MATERIALS AND METHODOLOGY
Map 3.1 Delhi Map showing Wazirpur industrial Area: And Soil Sampling Sites
Figure No. 3.2. Sketch of Wazirpur Industrial Area (not in scale)
3.1 Study Area:

We have selected Wazirpur Industrial Area as for waste collection and Chattarpur and JNU soil for disposal of this waste.

Wazirpur Industrial Area, which is situated in Northwest part of Delhi, covers an area of 210 acres, (figure 3.1). Northwestern ring road system surrounds this area. Initially this area had approximately 1000 industries among which only 424 were registered. But after 1998, as per strong recommendation of CPCB and DPCC a lot of unregistered industries are closed. The main polluting industries, which are still in working condition, are electroplating, rolling-pickling and textiles. The others are rubber, plastic, soap, electronic goods etc. Due to its large number of small-scale industries and their unmonitored level of pollution, now Wazirpur has emerged as one of the major polluted industrial zones of Delhi.

The entire area is divided into three industrial parts A, B and C (figure 3.2). Due to its industrial units, every day a huge amount of toxic wastes are spewing out of those units.

Though some of these industries are closed by CPCB, this area does not have a sound and satisfactory waste disposal system. This spreading of solid waste and effluents on the road side and dwelling places resulted in major health problems in this area.

The two sampling sites for soil are JNU campus and Chhattarpur. Both the sites are nearer to laboratory. JNU soil is free of any kind of perturbation by agricultural, industrial or any such activities, it is an educational cum residential area and situated in southeast part of Delhi. As the area comes under Aravali range, the soil here is still in developing stage. Moreover JNU is covered by thick greenery with rich flora and fauna. The soil by nature is shallow, loamy and rich in nutrients (N.B.S.S. Report 2002). Chhattarpur is a farmland. Therefore the soil collected from here has already been exposed to fertilizers, pesticides and many other pollutants. The soil found here is permanent type and blackish in colour. But the productivity of the soil is higher than JNU soil. Thus the two soils
represent the two different kinds of properties, JNU soil being in the natural state and that of Chhattarpur is disturbed by anthropogenic activities.

Below mentions description shows the types of soils as given by NBSS (1997).

**JNU Soil**

Soil of Hills terrains 25, 2050, (1.39)

<table>
<thead>
<tr>
<th>Mapping Unit</th>
<th>Description</th>
<th>Soil Taxonomy</th>
<th>Area(in ha) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>Shallow some what excessively drained loamy-skeltal soil on moderate slopes with loamy surface severe erosion and moderate stoniness.</td>
<td>Lithic Ustorthents</td>
<td>2773 (1.88)</td>
</tr>
</tbody>
</table>

**Chattarpur Soil**

<table>
<thead>
<tr>
<th>Mapping Unit</th>
<th>Description</th>
<th>Soil Taxonomy</th>
<th>Area (in ha) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>Deep some what excessively drained loamy-skeltal soil on moderate slopes with loamy surface severe erosion and moderate stoniness.</td>
<td>Lithic Ustorthents</td>
<td>2773 (1.88)</td>
</tr>
</tbody>
</table>

**3.2 Sampling**

The solid wastes were collected from open dumping grounds and roadside dumps from surface up to 30 cm deep, after removing a thin superficial layer. The collection was done using iron spade and the collected samples were kept in tight polythene bags. Ten samples were collected from each block thus counting 30 samples for the three blocks in each season.

**3.2.1 Collection of solid waste:**

Wazirpur industrial area is divided into three blocks A, B, and C. Samples were collected from ten different spots from each block (*Fig no. 3.2*). So we had thirty samples for each season. Samples were collected from an open dumping ground and roadside dump. The solid wastes were collected from the surface to 30 cm below. Then the samples were mixed and kept in airtight polythene bags.
3.2.2 Collection of soil:

Soil has been collected from JNU and Chhattarpur and homogenized separately.

