MATERIALS AND METHODS

*Spinacia oleracea* and *Trigonella foenum-graecum* were used for the biomonitoring purpose grown at three concentrations of urea i.e. 0.00, 1.44 (optimum) and 2.00 g (more than optimum) for *S. oleracea* and 0.0, 0.7 (optimum), 1.0 g (more than optimum) for *T. foenum-graecum*.

Above plants were exposed in chambers with NO, NO₂, O₃ alone and in combination (sequential exposure) in artificial exposure study. For field study, the other set of same spp. were fumigated in ambient atmosphere at Motinagar and at the same time measurement of NO, NO₂ and O₃ was carried out. The results were compared with that of control with respect to biochemical and physiological plant parameter such as ascorbic acid (AA), protein, ribonucleic acid (RNA), chlorophyll (Chl. a, b and total), nitrogen content (N) and phytomass (shoot, root and total dry wt). The observations were made at the interval of 15 days for 120 days with respect to plant parameters and pollution level.

Preparation of pots

Earthenware pots (20 cm dia) filled with local soil (sandy loam soil) mixed with manure (1:1 proportion). Soil and manure were thoroughly mixed before filling the pots. Three concentrations of urea were used for each plant spp. (optimum, more than optimum and no urea). Then half of the urea was added on the top of the soil surface in each pot.
and was mixed thoroughly. See Table 8b for detail regarding application of urea to the two species.

Table 8b Application of urea to S. oleracea and T. foenum-graecum

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Urea (g/pot)</th>
<th>Schedule of application of urea (g/pot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. oleracea</td>
<td>0.00 (no urea)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1.44 (optimum)</td>
<td>0.72 g at the time of sowing and 0.24 g at one month interval.</td>
</tr>
<tr>
<td></td>
<td>2.00 (more than optimum)</td>
<td>1 g at the time of sowing and 0.33 g at one month interval.</td>
</tr>
<tr>
<td>T. foenum-graecum</td>
<td>0.0 (no urea)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.7 (optimum)</td>
<td>0.35 g at the time of sowing and 0.12 g at the interval of one month.</td>
</tr>
<tr>
<td></td>
<td>1.0 (more than optimum)</td>
<td>0.5 g at the time of sowing and 0.17 g at the interval of one month.</td>
</tr>
</tbody>
</table>

Raising of seedlings

Seedlings were raised at JNU site. The seeds of S. oleracea and T. foenum-graecum were obtained from IARI. Before sowing they were pretreated with Captan for the prevention of any disease and soil was treated with Baslin (Co. 48 ml in 26 ml water). The seeds of T. foenum-graecum were sown on 1st Oct., 1986 and that of S. oleracea on 1st Feb., 1987. All the pots were watered daily with equal quantity of
water. The seeds were found to germinate after 10 days in *S. oleracea* and after 7 days in *T. foenum-graecum*.

**Plant species**

*S. oleracea* L. var. Pusa Joyti is commonly called as spinach. It's local name is palak. It belongs to the family Chenopodiaceae. Pod is 5-6 cm long. It is commonly grown and used as vegetable in India. It is of high nutritive value. Though it is a cold season plant, it can be grown throughout the year.

*T. foenum-graecum* L. var. Pusa Kasuri belongs to the family Papilionaceae. It is commonly called as fenugreek. Methi is it's local name. The flowers are bright orange yellow on long stalk having smaller sickle-shaped pods. The leaves are used as vegetable. It is a cold season plant. Its medicinal value consists of prevention of constipation, removal of indigestion, stimulation of spleen and liver and used as contraceptive, appetizer and for curing the diabetis.

