Materials & Methods
MATERIALS AND METHODS

For the study different materials and methods were employed in the following manners and experiments were divided into two major sections.

Source of Pollutants

Particulate air pollutants fly ash and brick-kiln dust used in the experiments were collected from different sources. Fly ash was obtained from the thermal power plant, Kasimpur 16 km away from Aligarh city. The power plant complex consists of three power houses namely ‘A’, ‘B’ and ‘C’ having a capacity of 90MW, 216 MW and 230MW electricity generation respectively. Brick-kiln dust was obtained from brick-kiln situated at Asna, 15km away from Aligarh city.

Extent of Pollution

The bituminous type of coal is used in Kasimpur Thermal Power Plant. The chemical constituents of coal are 2.93% moisture, 22.17% ash, 31.86% volatile matters including 0.48% sulphur, 5.61% hydrogen, 5.23% nitrogen, 20.3% oxygen and 42.47% fixed carbon on an average (Table 1). When huge amount of coal is subjected to high temperature (1200°C-1400°C) for combustion, it produces noxious gases,
Table 1: Chemical analysis of coal collected from some important collieries of India (Courtesy of AEE Thermal Powers Plant Complex, Kasimpur).

<table>
<thead>
<tr>
<th>Name of collieries</th>
<th>% Moisture</th>
<th>%Ash</th>
<th>%Volatile matters</th>
<th>%Fixed carbon (by difference)</th>
<th>%Sulphur</th>
<th>%Hydrogen</th>
<th>%Nitrogen</th>
<th>%Oxygen</th>
<th>Temperature of coal burning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Badjana</td>
<td>1.0</td>
<td>22.7</td>
<td>31.2</td>
<td>44.2</td>
<td>0.33</td>
<td>5.14</td>
<td>5.10</td>
<td>20.51</td>
<td>1400</td>
</tr>
<tr>
<td>Bejdihi</td>
<td>2.8</td>
<td>22.7</td>
<td>34.0</td>
<td>39.8</td>
<td>0.59</td>
<td>6.99</td>
<td>5.50</td>
<td>20.51</td>
<td>1400</td>
</tr>
<tr>
<td>Centre Salgram</td>
<td>5.8</td>
<td>19.6</td>
<td>31.2</td>
<td>43.0</td>
<td>0.35</td>
<td>5.21</td>
<td>5.16</td>
<td>20.32</td>
<td>1400</td>
</tr>
<tr>
<td>Hathara</td>
<td>2.1</td>
<td>20.6</td>
<td>30.6</td>
<td>46.1</td>
<td>0.56</td>
<td>5.14</td>
<td>4.96</td>
<td>19.86</td>
<td>1400</td>
</tr>
<tr>
<td>Methani</td>
<td>3.0</td>
<td>22.2</td>
<td>32.8</td>
<td>41.4</td>
<td>0.55</td>
<td>5.74</td>
<td>5.46</td>
<td>20.40</td>
<td>1400</td>
</tr>
<tr>
<td>Plidhi</td>
<td>2.8</td>
<td>25.1</td>
<td>31.4</td>
<td>40.2</td>
<td>0.56</td>
<td>5.41</td>
<td>5.23</td>
<td>19.95</td>
<td>1280</td>
</tr>
<tr>
<td>Average</td>
<td>2.9</td>
<td>22.7</td>
<td>31.8</td>
<td>42.5</td>
<td>0.48</td>
<td>5.60</td>
<td>5.23</td>
<td>20.30</td>
<td>1380</td>
</tr>
</tbody>
</table>
Fig. 1: Shows a Brick Kiln, situated at Asna, 15 Km away from Aligarh city, and coal used for burning of bricks.

Fig. 2: Shows the brick-kiln dust and prepared bricks.
**Fig. 3**: Shows the Thermal Power Plant, situated at Kasimpur 16 km away from Aligarh city.

**Fig. 4**: Shows the Fly Ash Pond where fly ash is collected through pipe from the source.
particulate matters and ash, released through the chimneys into the atmosphere.

For making bricks alluvial type of soil is used. When raw bricks are burned with coal at high temperature noxious gases specially hydrogen fluoride and huge amount of particulate matters are released. The particulate matters containing coal ash, wood ash and soil dust are called brick-kiln dust.

Selection of Site

The experiments were conducted in the glass houses fabricated at the Department of Botany of Aligarh Muslim University, Aligarh. The site is situated 16 Km away from the source of fly ash pollutant and 15Km away from brick-kiln dust pollutant.

Fly Ash/Brick-Kiln Dust Amendments

Soil collected from agricultural fields after scrapping of the surfaces, litters present if any. The soil used in the experiments was sandy loam containing 66% sand, 24% silt and 8% clay particles and 2% organic matter. The soil was autoclaved before incorporation. The fly ash in different concentration i.e. 0, 5, 15, 30, 45, 60, 75 and (100%) was thoroughly mixed with soil to ensure its homogeneous
distribution. These amended samples were crushed and passed through 2mm sieve. Three replicates of each treatment were used. The soil without fly ash in three replicates served as control. The brick-kiln dust was amended with soil in the similar manner as with fly ash.

