

The Effects of Atmospheric Nitrogen Dioxide and Sulphur Dioxide on the Biochemical Factors of Plant Tolerance

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Abstract

Atmospheric nitrogen dioxide (NO_2) and sulphur dioxide (SO_2) enter leaves through stomata and follow the same diffusion pathways as carbon dioxide (CO_2). During the exposure of these pollutants the plants experience changes in the levels of their biochemical constituents (such as, ascorbic acid, chlorophyll, leaf extract pH, and relative water content). Biochemical and Physiological changes in plant leaves are studied and quantified using air pollution tolerance index (APTI) formula. A plant with high APTI can be used as a tolerant, while a plant with a low APTI can be used as a crucial bio-monitor to track the level of air pollution. In the present study, APTI of 15 plants species were calculated. The leaves of the selected plants were sampled from six different locations in Punjab India. Simultaneously air quality data was collected from central pollution control board (CPCB) online monitoring portal. Among 15 species collected from Jalandhar, Amritsar, and Ludhiana, the *Ficus religiosa* has the highest APTI values (144.6, 176.7, and 280 respectively). Similarly in Sector 22, Sector 25 and Sector 53 of Chandigarh, *Ficus benghalensis* has been reported to have the highest APTI values. Moreover, the present study correlates the APTI and the concentration of gaseous air pollutants (SO_2 and NO_2) in the atmosphere. The APTI of the studied plant species has been found to have a negative correlation with SO_2 but no significant correlation has been found with NO_2 . The current study provides pollutant-specific information and insights that can potentially contribute in improvement in the APTI formula.

1. Introduction

Air quality has become a major focus of environmental policy due to rapid urbanization and economic growth in developing countries (Khaniabadi et al. 2017 a,b.) Atmospheric nitrogen dioxide (NO_2) and sulphur dioxide (SO_2) emissions have been increasing in many countries around the world during the past few decades, particularly in some Asian countries. The NO_x and SO_x concentrations will continue to rise, and the standard levels of these pollutants will continue to be exceeded, as a result of the growth of industrialized production and the continuous rise in automobile exhaust emissions (Sheng and Zhu 2019). The most toxic pollutants to plants are SO_2 and NO_2 , which enter leaves through stomata and follow the same diffusion pathways as carbon dioxide (CO_2) (Hamid and Jawaaid 2009). The SO_2 was one of the first air pollutants to cause harm to plant health and ecosystems. The amount of SO_2 in the air has significantly increased due to the combustion of fossil fuels (Wei et al. 2017). Due to its widespread distribution over the world has been reported to induce visible injury (Interveinal chlorosis, necrosis etc) to leaves and leads to reduction in photosynthetic pigments inhibition of metabolic processes and suppression of growth and yield of plants (Hamid and Jawaaid 2009). Until the 1970s, SO_2 was thought to be a major contributor to acid rain which damage the forests. Interestingly, when the Clean Air Act came into effect in the 1980s, the reduction of SO_2 in the atmosphere led to sulfur (S) deficiency in crops plants. This may be because plants are injured at high SO_2 concentrations which cause them to rapidly assimilate SO_2 and H_2S into low sulfur pools such as cysteine and sulphate (SO_4^{2-}) (Wei et al. 2017). After entering through the leaf stomata, SO_2 dissolves in the cells and oxidized to bisulphite (HSO_3^-) and

sulphite ions (SO_3^{2-}). SO_3^{2-} is highly toxic; chloroplasts convert small amounts of it into SO_4^{2-} (Hamid and Jawaid 2009). If they (SO_3^{2-} and SO_4^{2-}) accumulate in high concentration it causes SO_2 toxicity by inhibiting photosynthesis and energy metabolism (Wei et al. 2017). Generally, SO_3^{2-} beings 30 times more toxic than SO_4^{2-} (Thomas et al. 1943). As a result of it plants experience, intervenial chlorosis and necrosis in broad leaves and chlorotic spots and brown tips in pine conifers. Besides, it decreased leaf pH and disturbance of oxidation–reduction balancing in plant tissue that causes disruption of photosynthesis process at the enzymatic level of electron transportation and as a result in the decreased assimilation of CO_2 (Baciak et al. 2015).

