Journal of Applied and Natural Science
11(1): 193-198 (2019)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Stomatal Index of a few common tree species in the Kishangarh Marble Slurry Dump-yard area, Ajmer (Rajasthan): A case study

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Article Info
DOI: 10.31018/jans.v11i1.1977
Received: January 9, 2019
Revised: February 24, 2019
Accepted: February 28, 2019

How to Cite
Gahlot, L. and Khanna, V. (2019). Stomatal Index of a few common tree cpecies in the Kishangarh Marble Slurry Dump-yard area, Ajmer (Rajasthan): A case study. *Journal of Applied and Natural Science*, 11 (1): 193-198

Abstract
The size and number of the stomata present on the foliar epidermis of Tree species is directly influenced by the Suspended Particulate Matter. Marble dust is a very fine powder having approximately 40% particles below 75µm diameter of which approximately 30% are having a size less than 25µm present in the atmosphere. A comparative micro-morphological study of stomatal index of the dominant tree species*Albizialebbeck, Azadirachta indica, Callistemon lanceolatus* growing in the vicinity of Marble slurry dump-yard, Kishangarh (Ajmer) was done from the month of April 2014 to March 2015.The stomatal index of the lower epidermis of *A. indica* and *A. lebbeck* leaves from polluted sites was found to have greater value as compared to that with those of unpolluted site during the months of May to October while in case of the rough, leathery leaves of *Callistemon lanceolatus*, the stomatal index value in leaves from polluted site was less during these months. This may be attributed to the prevailing winds and weather conditions in the area of study which play a crucial role in the accumulated particulate matter on the phylloplane of the selected tree species viz.*A. lebbeck, A. indica,C. lanceolatus*

Keywords: *Albizia lebbeck, Azadirachta indica,Callistemon lanceolatus,* Marble-slurry and Stomatal index

INTRODUCTION

Rajasthan is known for mining of minerals and marbles. The State produces over 95% of the marble produced in India. Rajasthan State produces more than 65 types of mineral and rocks. The State is endowed with vast deposits of natural rocks known as- stones in local parlance and a few important ones amongst them are Granite, Marble, Sandstone, Limestone, Slate and Quartzite (MSME, 2017). A large number of mines and mining industrial process can produce particulate emission (Fennelly, 1975).
Rajasthan has around 4000 marble mines and about 1100 marble gang saws (processing units). Districts Ajmer (Makrana), Rajsamand, Udaipur, Chittorgarh, Banswara, Alwar, Sirohi, Jaipur etc are known for the mining of Marble and at the same time it has led to growth of many processing units engaged in cutting of marble in the form of Gang saw and cutters(Kushwah and Chaurasiya, 2015).
Dust particles form a major part of air pollutants arising due to industrial processes and pose serious threat to the ecosystem. (Shrivastava, 2017). Dust emission occurs from many processing units in the marble industries viz., cutting, buffing, polishing, loading and transportation. The processing waste of marble cutting plants comes out in the form of 'Marble Slurry'. Marble slurry is a suspension of marble fines in water, generated during processing and polishing. Marble dust is a very fine powder has approximately 40% particles below 75µm diameter of which approximately 30% are having a size less than 25µm (Central Pollution Control Board, Bhopal, 2011-12). Marble slurry has the maximum content of magnesium and calcium carbonate. This marble slurry is being dumped by the processing plants at the nearest site available or in the notified areas marked for dumping near the plants. When this slurry dries up, it leads to serious environmental pollution (Kushwah, 2014).The slurry not only pollutes the soil and ground water but also the air in the region is infested with the suspended particulate matter i.e. minute slurry particles. Rajasthan, a mining hub, has the second largest mineral reserves in the country. Applicant pay royalty for the marble to be produced from the mine, various applicable

taxes are paid thereby contributing to the regional revenue. The public revenue is further put for public expenditure as mentioned by the Rajasthan State Industrial Development and Investment Corporation Ltd. (Saxena *et al*., 2017).