Test Plants

Two test plants, *Brassica juncea* L. and *Linum usitatissimum* L. commonly grown as oilseed crops in Aligarh district, were selected for the study. Seeds of *B. juncea* var. Varuna and *L. usitatissimum* var. Neelam were obtained from Indian Agricultural Research Institute, New Delhi, India for the experiments.

SECTION-I

In this section, physico-chemical properties of different levels of fly ash and brick-kiln dust amended soil were studied before and after growing of *B. juncea* and *L. usitatissimum*.

Experiment-1

Analysis of Fly Ash Amended Soil Before Sowing

Some physico-chemical properties of fly ash amended soil before sowing were analysed to observe its fertility status.
A. Physical properties

**Bulk density**

The bulk density of soil is the mass of the soil per unit volume. For this weighing bottle of 50ml without the stopper was weighed for each sample. It was filled up with soil, flushed up to the brim tapping the bottle about 20 times and weighed again. The soil was removed from the bottle and filled with water by means of graduated pipette then the exact volume was noted. The bulk density was calculated as follows:

\[
\begin{align*}
\text{Weight of empty bottle} &= W_1 g \\
\text{Weight of bottle and soil} &= W_2 g \\
\text{Weight of soil} &= W_2 - W_1 \\
\text{Volume of the soil or volume of water needed to fill the bottle} &= V ml \\
\text{Bulk density} &= \frac{W_2 - W_1}{V} \text{ gm /cubic cm}
\end{align*}
\]

**Particle density**

The particle density of soil is the average density of soil particle. For this, 100ml specific gravity bottle was weighed and filled it with water completely. All moisture from outside the bottle wiped out and weighed again. The 10g air dried soil taken into a beaker and added few ml of water then boiled it to
expel all air and cooled at room temperature. Then water was removed from the bottle and filled it completely with the soil transferring it from the beaker with a jet of water. All moisture outside the bottle wiped out and weighed and calculated separately for each sample as follows:

Weight of empty S.G. bottle = \( W_{ig} \)

Weight of empty S.G. bottle + water = \( W_{ig} \)

Weight of water alone = \( W_{2} - W_{1} \)

Weight of soil taken = \( 10g \)

Weight of S.G. bottle + water + soil = \( W_{3}g \)

Weight of soil + water = \( W_{3} - W_{1} \)

Weight of water displaced by soil = \( (W_{3} - W_{1}) - (W_{2} - W_{1}) \) = \( W_{3} - W_{2} \)

Particle density of soil = \( \frac{10}{W_{3} - W_{2}} \) gm/cubic cm

**Pore space**

The portion of a given volume of soil, which is unfilled with solid matter, is termed pore space. The percentage of pore space in each sample of soil was calculated by the following formula:
Pore space (%) = \frac{\text{Particle density} - \text{bulk density}}{\text{Particle density}} \times 100

\text{Sticky point}

Ten gm of soil from each treatment was taken and separated out on a glass plate forming thin layer. Sprayed with a fine jet of distilled water until the soil was thoroughly wet and become sticky. Worked up the mass into a paste with a flexible steel spatula. The soil was scraped and kneaded in the fingers until it ceased to adhere to them or to the spatula. At this stage it was possible to cut, clear through the plastic mass without the soil adhering to the spatula, then it was weighed as quickly as possible in a weighing dish and then dried to a constant weight in the air oven and calculated the moisture content on oven dry basis. It represented the sticky point. Calculation was made as follows:

Weight of dry dish = W_1 g
Weight of dish + moist soil = W_2 g
Weight of dish + dry soil = W_3 g
Weight of moisture lost = W_3 - W_1

\text{Sticky point (%)} = \frac{W_2 - W_3}{W_3 - W_1} \times 100
Water holding capacity

For knowing the water holding capacity, air dried soil samples were crushed in a porcelain mortar and passed through a small sieve of 1.5mm holes. After complete crushing, the course particles from the sieve were removed and particles were thoroughly mixed. The weight of the circular brass box with perforated bottom having filter paper on bottom was taken \( (W_1) \). It was filled with soil. The box with soil was kept in hot air oven for complete drying of soil. The weight was taken again after drying \( (W_2) \). Now box was submerged in a petridish containing water \( \frac{1}{4} \) level and left for 12h. After the lapse of 12h, the box was gently taken out from the petridish and excess water was allowed to evaporate at room temperature and was weighed finally \( (W_3) \).

Calculation:

\[
\text{Water holding capacity} = \frac{W_3 - W_2}{W_2 - W_1} \times 100
\]

Saturation percentage

Saturation percentage was determined by taking 200g soil in a plastic container. Saturated paste was prepared by adding gradually distilled water from burette without stirring the soil, until water just wet the entire soil mass, then added few drops till the surface glistens. Water was moved down
slowly through the soil pores. The soil was mixed with spatula and added more drops of water if needed so that the paste slid freely and cleanly on tapping till it reflected light and glistens. A hole was made by spatula in the center of paste to check the flow of soil paste. This was a soil saturation stage. This saturated paste was left for an hour to attain equilibrium and checked again for stiffness or losing its shine. More water was added if needed and amount was recorded. Weight of a portion of saturated soil was taken by transferring it to a tared dish. Now it was dried in a hot air oven at 100-105°C overnight and found the weight again.

