Chapter III
Material and Methods
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MATERIAL AND METHODS

Water samples were collected from Yamuna river & soil samples collected from the Yamuna & Suburbs from all sampling stations. 50 samples of surface water and 50 soil samples were collected in the month of April and May (pre-monsoon) period and November and December (post-monsoon) period during the present study from different localities in the study area (Fig. 1).

Water Sampling Procedure :-

Sampling is one of the most important and foremost step in collection of representative water sample for water quality studies during the sampling programme. Information regarding the nature of rivers, irrigation and industries were also collected. Polythene bottles of one litre capacity, soaked in acid and rinsed with distilled water were used for the collection of water samples. Samples of water were collected by using a clean stainless steel water sampler which was introduced into the river with the help of rope and water was taken out. Prior to transferring the water samples, the bottle was rinsed thoroughly with the water to be sampled. A portion of sample was immediately filtered through Whatman filter paper No. 42 and acidified with 6N nitric acid (8ml/l). Analysis of these samples was done without adding any preservative.

Soil Sampling Procedure :-

All associated soil samples were collected in polythene and cloth bags of one kilogram capacity. A round nose trenching spade
is a convenient tool for sampling surface soil. A soil tube is useful for small surface soil samples. Soil should be air dried before shipping or storing for any extended length of time. Air dry soils that contain deliquescent salt may accumulate enough moisture during short storage period to decompose polythene and cloth bags. A container impervious to water vapour should be used for such soils. All samples should be air dried before storing into the container. The following precautions were taken into account in the selection of the soil samples.

1. Visible or suspected salt crusts on the soil surface were sampled separately and the approximate depth of sample recorded.

2. Wherever the soil showed evidence of profile development or distinct stratification, samples were taken from different horizons or layers.

3. In the absence of profile development or distinct stratification, surface samples including the surface crust were taken to the plough depth, usually to a depth of 6 to 7 inches.

4. Sometime soil samples taken for salinity and alkali determinations may be compositely to deduce the analytical work.

5. The size of sample was sufficiently large to allow various measurements to be made thereafter.
METHODS OF ANALYSIS

Water sample: -

The water sample for major and trace metal contents were analysed as per the standard methods of APHA (1992) and Trivedi and Goel (1984) in the Environmental Geology Laboratory, A.M.U., Aligarh. For trace elements, all water samples were concentrated at low heat in laboratory. The water samples for common cations, anions and trace metal contents were filtered through 0.45 μm membrane filters and 500 ml of the filtered sample were acidified with 10 ml concentrated HNO₃ (analytical grade HNO₃) before the concentration using evaporation methods (Parker, 1972). The concentrated samples were analysed for trace metal concentration by atomic absorption spectrophotometer (Varian Techtron N525). A blank sample was made for each spectrophotometer analysis to account for any analytical and instrumental errors.

Soil Sample: -

All soil samples were dried for 24 hours at 105° C, grounded in an agate and mortar, then passed through an 80 mesh sieve. 5 gm of the soil sample was digested for 0.5 hour in 25 ml of 1:3 H₂SO₄:HNO₃ mixture made upto 25ml with the double distilled water and filtered. The residue was again treated in a similar manner and filtered. Both the filtrates with washings were concentrated to a volume of 25 ml. The prepared samples were used for the heavy metals determination (Parker, 1972).
Trace metals in the digested water and soil samples were analysed by digital atomic absorption spectrophotometer. Operating parameters were adjusted for optimum response. Various metals were determined by taking respective hallow cathode lamps and using their most intense resonance lines. Background correlation was used for lead and cadmium. A flame rich in acetylene was used for chromium.

The name of the different instruments/equipments and their respective use in water analysis laboratory are listed below (Das, 1983).

<table>
<thead>
<tr>
<th>Name of the Instruments</th>
<th>Parameters determined</th>
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</thead>
<tbody>
<tr>
<td>1) pH meter</td>
<td>pH of the water and soil samples</td>
</tr>
<tr>
<td>2) Conductivity bridge</td>
<td>Electrical conductivity of the water and soil samples</td>
</tr>
<tr>
<td>3) Turbidity meter</td>
<td>Turbidity of the water samples</td>
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<tr>
<td>4) D.O. meter</td>
<td>Dissolved oxygen of the water body</td>
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<tr>
<td>5) Incubator, D.O. Meter</td>
<td>B.O.D.</td>
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<tr>
<td>6) Flame Photometer</td>
<td>Alkali metals</td>
</tr>
<tr>
<td>7) Atomatic absorption Spectrophotometer</td>
<td>Almost all metals</td>
</tr>
<tr>
<td>8) Specific ion meter with electrodes</td>
<td>F, Cl, NO₃, pH, metal &amp; non-metals etc.</td>
</tr>
<tr>
<td>9) Polarograph</td>
<td>Metallic ions specially in trade effluents</td>
</tr>
<tr>
<td>10) Fluorimeter</td>
<td>Fluorescent substances</td>
</tr>
<tr>
<td>11) Gas chromatograph</td>
<td>Dissolved gases, pesticides organic compound etc.</td>
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<tr>
<td>12) Total organic carbon analyser</td>
<td>Organic carbon</td>
</tr>
<tr>
<td>13) Neutron Activation Analyser</td>
<td>Analysis of trace quantity of pollutants</td>
</tr>
</tbody>
</table>
14) X-ray Fluorescence: -do-
15) Mass spectrometer: Gas content of water, inorganic gases, volatile inorganic impurities.
16) Emission spectrograph: Semi quantitative and quantitative determination of samples.

