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EFFECT OF AIR POLLUTION ON THE LEAF MORPHOLOGY AND ANATOMY OF COMMON PLANT SPECIES OF KATHMANDU VALLEY

Anjana Devkota*, S.D. Shrestha, PK Jha

Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal
**For correspondence: devkotaa@gmail.com*

Abstract.

The present study was mainly aimed to study the effect of air pollution on the morphological and anatomical characteristics of leaf of 6 common plant species viz., Bougainvillea glabra, Callistemon citrinus, Euphorbia pulcherrima, Jasminum mesnyi, Lagerstroemia indica *and* Nerium oleander *from different location of Kathmandu Valley. Results showed that all plant species exhibited significant (p<0.05) reduction at polluted site in their leaf length, width, and area when compared with the same plant species of non-polluted site. Results also showed that the overall reduction % in leaf length, width and area during different seasons at heavily polluted sites with respect to those of less polluted sites were found maximum during post monsoon than in pre-monsoon. Results also interpreted that maximum reduction in leaf length (25%) and leaf width(18.62%) was recorded in* E. pulchirrima*, and leaf area (28%) was recorded in* L. indica.*; while minimum decrease in leaf length (7.58%) in* N. oleander *and width(11.3%) as well as area (13.90%) in* C. citrinus. *was recorded. Likewise, the highest stomatal index (20.58%) and number of epidermal cells (30.4) were recorded in* Lagerstroemia indica*; while highest stomatal density (134.8mm^{-2}) and number of clogged stomata (8.33mm^{-2}) was recorded in* Bougainvillea glabra *and* Nerium oleander *respectively.*

Results further indicated that as the plants get ages, the reduction % of various leaf attributes of polluted plants also increased as compared with less polluted plant species. This could be mainly due to maximum exposure of plants to air pollutants come from various auto emission sources. This study showed that plants generally respond to air pollution with reduction in foliar features and the response is species specific.

Key words: *air pollution, foliar characters, leaf morphology, micromorphology*

Introduction

Air pollution is the major threat to developing world. Urban air pollution is a major environmental problem, mainly in the developing countries (Mage et al. 1996). Gaseous and particulates matters emitted either from vehicles or from different developmental activitiefigurs that can harm human health and the environment. The relationship between plants and different types of contaminants has been investigated by many researchers (Chukwuka & Uka 2014; Ahmed & Raof 2015 ; Ogunrotimi et al. 2017; Tra-Bi et al. 2015). Pollutants emitted from different sources not only affect human and animal health but also threaten plant life in any areas (Uaboi-Egbenni et al. 2009).

Leaf is the most sensitive part to be affected by air pollutants instead of all other plant parts such as stem and roots. The sensitivity rests on the fact that the major portions of the important physiological processes are concerned with leaf. Therefore, the leaf at its various stages of development, serves as a good indicator to air pollutants. Pollutants came from the auto emission can directly affect the plant by entering in to the leaf, destroying individual cells, and reducing the plant ability to produce food. Pollutants from automobile have long-term effects on plants by affecting the content of carbon dioxide, light intensity, temperature and precipitation. Therefore, plants need special protection because they are not only a source of food but are also helpful in cleaning the environment (Reig-Armiñana et al. 2004; Silva et al. 2005).

Effects of air pollutants in plants are studied in many types of research as one of plant environmental interference. This may be due to fact that plants are most living organisms that suffer from the effect of air pollution due to their stability within their environments and their long exposure to air pollutants when compared with other organisms (Abbasi et al.2004). The basic plants role in research related to air pollution was in several directions, including giving early pollution presence signal or warning in a given area and thus help in assessing air quality, and through the plants diversity can determine geographical distribution of air pollutants, and helps to concentrations estimate of those contaminants, if accumulated in plant tissues, and facilitate direct diagnosis of different pollutants depending on plant response types to contaminants (Wagh et al. 2006).

Various researchers investigated the effects of pollution on different plant species and reported that plants which are sensitive to air pollutants had showed changes in their morphology, anatomy, physiology and biochemistry (Reig-Armiñana et al. 2004; Silva et al. 2005). Most of the authors (Bhatti & Iqbal, 1988; Syed et al. 2008; Stevovic et al. 2010, Al-Obaidy et al . 2019) have reported the effects of air pollution on the morphology and anatomy of different plants species grown in different regions, but very little is known about the plants of Kathmandu valley.

Kathmandu is capital of Nepal. It is situated at an elevation of 1300 meters above sea level. Air pollution in Kathmandu valley is rising to an alarming state rapidly since the last few decades due to heavy automobile activities. Rapid increase in automobile activities and traffic congestion contributes most of air pollution problems, resulting in damage to the plants growth. Therefore, the present work was mainly designed to analyze the effects of air pollution, dominantly presented by automobile and dust, on morphology and anatomy of different plant species, growing near the roads of the city. The investigated plants are very common and widespread in the investigated area and these can be cultivated and also grows spontaneously. They have a wide distribution, which indicates a high ecologically plasticity and adaptability to different environmental conditions. Therefore, the main object of this study was to explore the morphological and anatomical features of plants as adaptability indicator. The goal was to prove the statement that the plants answer on environmental stress, from different anthropogenic and non-anthropogenic sites/ locations, by changing morphology and .anatomy of leaves.