Calculations:

Weight of the dish \( = W_1 \)g

Weight of dish+soil paste \( = W_2 \)g

Weight of dish+dry soil \( = W_3 \)g

Loss in weight of soil paste \( = W_2 - W_3 \)

Weight of oven dried soil \( = W_3 - W_1 \)

Saturation percentage \( = \frac{100 \text{ (loss in weight on drying)}}{\text{Weight of oven dried soil}} \)
Moisture content

Moisture content of the soil samples was determined by drying 10g of air dried soil samples separately in hot air oven at 105°C for 12-16 hours, then kept in a dessicator for cooling and weighed. The percent moisture content of soil was calculated as follows.

\[
\text{Weight of the soil taken} = W_1 g
\]
\[
\text{Weight of the soil after drying in oven} = W_2 g
\]
\[
\text{Moisture percent} = \frac{W_1 - W_2}{W_3 - W_1} \times 100
\]
\[
\text{Percent moisture content} = \frac{W_1 - W_2}{W_2} \times 100
\]

Ignition percentage

After determining the moisture, the dish containing the air dried soil (W₁) was transferred to a furnace then ignited it for 30-40 minutes at a bright red heat and cooled it in a dessicator. Later it was weight (W₂). Ignition percentage was calculated as above.
B. Chemical Properties

Soil reaction (pH)

Twenty gm soil sample was taken in a 100ml beaker and 40ml of double distilled water (DDW) was added. The suspension (1:2) was stirred at regular intervals for 30 minutes and the pH was recorded by glass electrodes.

Electrical conductivity

For this 50g soil sample was taken in 250ml conical flask and 100ml DDW was added. After shaking well flask was left for over-night, then soil suspension was filtered. Conductance was directly recorded by dipping the conductivity cell of conductivity meter in solution. Temperature was maintained and correlated with the table.

Cation exchange capacity

Cation exchange capacity (CEC) was measured by treating 10g soil sample with sufficient amount of 0.1N HCl. After half an hour soil solution was washed with DDW through filter paper till the removal of all acidity. Then soil was kept in saturated solution of KCl for 15min. After half an hour again it was filtered and filtrate was treated with standardized
0.1N NaOH solution. Cation exchange capacity was calculated by given formula.

\[
\text{CEC} = \frac{Y \times 100}{10} \text{ meq/100gm of soil}
\]

\(Y\) = volume of 0.1N NaOH used

**Sulphate content**

Three reagents, conditioning reagent, barium chloride and standard sulphate solution were used for estimation of sulphate content of different soil samples. Conditioning reagent was prepared by adding 75g NaCl, 30ml concentrated HCl, 100ml 95% ethyl alcohol in 300ml DDW. Then it was mixed thoroughly after addition of 50ml glycerol to this solution. Crystals of barium chloride \((\text{BaCl}_2)\) were used directly. Standard sulphate solution was prepared by dissolving anhydrous Na\(_2\)SO\(_4\) in DDW to make volume one litre. This solution contained 100mg/L of sulphate.

In 100ml soil solution \((\text{soil}:\text{water}=1:5)\) 50ml conditioning reagent was added. After stirring the solution \text{BaCl}_2\) crystals were added and stirred again for one minute. The readings were taken on a spectrophotometer at 420nm and the concentration of sulphate found out from the standard curve for 0.0-40mg/L at interval of 5mg/L.
\[
\text{SO}_4 \text{ Mg/L soil solution} = \frac{\text{Percent Sulphate}}{2000}
\]
\[\text{Mg/100g=values } (\%\text{SO}_4) \times 1000\]

*Carbonate and bicarbonate contents*

Carbonate and bicarbonate contents were estimated by Richards (1954) method. Fifty ml DDW was thoroughly mixed in 100g soil and after settling it was decanted in a conical flask. Five drops of phenolphthalein were added in the flask, thus pink colour developed. Then 0.1N H\textsubscript{2}SO\textsubscript{4} was added till the colour disappeared. Few drops of methyl red indicator was added in the flask and titrated till the colour changed from yellow to rose red. The carbonate and bicarbonate contents were calculated from the reading as follows:

\[V_1 = \text{volume of 0.1N H}_2\text{SO}_4 \text{ used with phenolphthalein.}\]
\[V_2 = \text{volume of 0.1N H}_2\text{SO}_4 \text{ used with methyl red.}\]

**Carbonates**

Normality of soil solution \((N_1) = \frac{2V_1 \times 0.1}{50}\)

Total carbonate = eq. Weight × Normality \((N_1)\) of CO\textsubscript{3}

**Bicarbonate**

\((N_2) = \frac{(V_2 - V_1) \times 0.1}{50}\)

Total bicarbonates = eq. Weight × Normality \((N_2)\) of HCO\textsubscript{3}
**Chloride content**

Three reagents, 0.02N sodium chloride, 0.02N silver nitrate and potassium chromate indicator were prepared separately. For 0.02 sodium chloride, 1.17g NaCl (AR grade, dried at 80°C for one hour) was dissolved in DDW to make volume one litre. Reagent 0.02N silver nitrate was prepared by adding 3.4 g silver nitrate in DDW to make volume one litre. This standardized against the standard NaCl solution and stored in amber coloured bottle away from light. Five percent aqueous solution (5%) of pure K$_2$CrO$_4$ was used as potassium chromate indicator.

