Urban climate and its effect on biochemical and morphological characteristics of Arjun (*Terminalia arjuna*) plant in National Capital Region Delhi

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This paper reports impacts of urban pollution on the biochemical and morphological characteristics of Arjuna (*Terminalia arjuna*) in particular the effects of urban industrial dustfall deposition on its foliar surface at a residential site (Jawaharlal Nehru University, JNU) and an industrial site (Sahibabad, SB) in Delhi region. Atmospheric dustfall fluxes were estimated for major anions and cations. Morphological analysis of foliar samples was carried out by using the scanning electron microscope. Biochemical parameters, namely chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, total soluble sugar, proline amino acid and ascorbic acid were also analysed in foliar samples. Results showed that the dustfall fluxes of ($SO_4^{2-} + NO_3^-$) at the industrial site were almost three times higher than that of the residential site. This can be attributed to the emissions of industrial activities and diesel-driven vehicular traffic in the area. It was observed that these elevated fluxes of $SO_4^{2-}$ and $NO_3^-$ had significant impacts on the biochemical constituents of the plant and foliar morphology. Concentrations of chlorophyll and carotenoids were recorded decreasing with increasing dustfall fluxes of ($SO_4^{2-} + NO_3^-$), whereas proline and ascorbic acid were found to be increasing with the increase in the dustfall fluxes of ($SO_4^{2-} + NO_3^-$) indicating the effect of pollution stress. The study showed that the deposition of dustfall was responsible for damage to stomata and leaf surface morphology, more significantly at the industrial site.

**Keywords:** air pollution; urban dust; dry deposition; biochemical parameters; stomatal damage

1. Introduction

It is widely accepted that high levels of atmospheric pollution can trigger severe environmental problems. Both gaseous as well as particulate pollutants play a very important role in various plant processes such as nutrient cycling, soil acidity and photosynthesis.\[1\] Different natural and industrial sources emit a considerable amount of particulate matter and gases such as $SO_2$ and $NO_2$, which finally settle down through wet and/or dry deposition processes.\[2\] Air particles have been reported to create serious environmental problems in industrial and urban areas.\[3\] According to WHO database, air pollution is a severe problem of low-income group countries including south Asian mega cities such as Delhi, Dhaka and Kathmandu.\[4\] Much of the air pollution in these cities is contributed by the transport sector due to large and continuously increasing density of the automobile vehicles. According to 2010 data, Delhi city has witnessed...
185% increase in the number of vehicles as compared to 1994.[5] There has been an increase of 76% in road length of Delhi since 1985–2011, indicating huge expansion of city and so the large emissions of particulate matter and gaseous pollutants from various anthropogenic activities.[5] Besides local sources, Delhi also receives air pollution through long-range transport as well as from nearby states especially agriculture residue burning emissions from Punjab and Haryana states and brick kilns emissions from nearby Uttar Pradesh states.[6–9]

It has been reported that air pollution stress limits the plant productivity and sometimes even survival of the plants.[10] Gases such as SO$_2$ and NO$_2$ are highly phytotoxic pollutants which enter through the stomata creating a stress to the plant.[11] Plant exposure to high levels of particulates may lead to different phytotoxic responses such as retarded rates of photosynthesis, flowering, yield, scavenging of reactive oxygen species (ROS) [12] and adaptation against climate changes.[13–17] Suspended soil dust and road dust are found abundant in the atmosphere in India. During prevailing dry weather conditions, deposition of particles through dustfall is the major pathway of removal of pollutants. Significant concentrations of sulphate and nitrate have been reported in dustfall at various sites in India.[18] Deposition of atmospheric dust with very high or very low pH may directly or indirectly affect the plant having a negative impact on photosynthesis and plant growth.[19] However, despite huge dustfall being deposited on the foliar surface several plant species survive because of the production of biochemicals such as ascorbic acid, proline and polyphenols which are considered as expressions of non-enzymatic resistance in plants against abiotic stresses.[20,21]

Considering the importance of dustfall for plant health, this study reports fluxes of dustfall and its major ionic species on the foliar of a medicinally important plant, that is, Arjun (Terminalia arjuna) at two sites of different characteristics in National Capital Region (NCR) of Delhi. As mentioned earlier, there have been several studies about SO$_2$ and NO$_x$ deposition on foliar but this paper highlights the harmful effects of dustfall fluxes of SO$_4^{2-}$ and NO$_3^-$ on various biochemical parameters and surface morphology of foliar.

