

Original Research Article

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Identification of Tolerant Plant Species Growing Alongside National Highway-21 in Himachal Pradesh, India

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ABSTRACT

Air pollution due to vehicular emissions has become a global tribulation for living organisms. Controlling air pollution is one of the complex environmental challenges. The only suitable ecological approach to control air pollution is constructing green belts in and around the pollution sources. A field study was conducted during the year 2016-17 to assess the air pollution tolerance of plants growing alongside National Highway-21 in Himachal Pradesh. The registered distribution pattern studies indicated that six tree species namely *Dalbergia sisso*, *Grewia optiva*, *Leucaena leucocephala*, *Toona ciliata*, *Morus alba* and *Ficus palmata* were found commonly growing alongside the selected highway. In addition, five shrub species namely *Adhatoda vasica*, *Vitex negundo*, *Murraya koenigii*, *Carissa opaca* and *Debregeasia hypoleuca* were also selected. Based on air pollution tolerance index (APTI), among commonly growing trees *Toona ciliata* registered highest APTI value of 12.53 followed by *Leucaena leucocephala* with value of 11.05. The tolerance level of these tree species was followed by *Dalbergia sisso* (10.56) > *Grewia optiva* (9.98) > *Ficus palmata* (9.94) > *Morus alba* (9.33). Also, tolerance level of shrubs was calculated which showed that among shrubs *Adhatoda vasica* and *Murraya koenigii* with APTI values (9.02 and 8.47) were found to be tolerant followed by *Debregeasia hypoleuca* (7.98) > *Carissa opaca* (7.96) > *Vitex negundo* (7.79). Overall results indicated that *Toona ciliata* and *Leucaena leucocephala* among tree species and *Adhatoda vasica* and *Murraya koenigii* among shrub species registered relatively higher tolerance level than other species. Therefore, should be recommended for the plantation alongside highways to reduce air pollution.

Keywords

Air pollution tolerance index, Biochemical parameters, Greenbelt, Indicators, Roadside plant species

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Introduction

Transport sector is a catalyst which leads to economic development of any nation and the rapid growth of motor vehicle impose a great

threat on environment. In India, 60–70% of the air pollution is caused by transport sector followed by industries (Khandar and Kosankar 2014; Sisodia and Dutta 2016). Air pollution due to vehicular emissions has become one of

the most serious problems in the whole world and has resulted in huge threat to both the environment and the health of living organisms. The major pollutants emitted from exhaust emission of gasoline fuelled vehicles are CO, HC, NO_x and Pb, while pollutant from diesel fuelled vehicles are particulate matter (including smoke), SO₂, PAH, which play a great role in determining ambient air quality of surrounding areas (Gidde and Sonawane 2012).

Continuous increase in the vehicular pollution has resulted in deterioration of fresh air and has exerted a deep influence on the morphological (leaf number, leaf area, stomata number, stomata structure, flowering, growth, and reproduction), biochemical parameters, such as the ascorbic acid content, chlorophyll content (Sharma *et al.*, 2017a) and physiological parameters (leaf extract pH and relative water content) of roadside plants (Seyyednejad *et al.*, 2011; Pandit *et al.*, 2017).

The degradation of air quality is a major environmental crisis that affects the surrounding regions. So, the only suitable ecological approach to control air pollution is plants. Plants are the prime receivers of different types of pollutants, act as a green belt component and a sink by cleaning the atmosphere (Sharma *et al.*, 2017b). Plant cleans the atmosphere through the mechanism of absorption, adsorption, accumulation and detoxification, which not only control air pollution but also improve air quality by providing sufficient amount of oxygen to the atmosphere (Mondal *et al.*, 2011; Kapoor and Bhardwaj 2016). Development of green belt, by plantation of tolerant plant species, can mitigate air pollution, prevent soil erosion and create aesthetic environment and for this, selection of plant species is an important factor to be considered. Screening of tolerant plant species was done on the basis of air pollution tolerance index (APTI) which

includes four parameters (ascorbic acid, total chlorophyll content, relative water content and pH).

Materials and Methods

Site description

The present study was conducted in National Highway-21 (now NH-154) also known as Kiratpur - Nerchowk Expressway during the year 2016-17. The present highway in Northern India connects Chandigarh to the popular tourist places like Kullu and Manali in Himachal Pradesh which has caused heavy traffic load causing air pollution. In order to assess the impact of expansion and construction activities on the present national highway a detailed survey was conducted and a uniform stretch of the highway from Garamoura in Bilaspur to Nerchowk in Mandi district was selected under the jurisdiction of Himachal Pradesh situated between North latitude of 31°21'64" to 31°38'56" and East longitude of 76°56'77" to 76°46'46".