The 5ml soil sample was taken in a porcelain dish and diluted with 25ml DDW. After adding 5-6 drops of K$_2$CrO$_4$ indicator, it was titrated with the standard AgNO$_3$ solution till the first brick red tinge appeared and calculated as follows:

1 ml of 0.02 N AgNO$_3$: 0.00071 g of chloride

Chloride = V×4 m.e./litre

Where V is the volume of 0.02N AgNO$_3$ used in titration.
Phosphorus

The available phosphorus in soil samples was estimated by Olsen’s method (Olsen et al., 1954). For preparation of Olsen’s reagent 42g NaHCO₃ was dissolved in DDW to make solution one litre. The pH was adjusted at 8.5 with small quantities of 10% NaOH. Dickman and Bray’s reagent was prepared by dissolving 15g of ammonium molybdate (AR) in 300ml of warm water (about 60°C). After cooling, 400ml 10N HCl was added to make volume one litre. For preparation of stannous chloride solution, 10g crystalline stannous chloride (LR) was dissolved in 25ml HCl by warming and stored in an amber coloured bottle, carefully avoiding all contact with air. This was 40% SnCl₂ stock solution. Just before use, 0.5ml was diluted with 66ml DDW. A piece of tin metal (AR) was added to keep the stock solution for long time.

Calculation:

Available phosphorus (μg) = R×(50/2.5) ×(25/5)

= R×100

R= μg phosphorus in the aliquot (To be seen from the standard curve)
For standard curve of phosphorus, potassium dihydrogen orthophosphate (KH$_2$PO$_4$) (Analytical grade) was dried in air oven at 60°C for 1 hr and after cooling, 0.439g was dissolved in half litre distilled water. Then 25ml of 7NH$_2$SO$_4$ (approx.) was added and made volume one litre with DDW. That was 100 ppm stock solution of phosphorus (100µg P per ml). From that stock solution, 2ppm phosphorus solution was made (50 times dilution). For preparation of the standard curve different concentrations of phosphorus (1, 2, 3, 4, 5 and 10ml of 2ppm P solution) were taken in 25ml volumetric flasks. To these, 5ml of extracted reagent (Olsen’s) was added, thus blue colour developed. The colorimetric reading was taken against 660nm (red filter just after 10 minutes). The curve was plotted by taking the colorimeter reading on the vertical axis and the amount of phosphorus (µg) in the horizontal one.

Soil sample 2.5g was taken in 100ml conical flasks and a little quantity of Darco G60 was added followed by 50ml of Olsen’s reagent. A blank was run without soil. The flasks were shaken for 30 minutes on a platform type shaker and the contents filtered immediately through dry filter paper (Whatman No.1) into clean and dry beakers. In the filtrate, phosphorus was estimated colorimetrically by Dickman and Bray’s (excess acid) procedure (Dickman and Bray, 1940).
Soil extract 5ml (filtrate obtained from shaking the soil in Olsen’s reagent) was pipetted into a 25ml volumetric flask. To this Dickman and Bray’s reagent was added drop by drop with constant shaking till the effervescence due to CO₂ evolution ceased. The neck of the flask was washed down with DDW and the volume was made approximately 22ml. Then one ml of the diluted stannous chloride solution (from 40% SnCl₂ stock solution) was added and volume was made up to 25ml. The intensity of blue colour was measured at 660nm using a spectrophotometer just after 10 minutes. The concentration of phosphorus was determined from the standard curve.

**Organic carbon**

Organic carbon was estimated by Walkley and Black (1934) rapid titration method. Five reagents were prepared: (i) Potassium dichromate (1N) was prepared by adding 49.04 g of AR grade K₂Cr₂O₇ in one litre DDW. (ii) Ferrous ammonium sulphate (0.5N) was prepared by adding 196g hydrated crystalline salt in one litre DDW containing 20ml concentrated H₂SO₄. (iii) For diphenylamine indicator, 0.5g diphenylamine was dissolved in a mixture of 20ml water and 100ml concentrated H₂SO₄ and stored in a coloured bottle. (iv) Concentrated sulphuric acid (sp. gr. 1.84) containing 1.25 per
cent silver sulphate and (v) Ortho-phosphoric acid (85%) were used.

A small quantity of soil sample was ground and completely passed through 0.2mm sieve then 1g was placed at the bottom of a dry 500ml conical flask. A 10ml of 1N K$_2$Cr$_2$O$_7$ was pipetted in and swirled a little. The flask was kept on asbestos sheet. Then 20ml H$_2$SO$_4$ (containing 1.25% Ag$_2$SO$_4$) was run in and swirled again two or three times. The flask was allowed to stand for 30 minutes and thereafter 200ml distilled water was added. After incorporation of 10 ml of phosphoric acid and 1ml of diphenylamine indicator the contents were titrated with ferrous ammonium sulphate solution till the colour flashed from blue-violet to green. A combination of H$_3$PO$_4$ and NaF was found to give a sharper end point simultaneously, a blank was run without soil and calculated as follows:

$$\text{Organic carbon (\%)} = \frac{10(B-T)}{B} \times 0.003 \times \frac{100}{\text{Weight of soil}}$$

Where $B$ = Volume (ml) of ferrous ammonium sulphate solution required for blank titration.

