Abstract: In the present study, 8 different plant species i.e. Ficus religiosa, Azadirachta indica, Sophora japonica, Tamarindus indica, Alistonia scholaris, Ficus benghalensis, Anacardium occidentalis, Delonix regia were taken from the highly polluted road side area (experimental Site) and the same species present in the residential colony which may be considered as control site, because it is free from any sort of pollution. The air pollution tolerance indices (APTI) of eight plant species were evaluated with the help of analysis of some biochemical parameters of the leaves i.e., ascorbic acid, chlorophyll, relative water content, and leaf-extract pH. The results showed that the species Delonix regia, (3.413) is most tolerant since it had the least percentage increase in APTI value and followed by Tamarindus indica, (10.712), Ficus benghalensis (11.336), Ficus religiosa, (14.046), Anacardium occidentalis, (18.000), are moderately tolerant, Sophora japonica (41.945), Alistonia scholaris (43.141), and Azadirachta indica, (59.029), are sensitive according to their air pollution tellerance index values. The results indicate that, all the biochemical parameters shows the deterioration with the increase of intensity of pollution.

Keywords: APTI, Ascorbic Acid, Chlorophyll, Relative Water Content, Leaf extract pH, sensitive, Tolerant.
Introduction

Air pollution is one of the severe problems world facing today. It deteriorates ecological condition and can be defined as the fluctuation in any atmospheric constituent from the value that would have existed without human activity. Over the years there has been a continuous increase in human population, road transportation, vehicular traffic and industries which has resulted in further increase in the concentration of gaseous and particulate pollutants. Environmental stress, such as air pollution, is among the factors most limiting plan productivity and survivorship. In urban environments, trees play an important role in improving air quality by taking up gases and particles. Plants provide an enormous leaf area for impingement, absorption and accumulation of air pollutants to reduce the pollutant level in the air environment, with a various extent for different species. Plants, act as a sink and as living filters to minimize air pollution by absorption, adsorption, detoxification, accumulation and/or metabolism without sustaining serious foliar damage or decline in growth, thus improving air quality by providing oxygen to the atmosphere. The use of plants as monitors of air pollution has long been established as plants are the initial acceptors of air pollution. They act as the scavengers for many air borne particulates in the atmosphere.

Plants differ markedly in their responses to pollutants, some being highly sensitive and others hardy and tolerant. Parameters used in defining sensitivity or resistance of plants towards different air pollutant concentrations are Ascorbic Acid content (AA), Relative Water Content (RWC), Chlorophyll content (TCh), and leaf extract pH. Categorization of plants as sensitive or tolerant is determined by the level of these parameters in plants, and thus plants show different susceptibility to different pollutants. Sensitive species are early indicators of pollution, and the tolerant species help in reducing the overall pollution load. Chlorophyll content decreases due to production of reactive oxygen species (ROS) in the chloroplast under water stress (ROSs are very small reactive molecules that can cause damage to cell structures during environmental stress). Higher ascorbic acid content of leaves might be an effective strategy to protect thylakoid membranes from oxidative damage under such water stress, as ascorbic acid is critically involved in the defense against ROS produced by the photosynthetic apparatus.

The present study provide an assessment of the use of biochemical parameters of trees as indicators of air pollution and used for the air quality monitoring in urban area.
MATERIALS AND METHODS

Area of study Selection of sampling area and sampling details

The research work was mainly confined in ponnur sorounding; the highly polluted road side 10 different plant species were taken from the highly polluted road side area(Experimental Site) and A site nearby with similar ecological conditions was selected as the control site (Control Site). The plants used for the study were those available in the experiment site. The leaf samples were collected at the lower most position of canopy at a height of 6-7ft from the ground surface. Samples were cleaned with distilled water and then refrigerated (22ºC) under suitable condition for further biochemical analysis.

