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# Bio-Monitoring of Air Quality Using Leaf Characteristics

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***Abstract*:** An evaluation of eight roadside ornamental trees was conducted for the purpose of evaluating their potential as bio-indicators of air quality. Tree leaves were analyzed to determine leaf area, stomatal density, and stomatal pore size. Leaf samples were collected from two sites: Ludhiana city (polluted site) and the university campus (control site). The mean stomatal density of selected tree species ranged from 200-412 mm$^{-2}$. In selected trees, mean stomatal density ranged between 246.88-351.56 mm$^{-2}$ (control > polluted). Among selected tree species, the mean stomatal length ranged from 8.61-14.40 µm. The location-wise order of mean stomatal length in selected trees ranged between 9.88-11.66 µm (control > polluted). The mean stomatal width of selected tree species ranged from 4.03-1.39 µm. The mean stomatal width of selected trees ranged between 2.47-2.87 µm (control > polluted). Polluted sites showed reduced stomatal density, length, and width. In light of these findings, it can be concluded that tree stomatal characteristics can provide practical information about air quality.

***Keywords*:** Air pollution, Ornamental trees, Stomatal parameters, Roadside, Bio-monitor

The air pollution levels have increased many folds during the last five decades which has led to adverse effects on plants in terms of specie loss or change in community composition Air quality is generally measured using physicochemical methods but this doesn't take into account the effect of air pollution on living entities viz., plants (Balasooriya et al 2009). Therefore using biomonitoring which is defined as the response of living entities to air pollution method instead of the physiochemical approach can address this problem (Nali and Lorenzini 2007). Plants play an important role in monitoring and maintaining the ecological balance by actively participating in the cycling of nutrients and gases like carbon dioxide, and oxygen and also provide a vast area for impingement, absorption, and accumulation of air pollutants to reduce the pollution level in the atmosphere (Escobedo et al 2008). Plants are continuously involved in gaseous exchange through stomata, they add oxygen into the atmosphere and in turn, take up $CO_2$ and other harmful gases thereby helping in ameliorating the environment's gaseous composition. Thus plants can help in combating the problem of air pollution but in this process get affected because of their continuous exposure to a particular environment. In the polluted environment change in plants' morphological, physiological, and biochemical parameters can be observed ((Verma and Singh 2006, Stevovi et al 2010, Wuytack et al 2010). Thus, a plant can be used as an important bio-monitor for studying air pollution levels by studying their morphological and physiological parameters.

The extent of plant susceptibility towards air pollution is variable as air pollution affects plants mainly through the uptake of pollutants into the leaves through stomata. And plant stomata response to pollution is variable from plant to plant, plant age, prevailing environmental conditions, and most important concentration of air pollution (Verma and Singh 2006, Stevovi et al 2010). Several studies suggested that low concentrations of air pollutants may influence stomata indirectly through damage to subsidiary cells or the epidermal cells or changes in cell wall structures (Abeyratne and Ileperuma, 2006). Plant's response to air pollution can be studied more specifically through parameters viz., morphological (specific leaf area) and anatomical (stomatal density & pore surface) (Balasooriya et al 2009, Kardel et al 2010, Chaturvedi et al 2013, Arriaga et al 2014). In the present studies, passive biomonitoring i.e. by using existing avenue plantations was performed with a major focus on leaf characteristics.

## MATERIAL AND METHODS

**Study areas:** The study area features a semi-arid climate with three distinct seasons; summer, monsoon, and winter. The average precipitation in the region is 809.3mm annually. Ludhiana is one of the worst polluted cities in India with particulate matter as high as six times normal with average maximum day temperature of 39.6°C and a minimum average temperature of 26.2°C. January is the coldest month followed by June as the hottest and November is the driest month in the region. National Highway 5 between

Chandigarh and Firozpur passing through Ludhiana city, Punjab (North latitude of 30°46'38" and the East longitude of 76°33'23") was taken as a study area (polluted site) while the Punjab Agricultural University campus was the control site to conduct present experiments.

