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Influence of cement dust pollution on leaf epidermal features of *Pennisetum purpureum* and *Sida acuta*

Clement Oluseye Ogunkunle*, A.A. Abdulrahaman, P.O. Fatoba

Department of Plant Biology, University of Ilorin, Ilorin, Nigeria

*Corresponding author, E-mail: ogunkunle.co@unilorin.edu.ng

Abstract

Anatomical features of leaves of two plants (*Pennisetum purpureum* Schumach and *Sida acuta* Burm. F.) growing around a cement factory were studied with the aim of examining leaf epidermal modifications that enhance their tolerance and continued survival in the presence of cement dust pollution. *P. purpureum* showed no anatomical modifications to the pollution, which may indicate that the dose-response level of the pollutants has not been reached in the grass. There were significant modifications in the stomatal size, density and index of leaves of *S. acuta* exposed to cement dust pollution. The observed modifications of stomatal features in the forms of reduced stomatal size and increased stomatal index in the leaves *S. acuta* from cement polluted area could be favourable anatomical adaptations to a polluted environment. These responses could be used as biological markers for the presence of cement dust pollutants in leaves of *S. acuta*.

Key words: cement particles, heavy metals, leaf epidermis, *Sida acuta, Pennisetum purpureum*.

Introduction

Air pollution in form of particulate matter is a serious problem affecting developed and developing countries (WHO 1997). One of the culpable industrial sectors is cement production with associated high dust particle emission (Gbadebo, Bankole 2007), which plays a significant role in the imbalance of the environment. The dust particles that escape during blasting of raw materials, grinding of cement clinker and packaging or loading of finished cement are often transported by wind and deposited in areas close and far away from the factory depending on the injection height of the particles, the terminal settling velocity of the particles, and the degree of atmospheric turbulence (CPCB 2007). Deposition of cement dust causes many several biochemical and physiological effects (Liu et al. 1997; Lepedus et al. 2003) in plants; and anatomical structure of plants are also distorted when pollutants in cement dusts are taken in by plants (Gostin 2009). Despite the detrimental effects of cement dusts on plants, some still remain tolerant to cement dust pollution, probably because of the genetic make-up or due to some biochemical/anatomical modifications during the stress periods (Erdal, Demirtas 2010). There is paucity of information on the anatomical modifications of leaves of plants exposed to cement dust pollution. Epidermis structures like trichomes have been reported to bear the brunt of damage from cement dust in *Cajanus cajanus* exposed to foliar dust application (Baralabai, Vivekanadan 1996). Reduction in stomata size and increase in stomatal density in leaves of *Trifolium* spp exposed to cement dust pollution has been reported (Gostin 2009), probably as a surviving strategy in polluted environment.

In this paper, anatomical study was carried out on the leaves of *Pennisetum purpureum* and *Sida acuta* growing around a cement factory, to assess their leaf adaptive responses to the influence of cement dust pollution. These weed species were prevalent in the vicinity of the cement factory, which suggests that these species have evolved modifications in their leaf anatomy for surviving the heavy cement dust pollution. We examined stomatal-related parameters of *Pennisetum purpureum* and *Sida acuta*, because stomata are the apparatuses that address the control of gas exchange required for plant metabolism and also serve as the gateway to the atmosphere.

Materials and methods

Collection of study material

Leaf samples of *Sida acuta* Schumach and *Pennisetum purpureum* Burm. F. were collected around the Lafarge-Cement WAPCO factory, Sagamu, south-western Nigeria (6°50' and 7° 00' N; 3°45' and 4°00'E) where heavy metal contamination from cement dust emissions has occurred (Ogunkunle, Fatoba 2012; Ogunkunle, Fatoba 2013). Control samples were collected at a distance of about 230 km from the cement factory where there was no record of cement dust pollution.

Description of diagnostic plants

S. acuta (Malvaceae) is a perennial erect shrub with height about 150 cm. It has glabrous leaf surfaces and is indigenous to pan tropical areas (Karou et al. 2005). *S. acuta* occurs on a wide range of soil types and reproduces by seed (Parsons, Cuthbertson 1992). *P. purpureum* (Poaceae) is native to the humid, tropical mainland of Africa (Burkill 1994) and grows on poorly drained clay to excessively drained sandy soil of pH range 4.5 to 8.2 (http://www.hort.purdue.edu/newcrop/duke_energy/Pennisetum_purpureum.html). Rainfall requirements of *P. purpureum* are 1500 mm per year, temperature for optimum growth is between 25 to 40 °C and fertilization is through cross pollination by wind (Skerman, Riveros 1990).

