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SELECTING ROADSIDE TREE SPECIES AND BIOCHEMICAL INDICATORS FOR AIR POLLUTION MONITORING

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Abstract

Roadside vegetation is widely used to monitor urban air quality, but two practical questions remain unsettled: which species should be planted along traffic corridors, and how few biochemical parameters are needed to detect pollution stress reliably. We measured eleven leaf biochemical parameters in seven roadside tree species at nine sites across three cities of north-western India, giving a balanced set of 693 determinations. All eleven parameters changed monotonically with pollution level in every species and city. No parameter differed significantly between the three cities, indicating a common regional response. Principal component analysis resolved the eleven parameters onto two nearly independent axes explaining 92.1% of total variance: a pollution-response axis (66.3%) carrying the antioxidants, malondialdehyde and leaf extract pH, and a pigment axis (25.8%) carrying chlorophyll, carotenoids and flavonoids. Ward cluster analysis placed the species in three tolerance classes, and this classification agreed exactly with an independent ranking by membrane damage. Ascorbic acid, proline and the three antioxidant enzymes were intercorrelated at $r = 0.985$ to 0.997 and therefore carry largely redundant information, whereas leaf extract pH was the only parameter correlating significantly with all ten others. We recommend Ficus benghalensis and Syzygium cumini for heavily trafficked corridors, and a graded monitoring protocol of one, two or three parameters according to available laboratory capacity.

Keywords: *Air pollution tolerance; Biomonitoring; Roadside vegetation; Leaf extract pH; Principal component analysis; Urban forestry*

1. Introduction

Roadside trees are planted along urban corridors for shade, amenity and the interception of vehicular emissions. In performing the last of these functions they absorb gaseous pollutants through the stomata and accumulate particulate matter on the leaf surface, and they pay a biochemical cost for doing so. Pollutant entry generates reactive oxygen species within the leaf, which degrade photosynthetic pigments and peroxidise membrane lipids. The plant responds by mobilising phenolic compounds, ascorbic acid and proline, and by inducing superoxide dismutase, catalase and peroxidase. These adjustments occur well before visible injury appears, which is why leaf biochemistry is a more sensitive indicator of exposure than leaf morphology.

Two practical questions follow from this, and neither is well settled. The first is which species to plant where pollution is heaviest. Species differ in their capacity to sustain the biochemical load, but published tolerance rankings are commonly derived from a single site or a single index and are seldom cross-checked against an independent criterion. The second question is how many parameters a monitoring programme actually needs. The literature reports a large number of candidate indicators, but rarely examines how much of the information they carry is redundant. For an agency with limited laboratory capacity this matters a great deal.

This paper addresses both questions using a balanced dataset of eleven biochemical parameters measured in seven roadside tree species at nine sites across three cities. We ask whether the response is consistent enough across cities to support a single regional protocol, how the eleven parameters group, whether a species tolerance classification is stable across independent criteria, and how far the parameter set can be reduced without losing diagnostic value.

2. Materials and methods

2.1 Study area and sampling design

Sampling was carried out in Sri Ganganagar (Rajasthan), Bathinda (Punjab) and Sirsa (Haryana), three cities of the semi-arid north-western plain of India. Three sites were selected in each city on the basis of traffic character established by pre-survey observation, and were classified as a control site with minimal vehicular movement, a moderately polluted site, and a highly polluted site at a major junction or arterial road (Table 1).

Table 1. Sampling sites and their classification.

Code	Site	Coordinates	Classification
A-0	Khalsa College, Sri Ganganagar	29.9395, 73.8630	Control
A-1	Bus Stand, Sri Ganganagar	29.9254, 73.8653	Moderately polluted
A-2	Sukharia Circle, Sri Ganganagar	29.9191, 73.8753	Highly polluted
B-0	Sanyas Ashram, Bathinda	30.2243, 74.8581	Control
B-1	Bathinda–Chandigarh Highway	30.2242, 74.9986	Moderately polluted
B-2	Mansa Road, Bathinda	30.2007, 74.9465	Highly polluted
C-0	Kelnian Village, Sirsa	29.5389, 74.9849	Control
C-1	Dabwali–Hisar Road Crossing, Sirsa	29.5329, 75.0352	Moderately polluted
C-2	Hisar–Barnala Road Crossing, Sirsa	29.5349, 75.0429	Highly polluted

