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STUDIES ON URBAN AIR POLLUTION AND ITS RELATION TO EPIDERMAL STRUCTURE OF *ZIZIPHUS MAURITIANA* IN REWA CITY

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ABSTRACT

The present study was undertaken to evaluate the impact of urban air pollution (air pollutants SPM, RSPM, SO_2 and NO_X) on epidermal structure of *Ziziphus mauritiana* growing along the road sides in Rewa city. The monitored values of SPM, RSPM, SO_2 and NO_X at the sampling sites clearly illustrates that the ambient air of Rewa city is primarily deteriorated by particulate matters (SPM, RSPM) and least by gaseous pollutants (SO_2, NOx). The light microscopic studies of Ziziphus mauritiana leaves exposed to urban air pollution showed marked alterations in the micro-morphological parameters. Increase in the number of epidermal cells and decrease in number of per unit area stomata number, length and width of epidermal and stomatal guard cells, stomatal frequency and stomatal index on both dorsal and ventral surface of leaves of polluted sites in comparison to those of control site was observed.These changes in epidermal traits could be an indicator of environmental stress caused due to urban air pollution.

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INTRODUCTION

Air pollution has acquired a great concern globally due to manifestation of technological and scientific innovations in various fields in addition to diverse activities of human beings for their satisfaction. It poses a worldwide threat to human ealth and environment. Air pollution in urban area is mainly due to emissions of industrial gases and traffic related particulates, which undergo dispersion, transport and chemical reaction in the atmosphere and are deposited as gaseous ions, solid and liquid particles. Currently, in India, air pollution is widespread in urban areas where vehicles are the major contributors and in a few other areas with a high concentration of industries and thermal power stations. Vehicular emissions are of particular concern since these are ground level sources and thus have the maximum impact on the general population. Also, vehicles contribute significantly to the total air pollution load in many urban areas. The industrialization, vehicular emissions and industrialization constitutes major sources of environmental pollution in Indian cities.

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Petrol and diesel engine driven motor vehicles release a wide variety of pollutants particularly benzene, carbon monoxide, organic compounds, oxides of nitrogen, sculpture 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, sculpture dioxide, nitrogen oxides and volatile organic compounds (Shrivastava *et al.,* 2013) . Plants are sinking for air pollution (Gaghate and Hassan1999) 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 (Emberson, 2004).The plants growing under the stressed conditions of pollution develop different micro- morphological (Aggarwal, 2000; Rai and Singh, 2015; Pawar, 2016), anatomical (Salgare and Acharekar, 1991; Giri et.al., 2013; Pawar, 2015) and biochemical (Pratibha and Sharma, 2000; Karthiyayiniet *et al.,* 2005; Ram krishnaiah and Somashekar, 2003; Gupta *et al.,* 2009) 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 (Kulshreshtha *et al.,* 1994a, 1994b;

Sharma and Roy, 1995; Carreras et al., 1996; Pal *et al.,* 2000; Rai and Kulshreshtha, 2006; Sher and Hussain, 2006; Amulya *et al.,* 2015).This study was undertaken to assess the changes caused by urban air pollution on number and size of stomata and epidermal cells in the leaves of a roadside tree species, *Ziziphus mauritiana.*

MATERIALS AND METHODS

Study area

The present study was conducted in Rewa city, which is situated on the north east border of Madhya Pradesh, centralpart 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 6,314kilometers 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 out viz; Transport nagar, New Bus Stand, District Hospital, PTS Square, Nehru Nagar, Mahajan Tola, Chirahula Colony, Khutehi, Model School, Civil Lines along with control site (APS University Campus) of Rewa City for one year (November 2016 to November 2017). Sampling was carried out at the eleven different locations using Respirable Dust sampler (Envirotech model APM 460BL-411) for 8 hours in a day at an average flow rate of 1.5LPM 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 respirablesuspended particulate matters (RSPM) were collected on the dust cup and glass fabric filter paper respectively. Leaf surface characteristics were studied with the help of optical microscope. Epidermal peel slides were made by the methods of making lasting impressions. In this method, fresh and mature leaf samples of *Ziziphus mauritiana* were collected from control and polluted sites and were properly washed from distilled water to remove dust and other pollutants from the upper and lower surfaces. 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 taken 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 dorsal and ventral surface. Leaf impression was examined under 400 x total magnifications by light microscope ("Motic Images plus2.0 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". Stomata index and Stomata frequency is calculated by the formula of Salisbury (1927):

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

Stomatal frequency = (Average number of stomata / Average number of epidermal cells) ×100

RESULTS

The percent contribution of each pollutant to the total estimated pollutants in the ambient of Rewa city has been computed from the basic data for one year. Results indicated (Figure - 1) maximum contribution of SPM (69.73 %) to the total pollutants of ambient air of Rewa city to be followed by RSPM (16.73 %), SO_2 (6.93 %) and NOX (7.18 %) during one year.

