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Research article

Impact of dust accumulation on three roadside plants and their adaptive responses at National Highway 37, Assam, India

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Abstract: Roadside plants are consistently exposed to dust. Deposition of dust on roadside plant and its impact on leaf epidermal traits as well as leaf pigment concentration, the percentage of carbon and nitrogen has been studied of three roadside plants *i.e. Cassia alata, C. tora* and *C. sofera* at National Highway - 37, Assam. Significant variation in terms of dust deposition with species specific result observed during the study. Declination of leaf pigment concentration, leaf area and nitrogen percentage along with increased leaf thickness indicate the dust pollution impact. Above findings may be helpful to find out some representative species for developing some model to cope with automobile generated dust pollution in future.

Keywords: Dust pollution - Roadside plant - Chlorophyll - Leaf epidermal traits - *Cassia* spp.

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INTRODUCTION

Dust is an important abiotic factor and has a key influence upon the organism. Sever pressure of dust may be responsible for altering bio-chemical as well as morphological setup of an organism which further initiated adaptive evolution to cope with the changing environment. Based on the source of generation as well as the structure of road, the role of dust may be variable (Farmer 1993, Anthony 2001). In an urban area, road dust contains a mainly huge amount of different metals as well as a small amount of clay and minerals too (Beckett *et al*. 2000). Vehicles are the prime source of dust generation for roadside plants. Poor road infrastructure, the frequency of running vehicles in terms wheel determine the rate of dust generation. Therefore, adaptive evolution of roadside plant species is more visible in poor road infrastructure with more running vehicles. The response of the plant to dust accumulation may vary according to different species, as dust deposition fluctuates with plant species due to leaf orientation, leaf surface geometry, phyllotaxy, epidermal and cuticular features, leaf pubescence, height and canopy of roadside plants (Davison & Blakemore 1976, Chaphekar *et al*. 1980, Farmer 1993, Chaturvedi *et al*. 2013) With the accumulation of dust, the roadside plant may exhibit adaptive response by changing morphological and physiological attributes.

A number of registered motor vehicles in India have reached from 0.3 million to 159.5 million from 1951 to 2012 (Report: Road Transport Year Book 2011–12, Government of India, Ministry of Road Transport and Highways Transport Research Wing, www.morth.nic.in). In Assam, vehicles are increased from 53, 4885 in 2000–01 to 1,98,4880 in 2013–14 indicating 73.05% boost of the growth of motor vehicle in the state. But the roads are limited and poor in condition (Source: Office of the Commissioner of Transport, Assam and Directorate of Economics and Statistics, Assam). National Highway - 37 (NH-37) is the backbone of road transport in Assam and the road starts from Panchatantra of Goalpara district of Assam to Roing of Arunachal Pradesh with a length of 740 km and considered one of the busiest road connecting all the major cities of the south of the Brahmaputra river.

In the present study, we emphasised the impact of dust deposition in terms of epidermal traits of leaf, chlorophyll contents, carbon and nitrogen percentage upon roadside plant and their adaptive responses to cope

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with the vehicular dust deposition. Three common shrub species of the genus *Cassia i.e. Cassia alata* L., *C. tora* L. and *C. sofera* L. selected to study the impact of vehicular dust and their adaptive response.

MATERIALS AND METHODS

CSIR-North East Institute of Science and Technology (CSIR-NEIST) is situated towards south to NH-37. A site on the NH-37 in front of CSIR-NEIST was selected to study vehicular load/ hour on NH-37 and the road was monitored continuously in a different time interval of the day up to one month. Total numbers of vehicles including two, three and >three wheelers (four wheelers and above) vehicles crossed from both sides has been counted and the average value was calculated per hour.