Dust particles form a major part of air pollutants arising due to industrial processes andpose serious threat to the ecosystem. (Shrivastava, 2017).The environmental degradation of the land due to marble mining is much less than the environmental degradation caused by the waste from marble processing plants. The processing waste of marble cutting plants comes out in the form of 'Marble Slurry'. This marble slurry is being dumped by the processing plants at the nearest site available or in the notified areas marked for dumping. When this slurry dries up, it leads to serious environmental pollution (Kushwah, 2014).

Pollutants have deleterious effect on stomata movements, foliar geometry, photosynthesis, membrane permeability and transportation of nutrients leading to stunted growth, poor yield and premature senescence in highly susceptible plants (Soni *et al.,* 2017). This suspended particulate matter not only affects the plant physiology and anatomy, thereby retarding the growth but also causes micromorphological alterations in the plant. Foliar surface of plants is the main receptor of dust as it is continuously exposed to the surrounding atmosphere (Rai and Panda, 2014).

The effect of dust pollution on plant body damage plant tissue which may reduce rate of photosynthesis. Deposition of dust particles exert stress on plant resulting in reduction of productivity of the plants (Supeand Gawande, 2015).The Air pollution caused by the dust generated during the marble crushing and mining can pose serious problems to overall physiology of plants by entering through leaves. Continuous exposure of trees to dust can lead to accumulation and integration of pollutants, thereby causing foliar injuries, stomata damage, premature senescence, chlorosis, yield reduction, change in photosynthesis and transpiration making them more sensitive.(Soni e*t al*., 2017).

Various tree species have been studied in their response to suspended particulate matter. Sett, (2017) describes the way the dust particles affect the plants and trees, and the response of the plants to the particulate-exposure. Impact of ambient air quality on micromorphological structure of leaves of the road side plant species, *Ficusbenjamina* has been reported by Shrivastava, (2017), while morphological and biochemical changes in *Azadirachtaindica* from coal combustion fly ash have been highlighted by Qadir, Sami Ullah *et al.* (2016).Chaudhary and Rathore, (2018) have reported the impact on castor (*Ricinus communis*L.) cultivars. The objective of the present study was to observe the changes caused by Marble slurry in stomatal index of three dominant tree species viz. *A. lebbeck, A. indica, C. lanceolatus* at the marble slurry dump yard in Kishangarh, Ajmer.

MATERIALS AND METHODS

Study area: Kishangarh is situated on the National Highway No. 8 about 30 K.M. on the North-East of Ajmer, Rajasthan.The area of the Tehsil falls between 26^0 15' to 27^0 0' North latitudes and 74^0 30' to 75^0 15' East longitudes. Kishangarh is the main area of marble industries (Dhanwar, 2012). It is the main market, particularly for the stone obtained from Makrana and Rajsam and region. Kishangarh is having about 523 marble gangsaws and 28 granite cutters in operation. Approximately slurry generation is 5500-6000MT per day. The generated slurry is being transported through tankers (capacity around 4000 litres) to the disposal site notified by Rajasthan State Industrial Development and Investment Corporation Ltd. (State Industrial Infrastructure Development Agency i.e. RIICO for Rajasthan). (Central Pollution Control Board, Bhopal, 2011-12).A dumping yard (Phase-I) has been developed in year 2005-06 of about 322 bigha area of 30-35 feets depth. This yard was designed to fulfill the 05 years of dumping requirement. Another site near to this yard has already been developed dumping yard phase-II in 532 bigha area to switch the dumping at this site. Green belt has been developed on the embankment of the dump-yards.

The route leading to this dump yard has also been lined with tree species particularly *A.indica* and various members of family Fabaceae. The ground flora on the route is negligible. The vegetation here is exposed to fine marble dust suspended in the environment.

The plants grown are continuously exposed to the Marble slurry pollution in atmosphere.