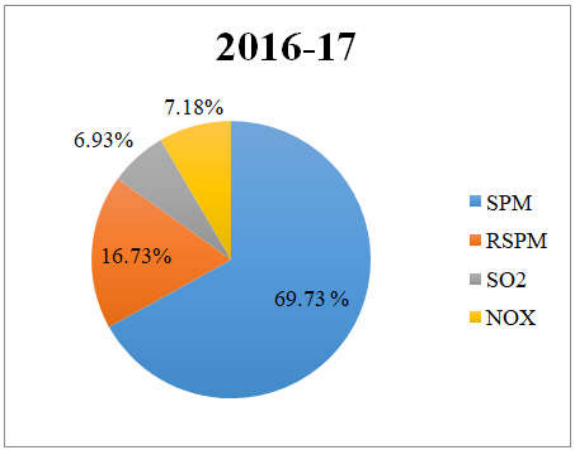

Fig .1. Percentage contribution of each pollutant to the ambient air of Rewa city observed during the year 2016-17

This result clearly illustrates that the ambient air of Rewa city is primarily deteriorated by particulate matters (SPM, RSPM) and least by gaseous pollutants (SO_2, NOx).

Table 1. Average length, width and number of epidermal cells (density) in leaves of *Ziziphus mauritiana* growing at different sites of Rewa city

S.No.	Site	Length (µm)		Width (µm)		Number (Density) $(mm)^2$	
		Dorsal Surface	Ventral Surface	Dorsal Surface	Ventral Surface	Dorsal Surface	Ventral Surface
1.	Transport Nagar	74.26+18.64	77.25+17.28	57.60+8.11	50.25+10.24	417.84+29.48	853.39+17.82
2.	New Bus Stand	69.12+10.86	56.35+10.59	49.78+8.95	38.54+5.64	442.63+38.45	880.72+13.56
3.	District Hospital	70.15+12.17	63.13+11.81	48.73+9.95	36.56+7.09	382.43+31.34	644.47+18.28
4.	PTS Square	70.02+9.14	69.93+14.21	52.96+9.08	51.32+5.65	375.60+34.08	619.68+13.56
5.	Nehru Nagar	83.56+13.24	78.23+10.68	60.48+9.48	53.45+7.86	336.75+30.50	610.75+16.60
6.	Mahajan Tola	88.95+14.28	79.87+10.54	60.62+6.55	58.32+8.51	330.16+27.35	617.45+15.72
7.	Chirahula Colony	90.20+9.11	75.32+14.26	68.25+9.11	59.82+9.57	333.47+26.90	621.62+15.40
8.	Khutehi	68.12+10.66	63.92+11.05	50.07+7.18	42.72+6.73	357.64+36.16	636.35+16.16
9.	Model School	75.18+12.65	65.42+12.70	56.54+8.32	44.32+6.09	350.45+33.66	635.07+15.88
10.	Civil Lines	95.45+13.67	93.42+12.52	65.12+6.28	66.05+6.46	313.50+26.05	578.36+15.45
11.	University Campus (Control site)	102.82+18.88	105.40+19.36	72.58+4.68	79.40+4.70	308.07+24.19	570.18+18.28

Table 2. Average length, width, number of stomata, stomatal frequency (SF) and stomatal index (SI) in leaves of *Ziziphus mauritiana* growing at different sites of Rewa City

