RESEARCH ARTICLE

Micromorphological Study of Shrubs from Roadsides in Kathmandu Valley

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Abstract

Urban air pollution is an environmental concern, particularly in developing countries and in their cities. The present study was mainly aimed to study the effect of air pollution on the micromorphological characteristics of the leaf of *Euphorbia pulcherrima* and *Jasminum mesnyi* from heavily polluted and less polluted areas of Kathmandu district. Different methods used to analyze the leaf structure (epidermal cells, stomatal frequency, and stomatal size). The stomatal study and observation from the leaf surface were completed under the compound microscope. Based on study results, in *E. pulcherrima*, the epidermal cells, stomatal pores, and the subsidiary cells were prominent in the anatomical structure of the leaf. In the winter season, the values were comparatively high for all different structures at less polluted sites. The values were comparatively equal for the stomatal index. The number of stomata, clogged stomata, subsidiary cells, epidermal cells, and the stomatal index was comparatively high. In *J. mesnyi,* the epidermal cells, stomata, were clear and many numbers in epidermal cells. The values were more in the less and moderately polluted sites both in spring and in the winter season. The number of stomata and stomatal index was high at less polluted sites and in the winter season. Within the two seasons, winter and in pre-monsoon, the values of stomatal size were comparatively high in the less polluted site. It was concluded that these two study plant species were categorized as good for plantation because their leaf micromorphological structure from air polluted areas have given suitable results.

Keywords: Leaf, Micromorphology, Pollution, Seasons, Stomata.

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Introduction

In urban environments, trees play an important role in improving air quality by taking up gases and particles (Woo and Je, 2006). Air pollution can directly affect plants via leaves or indirectly via soil acidification. When exposed to airborne pollutants, most plants experienced physiological changes before exhibiting visible damage to leaves (Liu and Dung, 2008).

Air pollution is one of the visible problems in Nepal. It has affected the plants in polluted areas. The leaf being the most exposed part of the plant is more sensitive and is affected more as compared to other plant parts. Contaminants present in the air are settled on the leaf, which results in disturbances in the metabolic activities of the leaf and affects the whole plant. Thus, usually, plants were affected by a little extra pollutant in the winter season (Kulshreshtha *et al.*, 1994).

Plants provide an enormous leaf area for absorption and accumulation of air pollutants to reduce the air pollutants in the polluted environment, with a various extent for different species (Liu and Ding, 2008). Since many studies have been focused on morphology and physiology, this study was based on the micromorphology of the leaves of the shrubs from the roadside of Kathmandu. The purpose of this research was to study the leaf micromorphology of plants from a heavily polluted and less polluted area of Kathmandu.

- Analysis of epidermal cells, stomatal frequency, and stomatal size from the micromorphological study of different leaves.
- Determine the air pollution effect on the leaves structure of plant species with seasons and sites.

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Materials and Methods

According to the report from the ministry of environment, the Nepal government and the ambient air quality analysis with some parameters such as particulate matter: PM_{10}, $PM_{2.5}$, total suspended particulates (TSP) were higher than National Ambient Air Quality Standards, 2012 for Nepal. Based on these reports and in my field study, the study sites were categorized into three different types these were as follows:

The study sites:

The study was carried out in the city area of Kathmandu district. The study was aimed to cover different sites:

A: Heavily polluted sites

Koteshwor-Airport area, Tudikhel-Ratna park area, Kalanki area

B: Moderately polluted sites

Bhudhanilkantha area

C: Less polluted sites

Suburban area, Tribhuvan University, Kirtipur campus area is taken as for the comparative study

Morphological Structure of Study Plants

The external or outer structure of leaves of study plants are completed, which are as follows:

- *Euphorbia pulcherrima* is a shrub or small tree and perennial habitat. It is in the Euphorbiaceae family (Figs 1A and B). The common name is 'poinsettia.' The plant bears dark green leaves. *E. pulcherrima* is a popular garden, roadside plant in tropical and subtropical areas. Because of its red upper part and green leaves, it is used in different celebrations and decorations.
- *Jasminum mesnyi* is a species of flowering plants in the family Oleaceae. *J. mesnyi* is an evergreen shrub growing with fragrant yellow flowers in winter (Fig. 1G). The form usually found in cultivation has semi-double flowers. It has a yellow color double flower. Flowers used to make a garland. It is a shrubby climber, trifoliate leaves, leaflets oblong-lanceolate, apex minutely pointed/ blunt, and green in color.

Structural Analysis of Leaves of Selected Plants

The micromorphological study was from the scope image program (SIP). The micromorphology of leaves of selected study plants was completed. The study plants were *Euphorbia pulcherrima* and *Jasminum mesnyi*. These plants were common and available in study sites. The external and anatomical (micromorphological) structure of leaves was completed by following different methods.