The atmosphere consist several number of nitrogen oxides (NO_x), including NO_2 , nitric oxide (NO), nitrous oxide (N_2O) and nitrogen trioxide (N_2O_3). The United States Environmental Protection Agency (USEPA) only regulates NO_2 , because it the most common type of NO_x produces anthropogenically (USEPA 1999). It has severe impact on plants either directly after being deposited on plants, soil or water, or indirectly through chemical reactions in the atmosphere (Oksanen and Kontunen-Soppela 2021). Further, it dissolves in cells and produce nitrite ions (NO_2^- , which are toxic at high concentrations), cell acidification (which lead to negative responses such as the generation of reactive oxygen species (ROS) and nitrate ions (NO_3^-). In spite of this it reduces the plant growth in high concentration and also inhibits photosynthesis (Hamid and Jawaid 2009; Sheng and Zhu 2019).

Plants absorb gaseous NO_2 more rapidly and NO_2 is known to be more toxic than other oxides of nitrogen. The visible symptoms resulting from NO_2 exposure are relatively large, irregular brown or black spots. Despite the fact that numerous policies, laws, and technological advancements have been made to address air pollution, the problem persists (Wei et al. 2017). Due to an increase in sources, sustainable development efforts to mitigate air pollution have turn out to be a long term solution Thus, increasing the sink is the only way out in this situation to mitigate the toxic emission into the air (Ram et al. 2015). The use of ecological methods such as plant absorption and catabolism of atmospheric NO_2 and SO_2 is more effective to reduce air pollutants concentration. Plants provide natural ways to reduce atmospheric pollution by absorbing gaseous pollutants (Bharti et al. 2018). Bioremediation of air pollutants can be done by using the ability of plant leaves. The aerial surfaces of plants especially leaves are able to absorb pollutants or biodegrade pollutants into less or nontoxic molecules (Wei et al. 2017). It helps to deal with the problem of air pollution, but in the process they also get affected due to their constant exposure to a particular environment. In the polluted environments, changes in morphological, physiological and biochemical parameters (ascorbic acid, chlorophyll, leaf extract pH and relative water content) are observed. Especially these biochemical parameters are analyzed to calculate the tolerance of plants typically by air pollution tolerance index method (APTI). Stress generated from the absorption of air pollutants on leaf surface affects the concentration of Ascorbic acid (AA), Total chlorophyll (TC), Relative water content (RWC) and pH of leaf extract indicating plants behaviour to air pollutants. The Chlorophyll content of plants decreases due to photochemical reactions like oxidation, reduction, reversible bleaching and generation of reactive oxygen species (ROS) in the chloroplast under polluted

area. During stress conditions, RWC in the plants balances the water uptake (Yadav and Pandey 2020). In addition, AA present in plants protects the thylakoid membranes from oxidative damage and also regulates cell expansion and cell division in stress conditions (Gupta et al. 2020). The APTI is an important method for classifying plants into tolerant, intermediate and sensitive categories. Thus, a plant with high APTI can be used as sink while a plant with low APTI can be used as important bio monitor to study air pollution levels (Verma et al. 2023).

The role of plants leaves and plants interaction is not well explored in remediation of air pollutants. It has been observed that APTI is an eco-friendly, cost effective method of remediation of air pollutants to evaluate tolerant plants. Previous research has documented plant responses to pollutants such as NO_x and SO₂ but the main aim of the research was to find out how plants were injured. However, the effect of SO₂ and NO₂ on plants tolerance has been studied in the present study. Besides, the current study provides pollutant-specific information and insights that can potentially contribute in improvement in the APTI formula.

