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RESEARCH ARTICLE

EFFECT OF CEMENT DUST POLLUTION ON FOLIAR EPIDERMAL STRUCTURE OF *POLYALTHIA LONGIFOLIA* AND *EUCALYPTUS GLOBULUS* GROWING IN THE CAMPUS OF JAYPEE CEMENT PLANT, REWA (M.P.) INDIA

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ABSTRACT

This study was undertaken to investigate the effect of cement dust pollution on foliar epidermal structure of two evergreen plant species viz. *Polyalthia longifolia* and *Eucalyptus globulus* growing in the campus of Jaypee cement plant, Rewa (M.P.). Increase in number of epidermal cells and stomata per unit area was observed in leaf samples collected from polluted sites than those from control site by light microscopic method. Results revealed that, as compared to the leaves from control, the length and width of epidermal cells and guard cells reduced considerably in leaves of polluted sites. The observed changes in the epidermal structure of leaves of both species are regarded as adoptive responses, aimed at combating the adverse effects of pollution.

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INTRODUCTION

Air pollution is a major environmental problem affecting both the developing and the developed countries of the world. Air pollution from man's industrial and domestic activities is an important problem which may have significant and occasionally devising effects on plants and soils under both, cultivated and natural conditions, and on human health. Air pollution is due to the discharge of toxic fumes, gases, smoke and dusts into the atmosphere (Park and Park, 1985). The cement industry plays a very important role in the growth of the national economy and the social development of a country. This industry is the largest user of limestone, the most extensively exploited, natural minerals. During recent years, the increasing demands of cement for industrial and urban development in India have resulted in rapid expansion of cement manufacturing industries which are increasing the problems of particulate emissions around the source. Cement dust, produced by cement manufacturing units is considered one of the most hazardous pollutants which affect the surrounding environment and cause progressive reduction in the photosynthetic ability of leaves, closure of leaf stomata and, mainly, a reduction in growth and productivity of plants.

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The leaf is the part of the plant which first and most obviously displays visible symptoms of injury. Various studies have been done noted reductions in leaf area and growth of plant species growing in higher polluted site as compared to non or less polluted site (Giri *et al*., 2013; Dubey *et al*., 1984; Mc Cune *et al*., 1967; Pawar *et al*., 2013). The impact of the cement dust on soil properties and plant production has been investigated by some researchers Schuhmacher *et al*., 2004; Zerrouqi *et al*., 2008 and Prakash and Mishra, 2003.Under polluted conditions, plants developed different physiological, morphological and anatomical changes has been studied by Inamdar and Chaudhari, 1984; Iqbal, 1985; Gupta and Ghouse, 1988; Gravano *et al*., 2003; Novak *et al*., 2003; Dineva, 2004; 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; Amulya *et al*., 2015; Pawar, 2016. The cement dust, produced by cement industries is considered one of the most hazardous pollutants in ambient atmosphere of the Rewa city. This study was undertaken to assess the changes caused by cement dust pollution on foliar epidermal structure i.e. number and size of stomata and epidermal cells in the leaves two tree species viz. *Polyalthia longifolia and Eucalyptus globulus.*

MATERIALS AND METHODS

Selection of Site-The campus of Jaypee Cement Plant, Rewa (M.P.) was selected as polluted site for the present study. The cement plant (JRP) is located about 15 km from Rewa city which is the district headquarters. JRP is located at village Naubasta, in tehsil Huzur, district Rewa of Madhya Pradesh. An area within radius of 10 km around the project site has been defined as the study area.

Selection and sample collection of plant- Two common tree species viz. *Polyalthia longifolia* and *Eucalyptus globulus* growing in the campus of JP Cement plant, Rewa and APS University campus as control site were observed for epidermal features of their leaves. During sampling, the new and old leaf samples were collected from upper, middle and lower canopy area of each plant species and kept in polythene bags. The leaf samples were brought to the laboratory for further study. The same sampling of leaf was done for each plant species growing at control site (University Campus).

