

Effect of Vehicular Exhaust Upon Roadside Plant, *Cassia tora* L. on NH-37, Assam, India

BITOPAN SARMA^{1,2}, SANJAY KUMAR CHANDA², MUNMI DUTTA³, PRANAB RAM BHATTACHARYA², MANTU BHUYAN*

¹Academy of Scientific and Innovative Research, CSIR-North East Institute of Science and Technology Campus, Jorhat, Assam 785006, India.

^{2,3} Medicinal Aromatic and Economic Plant Group, CSIR-North East Institute of Science and Technology, Jorhat, Assam 785006, India.

Emails: B. Sarma b.porasar@gmail.com; S.K. Chanda chandaihbt@yahoo.co.in; M. Dutta munmi.manu@gmail.com; P.R. Bhattacharya pranabramb@yahoo.com; M. Bhuyan mantubhuyan@gmail.com

* Author for correspondence

ABSTRACT

We studied the impact of vehicle exhausts on locally available common weed *Cassia tora* L. throughout the roadsides of National Highway (NH 37) in Assam, India. Results showed deterioration of pollen viability, stomatal density, chlorophyll content and specific leaf area. However significant increase was observed in leaf thickness and trichome density. Based on the consistent influence of vehicular exhaust upon some important leaf characteristics, the plant may be considered as an indicator to monitor such pollution in future.

Key Words: Roadside; Pollution; Vehicle Exhausts; Indicator; *Cassia tora*.

INTRODUCTION

Increasing numbers vehicles in most of the populated city creates havoc situation and a cause of concern for environmentalist. Vehicular emission is a major source of air pollution, contributing 57%–75% of total air pollution (WHO 2006). Air pollutants from motor vehicle exhausts have both direct and indirect effects on the metabolism of roadside plants (Viskari et al. 2000). Being immobile characteristics plants are the first victim under pollution stress condition and use of plants in monitoring air pollution also been established long day back (Darrall 1989). Roadside plants exposed to road traffic emissions exhibit changes in foliar anatomy and morphology (Joshi and Abhishek 2007, Verma et al. 2006). These changes may be an adaptive strategic response of roadside plant to consistent pressure of vehicular emission.

Number of registered motor vehicle in Assam has increased by 52% during 2005-06 to 2010-11, according to the Directorate of Economics and Statistics, Assam.

National Highway-37 of Assam is a major transport corridor of the State as it passes through all major cities of Assam including Guwahati and Jorhat. NH-37 bears the load of almost all vehicles of the region and hence, vehicular emission is significant in and around the highway. Being the populous cities, the vehicles are more concentrated in the highway close to Guwahati and Jorhat cities and are regarded as important traffic contaminated cities of Assam, causing heavy vehicular pollution in the air, containing various pollutants like SO₂, CO₂, hydrocarbons and solid particulate matter. Plants growing along the road sides are constantly exposed to these pollutants. Very little information is available on the impact of these pollutants on plants.

In the present study, we investigated the combined impact of CO₂ and SO₂ on *Cassia tora* L. (now recognised as *Senna tora* (L.) Roxb.) - a common roadside plant of Assam and examined its potential as a bio-indicator of vehicular exhaust. The study may help understand the adaptive responses of the plant under such conditions.

STUDY AREA

The present study was conducted in Guwahati (26° 06' 48" N, 91° 44' 42" E, 57 m above mean sea level (msl), Jagiroad (26° 07' 33" N, 92° 13' 33" E, 63.7 m above msl), and Jorhat (26° 45' 09" N, 94° 10' 57" E, 89.3 m above msl) along the NH-37, in Assam as polluted sites and at Cholahora - a site without vehicular exposure and located away from the NH-37, termed as control (26° 45' 47" N, 94° 11' 46" E, 91 m above msl). The different sites of NH-37 were selected based on traffic load. The National Highway 37 is one of the busiest roads of surface communication, connecting maximum numbers of cities of Assam. The road bears the vehicular load of an average of 580-600 vehicles including two- and three-wheelers besides cars and trucks per hour.

MATERIALS AND METHODS

The Plant

To examine the impact of vehicular exhaust, *Cassia tora*, a common roadside herb belonging to family Caesalpinaceae was selected. Low height of this plant is ideal for constant exposure to vehicular emission throughout its growing season. The plant is distributed all over India. It grows profusely during rainy season mostly in roadside and wasteland areas under subtropical or tropical climatic condition. This plant is about 30-90 cm in height with green leaves and produces pale yellowish flowers. In Assam, the plant survives from April to November and comes to bloom during June-July. Seeds mature during October- November.

