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Dust Pollution Effects On The Leaves Anatomy Of *Catharanthus Roseus* And *Nerium Oleander* Growing Along The Road Side Of Rewa City (M.P.)

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--*ABSTRACT*--
The present paper deals with the study of the effect of urban air pollution on anatomical structure of leaves of two shrub species viz; Catharanthusroseusand Nerium oleander growing along the road sides of Rewa city. The light microscopic studies of Catharanthusroseusand Nerium oleander leaves showed marked alteration in the anatomical parameters. Increase in the number of stomata and epidermal cells with decrease in length and width of guard cells and epidermal cells was recorded in the samples of polluted area on both upper & lower surface of leaf. The values of stomatal frequency and stomatal index were observed to be increasedin stressed area leaf samples on both the surfaces. The anatomical modifications occurred in leaves may potentially be used as biological markers for air pollution presence.
KEYWORDS: *Anatomical, Epidermal Cells, Guard cells,Stomatal index and Somatal frequency.*

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I. INTRODUCTION

Air pollution is a big 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 interactions between plants and different types of pollutants were investigated by many authors: most studies on the influence of environmental pollution focus on physiological and ultrastructural aspects (Heumann, 2002; Psaras and Christodoulakis, 1987; Velikova*et al.*, 2000). Studies concerning the anatomy of the vegetative organs under conditions of pollution have been also carried out (Alves*et al.*, 2008; Ahmad *et al.*, 2005; Silva *et al.*, 2005, 2006, Verma*et al.*, 2006). The reaction of different species to the altered environmental conditions is strongly correlated with their structural and functional features. Studies show that under the action of pollutants, plants develop different morphological and anatomical changes.

The road side plants play a significant role in assimilation and accumulation of pollutants and act as efficient interceptors of airborne pollutants. Urban air pollution adversely effects leaf structure of plants have been studied through various workers (Kulshreshtha et al; 1994a, 1994b; Carreras et al; 1996; Dineva, 2004; Pawar, 2013; Rai and Kulshreshtha, 2006; Sher and Hussain, 2006; Amulya et al; 2015).

Present study has been carried out to know the changes in the anatomical structure of leaves 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 two shrub species viz; *Catharanthusroseus*and *Nerium* growing along road sides of Rewa city with application of suitable statistical approaches.

II. 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.

Selection and sample collection of plant- For anatomical study road side shrub species viz. *Catharanthusroseus*and *Nerium*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: Leaf surface characteristics were studied with light microscope. The leaf epidermal peel slides were made by the method of lasting impressions. In this method fresh leaf samples of *Catharanthusroseus*and *Nerium*were collected from polluted and controlled sites during six months period January to June 2018and washed from distilled water to remove dust and other pollutants from the upper and lower surfaces, wiped out the water from the surfaces. A thick patch of clear nail polish was painted on the leaf surface of at least one square centimeter. Allowed the nail polish to dry completely then taped a piece of clear cellophane tape to the dried nail polish patch by carton sealing tape. Gently, peeled out the nail polish patch by pulling a corner of the tape 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 ("MagVision" software of Olympus optical microscope) and Size (Length and width) of epidermal cells and guard cells were measured with the help of this software. Number of stomata and epidermal cell were counted per square millimeter area and the stomatal frequency and stomatal index were calculated by using the following formulae (Salisbury, 1927):

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

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; *Catharanthusroseus*and *Nerium*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 average number of Stomata, length and width of guard cells of both dorsal and ventral surface of leaves of *Catharanthusroseus* growing in the polluted and conrol sites of Rewa city. The leaves of *Catharanthusroseus* have shown stomata on both dorsal and ventral surface collected from polluted and controlled site. Results 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 except the length of guard cell on ventral surface was insignificant (Table- 2). Similarly, the values of stomatal index and somatal frequency were also observed to be higher for the leaves sampled from polluted site.

Table-3 shows number of epidermal cells of both surface of leaves of *Catharanthusroseus* 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- 4).Average length and width of epidermal cells of both surface of leaves of *Catharanthusroseus* sampled at polluted and control sites are also given in table 3. 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 of epidermal cells in leaves of polluted site was significant on both dorsal and ventral surface of leaves where as insignificant in width of epidermal cells both dorsal and ventral surface of leaves (Table- 4).

