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PHYSIOLOGICAL, BIOCHEMICAL AND DEFENSE SYSTEM RESPONSES OF *PARTHENIUM HYSTEROPHORUS* TO VEHICULAR EXHAUST POLLUTION

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Abstract

Pollution caused by vehicular exhaust emissions detrimentally affect plants and other living beings. This investigation was carried out to evaluate the effects of vehicular exhaust pollutants on *Parthenium hysterophorus* at various sites along two major roads [Pindi Bhattian to Lillah (M-2) and Faisalabad to Sargodha (FSR)]in the Punjab, Pakistan. Control samples of *P. hysterophorus* were also collected from 100m away from the roads. Chlorophyll contents, photosynthetic rate, transpiration rate, stomatal conductance, substomatal CO_2 concentration, water use efficiency, total free amino acids and total antioxidant activity of *P. hysterophorus* were measured. The results depicted significant reductions in chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents of *P. hysterophorus*. Likewise, reduction in stomatal conductance was also recorded which resulted in lowered photosynthetic and transpiration rates. The overall reduction in photosynthetic rate of *P. hysterophorus* was 30.92% and 35.38% along M-2 and FSR roads, respectively. The limited photosynthesis resulted in increased levels of sub stomatal CO_2 concentration and water use efficiency. The elevated levels of free amino acids and total antioxidant activity were noted and could be attributed to activation of plant's defense system to cope with the deleterious effects of vehicular air pollutants. The significant correlations between various attributes of *P. hysterophorus* with traffic density signifies the stress caused by vehicular emissions.

Key words: Air pollution; Roadsides; Traffic density.

Introduction

Urban air pollution is a complex mixture of various pollutants, the concentration of which is not uniform, but the overall dominating feature of urban air pollution is vehicular traffic. The characteristics of pollution has changed significantly over the past decade. Pollution caused by vehicular traffic emissions has arisen as the main contributors of poor air quality (Aslam *et al.,* 2013). Over 90% of air pollution is attributed to vehicular emissions in cities (Anon., 2016). The major pollutants released by motor vehicles include nitrogen oxides (NOx), sulphur dioxide (SO_2), hydrocarbons (HC), carbon monoxide (CO), suspended particulate matter (SPM), volatile organic compounds (VOCs) and number of heavy metals (Wang and Xie, 2009; Bhandarkar, 2013). Previous studies have revealed that at higher concentrations, these pollutants present in exhaust emissions are toxic and have detrimental effects on plants, human health and ecology (Grantz *et al.,* 2003; Chen *et al*., 2015; Oyeleke *et al*., 2016).

Plants are the most important recipients of vehicular pollution, Since, they are static to their environment and continuously exposed to various pollutants from their surroundings. Many previous studies have reported a diversity of plant responses to vehicle emissions of different traffic densities. For example, photosynthetic activity and leaf senescence were greatly altered in *Quercus ilex* L. with increase in traffic density in Rome, Italy (Gratani *et al*., 2000). Similarly, a change in plant community composition was noted along roadsides in Germany (Bernhardt-Römermann *et al.,* 2006). In general, roadside pollutants greatly alter physiology of plants, leaf surface architecture (Verma & Singh, 2006) and cause reduction in growth, chlorophyll pigments, relative water content, ascorbic acid (Chauhan, 2010b), photosynthetic rate, transpiration rate, stomatal conductance (Nawazish *et al*., 2012) and total soluble proteins (Mir *et al*., 2008). Vehicular exhaust pollution also affects human health associated with headaches, asthma, respiratory and cardiovascular diseases (Kamal *et al*., 2015; Ierodiakonou *et al*., 2016; Requia *et al*., 2016). Ingestion or inhalation of particulate matter emitted by vehicles and consumption of plants produced in contaminated roadsides are two main factors responsible for human exposure to traffic pollution (Zhuang *et al*., 2008; Ogbonna & Okezie, 2011).

