Botanica Orientalis – Journal of Plant Science (2019) **13**: 13–21 ISSN 1726-6858

Research article

Effect of particulate matters on chlorophyll content in shrubby species growing along roadside of Kathmandu Valley

Shobha Dhyani, Anjana Devkota*, Sushila Devi Shrestha, Pramod Kumar Jha

Central Department of Botany, Tribhuvan University, Kathmandu, Nepal

Abstract

One of the most common impacts of air pollution on plant species is the gradual loss of chlorophyll and concomitant yellowing of leaves, which results in decrease in the capacity of photosynthesis. In the present study, effect of air pollutants on chlorophyll content of some selected plant species along the roadsides of Kathmandu Valley was assessed. Five commonly occurring plant species along the roadside, viz. *Bougainvillea glabra*, *Duranta erecta*, *Lantana camara*, *Ricinus communis* and *Sambucus chinensis* were selected for the study. The study revealed lowest concentration of chlorophyll "a", chlorophyll "b" and total chlorophyll in the leaf samples of all species collected from heavily polluted site (Thamel) when compared with the leaf samples collected from the control site (Tribhuvan University Area, Kirtipur). Chlorophyll content was insignificantly reduced in *Bougainvillea glabra* in heavily polluted site, but a significant reduction was recorded in *Sambucus chinensis*. Maximum reduction in chlorophyll content exhibited by *Sambucus chinensis* can be ascribed to sensitiveness of the species to air pollution, while least reduction in chlorophyll content by *Bougainvillea glabra* suggests that it is an air pollution tolerant species. This study clearly indicated that the vehicular air pollution affected the level of chlorophyll pigments in plants exposed to roadside.

Key-words: Air pollution, chlorophyll a, chlorophyll b, total chlorophyll.

Introduction

In recent times, there have been significant development activities in terms of industrialization and urbanization in almost all big cities. Such developmental activities are the main sources of pollutants discharged into the environment and introduce various harmful substances into the atmosphere. Air pollution has become an extremely serious problem for the modern industrialized world in both developing and developed countries. The increasing number of industries and automobile are continuously adding toxic substances, including gases (e.g., sulphur dioxide, nitrogen oxides, carbon monoxides and hydrocarbons), particulate matters (e.g., smoke, dust, fumes and aerosols), and radioactive materials to the environment causing significant human health problems. Apart from causing respiratory disorders in humans, the air pollutants have also caused various effects on plants. The particulates and gaseous pollutants, alone and in combination, can cause serious setbacks to the overall physiology of plants (Das and Prasad 2010), thus affecting patterns of their growth (Agbaire and Esiefarienrhe 2009). Pollutants' adverse effects on plant have long been known, air pollutants cause damage to leaf cuticles and affect stomatal conductance, and they can also have direct effects on photosynthetic systems, leaf longevity, and patterns of carbon allocation within plants (Wolfenden and Mansfield 1990).

Chlorophyll is found in the chloroplast of green plants and is called photoreceptor. There are different types of chlorophyll molecules, such as chlorophyll-a, chlorophyll-b, chlorophyll-c and chlorophyll-d. When plants are exposed to increased rates of environmental pollutants, the rate of photosynthesis also varies. The chlorophyll content of plants also varies from species to

*Corresponding author's email address: devkotaa@gmail.com

species, depending on age of leaf, pollution level, and presence of other biotic and abiotic conditions (Katiyar and Dubey 2001). Reduction in the concentration of leaf chlorophyll content in plants from polluted area was observed in different plant species (e.g., Giri *et al*. 2013; Amulya *et al*. 2015). A study conducted by Giri *et al*. (2013) indicated a decline in the chlorophyll content in trees growing in the industrial areas, suggesting that the reduction in chlorophyll content was due to degradation of chlorophyll into phaeophytin by the loss of magnesium ions. Similar changes in concentration of pigments were observed in six tree species exposed to air pollution due to vehicle emission (Joshi and Swami 2009). It was also observed that plants from polluted area had significantly lower leaf chlorophyll content than those present in the control or unpolluted site (Prajapati and Tripathi 2008; Rout *et al*. 2010; Stevovic *et al*. 2010).

Due to widespread road widening activities and the growing vehicular traffic, air quality of Kathmandu Valley has been a matter of serious concern. Further, a plant's response may alter under varying pollution stresses; however, until now, no study has been carried out in Kathmandu Valley to study the impacts of air pollution on roadside plants. In the present study, effect of air pollutants on chlorophyll content of commonly growing shrubby species along roadsides of Kathmandu Valley has been studied. The information generated under the study will help to understand how does pollution affects chlorophyll content.