Studies were conducted only on ventral surface of leaves of *Nerium oleander* for guard cells because stomata were absent on dorsal surface due to hypostomatous nature of the plant. Results indicated greater increase in number of stomata and decrease in length and width of guard cells on ventral surface of *Nerium oleander* leaves growing at polluted sites of the city than those of control sites during the study period (Table- 5). This decrease in length and width of guard cells was statistically significant (Table- 6) and the number of stomata also increased significantly at polluted sites as compared to control sites. The values of stomatal frequency and stomatal index were observed to be increased .Table- 7 reveals responses of epidermal cells to air pollution at different sites. It is obvious from the results that there was decrease in length and width of epidermal cells on both dorsal and ventral surfaces at polluted sites, as compared to control site plants. This decrease was observed to be significant except the length of epidermal cell on dorsal surface and number of epidermal cell on ventral surface was found to be insignificant (Table- 8).

Shapes of stomata were observed distorted on ventral surface of leaves of *Catharanthusroseus*and on both the surfaces of leaves of *Nerium oleander* at polluted sites (Figure-1 and Figure-2).

Table 1 - Average number of stomata (mm^2), length (μm) and width of guard cells (μm), stomatal frequency (%) and stomatal index (%) of *Catharanthusroseus***leaves growing at polluted and control sites of Rewa city.**

Leaf Surfaces \ Sites		Polluted	Controlled
DORSAL	LGC	44.147 ± 5.749	50.869 ± 6.047
	WGC	31.32 ± 5.320	43.038±5.606
	NOS	16.8±3.483	13.4±2.913
	SF	50.2240	51.519

	SI	14.23	13.52
VENTRAL	**LGC**	48.052±3.370	50.028±5.247
	WGC	25.425±7.047	34.605±3.858
	NOS	42.8±4.685	31.2±5.711
	SF	42.8900	37.905
	SI	30.01	27.486

Table 2- Values of 't' test between number of stomata and size of guard cells of *Catharanthusroseus*leaves growing at polluted and control sites of Rewa city.

Leaf Surfaces	**Stomata characteristics**	**t-test**	**P value**
DORSAL	**LGC**	2.550*	P=0.0201
	WGC	4.793***	P<0.0001
	NOS	2.368*	P=0.0293
VENTRAL	**LGC**	1.130	P=0.2734
	WGC	3.613**	P=0.0020
	NOS	4.966***	P<0.0001

Table 3- Average number (mm^2), length (µm) and width (µm) of epidermal cells of *Catharanthusroseus*leaves growing at polluted and control sites of Rewa city.

Sites / Leaf Surfaces		**Polluted**	**Controlled**
DORSAL	**LEC**	103.53±8.270	140.49±6.669
	WEC	45.469±4.281	48.80±7.471
	NEC	101.25±14.321	85.69±9.732
VENTRAL	**LEC**	84.244±6.261	144.508±5.8097
	WEC	41.691±6.3199	42.606±6.293
	NEC	99.79±9.512	82.309±4.280

Table 4- Values of 't' test for number and size of epidermal cell of *Catharanthusroseus*leaves growing at polluted and control sites of Rewa city.

Leaf Surfaces	**Epidermal cells**	**t-test**	**P value**
DORSAL	**LEC**	11.00***	**P<0.0001**
	WEC	1.123	**P=02.370**
	NEC	2.854*	**P=0.0105**
VENTRAL	**LEC**	22.31***	**P<0.0001**
	WEC	0.3244	**P=0.7494**
	NEC	5.275***	**P<0.0001**

Table 5- Average number of stomata (mm^2), length (µm) and width of guard cells (µm), stomatal frequency (%) and stomatal index (%) of *Nerium oleander* leaves growing at polluted and control sites of Rewa city.

Sites / Leaf Surfaces		**Polluted**	**Controlled**
DORSAL	**LGC**	-	-
	WGC	-	-
	NOS	-	-
	SF	-	-
	SI	-	-
VENTRAL	**LGC**	50.72±5.961	60.707±4.692
	WGC	33.436±5.790	39.141±3.690
	NOS	31.7±9.95	23.6±7.113
	SF	84.084	54.883
	SI	45.67	35.43

Table 6- Values of 't' test between number of stomata and size of guard cells of *Nerium oleander* leaves growing at polluted and control sites of Rewa city.