Based on the frequency of occurrence on the roadside of NH-37, 3 months old three plant species of the genus *Cassia i.e. Cassia alata* L. *Cassia tora* L. and *Cassia sofera* L. have been selected to study the accumulation of dust and its impact on the plants. Leaves were collected randomly of from plants with equal height from each species growing along with the roadside of NH-37, after the seasonal rainfall of Assam in April, 2015. Leaves from the three roadside plant species were collected in zipper pouches separately, in triplicate and brought to the laboratory for estimation of dust deposition. Each leaf was washed with distilled water using a spray bottle and suspended dust along with the water was collected carefully on pre-weighed Whatman's filter paper (pore size 110 mm). The filter papers were dried in an oven at 40–50 ºC for 2 hours to remove the water and weighed later to calculate the dust deposition in mg/cm^2 of the leaf. Washed leaves were blotted dry and then traced on graph paper to measure the total leaf area in mm^2 (Chaphekar *et al*. 1980, Vora & Bhatnagar 1986). Prominent secondary veins, as well as midrib of the leaf was avoided to reduce sample variation during measuring leaf area. Number of stomata and epidermal cell present per µm of each of the collected leaf from all the three plant species were calculated to estimate the Stomatal Index of the leaves. Mature leaf was fixed in FAA solution (acetic acid: alcohol: formalin: water = 2:5:1:12) for 24 hours followed by washing the leaf in 70% ethanol and then each leaf samples were cut out into circular form for easy peeling. Cut leaves were boiled in 5% aqueous solution of KOH for 5-10 minute and adaxial and abaxial surface was peeled and peeled section was stained with 1% safranin followed by temporary mounting in glycerin to observe the number of stomata as well as epidermal cell under the microscope (Leica DM-3000 LED, Made in Germany) (Satao *et al*. 1993). At least three microscopic observations at 20X were considered for counting. Stomatal index (I) was calculated by the following formula (Meidner & Mansfield 1968),

$$I = \frac{S}{(S + E)}$$

Where, (S) = Numbers of stomata, (E) = Numbers of epidermal cells present in a unit area^2

Leaf pigments *i.e.* chlorophyll a, b and total chlorophyll concentration was counted with the method described by (Arnon 1949) and the pigment concentration was expressed in $mg.g^{-1}$ tissue. Carbon and nitrogen percentage in leaf were determined by the Perkin Elemental CHN analyser. Microsoft Excel 2010 version is used for analysis. Data were entered and arranged accordingly to calculate Standard Deviation (SD) and Standard Error (SE) from different replicated samples (Federrer 1947). One way ANOVA is using with Sigma Plot statistical software v13 (Jandel Scientific, San Rafael, CA). All groups were compared using the Student-Newman-Keuls *post hoc* analysis method. The *p* value less than 0.05 was considered to be statistically significant.

RESULTS

National Highway - 37 is one the one of the busiest roads of communication, connecting maximum numbers of cities of Assam. The road bears the vehicular load of an average of 580 numbers of vehicles *i.e.* two, three and >three wheelers (four wheelers and above) per hour. More than three wheelers vehicles were more frequent followed by two and three wheeler vehicles on the NH-37 (Fig. 1A). Irrespective of plant species, deposition of dust on leaf of roadside plant was found to increase significantly with time. Amount of dust deposited in the leaf of *C. tora* was maximum followed by leaf of *C. sofera* and *C. alata* during 1st and 2nd week (Fig. 1B). However, during 3rd and 4th week dust deposition was found higher in *C. sofera* followed by *C. tora* and *C. alata*.

Leaf chlorophyll is an important index to measure the impact of dust on the roadside plant species. Leaf pigment concentration *i.e.* chlorophyll was found to decreased considerably in the roadside plant species compare to control. It is evident from table 1 that roadside plant produces less chlorophyll a, chlorophyll b and total

