in India have also reported similar effect of air pollution on micro-morphological parameters of different plant species at various places[3-11] (Palaniswamyet al., 1995; Morison, 1998; Aggarwal, 2000; Samal and Santra 2002; Raina and Sharma, 2006; Raina and Agrawal, 2004; Tiwari, 2012; Pawar, 2013 and Rai and Singh, 2015), anatomical[12-15] (Salgare and Rawal, 1990; Salgare and Acharekar1991; Giri et al., 2013; Pawar , 2015).

Present research work was undertaken to examine the effect of dust pollution on anatomical structure of leaves of two tree species viz; *Annona squamosa*and*Dalbergia sissoo* such as number of stomata and epidermal cells, length and width of epidermal cells and stomatal guard cells, stomatal frequency and stomatal index, etc. in some road side trees of Rewa city with application of suitable statistical approaches.

Materials and Methods:

Selection of Site- The present study was conducted in Rewa city, which is situated on the north-eastern part of Madhya Pradesh state, central part of India. The geographical area of Rewa district is 6,314 kilometers. Rewa is having three mega cement plants and some small scale industries at the outskirts, including a few stone crushers.

Selection and sample collection of plant- For anatomical study road side tree species viz. *Annona squamosa*and*Dalbergia sissoo*growing at polluted sites of Rewa city, M.P. India and APS University, Rewa campus as control site were selected. During sampling, mature leaf samples were collected from middle canopy area of each plant species.

Microscopy or Anatomical Study: Anatomicalcharacteristics of leaves were studied with optical microscope. By lasting impressions method the leaf epidermal peel slides were made. Leaf impression was examined under 400x magnification by light microscope ("MagVision" software of Olympus optical microscope) and size of epidermal cells and guard cells were measured with the help of this software. Stomatal number and epidermal cell number were counted per square millimeter area and the stomatal frequency and stomatal index were calculated by using the following formulae (Salisbury, 1927)[16]:

$$\text{Stomatal frequency (S.F.)} = \frac{S}{E} \times 100$$

$$\text{Stomatal Index (S.I.)} = \frac{S}{E+S} \times 100$$

where,S = Average number of stomata
E = Average number of Epidermal cells

Results: The present study was conducted to assess the effect of dust pollution on anatomical structure of leaves of two selected tree species viz; *Annona squamosa*and*Dalbergia sissoo* growing under ambient field conditions at various polluted sites located at different distances in, Rewa city (M.P.). Similar observations were also made for the respective tree species growing in the campus of A.P.S University, Rewa, a control site.

Table-1 shows an average stomatal number, length and width of guard cells of both dorsal and ventral of of*Annona squamosa*leaves growing in polluted and control sites. *Annona squamosa*has stomata only on ventral surface of the leaves as it is a hypostomatal species collected from both the sites (polluted and control site). Leaf samples of this species showed significantly increased number of stomata and reduced length and width of stomatal guard cell at polluted site (Table-2). Similarly, the values of stomatal index and stomatal frequency were also observed to be higher for the leaves sampled from polluted site.Table-3 shows average number of epidermal cells of both surface of leaves of *Annona squamosa* growing at polluted and control sites. Results indicated an increased number of epidermal cells per unit area (mm^2) on dorsal and ventral surface of leaves at polluted site than those of control site. However, this increase in number was statistically insignificant except for the ventral surface of leaves (Table-4). Average length and width of epidermal cells of both surfaces of leaves of *Annona squamosa* sampled at polluted and controlled sites are also given in table-5.3. There was significant decrease in the length and width of epidermal cells of both the surface of leaves of polluted site than those of control ones except the width of dorsal leaf surface which is insignificantly reduced (Table 3 and 4).