2. Methodology

2.1. Site description

The study was carried out at two sites located in the NCR of Delhi (i) Jawaharlal Nehru University (JNU) and (ii) Sahibabad (SB) which represent residential and industrial characteristics, respectively. The location of both the sampling sites has been shown in Figure 1.

2.1.1. Jawaharlal Nehru University

JNU campus is located in south Delhi (28°53′N, 77°34′E) (Figure 1). The campus has a vast green cover as ridge forest, which is dominated by Prosopis plant species. Other tropical plant species such as Morus, Acacia, Arjun, Cassia, Leucaena, Bauhinia species, etc. are also found in this forest. There is no industry in the vicinity of the site. However, vehicular traffic and construction activities are important sources of air pollution in and around the campus.

2.1.2. Sahibabad

Sahibabad is located 35 km east of central Delhi (28°67′N, 77°34′E) (Figure 1). In fact, this area falls under the Ghaziabad district of Uttar Pradesh state. It is an industrial area where several industrial units are located which include steel-processing, electrical, paints, plastic-processing
units, etc. The two important national highways, namely NH-24 and NH-58 pass through the site. The highways have a very high density of diesel-driven vehicles which contribute to significant emissions of $SO_2$, road dust and other particulates. This locality is very crowded having 3–4 times higher population density than that of the JNU campus.

2.2. Sample collection and analysis

2.2.1. Collection of foliar dustfall

Foliar dustfall was collected on 10 days exposure basis from the Arjun (T. arjuna) plant during winter season (November–January) of 2012–2013. Arjun tree is a large, deciduous/evergreen, tall (up to 15–25 m), often having a buttressed trunk, and a broad, oval crown with drooping branches. The tree has thin, smooth, shiny and greenish-grey bark which will peel off regularly. Around 150 trees/km$^2$ are planted in the vicinity of both the sites. The average age of the Arjun plant is approximately 15–20 years. The leaves were selected at around 3 m height off the ground from the road side. The selected leaves were tagged, cleaned and properly washed with distilled-deionised water using a sprayer. Four to five leaves were plucked after every 10 days and washed with 50 mL distilled-deionised water using a well-defined surface washing method.[22] Totally, seven samples of dustfall were collected during the period of study. Major anions ($Cl^-$, $F^-$, $NO_3^-$ and $SO_4^{2-}$) and cations ($Na^+$, $K^+$, $NH_4^+$, $Ca^{2+}$ and $Mg^{2+}$) were determined in the aqueous extract of dustfall by Ion Chromatography (Metrohm 883 Basic IC Plus) equipped with anion (Metrosep A SUPP 4, 250/4.0) and cation (Metrosep C4–100/4.0) columns. Anions were determined by using a mixture of 1.8 mmol/L $Na_2CO_3$ and 1.7 mmol/L $NaHCO_3$ as the eluent, while cations were determined by using a mixture of 1.7 mmol/L nitric acid and 0.7 mmol/L dipicolinic acid as the eluent. Calibration of methods and quantification of components were carried out using
Table 1. Calibration equation, correlation coefficient and analytical precision for different ionic species.

| Major ions | Calibration equation | Correlation coefficient ($r^2$) | Precision (%) |
|------------|-----------------------|----------------------------------|---------------|
| $F^-$      | $y = 0.1368x + 0.0481$ | 0.9999                           | 1.06          |
| $Cl^-$     | $y = 0.1081x + 0.0035$ | 0.9998                           | 3.37          |
| $NO_3^-$   | $y = 0.0552x - 0.0066$ | 0.9998                           | 1.14          |
| $SO_4^{2-}$| $y = 0.0858x + 0.0008$ | 0.9997                           | 3.07          |
| $Na^+$     | $y = 0.2138x - 0.0008$ | 0.9999                           | 2.59          |
| $NH_4^+$   | $y = 0.2527x - 0.0100$ | 0.9999                           | 2.84          |
| $K^+$      | $y = 0.1172x + 0.0025$ | 0.9999                           | 4.08          |
| $Ca^{2+}$  | $y = 0.2527x - 0.0100$ | 0.9999                           | 0.41          |
| $Mg^{2+}$  | $y = 0.3924x + 0.0088$ | 0.9999                           | 2.80          |

