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Interference of dust deposition on the physiology of roadside plants: screening bio-filters using a differential green exhaust absorption method

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Abstract

Plant leaves are the receptors of atmospheric gases and sunlight. Roadside dust and particulate pollutants deposited on the surfaces of plant leaves can impact plant physiology. The rate of dust deposition and the level of interference can also vary with species. The present sampling was conducted along the roadsides around Netaji Subhas Chandra Bose (N.S.C.B.) International Airport, Kolkata, India. The area is highly congested with a variety of motor vehicles that generate heavy gaseous and particulate pollutants into the surrounding air. We studied the differential effects of dust deposition on plant mesophyll cell-sap pH, relative water content, chlorophylls a and b and carotenoid, ascorbic acid, total protein, sugar contents. We also examined the differential reduction of oxygen release to the surrounding atmosphere. Dust deposition was found to be highest on Ficus benghalensis and lowest on Polyalthia longifolia out of the total number of roadside species studied (n = 5) in this research. Furthermore, Polyalthia longifolia was found to be least affected by dust deposition as estimated by our air pollution tolerance index (APTI) and in terms of oxygen emitted to the surrounding air. We determined a set of species that can cope best with dust deposition, which will help determine the optimal species to be used in the abatement of particulate roadside pollution and as natural filters.

Keywords: Air-pollution, Dust, Natural filters, Oxygen exhaust, Physiology, APTI.

Introduction

A significant part of urban air pollution is contributed by on-road vehicles. Automobiles produce volatile organic compounds (VOC), suspended particulate matter (SPM), oxides of sulphur (SO_X), oxides of nitrogen (NO_X), carbon monoxide (CO), etc. which can have adverse effects on surrounding ecosystem[1]. All these components and minute soil particles collectively can contribute to road dust and fly in air. Subsequently these gaseous and particulate pollutants can adversely affect adjacent vegetation if they persist for a long period[2]. The increasing load of on road vehicles in Kolkata megacity has caused a huge uprise in NOx, CO, HC, Pb, CO_2, SPM and RPM levels[3,4].

Roadside plants can mitigate polluted atmospheres and yet are also the most impacted by roadside pollutants. Impact of air pollution on different plant species is a major ecological issue. Wind erosion suspends large quantities of dust in the atmosphere that settles back to the earth's surface and is deposited on plant leaves when wind velocities decrease[5]. The climatic conditions, physicochemical properties of air pollutants and their residence time in the atmosphere can have different degrees of impact on surrounding plants, which in turn can depend on the plant species in question.

Selection of plant species that are tolerant of dust deposition can be effective methods of both indicating levels of pollution and as pollutant scavengers[6]. Through the course of evolution different pollution hazards have forced natural selection through which pollution resistant genotypes have evolved[7]. However, screening of different plants for their ability to resist pollution is needed to develop management strategies to mitigate dust and air pollution[8].

To screen plants for their sensitivity or tolerance level to air pollution, a proper selection measure of plants is of vital importance. The tolerance level of plants to dust and pollution can also depend on topography[9] and tolerance levels of different plant species are not uniform.

Physiological disorders are due to cumulative effects of the causal factors on plant growth and development[10] that can be revealed by comparative analysis of some biochemical properties of the plant in different conditions. Dust accumulation also provokes severe damage in the photosynthetic apparatus[11]. So, logically, loss of photosynthetic efficiency can result in reduced carbohydrate production and oxygen release.

Our present research studied the differential responses and tolerance levels of some roadside tree species to suspended air pollutants in terms of net oxygen release to the surrounding air. This research aims to help select optimal species from a set of common trees for roadside plantation as effective bio-filters.

Materials and methods

Selection of site: We selected a 'traffic congested site' at No.1 Airport traffic area beside Netaji Subhas Chandra Bose (N.S.C.B.) International Airport, Kolkata, India. This site represents a transition traffic zone (22°38'31.9"N; 88°25'52.8"E) between four heavily congested roads with a prevalence of private, public and goods transports. The plants near these locations are continuously exposed to flying dust and vehicular pollution. For a comparative part of our study we selected an almost pollution free site as a 'controlled site' (22°38'43.1"N 88°26'17.2"E) at the airport terminal area, with prohibited vehicular transport zone, which is situated ~2.5 km away from the polluted study site. The air quality of this location is considerably cleaner than the congested area as we pre-examined the amount of deposited dust on leaf surfaces of surrounding plant species.

