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ORIGINAL ARTICLE

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Breathing Plants: Analyzing Air Pollution's Effect on Leaf and Stomatal Morphology

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Urban air pollution has emerged as a critical environmental issue, posing significant risks to both human health and the natural environment. Among various environmental stressors, air pollution stands out due to its pervasive nature and detrimental impacts on urban flora. Plants, as integral components of urban ecosystems, are constantly exposed to pollutants such as particulate matter, nitrogen oxides, sulfur dioxide, and ozone. These pollutants can cause a range of physiological and morphological changes in plants, affecting their growth, development, and overall health. This study investigates the impact of air pollution on the morpho-anatomical characteristics and stomatal index of plant species growing in polluted and non-polluted areas. The research focuses on the adaptive responses of plants to air pollution by examining changes in leaf macro- and micro-morphological traits. Significant reductions in leaf length, breadth, L/B ratio, stomata size, stomata number, stomata index, and stomata frequency were observed. A lower stomata number is suggested to be an adaptation mechanism to minimize the absorption of gaseous pollutants from the air. Additionally, stomata clogging with occluded stomata pores, induced by air pollution, was identified in three plant species. These morphological alterations serve as indicators of environmental stress, providing valuable insights into the initial detection of urban air pollution. This comparative assessment highlights the importance of monitoring morpho-anatomical changes in plants as a tool for environmental pollution studies. The alterations in the leaf morpho-anatomical traits and stomatal characteristics were profound, emphasizing their potential use as reliable indicators of urban air pollution. By systematically comparing plants from polluted and non-polluted areas, the research highlights the crucial role of these morphological changes in the early detection and monitoring of environmental stress. This comparative assessment underscores the importance of integrating morpho-anatomical studies into urban air pollution monitoring programs. The findings contribute to a better understanding of how plants adapt to polluted environments, offering valuable insights for developing strategies to mitigate the impacts of air pollution on urban vegetation.

Key words: Stomatal index, Air pollution, Stress, Leaf morphology

Environmental pollution, especially the rapid decline in air quality generated by anthropogenic activities, has become an important problem in urban areas (Fenger, 2009). However, emissions of pollutants such as carbon monoxide (CO), nitrogen dioxide (NO_2), ozone (O_3), and especially the emission of particulate matter are threatening environmental sustainability and functionality and pose potential threats in urban areas. Air contamination has harmful impacts on plants species particularly growing on roads sides and bus stops (Rai, 2016; Krishnaveni *et al*., 2015). Air pollution affects both plant and human health, inducing physiological and biochemical responses. The World Health Organization (WHO) reported that air pollution can contribute to about four million deaths annually worldwide. Therefore, the prediction of air conditions and reduction in pollutants in urban areas are urgently needed (Vingarzan, 2004). As urban forests can improve environmental conditions, including air quality. Compared to humans and other animals, plants are more sensitive to environmental stress and have stronger physiological, biological, and morphological responses. Therefore, understanding the physiological link between tree parameters and environmental stress can be significant for gaining visions into urban forest health and functioning (Mukherjee and Agrawal, 2018).

Plants typically express phenotypic differences in response to environmental changes and under different environmental conditions, plants allocate biomass in several organs in order to capture optimum light, water, nutrient and carbon dioxide, for its maximum growth rate. Several studies have been done in anatomy of vegetative organs under polluted condition. Turgor changes in the guard cells determine the area of stomatal pore through which gaseous diffusion can occur, thus maintaining a constant internal environment within the leaf. In plants stomata play a major role in gaseous exchange and transpiration in which stomatal control is a critical process in plant adaptation in various environments (Leghari and Zaidi, 2013). The elevation of air pollution can cause both acute and chronic damage to the anatomical, morphological (leaf number, leaf area, stomata number), physiological (pH and relative weight content) and biochemical (pigments and ascorbic acid) characteristics of plant species (Karmakar and Padhy, 2019; Kaur and Nagpal, 2017). These gaseous contaminants reduce the stomatal conductance and photosynthesis (Adrees *et al.*, 2015).