The study area experiences sub-tropical climate and there is a considerable variation in the seasonal temperature. In general, May-June are the hottest months and December-January, are the coldest ones in the region. The average annual rainfall in the region is 1200 mm, the bulk of which is received during monsoon months (June-September) with few pre monsoon showers during early June period. The average maximum and minimum temperature varies from 22.50 to 38.77°C and 2.40 to 20.40°C.

Survey

A field survey was conducted alongside the NH-21 from Garamoura in Bilaspur to Nerchowk in Mandi. For studying the distribution of species, random points were selected on both sides of the highway and 10 x

10 m quadrates were laid in order to select the most commonly occurring species. Total, eleven commonly growing species were identified and selected for the study. Among all plant species, six were trees, viz. *Dalbergia sisso*, *Grewia optiva*, *Leucaena leucocephala*, *Toona ciliata*, *Morus alba* and *Ficus palmata* and five were shrubs, viz. *Adhatoda vasica*, *Vitex negundo*, *Murraya koenigii*, *Carissa opaca* and *Debregeasia hypoleuca*.

Experimental details

To complete the objectives of the present study the eleven identified commonly growing plant species were selected alongside from the edge of the National Highway. The influence of vehicular pollution on selected plants was studied in the month of October. The National Highway 21 was divided into four uniform segments and each was considered on replication. In total there were 11 treatments (11 x 1) which were replicated four times under factorial Randomised Block Design.

Leaf analysis

To assess the impact of vehicular pollution on various biochemical parameters of selected plant species fully matured leafs were collected in the morning hours. The collected leaf samples were carried to the laboratory for analysis in the ice box. The leaf samples were analysed for total chlorophyll, ascorbic acid, leaf extract pH and relative water content by using following standard procedures:

Total chlorophyll content

The leaf chlorophyll content was estimated by using method given by (Hiscox and Israelstam 1979). The fresh leaves were chopped to fine pieces under subdued light and 100 mg of chopped leaf samples was placed in vials containing 7 ml of dimethyl sulphoxide. These vials were incubated at 65°C for half an hour

and extract was transferred to graduated test tube and the final volume was made to 10 ml with dimethyl sulphoxide. Optical density values of the above extract was recorded on Spectrophotometer (Model-Spectronic-20) at 645 nm (A_{645}) and 663 nm (A_{663}) wavelength against dimethyl sulphoxide blank. Optical density of total chlorophyll content (T) is the sum of chlorophyll A_{645} density and chlorophyll A_{663} density. The total chlorophyll content was calculated by using formula:

$$\text{Total chlorophyll content (mg g}^{-1}\text{)} = \frac{20.0 A_{645} + 8.02 A_{663}}{a \times 1000 \times W} \times V$$

Where;

V = volume of extract made

a = length of light path in cell (usually 1cm)

w = weight of the sample taken

A_{645} is absorbance at 645nm

A_{663} is absorbance at 663nm

Ascorbic acid

The ascorbic acid content was estimated by using A. O. A. C. (1980) method. Fresh leaves (10 g) were homogenized in metaphosphoric acid solution. Volume was made to 100 ml. This solution was titrated against indophenols dye. Appearance of rose pink colour was the end point. The amount of ascorbic acid in milligrams per 100 g was calculated by using formula:

$$\text{Ascorbic Acid (mg g}^{-1}\text{)} = \frac{\text{Dye factor} \times \text{Titre reading} \times \text{Volume made}}{\text{Weight of leaves taken} \times \text{Volume taken for estimation}}$$

Dye factor

Dye factor was determined by using the method by Ranganna (2008). For estimation of dye factor 5 ml of the standard ascorbic acid solution was mixed with 5 ml of metaphosphoric acid solution. The prepared indophenols dye was then titrated to a pink colour which persisted for 15 seconds. The dye factor was determined by the amount of

ascorbic acid (mg) per ml of dye using the formula:

$$\text{Dye Factor} = \frac{0.5}{\text{Titre Value}}$$

Leaf extract pH

Recently matured leaves (5 g) were homogenized in 10 ml deionised water and supernatant obtained after centrifugation was collected for determination of pH by using pH meter (Model- ESICO 1013) with buffer solution of pH 4 and 9 (Barrs and Weatherly, 1962).

Relative water content

Relative water content of the samples was estimated using the method proposed by Singh (1977). Leaves were collected from different sites in polythene bags, then brought to the laboratory and were rinsed thoroughly and excess water was removed with filter paper.