Collection of leaf samples: During March-April 2015 we selected five tree species of common occurrence–*Ficus religiosa* L., *Neolamarckia cadamba* (Roxb.) Bosser, *Polyalthia longifolia* (Sonn.) Thwaites, *Ficus benghalensis* L., *Psidium guajava* L., from our roadside and controlled sites. Fresh leaf samples (in five replicates of selected trees) were collected from mature plants, found growing along the roadsides with very similar topography and common conditions at both study sites (about 5-8 feet height from ground level) sampled in the early morning. All these leaves were free from pathogens and disease. After collection, leaves were quickly transferred to the laboratory in polythene bags kept in an ice box for further analysis within 24 hrs of their harvesting.

Differential amounts of reduction in O_2 release due to dust deposition: We devised a method to track reduction of O_2 levels from plants due to dust deposition. Our protocol is a modification of that discussed by Millan-Almaraz *et al*[12]. Leaves (forth to fifth from the apex with optimum maturation) from both our polluted and control study sites were simultaneously used to perform this experiment. Two leaves of same plant were wrapped separately with colourless polythene bags and made air tight with rubber bands tied at petioles for 2 h (Figure-1). One set contained a perforated polythene box having KOH pellets and the other set contained similar box with anhydrous (blue) silica gel crystals. Silica gel crystals absorb water molecules and KOH pellets absorb both water and CO_2. Increase in weight of silica gel crystals in the experimental span of 2h is due to photosynthesis as well as transpiratory expulsion of water molecules from the leaves. Difference in the amount of expelled water from dust deposited and control leaves give an estimate of difference in photosynthesis rate under same environmental and experimental conditions.

Figure-1: Experimental setup to measure reduction in O_2 release due to dust deposition (A- with silica gel crystals, B- with KOH pellets)

SET 1: Bagging of leaves with Silica Gel crystals (SG): TYPE 1A: With dust deposited leaf - W_{dSGf}–W_{dSGi}= $\Delta SG_{dusted\ leaf\ set}$ = amount of water absorbed by $SG_{dusted\ leaf\ set}$= amount of water released from the dusted leaf due to photosynthesis and transpiration.

TYPE 1B: With control leaf: W_{cSGf}–W_{cSGi}= $\Delta SG_{control\ leaf\ set}$= amount of water absorbed by $SG_{control\ leaf\ set}$ = amount of water released from the control leaf due to photosynthesis and transpiration together.

$\Delta SG_{control\ leaf\ set}$ – $\Delta SG_{dusted\ leaf\ set}$ = reduction in photosynthetic water release as an effect of dust deposition on the leaf surface. This reduction in photosynthetic water release was converted into the reduction of photosynthetic oxygen donation rate to the environment by the green leaves.

SET 2: Bagging of leaves with anhydrous KOH pellets: TYPE 2A: With dust deposited leaf: W_{dKOHf} – W_{dKOHi} = $\Delta KOH_{dusted\ leaf\ set}$ = amount of CO_2 and water absorbed by $KOH_{dusted\ leaf\ set}$ = amount of CO_2 and water released from the dusted leaf due to respiration and transpiration respectively.

TYPE 2B: With control leaf: W_{cKOHf}– W_{cKOHi}= $\Delta KOH_{control\ leaf\ set}$ = amount of CO_2 and water absorbed by $KOH_{control\ leaf\ set}$ = amount of CO_2 and water released from the dusted leaf due to respiration and transpiration respectively.

$\Delta KOH_{dusted\ leaf\ set}$ –$\Delta KOH_{control\ leaf\ set}$ = amount of respiratory CO_2 that is less utilized by the dusted leaf in relation to control leaf = effect of deposited dust on CO_2 uptake for photosynthesis.
[W_{dSGf} - final weight of the SG used in the dust deposited leaf chamber, W_{dSGi} - initial weight of the SG used in the dust deposited leaf chamber, W_{cSGf} - final weight of the SG used in the control leaf chamber, W_{cSGi} - initial weight of the SG used in the control leaf chamber, W_{dKOHf} - final weight of the KOH used in the dust deposited leaf chamber, W_{dKOHi} - initial weight of the KOH used in the dust deposited leaf chamber, W_{cKOHf}- final weight of the KOH used in the control leaf chamber, W_{cKOHi}- initial weight of the KOH used in the control leaf chamber]

Chlorophyll –a and b: About 250 mg of deveined leaves from each sample were grounded in a chilled mortar pestle held in an ice tray with a pinch of quartz sand and extracted with a total of 10 ml absolute acetone. The extract was transferred into a 15 ml centrifuge tube and centrifuged at 2500 rpm for 10 minutes at 4°C. An aliquot of the clear leaf extract (supernatant) was transferred to a cuvette and absorbance was measured against a solvent blank in a UV-VIS spectrophotometer (Shimatzu UV 1800, Japan) at 663 and 645 nm wavelengths respectively.

