CHAPTER 6

EFFECT OF OZONE CONCENTRATION ON PLANTS
6.1 VEGETATION AS A BIOLOGICAL MONITOR OF AIR POLLUTANTS

The use of plants to monitor air pollution problems has often permitted the early detection of these problems, since plants are often the most sensitive biological receptor. Plants may develop characteristic symptoms when exposed to acute or chronic concentrations of certain air pollutants, and so they can serve as a useful tool in field surveys [Stern, 1977].

Field surveys have a significant place in the assessment of air pollution problems. Basic techniques originated in the nineteenth century in work involving sulfur dioxide [Weinstien, 1970]. The field survey attempts to answer the question: Does this area have a significant air pollution problem? Often this is somewhat in the category of confirming the obvious, since a survey would not be initiated unless there were subjective reasons for assuming that the answer would be affirmative. In such cases, the surveys are designed to produce objective data.

However, even characteristic symptoms are not necessarily definitive of cause, since disease, insects, cultural conditions, etc., singly or in combination, may produce similar, if not identical, injury patterns. Thus, considerable caution must be exercised in defining an air pollution problem.

If both the pollutant and its effect on vegetation are known, we can examine a field area for a relatively few characteristic symptoms. Even identifying the visible injury symptoms will not permit an investigator to
deduce with a high degree of confidence the amount, duration, or number of exposures to an injurious pollutant. A competent observer must make a series of inferences. Any individual judgment is subject to a high degree of uncertainty arising from the tissue age, the site, the weather conditions, and the history of the monitoring plant. But through a series of these inferences or judgments concerning different plants in different stages of growth, a competent observer is often able to say with a rather high degree of credibility, “This area has been fumigated with this pollutant.”

Plants used as a Biological monitors for a specific pollutant can be termed as “Passive Samplers”. Comparing these passive samplers with direct detection instruments, otherwise called Active monitors, there are certain advantages and disadvantages observed.

The advantage of passive samplers over the active monitors are such as their simplicity, low cost, independent of electricity, giving cumulative effect of the pollutant, which offer these passive samplers an attractive alternate of active monitors (Varshney, 2002). Thus by deploying these samplers it is possible to develop a wide spatial monitoring network in a highly cost effective manner.

At the same time there are certain disadvantages also observed. The active monitors would be specific in reaction to a pollutant, where as Bio-monitor may not be so. Also the instruments will give the result in a short period of exposure but the passive samplers may need a relatively long time exposure.
Anyway properly taking all the precautionary measures, the passive samplers can be deployed in field studies for a more realistic results.

It is also worth mentioning that a correlating effort is being carried-out to infer the concentration of polluting substance from the symptoms observed in the plants. By conducting both passive sampling and active monitoring simultaneously, their correlation could be obtained.

Components of photochemical pollution injure plants in most of the major metropolitan areas of the World, but scientists were slow to recognize these effects [Knabe, 1973]. Some phytotoxic oxidants have been isolated from the photochemical complex, and research in several laboratories suggests the presence of additional phytotoxicants. Ozone, the most important phytotoxicant in the complex [Heck, 1973], was first identified by Richards in 1958 as the cause of a stipple on the upper surface of grape leaves. The effect of ozone on plants which result in both visible and invisible injuries as well as damages are briefly discussed in the following section.

6.2 EFFECTS OF OZONE CONCENTRATION ON PLANTS

Acute symptoms of plant damage are sufficient to indicate the occurrence of adverse levels of a particular air pollutant; lower concentrations of the air pollutant may however be capable of causing chronic injury without visible damage. A more sensitive indicator of plant injury was therefore
proposed by De Koning and Jegier [1968], whereby the rates of apparent photosynthesis and respiration are related to the presence of air pollutants.

Some of the mechanisms of ozone actions on various plants have been outlined here.