3.2.3 Frequency of Sampling:

The soil samples and seasonal solid wastes were collected during the following period.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Date of sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monsoon waste</td>
<td>July 2003</td>
</tr>
<tr>
<td>Winter waste</td>
<td>Feb 2003</td>
</tr>
<tr>
<td>Summer waste</td>
<td>May 2003</td>
</tr>
<tr>
<td>JNU soil</td>
<td>Sept 2003</td>
</tr>
<tr>
<td>Chhattarpur soil</td>
<td>Oct 2003</td>
</tr>
</tbody>
</table>

Wazirpur industrial waste and both soils collected in three seasons because at different season waste and soil especially waste shows different pH and EC values. Waste showed highest pH in monsoon while lowest found in summer. Highest pH in monsoon waste is due to mixing of waste with rain water.

3.3 Preservation of Samples:

EC, pH and % Moisture Content of the samples were determined just after collection and for other parameters both the soil and solid waste samples were first air-dried and then kept in airtight polythene bags in dark cold room (4°C).
Figure 1. Natural soil, JNU.

Figure 2. Agricultural soil, Chhattarpur.
Figure 3: Washing of iron sheets after acid treatment

Figure 4: Roadside dumping of industrial waste

Figure 5: Common dumping yard near railway line, Block A.
3.4 Sample Processing:

Figure 3.3: Flow diagram of solid waste processing.
Materials And Methods

Both the solid wastes and soil samples were air-dried and then grinded by mortar and pestle and sieved using 2mm sieve. Solid waste samples of pH less than 3.5 from each season were taken and homogenized to make single representative composite samples by quadrate system. Similarly the collected soils also first air-dried and then smashed by using grinder and then homogenized after sieving and kept in airtight polythene bag.

3.5 Experimental Design:

3.5.1 Representative waste sample was first treated with lime (0%, 0.5% and 1%) and then mixed with homogenized JNU & Chhattarpur soil samples separately in different percentage of soils (10%, 20% and 30%) by maintaining 50% water holding capacity by adding known amount of distilled water. This is equal to 224, 448 and 672-mt hr\(^{1}\). The water requirement of the prepared waste treated soil was calculated considering water holding capacity and percent moisture content of the soils and solid wastes according to their. These waste amended soils were put in pots (2Kg/Pot). Control pots of both soils i.e. without waste amendment were also kept. Each treatment were replicated three times and kept in randomized order.

3.5.2 Collection of Wheat grains and Pea Seeds:

Wheat (*HD1553*) and Pea (*Arkel*) seeds were collected from National Seed Cooperation (NSC), Pusa, New Delhi. We have chosen Wheat (Gramineae) and Pea (Leguminoceae) because these crops are very common North Indian crops and grown at a very large scale in this area. Pea is a dicot while wheat is monocot crop and therefore has different behaviour in sludge-amended soil. Dicot plants uptakes more heavy metals in comparison to monocot sps. Wheat is also a very good phytoextractant for metals.

3.5.3 Preparation and sowing of Seedlings:

These Pea and Wheat seeds were first grown in Chattarpur and JNU pure soils for seven days respectively. Pea seeds were grown in the late October while wheat seeds were grown in the December last. These seven days seedlings were then
shifted to the pots having waste amended soils. We sow two plants per pots. Similarly seedlings were also grown in control soils. Distilled water was given to the plants to maintain 50% WHC of control and waste amended soils throughout the experiment. Whole the experiment was carried out in a glass chamber having a temperature around 22±2°C and moisture content around 50%.

3.6 Preservation of soil and plant samples:

Soil samples were taken from the experimental pots at different stages and preserved in dark cold room at 4°C in small airtight polythene bags, after recording their respective pH and EC.

Plant and soil samples were collected for analysis at different stages like seedling, tillering, flowering, grain/pod formation and at harvest stages. For storage of plants parts, plant samples were first washed with normal tap water than washed with deionized water. Root and shoot portion were then cutted by knife and wrapped in a brown paper and dried at 60°C for 48 hours. After drying these plants samples, these were removed from brown paper and grinded by mortar and pestle and the sieved by 0.2mm sieve. These sieved samples were then stored in polythene bags at 4°C for further analysis.