Materials and Methods

Study Site: The research work was done in along roadside of Kathmandu valley (27°42’14.40” N; 85°18’32.40” E), the capital city of of Nepal. It lies in central Nepal with mean temperature from minimum 10 (January) to maximum about 25°C(July) in different months . The area receives monsoon rain from June to September, which accounts about 80% of the total annual rainfall (2244 mm) (Figure 1). Rests of the months are dry with few showers of winter rain. Population of Kathmandu Valley is 2.17 million with annual population growth rate of 4.2% and population density 2,799.8/Km^2 (CBS 2011). Due to road construction activities and pipelines of Melampchi water supply causes dust pollution in Kathmandu Total suspended particulate matters (TSPMs) has been a major problem in roadsides of Kathmandu valley.

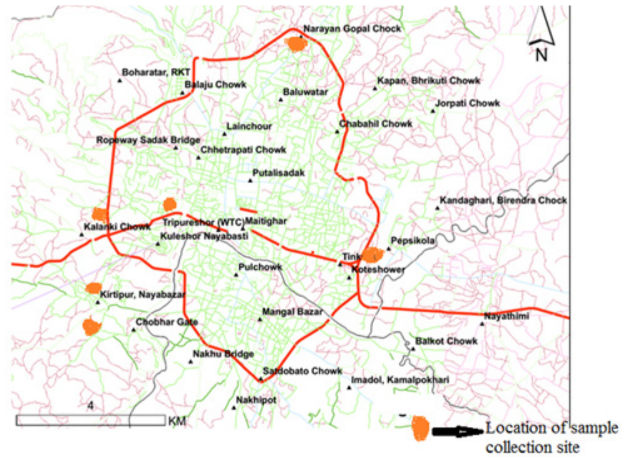

Figure 1. Map showing location of air quality monitoring site

Selection of study sites

The study area was divided into three on the basis of particulate materials) *viz.* highly polluted, moderately polluted, and less polluted (Tables 1). The quantity of particulate pollutants (PM $_{2.5}$, PM $_{10}$ and TSP) was measured by Gravimetric Method (CPCB 2013) . To see the impact of dust load on the roadside plants six sites were selected (Fig.1 – (1) heavily polluted (Koteshwor-Tinkunel area; Kalanki Bhatbhateni to Suichatar Area; and Tudikhel-Ratna Park Area; (2) Moderately polluted Budanilkantha 3. less polluted (Tribhuvan University campus area, Kirtipur and Matatirtha area) as the control site for the comparison. Characteristic feature of sample collection site is presented in table 2.

Table 1. Ambient air quality of study sites (Date of Sampling: March,7-17. 2018)

SN	Location	PM_{10}	$PM_{2.5}$	TSPM	Category of study site
			(μg/Nm^3)		
1.	Kalanki (Top of Traffic Police Post Building)	507.0	86.0	1390.0	Heavily polluted
2	Ratnapark	454.0	105.0	1107.0	Heavily polluted
3.	Koteshwor(inside compound of traffic police post)	229.0	72.0	813.0	Heavily polluted
4.	Budanilkantha(inside compound)	193.0	23.0	248.0	Moderately polluted
5.	Kirtipur (Coronation garden)	149.0	13.0	320.0	Less Polluted
6.	Machhegaun (Matatirtha)	127.0	28.0	240.0	Less Polluted
	National Ambient Air Quality Standards (NAAQS) 2012	120.0	40.0	230.0	

Table 2. Site description

SN	Site name	Characteristics
Site 1	Koteshwar—Tinkune	Junction, Commercial,Entrance from Arniko Highway
Site 2	Kalanki Bhatbhateni –Suichatar	Junction, Commercial,Entrance to city from outside
Site 3	Tudikhel-Ratnapark	Bus park, more flow of vehicles and people
Site 4	Budanilkantha	Away from city, settlement area, pilgrim
Site 5	Kirtipur-Coronation garden	University area, less flow of vehicles and movement
Site 6	Matatirtha-Machhegaun	Away from city area, less flow of vehicles

Morphological characteristics

Leaf morphological characters *viz*. leaf length, width and area were measured. Ten fully matured leaves from each individual from each site were collected during pre-monsoon (April-May) and post- monsoon season (October-November) at random for leaf character analysis. Leaves area, length and breadth were measured from Image J- Program (from leaves photographs). The average leaf area was utilized for calculating dust load on the leaves.

Anatomical studies

Leaf samples collected from all sites were processed following the method of Ahmed and Yunus (1974). The mature leaf samples were cut into small bits and taken in separate test tubes. Nitric acid solution (30%) was added to each test tube. The mixture was boiled in a water bath for 3 h till the leaf bits became transparent. The leaf bits were washed in distilled water and stained with 2% safranin. Excess stain was removed by washing with distilled water and mounted using gelatin jelly. A small amount of the jelly was taken on a slide and gently warmed using Bunsen burner. The leaf bits were placed to the jelly and a coverslip was mounted over it and observed under light microscope. Micromorphological characteristics such as stomata density and stomatal index were studied in 10 microscopic fields selected at random from each side and measured using ocular micrometer. Photomicrographs were taken with a digital camera at different magnifications. Micromorphological studies were carried out during pre-monsoon season only because these features do not vary with season.