Then three steps were followed: first, fresh weight (FW) was obtained by taking weight of fresh leaves; second, turgid weight (TW) was obtained by placing the leaves in a water filled Petri plate overnight, and in the morning the weight of turgid leaves was taken. In the third step, turgid leaves were dried in an oven at 70°C temperatures overnight and dry weight (DW) was recorded and was computed by using following equation:

$$\text{Relative water content (\%)} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

Where, FW- Fresh weight,
TW- Turgid weight,
DW- Dry weight of leaf samples

Air pollution tolerance index

The air pollution tolerance index (APTI) was estimated by considering four biochemical parameters namely ascorbic acid, total

chlorophyll, leaf extract pH and relative water content and was computed by using the following equation given by Singh and Rao (1983).

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

Where, A- ascorbic acid (mg g⁻¹ FW),
T- total chlorophyll (mg g⁻¹ FW),
P- leaf extract pH
R- relative water content (%) of the leaves

Analytical tools employed

The observations recorded for various biochemical parameters of plant species were subjected to statistical analysis under Randomized Block Design. Analysis of variance (ANOVA) was worked out and critical difference at 5 per cent level of significance was calculated as suggested by Cochran and Cox (1967).

Results and Discussion

The chlorophyll content

The plant species growing alongside the construction activities of National Highway were found to have significant variations in their leaf chlorophyll content (Table 1). The highest value of chlorophyll content (3.81 mg g⁻¹) in case of tree species was recorded in the leaves of *Toona ciliata*. This was followed by *Grewia optiva*, *Delbergia sisso*, *Leucaena leucocephala*, *Ficus palmata*, with respective values of 3.14 mg g⁻¹, 3.08 mg g⁻¹, 3.08 mg g⁻¹, 2.69 mg g⁻¹ and lowest value of 2.68 mg g⁻¹ in case of *Morus alba*. In case of shrubs maximum value of 2.89 mg g⁻¹ was observed in *Adhatoda vasica*, followed by *Debregeasia hypoleuca*, *Murraya koenigii* and *Carissa opaca*, with values of 2.20 mg g⁻¹, 2.17 mg g⁻¹ and 1.95 mg g⁻¹. While, minimum value of 1.65 mg g⁻¹ was observed in *Vitex negundo*.

Hence, total chlorophyll content of different plants varies from species to species due to

photosynthetic process of plants which depends on leaf age, biotic-abiotic conditions and the level of vehicular pollutants (Katiyar and Dubey 2001; Tak and Kakde 2017; Sen *et al.*, 2017). Higher values of chlorophyll content significantly reports that plant has high tolerance to air pollutants. Further, the high chlorophyll content may also be ascribed to the tolerance to the air pollutants. The results are in line with the findings of (Joshi *et al.*, 1993; Santosh *et al.*, 2008; Ninave *et al.*, 2001).

Ascorbic acid content

Ascorbic acid is a powerful antioxidant which maintains cell division and cell membrane stability in plants during stress conditions by scavenging cytotoxic free radicals and reactive oxygen species produced due to photo-oxidation of SO₂ to SO₃ (Jyothi and Jaya 2010; Sanghi *et al.* 2015). The plant species growing alongside the construction activities of National Highway were found to have significant variations in their leaf ascorbic acid content in the leaves (Table 2). The ascorbic acid content in the leaves of selected plants was in the range of 2.34 to 4.42 mg g⁻¹. The highest content of 4.42 mg g⁻¹ in case of tree species was recorded in *Toona ciliata* which was followed by *Delbergia sissio* (3.90 mg g⁻¹), *Leucaena leucocephala* (3.85 mg g⁻¹), *Ficus palmata* (2.93 mg g⁻¹), *Grewia optiva* (2.90 mg g⁻¹) and lowest in *Morus alba* (2.61 mg g⁻¹). In case of shrubs maximum value of 3.55 mg g⁻¹ was observed in case of *Adhatoda vasica* followed by *Debregeasia hypoleuca* (2.89 mg g⁻¹), *Carissa opaca* (2.81 mg g⁻¹), *Murraya koenigii* (2.55 mg g⁻¹), respectively, whereas the minimum value of 2.34 mg g⁻¹ was recorded for *Vitex negundo*.

High values of ascorbic acid content in the plant species prevent them from the harmful effects of air pollutants (Kuddus *et al.*, 2011). As Ascorbic acid content has inherent reducing power which is defensive against air

pollution. Hence, this quality of plant leaves having high ascorbic acid content makes them more tolerant to air pollution. The results are in similar line with (Gholami *et al.*, 2016; Ogunrotimi *et al.*, 2017).

