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Bioindicator responses and performance of plant species along a vehicular pollution gradient in western Himalaya

Rachit Kashyap · Rohit Sharma · Sanjay Kr. Uniyal

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Abstract Loss of green cover, and increasing pollution is a prime global concern. The problem calls for screening of pollution-tolerant tree species that can be integrated into plantation drives. Recognizing this, the study analyzed bio-indicator responses and performance of commonly occurring plant species along a pollution gradient in western Himalaya. Based on distance from the road, three sites viz., highly polluted (HP), moderately polluted (MP), and least polluted (LP), were identified. From these sites, leaves of commonly occurring 26 tree species were collected and analyzed for dust accumulation, total chlorophyll, relative water content (RWC), ascorbic acid, and pH using standard protocols. Later, assessment of Air Pollution Tolerance Index (APTI) and Anticipated Performance Indices (API) was carried out. The results revealed variations in biochemical characteristics. The pH, RWC, and total chlorophyll increased with decreasing pollution while ascorbic acid increased with increasing pollution. Dust capturing potential of *Ficus carica* (1.191 mg/m^2) and *Toona ciliata* (0.820 mg/m^2) was relatively higher. Based on the results of APTI, *Grevillea robusta* was classified as tolerant. It scored significantly higher values (21.06, 21.19, and 19.61 in LP, MP, and HP sites, respectively). *Quercus floribunda*, *G. robusta* (68.75% each), *Juglans regia* (68.7%), and *T. ciliata* (62.50%) were good performers in HP sites. *Acer caesium*, *Betula utilis*, and *Morus alba* that had low API scores (43.75%) were predicted as poor performers. Thus, *G. robusta*, *Q. floribunda, J. regia*, *T. ciliata*, and *F. carica* were evaluated as best performers. They could be integrated into plantations drives for environmental management.

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R. Kashyap · R. Sharma · S. K. Uniyal (✉)
High Altitude Biology Division, Institute of Himalayan Bioresource Technology, CSIR, Palampur, Himachal Pradesh 176 061, India
e-mail: suniyal@ihbt.res.in

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Introduction

Loss of forest cover and ever-increasing pollution, which are primarily a result of unplanned development, and unregulated human use, are contemporary global problems (Pandey et al. 2015; Wagh et al. 2006; Tang et al. 2007). Air pollution represents a major concern that has far-reaching implications on health and environment of a region (Ali and Athar 2008; Sharma and Uniyal 2016). Considering that no physical and chemical methods are available to ameliorate large-scale arrival of air pollutants, air pollution becomes a complex phenomenon (Dwivedi and Tripathi 2007; Mondal et al. 2011; Rai et al. 2013). This not only calls for reducing emissions, but also prioritized greening of the landscape. In developing countries such as India, urbanization and developmental activities that include expansion of road network and exponential growth in automobiles have been cited as prime reasons for increasing air pollution and decreasing greenery (Goyal et al. 2006; Prajapati and Tripathi 2008a, b; Kaler et al. 2016; Devi et al. 2015; Sharma and Uniyal 2016). Green cover along the roadsides not only helps in minimizing the effects of pollutants, but also acts as a barrier that limits dispersion of pollutants by decreasing airflow exchange (Jeanjean et al. 2017). Being static, plants are continuously exposed to pollutants and provide a large area for dust, particulate, and phytoaccumulation of air pollutants (Noor et al. 2015). Plants growing in a dusty environment generally have a higher particulate deposition on their leaves, and thus leaves are more sensitive to air pollution (Kaler et al. 2016). Different plant species respond differently to pollution, while some plants can tolerate fairly high levels of pollution, the others are sensitive (Nadgórska-Socha et al. 2016; Pathak et al. 2011; Radhapriya et al. 2012).

Susceptibility to air pollution varies among plants, which in turn is related to biochemical characteristics of plants such as leaf chlorophyll, ascorbic acid, leaf extract pH, and relative water content (Rai and Panda 2014; Lakshmi et al. 2009). Studying these parameters is therefore important for understanding pollution-induced changes in plants, and suggesting suitable plants for greening. In the early 1990s, Air Pollution Tolerance Index (APTI) approach that uses biochemical characteristics of plants was advocated to identify tolerance levels of different plant species (Singh et al. 1991). In the recent times, various workers have advocated the use of Anticipated Performance Index (API) for assessing performance of species growing in polluted environ(s) (Ogunkunle et al. 2015; Noor et al. 2015; Nagajyoti et al. 2010; Karbassi et al. 2016). For assessing API, information on biological and socioeconomic characteristics of species are also taken into consideration, and added to the results of APTI. Both these methods ultimately guide urban greening and plantation initiatives in polluted air environ(s).

The Himalayan state of Himachal Pradesh is rich in biodiversity and attracts nature, adventure, and religious tourists. Its scenic beauty, lush green forests, snow-bound mountains, and divine places have resulted in a boom of tourists from across the globe (Kuniyal 2002). One of the highly visited tourist places in the state is Rohtang pass. It is a gateway to the cold desert region Ladakh and motorable only for 6 months. For the rest 6 months, it is under snow. According to Himachal Pradesh Tourism Development Corporation, on an average, annually ~ 60 million people visit areas around Rohtang (HPTDC 2012; NGT 2013). It has resulted in heavy vehicular emissions, air pollution, and dust accumulation on the foliage of plants. Traffic congestion has further aggravated the problem. The National Green Tribunal (NGT), India has taken a serious note of the environmental hazards, and therefore imposed stringent regulations on movement of vehicles in the region (NGT 2013). Consequently, widening of National Highway (NH-21) on which this pass lies has already started (Sharma et al. 2011). Additionally, the Government is now focusing on the use of biodiesel, and compressed natural gas in vehicles that pass through this region (NGT 2013), and initiating suitable plantations. Consequently, the present study focused on (1) assessing dust accumulation on the leaves of tree species growing along NH 21, (2) analyzing biochemical characteristics of the leaves of these tree species, and (3) identifying bio-indicator responses and performance of naturally growing tree species along the NH-21 highway.

Materials and methods

Study area

The study was carried out along the 243-km stretch of national highway (NH-21) in Himachal Pradesh (India) that encompasses a gradient from plains to high altitude passes. In general, mean annual temperature varies from extremely cold (−3 °C) at 3300 m asl to 38 °C in areas around 500 m asl. The Rohtang pass experiences heavy snowfall during winter months (December to February), and is closed for vehicles from mid-November to mid-May (Sharma et al. 2011). Twenty-nine locations between 31°24′40.85″-71°51′38.81″ to 32°21′8.34″-77°13′32.63″ with altitude ranging from 500 m to 3300 m amsl were selected for sampling vegetation (Fig. 1). At all the sampling sites, starting from the fringe of the road, three pollution gradients were identified. These have been named as Highly Polluted (HP), i.e., area within 0–15 m distance from the road boundary, Moderately Polluted (MP) represents area 15–30 m away from the road boundary, while Least Polluted (LP) represents area > 45 m away from the road. A schematic representation is provided in Fig. 2.