Seven species present at all nine sites were sampled: Bougainvillea spectabilis Willd. (Bougainvel), Dalbergia sissoo Roxb. ex DC. (Shisham), Ficus benghalensis L. (Banyan), Ficus religiosa L. (Peepal), Melia azedarach L. (Chinaberry), Polyalthia longifolia (Sonn.) Thwaites (False Ashoka) and Syzygium cumini (L.) Skeels (Jamun). The design is fully crossed: every species was sampled at every site and every parameter was determined on every species–site combination, giving 63 combinations and 693 determinations with no missing cells.

2.2 Sampling and analysis

Sampling was carried out between 08:00 and 10:00 AM during the winter of 2022–2023, when atmospheric dilution is at a minimum. Mature, fully expanded leaves were taken from the middle canopy of trees standing 5–10 m from the edge of the carriageway. Five leaves were collected per plant, washed in distilled water and air-dried. A disc of 0.2 cm^2 was punched from each leaf and five discs were pooled to give a composite of 1 cm^2, which formed the analytical unit; a separate composite was prepared for each parameter. All values are therefore expressed per unit leaf area, which relates the response directly to the surface across which exposure occurs and allows species of very different leaf texture to be compared.

The eleven parameters were leaf extract pH, total chlorophyll and total carotenoids (Lichtenthaler and Buschmann, 2001), total phenolic content (Folin–Ciocalteu, 765 nm), flavonoid content (aluminium chloride, 415 nm), ascorbic acid (2,6-dichlorophenolindophenol titrimetric, 520 nm), malondialdehyde (thiobarbituric acid reactive substances, 532 and 600 nm; Heath and Packer, 1968), proline (acid-ninhydrin, 520 nm; Bates et al., 1973), superoxide dismutase (nitroblue tetrazolium photoreduction, 560 nm; Beauchamp and Fridovich, 1971), catalase (Aebi, 1984) and peroxidase (guaiacol oxidation, 470 nm). All determinations were made in triplicate.

2.3 Statistical analysis

Because composite sampling yields one value per species–site combination and group sizes are small, rank-based procedures were used. The Friedman test with the 21 species–city combinations as blocks tested for differences between pollution levels; the Wilcoxon signed-rank test examined each pairwise contrast; the Kruskal–Wallis test compared percentage change between cities; and an exact binomial sign test examined the uniformity of the direction of change. Pearson correlation was computed on the 63 observations. Principal component analysis was performed on the correlation matrix of the eleven standardised parameters, with components retained by the Kaiser criterion. Hierarchical clustering used Ward's minimum-variance linkage on Euclidean distances between standardised species means. Significance was assessed at $\alpha = 0.05$.

3. Results

3.1 The response is consistent across the three cities

All eleven parameters changed monotonically with pollution level in every species and every city; all 462 step comparisons fell in the expected direction. Leaf extract pH, chlorophyll and carotenoids declined by 15.6%, 25.6% and 15.5% respectively at the highly polluted sites, while the remaining eight parameters increased, from 37.9% for flavonoids to 707.0% for peroxidase. The Friedman test gave $\chi^2 = 42.00$ with Kendall's $W = 1.00$ and $p < 0.001$ for every parameter, and all three Wilcoxon contrasts gave $W = 231$ with $p < 0.001$.

The Kruskal–Wallis test found no significant difference in the magnitude of response between the three cities for any parameter. The closest approach to significance was leaf extract pH ($H = 4.797$, $p = 0.091$); all other parameters returned p between 0.280 and 0.922. The biochemical response is therefore a property of the regional vehicular pollutant load rather than of any city-specific factor, which supports the use of a single monitoring protocol across the region.