Leaf extract pH

The leaf extract pH plays a crucial role in regulating the pollution sensitivity in plants. (Das and Prasad 2010). The data pertaining to pH of plant species is cited in Table 3. A perusal of the data indicates that the selected plant species growing alongside the National Highway showed a significant variation in the leaf extract pH. The leaf extract pH in the selected plants was in the range of 5.63 to 6.11. The highest value of 6.11 in tree species was recorded in *Toona ciliata* which is followed by *Morus alba* (6.09), *Leucaena leucocephala* (6.04), *Delbergia sisso* (5.99), *Grewia optiva* (5.96) and lowest value of 5.93 in *Ficus palmata*. In case of shrubs maximum value of 5.97 was recorded in *Adhatoda vasica* followed by *Murraya koenigii* (5.95), *Vitex negundo* (5.90), *Debregeasia hypoleuca* (5.87) respectively and minimum value in *Carissa opaca* (5.63). The higher values of pH have significant role in the plants which offers tolerance level in the plants to air pollutants (Bakiyaraj and Ayyappan 2014; Bora and Joshi 2014).

The results showed pH of leaf extract was acidic, which may be due to diffusion of gaseous air pollutants like NO₂, CO₂ and SO₂ in the cell sap and when plants are suffering from air pollutants (especially SO₂) as their cellular fluid would produce massive H⁺ to react with SO₂, which enters through stomata and intercellular space from air, so that H₂SO₄ is generated and then leaf pH reduces. The present results are in line with (GHassanen *et al.*, 2016; Kaur and Nagpal 2017). It is also reported that, in the presence of an acidic pollutant (SO₂ and NO₂), the leaf pH is

reduced and the reducing rate is more in sensitive plants compared to that in tolerant plant species (Scholz and Reck 1977).

Relative Water Content

Relative Water Content (RWC) is an important factor with in plants which maintains its physiological balance under stressful conditions and enhances the tolerance capacity of the plants to the air pollution (Veni *et al.* 2014; Sen *et al.* 2017). The scrutiny of data in Table 4 revealed that the selected plant species growing alongside the National Highway showed a significant variation in RWC of the leaves. RWC in the plant leaves ranged from 56.64 to 81.48 %. Among the

plant species the highest relative water content (81.48 %) in tree species was recorded in *Toona ciliata*, which was followed by *Leucaena leucocephala* (75.43 %), *Grewia optiva* (73.32 %), *Dalberia sisso* (70.33 %), *Ficus carica* (67.97 %), respectively whereas lowest in *Moras alba* (67.33 %). In case of shrub species highest value was recorded in *Adhatoda vasica* (68.35 %) followed by *Murraya koenigii* (64.04 %), *Vitex negundo* (60.38 %) and *Carissa opaca* (58.28 %). The lowest content of 56.64 % was recorded in *Debregeasia hypoleuca*. The highest value of RWC in *Toona ciliata* may be ascribed due to its higher resistance capacity to stress conditions.

Table.1 Variation in total chlorophyll content (mg g⁻¹) of selected plant species growing alongside National Highway expansion

Species	Total Chlorophyll Content (mg g ⁻¹)	
	Mean	S.E.
Trees		
<i>Dalbergia sisso</i>	3.08	0.06
<i>Grewia optiva</i>	3.14	0.20
<i>Leucaena leucocephala</i>	3.08	0.15
<i>Toona ciliata</i>	3.81	0.13
<i>Ficus palmata</i>	2.69	0.11
<i>Morus alba</i>	2.69	0.07
Shrubs		
<i>Murraya koenigii</i>	2.17	0.29
<i>Vitex negundo</i>	1.65	0.24
<i>Adhatoda vasica</i>	2.89	0.07
<i>Carissa opaca</i>	1.95	0.22
<i>Debregeasia hypoleuca</i>	2.20	0.26
C.D. (0.05)	0.53	
SE(m)	0.18	
SE(d)	0.26	
C.V.	13.57	

Table.2 Variation in ascorbic acid (mg g^{-1}) of selected plant species growing alongside National Highway expansion

Species	Ascorbic Acid (mg g^{-1})	
	Mean	S.E.
Trees		
<i>Dalbergia sisso</i>	3.90	0.50
<i>Grewia optiva</i>	2.90	0.45
<i>Leucaena leucocephala</i>	3.85	0.28
<i>Toona ciliata</i>	4.42	0.22
<i>Ficus palmata</i>	2.93	0.21
<i>Morus alba</i>	2.61	0.27
Shrubs		
<i>Murraya koenigii</i>	2.55	0.31
<i>Vitex negundo</i>	2.34	0.58
<i>Adhatoda vasica</i>	3.55	0.12
<i>Carissa opaca</i>	2.81	0.13
<i>Debregeasia hypoleuca</i>	2.89	0.18
C.D. (0.05)	0.94	
SE(m)	0.32	
SE(d)	0.46	
C.V.	20.45	