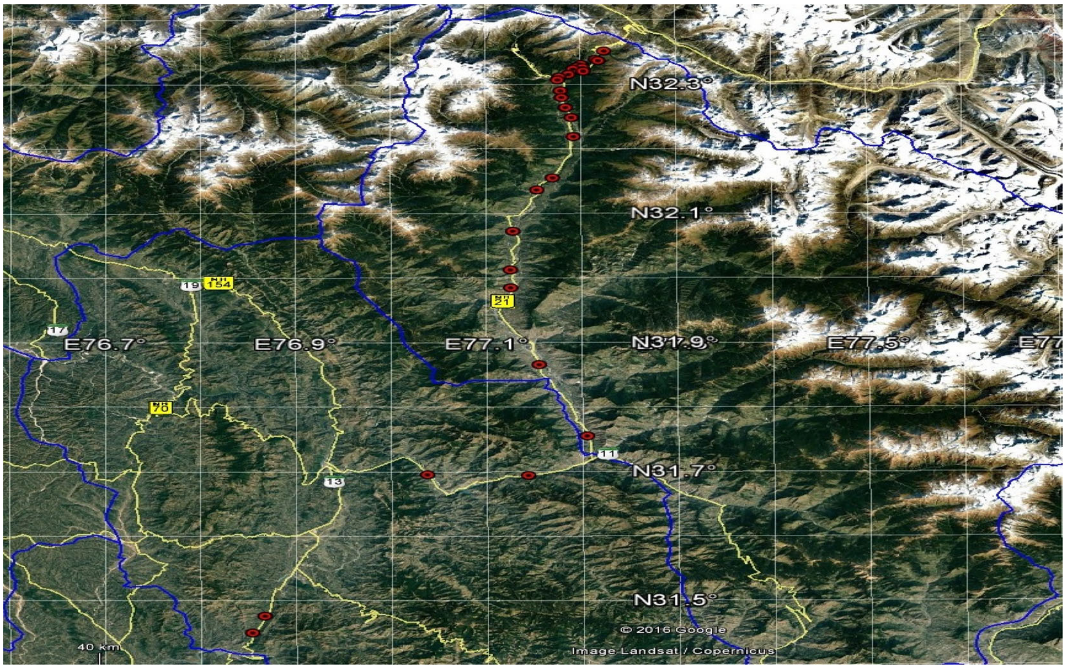

Fig. 1 Map showing twenty nine study sites along National Highway-21

Sample collection

In each of the three sites (HP, MP, and LP), quadrats (plots) of 10 × 10 m were laid, and their four corners marked with red paint. Twenty-six commonly occurring plant species namely *Abies pindrow* Royle, *Acacia catechu* Willd., *Acer caesium* wallich ex D. Don, *Betula utilis* D. Don, *Bombax ceiba* L., *Callistemon citrinus* Skeel, *Cedrus deodara* G. Don, *Dalbergia sissoo* Roxb.ex DC., *Ficus carica* L., *Grevillea robusta* A.Cunn. ex R.Br, *Grewia optiva* J.R. Drumm ex Burrett, *Juglans regia* L., *Mallotus philippensis* Mull.Arg, *Melia*

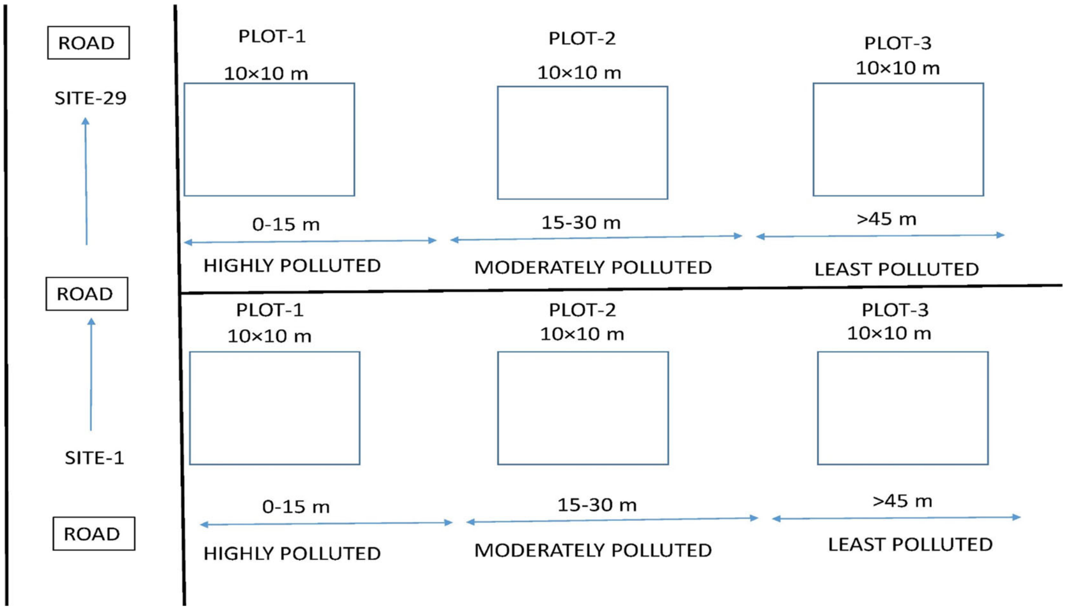

Fig. 2 Schematic representation of sampling plots along National Highway-21

azedarach L., *Morus alba* L., *Murraya koenigii* Spreng., *Picea smithiana* Bioss., *Pinus roxburghii* Sargent, *Populus deltoides* Bartr. ex Marsh., *Prunus cornuta* Steud., *Punica granatum* L., *Quercus floribunda* Lindl. ex A.Camus, *Robinia pseudoacacia* L., *Salix alba* L., *Toona ciliata* Roem., and *Zanthoxylum alatum* Roxb. were selected on the basis of their abundance in quadrats. The height of trees occurring in the quadrats was measured using a digital clinometer (Nikon make). Fully mature 12 to 14 leaf samples were collected from commonly occurring plants in each plot. The leaves along with their petioles were collected from roadside facing branches of plants from an average height of 1.5 to 2.5 m (Chen et al. 2017), and placed in thermoplastic vials (Tarson make). Vials were labeled with tough tags, marked, and immediately placed in a liquid nitrogen container (P-21-Biological, Indian oil make). For estimation of dust accumulation, fully mature plant leaves in LP, MP, and HP sites were thoroughly cleaned with a camel hair brush. Identification marks were put on them, and they were left as such for 24 h. Later, with the help of a brush, dust accumulated on the leaves was collected in butter paper bags. Bags were sealed with airtight seal.

After field sampling, leaf and dust samples were brought to the laboratory for analytical investigations. Vials containing leaf samples were transferred to CRYO-CUBE F570 (Eppendorf, India make) ultra-low temperature freezer (− 80 °C) in ice boxes, and dust sample was kept in a desiccator (SECADOR^{TM}, USA make).

Laboratory analyses

Dust estimation

Dust samples were weighed on a digital balance ME104 (Mettler-Toledo), and dust accumulation was calculated following Kaler et al. (2016) using the equation:

$$W = \frac{Wa - Wb}{A}$$

Where W is dust content (g/m^2), Wa initial weight of butter paper bag, Wb final weight of butter paper bag with dust, and A total area of the leaf (m^2).

Total chlorophyll

Chopped and crushed leaves (50 mg) were placed in vials containing 7 ml of dimethyle sulfoxide, and incubated at 65 °C for half an hour. After this step, centrifugation was performed, and extracted aliquot was transferred to another graduated tube, and final volume was made 10 ml in dimethyle sulfoxide. Samples were assayed using a spectrophotometer at 645 and 663 nm wavelength against a blank (Hiscox and Israelstam 1979).

$$\text{Total chlorophyll} = \frac{20.2 \times A645 + 8.02 \times A663}{a \times 1000 \times w} \times V$$

Where V is volume of aliquot, a length of the light path in the cell (1 cm), w weight of the sample taken, and A645 and A663 are an absorbance at 645 and 663 nm, respectively.