2. Materials And Methods

2.1 Description of the study area

The current work was conducted in Punjab during the period of August 2022 – October 2022 at six regions (Jalandhar, Ludhiana, Amritsar, and Chandigarh (Sector 22, Sector 25, and Sector 26) of Punjab. Ludhiana is one of the most polluted cities in Punjab state because of fast urbanization and heavy industrialization (Verma et al. 2023). Samples were collected from roadside which is heavily polluted by vehicle exhaust. Jalandhar is being considered as residential and commercial area. The sampling site is highly polluted as it is one of the main and busy roads in a city with a lot of traffic. Roadside plants of the site were selected for biochemical and physiological analysis. Amritsar (Golden temple) can be considered as a polluted place in Punjab due to its famous tourist place in Punjab and heavy traffic load (Kaur and Nagpal 2017). In Chandigarh, Sector 22 sector 25 and sector 53 are less polluted sites due to low traffic load and more green belts with large number of canopy trees around the roads.

2.1.1 Visible effects of air pollution on plants

Plant species depicted some common visual effects due to vehicle exhaust/industrial pollution. Necrosis and chlorosis were the most common effects observed. Necrosis was observed in plants as dry and brownish to black coloured spots on the tips and surfaces of leaves (Figure a, c, d) due to collapse of mesophyll cells. Chlorosis was diagnosed as yellowing of green leaves (Figure a, b, d) due to lack of chlorophyll.

2.2 Collection of leaf sample

The leaf samples of 15 chosen plant species, namely *Ficus benghalensis*, *Ficus religiosa*, *Murraya koenigii*, *Yellow oleander*, *Melia azedarach*, *Psidium guajava*, *Ziziphus mauritiana*, *Ocimum*

sanctum, *Mentha piperita*, *Syzygium cumini*, *Mangifera indica*, *Polyalthia longifolia*, *Morus alba*, *Alstonia scholaris* and *Moringa oleifera* were sampled. Plants were selected based on their prevalence across six different places (Amritsar, Jalandhar, Ludhiana, Chandigarh sector 23 sector22 sector25) in Punjab India (during August 2022 – October 2022). The use of multiple species had increased the chance of selecting the appropriate pollution-tolerant plants with higher probability than using single or few species. The matured leaves of the plants were collected in black polythene during morning hours. 10–15 fully developed leaves from shrubs and 5 leaves from trees of each plant were taken randomly from all sides of a crown and leaves were mixed to form a mixed representative sample. After collection, fresh leaves were packed in polyethylene bags and taken to the laboratory and washed properly, first with tap water and then with distilled water for analyzing various biochemical parameters, and stored in refrigerator for further analysis.

2.3 Estimation of APTI

The fresh leaf samples were analysed for four biochemical parameters namely pH, RWC, TC, and AA for determining APTI by following standard procedure (Singh and Rao 1983)

2.3.1 Estimation of leaf extracts pH

pH determination was done following Pandey et al. (2015). A total 0.5 g of leaf sample was macerated in 10 ml of deionised distilled water. The leaf extract was filtered and measured for using a calibrated glass electrode pH meter.

2.3.2 Estimation of RWC

The relative water content of the leaves was calculated by adopting the method suggested by Singh (1977). Fresh leaves of selected species were cleaned and weighed. The leaves were then immersed in beaker all-night, took blotted dry weight next day and then leaves were dried at 80°C for 24 hour and weighed again. Then RWC were calculated by [Eq. 1]:

$$RWC = \frac{(Freshweight - Dryweight)}{(Turgidweight - Dryweight)} \times 100 \dots [Eq. 1]$$

2.3.3 Estimation of TC

Total chlorophyll content of the leaves has been extracted without maceration using the method of Hiscox and Israelstam (1979). A 5gm of fresh leaves were immersed in a test tube containing 20 ml DMSO and heated on water bath for 30 min. The extracted liquid was scanned for optical density values at 663nm and 645nm on spectrophotometer at blank solution. The quantitative estimation of TC has been calculated by using formula [Eq. 2]

$$TC = \frac{(2.12 \times O.D_{645}) + (8.02 \times O.D_{663})}{W \times 1000} \dots [Eq. 2]$$