Quantification of pigments (for 100% acetone) was done using the formula given by Lichtenthaler[13].
Chl-a (µg/ml) = $11.24\ A_{661.6} - 2.04\ A_{644.8}$
Chl-b (µg/ml) =$20.13\ A_{644.8} - 4.19\ A_{661.6}$

Relative leaf water content (RWC): RWC of all the sample leaves were determined following the method described by Liu and Ding[14]. Accordingly, RWC = (wf – wd) × 100 / (wt – wd) where: wf - fresh weight of the leaf , wt - turgid weight of the leaf after immersing into water overnight and the wd- dry weight of the leaf.

Ascorbic acid (AA) content: Ascorbic acid contents in the targeted leaves were determined using the method of Roe and Kuether[15].

Leaf extract pH: Leaf samples (500 mg) were crushed and homogenized in 50 ml double distilled water, the mixture was centrifuged and supernatant was collected for detection of pH using Digital pH Meter (Systronics335).

Amount of dust deposition: The amounts of dust deposited on the unit areas of the leaves sampled were calculated by taking the initial and final weight of the container in which they were washed, followed by complete evaporation. It was calculated using the formula given by Keller and Lamprech[16].

Estimation of protein: The amounts of total proteins were estimated following the standard method given by Lowry et al.[17].

Estimation of soluble sugar: Total soluble sugar contents were estimated using Anthrone reagent following the method described by Pons et al.[18].

Air Pollution Tolerance Index (APTI): Air Pollution Tolerance Indices of tree species were calculated by the formula given by Singh and Rao[9].

According to the method, APTI = {A(T+P)+R}/10

Where: A = Ascorbic acid content of leaf in mg/gm fresh weight, T = Total chlorophyll content of leaf in mg/gm fresh weight, P = Leaf extract pH, R = Percent relative water content of leaf.

Statistical Analysis: Each experimental result was based on five replicates. The standard errors of mean values were calculated and to determine the significance level paired t-tests were performed using Microsoft Office Excel package.

Results and discussion

The highest amounts of dust were observed on leaves from *Ficus benghalensis* (0.187±0.021 mg/cm^2) and the lowest were on leaves from *Polyalthia longifolia* (0.075±0.005 mg/cm^2) (Table-1). An increasing trend of foliar surface dust content was observed among our other studied species in the order: *Neolamarckia cadamba* (0.131±0.021 mg/cm^2) <*Ficus religiosa* (0.142±0.011 mg/cm^2) <*Psidium guajava* (0.164±0.031 mg/cm^2). Reduction in the amount of released O_2 was found to be statistically significant with species with a maximum observed for *Psidium guajava* (0.13±0.010 mg/cm^2/hr) and minimum for *Polyalthia longifolia* (0.03±0.006 mg/cm^2/hr) among the investigated species. There was a significant decrease in amount of photosynthetic pigments like chlorophyll-a, b and carotenoid found in the polluted site as compared to the control site (Table 1). Degradation of chlorophyll was observed to be at a maximum in *Psidium guajava* (35.06%) and a minimum in *Polyalthia longifolia* (16.66%). All the physiological parameters studied became considerably lower and the mesophyll cell pH was acidic in the polluted roadside dust containing members (Table-1). The lowest amount of reduction in Relative water content (8.95%), Ascorbic acid (22.36%), and total protein (23.84%) had been found in *Polyalthia longifolia* when comparing our polluted and control roadside sites. The total soluble sugar production was least affected (Table-1) in case of *Neolamarckia cadamba* (12.69%) and was greatly reduced in *Ficus benghalensis* (54.68%). Leaf cell pH also became comparatively less acidic in *Polyalthia longifolia*. The Air Pollution Tolerance Index (APTI) was found to be highest from *Polyalthia longifolia* (7.44) and the lowest value was from *Ficus religiosa* (5.19). Comparative statistical analysis using a t-test showed significant variation (mostly P<0.05) in the estimated amount of biochemical parameters among control and vehicle loaded sites excluding only *P. Longifolia.*

Table-1(a): Effect of dust deposition on some biochemical and physiological parameters of selected roadside plants (Mean ±SE)