$T$ = Volume of ferrous ammonium sulphate needed for soil sample.
Nitrogen

For determining the nitrogen in soil samples alkaline permanganate method (Subbiah and Asija, 1956) was employed. Six reagents were prepared. Potassium permanganate solution of 0.32%, sodium hydroxide of 2.5%, liquid paraffin (extra pure), 0.02N sulphuric acid of 0.02 N and boric acid solution of 2% containing 20ml of mixed indicator per litre were used. For mixed indicator, 0.066g methyl red and 0.099 g bromocresol green were dissolved in 100ml of 95% alcohol.

About 20g soil sample was taken in a 800ml dry Kjeldahl flask and 20ml water was added followed by 100ml each of 0.32% KMnO₄ and 2.5% NaOH solutions. The frothing during boiling was prevented by liquid paraffin (1ml) and bumping by adding a few glass beads. The contents were distilled in a Kjeldahl assembly at a steady rate and the liberated ammonia was collected in a conical flask containing 20ml of boric acid solution (with mixed indicator). With the absorption of ammonia the pinkish colour turned to green. Nearly 100ml of distillate was collected in 30 minutes which was titrated with 0.02N H₂SO₄ till the original shade (Pinkish) came. Blank correction (without soil) was made for final calculation.

\[ N \text{ (ppm)} = R \times 0.05 \times 0.014 \times 10^6 \]
**Heavy metal estimation**

Heavy metals i.e. Zn, Mg, Mn and Pb in soil samples were determined by mixed acid digestion using concentrated HNO₃, H₂SO₄ and HClO₄ followed by atomic absorption spectrophotometer (Allen et al. 1974). Potassium was determined by flame photometer.

**Experiment-2**

**Analysis of Brick-Kiln Dust Amended Soil Before Sowing**

Brick-kiln dust collected from the brick-kiln established around Aligarh city was mixed thoroughly with field soil in different concentrations i.e. 0, 5, 15, 30, 45, 60, 75 & 100%. After grinding homogenous mixtures were prepared and passed through 2mm sieve for further use. All physico-chemical properties were analysed similarly as given in experiment 1.

**Experiment-3**

**Analysis of Fly Ash Amended Soil After Harvesting of B.juncea**

*B. juncea* L. var. Varuna was grown in fly ash amended soil receiving concentrations 0, 5, 15, 30, 45, 60, 75 & 100% of fly ash. After harvesting on maturity the soil samples were
collected in polythene bags for their phyico-chemical analysis.
For analysis of physico-chemical characteristics same methods
were opted as done in experiment-1.

Experiment-4

Analysis of Fly Ash Amended Soil After Harvesting of
*L. usitatissimum*

Soil samples were taken from the fly ash amended soil of
different concentrations i.e. 0, 5, 15, 30, 45, 60, 75 & 100% after harvesting of *L. usitatissimum* in four months. The physico-chemical characteristics of soil were assessed as done in previous experiments.

Experiment-5

Analysis of Brick-Kiln Dust Amended Soil After Harvesting
of *B. juncea*

Brick-kiln dust was mixed with field soil in different concentrations viz. 0, 5, 15, 30, 45, 60, 75 & 100%. *B. juncea* was grown in these amendments for three months. After harvesting the soil samples were collected in polythene bags and physico-chemical properties were analysed as opted in experiment 1.
Experiment-6

Analysis of Brick-Kiln Dust Amended Soil After Harvesting of *L. usitatissimum*

*L. usitatissimum* was grown in different concentrations of brick-kiln dust amended soil. After four months plants were harvested and the soil samples were collected separately in polythene bags. Physico-chemical analysis was done as opted in previous experiments.

Section II

This section includes the evaluation of plant growth and yield of *B. juncea* and *L. usitatissimum* as well as analysis of NPK, heavy metals, total protein, photosynthetic pigments and oil properties in different concentrations of fly ash and brick-kiln dust.

Culturing of Plants

Two oilseed crops- *B. juncea* and *L. usitatissimum* were grown for experiments during 'rabi' season (October to January for *B. juncea* and October to February for *L. usitatissimum*) in following manner-

*B. juncea*: The seeds of *B. juncea* L. var. Varuna were surface sterilized with 0.1% HgCl₂ and sown (10 seeds/ pot) directly
in autoclaved clay pots of 25cm sized having eight different fly ash amended soils, in the month of October, 2000. Each treatment was replicated five times (7 treatments x 5 replicates = 35 pots) for each experiment. The pots were arranged in randomized block design in the glass house. The plants were irrigated 2 or 3 times in a week with required amount of water from the side of pots. Thinning was done after three weeks of sowing to retain one healthy plant per pot. The crop was harvested after 90 days just before maturation and sampling was done carefully for plant growth and yield.

*L. usitatissimum*: Seeds (10 seeds/ pot) of *L. usitatissimum* L. var. Neelam was sown in a same way as done in *B. juncea*. Thinning was done at four leaf stage (3 weeks old plants) and plants were allowed to grow for 120 days. After harvesting sampling was done for estimating plant growth and yield. Data were analysed statistically.