Table- 5 Shows average stomatal number, length and width of guard cells of both dorsal and ventral surface of leaves of *Dalbergia sissoo* growing in the polluted and conrol sites of Rewa city. The leaves of *Dalbergia sissoo*have shown stomata on both dorsal and ventral surface collected from polluted and controlled site. Results (Table-5) clearly indicated an increased number of stomata and decreased length and width of stomatal guard cells of leaves of this species on both dorsal and ventral surfaces at polluted sites as compared to control site. This increase in stomata number and decrease in length and width of guard cells at polluted site was significant (Table-5.6). Similarly, the values of stomatal index and somatal frequency were also observed to be higher for the leaves sampled from polluted site.Table-7 shows number of epidermal cells of both surface of leaves of *Dalbergia sissoo* growing at polluted and control site. Results indicated that number of epidermal cells per unit area (mm^2) at polluted site increased significantly on both the surface than those of control site (Table-8).Average length and width of epidermal cells of both surface of leaves of *Dalbergia sissoo* sampled at polluted and control sites are also given in table 5.7. Results revealed that there was decrease in size of length and width of epidermal cells of both the surface of leaves of polluted site than those of control ones. However, this decrease in length and width of epidermal cells in leaves of polluted site was significant on both dorsal and ventral surface of leaves (Table-8).

Figure-1 and Figure-2 showed shapes of stomata and epidermal cells were observed on ventral surface of leaves of *Annona squamosa* and on both the surfaces of leaves of *Dalbergia sissoo* at polluted sites ().

Table 1 - Average number of stomata (mm^2), length (µm) and width of guard cells (µm), stomatal frequency (%) and stomatal index (%) of *Annona squamosa* leaves.

Leaf Surfaces \ Sites		Polluted	Controlled
DORSAL	LGC	-	-
	WGC	-	-
	NOS	-	-
	SF	-	-
	SI	-	-
VENTRAL	LGC	30.583 ± 6.622	39.178 ± 6.439
	WGC	25.244 ± 3.587	32.611 ± 9.414
	NOS	13.7 ± 2.907	3.7 ± 1.880
	SF	13.40	10.37
	SI	11.82	9.39

Table 2- Values of 't' test between stomatal number and size of guard cells of *Annona squamosa* leaves.

Leaf Surfaces	Stomata characteristics	t-test	P value
DORSAL	LGC	-	-
	WGC	-	-
	NOS	-	-
VENTRAL	LGC	2.943**	P=0.0087
	WGC	2.312*	P=0.0328
	NOS	9.134***	P<0.0001

Table 3- Average number (mm²), length (µm) and width (µm) of epidermal cells of *Annona squamosa* leaves.

Leaf Surfaces \ Sites		Polluted	Controlled
DORSAL	LEC	59.989 ± 10.455	78.662 ± 11.991
	WEC	41.068 ± 12.628	43.897 ± 7.67
	NEC	138.7 ± 11.677	132.7 ± 5.518
VENTRAL	LEC	55.81 ± 20.688	76.4 ± 12.276
	WEC	26.774 ± 7.922	39.498 ± 16.392
	NEC	132.10 ± 24.902	102.20 ± 21.401

Table 4- Values of 't' test for number and size of epidermal cell of *Annona squamosa* leaves.

Leaf Surfaces	Epidermal cells	t-test	P value
DORSAL	LEC	3.712**	P=0.0016
	WEC	0.4119	P=0.6853
	NEC	1.469	P=0.1591
VENTRAL	LEC	2.707*	P=0.0144
	WEC	2.210*	P=0.0403
	NEC	2.880**	P=0.3295

Table 5- Average number of stomata (mm²), length (µm) and width of guard cells (µm), stomatal frequency (%) and stomatal index (%) of *Dalbergia sissoo* leaves.

Leaf Surfaces \ Sites		Polluted	Controlled
DORSAL	LGC	39.569 ± 6.93	113.205 ± 12.215
	WGC	21.931 ± 2.409	62.425 ± 4.176
	NOS	1.70 ± 0.496	0.7 ± 0.767
	SF	2.106	1.75
	SI	2.063	1.719
VENTRAL	LGC	29.03 ± 10.047	60.998 ± 20.642
	WGC	29.572 ± 4.337	93.508 ± 3.703
	NOS	18.3 ± 4.580	4.8 ± 3.008
	SF	22.704	8.823
	SI	18.635	8.108

Table 6- Values of 't' test between stomatal number and size of guard cells of *Dalbergia sissoo* leaves.

Leaf Surfaces	Stomata characteristics	t-test	P value
DORSAL	LGC	16.58***	P<0.0001
	WGC	26.56***	P<0.0001
	NOS	3.462**	P=0.0028
VENTRAL	LGC	5.643***	P<0.0001
	WGC	35.45***	P<0.0001
	NOS	7.791***	P<0.0001

Table 7- Average number (mm^2), length (µm) and width (µm) of epidermal cells of *Dalbergia sissoo*leaves.