Sampling and isolation of leaf epidermal layers

Ten plant samples of each of *S. acuta* and *P. purpureum* were collected randomly and three mature, photosynthesizing leaves from each of the plant samples were prepared as specimens for anatomical study. Leaf segment of an area of 1cm2 from each specimen was cut and immersed in concentrated solution of nitric acid for 5 to 10 min. The upper (adaxial) and the lower (abaxial) surfaces were separated with dissecting needle and forceps and rinsed with clean water. Each specimen was stained with 1% aqueous safranin for 5 to 30 min and rinsed in water (modified from Olofinobinu, Oladele 1997). The samples were then mounted on glycerine jelly for microscopic observation using a Olympus research microscope fixed with an Amscope camera (FM A050). Sample field of 35 was used for all examinations. The following anatomical characteristics were determined: epidermal cell wall, anticlinal cell wall, stomatal size, stomatal density, stomata index and stomatal complex-types (methods after Salisbury 1927; Stace 1965; Franco 1939; Weyers, Meidner 1990). Terminologies for naming stomatal complex types followed those of Dilcher (1974), Metcalfe and Chalk (1988), Weyers and Meidner (1990). Each set of parameters was subjected to a Student t-test at the 95% probability level using the Statistical Package Social Sciences (SPSS) version 16.

Results

There was no difference in epidermal cells and anticlinal cell walls of *P. purpureum* from cement polluted and the control sites (Table 1). Trichomes were absent in *P. purpureum* from both sites (Table 1; Figs. 1 and 2). Epidermal cell and

Table 1. Epidermal cells, anticlinal cell wall patterns and trichome-type of *Pennisetum purpureum* from cement polluted and the control sites

Parameter	Cement-polluted site	Control site
Epidermal cell	Elongated, rectangular	Elongated, rectangular
Anticlinal cell wall	Wavy	Wavy
Trichome type	Absent	Absent

anticlinal cell walls of *S. acuta* from both cement polluted and the control sites were straight and wavy. Trichomes were present in *S. acuta* from the two sites but with different frequency, density and index except for long glandular and stellate trichomes that had the same trichome density (Table 2; Figs. 3 and 4).

Table 3 shows the stomatal complex-type in *P. purpureum* and *S. acuta* from the cement polluted site (Lafarge-Cement WAPCO factory) and the control (University of Ilorin campus). *P. purpureum* from the cement polluted site had three different stomatal complex-types (tetracytic, anisocytic and paracytic); the adaxial surface had a tetracytic and anisocytic stomatal complex-types, while the abaxial surface had tetracytic and paracytic complex-types (Table 3). The control site had two different stomatal complex-types, paracytic and tetracytic, both on the adaxial and abaxial surfaces. The frequency of the different stomatal complex-types in both leaf surfaces differed between the two sites (Table 3). *S. acuta* from the cement polluted site had three stomatal complex-types, anisocytic, paracytic and laterocytic on both the adaxial and abaxial surfaces. The stomatal complex-types in *S. acuta* from the control had four stomatal complex-types (anisocytic, paracytic, laterocytic and diacytic) on the adaxial surface while there were three stomatal complex-types on the abaxial surface (anisocytic, paracytic and laterocytic). There was varied frequency of stomatal complex-types between the *S. acuta* from cement polluted and the control sites, except for the paracytic stomatal complex-type on the adaxial surface (Table 3).

Mean values of stomatal features of *P. purpureum* from the cement-polluted site and the control site are presented in Table 4. There was no significant difference in stomatal density and stomatal index in the leaf surfaces (adaxial and abaxial) between *P. purpureum* from the cement-polluted and the control sites ($p < 0.05$; Table 4). Stomatal size of the adaxial surface in *P. purpureum* showed no significant difference between the two sites, while there was a significant difference on the abaxial surface ($p < 0.05$).

There was a significant difference in stomatal density on both surfaces between *S. acuta* from cement polluted and the control sites ($p < 0.05$; Table 5). The stomatal index of adaxial surface between the two sites showed significant difference while no significant difference was recorded for the abaxial surface ($p < 0.05$). The difference was also significant ($p < 0.05$) for stomatal size in the abaxial surface, while no significant difference was recorded for the adaxial surface (Table 5). It is important to note that *P. purpureum* and *S. acuta* from the two sites are amphistomatic i.e. they have stomata on both leaf surfaces.