Ascorbic acid

Chopped and crushed leaves (10 g) were homogenized in standard meta-phosphoric acid solution, and final volume was made to 100 ml. This solution was titrated against indophenol dye. The appearance of rose pink color was the endpoint. The procedure of AOAC (1980) was followed, and ascorbic acid calculated using the formula given below:

$$\text{Ascorbic acid} = \frac{DF \times TR \times v}{w \times V}$$

Where DF is volume of dye used, TR titration reading, v volume made, w weight of sample taken, and V volume used for titration.

Leaf extract pH

Chopped and crushed leaves (5 g) samples were mixed with 10 ml double distilled water. The supernatant obtained after centrifugation was subjected to estimation of pH by digital pH meter (0.001) (Singh et al. 1991).

Relative water content

For relative water content (RWC), after taking fresh leaf weight, the leaf was immersed in water and left overnight. Next day, it was blotted dry to get the turgid weight. Leaf was then dried overnight in an oven at 60 °C, and reweighed to obtain dry weight (Govindaraju et al. 2012).

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

Where FW is fresh weight, DW dry weight, and TW turgid weight of leaves.

For all analytical work, quality assurance and quality control (QA/QC) standard protocols were followed to validate the accuracy and repeatability of results. Double distilled water and reagents of analytical (AR) grade procured from Central Drug House (P) LTD, India, an ISO 9001: 2008 certified company, were used. All glassware was thoroughly cleansed with 1% HCl, and dried overnight in an oven at 60 °C. For maintaining calibration and precision of spectrophotometer, standards were periodically run after ten test sample analysis. Errors of instrumental readings were checked and corrected by running reagent blanks after every five test sample determinations.

The APTI was determined by incorporating values of the above-mentioned parameters in the formula used by Govindaraju et al. (2012) and Ogunkunle et al. (2015):

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

Where A is ascorbic acid (mg/g), T total chlorophyll (mg/g), P leaf extract pH, and R relative water content of leaves.

Bio-indicator response of plant species was recorded based on APTI ratings (< 11 is sensitive, 11–17 is intermediate, and > 17 is tolerant) obtained by the plant species.

By combining grades of APTI with biological and socioeconomic characters (plant habit, type of plant, canopy structure, size of the plant, leaf structure, and hardiness), API index was worked out. All studied plants were scored with respect to the obtained grades. Gradation scale obtained by both methods is presented in Table 1. Socioeconomic importance of plants was noted from available literature, and also recorded during our field surveys.

Grades (0 to 7) and scores (0 to 100) obtained by the species help in determining their performance in polluted environments (Padmavathi et al. 2013) (Table 2).

Statistical analyses

Descriptive statistics, two-way ANOVA (detailed results are given in supplementary material), and multiple regression (linear function model) were used for data analyses of biochemical parameters using XL-STAT-2017 (version-2017.1). Multiple regression predicts the estimates of dependent variables from original values of independent variables. A best-fit regression line is always used to accomplish this estimation. The functional form of this line is known as linear function model of multiple regression. Further validation of estimates provided by regression coefficients called coefficients of multiple determination (R^2). Certain errors involved during this prediction, estimated by the standard error of estimates.

This model for the multiple regression is as follows (1):

$$Y = f(X1, X2, X3, X4, e) \quad (1)$$

Where Y is APTI of plant species, X1 is leaf extract pH, X2 is total chlorophyll (mg/g), X3 is relative water content (%), X4 is the ascorbic acid (mg/g), and e is a stochastic error term.

A linear functional form of the above model was used to assess absolute changes in APTI (dependent variable) governed by biochemical parameters (independent variables) of plant species, and altered along vehicular air pollution gradient as follows (2):

$$Y = \beta o + \beta 1X1 + \beta 2X2 + \beta 3X3 + \beta 4X4 + e \quad (2)$$

Where Y is a linear function of APTI, βo is slope factor, and β1X1, β2X2, β3X3, β4X4 are regression coefficients of biochemical characteristics explained in Eq. 1.

Results and discussion

Density, height, and dust capturing potential of plant species

Tree density and height of the studied plant species in HP, MP, and LP sites ranged from 1 to 6 (individuals/10 m^2) and 2 to 31 m, respectively. Dust accumulation loads varied among the species. In HP site, maximum dust load was noticed on leaves of *F. carica* (1.191 mg/m^2) followed by *T. ciliata* (0.820 mg/m^2), *P. cornuta* (0.308 mg/m^2), *J. regia* (0.249 mg/m^2), and *B. ceiba* (0.116 mg/m^2) (Table 3). Negligible dust accumulation was noted on the leaves of *C. citrinus*, *D. sissoo*, and *C. deodara* (0.001 mg/m^2 each). Similar trends were

Table 1 Allocation of grades to plant species based on air pollution tolerance index (APTI) and considered biological and socioeconomic traits

S no.	Grading character		Pattern of assessment	Grading allotted*
1	Tolerance	APTI	2.0–6.0	+
			6.1–10.0	++
			10.1–14.0	+++
			14.1–18.0	++++
			18.1–22.0	+++++
2	Biological and socioeconomic	Plant habit	Small	−
			Medium	+
			Large	++
		Canopy structure	Sparse/irregular/globular	−
			Spreading crown/open/semi-dense	+
			Spreading dense	++
		Type of plant	Deciduous	−
			Evergreen	+
		Size	Small	−
			Medium	+
			Large	++
3	Laminar structure	Texture	Smooth	−
			Coriaceous	+
		Hardiness	Delineate	−
			Hardy	+
		Economic value	Less than 3 uses	−
			3 or 4 uses	+
			5 or more uses	++

*Maximum 16 grades can be obtained by a plant

noticed in MP, where dust load ranged from 0.001 to 0.099 mg/m^2. Leaves of plants in LP sites accumulated minimal dust when compared to leaves of plants in the MP and HP sites. Highly polluted sites were relatively more disturbed due to the continuous movement of vehicles, and thus a large amount of dust was captured by the plant species growing in this site (Joshi and Swami 2007). On the other hand, among species, broad and hairy leaves of *F. carica* facilitated higher dust accumulation. Thakar and Mishra (2010) also reported that leaves which are broad, rough, and hairy accumulate more dust. Height could also be an important factor governing patterns of dust accumulation on the foliage of plant species (Kaler et al. 2016).