The impact of roadside pollutants on plants can be determined by biomonitoring, which is an important tool to evaluate the level of pollution in ecologically sensitive areas. Plants show different type of responses to various air pollutants. They show early warning signals of pollution trends, henceforth, they can be used to evaluate the air quality. For biomonitoring studies, previous researchers have used various plants species e.g. *Rosa rugosa* (Calzoni *et al*., 2007), *Mangifera indica* (Bamniya *et al*., 2012), *Eucalyptus camaldulensis* (Seyyednejad & Koochak, 2011) and other tree species (Carneiro *et al*., 2011). In the present study, *P. hysterophorus* was used to investigate the effects of roadside pollution due to its ubiquitous presence at all the site along M-2 (Pindi Bhattian to Lillah) and FSR (Faisalabad-Sargodha road), which are two major and heavily trafficked highways in the Punjab province. Therefore, the objectives of this study were to evaluate the adverse effect of roadside pollution on physiology of *P. hysterophorus.*

Materials and Methods

Description of study sites: Two heavily trafficked roads in the Punjab, Pakistan, i.e. Faisalabad Sargodha road (FSR) and Pindi Bhattian to Lillah road (M-2) were selected in order to evaluate the effect of air pollutants released by vehicles (Fig. 1). Five sites on each road were selected at a mean distance of 10 km between them. Roads were varied in the level of traffic density and vehicular type. The traffic on M-2 was comprised of buses, vans and cars, whereas, FSR remained busy round the clock with trucks, container loaders, buses, cars, rickshaws and animal carts. Therefore, the vehicular emissions are expected very high along both of these roads.

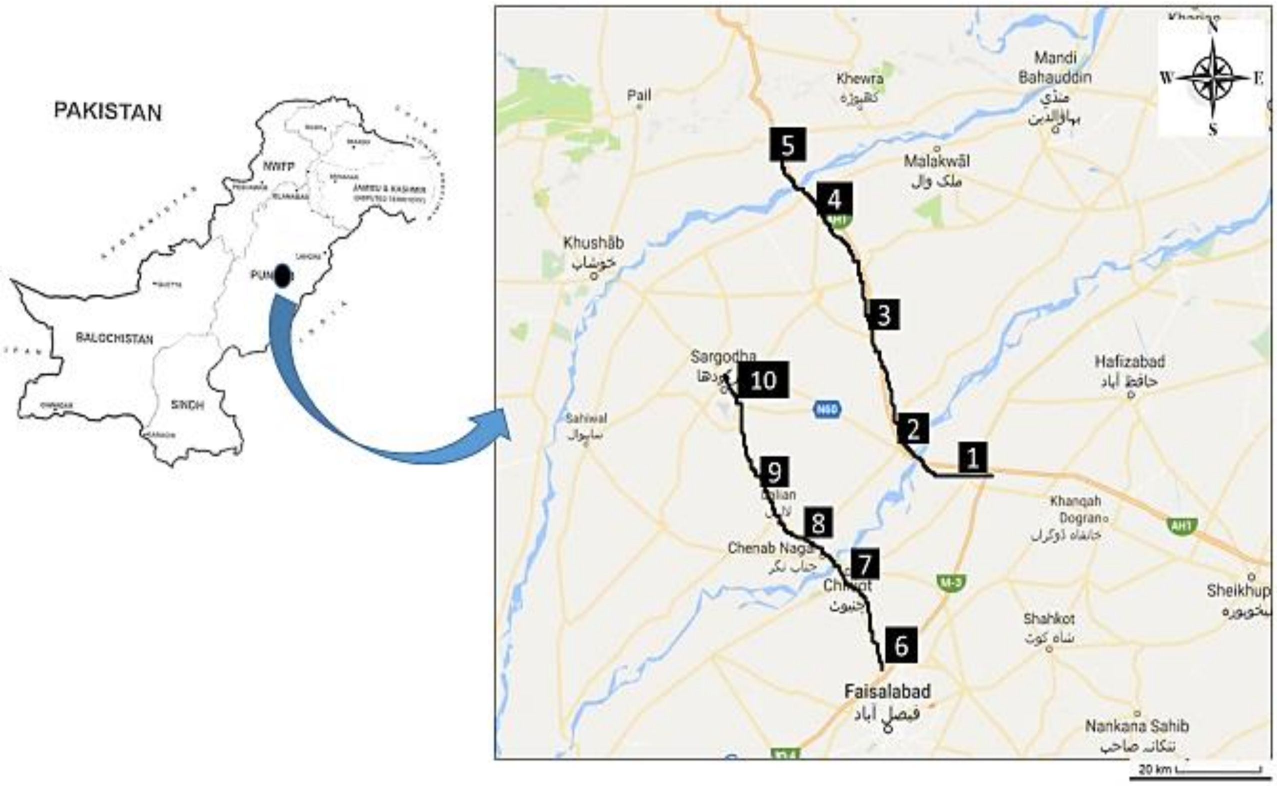

Fig. 1. Map showing sites on M-2 and FSR, where, 1. Pindi Bhatian; 2. Kot Momin; 3. Salim; 4. Bhera; 5. Lillah; 6. Pull Dingro; 7. Chiniot; 8. Chenab Nagar; 9. Adda-46; 10. Pull-111.

