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Impact of Cement Dust Pollution on Leaf Anatomical Features of *Lantana camara* and *Calotropis procera*

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ABSTRACT

Cement industries are major reservoirs of greenhouse gas emissions and suspended particle matter (SPM) with adverse impact on environment. In this study, impacts of cement dust on surrounding vegetation were assessed. The evaluation of anatomical characters of new and old leaves of two common shrubs *Lantana camara* and *Calotropis procera* growing in the vicinity of a cement factory presented a significant variation in epidermal and stomatal features in polluted as compared to control site. The leaves of *L. camara* show presence of trichome with reduced features in polluted site. Further, observation discloses modification in epidermal and stomatal features in both the species exposed in cement dust area. The alterations in anatomical features of these species are mainly an adaptation to resist against cement dust and for continuous survival, thus acting as a biomonitoring species for assessing air pollutant in the environment.

Key words: Air pollutant, cement dust, leaf anatomy, biomonitoring

Introduction

Cement industries are considered major sources of GHG emission and contributors of extremely large amounts of dust (Ghada and Ahmed, 2016; Magiera *et al.,* 2013) and fly ash with an alkaline nature of pH>7.2 and significant amount of trace elements (Magiera *et al*., 2013). Of the total global GHG emission, fossil fuel and industrial processes accounts for nearly 65% carbon emission (IPCC, 2014), with cement industry share of nearly 6% (CO_2.Earth GCE, 2014). In most of the countries, the environment has reached its carrying capacity in terms of air pollutants like sulfur dioxide (SO_2), nitrous oxide (NOx), carbon monoxide (CO), carbon dioxide (CO_2), suspended particles and toxic heavy metals (Radhapriya *et al*., 2012). India the second largest cement producer in the world, with a share of around 6% of the world's cement production (PCI report, MOC, 2011), and central India being the third largest producer of cement in the country provides up to 13% of national demand (IBEF, 2016). The processes of cement production like calcinations of limestone, combustion of fossil fuel and electricity consumption causes CO_2 emission. Overall, one tonne production of cement emits 0.85 tonnes of CO_2, 0.234 kg of particulate matter, 1.5 kg of sulphur dioxide and 3 kg of oxides of nitrogen (PCI report, MOC, 2011). Moreover, the cement production requires massive amount of energy and during storage, milling, packing and transportation huge amount of fly ash and dust is generated (Dubey, 2013).

In general, cement production sector has developed as a potential threat to the natural environment and living organisms (Magiera *et al*., 2013). The Kymore plateau in central India with highest number of cement factories is highly influenced by the air pollutant showing adverse impact on vegetation. The cement dust strongly influence the physiology and morphology including plant height, leaves area and number, chlorophyll fluorescence, leaf mass per area fresh and dry weight of shoot and root systems, reductions in chlorophyll and carotenoid, non-reducing and total sugars, protein and total lipid contents of the plant (Salama *et al*., 2011). Thus the plants surrounding the cement industry with high pollution exposure (Radhapriya *et al*., 2012) can not only be recommended as dust tolerant but can also be used as an efficient biomonitoring plant species. Therefore this

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research was carried out to evaluate the anatomical modification in leaves of common plant species for tolerance and continuous survival in response to cement dust pollution.

Materials and Methods

Collection of Plant material

Plant samples of two shrub species *Lantana camara* and *Calotropis procera* were collected from Jay pee Cement Plant (latitude 24^0 33'47'' North and longitude $81^0$11'41'' East) located 15 km North-West of Rewa town, Madhya Pradesh, India. The samples were collected from heavy dust contaminated area. The Rewa University campus which is located at a distance of 20 km from the cement factory was considered the control site. Ten leaf samples of each plant sps. were collected randomly from polluted and control site and brought to the Environmental Biology Department laboratory, Rewa University for anatomical study.