MERCK reference standards (CertiPUR). In the case of anions, 1, 2 and 5 ppm standards were used to achieve the calibration curve of each component, whereas the calibration curve of each cation was achieved by using 1, 2, 5 and 10 ppm standards. The calibration equations along with precision and values of correlation coefficients ($r^2$) for all the components are given in Table 1.

2.2.2. Calculation of dustfall fluxes

The dustfall fluxes were calculated by using the gravimetric method where the weight difference of leaf before and after exposure was taken to obtain the total deposition on the foliar surface. After the collection, each leaf was immersed in distilled-deionised water in a pre-weighed petridish ($m_1$) for about 20 min to leach out the deposited material from the leaf. Then the water was evaporated by putting the petridish on a hot plate for about 20–30 min at 110–120°C and then after cooling the petridish was weighed ($m_2$). The total dustfall weight ($m_2 - m_1$) was used to calculate the deposition fluxes as follows:

$$\text{DF} = \frac{m_2 - m_1}{A \times d},$$

where DF is the dustfall flux (mg/m²/d), $m_1$ is the initial weight of petridish, $m_2$ is the final weight of the petridish, $A$ is the surface area of the selected leaves (m²) and $d$ is the number of days. Area of the leaf was calculated by the graph sheet drawing method. In this method, the leaf is drawn on 1 cm² graph sheet. The external boundaries of the sample leaf are outlined on the graph sheet with the help of a pencil. Then the numbers of squares are counted which gives the area of the leaf. This method is found to be simple and reliable having an accuracy of calculation around ±2%.

2.2.3. Analysis of biochemical parameters

The foliar samples were processed and analysed for chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, soluble sugar, proline amino acid and ascorbic acid content. Chlorophyll and carotenoids were determined by using Hiscox and Israealtm’s [23] method. The soluble sugar, proline amino acid and ascorbic acid were determined by using methods suggested by Dubois et al., [24] Bates et al. [25] and Keller and Schwager, [26] respectively. The foliar samples were analysed in triplicate to ensure authenticity of the results (Table TS1).
2.2.4. Morphological analysis of leaves

Surface morphological characteristics of the two selected leaves (one from each site) were studied by using Scanning Electron Microscopic studies (SEM) (Carl Zeiss EVO 40, Germany) at the Advance Instrumentation Research Facility (AIRF), JNU. The collected foliar samples were washed with distilled-deionised water. Then four small pieces were cut from this leaf (two for adaxial and two for abaxial surface study), which were dehydrated and mounted on the aluminium stubs with carbon tape and coated with a thin layer of gold and then observed under SEM. Images obtained from SEM are given in Section 5.

2.2.5. Statistical analysis

In order to know the distribution of data points and the relationship of the various components between and within the site, statistical analyses were performed using the Statistical Package for Social Sciences (SPSS Ver.16). The correlation and regression analyses were attempted to estimate relationship, cause and effects of the variation in dustfall and its ionic components along with biochemical constituents of the plant. Data normality was checked by the one-sample Kolmogorov–Smirnov test.

3. Results and discussion

3.1. Dust deposition fluxes on foliar surfaces

Average dustfall flux on foliar surface was estimated as 281 and 113 mg/m²/d, respectively, at SB and JNU sites. The SB site showed almost 2.5 times higher deposition of dustfall particles as compared to the JNU site. Similarly, the fluxes of all the ionic components (Cl⁻, F⁻, NO₃⁻, SO₄²⁻, Na⁺, NH₄⁺, K⁺, Ca²⁺ and Mg²⁺) were found to be higher at the SB site as compared to the JNU site (Figure 2). The order of these ionic fluxes was SO₄²⁻ > Ca²⁺ > Cl⁻ > Na⁺ > Mg²⁺ > K⁺ > NH₄⁺ > NO₃⁻ > F⁻ and Ca²⁺ > SO₄²⁻ > NO₃⁻ > Cl⁻ > K⁺ > Mg²⁺ > NH₄⁺ > Na⁺ > F⁻ at SB and JNU sites, respectively. Higher values of fluxes at SB are obvious due to the industrial nature of the site. In addition, unpaved roads and poor

Figure 2. Dustfall fluxes of major ions.
road conditions also contribute to huge amounts of particulate matter which enhances the fluxes of dustfall at the SB site.