***Parthenium hysterophorus*:** *P. hysterophorus* L. is an annual herb which aggressively invades disturbed lands and roadsides. It is invasive species in Pakistan and native to North America and Mexico. It is a strong competitor, highly drought tolerant, thermo insensitive and have high seed producing ability. Due to its properties and wide adaptability along roadsides makes it an excellent tool to determine the effect of roadside pollution.

Collection of samples: Three leaves were selected from the middle of the plant randomly at a distance of 1-2m away from the road edge. Control plants samples were also collected 100 m away from road (Ma *et al*., 2009). The plant samples were packed in labelled plastic bags, placed immediately in an iced cooler and brought to the laboratory for further analysis.

Photosynthetic pigments: The photosynthetic pigments i.e. chlorophyll *a*, *b* and total chlorophyll contents were calculated according to the method of Arnon (1949) and carotenoid contents were measured by following Davis (1976). Measurements were taken on UV-visible spectrophotometer (IRMECO U2020).

Gas exchange characters: Gas exchange parameters i.e. photosynthetic rate (*A*), stomatal conductance (g_s), transpiration rate (*E*), and sub stomatal CO_2 concentration (*Ci*) were measured from fully expanded young leaves using LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddeson, England. Model C1-340). All the measurements were taken between 10:00 am - 02:00 pm. The adjustments/ specifications of IRGA were as follows: leaf surface area 11.35 cm^2, temperature of leaf chamber (Tch) varied from 29.2 to 37.5°C, ambient CO_2 concentration (Cref) 349.12 $\mu molmol^{-1}$, ambient temperature ranged from 31-36°C, leaf chamber volume gas flow rate (v) 397 ml min^{-1}, water vapor pressure in chamber ranged from 6-9.0 m bar, molar flow of air per unit leaf area (Us) 401.06 $molm^{-2}sec^{-1}$,ambient pressure (P) 99.95 KPa, PAR (Q leaf) at leaf surface was upto 1515 $\mu molm^{-2}$.

Total free amino acids and Total antioxidant activity: Total free amino acids were analyzed by following Hamilton and Van- Slyke (1943). A standard curve was made with leucine. On spectrophotometer (Hitachi 220, Japan), optical density of the solution was noted at 570 nm and total free amino acids were calculated by the following formula given below:

$$\text{Total amino acid} = \frac{\text{Graph reading of sample X volume of sample X dilution factor}}{\text{Weight of fresh tissue X 1000}}$$

For measuring total antioxidant activity, dried plant sample (1 g) was taken in test tube and 20 mL of 0.45% salt solution was added to it. Heated in the water bath at 40°C for 20 mins. The solution was centrifuged for 30 minutes at 3000 rpm. Supernatant was collected and stored at -20°C before commencing the experiment. Ferric Thiocyanate (FTC) method was used for estimation of total antioxidant activity by following Rahmat *et al.* (2003).

Traffic density: Traffic density was noted at each study site along M-2 and FSR roads for two hours. Traffic data was also obtained from toll plazas along both roads.

Statistical analysis: One-way Analysis of Variance (ANOVA) was used to analyze data through COSTAT software (Cohort software, Berkeley California, USA). LSD test at 0.05 significance level was used to compare means (Liu *et al*., 2014). Pearson's coefficient was used to determine correlation between traffic density and various plant attributes (2-tailed test).

Results

A significant reduction ($p<0.01$) in chlorophyll *a* content of *P. hysterophorus* was recorded along both roads (Fig. 2). Along M-2, the maximum reduction (18.39%) in chlorophyll *a* of *P. hysterophorus* was recorded at Kot Momin site followed that (14.37%) recorded at Pindi Bhattian site. Similarly, along FSR, *P. hysterophorus* showed maximum (13.78%) reduction in chlorophyll *a* content at Adda-46 site as compared to control. Chlorophyll *b* contents were also got reduced significantly ($p<0.01$) in *P. hysterophorus* along both roads (Fig. 2a). At Kot Momin site along M-2, 38.03% reduction in chlorophyll *b* was noticed as compared to control, whereas, along FSR, maximum reduction (36.92%) in chlorophyll *b* content of *P. hysterophorus* was found at Adda-46 site followed by 27.80% reduction noticed at Pull-111 site. Likewise, highly significant ($p<0.01$) reductions in total chlorophyll and carotenoid contents were also noted in *P. hysterophorus* along both roads (Figs. 2b, c & d).