Discussion

Leaf anatomical studies of *P. purpureum* and *S. acuta* collected from the cement dust polluted site (Lafarge-

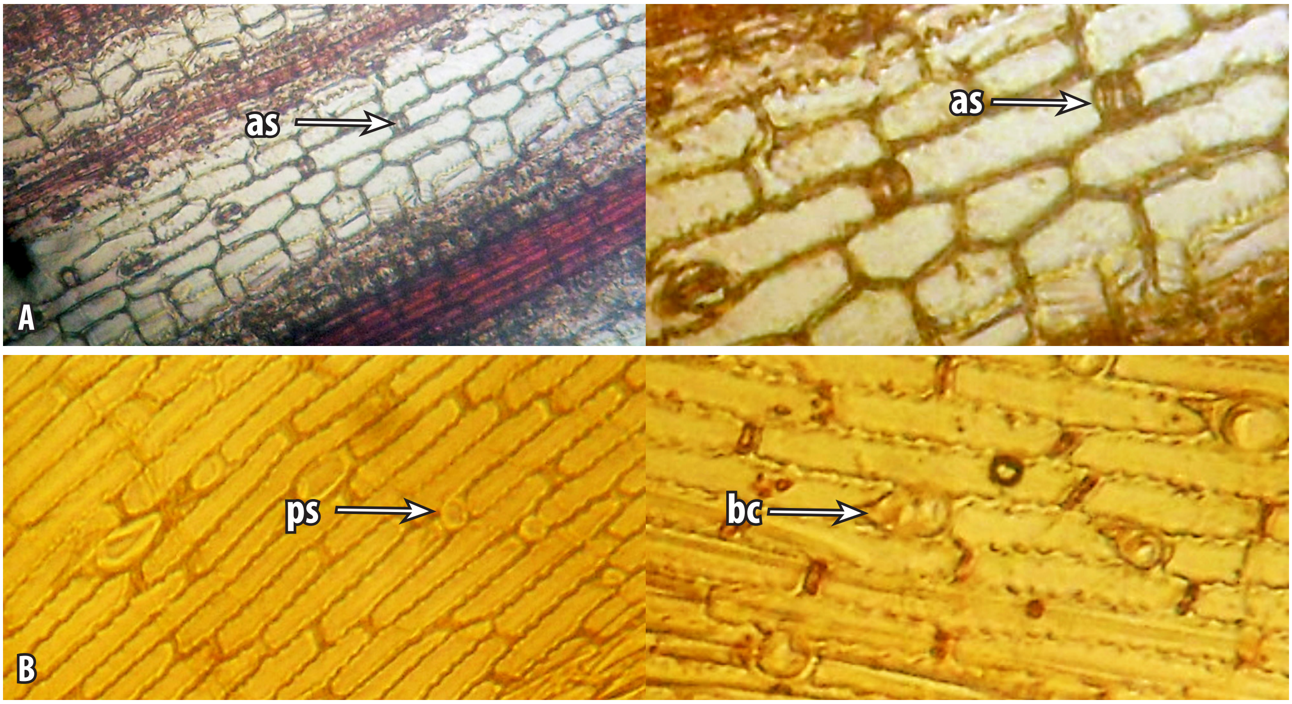

Fig. 1. Adaxial leaf epidermis of *Pennisetum purpureum* exposed to cement pollution (A) showing anisocytic stomata (as) (absence of trichomes), and control conditions (B) showing paracytic stomata (ps) and bulliform cells (bc).