**Fumigation materials**

For fumigation of plants, fumigation chambers were designed in our laboratory (Plate 1-4). Each of them is made up of wooden frame with net (except at lower side). Both the sides of the net (inner and outer) were wrappened with polyethylene sheets. On the five sides of each chamber the crossed plywood streeps were fixed (dia 5 cms approx). The purpose of the net and streeps was to give the protection to the polyethylene sheets from wind. Thus four chambers of equal size (1m³) were fabricated.
For the uniform distribution of gas, plenum was used. The gas plenum consists of 3 glass tubes connected at right angle to each other having the holes at the distance of 10 cms of a dia ranging from 0.1 cm (near inlet) - 0.3 cm (towards close end of both the tubes) (Fig. 2). The opening of one tube was joined with that of other with a piece of polyethylene pipe. The other ends of two tubes parallel to each other were sealed. The holes were facing inner side of the chamber. The dia of tube was 2 cms. From the centre of the tube which is common to the two parallel tubes, one small glass tube of 3 cms length and 3.5 cms dia was connected (for NOx and O3 gases).

Field site selection

Before starting the actual experiment two sites were selected for the study. The basis of the selection of the sites were the extent of the pollution level at both the sites. Thus the Jawaharlal Nehru University, New Campus, (School of Life Sciences building) was taken as reference site which is relatively free from pollution and Motinagar (Police Station) as polluted site, suffering from visible pollution due to industrial complexes and vehicular pollution (Fig. 3).

The JNU New Campus is situated in the south Delhi on Aravali Hill belt, near Qutab Minar. It is characterised by the presence of rocks and sandy soil. Therefore the vegetation is sparse one.
The Motinagar is situated in NWN direction to that of JNU. In this area many types of air polluting industries are present. It has industrial and housing complex. The main polluting sources are industries and automobiles. The possible sources of polluting industries are Food Processing, Hindustan Insecticides Ltd., Foundries, Chemicals, Bakeries, Chemicals and Rubber.

Fumigation scheme

The study was carried out in the following manner:

\[
\begin{align*}
\text{Fumigation of plant spp. with exposure chambers at JNU:} \\
\text{With urea} & \quad \text{Without urea} \\
\text{NO} & \quad \text{With urea} \\
\text{NO}_2 & \quad \text{Without urea} \\
\text{NO}_2 + O_3 & \quad \text{With urea} \\
\text{Fumigation of plants spp. at Motinagar:} \\
\text{With urea} & \quad \text{Without urea}
\end{align*}
\]

The study consists of two sections. First section consists of artificial fumigation of the two species i.e. *S. oleracea* and *T. foenum-graecum* in fumigation chambers, at JNU site (New Campus)
and maintaining the control at the same site for the same species. Simultaneously, the other part of the study consists of the field fumigation of the other set of above species at Motinagar site of which the control plants were maintained at JNU site.

The scheme followed is:

**In case of artificial fumigation**

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of pots per concentration of urea treatment</td>
<td>2</td>
</tr>
<tr>
<td>in each fumigation chamber</td>
<td></td>
</tr>
<tr>
<td>No. of urea concentrations</td>
<td>3</td>
</tr>
<tr>
<td>No. of pots in each chamber</td>
<td>6 (3x2)</td>
</tr>
<tr>
<td>No. of fumigation chambers</td>
<td>4</td>
</tr>
<tr>
<td>No. of pots in four fumigation chambers</td>
<td>24 (6x4)</td>
</tr>
<tr>
<td>No. of pots for control species</td>
<td>24</td>
</tr>
<tr>
<td>Total no. of control and polluted pots</td>
<td>48 (24+24)</td>
</tr>
<tr>
<td>in artificial fumigation</td>
<td></td>
</tr>
</tbody>
</table>

**In case of field fumigation**

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of pots of each species at JNU site</td>
<td>6</td>
</tr>
<tr>
<td>No. of pots of each species at Motinagar</td>
<td>6</td>
</tr>
<tr>
<td>Total no. of pots in field fumigation</td>
<td>12 (6+6)</td>
</tr>
</tbody>
</table>

Grand Total of all the pots of *S.oleracea* 60 (48+12)

Grand Total of all the pots of *T.foenum-graecum* 60

In artificial fumigation plants were exposed daily for 1.5 hrs with NO, NO₂, O₃ alone and sequentially with NO+NO₂+O₃
combination (NO, 8-9.30 a.m., NO₂, 10-11.30 a.m., O₃, 12-1.30 a.m. alone in one chamber and sequentially in the other).