Table 2 Anticipated performance index (API) of plant species (Padmavathi et al. 2013)

Grade	Score (%)	Assessment category
0	Up to 30	Not recommended
1	31–40	Very poor
2	41–50	Poor
3	51–60	Moderate
4	61–70	Good
5	71–80	Very good
6	81–90	Excellent
7	91–100	Best

Variations in biochemical characteristics of plant species

The biochemical characteristics of the studied plant species varied significantly ($P < 0.05$) among themselves, and the sites (see supplementary table). In all the three sites, *B. ceiba* reported highest leaf extract pH, i.e., 6.35 ± 0.006, 7.57 ± 0.017, and 7.72 ± 0.012 in HP, MP, and LP sites, respectively. On the other

Table 3 Density, height, and dust capturing potential of plant species along pollution gradients

Plant species (family)	Density (individuals/10 m^2)			Average height (m)			Dust accumulation (mg/m^2)		
	HP	MP	LP	HP	MP	LP	HP	MP	LP
Abies pindrow (Pinaceae)	4	1	1	9.37	26.00	18.00	0.005	0.002	np
Acacia catechu (Mimosaceae)	3	5	3	3.80	3.76	5.00	0.003	0.002	0.001
Acer caesium (Aceraceae)	4	4	1	6.62	6.75	4.00	0.014	0.005	np
Betula utilis (Betulaceae)	2	1	3	7.25	12.00	7.00	0.002	0.001	np
Bombax ceiba (Bobacaceae)	2	3	2	11.00	12.66	15.50	0.116	0.002	0.002
Callistemon citrinus (Myrtaceae)	4	1	2	5.00	5.00	12.25	0.001	0.004	np
Cedrus deodara (Pinaceae)	3	4	6	15.66	8.50	9.43	0.001	0.001	np
Dalbergia sissoo (Fabaceae)	5	1	1	5.60	2.00	6.00	0.001	0.003	np
Ficus carica (Moraceae)	3	1	1	5.50	5.66	8.00	1.191	0.099	0.002
Grevillea robusta (Proteaceae)	4	3	3	5.20	4.86	11.66	0.002	0.002	np
Grewia optiva (Tiliaceae)	1	2	4	8.00	11.00	6.25	0.016	0.006	0.001
Juglans regia (Juglandaceae)	1	3	4	18.00	11.33	8.12	0.249	0.005	0.002
Mallotus philippensis (Euphorbiaceae)	2	3	5	3.50	4.50	4.58	0.043	0.036	0.001
Melia azedarach (Meliaceae)	1	1	4	5.00	5.00	8.25	0.010	0.002	0.005
Morus alba (Moraceae)	1	4	1	4.00	5.05	5.00	0.028	0.004	0.001
Murraya koenigii (Rutaceae)	1	5	3	8.00	7.20	6.00	0.039	0.016	0.008
Picea smithiana (Pinaceae)	1	3	1	31.00	11.66	14.00	0.003	0.001	np
Pinus roxburghii (Pinaceae)	3	1	1	10.33	20.00	20.00	0.002	0.001	np
Populus deltoides (Salicaceae)	3	1	1	20.00	3.00	12.00	0.002	0.002	np
Prunus cornuta (Rosaceae)	2	2	1	8.60	7.25	8.00	0.308	0.087	0.004
Punica granatum (Punicaceae)	1	1	2	6.00	8.00	7.00	0.091	0.001	np
Quercus floribunda (Fagaceae)	3	2	6	11.33	9.00	9.13	0.004	0.001	np
Robinia pseudoacacia (Fabaceae)	3	3	1	9.50	9.00	6.00	0.002	0.002	np
Salix alba (Salicaceae)	2	1	6	8.50	6.00	8.05	0.002	0.002	np
Toona ciliata (Meliaceae)	2	1	3	7.50	4.00	7.90	0.820	0.003	0.002
Zanthoxylum alatum (Rutaceae)	3	2	2	2.83	4.00	2.00	0.013	0.003	np

HP highly polluted, *MP* moderately polluted, *LP* least polluted, *np* not present

hand, minimum pH in all the three sites was recorded for *C. deodara*, i.e., 3.05 ± 0.002, 3.12 ± 0.005, and 3.16 ± 0.008 in HP, MP, and LP sites, respectively. Leaf extract pH was recorded to vary significantly between the sites ($P < 0.05$), and in the majority of the species, it ranged between 4 to 6 (Table 4). In general, for all the species, pH increased with decreasing pollution. In HP sites, *Z. alatum* (3.31 ± 0.006), *P. smithiana* (3.50 ± 0.008), *P. granatum* (3.64 ± 0.010), *P. roxburghii* (3.68 ± 0.008), and *B. utilis* (3.71 ± 0.007) had higher leaf extract pH values. In MP sites, pH of *Z. alatum*, *P. smithiana*, *P. granatum*, *P. roxburghii*, and *B. utilis* was 3.77 ± 0.006, 3.88 ± 0.003, 4.39 ± 0.012, 4.05 ± 0.010, and 4.17 ± 0.015, respectively, that was significantly higher ($P < 0.05$) than their respective values in HP sites. For other species also, similar trends were noted. In plant species, pH of leaf extract acts as an indicator of sensitivity to air pollution. Plants growing in polluted sites are reported to have acidic pH (Tripathi and Gautam 2007). We also observed higher acidic pH in plants growing in HP sites as compared to those growing in the LP sites (Table 4). Plants growing in highly polluted sites have high particulate matter deposition that puts them under stress, and thus higher acidic pH of leaf extract indicates higher air pollution (Tripathi and Gautam 2007).

Table 4 Comparative account of air pollution tolerance index of studied plants and their bio-indicator responses along pollution gradients