3.2 The eleven parameters resolve onto two axes

Two components were retained under the Kaiser criterion (eigenvalues 7.29 and 2.83), together explaining 92.1% of total variance. The first component (66.3%) loads positively on ascorbic acid, proline, superoxide dismutase, catalase, peroxidase, total phenolics and malondialdehyde, and negatively on leaf extract pH. These are the parameters that track exposure, and this axis is interpreted as the pollution-response dimension. The second component (25.8%) loads on chlorophyll, carotenoids and flavonoids, and represents constitutive properties of the species (Table 2, Fig. 1).

Table 2. Loadings of the eleven parameters on the first two principal components.

Parameter	PC1 (66.3%)	PC2 (25.8%)
Superoxide dismutase	+0.369	−0.007
Proline	+0.368	−0.016
Catalase	+0.368	+0.026
Ascorbic acid	+0.366	+0.033
Peroxidase	+0.364	−0.020
Total phenolic content	+0.331	+0.134
Flavonoid content	+0.279	+0.334
Malondialdehyde	+0.231	−0.302
Total carotenoids	+0.037	+0.583
Total chlorophyll	−0.011	+0.586
Leaf extract pH	−0.291	+0.305
Parameters are ordered by descending PC1 loading. PC2 is oriented so that pigment content increases upward.		

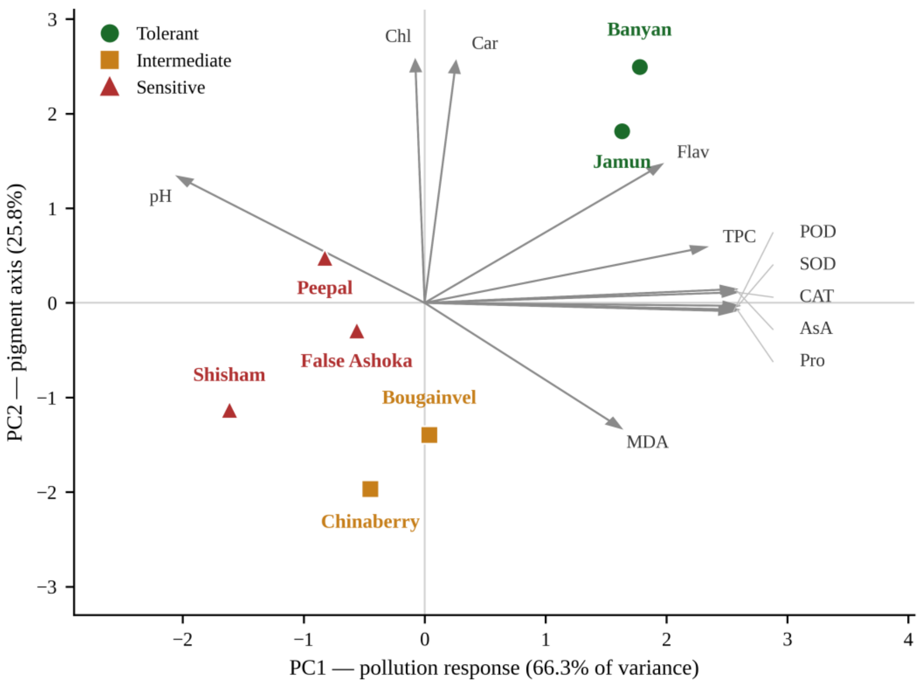

Fig. 1. Principal component biplot of the eleven biochemical parameters (grey vectors) and the seven species (symbols) positioned by their mean scores. Symbol shape and colour denote the tolerance class obtained independently by cluster analysis.

Two features of this structure have practical consequences. First, ascorbic acid, proline and the three enzymes were intercorrelated at r = 0.985 to 0.997, so they behave as a single co-regulated module and carry largely redundant information. Second, flavonoids group with the pigments rather than with the soluble antioxidants: flavonoid content ranks the species almost identically to chlorophyll (ρ = +0.964) but not to peroxidase (ρ = +0.393). Flavonoids in these species are therefore better regarded as constitutive leaf constituents than as an inducible defence pool.

Leaf extract pH was the only parameter correlating significantly with all ten others across the 63 observations, with coefficients ranging from r = −0.782 against malondialdehyde to r = +0.394

against carotenoids (all $p < 0.01$). It is the single parameter that responds to both axes of the structure.