2.3.4 Estimation of AA

The AA content was estimated by Titrimetric analysis using DCPIP (dichlorophenyl indophenol) was conducted using leaf extract, ground in 4% oxalic acid and the endpoint noted as V2 when the solution turns light permanent pink colour which persists after continuous shaking (Watson and Bai 2021). The same process was done using pure ascorbic acid solution with known concentration and the endpoint was noted as V1 [Eq. 3]

$$\text{Amount of AA (mg/100mg)} = \frac{0.5\text{mg}}{v1\text{ml}} \times \frac{v2}{5\text{ml}} \times \frac{100\text{ml}}{\text{Sampleweight}} \times 100 \dots [\text{Eq. 3}]$$

2.3.5 APTI calculations

Air pollution tolerance index was calculated by incorporating the four biochemical parameters of the leaf (AA content, leaf extract pH, total chlorophyll content and RWC). The APTI was calculated using the following formula [Eq. 3] Singh and Rao (1983).

$$\text{APTI} = \frac{A(T+P)+R}{10} \dots [\text{Eq. 3}]$$

Where, A is the AA content in mg g^{-1} of fresh tissue; T is the total chlorophyll in mg g^{-1} of fresh tissue; P is the pH of leaf extract and R is the relative water content in percentage.

2.4 Collection of air quality data

The concentration of air pollutants (SO_2 and NO_2) in the ambient air at the time of sampling was collected from CPCB online monitoring portal.

3. Results And Discussion

The vegetation of the sampling area was exposed to dust pollution and chronic concentrations of gaseous pollutants, which affect the biochemical make up, and also affect the tolerance of plants to the air pollution. The biochemical characteristics and the APTI for plants from six different places are shown in Tables 1, 2, 3, 4, 5, 6 respectively.

3.1 Differences in APTI values

The Plant species under study showed significant variation in their APTI values at study sites (Tables 1–6). The variation in the plant species and pollutant concentration was found to have a significant effect on APTI values. Table 1 presents the APTI results for all the fifteen species examined at Jalandhar city. The APTI ranged from 8 to 35. Higher APTI values were recorded for *Ficus religiosa* (35.7) followed by *Morus alba* (16.6). Intermediate tolerance index values were recorded in *Ficus benghalensis* (15.06) and lowest APTI values were observed in *Melia azdearch* (8.9) and *Psidium gujava* (9.8).

Table 1
Variation in APTI and biochemical parameters of selected Plant species at SO₂ and NO₂ 14(µg/m³) and 7(µg/m³) respectively in Jalandhar city.

Plants	RWC	pH	AA	TC	APTI
<i>Ficus benghalensis</i>	131.3	6.8	2.1	2.4	15.06
<i>Ficus religiosa</i>	144.6	7.2	1.8	0.65	35.75
<i>Polyalthia longifolia</i>	111.7	7.2	4.5	0.22	14.41
<i>Mangifera indica</i>	102	5.9	6.9	0.77	14.27
<i>Alstonia Scholaris</i>	104.3	6.1	1.5	0.34	11.34
<i>Moringa oleifera</i>	102.2	6.5	2.4	0.22	11.78
<i>Yellow oleander</i>	93.6	7.4	3.3	0.12	11.80
<i>Ocimum sanctum</i>	129.2	6.8	2.1	0.21	14.34
<i>Ziziphus maurtiana</i>	91.4	6.2	1.8	0.13	10.25
<i>Methna Piperita</i>	98.3	6.1	3.9	0.16	12.20
<i>Syzygium cumini</i>	105.3	5.03	3	1.9	12.03
<i>Murraya koenigii</i>	95.2	4.9	3.9	0.27	11.43
<i>Melia azaderach</i>	66.6	6.3	3.6	0.18	8.92
<i>Psidium guajava</i>	84.4	6.5	2.1	0.21	9.80
<i>Morus alba</i>	133.3	7.4	4.5	0.33	16.6

Table 2
Variation in APTI and biochemical parameters of selected
Plant species at SO₂ and NO₂ 20(µg/m³) and 58(µg/m³)
respectively in Amritsar city.

