ASSESSMENT OF AIR POLLUTION IMPACT ON MICRO-MORPHOLOGICAL AND BIOCHEMICAL PROPERTIES OF *CALLISTEMON CITRINUS* (CURTIS) SKEELS AND *LAGERSTROEMIA INDICA* L.

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Abstract: The urban air pollution is a major environmental concern, particularly in the developing countries and in their major cities. In the present study an attempt has been made to assess the air pollution effect on micromorphological and biochemical parameters of *Callistemon citrinus* and *Lagerstroemia indica*. Biochemical parameters were analyzed by using standard protocol. one-way analysis of variance was performed with SPSS software, v. 20.0, and Duncan test ($p \leq 0.05$) was used to compare averages of all measured biochemical parameters and micromorphologlcal features. There was a decrease in number of stomata of both species at highly polluted site compared to less polluted site (control). Stomatal index of both species was found to be reduced in polluted site when compared to control. The number of clogged stomata was less in control area samples when compared to polluted sample. A number of epidermal cells were significantly decreased at heavily polluted site. Total chlorophyll content and leaf extract pH in both the plants were found to be significantly higher in control sites than in heavily polluted sites. Ascorbic acid, relative water content, and Air Pollution Tolerance Index (ATPI) was found to be significantly higher at more polluted plants than at control. Based on the present study results , *Callistemon citrinus* emerged as the most tolerant species with the highest APTI. Hence, *Callistemon citrinus* can be suggested for plantations along the roadside of urban areas for green belt development.
Keywords: APTI; Ascorbic acid; Clogged stomata; Chlorophyll content and leaf extract pH.

INTRODUCTION

Industrialization, the growth in number of vehicles in an urban area and the burning of biofuels have led to the rapid deterioration of air quality. In urban areas the transport sector causes the most pollution, producing 74% of the $C0_2$ and all the lead emitted. The number of vehicles in Kathmandu Valley has been increasing leading to increase in pollution and plants being static i.e. immobile are therefore more exposed to dangers of road side due to intense vehicular traffic emission and their leaves are in constant exchange of gases with the atmosphere through transpiration by stomata[1]. Air pollution stress leads to stomatal closure, which reduces carbon dioxide availability to leaves and inhibits carbon fixation. The road side plants play a significant role in assimilation and accumulation of pollutants and act as efficient interceptors of airborne pollutants. Pollutants can also cause leaf injury, stomatal damage, decrease photosynthetic rate, disturb membrane permeability and reduce growth and yield in pollution sensitive plant species[1]. Several studies show that under polluted conditions, plants develop different morphological, physiological and anatomical changes [2-4].Modification of epidermal trait could be indicative of environmental pollution and could, therefore be used as bio-indicator of air pollution [5]. Adverse impacts of urban air pollution on leaf structure of plants have been studied by various workers [4, 6, 7]. Vehicular exhausts are the main source of pollution in ambient atmosphere of the Kathmandu Valley. This study was undertaken to assess the changes caused by vehicular air pollution on number and size of stomata, and epidermal cells in the leaves of a roadside shrub species, *Callistemon citrinus* and *Lagerstroemia indica.* This study has been carried out along roadside of Kathmandu Valley during 2017.

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Received: 25 Dec 2020; First Review: 2 Jan 2021; Second Review: 5 Jan 2021; Accepted: 7 Jan 2021.
Doi: https://doi.org/10.3126/sw.v14i14.35024

MATERIALS AND METHODS

Study area

The present study was conducted in Kathmandu Valley (27^0 37'30" and 27^0 45'0" N latitude, and 85^0 15'0" and 85^0 22'30" E longitude) in central Nepal (Fig.1). It is about 1400 m above mean sea level, surrounded by high mountains, such as Phulchowki Hill (3132 m) in the south-east, Shivapuri (2713 m) in the north, Champa Devi (2400 m) in the south-west and Nagarjun (2100 m) in the north-west. Due to this, the valley has a unique bowl-shaped topographic structure where the air pollutants become trapped and accumulated without dilution by vertical dispersion [8]. Accumulation of total suspended particulate matters (TSPMs) has been a major problem along roadsides of Kathmandu Valley.