3.7 Parameters to be analyzed:

Physico–chemical characterisation of soils, wastes, waste amended soils and plants will be carried out according to international standard methods. The list of methods for plants samples and soil samples are given as below

In Soil:

pH - Digital pH meter (Thomas, 1967)
EC - Digital conductivity meter (Hess, 1972)
MC (%) - Water loss from fresh sample at 105°C during 48 hours (Singh, 1989)
WHC - Water loss from saturated sample at 105°C during 48 hours (Salter and William, 1967)
CEC - Direct Distillation Method (Jackson 1958)
Organic Carbon - Walkley and Black Method (1934)
Available P - Olsen’s Method (1954)
Bray’s No1 Method (1945)
Total P - Block digestion followed by ascorbic acid method with no pH adjustment (1965)
Available Heavy Metals – DTPA method (Lindsy and Narwell, 1978)
Total Heavy Metals- closed digestion

In Plants:

Total P – Wet Digestion Method (Diacid Digestion Method)
Total Heavy Metals – Wet Digestion Method (Diacid Digestion Method)

3.8: Analysis of Physico-Chemical Parameters of Soil

3.8.1 pH (Thomas, 1967)

Soil pH is a measure of the activity of ionized H in the soil solution. The measurement of pH is normally done by either a colorimetric or an electrometric method. In present study electrometric methods had been followed. A solution of soil and double distilled water is prepared in the ratio of 1:10 in a 100 ml beaker by stirring it with a magnetic stirrer for 10 minutes and pH values were measured after half an hour using a pH meter after standardization.

3.8.2 Electrical Conductivity (Hesse, 1971)

Electrical conductivity of a solution is the conductance of the solution at 25°C temperature between electrodes of 1 cm sq. and 1 cm apart. It measures the dissolved salts in a soil solution (Holden, 1970). Electrical conductivity is a measure of salt concentration and is measured in mS/cm. Conductivity depends on dilution of the sample. However sample/water ratio is 1:5, 1:10 are most common for conductivity study to reduce microbial influence. A solution of soil
and double distilled water is made in the ratio of 1:10 in a 100 ml beaker by stirring it with a magnetic stirrer for 10 minutes. EC values of solution thus prepare are measured after half an hour using an Electrical-conductivity meter after standardization. For soil solution it is more usual to express results of conductivity as millisiemens per cm (mS/cm).

\[ C = \frac{1}{R} \]

\( C \) = Electrical conductance in Ms/cm and \\
\( R \) = Resistance

3.8.3 Moisture Content (Singh, 1989)

The moisture content of the samples (soils, waste and waste amended soils) at any time more or less depends on its water holding capacity and environmental condition. 10g of fresh solid wastes and soils (JNU and Chhattarpur) were kept in hot-air oven at 105 °C in clean-dry petridishes separately. After 24 and 48 hrs they were weighed. The moisture content is normally expressed in percentage on weight basis (g of water/100 g oven dry soil).

\[ \% \text{ of moisture on dry wt basis} = \frac{W_a - W}{W} \times 100 \]

3.8.4 The Water Holding Capacity (Salter and William, 1967)

Water holding capacity is defined as the maximum amount of water that freely obtained sample can hold.

About 20g of the processed soil samples each of JNU and Chhattarpur and solid waste samples were flooded for 2 hrs in 100 ml beakers separately. Filtered for the last drop of water using filter paper (Whatman no.1), these saturated soils were transferred on previously weighed petridishes and weighed. Then they were kept in a hot air oven at 105 °C and weighed after 24 and 48 hrs. WHC is expressed as:
Materials And Methods

\[ \text{Weight \% of WHC} = \left( \frac{\text{Water saturated soil} - \text{Oven dried soil}}{\text{Oven dried soil}} \right) \times 100 \]

3.8.5 Organic Carbon (Walkley and Black Method, 1934)

Organic carbon contains soil organic fraction which consist of the cells of microorganisms, plant and animal residues at various stages of decomposition, stable 'humus' synthesized from residues & highly carbonized compound such as charcoal, graphite & coal.