Plants	Chl-a (mg/gm)			Chl-b (mg/gm)			Total chl (mg/gm)			Relative water content (%)		
	Polluted	Control	t test P	Polluted	Control	t test P	Polluted	Control	t test P	Polluted	Control	t test P
Ficus religiosa	0.29± 0.0115	0.39± 0.0173	0.003317	0.18± 0.0103	0.25± 0.0098	0.000136	0.47± 0.01	0.64± 0.02	0.037405	49.35± 2.55	63.62 ± 1.76	0.068879
Neolamarckia cadamba	0.31± 0.0115	0.38± 0.0057	0.05612	0.17± 0.0078	0.23± 0.0133	0.008253	0.48± 0.011	0.61± 0.012	0.004897	59.58± 1.98	69.14 ± 2.31	0.021967
Polyalthia longifolia	0.19± 0.0104	0.23± 0.0057	0.130572	0.11± 0.0063	0.13± 0.0075	0.003317	0.3± 0.013	0.36± 0.018	0.303599	73.11± 2.35	80.30 ± 1.69	0.058275
Ficus benghalensis	0.3± 0.0086	0.41± 0.0115	0.000688	0.19± 0.0109	0.27± 0.0119	0.07319	0.49± 0.014	0.68± 0.015	0.003351	56.14± 2.26	74.52 ± 2.42	0.005542
Psidium guajava	0.28± 0.0028	0.45± 0.0086	0.004582	0.22± 0.0127	0.32± 0.0132	0.000033	0.5± 0.02	0.77± 0.04	0.047071	59.26± 1.49	85.32 ± 1.58	0.074653

Table-1(b): Effect of dust deposition on some biochemical and physiological parameters of selected roadside plants (Mean ±SE)

Plants	Ascorbic acid content (mg/gm)			Leaf extract pH			Amount of deposited dust (mg/cm^2)			Total protein content (mg/gm)		
	Polluted	Control	t test P	Polluted	Control	t test P	Polluted	Control	t test P	Polluted	Control	t test P
Ficus religiosa	0.43 ±0.029	0.57 ±0.034	0.023568	5.52 ±0.21	7.56 ±0.42	0.003514	0.142 ±0.011	0.049 ±0.006	0.000962	12.35 ±0.92	16.72 ±1.21	0.001465
Neolamarckia cadamba	0.59 ±0.031	0.76 ±0.041	0.037405	4.73 ±0.53	5.86 ±0.48	0.000652	0.131 ±0.021	0.067 ±0.008	0.013476	11.21 ±0.86	14.96 ±0.95	0.000192
Polyalthia longifolia	0.23 ±0.016	0.32 ±0.019	0.024883	5.72 ±0.61	6.39 ±0.49	0.010524	0.075 ±0.005	0.036 ±0.004	0.000219	10.86 ±1.01	14.26 ±2.01	0.027645
Ficus benghalensis	0.5 ±0.021	0.66 ±0.036	0.059509	5.03 ±0.38	6.96 ±0.86	0.020001	0.187 ±0.021	0.061 ±0.017	0.000336	12.53 ±0.87	18.36 ±2.16	0.015931
Psidium guajava	0.7 ±0.031	0.97 ±0.041	0.024699	5.63 ±0.44	6.54 ±0.81	0.050933	0.164 ±0.031	0.052 ±0.007	0.014963	13.91 ±0.99	18.96 ±1.22	0.000691

Table-1(c): Effect of dust deposition on some biochemical and physiological parameters of selected roadside plants (Mean ±SE)

Plants	Total sugar content (mg/gm)			Air pollution tolerance index (APTI)		Reduction in oxygen exhaust (mg/cm^2/hour)
	Polluted	Control	t test P	Polluted	Control	
Ficus religiosa	1.06±0.12	1.81±0.18	0.002127	5.19	6.82	0.09±0.005
Neolamarckia cadamba	1.65±0.15	1.89±0.17	0.002307	6.26	7.4	0.07±0.007
Polyalthia longifolia	1.56±0.18	2.16±0.21	0.000832	7.44	8.24	0.03±0.006
Ficus benghalensis	0.87±0.08	1.92±0.25	0.008625	5.89	7.95	0.11±0.011
Psidium guajava	1.24±0.12	2.64±0.34	0.008131	6.35	9.24	0.13±0.010