Leaf Surfaces \ Sites		Polluted	Controlled
DORSAL	LEC	51.404 ± 20.877	86.761 ± 5.594
	WEC	46.835 ± 9.292	60.749 ± 5.594
	NEC	80.7 ± 16.152	40.00 ± 6.429
VENTRAL	LEC	30.269 ± 36.344	63.037 ± 7.767
	WEC	35.974 ± 11.700	66.809 ± 6.653
	NEC	80.6 ± 18.21	54.4 ± 24.806

Table 8- Values of 't' test for number and size of epidermal cell of *Dalbergia sissoo* leaves.

Leaf Surfaces	Epidermal cells	t-test	P value
DORSAL	LEC	5.173***	P< 0.0001
	WEC	4.057***	P= 0.0007
	NEC	7.403***	P< 0.0001
VENTRAL	LEC	2.788*	P= 0.0121
	WEC	7.245***	P< 0.0001
	NEC	2.692*	P= 0.0149

NOS= Number of Stomata

LGC= Length of Guard cell

WGC= Width of Guard cell

SI= Stomatal Index

SF= Stomatal Frequency

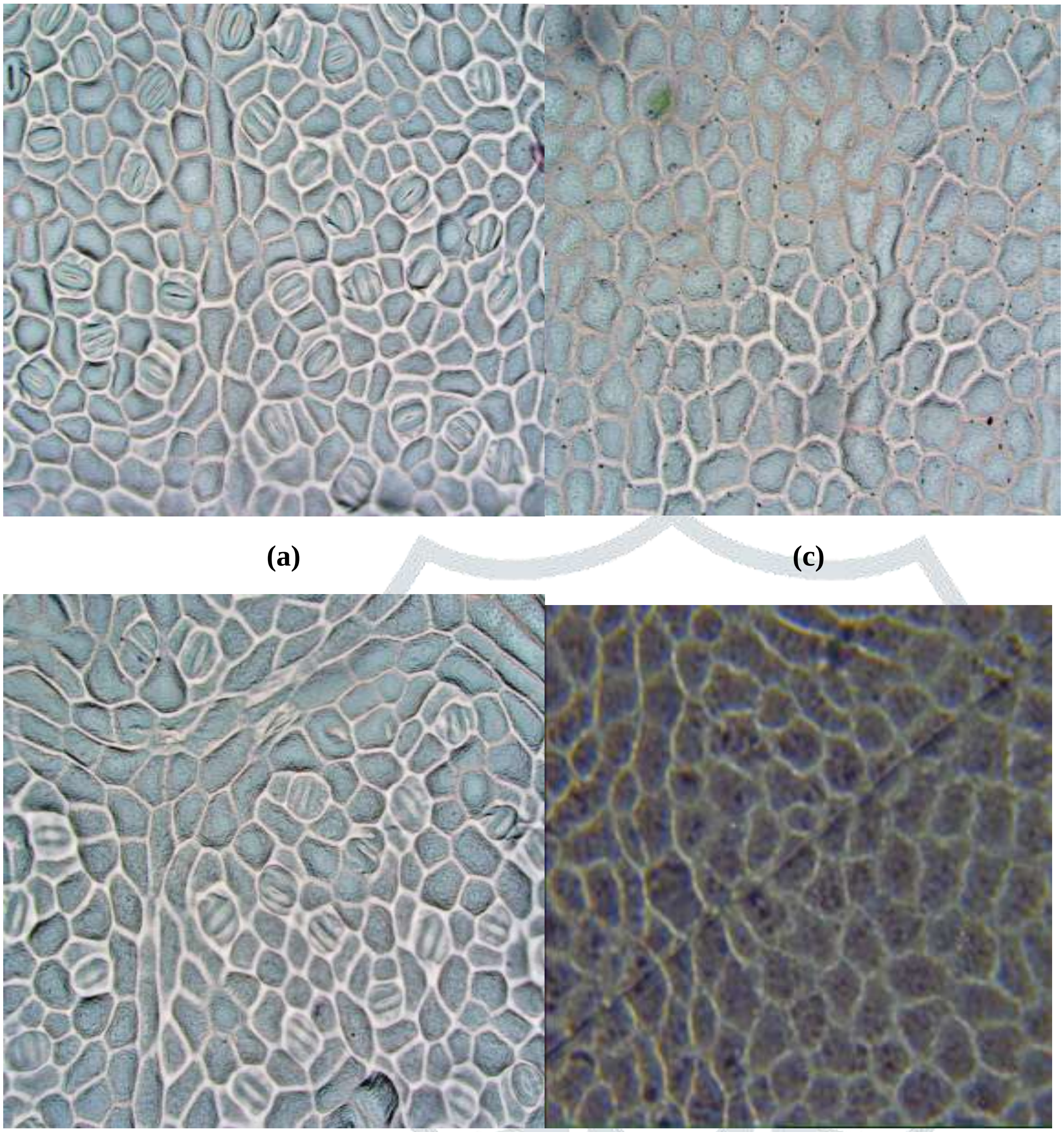
LEC= Length of Epidermal cell

WEC= Width of Epidermal cell

NEC= Number of Epidermal cells

*** Significant**

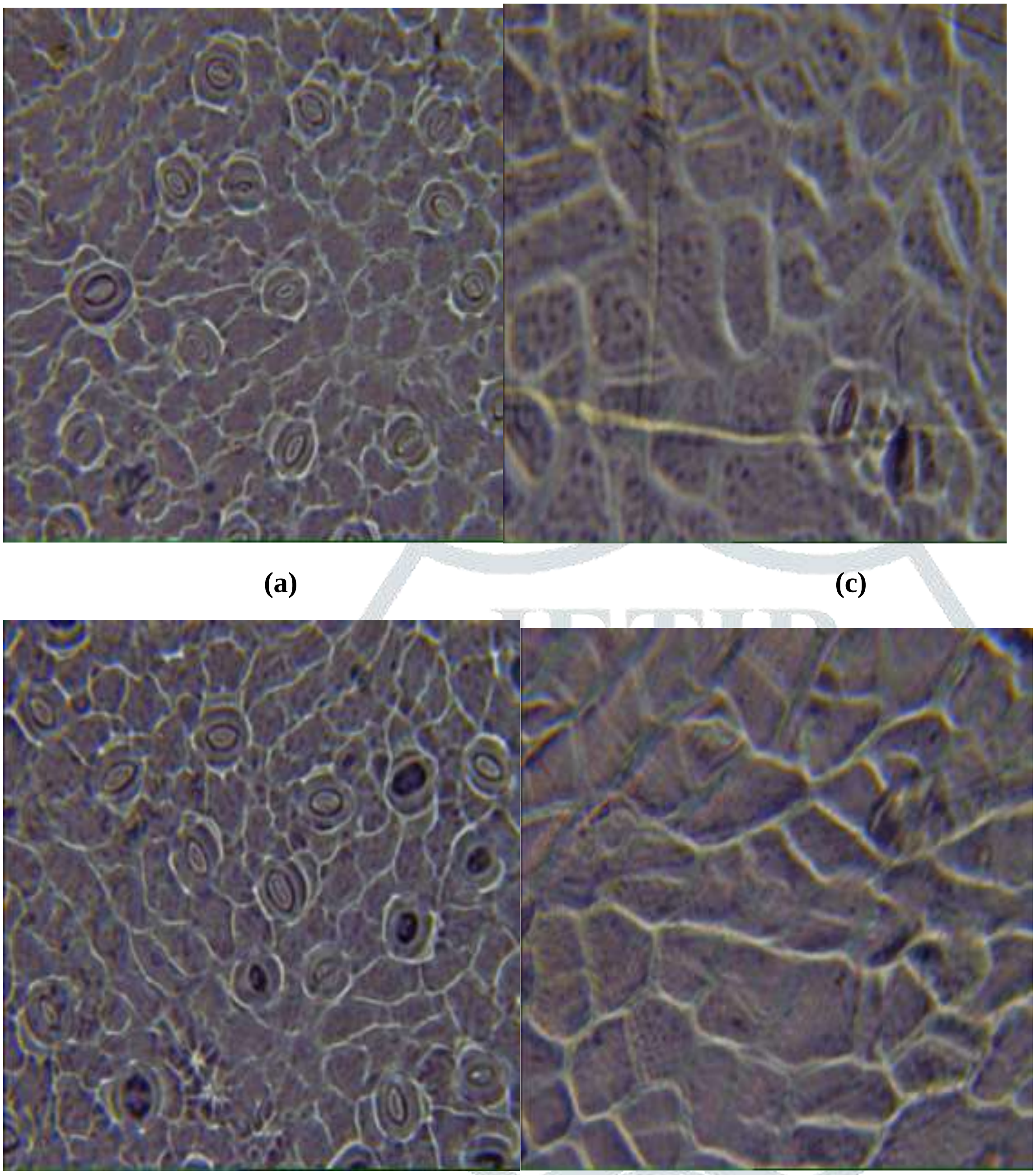
't' value at 18 d.f. on 0.05% level is 1.734



