CHAPTER 3

MATERIALS AND METHODS

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CHAPTER-3
MATERIALS AND METHODS

To fulfil the aims and objectives mentioned earlier (Chapter 1), five field experiments were conducted to test the suitability of wastewater, taking various varieties of triticale, namely Delfin, Juppa'S', Mula'S', Tigre'S' (all Mexican) and TL-419 (bred from Mexican parents and released by PAU, Ludhiana, India) and one local high yielding cultivar of wheat i.e. HD-2204 as check. These were irrigated with sewage wastewater and ground water, supplemented with different doses of nitrogen and phosphorus and a fixed dose of potassium under local agro-climatic conditions. These trials were conducted during the 'rabi' (winter) season of 1991-1993 at the University Farm of Aligarh Muslim University, Aligarh, India.

3.1 Agro-climatic conditions

Aligarh is one of the sixty eight districts of Uttar Pradesh (North India). It has an area of 5,024 sq km and is situated at 27° 52'N latitude, 78°51' E longitude and 187.45 m altitude. Its climate is semi-arid and sub-tropical, with hot dry summers and cold winters. The winter extends from the middle of October to the end of March. The mean temperature for December and January, the coldest months, is
about 15°C and 13°C and the extreme minimum record for any single day is 2°C and 5°C respectively. The summer extends from April to the end of June. The average temperature for May is 34.0°C and for June, 34.5°C, whereas, the extreme maximum record is 45°C and 45.5°C respectively (Fig. 1). The average annual rainfall is 647.3 mm. More than 85 percent of the total rainfall occurs during June to September and some 10 percent, in the winter (Fig. 2). The winter rainfall is useful for the 'rabi' crops. The meteorological data for the period of present investigation were recorded at the Meteorological Observatory, Department of Physics, Aligarh Muslim University, Aligarh.

Aligarh district has the same soil composition and appearance as that found generally in western Uttar Pradesh. Different types of soils such as sandy, loamy, sandy loam and clayey loam are found in the district. The soil of the experimental field was sandy loam.

3.2 Preparation of experimental field

Before the start of each experiment, the field was thoroughly ploughed to ensure proper aeration along with the elimination of weeds. Plot size was kept 10 sq m. One light irrigation was given before each sowing to provide proper moisture for maximum germination.

3.3 Field experiments

The following field experiments were conducted according
DATA BASED ON RECORD FROM 1901

INDEX
- Extreme Maximum Record
- Mean Daily Maximum
- Mean Monthly
- Mean Daily Minimum
- Extreme Minimum Record
- Yearly Mean Maximum
- Yearly Mean Temperature
- Yearly Mean Minimum

FIG.1 MONTHLY TEMPERATURE VARIATION AT ALIGARH
FIG 2 AVERAGE ANNUAL RAINFALL AT ALIGARH
to the scheme of treatment given below. Experiment 1 and 2 were conducted in the first 'rabi' season while 3, 4 and 5 were laid out in the next 'rabi' season.

3.3.1 Experiment 1

This experiment was conducted during the 'rabi' season of 1991-92 to test the comparative utility of sewage wastewater and ground water used as irrigant, on the basis of growth, yield and quality of five triticakes, including Delfin, the best yielding Mexican variety among those already tested at Aligarh, Juppa'S', Mula'S', Tigre'S' and TL-419, and one wheat (HD-2204) check. Three irrigations were given either from tubewell for ground water (control) or as sewage wastewater discharged from the municipal sewage pump, Aligarh. The design of the experiment was split plot, with three replications. Urea @ 120 kg N/ha., monocalcium superphosphate @ 60 kg P/ha., and muriate of potash @ 60 kg K/ha., were used as the sources of nitrogen, phosphorus and potassium respectively. This dose was selected on the basis of earlier fertilizer trials conducted on triticakes at Aligarh. The seed rate was 150 Kg/ha and the sowing was done on 10th November, 1991 by 'behind the plough' method. Weeding was done twice at tillering and heading stages and harvesting, on 20th April, 1992.

3.3.2 Experiment 2

This experiment was conducted simultaneously with
Experiment 1. The aim of this experiment was to compare the effect of two irrigants (sewage wastewater and ground water) and of six doses of basal nitrogen on the growth, yield and quality of triticale variety TL-419 because of its origin in India. Like Experiment 1, irrigation water was obtained from sewage pump and tubewell. A uniform basal dose of phosphorus and potassium @ 60 kg P and 60 kg K/ha each was applied. The nitrogen doses were \( N_{60}, N_{90}, N_{120}, N_{150}, N_{180} \) and \( N_{210} \). The design of the experiment was split plot with three replications. The agricultural practices, like sowing date, seed rate, sowing method, weeding, sources of irrigation water, irrigation schedule, sources of nitrogen, phosphorus and potassium, sowing and harvesting, were the same as in Experiment 1.

3.3.3 Experiment 3

This experiment was conducted in the following 'rabi' season (92-93). The aim of this experiment was to study the effect of sewage wastewater alone together with four nitrogen doses \( (N_0, N_{60}, N_{90}, N_{120}) \) on comparative performance of two varieties of triticale, namely TL-419 and Juppa'S' and one of wheat (HD-2204). A uniform basal dose of phosphorus and potassium @ 60 kg P/ha and 60 kg K/ha each was applied at the time of sowing. The design of the experiment was factorial randomized. The sources of nitrogen, phosphorus and potassium were urea, monocalcium
superphosphate and muriate of potash respectively. The agricultural practices were adopted as in earlier experiments. The crop was sown on 15th November, 1992 and harvested on 18th April, 1993.