Plants	RWC	pH	AA	TC	APTI
<i>Ficus benghalensis</i>	102	6.9	1.8	0.52	11.5
<i>Ficus religiosa</i>	176.7	8.6	6.5	0.68	23.70
<i>Polyalthia longifolia</i>	53.7	7.4	4.5	1.2	9.24
<i>Mangifera indica</i>	108.8	7.4	4	1.86	14.58
<i>Alstonia scholaris</i>	105.1	6.3	4.6	0.17	13.48
<i>Moringa oleifera</i>	93.1	7.5	6	0.64	14.19
<i>Yellow oleander</i>	79.1	7.6	3.3	0.64	10.62
<i>Ocimum sanctum</i>	103.2	7.6	4.3	0.1	13.63
<i>Ziziphus mauritiana</i>	100	7	5	0.1	13.55
<i>Mentha piperita</i>	107.6	7.1	6	0.61	15.38
<i>Syzygium cumini</i>	68.1	6.9	5	0.9	10.71
<i>Murraya koenigii</i>	100	7.5	6	0.64	14.88
<i>Melia azaderach</i>	89.1	8	6	0.48	13.99
<i>Psidium guajava</i>	100	7.8	5.5	0.19	14.39
<i>Morus alba</i>	80.4	7.9	5	2.1	13.04

At Amritsar city, plants showed APTI ranged from 9 to 23 (Table 2). Higher APTI value was recorded in *Ficus religiosa* (23.7) followed by *Mentha piperita* (15.9). Intermediate tolerance was observed in *Murraya koenigii* (14.8) *Psidium guajava* (14.3), *Mangifera indica* (14.5) and *Moringa oleifera* (14.1). Lowest APTI values were observed in *Polyalthia longifolia* (9.2). Similar plant species show significant variation at different sites. The APTI values were found to be in the range of 6 to 31 (Table 3) in Ludhiana city. The highest APTI value was recorded for *Ficus religiosa* (31.4) followed by *Morus alba* (19.3). Among the plants studied, *Ficus religiosa* showed high APTI values at three (Jalandhar, Amritsar and Ludhiana) sites. Besides, the lowest APTI was observed in *Ziziphus mauritiana* (6.4).

Table 3
Variation in APTI and biochemical parameters of selected
Plant species at SO₂ and NO₂ 14(µg/m³) and 22(µg/m³)
respectively in Ludhiana city

Plants	RWC	pH	AA	TC	APTI
<i>Ficus benghalensis</i>	117.4	6.8	2.3	0.67	13.45
<i>Ficus religiosa</i>	280	7.5	4.5	0.22	31.47
<i>Polyalthia longifolia</i>	85.3	7.5	5	0.19	12.37
<i>Mangifera indica</i>	128.8	5.1	3	0.26	14.48
<i>Alstonia scholaris</i>	93.9	7.6	6.5	0.12	14.40
<i>Moringa oleifera</i>	31.8	7.7	6	0.37	8.02
<i>Yellow oleander</i>	64.8	6.6	5.5	0.37	10.31
<i>Ocimum sanctum</i>	109.3	7.7	3	0.57	13.41
<i>Ziziphus mauritiana</i>	31.8	7	4.5	0.19	6.41
<i>Mentha piperita</i>	110.4	6.5	3.1	0.78	13.29
<i>Syzygium cumini</i>	125	6.5	5	0.98	16.24
<i>Murraya koenigii</i>	53.5	7.5	5	0.11	9.15
<i>Melia azaderach</i>	98.5	7.6	3.5	0.15	12.56
<i>Psidium guajava</i>	103.4	7.1	5.5	0.16	14.33
<i>Morus alba</i>	140.8	7.8	6	0.9	19.3

Similarly, APTI value of plants from Chandigarh sector 22 range from 11 to 17 (Table 4). The highest APTI was found to be *Ficus benghalensis* (17.7) followed by *Murraya koenigii* (16.4) and *Morus alba* (16.1). Most of the plants displayed intermediate tolerance value and even none of the plants had value less than 10. This means that the plants in Chandigarh sector 22 are great performers and acts as good sink for both SO₂ and NO₂.