The aim of the present study was to investigate and compare the effect of air pollution on the leaf morphology, anatomy and stomatal index of some common plants growing around the areas of polluted areas and the same plant species growing in the non-polluted region. In this study the investigation was carried out to know the effect of environmental pollution especially on leaf properties of plants in urban and rural area because leaf is the most sensitive and exposed part to be affected by air pollutants instead of all other plant parts such as stems and roots. The information generated under the study will help to understand how pollution affects morphology and anatomy of selected plants.

MATERIALS AND METHODS

DESCRIPTION OF THE STUDY AREA

The non- polluted area under study is in an around Vadakkumpuram (Fig 1a). It is area which is rich in vegetation and biodiversity. The polluted area selected for the present study is area in and around Aluva metro station (Fig. 1b). Comparing to the metro station the respective area is less effected by air pollution and thus it is selected as the control site for this study. Plant twigs and leaf samples of five selected plant species are collected from area around Aluva metro station and alternative samples are also collected from Vadakkumpuram regions to investigate and compare the effect of air pollution on the leaf morphology and anatomy of the selected plant species.

PLANT SPECIES SELECTION AND SAMPLE COLLECTION

The present study was carried out at Department of Botany, Union Christian College, Aluva. The study mainly focuses on the comparative analysis on impact of air pollution on leaves of plants growing in the polluted areas with those growing in control sites (non-polluted area). Different plants were noted and listed from the polluted site and only few plants were taken for comparative analysis of pollution effects with non-polluted plants. Plant species selection was based on direct observation regarding their dominance and occurrence in both sites. The plants under investigation were *Mangifera indica* L., *Tridax procumbens* L., *Chromolaena odorata* (L.) R. M. King & H. Rob., *Gliricidia sepium* (Jacq.) Walp.., *Clerodendrum Infortunatum* L. The plant twigs and leaves of these plants were collected from polluted as well as nonpolluted areas. The study was conducted during the period of February – April of 2022.

MORPHOMETRY STUDIES

Various macroscopic characters of the leaves of fresh plants were recorded. Leaves were collected in replicates from trees which were having uniform height and growth form. Colour, texture, and physical appearance of the leaves were analysed with naked eyes. Leaf shape and size variation is also noted. Length and breadth of leaves were measured using a ruler. Length to breadth ratio (L/B) were also calculated. The measurement of leaf length was from leaf tip along the midrib of the leaf lamina to the leaf base at the point of attachment of the lamina to the petiole. The leaf breadth was measured along the widest breadth across the lamina.

MICROSCOPIC EVALUATION

Stem anatomy

Fine sections of petioles of tree species of the selected plants were taken and stained with safranin and mounted in glycerine.

Determination of stomatal structure and stomatal index

To study the epidermal morphology, stomatal number and stomatal index of leaf, the leaf was subjected to epidermal peeling. The epidermis was peeled by hand and to prepare the abaxial surface, the leaf blade was placed on a tile or a cutting plate with its adaxial surface facing upwards. The adaxial surface was carefully scrapped with sharp razor until only the abaxial surface was left behind and all the above materials were removed with the help of camel hairbrush. The peels washed with water, placed on a clean glass slide in a drop of glycerine and followed by a cover slip over it. The slide with cleared leaf was placed on the stage of the microscope and examined under 45X objective and 10X eye piece. The number of stomata present in the area of 1sq.mm including the cell or at least half of its area within the square was counted. The average number of stomata per sq.mm was determined and their values are tabulated. Number of stomata and epidermal cell were counted per square millimeter area and the stomatal frequency and stomatal index were calculated by using the following formulae (Salisbury, 1927):

$$\text{Stomatal index (SI)} = \frac{S}{(E+S)} \times 100$$

Where, SI - stomatal index

S- The number of stomata in 1sq.mm area of leaf

E- the number of epidermal cells in 1sq.mm area of leaf

$$\text{Stomatal frequency (S.F.)} = \frac{S}{E} \times 100$$

S.F – Stomatal frequency

S- The number of stomata in 1sq.mm area of leaf

E- the number of epidermal cells in 1sq.mm area of leaf

RESULTS

Plants from polluted and non-polluted areas were collected, identified and described based on literatures. Photographs of plants collected from polluted and non-polluted areas were shown in Fig. 2 and 3.