A highly significant reduction ($p<0.01$) in photosynthetic rate of *P. hysterophorus* was recorded at all the sites along both roads (Fig. 3a). Along M-2, the maximum reduction (43.46%) in photosynthetic rate of *P. hysterophorus* was noted at Salim site. In case of FSR, *P. hysterophorus* collected from Pull-111 and Adda-46 sites showed highest reductions in photosynthetic rates i.e., 49.71% and 43.75%, respectively. The reduction in transpiration rate of *P. hysterophorus* was however, non-significant along M-2, but along FSR, it showed highly significant ($p<0.01$) reduction with maximum (44.05%) reduction seen at Chenab Nagar site (Fig. 3b). Stomatal conductance in *P. hysterophorus* also got reduced highly significantly ($p<0.01$) along both roadsides as compared to control (Fig. 3c). However, a significant increase ($p<0.01$) in sub stomatal CO_2 concentration of *P. hysterophorus* was recorded along both roads (Fig. 3d). Along M-2, highest increase (62.41%) in sub stomatal CO_2 of *P. hysterophorus* was noted at Bhera site followed by a slight difference seen at Lillah site (59.07%). Likewise, along FSR, *P. hysterophorus* at Adda-46 and Pull-111 sites showed maximum increase in sub stomatal CO_2 concentration i.e. 64.30% and 62.95%, respectively. For water use efficiency of *P. hysterophorus*, sites along M-2 showed non-significant variation, whereas, a significant ($p<0.01$) variation among sites was found along FSR road (Fig. 3e).

P. hysterophorus at various sites along both roads showed highly significant ($p<0.01$) increase in free amino acids concentration (Fig. 4a). However, a higher free amino acids concentration was noticed in *P. hysterophorus* at sites along FSR as compared to M-2. Total antioxidants in *P. hysterophorus* were also got increased at different sites along both roads, though, the increase was statistically non-significant along M-2, whereas, it was highly significant ($p<0.01$) for FSR (Fig. 4b).

Strong negative correlation ($r= -0.942$) was found between photosynthetic rate of *P. hysterophorus* and traffic density on FSR road (Table 1). Similarly, chlorophyll pigments also showed negative correlation with traffic density on FSR. A significant positive correlation was seen for free amino acids, total antioxidants activity and sub stomatal CO_2 concentration with traffic data on FSR road. However, we did not find significant correlation between plant attributes with traffic density for M-2 road.

Discussion

Vehicular pollution imposed a negative impact on *P. hysterophorus* and significantly affected its physiology in the present study. Chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid contents were got reduced significantly at all the sites along both roads. Previous researchers have also reported reductions in chlorophyll pigments of *Eucalyptus citriodora*, *Mangifera indica*, *Shorea robusta*, *Tectona grandis* (Joshi & Sawami, 2007), *Duranta repens* (Raina & Bala, 2011), *Prosopis juliflora* (Seyyednejad & Koochak, 2011) and *Artemisia maritima* (Laghari *et al*., 2015) due to the effects of environmental pollutants. Chlorophylls and carotenoid pigments take part in photosynthetic mechanism, but heavy metals and other pollutants present in vehicular exhausts significantly reduce these contents, thereby, affecting photosynthetic activity (Chauhan & Joshi, 2008). Reduction in various photosynthetic pigments has been regarded as the common indication of metal toxicity in several plant species (Chauhan, 2010a; Pooja *et al*., 2012). Among various sites along FSR, the maximum reductions in chlorophyll pigments were observed at Adda-46 and Pull-111 sites, identified as the most polluted sites. Whereas, along M-2, a clear trend of reduction was not found but instead, various sites showed overall reductions in chlorophyll pigments. The heavy metals present in vehicular exhausts could inhibit the normal functioning of biosynthesizing enzymes of chlorophyll, thereby, disrupting the structure and function of chloroplasts (Thapar *et al*., 2008; Sengar *et al*., 2008; Sharma & Tripathi, 2009; Iqbal *et al*., 2010). Carotenoids act as photo protective agents within chloroplasts as they provide protection to chloroplast machinery against photo oxidative destruction. Therefore, reduction in the carotenoid contents due the effect of various pollutants leads to pigment degradation and cellular destruction of chloroplast (Sharma & Tripathi, 2009). Panda *et al*. (2015) in a previous study, reported reductions in photosynthetic pigments of plants due to soot based environmental pollution. Baycu *et al*. (2006) stated that traffic pollution badly affects the chlorophylls pigments in plants growing along roads. Similarly, Iqbal *et al*. (2015) stated a significant reduction in chlorophyll *a*, chlorophyll *b* and total chlorophyll contents in some plant species growing along roadsides in Karachi city, Pakistan.