3.2. Dustfall fluxes of $\text{SO}_4^{2-}$ and $\text{NO}_3^-$ on foliar surfaces

$\text{SO}_4^{2-} + \text{NO}_3^-$ fluxes were recorded higher at the SB site as compared to the JNU site. Interestingly, the fluxes of $\text{SO}_4^{2-}$ were much higher than the $\text{NO}_3^-$. Very high fluxes of $\text{SO}_4^{2-}$ can be attributed to the preferred oxidation of $\text{SO}_2$ than $\text{NO}_2$ in polluted and dusty atmosphere.[27–29]. Direct deposition of $\text{SO}_2$ onto foliar surface as well as its adsorption onto the dust particle already settled on the foliar surface can give rise to $\text{CaSO}_4$.[30] $\text{CaCO}_3$-rich soil dust in India significantly scavenges atmospheric $\text{SO}_2$.[27] Moreover, the oxidation of $\text{SO}_2$ is catalysed by $\text{NO}_2$ favouring $\text{SO}_4^{2-}$ formation.[28] Also, the $\text{NO}_3^-$ already present on the leaf surface (through NOx oxidation) increases the hygroscopicity of mineral deposited on the surface which further increases the oxidation of $\text{SO}_2$ to $\text{SO}_4^{2-}$. The SB site can be considered as $\text{SO}_2$-rich location as compared to the JNU site. Hence, the fluxes of $\text{SO}_4^{2-}$ are noticed much more higher than the $\text{NO}_3^-$ in the highly polluted conditions. Calculation of gas/aerosols partition coefficient as reported by Singh [31] also showed greater existence of $\text{NO}_2$ in the gas phase as compared to the aerosol phase ($\text{NO}_3^-$). A similar range of ionic fluxes has been reported by Kumar and co-workers [32] for vegetative surfaces of the Agra region. Apart from NOx oxidation, there is a possibility that $\text{NO}_3^-$ is contributed by local soils.[18]

In order to find out the balance between acidic and basic components, $(\text{SO}_4^{2-} + \text{NO}_3^-)/(\text{Ca}^{2+} + \text{Mg}^{2+} + \text{NH}_4^+)$ ratios were calculated which are shown in Figure 3. The ratio values indicated a higher level of acidity at the SB site due to greater deposition of $\text{SO}_4^{2-}$ and $\text{NO}_3^-$. This is corroborated by elevated values of pH of water-soluble extracts of dustfall. The pH value of the dustfall extract at the JNU site has been recorded as 7.41 and while at the SB site as 7.28. Foliar extract also had very similar values of pH. The pH of the leaf extract at the SB site was recorded as 5.90 as compared to 6.15 at the JNU site. Higher acidity of the foliar extract along with higher ratios of fluxes of $(\text{SO}_4^{2-} + \text{NO}_3^-)/(\text{Ca}^{2+} + \text{Mg}^{2+} + \text{NH}_4^+)$ also indicated that air was more polluted at the SB site posing greater risks of foliar damage. The average dustfall fluxes of $(\text{SO}_4^{2-} + \text{NO}_3^-)$ were recorded almost three times higher at the SB site than that at the JNU site.
4. Variation of biochemical parameters in relation with dustfall fluxes of SO$_4^{2-}$ and NO$_3^-$

Atmospheric dust and gaseous pollutants on foliar surface block stomata and interfere with photosystem II.[33] NO$_3$ is dissolved in cells forming nitrite (NO$_2^-$) and nitrate (NO$_3^-$) and participate in nitrogen metabolism. In some cases, exposure to polluting gases, particularly SO$_2$, causes stomatal closure. This action protects the leaf against the further entry of the pollutant and also hinder the photosynthesis process. In the cells, SO$_2$ is dissolved to give bisulphite and sulphite ions. Sulphite is toxic, but at low concentrations it is metabolised by chloroplasts to sulphate, which is not toxic.[33] At very low concentrations, bisulphite and sulphite are efficiently detoxified by plant making the availability of sulphur (S) as a nutrient to the plant.[34] In addition, the plasma membranes of leaf cells have proton/sulphate co-transport pump which assists in the uptake of SO$_4^{2-}$ directly from the environment.[35] Changes in biochemical parameters due to total dust SO$_4^{2-}$ and NO$_3^-$ fluxes have been discussed in the next section.

