Octa Journal of Environmental Research *Jan. – Mar., 2021*
International Peer-Reviewed Journal ISSN 2321 3655
Oct. Jour. Env. Res. Vol. 9(1): 001-012
Available online http://www.sciencebeingjournal.com

Research Article

Octa Journal of Environmental Research
(Oct. Jour. Env. Res.) ISSN: 2321-3655
Journal Homepage: http://www.sciencebeingjournal.com

COMPARATIVE ASSESSMENT OF BIOCHEMICAL CHARACTERISTICS OF PLANTS FROM POLLUTED AND NON-POLLUTED REGIONS OF URBAN AREAS

Sarita Sapkota and Anjana Devkota*
Central Department of Botany, Tribhuvan University, Kathmandu, Nepal
Corresponding Author's Email: **devkotaa@gmail.com**
Received: 24th Jan. 2021 **Revised:** 16th Feb. 2021 **Accepted:** 24th Mar. 2021

Abstract: Air pollution is major threat to urban areas. Aerial parts of pants mainly leaf surface acts as a sink for deposition of air pollutants in the urban and industrial environment and is considered as ecologically sustainable cost-effective strategies to mitigate adverse effects of air pollution. In this condition, assessment of biochemical characteristics total chlorophyll content, leaf extract pH, ascorbic acid content and relative water content and computed air pollution tolerance index. Air pollution tolerance index (APTI) offers simple tool for screening and identifying plants that tolerate air pollution. Five plants commonly growing in the roadside of Lalitpur district were selected for assessing the biochemical characteristics and air pollution tolerance response. There were significance differences in biochemical characteristics between polluted and non-polluted sites. APTI indices of *Buddleja asiatica* Lour., *Ficus religiosa* L., *Lecosceptrum canum* Sm, *Tecoma stans* (L.) Juss. ex Kunth. and *Nyctanthes arbor-tristis* L. were assessed and compared with the plants grown in non-polluted sites of Lalitpur to analyze the response to air pollution. Among the plants evaluated, high APTI of >11 was recorded in *F. religiosa* indicating that the plant is tolerant to air pollutants at the collected sites. *B. asiatica* recorded lowest APTI of 8.30, indicating that this plant is sensitive to air pollutants and can be used for biomonitoring the air pollution.

Keywords: Total Chlorophyll Content, Relative water content, Ascorbic acid content, Leaf extract pH

Postal Address: Central Department of Botany, Tribhuvan University, Kathmandu, Nepal

INTRODUCTION

Atmospheric pollution has emerged as one of the leading problems that have resulted from the rising human population and the industrialization (Odilara et al., 2006). Air pollutants including the particulate matter (PM), the vehicular exhausts as well as the industrial emissions result in deteriorating health effects in humans, create disturbances in plant ecosystem in addition to the global estimates, that air pollution results in around a million premature infant deaths around the world (Litchfield et al., 2018). Out of several mitigation measures adopted to reduce the impact of air pollution, one such mitigation measure and management of air pollution is the large scale plantation of trees and other plants in polluted areas as well as in degraded lands. Aerial parts of plants provide sufficient area for absorption and accumulation of air pollutants to reduce the pollution level in the environment (Escobedo et al., 2008). Leaf surface of plants acts as a descend for deposition of air pollutants in the urban and industrial environment. Trees act as a natural sink, removing a significant amount of toxic air pollutants (Shannigrahi et al., 2003). It is reported that 1261.4 tons of air pollutants were removed annually by urban trees in Beijing (Yang et al., 2005). Tolerance capacity of plants to air pollution and particulate matters also varied; which also considered as eco-

sustainable tool for air pollution mitigation (Prabhat, 2016). Sensitivity and tolerance capacity of plants to air pollution varied species to species Screening and identification of plants that are adaptive to the native environment of polluted sites provide ecological restoration strategies to mitigate the impact of air pollution. Air Pollution Tolerance Index (APTI) is an inherent quality of plants to encounter air pollution stress. Owing to the contributions of parameters like ascorbic acid, the chlorophyll content, relative water contents as well as the leaf-extract pH in the pollution tolerance in plants, these parameters were computed together in a formulation to obtain an empirical value signifying the air pollution tolerance index (APTI) of species (Singh and Rao,1983; Aghaiee et al., 2019; Bellini and Tullio, 2019; Manjunath and Reddy, 2019).

To measure the air pollution tolerance indices of different plant species has become necessary owing to the increasing air pollution in urban localities. The categorization of pants species as sensitive and tolerant has great importance as the susceptible plant species can be used to serve as indicators while as the tolerant ones can work as sinks to monitor the air pollution in urban areas (Singh et al., 1999; Aghaiee et al., 2019). World-Wide significant work has been done to identify the plants suitable for phytomonitoring the vehicular pollution but nothing significant has been done to see the phytomonitoring capacity of urban areas. So this research aims to access biochemical characteristics and screening the Air Pollution Tolerance of common flora of urban areas (Lalitpur area) to know about the susceptible and tolerant plant species.

EXPERIMENTAL

Study Area: The present research was carried out in Lalitpur District in Nepal**.** The district, with Lalitpur as its district headquarters, covers an area of 385 km² (149 sq m). The study area (27.5420° N, 85.3343° E) is one the elevated tract of land in Kathmandu valley on the southern side of Bagmati River, which separates it from the city of Kathmandu on the northern and western side. For evaluation of air pollution tolerant index of plant species, plant samples on the roadside of two locations viz. Godawari and Lagankhel of Lalitpur districts were selected for the comparative study (Table 1). On the basis of particulate matters study site were categorized as polluted (Lagankhel) and non-polluted (control) (Godawari) (Table 2).

Selection of plant species: Plants were selected because of their religious purpose, common occurrence, easy accessibility and abundance at selected sites of the study. The physiology and botanical characters of plants under study are given in Table 3.

Table 1. Site Description

Location	PM 2.5 (µg/m^3)	PM 10 (µg/m^3)	TSPM (µg/m^3)
Lagankhel (Polluted)	195	362	838
Godawari Herbarium (non-polluted) (Control)	21	72	115

Table 2. Ambient air quality of study sites (24 hours' average data May 2017, Source: GoN 2017).