Before fumigation plants were watered (for raising the sensitivity to pollutants). To avoid the chamber effect, the position of pots inside the chamber was changed and control plants were also covered with exposure chamber (Plate 3).

Meteorological parameters i.e. temperature and relative humidity inside each chamber were noted (daily) during fumigation.

Soil was aerated twice in a month during exposure period.

**Generation of NOₓ and O₃**

NO was generated by the action of copper coiling and nitric acid placed in a gas generator placed in water bath at constant temp. i.e. 30°C and introduced inside the chamber by bubbling
with High Volume Sampler at the rate of 1 lit/min. via polyethylene tube (Fig. 4) by using the method of pitot meter. Inlet end of the bubbler was sucking the air coming from the blower while the outlet discharging the generated gas downstream to air flow.

$\text{NO}_2$ was generated by heating the $\text{NaNO}_2$. Rest of the procedure followed was as above (Fig. 4) for $\text{NO}_2$ generation (Plate 6).

The generation of ozone was achieved with ozone generator, Model oxygen 100. The gas was introduced inside the fumigation chamber by putting the nozzle of Y-shaped glass tube inside the nozzle of ozone generator. The other end of glass tube was connected with the polyethylene tube which in turn was connected with blower (Fig. 5) of High Vol Sampler (Plate 7) with filter paper (Plate 5).

In all the above cases the flow rate of ambient air coming inside the chambers was 25 lit/min. To fill up the chamber having 1000 lits of capacity, 40 mins. were required ($25 \times 40 = 1000$ lits). That means the air inside the chambers was replenished after every 40 mins.

**Monitoring of oxides of nitrogen ($\text{NO}$ and $\text{NO}_2$) and ozone**

A concentration of $\text{NO}$, $\text{NO}_2$ and $\text{O}_3$ inside all the exposure chambers were measured daily during the exposure period and mean value was worked out for each pollutant. The values of above pollutants were also measured for the field study at Motinagar.
and JNU site at the interval of 15 days for 120 days. For artificial exposure study, the NO\textsubscript{x} conc. was measured during the exposure period with Air Monitor while O\textsubscript{3} was measured by Impinger method. On the other hand for field study, the 24 hrs measurement was done at a fixed time i.e. 11 a.m. for all the pollutants by Impinger method. The monitoring of pollutants inside the chambers was done by connecting the Air Monitor and Impingers with exposure chambers (Plate 8).

Oxides of nitrogen (artificial exposure)

The measurement of NO\textsubscript{x} inside the fumigation chambers was done by Automatic CEA 555 Air Monitor (Plate 4). Sodium nitrite was used as a standard for NO and NO\textsubscript{2} measurement which is based on the method of APHA, 1977.

Before starting the experiment, the connections were made as shown in the Fig. 6. Then the entire unit was flushed with the distilled water for half an hour. Air flow rate was adjusted to 0.5 lit/min and that of sodium nitrite to 0.27 ml/min. Then the dilution factor was worked out which was found to be 2.43 which is the ratio between \( \mu g \) NO\textsubscript{2}/ml in air and \( \mu g \) NO\textsubscript{2}/ml in NaNO\textsubscript{2}. With the help of this dilution factor, concentrations of NO\textsubscript{2} ranging from 0-200 \( \mu g/m^3 \) was obtained and standard graph was plotted by taking NO\textsubscript{2} on X-axis and full scale per cent on Y-axis. It was found out that 100 \( \mu g/m^3 \) NO\textsubscript{2} intersects the 50 per cent of the full scale. Beyond 100 \( \mu g \), the curve was not following (Fig. 7) Beer's law. Thus 50 per cent on the digital
display was found equivalent to 100 μg/m³ NO₂ which is the upper limit of the instrument (under above condition). Therefore 1 per cent is equal to 2 μg NO₂/m³.