(a) (c)

(b) (d)

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



(a) (c)

(b) (d)

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

Discussion: Today air pollution is one of the most severe problem in modern society. Even though air pollution is usually a greater problem in cities, pollutants contaminate air everywhere. These substances include various gases and tiny particles, or particles that can harm human health and damage the environment. The pollutants emitted by the vehicles interact with plants growing along roadsides and these air pollutants strongly influence or alter the normal functioning of the plants. It affects plant anatomy in a variety of ways and the morphological changes induced by different pollutants serve as useful indicators of air pollution. This text discusses the effect of dust pollution on the anatomical structure of leaves of two tree species growing near the road side of Rewa city. An attempt has been made to reveal the responses of higher plants to urban air pollution, particularly the toxic effect of particulate matters and gases on the anatomical characters of leaves of five trees species.The epidermal surface of plant species, particularly trees, growing along the roadsides of urban areas is the most important receptor of atmospheric pollutants where they cause several structural and functional changes and also absorb the pollutants at their foliar surface function as a filter for atmospheric deposition, which significantly reduces the toxic effects of pollutants and mitigate the impact of other stress factors in such environment.

Study indicated significant alternations in anatomical structures of selected plant species due to vehicular air pollution in Rewa city. An intensive and continuous deposition of toxic substances causes disturbances in the plant physiological processes, visible damage symptoms and micro-morphological and anatomical structures of leaves. Anatomy and morphology of a leaf is useful to assess the impact of pollutants released from vehicular exhaust.[17]

Keeping above view in mind, the present work is undertaken to assess the effect of dust pollution on the anatomical structure of leaves of two trees species such as *Annona squamosa* and*Dalbergia sissoo*growing along the road side of Rewa city. The findings on anatomical traits of these species growing at polluted sites have been compared with the findings of respective plant species growing at University campus, considered as control site in this study. Boththe species of selected study sites registered significant increase in epidermal cells number and stomata number on their leaf surfaces, as compared to control areas. On the other hand, the plant species of polluted sites have shown significant reduction in the size of epidermal and guard cells in comparison to respective control plants. Similar results were observed by various workers.[18-26] Noticeable changes were recorded in stomatal frequency and stomatal index for the leaves of plant species growing at polluted sites in comparison to those of control sites.

These changes in micro-morphological structures could be an indicator of environmental stress caused due to urban or vehicular air pollution. The marked alternations in the size and number of foliar epidermal and guard cells, stomatal frequency and stomatal index in both dorsal and ventral surfaces of plant species under this study are not unexpected. In the present study both the species have registered significant increase in stomatal number and significant reduction in size of stomatal guard cells in leaves at most of the polluted sites.

This study demonstrates that anatomical characters in selected tree species growing near road sides of Rewa city are modified due to the stress of vehicular exhaust emission with high traffic density and construction works going on in the city. In fact, most pollution resistant species serve as both scavengers as well as indicators of pollution, as on one hand, they absorb the pollutants and on other hand through significant changes they indicate the presence of pollutants in the environment. The adverse effect of traffic emissions on plants was greater in the area receiving higher pollution load due to higher vehicle density and vice-versa.It is evident from the above discussion that the pollutants such as RSPM, SPM, SO_2 and NO_2 from automobile exhaust not only cause bad air quality condition around nearby areas but also cause significant reduction in number of stomata and epidermal cells of trees growing near the road sides of Rewa city.

Conclusion: From the present study it may be concluded that Leaf surface characteristics are sensitive to the air pollution. Response of leaf character to air pollution will indicate the adverse effects of air pollution, which thus can be used as bio-indicators of environmental stress. This work was designed to monitor the effect of dust pollution on the anatomical structure of leaves of some trees growing along the road side of Rewa city.

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