Ascorbic acid (AA) content analysis

Ascorbic acid is a strong reducing agent. Ascorbic acid can be measured by means of its reducing property. It is oxidized in presence of coloured dye 2,6 dichlorophenol indophenol to de hydro ascorbic acid. At the same time, the dye is reduced by AA to colourless compound. So the end point of the reaction can be easily determined.

The dye is decoloured by other compounds as well as AA, but the specificity can be increased by carrying out the reaction in an acid solution where interfering substances react slowly.

Materials:

2,6, dichlorophenol indophenols: 250mg/1000mlDW
Stock AA solution : 100mg/100mlDW
Ascorbic Acid (Standard solution): 10mg/100ml 5%oxalic acid.
Oxalic acid(5%): 5g/100mlDW

Procedure

10ml of standard AA solution is taken and titrated with 2,6 dichlorophenol indophenols dye. The appearance of pink colour indicated the end point. Similarly, 10ml of unknown solution is taken and titrated with the dye. For blank 10ml of 5% oxalic acid is taken and titrated with the dye. The AA present in the unknown sample is calculated as follows:

Calculation:

1. Working standard AA 10mg/100ml DW.
2. Volume taken for estimation 10ml.
3. Total volume of unknown solution 10ml.

Amount of Vit. C in 100ml of unknown solution.

<table>
<thead>
<tr>
<th>Titre value of unknown U.T.</th>
<th>− Blank(B1)</th>
<th>Amount of Vit.C</th>
<th>X</th>
<th>Total volume of unknown</th>
</tr>
</thead>
</table>

Titrate value of AA solution − B1

\[
\text{mg/100ml} = \frac{(\text{U.T.} - \text{B1}) \times 1\text{mg} \times 100}{\text{S.T.} - \text{B1} 	imes 10}
\]

**Unknown solution preparation from plant tissue:**

Many plant tissues contain AA (Vit.C) in varying amounts. The tissues can be homogenized by grinding in a pestle with a mortar. Many plant tissues also contain the enzyme ascorbic acid oxidase. The enzyme catalyses the oxidation of AA to dehydro ascorbic acid. When the cells were disrupted by grinding, the activity of AA oxidase may be sufficient to catalyze the oxidation of AA in the tissue. In order to avoid such a loss of AA, extract the tissue with 5% oxalic acid (or 5% metaphosphoric acid). The treatment will inactivate the oxidase. Hence, grind the tissue with the acid and filter the homogenate. The volume is made up to 100ml with 5% oxalic acid. Ascorbic acid is estimated as described above.

i) Blank titration.

ii) Titration of standard AA.

iii) Titration of unknown sample.

iii) Titration of unknown sample.

**Total Chlorophyll Content (TCh) estimation:**

Chlorophyll is an indicator of plants biomass. All green plants contain chlorophyll a, b, xanthophylls and carotenoids. The chlorophyll consents works as a good measurement to estimate the tolerance limits by plants against air pollution.

**Required Chemicals:** 80% acetone; magnesium carbonate.

**Procedure:** One gram of the greenest leaves of the plants were selected and cleaned thoroughly with water and dried in room temperature. Macerated these leaf material in a pestle with mortar adding 20-25ml of 80% acetone. A pinch of magnesium carbonate is also added to the leaf material while grinding. Centrifuged the contents at 2000 r.p.m. for 15 mints. Transferred the
extract to a volumetric flask and made up to the volume of 50 ml using 80% acetone. The absorbance of this green solution is read at 645 and 663, and the total chlorophyll is calculated with the following formula.

\[20.2 \times A_{645} + 8.02 \times A_{663}\]

**Determination of pH of Leaf Extract**

pH is the measure of hydrogen ion activity and depends largely on the relative amounts of the adsorbed hydrogen and metallic ions. It is a good measure of the intensity of acidity and alkalinity of suspension.

**Required Chemicals:** pH papers, Distilled water.

**Procedure:** One gram of leaf material is taken into a pestle and mortar and perfectly morated adding little quantity of water. After 20ml of DW is added and allowed the solution to settle for 15-20 minutes. From supernatant solution, the exact pH is determined with the help of pH papers.