Statistical analyses: Data were analyzed by SPSS version 20 using Two-way ANOVA. Comparison of mean of morphological and anatomical characteristics, among plant species was carried out by Two way interaction among plant species, and location (pollution level).

Results

Morphological characteristics

In morphology, leaf length, width and area of selected species were measured during pre- and post-monsoon seasons.

Leaf length

There was significantly difference in leaf length among different species at different location. The leaf length varied from 4.05 to 14.59 cm (Table 3, Fig.2). Maximum leaf length (14.59 cm) was recorded in *Nerium oleander* whereas minimum (4.05 cm) for *Lagerstroemia parviflora*. The leaf length of selected species followed the order of *Nerium oleander* (14.59 cm) > *Callistemon citrinus* (8.07 cm) > *Euphorbia poinsettia* (11.07 cm)> *Bougainvillea glabra* (7.61 cm) > *Jasminum mesnyi* (4.33 cm) > *Lagerstroemia parviflora* (4.05 cm) . There was marginal difference (p=0.052) in leaf length of the plants species with location of sample collection (Table 3). The leaf length of selected plants growing at different location along roadside ranged from 7.32cm – 9.19cm. Highest leaf length (9.19 cm) was recorded at less polluted site, whereas relatively less leaf length (7.32 cm) was observed at highly polluted site. The season of the year also significantly influenced the leaf length with longer leaf during post monsoon (9.13 cm) (Fig. 2).

The overall average reduction % of leaf length at polluted site with respect to less polluted site (control) was found in the range of 7.58- 25.16% % lowest to highest in the leaf of N. indicum and Euphorbia poinsettia respectively at heavily polluted site (Fig.3). The reduction in leaf length of selected species followed the order of E. poinsettia (25.16%)>J. mesnyi (22.92%)> Lagerstroemia indica (21.21%)> Bougainvillea glabra (15.05%)> Callistemon citrinus (11.53%) and N. oleander (7.58%).

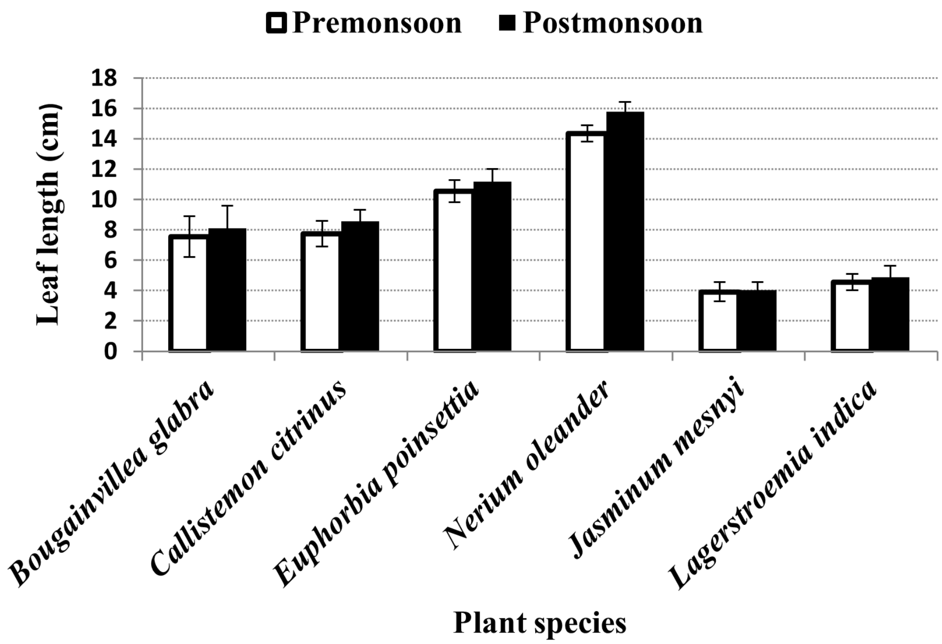

Figure 2. Seasonal variation of leaf length (cm) (mean) of selected species., error bar represents standard deviation . (N= 180) .

Table 3. Seasonal variation in leaf length (cm) of selected species at growing at different location

Plant species		Location		
	Less polluted (Control)	Moderately polluted	Heavily Polluted	Mean
Bougainvillea glabra	8.84±0.13	8.51±0.22	7.51 ±0.15	7.61±0.17
Callistemon citrinus	8.84±0.23	8.56±0.21	7.82±0.23	8.07±0.22
Euphorbia poinsettia	12.04±0.213	12.0±0.23	9.01±0.34	11.07±1.15
Nerium oleander	16.21±2.63	15.99±1.14	14.98±2.23	14.59±2.03
Jasminum mesnyi	4.12±0.203	3.92±0.27	3.18 ±0.32	4.33±0.45
Lagerstroemia Indica	5.09±0.23	5.00±0.202	4.01±0.15	4.05±0.182
Mean (location)	9.19±0.53	9.04±0.23	7.32 ±0.13	Mean (season)
Location	P- value 0.052	F=7.34		
Species	P -value <0.001	F=53.3		

Leaf width

Leaf width of different plant species varied from 0.81 cm to 10.00 cm (Figure 3). There was significant difference in leaf width at different species and location (Table 4). Maximum width of the leaf (10.00 cm) was exhibited by *Euphorbia poinsettia* and minimum (0.81cm) by *Callistemon citrinus.* Width of leaves of species varied significantly (p=0.04) with location of collection site . It was higher (3.91 cm) at less polluted site and lower (3.62cm) at highly polluted site. Season of collection also affect leaf width. It was significantly (p=0.051) higher at postmonsoon season than in premonsoon season (Table 4, Fig.4).