4.1. Photosynthetic pigment versus dustfall fluxes of SO$_4^{2-}$ and NO$_3^-$

The photosynthetic pigments of higher plants fall into two classes, the chlorophyll (Chl) and carotenoids (Car). Chlorophyll is the pigment primarily responsible for harvesting light energy used in photosynthesis. In this study, average concentrations of Chl a and Chl b have been recorded as 1.69 ± 0.09 and 0.5 ± 0.05 mg/g (f.w), respectively, at JNU and 1.38 ± 0.06 and 0.33 ± 0.019 mg/g (f.w), respectively, at the SB site. As shown in Figure 4 (a)–(d), the concentrations of Chl a and Chl b at the SB site decreased with an increase in total fluxes of dust SO$_4^{2-}$ and NO$_3^-$. Trendlines in these plots are more negative, clear and prominent for the SB site, clearly showing a reduction in pigment levels.

Chlorophyll is considered as an indicator of plant injury due to air pollutants.[36] Pollution stress might affect Chl through oxidation, reduction, pheophytinisation and reversible bleaching processes.[37] This further influences the morphological, physiological and biochemical characteristics of the plant. The decrease in chlorophyll content is associated with the development of injury symptoms in leaves. Agrawal and co-workers reported that exposure of rice plants to low concentration of SO$_2$ showed foliar injury at different levels.[38] White et al. [39] have observed inhibition of net photosynthesis in *Medicago sativa* exposed to SO$_2$ concentration greater than 0.2 ppm. However, normal ambient levels of SO$_2$ in Delhi are much below such limits because of its scavenging by CaCO$_3$ particles forming CaSO$_4$.[27] These particles are directly settled on the leaves through sedimentation. The soluble components are mobilised into the leaf cells. The entry pathway(s) of such particles may be the same as gaseous SO$_2$, but the contribution of acidity level will be different as dust aerosols are more alkaline than the acidity generated by the direct exchange of SO$_2$ on the foliar surface.[30] Ma and co-workers explained that the exposure to moderate dose of NO$_2$ had a favourable effect on plants, whereas the exposure to high NO$_2$ concentrations caused a reduction in the total chlorophyll content.[40] In the present study, levels of Chl a were found to be more affected than Chl b. Chl a is degraded to pheophytin through replacement of Mg$^{2+}$ ions from chlorophyll molecules, but degradation of Chl b by SO$_2$ leads to the formation of chlorophyllide b due to the removal of the phytol group of the Chl b molecules.[41]

In plants, carotenoids (Car) represent group of lipophilic antioxidants which detoxify various forms of ROS[42] and also protect chlorophyll from photooxidation.[43] In this study, average concentrations of Car have been recorded as 0.86 ± 0.04 and 0.76 ± 0.03 mg/g (f.w) at JNU and SB sites, respectively. Similar to chlorophyll, carotenoid content also decreased with increasing pollution load in the environment (Figure 4 (e) and 4(f)). The major task of carotenoids in plants
Figure 4. Relationship of dustfall flux of (SO$_4^{2-}$ + NO$_3^-$) with photosynthetic pigments and soluble sugar of Arjun at JNU (a, c, e, g) and SB (b, d, f, h) sites.

is absorbing visible light (400–550 nm) wavelength and transfer its high energy to Chl to be used in photosynthesis.[44] As an antioxidant, it scavenges $^1$O$_2$ to inhibit oxidative damage and quenches triplet sensitiser (3Chl$^*$) and excited chlorophyll (Chl$^*$) molecule to control $^1$O$_2$ formation which helps in safeguarding the photosynthetic apparatus.
4.2. Soluble sugars versus dustfall fluxes of $\text{SO}_4^{2−}$ and $\text{NO}_3^{−}$