**Sampling Technique**

For study the physico-chemical and biochemical aspects, plants were sampled just before maturation. Plants were uprooted carefully, keeping the root system intact and thoroughly washed to remove the soil particles. Length, fresh weight, dry weight of roots and shoots; photosynthetic
pigments; total protein; N, P, K and heavy metals; seed weight and number of seeds; and oil properties were estimated separately.

**Experiment-7**

**Impact of Fly Ash on Plant Growth, Productivity and Biochemical Properties of *B. juncea***

The above mentioned parameters were observed to study the impact of fly ash amended soil on plant growth and productivity of *B. juncea*. The readings of proposed parameters were recorded by their standard methods.

**Plant Growth**

*Growth activity*

The shoot and root lengths of *B. juncea* L. var. Varuna were measured in centimeter. The shoot length of the plant was considered from the ground level to the upper most growing tip of the main axis. The root length was measured from the ground to the last point of root tip. Fresh weight of roots and shoots was taken in grams. After taking fresh weight, plant parts were kept in hot air oven at 80°C for 48h to find out dry weights of root and shoot. Phytomass of the plant was calculated by following formula-
Dry weight of whole plant

Phytomass = \frac{\text{Age of plant}}{\text{Green area}}

Green area

Number of branches /plant: At interval of 7 days, the number of branches in each plant with five replicates was counted.

Number of leaves /plant: The number of fully opened leaves was recorded regularly at 7 days intervals of five replicates.

Leaf area

For leaf area, three leaves from each replicate were determined by tracing the leaves on tracing paper. The areas occupied by these drawings were measured with the help of a planimeter.

Total green area/plant: The average leaf area was multiplied by total number of leaves per plant to obtain the total green area of leaf in cm².

Productivity

Reproductive growth

Flowers and fruits on their appearance were counted regularly at 7 days interval. Length of pods, seeds per pod and seed per plant of selected individuals were counted. The 100
seeds were sun dried for a week and weighed on a chemical balance in grams.

**Biochemical Properties**

**Photosynthetic Pigments**

Photosynthetic pigments were estimated by MacLachlan and Zalik (1963) method. One gram fresh leaves were ground in 40ml of 80% acetone with the help of mortar and pestle. The suspension was decanted in Buchner funnel having two whatman No. 1 filter paper No. 1 and filtration was done with the help of suction pump. The residue was ground thrice by adding 30, 20 & 10ml acetone. The suspension was decanted in Buchner funnel and filtered in vacuum. At last mortar and pestle were rinsed with 80% acetone, transferred in Buchner funnel and filtered in vacuum. The filtrate was transferred in 100ml volumetric flask and volume was made upto the capacity by adding acetone. Optical density (O.D.) in spectrophotometer was read at 480nm & 510nm for carotenoid and 645nm & 663nm for chlorophylls. Carotenoid and chlorophyll contents were calculated according to the formulae given below.

\[
\text{Carotenoid} = \frac{7.6(O.D. \ 480) - 1.49(O.D. \ 510)}{D \times 1000 \times W}
\]
Chl.a = \(12.7 \text{(O.D. 663)} - 2.69 \text{(O.D. 645)}\) \(\times \frac{V}{1000 \times W}\)

Chl.b = \(22.9 \text{(O.D. 645)} - 4.68 \text{(O.D. 663)}\) \(\times \frac{V}{1000 \times W}\)

Total ch. = \(20.2 \text{(O.D. 645)} + 8.02 \text{(O.D. 663)}\) \(\times \frac{V}{1000 \times W}\)

Where

D = length of the light path

V = total volume of the chlorophyll solution

W = Fresh weight of leaf

Total Protein Estimation

Total protein of the seeds of \(B.\text{juncea}\) was estimated by Lowry \textit{et al.} (1951) method. Following five reagents were used:

Reagent A- 2.5% sodium carbonate in 0.1N NaOH in ratio of 1:1.

Reagent B- 0.5% \(\text{CuSO}_4\) in 1% sodium tartrate in ratio of 1:4.

Reagent C- 50ml Reagent A + 1ml Reagent B (alkaline \(\text{CuSO}_4\)).
Reagent D- 50ml of 2% sodium carbonate in 1ml Reagent B
(carbonate copper sulphate solution)

Reagent E- Follin’s reagent diluted to make 1N acid
(diluted Follin’s reagent)

Standard curve

Before actual estimation standard curve was prepared by dissolving 40mg of egg albumin in 0.1N NaOH solution. The volume was made upto 100ml. From this solution, aliquots of 0.1ml to 1ml were taken in 10 test tubes. Reagent A was added to the test tubes. After 10 minutes 0.5ml reagent E was added to the test tubes. The optical density was read at 770nm and standard curve was drawn between optical density and concentration.

Soluble protein estimation

The 50mg dry powder of seeds was ground in 5 ml of double distilled water. The water extract was decanted in centrifuged tube for centrifugation at 4000 RPM for 10 minutes. Then supernatant was collected in 50ml volumetric flask and residue was retained in centrifuge tube for estimating insoluble proteins after making the volume upto 50ml. One 1ml of water extract was transferred in 10ml test tube followed by addition of 5ml of Reagent C. This solution
was mixed and left as such for 10 minutes. Then 5ml of reagent E was added and mixed immediately. The control was run along with experimental set. After half an hour the per cent transmittancy was read at 660nm and corresponding protein content was determined by using standard curve.