Materials and Methods

STUDY AREA

The present study was conducted in Kathmandu Valley (27^0 37'30" N and 27^0 45'0" N latitude, and 85^0 15'0" E and 85^0 22'30" E longitude) in Central Nepal. It is about 1400 m above 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 (Sapkota and Dhaubadel 2002). The mean temperature ranges from 10°C (in January) to about 25°C (in July). 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. Human population of Kathmandu Valley is 2.5 million with annual population growth rate of 4.2% and population density of 2,799.8 km^{-2} (CBS 2011). Accumulation of total suspended particulate matters (TSPMs) has been a major problem along roadsides of Kathmandu Valley.

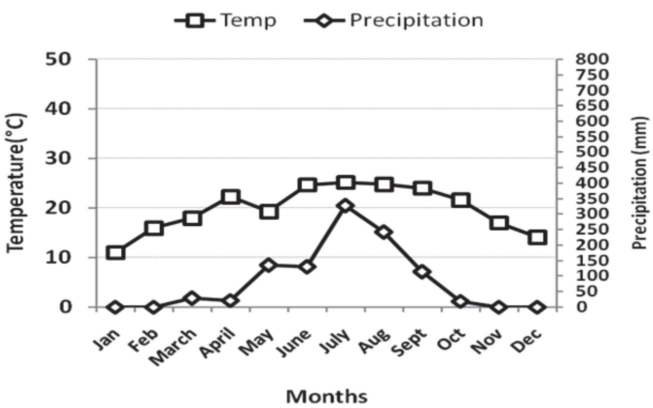

Figure 1. Average monthly mean temperature and precipitation of Kathmandu Valley (2012–2017). *Source*: Department of Hydrology and Meteorology, Government of Nepal, Kathmandu.

Four sites, Thamel, Lazimpat, Bhaktapur 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, less polluted and least polluted (Table 2). The least polluted site was considered as control.

Table 1. Characteristics of study sites.

SN	Study site	Characteristics
Site 1	Thamel area	Crowded, touristic area, high flow of vehicles
Site 2	Lazimpat	High flow of vehicle, road connecting the northern part of the Kathmandu Valley
Site 3	Bhaktapur	Residential area
Site 4	Kirtipur	University Campus area (within Coronation Garden), less flow of vehicles

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

Study site	TSPMs ($\mu g/m^3$)	PM2.5 ($\mu g/m^3$)	PM10 ($\mu g/m^3$)
Thamel (heavily polluted)	1289	147	696
Lajimpat (moderately polluted)	467	95	278
Bhaktapur (less polluted)	321	11	178
Kirtipur (least polluted; control)	305	13	149

Table 3. Chlorophyll-a content (mg g-1) of selected plant species at different locations. The data were expressed as mean ± SD and statistical analysis using two-way ANOVA for obtaining *F*- and *p*-values. 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	Location				
	Heavily polluted	Moderately polluted	Less polluted	Least polluted (Control)	Mean
Bougainvillea glabra	1.90 ± 0.08^{a}	1.89 ± 0.23^{a}	1.92 ± 0.34^{a}	1.97 ± 0.32^{a}	1.92±0.31
Duranta erecta	1.33 ± 0.12^{a}	1.37 ± 0.12^{b}	1.35 ± 0.12^{b}	1.47 ± 0.12^{c}	1.38±0.12
Lantana camara	1.63 ± 0.05^{a}	1.62 ± 0.10^{a}	1.60 ± 0.12^{a}	1.64 ± 0.15^{a}	1.62±0.13
Ricinus communis	1.75 ± 0.02^{a}	1.78 ± 0.21^{a}	1.82 ± 0.56^{ab}	1.96 ± 0.11^{b}	1.82±0.42
Sambucus chinensis	1.45 ± 0.07^{a}	1.46 ± 0.03^{a}	1.65 ± 0.35^{b}	1.80 ± 0.24^{c}	1.59±0.21

F-value. Plant spp.: $F = 16.887$; location: $F = 21.117$
p-value. Plant spp.: $p<0.001$; location: $p<0.001$

SELECTION OF PLANT SPECIES

Five plant species (*Bougainvillea glabra* Choisy, *Duranta erecta* L., *Lantana camara* L., *Ricinus communis* L. and *Sambucus chinensis* Lindl.) widely grown along roadside of Kathmandu Valley were selected for the present study. The species were selected on the basis of availability at all sites.

COLLECTION OF SAMPLES

To include the effect of seasons on plant response to pollution, two seasons, *viz.* winter (December-January) and summer (June-July) were considered. In order to study the impact of dust and other pollutants generated by vehicular activities on chlorophyll content 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 isoecological environments 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. Fully mature leaves were collected in the morning hours (8 to 10 AM) from branches located at breast height (ca.1.3 m). 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.