Table.3 Variation in pH of selected plant species growing alongside National Highway expansion

Species	pH	
	Mean	S.E.
Trees		
<i>Dalbergia sisso</i>	5.99	0.03
<i>Grewia optiva</i>	5.96	0.02
<i>Leucaena leucocephala</i>	6.04	0.02
<i>Toona ciliata</i>	6.11	0.01
<i>Ficus palmata</i>	5.93	0.03
<i>Morus alba</i>	6.09	0.06
Shrubs		
<i>Murraya koenigii</i>	5.95	0.03
<i>Vitex negundo</i>	5.90	0.02
<i>Adhatoda vasica</i>	5.97	0.03
<i>Carissa opaca</i>	5.63	0.21
<i>Debregeasia hypoleuca</i>	5.87	0.04
C.D. (0.05)	0.21	
SE(m)	0.07	
SE(d)	0.10	
C.V.	2.43	

Table.4 Variation in relative water content (%) of selected plant species growing alongside National Highway expansion

Species	Relative Water	
	Mean	S.E.
Trees		
<i>Dalbergia sisso</i>	70.33	0.74
<i>Grewia optiva</i>	73.32	1.23
<i>Leucaena leucocephala</i>	75.43	2.11
<i>Toona ciliata</i>	81.48	2.22
<i>Ficus palmata</i>	67.97	2.34
<i>Morus alba</i>	67.33	2.21
Shrubs		
<i>Murraya koenigii</i>	64.04	3.70
<i>Vitex negundo</i>	60.38	0.57
<i>Adhatoda vasica</i>	68.35	0.42
<i>Carissa opaca</i>	58.28	2.38
<i>Debregeasia hypoleuca</i>	56.64	2.33
C.D. (0.05)	6.08	
SE(m)	2.09	
SE(d)	2.96	
C.V.	6.20	

Table.5 Variation in air pollution tolerance index of selected plant species growing alongside National Highway expansion

Species	APTI	
	Mean	S.E.
Trees		
<i>Dalbergia sisso</i>	10.56	0.46
<i>Grewia optiva</i>	9.94	0.34
<i>Leucaena leucocephala</i>	11.05	0.40
<i>Toona ciliata</i>	12.53	0.19
<i>Ficus palmata</i>	9.33	0.22
<i>Morus alba</i>	9.02	0.24
Shrubs		
<i>Murraya koenigii</i>	8.47	0.31
<i>Vitex negundo</i>	7.79	0.41
<i>Adhatoda vasica</i>	9.98	0.07
<i>Carissa opaca</i>	7.96	0.37
<i>Debregeasia hypoleuca</i>	7.98	0.16
C.D. (0.05)	0.917	
SE(m)	0.316	
SE(d)	0.447	
C.V.	6.643	

Table.7 Pearson’s correlation coefficient (r) between biochemical parameters of leaves of eleven species collected alongside of National Highway

		Pearson's correlation coefficient			
Plant Species		T	A	R	Ph
<i>Dalbergia sisso</i>	<i>Dalbergia sisso</i>	-0.950	1		
	R	-0.551	0.308	1	
	pH	0.368	-0.296	0.114	1
	APTI	-0.973	0.990*	0.438	-0.236
<i>Grevia optiva</i>	<i>Grevia optiva</i>	-0.997	1		
	R	0.073	-0.063	1	
	pH	0.775*	-0.741	0.563*	1
	APTI	-0.931	0.940*	0.280	-0.506
<i>Leucaena leucocephala</i>	<i>Leucaena leucocephala</i>	0.448*	1		
	R	-0.241	0.405	1	
	pH	0.454*	-0.216	-0.972	1
	APTI	0.319	0.910*	0.731*	-0.563
<i>Toona ciliata</i>	<i>Toona ciliata</i>	0.327	1		
	R	-0.872	-0.530	1	
	pH	0.531*	0.968*	-0.634	1
	APTI	-0.320	0.650*	0.285	0.557*
<i>Ficus palmata</i>	<i>Ficus palmata</i>	0.596*	1		
	R	0.244	-0.592	1	
	pH	-0.849	-0.839	0.275	1
	APTI	0.877*	0.251	0.631*	-0.492
<i>Morus alba</i>	<i>Morus alba</i>	-0.067	1		
	R	0.209	-0.425	1	
	pH	-0.612	-0.691	0.436*	1
	APTI	0.166	0.528	0.544	-0.255
<i>Murraya koenigii</i>	<i>Murraya koenigii</i>	-0.200	1		
	R	0.776*	-0.750	1	
	pH	-0.810	-0.161	-0.511	1
	APTI	0.976*	-0.097	0.729*	-0.917
<i>Vitex negundo</i>	<i>Vitex negundo</i>	-0.382	1		
	R	0.925*	-0.275	1	
	pH	0.774*	-0.815	0.776*	1
	APTI	-0.176	0.976*	-0.060	-0.673
<i>Adhatoda vasica</i>	<i>Adhatoda vasica</i>	-0.783	1		
	R	0.993*	-0.850	1	
	pH	0.921*	-0.539	0.873*	1
	APTI	-0.160	0.739*	-0.275	0.143
<i>Carissa opaca</i>	<i>Carissa opaca</i>	-0.142	1		
	R	0.217	0.854*	1	
	pH	0.965	-0.205	0.246	1
	APTI	0.429	0.783*	0.972*	0.430
<i>Debregeasia hypoleuca</i>	<i>Debregeasia hypoleuca</i>	-0.769	1		
	R	-0.045	-0.484	1	
	pH	0.026	0.253	-0.875	1
	APTI	-0.254	-0.162	0.926*	-0.958