3.3.4 Experiment 4

This experiment was carried out together with experiment 3. The aim of this experiment was to study the performance of the same two varieties of triticale and one of wheat, namely TL-419, Juppa'S' and HD-2204, as taken in Experiment 3 under four basal phosphorus regimes, i.e. $P_0$, $P_{20}$, $P_{40}$ and $P_{60}$. A uniform basal dose of nitrogen and potassium (120 kg N/ha and 60 kg K/ha) was applied before sowing. As in Experiment 3, the plants were irrigated with sewage wastewater. The design of the experiment was factorial randomized with three replications. The agricultural practices were the same as in previous experiments. The crop was sown on 16th November, 1992 and harvested on 19th April, 1993.

3.3.5 Experiment 5

This experiment was performed together with Experiments 3 and 4. The aim of the study was to find out the best combination of nitrogen and phosphorus doses for the cultivation of the Indian variety of triticale, namely TL-419 selected on the basis of its performance in earlier experiments. Juppa'S’ being a low yielding variety of
triticale (Experiment 1) was included for contrast. The plants were irrigated with sewage wastewater only. The different combinations of nitrogen and phosphorus were $N_0P_0$, $N_{60}P_{20}$, $N_{90}P_{20}$, $N_{120}P_{20}$, $N_{60}P_{40}$, $N_{90}P_{40}$, $N_{120}P_{40}$, $N_{60}P_{60}$, $N_{90}P_{60}$ and $N_{120}P_{60}$. A uniform basal dose of potassium @ 60 kg K/ha was applied simultaneously. The design of the experiment was factorial randomized. The cultural practices were the same as in 3 and 4 experiments. The crop was sown on 17th November, 1992 and harvested on 20th April, 1993.

3.4 Sampling of materials

Soil, water and plant samples were collected according to the requirement of each experiment undertaking the following procedure.

3.4.1 Soil

Samples of soil were collected for analysis before sowing for each experiment. To obtain a composite sample, small quantity of soil was collected from a depth of 15 cm from ten well distributed spots. These were thoroughly mixed on a polythene sheet. Only 500 g of each composite sample was kept in polythene bags with discription and identification for analysis.

3.4.2 Water

Samples of water were collected before irrigation for analysis in 5 litre plastic containers. Wastewater (sewage)
and groundwater (tubewell) were stored separately at low temperature (4°C) and analysis was completed within 24 hr.

3.4.3 Plant

In order to assess the effect of sewage wastewater irrigation and fertilizer application on crop varieties, plant samples were collected randomly at tillering, heading and milky grain stages corresponding to 70, 100 and 120 days after sowing (DAS) respectively. Three plants were collected from each plot for the study of various physiomorphological characteristics.

3.5 Growth characteristics

The following growth characteristics were observed at tillering, heading, and milky grain stages.

1. Shoot length/plant (cm)
2. Leaf number/plant
3. Tiller number/plant
4. Fresh weight/plant (g)
5. Dry weight/plant (g)
6. Leaf area/plant (sq cm)
7. Net assimilation rate (g/m²/day)

Whereas fresh weight and dry weight account for total productivity in terms of increase of weight, volume and dry matter accumulation, leaf number indicates a measure of differentiation and tiller number for meristematic activity. To assess dry weight of shoot, the three plants already
evaluated for their various growth parameters were dried for about 72 hr in an oven maintained at 80° C.

3.5.1 Leaf area

The area of each leaf was noted by drawing its outline on a graph paper which was then measured by counting the blocks. Three leaves were taken randomly from each plant for calculating average (Yahiya, 1993).

3.5.2 Net assimilation rate (NAR)

It was calculated according to the formula of Mil thrope and Moorby, (1979) and is given below:

\[
NAR = \frac{(W_2 - W_1)}{(t_2 - t_1)} \times \frac{2.303(\log_{10}L_2 - \log_{10}L_1)}{(L_2 - L_1)}
\]

where, 
- \(W_1\) = dry weight of plant at 1st sampling
- \(W_2\) = dry weight of plant at subsequent sampling
- \(L_1\) = leaf area of plant at 1st sampling
- \(L_2\) = leaf area of plant at subsequent sampling
- \(t_1\) = days of sampling at 1st sampling
- \(t_2\) = days of sampling at subsequent sampling
- \(\log_{10}\) = logarithm to base 10

Leaf area and NAR reflect the photosynthetic efficiency, rate of differentiation and accumulation of metabolic products in plants.
3.6 Yield characteristics

Three plants were sampled randomly from each bed at the time of harvest and the following yield characteristics were observed:

1. Ear number/plant
2. Ear weight/plant (g)
3. Length/ear (cm)
4. Spiklet number/ear
5. Grain number/ear
6. 1,000 grain weight (g)
7. Grain yield (q/ha)
8. Straw yield (q/ha)

After harvesting, the total produce (grain plus straw) of each plot was allowed to dry for a few days and its weight recorded. The grain in the total yield of each plot was threshed out manually and its weight recorded. The straw yield was obtained by subtracting the grain yield from the weight of the total produce recorded before threshing.

3.7 Chemical analyses

Chemical analyses of leaves (at each growing stage), grain (at harvest), soil and water were carried out as follows:

3.7.1 Leaf

Leaves were analyzed according to the method of Lindner (1944). Healthy looking leaves from dried plant shoots were
removed and powdered with mortar and pestle and passed through a 72 mm mesh screen. The leaf powder of each sample was kept overnight at 70°C before digestion. 100 mg of the leaf powder was carefully transferred to a 50 ml Kjeldhal flask and 2 ml of chemically pure sulphuric acid was added. Digestion was continued on a heating mantle for 2 hr to allow complete reduction of nitrates present in the plant material. When the colour turned to brownish black, the flasks were cooled for 15 min, followed by dropwise addition of 0.5 ml of chemically pure 30% hydrogen peroxide. The solution was heated for about half an hour till its colour changed from brownish black to light yellowish. It was then cooled and an additional amount (3-4 drops) of hydrogen peroxide was added, followed by gentle heating for another 15 min to get a clear and colourless solution. It was transferred to a 100 ml volumetric flask, and the volume was made up to the mark with double distilled water, for the estimation of nitrogen, phosphorus and potassium contents.