Slide Preparation: - Fresh samples of leaves ware washed and slides were prepared as per lasting impression method for counting of stomata and epidermal cells. A small patch of transparent nail paint was applied on leaf surfaces and the epidermal peels were peeled out with the help of cellophane tape after 30 minutes and sticked on the clear glass slides at room temperature. The stomata and epidermal cells were counted per mm unit area with the help of microscope ("Motic Images plus 2.0 ML" software).

Measurement of Cell Size: - Leaf impression was examined under 400 x total magnifications by light microscope. Number of stomata, epidermal cells and trichomes were counted per square millimeter area. Length and width of epidermal cells, stomata guard cells and trichomes of a leaf were measured in μm with the help of ocular micrometer under high power magnifications by micrometry i.e. "Stage-ocular micrometry" and optical microscope.

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
E= Average number of epidermal cells.

RESULTS

The leaves of two plant species *Polyalthia longifolia and Eucalyptus globulus* growing in the campus of Jaypee Cement plant, Rewa (M.P.) were observed for their number of stomata and epidermal cells, length and width of guard cells and epidermal cells. Similar observations were also made for the respective plant species growing in the campus of A.P.S University, Rewa, a control site Table-1 shows an average number of stomata, length and width of guard cells of both dorsal and ventral surface of new and old leaves of *Polyalthia longifolia* growing in polluted and control sites. *Polyalthia longifolia* is a hypostomatal species i.e. it has stomata only on ventral surface of the leaves collected from both the sites (polluted and control site). Old leaf samples of this species showed insignificantly increased number of stomata, length and width of stomatal guard cell at polluted site. On the other hand, the new leaves showed insignificantly increased number of stomata at control ones (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 *Polyalthia longifolia* 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 new and old leaves at polluted site than those of control site. However, this increase in number was statistically insignificant except for the ventral surface of old leaves (Table 4). Average length and width of epidermal cells of both surfaces of new and old leaves of *Polyalthia longifolia* sampled at polluted and controlled sites are also given in table 3. Results revealed that there was insignificant decrease in the length of epidermal cells of both surfaces of new leaves of polluted site than those of control ones (Table 3 and Table 4). Contrarily, old leaves showed statistically significant increase in the length of epidermal cells of both surfaces of polluted site. On the other hand, old leaves showed statistically significant increased but new leaves showed insignificant decrease in the width of epidermal cells on dorsal surface at polluted site. Contrarily, new and old leaves exhibited insignificant increase in the width of epidermal cells on ventral surface at polluted site.

Table-5 shows an average number of stomata, length and width of guard cells of *Eucalyptus globulus* of both dorsal and ventral surface of old and new leaves growing in polluted and controlled sites. The old and new leaves of this species collected from both the sites (polluted and control site) have reported stomata on both dorsal and ventral surface. A light microscopic study showed that there was decrease in number of stomata on both (dorsal and ventral) surface of new and old leaves at polluted site in comparison to those of control site. This decrease in stomata number was statistically significant (Table 6). Table-5 also shows average length and width of guard cells, stomatal index and stomatal frequency for the leaves of this species collected from polluted as well as control site. Results indicated reduction in size of length and width of guard cells of new and old leaves collected from polluted site in comparison to those of control site. However, length and width of guard cells have reduced significantly in new leaves and insignificantly in old leaves (Table 6). Similarly, the values of stomatal index and stomatal frequency were also observed to be higher for the leaves sampled from control site.
Table-7 shows average number, length and width of epidermal cells of *Eucalyptus globulus*. Results indicated that dorsal surface exhibited increased number of epidermal cells in new and old leaves of polluted site, as compared to leaves of control plant. The new leaves showed significantly increased number of epidermal cells on dorsal at polluted sites. Whereas, this increase was insignificant in old leaves (Table 8). Contrarily, ventral surface exhibited decreased number of epidermal cells in both new and old leaves at polluted sites, as compared to control ones. However, this decrease in epidermal cell number on ventral surface was statistically significant for old leaves but insignificant for new leaves (Table 8). Both the leaves demonstrated decreased length and width of epidermal cells on both the surfaces at polluted sites, as compared to control site (Table 7). This measured decreased length at polluted sites was observed to be significant for the epidermal cells of dorsal surface of new and old leaves and ventral surface of new leaves.