6.2.1 PHOTOSYNTHESIS

Photosynthesis is a complex chain of oxidation-reduction reactions, with carefully balanced equilibria, leading to the reduction of carbon dioxide to form high-energy sugars. The intake of even a small quantity of a strong oxidant such as ozone may therefore result in a change of the rate of apparent photosynthesis. It is intimately linked to productivity and has deservedly received the most attention [Treshow, 1989].

It is easy to say that ozone affects photosynthesis, but many factors enter into photosynthesis. Which of these is most sensitive to ozone? And which is the first to be affected and how might this influence subsequent interactions?

Photosynthesis in the sensitive flag leaves of oats, for instance, is suppressed at concentration of 70 ppb [Dempster, 1988]. White pine proved even more sensitive, and net photosynthesis was reduced 15 - 20% following 3 months exposure to as low as 40 ppb ozone. No change was reflected in the dry weight of the pine seedlings.
Photosynthesis and related processes take place in the chloroplast. Ozone affects so many related processes; it is difficult to decide which comes first. One vital process inhibited is electron transport in the water-splitting light reaction, whereby oxygen is released and energy is made available to drive the so-called dark reaction, in which carbon dioxide is reduced (hydrogen is added) and carbohydrates are formed. This is accomplished by the coenzyme nicotinamide adenine dinucleotide phosphatase (NADP), which after capturing an electron from chlorophyll can accept a hydrogen ion from the splitting of water molecules, becoming NADPH or reduced NADP. NADPH then takes part in the sugar-building reactions of the carbon cycle. This inhibition is demonstrated in isolated spinach chloroplasts. In this process Adenosine Tri Phosphate (ATP) production declined concurrently with both electron transport and the H+ gradient. This was the first detectable effect of ozone on photosynthesis; then came inhibition of electron transport between the photosystems [Treshow, 1989].

Ozone also affects and can destroy chlorophyll, leading to reduced photosynthesis, but mostly at higher concentrations that involve visible injury [Krupa, 1988]. While net photosynthesis can be impaired without the development of visible symptoms, research first suggested that photosynthesis tends to return to normal when the exposure stops [Koziol, 1984]. This is not always the case though, and exposure of ponderosa pine trees to 150 ppb ozone for 30 days reduced net photosynthesis 10% over controls in the absence of visible symptoms. Reductions in photosynthesis were also demonstrated in citrus [Krupa, 1988]
6.2.2 RESPIRATION

Ozone apparently evokes much the same response as other stresses in causing an increase in the rate of respiration [Koziol, 1984]. One way in which ozone may act is in inhibiting phosphorylation of leaf mitochondria. ATP and total adenylate increased immediately following ozone exposure. It has been postulated that the increased energy comes from lipids and proteins in the cell membranes once the normal carbohydrate reserves are exhausted [Treshow, 1989].

6.2.3 CARBOHYDRATE AND RELATED METABOLISM

Following the probable initial effects of ozone on membranes and photosynthesis, a number of secondary responses might be expected. Both increases and decreases in sugars have been reported, depending largely on the ozone concentrations. An increase in soluble sugar content of ponderosa pine needles followed by a decrease in the roots may be especially important. Changes related to the activity of enzymes in the glycolytic pathway and stimulation of the pentose phosphate pathway may be responsible [Tingey, 1985].

Ozone can also affect polyunsaturated fatty acids by oxidative mechanisms; these oxidations in turn can change the properties of membranes [Koziol, 1984].
Accumulation of isoflavonoids in ozone-treated plants is reported, and it is suggested that this might involve a general stress response as known to be associated with pathogenic infections [Krupa, 1988]. The build-up increased with the appearance of visible symptoms. Enzymes involved in phenol metabolism may increase following ozone exposure together with peroxidase activity [Tingey, 1985].

6.2.5 NITROGEN METABOLISM

Ozone has been found to inhibit both nitrate reductase, and especially nitrite reductase, activity [Tingey, 1985]. This could influence the nitrogen available for photosynthesis as well as the photosynthetic efficiency. An increase in free amino acids in foliage of several plant species following ozone exposure also has been demonstrated [Treshow, 1989]. This surplus infers that protein synthesis is impaired.