3.7.1.1 Nitrogen

The method of Lindner (1944) was adopted for estimating leaf nitrogen content. A 10 ml aliquot of the peroxide digested material was taken in a 50 ml volumetric flask and to this 2 ml of 2.5 N sodium hydroxide was added to neutralize the excess of acid. In order to prevent turbidity, 1 ml of 10% sodium silicate solution was added.
and the volume was made up to the mark with distilled water. 5 ml of this solution was taken in a 10 ml graduated test tube and 0.5 ml of Nessler’s reagent was added dropwise and mixed thoroughly after each drop. The final volume was made up with distilled water and the tube was allowed to stand for about 5 min for maximum colour development.

The solution was transferred to a colorimetric tube and the optical density of the solution was determined at 525 nm on a Bausch and Lomb spectrophotometer (Spectronic 20). A blank consisting of distilled water and Nessler’s reagent was run simultaneously. A calibration curve was obtained by using known dilutions of a standard ammonium sulphate solution and the reading of each sample was compared. Nitrogen in the leaves was determined in terms of percentage on dry weight basis.

3.7.1.2 Phosphorus

Phosphorus was estimated according to the method of Fiske and Subba Row (1925). A 5 ml aliquot of the peroxide digested material was taken in a 10 ml graduated test tube and 1 ml of molybdic acid (2.5% ammonium molybdate in 10 N sulphuric acid) was added carefully, followed by the addition of 0.4 ml of 1,2,4-aminonaphthalene sulfonic acid. The colour of the solution turned blue. Distilled water was used to make the volume up to the mark. The solution was kept for 5 min to allow colour development and then transferred to a
colorimetric tube. The optical density of the solution was read at 620 nm, using the same type of spectrophotometer as employed for nitrogen estimation. A blank was run simultaneously with each determination. A standard calibration curve was prepared by using known concentration of monobasic potassium phosphate solution. The reading in each sample was compared with this curve and phosphorus content in the leaves was computed in terms of percentage on dry weight basis.

3.7.1.3 Potassium

It was estimated flame photometrically. A 10 ml aliquot of the digested material was taken in small tubes and read after proper dilution. A blank containing only distilled water was run side by side. The reading was compared with a calibration curve plotted for different dilutions of standard potassium sulphate and potassium content was calculated in terms of percentage on dry weight basis.

3.7.2 Grain

The grain of each sample was chemically analyzed for its carbohydrate and protein content. The dry grain samples were ground to fine powder and passed through a 72 mm mesh sieve. The powder was stored in polythene bags. It was dried overnight in an oven at 80°C before analysis.
3.7.2.1 Carbohydrate

Soluble and insoluble carbohydrates were extracted according to the method of Yih and Clark (1965) and estimated by the method of Dubois et al. (1956).

For extraction of soluble carbohydrates, 50 mg powder of each sample was transferred to a glass centrifuge tube, 5 ml of 80% ethyl alcohol after pipetting into the test tube, was heated on a water bath at 60°C for 10 min. The samples were cooled and centrifuged at 4,000 rpm for 10 min. The supernatant was poured into a 25 ml volumetric flask with three washings and the final volume was made up with 80% ethyl alcohol. The residue was left in the test tube for estimation of insoluble carbohydrates. 1 ml of this extract was transferred to a test tube and evaporated to dryness on a water bath. The test tubes were cooled and 2 ml of distilled water was added. The extract was used for the estimation of soluble carbohydrates.

For the extraction of insoluble carbohydrate the residue was taken, to it 5 ml of 1.5 N sulphuric acid (Appendix) was added and heated on a water bath at 100°C for 2 hr. This digested sample was centrifuged, after cooling, at 4,000 rpm. The supernatant was collected in a 25 ml volumetric flask with three washings. The final volume was made up with distilled water. 1 ml of the extract and 1 ml of distilled water was taken into a test tube to estimate insoluble carbohydrates.
For estimation of soluble and insoluble carbohydrates, 5 ml of 5% distilled phenol was pipetted into each test tube, containing the extract of soluble and insoluble carbohydrates, followed by the addition of 5 ml concentrated sulphuric acid. The test tube was shaken well so that the colour turned yellowish orange. Then, the test tube was cooled by placing it in chilled water. After 30 min, the solution was transferred to a colorimetric tube and its optical density was measured at 490 nm, using the same type of spectrophotometer as employed earlier. A blank was run simultaneously. The carbohydrate content was calculated by comparing the optical density of the sample with a calibration curve plotted by taking known dilutions of a standard solution of pure glucose.

3.7.2.2 Protein

Protein was estimated following the method of Lowry et al. (1951). For the extraction of soluble and insoluble protein, grain powder was kept overnight in an oven at 80°C. Then, it was cooled and 50 mg sample was transferred to a mortar to which 1 ml of distilled water was added. The powder was ground well and transferred to a centrifuge tube with repeated washings and volume was made up to 5 ml with distilled water. The extract was then centrifuged at 4,000 rpm for 5 min and the supernatant was collected for soluble protein.

To the residue 5 ml of 5% trichloroacetic acid was
added. The solution was allowed to stand at room temperature for 30 min with thorough shakings. It was then centrifuged at 4,000 rpm for 10 min and the supernatant was discarded. 5 ml of 1 N sodium hydroxide (Appendix) was added to the residue and mixed well by shaking. The residue was allowed to stand in a water bath at 80° C for 30 min. Then, it was cooled and centrifuged at 4,000 rpm. The supernatant, together with three washings with 1 N sodium hydroxide, was collected in a 25 ml volumetric flask. The volume was made up to the mark with 1 N sodium hydroxide and used for estimation of insoluble protein.