Slide Preparation

Fresh samples of leaves were rinsed with clean water and slides were prepared as per lasting impression method for counting of stomata and epidermal cells. To prepare an epidermal impression a small patch of transparent nail paint was applied on leaf surfaces and after 30 minutes the epidermal peels were peeled out with the help of sellotape 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, guard cells and trichomes of the leaf sample were measured in µm with the help of ocular micrometer under high power magnifications by micrometry i.e. "Stage-ocular micrometry" and optical microscope. The stomatal index and stomatal frequency were calculated by following equation

$$\text{Stomatal Index} = \frac{\text{number of stomata}}{\text{number of stomata} + \text{number of epidermal cells}} \times 100$$ (Salisbury (1927)

$$\text{Stomatal frequency} = \frac{\text{Average number of stomata}}{\text{Average number of epidermal cells}} \times 100$$

Standard t-test at multiple probability level using the Statistical Package Social Sciences (SPSS) version 16 software were applied for statistical calculations.

Results

The trichomes, stomatal characteristics and epidermal cells of new and old leaves of *L. camara* and *C. procera* were evaluated from polluted and control sites. Trichomes were absent in *C. procera* leaves in both sites. Both the species were noted to be amphistomatic with the presence of stomata on dorsal and ventral surfaces.

Trichomes observed in old and new leaves of *L. camara* on both ventral and dorsal surface shows significant differences in mean length, width and number (Table 1). The newer leaves shows decrease in number, whereas in older leaves the number of trichomes increases in dorsal while decreases in ventral surfaces in polluted sites. However, this decrease in numbers of trichome at polluted sites was statistically insignificant. Similarly, there was insignificant decreased in the length and width of trichomes in new and old leaves on dorsal surface whereas in older leaves only the dorsal surface shows decrease in size, whereas only ventral surface witnessed increase in width of trichomes in polluted sites The't' test value for number and size of trichomes in new and old leaves of both the sites shows significant differences ($p \leq 0.05$).

The characteristics of epidermal cells of both surfaces of *L. camara* and *C. procera* leaves growing at polluted and control sites are presented in Table 2. In *L. camara* there was an increase in number of epidermal cells per unit area (mm^2) on both dorsal and ventral surfaces of new and old leaves in polluted site compare to control site. However, this increase was statistically insignificant for old and new leaves surfaces, except on dorsal surface of old leaves where significant increase was observed (Table 2).

Table 1: Average number (mm^2), length (µm) width (µm) of trichomes of *Lantana camara* leaves growing at polluted and control sites

Leaf Surface	Trichomes characteristics	Polluted		Controlled		't' value	
		New	Old	New	Old	New	Old
Dorsal	Length	101.4 ±13.99	91.5 ±12.83	129.9 ±49.85	109.8± 96.27	1.741 P=0.0988	0.5959 P=0.5587
	Width	44.4 ±5.80	45.3 ±7.28	48.6 ±10.28	46.2 ± 6.68	1.125 P=0.2753	0.2881 P=0.7766
	Number	2.1 ±1.2	3.1 ±1.2	3.1 ±1.11	2.3± 1.26	1.935 P=0.689	1.454 P=0.1632
Ventral	Length	106.8 ±9.92	107.4 ±10.85	125.7 ±33.02	158.1± 58.76	1.733 P=0.1001	2.683* P=0.0152
	Width	53.1 ±11.05	48.3 ±4.99	53.7 ±12.29	46.2± 6.74	0.1148 P=0.9099	0.7919 P=0.4383
	Number	2.1 ±1.2	1.2 ±1.04	2.1 ±1.2	1.7 ± 0.94	1.715 P=0.1035	1.128 P=0.2743

Table 2: Average number (mm^2), length (µm) and width (µm) of epidermal cells of *L. camara* and *C. procera* leaves at polluted (p) and control (c) site