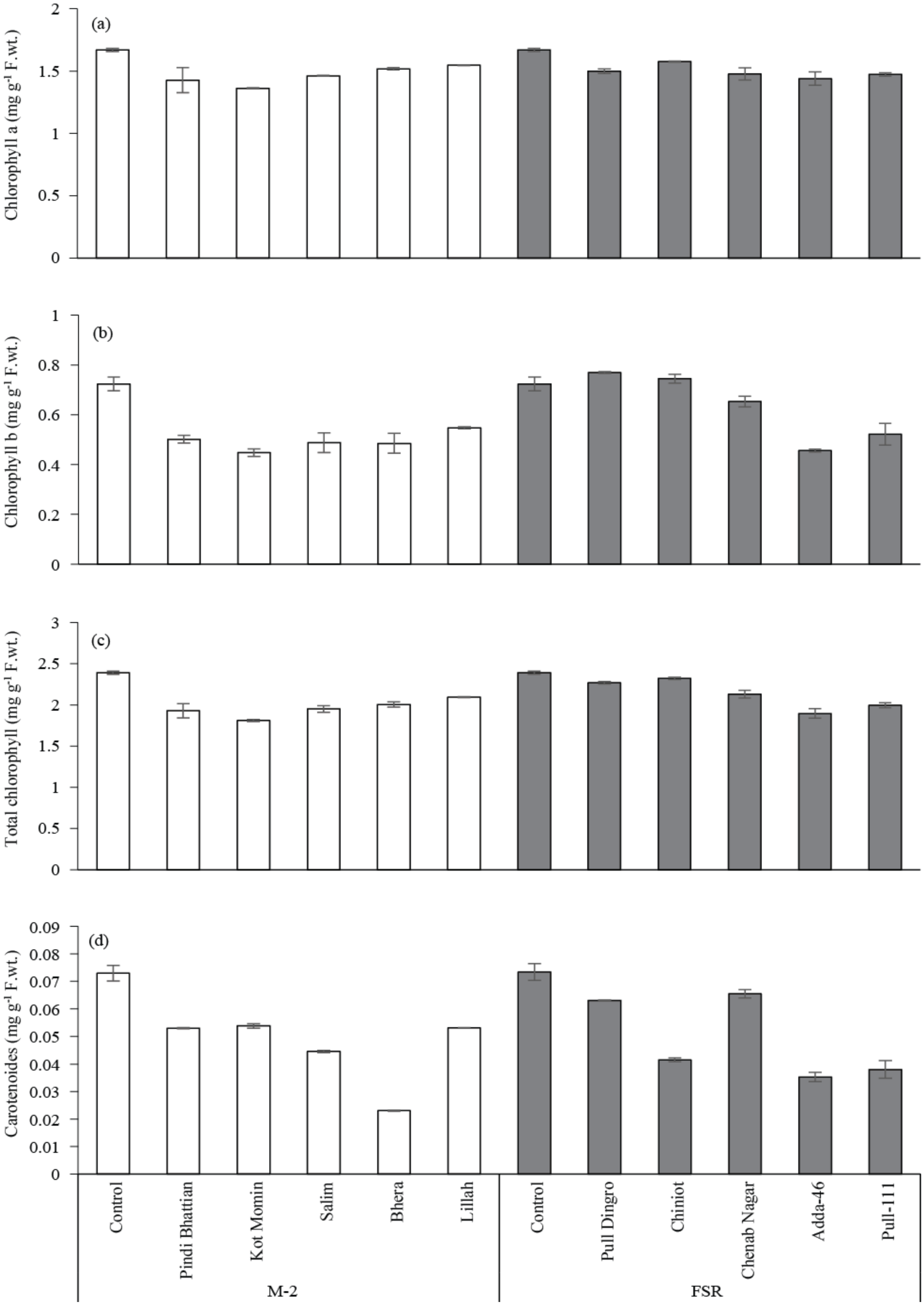

Fig. 2. Chlorophyll contents in *P. hysterophorus* at various sites on M-2 and FSR roads. Where, a. chlorophyll *a*; b. chlorophyll *b*; c. total chlorophyll; d. carotenoids