**Insoluble protein**

The residue in the centrifuged tube was mixed with 5ml of 5% trichloroacetic acid. After half an hour, it was centrifuged at 4000 RPM for 10 minutes. The supernatant was discarded. Five ml of 1N NaOH was added in the residue with vigorous shaking. After half an hour it was again centrifuged and supernatant was collected in 50 ml volumetric flask and volume was made upto within 1N NaOH. One ml of this solution was taken in test tube with 5ml of Reagent D followed by mixing after 10 minutes. Then 0.5 ml of Reagent E was added with immediate mixing. The 1N NaOH was used in control. The per cent transmittance was recorded at 660nm after 30 minutes. The protein content was calculated by using the standard curve.

**Oil Estimation**

Fats are fatty acid esters of glycerol. The liquid fats are called oil. Oil was estimated by the following method given by
Cox and Pearson (1962). Powdered seeds were kept in a filter paper, folded in such a way to hold the seed meal. A second filter paper was wrapped around which was left open at the top like a thimble. A piece of the cotton wool was placed at the top to evenly distribute the solvent as it drops on the sample during extraction. The sample pocket was placed in the built tubes of the soxhlet extraction apparatus and extracted with petroleum ether (150 drops/min.) for 6h without interruption by gentle heating. Then it was allowed to cool and dismaluted the extraction flask. It was cooled at room temperature after evaporation the ether on water bath until no odour of the ether remains. The dirt outside the flask was carefully removed and weighed the flask. The heating was repeated until the constant weight was recorded. Oil percentage was calculated by following formula.

\[
\text{Oil in ground sample (\%)} = \frac{\text{Wt. of oil (g)}}{\text{Wt. of sample (g)}} \times 100
\]

Estimation of saponification value

Saponification is the process by which the fatty acids in the glycerides of the oil are hydrolysed by an alkali. Saponification value is the amount (mg) of alkali required to saponify a definite quantity (lg) of an oil or fat. This value is useful for a comparative study of the fatty acid chain length in
oils. Saponification values were determined by methods of William (1975). The sample was filtered through filter paper to remove any impurities and the last traces of moisture. 4-5 g sample was weighed, later 50ml of alcoholic KOH was added from burette by allowing it to drain for definite period of time. A blank was also prepared by taking only 50ml of alcoholic KOH allowing it to drain at the same duration of time. Air condenser was connected to the flasks and boiled them gently for about 1h. After the flask and condenser get cooled it was rinsed down inside of the condenser with a little distilled water and then removed the condenser. About 1ml of phenolphthalein indicator was added and titrated against 0.5N HCl until the pink colour just disappeared and saponification value was calculated as follows:

$$\text{Saponification value} = \frac{28.05 \times (\text{titre value of blank} - \text{titre value of sample})}{\text{Wt. of sample (g)}}$$

**Estimation of acid value**

A small quantity of free acids is usually present in oils along with the triglycerides. The free fatty acid content is known as acid value. For determining acid value 1-10g of oil was dissolved in 50ml of the neutral solvent in a 250ml conical flask and few drops of phenolphthalein were added. Then the contents were titrated against 0.1N potassium hydroxide.
hydroxide until a pink colour which persists for fifteen seconds was obtained. Value was calculated by following formula:

Acid value (mg KOH/g) = \frac{\text{Titre value} \times \text{Normality of KOH} \times 50}{\text{Wt. of sample (g)}}

1 ml N/10 KOH = 0.028 g oleic acid

Estimation of iodine value

Iodine value of oil was estimated by method of William (1975).

Reagent 1- For Hanus iodine solution 13.6 g of iodine was weighed and dissolved in 825 ml glacial acetic acid by heating, then cooled and 25 ml of this solution was titrated against 0.1N sodium thiosulphate. Another portion of 200 ml of glacial acetic acid was measured and 3 ml of bromine added to it. To 5 ml of this solution 10 ml of 15% potassium iodide solution was added and titrated against 0.1N sodium thiosulphate. The value of bromine solution was calculated, to double halogen content of the remaining 800 ml of the above iodine solution as follows- \( X = \frac{B}{C} \), where \( X = \text{ml of bromine solution} \) as follows-double the halogen content, \( B = 800 \times \text{thiosulphate equivalent of 1 ml of iodine solution} \) and \( C = \text{thiosulphate equivalent of one ml of bromine solution} \).
Reagent 2- 15% potassium iodide solution,

Reagent 3- 0.1% sodium thiosulphate and

Reagent 4- 1% starch

About 0.25g of oil was taken into an iodine flask and dissolved in 10ml of chloroform, mixed well with 25ml of Hanus iodine solution with the help of pipette by draining in a definite time and allowed to stand in dark for exactly 30 min with occasional shaking. It was shaked thoroughly by adding 10ml of 15% KI and 100ml of fresh boiled and cooled water, washing down any free iodine on the stopper, titrated against 0.1N sodium thiosulphate until yellow solutions turned almost colourless. Then a few drops of starch was added as an indicator and titrated until the blue colour completely disappeared. Towards the end of titration flask was stoppered and shaked vigorously so that any iodine remaining in solution in CHCl$_3$ was taken up by potassium iodide solution. A blank was run.