The overall average reduction % of width at polluted site with respect to less polluted site (control) was found in the range of 11.3- 18.62% % lowest to highest in the leaf of C. citrinus and Euphorbia poinsettia respectively at heavily polluted site (Fig. 5). The reduction in leaf width of selected species followed the order of E. poinsettia (18.62%)> Lagerstroemia indica (17.3%)> Bougainvillea glabra (17.13%)> N. oleander (12.66%)> J. mesnyi (12.0%)> Callistemon citrinus (11. 3%).

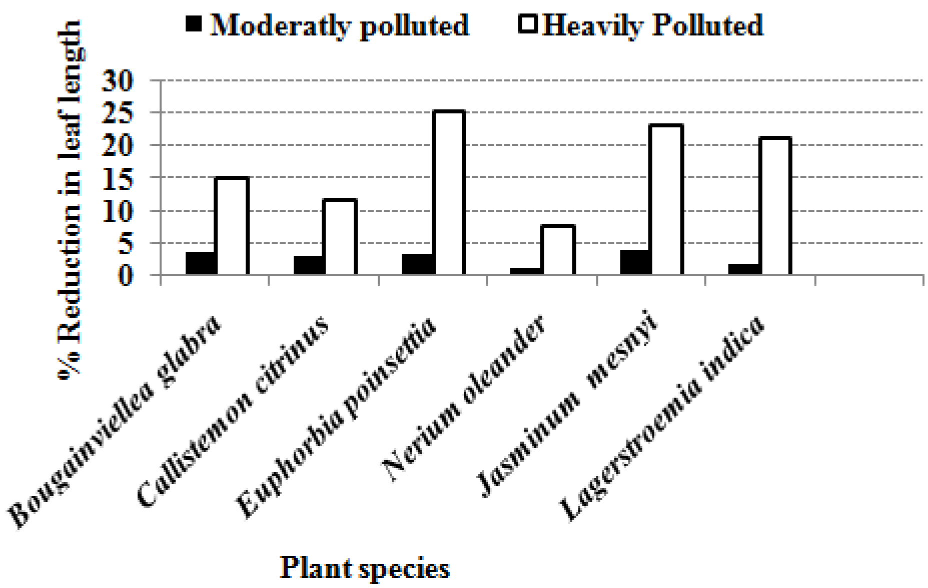

Figure 3. Overall average reduction % of leaf length of studied plant species at different location. (N=180).

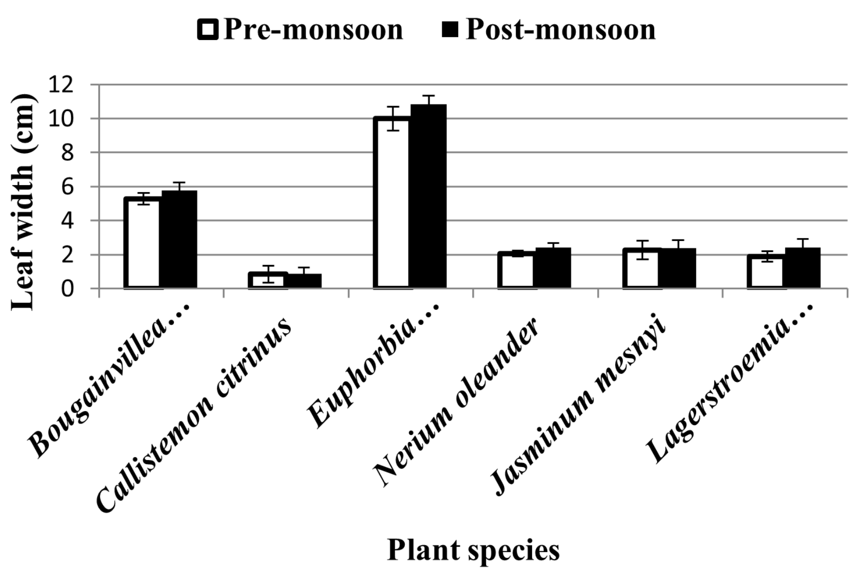

Figure 4. Variation of leaf width (cm) of selected species. Significant difference between mean among species indicated by different letters (Duncan homogeneity test, α = 0.05). F and P values were obtained by two way analysis of variance (ANOVA). (N=180).