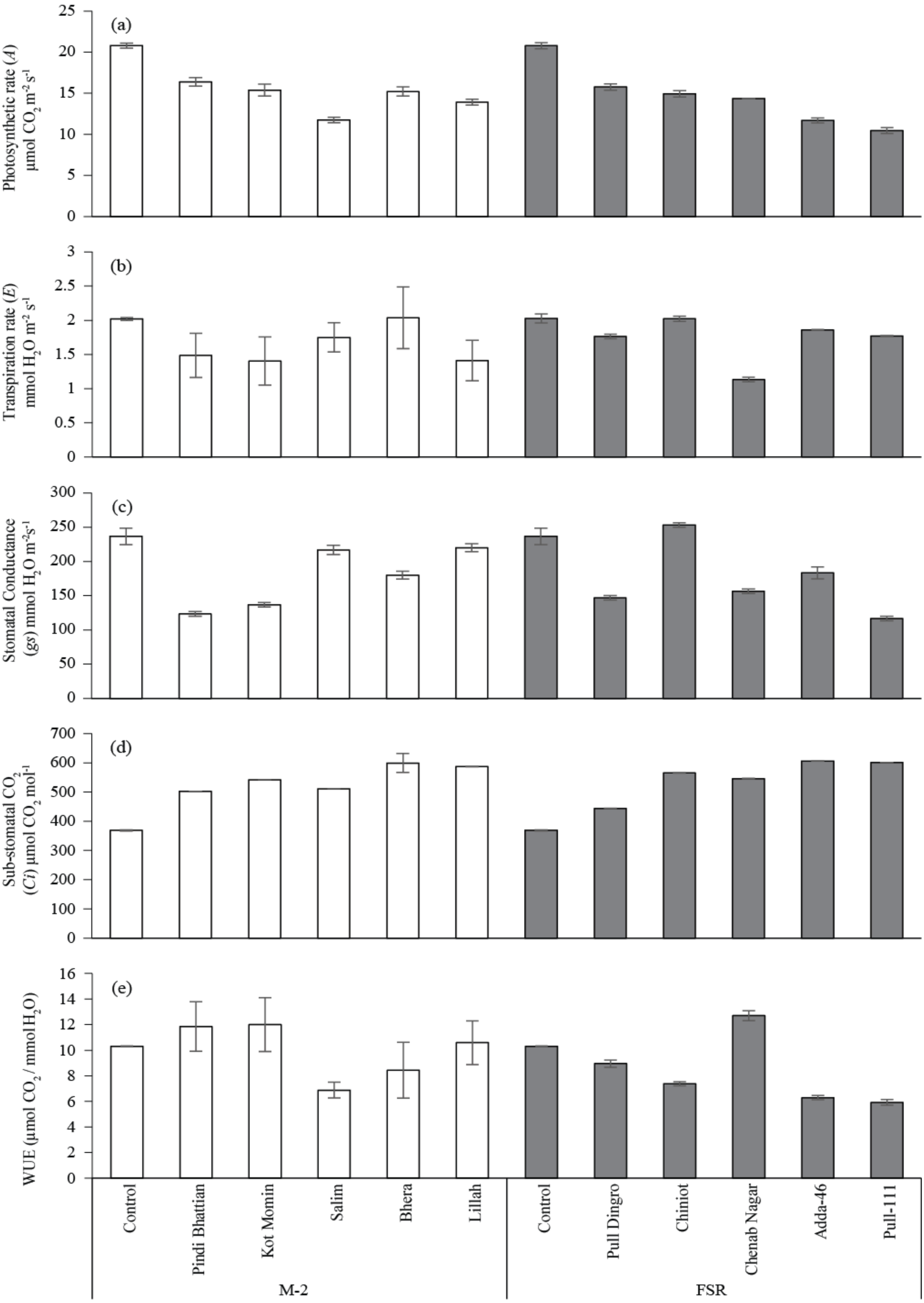

Fig. 3. Gas exchange characters of *P. hysterophorus* at various sites on M-2 and FSR roads. Where, a. photosynthetic rate; b. transpiration rate; c. stomatal conductance; d. sub stomatal CO_2 conc.; e. water use efficiency.