1.0g of each sample has been taken in a 500 ml conical flask and shaken well with 10ml 1N \( \text{K}_2\text{Cr}_2\text{O}_7 \). 20 ml of conc. \( \text{H}_2\text{SO}_4 \) was added along the sides of the flask in each sample and kept for 30 minutes to complete the reaction. Then added 200ml distilled water was added in each conical flask followed by 10 ml \( \text{(H}_3\text{PO}_4 \) orthophosphoric acid and stirred vigorously. The excess \( \text{K}_2\text{Cr}_2\text{O}_7 \) is titrated against 0.1 N \( \text{Fe(NH}_4\text{)}_2\text{SO}_4 \) in presence of diphenylamine as indicator. At the end point, the colour changes from blue to scarlet green. A blank sample was run in exactly similar way but without any soil. Mathematical representation is as:

\[ \text{Organic Carbon (\%)} = \left( \frac{3.951}{\text{dry wt of soil}} \right) \times \left( 1 - \frac{T}{S} \right) \]

3.8.6 Cation Exchange Capacity (Direct Distillation Method, Jackson 1958)

Cation exchange capacity is usually expressed in meq./100gm.of soil. It is a measure of the quantity of readily exchangeable cations neutralizing negative charges in the soil (Bolt et Al., 1976). A soil leached with a salt solution (1M) has the power to absorb the cations of the percolating solution and to liberate an equivalent amount of other cations.

5.0g of soil sample was shaken well in ammonium acetate solution and kept overnight, covered with a watch glass. Next day samples was filtered to excess off \( \text{NH}_4\text{OAc} \) (ammonium acetate, pH-7.0) and then washed with 95% ethanol till the filtrate shows the presence of \( \text{NH}_4 \) with Nesseler's reagent and was then left
for half an hour to drop out ethanol. Now, the sample along with the filter paper in 400 ml double distilled water and 25 ml 45% NaOH was digested in Kjeldahl flask in presence of glass beads and liquid paraffin. About 200 ml of distillate was collected in 20 ml 2% boric acid mixed indicator solution in a 250 ml conical flask and titrated against 0.1N HCl. CEC can be expressed as

\[ CEC = \frac{10 T}{D} \]

3.8.7 Available Phosphorus:

Available P is present in soil with the mixture of aluminium, iron and calcium phosphate. The relative percentage among these three forms is a function of soil pH, and the higher percentage of Al and Fe phosphates found in acid soils, while higher percentage as calcium phosphate found in neutral to alkaline soils.

There are two methods by which available P could be measure for different type of samples. These are

a) **Reagents for Olsen's method (for alkaline and neutral samples):**

i) **Reagents for sample extract:**

NaHCO₃ of 0.5 M (pH-8.5) was prepared by dissolving 4.2 g of NaHCO₃ in one liter of distilled water. The pH (8.5) was adjusted by adding 10% NaOH.

ii) **Reagents for colour development:**

15 g of ammonium molybdate (AR) was dissolved in 300 ml of distilled water by warming at 60°C. After cooling 350 ml of 10 N HCl was adjusted correctly by titration.

iii) **Stannous Chloride:**

10 g of crystalline SnCl₂ (AR) was dissolved in 25 ml of concentrated HCl by keeping airtight while on warming and stored in amber coloured bottle carefully
avoiding all contact with air. Just before use 1 ml was diluted to 66 ml with distilled water.

b) Reagents for Bray's No.1 Method (for acidic samples)

i) Reagents for sample extract.

2.775 g of NH₄F was added to 2.5 liter of 0.025 N HCl. The normality of HCl was checked with NaOH solution.