Plant species	Pollution gradients	pH	Relative water content (%)	Total chlorophyll (mg/g)	Ascorbic acid (mg/g)	APTI	Bio-indicator response
Abies pindrow	HP	4.17 ± 0.011	8.64 ± 0.084	3.86 ± 0.012	3.31 ± 0.034	3.52	S
	MP	4.58 ± 0.003	12.82 ± 0.441	4.38 ± 0.013	2.15 ± 0.012	3.21	S
	LP	4.58 ± 0.003	17.16 ± 0.227	6.81 ± 0.023	1.91 ± 0.002	3.90	S
Acacia catechu	HP	4.94 ± 0.020	47.41 ± 0.157	2.22 ± 0.265	3.20 ± 0.009	7.03	S
	MP	5.04 ± 0.020	58.62 ± 0.444	3.29 ± 0.134	2.80 ± 0.010	8.19	S
	LP	5.12 ± 0.022	86.15 ± 0.292	3.81 ± 0.008	2.10 ± 0.006	10.49	S
Acer caesium	HP	4.82 ± 0.062	22.47 ± 0.123	4.24 ± 0.023	1.50 ± 0.013	3.61	S
	MP	5.34 ± 0.056	27.77 ± 0.104	4.38 ± 0.026	0.94 ± 0.010	3.69	S
	LP	5.52 ± 0.055	48.03 ± 0.232	4.91 ± 0.055	0.64 ± 0.015	5.47	S
Betula utilis	HP	3.71 ± 0.007	32.26 ± 0.128	4.03 ± 0.020	1.46 ± 0.013	4.36	S
	MP	4.17 ± 0.015	34.94 ± 0.224	4.18 ± 0.025	1.42 ± 0.014	4.68	S
	LP	4.53 ± 0.028	40.33 ± 0.288	4.31 ± 0.025	1.16 ± 0.010	5.05	S
Bombax ceiba	HP	6.35 ± 0.006	37.30 ± 0.170	3.30 ± 0.003	5.05 ± 0.009	8.61	S
	MP	7.57 ± 0.017	48.74 ± 0.127	4.22 ± 0.002	0.35 ± 0.002	5.28	S
	LP	7.72 ± 0.012	50.11 ± 0.188	6.00 ± 0.009	1.37 ± 0.004	6.90	S
Callistemon citrinus	HP	5.26 ± 0.003	25.40 ± 0.217	3.18 ± 0.012	3.97 ± 0.008	5.89	S
	MP	5.58 ± 0.007	67.94 ± 0.357	4.16 ± 0.014	0.54 ± 0.014	7.32	S
	LP	5.84 ± 0.005	66.84 ± 0.424	5.96 ± 0.008	1.40 ± 0.004	8.33	S
Cedrus deodara	HP	3.05 ± 0.002	15.87 ± 0.127	4.30 ± 0.004	1.78 ± 0.014	2.90	S
	MP	3.12 ± 0.005	15.20 ± 0.066	4.49 ± 0.027	3.04 ± 0.033	3.83	S
	LP	3.16 ± 0.008	19.53 ± 0.136	5.81 ± 0.018	0.31 ± 0.001	2.23	S
Dalbergia sissoo	HP	4.56 ± 0.009	23.34 ± 0.228	2.25 ± 0.031	2.48 ± 0.022	4.02	S
	MP	5.68 ± 0.008	42.38 ± 0.321	3.15 ± 0.007	2.36 ± 0.017	6.32	S
	LP	6.63 ± 0.015	75.66 ± 0.142	4.03 ± 0.011	5.32 ± 0.010	13.24	I
Ficus carica	HP	4.49 ± 0.005	96.57 ± 0.095	5.79 ± 0.011	1.74 ± 0.004	11.45	I
	MP	6.44 ± 0.004	109.61 ± 0.202	5.57 ± 0.002	0.97 ± 0.011	12.13	I
	LP	7.59 ± 0.002	117.63 ± 0.141	6.69 ± 0.004	2.55 ± 0.001	15.40	I
Grevillea robusta	HP	5.97 ± 0.014	94.29 ± 0.063	4.56 ± 0.043	9.66 ± 0.008	19.61	T
	MP	6.26 ± 0.012	106.44 ± 0.091	5.22 ± 0.027	9.18 ± 0.014	21.19	T
	LP	6.57 ± 0.014	106.22 ± 0.168	5.44 ± 0.026	8.69 ± 0.015	21.06	T
Grewia optiva	HP	5.74 ± 0.034	74.06 ± 0.190	1.85 ± 0.079	4.96 ± 0.001	11.17	I
	MP	5.90 ± 0.028	92.37 ± 0.264	2.43 ± 0.014	4.53 ± 0.015	13.01	I
	LP	6.41 ± 0.031	97.95 ± 0.542	2.52 ± 0.009	3.65 ± 0.017	13.05	I
Juglans regia	HP	4.50 ± 0.010	26.72 ± 0.247	2.66 ± 0.013	1.87 ± 0.005	4.01	S
	MP	6.52 ± 0.006	62.18 ± 0.171	3.37 ± 0.007	1.45 ± 0.026	7.65	S
	LP	7.33 ± 0.005	86.13 ± 0.121	4.95 ± 0.010	1.12 ± 0.004	9.99	S
Mallotus philippensis	HP	4.34 ± 0.008	17.15 ± 0.275	3.60 ± 0.007	3.01 ± 0.004	4.10	S
	MP	5.38 ± 0.016	23.24 ± 0.197	5.62 ± 0.006	0.61 ± 0.006	2.99	S
	LP	6.03 ± 0.076	30.36 ± 0.301	6.04 ± 0.010	0.96 ± 0.005	4.19	S
Melia azedarach	HP	5.32 ± 0.004	37.47 ± 0.194	3.92 ± 0.006	3.02 ± 0.006	6.54	S
	MP	6.78 ± 0.008	43.92 ± 0.405	5.56 ± 0.011	1.35 ± 0.009	6.06	S
	LP	7.42 ± 0.007	57.27 ± 0.236	4.81 ± 0.012	4.07 ± 0.006	10.70	S
Morus alba	HP	5.45 ± 0.004	28.30 ± 0.332	2.63 ± 0.003	2.79 ± 0.009	5.08	S
	MP	6.39 ± 0.011	45.14 ± 0.211	3.95 ± 0.026	0.98 ± 0.004	5.53	S

Table 4 (continued)

Plant species	Pollution gradients	pH	Relative water content (%)	Total chlorophyll (mg/g)	Ascorbic acid (mg/g)	APTI	Bio-indicator response
	LP	7.45 ± 0.007	51.73 ± 0.210	4.75 ± 0.009	1.57 ± 0.005	7.09	S
Murraya koenigii	HP	5.30 ± 0.005	43.81 ± 0.299	2.43 ± 0.027	3.95 ± 0.012	7.43	S
	MP	5.58 ± 0.015	70.17 ± 0.524	3.59 ± 0.014	1.89 ± 0.004	8.75	S
	LP	6.49 ± 0.012	80.88 ± 0.275	4.98 ± 0.016	1.29 ± 0.008	9.57	S
Picea smithiana	HP	3.50 ± 0.008	14.74 ± 0.075	3.14 ± 0.014	2.25 ± 0.019	2.97	S
	MP	3.88 ± 0.003	24.72 ± 0.056	6.38 ± 0.008	1.17 ± 0.007	3.67	S
	LP	4.06 ± 0.006	26.17 ± 0.149	7.87 ± 0.011	0.37 ± 0.007	3.06	S
Pinus roxburghii	HP	3.68 ± 0.008	18.06 ± 0.114	2.20 ± 0.027	3.01 ± 0.012	3.57	S
	MP	4.05 ± 0.010	25.56 ± 0.087	2.45 ± 0.027	2.67 ± 0.008	4.29	S
	LP	4.36 ± 0.012	29.39 ± 0.071	2.88 ± 0.025	2.52 ± 0.009	4.76	S
Populus deltoides	HP	5.60 ± 0.008	30.46 ± 0.098	2.61 ± 0.035	5.45 ± 0.012	7.52	S
	MP	5.82 ± 0.007	42.93 ± 0.107	2.87 ± 0.035	4.72 ± 0.016	8.40	S
	LP	6.18 ± 0.009	58.36 ± 0.232	3.17 ± 0.034	1.07 ± 0.009	6.84	S
Prunus cornuta	HP	3.87 ± 0.004	56.99 ± 0.138	1.32 ± 0.004	4.70 ± 0.017	8.13	S
	MP	4.71 ± 0.004	63.67 ± 0.194	6.41 ± 0.013	2.07 ± 0.005	8.67	S
	LP	7.22 ± 0.006	64.74 ± 0.131	7.30 ± 0.005	0.91 ± 0.002	7.80	S
Punica granatum	HP	3.64 ± 0.010	12.70 ± 0.156	4.31 ± 0.003	3.11 ± 0.008	3.74	S
	MP	4.39 ± 0.012	15.68 ± 0.114	3.35 ± 0.003	2.30 ± 0.003	3.35	S
	LP	5.26 ± 0.024	22.96 ± 0.156	7.10 ± 0.009	1.65 ± 0.016	4.34	S
Quercus floribunda	HP	4.57 ± 0.015	44.24 ± 0.148	7.12 ± 0.022	4.74 ± 0.044	9.96	S
	MP	4.66 ± 0.014	58.73 ± 0.117	7.24 ± 0.020	4.01 ± 0.037	10.64	S
	LP	4.76 ± 0.012	61.67 ± 0.089	7.26 ± 0.020	3.67 ± 0.036	10.58	I
Robinia pseudoacacia	HP	6.36 ± 0.036	84.61 ± 0.116	1.72 ± 0.010	6.44 ± 0.032	13.66	I
	MP	6.56 ± 0.036	93.81 ± 0.193	1.98 ± 0.012	2.45 ± 0.011	11.47	I
	LP	7.01 ± 0.028	97.42 ± 0.173	2.41 ± 0.041	1.24 ± 0.020	10.91	S
Salix alba	HP	6.26 ± 0.031	75.12 ± 0.049	2.52 ± 0.017	6.86 ± 0.006	13.54	I
	MP	6.50 ± 0.032	83.66 ± 0.087	2.83 ± 0.016	6.64 ± 0.008	14.57	I
	LP	6.88 ± 0.025	87.69 ± 0.089	3.02 ± 0.016	5.83 ± 0.009	14.54	I
Toona ciliata	HP	4.62 ± 0.013	60.36 ± 0.192	5.06 ± 0.027	2.20 ± 0.009	8.16	S
	MP	5.32 ± 0.010	65.00 ± 0.207	4.44 ± 0.004	2.82 ± 0.023	9.25	S
	LP	5.73 ± 0.009	69.65 ± 0.166	6.89 ± 0.012	2.52 ± 0.008	10.15	S
Zanthoxylum alatum	HP	3.31 ± 0.006	16.43 ± 0.114	1.94 ± 0.029	3.90 ± 0.010	3.69	S
	MP	3.77 ± 0.006	50.19 ± 0.334	2.21 ± 0.005	1.10 ± 0.013	5.68	S
	LP	4.17 ± 0.012	63.70 ± 0.334	4.08 ± 0.019	1.13 ± 0.006	7.30	S