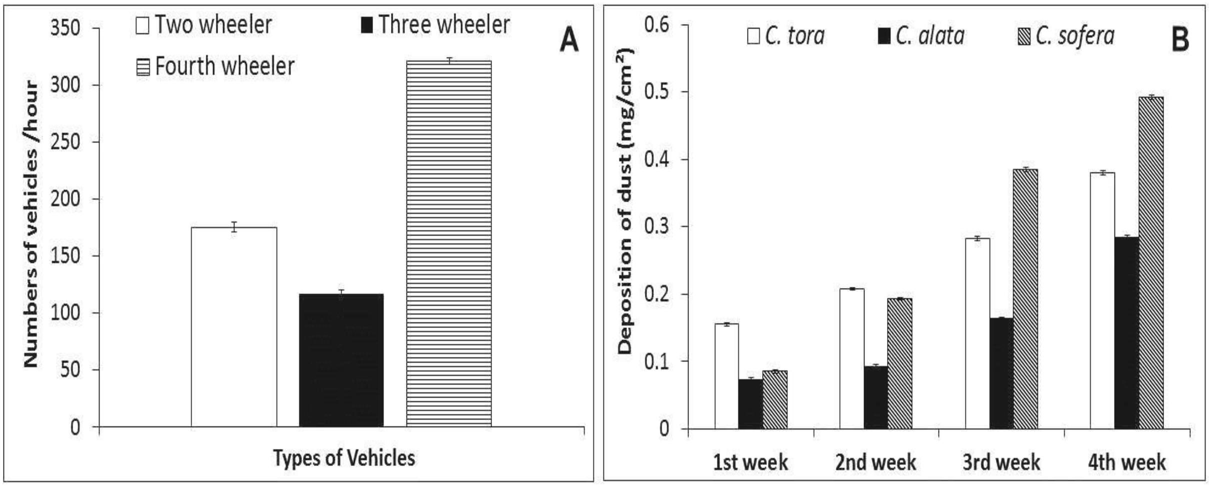

Figure 1. A, Average numbers of different vehicles per hour at NH-37; **B,** Dust deposition (mg/cm^2) on the leaf of *Cassia tora, C. alata* and *C. sofera*.

Table 1. Chlorophyll (chlorophyll a, b and total) contents of leaves of three different *Cassia* species in different sites.

Species	Site	Chlorophyll a (mg.g^{-1})	Chlorophyll b (mg.g^{-1})	Total
Cassia sofera	**Control**	1.87^{a}±0.026*	0.89^{A}±0.012*	2.47^{aa}±0.023*
	NH-37	1.57^{b}±0.012	0.87^{A}±0.022	2.21^{bb}±0.025
	Reduction (%)	16.04	2.24	10.52
Cassia tora	**Control**	1.88^{c}±0.041	1.05^{B}±0.032	2.62^{cc}±0.030
	NH-37	1.43^{d}±0.020	0.86^{C}±0.008	2.12^{dd}±0.032
	Reduction (%)	23.93	18.09	19.08
Cassia alata	**Control**	1.94^{e}±0.027	0.92^{D}±0.010	2.91^{ee}±0.011
	NH-37	1.55^{f}±0.031	0.73^{E}±0.009	2.31^{ff}±0.013
	Reduction (%)	20.1	20.62	20.61

Note: *Values are mean ± SE (n = 10). Each rows having different letters are significant at p<0.05 level.

chlorophyll than the control. The percentage of reduction of chlorophyll content was found highest in *C. alata* plant species. Reduction of Specific Leaf Area (SLA), Stomatal index (adaxial and abaxial) and Leaf Dry Matter Content (LDMC) were observed in all the three plant species growing along the roadside of NH-37 compare to control (vehicle free zone) (Fig. 2A–D). All those parameters were different among all the three species even in control. However irrespective of plant species, the above mentioned leaf parameters were recorded lower in the leaf of roadside plant species. Maximum percentage of reduction of SLA was observed in the leaves of *C. sofera* (Fig. 2A), but highest percentage reduction of the stomatal index for both abaxial and adaxial surface of the leaves was recorded in *C. tora* plant. Similarly, *C. tora* also exhibited maximum percent reduction of LDMC (Fig. 2B–D).

On the other hand, leaf thickness of each plant species from roadside was found to increase in compare to control (Fig. 2E). Leaf thickness of *C. tora* was significantly higher than rest of the two plant species, but highest percentage increase was recorded in *C. sofera*. Nitrogen content of plant is a limiting resource for plant growth, reproduction and defence. Roadside plant exhibited less nitrogen with highest reduction in *C. sofera* followed by *C. alata* and *C. tora* (Fig. 3A). However, dust exposure roadside plant presenting higher accumulation of carbon content in all studied plant and highest percentage of increase was recorded in *C. sofera* plant followed by *C. alata* and *C. tora* (Fig. 3B). It is evident from (Fig. 3A–B) that highest reduction of nitrogen of the plant is associated with the highest increase of carbon (*C. sofera*) and lowest nitrogen of the plant associated with lowest carbon (*C. tora*).