Sampling Methodology

CO₂, SO₂, temperature and humidity were measured at all study sites from May to October 2014. Measurements were taken by SO₂ Mobile Sensor, CO₂ Mobile Sensor and hygrometer (Testo, Germany). Total chlorophyll content of the selected plant was estimated by the method of (Arnon 1949). One gram fresh leaf was macerated with 80% (v/v) chilled acetone and a pinch of magnesium carbonate in a pre-chilled pestle and mortar. The extract was centrifuged at 5000 rpm for 10 minutes. The process was repeated till the extract becomes colourless and the extracts were pooled and the volume was made up to 10 mL. All operations were carried out in the ice bath under dark condition. The absorbance was

measured at 645 nm and 663 nm using spectrophotometer (Shimadzu UV 220 Spectrophotometer). Total chlorophyll was calculated using the following formula:

$$\text{Total Chlorophyll} = 20.2(A_{645}) + 8.02(A_{663}) \times V \div (1000 \times W),$$

where V = 10 mL and W = 1g

Leaves of the plant were collected randomly from the four study sites. Leaf epidermis were removed by peeling method and observed under the microscope at 40X resolution (Leica DM 3000 LED). The number of stomata and the number of epidermal cells per unit area of leaf epidermal peels were counted under the microscope. Stomatal index (SI) was calculated as per the following formula (Salisbury 1972)"

$$\text{SI} = \left[\frac{\text{Stomatal Density}}{\text{Epidermal cell density} + \text{Stomatal Density}} \right] \times 100$$

For the measurement of Specific Leaf Area (SLA), Leaf Dry Matter Content (LDMC) and leaf thickness, twigs with leaves of *C. tora* were collected at a fixed time, i.e. at 9 o'clock. in the morning from all the sites. Collected twigs were covered within polybags and transferred to the lab as soon as possible. The cut end of the twigs was then dipped in distilled water for 6 hour at 15°C. To estimate SLA, individual leaves were placed on graph paper and leaf area of each leaf was calculated on the basis of area (mm²) covered. SLA was calculated as the ratio between leaf area and LDMC which was calculated as the ratio between leaf dry mass and leaf fresh mass. We used standardized protocol provided by Garnier et al. (2001). For the measurement of trichomes, leaves were collected randomly from the study sites. A leaf disc (area = 0.29 cm²) was taken by hole punch from the centre of the leaf blade and the number of adaxial trichomes was counted under a dissecting microscope (Zeiss, Jena, Germany). Trichome density (TD) was calculated as the trichome number per square of millimetre. The mean values of TD were calculated from five replications of each leaf, and then averaged to determine the mean values for each plant. To study pollen viability, flower as well as flower buds were collected at 9-11 AM from the study sites and anthers were dissected out under stereo zoom microscope and fixed for 3 days in FAA (formaldehyde: glacial acetic acid : 50% ethanol in 1:1:18) solution. After fixation, anthers were washed thoroughly in water and pollens were taken in a cavity

slide by puncturing the anther lobes. Pollens were then stained with Alexander stain for determining the viability. Slides were observed under microscope (Leica DM 3000 LED) and photographs were taken to calculate pollen viability. The pollen viability was calculated as percentage from the mean values obtained by sampling three replications from each site.

Statistical Analysis

Microsoft Excel 2010 version is used to calculate Standard Deviation (SD) and Standard Error (SE) from replicated samples. One way ANOVA was computed using Sigma Plot v13 (Jandel Scientific, San Rafael, CA). All groups were compared using the Student-Newman-Keuls *post hoc* analysis method. The *p* value <0.05 was considered to be statistically significant.

RESULTS

Air Quality

Air quality varies according to the number of vehicles. Highest aerial CO₂ concentration was observed in Guwahati (620 ppm) followed by Jagiroad and Jorhat, against only 393 ppm at the control site. With the increasing trend of CO₂, temperature also showed positive correlation at each site (Table 1). Guwahati was the most SO₂ emitting site (6.1 ppm) followed by Jagiroad as compared to control.

Biochemical, Morphological and Physiological Traits of Leaf

Total chlorophyll content of leaf (Table 2) was observed to decrease the most in Guwahati and Jagiroad site (1.8 mg g⁻¹) followed by Jorhat (3.1 mg g⁻¹) as compared to

the control site (3.3 mg g⁻¹). Thickness of leaf also varied between sites (Table 2), with the thickest leaf (0.274 mm) at Jagiroad site. Pollen viability was lower at the industrialized and busy areas like Jagiroad, Guwahati and Jorhat (Figure 2) than at the control site (Table 2). Highest reduction of SLA was observed (Figure 1A) in Guwahati site followed by Jagiroad and Jorhat. Figure 1B shows that SI was higher in Guwahati site (53.7) than at the control site (28.40). Trichome density was maximum in Guwahati roadside areas (Figure 1C) followed by Jagiroad and Jorhat sites (Figure 1D). LDMC was higher (Figure 1D) at the Jagiroad site (0.341) followed by Guwahati and Jorhat than at the control site.