Leaf Surfaces	**Stomata characteristics**	**t-test**	**P value**
DORSAL	**LGC**	-	-
	WGC	-	-
	NOS	-	-
VENTRAL	**LGC**	4.163***	P=0.006
	WGC	2.628*	P=0.00171
	NOS	2.094*	P=0.050

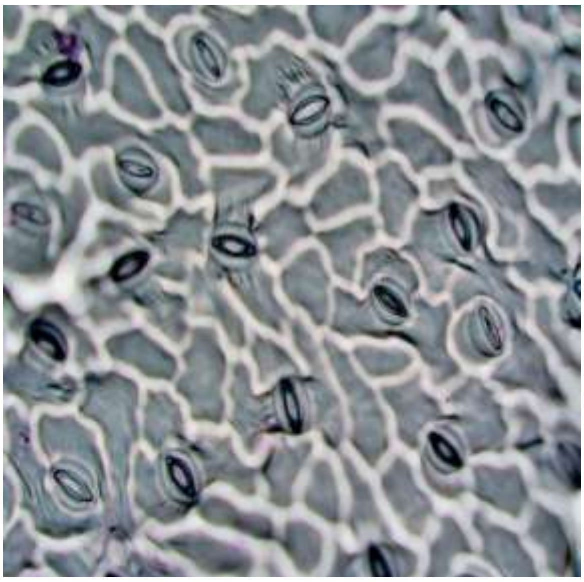
Table 7- Average number (mm^2), length (µm) and width (µm) of epidermal cells of *Nerium oleander* leaves growing at polluted and control sites of Rewa city.

Leaf Surfaces \ Sites		Polluted	Controlled
DORSAL	LEC	177.71 ± 13.37	183.03 ± 5.12
	WEC	79.20 ± 5.91	88.98 ± 4.53
	NEC	31.70 ± 7.0	21.40 ± 7.06
VENTRAL	LEC	92.02 ± 3.31	153.22 ± 6.41
	WEC	48.34 ± 3.87	57.34 ± 3.14
	NEC	43.00 ± 9.94	37.7 ± 5.38

Table 8- Values of 't' test for number and size of epidermal cell of *Nerium oleander* leaves growing at polluted and control sites of Rewa city.

Leaf Surfaces	Epidermal cells	t-test	P value
DORSAL	LEC	1.175	P=0.255
	WEC	4.146***	P=0.0006
	NEC	3.276**	P=0.0042
VENTRAL	LEC	2.677***	P=0.0001
	WEC	5.705***	P=0.0001
	NEC	1.182	P=0.11556

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



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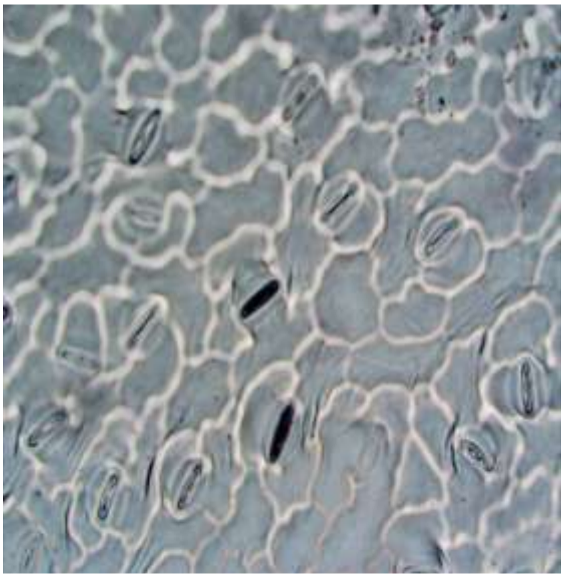

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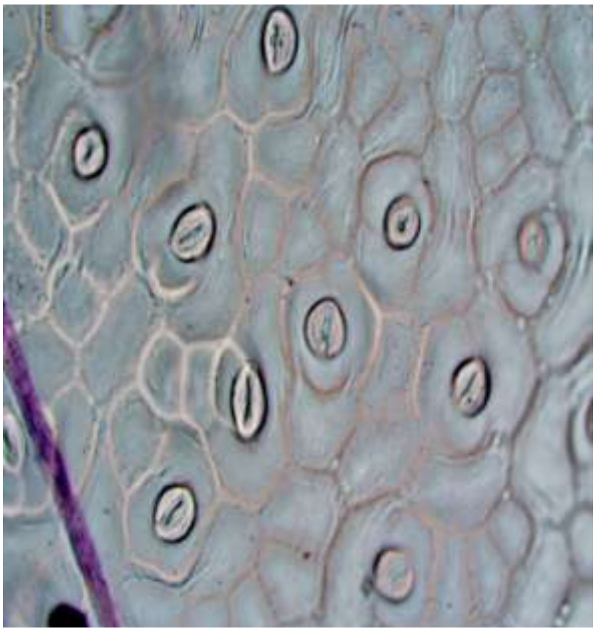