Anatomical Structure of Study Plants

Different methods were applied for the micromorphological study of the leaves as follows (Amulya *et al*., 2015): 1. Leaves were collected, cleaned, and made leaf bits which kept in separate test tubes containing 30% nitric acid. Boil the sample tubes for 2–3 hours (till leaf green pigments dissolve and leaf bits become transparent) in a water-containing beaker (Ahmad and Yunus, 1974). Wash the boil samples in distilled water and mount it in the slide. Observed the slides and completed the research work and photographs. 2. Fresh leaves have collected and wash it. Colorless nail polish used on the upper/lower surface of clean leaves. Stick the cello tape (transparent tape) on the dried polish. The tape part was taken out gently with the epidermal part and prepared slides for observation. Observed the slides and completed the research work and photographs. 3. ScopeImage 9.0 program was used in the computer for the micromorphological study of the leaves. First prepared the slides from the different process and observed under the microscope with the connection of this Scope Image 9.0 program (Acrobat Reader 7) which should be calibrated before the slides observation, micromorphological characteristics such as epidermal cells, stomata, stomatal index, guard cells, and are studied in 40 microscopic fields selected at random from each side and measured using an ocular micrometer. Microphotographs of different slides are taken from this SIP on the computer.

Results and Discussion

Structural analysis of leaves of selected two plants: morphological structure; leaf description of the study plants.

- *Euphorbia pulcherrima*

Leaves are large alternate, with or without teeth on the margin. Its oval-shaped, pointed, green leaves. It has dark green oblong smooth-textured leaves.

- *Jasminum mesnyi* Hance

Leaves are opposite, trifoliate, pinnately compound, 3-leaflets, each elliptic-oblong to lanceolate, glossy light, or dark green.

Micromorphological Structure

- *Euphorbia pulcherrima*

The epidermal cells and stomata were seen very clearly, which were numerous in number. Stomata were covered by guard cells. Guard cells were bean shape. The cells surrounding the guard cells (subsidiary cells) were also prominent in the anatomical structure of the leaf. Some trichomes were present in the cell photographs (Figs 1C and D from polluted sites and Figs 1E and F from less polluted sites).

- *Jasminum mesnyi*

The epidermal cells and stomata were seen very clearly. Stomata were covered by guard cells. Guard cells were bean shape. The cells surrounding the guard cells (subsidiary cells) were also prominent in the anatomical structure of the leaf. Trichomes were not present in the cell photographs (Figs 1H and J from polluted sites and Figs 1I and K from less polluted sites).

Analysis of Leaf Structure (Micromorphological)

The micromorphological studies were made comparatively in two seasons, namely in winter and in spring. The study was conducted on the leaves from the different study sites in the following structures are, the number of stomata, clogged stomata, stomata (stomatal pore) length (L), stomatal breadth (B), subsidiary cells, epidermal cells, and stomatal index were included in the study of the anatomical structure.

It was made to assess the air pollution effects on micromorphological parameters of *Tabernaemontana divaricata* (Gentianales: Apocynaceae) and *Hamelia patensi* (Gentianales: Rubiaceae). In the polluted area, the number of stomata and clogged stomata were found to be higher than in control, whereas the number of unclogged stomata are found to be very less in the control site. The stomatal breadth and pore length were found to be decreased in the polluted area in both the plants when compared to control. The number of subsidiary cells, trichome length values was found to be less than control plants. However, trichomes are absent in *T. divaricata*. The stomatal index was found to be higher in both the plants is when compared to control (Amulya *et al*., 2015).

In the present investigation, the results were given in the different tables with some photographs. The experimental values, as a result, presented in the data tables from the leaf micromorphology were low in some heavily polluted sites rather than in less polluted sites. It was concluded that study plants were good and healthy in pollutant areas. These plants are well adapted in the urban areas of Kathmandu.

There are four tables and one photo plate of the experimental results of *Euphorbia pulcherrima* and *Jasminum mesny*. The values from Tables 1 and 2 and Figs 1C and F were high in less polluted and moderately polluted sites. The high values were in the spring season rather than in the winter season. The number of stomata and stomatal index was high