DISCUSSION

Vehicular emissions released due to burning of fossil fuels add CO₂ and SO₂ in to the air. Dispersion pattern of pollutants relay the concentration of pollutant depends on the numbers of vehicles involved, with increasing numbers of vehicle the load of emission increased significantly causing serious threat to the environment. Air of metropolitan cities in developing countries is more vulnerable to vehicular pollution due to numbers of vehicles, traffic jam and lower level of regulations and monitoring.

Dispersion pattern and concentration of pollutants depends upon the numbers of vehicles run on the road and with increasing numbers of vehicle the load of emission increased significantly. Air of metropolitan cities in developing countries are more vulnerable to vehicular pollution due to traffic jam, poor quality of road and lack of proper regulations monitoring system. In present study, aerial carbon dioxide and sulphur dioxide throughout the NH-37 from Jorhat to Guwahati were documented above the ambient level (Table 1).

Table 1. Carbon dioxide, sulphur dioxide concentration, humidity and temperature of the air of different study sites

Study sites		CO ₂ (ppm)	SO ₂ (ppm)	Humidity (%)	Temperature °C
N 26°45' 47", E 94°11' 46"	Control	393 ^a ±0.023	0.1 ^a ±0.004	63.7 ^a ±0.11*	21.2 ^a ±0.22
N 26°45' 09", E 94°10' 57"	Jorhat	438 ^b ±0.12	0.2 ^b ±0.004	56.8 ^b ±0.14	23.2 ^b ±0.20
N 26°07' 33", E 92°13' 33"	Jagiroad	588 ^c ±0.45	2.7 ^c ±0.008	52.8 ^c ±0.14	24.1 ^c ±0.20
N 26°06' 48", E 91°44' 42"	Guwahati	620 ^d ±0.21	6.1 ^d ±0.002	48.2 ^d ±0.19	26.7 ^d ±0.24

*Data represented as a mean ± standard error (SE) and each column having different letters are significant at *p*<0.05 level.

Table 2. Morpho-physiological characteristics of *Cassia tora* at different study sites

Location	Total chlorophyll (mg g ⁻¹)	Thickness (mm)	Stomatal density (No. mm ⁻²)	Pollen viability (%)
Control (Non-polluted)	3.3 ^a ± 0.022	0.205 ^a ± 0.001	183	100
Jorhat	3.0 ^b ± 0.049*	0.223 ^b ± 0.007	246	26.25
Jagiroad	1.8 ^c ± 0.008	0.274 ^c ± 0.008	300	20.75
Guwahati	1.8 ^c ± 0.046	0.232 ^d ± 0.006	383	21.25

*Data represented as a mean ± standard error (SE) and each column having different letters are significant at p<0.05 level.

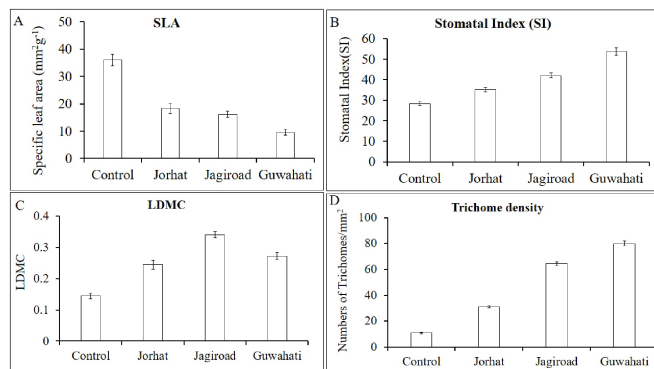


Figure 1. A. Specific Leaf Area (mm² g⁻¹), B. Stomatal Index, C. Trichome Density (no. mm⁻²), and D. Leaf Dry Matter Content of *C. tora* at four roadside sites in Assam. Vertical lines on bars represent ± SE.