Collection of leaves: Leaves of *A. indica, A. lebbeck* and *C. lanceolatus* were collected from the vicinity of marble slurry dump yard and control samples of leaves were collected from the Botanical Garden of Samrat Prithviraj Chouhan, Government College Ajmer, which is nearly 27 km from the marble-slurry dumpyard.

Methodology: The leaf samples collected from control and polluted sites were thoroughly washed with tap water followed by distilled water and fixed in F.A.A. (5ml formalin + 5 ml acetic acid + 90 ml 70% ethyl alcohol). Leaves of each species were washed carefully with water and boiled in conc. nitric acid (HNO_3) solution for 2-3min. Boiled leaves were washed thoroughly with water in watch glass and abaxial epidermis was peeled. The peels were stained with safranin and mounted in glycerine on slides. Number of stomata and epidermal cells were noted using a light microscope under (15×40) magnification. The stomatal index [calculated by formula given by Salisbury

(1927)] has been considered in the present paper.
{Stomatal index= (S / E+S) 100 ……Eq. 1
Where, S = number of stomata per unit microscopic area, E = number of epidermal cells per same unit microscopic area.}

RESULTS AND DISCUSSION

Study area: At the marble-city, Kishangarh, Ajmer, the slurry is transported to the dump-yard on whose embankment, plantation has been done.

Table 1. Stomatal index of the abaxial surface of *A. lebbeck* from the control and polluted slurry dumpyard sites of Kishangarh area.

Month	Control Area			Polluted Area		
	No. of Stomata	No. of Epidermal cells	S.I.	No. of Stomata	No. of Epidermal cells	S.I.
Apr-14	19.6	48.25	28.89	19.14	52.8	26.61
May-14	24.25	83.35	22.54	21.6	76.33	22.06
June-14	24.5	100.33	19.63	17.5	73.5	19.23
July-14	26	76.2	25.44	21	84.5	19.91
Aug-14	19	85.67	18.15	19.4	66.4	22.61
Sep-14	21.66	95	18.57	22	71.5	23.53
Oct-14	19.4	87.4	18.16	21.2	85.6	19.85
Nov-14	22	87	20.13	20	92.8	17.73
Dec-14	20.5	101.5	16.8	22.33	80.33	21.75
Jan-15	24.5	107.5	18.56	30	85	26.09
Feb-15	25	108	18.8	23.5	92	20.35
March-15	26.4	78	25.29	25.25	97	20.65

S.I.: Stomatal index

Table 2. Stomatal index of the abaxial surface of *A.indica* from the control and polluted slurry dumpyard sites of Kishangarh area.

Month	Control Area			Polluted Area		
	No. of Stomata	No.of Epidermal cells	S.I.	No. of Stomata	No.of Epidermal cells	S.I.
Apr-14	16.8	291.11	5.46	21	322.22	6.12
May-14	19.33	303.7	5.99	17.6	328	5.09
June-14	17	279.11	5.74	20.33	318.52	6.00
July-14	13.6	287.11	4.52	24	322.22	6.93
Aug-14	14	336.89	3.99	18.4	309.78	5.61
Sep-14	13.4	280	4.67	15.2	261.33	5.5
Oct-14	15.2	346.67	4.2	20.2	277.78	6.78
Nov-14	20.75	337.78	5.79	13.4	284.89	4.5
Dec-14	17	302.22	5.33	16	392.89	3.91
Jan-15	22	333.33	6.19	17.2	352	4.66
Feb-15	14.8	292	4.82	18.5	333.33	5.26
March-15	17.4	337.33	4.91	20.5	350	5.53

S.I.: Stomatal index

Table 3. Stomatal index of the abaxial surface of *C. lanceolatus* from the control and polluted slurry dumpyard sites of Kishangarh area.