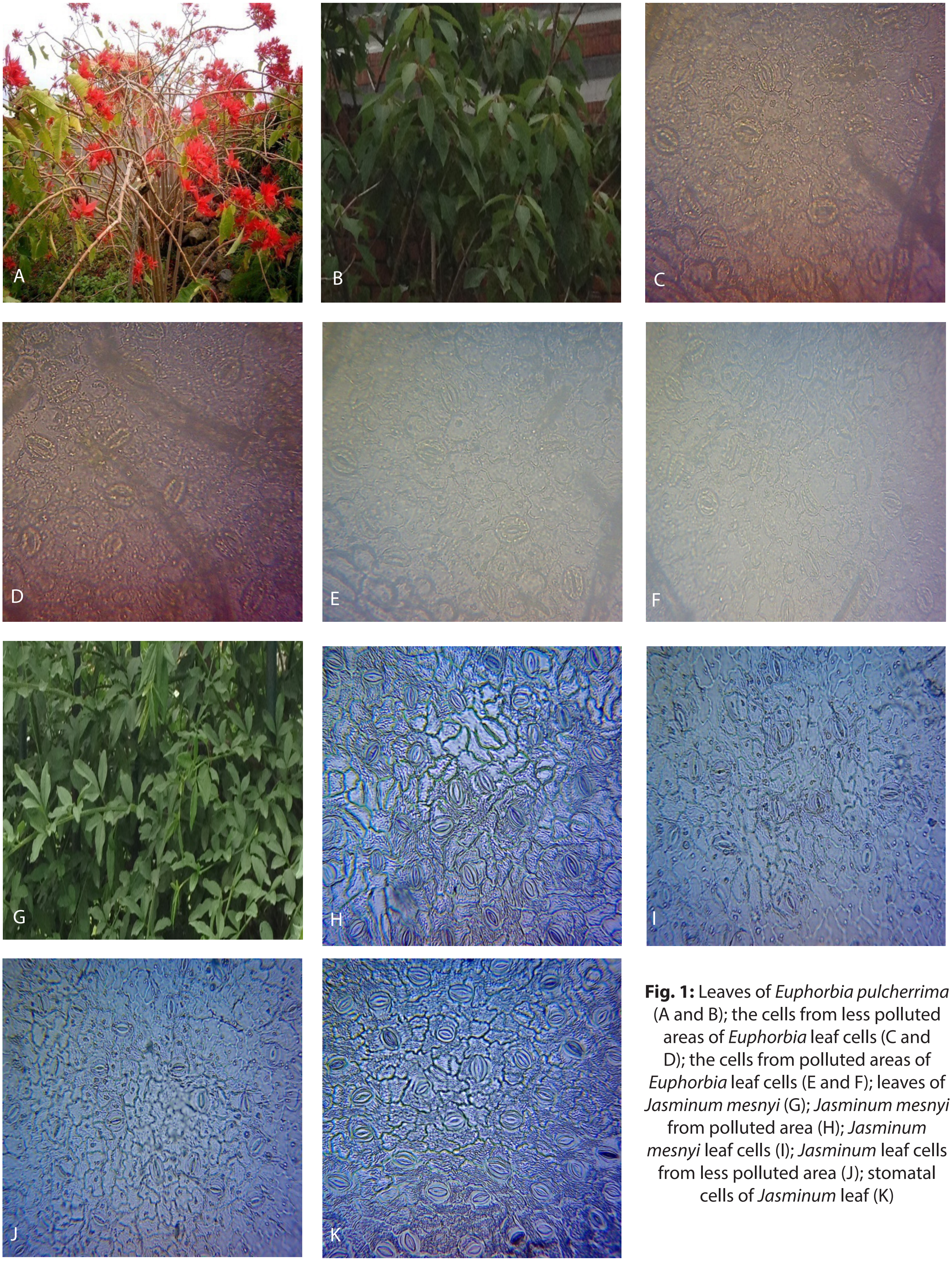

Fig. 1: Leaves of *Euphorbia pulcherrima* (A and B); the cells from less polluted areas of *Euphorbia* leaf cells (C and D); the cells from polluted areas of *Euphorbia* leaf cells (E and F); leaves of *Jasminum mesnyi* (G); *Jasminum mesnyi* from polluted area (H); *Jasminum mesnyi* leaf cells (I); *Jasminum* leaf cells from less polluted area (J); stomatal cells of *Jasminum* leaf (K)

Table 1: Impact of pollution on micromorphological features of *Euphorbia pulcherrima* in winter season

S. No.	Sites	No. of stomata	Clogged stomata	Stomata size length (L)	Stomata size breadth (B)	Subsidiary cells	Epidermal cells	Stomatal index
1	Less polluted	25 ± 0.91	4.5 ± 0.65	5.62 ± 0.04	1.847 ± 0.04	4.583 ± 0.159	153.25 ± 6.47	14.48 ± 0.86
2	Moderately polluted	12.75 ± 1.44	2.25 ± 0.25	5.61 ± 0	1.93 ± 0	5 ± 0	167.5 ± 4.031	7.05 ± 0.66
3	Heavily polluted	20 ± 3.42	3.56 ± 0.40	5.38 ± 0.201	1.797 ± 0.11	4.815 ± 0.098	173.44 ± 11.56	11.11 ± 1.58