Higher level of carbon dioxide was recorded at Guwahati area. Chlorophyll content of the plant was found less in the study site, indicating lower photosynthesis and lower absorption of CO₂ from air by the plant. Guwahati is the largest city of North East with high intensity of vehicular activities. Presence of higher level of aerial sulphur dioxide in and around Guwahati reflects the vehicular load in the city. Temperature was also recorded higher in all the study area. Carbon dioxide is associated with elevated temperature as the gas absorbs infrared part of solar radiation. Present investigation revealed positive correlation of temperature with respect to elevated carbon dioxide concentration. Relative humidity was found lower in the study areas in comparison to control. Survival of plant is very critical under such abiotic stresses. Plant may adapt strategically by altering their physiological, morphological and biochemical setup in such situation. Present study revealed lower chlorophyll content in all the polluted sites i.e. 45% reduction of chlorophyll content in plant growing around Guwahati and Jagiroad in comparison to control site (3.3 mg g⁻¹). Reduction of chlorophyll concentration effects directly on plant growth (Pawar and Dubey 1985). It can be

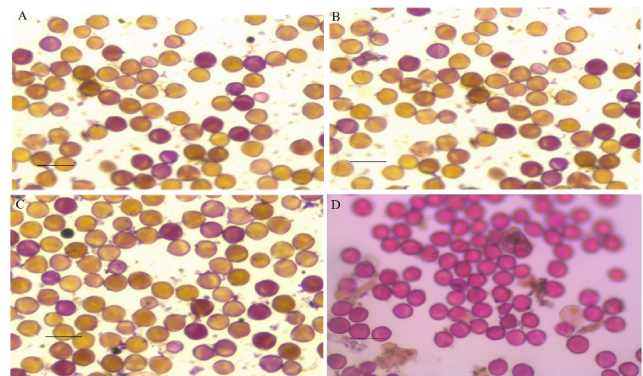


Figure 2. Viable (red) and non-viable (pale) pollen of *C. tora* at Jagiroad (A), Guwahati (B), Jorhat (C) and control (D) sites. Scale bar = 100 µm

assumed that higher aerial sulphur dioxide in the air of those sites may produce superoxide radicals by the reaction of sulphite with chlorophyll under illumination (Shimazaki et al. 1980). Byres et al. (1992) reported that reduced photosynthetic capacity and cell elongation mechanism in plant was found due to decrease in leaf area under the stress of air pollutants. Specific leaf area (SLA) of the plant was observed lower in all the polluted sites over control. Plants that were growing around Guwahati possess highest reduction (63%) of SLA followed by Jorhat and Jagiroad (Figure 1A).

It was widely reported (Pandey et al. 1994, Tiwari et al. 2006) that SLA decreases under pollution stress. Jahan and Iqbal (1992) also reported a reduction of leaf area as well as leaf length in some plants such as *Ficus benghalensis*, *Eucalyptus* sp. etc., growing on roadsides. Species of highly productive habitats have higher SLA that those in low productive habitats, although individual species sometimes deviated substantially from this general trend (Poorter et al. 1999). It can be summarised that vehicular emissions reduce the productivity of a habitat. Plants growing in roadside area of Jagiroad had highest leaf thickness (0.25 mm) while the leaf thickness

was lowest at the control site. Thicker leaves are formed due to development of palisade layers induced by elevated CO₂ and temperature (Thomas et al. 1983, Ferris et al. 1996, Pandey et al. 2007). Lower SLA and increased leaf thickness may enhance dry matter content of leaf. In our observation, increased LDMC was seen in every location other than the control. LDMC was observed to increase under elevated CO₂ due to the partitioning of greater amounts of assimilated carbon towards the growing organs and the extra carbon fixed by the plants due to CO₂ enrichment translocate to the growing axis (Overdieck et al. 1986, Sharma et al. 1990). SD is proportional to photosynthesis rate of plant and photosynthesis rate increases with elevated CO₂ concentration (Porter 1993, Zhu et al. 1998). In our study also, the SD and SI increased at the polluted sites (Table 2, Figure 1B).

Being more sensitive pollen grains are supposed to be the indicator of aerial pollutants. Gottardini et al. (2004) reported that pollen can measure the biological impact of pollutants. Significant decline of pollen viability in *C. tora* at the polluted sites (only 20.7% viability at Jagiroad) indicates a negative effect upon reproductive biology of the plant. Higher concentration of SO₂ in the air along with other vehicular pollutants may be responsible for this decline (Keller et al. 1984). Leaf trichome (LT) is considered as a defence entity in plants (Levin 1975). Increase in the number of trichomes would protect the leaf from direct exposure to pollutants. LT might act as a barrier to restrict the entry of dust particles into the mesophyll tissue through stomata. We observed the numbers of LT to be higher at polluted sites than the control site (Figure 1). Elevated levels of CO₂ may be responsible for the changes in LT numbers as greater amount of carbon will be available as a resource to the plants.

In conclusion, the adaptive responses of *C. tora* to vehicular exhaust indicate that the plant is suitable for monitoring air quality, particularly from roadside pollution. Widespread distribution of the plant in roadside areas offers an advantage of the plant being a bioindicator of such pollution.

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