For the estimation of soluble protein, 1 ml of water extract was transferred to a 10 ml test tube and 5 ml of reagent C (Appendix) was added. The solution was mixed well and allowed to stand for 10 min at room temperature. 0.5 ml of reagent E (Appendix) was added rapidly with immediate mixing. After 30 min, the blue coloured solution was transferred to a colorimetric tube and its optical density was read at 660 nm, using a Bausch and Lomb "Spectronic 20" spectrophotometer. A blank was run simultaneously. The soluble protein content was estimated by comparing the optical density of each sample with a calibration curve plotted by taking known dilutions of a standard solution of egg albumin.

For the estimation of insoluble protein, 1 ml of sodium hydroxide extract was transferred to a 10 ml test tube and 5
ml of reagent D (Appendix) was added rapidly. After keeping it for 10 min at room temperature, 5 ml reagent E (Appendix) was mixed. After 30 min, the intensity of the blue colour was measured at 490 nm, using a Bausch and Lomb "Spectronic 20" spectrophotometer.

3.7.3 Soil

The soil sample was spread in the laboratory on a sheet of paper to break large lumps with a wooden pestle, and passed through a 2 mm sieve for determining the following physico-chemical properties (Table 1).

3.7.3.1 Soil texture

Texture refers to the relative proportion of sand, silt and clay. It is an important soil property because it is closely related to the rate of water intake, water supplying power and the fertility, erosion, aeration and energy required to till the soil. It was determined through a rapid procedure by rubbing the soil between the thumb and the index finger. For this a small quantity of the dry soil was moistened and mixed thoroughly in a glass dish to form a soft ball and then worked till stiff and squeezed out between thumb and index finger.

3.7.3.2 Cation exchange capacity (CEC)

CEC of the soil samples was determined by the method of Ganguly (1951). To 10 g soil, 0.2 N HCl was added till the
TABLE 1. Soil Characteristic before sowing. All determinations in kg/ha 1:5 (soil water extract) or as specified.

<table>
<thead>
<tr>
<th>Determinations</th>
<th>Expt.1</th>
<th>Expt.2</th>
<th>Expt.3</th>
<th>Expt.4</th>
<th>Expt.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Texture</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sandy loam</td>
</tr>
<tr>
<td>CEC&lt;sub&gt;a&lt;/sub&gt;</td>
<td>2.80</td>
<td>2.80</td>
<td>3.40</td>
<td>3.30</td>
<td>3.27</td>
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<tr>
<td>pH</td>
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<td>8.20</td>
<td>7.80</td>
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<td>7.90</td>
</tr>
<tr>
<td>Organic C%</td>
<td>0.28</td>
<td>0.28</td>
<td>0.80</td>
<td>0.95</td>
<td>0.91</td>
</tr>
<tr>
<td>EC&lt;sub&gt;b&lt;/sub&gt;</td>
<td>375</td>
<td>359</td>
<td>442</td>
<td>425</td>
<td>437</td>
</tr>
<tr>
<td>Total availableN</td>
<td>138</td>
<td>144</td>
<td>170</td>
<td>189</td>
<td>183</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>17.4</td>
<td>18.1</td>
<td>19.9</td>
<td>21.4</td>
<td>21.6</td>
</tr>
<tr>
<td>Potassium</td>
<td>280</td>
<td>278</td>
<td>308</td>
<td>333</td>
<td>326</td>
</tr>
<tr>
<td>Calcium</td>
<td>622.5</td>
<td>555</td>
<td>693.75</td>
<td>768</td>
<td>641.25</td>
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<tr>
<td>Magnesium</td>
<td>326.25</td>
<td>318.75</td>
<td>393.75</td>
<td>375</td>
<td>356.25</td>
</tr>
<tr>
<td>Sodium</td>
<td>506.25</td>
<td>435</td>
<td>495</td>
<td>487.5</td>
<td>468.75</td>
</tr>
<tr>
<td>Cadmium&lt;sub&gt;c&lt;/sub&gt;</td>
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<td>0.14</td>
<td>0.21</td>
<td>0.25</td>
<td>0.20</td>
</tr>
<tr>
<td>Copper&lt;sub&gt;c&lt;/sub&gt;</td>
<td>31</td>
<td>36</td>
<td>38</td>
<td>27</td>
<td>28</td>
</tr>
<tr>
<td>Ferrous&lt;sub&gt;c&lt;/sub&gt;</td>
<td>64</td>
<td>58</td>
<td>86</td>
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<td>75</td>
</tr>
<tr>
<td>Manganese&lt;sub&gt;c&lt;/sub&gt;</td>
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<td>19</td>
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<td>40</td>
</tr>
<tr>
<td>Nickle&lt;sub&gt;c&lt;/sub&gt;</td>
<td>0.76</td>
<td>0.64</td>
<td>0.86</td>
<td>1.05</td>
<td>1.01</td>
</tr>
<tr>
<td>Plumbous&lt;sub&gt;c&lt;/sub&gt;(Pb)</td>
<td>0.59</td>
<td>0.63</td>
<td>1.0</td>
<td>1.20</td>
<td>1.10</td>
</tr>
<tr>
<td>Zinc&lt;sub&gt;c&lt;/sub&gt;</td>
<td>2.60</td>
<td>2.20</td>
<td>3.50</td>
<td>4.50</td>
<td>4.30</td>
</tr>
</tbody>
</table>

a = meq/100g soil  
b = micro mos/cm  
c = ppm or micro gm/gm
soil became acidic. It was shaken for 30 min, then filtered and washed with distilled water, till it became free from chloride ions, which was checked with AgNO₃. The residue was transferred from the filter paper to a beaker and a suspension of known concentration was prepared. It was then treated with the same volume (10 ml) of standard KCl solution, shaken for 30 min and left overnight. It was titrated with 0.1 N NaOH (Appendix), using phenolphthalein as indicator. From the amount of sodium hydroxide required, the cation exchange capacity of the soil samples was calculated as follows:

\[
\text{CEC} = \frac{\text{volume of 0.1 N NaOH} \times \text{N of NaOH}}{\text{weight of the soil sample}}
\]

3.7.3.3 Soil pH

It is an important chemical property of the soil because the essential nutrients availability to the plants is highly dependent upon the pH of the soil. It was measured with the help of pH meter. To 10 g of the soil, 25 ml of distilled water was added and shaken thoroughly. After a 30 min lapse, the pH of the suspension was observed. The pH meter was calibrated with a standard buffer of known pH (Jackson, 1973).