Leaf surface	Site	Nature of leaves	Epidermal cells characteristics					
			Length		Width		Number	
			L. camara	*C. procera*	*L. camara*	*C. procera*	*L. camara*	*C. procera*
Dorsal	P	New	63.0±8.49	30.0±6.63	27.6±6.76	13.8± 4.28	29.2±5.62	133.6±36.03
		Old	66.0±6.49	39.0± 5.46	27.3±5.74	27.9± 5.66	31.6±5.09	237.4±52.15
	C	New	36.0±11.54	27.9±4.49	15.3±5.74	16.8±4.74	26.6±3.72	176.1±9.59
		Old	80.4 ±6.76	23.4±11.56	44.1±5.67	17.7±7.80	26.3 ±6.15	167.6±8.28
Ventral	P	New	35.7±10.82	44.1± 5.66	24.6±10.08	20.4± 7.04	27.1±4.75	178.6±13.92
		Old	42.3 ±6.08	36.0±4.89	20.4 6.30	13.8±4.93	27.6 ±6.01	145.2±27.74
	C	New	78.0±4.48	23.7±4.99	39.0±4.48	12.9 ±3.48	26.7 ±4.97	184.9±8.36
		Old	45.6 ±6.30	36.0± 4.48	27.9±5.67	12.9± 3.48	23.8±5.52	185.8±7.24

Average length and width of epidermal cells of both surface of new and old leaves sampled at polluted and control sites revealed that new leaves exhibit increase in length and width on dorsal surface and decreased size of width of epidermal cells on ventral surfaces in polluted site than those of control ones. Contrarily, old leaf shows decreased size of length and width on dorsal surface whereas in ventral surface length of epidermal cell increases whereas width decreases in polluted site. This change in length and width was statistically significant on both surfaces of new and old leaves, except on ventral surface of old leaves (Table 3). *C. procera* leaves shows mixed results in regard to number of epidermal cells of both surface of old as well as new leaves growing at polluted and control site (Table 2). The number of epidermal cells decreased on dorsal and ventral surface of new leaves and also ventral surface of old leaves at polluted sites, except dorsal surface of older leaves.

However, this decrease was statistically significant for dorsal and ventral surface of new and old leaves respectively, and significant for ventral surface of new leaves (Table 3). An increase in length of epidermal cells of both surfaces of new and old leaves has been observed at polluted sites. This increase was statistically significant for dorsal and ventral surface of old and new leaves respectively (Table 3). The width of the epidermal cells was also reported to be increased on both surfaces of new and old leaves at polluted sites, except on dorsal surface of new leaves where

decrease in width was observed. The increase in width size was statistically significant for dorsal and ventral surface of old and new leaves respectively (Table 3). Significant difference was observed in stomatal characteristics of *L. camara* and *C. procera* leaves from the polluted and the control site (Table 4). The result reveals insignificantly decreased number of stomata on both surfaces of new leaves in *L. camara* at polluted sites. Contrarily, increased numbers of stomata were observed in old leaves on ventral surfaces and this modification was statistically significant on ventral surface and insignificant on dorsal surface of old leaves at polluted sites (Table 5). The reduction in length and width of guard cells of the leaves (new and old) collected from polluted site in comparison tothose of control site, except an increase in length of guard cells in dorsal surface of new leaves, was observed. The decrease in length and width of guard cells of both surfaces of old and new leaves at polluted sites was almost statistically significant (Table 5). Similarly, the Stomatal Frequency (SF) and Stomatal Index (SI) were also observed to be higher for the leaves sampled from control site, except SI increases in ventral surface of old leaves (Table 4).

Table 3: 't' test value between number and size of epidermal cells of *L. camara* and *C. procera* leaves at polluted and control site

Leaf Surface	Epidermal characteristics	Nature of leaves			
		New		*Old*	
		L. camara	*C. procera*	*L. camara*	*C. procera*
Dorsal	Length	5.96**** P<0.0001	0.8293 P=0.4178	4.859*** P=0.0001	3.859** P=0.001
	Width	4.386*** P=0.0004	1.485 P=0.1547	6.585**** P<0.0001	3.347** P=0.0036
	Number	1.22 P=0.2382	3.605** P=0.0020	2.099* P=0.0501	4.18*** P=0.0006
Ventral	Length	11.42**** P<0.0001	8.549**** P<0.0001	1.192 P=0.2488	0.09537 P=0.9251
	Width	4.128**** P=0.0006	3.02** P=0.0074	2.798* P=0.0119	0.4716 P=0.6429
	Number	0.184 P=0.8561	1.227 P=0.2357	1.473 P=0.1581	4.478*** P=0.003

**Indicate statistically significant differences (* P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, ****P ≤0.0001)*