DESCRIPTION ABOUT THE SELECTED PLANTS

Tridax procumbens L., *Chromolaena odorata* (L.) R. M. King & H. Rob., *Gliricidia sepium* (Jacq.) Walp., *Mangifera indica* L., and *Clerodendrum Infortunatum* L. were selected for our study. Plants were identified based on the herbarium specimens, expert opinions and salient features described in the species protologues.

Tridax procumbens L.

Kingdom	-Plantae
Division	-Tracheophyta

Class	-Magnoliopsida
Order	-Asterales
Family	-Asteraceae
Genus	-*Tridax* L.
Species	-*Tridax procumbens* L.

Tridax procumbens is a prostrate herbaceous plant, whose flowering axis rises up to 40 cm high. It is abundantly covered with erect stiff hairs. The leaves are opposite, simple, and with dense hairs. At the end of the long stems, is a capitulum inflorescence. The fruit is topped with a tuft of white hairs.

***Chromolaena odorata* (L.) R. M. King & H. Rob.**

Kingdom	-Plantae
Division	-Tracheophyta
Class	-Magnoliopsida
Order	-Asterales
Family	-Asteraceae
Genus	-*Chromolaena*
Species	-*Chromolaena odorata* (L.) R.M. King & H. Rob.

C. odorata is a bushy plant with woody stem, and spread much branched. It can reach up to 7 m high. It is entirely covered with a pubescence. The leaves are simple and opposite. As indicated, the name of this species, its leaves give off a strong odour when crumpled. The terminal inflorescence consists of small and narrow cylindrical flower heads with white florets.

Gliricidia sepium (Jacq.) Walp

Kingdom	-Plantae
Division	-Tracheophyta
Class	-Magnoliopsida
Order	-Fabales
Family	-Fabaceae
Genus	-*Gliricidia*
Species	-*Gliricidia sepium* (Jacq.) Walp.

A perennial, medium-sized (2-15 m high) legume tree. Leaves are imparipinnate; leaflets (5- 20) are ovate. The bright pink flowers is arranged in clustered racemes. The fruits are dehiscent pods, 10-18 cm long and 2 cm broad, that contain 8 to 10 seeds.

Mangifera indica L.

Kingdom	-Plantae
Division	-Tracheophyta
Class	-Magnoliopsida
Order	-Sapindales
Family	-Anacardiaceae
Genus	-*Mangifera*

Evergreen trees, to 30 m high. Leaves simple, alternate, clustered at the tips of branchlets, lamina elliptic, elliptic-lanceolate, margin entire, glabrous, shiny. Flowers polygamous, in terminal panicles. Fruit a drupe, 5-15 cm long, mesocarp fleshy, endocarp fibrous.

Clerodendrum Infortunatum L.

Kingdom	-Plantae
Division	-Tracheophyta
Class	-Magnoliopsida
Order	-Lamiales
Family	-Lamiaceae
Genus	-*Clerodendrum*
Species	-*Clerodendrum infortunatum* L.

Clerodendrum infortunatum is a shrub that can grow from 1 - 5 metres tall. Leaves are simple, opposite; both surfaces' pubescent, inflorescence in terminal, peduncled, few-flowered cyme; flowers white with purplish pink throat. Fruit berry, globose, turned bluish-black or black when ripe, enclosed in the red accrescent fruiting-calyx. The fruits are drupes.