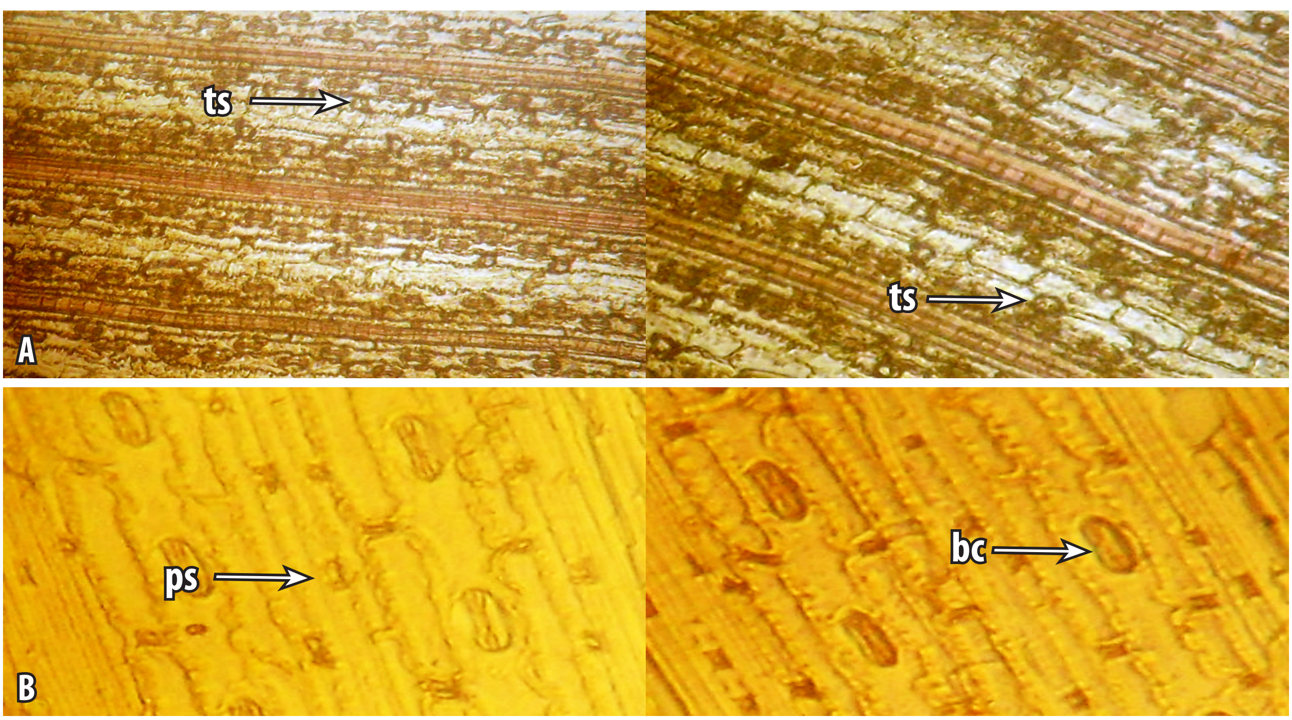

Fig. 2. Abaxial leaf epidermis of *Pennisetum purpureum* exposed to cement pollution (A) showing tetracytic stomata (ts) (absence of trichomes), and control conditions (B) showing paracytic stomata (ps) and bulliform cells (bc).

Cement WAPCO factory site) in Sagamu, south-west Nigeria was compared to the same species collected from another environment where the environment was free from cement dust pollution. The latter samples were used as the controls in order to evaluate the leaf epidermal responses of the diagnostic species to cement dust pollution in the study area. Several researchers have reported that the air and soil media around the Lafarge-Cement WAPCO factory in Sagamu is polluted, especially with heavy metals deposited along with the cement dust (Bankole, Gbadebo