3.7.3.4 Organic carbon

It was estimated according to the method given by
Walkley and Black (1934). 2 g of soil was taken in a 500 ml conical flask. To this, 10 ml of 1 N potassium dichromate solution (Appendix) and 20 ml of concentrated sulphuric acid were added. After shaking for about 2 min, it was kept on an asbestos mat for 30 min. 200 ml of distilled water, 10 ml of phosphoric acid (Appendix) and 1 ml of diphenylamine indicator (Appendix) were added. A deep violet colour appeared which was titrated with 0.5 N ferrous ammonium sulphate solution (Appendix) till the colour changed to purple and finally green. Simultaneously, a blank was run without soil.

3.7.3.5 Electrical conductivity

It is a numerical expression of the ability of sample to carry electric current which depends on the total concentration of the ionised substances dissolved and the temperature at which the measurement is made. 10 g of soil was shaken intermittently with 40 ml of distilled water in 150 ml conical flask for 1 hr and allowed to stand. The conductivity of the supernatant liquid was determined with the help of conductivity meter. The apparatus was adjusted to a known temperature (25°C) of the solution (Jackson, 1973).

3.7.3.6 Nitrate nitrogen

It was estimated according to the method of Ghosh et al. (1983). 20 g of soil was shaken continuously with 50 ml of
distilled water for 1 hr in a 100 ml conical flask fitted with rubber stopper. A pinch of CaSO$_4$ was added and shaken. Then the contents were filtered through a filter paper (Whatman No.1), 20 ml of the clear filtrate was transferred to a 50 ml porcelain dish and was evaporated to dryness on a steam bath. Then it was cooled and 3 ml of phenol disulphonic acid (Appendix) was added and allowed to react for 10 min. 15 ml of distilled water was added and stirred with a glass rod until the residue was dissolved. After cooling, the contents were washed down into a 100 ml volumetric flask, to this 1:1 ammonia (Appendix) was added slowly and mixed well, till the solution became alkaline which was indicated by the yellow colour due to the presence of nitrate. Then, another 2 ml of ammonia was added and finally the volume was made upto 100 ml with distilled water. The intensity of yellow colour was read with "Spectronic 20" spectrophotometer.

For preparing the standard curve, a stock solution containing 100 ppm nitrate was prepared by dissolving 0.7215 g of potassium nitrate in water and the volume was made upto 1 L. This was distilled ten times to give a 10 ppm NO$_3^-$-N solution. Aliquots (2, 5, 10, 15, 20 and 25 ml) were evaporated on water bath to dryness in small porcelain dishes. After cooling, 3 ml of phenol sulphonylic acid was added and yellow colour was read as described above. Simultaneously, a blank was also run.
3.7.3.7 Phosphorus

To 2.5 g of soil in a 100 ml conical flask a pinch of Draco G 60 was added followed by 50 ml of Olsen's reagent (Appendix). A blank was run without soil. The flask was shaken for 30 min on a shaker and then the contents were filtered through a Whatman No.1 filter paper. In the filtrate, phosphorus was estimated spectrophotometrically by Dickman and Bray's (1940) method.

5 ml of soil extract was pipetted into a 25 ml volumetric flask and 5 ml of Dickman and Bray's reagent (Appendix) was poured drop by drop with constant shaking till the effervescence due to CO₂ evolution ceased. The inner wall of the neck of the flask was washed with distilled water and the contents diluted to about 22 ml. Then 1 ml of stannous chloride solution (Appendix) was added and the volume was made upto the mark. The intensity of blue colour was read at 660 nm on "Spectronic 20" spectrophotometer. 0.439 g of potassium dihydrogen orthophosphate (KH₂PO₄) was dissolved in about half a litre of distilled water. To this, 25 ml of 7 N H₂SO₄ (Appendix) was added and volume was made upto 1 l with distilled water, giving 100 ppm stock solution of P (100 μg P per ml). From this, 2 ppm P solution was made after 50 times dilution. For the preparation of the standard curve, different concentrations of P (1, 2, 3, 4, 5 and 10 ml of 2 ppm P solution) were taken in 25 ml volumetric flasks. To
these, 5 ml of extracting reagent (Olsen's reagent) was added. The colour was developed by adding Dickman and Bray's reagent and stannous chloride and read at 660 nm. The curve was plotted by putting the colorimeter reading on the vertical axis and the amount of P (in ug) on the horizontal one.

3.7.3.8 Potassium

5 g of soil was shaken with 25 ml neutral normal ammonium acetate (Appendix) for 5 min and was filtered immediately through a dry Whatman No.1 filter paper. Potassium concentration in the extract was determined flame photometrically. Stock solution of 1,000 ppm K was prepared by dissolving 1.908 g KCl in 1 L of distilled water. From the stock solution, aliquots were diluted in 50 ml volumetric flask with ammonium acetate solution to give 10 to 40 ppm of K. These were read with the help of a flame photometer after setting zero for the blank and at 100 for 40 ppm of K. The curve was obtained by plotting the readings against the different concentrations (10, 15, 20, 25, 30, 35, 40 ppm) of K.