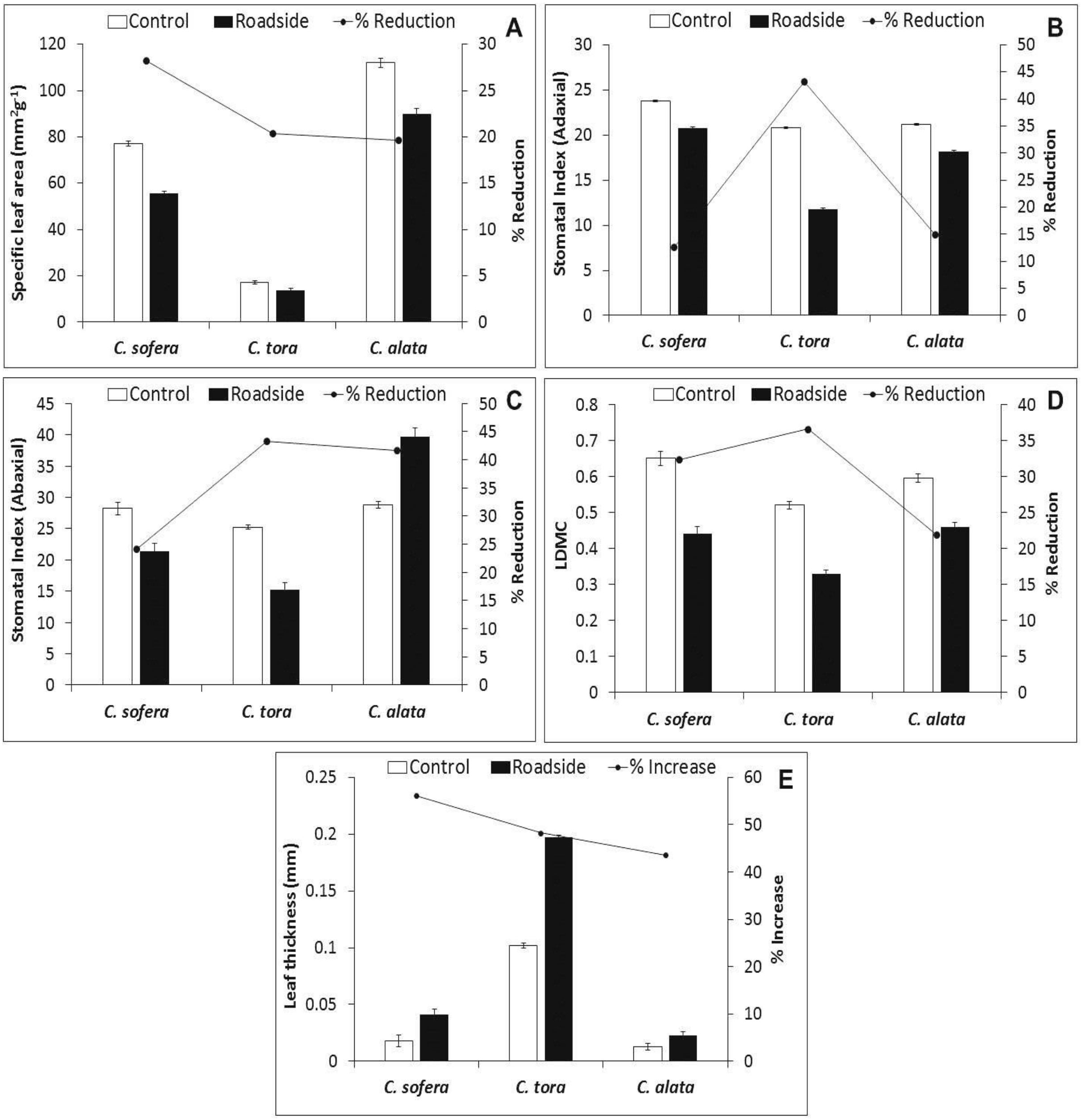

Figure 2. A, Specific leaf area (SLA); **B,** Stomatal index (Adaxial); **C,** Stomatal index (Abaxial); **D,** Leaf dry matter content (LDMC); **E,** Leaf thickness of leaf of the control and roadside plant of *Cassia tora*, *C. alata* and *C. sofera*.