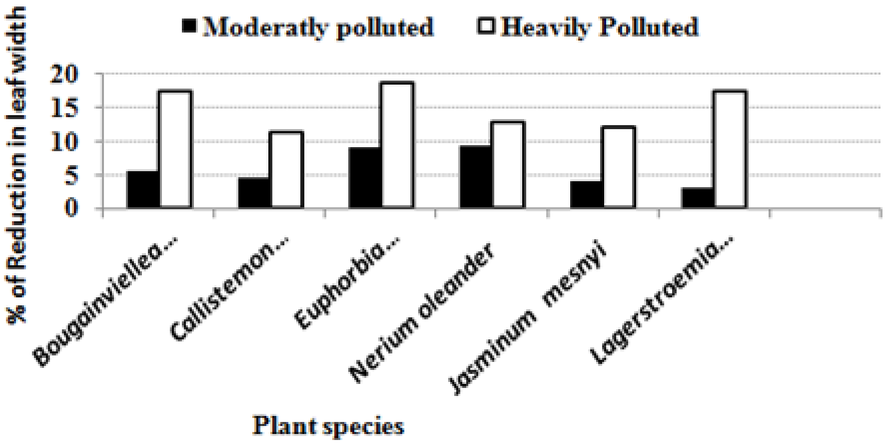

Figure 5. Overall average reduction % of leaf width of studied plant species at different location(N=180).

Table 4. Seasonal variation in leaf width (cm) of selected species at growing at different location

Plant species		Location		
	Less polluted (Control)	Moderatly polluted	Heavily Polluted	Mean
Bougainvillea glabra	6.01±0.13	5.68±0.22	4.78±0.45	5.49 ±0.86
Callistemon citrinus	0.88±0.03	0.84±0.01	0.72±0.13	0.81±0.72
Euphorbia poinsettia	10.02±0.213	10.02±0.23	10.01±0.34	10.00±1.15
Nerium oleander	2.29±0.203	1.80±0.14	2.19±0.203	2.09 ± 0.83
Jasminum mesnyi	2.25±0.203	2.36±0.27	2.04±0.32	2.21 ±0.51
Lagerstroemia indica	2.08±0.23	2.12 ±0.42	1.80 ±0.15	2.0 ±0.62
Mean (location)	3.91±0.53	3.80±0.23	3.62±0.13	Mean (season)
Location	P- value <0.001	F=114.34		
Species	P -value <0.001	F=78.13		

Leaf area

There was significant difference ($p<0.001$) in leaf area of collected samples from different location (Table 5). Leaf area of selected species ranged from 6.06 cm^2 to 143.60 cm^2 (Fig.6). Maximum leaf area (143.60 cm^2) was exhibited by Euphorbia poinsettia and minimum area (6.08 cm^2) by Lagerstroemia parviflora. Leaf area of plant species also varied significantly ($p<0.001$) among different location (Fig. 6). The effect of changing seasons on the leaf area was also significant ($p<0.001$) (Fig.6). The leaf area of the selected plants was found to be higher with the value of 38.37 cm^2 during post monsoon than the value of 34.98 cm^2 in pre monsoon season.

The overall average % reduction of leaf area at polluted site with respect to less polluted site (control) was found in the range of 13.90- 28.0% % lowest to highest in the leaf of C. citrinus and Lagerstroemia indica respectively at heavily polluted site (Fig. 7). The reduction in leaf area of selected species followed the order of Lagerstroemia indica (28) > Euphorbia poinsettia (26.53) > Jasminum mesnyi (22.57) > Bougainvillea glabra (22.40) > Nerium oleander(18.75) > Callistemon citrinus (13.90).

Micromorphological Characters

Micromorphological characteristic *viz*. stomatal density, stomatal indices, number of clogged stomata and epidermal cells on leaf surface of selected species were recorded.

Stomatal Density

There was a significant variation in stomatal density among the plant species at different location (Table 6). The stomatal density on the adaxial surface of the leaves varied among the species growing at different location. The stomatal density of *Bougainvillea glabra* was the highest (134.8 mm^{-2}). The lowest density (120.70 mm^{-2}) was recorded in *Euphorbia poinsettia*. With the declining pollution level, the stomatal density increased in the following order: highly polluted (123.73 mm^{-2} < moderately polluted (130.01 mm^{-2}) < least polluted site (130.75 mm^{-2}) (Table 6).