3.7.3.9 Sodium

The ratio of sodium to total cations is important in agriculture. Soil permeability is harmed by a high sodium ratio. The determination of sodium was carried out directly from the soil extract (1:5) with the
help of flame photometer, using appropriate filter and standard curve was prepared by taking known concentrations of sodium.

5.845 g of NaCl was dissolved in distilled water and the volume was made upto 1 L which gave 100 milli-equivalents per L of Na. From this stock solution, dilutions containing 5, 10, 20, 30, 40, 50 meq Na/L were prepared. The curve was drawn by plotting the flame photometer readings on the vertical axis against concentration of Na on the horizontal axis. Na in the unknown sample was read from the curve.

3.7.3.10 Preparation of extract for Ca and Mg

100 g soil was transferred to a 750 ml flask. To this 500 ml distilled water was added and the flask was shaken for about 1 hour. The contents were then filtered through Buchner funnel.

Calcium

Calcium was estimated according to the method of Chopra and Kanwar (1982). To 25 ml extract, 2-3 crystals of carbamate and 5 ml of 16% NaOH solution were added. Then, it was titrated with 0.01 N EDTA (Appendix), using murexide indicator powder (Appendix) till colour changed from orange red to purple.
Magnesium

To 25 ml extract, 1 ml of NaCN (2 %) was added. Then, 5 ml ammonium chloride-ammonium hydroxide buffer was added, followed by titration with 0.01 N EDTA (Appendix); using EBT as indicator, the colour changing from green to wine red (Chopra and Kanwar, 1982)

3.7.4 Physico-chemical analysis of water

Samples of irrigation water were analyzed in accordance with irrigation water quality criteria. As irrigation results in water logging, salinity and alkalinity, it is very important to know the quality of irrigation water. The following parameters were, therefore, studied (Table 2):

3.7.4.1 Electrical conductivity

Samples were directly read using a conductivity meter by putting the samples in the beaker. The apparatus was adjusted to a known temperature (25° C) of the solution.

3.7.4.2 Measurement of pH

It was determined with the help of pH meter. The pH meter was checked before use with standard buffer of a known pH.

3.7.4.3 Biological oxygen demand

It is widely used to determine the pollution power or
### TABLE 2. Irrigation water quality (Groundwater-GW, Sewage waste water WW) all determinations in mg/L or as specified.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>EC (mmhos/cm)</td>
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<td>1168</td>
<td>567</td>
<td>1220</td>
</tr>
<tr>
<td>pH</td>
<td>7.8</td>
<td>7.9</td>
<td>7.8</td>
<td>6.9</td>
</tr>
<tr>
<td>BOD</td>
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<tr>
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<tr>
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<td>87.3</td>
</tr>
<tr>
<td>Mg</td>
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<td>16.10</td>
<td>26.0</td>
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</tr>
<tr>
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<td>27.60</td>
<td>11.5</td>
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<td>160</td>
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<td>180</td>
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<tr>
<td>Bicarbonate</td>
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<td>109</td>
<td>244</td>
</tr>
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<td>Carbonate</td>
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<td>364</td>
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<td>404</td>
</tr>
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<td>5.20</td>
</tr>
<tr>
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<tr>
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<td>0.87</td>
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</tr>
<tr>
<td>Flouride</td>
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<td>0.98</td>
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**Heavy Metals**

<table>
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<tr>
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<th>1991-92</th>
</tr>
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<tbody>
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<td>0.0050</td>
</tr>
<tr>
<td>Cr</td>
<td>0.0250</td>
<td>0.0264</td>
</tr>
<tr>
<td>Cu</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Fe</td>
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</tr>
<tr>
<td>Mn</td>
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</tr>
<tr>
<td>Ni</td>
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</tr>
<tr>
<td>Pb</td>
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<td>0.0264</td>
</tr>
<tr>
<td>Zn</td>
<td>0.0124</td>
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</table>
strength of sewage and industrial waste in terms of the oxygen that microorganisms would require for complete stabilization.

Different volumes of the effluent samples were placed in BOD bottles (300 ml) to get several dilutions of the samples to obtain the required depletions ranging between 0.1 and 1.0%. These bottles were filled with distilled water, stoppered and incubated for five days in an incubator maintained at 20° C. The dissolved oxygen of these samples was determined by first adding 2 ml of manganous sulphate solution (Appendix), followed by 2 ml alkali azide iodide (Appendix) by means of a graduated pipette by dipping its end well below the surface of the liquid. The bottles were stoppered and mixed well by inverting them. The bottles were allowed to stand till the precipitate settled half way, leaving a clear supernatant above the manganese hydroxide flocs. The stopper was removed and 2 ml of H₂SO₄ was immediately added. Each bottle was restoppered and the contents were mixed by gentle inversion until dissolution was complete. 203 ml of the sample was taken in a 500 ml conical flask, 2 ml starch indicator was added and titrated against 0.025 N sodium thiosulphate solution till the disappearance of the blue colour. The reading of sodium thiosulphate used up was indicative of the dissolved oxygen of the sample in mg/L. BOD was calculated using the following relationship;
mg/L BOD = \frac{D_1 - D_2}{P}

where $D_1$ and $D_2$ are the dissolved oxygen of the diluted samples 15 min after the preparation of the sample and after 5 days of incubation respectively and $P$ is the decimal fraction of the sample used.

3.7.4.4 Chemical oxygen demand

It is a measure of oxygen equivalent of that portion of the organic matter in a sample which is susceptible to oxidation by a strong chemical oxidant.