CHLOROPHYLL CONTENT

Chlorophyll content in the leaves was estimated following the method of Arnon (1949) by using a spectrophotometer. The fresh and clean leaves (0.25 gm) of selected plants were blended in 80% acetone using mortar and pestle. The mixture was filtered and the filtrate was made to 50 ml. Absorbance of the extract was measured at 645 nm, 663 nm and 652 nm using spectrophotometer (Spectronic 21, Milton Roy Company). The following formulae were used to calculate contents (mg g^{-1}) of chlorophyll-a (Cha a), chlorophyll-b (Cha b) and total chlorophyll (Arnon 1949).

$$\text{Chl a} = \frac{12.7\,(A\,663) - 2.69\,(A\,645) \times V}{1000 \times W}$$

$$\text{Chl b} = \frac{22.9\,(A\,645) - 4.68\,(A\,663) \times V}{1000 \times W}$$

$$\text{Total Chl} = \frac{20.2(A\,645 + 8.02(A663) \times V}{1000 \times W}$$

Where, A = absorbance at specific wavelength; V = final volume of chlorophyll extract in 80% acetone; W = fresh weight of leaf extracted.

STATISTICAL ANALYSIS

Data were analyzed by SPSS version 17 using two-way ANOVA. Comparison of mean of chlorophyll-a, chlorophyll-b and total chlorophyll contents among plant species was carried out by two-way interaction among plant species and location (pollution level). Comparison between the pigments of two seasons was done by using t-test. For significant factors, multiple comparison tests, using Duncan multiple comparison statistics, were used.

Results

CHLOROPHYLL-A CONTENT

The leaf chlorophyll-a content of the selected plant species varied from 1.38 to 1.92 mg g^{-1} (Table 3). Among the five species studied, *Bougainvillea glabra* had maximum leaf chlorophyll-a content of 1.92 mg g^{-1}. This was followed by *Ricinus communis*, *Lantana camara*, Sambucus chinensis and Duranta erecta, with respective values of 1.82, 1.62, 1.59 and 1.38 mg g^{-1}. Further, the variations in leaf chlorophyll-a content of all plant species varied significantly ($p<0.001$) with the pollution status of the area with higher value at less polluted site and least at heavily polluted site (Table 3). Season of collection also significantly affected the chlorophyll-a content (Figure 2). The interaction between species and seasons exerted a significant influence on the content of leaf chlorophyll. Among the selected species, *Bougainvillea glabra* leaves contained highest chlorophyll-a content of 2.01 mg g^{-1} during the summer season. The lowest value of 0.56 mg g^{-1} was recorded in *Duranta erecta* during winter season (Figure 2).

Overall average reduction of chlorophyll-a content ranged 0.60% (*Lantana camara*) to 19.44% (*Sambucus chinensis*) at heavily polluted site when compared with less polluted site (control) (Figure 3). The reduction in leaf chlorophyll-a content of selected species followed the order of *Sambucus chinensis* (19.44%) > *Ricinus communis* (10.71%) > *Duranta erecta* (9.52%) > *Bougainvillea glabra* (3.55%) > *Lantana camara* (0.60%).

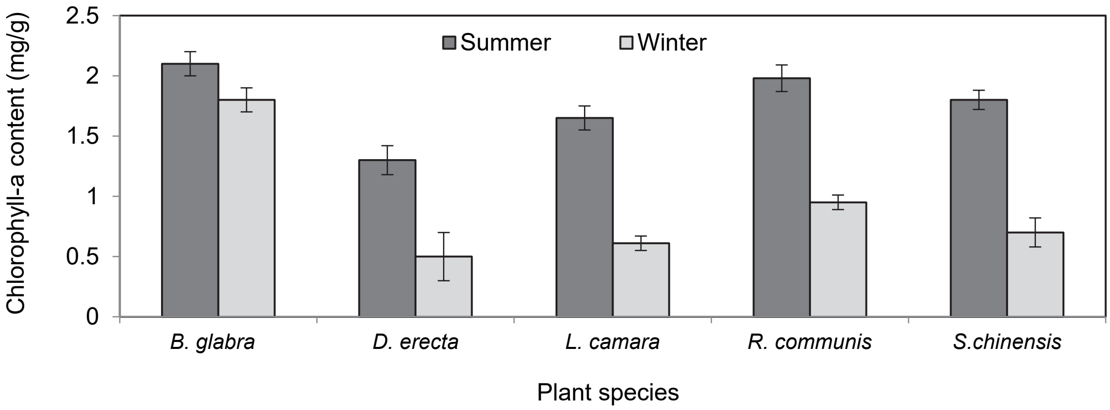

Figure 2. Seasonal variation in the chlorophyll-a content (mean with error bar) of the studied shrub species (*Bougainvillea glabra*, *Duranta erecta*, *Lantana camara*, *Ricinus communis* and *Sambucus chinensis*) ($p<0.001$).