HP highly polluted, *MP* moderately polluted, *LP* least polluted, *S* sensitive, *I* intermediate, *T* tolerant

With respect to RWC, in comparison to other species, *F. carica* reported the highest RWC values in all the three sites, i.e., 117.63 ± 0.141, 109.61 ± 0.202, and 96.57 ± 0.095% in LP, MP, and HP sites, respectively. The needles of *A. pindrow* retained less water when compared to all other species, and therefore reported significantly lower ($P < 0.05$) RWC values in LP (17.16 ± 0.227%), MP (12.82 ± 0.441%), and HP sites (8.64 ± 0.084 %) (Table 4). Alike pH, RWC also increased with decreasing pollution (Table 4). Relatively lower pollution in LP was a testimony to higher RWC in plants at this site when compared to RWC in plants growing in HP sites. Other species that recorded high RWC in LP were

G. robusta (106.22 ± 0.168%), *G. optiva* (97.95 ± 0.542%), *R. pseudoacacia* (97.42 ± 0.173%), and *S. alba* (87.69 ± 0.089%). In HP, *G. robusta* (94.29 ± 0.063%), *G. optiva* (74.06 ± 0.190%), *R. pseudoacacia* (84.61 ± 0.116%), and *S. alba* (75.12 ± 0.049%) reported significantly lower values ($P < 0.05$). In HP sites, owing to dust on leaves, stomata generally remain closed during most of the time. This reduces transpiration thereby decreasing the RWC (Thawale et al. 2011). Similar results of low RWC values in dust-laden leaves have also been reported by Joshi and Swami (2007) from Haridwar, India.

It was revealed that, in all the targeted plant species, total chlorophyll increased with decreasing pollution (Table 4). In LP site, maximum total chlorophyll (7.87 ± 0.011 mg/g) was recorded in *P. smithiana*, followed by *P. cornuta* (7.30 ± 0.005 mg/g), and *Q. floribunda* (7.26 ± 0.020 mg/g). Minimum total chlorophyll content in HP sites was recorded in *P. cornuta* (1.32 ± 0.004 mg/g) followed by *R. pseudoacacia* (1.72 ± 0.010 mg/g), *G. optiva* (1.85 ± 0.079 mg/g), and *Z. alatum* (1.94 ± 0.029 mg/g). The difference was found to be significant ($P < 0.05$). The degree of pollution plays an important role in guiding total chlorophyll content in plant species. In polluted sites, dust load on leaves and high concentration of noxious gasses have been cited to result in degradation of photosynthetic pigments (Rai and Panda 2014). Individuals of same species growing in polluted sites have been reported to have less chlorophyll as compared to individuals growing in relatively less polluted sites (Agbaire 2009). Thus, higher chlorophyll in plants strengthens tolerance to environmental pollutants whereas less chlorophyll makes them sensitive (Das and Prasad 2010; Govindaraju et al. 2012).

When compared to total chlorophyll, a reverse trend was noticed for ascorbic acid. Ascorbic acid increased with increasing air pollution. In each of the three sites, *G. robusta* and *S. alba* reported higher values of ascorbic acid. In HP, *G. robusta* and *S. alba* had 9.66 ± 0.008 and 6.86 ± 0.006 mg/g of ascorbic acid, respectively. Their respective values in MP were 9.18 ± 0.014 and 6.64 ± 0.008 mg/g, respectively, whereas in LP the same was 8.69 ± 0.015 and 5.83 ± 0.009 mg/g, respectively (Table 4). *C. deodara*, on the other hand, had minimum ascorbic acid (0.31 ± 0.001 mg/g) in LP site. The high ascorbic acid in *G. robusta* and *S. alba* in HP sites indicated their tolerance towards air pollutants. In a polluted environment, ascorbic acid inhibits oxidation of tree leaves, and also plays an important role in preventing early aging of tree leaves (Prajapati and Tripathi 2008a, b). Thus, for maximizing defense, species growing in HP sites were found to have higher levels of ascorbic acid as compared to their respective values in less polluted sites. Tolerant plants are reported to contain higher ascorbic acid in comparison to sensitive ones (Mondal et al. 2011).

Linear function model of multiple regression

With respect to statistics, the computed R^2 value was 0.995 meaning that 99.5% of the variability in the study could be explained by linear function model. The coefficients of determination (β-coefficients) for leaf extract pH, total chlorophyll, RWC, and ascorbic acid were positive, and significantly related with APTI ($P < 0.05$) (Table 5). One unit change in pH, total chlorophyll, RWC, and ascorbic acid was increasing APTI values of plant species by 0.16, 0.10, 0.31, and 1.03 times, respectively. These results are in line with the findings of Rai and Panda (2014), and Mondal et al. (2011) who studied variations in biochemical characteristics in relation to APTI by employing regression linear function model. Linear functional model bi-plots of APTI with each biochemical parameter are shown in Figs. 3, 4, 5, and 6. Interestingly positive relationship existed between APTI with pH ($R^2 = 0.3265$), total chlorophyll ($R^2 = 0.4180$), relative water content ($R^2 = 0.5032$), and ascorbic acid ($R^2 = 0.7997$), respectively. This indicates the importance of biochemical characteristics that ultimately define the tolerance of the plant species.

Bio-indicator responses and APTI of plant species

Bio-indicator response of the studied species (Table 4), and its interpretation based on economic value, morphological traits along with APTI values (Tables 6 and 7), showed that *G. robusta* had significantly ($P < 0.05$) higher values, i.e., 21.06, 21.19, and 19.61 in LP, MP, and HP sites, respectively. The bioindicator response of *G. robusta* (> 17) indicated that it was tolerant to air pollution in

Table 5 Linear function model (multiple regression) of air pollution tolerance index determinants

Regression coefficients	Values of coefficients	Standard error	t Stat	*P* value
β0	− 2.60	0.20	− 12.95	1.4E−20*
β1	0.16	0.04	4.19	7.6E−05*
β2	0.10	0.01	59.28	1.7E−63*
β3	0.31	0.02	13.30	3.4E−21*
β4	1.03	0.02	52.07	1.8E−59*

*$P<0.05$ level

each of the sites. It has been pointed out that species with high values of APTI (> 17) are ideal for plantations in urban areas (Babu et al. 2013; Ogunkunle et al. 2015), and can form an important component of green belt around polluted sites such as the present area. In the present study, minimum APTI value was recorded for *C. deodara* (2.23) in LP sites. This points to its sensitivity towards air pollution. With low APTI values (< 11) (Table 6), bio-indicator response of *P. deltoides*, *P. roxburghii*, *J. regia*, *A. caesium*, *A. pindrow*, *P. smithiana*, and *B. utilis* puts them under sensitive category in each of the sites. *G. optiva*, *F. carica*, and *S. alba* showed an intermediate response, with APTI values lying between 12 to 16. On the other hand, *D. sissoo*, and *Q. floribunda*, gave mixed responses, i.e., they were sensitive in HP sites, and intermediate in LP sites.