MACROSCOPIC EVALUATION

The morphological parameters of plant species collected from the contaminated sites were significantly affected due to the vehicular emissions. The size of leaves is regularly declining because vehicular emissions in the study area is increasing day by day. The natural colour of the plants was also affected. Plants in polluted area was looking like a dry dead leaf with full of lesions and other patch marks, they were also covered by dust and dirt (Fig 3).

The plants in non-polluted area where fresh looking and more appealing with lesser number of lesions. The natural fragrance of the plant was much more experienced from the plants collected from non- polluted sites than in polluted sites. Plants in polluted areas seems to have lower growth than the ones in non-polluted area. The leaves where sometimes teared and rolled inwards. The strength of stem was very less in plants in polluted area than in non-polluted area. They were looking dull and tired but the plants of non-polluted sites were looking healthy and strong. The flowers in non-polluted site plants have bright colour and fragrance than the plants in polluted area.

Leaf characteristics such as L/B ratio, and length and breadth of leaves are important factors in the assessment of air pollution. Among the selected polluted and non-polluted plants there were reduction in the width of leaf in the polluted plants. The leaf length was measured and the results revealed that there was reduction in leaf length in polluted plants when compared to non-polluted plants.

Dust particles were present on the leaf surface of plants growing in polluted area. Plant species are regularly manifested in the atmosphere and consume, store and integrate pollutants on their leaves. Therefore, the morphological parameters of the plants collected from control site showed better growth than the polluted sites. This study showed that air pollution adversely affects plant morphology. The morphological analysis showed that the contaminated plant species undergo physical and probably functional changes to tolerate air pollution. Both the polluted and non-polluted site maximum L/B ratio was observed in *Mangifera indica* and minimum observed in *Cleodendrom infortunatum* (Table 1).

Figure 1. (**A**) The non-polluted site selected for the study; (**B**) The polluted site selected for the study.

Figure 2. Showing plants from non-polluted site a) *Tridax procumbens* b) *Chromolaena odorata* c) *Gliricidia sepium* d) *Mangifera indica* e) *Clerodendrum infortunatum*

Figure 3. Showing plants from polluted site a) *Tridax procumbens* b) *Chromolaena odorata* c) *Gliricidia sepium* d) *Mangifera indica* e) *Clerodendrum infortunatum*

Table 1: Showing different morphometric characters of plants growing in polluted and non-polluted

	Tridax procumbens		***Gliricidia sepium***		***Cleodendrom infortunatum***		***Mangifera indica***		***Chromolaea odorata***	
	NP	P	NP	P	NP	P	NP	P	NP	P
LL (cm)	5.2	2.5	10	7	20	14	30	26	12	9
LB (cm)	2.5	1.5	5	3.6	17	13	8	5	8	6
L/B ratio	2.08	1.66	2	1.18	1.18	1.11	3.75	5.2	1.5	1.5

NP: Non-polluted; P: Polluted; LL: Leaf Length; LB: Leaf Breadth; L/B ratio: Length/Breadth ratio

Table 2: Showing stomatal characteristics of non- polluted and polluted plants

Plant species	**Stomatal type**	**stomata**		**No. of epidermal cells**		**Stomatal index**		**Stomatal frequency (%)**	
		NP	**P**	**NP**	**P**	**NP**	**P**	**NP**	**P**
Tridax procumbens	Anomocytic	15	12	23	38	39.47	24	65.21	31.57
Chromolaena odorata	Anomocytic	5	7	52	137	8.77	4.86	9.61	5.109
Gliricidia sepium	Anomocytic	15	9	85	82	15	9.89	17.64	8.53
Mangifera indica	Anomocytic	86	48	268	342	24.29	12.3	32.08	14.03
Clerodendrum infortunatum	Diacytic and anomocytic	25	14	109	139	18.65	9.15	22.93	10.07