**Sampling:** In the survey, the distribution of tree species along both sides of the Highway was recorded during 2019-2020. Out of the total, eight commonly growing trees (*Acacia auriculiformis, Alstonia scholaris, Cassia fistula, Cassia siamea, Chukrasia tabularis, Dalbergia sissoo, Heterophragma adenophyllum,* and *Putranjiva roxburghii*) were identified and chosen for the study with three replications of each treatment and each replication comprises of three trees at each polluted (NH-5) and control location (Punjab Agricultural University campus). To keep homogeny, trees of the same age, size, and spread were selected. In total seven tree species were selected for study with three replications of each treatment and each replication comprises three trees at each polluted and control site

**Methodology**

**Leaf area (cm$^2$):** The dimension of leaf length was measured from the leaf tip down the midrib of the leaf lamina to the leaf base point where the petiole attaches to lead. The leaf breadth was considered along the widest width crossways the lamina. Ten leaves were selected per tree for calculation.

**Stomatal density (mm$^{-2}$):** The rightly healthy leaves were collected from selected trees from experimental locations. Leaves were dried and fixed on aluminum stubs with the help of double-sided carbon tape followed by gold plating. Leaf samples were then observed under a scanning electron microscope (S-3400 N) by Hitachi. The stomatal density was estimated by counting the number of stomata per unit area and expressed in mm$^{-2}$.

**Stomatal pore length (µm):** Pore length was measured by stretching the cursor over the length of the stomatal pore.

**Stomatal pore width (µm):** Pore width was measured by stretching the cursor across the width of the stomatal pore.

**Statistical analysis:** The data were analyzed using factorial randomized block design. Mean comparison was performed by Tukey's test using SAS software computer version 9.2.

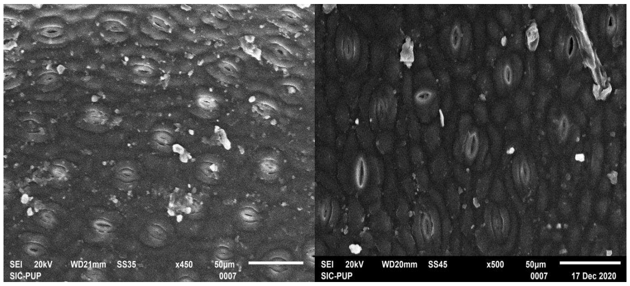
## RESULTS AND DISCUSSION

The stomatal length, width, and density of selected trees were compared under controlled and polluted conditions (Table 1. Fig. 1-8). *Dalbergia sissoo* (15.23 µm) has the longest stomata at control location followed by with shortest in *Putranjiva roxburghii* (7.83 µm) at polluted location; stomatal width was recorded highest for *Chukrasia tabularis* (4.37 µm) at control location with least in *Cassia fistula* (1.30 µm) at polluted location . Stomatal density was highest in *Heterophragma adenophyllum* (475 mm$^{-2}$) at control location with lowest in *Dalbergia sissoo* (200 mm$^{-2}$). Overall mean higher stomatal length, stomatal width & stomatal density (11.66 µm, 2.87 µm & 351.56 mm$^{-2}$) was in leaves of trees growing at the control location as compared to the polluted location (9.88 µm, 2.47 µm and 246.88 mm$^{-2}$). In line with Wuytack et al (2010), observed that selected tree species were resistant to the polluted environment. The tree with the most resistance to polluted environment was *Cassia fistula* followed by *Heterophragma adenophyllum, Cassia siamea, Putranjiva roxburghii, Alstonia scholaris, Dalbergia sissoo, Acacia auriculiformis*and *Chukrasia tabularis*. Among different tree species, stomatal characteristics vary based on genetics or their adaptability to environmental conditions, but plants with higher stomatal frequencies / resistances are considered to be better adapted or tolerant of air pollution (Al

**Table 1.** Stomata pore length, width (µm) and stomatal density of selected roadside tree species at the control and pollution location