Table 1. Average number of stomata (mm^2), length (µm) and width of guard cells (µm), stomatal frequency (%) and stomatal index (%) of *Polyalthia longifolia* leaves growing in the campus of jaypee Cement plant and control site of Rewa

Leaf Surface	Stomata characteristics	Polluted		Controlled	
		Nature of leaves		Nature of leaves	
		New	Old	New	Old
Ventral	NOS	27.4±5.96	35.9±14.11	28.9±7.76	26.2±8.96
	LGC	18.3±3.86	15.9±4.01	19.5±4.52	14.7±2.62
	WGC	9.9±2.84	8.4±2.36	10.5±4.30	7.5±1.58
	SI	20.07	20.37	20.07	19.20
	SF	25.11	25.58	25.11	23.77

NOS= Number of Stomata
LGC= Length of Guard cell
WGC= Width of Guard cell
SI= Stomatal Index
SF= Stomatal Frequency

Table 2. Values of 't' test for number of stomata and size of guard cells of *Polyalthia longifolia* leaves growing at polluted and control sites

Leaf Surface	Stomata characteristics	Nature of leaves	
		New	Old
Ventral	LGC	0.6384 P=0.5312	0.7922 P=0.4386
	WGC	0.3682 P=0.7170	1.002 P=0.3296
	NOS	0.4848 P=0.6337	1.835 P=0.0831

LGC= Length of Guard cell
WGC=Width of Guard cells
NOS= Number of Stomata
* Significant
't' value at 18 d.f. on 0.05% level is 1.734
't' value at 18 d.f. on 0.01% level is 2.552

Table 3. Average number (mm^2), length (µm) and width (µm) of epidermal cells of *Polyalthia longifolia* leaves growing in the campus of Jaypee Cement plant and control site of Rewa

Leaf Surface	Epidermal cells	Polluted		Controlled	
		Nature of leaves		Nature of leaves	
		New	Old	New	Old
Dorsal	NEC	159.0±27.55	113.7±27.55	160.2±26.81	134.5±2.70
	LEC	10.8±2.52	20.1±4.01	11.1±2.84	10.5±3.53
	WEC	5.1±2.02	11.7±2.98	5.7±2.21	4.8±1.61
Ventral	NEC	109.1±10.71	140.3±20.83	113.0±10.87	110.2±10.77
	LEC	16.8±4.41	27.3±3.86	18.6±4.19	19.5±3.80
	WEC	11.7±4.11	13.2±3.52	11.1±4.48	10.5±3.80

NEC= Number of Epidermal cells
LEC= Length of Epidermal cell
WEC= Width of Epidermal cell

Table 4. Values of 't' test for number and size of epidermal cells of *Polyalthia longifolia* leaves growing at polluted and control sites

Leaf Surface	Epidermal cells	Nature of leaves	
		New	Old
Dorsal	LEC	0.2499 P=0.805	5.682**** P<0.0001
	WEC	0.6337 P=0.5342	6.442**** P<0.0001
	NEC	0.0987 P=0.9225	1.909 P=0.0724
Ventral	LEC	0.9357 P=0.3618	4.554*** P=0.0002
	WEC	0.3121 P=0.7586	1.648 P=0.1166
	NEC	0.8082 P=0.4295	4.059*** P=0.0007

LEC= Length of Epidermal cells
WEC= Width of Epidermal cells
NEC= Number of Epidermal cells
* Significant
't' value at 18 d.f. on 0.05% level is 1.734
't' value at 18 d.f. on 0.01% level is 2.552

Table 5. Average number of stomata (mm^2), length (µm) and width of guard cells (µm), stomatal frequency (%) and stomatal index (%) of *Eucalyptus globulus* leaves growing in the campus of jaypee Cement plant and control site of Rewa

Leaf Surface	Stomata characteristics	Polluted		Controlled	
		Nature of leaves		Nature of leaves	
		New	Old	New	Old
Dorsal	NOS	7.1±1.85	8.9±2.13	14.5±5.10	13.2±2.39
	LGC	20.9±4.14	18.3±3.59	26.4±3.94	19.5±3.80
	WGC	12.0±3.16	12.0±3.46	14.4±3.94	11.7±3.59
	SI	5.75	7.81	13.52	14.24
	SF	6.11	8.47	15.63	16.61
Ventral	NOS	11.2±1.98	10.4±3.16	19.7±3.56	22.1±3.07
	LGC	21.0±4	15.6±2.74	31.2±3.22	17.1±4.01
	WGC	13.8±3.22	9.9±2.9	19.2±3.20	10.8±3.79
	SI	8.86	8.63	13.52	14.20
	SF	9.73	9.45	15.63	16.55