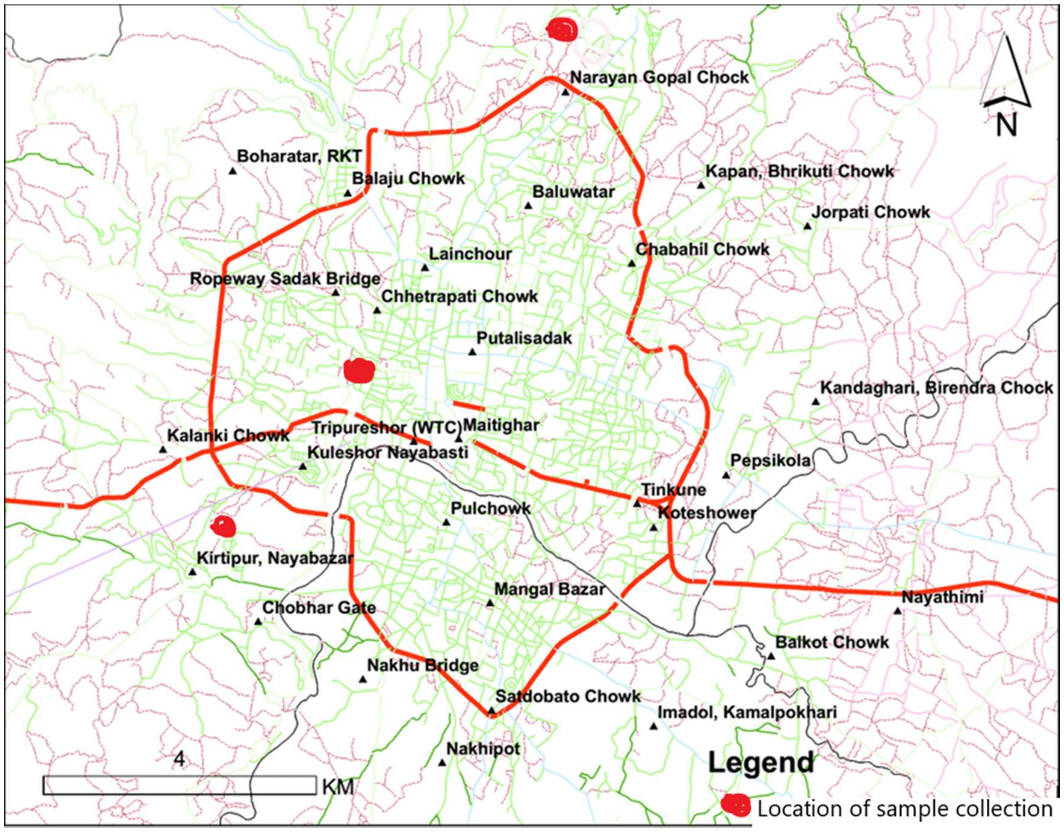

Figure 1: Map showing location of sample collection site

Assessment of Ambient Air Quality of the sampling sites

Assessment of Ambient Air Quality of study sites was carried out by hiring the expertise and instrumentation of Water Engineering & Training Centre P. Ltd., (WETC) Ratopul, Kathmandu. Ambient air quality and noise quality monitoring were carried out by monitoring three different parameters *viz.* particulate matter less than 10 Micron (PM_{10}), particulate matter less than 2.5 micron ($PM_{2.5}$), and total suspended particulate matters of ambient air of study sites were measured for categorization of study sites.

Three sites, Ratanapark, Budanilkantha, and Tribhuvan University Campus area in Kirtipur were selected for plant sampling (Table 1). Based on the presence of particulate matters, the study sites were categorized as heavily polluted, moderately polluted and less polluted (Table 2). The less polluted site was considered as control.

Table 1. Characteristics of study sites.

S.N.	Study site	Characteristics
Site 1	Ratnapark	Crowded, Bus Park area, high flow of vehicles
Site 2	Budanilkantha	Relatively Less flow of vehicle, Residential Area
Site 3	Kirtipur (Tribhuvan University Campus area)	University area (within Coronation Garden), less flow of vehicles

Table 2. Ambient air quality of study sites (24 hours average data, May 2018).