Soluble sugars (SS), especially sucrose, glucose and fructose, play a very important role in plant structure and metabolism at the cellular and whole organism levels.\textsuperscript{[45]} SS act as a source of energy for all living organisms. In plant tissues, SS has osmoprotectant and cryoprotectant roles and their presence is important for plasma membrane integrity\textsuperscript{[46]} as well as detoxification of foreign substances.\textsuperscript{[47]} On an average, the concentrations of total soluble sugar were noticed as 124.64 ± 4.0 and 90.51 ± 5.2 mg/g (d.w) at JNU and SB sites, respectively. The total soluble sugar decreased significantly ($p \leq .05$) at the SB site with the increase in total fluxes of dust ($\text{SO}_4^{2−} + \text{NO}_3^{−}$) as compared to the JNU site (Figure 4(g) and 4(h)). The reduction in the total soluble sugar content of stress leaves probably corresponds with the photosynthetic inhibition or stimulation of respiration rate. Lorenc-Plucinska\textsuperscript{[48]} has reported a higher respiration rate in tree species resistant to air pollution. He has established higher inhibition rate of photosynthesis and photorespiration in $\textit{Pinus sylvestris}$ which is a less resistant plant. Tripathi and Gautam\textsuperscript{[49]} found significant loss of soluble sugar due to pollution. Reduction in soluble sugar is an indicator of an increased rate of respiration and decreased CO$_2$ fixation.\textsuperscript{[50]} Carbohydrate can also degrade sulphite reaction with aldehydes and ketones of carbohydrates\textsuperscript{[49]}.

4.3. Ascorbic acid (AsA) versus dustfall fluxes of $\text{SO}_4^{2−}$ and $\text{NO}_3^{−}$

Ascorbic acid (AsA) is a crucial component which is produced by the plant for its defense against oxidative stress. It is found abundant in chloroplasts and other cellular compartments. AsA is considered as the most powerful ROS scavenger because of its ability to donate electrons in a number of enzymatic and non-enzymatic reactions. It can provide protection to the membranes by directly scavenging the $\text{O}_2^·$ and $\text{OH}^·$ and by regenerating $\alpha$-tocopherol (Vitamin E) from tocopheroxy radical. In chloroplast, it helps in sustaining dissipation of excess excitation energy by acting as a cofactor of violaxantin de-epoxidase.\textsuperscript{[52]} The average AsA content of foliar was estimated to be 0.91 ± 0.06 and 1.35 ± 0.05 mg/g (f.w) at JNU and SB sites, respectively. Results showed that AsA content increased significantly ($r = 0.825$, $p \leq .05$) with the increase in dustfall fluxes of ($\text{SO}_4^{2−} + \text{NO}_3^{−}$) at the SB site as compared to the JNU site (Figure 5(a) and 5(b)). In a study, Sharma and Tripathi\textsuperscript{[53]} have also reported increased levels of ascorbic acid in the plants exposed to air pollution.

4.4. Proline (Pro) amino acid versus total dust fluxes of $\text{SO}_4^{2−}$ and $\text{NO}_3^{−}$

The average proline (Pro) content was estimated to be 0.79 ± 0.06 and 1.02 ± 0.05 mg/g (f.w.) at JNU and SB sites respectively. Results showed that Pro content increased with the increase in total fluxes of ($\text{SO}_4^{2−} + \text{NO}_3^{−}$) at the SB site as compared to the JNU site (Figure 5(c) and 5(d)). Pro is considered as a potent antioxidant and potential inhibitor of programmed cell death. The function of proline in stressed plants is often explained by its property as an osmolyte, ability to balance water stress.\textsuperscript{[54]} In addition, other possible positive roles of proline under stress have been proposed with greater or lesser convictions, which include stabilisation of proteins,\textsuperscript{[55]} scavenging of hydroxyl radicals,\textsuperscript{[56]} regulation of the cytosolic pH\textsuperscript{[57]} and regulation of the NAD/NADH ratio.\textsuperscript{[58]} Accumulation of free proline in plants has often been reported as a consequence of a wide range of environmental stress, Assadi et al.\textsuperscript{[59]} confirmed proline increase in plants growing in the air-polluted region.
5. Correlation of biochemical parameters