Estimation of NPK (Nitrogen, Phosphorus and Potassium)

Digestion of plant leaves

About 100ml of the oven dried powder of each leaves sample was transferred to a 50ml Kjeldahl flask to which 2ml
sulphuric acid was added. The contents of the flask were heated on temperature controlled assembly for about 2h, to allow complete reduction of nitrates present in the plant material by the organic matter itself. As a result the contents of the flask were turned black. After cooling the flask for about 15 minutes, 0.5ml of 30% hydrogen peroxide was added drop by drop and the solution was heated again till the colour of solution changed from black to light yellow. After cooling for 30 minutes an additional 3-4 drops of 30% hydrogen peroxide was added, followed by heating for another 15 minutes. The addition of 30% hydrogen peroxide, followed by heating was repeated if the flask did not become colourless. The peroxide digested material was transferred from the Kjeldahl flask to 100ml volumetric flask with three washings each with 5ml DDW. Volume of the volumetric flask was made upto the mark with DDW.

Estimation of Nitrogen

Nitrogen was estimated according to the method of Linder (1944). A 10ml aliquot of the digested material was taken in a 50ml volumetric flask and 2ml of 2.5N sodium hydroxide and 1ml of 10% sodium silicate solution were added to neutralise excess of acid and to present turbidity respectively. The volume of the solution was made upto the
mark with distilled water. A 5ml aliquot of this solution was taken in a 10ml graduated test tube and 0.5ml of Nessler’s reagent was added. The final volume was made with distilled water. The content of the tube was allowed to stand for 5 minutes for maximum colour development and the optical density was read at 525nm with the help of a spectrophotometer.

**Standard curve:** Ammonium sulphate 50mg was dissolved in 1 lit DDW. From this solution 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml were pipetted to ten different test tubes separately. The solution in each test tube was diluted with 5ml DDW. In each test tube, 0.5ml Nessler’s reagent was added. After 5 minutes, the optical density was read at 525nm on spectrophotometer. A blank was run with each set of determination. Standard curve was prepared using different dilution of ammonium sulphate solution versus optical density and with the help of the standard curve the amount of nitrogen present in the sample was determined.

**Estimation of Phosphorus**

Total phosphorus in the sulphuric acid peroxide digested material was estimated by the method of Fiske and Subba Row (1925). A 5ml aliquot was taken in a 10ml graduated test tube.
and 1ml of molybdic acid (2.5 per cent ammonium molybdate in 10N sulphuric acid) was added carefully, followed by the addition of 0.4 ml of 1-amino 2-naphthol-4-sulphonic acid. The colour was turned blue. Distilled water used to make up the volume upto 10ml. The solution was shaken for 5 minutes and then transferred to a colorimetric tube. A blank was used simultaneously with each determination. The optical density was read at 620nm on a spectrophotometer.

**Standard curve:** Potassium dihydrogen orthophosphate 351g was dissolved in sufficient DDW to which 10ml NH₂SO₄ was added and the final volume was made upto 1 litre with DDW. From this solution 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml were taken in ten different test tubes separately. The solution in each test tube was diluted with 5ml DDW. In each tube, 1ml molybdic acid and 0.4ml 1-amino-2-naphthol-4-sulphonic acid were added. After 5 minutes the optical density was read at 620nm on spectrophotometer. A blank was run with each set of determination. Standard curve was prepared using different dilutions of potassium dihydrogen orthophosphate solution versus optical density and with the help of standard curve, the amount of phosphorus present in the sample was determined.
**Estimation of Potassium**

Potassium was estimated with the help of flame photometer. After adjusting the filter for potassium in the photometer, 10ml peroxide digested material was run. A blank was also run side by side with each set of determination.

**Standard curve:** Potassium chloride 1.91g was dissolved in 100 ml of DDW, of which 1 ml solution was diluted with DDW to make 1 litre. The resulting solution was of 10ppm potassium. From this 10ppm, potassium solution, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10ml solution was transferred to 10 vials separately. The solution in each vial was diluted to 10ml with DDW. The diluted solution of each vial was run separately. A blank was also run with each set of determination. Standard curve was prepared using different dilution of potassium chloride solution versus readings on the scale of the galvanometer. The amount of potassium present in sample was determined with the help of standard curve.

**Heavy Metal Estimation**

Heavy metals present in the seeds' were estimated with the help of Atomic Absorption Spectrophotometer by the method of Jackson (1973). The plant material was ground and weighed 0.5g. Powdered plant material was kept overnight in
5ml of conc. HNO₃. Next day 10ml of tri acid mixture (HNO₃: HClO₄: H₂SO₄=10:4:1) was added to it for digestion. After cooling, 5ml of 6N HCl and 50ml distilled water were added and the content was filtered to a 100ml volumetric flask. This solution was directly fed to an Atomic Absorption Spectrophotometer after calibrating the instrument with standard solution of different dilutions.

**Experiments-8, 9, 10**

Plant growth, productivity and biochemical properties of *L. usitatissimum* with fly ash, *B.juncea* and *L. usitatissimum* with brick-kiln dust were studied in experiments 8, 9 and 10 respectively. The concentration taken and parameters measured were same as taken in experiment-7.