S.No.	Site name	Characteristics
Site 1	Lagankhel	Dusty Crowded, bus park area, flow of vehicles high
Site 2	Godawari garden area	Garden area, less flow of vehicles

Table 3. Botanical characters of Selected plants

Botanical name	Family	Common name	Uses
Buddleja asiatica Lour.,	Scrophulariaceae /Loganiaceae	Dhursilo, Bhimsen pati	Ornamental, religious and medicinal purpose
Ficus religiosa L.,	Moraceae	Peepal	Medicine, edible, ornamental,fodder and religious tree
Leucosceptrum canum Sm.,	Lamiaceae	Bhusure	Ornamental,folk medicine

Nyctanthes arbor-tristis L	Oleaceae	Parijat, Night flowering jasmine	Ornamental, medicinal and religious tree,fooder
Tecoma stans (L.) Juss. ex Kunth	Bigoniaceae	Yellow trumpet bush, yellow bells	Medicinal and ornamental

Collection of plant materials: Leaves of selected plants species were collected from polluted (Lagankhel) and non-polluted (control) (Godawari) sites of Lalitpur district. In order to study the impact of air pollutants generated by vehicular activities on physiological characteristics *viz*. total chlorophyll content, ascorbic acid content, relative water content of leaves and leaf extract pH of plants, the horizontal distance of 0-10 m were selected from both sides of the road. The plants growing at selected sites on both sides of the road at iso-ecological sites having approximately same height and canopy were considered to maintain the uniformity. Five leaves from each of the three selected individuals at each location were collected 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

Biochemical analysis: The fresh leaf samples were analyzed for total chlorophyll, ascorbic acid, leaf extract pH, and relative water content for determining APTI by following standard procedure. Relative water content (% RWC) of the leaf was determined using the method of Barr and Weatherley (1962) as follows:

Relative Water Content (RWC) (%)

$$= \frac{FW - DW \times 100}{TW - DW}$$

where, FW is the fresh weight, DW is the dry weight of turgid leaves after oven-drying at 115◦C for 24 h, and TW is the turgid weight after immersion in water overnight. Total chlorophyll content was determined using the spectrophotometric method (Arnon, (1949), 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 (Bajaj and Kaur 1981). 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 (1983) was determined by the formula:

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

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: Statistical analysis was carried out using software SPSS-17. Significance of difference of selected plants from polluted and non- polluted areas was measured in independent trials using Student's *t*-test. Difference in the plants' biochemical parameters under pollution effects were compared using ANOVA with means separation by Duncan's test at a significance level of $P \leq 0.05$. All data are means of fifteen replicates.

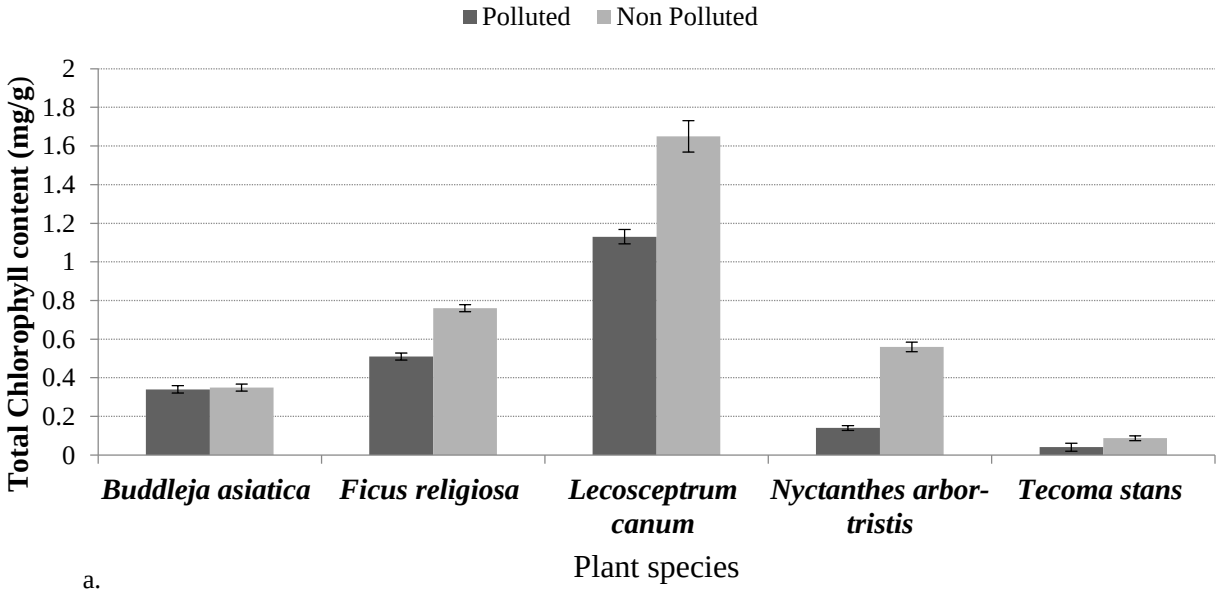
RESULTS AND DISCUSSION

Total Chlorophyll Content

Total chlorophyll content of selected plants from polluted (Lagankhel) and non -polluted (Godawari) areas is summarized in Figure 1a. There was significant different in total chlorophyll content ($p>0.001$) among plant species (Table 4).

The mean chlorophyll content of polluted plants was significantly reduced ($P < 0.01$) at 2.33 mg/g compared to 2.68 mg/g (Figure 1b). Highest and lowest total chlorophyll content of 3.65mg/g and 1.041mg/g was recorded in *Leucosceptrum canum* from non- polluted area and *Tecoma stans* from polluted area, respectively. Highest impact of air pollution on total chlorophyll content was recorded in *L. canum* where 14.24% reduction in chlorophyll was observed. In *L. canum* total chlorophyll content of 3.13 mg/g was observed in polluted compared to 3.65 mg/g observed in non – polluted area. Least impact of pollution on total chlorophyll was recorded in *Buddleja asiatica*; where only 2.60% reduction in chlorophyll was observed.