**pH Determination of Water:**

pH of water is normally determined electrometrically using pH meter with glass and reference electrodes.

**Reagents:**

Buffer solutions of pH 4, 7 and 9 which may be prepared from the respective buffer tablets.

**Procedure:**

Connect the electrodes with the pH meter. It is then connected with mains and switch on the instrument for 10 minutes. Note the temperature of the buffer solution and set the instrument to that temperature. Standardize the instrument immersing the electrodes in respective buffer solution. Standardization should be carried out either at pH 7 and 4 or pH 7 and 9 depending upon the acidic or alkaline character of water sample to be measured. Now remove buffer solution, wash electrode with distilled water and finally with water whose pH is to be determined. Immerse the electrodes in the sample and measure the pH value. When the determinations are over, wash the electrode thoroughly and keep them immersed in distilled water.
pH Determination of Soil:-

Appratus:-

Container of 250 ml capacity or greater such as a cup of moisture box was used.

Procedure:-

The pH of the soil sample was recorded with Backman pH meter using glass electrode and saturated calomel electrode assembly. A 1:5 soil water ratio suspension was used for measuring the pH (Jackson, 1973).

Determination of Electrical Conductivity in Water:-

Electrical conductivity is a measurement of water's capacity for conveying electric current and is directly related to the concentration of ionized substances in the water. It is the measure of the mineralization and indicative of the salinity of groundwater. The EC of the water was measured with HACH model DR-EL/4 conductivity meter.

The electrical conductivity with 400 micro mhos/cm at 25°C is considered suitable for human consumption.

Determination of Electric Conductivity in Soil:-

The electric conductivity of the soil was measured with HACH model DR-EL/4 conductivity meter. A 1:5 soil : water ratio suspension was used for these measurements (Jackson, 1973).

Atomic Absorption Spectrophotometer:-

Sodium, potassium, magnesium, calcium, iron, copper, manganese, zinc, cadmium chromium, lead, nickel and cobalt, etc.
were determined by atomic absorption spectrophotometer (Perkin Elmer model, 372).

All metal contents were determined in filtered and acidified water and soil samples. Various metal contents were determined by taking hollow cathode lamps.

Atomic absorption spectrophotometer is almost similar to flame emission photometer in which water sample is sprayed into air acetylene flame in order to excite the spectral lines of desired element. Flame photometer, however, measures the amount of light emitted whereas in digital atomic absorption spectrophotometer a light beam is directed through the flame into a monochromometer on to a detector that measures the amount of light absorbed. In many instances absorption is more sensitive because it depends upon the presence of free unexcited atoms and generally the ratio of unexcited atoms at a given moment is very high. As the wavelength of light beam is characteristic of only the metal being determined, the light energy absorbed by the flame is a measure of concentration of the metal in the sample.

Simplicity, sensitivity, specificity and other numerous advantages have caused this instrument to be adopted as a standard analytical method for routine determination of major and minor trace metals in water and soil sediments. However, chemical interference gives some trouble in atomic absorption spectrophotometer which may be caused by lack of absorption of metal atoms bound in molecular combination in the flame phenomenon. The chemical interference may occur when the flame
is not sufficiently hot to dissociate the molecule, as the case of phosphate interference with magnesium. Addition of lanthanum will overcome the phosphate interference in magnesium and calcium determinations.

**Apparatus:-**

**Atomic Sbsorption Spectrophotometer:-**

Atomic absorption instrument having a source of energy, an automizer burner system, a monochromator and a detector.

**Glass ware:-**

All glass wares should be washed with detergent, rinsed with hydrochloric acid (HCl), tap water and deionised distilled water simultaneously.

**Burner:-**

The burner recommended by particular instrument manufacturer should be used. Nitrous oxide burner is required for some trace elements.

**Analytical Procedure:-**

According to metal concentration, select the hollow cathode lamps for chemical analysis, the lamp should be allowed to warm minimum fifteen minutes. Now, set the position of monochromator at the correct wave length, select proper monochromator slit width and adjust the hollow cathode simultaneously adjust the burner and nebulizer flow rate for maximum percent absorption and stability and balance photometer, light the flame and regulate the flow of fuel and oxidant. Run a series of standards of metals under
chemical analysis. Computerized values of metal concentration are printed on paper sheet automatically. It is, therefore, important to run standards each time for a sample or series of samples.

Concentration of all trace metals has been calculated in milligram/litre or parts per million (PPM) in water and soil samples by using the following formula.

\[
\text{Sample absorbance} \times \text{concentration of standard} \over \text{Standard absorbance} = \text{Mg/l}
\]

Scope and Application:-

1) The method is simple, rapid and applicable to a large number of metals in surface water, groundwater, domestic and industrial wastes, saline and alkali soils. Metal contents are readily determined by atomic absorption spectrophotometer.

2) Detection limit, sensitivity and optimum range of the metals will vary with various models of atomic absorption spectrophotometers. Concentration shown below may be extended much lower with scale expansion and conversely extended upwards by using a less sensitive wave length. Detection limits may also be extended through the concentration of the sample or through solvent extraction techniques.