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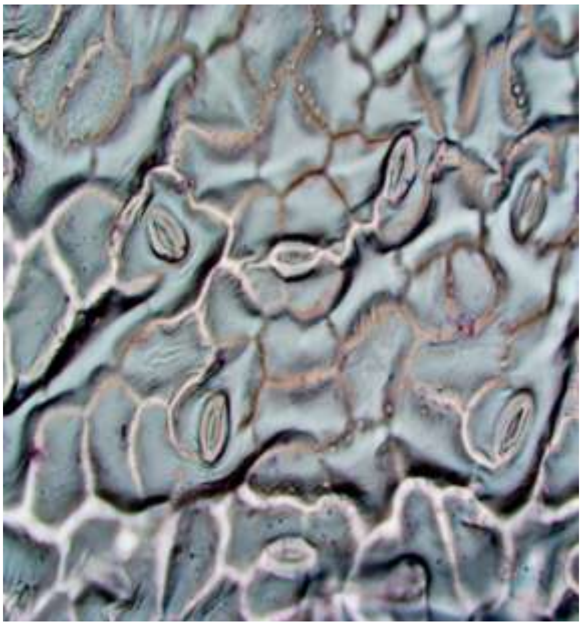
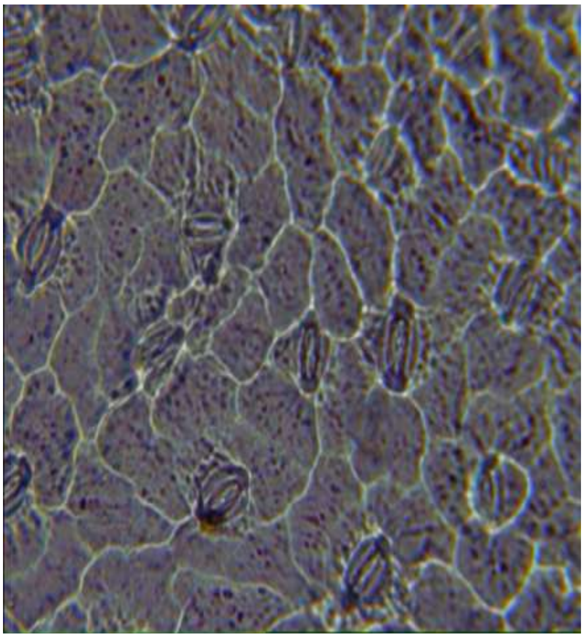
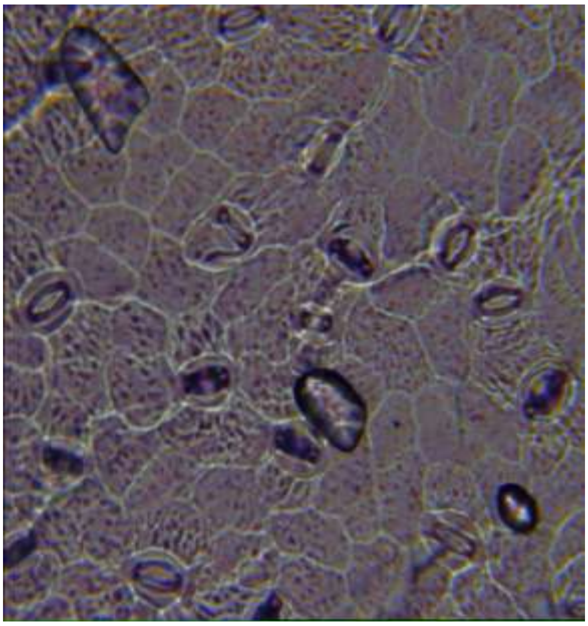


(d)