For the measurement of NO, a train was used (Fig. 8) which consists of NO₂ absorber, humidity regulator and oxidizer. This assembly was connected to CEA 555 Air Monitor for measuring the NO. NO₂ absorber absorb NO₂ gas from the incoming air current inside the gas monitor, which also contains NO. Thus NO passes unabsorbed which finally oxidized to NO₂ in oxidizer. The amount of NO₂ measured will be equal to the amount of NO oxidized to NO₂. Thus the concentration of NO was worked out.

**NO₂ absorber**: A polyethylene tube (20mm ID x 50 mm long) with connecting caps at both the ends, was fitted with nitrogen dioxide absorbent (Fig. 8). For the preparation of NO₂ absorbent, a 20 mesh porous inert material i.e. firebrick was soaked in 20 per cent triethanol amine. Then it was spread up in wide petri-dish after draining and was dried for 60 min at 95°C in oven. The free floating pellets were then taken in NO₂ absorber tube held in place with glass wood plugs.

**Humidity regulator**: A 20 mm ID x 50 mm long polyethylene tube was filled as above with constant humidity buffer mixture held in place with glass wool plugs (Fig. 8) which was prepared by adding drop by drop a 30 ml of water in 40 g of anhydrous sodium acetate in beaker to get coarse grained crystal pellets. Humidity regulator provide 40-70 per cent relative humidity steadily for efficient working of NO₂ absorber.
Oxidizer: It was made up of a glass tube (15 mm ID) having connecting ends on both the sides. The oxidizer was prepared by soaking alumina (mesh size 15 mm) in a solution of 17 g chromium trioxide and 100 ml water. It was then taken in petridish in the form of thin film after draining. After drying in an oven at 105°C, it was exposed to 70 per cent relative humidity which was achieved in a dessicator with saturated solution of sodium acetate prepared as above. Glass tube was then filled with oxidizer between two glass wool plugs. Golden orange colour was obtained after equilibration (Fig. 8).

Oxides of nitrogen (field exposure)

$\text{NO}_x$ of ambient air was measured by APHA method (1977). Principle, apparatus and reagents required are the same as used for measuring the NO by Air Monitor CEA 555.

For the preparation of liquid absorber, to a 15 g of triethanolamine, 500 ml of distilled water was added. This was followed by the addition of 3 ml of butanol. This was then diluted to 1 lit after mixing it thoroughly. This solution was kept in refrigerator which was found to be stable for at least two months.

Hydrogen peroxide solution was prepared by diluting 0.2 ml of 30% hydrogen peroxide to 250 ml with distilled water.

Sulphanilamide solution was made by dissolving 10 g of sulphanilamide in 400 ml of distilled water. It was then made
upto 500 ml after the addition 25 ml of conc. phosphoric acid. This solution was found to be stable for one year when stored in refrigerator.

N-(1-Naphthyl)-ethylenediamine dihydrochloride was prepared by dissolving 0.1 g of NEDA in 100 ml of distilled water. When refrigerated, this solution was stable at least for a month.

For standardization, nitrite stock was prepared by diluting 0.135 g of sodium nitrite with distilled water upto 1 lit. From this stock, working solution was obtained when 1.0 ml of stock was diluted upto 50 ml. To a series of 25 ml graduated tubes, 0, 1, 3, 5, 7 and 9 ml of nitrite working solution was taken and was diluted to 10 ml with absorbing solution. This solution was then transferred to 25 ml graduated glass cylinders. To each of these cylinders 1.0 ml of dilute hydrogen peroxide was added followed by mixing. This was followed by the addition of 10 ml of sulphanilamide and 1 ml of HEDA solution. After proper mixing, the OD was taken at 540 nm after 10 mins against the blank solution which was obtained in a similar way as that of sample but in the absence of nitrite solution. Then the standard curve was prepared between NO₂ conc. and absorbance.

For the measurement of NO, nitric oxide assembly (see NOₓ measurement by CAA 555 Air Monitor) was connected to the two impingers containing absorbing solution already connected in series (Plate 9-10) which in turn were connected to Aimyl pump. The air sample was drawn at the rate of 0.1 lit/min for 24 hrs in the absorbers in two impingers and thus the
Measurement of NO as NO₂ was carried out as above by converting the NO to NO₂. The vol. of 10 ml absorbers in impingers was made (if there was any loss) by distilled water. This sample was then treated as above (see standardization) for colour development and the OD was taken at 540 nm after 10 min. of colour development against the blank solution. Then the NO (in terms of NO₂) was determined from the calibration curve.