Study site	TSPMs ($\mu g/m^3$)	PM2.5 ($\mu g/m^{3)}$)	PM10($\mu g/m^{3)}$)
Ratnapark (heavily polluted)	1107	105	454
Budanilkantha (Moderately polluted)	321	23	149
Kirtipur (least polluted; control)	248	13	193

Collection of plant materials

Leaves of *Callistemon citrinus* (Curtis) Skeels and *Lagerstroemia indica* L were collected from three different location of Kathmandu valley. In order to study the impact of air pollutants generated by vehicular activities on micro morphological and physiological characteristics viz. total chlorophyll content, ascorbic acid content, relative water content of leaves , and leaf extract pH of plants, the horizontal distance of 0-10m were selected from both sides of the road. The plants growing at selected sites on both sides of the road at isoecological sites having approximately same height and canopy were considered to maintain the uniformity. Five leaves from each of the three selected individual at each location were collected for study. Fully mature leaves were collected in the morning hours (8 to 10 AM) at breast height (ca.1.3m). The collected leaf samples were then transported to the laboratory in zipper plastic and washed with tap water and then with 0.1N HCl followed by washing with distilled water.

Micromorphological studies

Leaf sample collected from study sites were processed for micro morphological studies following the method of Ahmad and Yunus [9]. The collected leaf samples were cut into small bits and taken in separate test tubes. 30% of Nitric acid solution was added to each test tube. Then it was boiled in a boiling water bath for 5–10 minutes. Then 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 glycerin jelly. The following micro-morphological parameters were studied which includes stomatal density, stomatal index, number of epidermal cells, number of clogged stomata and size of the stomatal aperture.

Biochemical analysis

The fresh leaf samples were analyzed for total chlorophyll, ascorbic acid, leaf extract pH, and relative water content for determining Air Pollution Tolerance Index (APTI) by following standard procedure.

Relative water content (% RWC) of the leaf was determined using the method of Barr and Weatherley [10]as follows:

Relative water content (RWC) (%) = $\frac{FW-}{TW-D} x100$

where, FW is the fresh weight, DW is the dry weight of turgid leaves after oven-drying at 115°C for 2 h, and TW is the turgid weight after immersion in water overnight.

Total chlorophyll content was determined using the spectrophotometric method[11] and leaf extract pH was determined using a glass electrode pH meter (PHS-3C model) by homogenizing 2.5 g of the fresh leaf sample in 10 ml distilled water; the pH was determined after pH calibration with a buffer at 4 and 9.

Ascorbic acid (AA) content of leaves was determined using the spectrophotometric method[12]. Four milliliters of oxalic acid-EDTA, 1 ml of orthophosphoric acid, 1 ml of 5 % tetraoxosulphate (VI) acid, 2 ml of ammonium molybdate and 3 ml of water were used as extractants for 1 g of the fresh leaves in a test tube. The solution was allowed to stand for 15 min and the absorbance read at 760 nm. The concentration of ascorbic acid in the sample was then extrapolated from a standard ascorbic acid curve.

The APTI proposed by Singh and Rao [13] was determined by the formula:

$$\frac{\mathrm{APTI} = [\mathrm{A}(\mathrm{T}+\mathrm{P})] + \mathrm{R}}{10}$$

where A is the ascorbic acid content (mg/g), T is the total chlorophyll (mg/g),

P is leaf extract pH of leaf sample,

R is relative water content (%) of leaf sample.

Statistical Analysis

A one-way analysis of variance was performed with SPSS software, v.20.0, and Duncan test ($p \leq 0.05$) was used to compare averages of all measured parameters.

RESULTS

Air pollution directly affects the micro morphological parameters of the leaf (Table 3). The present study showed that air pollution causes significant changes in leaf micromorphology of two plant species *Callistemon citrinus* (Curtis) Skeels and *Lagerstroemia indica* L growing at heavily polluted sites in Kathmandu Valley when compared with the same plant species growing at less polluted site. There was a decrease in stomatal density in both plant species *at* heavily polluted site compared to less polluted site . Stomatal density of *C. citrinus* was 133. 7 per mm^2 in less polluted site which was reduced to 123.1 per mm^2 in heavily pollued site. Likewise stomatal density of *L. indica* was 130 per mm^2 at less polluted sited and it was reduced to 128 per mm^2 at heavily polluted site. Stomatal index is an important parameter to determine the physiological changes in the plant due to air pollution. Stomatal index of both species is reduced in polluted site. Stomatal index of *Callistemon citrinus.* in less polluted site was 16.90, it has been reduced to 14.0 in the heavily polluted site. Similarly in *Lagerstroemia indica* stomatal index was 21.1 at less polluted site (control) and 16.70 at heavily polluted site. In both the species clogged stomata (Table 3) were observed, number of clogged stomata were less in less polluted (control area) site samples when compared to heavily polluted sample.