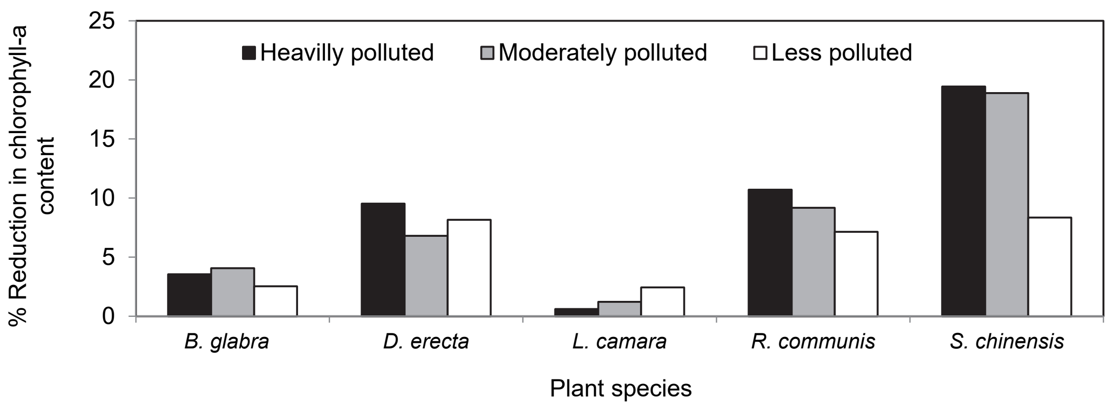

Figure 3. Overall average reduction of chlorophyll-a content of studied shrub species (*Bougainvillea glabra*, *Duranta erecta*, *Lantana camara*, *Ricinus communis* and *Sambucus chinensis*) at sites with different pollution levels (the reduction was calculated with respect to less polluted site, i.e., control).

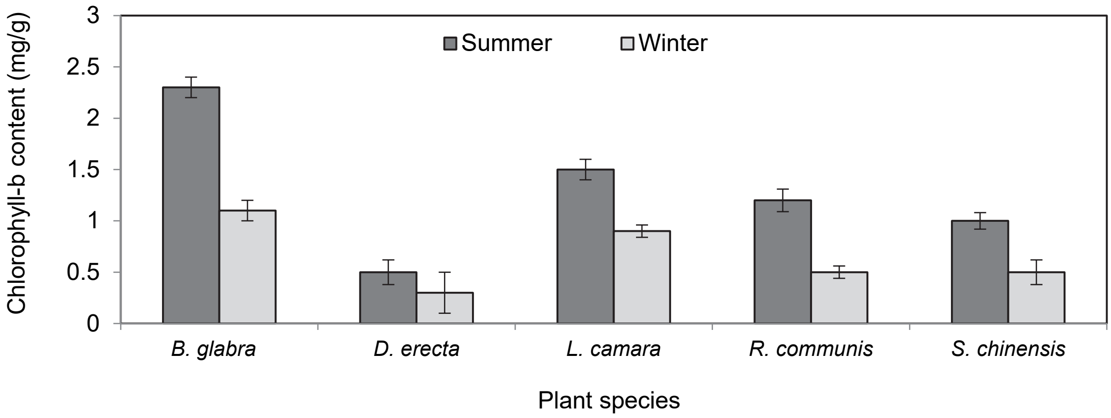

Figure 4. Seasonal variation in the chlorophyll-b content (mean with error bar) of the studied shrub species (*Bougainvillea glabra*, *Duranta erecta*, *Lantana camara*, *Ricinus communis* and *Sambucus chinensis*) (p<0.001).