Table 2: Impact of pollution on micromorphological features of *Euphorbia pulcherrima* in pre-monsoon/ spring

S. No.	Sites	No. of stomata	Clogged stomata	Stomata size length (L)	Stomata size breadth (B)	Subsidiary cells	Epidermal cells	Stomatal index
1	Less polluted	27.25 ± 1.031	4.75 ± 0.48	5.62 ± 0.04	1.84 ± 0.023	4.5 ± 0.07	155.75 ± 4.27	14.48 ± 0.86
2	Moderately polluted	14.75 ± 0.85	3 ± 0.41	5.62 ± 0.07	1.92 ± 0.004	5.25 ± 0.25	170.25 ± 2.78	8.25 ± 0.48
3	Heavily polluted	13.67 ± 2.19	3 ± 0.5	4.85 ± 0.34	1.88 ± 0.12	5 ± 0	200 ± 5.86	6.33 ± 1.33

Table 3: Impact of pollution on micromorphological features of *Jasminum mesnyi* during the winter season

S. No.	Sites	No. of stomata	Clogged stomata	Stomata size length (L)	Stomata size breadth (B)	Subsidiary cells	Epidermal cells	Stomatal index
1	Less polluted	47.4 ± 0.93	9.8 ± 0.36	5.65 ± 3.44	1.85 ± 8.60	5 ± 0	143.6 ± 6.87	25.03 ± 1.30
2	Moderately polluted	39.2 ± 1.77	8.4 ± 0.6	5.68 ± 0.23	2.09 ± 0.07	4.8 ± 0.06	166.2 ± 3.39	19.3 ± 0.8
3	Heavily polluted	42.3 ± 5.44	6.6 ± 0.1	4.78 ± 0.59	2.02 ± 0.28	4.5 ± 0.25	172.88 ± 13.9	20.05 ± 2.82

Table 4: Impact of pollution on micromorphological features of *Jasminum mesnyi* during pre-monsoon/ spring season

S. No.	Sites	No. of stomata	Clogged stomata	Stomata size length (L)	Stomata size breadth (B)	Subsidiary cells	Epidermal cells	Stomatal index
1	Less polluted	47.4 ± 0.75	9.2 ± 1.16	5.54 ± 0.01	1.82 ± 0.003	5.2 ± 0.2	146.4 ± 6.05	24.6 ± 0.98
2	Moderately polluted	39.2 ± 1.88	8.4 ± 0.51	5.76 ± 0.28	2.09 ± 0.07	4.8 ± 0.063	166.2 ± 3.4	19.4 ± 0.75
3	Heavily polluted	33.25 ± 1.65	6.5 ± 0.65	3.82 ± 0.03	1.49 ± 0.02	4.59 ± 0.058	209 ± 6.82	14.0 ± 0.71

at less polluted sites and in the spring season. The clogged stomata were counted high at less polluted sites in the spring season. The stomata size was large at less polluted sites in both spring and winter. Within winter and pre-monsoon season, winter season showed comparatively equal values for the stomatal index. From winter and pre-monsoon season, the values were comparatively high in pre-monsoon season for all different results. The high values were at less polluted sites in both the spring and winter seasons. The number of stomata and stomatal index was high at the less polluted sites in the winter season, almost similar values in the spring season as well. The clogged stomata were high at less polluted sites in spring. The stomata size was large at a moderately polluted area in the spring season.

In *Jesminum*, within winter and pre-monsoon, the values of the number of stomata, stomatal size, subsidiary cells, and epidermal cells were comparatively high in pre-monsoon season Tables 3 and 4 and Figs 1H and K. Both plants were well in structure. The plant activities were also conducted properly in different sites and in seasons. The leaves structure (epidermal cells, stomata, guard cells, and subsidiary cells) were clear in their shape and size in the presence of data tables and photographs for both the plants. The effects of seasons and sites in the micromorphology were given in the leaves of both plants with some differences. The clogged stomata values were also given. The seasons and sites showed an impact on the micromorphology and activities of the plants.

Conclusions

The two study plants were able to give the expected results. The obtained results were analyzed and can be concluded that a little difference in results following the effect of seasons and study sites in the leaf micromorphology. This could be mainly due to plant physiological and biochemical activities, even getting exposure of plants with air pollutants come from various auto emission sources in the roadsides. The micromorphological results were good structure, a good number of different study and involvement in plant life activities clearly. Due to unwanted pollutants, vital processes occurring in the plants are disturbed as a result of which changes may occur in the morphology, physiology, and anatomy of plants.

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