NP: Non- Polluted; P: Polluted

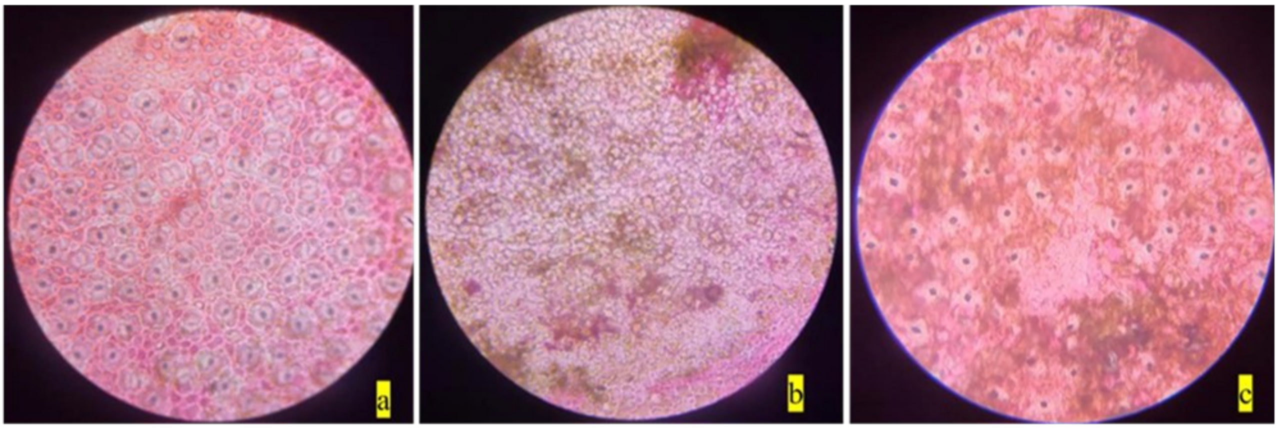

Figure 4. Stomata of *Mangifera indica* : a) non-polluted site; b) polluted site; c) polluted site magnified view.

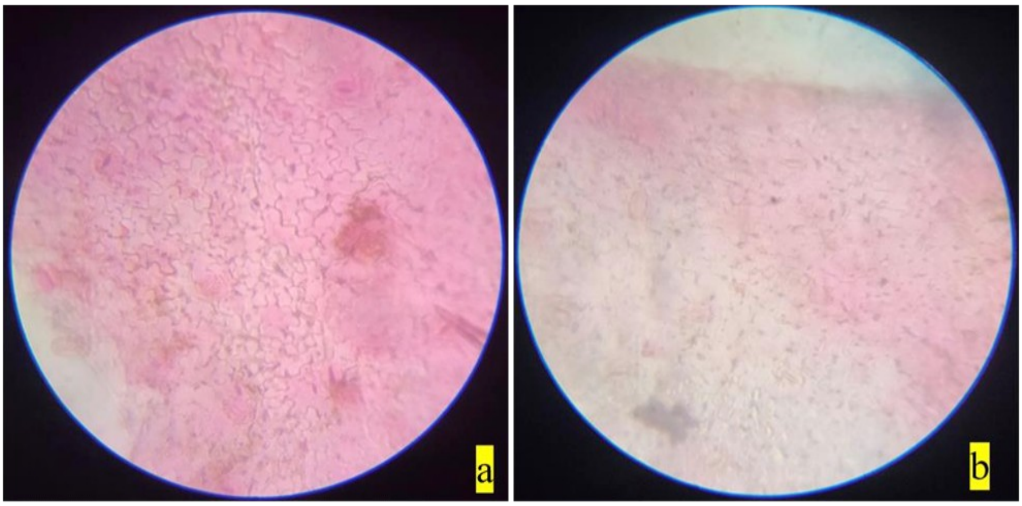

Figure 5. Stomata of *Clerodendrum infortunatum* : a) non-polluted site; b) polluted site