3.3 Three tolerance classes

Ward clustering on the standardised species means separated the seven species into three groups (Fig. 2). Banyan and Jamun joined at a linkage distance of 2.24 and remained apart from the remaining five until the final join at 9.44, the largest separation in the dendrogram. Bougainvel and Chinaberry joined at 1.17, the closest pair. Shisham, Peepal and False Ashoka formed the third group.

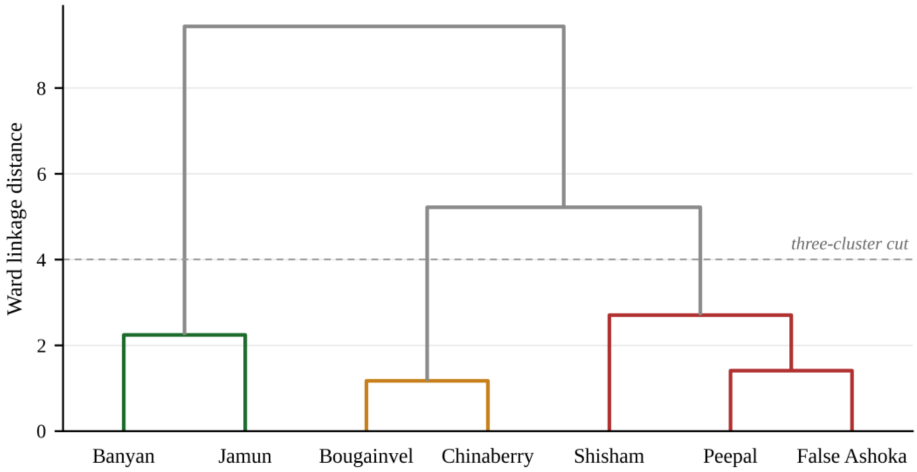

Fig. 2. Dendrogram of the seven species from Ward's minimum-variance linkage on the eleven standardised parameters. The dashed line marks the three-cluster cut.

This classification was obtained without reference to the pollution gradient, so its agreement with a damage-based ranking is an independent check rather than a restatement. Ranking the species by mean malondialdehyde content gives exactly the same three groups, and the ordering is reproduced perfectly by the mean first-component score ($\rho = 1.000$) and closely by the mean rank across all eleven parameters ($\rho = +0.893$) (Table 3).

Table 3. Tolerance classification of the seven species, with the parameters most relevant to selection.

Species	Rank	Class	MDA	Ascorbate	Chlorophyll	Leaf pH
Ficus benghalensis	1	Tolerant	0.99	216.2	52.18	6.51
Syzygium cumini	2	Tolerant	1.11	200.1	46.27	6.20
Bougainvillea spectabilis	3	Intermediate	1.25	160.6	22.93	6.07
Melia azedarach	4	Intermediate	1.47	144.7	19.83	6.07
Polyalthia longifolia	5	Sensitive	1.64	133.2	33.26	6.29
Ficus religiosa	6	Sensitive	1.83	117.8	41.10	6.32
Dalbergia sissoo	7	Sensitive	2.11	98.8	27.12	6.39

Values are means of the nine site values. MDA in nmol cm^{-2}; ascorbate and chlorophyll in µg cm^{-2}. Rank 1 denotes the least membrane damage sustained.

4. Discussion

4.1 Which species to plant

Ficus benghalensis and Syzygium cumini are the clear recommendations for heavily trafficked corridors. Both combine a large antioxidant reserve with high pigment retention and sustain the least membrane damage, and they occupy the favourable end of both axes in Fig. 1. Bougainvillea spectabilis and Melia azedarach are adequate as supplementary species: their antioxidant capacity is sufficient, but their low constitutive pigment content limits their value where canopy density is also an objective.