Month	Control Area			Polluted Area		
	No. of Stomata	No.of Epidermal cells	S.I.	No. of Stomata	No.of Epidermal cells	S.I.
Apr-14	12	152.33	7.3	10	166.67	5.66
May-14	13.67	156.67	8.02	13.67	165	7.65
June-14	9.67	110	8.08	15.67	137.33	10.24
July-14	13.33	128.67	9.39	18	125	12.59
Aug-14	13	145.67	8.19	10	123.33	7.5
Sep-14	21.64	145	12.98	10.33	130.67	7.33
Oct-14	12.67	173	6.82	10	154.25	6.09
Nov-14	13	157	7.65	10	135	6.9
Dec-14	13	179	6.77	8.33	149	5.3
Jan-15	11.8	132.8	8.16	12	114	9.52
Feb-15	12	131.5	8.36	11.5	116.75	8.97
March-15	12	142.75	7.75	13.5	155.75	7.98

S.I.: Stomatal index

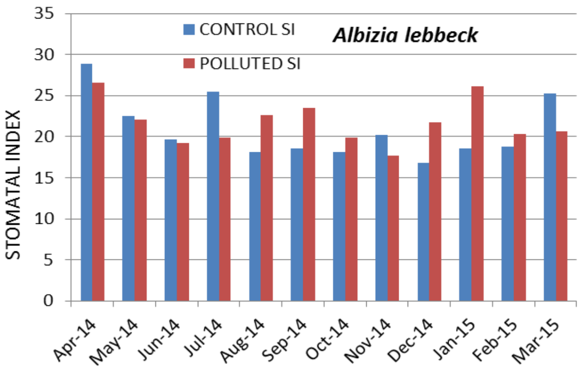

Fig.1. *Stomatal index of A .lebbeck during the study period.*

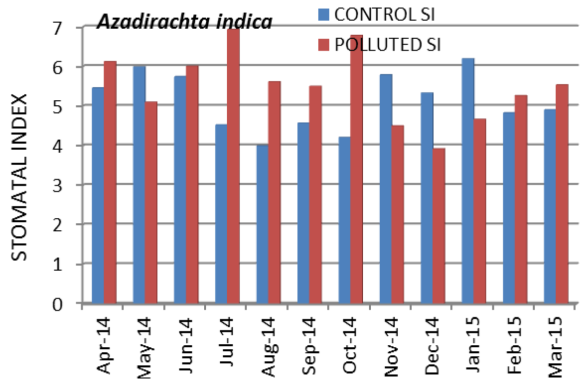

Fig. 2. *Stomatal index of A.indica during the study period.*

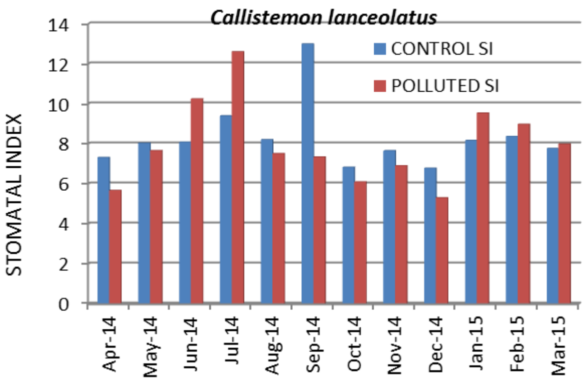

Fig. 3. *Stomatal index of C. lanceolatus during the study period.*

The vegetation of the green belt developed on the embankments is exposed to the fine dust of marble which deposits on the leaves. The suspended particulate matter is trapped by the phyllo-plane, which in turn alters the stomatal characteristics, including the stomatal index. Dust emission occurs from various primary, secondary and tertiary processes and transportation during mining and processing operations. It has been established that aerial parts of a plant especially leaves act as persistent absorbers in a polluted environment (Samal and Santra, 2002).

Plants are the natural air purifiers. They are directly affected by the suspended particulate matter present in their environment. Trees are planted for mitigating the air pollution by filtering, intercepting and absorbing pollutants in a sustainable manner

Pollution stress altered the stomatal characteristics of the three investigated species, *viz. A. indica, A. lebbeck* and *C. lanceolatus.* Nevertheless, all these species are quite resistant to air pollutant actions as is obvious from the seasonal variations in the stomatal index, observed during the study period. Despite the observed modifications they continue to grow and reach sexual maturity, though the flowering was reduced considerably.