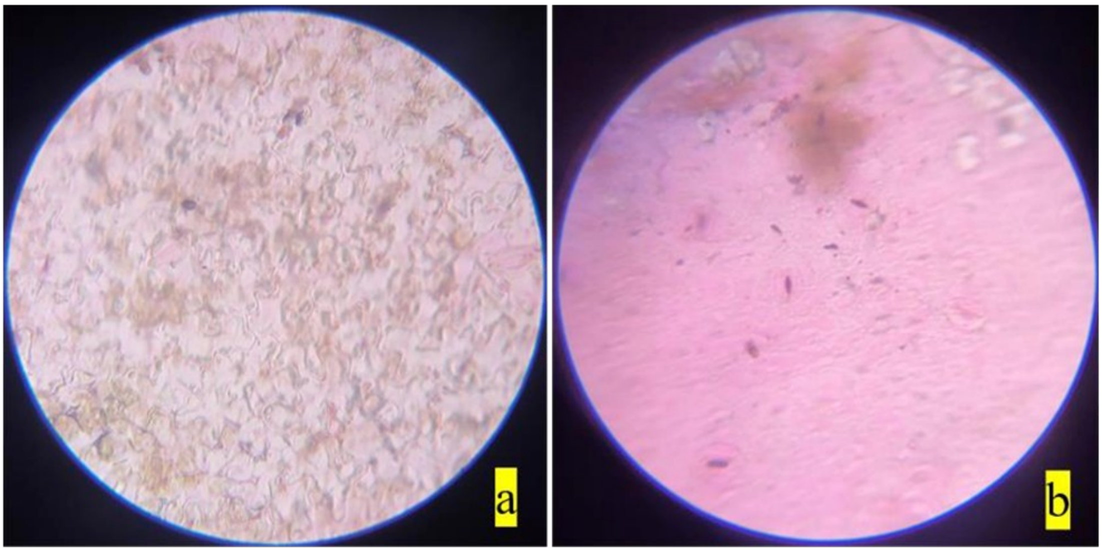

Figure 6. Stomata of *Chromolaena odorata* : a) non-polluted site; b) polluted site

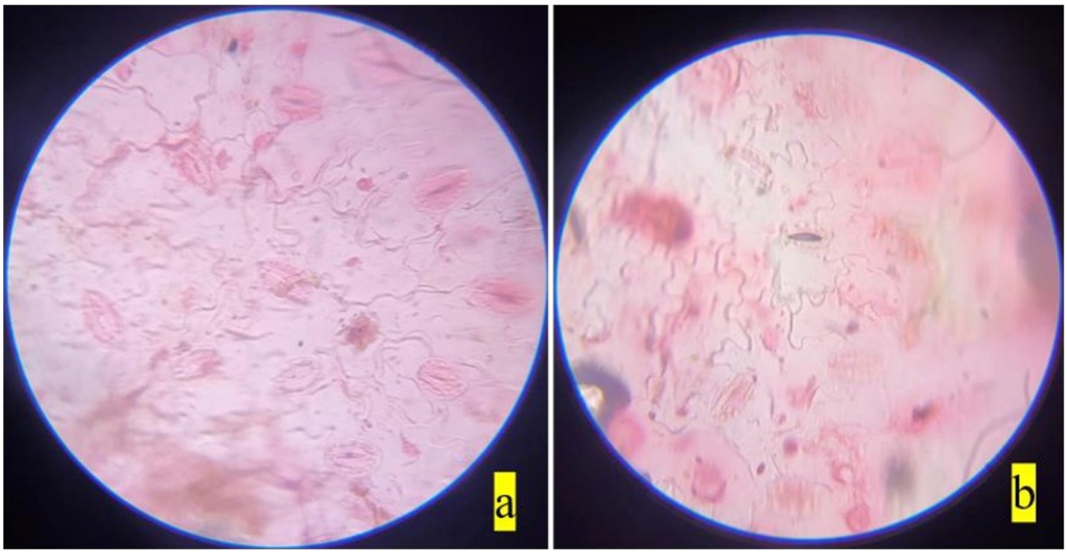

Figure 7. Stomata of *Tridax procumbens* : a) non-polluted site; b) polluted site

