CHAPTER-III

MATERIALS AND METHODS

3.1 Materials and Methods

Muddy heterogeneous wastes generated from Wazirpur industrial area and two soil samples (one contaminated and another uncontaminated) were collected respectively from Chhatrapur, Delhi and JNU new campus.

3.1.1 Site of Waste Samples collection

Materials were collected from 30 representative sites of three different blocks of Wazirpur industrial area of Delhi (fig.3.1). These sites were identified numbers of heaps of wastes. Which were drained out and dumped near the drains coming from the industries. The waste heaps of 1-2 feet were possibly mixed with soil, garbage rubbish and night soil from near by residences. Sometimes, the wastes were seen to be containing high moisture since these were drained out from the flowing liquid wastes of the drains.

3.1.2 Frequency of collection

Samples were collected on seasonal basis viz. summer, monsoon and winter for three years, 1998, 1999 and 2000 respectively.

3.1.3 Collection, Processing and Storage

The wastes generated in this area were heterogeneous in nature and no detailed informations on chemical properties were available. Hence, simple random sampling strategies (Fuentes and Simmon 1991) was used to identify 30 sampling stations (10 from each block fig. 3.2). Then representative samples with 5 replicates were collected by the help of trowel and mixed proportionately to form one representative sample. Then samples were packed tightly in polythene bags and taken to processing laboratory. After determination of EC, pH and MC, samples were air dried at room temperature till it become dusty powder. To minimize chemical and microbial changes samples were
Fig.: 3.1 Delhi Map Showing Wazirpur Industrial Area
Fig.: 3.2 Wazirpur Industrial Area Showing Sampling Stations
invariably dried by spreading the bulk after removal of stone, bricks, glasses and other materials. After drying samples were lightly crushed to break the aggregates and sieved through 2.0 mm opening. Then samples were stored in cold room at 4°C for future studies.

3.1.4 Sites of Soil Samples Collection

Depending on the specific purposes of study 2 types, one contaminated Chhatrapur and another uncontaminated JNU soil was selected.

3.1.5 Period of Soil Samples Collection

For 3rd pilot study the JNU soil sample was collected on 14/10/99 and Chhatrapur soil samples was collected on 18/02/ 2000. Before one week of final incubation study Chhatrapur soil sample was collected on 27/ 12/2000 and JNU soil sample was collected on 28/12/2000. Soil samples were collected following standard method (Jackson, 1986).

3.1.6 Preparation and Storage of Soil Samples

To minimize microbial changes and to remove excess moisture samples were air dried by spreading in bulk samples after removing obvious stones and root fragments. Then samples were sieved (< 2.0 mm) and kept moist and stored in aerobic condition for seven days to stabilize microbial activity (Johnson et al, 1998). Then sub-samples were taken for moisture and WHC measurement.

3.2 Physicochemical Methods and Experimental Techniques

All experiments were performed in laboratory condition. Double distilled water, borosil glass apparatus and analytical grade reagents were used for preparation of all standards solutions. Physicochemical analysis carried out according to international standard methods, which are briefly discussed below. The readings were recorded as an average of 3 replicate samples.
3.2.1 pH [Zener Made Single (Combined) Digital pH Meter]

Digital pH meter was kept on for 30 minutes in room temperature. pH meter was calibrated with three different buffer (pH 9.2, 7.0 and 4.0). Then wastes and double distilled water (DDW) ratio 1:10 and soil water ratio 1:5 (w/v) was stirred for 10 minutes and kept standing for 30 minutes. Then pH of the suspension was noted once again after stirring for 2 minutes.

3.2.2 Electrical Conductance (Zener Made Electrical Conductivity Meter)

Conductivity meter with a conductivity cell was kept on for 30 minutes and cell constant was adjusted. Instrument was calibrated with standard KCl (0.7456g/l = 1.412 m S/cm at 25° C). Then conductivity of the supernatant liquid (prepared for pH study) was measured.

3.2.3 Moisture Content (Allen, 1989)

Moisture content was measured as the amount of water lost in weight on drying at 105° C to constant weight it was expressed in percentage with respect to fresh weight of the sample.

3.2.4 Water Holding Capacity (Allen, 1989 with Slight Modification)

Water holding capacity was measured as the amount of water lost in weight on the saturated sample at 105° C to constant weight of oven dried soil. WHC was expressed in percentage with respect to dry weight of the sample.