NOS= Number of Stomata LGC= Length of Guard cell
WGC= Width of Guard cell SI= Stomatal Index
SF= Stomatal Frequency

Table 6. Values of 't' test for number of stomata and size of guard cells of *Eucalyptus globulus* leaves growing at polluted and control sites

Leaf Surface	Stomata characteristics	Nature of leaves	
		New	Old
Dorsal	LGC	3.043**	0.7259
		P=0.0070	P=0.4772
	WGC	1.503	0.1903
		P=0.1503	P=0.8512
	NOS	4.313***	4.346***
		P=0.0004	P=0.0004
Ventral	LGC	6.281****	0.9767
		P<0.0001	P=0.3417
	WGC	3.762**	0.5964
		P=0.0014	P=0.5583
	NOS	6.598****	8.398****
		P<0.0001	P<0.0001

LGC= Length of Guard cell WGC=Width of Guard cells
NOS= Number of Stomata * Significant
't' value at 18 d.f. on 0.05% level is 1.734
't' value at 18 d.f. on 0.01% level is 2.552

Table 7. Average number (mm^2), length (µm) and width (µm) of epidermal cells of *Eucalyptus globulus* leaves growing in the campus of jaypee Cement plant and control site of Rewa

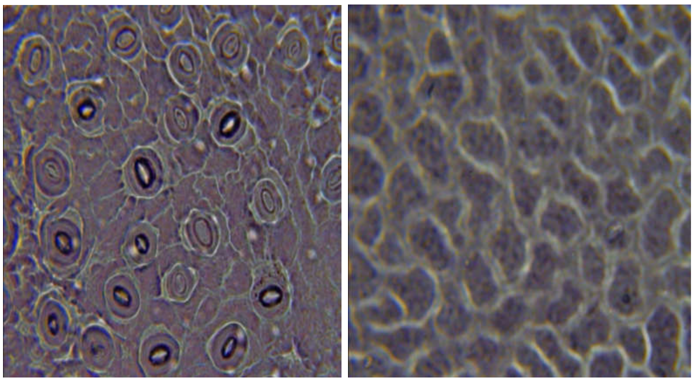
Leaf Surface	Epidermal cells	Polluted		Controlled	
		Nature of leaves		Nature of leaves	
		New	Old	New	Old
Dorsal	NEC	116.2±15.33	105.0±14.21	84.5±10.28	94.7±14.83
	LEC	21.0±3.74	21.0±3.74	33.3±4.34	31.2±3.79
	WEC	12.6±3.40	12.0±2.44	22.5±5.52	19.8±7.54
Ventral	NEC	115.1±17.53	110±21.01	126.0±21.30	133.5±10.89
	LEC	25.8±4.28	26.01±3.17	38.4±4.27	27.9±4.01
	WEC	20.4±6.60	15.3±2.62	27.9±4.01	12.9±4.48

NEC= Number of Epidermal cells LEC= Length of Epidermal cell WEC= Width of Epidermal cell

Table 8. Values of 't' test for number and size of epidermal cells of *Eucalyptus globulus* leaves growing at polluted and control sites.