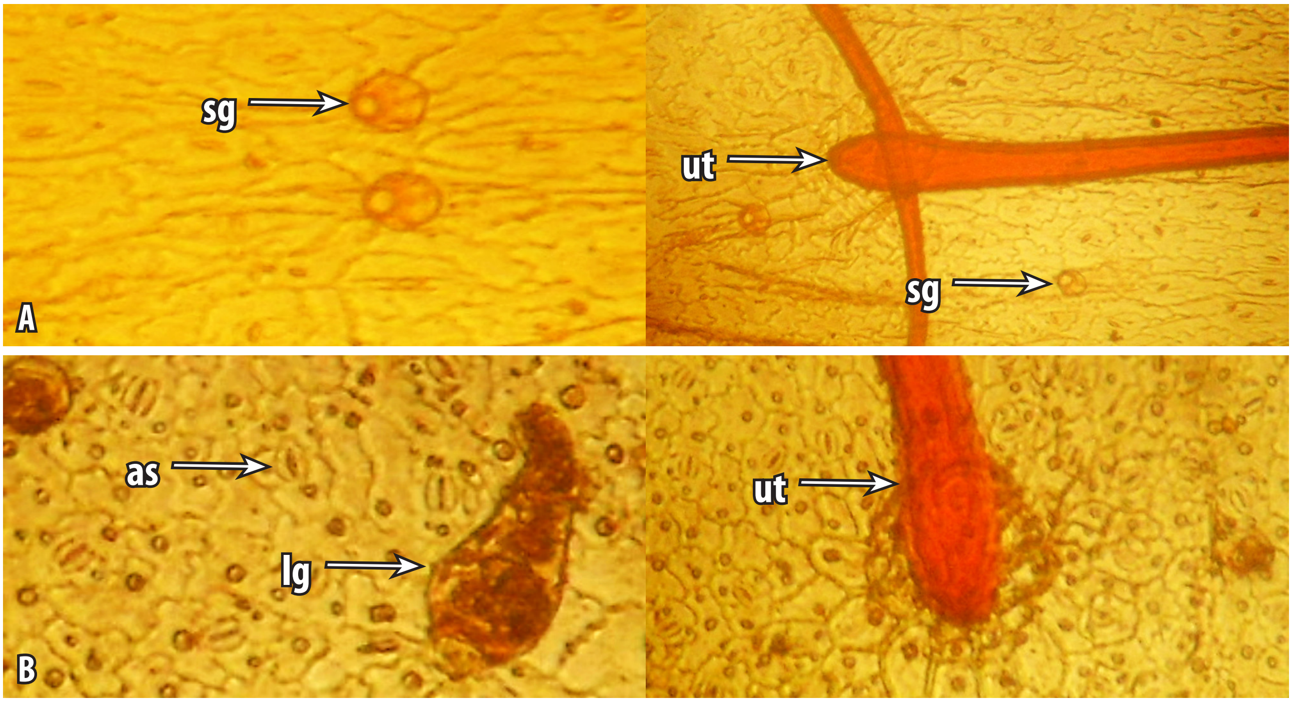

Fig. 3. Adaxial leaf epidermis of *Sida acuta* exposed to cement pollution (A) showing short small glandular (sg) and unicellular trichomes (ut), and control conditions (B) showing anisocytic stomata (as), large glandular trichome (lg) and unicellular trichome (ut).

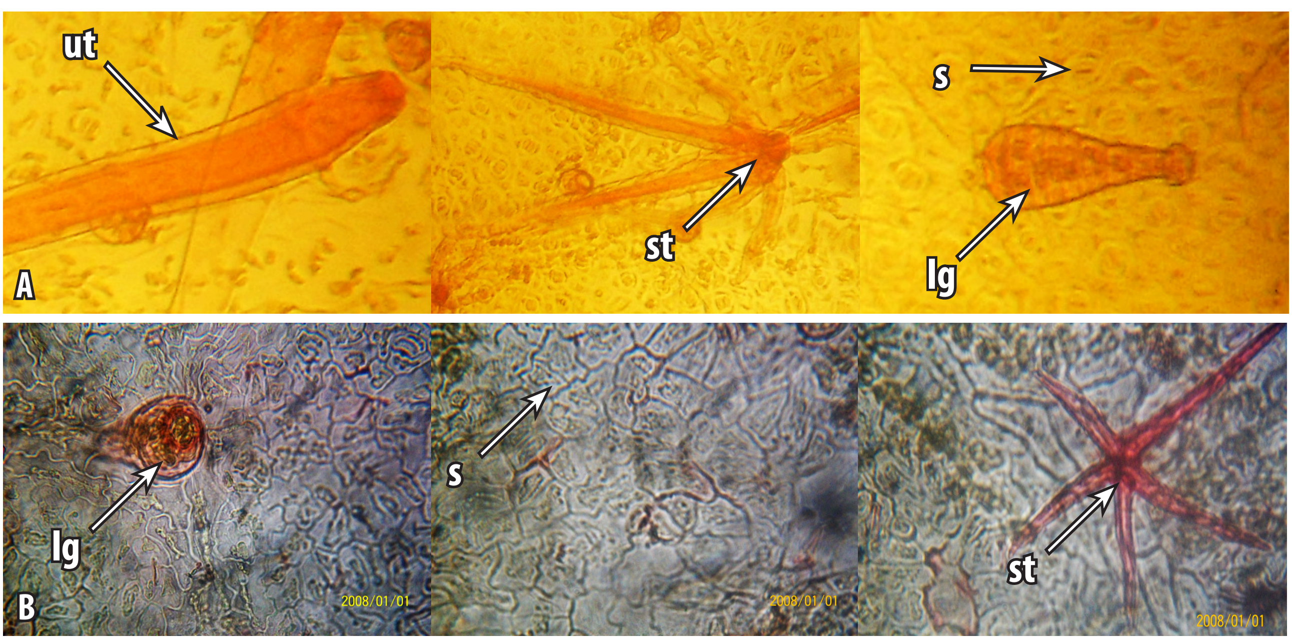

Fig. 4. Abaxial leaf epidermis of *Sida acuta* exposed to cement pollution (A) showing unicellular trichome (ut), stellate trichome (st), stomata (s) and large glandular trichome (lg), and control conditions (B) showing stomata (s), large glandular (lg) and stellate (st) trichomes..