For knowing the NO₂ conc. in the ambient atmosphere (J.N.U. and Motinagar site), the NO₂ was collected as above in 50 ml absorber at the rate of 150 ml/min for 24 hrs and any loss was made up with distilled water. Rest of the procedure was followed as above. The formula adopted for the calculation of NO₂ is as follows:

$$\mu g \text{ NO}_2/m^3 = \frac{\mu g \text{ NO}_2 \times 10^3}{r \times t \times K}$$

where:
- \( r \) = Sampling rate in lit/min.
- \( t \) = Sampling time in min.
- \( K \) = Dilution factor
- \( 10^3 \) = Conversion factor, lit/m³

**Ozone**

Ozone was determined by APHA method (1977). All the chemicals used were of reagent grade. A 13.6 g of potassium dihydrogen phosphate, 14.2 g of dihydrogen phosphate and 10.0 g of potassium iodide was added sequentially, for the preparation of absorbing solution i.e. 1 per cent KI in 0.1 M phosphate buffer. The volume was made upto 1 litre with double distilled water. This solution
was kept for aging at least for one day before the actual use in brown bottle for the protection from sunlight. It was stable for several months. The pH of the solution was always kept constant i.e. 68±0.2 by adjusting with NaOH. The solution was discarded whenever the mold was seen.

A 0.025 M\textsubscript{2} stock solution was prepared by dissolving 16 g of potassium iodide and 3.173 g of resublimed iodine in succession and diluting the same to 500 ml with double distilled water. This was also kept for aging for at least one day before the use of this solution.

For the preparation of 0.001M\textsubscript{2} solution, a 4.00 ml of the above stock solution of I\textsubscript{2} was diluted upto 100 ml. It was protected from sunlight and was discarded after use.

For the preparation of working solution (calibrating iodine solution), 4.09 ml of 0.001M\textsubscript{2} was made upto 100 ml with double distilled water to make the final concentration equivalent to 1 \textmu l of O\textsubscript{3}/ml (or 4.09 ml I\textsubscript{2} = 100 \textmu l O\textsubscript{3}). This solution was also discarded after use.

The micro amount of O\textsubscript{3} and other gases liberates equivalent amount of I\textsubscript{2} when absorbed in 1 per cent potassium phosphate buffer solution of pH 6.8±0.2. The optical density of the product triiodide was measured spectrophotometrically at 352 nm for the determination of equivalent amount of I\textsubscript{2}.

For standardization of O\textsubscript{3}, the working calibrating solution was taken upto 25 ml (0-25 ml) to obtain graduated range of concentration of I\textsubscript{2} solution. This was then diluted upto 25 ml with the phosphate buffer solution. Then the OD was taken with spectronic 1001 spectrophotometer after 30 minutes at 352 nm. against the absorbing solution. The observations were made in triplicate. The standard curve was plotted by taking OD on Y-axis and concentration of O\textsubscript{3} in \textmu l/10 ml absorbing solution on X-axis.

This method is subjected to interference by sulphur dioxide and nitrogen dioxide. The former is corrected by keeping sulphur
dioxide absorber upstream in the gas measuring assembly. The SO\textsubscript{2} absorber was prepared by adding 15 ml aqueous solution (containing 2.5 g of chromium trioxide and 0.7 ml of conc. sulphuric acid) on 400 cm\textsuperscript{2} flash-fired glass fibre paper. It was then dried in oven for 1 hr at 80-90°C and was stored in dissicator. A 6x12 mm strip of this paper was folded in V-shape and was packed in 85 ml U-tube. The dry air was passed through this tube for about one night. This absorber was found to be active for 25 days. Later on it turned brown which was discarded and fresh absorber was used. The interference due to nitrogen dioxide was corrected by analysing the NO\textsubscript{2} and subtracting one-tenth of this from the ozone value.