6.2.6 STRESS AND SENESCENCE

Plants subjected to stress produce elevated levels of ethylene. Ozone evokes the same response. Ethylene production precedes the appearance of visible symptoms but appears to be associated with many of the phenol and pigment accumulations. Premature senescence associated with ozone is characterized by increases in reactions such as loss of starch,
proteins and chlorophyll, and increases in anthocyanin and polyphenols, respiration and ethylene production [Krupa, 1988]. Premature leaf abscission and fruit drop also occur [Treshow, 1989]. The effects of ethylene in plants include in the promotion of both abscission and fruit ripening.

6.2.7 ASSIMILATE PARTITIONING

The impact of ozone in altering the partitioning of carbohydrates in the plant may be its most significant role [Cooley & Manning, 1987]. If a plant is to be healthy, the photosynthetic products must be properly distributed. Ozone generally reduces the amount of dry matter produced, but even if the amount of photosynthate were not reduced the available assimilate is diverted to the leaves and stem at the expense of the root and crown.

The amount of starch accumulating in roots tends to decrease with increasing ozone exposure. Ponderosa pine seedlings exposed to 100 ppb ozone 6 hours per day during the growing season were found to have lower root reserves of soluble sugars and starch in the autumn, which had the potential for restricting initiation of new growth the following spring [Tingey, 1985].

At ozone concentrations above about 100 ppb, assimilate accumulation becomes greatly depressed and effects on partitioning are not obvious. At lower ozone concentrations of 50 to 100 ppb, storage organs, including roots of trees, are most affected.
6.3 OBJECTIVE OF THE STUDY

Reduction of photosynthesis following exposure to ozone could be related to a loss of chlorophyll \(b\). Studies with algae mutants lacking chlorophyll \(b\) support the theory that chlorophyll \(b\) acts as an accessory pigment by preventing the permanent oxidation of chlorophyll \(a\). Loss of chlorophyll \(b\) will therefore lead to a reduction of the rate of photosynthesis.

In this study, it is proposed to have plant as a monitor of a specific air pollutant. In the earlier two chapters, a concept of concentration gradient from coastal to inland sites is observed and checked. Here in this experiment the same is put to re-check with the help of a "Bio-monitor".

6.3.1 PRINCIPLE:

As ozone concentration increases photosynthesis reduces which could be related to the reduction of chlorophyll \(\cdot b\). This principle was tested by many scientists using macro and micro plants [Allen, 1958].

One interesting experiment was done by De Koning and Jegier [1965] using *Euglena gracillis*, a unicellular algae as a reactant. *Euglena gracillis*, after the exposure of ozone concentration showed no decrease in chlorophyll \(a\); however showed a small but significant decrease (5%) of chlorophyll \(b\).
The quantitative relationship between reductions of the rate of photosynthesis to the various ozone concentrations (Fig. 6.1) can be presented mathematically [De Koning and Jegier, 1968b] as follows:

\[ \log Y_{O_3} = 1.392 X_{O_3} \]  

(1)

Where \( Y_{O_3} \) = percentage reduction of photosynthesis and \( X_{O_3} \) = ozone concentration (ppm), during 1 hour.

Equation (1) permits the calculation of the effect of 0.5 ppm \( O_3 \) on the rate of photosynthesis: a decrease of 5.0 per cent.

For the estimation of chlorophyll \( a \) and chlorophyll \( b \), a known volume of *Euglena* suspension is diluted with acetone to a final concentration of 80% acetone. The optical densities of the resulting chlorophyll solution at 663 nm and 654 nm permit the calculation of the quantities of chlorophyll \( a \) and chlorophyll \( b \) using the formulas of McKinney [Smith, 1955]. The reduction of photosynthesis is calculated as a percentage of respective control which is taken as 100 percent.