Stomatal pore size showed a significant difference in both the species. The length and breadth of stomatal pore and epidermal cell number were decreased in polluted areas of *C. citrinus* and *L indica* compared to control plants. In *C. citrinus* the stomatal pore length and breadth decreased from 3.85 to 2.92 and 1.61 to 1.45 from less polluted site to heavily polluted site respectively likewise, in *L. indica* the stomatal pore length and breadth decreased from 5.45 to 4.02 and 3.61 1to.72 respectively, The number of epidermal cells in *C. citrinus* decreased from14.55 to 12.40 at heavily polluted site to less polluted site respectively, while in *L. indica*, the number of epidermal cells at heavily polluted site to less polluted site decreased from 34.8 to 25.8. The subsidiary cells were found to be decreased in number in both the species growing at heavily polluted area (6.74 and 6.24) compared to less polluted area (10.8 and 8.61) (Table 3).

Biochemical parameter: total chlorophyll, ascorbic acid content, relative water content and pH are presented in Table 4. Chlorophyll Content: There was significant difference in total chlorophyll content in both species. Further, the variations in total chlorophyll content of plant species varied significantly with the pollution status of the area with higher value at less polluted site and least at heavily polluted site.

Ascorbic acid content: Ascorbic acid content in both the plant species of control and polluted area are presented in Table 4. In both species ascorbic acid content was increased at heavily polluted site in comparison to less polluted site (control). In *C. citrinus* there was increase in the ascorbic acid content of heavily polluted site (4.36 mg g^{-1}) sample compared to the less polluted site (3.93 mg g^{-1}) likewise in *L. indica* increase of the ascorbic acid content was observed at heavily polluted area (1.36 mg g^{-1}) when compared to less polluted (1.13 mg g^{-1}) area.

Table 3. Effect of air pollution on the micromorphological structures present on the leaves of Callistemon citrinus and Lagerstroemia indica. The data were expressed as mean±SD and statistical analysis using one-way ANOVA. Significant difference between mean values for each species among different pollution levels are indicated by different superscript letters (Duncan multiple comparison test, p< 0.05).

Plant Species	Sites	Stomatal density (per $mm^{2)}$	Stomatal index (%)	Number of clogged stomata	Number of epidermal cells (mean ± SD) per unit area	Stomatal pore size length	Stomatal pore size breadth	Number of Subsidiary cells
Callistemon citrinus	Less polluted	$133.7±1.15^b$	**$16.90±1.0^c$**	$1.09 ±0.14^a$	$14.55 ±2.50^b$	$3.85±0.13^b$	$1.61±0.12^b$	$10.8±1.21^c$
	Moderately polluted	$126.0±1.13^a$	$15.40±1.02^b$	$1.6 ±0.42^b$	$14.13±2.00^b$	$3.18±0.06^b$	$1.60±0.11^b$	$9.40±2.14^b$
	Heavily Polluted	$123.1±1.12^a$	$14.0±0.45^a$	$1.65 ±0.23^b$	$12.70± 2.05^a$	$2.92±0.2^a$	$1.45±0.30^a$	$6.74±1.13^a$
Lagerstroemia indica	Less polluted	$130.3±1.05^b$	$21.10±1.23^c$	$3.4 ±0.96^a$	$34.8 ± 2.78^c$	$5.45±0.13^b$	$3.61±0.10^b$	$8.61±1.42^c$
	Moderately polluted	$131.7±1.3^c$	$19.50±2.0^b$	$4.15 ±0.34^b$	$30.6 ±2.09^b$	$4.98±0.06^a$	$1.76±0.2^a$	$7.80±2.10^b$
	Heavily Polluted	$128.1±1.12^a$	$16.70±1.02^a$	$4.35 ±0.87^b$	$25.8 ±2.09^a$	$4.02±0.2^a$	$1.72±0.12^a$	$6.24±1.54^a$

Table 4. Comparative data of foliar biochemical parameters and Air pollution tolerance index (APTI) of selected plants collected from the different sites. The data were expressed as mean±SD and statistical analysis using one-way ANOVA.. Significant difference between mean values for each species among different pollution levels are indicated by different superscript letters (Duncan multiple comparison test, p< 0.05).