Table 4: Average number of stomata (mm^2), length (µm) and width of guard cells (µm), stomatal frequency (%) and stomatal index (%) of *L. camara* and *C. procera* leaves growing at polluted and control site

Leaf surface	Site	Nature of leaves	Stomatal characteristics									
			Length		Width		Number		Frequency		Stomatal Index	
			L. camara	*C. procera*	*L. camara*	*C. procera*	*L. camara*	*C. procera*	*L. camara*	*C. procera*	*L. camara*	*C. procera*
Dorsal	P	New	17.1 ±5.67	20.1 ±5.66	6.6 ±3.10	45.0 ±4.47	2.8 ±1.48	6.6 ± 2.22	9.59	4.95	8.75	4.71
		Old	18.3 ±6.08	16.8 ± 6.81	7.8 ±2.90	13.8 ± 4.2	4.4 ±1.84	5.9 ±2.51	13.93	2.49	12.23	2.43
	C	New	12.9 ±3.48	15.3 ± 4.99	10.2 ±4.05	45.0 ± 4.47	3.1 ±1.53	10.6 ± 1.78	11.66	6.02	10.43	5.68
		Old	25.8 ±5.14	19.2 ± 5.69	20.4 ±7.05	12.0 ± 3.16	3.7 ±1.34	8.5 ±2.45	14.07	5.08	12.33	4.83
Ventral	P	New	14.7 ±5.38	26.1 ± 6.33	9.9 ±4.49	15.3 ± 4.98	3.5 ±1.59	11.8 ±2.29	12.91	6.61	11.44	6.20
		Old	15.9 ±6.01	19.2 ± 8.02	12.3 ±4.99	13.2 ±4.04	15.3 ±4.14	7.0 ± 2.10	55.43	4.82	35.66	4.60
	C	New	24.6 ±6.89	25.5 ± 5.53	16.2 ±6.20	13.5 ±3.81	4.3 ±2.31	12.9 ± 3.29	16.10	6.98	13.87	6.53
		Old	36.2 ±6.77	25.5 ± 4.72	36.2 ±6.77	12.3 ±3.59	10.1 +±2.61	11.7 ±2.31	42.44	6.30	29.79	5.93

The stomatal characteristics of *C. procera* leaves presented in Table 4 shows reduction in the number of stomata on dorsal and ventral surface of both new and old leaves at polluted site as compare to control site. This decrease in stomata numbers was statistically significant, except at ventral surface of new leaves (Table 5). The result reveals increased length of guard cells of new leaves on both surfaces at polluted site, where as a decrease in length of guard cells on both surfaces of old leaves (Table 4). The increased length of guard cells on both surfaces of new leaves of polluted sites was statistically not found to be significant. The decreased length of guard cells was statistically significant on ventral surface of old leaves but insignificant on dorsal surface of the leaves at polluted sites (Table 5). The guard cells of both surfaces of new and old leaves have registered an increase in width at polluted sites. Statistical analysis shows insignificant increase in the width of guard cells of both surfaces of new and old leaves, except for the guard cells of both surfaces of new leaves where t-test value observed to be significant (Table 5). The stomatal features like frequency and stomatal index decreases in both the surfaces of new and old leaves in polluted as compared to control site (Table 4).

Table 5: 't' test value between number of stomata and size of guard cells of *L. camara* and *C. procera* leaves at polluted and control site

Leaf surface	Stomata characteristics	Nature of leaves			
		New		*Old*	
		L. camara	*C. procera*	*L. camara*	*C. procera*
Dorsal	Length	1.996 P=0.0612	2.012 P=0.0595	2.979** P=0.0080	0.8552 P=0.4037
	Width	2.232* P=0.0386	7.799**** P<0.0001	5.227**** P<0.0001	1.083 P=0.2931
	Number	0.4457 P=0.6612	4.445*** P=0.0003	0.9725 P=0.3437	2.344* P=0.0307
Ventral	Length	3.581** P=0.0021	0.2257 P=0.8240	7.091**** P<0.0001	2.141* P=0.0462
	Width	2.603* P=0.0180	0.9078 P=0.3760	0.1491 P=0.8832	0.5266 P=0.6041
	Number	0.902 P=0.3789	0.8678 P=0.3969	3.36** P=0.0035	4.0761*** P=0.0004