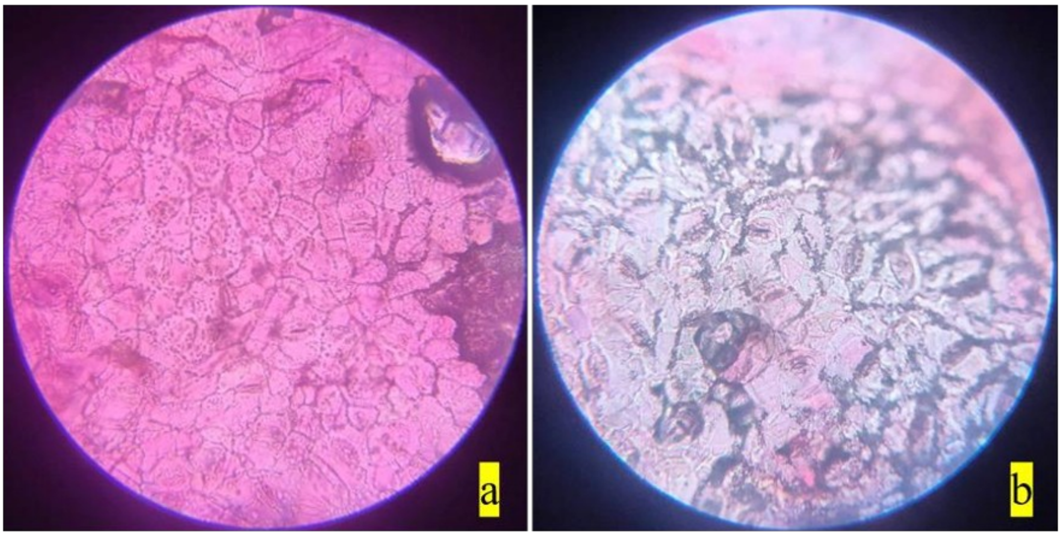

Figure 8. Stomata of *Gliricidia sepium* : a) non-polluted site; b) polluted site

The petiole anatomy of *Mangifera indica* and *Gliricidia sepium* were studied but no differences were noticed. The anatomical structure of the leaves of selected specimens as influenced through air pollution demonstrated a reduction in stomatal size and number control site. Occluded or closed stomata were observed in *Mangifera indica*, *Chromolaena odorata* and in *Tridax procumbens* (Fig. 4 — 8). Stomatal characteristics observed were shown in table 2.

DISCUSSION

Leaf is the most sensitive and exposed parts to be affected by air pollutants instead of all other plant parts such as stem and roots (Leghari and Zaidi, 2013). Plants leaf acts as the scavangers for many air borne particulates in atmosphere (Joshi and Swami, 2009). Air pollution is one of the severe problems facing the world today. It deteriorates the ecological condition (Tripathi and Gautam, 2007). In a study it has been observed that plant species at the contaminated sites showed reduction in the leaf L/B ratio as compared to the controlled sites. This reduction in the contaminated sites might be due to the fact that air pollution affects the photosynthetic activity and ultimately reduced the leave growth and size.

In another study it has been observed that the leaf length of *Thevetia nerifolia*, *Cassia siamea* and *Ficus bengalensis* were significantly reduced due to the air pollution (Bhatti and Iqbal, 1988). The finding of this study demonstrates that plant species at the contaminated site has less growth than the controlled site. Therefore, these results observed the impacts of vehicular emission on morphological parameters and provide a clear image that the vehicular emitted pollutants significantly affect the growth of plant species (Liu and Ding, 2008). Air pollutants cause leaf injury, stomata damage, premature senescence, decrease photosynthetic activity, disturb membrane permeability, reduce growth and yield in sensitive plant species (Tiwari *et al*., 2006).