0.4 g of mercuric sulphate was placed in a refluxing flask and 20 ml of the sample was added. Both were mixed well and 10 ml of 0.25 N potassium dichromate solution (Appendix) was added followed by 30 ml of sulphuric acid and a small amount of silver sulphate. A blank was run using distilled water instead of the sample. These were subjected to reflux for 2 hr, cooled and then diluted to about 100 ml with distilled water. The contents were then titrated against standard ferrous ammonium sulphate solution (Appendix), using ferroin as indicator (Appendix). The COD was calculated by the following relationship.

\[ \text{mg/L COD} = \frac{(A-B) \times C \times 8,000}{\text{ml sample}} \]

where

$A = \text{ml of ferrous ammonium sulphate used for blank titration}$
B = ml of ferrous ammonium sulphate used for sample titration.

C = normality of ferrous ammonium sulphate solution.

3.7.4.5 Calcium

In a conical flask, 50 ml of water sample was taken and neutralized with acid. It was boiled for 1 min and then cooled. Then, 2 ml of 1 N sodium hydroxide solution (Appendix) were added to maintain the pH at 12-13. After the addition of 1-2 drops of ammonium purpurate indicator (Appendix), it was titrated slowly with 0.01 M EDTA (Appendix) and calculated as follows:

\[
mg Ca/L = \frac{A \times B \times 400.8}{ml of sample}
\]

where A = ml titration for sample

B = mg CaCO₃ equivalent to 1.0 ml EDTA titrant at the calcium indicator end point.

3.7.4.6 Magnesium

It was estimated from the EDTA and hardness titration (taken from total hardness estimation)

\[
mg/L \ mg = \text{Total hardness (as mg CaCO}_3/\text{L)} - \text{Calcium hardness} \times 0.244 \text{ (as mg CaCO}_3/\text{L)}
\]

3.7.4.7 Potassium

Potassium determination was carried out with flame photometer, using appropriate filter and a standard curve by taking known concentration of potassium. A stock
solution of 1,000 ppm K was prepared by dissolving 1.908 KCl in 1 L of distilled water. Dilute solutions containing 2, 5, 10, 15 and 25 ppm were prepared from the stock solution. The standard curve was prepared by plotting the flame photometer (Systronics) readings against concentrations of K.

3.7.4.8 Sodium

It was carried out directly with the help of flame photometer using appropriate filter and standard curve by taking known concentrations of a sodium salt. For standard curve, 5.845 g of NaCl was dissolved in water and the volume, maintained at 1 L. This gave 100 milli-equivalents per L of Na. From this stock solution dilutions containing 5, 10, 20, 30, 40 and 50 meq Na/L were prepared. A curve was drawn by plotting the flame photometer readings on y-axis against concentrations of sodium on x-axis. The concentration of sodium in the unknown sample was read from the curve.

3.7.4.9 Carbonates and bicarbonates

Estimation of carbonates and bicarbonates was done following the method of Richards (1954).

50 ml water sample was taken in a clean flask. To this, 5 drops of phenolphthalein indicator (Appendix) were added. The appearance of pink colour indicated the presence of carbonates. Then it was titrated against 0.1 N sulphuric acid (Appendix) till the solution became colourless. To the
colourless solution from the above titration, 2 drops of methyl red indicator (Appendix) were added. It was again titrated against 0.01 N sulphuric acid till the colour changed from yellow to rose red. This indicated the bicarbonate presence.

Calculations:

a) carbonates (meq/L)

\[
\text{carbonates} = 2Y \times \text{normality of } \text{H}_2\text{SO}_4 \times \frac{1000}{\text{ml aliquot}}
\]

\[
= 2Y \times 2
\]

b) bicarbonates (meq/L)

\[
\text{bicarbonates} = (Z-2Y) \times \text{normality of } \text{H}_2\text{SO}_4 \times \frac{1000}{\text{ml aliquot}}
\]

\[
= (Z-2Y) \times 2
\]

where \(Y\) = reading of burette for the titration of carbonates

\(Z\) = reading of burette for the titration of bicarbonates

3.7.4.10 Chloride

50 ml of water sample was taken in a flask and 0.5 ml potassium chromate indicator (Appendix) was added. It was titrated against 0.0141 N silver nitrate solution (Appendix). Chloride concentration in the sample was calculated as follows:

\[
\text{mg/L Cl} = \frac{(A-B) \times 0.0141 \times 35,450}{\text{ml sample}}
\]

where \(A\) = ml titrations for sample
3.7.4.11 Sulphate

50 ml of sample was taken in a flask and 2.5 ml conditioning reagent (Appendix) and a small amount of barium chloride were added. After shaking for 1 min, it was read with the help of a nephelometer.

Standard sulphate solution was made by dissolving 147.9 g sodium bisulphate (NaHSO₄) in sufficient distilled water and making up to 100 ml. From this 10, 20, 30, and 40 ppm dilutions were prepared. Turbidity was developed by adding sufficient barium chloride. A standard curve was prepared by plotting the readings for each dilution, using a nephelometer.

3.7.4.12 Phosphate

To a 100 ml sample containing not more than 0.2 mg phosphorus and free from colour and turbidity, 0.05 ml phenolphthalein indicator was added. If the sample turned pink, strong acid solution was added dropwise to discharge the colour. If more than 0.25 ml was required, smaller sample was taken and diluted to 1,000 ml with distilled water. After discharging the pink colour with acid, 4 ml of molybdate reagent was added. After 10 min, the colour was measured spectrophotometrically at 690 nm and comparison with the calibration curve was made, using a distilled water blank.
\[ \text{mg/L P} = \frac{\text{mg P} \times 1,000}{\text{ml sample}} \]

3.7.4.13 Nitrate and ammonium nitrate

First nitrate standard was prepared in the range 0.1 to 1 mg/L N by diluting 1, 2, 4, 7 and 10 ml standard nitrate solution to 10 ml with distilled water. Samples containing residual chlorine were removed by adding 1 drop (0.05 ml) sodium arsenite solution for each 0.10 mg Cl and mixed. One drop was added in excess to 50 ml portion. For colour development, a number of reaction tubes were set in a wire rack, and so spaced that each tube was surrounded by empty space. To each tube 10 ml sample was added. The rack was placed in a cool water bath and 2 ml NaCl solution was added and was mixed well. Then 10 ml \( \text{H}_2\text{SO}_4 \) was mixed and cooled. 0.5 ml brucine sulfanilic acid reagent was added and the tubes swirled to mix, and then placed in a water bath at not less than 95°C. After 20 min, it was taken out and cooled in a cold water bath. Reading was taken against a reagent blank at 410 nm with a "Spectronic 20" spectrophotometer.