2007; Ogunkunle, Fatoba 2012; Ogunkunle, Fatoba 2013). Foliar uptake through the stomata or leaf cuticle or both may be the principal route for accumulation of these air-borne pollutants in plants growing around such polluted areas. Pollution stress can alter structure of plant leaves exposed to air pollution, but nevertheless, some are quite resistant to air pollutants and grow to maturity with several modifications (Gostin 2009).

There was no significant difference in the leaf epidermal features of *P. purpureum* on both leaf surfaces except stomatal size in the abaxial surface. This implies that the leaf epidermal features of *P. purpureum* were not affected

Table 2. Epidermal cells, anticlinal cell wall patterns and trichome-type of *Sida acuta* from cement polluted and the control sites

Site	Epidermal cell	Anticlinal cell wall	Trichome type	Trichome frequency (%)	Trichome density (per mm²)	Trichome index
Cement-polluted	Straight	Wavy	short glandular	50	3	3.93
			long glandular	23	1	1.34
			unicellular	20	2	2.65
			stellate	7	1	1.07
Control	Straight	Wavy	short glandular	70	3	4.64
			long glandular	15	1	1.61
			unicellular	10	3	4.64
			stellate	5	1	1.50

Table 3. Stomatal complex-types in *Pennisetum purpureum* and *Sida acuta* from cement-polluted and the control sites

Species	Cement-polluted site			Control site		
	Surface	Stomatal complex-type	Frequency (%)	Surface	Stomatal complex-type	Frequency (%)
P. purpureum	Adaxial	Tetracytic	57	Adaxial	Tetracytic	30
	Adaxial	Anisocytic	43	Adaxial	Paracytic	70
	Abaxial	Tetracytic	62	Abaxial	Tetracytic	25
	Abaxial	Paracytic	37	Abaxial	Paracytic	75
S. acuta	Adaxial	Anisocytic	85	Adaxial	Anisocytic	5
	Adaxial	Paracytic	10	Adaxial	Paracytic	10
	Adaxial	Laterocytic	5	Adaxial	Laterocytic	60
		Absent	–	Adaxial	Diacytic	25
	Abaxial	Anisocytic	10	Abaxial	Anisocytic	5
	Abaxial	Paracytic	10	Abaxial	Paracytic	20
	Abaxial	Laterocytic	80	Abaxial	Laterocytic	75

by the pollution level of the environment. It is possible that the level of atmospheric pollutants has not reached the threshold that could initiate leaf epidermal modification or response in *P. purpureum*. The high frequency of tetracytic and anisocytic stomatal complex-types in *P. purpureum* might suggest that it needs to transpire faster than normal to carry out biochemical activities, due to the presence of cement dust clogging some of the stomatal pores. Earlier studies by Obiremi and Oladele (2001) and Oyeleke et al. (2004) confirmed that more subsidiary cells surrounding the guard cells lead to faster opening of the stoma and vice versa. Also, the presence of the tetracytic stomatal complex-type in high frequency may play a role in reducing the amount of toxic gases accumulating in the leaves, as AbdulRahman and Oladele (2008) suggested that plants that possess stomata with many subsidiary cells (e.g. tetracytic and anomocytic types) play an important role in reducing greenhouse gases.

Several leaf epidermal modifications in trichome density, stomatal density, stomatal index and stomatal size of *S. acuta* is a good indication that the level of atmospheric pollutants has become hazardous to the species. These responses of *S. acuta* could be adaptive features to tolerate the high cement dust pollution of the area. Increased number/

Table 4. Mean values of stomatal features of *Pennisetum purpureum* from cement-polluted site and the control site. *Significant at $p < 0.05$