The ozone measurement assembly (Fig. 9 ) consists of U-tube, two impingers connected in series and Aimyl pump. All connections were of Borosil glass. A 10 ml of absorbing solution was taken in each Impinger. The sampling was done for 1 hr. A 30 litres of gas, at the rate of 0.5 lit/min; was obtained. The flow rate was measured with dry gas rotameter. While sampling the gas, assembly was protected from sunlight by housing it in wooden box. Any loss in volume of absorber was made up with the distilled water. After bringing in the laboratory, analysis of the gas was done as above.

A \( \text{mg O}_3 \) was read from the calibration curve. Following formula was adopted for the calculation of ozone:

\[
\text{mg O}_3/m^3 = \frac{\text{mg O}_3 \text{ from calibration curve}}{RT}
\]

Where, \( R \) = Air sampling rate in litres

\( T \) = Sampling period
Sampling of leaf

Leaf samples were collected in an ice bucket after the measurement of gases for the estimation of biochemical and physiological parameters. A 4th and 6th fully expanded leaf from the top for S. oleracea and T. foenum-graecum L. respectively were used. Biochemical parameters of leaf tissue includes ascorbic acid, protein and RNA; physiological parameters are chlorophyll (Chl a, b and total), nitrogen content and phytomass (shoot, root and total). The measurement of these parameters were done at the interval of 15 days for 120 days.

Measurement of ascorbic acid

The AA was estimated with Keller and Schwager (1977) method which was specially developed for the measurement of AA in the plant spp. subjected to air pollution stress.

The solutions for the extraction of AA were prepared by diluting 5.0 g of oxalic acid and 0.75 g of sodium salt of ethylene diamine tetra chloro acetic acid (NaEDTA) in 1000 ml distilled water. A stock solution of the dye 2,6-dichlorophenol-indophenol blue (DCPIP) was prepared by dissolving 100 mg of NaDIP in 500 ml distilled water. This solution was heated at 80°C for 5 minutes. It was subjected to cooling for 10 minutes at room temp. and filtering with Whatman filter paper. Then it was made up to 100 ml with distilled water. The stock solution so prepared was stored in fridge for one week at 2°C.
The working DCPIP solution was prepared daily by diluting 40 ml of stock solution to 200 ml with distilled water.

A 0.1 g of fresh leaf tissue was homogenised in 20 ml of extracting solution with ice-chilled pestle and mortar, and then it was centrifuged at 20,000 x g for 15 minutes.

Out of the extract obtained as above, 1 ml extract was taken and to this 5 ml of DCPIP solution was added. This was mixed by shaking it vigorously. The extracting solution and DCPIP prepared as above was used as blank. Then the OD was taken at 520 nm with spectronic 1001 spectrophotometer. The calibration curve was prepared using the pure AA obtained from Sigma Chemical Company.

The formula used for the calculations is as:

\[(E_0 - E_s - E_t) \times \text{Factor } f = \text{mg/g}\]

where,

- \(E_0\) = OD of extracting solution + DCPIP
- \(E_s\) = OD of extract + DCPIP
- \(E_t\) = OD of turbidity (one drop of 1 per cent AA + extracting solution + DCPIP)

The factor \(f\) was calculated by creating the artificial sample conc. of the AA as used for the preparation of standard curve and measuring the OD after subjecting this solution similarly as used for the preparation of standard curve. Each of these \(ODS'\) were divided with the corresponding \(ODS'\) from the standard curve. The mean of these resultant \(ODS'\) was treated as multiplying factor \(f\).
Measurement of protein

Total protein was measured with modified method of Bradford (1976) by Read and Northcote (1981). This procedure remove much variation observed in the previously described methods for protein estimation e.g. Lowry et al. (1951) and even Bradford's method. It involves either increasing the conc. of dye or decreasing the conc. of phosphoric acid in the assay solution. In spite of this it retains the features of Bradford's method of simplicity and rapidity but increased the sensitivity of the protein assay.