The correlation coefficients were calculated among biochemical parameters, that is, Chl a, Chl b, total Chl, carotenoids, soluble sugar, ascorbic acid and proline with dustfall fluxes of $\text{SO}_2^{2-}$ and $\text{NO}_3^-$ (Tables 2 and 3) at JNU and SB sites. Dustfall fluxes of $\text{SO}_2^{2-}$ and $\text{NO}_3^-$ showed a negative correlation with Chl a, Chl b, total Chl, carotenoids, soluble sugar, while ascorbic acid and proline showed a positive correlation at both the sites. The concentrations of SS decreased with increasing concentrations of ascorbic acid as the soluble sugar is involved in the biosynthesis of ascorbic acid.[60] Ascorbic acid also showed a negative correlation with total chlorophyll and carotenoids, probably due to the formation of ROS during stress conditions which adversely affect the formation of chlorophyll and carotenoid and increase the

Table 2. Correlation matrix of dustfall fluxes of $\text{SO}_2^{2-}$ and $\text{NO}_3^-$ with biochemical parameters of Arjun at the JNU site.

|         | SO$_4$ | NO$_3$ | Chl a | Chl b | Total Chl | Carotenoids | Soluble sugar | Ascorbic acid | Proline |
|---------|--------|--------|-------|-------|-----------|-------------|---------------|--------------|--------|
| SO$_4$  | 1      |        |       |       |           |             |               |              |        |
| NO$_3$  | 0.75*  | 1      |       |       |           |             |               |              |        |
| Chl a   | -0.54  | -0.52  | 1     |       |           |             |               |              |        |
| Chl b   | -0.53  | -0.41  | 0.74  | 1     |           |             |               |              |        |
| Total Chl| -0.57  | -0.52  | 0.97**| 0.87**| 1         |             |               |              |        |
| Carotenoids| -0.47  | -0.38  | 0.56  | 0.60  | 0.61      | 1           |               |              |        |
| Soluble sugar| -0.46  | -0.75  | 0.06  | 0.63  | 0.06      | 0.06        | 1             |              |        |
| Ascorbic acid| 0.60  | 0.74   | -0.67 | -0.36 | -0.61     | -0.69       | -0.30         | 1            |        |
| Proline | 0.48   | 0.65   | -0.14 | -0.57 | -0.30     | -0.26       | -0.79*        | 0.13         | 1      |

*Correlation is significant at $p \leq .05$.
**Correlation is significant at $p \leq .01$. 

Figure 5. Relationship of dustfall flux of ($\text{SO}_2^{2-} + \text{NO}_3^-$) with ascorbic acid and proline at JNU (a, c) and SB (b, d) sites.
Table 3. Correlation matrix of dustfall fluxes of SO$_4^{2-}$ and NO$_3^-$ with biochemical parameters of Arjun at the site.

|        | SO$_4$ | NO$_3$ | Chl a | Chl b | Total Chl | Carotenoids | Soluble sugar | Ascorbic acid | Proline |
|--------|--------|--------|-------|-------|-----------|-------------|---------------|--------------|---------|
| SO$_4$ | 1      |        |       |       |           |             |               |              |         |
| NO$_3$ | 0.98** | 1      |       |       |           |             |               |              |         |
| Chl a  | -0.69  | -0.58  | 1     |       |           |             |               |              |         |
| Chl b  | -0.61  | -0.66  | 0.30  | 1     |           |             |               |              |         |
| Total Chl | -0.78* | -0.70  | 0.96**| 0.55  | 1         |             |               |              |         |
| Carotenoids | -0.72  | -0.73  | 0.27  | 0.85* | 0.48      | 1           |               |              |         |
| Soluble sugar | -0.82* | -0.77* | 0.87**| 0.39  | 0.83**    | 0.33        | 1             |              |         |
| Ascorbic acid | 0.82*  | 0.83*  | -0.64 | -0.19 | -0.62     | -0.26       | -0.83*        | 1           |         |
| Proline | 0.72   | 0.68   | -0.85*| -0.26 | -0.82*    | -0.13       | -0.82*        | 0.80*        | 1       |