3.2.5 Available Nitrogen (Subbiah and Asija, 1956)

The readily oxidizable and reactive forms of soil nitrogen of the same order as available nitrogen was estimated following distillation with alkaline (2.5% NaOH) potassium permanganate (3.2%) solution and sample in Kjeldahl assembly. About 100 ml of distillate with liberated ammonia was collected in conical flask containing boric acid indicator solution and titrated against 0.02N H2SO4 to original pink color. Blank experiment was conducted for final calculation.
3.2.6 Total Nitrogen (Okalebo et al., 1993)

This method was followed with complete wet acid oxidation of organic matter on Kjeldahl oxidation at 360°C for two hours to leave a sulfuric acid solution. 450 ml of H₂SO₄ was added, 350 ml of H₂O₂ (%), 0.42 g Selenium powder and 14.0 Li₂SO₄·H₂O as additional oxidizing agent, catalyst and increasing boiling point of mixture respectively. In the 2nd step distillation of digested aliquot was done in presence of excess alkali (40% NaOH) into conical flask containing boric acid indicator solution. Then distillate was titrated against N/140 HCl from green through gray to definite pink. Blank experiment was conducted for final calculation. Occasionally satisfactory distillation recovery was checked up by taking an aliquot (5 ml) of standard ammonium sulfate solution in place of sample.

3.2.7 Available Phosphorus (Olsen et al., 1954; Bray et al., 1945)

1st step in Olsen method of available phosphorus, 0.5M NaHCO₃ was used as an extracting reagent. pH of NaHCO₃ solution was adjusted by adding 2M NaOH. NaHCO₃ solution was designed to control ionic activity of Ca through solubility product of CaCO₃ in alkaline and neutral sample solution resulting the increase of the most reactive form phosphorus of iron aluminum and calcium. A little activated charcoal (repeatededly washed with Olsen regent and warm distilled water) was added as an adsorbent to reduce strong coloration due to organic matter. Boric acid was added to reduce fluoride interference in molybdenum blue reaction. The combination of NH₄F (0.03N) in HCl (0.02N) as extracting reagent in Bray No-1 method to remove adsorbed and easily acid soluble forms of phosphorus in acidic samples largely the calcium phosphate and portion of Al and Fe phosphates by formation of complex metals ion with NaF. The 2nd step of both the method was followed with colorimetric method determination of available phosphorus using 660 m micron filter in spectrophotometer by chlorostenous reduced molybdophosphoric blue in hydrochloric acid system. The concentration of phosphorus was determined from the standard curve just after 10 minutes of the development of color.
since it started fading after sometimes. Glasswares were cleaned with chromic acid thoroughly.

3.2.8 Total Phosphorus (Okalebo et al., 1993)

In this method digested aliquot (prepared in case of total nitrogen determination) was taken for colorimetric analysis of phosphorus following ascorbic acid method-no pH adjustment. A stock solution of ammonium molybdate (12.0g in 250 ml of D.D.W at 50°C) and antimony potassium tartarate solution (0.291 in 100 ml D.D.W) was mixed in 1000 ml of 5N H₂SO₄ and diluted to 2 litres. The ascorbic acid reducing agent (2.108g C₆H₅O₆/400 ml of antimony potassium tartarate and ammonium molybdate stock solution) was prepared on the day of analysis. Then 5ml of digested was mixed with 10 ml of ascorbic acid reducing agent and diluted to 50 ml and left for one hour for full development of color. Phosphorus standard was also mixed with 10 ml of ascorbic acid for development of blue color. Then phosphorus concentration in the aliquot was determined from standard curve. The final concentration of the sample was calculated taking size and volume of the sample.

3.2.9 Organic Carbon (Okalebo et al., 1993; Ghosh et al., 1983)

In this method organic carbon in the finely ground (<0.2 mm) sample was oxidized by chromic acid (potassium dichromate 1N plus conc. H₂SO₄) utilizing the heat of dilution of sulfuric acid. The unreacted dichromate was determined by back titration with ferrous ammonium sulfate (redox titration) to the end point of violet blue to green. A combination of H₃PO₄ and NaF was used which shown sharp end point. Ag₂SO₄ was used to remove chloride interference. Blank correction was made for final calculation.