Reports have appeared from time to time relating dose injury response of plants to ozone concentrations. Dugger [1965] have made a detailed study of the effect of peroxyacetyl nitrate (PAN), a component of smog, on certain photosynthesis reactions.
FIGURE 6.1 RELATION BETWEEN PERCENTAGE REDUCTION OF PHOTO SYNTHESIS AND OZONE CONCENTRATION
The above explained reference of this ozone–plant interaction had been tested many times and proved as a useful tool in air pollution monitoring. Hence, in this study it is planned to observe the response of a macro plant grown at various environments of different surface ozone concentration.

This results could be compared with the already available ozone data at some places. Ultimately the effect of ozone on the plant as well as the comparative ozone concentration abundance of places considered could be established.

6.4 EXPERIMENTAL PROCEDURE AND DATA COLLECTION

Based on the objective formulated in the previous section suitable experimental procedure was adopted and the study was conducted. Details of the procedure are given below.

6.4.1 SELECTION OF SAMPLE

In the previous section, it is explained that there is a loss of chlorophyll \( b \) in the micro plant i.e. *Euglena gracilis* due to ozone concentration. Similarly, to observe the effect in Macro plant, we could select a ozone sensitive plant. Here are some ozone sensitive plants listed [Treshow, 1989] below:—
Tomato *[Solanum Lycopersicum, family Solanaceae]* was the plant selected for the study. It is well acclimatized to our climatic conditions and grows easily. This plant has also got economical importance. Same type and same size tomato seeds were selected from a particular plant.

### 6.4.2 SELECTION OF PLACE

Ozone concentration will never be equal in all the places. Therefore we have selected different places like coastal, inland, rural and urban environments randomly.

The places selected for the study, their distance from the sea and their major characteristics are given in Table 6.1. A location map illustrating all these observational points is given as Figure 6.2

### 6.4.3 SELECTION OF PERIOD

Tomato plants are capable growing in almost all seasons. But, in summer and pre-monsoon period, the growth will be remarkable. Also, in these two seasons, the ambient ozone concentration is also observed remarkably high.
# TABLE 6.1 PLACES OF PLANT - OZONE INTERACTION STUDY

<table>
<thead>
<tr>
<th>S.No</th>
<th>Place</th>
<th>Distance from sea in Km</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pondicherry</td>
<td>0</td>
<td>Coastal, urban</td>
</tr>
<tr>
<td>2</td>
<td>Tranquebar</td>
<td>0</td>
<td>Coastal, rural</td>
</tr>
<tr>
<td>3</td>
<td>Porayar</td>
<td>02</td>
<td>Coastal, rural</td>
</tr>
<tr>
<td>4</td>
<td>Vaidheeswaran koil</td>
<td>20</td>
<td>Inland, rural</td>
</tr>
<tr>
<td>5</td>
<td>Kumaratchi</td>
<td>35</td>
<td>Inland, rural</td>
</tr>
<tr>
<td>6</td>
<td>Vandavasi</td>
<td>55</td>
<td>Inland, rural</td>
</tr>
</tbody>
</table>
Vandavas

Pondicherry

Kumaratchi

Vaitheeswaran Koil

Porayar

Tranquebar

FIGURE 6.2 LOCATION MAP
these two seasons, the ambient ozone concentration is also observed remarkably high.

So, we have selected 40 days each from both the season of Summer 2000 and Pre-Monsoon 2000. The actual seedling started for the first exposure on 12 April 2000 and sampling was done on 21 May 2000. The second trial was started on 25 July 2000 and completed on 02 September 2000.

6.4.4 SAMPLING PROCEDURE

To sow the tomato seeds we have taken three small pots for every place and filled it with the mixture of clay, sand and fertilizer in the ratio of 2:1:1 respectively. The pots were placed in the sampling stations. Seeds were sown in same time at all the places. The growth of the plants were monitored carefully. After the period of 40 days fully-grown secondary leaves were collected as specimen from each plant.