Similarly, in *Nyctanthes arbor-tristis* plant, total chlorophyll content of 2.14mg/g was observed in polluted compared to 256 µg/g observed in non- polluted area. In other plants from polluted area, 2.6–14.24% reduction in total chlorophyll content was noted. Total chlorophyll content is an important biochemical factor that impacts photosynthetic activity which determines the plant growth. Total chlorophyll content is considered frequently to evaluate the impact of air pollution on plants[9]. Several studies have reported reduction in total chlorophyll content in plants exposed to air pollutants. Effect of PM in polluted area on reduction of chlorophyll content at the enzyme level and correlated with increased activity of chlorophyllase enzyme activity was studied (Prajapati, 2012; Manjunath and Reddy,2019). Reduction in total chlorophyll content to reduce gaseous exchange due to blockage of stomatal opening in response to air pollutants was analyzed (Leghari and Zaidi, 2013). The study on inverse relationship of total chlorophyll content with dust accumulation on leaves in polluted environment was reported (Manjunath and Reddy, 2019). In our study reduction in total chlorophyll content in samples of polluted site could be due to heavy load of dust in study area (Table 2). Further, detailed correlation analysis on influence of various physicochemical factors on photosynthetic pigments synthesis and activity should help in identifying adaptive plants with sustained growth in polluted environments.



a.

Figure 1a. Total chlorophyll content of selected plants from polluted and non- polluted area

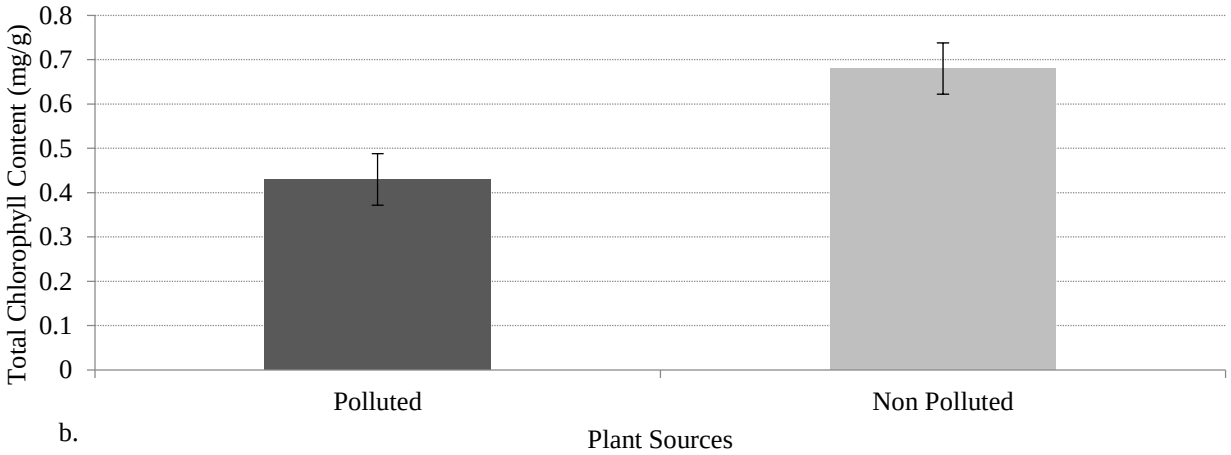


Figure 1b. Total chlorophyll content of selected plants from polluted and non- polluted area

Table.3. Air Pollution Tolerance Index (APTI) of selected plants from polluted (Lagankhel) and non-polluted area (Godawari) ((n=15) (mean ±SD)

Plant	AA		pH		RWC		Total Chl		APTI	
	P	NP	P	NP	P	NP	P	NP	P	NP
Buddleja asiatica	1.39±0.02	0.85±0.12	6.73±0.11	6.21±0.13	70.62±4.76	61.21±8.25	2.24±0.019	2.35±0.018	8.30±0.45	6.84±0.24
Ficus religiosa	2.69±0.50	2.34±0.036	7.73±0.17	6.52±0.13	89.09±1.96	90.15±1.11	2.51±0.018	2.76±0.018	11.66±0.52	10.250.12
Leucosceptrum canum	1.42±0.019	1.36±0.050	6.14±0.088	6.65±0.074	79.80±7.90	63.79±4.99	3.13±0.037	3.65±0.081	9.29±0.79	8.47±0.48
Tecoma stans	2.36±0.33	2.34±0.715	7.15±0.097	6.96±0.13	74.62±2.45	71.62±2.99	1.041±0.001	1.087±0.03	9.39±0.37	9.04±0.72
Nyctanthes arbor-tristis	*1.60±0.04*	1.04±0.07	6.07±0.087	7.07±0.06	76.51±2.45	75.25±2.44	2.14±0.013	2.56±0.025	8.96±0.25	8.57±0.25
Mean	1.89±0.14	1.35±0.16	6.98±0.093	6.48±0.068	78.13±2.13	68.40±2.76	2.33±0.058	2.68±0.082	9.57±0.26	8.07±0.32

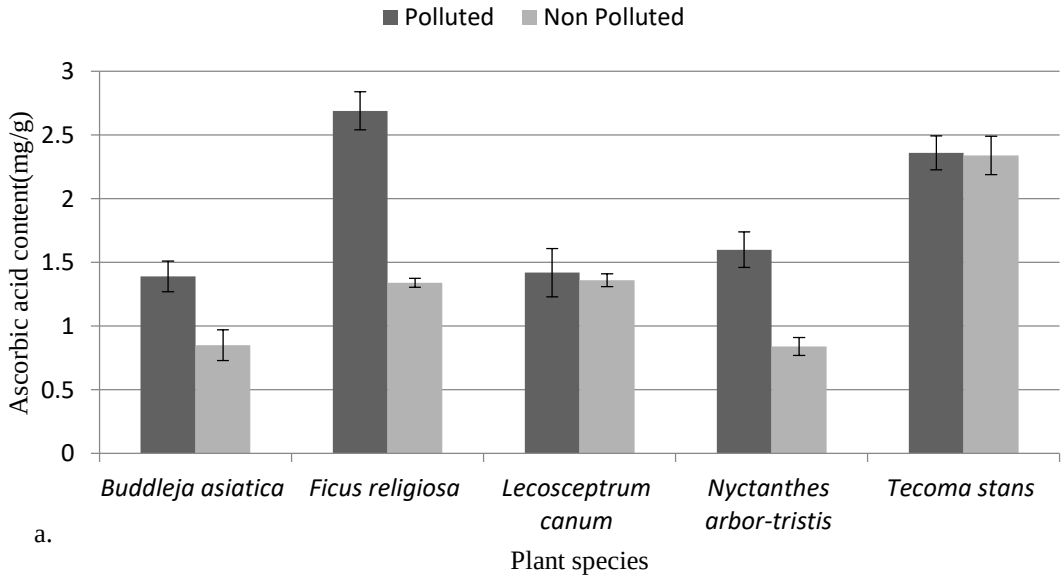


Figure 2a. Ascorbic acid content of selected plants from polluted and non -polluted area