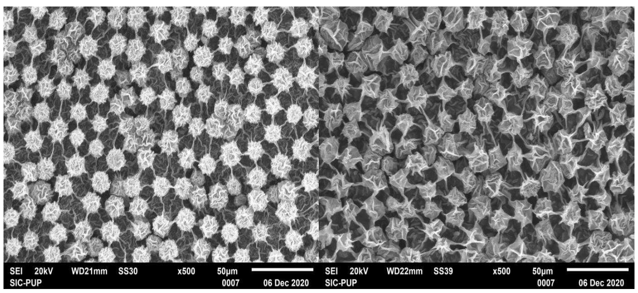
| Tree species | Pore length (µm) | | Pore width (µm) | | Stomata density (mm$^{-2}$) | |
|---|---|---|---|---|---|---|
| | Control | Polluted | Control | Polluted | Control | Polluted |
| *Acacia auriculiformis* | 11.88 | 9.85 | 2.65 | 1.98 | 350.00 | 225.00 |
| *Alstonia scholaris* | 11.53 | 8.88 | 4.06 | 3.88 | 237.50 | 175.00 |
| *Chukrasia tabularis* | 11.25 | 7.85 | 4.37 | 3.68 | 400.00 | 300.00 |
| *Cassia fistula* | 11.61 | 10.63 | 1.48 | 1.30 | 325.00 | 225.00 |
| *Cassia siamea* | 13.17 | 12.51 | 4.12 | 3.78 | 400.00 | 275.00 |
| *Dalbergia sissoo* | 15.23 | 13.57 | 1.72 | 1.34 | 250.00 | 150.00 |
| *Heterophragma adenophyllum* | 9.29 | 7.97 | 2.20 | 2.04 | 475.00 | 350.00 |
| *Putranjiva roxburghii* | 9.38 | 7.83 | 1.98 | 1.77 | 375.00 | 275.00 |
| Overall mean (Location) | 11.66$^a$ | 9.88$^b$ | 2.82$^a$ | 2.47$^b$ | 351.56$^a$ | 246.88$^b$ |

Different letters in each column are significantly different at P≤0.05 by Turkey's Test



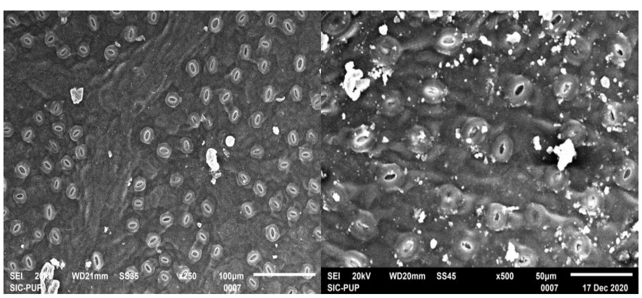
A) Control location B) Polluted location

**Fig. 1.** Stomata in leaves of *Acacia auriculiformis* at control and polluted locations



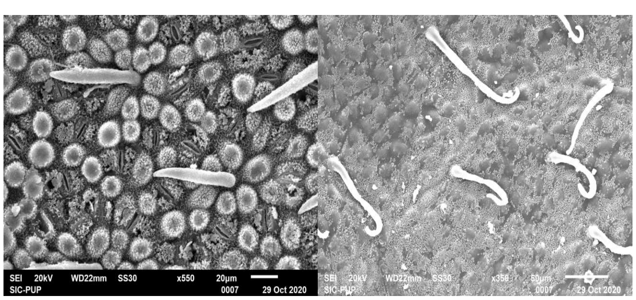
A) Control location B) Polluted location

**Fig. 2.** Stomata in leaves of *Alstonia scholaris* at control and polluted locations



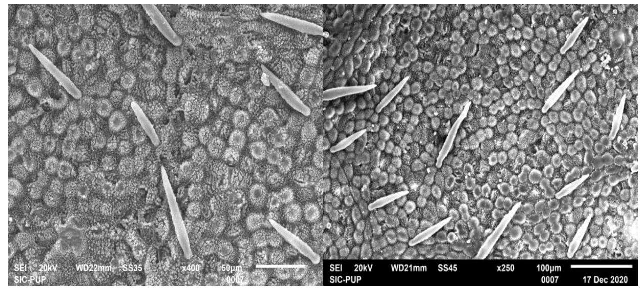
A) Control location B) Polluted location