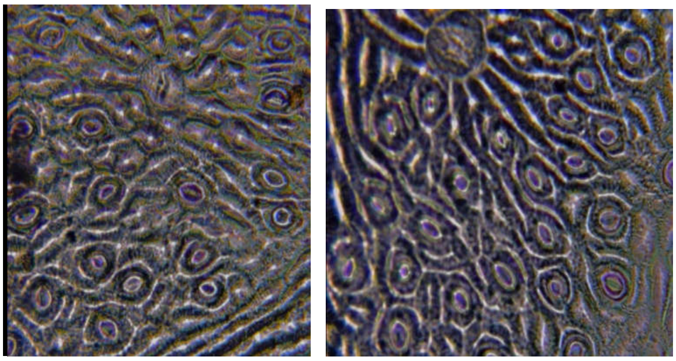
Leaf Surface	Epidermal cells	Nature of leaves	
		New	Old
Dorsal	LEC	6.789****	6.058****
		P<0.0001	P<0.0001
	WEC	4.829***	3.1112**
		P=0.0001	P=0.0060
	NEC	5.431****	1.586
		P<0.0001	P=0.1302
Ventral	LEC	6.591****	1.114
		P<0.0001	P=0.2801
	WEC	3.071**	1.462
		P=0.0066	P=0.1609
	NEC	1.25	3.14**
		P=0.2275	P=0.0057

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
't' value at 18 d.f. on 0.01% level is 2.552



(a) (b)

Fig. 1. *Polyalthia longifolia*: (a) Adaxial layer (b) Abaxial layer



(a) (b)

Fig. 2. *Eucalyptus globulus:* (a) Adaxial layer (b) Abaxial layer

The epidermal cell lengths of ventral surface in old leaves have decreased insignificantly at polluted sites (Table 8). Similarly, there was significant decrease in the width of epidermal cells on the dorsal and ventral surface of old and new leaves respectively. The dorsal and ventral surfaces of new and old leaves have reported insignificant decrease in width of epidermal cells respectively (Table 8).

DISCUSSION

The foliar surface is the most important receptor of atmospheric pollutants where they cause several structural, biochemical and functional changes. Mixed results have been observed with respect to responses of epidermal cells and stomata of leaves of selected plant species under present investigation. It was observed that cement dust pollution showed marked alterations in foliar surface architecture of selected plant species in cement plant. In present study *Polyalthia longifolia,* registered decreased number of epidermal cells in leaves of polluted site where as leaves of *Eucalyptus globulus* showed increased number of epidermal cells at polluted site as compared to control areas. Similarly, the plant species have shown significant alterations (decrease and increase) in the size of epidermal and guard cells in comparison to respective control plants. 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. The marked alterations in the size and number of foliar epidermal and guard cells, stomatal frequency and stomatal index in both dorsal abaxial and adaxial surface of plant species under this investigation are not unexpected. Several researchers investigating the effect of cement dust pollution on micro-morphological parameters of leaves of different plant species have reported similar results for Indian cities. (Jacobson *et al*., 1971; Sai *et al*., 1986; Salgare and Acharekar 1991; Gupta and Mishra 1993; Palanisway *et al*., 1995; Raina and Sharma 2006; Rai and Kulshrestha 2006; Shrivastava *et al*., 2013; Giri *et al*., 2013) Clogged stomata were observed in plants to expose to cement dust pollution in all cement industries. This might have been due to presence of high soot contents and other harmful constituents in exhausts of cement dust. The distorted shapes of stomata might have resulted due to lowering of pH in the cytoplasm of guard cells and thus a change in turgour relations of stomatal complex (Kondo *et al*. 1980) and due to physiological injury within the leaf (Ashenden, 1978). The decrease in number, length and width of stomata in the present study suggest that the species are more sensitive and does not have adoptive measures for mitigating the pollutants present in the air. However overall decrease in the number and size of stomata may be helpful in preventing the entry of pollutants into leaves, which can otherwise cause injury and death of the tissue of the leaves.

Conclusion

It may be concluded that trees can capture significant quantities of dust particles from the atmosphere with the potential to improve local air quality. Leaf surface characters, including epicuticular wax, cuticle and epidermal cell in plants growing in the campus of cement plant are significantly modified due to the stress of dust emission with high cement density in that area. The observed changes in the epidermal structure of leaves of both species are regarded as adoptive responses, aimed at combating the adverse effects of pollution. The present study reveals that the sticky particulate matter emitted from the cement plant is the major constituent of particulate pollution, which is deposited on the leaf surface of common plants growing in the campus. These plants have shown characteristics cuticle injury and decrease or increase in the number of epidermal cells and stomata, length and width of epidermal and guard cells this cultivar can be recommended for plantation in urban and industrial areas where particulate is problem.

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