**Estimation of water content in Leaf material (Relative Water Content RWC):**

Leaf samples were collected from the field and transported as quickly as possible to the laboratory. 10 grams of the leaf material is weighed and dried at 60-700C in a properly ventilated hot air oven for a period of 3 hours. The weight of the leaf material is measured and the percentage of water content in the leaf material is calculated.

**Determination of APTI**

The air pollution tolerance indices of ten common plants were determined by the following method. The formula of APTI is given as

\[\text{APTI} = \frac{[A \times (T+P) + R]}{10}\]

Where A = Ascorbic acid content (mg/g),

T = total chlorophyll(mg/g),

P = pH of leaf extract, and

R = relative water content of leaf (%).

The APTI values help to identify the sensitive species to be used for bio monitoring of air pollutants and to determine the air pollution tolerant species.
Results and Discussion

APTI is calculated for 8 different plant species taken from the highly polluted road side area (experimental Site) of Warangal town and the same species present in a residential colony which is free from any sort of pollution (control site).

The APTI and the observed values of different biochemical parameters of the leaves i.e, ascorbic acid, chlorophyll, relative water content, and leaf-extract pH is presented in Table:1. According to the observed APTI values different plants responded differently to the air pollutants. The results revealed that of the 8 species studied Delonix regia (Gulmohar, Flame Tree) (0.57) is most tolerant followed by Ficus benghalensis banyan (1.51), Tamarindus indica (1.76), Ficus religiosa, Peepal (raavi) tree (2.18), Anacardium occidentalis Badam (2.70), Sophora japonica, Pogada chettu, Japanese pagoda (5.26), Alistonia scholaris, Devil’s Tree (5.49), VEPA, Azadirachta indica (6.57).