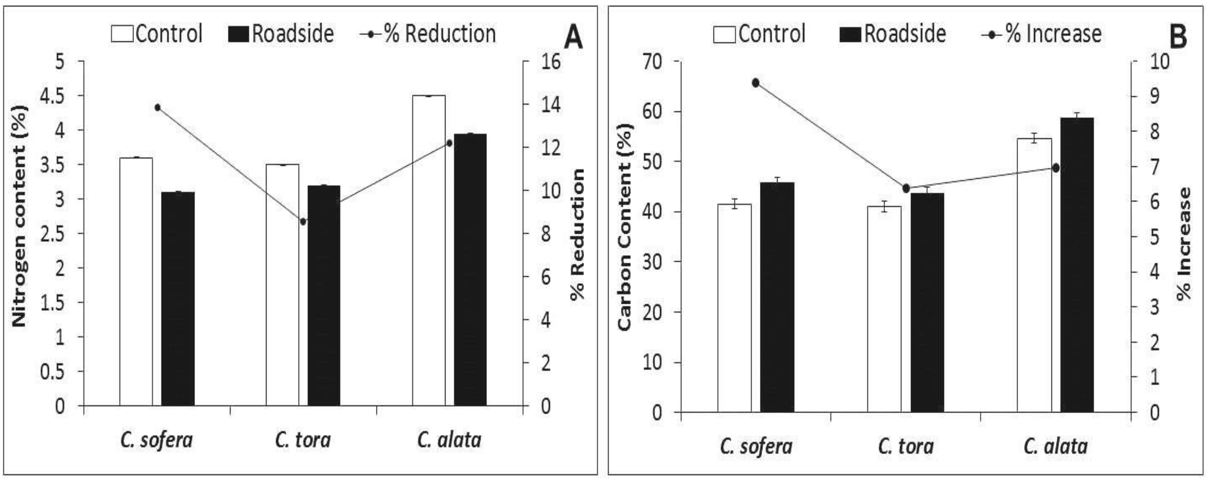

Figure 3. A, Nitrogen content (%); **B,** Carbon content (%) of the leaf of the control and roadside plant of *Cassia tora*, *C. alata* and *C. sofera*.

DISCUSSION

Rolling vehicles put force to the road surface and pulverizes the roadbed materials in the form of dust; eject it in shearing force and turbulent vehicle force (Khan *et al*. 2015). The pulverization of roadbed materials will be more in the poor road with more vehicles. NH-37 in Assam is poor in quality and >three wheelers (four wheelers and above) are dominant on the road. In poor road, more numbers wheels can faster dust generation. Roadside plants *i.e. Cassia sofera, C. tora* and *C. alata* of NH-37 are consistently exposed to dust. The dust particles settled on the leaf surfaces every day, but deposition was not uniform in all the plant species. Settlement of dust particle on the leaf surface mainly depends on the phylotaxy of leaf, smoothness of the leaf surface, shape of leaf, petiole length as well as its position in the plant (Davison & Blakemore 1976). On the basis of leaf structure and texture, local air movement influence to dislodge deposited dust from the leaf. However, during the study period, local air movement was found to be insignificant. Therefore, variation of dust deposition was observed in leaves of different plant species. Dust deposition was also found to increase with time. Dust generated from day to day traffic activity will be accumulated on the leaf up to a limit. Hence, irrespective of plant species, dust deposition was found to increase in different time intervals. It was also observed that during the first two weeks, dust deposition was found highest on the leaf of *C. tora* followed by *C. sofera* and *C. alata*. But after 2nd week the pattern of dust deposition changed and highest accumulation was recorded in *C. sofera* then to *C. tora* and *C. alata*. Leaf structure and texture determine the limit of dust deposition. According to plant species, dust load per day may very *i.e.* initial rate of dust deposition may increase in some plant species and deposition rate decrease with time. Here the size of leaf and overall height of the plant species may also contribute. Leaf petiole and surface characteristics may be responsible for the higher rate of dust deposition in *C. sofera* during 3rd and 4th week. In addition to the leaf surface characteristics, the position of leaves on the plant and their orientation also crucial in terms of dust deposition.