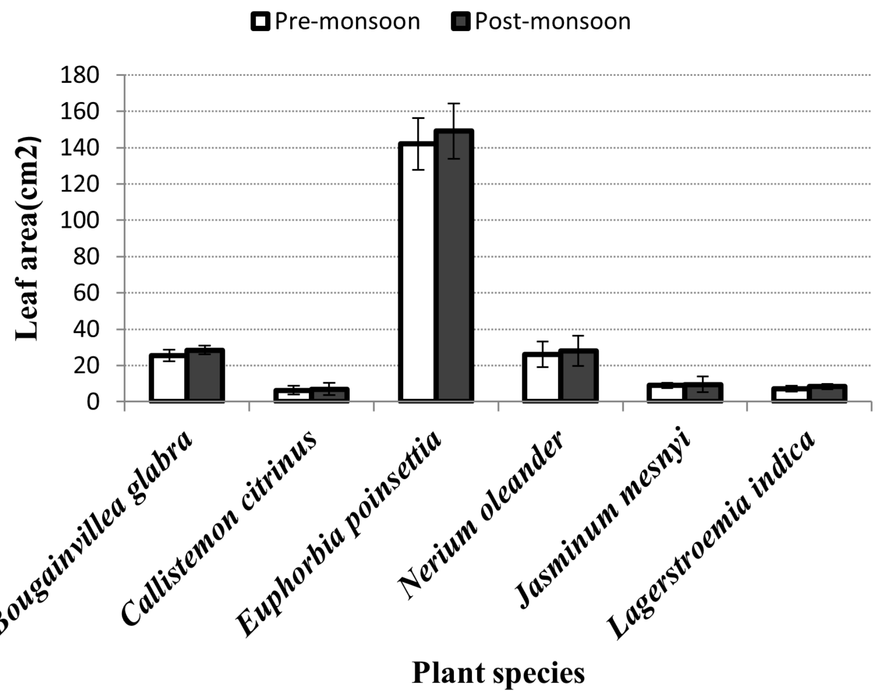

Figure 6. Seasonal variation of leaf area of selected species.

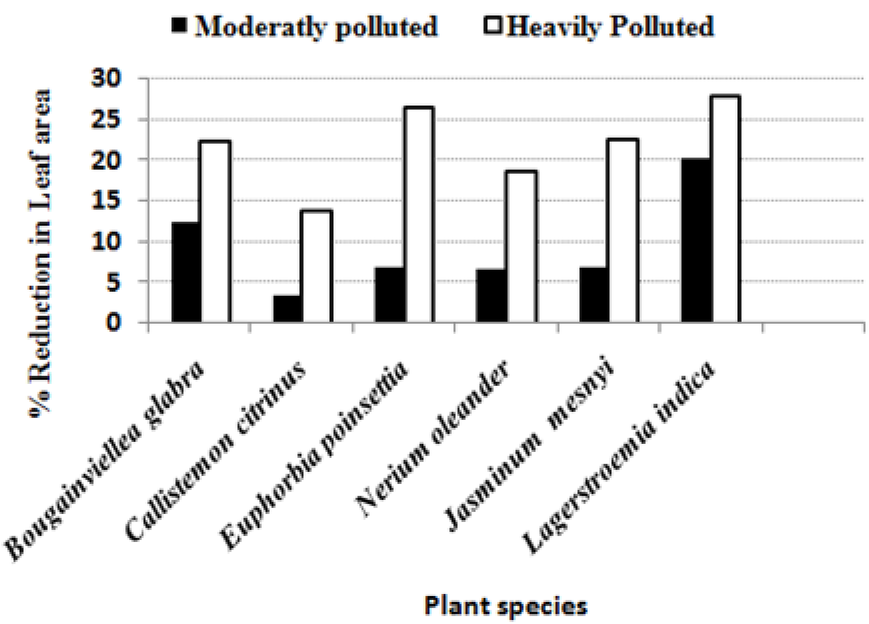

Figure 7. Overall average reduction % of leaf area of studied plant species at different location (N=180).

Table 5. Seasonal variation in leaf area (cm^2) of selected species at growing at different location

Plant species		Location		
	Less polluted (Control)	Moderatly polluted	Heavily Polluted	Mean
Bougainvillea glabra	31.77±0.13	21.105±0.22	19.83±0.45	24.23`±0.23
Callistemon citrinus	6.76±0.23	6.52±0.21	5.82±0.23	6.37±0.45
Euphorbia poinsettia	147.92±3.21	137.69±3.23	118.34±5.34	143.60±0.15
Nerium oleander	31.81±1.63	24.67±1.14	24.36±2.23	26.14±1.13
Jasminum mesnyi	9.17±1.203	8.53±1.27	7.10±0.92	8.81±1.45
Lagerstroemia indica	8.02±0.23	6.38±1.02	4.76±0.15	6.08±0.12
Mean (location)	39.24 ± 4.53	34.10 ±3.23	30.035 ±0.13	Mean (plant species)
Location	P- value <0.001	F=92.34		
Species	P -value <0.001	F=58.13		

Table 6. Variation in leaf stomatal density (per mm^2)(mean±SD) on leaf surface of selected plant species growing at different location of roadside of Kathmandu valley. *p*- value and F- value obtained through two way ANOVA.(N=10).

Plant species		Location		
	Less polluted (Control)	Moderatly polluted	Heavily Polluted	Mean (Species)
Bougainvillea glabra	135.7±1.05	136.4±1.12	128.3±1.10	134.80±1.09
Callistemon citrinus	133.7±1.15	126.0±1.13	123.1±1.12	127.60±0.65
Euphorbia poinsettia	122.3±2.01	119.8±1.12	120.0±1.13	120.70±0.62
Nerium oleander	131.6±1.12	131.4±1.01	120.7±1.13	128.56±0.65
Jasminum mesnyi	128.9±2.13	122.9±2.13	122.5±0.9	124.8 ±0.62
Lagerstroemia indica	130.3±1.05	131.7±1.33	128.1±1.12	130.36±0.62
Mean (location)	130.75±0.46	130.01±0.47	123.75±0.46	
Location	P- value <0.001	F=112.34		
Species	P -value <0.001	F=98.13		

Stomatal index

The stomatal index of the leaves varied among the selected plant species. There was a significant variation in the plants growing at different location ($p<0.001$) (Table 7). The highest index (20.58%) was recorded in *Lagerstroemia indica* and the lowest (11.91%) in *Euphorbia poinsettia*. This parameter was found the highest (18.78 %) at the least polluted site which was followed by the plants at moderately polluted (15.76%) and highly polluted sites (11.86%) (Table 7).