Table:1. Ascorbic Acid (AA), Total Chlorophyll (TCh), Leaf-extract pH, Relative Water Content (RWC), and APTI values of trees.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Species</th>
<th>Site</th>
<th>AA</th>
<th>TCh</th>
<th>pH</th>
<th>RWC</th>
<th>APTI</th>
<th>Difference/ Increase in APTI%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ficus religiosa Peepal (raavi) tree</td>
<td>Experimental</td>
<td>1.161</td>
<td>71.01</td>
<td>5.39</td>
<td>88.3</td>
<td>17.70</td>
<td>2.18 14.046</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>1.243</td>
<td>55.10</td>
<td>5.50</td>
<td>79.9</td>
<td>15.52</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Azadirachta indica Vepa</td>
<td>Experimental</td>
<td>1.261</td>
<td>72.61</td>
<td>5.78</td>
<td>78.2</td>
<td>17.70</td>
<td>6.57 59.029</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>1.131</td>
<td>58.99</td>
<td>5.93</td>
<td>37.9</td>
<td>11.13</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Sophora japonica Japanese pagoda</td>
<td>Experimental</td>
<td>1.173</td>
<td>70.81</td>
<td>7.53</td>
<td>86.1</td>
<td>17.80</td>
<td>5.26 41.945</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>1.257</td>
<td>56.66</td>
<td>5.80</td>
<td>46.9</td>
<td>12.54</td>
<td></td>
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<tr>
<td>4</td>
<td>Tamarindus indica Chinta</td>
<td>Experimental</td>
<td>1.350</td>
<td>72.50</td>
<td>5.60</td>
<td>76.5</td>
<td>18.19</td>
<td>1.76 10.712</td>
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<tr>
<td></td>
<td></td>
<td>Control</td>
<td>1.241</td>
<td>65.84</td>
<td>5.36</td>
<td>76.0</td>
<td>16.43</td>
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</tr>
<tr>
<td>5</td>
<td>Alistonia scholaris Devil’s Tree</td>
<td>Experimental</td>
<td>1.151</td>
<td>71.93</td>
<td>5.77</td>
<td>90.9</td>
<td>18.03</td>
<td>5.49 43.141</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>1.257</td>
<td>56.66</td>
<td>5.80</td>
<td>46.9</td>
<td>12.54</td>
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<tr>
<td>6</td>
<td>Ficus benghalensis banyan</td>
<td>Experimental</td>
<td>0.928</td>
<td>72.54</td>
<td>5.64</td>
<td>75.7</td>
<td>14.83</td>
<td>1.51 11.336</td>
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<td></td>
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<td>Control</td>
<td>1.122</td>
<td>72.43</td>
<td>5.52</td>
<td>45.7</td>
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<tr>
<td>7</td>
<td>Anacardium occidentalis Badam</td>
<td>Experimental</td>
<td>1.261</td>
<td>72.61</td>
<td>5.78</td>
<td>78.2</td>
<td>17.70</td>
<td>2.70 18.000</td>
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<tr>
<td></td>
<td></td>
<td>Control</td>
<td>1.257</td>
<td>61.30</td>
<td>5.66</td>
<td>65.8</td>
<td>15.00</td>
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<tr>
<td>8</td>
<td>Delonix regia Gulmohar Flame Tree</td>
<td>Experimental</td>
<td>1.181</td>
<td>72.54</td>
<td>5.66</td>
<td>80.4</td>
<td>17.27</td>
<td>0.57 3.413</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>1.192</td>
<td>67.60</td>
<td>5.55</td>
<td>79.8</td>
<td>16.70</td>
<td></td>
</tr>
</tbody>
</table>
The results are as shown on table 1. Plants that are constantly exposed to environmental pollutants absorb, accumulate and integrate these pollutants into their systems, depending on their sensitivity level; plants show visible changes which would include alteration in the biochemical processes or accumulation of certain metabolites. In this study, changes in parameters such as ascorbic acid, total chlorophyll, relative water content, pH of leaf extract were used in evaluating the degree of tolerance to air pollution by the plant species. Under experimental conditions, the ascorbic acid concentration is higher than those of the control site.
Ascorbic acid is a strong reductant and it activates many physiological and defense mechanism. Its reducing power is directly proportional to its concentration. However, its reducing activity is pH dependent, being more at higher pH levels.

Chlorophyll is an index of productivity of plant. Whereas certain pollutants increase the total chlorophyll content, other decreases it. In the present study, it has been observed that plants from experimental site contain more chlorophyll compared with those from the control site. The chloroplast is the primary site of attack by air pollutants. Air pollutants make their entry into the tissues through the stomata and cause partial denaturation of the chloroplast and decreases pigment contents in the cells of polluted leaves.

The relative water content in a plant body helps in maintaining its physiological balance under stress conditions of air pollution. This is responsible for the higher level of relative water content in the plants in the experimental site than the control site.
An overview of the entire result obtained from this study reveals that different plants respond differently to air pollution; hence the different indices it is observed that plants growing in apparently polluted environment have higher APTI than less from less polluted environment. From the result obtained, it has been observed that *Delonix regia*, (3.413), is most tolerant since it had the least percentage increase in APTI value and followed by *Tamarindus indica*, (10.712), *Ficus benghalensis* (11.336), *Ficus religiosa*, (14.046), *Anacardium occidentalis*, (18.000), are moderately tolerant, *Sophora japonica* (41.945), *Alistonia scholaris* (43.141), and *Azadirachta indica*, (59.029), are sensitive according to their air pollution tellerance index values.

**Conclusion:**

It is concluded that this study is most important, useful for the better understanding and management of air quality as well as in selection of suitable plant species (with high APTI) for plantation in highly polluted road side areas, industrial areas to reduce the air pollution and use of plants, as bio indicators is inexpensive and easy technique. Determination of APTI is important because the increase in industrialization, danger of deforestation due to air pollution threatening the environment. Therefore results of such studies are handy for future planning. It is worth noting that combining a variety of parameters gave a more reliable result than when based on a single biochemical parameter.

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