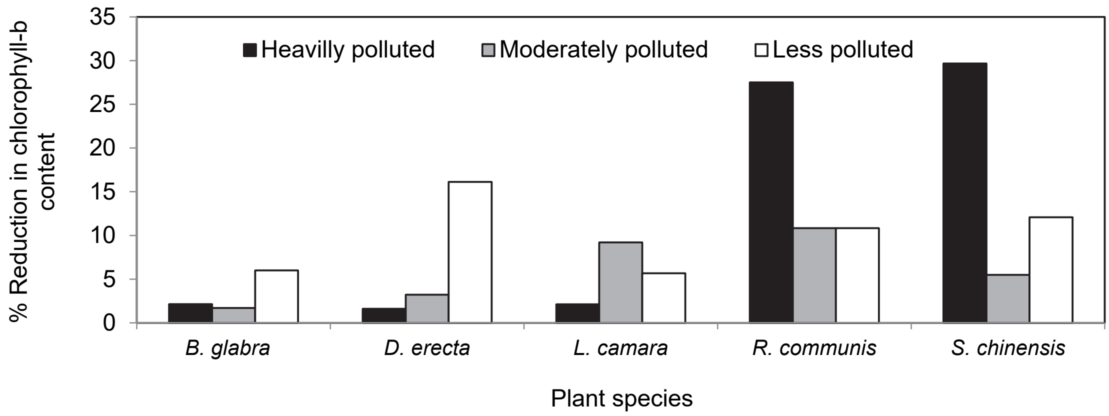

Figure 5. Overall average reduction of chlorophyll-b content of studied shrub species (*Bougainvillea glabra*, *Duranta erecta*, *Lantana camara*, *Ricinus communis* and *Sambucus chinensis*) at sites with different pollution levels (the reduction was calculated with respect to less polluted site, i.e., control).

CHLOROPHYLL-B CONTENT

The leaf chlorophyll-b content of the selected plant species ranged 0.60–2.27 mg g^{-1} (Table 4). Among the five species studied *Bougainvillea glabra* had maximum leaf chlorophyll-b content of 2.27 mg g^{-1}. This was followed by *Lantana camara*, *Ricinus communis*, *Sambucus chinensis* and *Duranta erecta*, with respective values of 1.35, 1.05, 0.80 and 0.60 mg g^{-1}. Further, the variations in leaf chlorophyll-b content of plant species varied significantly (p>0.001) with the pollution status of the area with higher value at less polluted site and least at heavily polluted site (Table 4).

Season of collection also significantly affected chlorophyll-b content of samples (Figure 4). The interaction between species and seasons exerted a significant influence on the leaf chlorophyll-b content (Figure 4). Among the studied species, *Bougainvillea glabra* leaves were found to contain highest chlorophyll-b content of 2.29 mg g^{-1} during the summer season. The lowest leaf chlorophyll-b content (0.522 mg g^{-1}) was recorded in *Sambucus chinensis* during winter season.

Overall average reduction of chlorophyll-b content at polluted site with respect to less polluted site (control) was found in the range of 1.61 %–29.67%, lowest to highest in the leaf of *Duranta erecta* and *Sambucus chinensis*, respectively at heavily polluted site (Figure 5). The reduction in leaf chlorophyll-b content of selected species followed the order of *S. chinensis* (29.67%) > *Ricinus communis* (27.5%) > *Bougainvillea glabra* (2.14%). *Lantana camara* (2.12%) > *Duranta erecta* (1.61%).

TOTAL CHLOROPHYLL CONTENT

There was significant difference (p<0.001) in total chlorophyll content among the samples at different pollution level. The total chlorophyll content of species ranged 1.55–3.40 mg g^{-1} (Table 5). Among the five species studied, Bougainvillea glabra had maximum total chlorophyll content of 3.40 mg g^{-1} in their leaves.

Table 4. Chlorophyll-b content (mg g-1) of selected plant species at different locations. The data were expressed as mean ± SD and statistical analysis using two-way ANOVA for obtaining *F*- and *p*-values. 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	Location				
	Heavily polluted	Moderately polluted	Less polluted	Least polluted (Control)	Mean
Bougainvillea glabra	$2.28±0.04^b$	$2. 29±0.21^b$	$2.19±0.34^a$	$2.33 ±0.14^b$	2.27±0.17
Duranta erecta	$0.61±0.13^a$	$0.60±0.09^a$	$0.59±0.08^a$	$0.62± 0.06^a$	0.60±0.12
Lantana camara	$1.38±0.61^b$	$1.28±0.43^a$	$1.33±0.05^a$	$1.41±0.71^b$	1.35±0.56
Ricinus communis	$0.87±0.12^a$	$1.07 ±0.02^b$	$1.07±0.04^b$	$1.20± 0.04^b$	1.05±0.05
Sambucus chinensis	$0.64±0.06^a$	$0.86±0.02^b$	$0.80±0.05^b$	$0.91±0.04^c$	0.80±0.03

F-value. Plant spp.: $F = 16.42$; location: $F = 37.41$
p-value. Plant spp.: $p<0.001$; location: $p<0.001$