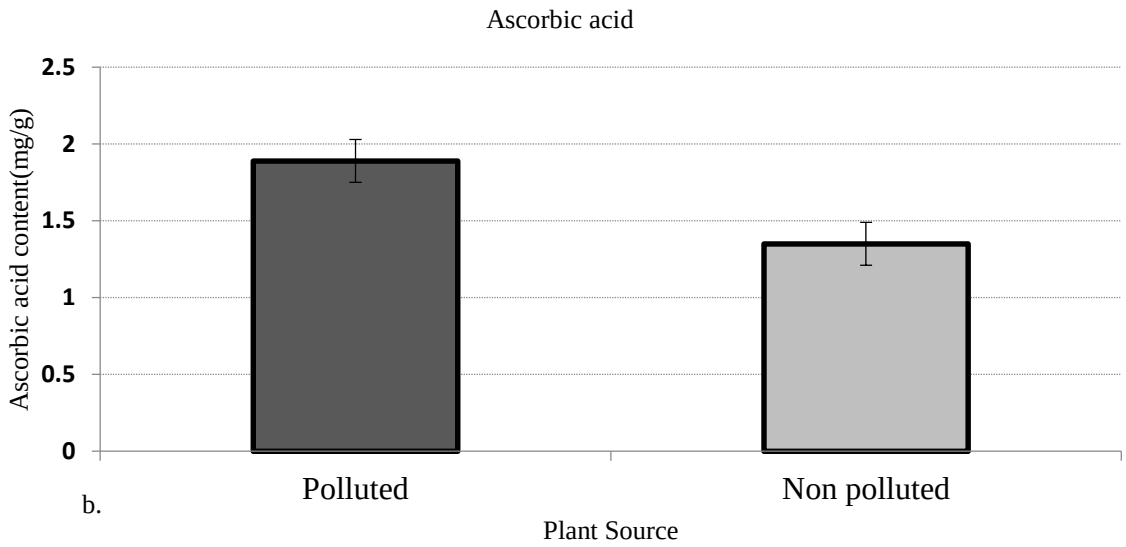


Figure 2b. Ascorbic acid content of selected plants from polluted and non -polluted area

Table 4. Comparison of biochemical characters (Mean± SD, n=30) in leaves between different plants grown at different pollution level. Post-Hoc Duncan- test: no differences between groups with the same letter.

Plant species	Ascorbic acid	Total chlorophyll	pH	RWC	APTI
B. asiatica	1.14± 0.11 a	0.35±0.12ab	6.50±2.12ab	55.69±9.10a	6.24±2.1a
F. religiosa	2.15± 0.98b	0.636±0.21b	7.14±1.20c	89.72± 12c	10.44±2.56c
L. canum	1.40±0.14a	1.22±0.12c	6.34±1.34a	73.39± 13b	8.32±1.13b
N. arbor-tritis	1.17 ± 0.23a	0.36±0.20ab	6.73±1.0ab	75.75±11b	8.46±1.21b
T. stans	2.36 ±1.01b	0.079±0.01a	7.02±2.01bc	72.74 ±9.0b	9.028±1.13b
F- value	10.738	8.327	3.145	12.327	14.202
P- value	0.000	0.000	0.037	0.000	0. 000

Ascorbic Acid Content

Ascorbic acid content of selected plants from polluted (Lagankhel) and non- polluted areas (Godawari) is summarized in Figure 2a. The mean ascorbic acid content of polluted plants was significantly increased ($P < 0.05$) at 1.89 mg/g compared to 1.35 mg/g in non- polluted areas (Figure 2b). Highest and lowest total ascorbic acid content of 2.69 mg/g and 0.85 mg/g recorded in *Ficus religiosa* from polluted area (Lagankhel) and *Buddleja asiatica* from non-polluted area (Godawari), respectively. Highest impact of air pollution on total ascorbic acid content was recorded in *B. asiatica* (1.39 mg/g in polluted v/s 0.85 in NP area) and *Nyctanthes arbortristis* (1.60mg/g in polluted vs. 1.04 µg/g in non-polluted area) with 63.52% and 53.84% increase, respectively, in ascorbic acid content was observed. Moderate impact of air pollution on ascorbic acid content was observed in *F religiosa* (2.69mg/g in polluted vs.2.34 mg/g in non-polluted samples) and *L. canum* (1.42mg/g in polluted samples and 1.36 in non-polluted samples) where 4.41–14.95 % increase in ascorbic acid content was recorded (Table 3). Least impact of air pollution on total ascorbic acid content was recordedin *T. stans,* where only 0.85% increase in ascorbic acid was observed (2.36 mg/g in polluted v/s 2.34 mg/g in non-polluted samples*).* There was significant different in ascorbic acid content (p>0.001) among plant species. (Table 4). Ascorbic acid is an important antioxidant which provides resistance from stress in plants by neutralizing the free radicals generated that can affect the biochemical and physiological activity (Keller, 1986; Conklin, 2001). Thus, ascorbic acid is an important determinant that decides the plants ability to nullify and withstand the toxic effects of air pollutants (Singh et al., 1991; Odilara et al.,

2001). Several researchers have studied and reported positive correlation of air pollution on ascorbic acid content (Pandey et al., 2015; Manjunath and Reddy, 2019).

pH of Leaf Extracts

The pH of leaf extractives of the leaves from polluted and non-polluted areas is summarized in Figure 3a. There was significant different in leaf extract pH (p=0.037) among plant species. (Table 4). The pH in plants from polluted (Lagankhel) and non-polluted (Godawari) plants ranged from 6.07 to 7.73 and 6.21 to 7.07, respectively. There were significant ($P < 0.05$) variations observed in mean pH of plants from polluted and non-polluted areas (6.98 in polluted v/s 6.48 Figure 3b). However, marked decrease in pH was observed in *F. religiosa* (−1.21units) followed by *B. asiatica* (-0.52 units) and *T stans* (−0.19). In *N. arbortris-tis* and *L. canum* moderate increase in pH of 1.00 and 0.51 units was recorded in plants collected from polluted areas (Table 3). The presence of acidic pollutants such as SO_2 and NO_2 in air cause lowering of pH. Higher tolerance of plants with higher pH of leaf extractives against pollutants was reported (Agrawal, 1988). Higher pH of leaf extractives provides optimal pH for synthesis or reducing activity of important antioxidants such as ascorbic acid, thereby protects enzymes involved in CO_2 fixation cycle and chlorophyll inactivation from the oxidative stress induced by pollutants (Choudhary and Rao,1977; Tanaka et al., 1982). Photosynthesis efficiency of plants is pH dependent and at lower acidic pH photosynthesis efficiency of plants gets reduced (Manjunath and Reddy, 2019). Thus, the resistance of plants to the air pollutant-induced pH changes could determine the tolerance to air pollution. In this study, in *F. religiosa* with pH of 7.12 recorded higher air pollution tolerance index (Table 3).