Anticipated performance index of plant species

API is an advancement over APTI (Table 7). In this method, a plant species can obtain a maximum of up to 16 grades (Mondal et al. 2011; Prajapati and Tripathi 2008a, b). Here socioeconomic and biological characters of the targeted plant species along with their APTI values revealed that *G. robusta* (68.75%), *J. regia* (68.75%), *Q. floribunda* (68.75%), and *T. ciliata* (62.50%) were good performers in HP sites along the national highway. Performance of *P. smithiana*, *C. deodara*, and *P. deltoides* was moderate in each of the sites. *A. pindrow* (50.00%), *A. caesium* (43.75%), *B. utilis* (43.75%), *G. optiva* (43.75%), *M. philippensis* (50.00%), *M. alba* (43.75%), and *M. koenigii* (43.75%) having low API scores were poor performers in their respective sites (Table 7).

Along the pollution gradient from LP to HP, the performance of *F. carica* and *M. azedarach* dropped from moderate (56.25%) to poor (50.00%) performer. On the other hand, *J. regia* and *Q. floribunda* were found to be good performers (68.75%) in HP site, and very good performers (75.00 %) in MP, and LP sites (Table 7). Pandey et al. (2015) have also shown that performance of a species may change with pollution loads. Pathak et al. (2011) and Rao et al. (2004) reported that plants exhibiting maximum API scores should be recommended for greening in air-polluted

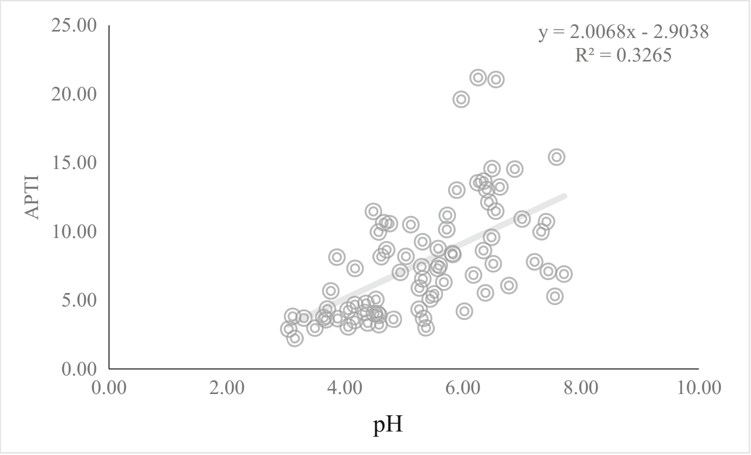

Fig. 3 Linear function model of APTI with leaf extract pH

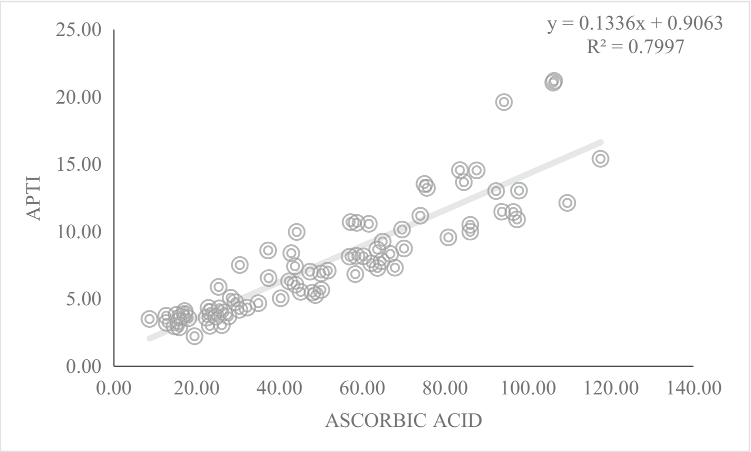

Fig. 4 Linear function model of APTI with ascorbic acid

areas. In the present study, API appraisal of studied plant species showed that *G. robusta*, *J. regia*, *Q. floribunda*, and *T. ciliata* are tolerant, and thus perfect species for green belt development along the NH 21. Due to their dense/semi-dense canopy and high socioeconomic value, these plant species may be preferred for plantations, especially along the roadside.

Overall, the study thus shows that APTI is a good method to study sensitivity and tolerance of plant species based on their evaluated responses. The plant species which are more sensitive act as indicators of air pollution, whereas tolerant species can be used to mitigate air pollution. This method also helps to determine anthropogenic stresses faced by plants at biochemical levels in severely polluted environ(s). The method further screen large number of plants with respect to their susceptibility. While, API provides an improvement over APTI and incorporates the use of biological and socioeconomic uses along with biochemical parameters. Based on the performance, API evaluates the potential and capability of plant species to mitigate air pollution. With the help of these two indices, most suitable plant species for green belt development along roadsides can be identified and recommended.

Conclusions

The present study concludes that plant species vary in their responses towards air pollution, and APTI alone may not be ideal for recommending plantations around polluted areas. On the other hand, APTI values integrated with API could be of great importance in meeting the current requirements of suggesting suitable plants for greening. Significant values of

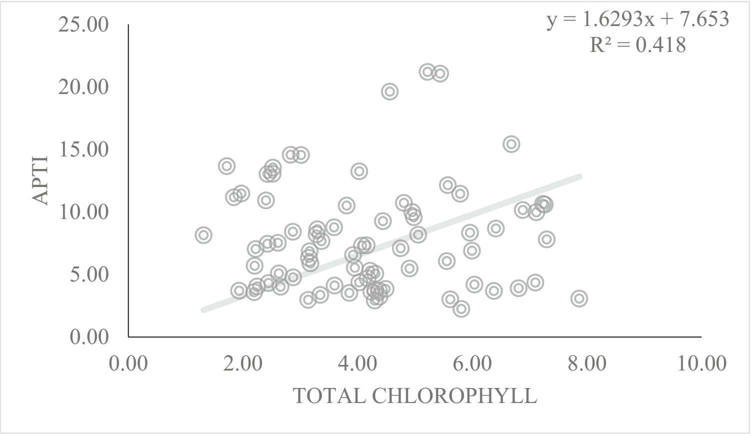

Fig. 5 Linear function model of APTI with total chlorophyll

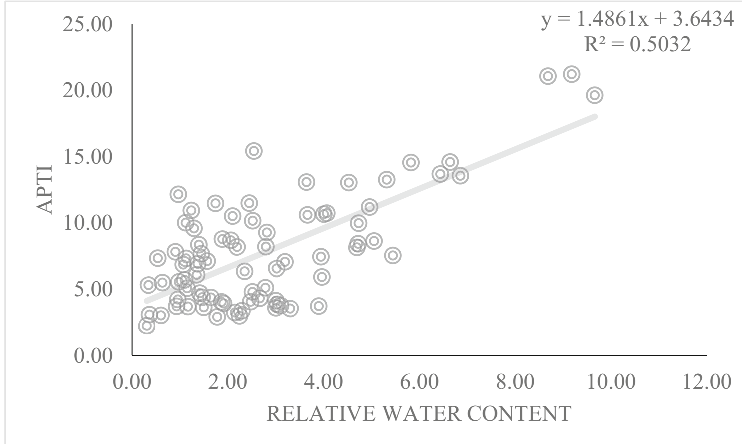

Fig. 6 Linear function model of APTI with relative water content

Table 6 Allocation of APTI grades, socioeconomic and morphological traits of studied plant species along pollution gradients