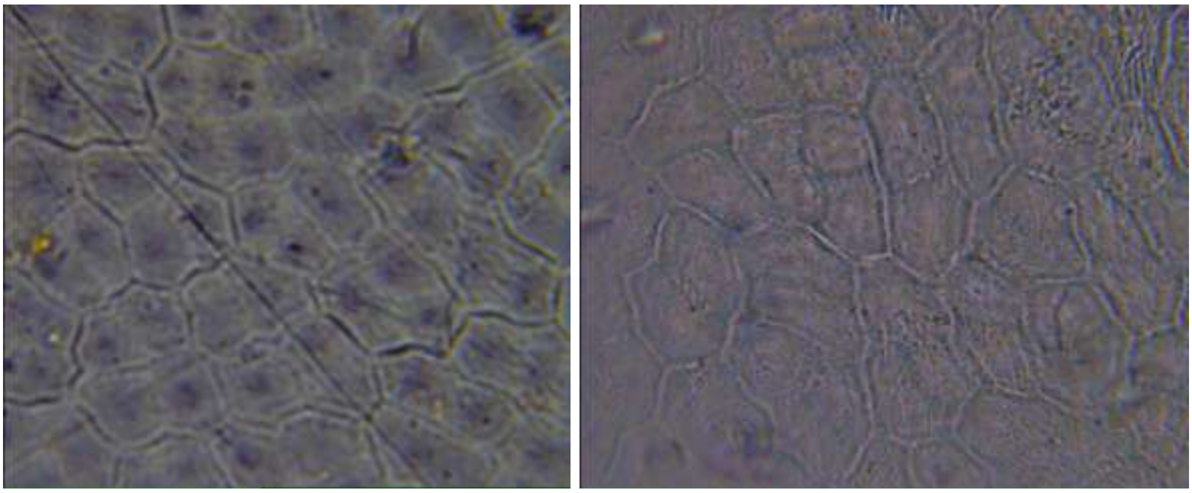
Figure-1: Lower foliar surface of ***Catharanthusroseus*****showing stomata at control (a) and polluted (b) site; upper foliar surface showing waxy-walled epidermal cells at control(c) and polluted (d) site.**



(a)



(c)



(b) (d)

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

III. DISCUSSION

In developing countries, urbanization has brought as a consequence greater emissions in to atmosphere. This is mainly produced by the increase of vehicular traffic, a problem exacerbated by the tendency in these countries to have a stock of old and badly maintained vehicles. At the same time, the number of vehicles in circulation has increased as well. These factors have produced far-reaching changes in air quality in urban areas. Shrub species, growing along the roadsides of urban areas 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. Plants react to vehicular air pollution induced stress with a variety of active morphological and anatomical responses, which includes the effect on size and frequency of stomata and epidermal cells and also the stomatal index (Samal and Santra 2002; Raina and Sharma, 2006; Raina and Agrawal, 2004; Raina and Bala, 2011; Tiwari, 2012). Keeping above views in mind, the present work is undertaken to assess the effect of dust pollution on the anatomical structure of leaves of two shrub species such as *Catharanthusroseus*and *Nerium oleander* andgrowing 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.

Results revealed reductions in length and width of epidermal and stomatal guard cells on dorsal and ventral surface of leaves of all the selected plant species growing at different polluted sites, as compared to University campus of Rewa city was observed. Similar reduction in stomatal size and epidermal cells at polluted sites of urban areas as compared to that at reference site is observed by various workers (Kulshreshtha*et al.* 1980, 1994; Sharma and Roy, 1995; Aggarwal, 2000; Snehlata*et al.* 2010; Saadabi, 2011; Saadabi and Al-Nur-El-Amin 2011; Rai and Mishra, 2013;Shrivastava and Mishra, 2018).

This reduction in stomata size could be considered as an adaptive response of these plants to avoid entry of harmful constituents of vehicle exhaust which can otherwise cause adverse effects (Salgare and Thorat, 1990). Distorted shapes of stomata observed in leaves of studied plants of present investigation might have resulted due to lowering of pH in cytoplasm of guard cells and thus a change in turgor relations of the stomata complex (Kondo *et al.* 1980) and due to physiological injury within leaf (Ashenden and Mansfield, 1978).