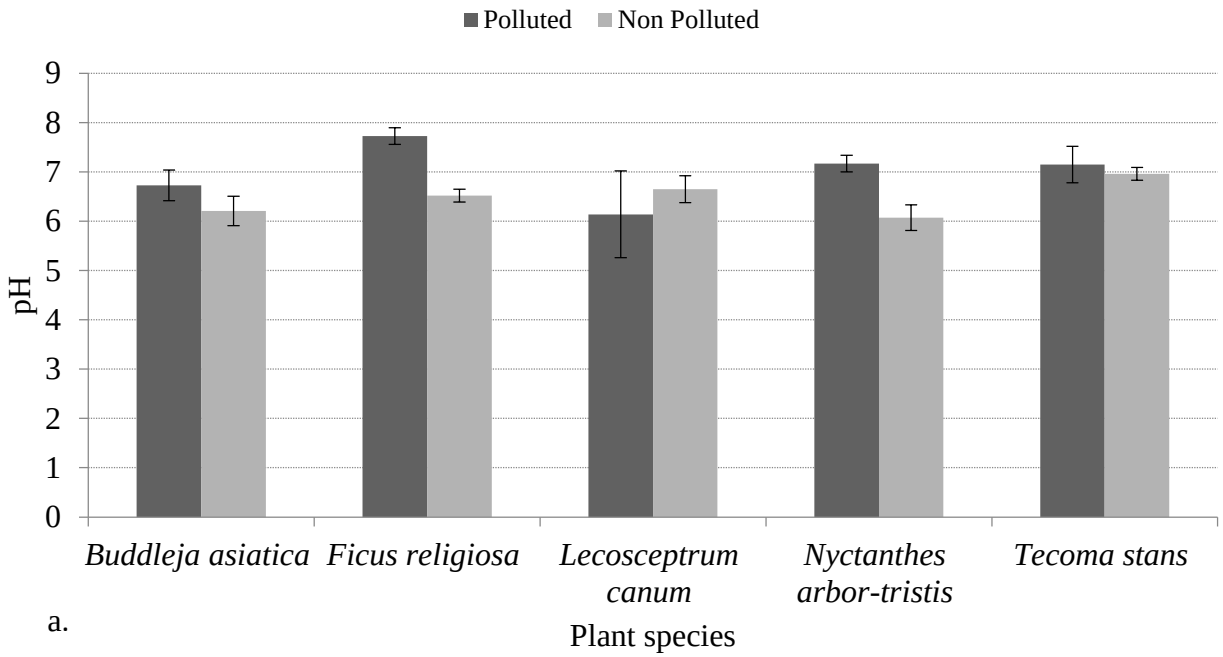
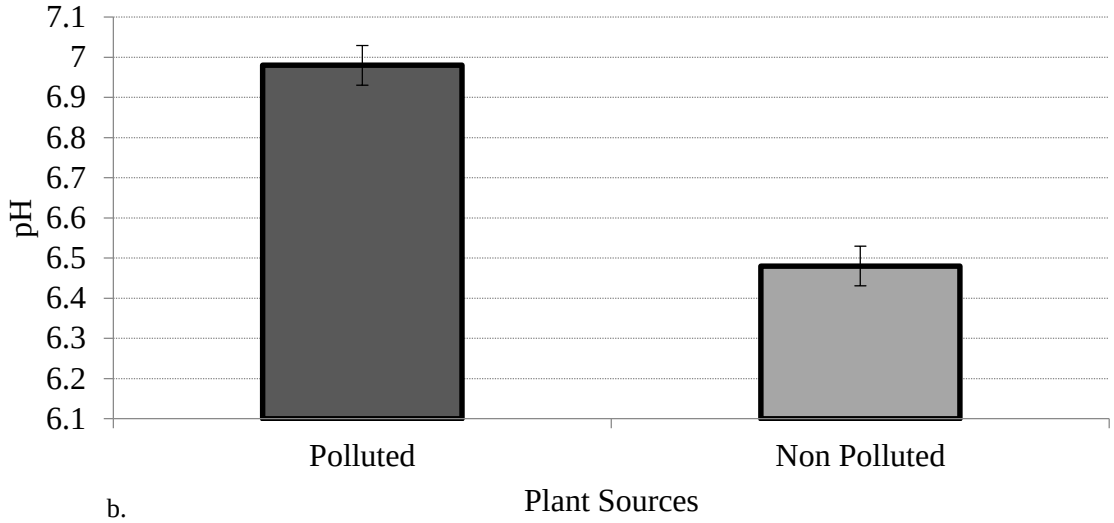


Figure 3a. Leaf extract pH content of selected plants from polluted and non-polluted area



b.