Number of clogged stomata

There was significant difference ($p<0.001$) in number of clogged stomata among the species at different sites. Number of clogged stomata among the plant species at different location ranged from 1.45 to 8.33 mm^{-2}. The maximum number of clogged stomata was recorded in *Nerium oleander* (8.33mm^{-2}) and a minimum (1.45 mm^{-2}) in *Callistemon citrinus*. Number of clogged stomata within the species also varied with location of collection site which ranged from 3.78 at less polluted site to 5.34 at highly polluted site (Table 8).

Table 7. Variation in leaf stomatal index (%) (mean±SD) on leaf surface of selected plant species growing at different location of roadside of Kathmandu valley. *p*- value and F- value obtained through two way ANOVA.(N=10).

Plant species	Location			
	Less polluted (Control)	Moderately polluted	Heavily Polluted	Mean (Plant spp)
Bougainvillea glabra	18.00±1.0	14.01±1.32	13.67±1.21	15.22±1.01
Callistemon citrinus	16.90±1.00	15.40±1.02	14.0±0.45	15.43±1.02
Euphorbia poinsettia	15.50±0.91	12.30±0.89	7.93±1.01	11.91±1.01
Nerium oleander	19.90±1.00	14.80±2.1	15.30±1.2	16.67±0.56
Jasminum mesnyi	21.30±2.1	18.60±2.10	14.50±1.01	18.14±0.67
Lagerstroemia indica	21.10±1.23	19.50±2.00	16.70±1.02	19.50±0.45
Mean (location)	18.78±2.10	15.76±2.13	11.86±0.87	
Location	p- value <0.01	F=54.23		
Species	p- value- 0.04	F=4.21		

Table 8. Variation in number of clogged stomata (mean±S.D) on leaf surface of selected plant species growing at different location of roadside of Kathmandu valley. *p*- value and F- value obtained through two way ANOVA.(N=10).

Plant species	Location			
	Less polluted (Control)	Moderately polluted	Heavily polluted	Mean (species)
Bougainvillea glabra	3.32 ±0.80	3.72 ±0.56	4.14 ±0.34	3.72 ±0.43
Callistemon citrinus	1.09 ±0.14	1.6 ±0.42	1.65 ±0.23	1.45 ±0.21
Euphorbia poinsettia	2.28 ±0.18	3.21 ±0.70	3.95 ±0.35	3.14±0.43
Nerium oleander	5.6 ±0.01	8.5 ±1.2	10.9 ±1.24	8.33 ±1.20
Jasminum mesnyi	4.99 ±0.92	6.18 ±0.92	7.1 ±1.02	6.09 ±1.22
Lagerstroemia indica	5.4 ±0.96	4.85 ±0.34	4.35 ±0.87	4.86 ±0.78
Mean (location)	3.78 ±0.65	4.67 ±0.71	5.34 ±0.89	
Location	p-value =0.02	F=5.62		
Species	*p-value* <0.001	F=37.23		

Table 9. Variation in number of epidermal cells (mean ± SD) per unit area on leaf surface of selected plant species growing at different location of roadside of Kathmandu valley. *p*- value and F value obtained through two way ANOVA. (N=10).

Plant species	Location			
	Less polluted (Control)	Moderately polluted	Heavily polluted	Mean (species)
Bougainvillea glabra	15.31 ±0.85	17.25 ±1.01	19 ± 1.01	17.18 ± 0.23
Callistemon citrinus	12.70± 2.05	14.13±2.00	14.55 ±2.50	13.79 ± 0.24
Euphorbia poinsettia	7.51 ±1.05	12.63 ±1.45	14.35 ± 3.01	11.34 ±0.23
Nerium oleander	26.4 ± 2.27	33.4 ± 3.21	37.1 ±2.54	32.3 ±0.32
Jasminum mesnyi	16.35 ±1.63	18.5 ±1.45	22.4 ± 1.34	19.08±0.23
Lagerstroemia indica	25.8 ±2.09	30.6 ±2.09	34.8 ± 2.78	30.4 ±0.24
Mean (location)	17.34±0.32	21.13±0.34	23.73± 0.32	
Location	p-value <0.001	F= 78.09		
Species	p-value <0.001	F=56.12		

Number of epidermal cells

There was significant difference ($p<0.001$) in number of epidermal cells per unit area in leaf surface among the species at different location (Table 9). Number of epidermal cells among the plant species ranged from 11.34 to 30.4. The maximum number of epidermal cell was recorded in *Lagerstroemia indica* (30.4) and minimum in *Euphorbia poinsettia* (11.34). The number of epidermal cells varied significantly ($p<0.001$) in the samples from different location; with higher number (23.73) at highly polluted site and less number (17.34) at control (less polluted) site (Table 9).