6.4.5 CHEMICAL TREATMENT

This chlorophyll determination method was invented by Moran and Porath in 1980. From the leaves, a uniform area of leaf surface could be cut using any device. Then these three test tubes were filled with 7ml to 10 ml of Di Methyl Formamide (DMF) acid. The leaf samples are immersed in the test tube chemicals and the chlorophyll contents are washed into the DMF acid. This chemical treatment had been done for all the environments. The test tubes were then preserved in 4°C for about 24 hours before the spectrophotometric analysis.
6.4.6 SPECTROPHOTOMETRIC ANALYSIS

The solutions were transferred to a clean cell or cuvette. The absorbance readings could be taken for the wavelength of 645 nm and 663 nm. Before that we should keep absorbance readings in 0.000 by filling in "blank" DMF. The wavelength considered above correspond to the following contents.

1. 645 nm → used to calculate the chl. a &  
2. 663 nm → used to calculate the chl. b

6.4.7 ESTIMATION OF CHLOROPHYLL "a" AND CHLOROPHYLL "b"

Formulae for the Estimation of Chlorophyll a and Chlorophyll b were suggested by Inskeep and Bloom (1965). We can calculate the chlorophyll a and chlorophyll b with the help of the formula given below:

\[
\text{Chlorophyll } a \,(\text{mg/L}) = 12.7 \,(A_{663}) - 2.69 \,(A_{645})
\]
\[
\text{Chlorophyll } b \,(\text{mg/L}) = 22.9 \,(A_{645}) - 4.68 \,(A_{663})
\]

Where,  A is the absorbance value
6.5 RESULT AND DISCUSSION

6.5.1 FIELD STUDY

The field study was carried out as explained in the previous section. The spectrophotometer readings of two seasons were tabulated and Chlorophyll $a$ and $b$ values were calculated for these observations using Inskeep and Bloom (1985) formulae. The results were presented in Tables 6.2 and 6.3.

6.5.2 DISCUSSION

From the results obtained the following inferences could be made:

1. As the distance varies from seashore, there is no remarkable change in chlorophyll $a$ concentration.

2. As the distance varies from the seashore, there is a noticeable change in chlorophyll $b$.

3. Chlorophyll concentration is high at Kumaratchi and low at Pondicherry.

4. As we move from seashore, the chlorophyll $a$ concentration remains much unaltered and chlorophyll $b$ concentration is gradually decreases except for the place Vandavasi. (Fig6.3 & 6.4)
5. This decrease is qualitatively linear with the relationship evaluated between ozone concentration and chlorophyll reduction by De Koning and Jegier, 1968b

6. The decrease of chlorophyll \( b \) shows a similar pattern for both the seasons. However, the variation (standard deviation) is slightly higher in pre-monsoon than in summer seasons (Fig 6.5).

6.6 CONCLUSIONS

From the above inferences we could reach the following conclusions:

1. Chlorophyll \( b \) content is lower at Pondicherry and higher at Kumaratchi and also shows a slight increment as we move from seashore; may be due to the slight decrease of ozone concentration towards the inland locations.

2. The reason for higher ozone concentration at Pondicherry may be attributed to its conducive atmosphere for more ozone production. As Pondicherry is a industrialized urban place located at tropical coast, ozone precursor concentrations must be high. This will cause photochemical production of ozone in daytime.