As is obvious from the tables, quantitative analysis of stomata parameters showed that leaves from the polluted site had higher number of stomata per unit area than those from control site. Similar results were reported by Ogbonna *et al*. (2018) for broad leaved trees and fruit trees (Ogbonna *et al*., 2017).

Shrivastava and Mishra (2018) reported higher values of stomatal index on both dorsal and ventral surface at polluted sites as compared to control sites. On the contrary, Pawar (2016) recorded decline in stomatal Index in stressed area leaf samples on both the surfaces. Air pollution is known to affect the stomatal index and it has been reported to decrease in some plants viz. the shrub species (Tiwari, 2012); *Santalum album* L. and *Ficus religiosa* L.(Pavana *et al.,* 2014), *Tabernaemontana divaricata* L. (Gentianales: Apocynaceae) and *Hamelia patens* Jacq. (Gentianales: Rubiaceae)(Amulya *et al.,* 2015) and *Muntingiacalabura*L. and *Ixora coccinia* L (Thara *et al.,* 2015).

Stomatal index recorded an increase in the lower epidermis of *A. aindica* and *A. lebbeck* leaves from polluted sites as compared to that with those of unpolluted site during the months of May to October. The climatic conditions of the region during these months are generally either windy or when there are monsoon showers the marble slurry is washed off the surface of the leaves. However during winter months when the slurry deposition on the leaves in particular, increases, the value of stomatal index of these leaves exhibited a decrease.

Stomatal index has been proven to be an indicator of environmental stress (Gostin, 2009). Decreased rate of photosynthesis and alteration of stomatal conductance are responsible for reduction of the stomatal index as well as dry matter content of leaf (Khan *et al*., 2015)

The modification of the frequency and subsequently the stomatal index in response to the environmental stress is an important means of controlling the absorption of pollutants by plants. These alterations may also be attributed to the deposition of particulate matter on the phyllo-plane in case of the present study. The use of epidermal traits of plants as indicators of environmental pollution has been emphasized.

In case of the rough, leathery leaves of *C. lanceolatus*, stomatal index in leaves from polluted site was more during the winter months, probably because the leaf anatomy of this tree species and its canopy architecture which reduces the accumulation of the slurry dust on the phylloplane.During the windy summer months the particulate matter remain trapped on the leaf surface and influences the characteristics of the leaf stomata.

According to Sharma and Butler (1973) high pubescence would reduce the amount of solar radiation incident upon the leaf surface. This alteration in the energy of leaf decreases its temperature and may alter the response to the accumulated particulate matter. Verma *et al.*(2006) find a significant decrease of stomatal density and stomatal index in *Ipomea pes-tigridis* grown under various degrees of environmental stresses (coal-smoke pollutants). This could be adaptive. The effect of pollution on stomatal frequency is species specific.

Conclusion

Of the three species under consideration, the value of stomatal index was observed to be highest for *A. lebbeck* , 22.06 during May and 26.08 during January followed by that for *C. lanceolatus* (7.6 and 9.5 respectively during May and January); the least being for*A.indica* (5.09 and 4.63 respectively during May and January). Higher values of stomatal index in the leaves of trees of *A. lebbecka* growing on the marble-slurry dumpyard particularly during winter and summer months, as compared to *C.lanceolatus and A.indica,* clearly indicated that it is better adapted to the prevailing abiotic stress and may be used in the green belt plantation of other marble-slurry dumpyards also.

ACKNOWLEDGEMENTS

This research was supported by University Grant Commission (UGC) – Rajiv Gandhi National Fellowship for SC Candidates, New Delhi. We are thankful to the College administration and the Department of Botany Samrat Prithviraj Chouhan Government College, Ajmer, for providing the desired support and basic infrastructure facilities that greatly assisted the present endeavour.

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