Discussion

There was significant effect of pollution on leaf length, width and area. Among six species maximum reduction in leaf area (28%) was recorded in Lagerstroemia indica (Fig. 6). The reduction in leaf area of selected species followed the order of Lagerstroemia indica >Euphorbia poinsettia> Jasminum mesnyi > Bougainvillea glabra > Nerium oleander> Callistemon citrinus . The reduction % in leaf size ascribed to the tolerance and sensitiveness of the individual species. The reduction in leaf size of plants growing on more polluted site might be due to adverse effect of vehicular pollution on the foliar morphology.The reduction of plant leaves characteristics length, width and area as one of plant adaptation mechanisms to survive under severe environmental stress as exhausts air pollution (Efe & Ozbay 2000) . As well as this reduction in leaf size may be due to the air pollution effect on leaf growth and elongation (Pourkhabbaz & Rastin. 2010). Moreover reduction in leaf size at roadside polluted site might be due to the pollutants like SO_2 and NO_x in ambient air emitted by high automobile activity. Reduction percentage of leaf length, width and area at heavily polluted site as compared to less-polluted site might be due to effect of air pollution at that site which reduces the gases exchange for photosynthesis and productivity of leaf. Leaves area, length and width reducing may lead to reduced contact between air pollutants and plant leaves and thus protect the plant from pollution (Assadi et al. 2011). This decrease in morphological properties is explained as one of plant leaves resistance ways to air pollutants and maintain the water balance of plant leaf tissues by reducing leaf area; plant leaves area decreases with more plant leaves the stress and little water leaves loss and increases plant resistance (Seyyednejad et al. 2009).

Similar observation was also made by Bhatti & Iqbal (1988). They found significant decline in leaf size of Ficus bengalensis at the polluted sites. More over other workers (Iqbal & Shafiq 1999; Shafiq & Iqbal 2003, 2005; Al-Obaidy et al. 2019) also reported that plants growing adjacent to roadsides exhibited considerable damage in response to automobile exhaust emission. They also reported that atmospheric pollutants after making their entry through stomata of leaf causes reduction in leaf size of plants due to damage of photosynthetic tissues;since plant growth and production depend on phyotosynthetically functional leaf size of the species. Reduction in dimension of leaf blade of several tree species in the vicinity of heavy dust and SO_2 pollution was also reported by Jahan & Iqbal (1992) ; Syed et al. (2008). The reduced leaf area results in reduced absorption of radiations and subsequently in reduced photosynthesis (Tiwari et al. 2006). Reduction in leaf area under polluted environment has been also reported by Dubey et al. (1984), Dineva (2004) ; Tiwari et al. (2006); Jahan & Iqbal (1992) reported changes in area of leaflets and length of petiole of G officinale in polluted air. Preeti (2000) also observed that leaves of Thevetia nerifolia and Cassia siamea growing in the polluted environment showed significant reduction in their growth.

The present study results show a decrease in the stomatal density and stomatal index in most plant leaves when compared between polluted and less polluted regions. While number of clogged stomata in leaves showed an increase in polluted regions compared with less polluted region. This variation in results may be due to plant response degree to air pollutants and also their differences between studied species.The decrease in stomatal density and stomatal index in plant leaves may be due to reduced leaf area (Abdulmoniem 2011). These changes explained that urban environmental conditions have an effect on plant leaf morphological and anatomical characteristics. And also considered as a plant resisting means to drought or air pollutants because of low leaf area, low stomata number and low water loss rate by transpiration and low plant leaf gas exchange rate and thus reduced polluting gases penetration rate into leaf (Pourkhabbaz & Rastin 2010). Stomatal index can be considered as one of good anatomical adaptations shown by the plant to air pollution (Ogunkunle et al. 2013). 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 (Verma et al. 2006).

The stomata were slightly raised from rest of the cells, were often filled with dust particles and at some place were also clogged. The number of clogged stomata and epidermal cells of leaves of plant species also varied significantly among different location. The value of number of clogged stomata and epidermal cells was found higher on the leaves of the selected plants growing at highly polluted site as compared to the less polluted (control) site. The fine particles clog the stomata, affecting the gaseous exchange process and in turn affecting photosynthesis, water retention, respiration, and overall growth of plants. As the roadside plants covered with dust also suffered from water loss, water deficiency and any change in the original morphological structure make those plants more sensitive to water loss (Saneoka & Ogata 1987).

Conclusion

It is clear from this study that the foliar morphology and anatomy of selected plant species are affected by air pollution and that these effects are species specific. The differences observed in the morphological features occurred in all the foliar parameters investigated across the three locations and between the two seasons. This shows that the effect do not occur by chance, rather it is due to the varying levels of pollutants concentrations in the three locations. Thus, the study of leaves morphological and anatomical characteristics gives a more reliable description of environmental effects on the plant. The plant's response to air pollution is different, so it is possible to count plants as quantitative and qualitative indices for air pollution.It will also help to better our understanding of the impact of pollution on foliar characters as well as serving as a basis of future studies in this area.

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