A Ascorbic Acid, R Relative water content, T Total chlorophyll content, APTI air pollution tolerance index
 *Significant at p ≤ 0.05.

Figure.1 Location map of the study area

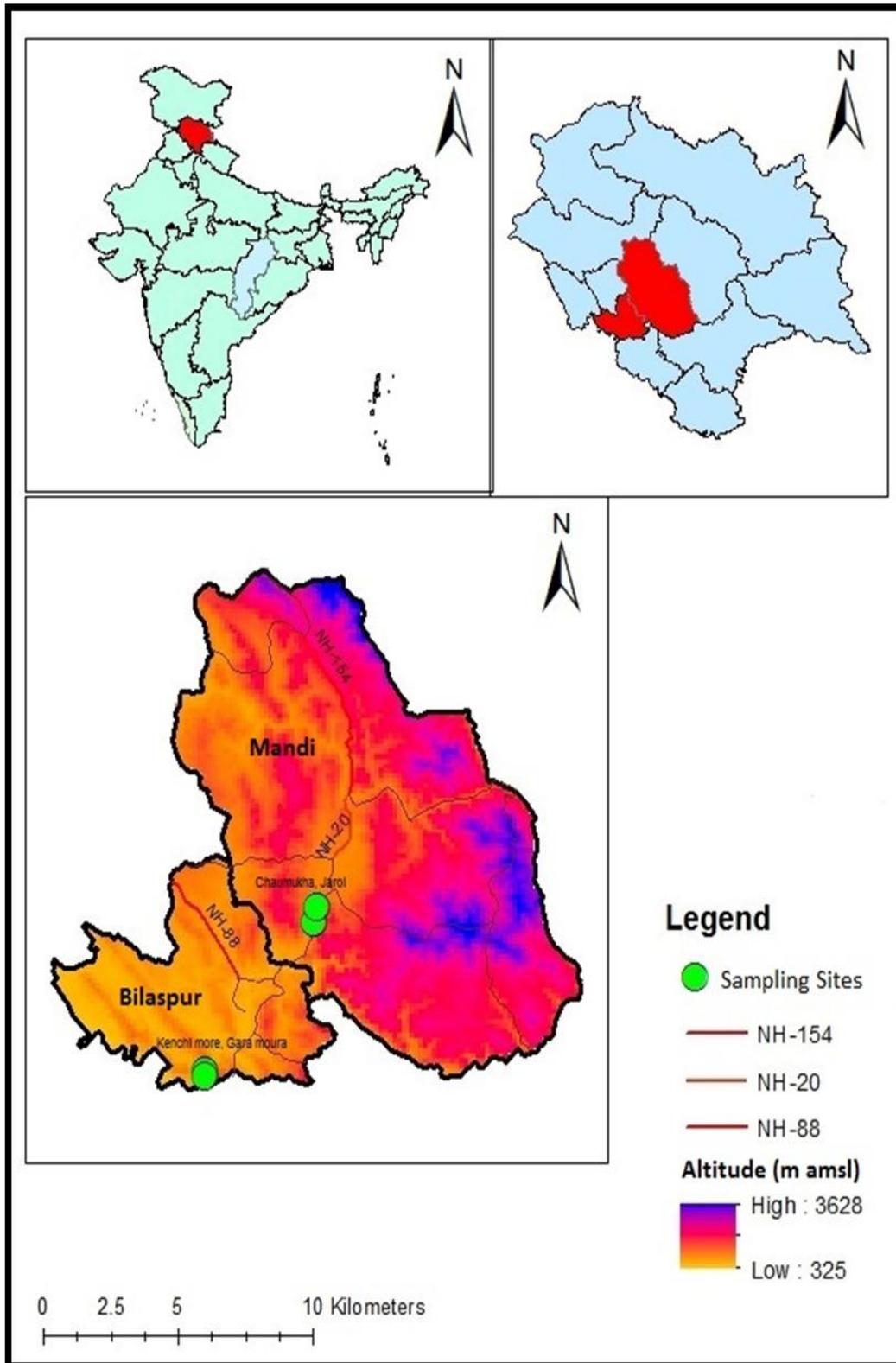
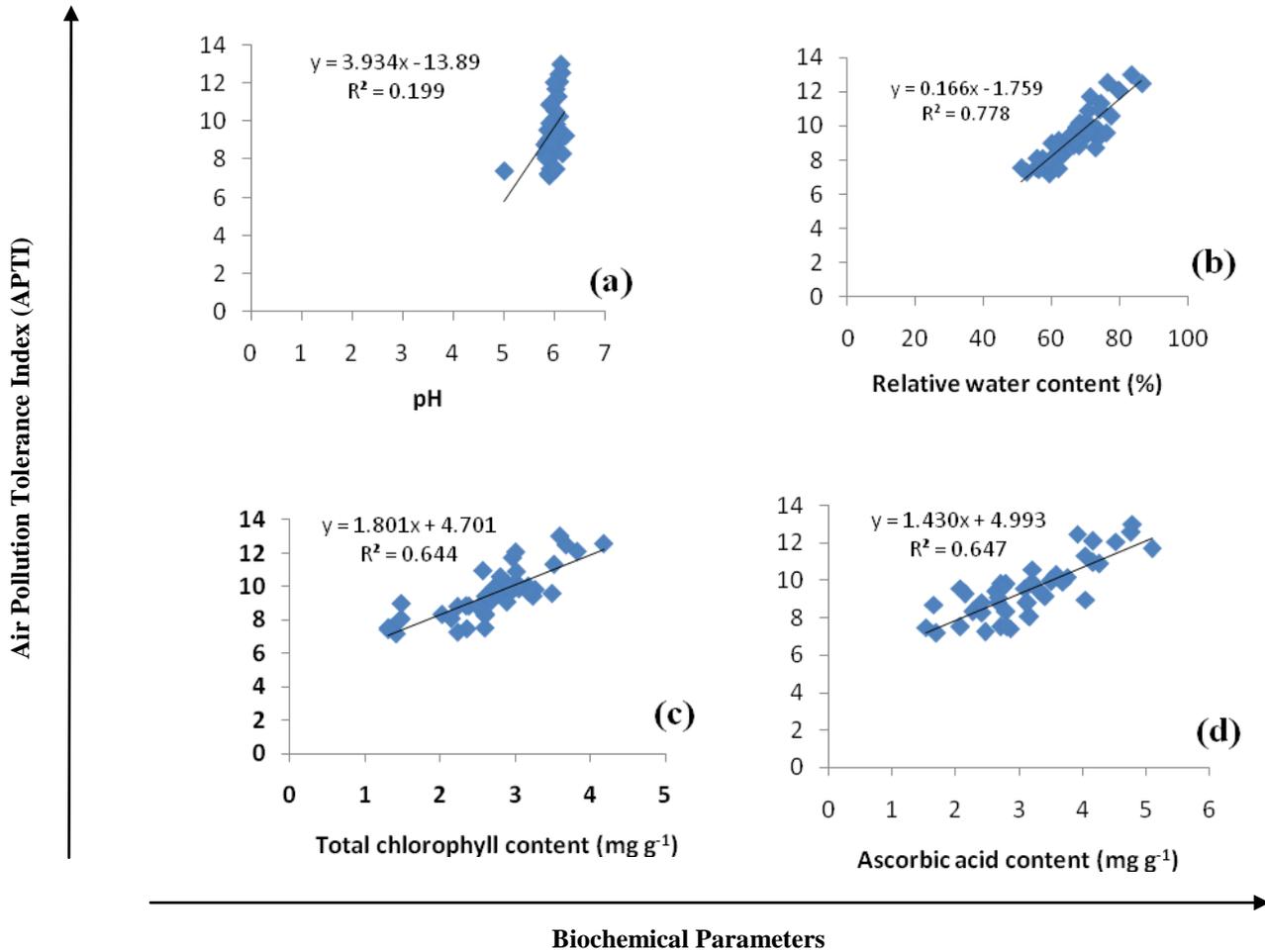