Plant Species	Sites	Leaf chlorophyll content (mg g-1)	Leaf extract pH (mean± SD)	Relative water content (%) (mean±SD)	Leaf ascorbic acid content (%) (mean± SD	Air Pollution Tolerance Index (APTI
Callistemon citrinus	Less polluted (Control)	2.52±0.02b	6.73±0.12b	89.30±1.32a	3.93±0.102a	12.43±0.63b
	Moderately polluted	1.92±0.01a	6.34±0.04b	91.18±4.31b	3.76±0.02a	10.46±0.14a
	Heavily Polluted	1.21±0.03a	5.42 ±0.13a	91.36±2.13b	4.36 ±0.02b	11.64±0.23a
Lagerstroemia indica	Less polluted (Control)	2.61±0.0b	6.71±0.11b	77.76±2.43b	1.13±0.002a	8.78±0.23a
	Moderately polluted	2.52±0.03ab	5.26±0.04a	74.61±3.45a	1.17±0.03a	8.37±1.02a
	Heavily Polluted	2.03±0.11a	5.11±0.03a	79.17±3.42c	1.36±0.02b	8.87±0.15a

Leaf extract pH: The pH value of both the plants of polluted and control sites are found to be significantly different (Table 4). In *Callistemon citrinus* pH value was decreased in the polluted sample (5.42) compared to control sample (6.73) likewise in *Lagerstroemia indica* also pH value was decreased in the polluted sample (5.11) compared to control (6.71) respectively.

Relative water content: The relative water content in both the plants differed considerably (Table 2). Relative water content was increased in polluted (91.36 %) sample of *C. citrinus* compared to control (89.30%) site likewise in *L. indica* relative water content was increased in polluted samples (79.17%) as compared to that of control (77.76%) samples.

Air pollution tolerance index: *Callistemon citrinus* showed APTI values of 10.46 and 12.43 in less polluted and heavily polluted sample respectively and *Lagerstroemia indica* did not show significant difference among the plants of polluted (8.78) and control (8.87) area.

DISCUSSION

The response of leaf characters to air pollution indicates the adverse effect of air pollution, which can be used as bioindicator. Both plant species *Callistemon citrinus* and *Lagerstroemia indica* in the present study showed micromorphological changes in leaf characters . The stomatal index was found to be decreased in both the plant species under heavily polluted sites as compared to less polluted site (control site). Vehicular pollution is known to affect the stomatal index and it has been reported to decrease in some plants [3], [7]. Verma *et al.* [14] found significant reduction in the stomatal index of *Ipomoea pestigridis* to be a response to environmental stress (coalsmoke pollution). Chauhan *et al.* [15] also suggested 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. The length and breadth of stomata and number of epidermal cells in two plant species decreased in polluted plants when compared to control areas plants, which may be helpful in preventing the entry of the pollutants into the leaves, that can otherwise cause injury and death of the tissues of leaves [16].The increase or decrease of epidermal cells might be to accommodate the decrease or increase of the stomata. Similar reduction in stomatal size and epidermal cells at polluted sites of urban areas as compared to that at reference site is observed by various workers [17-19]. This reduction in stomata size could be considered as an adaptive response of these plants to avoid entry of harmful constituents of vehicle exhaust which can otherwise cause adverse effects([20,21]). Distorted shapes of stomata observed in leaves of studied plants of present investigation might have resulted due to lowering of pH in cytoplasm of guard cells and thus a change in turgor relations of the stomata complex [22] and due to physiological injury within leaf [23]. Gaseous pollutants enter the leaves through stomata following the same diffusion pathway as CO_2. This may be an adaptation of these plants to increase the area for proper gas exchange, as suggested by Raina and Bala [24] and also by various other workers [19,25] .

Clogging of stomata is also affected by air pollution. The number of clogged stomata was more in polluted site compared to control site in both species. 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 [26].

Chlorophyll content of the plants signifies its photosynthetic capacity along with its growth and development. According to Achakzai *et al.* [27] degradation of photosynthetic pigments was widely used as an indicator of pollution stress. It is well evident that chlorophyll content varies with tolerance as well as the plant species' sensitivity. In other words, the higher the sensitive nature of the plant to pollution stress, the lower the chlorophyll content[28]. Reduction in the concentration of chlorophyll content in leaves of the polluted area was observed in both the plant species. Similar changes in the concentration of pigments were also observed in leaves of six tree species exposed to air pollution due to vehicle emission by Joshi and Swami [29]. Leaves from the polluted area had significantly lower chlorophyll content than control [30], [31]. According toElloumi *et al.* [32], the decrease of chlorophyll content in *C. citrinus* and *L. indica* , exposed to air pollution stress, could be due to: (a) the inhibition of important enzymes, such as δ-aminolevulinic acid dehydratase and protochlorophyllide reductase, involved in chlorophyll biosynthesis; (b) Mg and Zn deficiency, required for synthesis of chlorophylls; and/or (c) deposition of dust on the leaf, which adversely affects metabolic activity. Furthermore, substitution of the central Mg in chlorophyll with fluoride was an important cause of chlorophyll damage in plants, growing in heavy-metal-contaminated environments [33]. This substitution may prevent the capture of photosynthetic light, resulting in a breakdown of photosynthesis in common fig and white Mulberry plants [33].