In Chandigarh sector 25, the APTI range varies from 11 to 20 (Table 5). The highest APTI was found to be *Ficus benghalensis* (20.7) followed by *Moringa oleifera* (17.7), *Ficus religiosa* (17.3) *Mangifera indica* (17.2) and *Syzygium cumini* (17.2), *Ocimum sanctum* (15.4), *Murraya koenigii* (15.6) and *Morus alba* (15.7).

In Chandigarh sector 53, the APTI range varies from 10 to 17 (Table 6). The maximum APTI was observed in *Syzygium cumini* (18.06) followed by *Mentha piperita* (17.5) and *Ficus benghalensis* (17.4).

Intermediate tolerance value was recorded in *Murraya koenigii* (14.8) and *Melia azaderach* (14.2). The minimum APTI value was recorded in *Moringa oleifera* (10.07).

The tolerance of the same plant species to the same air pollutants has been observed to vary between different locations. Plants with lower values of APTI can act as bio indicators, while those with higher values act as sinks of atmospheric pollution in polluted environments.

Table 4
Variation in APTI and biochemical parameters of selected
Plant species at SO₂ 8(µg/m³) and NO₂ 7(µg/m³)
respectively in Chandigarh sector 22

Plants	RWC	pH	AA	TC	APTI
<i>Ficus benghalensis</i>	159.8	7.1	2	1.5	17.7
<i>Ficus religiosa</i>	145	6.8	2.7	0.98	16.60
<i>Polyalthia longifolia</i>	125	7.1	2.5	0.76	14.46
<i>Mangifera indica</i>	140	7.5	2.1	1.3	15.84
<i>Alstonia scholaris</i>	110	7.3	2.5	0.76	13.01
<i>Moringa oleifera</i>	98	7	2.6	1.5	12.01
<i>Yellow oleander</i>	110	6.9	3.2	0.26	13.29
<i>Ocimum sanctum</i>	138	7.5	1.7	1.9	15.39
<i>Ziziphus mauritiana</i>	132	7.1	1.8	2.1	14.85
<i>Methna Piperita</i>	141	6.8	1.5	1.9	15.40
<i>Syzygium cumini</i>	125	6.5	2.4	1.7	14.46
<i>Murraya koenigii</i>	148	6.5	2.5	0.23	16.48
<i>Melia azaderach</i>	98.4	6.7	2.6	1.6	11.99
<i>Psidium guajava</i>	123.4	7.1	2.6	2.1	14.73
<i>Morus alba</i>	137.8	7.5	2.6	1.8	16.19

Table 5
Variation in APTI and biochemical parameters of selected Plant
species at SO₂ and NO₂ 3(µg/m³) and 42(µg/m³) respectively in
Chandigarh sector 25

Plants	RWC	pH	AA	TC	APTI
<i>Ficus benghalensis</i>	187.6	7.5	2.1	1.9	20.07
<i>Ficus religiosa</i>	154.6	7.4	2.1	1.6	17.35
<i>Polyspatha longifolia</i>	123.5	6.8	1.8	1.5	13.84
<i>Mangifera indica</i>	156	7.1	2.1	0.98	17.29
<i>Alstonia scholaris</i>	112	6.8	1.5	1.7	12.47
<i>Moringa oleifera</i>	165.5	6.9	1.4	1.5	17.72
<i>Yellow oleander</i>	87.6	7.5	3.2	1.6	11.67
<i>Ocimum sanctum</i>	145.5	7.1	0.98	1.8	15.42
<i>Ziziphus mauritiana</i>	128.5	7	1.8	2.1	14.48
<i>Methna piperita</i>	132.5	6.5	1.5	1.9	14.51
<i>Syzygium cumini</i>	156.4	6.9	2.1	0.89	17.27
<i>Murraya koenigii</i>	134.5	7.1	2.8	0.78	15.65
<i>Melia azadirach</i>	110.4	6.5	1.9	1	12.46
<i>Psidium guajava</i>	124.5	7.5	1.6	0.98	13.80
<i>Morus alba</i>	140.9	6.9	2.1	0.78	15.70