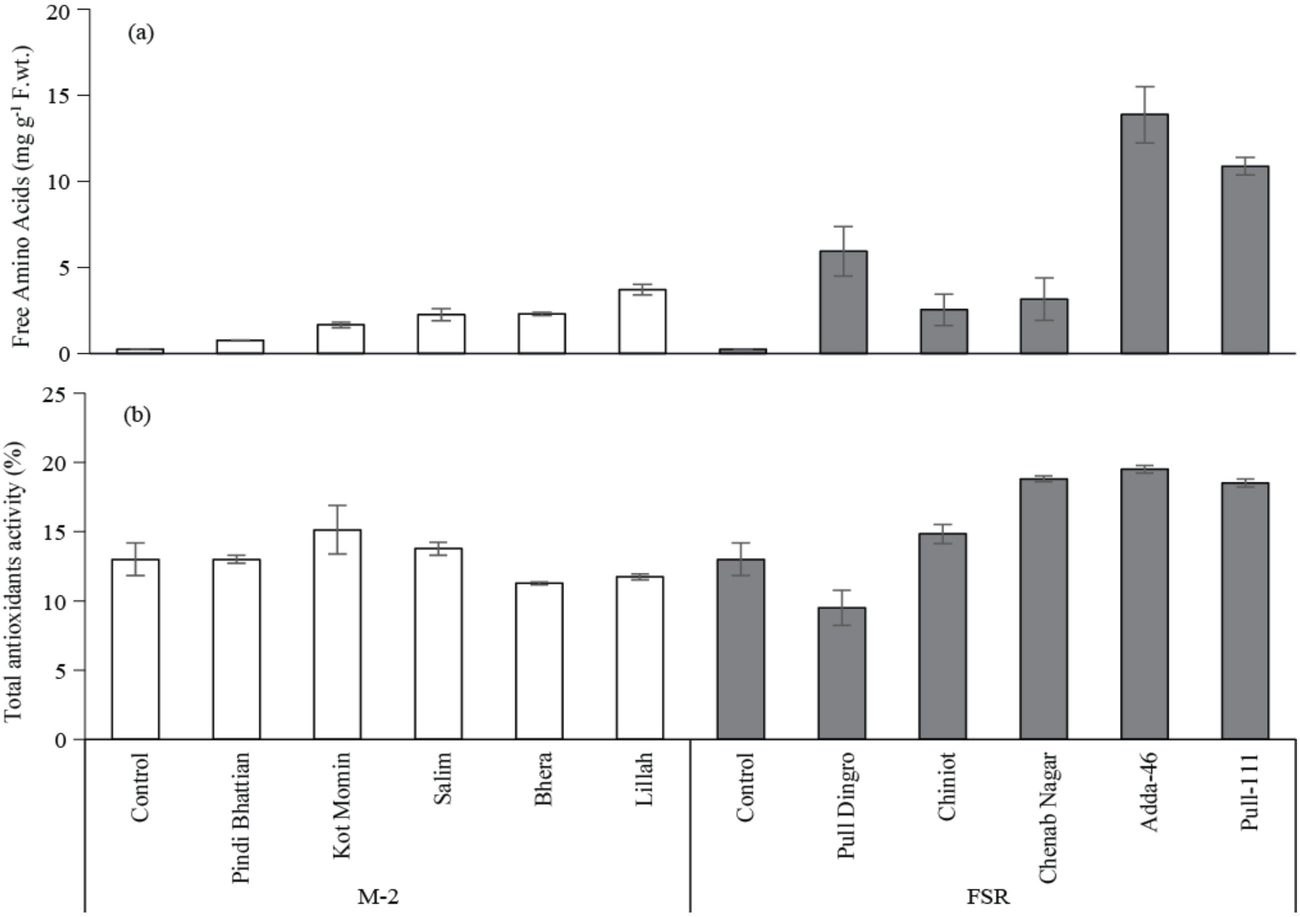

Fig. 4. Total free amino acids (a) and total antioxidants activity (b) in *P. hysterophorus* at various sites on M-2 and FSR roads.

Table 1. Pearson's correlation coefficient between traffic density and various plant attributes.

Traffic density	Chl. *a*	Chl. *b*	Total chl.	Carotenoids	*A*	*E*	*Ci*	*gs*	WUE	Free amino acids	Antioxidants
M-2	0.567	0.562	0.593	-0.094	0.437	-0.128	0.793*	0.092	0.399	0.516	-0.667
FSR	-0.713*	-0.961***	-0.939***	-0.553	-0.942***	-0.152	0.826**	-0.415	-0.332	0.739*	0.889**

*, ** and *** = significant at 0.1, 0.05 and 0.01 levels, respectively

Among physiological attributes of plants, gas exchange features have high importance (Ashraf, 2009). During the present study, a general reduction was recorded in transpiration rate, photosynthetic rate and stomatal conductance, whereas, increase in water use efficiency and internal CO_2 concentrations were recorded in *P. hysterophorus* under the stress of roadside pollutants at various sites along M-2 and FSR roads. Similar findings have been reported by previous scientists (Bell *et al.*, 2011). Bao *et al.* (2015) reported reduction in gas exchange parameters with 16% reduction in photosynthetic rate in *Sophora japonica* due to roadside toxic pollutants. Similarly, gas exchange and chlorophyll pigments were altered in lettuce plants at some urban areas in Jeddah city, Saudi Arabia, which showed significant correlation with heavy metal pollutants (Hassan *et al.*, 2013). Muhammad *et al.* (2014) also recorded a reduction in transpiration rate, photosynthetic rate, stomatal conductance and photosynthetic pigments of *Alstonia scholaris* along some heavily trafficked roads in Lahore, Pakistan.