*Correlation is significant at $p \leq .05$
**Correlation is significant at $p \leq .01$

Figure 6. SEM images of foliar showing upper epidermal hairs and (a, b – adaxial surface); dust particle (c, d – abaxial surface), clogged stomata and ruptured guard cell (e, f – abaxial surface) and difference in size of stomatal pores (g, h – abaxial surface) at JNU and SB sites.
formation of ascorbic acid. Reduction of total chlorophyll affects carbohydrate synthesis and thus the availability of SS. Positive correlation of SS with total chlorophyll also corroborated these findings.

6. Changes in foliar surface morphology

Leaves are directly exposed to ambient air and hence act as pollution absorbers.[61] In fact, the leaves are helpful in reducing dust concentrations in ambient air. Dust removal efficiency of a leaf depends on various factors such as height, canopy, surface geometry, phyllotaxy, epidermal and cuticular features, leaf pubescence, etc. [62] On the other hand, morphology and internal structure of leaves are altered by the heavy load of dust pollutants [63] which can cause many lethal effects on plants such as stomatal clogging, reduced photosynthetic activity, leaf fall and death of the tissues.[64] Surface morphology of the collected foliar samples was studied using a SEM. The surface of leaves is seen more rough at the SB site as compared to the JNU site and reductions in number of epidermal hairs at adaxial surface are shown in Figure 6(a) and 6(b). As shown in Figure 6(c) and 6(d), the dustfall deposition at the SB site is seen to be higher as compared to the JNU site. The size of stomatal pore is seen to be larger (13.5 µm × 4.74 µm) at the JNU site as compared to the SB site (9.52 µm × 2.43 µm) (Figures 6(g) and 6(h)). Similarly, guard cells are more damaged at the SB site as compared to the JNU site. Ruptured guard cells and the epidermal cell at the abaxial surface are shown in Figures 6(e)–(h) at both the sites. Dust particles are deposited in and around stomatal pores on abaxial and adaxial leaf surfaces as shown in Figures 6(a)–(h). These particles clog the stomatal pores as seen in Figure 6(f). Cuticle and epicuticular wax restructuring was observed from the abrasive effect of the particulate deposition on the leaf surface.[65] All these features indicated greater damage of surface of foliar at the SB site.

7. Conclusion

It is concluded from this study that the industrial sources are responsible for elevated fluxes of dustfall and its chemical constituents such as Ca\(^{2+}\), SO\(_4^{2-}\) and NO\(_3^-\). Unpaved roads and poor road conditions also contribute to these fluxes by adding large amounts of particulate matter in the atmosphere. Industrial site had almost two and half times higher loadings of atmospheric dust as compared to the residential site, which is due to the influence of road dust and industrial activities. Higher dustfall fluxes SO\(_4^{2-}\) have been attributed to the two processes: (i) dry deposition of SO\(_2\) onto the foliar surface where it reacts with dust particles already settled on the foliar and (ii) gravitational settling of atmospheric CaSO\(_4\) particles formed through SO\(_2\) oxidation onto the dust particle in air. NO\(_3^-\) fluxes are primarily contributed by the oxidation of NO\(_x\) emitted from automobiles. However, a small fraction of NO\(_3^-\) is also contributed by the soils of the region. Study suggested that the higher dustfall fluxes of (SO\(_4^{2-}\) + NO\(_3^-\)) had a significant impact on the biochemical constituents of the plant. The concentrations of chlorophyll and carotenoids were decreasing, whereas proline and ascorbic acid were found to be increasing with the increase in fluxes of (SO\(_4^{2-}\) + NO\(_3^-\)) indicating their air pollution stress effect. Foliar morphology studies showed that the deposition of dustfall was responsible for the damage to the surface morphology of the leaf. This damage was noticed greater at the industrial site (SB) as compared to the residential site (JNU). More roughness of foliar surface was seen at the SB site due to higher dustfall. The clogged stomata and smaller size of the stomatal pores were seen at the SB site where pollution is higher as compared to the JNU site.
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Supplemental data

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