In the present investigation increase in ascorbic acid content in both studied species in polluted site gives an idea about variation in the ascorbic acid content under air pollution stress and their tolerance to air pollution. According to Zhang *et al.* [34] ascorbic acid imparts tolerance to air pollution in plants, since it activates many physiological and defense mechanisms. It can act as: (i) an electron donator to various enzymatic and non-enzymatic reactions, (ii) a reducing agent, and (iii) an antioxidant. Esfahani *et al.* [35]observed that the reducing power of ascorbic acid depends directly on its concentration; therefore, plants that maintain high ascorbic acid levels even under polluted conditions are considered tolerant. Increase in ascorbic concentration with respect to the control leaves was also reported by Jyothi and Jaya[36].

The pH plays a significant role in plants' physiological processes. Most of the enzymes, involved in biological activities of the organism, require relatively high pH for their effective functions. Consequently, plants with relatively low pH are more susceptible, while those whose pH is around 7 are tolerant [27]. The leaf pH values decreased in both plant species in the polluted area compared with that of control. Similarly decreased pH values in plants growing at the polluted site were also observed by Patel and Kousar [37].

Leaf Relative Water Content (LRWC) is a useful indicator of plant's water status. Under stressful conditions, a large quantity of water in plant tissue helps maintaining its physiological balance [29]. The increased relative water content in both plant species was observed in polluted sites. Similar increased relative water content was observed by [Patel] and Kousar[37]. According to Rai and Panda[28] high LRWC under stress condition is an indication of plant species' tolerance. Variation in four physiological and biochemical aspects (LRWC, chlorophyll content, pH, and ascorbic acid content) of plant species resulted in variations of APTI values. Air Pollution Tolerance Index (APTI) has been used as a more precise parameter for landscapers to identify and select both tolerant and sensitive plant species to air pollution [38]. The sensitive species can serve as biological indicators for air pollution, whereas, the tolerant ones are considered as sink, able to be used to combat pollutants' level in that specific environment[30],[39]. Among two plant species investigated for APTI *Callistemon* showed more tolerance to air pollution than *L. indica.* Different plant species shows considerable variation in their susceptibility to air pollution. The plants with high and low APTI can serve as tolerant and sensitive species respectively. Also, the sensitivity levels of plants to air pollutants differ in from shrubs and trees with identical values, a tree may be sensitive but a shrub may be tolerant to given pollutant. Therefore, the indices for different plant types should be considered separately. According to Zhang *et al.* [34], not only do tolerant plants help to mitigate air pollution, they also maintain the ecological balance, controlling soil erosion and improving aesthetic aspects of such polluted areas.

CONCLUSION

In the present study an attempt has been made to assess the air pollution effect on micromorphological and biochemical parameters of *Callistemon citrinus* and *Lagerstroemia indica*. There was a decrease in number of stomata of both species at highly polluted site compared to less polluted site (control). Stomatal index of both species was found to be reduced in polluted site when compared to control. The number of clogged stomata was less in control area samples when compared to polluted sample. A number of epidermal cells were significantly decreased at heavily polluted site. Total chlorophyll content and leaf extract pH in both the plants were found to be significantly higher in control sites than in heavily polluted sites. Ascorbic acid, relative water content, and Air Pollution Tolerance index was found to be significantly higher at more polluted plants than at control.

Marked alterations were observed both in the physiological status and in the foliar surface ultrastructural configuration of both *Callistemon citrinus* and *Lagerstroemia indica* plants growing at highly polluted site in comparison to less polluted site. Therefore, these plant species may be used as biomarkers and mitigators of pollutants coming out of the automobile exhaust. Significant changes were recorded in *C. citrinus* in comparison to *L. indica* in the studied parameters. It was noticed from the above result that *C. citrinus* was found to be more tolerant compared to the *L. indica*.

ACKNOWLEDGEMENT

We are thankful to Prof Mohan Siwakoti, former head of Central Department of Botany, TU for providing laboratory facility. This work was supported by the University Grants Commission, Kathmandu, Nepal.

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