The particulate pollutants present in vehicle exhaust emissions might be involved in suppression of stomatal conductance, leading to increase in sub stomatal CO_2 concentration due to reduced photosynthesis. Furthermore, closure of stomata also causes reduction in photosynthesis. The exposure of plants to NOx present in vehicular pollution reduces chlorophyll contents and stomatal conductance and influences photophosphorylation and electron flow. Membrane and biochemical injury produced from increased NOx concentrations inhibit photosynthesis by inducing structural alterations and uncoupling of electron transport (Kulshrestha & Saxena, 2016). Chaturvedi *et al.* (2013) also reported a suppressed photosynthetic rate and stomatal conductance in some tree species along roadsides. Environmental pollutants reduced the stomatal conductance which caused an increase in internal CO_2 concentration and decline of photosynthetic rate in *Azadirachta indica* (Qadir *et al.*, 2016). The increase in water use efficiency of *P. hysterophorus* along roadsides in present study might be due to water storage in reserves by plants when various environmental pollutants and stresses limit water uptake efficiency by roots (Veselov *et al*., 2003). Reduction in gas exchange parameters of plants along roadsides is due to Cd and Pb deposition which might be involved in blocking stomatal aperture resulting in reduced photosynthetic rate (Nawazish *et al*., 2012).

An elevated concentration of total free amino acids and total antioxidant activity were measured in *P. hysterophorus* along both roads as compared to control. These findings are in conformity with many previous studies, where increase in total free amino acids and antioxidants were found due to the effect of air pollutants (Nadgorska-Socha *et al*., 2013; Zemanova *et al*., 2013; Almohisen, 2014). Heavy metals caused stress result in accumulation of variety of metabolites such as proline, betaine, antioxidants, polyamines, nicotianamine and other free amino acids in plants. These metabolites bind to metallic particles and help in reducing stress. They accumulate due to the activation of defense system of plants (Sharma & Dietz, 2006). The increase in amino acids level in the present study could be attributed to increased metals level in their environment, as free amino acids increase in plants due to metal toxicity (Clemens, 2001). Antioxidant enzymes are normally produced in plants, but, under the effect of environmental abiotic stresses, their activities tend to increase and enable plants to survive against the stress (Srivastava *et al*., 2011; Eraslan *et al*., 2016). Oxidative stress takes place in response to accumulation of excessive heavy metal in plant tissues, which leads to production of reactive oxygen species (ROS) (Petrov *et al*., 2015). Sulphur dioxide (SO_2) is very harmful pollutant present in vehicular exhausts. It also stimulates the production of ROS and increase antioxidant activity (Li & Yi, 2012). In the present investigation, enhanced level of air pollutants resulted in production of ROS and eventually increased antioxidant enzymes activation to overcome the stress.

The significant negative correlation between chlorophyll pigments and photosynthetic rate with traffic density along FSR indicates that increase in intensity of negative effects on *P. hysterophorus* was due to increase of vehicular traffic load and associated air pollution. Similarly, occurrence of significant positive correlation between free amino acids and antioxidant activity with vehicular density on FSR further strengthens the results. However, existence of non-significant correlations between various attributes of *P. hysterophorus* with traffic on M-2 might be due to a nearly constant or slightly variable traffic volume at sites over there.

Conclusions

This study has demonstrated a variety of responses showed by *P. hysterophorus* to vehicular exhaust pollutants at various sites along two roads. The results clearly indicated the potentially detrimental changes caused by urban roadside pollution on physiology of *P. hysterophorus*. The magnitude of impacts produced by both roads on *P. hysterophorus* were however, varied by level. Though, *P. hysterophorus* showed reduction in photosynthetic pigments, photosynthetic rate, transpiration rate and stomatal conductance with an enhanced level of sub stomatal CO_2 concentration, free amino acids and total antioxidant activity at various sites along both roads. The physiological attributes measured in this study clearly indicate that they could be used as biomarker basis for monitoring and prediction of early effects of air born vehicular exhaust pollution.

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