Two negative recommendations are worth stating explicitly because both run against common practice. Dalbergia sissoo is widely planted along highways in the region and is regarded as a hardy tree, yet it recorded the lowest antioxidant capacity of the seven species and the highest membrane damage at every site. Its large proportional induction — peroxidase activity rose by 965.4%, the largest response in the study — should not be mistaken for tolerance, because the absolute activity it reaches at the most polluted sites (9.2 μmol min^{-1} mg^{-1}) remains below what F. benghalensis maintains at merely moderately polluted sites (11.3 μmol min^{-1} mg^{-1}). Tolerance here rests on the constitutive size of the antioxidant pools rather than on the extent to which they can be induced.

The second is that Ficus religiosa cannot be assumed to share the tolerance of F. benghalensis. The two congeners are morphologically similar and are planted for the same purposes, but they fall in different classes: F. religiosa carries high chlorophyll but the second-lowest ascorbate and enzyme activities of the seven species, and sustains the second-highest damage. Fig. 1 shows why — it sits high on the pigment axis but well to the negative side of the pollution-response axis.

4.2 A Graded Monitoring Protocol

The redundancy within the antioxidant module means that a monitoring programme does not need all eleven parameters. We suggest the following graded scheme, matched to available laboratory capacity.

One parameter: leaf extract pH. It is the only parameter correlating with all ten others, it responds to both axes of the structure, and it is by a wide margin the cheapest and fastest determination.

Two parameters: add total chlorophyll. Together with pH this spans both principal components, so the two dimensions of the response are covered.

Three parameters: add ascorbic acid. Its response is the largest of the non-enzymatic antioxidants (+184.9%), which gives good discrimination relative to analytical error, and it permits comparison with the extensive Air Pollution Tolerance Index literature.

Where an enzyme assay is affordable, one suffices. Peroxidase gives the steepest gradient of the eleven parameters and therefore the greatest discrimination between pollution levels.

Two constraints apply to any reduced protocol. Comparisons must be made within a species against its own control, because species differ by up to seven-fold in absolute values, so a single leaf sample cannot be interpreted without knowing its species. And determinations should be made on a standardised leaf-area basis, which removes variability arising from differences in leaf thickness and specific leaf area.

4.3 Relation to the Air Pollution Tolerance Index

The Air Pollution Tolerance Index, which combines ascorbic acid, chlorophyll, leaf pH and relative water content, is the most widely used composite for this purpose. Applied to the present species means, an index of this form ranks the two extremes correctly but agrees only moderately with the damage-based ordering ($\rho = +0.679$). The disagreement is instructive rather than incidental.

The index misplaces Ficus religiosa, ranking it third of seven where the damage marker places it sixth. This happens because the index rewards high chlorophyll and does not include any measure of damage sustained, and F. religiosa combines high pigment content with low antioxidant capacity. The two-component structure recovered here explains the failure directly: a composite drawing mainly on the pigment axis will misrank any species whose position on the two axes is discordant. Where an index is used for species selection, we suggest it be checked against a damage marker such as malondialdehyde, or against a cluster classification computed on the full parameter set.

4.4 Limitations

Composite sampling yields one value per species–site combination, so no independent within-site error term is available and the analysis is confined to rank-based procedures. Sampling covered a single winter season, so seasonal variation in the response is not characterised. Site classification rests on traffic character rather than measured ambient pollutant concentrations, so the study cannot attribute the response to any individual pollutant. The three cities lie within one semi-arid region, and the finding that they are statistically indistinguishable is a statement about that region rather than a general one.

5. Conclusions

The biochemical response of roadside trees to vehicular pollution was uniform in direction across seven species, nine sites and three cities, and did not differ significantly in magnitude between cities. The eleven parameters measured resolve onto two nearly independent axes accounting for 92.1% of variance, one representing the response to exposure and the other the constitutive pigment status of the species. Species fall into three tolerance classes that are reproduced by independent criteria.

For practical purposes two conclusions follow. Ficus benghalensis and Syzygium cumini should be preferred for heavily trafficked corridors, and Dalbergia sissoo should not be selected on an assumption of tolerance despite its reputation and its large induction response. And a monitoring programme can be reduced to one, two or three parameters — leaf extract pH, then total chlorophyll, then ascorbic acid — with little loss of diagnostic value, provided comparisons are made within a species against its own control.

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