Coomassie brilliant blue G-250 was obtained from Sigma Chemical Company. The stock solution containing 0.333 per cent (w/v) dye was made in the mixture of phosphoric acid (88 per cent w/w, 16 M) and ethanol (93.5 per cent v/v, 16M). The ratio of phosphoric acid and ethanol was 2:1. The dye was soluble in the above mixture and was found to be stable for long duration at room temperature. This solution was then filtered using Whatman filter paper No. 1. At this stage the solution contains 0.001-0.02 per cent dye, 0.8-3.2 M phosphoric acid and 0.17-0.80M ethanol.

In our experiment, we have reduced the quantity of phosphoric acid. The standard dye reagent was prepared (0.005 per cent Brilliant blue - G250 in 0.8 M phosphoric acid/0.8 M ethanol) by taking 15 ml of the above stock solution of dye. To this 40 ml of 16 M phosphoric acid and 42 ml of absolute
ethanol were added. It was then made up to 1 l with distilled water after mixing it thoroughly. This solution also was filtered with Whatman filter paper No. 1 and was stored in dark coloured bottle which was found to be stable for several weeks.

A 0.1 g of leaf tissue was homogenised in 10 ml of 10 per cent trichloro acetic acid (TCA) with pestle and mortar under ice chilled condition. It was then centrifuged with Remi centrifuge at 1500xg for 20 min. The pellets were dissolved in 1N NaOH, centrifuged again and supernatant so obtained was used for further assay. In all cases the vol of supernatant was always maintained at 15 ml with 1N NaOH. This was mixed with NaCl/phosphate buffer.

For assay, to a 0.05 ml of the above solution, 0.95 ml of the dye was added and the OD was taken at 595 nm within 30 min. A 0.05 ml of NaCl phosphate buffer in 0.95 ml of the same dye reagent was used as blank. BSA was used as standard.

**Estimation of RNA**

RNA was estimated according to the method of Malic and Singh (1980). A 0.1 g of leaf tissue was homogenised in 10 per cent perchloric acid (PCA) and centrifuged at 3000 xg using Remi centrifuge. Supernatant was discarded and the residue was suspended in cold 5 per cent PCA and centrifuged again. Then supernatant was discarded. The residue was subjected to washing with 70 per cent ethanol, 95 per cent ethanol, 3:1 boiling ethanol-ether (twice) and 0.2 N cold PCA. It was centrifuged immediately.
The residue was suspended in 2N cold PCA and was stored in fridge for 18 hrs. After that it was centrifuged and thus supernatant I was collected. The remaining residue was again washed with 2N cold PCA and was centrifuged. The supernatant II thus obtained was combined with that of I and the vol was made upto 20 ml with distilled water. It contained the RNA fraction.

The RNA was estimated by Orcinol method. To a 0.5 ml of sample obtained as above, 1.5 ml orcinol reagent A was added and it was then heated for 20 min. in boiling water bath and was cooled at room temperature in cold water. Then the OD was taken at 660 nm with spectronic 1001 spectrophotometer.

The reagents A and B were prepared as follows:
Reagent A : To a 1 g orcinol, 375 mg FeCl₃6H₂O was added. It was then diluted to 25 ml with distilled water and was cooled in an ice bath. To this 475 ml HCl reagent B was added.

Reagent B : To 500 ml conc. HCl, 100 ml distilled water was added. Pure RNA was used as a standard which was obtained from Sigma Chemical Company.

Measurement of chlorophyll

The chlorophyll was determined by the method of Arnon(1949). A 0.1 g of leaf tissue was grinded in chilled 80 per cent acetone (analytical grade) with ice chilled pestle and mortar. This
operation was done in dim light. It was then centrifuged at 3000xg with Remi centrifuge for 15 min. The supernatant was collected and the residue was again grinded as above. The supernatant so obtained was mixed with the previously collected supernatant and the volume was made upto 20 ml with acetone. The OD was taken at 663 and 645 nm for the estimation of Chl a and Chl b. The values of Chl a and Chl b were added to obtain total Chl. The following formula given by M’Clachlan and Zalic (1963) was used for the determination of Chl a and Chl b.