Structural properties of the leaf are influenced by deposition of dust, which may lead to lower photosynthesis activity (Pourkhabbaz *et al*. 2010). All the studied roadside plant species showed reduction of chlorophyll contents (chlorophyll a, b and total chlorophyll) in compare to vehicle free site (control). Dust particles physically obstruct sunlight as well as block stomatal pore of leaf and thus dust deposition hampered photosynthetic activities of plant (Nicholson *et al*. 1989, Keller & Lamprecht 1995, Pandey & Kumar 1996, Hope *et al*. 1999, Manno *et al*. 2006). Shading effect of dust as well as alkaline condition caused by dissolution of dust particles in cell sap degrade leaf pigment concentration has been reported earlier (Prajapati & Tripathi 2008a). Degradation of chlorophyll contents in the leaf is a common phenomenon from dust deposition on leaf (Prajapati & Tripathi 2008b). A plenty of studies (Satao *et al*. 1993, Prusty *et al*. 2005, Gostin 2009) reviewed declination of chlorophyll concentration due to deposition of dust in a number of the annual non-leguminous crop.

SLA which is a supposed to be an indicator of plant morphology was found to decrease in roadside plant species in compare to control. Among the roadside plant species, *C. sofera* exhibited maximum reduction in SLA. Reduction in SLA specifies poor physiological as well as the biochemical status of the plant. Strong negative correlation has been reported earlier in between dust load and SLA (Raupach *et al*. 2001, Chaturvedi *et al*. 2013). Continuous exposure to dust in leaf surface leading to the formation of dense dust layers, which reduce light capturing capacity of plants (Prajapati & Tripathi 2008a, Pourkhabbaz *et al*. 2010) and finally hamper plant photosynthetic activity. Light capturing capacity of leaf via lowering photosynthetic rates, clogged stomata and increased foliage temperature are the cause behind dust deposition reported earlier (Anthony 2001). Stomatal index has been proven to be an indicator of environmental stress (Gostin 2009). Decreased rate of photosynthesis and alteration of stomatal conductance are responsible for reduction of the stomatal index as well as dry matter content of leaf (Khan *et al*. 2015). Reduction of the stomatal index (abaxial and adaxial) of the roadside plant may be due to shading effect of dust layers, which may block the stomata and reduce the photosynthesis rate of roadside plants. The Same impact also reflected on reduced leaf dry matter content. Reduction percentage of the stomatal index and leaf dry matter were found higher in *C. tora* plant compared to other two roadside plant species. It was already evident that leaf of *C. tora* accommodated more dust in first two weeks, hence initial response of reduction of stomatal index and leaf dry matter may be higher than the other two plant species. Leaf thickness was found to increase in all the roadside plants; however, maximum leaf thickness was observed in *C. sofera* than other two plant species. It is also evident that plant under the environmental stress, produces thicker leaves as an adaptive response (Satao *et al*. 1993, Hope *et al*. 1999, Gostin 2009) and to cope with the stress of vehicular dust, roadside plants may produce thicker leaves.

Roadside plants indicate a reduction in the percentage of nitrogen in compare to control. Actually, dust deposition alters the photosynthesis capacity and hampered the overall growth and development of the plant (Thomson *et al*. 1984). Therefore, those plants are unable to uptake required nitrogen under such condition. Accumulation of carbon was more resulting higher carbon percentage in plant tissue. It may be a part of plant defence mechanisms because under stress condition plant produces more carbon based secondary metabolites. Finally, present study suggested that alteration of leaf chemistry may be the result of heavy dust load which lowered the nitrogen concentration in plant tissue and greater carbon uptake as a part of the adaptive response.

CONCLUSION

Present work provides basic information about the variation in dust accumulation of three roadside plant species *i.e. Cassia sofera* then to *C. tora* and *C. alata*. Variation of dust loads positively correlated with the alteration of biochemical and epidermal constituents of the leaf. Significant variation in terms of dust deposition with species specific effect observed during the study. In future, to monitor dust fall at roadside areas these representative plant species can be used as an indicator.

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