Table 6
Variation in APTI and biochemical parameters of selected
Plant species at SO₂ and NO₂ 9(µg/m³) and 28(µg/m³)
respectively in Chandigarh sector 53

Plants	RWC	pH	AA	TC	APTI
<i>Ficus benghalensis</i>	156.7	6.8	2.3	1.1	17.48
<i>Ficus religiosa</i>	140.7	7.5	1.8	1.5	15.69
<i>Polyalthia longifolia</i>	134.5	7.6	1.9	1.1	15.10
<i>Mangifera indica</i>	143.1	7.1	2.1	1	16.01
<i>Alstonia scholaris</i>	115.4	7	1.4	1.5	12.73
<i>Moringa oleifera</i>	92.1	6.9	1	1.7	10.07
<i>Yellow oleander</i>	123.5	6.8	1.1	1.7	13.28
<i>Ocimum sanctum</i>	135.6	7.9	1.5	2.1	15.06
<i>Ziziphus mauritiana</i>	145.6	7.6	1.5	0.98	15.84
<i>Mentha piperita</i>	158	6.1	2.1	2	17.50
<i>Syzygium cumini</i>	167.7	6.8	1.7	0.81	18.06
<i>Murraya koenigii</i>	135.5	6.4	1.8	1	14.88
<i>Melia azaderach</i>	128.7	7.5	1.5	1.5	14.22
<i>Psidium guajava</i>	143.4	6.8	1.1	0.76	15.17
<i>Morus alba</i>	125.5	6.9	1.1	0.89	13.40

3.2 Statistical analysis

The SO₂ and NO₂ concentration were correlated with APTI values of fifteen different plants species using Pearson correlation matrix. Correlation coefficient (r) was calculated between independent variables atmospheric concentration of SO₂ and NO₂ and dependent variable APTI to determine the degree of correlation between the variables. Significance was tested 5% level of significance (i.e. p = 0.05).

Table 7
Correlation coefficient (r) between APTI of plants species and SO₂ and NO₂ collected from six different sites of Punjab.

Plants	SO ₂	NO ₂
<i>Alstonia scholaris</i>	0.28	0.31
<i>Ficus benghalensis</i>	-0.98	-0.2
<i>Ficus religiosa</i>	0.5	0.25
<i>Mangifera indica</i>	-0.89	0.18
<i>Melia azedearch</i>	0.02	0.67
<i>Mentha piperita</i>	-0.21	0.34
<i>Moringa oleifera</i>	-0.35	0.51
<i>Morus alba</i>	-0.1	-0.57
<i>Murraya koeniggi</i>	-0.43	0.28
<i>Ocimum sanctum</i>	-0.87	-0.24
<i>Polyalthia longifolia</i>	-0.73	-0.72
<i>Psidium guajava</i>	-0.17	0.39
<i>Syzygium cumini</i>	-0.75	-0.1
<i>yellow oleander</i>	-0.57	-0.46
<i>Ziziphus mauritiana</i>	-0.42	0.26

The significant correlations between concentration of SO₂ and NO₂ in the ambient air and APTI values of studied plant species are shown in table(7). It is worth noting that APTI for all the studied species had significant negative correlation with SO₂ (r range:-0.5to-0.9). Among studied species, *Ficus bengalensis* has the highest negative correlation with SO₂ (r=-0.98), followed by *Mangifera indica* (r=-0.89), *Polyalthia longifolia* (r=-0.73) and *Syzygium cumini* (r=-0.75). In addition, *Ziziphus mauritiana* (r=-0.42) and *Murraya koenigii* (r=-0.43) have been shown to have comparatively weak negative correlations with SO₂. It is also possible that morphological parameters somewhere influence the tolerance behaviour of plant to air pollutants. However, according to Bharti et al. (2018) large leaf surface area leads to higher absorption of gaseous pollutants by the plant which lowers the APTI of the plant. Similarly, in the present study, it is observed that plants with larger leaf area are showing negative correlation with SO₂. Further, low concentration of SO₂ can induce stomatal opening, while stomatal closure is facilitated by high dose of SO₂. It has been found that studied plant species differ in the degree of injury which they sustain from