**Fig. 3.** Stomata in leaves of *Chukrasia tabularis* at control and polluted locations



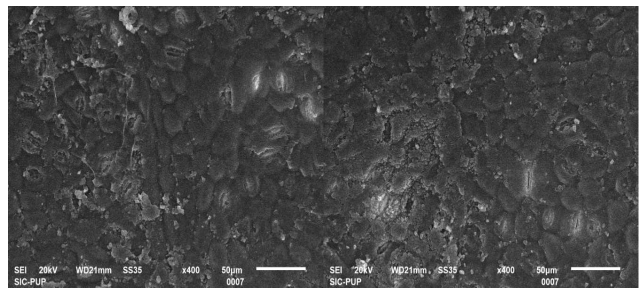
A) Control location B) Polluted location

**Fig. 4.** Stomata in leaves of *Cassia fistula* at control and polluted locations



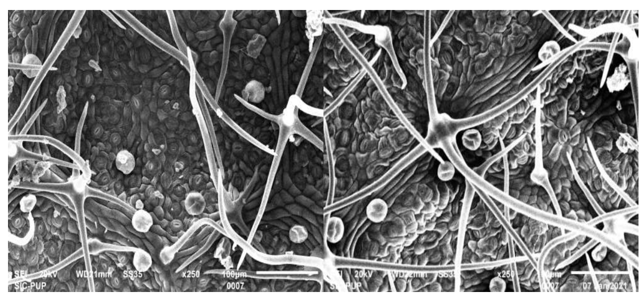
A) Control location B) Polluted location

**Fig. 5.** Stomata in leaves of *Cassia siamea* at control and polluted locations



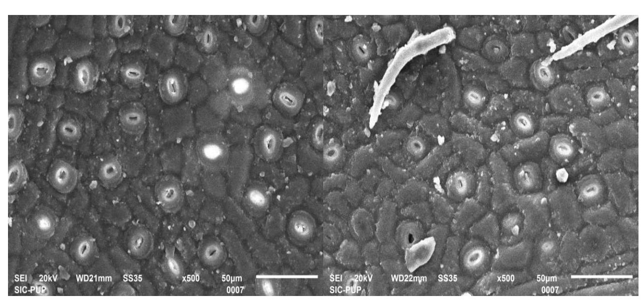
A) Control location B) Polluted location

**Fig. 6.** Stomata in leaves of *Dalbergia sissoo* at control and polluted locations



A) Control location B) Polluted location

**Fig. 7.** Stomata in leaves of *Heterophragma adenophyllum* at control and polluted locations



A) Control location B) Polluted location

**Fig. 8.** Stomata in leaves of *Putranjiva roxburghii* at control and polluted locations

Afas et al 2006, Raina et al 2008, Ha and Martinez 2018). The location of the trees had a significant impact on these parameters.In many previous studies, leaf stomatal characteristics were also reported to decrease as a result of pollution (Pourkhabbaz et al 2010, Wuytack et al 2010, Khushwaha et al 2018).The stomatal characteristics of plants are determined not only by their genetic makeup but also by the environmental conditions surrounding them.These trees can therefore be used as an indicator of air pollution based on their stomatal characteristics. The high $CO_2$ concentration in a polluted environment causes low reactivity of stomata. Therefore, reduction in stomatal density at the polluted site can be seen as an adaptation of plants for controlling pollutant absorption but cause a reduction in photosynthesis at the same time (Verma et al 2006).

## CONCLUSION

The trees growing in the more polluted area (roadside) adapts themselves by a reduction in pore size and the number of stomata, which will enhance stomatal resistance and alternatively increases plant survival potential in the polluted environment. Therefore, can conclude that roadside trees' stomatal characteristics can be used as good bio-indicators for monitoring air quality and as resistance trees for developing greenbelts in polluted urban areas. *Cassia fistula* is the most tolerant tree for NH-5.

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