Figure 3b. Leaf extract pH content of selected plants from polluted and non-polluted area

Relative Water Content (RWC)

Relative water content of leaves from polluted and non-polluted source is presented in Figure 4. Mean RWC of plants from non-polluted areas (Godawari) was 68.40 % which was significantly lower ($P < 0.05$) compared to plants from polluted area (78.13 %, Figure 4b). Effect on air pollution on water holding capacity of leaves varied with different plants. In *L. canum collected* from polluted area, there was maximum 25% increase in RWC when compared to that of non-polluted area (79.80% in polluted v/s 63.79 % in non-polluted areas). A moderate effect of air pollution assessed by 15% reduction in RWC was recorded in *B. asiatica* (70.62% in polluted vs 61.21% in non-polluted areas*),* In *T. stans* and *N. arbortristis* plants from polluted area showed lesser rise in RWC of 1.49% (74.62 in polluted and 71.62 in non-polluted) and 1.67 % (76.51 in polluted v/s 75.25) in non-polluted areas), respectively, compared to that from non-polluted area. In *Ficus religiosa*, RWC of samples was 1.19% lower (in polluted samples than in non-polluted samples (89.09 in polluted and 90.15 in non-polluted areas) and plants with higher RWC are having better air pollution, tolerance capacity was reported (Paulsamy et al., 2000). Similarly, in present study, *F. religiosa* from non-polluted area with highest RWC of 90.15% showed highest APTI of 11.66 (Table 3). There was significant different in relative water content (p>0.001) among plant species. (Table 4). Airborne pollutants are extensively reported to increase the loss of water and nutrients from plant leaves affected by increased protoplasmic permeability, leading to senescence (Keller and Schwager, 1977). Higher RWC helps plant in regulating the physiological functions under stress induced by airborne pollutants (Tsega and Prasad, 2014). Higher water holding capacity of leaves under polluted environment may impart tolerance against the toxic airborne pollutants.

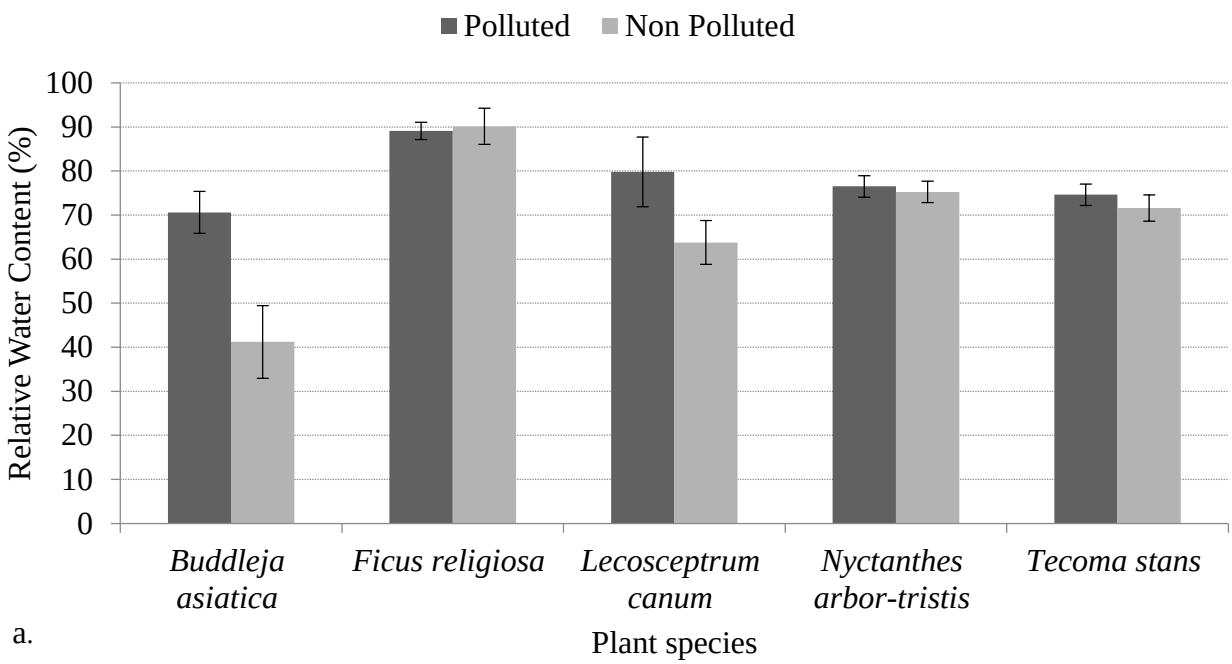
Air Pollution Tolerance Index (APTI)

APTI of selected plants from polluted and non-polluted areas is summarized in Table 3. There was significant different in air pollution tolerance index (p>0.001) among plant species (Table 4). APTI of plants from polluted area ranged from 8.30 to 11.66. APTI of plants from non-polluted area (Godawari) ranged from 6.84 to 10.25. Lowest APTI was recorded for *B. asiatica* selected from both polluted (Lagankhel) and non-polluted area (Godawari) at 8.30 and 6.84, respectively, indicating that it is sensitive to air pollution. Highest APTI of 11.66 was recorded for *Ficus religiosa.* The higher APTI of *F. religiosa* may be attributed to its higher tendency to synthesize ascorbic acid and maintain high RWC during pollution stress conditions (Kuddus *et al.,*

2011). It was followed by in polluted sites as *T. stans* (ATPI 9.39)> *L canum* (ATPI 9.29)> *N. arbortristis* (ATPI 8.96) > *B. asiatica* (ATPI 8.30). Among the plants selected from non-polluted areas, the APTI score was in the order of *F. religiosa* (APTI 10.25) >*T. stans (*APTI 9.04) > *N. arbortristis* (APTI 8.57) > *L. canum* (APTI 8.47). > *B.asiatica* (APTI 6.84). APTI indices in plants reveal that the plants vary in their response determined by their ability to undergo physicochemical adaptation to either prevent pollutants or mitigate the stress induced by pollutants through the antioxidant. Ascorbic acid content in the plants can be used for screening of plants with favorable tolerance to air pollution. Susceptibility to air pollution varies from species to species (Radhapriya et al., 2011; Chouhan et al., 2012). All the plants found in more polluted sites have higher ATPI value than in less pollutes sites (Table 3). The higher APTI of *F. religiosa* and *T. stans* proved these plants be of resistant trees, their dominance in polluted site is an indication of its tolerant nature. The plants with higher APTI were found to be resistant and also act as a bioaccumulator for air pollutants (Prasanna et al., 2005). The findings indicate that the plants *F. religiosa* and *T. stnas* can be selected for growing in polluted environments of urban areas. Being resistant, both species can be grown around the roadside and along road sides as avenue trees.

Correlation of Biochemical Parameters with ATPI

The linear regression plots of four biochemical parameters with APTI (Figure 5) showed a high positive correlation between APTI and ascorbic acid (R^2= 0.852) (Figure 5a). Low positive correlation existed between APTI and RWC (R^2 = 0.3677). An insignificant correlation was found between ATPI and chlorophyll content (R^2= 0.0014) (Figure 5b) and between ATPI and leaf extract pH (R^2=0.144) (Figure 5c and 5d).



a.