Gaseous pollutants enters the leaves through stomata following the same diffusion pathway as CO_2. This may be an adaptation of these plants to increase the area for proper gas exchange, as suggested by Raina and Chand Bala (2011) and also by various other workers (Shrivastava and Prakash, 2017; and Shrivastava and Mishra 2018) .

Conclusion: This work was carried out to monitor the dust pollution effect on the anatomical structure of leaves of some shrubs growing along the road side of Rewa city.It is evident from the above discussion that Leaf surface characteristics of all investigated species indicated a significant potential for resistance to air pollutants. The anatomical modifications occurred in leaves may potentially be used as biological markers for air pollution presence.

REFERENCES

[1]. Aggarwal, P. (2000). The effect of auto-exhaust pollution on leaf surface of *Cassia siamea*(L.), a road side tree. *ActaEcologica*, 22: 101-106.
[2]. Ahmad, S. H., Z. Reshi, J. Ahmad and M. Z. Iqbal (2005). Morpho-anatomical responses of *Trigonellafoenumgraecum*Linn. to induced cadmium and lead stress. Journal of Plant Biology. 48 (1):64-84.

[3]. Alves, E. S., M. BaêssoMoura and M. Domingos (2008). Structural analysis of *Tillandsiausneoides*L. exposed to air pollutants in São Paulo City–Brazil, Water Air and Soil Pollution. 189 (1-4):61-68.

[4]. Amulya, L. Hemanth K.N. K. and Jagannath, S. (2015).Air pollution impact on micromorphological and biochemical response of Tabernaemontanadivaricata L. (Gentianales: Apocynaceae) and Hamelia patens Jacq. (Gentianales: Rubiaceae). Brazilian Journal of Biological Sciences, v. 2, n. 4, p. 287-294.

[5]. Ashenden, T.W. and Mansfield, T.A. (1978). Extreme pollution sensitivity of grasses when SO2 and NO2 are present in atmosphere together. Nature, 273: 142-143.

[6]. Carreras, H.A., Canas, M.S. and Pagnate, M.L. 1996. Differences in responses to urban air pollutants by LigustrumlucidumAit. F. tricolor (Rehd.). Environmental Pollution 2: 211-218.

[7]. Dineva, S. (2006). Development of leaf blades of *Acerplatanoides*in industrially contaminated environment.*Dendrobiology*, 55: 25-32.

[8]. Heumann, H. G. (2002). Ultrastructural localization of zinc in zinc-tolerant *Armeria maritime* ssp. *halleri*by autometallography. Journal of Plant Physiology. 159(2):191-203.

[9]. Kondo, N.; Maruta, I. and Sugahara, K. (1980). Research report from the National Institute for Environmental Studies, Yatabe, Japan 11: 127-136.

[10]. Kulshreshtha, K., Farooqui, A., Srivastava, K., Singh, S.N., Ahmad, K.J. and Behl, H.M. (1994a). Effect of diesel pollution on cuticular and epidermal features of Lantana camara Linn. andSyzygiumcumini Linn. (Skeels). Journal of Environmental Science and Health. 29 (2): 301-308.

[11]. Kulshreshtha, K.; Srivastava, K. and Ahmad, K. J.(1994b). Effect of automobile exhaust pollution on leaf surface structures of Calotropisprocera L. and Neriumindicum L. Journal of Botanical Taxanomy and Geological Botany, v. 105, p. 185-189.

[12]. Kulshreshtha, K., Yunus, M., Dwivedi, A.K. and Ahmad, K.J. (1980). Effect of air pollution on the epidermal traits of *Jasminumsambac*Ait. *New Botanist*, 7: 193-197.

[13]. Pawar, A. (2013). Impact on the phenological events of plants under stress conditions of auto-exhaust pollution. *Journal ofPure & Applied Sciences & Technology*, Vol. 3(2), pp. 1-4.

[14]. Psaras, G. K. and N. S. Christodoulakis (1987). Air pollution affects on the ultrastructure of *Phlomisfruticosa*mesophyll cells. Bulletin of Environmental Contamination and Toxicology. 38(4):610-617.

[15]. Rai, A. and Kulshreshtha, K. (2006). Effect of particulates generated from automobile emission on some common *50151 International Journal of Current Research, Vol. 9, Issue, 05, pp. 50147-50152, May, 2017* plants. *Journal of food, Agriculture and Environment* 4(1):253-259.