**indicate statistically significant differences (* P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, ****P ≤0.0001)*

Discussion

The shrubs *L. camara* and *C. procera* collected from cement polluted site shows remarkable differences in leaf epidermal and stomatal features, when compared with control site. Several studies have reported the impact of cement dust pollution on micro morphological characteristics of leaves in different plant species either locally (Shukla *et al*., 2008; Chauhan, 2014; Rai and Mishra, 2015) or other regions in India (Raajasubramanian *et al*., 2011; Kumar and Thambavani 2012; Ramesh *et al*., 2014; Kumar *et al., 2015).*

In present study *L. camara* leaves exhibited a modification in number, length and width of trichomes in polluted sites. It makes plant resist against cement dust pollutant. The modification in trichome frequency in polluted plants have been reported by Prasad and Inamdar (1990) Ayanbamiji and Ogundipe (2010). The findings are in close conformity with Amulya *et al*. (2015) who reported decrease in trichome length in plant leaves in cement dust polluted site. The modifications in trichomes are adaptive features that can favour survival to the cement dust polluted environment (Ogunkunle *et al*., 2013). The presence of trichomes enhances the scavenging and retention of airborne particulate and are useful in biomonitoring (Baldantoni *et al*., 2014).

The leaf epidermal characteristics of *L. camara* registered an increase in number of epidermal cell in dorsal and ventral surface of new and old leaf in polluted sites. However, length and width of epidermal cell in dorsal surface increases and in ventral surface it decreases whereas in old leaves the modification in epidermal structure follows opposite trend. The *C. procera* leaves shows decrease in number and increase in length and width of epidermal cells in polluted site. This indicates that some epidermal features of *L. camara* and *C. procera* leaves were not affected by the pollution level and

cement dust pollutants has not reached the threshold limit. Earlier studied confirmed that there are certain plants that are tolerant to dust pollutants and shows good survival at cement polluted sites (Shafiq and Iqbal 1998; Iqbal and Shafiq 2001; Ogunkunle *et al*. 2013 and Siqueira-Silva *et al*. 2016 a). The deposition of cement dust and heavy metals to some extent alters anatomical features of the leaf epidermis in plants as reported in previous works (El-Khatib *et al*., 2012; Ghada and Ahmad, 2016). The cement dust shows a wider impact on structural organization and physiological traits resulting reduction in epidermal cell turgidity and obstructed stomata (Siqueira-Silva *et al*. 2016b).

Stomatal charaterstics of the *L. camara* and *C. procera* represents significant variation in polluted site compare to control. *L. camara* shows reduction in number, size, frequency and stomatal index in polluted site. On contrary the stomatal features of *C. procera* exhibited a decrease in number, frequency and stomatal index however an increase in length and width of guard cell in polluted site. This modification was not significant in response to plant survival. Several studies observed alteration in the stomatal features in plant leaves exposed under cement dust environment (Gostin 2009; Ayanbamiji and Ogundipe 2010; Amulya *et al*., 2015; Siqueira-Silva *et al*., 2016a, 2016b) This modification is a necessary adaption in response to the pollution stress and helps in restricting the dust pollutant entering the plants. The cement dust deposition lead to modification in stomatal frequency which is an adaptive mechanism to counter the blockage and proper functioning of gaseous exchange and transpiration rate (El-Khatib *et al*., 2012). Gostin (2009) reported decrease in size and increase in stomatal density in the leaves in polluted sites. Siqueira-Silva *et al*., (2016a) observed that the exposure of plant to cement dust polluted environment leads to a mild morphoanatomic alteration in leaves making it more resistant to the pollutant.

Conclusion

In conclusion the cement dust pollution stress results in changes in anatomical features of the plant leaves. The present investigation shows a remarkable impact on epidermal and stomatal characteristics of the two shrub species in cement polluted compare to control site. This anatomical variation will help in biomonitoring and selecting suitable dust tolerant species with prolongs survival potentiality.

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