Discussion: Our research included a simplified way of measuring the effect of the leaf dust deposition on net photosynthetic release of oxygen to the atmosphere. Our experiment was devised from the concept and stoichiometry of photosynthesis within closed colourless chambers. In a closed colourless chamber the only source of CO_2 for photosynthetic uptake by a leaf is its respiratory CO_2 expelled from the leaf. Dust deposition on leaf surface reduces the photosynthetic CO_2 uptake in comparison with a control or reference leaf under same condition of a colourless closed chamber. With passage of time a certain amount of CO_2 is surplus in the dust deposited leaf chamber in relation to reference leaf chamber. This amount indicates proportionate reduction in photosynthesis or net O_2 donation to the surroundings (mg/cm^2 of leaf area/hour) due to dust deposition on leaf surface. Following the above experiment used perforated KOH box kept (as surplus CO_2 absorbent) in a closed colourless polythene chamber enclosing a leaf attached with the mother plant. Two separate sets were used for dusted and control leaves. KOH also absorbs moisture exhausted from the leaves within the closed chamber, but it is nullified during subtraction of calculation in the direction of the present experiment. A similar retarding effect of foliar dust deposition on photosynthesis rate can also be obtained using a suitable moisture absorbent (like anhydrous silica gel crystals) kept within colourless closed chambers as stated above. The amount of water vapour released during photosynthesis decreases due to dust deposition in relation to reference leaf. Both the experimental setup with common objective to determine the effect of dust deposition on the rate of photosynthesis or O_2 release to the surrounding atmosphere has been designated in the present study as ***'differential green exhaust absorption method'***.

Differential values of dust content could be considered as a species-specific holding capacity. This specificity depends on their surface geometry, phyllotaxy and leaf exo-morphic characters such as hairs, cuticle, length of petiole, height and canopy etc.[19-22]. Therefore in order to screen species with comparatively lower dust retention and higher exhaust absorption capacity we included two experimental sites (i.e. control and vehicular transport loaded) with different environmental conditions. Weather condition, direction and speed of wind also affect the dust interception capacity of plants. The highest dust accumulation we observed in *Ficus benghalensis,* which may be due to its shiny, waxy coating and rough surface with short petioles[23], whereas in case of all other species, lower dust accumulation was due to their relatively rough surface with slightly folded margin. The lowest values were observed in *Polyalthia longifolia,* which was most likely a result of its specialized canopy structure and leaf arrangement strategy.

Due to dust deposition on leaves, photosynthesis rates were reduced. Hence the amount of oxygen production was also decreased among the roadside plants in that polluted site. As the species *Polyalthia longifolia* had the lowest amount of dust accumulation on its leaves it also had less severity in pigment damage and there in its oxygen release rate was also reduced.

We found that for all the plant species we investigated had lowered the amount of photosynthetic pigment with dust deposition. This is in congruence with previous research that found that dust accumulation caused severe damage in the photosynthetic apparatus[11] and decreased chlorophyll content[5,24]. Deposited dust on leaf alters its optical properties particularly the surface reflectance[25] and interrupts sunlight from reaching photosynthetic apparatus and subsequently affects plant growth.

The pH change in the leaf cell sap is mostly caused by the pollutant particles accumulated on leaves. We found that for all the plant species we investigated were there was a shift to a more acidic pH in the more polluted study site. In a previous report[23]*Artocarpus heterophyllus* leaf extracts also had alower pH due to dust deposition, which was ascribed to acidic pollutants being present on leaves. Furthermore, roadside dust accumulation becomes a barrier in all growth condition[26]. For this reason the relative water content, sugar and protein production was found to be affected in plants. Mesophyll cell water maintains the physiological balance under physiologically stressful conditions of air pollution, when transpiration rates are usually high. Ascorbic acid is a strong reductant that protects chloroplasts against SO_2 induced H_2O_2, O_2 and OH– accumulation and thus protects the enzymes associated with CO_2 fixation and chlorophyll for inactivation together with leaf pH. Thus, plants maintaining high ascorbic acid under polluted conditions are considered to be tolerant to air pollution.

An air pollution tolerance index is a value determined by several biochemical parameters that indicate plants adaptability towards tolerance. A very similar and notable trend was followed by the plant *Polyalthia longifolia* having the highest APTI throughout our study. In addition, a very small change occurred in this species regarding biochemical properties after dust deposition. From a number of viewpoints this species had both pollution avoidance (due to exo-morphic adaptations to pollution) and tolerance (less effect of dust deposition on physiological and biochemical parameters) capacity. This was also found in a previous study[27], which was conducted with a different group of species. *Polyalthia longifolia* bears the characters of an effective bio-filter in highly vehicle loaded polluted roadside.

Conclusion

Atmospheric pollution has differential effects on the plant species investigated in this study. Dust deposition induces changes in the physiological and biochemical parameters. Dust deposition corresponds significantly with a reduction in photosynthetic O_2 release to the environment. The extent of such changes depends on plant specific tolerance and avoidance towards dust. Proper screening of the plants which can thrive in the pollution effect made possible to utilize them for pollution mitigation. On the basis of our research findings *Polyalthia longifolia* could serve as an ecological model to ensure a relatively pollution free environment. We also found that the ‘differential green exhaust absorption method’ can be applied for rapid screening of efficient bio-filters against suspended air pollution.

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