3. As Tranquebar stands next due to the reason that it is similar to Pondicherry atmosphere but for its rural location. So the pollution caused by industries are not influencing this atmosphere. Nevertheless this coastal
<table>
<thead>
<tr>
<th>Place</th>
<th>Distance from sea in Km</th>
<th>Spectrophotometric Absorbance at 645nm</th>
<th>Spectrophotometric Absorbance at 663nm</th>
<th>Chlorophyll concentration in mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>PONDY</td>
<td>0</td>
<td>0.137</td>
<td>0.316</td>
<td>3.645</td>
</tr>
<tr>
<td>TQR</td>
<td>0</td>
<td>0.138</td>
<td>0.302</td>
<td>3.464</td>
</tr>
<tr>
<td>PYR</td>
<td>02</td>
<td>0.142</td>
<td>0.297</td>
<td>3.389</td>
</tr>
<tr>
<td>V.KOIL</td>
<td>20</td>
<td>0.139</td>
<td>0.293</td>
<td>3.347</td>
</tr>
<tr>
<td>KUM</td>
<td>35</td>
<td>0.155</td>
<td>0.265</td>
<td>2.948</td>
</tr>
<tr>
<td>VANDV</td>
<td>55</td>
<td>0.164</td>
<td>0.280</td>
<td>3.114</td>
</tr>
<tr>
<td>Palace</td>
<td>Distance from Sea in Km</td>
<td>Spectrophotometric Absorbance at</td>
<td>Chlorophyll concentration in mg/L</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>-------------------------</td>
<td>----------------------------------</td>
<td>----------------------------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>645 nm</td>
<td>663 nm</td>
<td>a</td>
</tr>
<tr>
<td>PONDY</td>
<td>0</td>
<td>0.247</td>
<td>0.487</td>
<td>5.52</td>
</tr>
<tr>
<td>TQU</td>
<td>0</td>
<td>0.256</td>
<td>0.491</td>
<td>5.547</td>
</tr>
<tr>
<td>PYR</td>
<td>02</td>
<td>0.266</td>
<td>0.495</td>
<td>5.57</td>
</tr>
<tr>
<td>V.KOIL</td>
<td>20</td>
<td>0.288</td>
<td>0.465</td>
<td>5.13</td>
</tr>
<tr>
<td>KUM</td>
<td>35</td>
<td>0.306</td>
<td>0.513</td>
<td>5.691</td>
</tr>
<tr>
<td>VAND</td>
<td>55</td>
<td>0.303</td>
<td>0.517</td>
<td>5.750</td>
</tr>
</tbody>
</table>
FIGURE 6.3 CHLOROPHYLL CONCENTRATION AT VARIOUS PLACES IN SUMMER
FIGURE 6.4 CHLOROPHYLL CONCENTRATION AT VARIOUS PLACES IN PRE-MONSOON
**FIGURE 6.5 COMPARISON OF STANDARD DEVIATION OF CHLOROPHYLL “b” CONCENTRATION AT TWO SEASONS**
location has got adequate precursor concentration for the favourable photochemical production of surface ozone.

4. Porayar is a place which shows near similar characteristics with Tranquebar, whereas Vaitheeswaran Koil would be having medium range chlorophyll b and hence lesser ozone concentration.

5. Incase of Kumaratchi, which is located at 35km from the seashore, represents a clean, rural, inland atmosphere, records the highest chlorophyll b value means having the lowest ozone concentration.

6. Vandavasi, which is located in remote inland area, and expected to give high chlorophyll b value contrarily gives medium range value. This is not surprising because this place is a semiurban location which is moderately polluted, will definitely be having higher potential to produce surface ozone. This may be the reason for its higher ozone level when compared to the other inland locations like Vaitheeswaran Koil and Kumaratchi.

7. According to DE KONING and JEGIER (1968), there is a relationship has been established between ozone concentration and reduction of photosynthesis. Therefore as Ozone concentration increases chlorophyll "b" concentration decreases there by there is a reduction observed in photosynthesis. The reduction in photosynthesis will lead to the reduced food production crop yielding and consequently the economic loss.
8. While comparing variation trends of chlorophyll b in both the seasons observed; Summer season shows minimum and Pre-monsoon maximum. Also the average concentration of summer is lower than Pre-monsoon. This may be due to the high temperature of summer season, which is conducive for production of ozone.

9. Two trials of this field study at two different seasons have obviously proved that the Chlorophyll b content is reduced due to the variation of ozone concentration between Inland and coastal environments.

Hence the Production of Photo chemically dominated surface ozone concentration at tropical, polluted, coastal urban environments, would affect the growth of this macro plant, Tomato.