S.No	Site	Guard cell length (μm)		Guard cell width (μm)		Stomata number $(mm)^2$		Stomatal Frequency (%)		Stomatal Index (%)	
		Dorsal Surface	Ventral Surface	Dorsal Surface	Ventral Surface	Dorsal Surface	Ventral Surface	Dorsal Surface	Ventral Surface	Dorsal Surface	Ventral Surface
1.	Transport Nagar	68.24+9.45	68.52+8.28	28.50+9.45	28.72+4.54	102.69+7.08	251.41+13.56	24.57	29.46	19.72	22.75
2.	New Bus Stand	46.70+7.66	50.20+8.82	20.22+7.70	17.16+3.45	92.06+8.17	194.75+7.08	20.79	22.11	17.21	18.10
3.	District Hospital	60.22+6.45	59.15+7.44	21.05+5.67	19.70+3.24	120.39+27.12	258.49+7.08	31.48	40.10	23.94	28.30
4.	PTS Square	62.33+6.84	65.35+10.29	25.53+8.39	23.50+2.95	148.72+8.17	277.45+24.69	39.59	44.77	28.36	30.92
5.	Nehru Nagar	76.20+10.17	68.23+8.06	29.46+6.83	19.70+2.64	154.25+9.63	270.62+16.54	45.80	44.30	31.41	30.70
6.	Mahajan Tola	59.79+8.61	61.58+7.47	29.02+5.43	19.67+1.69	162.38+8.71	266.58+17.30	49.18	43.17	32.96	30.15
7.	Chirahula Colony	69.23+7.76	63.27+8.71	26.64+6.39	19.88+2.07	153.66+8.88	272.45+19.37	46.07	43.82	31.54	30.47
8.	Khutehi	50.48+8.90	57.48+5.67	20.26+8.80	20.30+2.82	144.30+7.06	228.06+18.18	40.34	35.83	28.74	26.38
9.	Model School	59.97+8.26	59.80+6.72	22.25+5.32	28.78+2.86	149.69+8.44	230.17+17.90	42.71	36.24	29.92	26.60
10.	Civil Lines	72.18+10.80	74.82+9.85	31.25+8.60	29.15+2.68	178.64+10.15	281.62+14.54	56.98	48.59	36.29	32.74
11.	University Campus (Control Site)	88.10+14.19	86.15+11.50	34.06+8.72	33.72+1.88	190.20+14.16	298.69+17.47	61.73	52.38	38.17	34.37

Table 3 Average increase or decrease in number of epidermal cells and stomata, length and width of guard and epidermal cells of leaves of *Ziziphus mauritiana* growing at polluted sites of Rewa city

Epidermal Attributes	Leaf surface	Average at control sites	Average at polluted sites	Average increase (+) or decrease (-) at polluted sites (%)	Average range of increase (+) or decrease (-) at polluted sites (%)
Number of epidermal cells (mm^2)	Dorsal	308.07	364.04	(+) 18.16	(+) 1.76-43.67
	Ventral	570.18	669.78	(+) 17.46	(+) 1.43-54.46
Epidermal cells length (μm)	Dorsal	102.82	78.49	(-) 23.66	(-) 7.16-33.74
	Ventral	105.40	72.28	(-) 31.42	(-) 11.36-40.10
Epidermal cell width (μm)	Dorsal	72.58	57.01	(-) 21.45	(-) 10.27-32.86
	Ventral	79.40	50.13	(-) 36.86	(-) 16.81-53.95
Stomata number (mm^2)	Dorsal	190.20	140.67	(-) 26.07	(-) 6.07-51.59
	Ventral	298.69	253.16	(-) 15.24	(-) 5.71-34.79
Guard cell length (μm)	Dorsal	88.10	62.53	(-) 29.02	(-) 18.02-46.99
	Ventral	86.15	62.85	(-) 27.04	(-) 13.15-38.24
Guard cell width (μm)	Dorsal	34.06	25.41	(-) 25.39	(-) 8.25-40.63
	Ventral	33.72	22.65	(-) 32.82	(-) 13.55-49.11

The leaves of spiny and evergreen shrub species *Ziziphus mauritiana* growing at polluted sites and controlled of Rewa city were observed for number of stomata and epidermal cells, length and width of guard cells and epidermal cells. Table – 1 and Table - 2 illustrates average number of stomata and epidermal cells, length and width of epidermal and stomatal guard cells, stomatal frequency and stomatal index observed for dorsal and ventral surface of leaves of *Ziziphus mauritiana* growing at ten polluted sites, along with control site of Rewa city. Results demonstrated an increase in number of epidermal cells and decrease in number of per unit area stomata number, length and width of epidermal and stomatal guard cells, stomatal frequency and stomatal index on both dorsal and ventral surface of leaves of polluted sites in comparison to those of control site.

Table - 3 shows percent range of increase or decrease in various micro-morphological parameters of leaves of this species growing at polluted sites. The epidermal cells of dorsal and ventral leaf surface of polluted sites had increased in the range of 1.76 to 43.67% (average 18.16%) and 1.43 to 54.46% (average 17.46%) respectively. On the other hand, there was a decrease in length of epidermal cells of dorsal and ventral leaf surface collected from the polluted sites in the range of 7.16 to 33.74% (average 23.66%) and 11.36 to 40.00% (average 31.42%) respectively. Similarly, the width of epidermal cells on both the surface was observed to be reduced in the range of 10.27 to 32.86% (average 21.45%) and 16.81 to 53.95% (average 36.86%) at polluted sites respectively.