[16]. Rai, P. and Mishra, R.M. (2013). Effect of urban air pollution on epidermal traits of road side tree species, *Pongamiapinnata* (L.) Merr. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, pp.04-07.

[17]. Raina, A.K and Bala, C. (2011). Effect of vehicular pollution on Durantarepens L. in Jammu city. *Journal of Applied andNatural Science*, Vol. 3(2), pp. 211-218, 2011.

[18]. Raina, A.K. and Aggrawal, B. (2004). Effect of vehicular exhaust on some trees in Jammu.*Journal of IndustrialPollution Control*, Vol. 20(2), pp. 229-232.

[19]. Raina, A.K. and Sharma, A. (2006). Assesment of air pollution and its impact on the leaves of some plant species. *Poll.Res*., Vol. 25(3), pp. 543-547.

[20]. Saadabi, A. M. A. (2011). Effects of auto-exhaust pollution on the micro-morphology and leaf epidermal features of ornamental plants in Khartoum, Sudan. Journal of Applied Sciences Research, v. 7, n. 3, p. 270-273.

[21]. Saadabi, A.M.A., El-Amin, A. (2011). Effects of Environmental Pollution (Auto-Exhaust) on the MicroMorphology of Some Ornamental Plants from Sudan. Environmental Research Journal, 5(2): 38-41.

[22]. Salisbury, E.J. (1927). On the causes and ecological significance of stomatal frequency, with special reference to the woodland fora. *Philos. Trans. R. Soc. London B* 216, 1-65.

[23]. Salgare, S.A. and Thorat, V.B. (1990). Effect of auto exhaust pollution at Andheri (West), Bombay on the micromorphology of some trees. Journal of Ecobiology, 2(4): 267-272.

[24]. Samal, A.C. and Santra S.C. (2002). Air quality of Kalyani Township (Nadia, West Bengal) and its impact on surrounding vegetation. *Indian J. Environ. Health*, Vol. 44(1), pp. 71-76.

[25]. Sharma, M. and Roy, A.N. (1995). Effect of automobile exhauston the leaf epidermal features of *Azadirachtaindica*and*Dalbergiasissoo*.*Int. Journal of Mendel*, 12 (1-4): 18-19.

[26]. Sher, Z. and Hussain, F. 2006. Effect of automobile traffic on some cultivated trees along road side in Peshawar. Pakistan Journal of Plant Science 12(1): 47-54.

[27]. Shrivastava, R. and Mishra, A. (2018). Air pollution induced changes in foliar miro-morphology of road side shrub species *Thevetiaperuviana*and*Plumeriaalba*in Rewa City, MP, India. *International Research Journal* Vol. 7(3), 1-7.

[28]. Shrivastava, R. and Prakash, J. (2017). Effect of Vehicular Pollution on Micro-Morphological Structure of Hibiscus Rosa Sinensis. International Journal of Engineering Technology Science and ResearchVolume 4(12), 521-527.

[29]. Silva, H. G. de O. ; Pires, A. J. V. ; Silva, F. F. da ; Veloso, C. M. ; Carvalho, G. G. P. de ; Cezario, A. S. ; Santos, C. C., (2005). Effects of feeding cocoa meal (*Theobroma cacao* L.) and palm kernel cake (*Elaeisguineensis*, Jacq) on milk intake and yield for lactating goats. Rev. Bras. Zootec., 34 (5): 1786-1794.

[30]. Silva, L. C., M. A. Oliva, A. A. Azevedo and J. M. Araújo (2006). De Responses of restinga plant species to pollution from an iron pelletization factory. Water Air and Soil Pollution. 175(1-4):241-256

[31]. Snehlata, D. Shah and Poonam (2010). Comparative ecomorphological studies on Daturaalbalinn. Effects of aiming along roadsides and railway tracks. Environ. We Int. Sc. Tech. 5 : 156-162.

[32]. Verma, A. and Singh, S. N. (2006). Biochemical and ultrastructure changes in plant foliage exposed to autopollution. Environ. Monit. Assess., 120 : 585-602.

[33]. Velikova, V., I. Yardanov and A. Edreva (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Plant Science. 151(1):59-66.

[34]. Tiwari, S. (2012). Air Pollution Induced changes in Foliar Morphology of two shrub species at Indore city, India. *Research Journal of Recent Sciences*, Vol.2, 195-199.

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