Parameter	Leaf surface	Cement polluted	Control	*t*-Value	*P*-Value
Stomatal density (per mm²)	Adaxial	13.0 ± 1.826	13.0 ± 0.632	0.334	0.074
	Abaxial	17.75 ± 3.096	17.14 ± 0.890	0.387	0.723
Stomatal index	Adaxial	27.62 ± 3.470	27.52 ± 0.502	0.059	0.957
	Abaxial	43.25 ± 5.188	43.14 ± 0.090	1.546	0.219
Stomatal size (μm²)	Adaxial	96.97 ± 10.781	75.05 ± 7.467	3.148	0.051
	Abaxial	100.30 ± 14.681	68.14 ± 6.267	5.864*	0.031

Table 4. Mean values of stomatal features of *Sida acuta* from cement-polluted site and the control site. *Significant at $p < 0.05$

Parameter	Leaf surface	Cement polluted	Control	*t*-Value	*P*-Value
Stomatal density (per mm²)	Adaxial	14.00 ± 1.410	11.70± 2.165	1.877*	0.042
	Abaxial	21.75 ± 2.75	7.66 ± 4.490	13.666*	0.001
Stomatal index	Adaxial	37.66 ± 1.957	97.92 ± 2.083	5.322*	0.013
	Abaxial	61.71 ± 4.046	45.10± 32.396	0.883	0.468
Stomatal size (μm²)	Adaxial	54.11 ± 19.392	61.22 ± 10.063	0.247	0.954
	Abaxial	47.40 ± 0.001	67.67 ± 12.570	3.225*	0.048

frequency of trichomes in *S. acuta* can favour adaptation/survival to the cement dust polluted environment. Sharma and Davis (2001) have reported increased trichome density and reduction in epidermal wall undulation as a response of *Parthenocissus quinquefolia* to cement pollution. The increased number of trichomes may aid *S. acuta* in filtering out particulate matter and insulating the leaflet surface from detrimental pollutants, which otherwise may enter the leaf and disrupt metabolic activities in plant tissues. Thus, increased number of trichomes with significant occurrence of stomatal complex-types with large number of subsidiary cells on the leaf of *S. acuta*, may be an adaptation to the stress of cement dust pollution. Stomata with many subsidiary cells tend to open more often than those with small number of subsidiary cells (AbdulRahaman, Oladele 2009) and this opening process may facilitate required gas exchange for metabolism in the pores that are still unblocked by cement dusts. This can explain the reason for the absence of the diacytic stomatal complex-type in *S. acuta* from the cement dust polluted environment, as the function of this complex in such an environment would be an added burden to the already stressed plant species.

Modifications in the frequency and sizes of stomata have been reported to be responses to environmental stress, and seem to be an important strategy of controlling the leaf absorption of pollutants by plants (Gostin 2009). Verma et al. (2006) asserted that decrease of stomatal size may be an avoidance mechanism against the inhibitory effect of pollutants on physiological activities, such as photosynthesis, and also result in quicker response to external stimuli (Hetherington, Woodward 2003).

The significantly high stomatal density observed in *S. acuta* from the cement polluted site is another adaptive response to the cement dust pollution. Studies have shown that this anatomical characteristic favours survival of plants in harsh environments. Increased stomatal density is considered as adaptability indicator to a polluted environment (Kapitonova 2002; Gostin 2009). According to Yunus and Ahmad (1979), high stomatal density of leaf in plants around polluted environments is due to the response of the plants to the loss of mature and healthy stomata, through the process of degradation caused by air pollution. The presence of stomata on adaxial and abaxial surfaces of leaf is also an important feature that could be responsible for the survival of *S. acuta* in the presence of the cement dust pollution. Amphistomatic leaves have been considered a reason for ecological success of *Rosa* sp. (Nawaz et al. 2011)

Reduced stomatal index recorded in *S. acuta* is another leaf epidermal response to air pollution. Verma et al. (2006) found significant reduction in the stomatal index of *Ipomea pes-tigridis* to be a response to environmental stress (coal-smoke pollution). Chauhan et al. (2004) also suggested that reduction in stomatal index could be considered as a favourable adaptation to air pollution, as it might help in reducing the absorption of gaseous pollutants.

In conclusion, the anatomical structures of the leaves of *P. purpureum* showed no anatomical/epidermal modifications to the cement dust pollution around the studied area. *P. purpureum* may be a very resilient grass to cement dust pollution. *S. acuta* appeared to be sensitive to the cement dust pollution and had various anatomical modifications of the leaf epidermis. These responses/modifications reported in *S. acuta* may be used as biological markers for the presence of cement dust pollutants in *S. acuta* leaves.

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