The number of stomata on dorsal and ventral surface of leaf sampled from polluted sites was found to be decreased in the range of 6.07 to 51.59% (average 26.04%) and 5.71 to 34.39% (average 15.24%) respectively. A range of 18.02 to 46.99% (average 29.02%) and 13.15 to 38.24% (average 27.04%) has been observed for reduction in the length of stomatal guard cells on upper and lower surface of leaves respectively. The width of stomatal guard cells on both the leaf surface collected from polluted sites was recorded to be reduced in the range of 8.25 to 40.63% (average 25.36%) and 13.55 to 49.11% (average 32.82%) respectively.It is concluded from the above study that the selected plant species *Ziziphus mauritiana* have exhibited increased number of epidermal cells and varying degree of reduction in number of stomata on both the surface of leaves at polluted sites in comparison to respective leaf samples collected from the University campus.

DISCUSSION

Quality of air is one of the basic indicators of the overall quality of the environment. In India, urban air pollution is the result of emissions from a multiplicity of sources, mainly stationary, industrial and domestic fossil fuel combustion, motor vehicles emissions and ineffective environmental regulations. Rewa has insufficient transport infrastructure. Due to increasing urban pollution, use of personalized vehicles, mainly two wheelers and intermediate public transport are growing at a rapid rate. Due to encroachment at parking, public and also at street places, the traffic flow becomes restricted and result is more emission of pollutants. In most part of city, due to bad quality of road surface and unavailability of traffic rotaries and light signals at the intersections has increased the emission of pollutants in the environment. The ambient air of seventeen sampling sites of Rewa has been monitored and analyzed for SPM, RSPM, SO_2 and N Ox concentrations. Plant species, particularly trees and shrubs, 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. At the same time atmospheric pollution represents a major and serious challenge for plants.

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. 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 (Raina and Sharma, 2003, 2006; Raina and Agrawal, 2004; Rai and Kulshreshtha, 2006; Raina and Bala, 2007, 2011; Saadabi, 2011; Tiwari, 2012). Dimensions of micro-morphological attributes of leaves can influence physiological response to changes in environment over time. Linking micro-morphological attributes of leaves to site conditions provide a clear understanding of changes in anatomy and morphology of species when exposed to pollution in urban landscapes. Thus, different plant species vary in their sensitivity to pollution stress. It is therefore, useful to consider the anatomy and morphology of a leaf to assess the impact of pollutants released from vehicular exhaust (Pal *et al.* 2002). Keeping above views in mind, the present work is undertaken to assess the impact of vehicular pollution on epidermal traits of leaves shrub *Ziziphus mauritiana* growing at different sites along the road sides of Rewa city.

The findings on epidermal 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 indicated marked alteration in epidermal traits, with varying degree of reductions in length and width of epidermal and stomatal guard cells on dorsal and ventral surface of leaves of the selected plant species growing at different polluted sites, as compared to University campus of Rewa. Similar reduction in size of stomata 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; Kaur, 2004; Rai and Kulshreshtha, 2006; Snehlata *et al.* 2010; Saadabi and Al-Nur-El-Amin 2011; Raina and Chand Bala, 2011; Rai and Mishra, 2013).This study also supports the findings of Raina and Bala (2011). These workers have reported significant reduction in size of stomata and length and width of epidermal cells in leaves of *Durantarepens* at ten polluted sites of Jammu city. The reduction in size of stomata and epidermal cells under present study is consistent with the findings of Raina *et al* ., (2010) while studying the effect of particulate pollution on leaf structure of ten annual plants.

The remarkable reduction in the size of epidermal cells and stomata resulted due to inhibited cell elongation, leaf area and consequently the increase in cell frequency, as suggested by Rai and Kulshreshtha (2006). 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 (Satyanarayana *et al.,* 1990; 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).This study demonstrated not only reduction in length and width of stomatal guard cells but also decrease in number of stomata on both dorsal and ventral surface of leaf *Ziziphusmauritiana*.

The leaves of *Ziziphus mauritiana* have shown increased number of epidermal cells on both dorsal and ventral surface at polluted sites, as compared to those of control site. Similar increase in epidermal cells has been reported in *Jasminumsambac* (Kulshreshtha *et al.,* 1980), *Calotropisgigantia* (Ramanathan and Kanabiran, 1989), *Azadirachtaindica* and *Dalbergiasissoo* (Sharma and Roy, 1995), *Azadirachtaindica* and *Polyalthialongifolia* (Pal *et al.,* 2000), *Cassia siamea* (Aggarwal, 2000) and *Nyatanthese arbor tristis*, *Quisqualisindica* and *Terminaliaarjua* (Rai and Kulshreshtha, 2006), nine ornamental plants in Khortoum ,Sudan (Saadabi, 2011). It is suggested that as the number of stomata decreases, consequently the number of epidermal cells increases.

Conclusion

This study demonstrates that leaf surface characters, including stomata and epidermal cells, in selected plant species *Ziziphus mauritiana* growing along road sides of Rewa city are considerably modified due to the stress of automobile exhaust emission with high traffic density. These changes could be considered as indicator of environmental stress.

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