Standard curve was prepared from the absorbance values of the standards run together with the samples and corrected by subtracting their "sample blank", values from their final absorbance values. The concentrations of \( \text{NO}_3\text{-N} \) were read directly from the standard curve.
For the estimation of ammonia nitrogen, first a preliminary distillation was performed. 500 ml of ammonia-free water was added to 20 ml borate buffer and the pH was adjusted to 9.5 with 6 N NaOH solution. A few glass beads were added to this and the mixture was used to steam out the distillation apparatus until the distillate showed no traces of ammonia. For ammonia nitrogen content of less than 100 ug/L, a sample volume of 4,000 ml was used. Residual chlorine was removed in the sample by adding a dechlorinating agent, 25 ml borate buffer was added and the pH was adjusted to 9.5 with 6 N NaOH, using a pH meter. Distillation of sample was done. The steaming out flask was disconnected and immediately the sample was transferred to the distillation apparatus. It was distilled at a rate of 6 to 10 ml/min with the tip of delivering tube submerged. The distillate was collected in a 500 ml Erlenmeyer flask, containing 50 ml boric acid solution. At least 300 ml of distillate was collected. It was diluted to 500 ml with ammonia free water. 100 ml of the sample was taken in a 500 ml Kjeldhal flask with ammonia-free distilled water and diluted to 250 ml. Again it was distilled as before with a few pieces of paraffin wax added to the distillation flask and 100 ml of distillate was collected.

Ammonia in the distillate was titrated against standard 0.02 H$_2$SO$_4$ titrant until the indicator turned a pale
lavender. A blank was run through all the steps of the procedure.

\[
(A-B) \times 280
\]

\[
\text{mg/L ammonnia N} = \frac{\text{A}}{\text{ml of sample}}
\]

where \( A = \text{ml H}_2\text{SO}_4 \text{ titration for sample} \)

\( B = \text{ml H}_2\text{SO}_4 \text{ titration for blank} \)

3.7.4.14 Fluoride

For the estimation of fluoride, first a preliminary distillation was done. For this, 400 ml distilled water was placed in the distilling flask and 200 ml concentrated \( \text{H}_2\text{SO}_4 \) was added carefully. The contents were swirled until homogeneity. 25 to 35 glass beads were placed at the bottom and the apparatus was connected making sure that all joints were tight. Heating was allowed as long as the efficiency of the condenser permitted the distillate to cool down, keeping the temperature of the contents of flask at 80° C. The distillate was discarded. This process removed fluoride contamination and helped to adjust the acid water ratio for subsequent distillations. After cooling the acid mixture, 300 ml of the sample was taken and mixed thoroughly with \( \text{Ag}_2\text{SO}_4 \) and added to the distilling flask at the rate of 5 mg/mg Cl, when high chloride samples were distilled. Sulphuric acid solution in the flask was used repeatedly until the contaminants from the samples accumulated to
such an extent that the recovery was effected or interferences appeared in the distillate. Suitability of the acid was checked periodically by distilling standard fluoride samples. After the distillation of high fluoride samples, the still was flushed with 300 ml of distilled water and the two fluoride distillates were combined. Next, standard curve was prepared. For it, fluoride standards were prepared in the range of 0 to 1.40 mg/L by diluting appropriate quantities of the standard fluoride solution to 50 ml with distilled water. 5 ml each of SPANDS solution and zirconyl acid reagent were pipetted to each standard and mixed well. The spectrophotometer was set at zero absorbance with the reference solution and the absorbance readings of the standard were taken immediately. A curve for the fluoride absorbance relationship was plotted.

Taking a 50 ml sample, its temperature was adjusted to that for standard curve. 5 ml of SPANDS solution and 5 ml zirconyl acid reagent were added. It was mixed well and its absorbance was read immediately;

\[
\text{mg/L F} = \frac{A}{B} \times \frac{C}{\text{ml sample}}
\]

where \( A = \text{ug F determined photometrically.} \)

The ratio \( B/C \) applied only to sample diluted to a volume \( B \), and a portion \( C \) taken from it for colour development.
3.7.5 Heavy metals

To check the presence of heavy metals in water and their accumulation in soil it was decided to estimate the following heavy metals: cadmium, copper, chromium, iron, lead, manganese, nickel and zinc.

3.7.5.1 Water samples

20 ml water was taken in a conical flask. To this, 10 ml of nitric acid was added. It was placed on a hot plate for digestion. After complete digestion, total volume was made upto 100 ml. It was stored in polythene bottles after filtering through Whatman filter paper No 42 and analysed for heavy metals with the help of GBC 902 atomic absorption spectrophotometer.

3.7.5.2 Soil samples

1 g of soil sample was taken in a conical flask. To this, 10 ml of nitric acid was added. It was placed on a hot water plate for digestion. After 12 hr of digestion, 5 ml of perchloric acid was added for complete digestion. After cooling it was filtered and the volume was made upto 100 ml with double distilled water. After filtering through Whatman filter paper No.42, it was stored in polythene bottles and analysed for heavy metals with the help of GBC-019 atomic absorption spectrophotometer.
3.8 Statistical analysis

All the data were analyzed statistically according to Panse and Sukhatme (1985). The most rigorous "F" test were followed in which the error due to replicates was also determined. When "F" value was found to be significant at 5 percent level of probability, critical difference (CD) was calculated.