the same level of SO₂ exposure. The differences in sensitivity to SO₂ could reflect differences in stomatal resistance to SO₂ or biochemical detoxification of absorbed SO₂ Prasad and Rao (1982). Besides, stomatal closure at high SO₂ dose may be associated with CO₂ accumulation in the sub stomatal cavities due to decline in photosynthesis, which leads to reduced of total chlorophyll, relative water content, and ascorbic acid content that ultimately reduce APTI (Thawale et al. 2010). Some plant species exhibit strong resistance to air pollutants by modifying their morphological and physiological functions. As an example, according to Permana et al. (2022), *Sanseveria* plant has thick leaves that can absorb lethal pollutants gases such as carbon monoxide (CO), benzene, formaldehyde and CO₂ released by incomplete combustion in motor vehicles. It has a thick wax coating on the leaves which is one of the factors preventing leaf damage due to air pollution. In addition, the thick coating retains water content, reduces the loss of nutrients and metabolites, gas exchange and protect against reactive pollutants such as CO and O₃.

On the other side, most of the plants species are showing positive correlation with NO₂ *Ocimum sanctum* (0.79) followed by *Melia azaderach* (0.67) and *Moring oleifera* (0.57). Similarly, *Psidium guajava* (0.39), *Mentha piperita* (0.34), *Alstonia scholaris* (0.31) are showing weak positive correlation with NO₂. It may be because NO₂ is not always act as pollutant. Similarly, in the present study, it is showing positive correlation with APTI of plants species. Instead of acting as pollutant, NO₂ is absorbed through stomatal uptake or deposition on the leaf surface and is involved in other activities within plants such as higher growth and aboveground biomass production, nutrient uptake, leaf area as well as flower and fruit production (Oksanen and Kontunen-Soppela 2021).

4. Conclusion

Random planting of some plants around the polluted area may not be an effective way to combat air pollution. Selecting the right species of plants and having a sustainable landscape management strategy should be essential. In the present study *Ficus religiosa* and *Ficus benghalensis* has high APTI value and can be considered as tolerant plants species and suitable sink for air pollution. Furthermore, it has been found that there is a negative correlation of plant APTI with SO₂ but no significant correlation has been found with NO₂. Plants with a negative correlation with SO₂ may indicate plant's susceptibility to SO₂. On other side, they may be tolerant to NO₂ because no significant change is observed for APTI due to NO₂ concentration. However, daily or weekly sampling of plants from more polluted sites (especially SO₂ and NO_x polluted) would provide better correlation of APTI with ambient SO₂ and NO_x. In the present study, three months of data were taken, but a larger database would be more reliable in identifying resistant and indicator species for effective air pollution management program

In the present study, three months of data were taken, but a larger database would be more reliable in identifying resistant and indicator species for an effective air pollution management programme. Besides, the present findings will provide theoretical basis for developing leaf based bioremediation techniques to reduce air pollutants in atmosphere. The highly tolerant plant species to SO₂ and NO₂ can

be chosen for large scale planting programs as a part of social forestry in urban areas, while sensitive plants can be used as bio indicators of air pollution sensitive zones.

Declarations

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iv. Author Contributions

- a. Prasenjit Adak: Conceptualization, Supervision
- b. Navneet Kour: Data curation, Writing- Original draft preparation.

v. Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures



a



b



c



d

Figure 1

Examples of yellowing and dry brownish to black coloured spots on the tips and surfaces of leaves.