Table 5. Total leaf chlorophyll content (mg g-1) of selected plant species at different locations. The data were expressed as mean ± SD and statistical analysis using two-way ANOVA for obtaining *F*- and *p*-values. 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	Location				
	Heavily polluted	Moderately polluted	Less polluted	Least polluted (Control)	Mean
Bougainvillea glabra	$3.43±1.01^a$	$3.43±0.98^a$	$3.30 ±1.21^a$	$3.47±1.82^a$	3.40± 1.00
Duranta erecta	$1.60±0.12^a$	$1.50±0.11^b$	$1.47 ±0.56^b$	$1.63±0.21^a$	1.55±0.35
Lantana camara	$2.82±0.23^b$	$2.78 ±0.23^a$	$2.75 ±0.21^a$	$2.86±0.23^b$	2.80± 0.12
Ricinus communis	$2.49±0.31^b$	$2.39±0.23^a$	$2.46 ±0.12^b$	$3.41±0.34^c$	2.69±0.25
Sambucus chinensis	$1.68±0.03^a$	$2.38±0.36^c$	$2.14 ±0.21^b$	$2.65±0.31^d$	2.21±0.28

F-value. Plant spp.: $F = 33.896$; location: $F = 45.735$
p-value. Plant spp.: $p<0.001$; location: $p<0.001$

This was followed by *Lantana camara*, *Ricinus communis*, *Sambucus chinensis*, and *Duranta erecta*, with respective values of 2.80, 2.69, 2.21 and 1.55 mg g^{-1}. Further, the variations in total chlorophyll content of plant species varied significantly ($p>0.01$) with the pollution status of the area with higher value at less polluted site and least at heavily polluted site. Season of collection also significantly affected the total chlorophyll content of samples (Figure 6). The interaction between species and seasons exerted a significant influence on the leaf chlorophyll content. Among the selected species, *Bougainvillea glabra* leaves were found to contain highest total chlorophyll content of 3.96 mg g^{-1} during the summer season. The lowest value of 1.0 mg g^{-1} was recorded in *Duranta erecta* during winter season (Figure 6).

Overall average reduction of total chlorophyll content at polluted site with respect to less polluted site (control) was found in the range of 1.15–36.28%, lowest to highest in the leaf of *Bougainvillea glabra* and *Sambucus chinensis*, respectively at heavily polluted site (Figure 7). The reduction in leaf total chlorophyll content of selected species followed the order of *S. chinensis* (36.28%) > *Ricinus communis* (26.75%) > *Duranta erecta* (2.45%) > *Lantana camara* (1.39%) > *Bougainvillea glabra* (1.15%).

Discussion

Chlorophyll content of plant signifies its photosynthetic activity as well as the growth and development of biomass. Chlorophyll content varies from species to species with age of leaf and also with the pollution level along with other biotic and abiotic conditions. There was significant difference in chlorophyll-a, chlorophyll-b and total chlorophyll content of collected samples in present

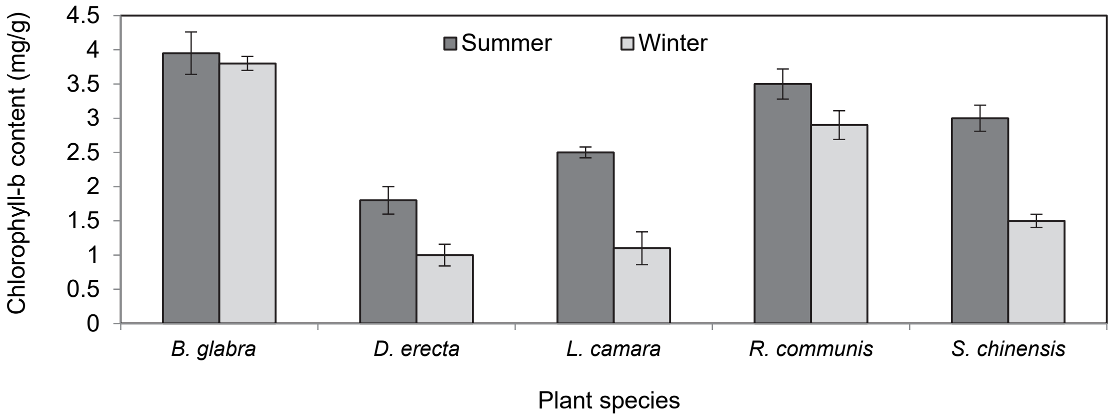

Figure 6. Seasonal variation in the total chlorophyll content (mean with error bar) of the studied shrub species (*Bougainvillea glabra*, *Duranta erecta*, *Lantana camara*, *Ricinus communis* and *Sambucus chinensis*) (p<0.001).