(ii) Reagent for Colour development

15 g of ammonium molybdate was dissolved in 300 ml of distilled water by warming at 60°C. Then 350 ml of 10N HCI was added and made up to 1 liter. The normality of HCl was adjusted with NaOH solution.

(iii) Stannous Chloride Solution (Same as Olsen's method)

*Phosphorus Stock solution (100 ppm)*:

0.439 g of dried KH₂PO₄ was dissolved in 500 ml of distilled water by adding 25 ml of 7N H₂SO₄. Then total content was diluted to 1 liter.

Procedure:

a) Sample extract for Olsen's Method:

2.5 g of sample in 100 ml of conical flask with a little activated carbon (free of phosphorus) was added followed by 50 ml of Olsen's reagent. A blank was run without the sample. The flasks were shaken for 30 min on a platform type shaker and the contents filtered immediately through Whatman no.1 filter paper into dry clean beaker.

b) Samples Extract for Bray's No.1 Method:
Materials And Methods

5 g of soil and 50 ml of reagents were taken in 100 ml of flask and shaken exactly for 5 min. and filtered by Whatman no.1 filter paper. To avoid interference 7.5 ml of 0.8N boric acid was added to 5 ml of extract.

c) Preparation of phosphorus Standard Solution:

First 2 ppm of working standard was prepared by dissolving 20 ml of 100 ppm phosphorus stock solution in 1000 ml. 0, 1, 2, 3, 4, 5 and 10 ml of 2 ppm phosphorus were pipetted respectively only into 50 ml volumetric flask followed by 2 ml of SnCl₂. Addition of SnCl₂ in standard and samples were done simultaneously and then after 10 min, absorbance were measured by spectrophotometer.

d) Sample Analysis:

First 5 ml of extract was taken in 50 ml volumetric flask. Then 5 ml of Bray's reagent was added. Olsen's Reagent added drop wise to remove CO₂. Then the neck of the flask was down and content was diluted up to 20 ml and was added with 2 ml of SnCl₂ solution. Lastly the volume was made up to mark and absorbance noted at 660 nm wavelength.

Calculation:

\[ \text{Conc. of } P \,(\text{ppm}) = C_d \times V_d \times \frac{V_a}{V_a} \times \frac{1}{W} \]

Where

- \( C_d \) – conc. (ppm) in diluted aliquot
- \( V_a \) – Volume of the aliquot
- \( W \) - Weight of the sample in grams sample
- \( V_s \) – Volume of the digested
- \( V_d \) – Volume of the diluted aliquot

3.8.7: Total Phosphorus:

Analysis of total phosphorus needs the complete oxidation of organic matter. However complete oxidation can be achieved by several methods. In weight
oxidation method oxidation finally leaves a sulfuric acid solution. Hydrogen peroxide is added as an additional oxidizing agent, selenium is used as catalyst while \( \text{K}_2\text{SO}_4 \) is added to raise the boiling point of the mixture. The main advantages of this method are that a single digestion is required for both plant and soil material to bring nearly all nutrients into solution. All organic phosphorus converted into inorganic form. The total phosphorus is then colorimetrically determined by ascorbic acid reduced molybdenum blue in acidic medium.

**Reagents:**

i) Digestion mixture  
ii) Selenium powder  
iii) Potassium sulfate  
iv) Hydrogen peroxide  
v) \( \text{H}_2\text{SO}_4 \) (concentrated)

Selenium powder 0.42g and 14g \( \text{K}_2\text{SO}_4 \) were added to 350 ml of \( \text{H}_2\text{O}_2 \) (30%) and mixed well. Then 420 ml of concentrated \( \text{H}_2\text{SO}_4 \) was added slowly with care while cooling the solution in an ice bath. This mixture is stored at 2°C.

a) **Reagents for Spectrophotometric Analysis of Ascorbic acid method - no pH adjustment:**

i) \( \text{H}_2\text{SO}_4 \) 5N: 148 ml concentrated \( \text{H}_2\text{SO}_4 \) was added to 500 ml of distilled water while cooling in an ice bath. Then the volume is diluted to 1 liter.