Figure 4a. Relative water content of selected plants from polluted and non-polluted area

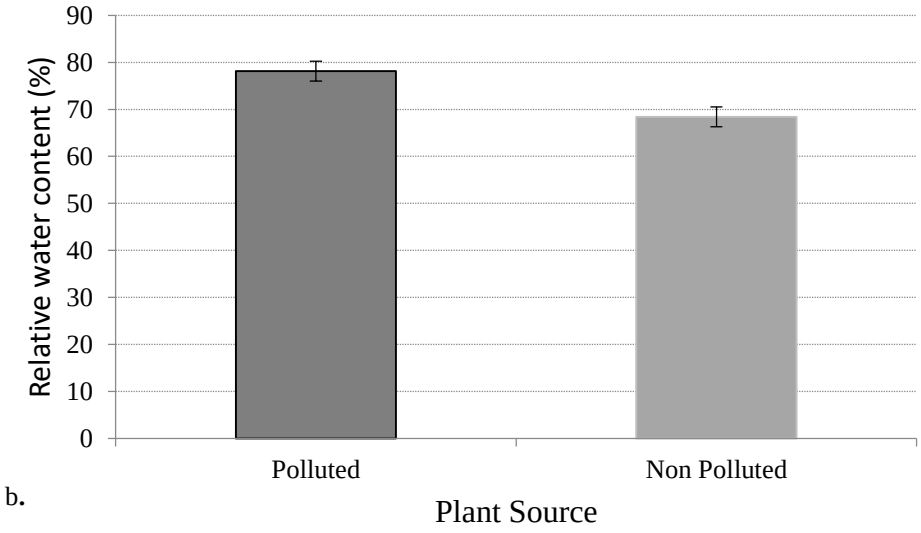
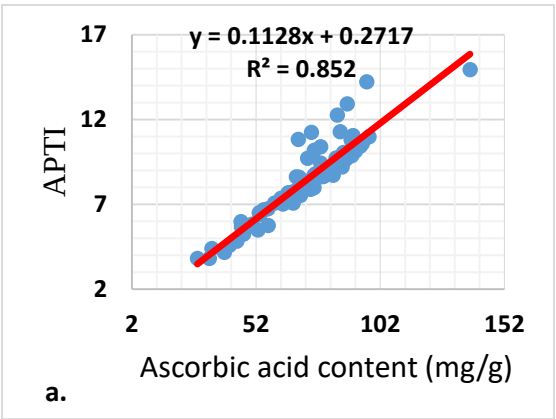


Figure 4b. Relative water content of selected plants from polluted and non-polluted area



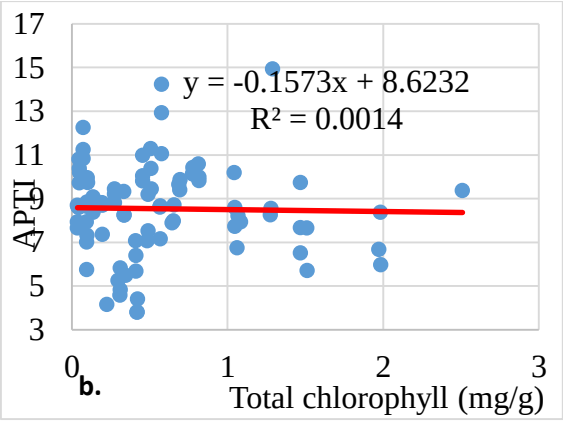
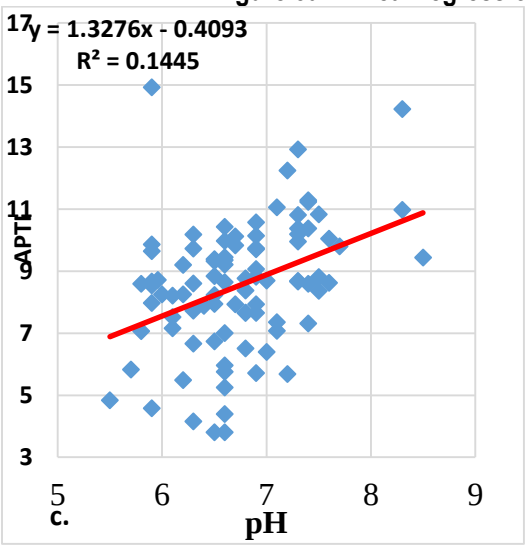


Figure 5a. Linear regression between APTI and biochemical parameters



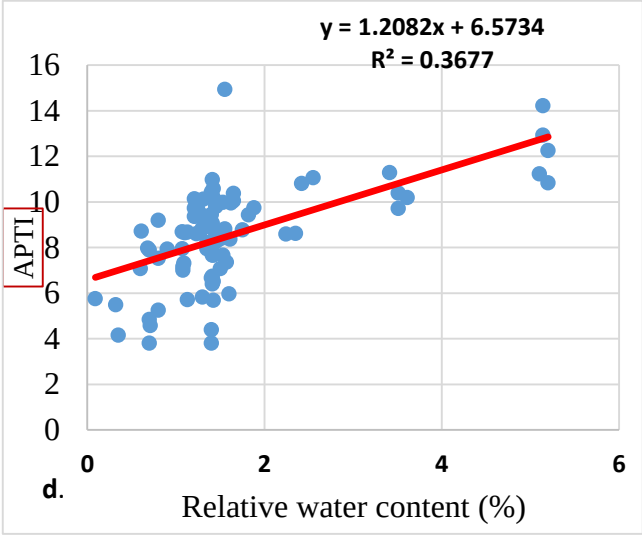


Figure 5b. Linear regression between APTI and biochemical parameters

CONCLUSION

In the present study, comparative assessment of biochemical characteristics and air pollution tolerance indices of five common plants growing in the polluted areas and non-polluted (control) area in Lalitpur district was analyzed as a biomonitoring tool to assess the response of plants to air pollution-induced stress. There were significance differences in biochemical characteristics between polluted and non-polluted sites. The study findings reveal that *Ficus religiosa* and *Tecoma stans* can be effectively used for the improvement of air pollution effects. Further, efforts to evaluate these plants to ameliorate air pollutants at heavily polluted urban environments should facilitate exploring these plants for restoration of green urban ecosystem.