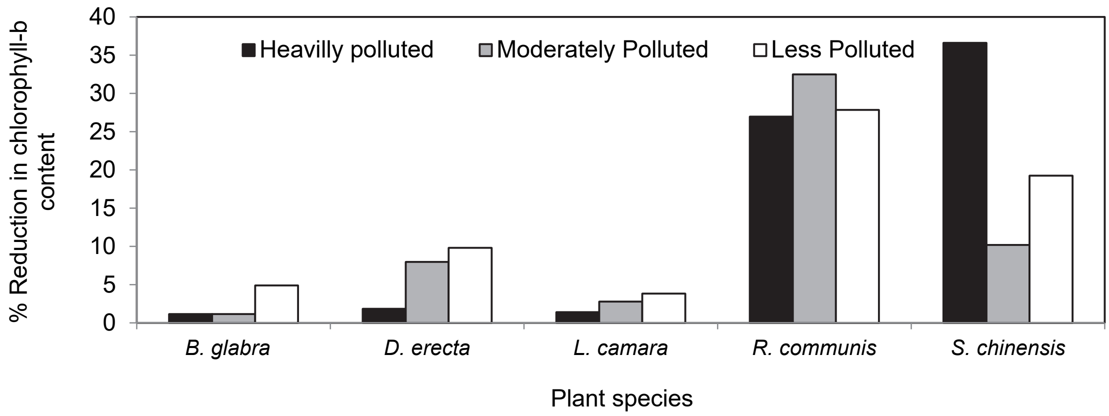

Figure 7. Overall average reduction of total chlorophyll content of studied shrub species (*Bougainvillea glabra*, *Duranta erecta*, *Lantana camara*, *Ricinus communis* and *Sambucus chinensis*) at sites with different pollution levels (the reduction was calculated with respect to less polluted site, i.e., control).

study (Tables 3–5). Highest content for all pigments was recorded in Bougainvillea glabra and lowest in *Duranta erecta*. This variation of chlorophyll content may be attributed to the genetic variations among the plant species. Further, the variations in leaf chlorophyll content may also be ascribed to the tolerance as well as the sensitivity of the species to the pollution load (Singare and Talpade 2013). Plants growing in different pollution conditions also exhibited significant variation in their leaf chlorophyll content. Interestingly, plants growing at least polluted site had the highest chlorophyll content, which was followed by those growing at less polluted, moderately polluted and heavily polluted sites (Tables 3–5). The results are in conformity with the findings of Mir *et al*. (2008), who pointed out high level of automobile pollution is the cause of decrease in chlorophyll content of plants near roadside. Several researchers have recorded reduction in chlorophyll content under air pollution (Joshi and Swami 2009). There are several possible reasons for reduction in chlorophyll content due to increased air pollution, which are related to photochemical reactions such as oxidation, reduction and reversible bleaching of the photosynthetic pigments under stressed condition (Rout et al. 2010). Another possible reason may be due to the alkaline condition created by the dissolution of chemicals present in dust particles in cell sap, which is responsible for chlorophyll degradation. The deposition of dust particles in the stomata interfere with the gaseous exchange that consequently retard the chlorophyll. Reduction in chlorophyll content usually results in conversion of chlorophyll into phaeophytin, which results due to loss of magnesium ions (Giri *et al*. 2013).

All the plant species studied had significantly higher content of chlorophyll-a, chlorophyll-b and total chlorophyll (Figures 2, 4 and 6) during summer season (June-July), which may be attributed to the availability of better growing conditions in this season. This view is supported by Kershaw and Webber (1986) and Mishra *et* *al*. (2013), who also reported highest value of chlorophyll during this period. This may also be attributed to the fact that chlorophyll concentration in leaves decreases in response to low temperature and short-day conditions as are found in the present study area and increases with increase in temperature (Ottander *et al*. 1995; Weng *et al*. 2005).

The present study showed reduction in the chlorophyll content with increase in air pollution as shown in Tables 3–5. The reduction in leaf chlorophyll content of selected species generally followed the order of *Sambucus chinensis* > *Ricinus communis* > *Duranta erecta* > *Lantana camara* > *Bougainvillea glabra*. The reduction in leaf chlorophyll content ascribed to the tolerance and sensitiveness of the individual species. The reduction in leaf chlorophyll content of plants growing on more polluted site might be due to adverse effect of vehicular pollution on the plant physiology. Maximum reduction in leaf chlorophyll content exhibited by *Sambucus chinensis* can be ascribed to sensitiveness of the species to particulate pollutants, while least reduction in chlorophyll content by *Bougainvillea glabra* suggests that it is a tolerant species and can tolerate the air pollutants.

Conclusions

The five shrub species growing along the roadside of Kathmandu Valley differ in their responses to the particulate dust pollution. The decrease in chlorophyll content in samples collected from polluted site as compared to those collected from the control site indicates that plants along the roadside of Kathmandu Valley are greatly influenced by air pollution. The dust and vehicular emissions have differentially influenced chlorophyll content (chlorophyll-a, chlorophyll-b and total chlorophyll) of plant species growing at different polluted sites in different seasons. Plants growing at the heavily polluted site exhibit highest effects of pollution. However, plants in such areas exhibit higher leaf chlorophyll content during favorable growing conditions of summer season. Out of five species, *Bougainvillea glabra* is tolerant and *Sambucus chinensis* is more sensitive to air pollution.