ii) **Ammonium Molybdate/Antimony potassium tartrate solution:** 12 g of ammonium molybdate was dissolved in 250ml of warm (50°C) distilled water. Separately 0.291g antimony potassium tartrate (K\text{Sb}_5\text{C}_8\text{H}_4\text{O}_6) was dissolved in 100 ml of distilled water. Then the both solutions are added to above mention 1 liter of 5 N \( \text{H}_2\text{SO}_4 \) above. This volume was then diluted to 2 liter and kept in a cool and dark place.

iii) **Ascorbic Acid Reducing Agent:**
Materials And Methods

2.108 g of ascorbic acid \((C_6H_8O_6)\) was dissolved in 400 ml of ammonium molybdate/potassium antimony tartrate solution and was mixed properly. This was made at time of analysis.

iv) **Standard Phosphorus stock solution (1000 ppm):**

1.0967 g of oven dry \(KH_2PO_4\) was dissolved in 250ml of distilled to get 100ppm phosphorus. From this 10 ppm of working solution was prepared by taking 10ml of 1000 ppm and diluting it to 1 liter.

**Procedure for estimating total P:**

a) **Digestion:**

First 0.3g of each dry processed sample was taken in dry and clean digestion tube. Then 4.4ml digestion mixture to each test tube of the samples and blank was also run without samples. Samples were digested at 360°C for 3hr then the colourless sand white solution were taken out and cooled by adding about 25 ml of distilled water. Lastly the volume of the solution was made up to 50 ml after transferring into volumetric flask and allowed to settle.

b) **Preparation of working Standard:**

Working solution of 0, 1, 2, 3, 4, 5 and 10 ml were pipette into 50 ml of volumetric flask. 10 ml of ascorbic acid reducing agent was then added to each flask and the volume was made up to the mark and left for 1 min. to develop colour.

5ml of the supernatant clear wet ashed digested solution were taken into 50ml volumetric flask. Then 20ml of distilled water was added in each flask and followed by 10ml of ascorbic acid reducing agent to each flask. Then the volume is made up to the mark of the flask. However addition of ascorbic acid to standard was done at same time. Finally after 1 min absorbance was measured at 880nm-wave length.
c) Calculation:

\[ \text{Total P} \% = C_d \times V_d \times \frac{V_s}{V_a} \times \frac{10^{-4}}{W} \]

Where

- \( C_d \) – conc. (ppm) in diluted aliquot
- \( V_d \) – Volume of the diluted aliquot
- \( V_s \) – Volume of the digested sample
- \( V_a \) – Volume of the aliquot
- \( W \) – Weight of the sample in grams

3.8.9: Available Heavy Metals

DTPA Extraction:

The DTPA chelation procedure offers a favorable combination of stability constant for the simultaneous complexing of Cadmium (Cd), Copper (Cu), Nickel (Ni), and Zinc (Zn) (Lindsay 1979). The theoretical basis for the DTPA extraction is the equilibrium of the metal in the soil with the chelating agent. The 7.3 pH, which is buffered with triethanolamine (TEA), prevents excess dissolution of the trace (heavy) metals.

(i) Reagents:

(iia) Extraction reagent: [DTPA (diethylenetriaminepentaacetic acid)]: After weighing 1.967g of DTPA in 1000mL of volumetric flask, 1.33ml triethanolamine (TEA) was added and made the volume up to 950 ml with water. Further 1.47g of CaCl₂·2H₂O was added to it. Made up the volume to 1000mL with distill water while adjusting the pH to exactly 7.3 with 6N HCl.

(iib) Standards for Zinc: 1.0g of pure Zn metal was weighed in 1000mL of volumetric flask and added 10mL of conc. HCl to it. This was evaporated it to almost dryness and then diluted to 1000mL with DTPA. Working standards were prepared by diluting aliquots of stock solution with DTPA reagent.
All other metals standards were made in similar way.