3.2.10 Exchangeable Bases (Okalebo, et al., 1993; Ghosh et al., 1983)

In this method maximum exchange between NH₄ and cations occupying exchange sites were performed by shaking sample and extracting reagent, 1:20 for 30 minutes. Adding lanthanum 2000 and strontium 100ppm in sample extract during aspiration (in air
3.2.11 Cation Exchange Capacity (Dewis, et al. 1984; Jackson, 1973)

To ensure saturating cation (NH₄⁺) displaces maximum exchangeable cation originally occupying exchange sites, samples were treated with 1N NH₄OAc (pH = 7.0) at 1:20 (W/V) for 30 minutes. The excess salt was removed with ethanol containing 5% water. The efficacy of washing out ammonium ion was Nesslerized from brown precipitate through yellow tint to colorless. Immediately after washing, exchanged ammonium was measured by direct distillation from alkaline solution (45% NaOH) and absorption in boric acid indicator and titration with 0.1N H₂SO₄ to pink end point. Blank correction was made for final calculation CEC was expressed in milliequivalent per 100gm.

3.2.12 DTPA (Diethylene Triamine Penta Acetate) Extractable Metal (Lindsay and Norvel, 1978)

In first step, method was followed by preparation of each 2 litres of extracting solution with 0.005M DTPA (3.934g), 0.1M TEA (Triethanolamine) (29.84ml), 0.01M CaCl₂.2H₂O (2.94g) in 50 ml water (DDW) and diluted to 1800 ml. The pH was adjusted to 7.30 with 1:1 HCl while stirring and diluted to 2 litres. Then treating sample to solution ratio 1:2 for two hours shaking followed 2nd step. DTPA 0.00M and CaCl₂.2H₂O 0.02 M was designed to a full association of DTPA ligand with Ca (CaL³⁺ and Ca₂L⁻ where L⁻ as free DTPA⁻ ligand) and balance Ca²⁺ was desired to suppress dissociation of CaCO₃ to avoid excess release of occluded micronutrients. The pH 7.3 was selected partly to suppress dissociation of CaCO₃. TEA-0.1M at pH 7.3 was preferred as buffer to supply excess proton (HTEA⁺) to exchange with Ca⁺ and Mg²⁺ from soil exchange sites and because it burnt clearly during flame atomization of atomic absorption spectrophotometer (AAS). In the extract elements were estimated by setting instrument for each element and using respective standard curve.
3.2.13 Total Concentration of Metals (Lorring and Rantaks, 1992; Lim and Jackson, 1982)

The method was followed by the total decomposition of 500 mg sample in closed Teflon bomb with 1-ml aqua regia and 6 ml HF (added very slowly) at 140°C in oven for three hours. 5.0 to 6.0g of H₃BO₃ and 20 ml of water was added to the digested solution after transferring to polypropylene flask (100 ml) and diluted to 100 ml following cooling. Then solution was left undisturbed into polypropylene bottle until gelatinous precipitate of borosilicate settled. A longer period (3 hours) of digestion was found effective to digest refractory compounds. H₃BO₃ was added to effect the dissolution of precipitated metal hexafluorides. The stable matrix system (HBF₄⁻ and H₃BO₃⁻ ionic constituents of silicates) was providing a salt free single matrix which greatly diminished the chemical ionization, matrix and instrumental interference for AAS determination. In the single digested solution all elements were estimated by setting instruments and respective standards following Lorring et al., (1992) procedure.

3.2.14 Particle Size Analysis (Hydrometer Method: Okalebo, et al., 1993)

The hydrometer method of particle size analysis based on differential settling velocity (Stock’ law) within water column. The 1st step of this method was followed with dispersion of soil (2.0 mm) into individual particles e.g. Sand (2.0-0.05 mm), silt (0.05-0.002 mm) and clay (<0.002mm) fractions 10% sodium hexametaphosphate (calgon) solution and water in a bottle for shaking over night. Then the suspension was transferred into graduated cylinder and diluted to the level (marked before with 1000 ml D.D.W + hydrometer) inserting hydrometer and then hydrometer was removed. Then suspension was mixed by inverting cylinder 10 (ten) times while keeping cylinder air tight with a rubber cork and time was noted. Quickly 2-3 drops of amyl alcohol was added to remove frothing and after 20 seconds hydrometer was gently inserted and at 40 seconds hydrometer reading and suspension temperature was noted which shown percentage of silt and clay. The mixing was again repeated 10 times and after 2 hours the second reading and suspension temperature was noted which gave percentage of clay. Temperature correction was made for final calculation.