Figure.3 Linear regression analysis of individual variables (biochemical parameters) with APTI values. a) pH vs APTI, b) Relative water content (%) vs APTI, c) Total chlorophyll content (mg g⁻¹) vs APTI and d) Ascorbic acid content vs APTI



High RWC content in plants ensures the maintenance of the physiological balance under stresses such as air pollution (Buchchi *et al.*, 2013; Sharma *et al.*, 2017a). Also, higher water content in plants can dilute acidity inside the leaf cell sap and improve its drought tolerance (Palit *et al.*, 2013; Rai *et al.*, 2013). Therefore, it is likely valedictory that the plant species having high RWC have high capacity of tolerance of air pollution.

Air pollution toleration index (APTI)

The selected plant species growing alongside

the national highway expansion were found to have significant variations in the air pollution tolerance index (Table 5). The APTI of selected tree species followed the order of *Toona ciliata* > *Leucaena leucocephala* > *Dalbergia sisso* > *Grevia optiva* > *Ficus palmata* > *Morus alba* with their respective values of 12.53, 11.05, 10.56, 9.94, 9.33 and 9.02. Also, the APTI of most shrubs was higher than those tree species such as *Adhatoda vasica* > *Murraya koenigii* > *Debregeasia hypoleuca* > *Carissa opaca* > *Vitex negundo* with their respective values of 9.98, 8.47, 7.98, 7.96 and 7.79 suggesting that

shrubs in general, were more tolerant to air pollution than trees. Higher values of APTI represent the potential of plants to facilitate in polluted areas and contribute as an air pollution synthesizer (sink) (Joshi and Swami, 2007; Sharma *et al.* 2017b; Kumari and Deswal 2017). The variation in the tolerance of the plant species of a region to stress conditions has also been reported by (Agbaire and Esiefarienrhe 2009; Kapoor and Bhardwaj 2016; Pandit *et al.* 2017).

Linear regression analysis

APTI of plant species was found to increase, which is mainly due to increase in the values of pH, relative water content, total chlorophyll content and ascorbic acid content of plant leaves.

Therefore a linear regression analysis was performed to ascertain which of leaf biochemical parameter had a greater influence on APTI of plant species. Analysis showed significantly strong positive impact of ascorbic acid content ($R^2 = 0.647$) on APTI. However, it was concluded that the higher values of ascorbic acid were found in all plant species as compared to pH, relative water content and total chlorophyll content, which indicated that ascorbic acid content impacted on the APTI for screening out the most tolerant plant species (Figure 3).

Pearson's correlation coefficient analysis

The correlation analysis shows the relationship between the four biochemical parameters and APTI for each plant species. The scrutiny of analysis presented in Table 6 revealed a significant positive correlation between APTI and ascorbic acid content in case of *Dalbergia sisso* ($r = 0.990$), *Grevia optiva* ($r = 0.940$), *Leucaena leucocephala* ($r = 0.910$), *Toona ciliata* ($r = 0.650$), *Vitex negundo* ($r = 0.976$), *Adhatoda vasica* ($r = 0.739$) and *Carissa opaca* ($r = 0.783$).

APTI of *Ficus palmata* ($r = 0.631$), *Leucaena leucocephala* ($r = 0.731$), *Murraya koenigii* ($r = 0.729$), *Carissa opaca* ($r = 0.972$), *Debregeasia hypoleuca* ($r = 0.926$) showed a significant positive correlation at $p \leq 0.05$ with relative water content.

Also, a significant positive correlation at $p \leq 0.05$ between APTI and total chlorophyll content in case of *Ficus carica* ($r = 0.877$) and *Murraya koenigii* ($r = 0.729$).

Overall analysis stated that each parameter plays a significant role in the evaluation of air pollution tolerance index of particular plant species. Especially ascorbic acid content and relative water content of leaves had a special affect in the higher resistance to stress conditions.

In conclusion, the present study inferred that tree species *Toona ciliata* and *Leucaena leucocephala* and shrubs namely *Adhatoda vasica* and *Murraya koenigii* emerged as tolerant species growing alongside the NH-21. It was concluded that air pollution tolerance index increased with increase in biochemical parameters such as total chlorophyll content, ascorbic acid, relative water content and pH and hence these plant species need to be recommended for plantation alongside the national highway falling in sub-tropical region of Himachal Pradesh.

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