REFERENCES

Aghajanzadeh, T.; Hawkesford, M.J., and De Kok, L.J. (2016). Atmospheric H_2S and SO_2 as sulfur sources for *Brassica juncea* and *Brassica rapa*: Regulation of sulfur uptake and assimilation. *Environmental and Experimental Botany*; 124:1-10.

Aghaiee, N.; Zarei, L., and Cheghamirza, K. (2019). Evaluation of some morpho-physiological characteristics in strawberry under different moisture stress regimes. *Journal of Berry Research* (Preprint), 1-11.

Agrawal, A.L. (1988). Air Pollution Control Studies and Impact Assessment of Stack and Fugitive Emissions from CCI Akaltara Cement Factory. Project Report, Project Sponsored by M/s. CCI Akaltara Cement Factory. Nagpur: NEERI.

Arnon, D.I(1949). Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. *Plant Physiology* 24:1-15.

Bajaj, K.L.and Kaur, G. (1981). Spectrophotometric determination of Ascorbic acid in vegetation and fruits. *Analyst*. 106:117-120.

Barr, H.D. and Weatherley, P.E. (1962). A reexamination of the relative turgidity technique for estimating water deficit in leaves. *Australian Journal of Biological Science* 15:413-428.

Bellini, E., and De Tullio, M.C. (2019). Ascorbic acid and ozone: Novel perspectives to explain an elusive relationship. *Plants*, 8(5):122.

Choudhary, C.S. and Rao, D.N. (1977). Study of some factors in plants controlling their susceptibility to SO_2 pollution. *Proceedings of the National Academy of Sciences*, *India*, 43:236-41.

Chouhan A., Iqbal S.and Maheshwari R.S. (2012). A study of air pollution tolerance index of plants growing in Pithampur industrial area sector 1, 2 and 3. *Research Journal of Recent Sciences*. 1:172.

Conklin, P.L. (2001). Recent advances in the role and biosynthesis of ascorbic acid in plants. *Plant Cell and Environment,*24:383-94.

Escobedo, F. J., Wagner, J. E., Nowak, D. J., De la Maza, C. L, Rodriguez, M. and Crane, D. E. (2008). Analyzing the cost effectiveness of Santiago, Chile's policy of using urban forests to improve air quality. *Journal of Environmental Management*, 86(1):148–157.

Keller, T. (1986). The electrical conductivity of Norway spruce needle diffusate as affected by air pollutants. *Tree Physiology*, 1:85-94.

Keller, T. and Schwager, H. 1977.Air pollution and ascorbic acid. *European Journal of Forest Pathology* ,7:338-50.

Leghari, S.K and Zaidi, M.A. (2013). Effect of air pollution on the leaf morphology of common plant species of Quetta city, *Pakistan. Pakistan Journal of Botany* ,45:447-54.

Litchfield, I.J., Ayres, J.G., Jaakkola, J.J.K. and Mohammed, N.I (2018). Is ambient air pollution associated with onset of sudden infant death syndrome: a case-crossover study in the UK. BMJ Open 8:e018341. doi:10.1136/ bmjopen-2017-018341.

Manjunath, B.T. and Reddy J (2019). Comparative evaluation of air pollution tolerance of plants from polluted and non-polluted regions of Bengaluru. *The Journal of Applied Biology & Biotechnology,*7(03);63-68. DOI: 10.7324/JABB.2019.70312.

Odilara, C.A., Egwaikhide, P.A., Esekheigbe, A. and Emua, S.A. (2006). Air pollution tolerance indices (APTI) of some plant species

around Ilupeju industrial area, Lagos International *Journal* of *Engineering Science* 4(2):97-101.

Pandey, A.K., Pandey, M., Mohan, S., Tiwary, M. and Tripathi, B.D. (2015). Air pollution tolerance index and anticipated performance index of some plant species for development of urban forest. *Urban Forestry and Urban Greening*.14:866-71.

Paulsamy, S., Sivakumar, R. and Latha, N. (2000). Evaluation of air pollution tree species in Coimbatore city. J Ecol Res Biocon,1:20-3.

Prabhat, K.R. (2016). Biodiversity of roadside plants and their response to air pollution in an Indo-Burma hotspot region: Implications for urban ecosystem restoration. *Journal of Asia-Pacific Biodiversity*,9:47-55.

Prajapati, S.K. (2012) Ecological effect of airborne particulate matter on plants. *In*: *Environmental Skeptics and Critics* 1(1): 12-22.

Prasanna, R.T., Despande, U.K. and Awaid, U.G. 2005. Effect of sulphur dioxide on protein and carbohydrate of some macrophytes. *Pollution Research,* 24(2):427-430.

Radhapriya, P., Gopalkrishnan, A.N., Malini, P. and Ramachandran, A. 2011. Assessment of air pollution tolerance levels of selected plants around cement industry, Coimbatore, India. *Journal of Environmental Biology*, 33:635-641.

Shannigrahi A, Sharma R, Fukushima T (2003) Air pollution control by optimal green belt development around the Victoria Memorial monument Kolkata India. *International Journal of Environmental Studies*. 60:241-249.

Singh, S.K. and Rao D.N(1983). Evaluation of plants for their tolerance to air pollution. In: *Proceeding of Symposium on Air Pollution Control*. Vol. 1. New Delhi: Indian Association of Air Pollution Control; pp. 218-224.

Singh, S.K.; Rao, D.N.; Agrawal, M.; Pandey, J. and Narayan, D. (1991). Air pollution tolerance index of plants. *Journal of Environmental Management*, 32:45-55.

Tanaka, K.; Otsubo, T. and Kondo, N. (1982). Participation of hydrogen peroxide in the inactivation of Calvin-cycle SH enzymes in SO_2-fumigated spinach leaves. *Plant and Cell Physiology* 28:1009-18.

Tsega, Y.C. and Prasad, A.D. 2014. Variation in air pollution tolerance index and anticipated performance index of roadside plants in Mysore, India. *Journal of Environmental Biology*, 35:185-90.

Yang, J., Bride, J., Zhou, J.X and Sun, Z.Y. (2005). The urban forest in Beijing and its role in air pollution reduction. *Urban Forestry and Urban Greening* 3:65-78.

Source of Financial Support: University Grants Commission Nepal.
Conflict of Interest: None, Declared.