Acknowledgements

We are grateful to Prof. Mohan Siwakoti, former Head of Central Department of Botany, Tribhuvan University for providing laboratory facilities. This work was supported by the University Grants Commission, Kathmandu, Nepal.

References

Agbaire P.O. and Esiefarienrhe E. 2009. Air pollution tolerance indices (APTI) of some plants around Otorogun gas plant in Delta State, Nigeria. *Journal Applied Science Environment Management*, 13: 11–14.

Amulya L., Kumar N.K.H. and Jagannath S. 2015. Air pollution impact on micro-morphological and biochemical response of *Tabernaemontana divaricata* L. (Gentianales: Apocynaceae) and *Hamelia patens* Jacq. (Gentianales: Rubiaceae). *Brazilian Journal of Biological Sciences*, 2: 287–294.

Arnon D.J. 1949. Copper enzyme in isolated chloroplast polypheoloxidase in *Beta vulgaris*. *Plant Physiology,* 24: 1–15.

CBS. 2011. *Population Census 2011 of Nepal*: *National Report.* Central Bureau of Statistics, Kathmandu, Nepal.

Das S. and Prasad P. 2010. Seasonal variation in air pollution tolerance indices and selection of plant species for industrial area of Rourkela. *Indian Journal of Environment protection,* 30: 978–988.

Giri S., Shrivastav D., Deshmukh K. and Dubey P. 2013. Effect of air pollution on chlorophyll content of leaves. *Current Agriculture Research Journal*, 1: 93–98.

GoN. 2017. Air *Quality Management Action Plan for Kathmandu Valley*. Department of Environment, Ministry of Population and Environment, Government of Nepal, Kathmandu.

Joshi P.C. and Swami A. 2009. Air pollution induced changes in the photosynthetic pigments of selected plant species. *Journal of Environmental Biology*, 30: 295–298.

Katiyar V. and Dubey P.S. 2000. Growth behavior of two cultivars of maize in response to SO_2 and NO_2. *Journal of Environmental Biology*, 21: 317–323.

Kershaw K.A. and Webber M.R. 1986. Seasonal changes in the chlorophyll content and quantum efficiency of the moss *Brachythecium rutabulum*. *Journal of Bryology*, 14: 151–158.

Mir Q.A., Yazdani T., Kumar A., Narain K and Yunus M. 2008. Vehicular pollution and pigment content of certain avenue trees. *Pollution Research*, 27: 59–63.

Mishra S.S., Mishra K.N.and Mahananda M.R. 2013. Chlorophyll content studies from inception of leaf buds to leaf-fall stages of teak (*Tectona grandis*) of Kapilash Forest Division, Dhenkanal, Odisha. *Journal of Global Bioscience*, 2: 26–30.

Ottander C., Campbell D. and Öquist G. 1995. Seasonal changes in photosystem II organization and pigment composition in *Pinus sylvestris*. *Planta*, 197: 176–183.

Prajapati S.K. and Tripathi B.D. 2008. Seasonal variation of leaf dust accumulation and pigment content in plant species exposed to urban particulates pollution. *Journal of Environmental Quality*, 37: 865–870.

Rout J., Dubey U. and Upreti D.K. 2010. A comparative study of total chlorophyll content and chlorophyll degradation of some lichens in disturbed and undisturbed sites along town, West Siand District, Arunachal Pradesh. *Biological and Environmental Sciences*, 6: 46–51.

Sapkota B.K. and Dhaubhadel R. (2002). Atmospheric turbidity over Kathmandu Valley. *Atmospheric Environment,* 36: 1249–1257.

Singare P.U. and Talpade M.S. 2013. Physiological responses of some plant species as a bioindicator of roadside automobile pollution stress using the air pollution tolerance index approach. *International Journal of Plant Research*, 3: 9–16.

Stevovic S., Mikovilovic V.S. and CalicDragosavac D. 2010. Environmental impact on morphological and anatomical structure of Tansy. *African Journal of Biotechnology*, 9: 2413–2421.

Weng J.H., Liao T.S., Sun K.H., Chung J.C., Lin C.P. and Chu C.H. 2005. Seasonal variations in photosynthesis of *Picea morrisonicola* growing in the subalpine region of subtropical Taiwan. *Tree Physiology*, 25: 973–979.

Wolfenden J. and Mansfield T.A. 1990. Physiological disturbances in plants caused by air pollutants. *Biological Sciences*, 97: 117–138.