(ii) Procedure:

Weighed 2g of soils, waste and waste amended soil samples of different stages in 100mL extraction vials, 40mL of extraction reagent has added, followed by shaking it on a reciprocating shaker for two hours. Now the samples were filtered through Whatman 42 filter paper and filtrate were used to determine different elements by AAS (model no.AA 6800).

(iii) Calculation:

\[
\text{Conc . (ppm) = Sample conc . (ppm) } \times \frac{40}{\text{wt of sample}}
\]

3.8.10 Total Heavy Metals in soil:

(i) Reagents:

(a). HF acid

(b). HNO₃ acid

(c). HClO₄ acid

(ii) Procedure:

Weighed 0.2g of soil waste and waste amended soil samples of different stages in Teflon Bomb. Now added 6.0mL of HF acid, 4.0mL of HNO₃ and 1.0mL of HClO₄ in it. Now kept all Teflon bomb in hot air oven for 5 hours. After cooling all bomb were taken out and volume was made to 100mL with water by adding 4.8g of Boric acid in plastic vials. These vials were left for 15 days, after they were filtered through Whatman 42 filter paper, and filtrate were used to determine different elements by AAS (model no. AA 6800) like available heavy metals.
(iii) Calculation:

\[
\text{Conc. (ppm)} = \frac{\text{Sample conc. (ppm)} \times 100}{\text{wt of sample}}
\]

3.9: Total Phosphorus Total Metals in Plant (Diacid Digestion):

a) Reagent: 9:4 mixtures of HNO₃ and HClO₄ is used in diacid digestion mixture.

b) Procedure:

0.2g plant material was placed in 50mL volumetric flask. To this, 10mL of acid mixture was added and the content of the flask is mixed by swirling. The flasks were placed on heated hot plate in a digestion chamber. Then, the flasks were heated at higher temperature until the production of red fumes of NO₂ ceases. The contents were further evaporated until the volume was reduced to about 3-5mL but not to dryness. Digestion was completed when the contents became to colourless.

After cooling the flask, volume was made up with distilled water to 50mL and the solution was filtered through Whatman 42 filter paper. Aliquots of this solution were used for the determination of P, Fe, Mn, Zn, Cu and other metals.

3.9.1: Phosphorus estimation in acid digests:

a) Reagents:

Ammonium molybdate-ammonium vandate in HNO₃: Dissolved 22.5g of amm.molybdate \([(NH₄)₆Mg₇O₂₄·4H₂O]\) in 400mL of distilled water. Dissolved 1.25g of ammonium vanadate in 300mL boiling distilled water. Added the vanadate solution to the molybdate solution and cool to room temperature. Now added 250mL of HNO₃ and diluted to 1.0 litre.

Phosphate Standard Solution: Dissolved 0.2195g of analytical grade KH₂PO₄ and diluted to 1.0 liter (50ppm of P).

b) Preparation of Standard Curve:
Materials And Methods

Transferred 0, 1, 2, 3, 4, and 5mL of Standard solution to 50mL volumetric, this solution had 0, 1, 2, 3, 4 and 5 ppm of P respectively. Now added 10mL of vanedomolybdate reagent to each flask. Volume was made upto 50mL with distilled water and absorbance was taken after 30 minutes at 420nm with spectrophotometer.

c) Estimation of P in Plant Extract

The plant digest containing P was transferred to 50mL volumetric flask and the same procedure followed as for the standards samples.

Calculation:

\[
P(\text{ppm}) = \frac{1}{\text{wt of sample}} \times \frac{50}{\text{Final Volume}} \times \text{wt of sample aliquote (ml)}
\]

3.9.2: Heavy metals in Plant Digest:

Filtrate diacid digestion was directly used to determine different elements by AAS (model no. AA 6800)

Calculation:

\[
P(\text{ppm}) = \frac{1}{\text{wt of sample}} \times 50
\]