\[
\begin{align*}
\text{Chl a} &= \frac{12.3D_{663} - 0.86D_{645}}{d \times 1000 \times W} \times V \\
\text{Chl b} &= \frac{19.3D_{645} - 3.6D_{663}}{1000 \times W} \times V
\end{align*}
\]

Where, 
\(D_{645}\) = OD at 645 nm
\(D_{663}\) = OD at 663 nm
\(V\) = Total vol of extract
\(W\) = Wt of leaf tissue taken
\(d\) = length of light path (1 cm)

**Measurement of nitrogen**

It was determined according to the method given by Bergersen (1980). The leaf tissue was dried in oven at 80°C overnight. It was then subjected to powdering and sieving. For estimation, 1 g of leaf powder was taken in Kjeldahl flask. To this, 5 ml of 5 per cent salicylic sulphuric acid (prepared
in conc. sulphuric acid) was added. After shaking it, a 50 mg of sodium thiosulphate was added to it. After heating for few minutes it was cooled and 100 mg sulphate mixture containing powder of 10 part of potassium sulphate, 1 part of cupric sulphate and 0.1 part of selenium metal was added to the Kjeldahl flask and was strongly heated. Finally the vol was made upto 100 ml with distilled water.

For further treatment, 50 g/lit phenol, 0.25 g/lit sodium nitroprusside, 40 g/lit sodium hydroxide and 2.1 g/lit sodium hypochlorite were used. To 1 ml digestion mixture, 5 ml phenol and sodium nitroprusside in 1:4 ratio and 5 ml of sodium hydroxide and sodium hypochlorite in the same ratio (1:4) was added. The OD was taken at 625 nm with spectronic 1001 spectrophotometer. Similar procedure was followed for the estimation of nitrogen in the soil. But here 5 g of soil sample was analysed and the reagents were increased proportionately.

The standard curve was prepared in 5% sulphuric acid using ammonium sulphate.

**Measurement of phytomass**

Each plant was rooted out from the pot with intact root system. The soil particles on the roots were removed carefully by washing with the jet of water. Then the whole plant was cut for obtaining root system and shoot system (with leaves). The roots and above ground parts were oven dried separately for 24
hrs. and after that they were weighed with Metler electric balance.

**Standard deviation**

Standard deviation was worked out with the following formula (for all parameters):

\[
\sqrt{\frac{\sum x^2 - \left(\frac{\sum x}{n}\right)^2}{n - 1}}
\]

Where,

\(\Sigma x = \) algebraic sum

\(n = \) no. of observations
Plate 1    Chambers and earthenware pots before fumigation of plants.

Plate 2    Exposure system during fumigation.
Plate 3  Control plants inside the bigger chamber in the exposure system.

Plate 4  High Vol Samplers, and NO$_x$ and O$_3$ monitoring assembly.
Plate 5  High Vol. Sampler with glass fibre filter paper.

Plate 6  Connection of fumigation pipes and NO\textsubscript{x} generation.
Plate 7  Ozone generation.

Plate 8  Fumigation system with glass tubes for monitoring the NO\textsubscript{x} and O\textsubscript{3}. 
Plates 9 and 10. NO\textsubscript{x} and O\textsubscript{3} monitoring of ambient air.
Fig. 2. PLENUM (GLASS)
Fig 3 LOCATION OF SAMPLING STATIONS (DELHI)
Fig. 4. ARTIFICIAL EXPOSURE SYSTEM (NOX)
Fig. 5. OZONE FUMIGATION ASSEMBLY
Fig. 6 Liquid calibration and operation system set up for NO\textsubscript{x} measurement.
Fig. 7. AIR MONITOR STANDARDIZATION FOR NO\textsubscript{X} MEASUREMENT (STANDARD CURVE FOR (A) NO\textsubscript{X} (B) RANGE OF NO\textsubscript{X} DETERMINATION.)
Fig. 8. NO MONITORING ASSEMBLY
Fig. 9. OZONE MONITORING SYSTEM