The decrease in stomata number arising from vehicular air pollutants is an indication that the quantity of gaseous pollutants entering the leaves via the stomata is reduced and the plant becomes tolerant to the pollution. A slight modification for general gas exchange control and pollutant entry through the stomata singularly is initiated through reduction of pollutant uptake by plants by merely decreasing their stomata number. Stomatal size was decreased significantly in all analysed species and increase of absorbing capacity of the pollutant deposition. The decrease of the stomatal size may be an avoidance mechanism against the inhibitory effect of a pollutant on physiological activities such as photosynthesis.

Stomata index is the ratio of stomatal number and number of both epidermal and stomatal cell. In this case, the dividend is the stomata number, while the divisor is summation of epidermal cell number and stomata number. When compared with the control, it indicates variations in number of stomata and in number of epidermal cells. It has been opined 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.

There was increased epidermal cell number in all the five studied tree species at the road sites. The increase in epidermal cells could be a favourable adaptation in plants found in road sites for pollutant detoxification. The epidermis is the major site air pollutants are first opposed upon by free radical scavengers (Pawar, 2016). Epidermal cells increase ensures greater quantity of antioxidants, thereby enhancing the detoxification of pollutants (Shah *et al*., 2000). The modification of the frequency and sizes of stomata as a response to the environmental stress is an important manner of controlling the absorption of pollutants by plants.

In this study, clogging of stomata pores resulting from the effect of vehicular pollution were noticed in the three species *Mangifera indica*, *Chromolaena odorata* and *Tridax procumbens*. This could be due to high concentrations of particulate matter arising from vehicular emissions that is subsequently deposited on the stomatal pores. Aerosols larger than the pore of stomata are largely accumulated on the pore opening and thus interfere with exchange of gas, photosynthesis and a reduction in plant growth. Verma and Chandra (2014) had similar observations regarding the clogging of stomata on the leaves of *Sida cordifolia* and *Catharanthus roseus* receiving the burden of auto pollution.

The response of the plant to dust accumulation may vary according to different species, as dust deposition fluctuates with plant species due to leaf orientation, leaf surface geometry, phyllotaxy, epidermal and cuticular features, leaf pubescence, height and canopy of roadside plants. With the accumulation of dust, the roadside plant may exhibit adaptive response by changing morphological and physiological attributes. Heavy metals released from automobiles are extremely toxic and reduces plant growth and morphological parameters.

Air pollution due to vehicular emission mostly arises from cars, buses, mini-buses, wagons, rickshaws, motorcycles and trucks. These resources introduce varieties of pollutants (oxides of nitrogen, sulphur, hydrocarbon, ozone, particulate matters, hydrogen fluoride, peroxyacyl nitrates, etc.) into the environment which not only put an adverse effect on the health of human beings, and animals, but seriously treating the trees and crops of such areas. Pollutants can cause leaf injury, stomatal damage, premature senescence, decrease photosynthetic activity, disturb membrane permeability and reduce growth and yield in sensitive plant species. Leaf is the most sensitive part to be affected by air pollutants instead of all other plant parts such as stem and roots. Therefore, the leaf at its various stages of development serves as a good indicator of air pollution.

CONCLUSION

In this study, the plant species response to air pollution was by adjusting its leaf macro- and micro-morphological characteristics and such there was reduction in leaf length, breadth, L/B ratio, stomata size, stomata number, stomata index and stomata frequency. Low stomata number has been considered to be a sign of plant adaptation to air pollution, because of lower stomata number, lower of gaseous pollutants absorption from the air. In this study, stomata clogging with occluded stomata pores resulting from the effect of air pollution were noticed in three plant species. These alterations can be considered as indicators of environmental stress for initial revelation of urban air pollution.

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CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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