Plant species	Obtained APTI grades			Plant habit	Canopy structure	Tree type	Laminar structure			Economic importance
	HP	MP	LP				Size	Texture	Hardiness	
Abies pindrow	+	+	+	+	++	+	−	+	+	+
Acacia catechu	++	++	+++	−	+	−	−	−	−	++
Acer caesium	+	+	+	+	++	−	++	+	−	−
Betula utilis	+	+	+	+	+	−	+	+	+	+
Bombax ceiba	++	+	++	++	+	−	++	+	+	+
Callistemon citrinus	+	++	++	−	−	−	−	−	+	−
Cedrus deodara	+	+	+	++	++	+	−	+	+	+
Dalbergia sissoo	+	++	+++	+	+	−	++	+	+	+
Ficus carica	+++	+++	++++	−	+	−	++	+	+	−
Grevillea robusta	+++++	+++++	+++++	+	−	+	++	+	−	+
Grewia optiva	+++	+++	+++	−	−	−	+	+	+	+
Juglans regia	+	++	++	++	++	−	++	+	+	++
Mallotus philippensis	+	+	+	+	+	−	++	+	+	+
Melia azedarach	++	+	+++	+	++	−	+	−	+	+
Morus alba	+	+	++	+	+	−	++	−	−	++
Murraya koenigii	++	++	++	−	+	−	+	+	+	+
Picea smithiana	+	+	+	++	++	+	−	+	+	+
Pinus roxburghii	+	+	+	++	−	+	−	−	−	++
Populus deltoides	++	++	++	++	−	−	+	+	+	++
Prunus cornuta	++	++	++	+	−	−	+	+	+	+
Punica granatum	+	+	+	−	−	−	−	−	+	+
Quercus floribunda	++	+++	+++	++	++	+	+	+	+	+
Robinia pseudoacacia	+++	+++	+++	+	−	−	−	−	−	−
Salix alba	+++	++++	++++	+	+	−	+	+	+	+
Toona ciliata	++	++	+++	++	+	−	+	+	+	++
Zanthoxylum alatum	+	+	++	−	−	−	−	+	+	+

HP highly polluted, *MP* moderately polluted, *LP* least polluted, *(+)* is one grade, *(−)* represents no grade

Table 7 Allocation of API grades and performance of plant species along pollution gradients

Plant species	Plots	Grade allotted		API grade	Performance assessment
		Total plus	%Score		
Abies pindrow	HP	8	50.00	2	Poor
	MP	8	50.00	2	Poor
	LP	8	50.00	2	Poor
Acacia catechu	HP	5	31.25	1	Very poor
	MP	5	31.25	1	Very poor
	LP	6	37.50	1	Very poor
Acer caesium	HP	7	43.75	2	Poor
	MP	7	43.75	2	Poor
	LP	7	43.75	2	Poor
Betula utilis	HP	7	43.75	2	Poor
	MP	7	43.75	2	Poor
	LP	7	43.75	2	Poor
Bombax ceiba	HP	10	62.50	4	Good
	MP	9	56.25	3	Moderate
	LP	10	62.50	4	Good
Callistemon citrinus	HP	2	12.50	0	Not recommended
	MP	3	18.75	0	Not recommended
	LP	3	18.75	0	Not recommended
Cedrus deodara	HP	9	56.25	3	Moderate
	MP	9	56.25	3	Moderate
	LP	9	56.25	3	Moderate
Dalbergia sissoo	HP	8	50.00	2	Poor
	MP	9	56.25	3	Moderate
	LP	10	62.50	4	Good
Ficus carica	HP	8	50.00	2	Poor
	MP	8	50.00	2	Poor
	LP	9	56.25	3	Moderate
Grevillea robusta	HP	11	68.75	4	Good
	MP	11	68.75	4	Good
	LP	11	68.75	4	Good
Grewia optiva	HP	7	43.75	2	Poor
	MP	7	43.75	2	Poor
	LP	7	43.75	2	Poor
Juglans regia	HP	11	68.75	4	Good
	MP	12	75.00	5	Very good
	LP	12	75.00	5	Very good
Mallotus philippensis	HP	8	50.00	2	Poor
	MP	8	50.00	2	Poor
	LP	8	50.00	2	Poor
Melia azedarach	HP	8	50.00	2	Poor
	MP	7	43.75	2	Poor
	LP	9	56.25	3	Moderate
Morus alba	HP	7	43.75	2	Poor

Table 7 (continued)

Plant species	Plots	Grade allotted		API grade	Performance assessment
		Total plus	%Score		
	MP	7	43.75	2	Poor
	LP	8	50.00	2	Poor
Murraya koenigii	HP	7	43.75	2	Poor
	MP	7	43.75	2	Poor
	LP	7	43.75	2	Poor
Picea smithiana	HP	9	56.25	3	Moderate
	MP	9	56.25	3	Moderate
	LP	9	56.25	3	Moderate
Pinus roxburghii	HP	6	37.50	1	Very poor
	MP	6	37.50	1	Very poor
	LP	6	37.50	1	Very poor
Populus deltoides	HP	9	56.25	3	Moderate
	MP	9	56.25	3	Moderate
	LP	9	56.25	3	Moderate
Prunus cornuta	HP	7	43.75	2	Poor
	MP	7	43.75	2	Poor
	LP	7	43.75	2	Poor
Punica granatum	HP	3	18.75	0	Not recommended
	MP	3	18.75	0	Not recommended
	LP	3	18.75	0	Not recommended
Quercus floribunda	HP	11	68.75	4	Good
	MP	12	75.00	5	Very good
	LP	12	75.00	5	Very good
Robinia pseudoacacia	HP	4	25.00	0	Not recommended
	MP	4	25.00	0	Not recommended
	LP	4	25.00	0	Not recommended
Salix alba	HP	9	56.25	3	Moderate
	MP	10	62.50	4	Good
	LP	10	62.50	4	Good
Toona ciliata	HP	10	62.50	4	Good
	MP	10	62.50	4	Good
	LP	11	68.75	4	Good
Zanthoxylum alatum	HP	4	25.00	0	Not recommended
	MP	4	25.00	0	Not recommended
	LP	5	31.25	1	Very poor

HP highly polluted, *MP* moderately polluted, *LP* least polluted

β-coefficients showed that biochemical characteristics of plants had a pronounced positive effect on air pollution tolerance of plant species. We found that out of the 26 plant species growing naturally, *G. robusta*, *J. regia*, *Q. floribunda*, and *T. ciliata* were good performers and tolerant. Additionally, plant species such as *F. carica* and *P. cornuta* had higher dust accumulation potential. Thus, these plants species may be integrated into plantation programs in the present area.

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