Chapter 3

Material and Methods
MATERIALS AND METHODS

3.1 Materials

Materials used in this study includes different adsorbents viz., cow dung ash (CDA), mango stone ash (MSA), corn cob ash (CCA), orange peel powder (OPP) and granulated activated carbon (GAC). Metal ions used in this study were Cr(VI), Ni(II), Cu(II) and Co(II) and their chemical source were potassium dichromate (K$_2$Cr$_2$O$_7$, Merck), nickel sulphate (NiSO$_4$.6H$_2$O, CDH), copper sulphate (CuSO$_4$.5H$_2$O, CDH) and cobalt acetate (CDH) respectively. Other chemicals used for the estimation of the metal ion concentration were 1,5-diphenyl cabazide, hydroxylamine hydrochloride (NH$_2$OH.HCl), sodium citrate (Na$_3$C$_6$H$_5$O$_2$.2H$_2$O), acetone, disodium bathouproine disulfonate (C$_6$H$_4$N$_2$(CH$_3$)$_2$(C$_6$H$_4$)$_2$(SO$_3$Na)$_2$), sodium acetate (CH$_3$COONa), nitroso-R-Salt, dimethyl glyoxime, iodine, potassium iodide, ammonia, hydrochloric acid, sulfuric acid and sodium hydroxide etc. All the chemicals used in the experimental were of AR grade and the distilled water was used for the solution preparation.

3.2 Adsorbents Preparation

Adsorbents used in this study are CDA, MSA, OPP, CCA which are agro waste materials and easily available and a commercial grade granulated activated carbon (GAC) with varying surface characteristics such as surface area, pore size distribution, particle size and associated with varying amount of surface chemical groups.

3.2.1 Preparation of Cow Dung Ash (CDA)

Cow dung cakes were collected from the near by villages and were burned in air like a fuel to obtain cow dung ash and this CDA was stored in an air tight container for further use.

3.2.2 Preparation of Mango Stone Ash (MSA)

Mango stones were collected from the near by juice vendors in Chandigarh city. These were washed properly and cleaned from any dust and pulp of mango. Then these stones were dried at room temperature and after drying these mango stones were burned in the presence of air. To reduce their
Chapter 3: Materials and Methods

particle size, burned mango stones were grinded in pestle and mortar up to desired level.

3.2.3 Preparation of Orange Peel Powder (OPP)

Orange peels were collected from the near by juice vendors as it is considered as a waste by them. They were dried at room temperature and then washed well with water and again dried. Then orange peels were grinded to give them desired particle size.

3.2.4 Preparation of Corn Cob Ash (CCA)

Corn cobs were collected from the near by villages, dried and washed with water and then with distilled water to remove any dust. Then these were burned in air like a fuel to obtain corn cob ash.

All the above mentioned adsorbents were sieved through I.S.70 mesh screen prior to their use. After sieving, these biomasses were kept in an air tight container for further use. Prior to their use these adsorbents were activated by placing them into an oven at 100°C temperature for 4 h so that in any case any water molecules or environmental gases get adsorbed on the surface of these materials can be removed.

3.3 Characterization of the Adsorbent

The biosorbents used in this study are found to be amorphous in nature which was confirmed by X-ray diffraction study. For the characterization of these materials i.e. to find out the available functional groups and the surface morphology of the materials, Fourier transformation infra red spectroscopy (FTIR) and scanning electron microscopic (SEM) studies were carried out on all the five materials used as adsorbents in this study.

3.3.1 Fourier Transformation Infrared Spectroscopy (FTIR)

Fourier transformation infrared spectroscopy (FTIR) is widely used to find out the presence of various functional groups present in a particular substance. For the characterization of the adsorbents used in this study, it is very important to find out various functional group associated with the adsorbent, so that possible interactions between the adsorbent and metal ions can be explained.
Therefore FTIR spectroscopy was performed for all the adsorbents by using a FTIR spectrophotometer PerkinElmer RXI spectrum. About 150 mg of KBr disks containing approximately 2% of the adsorbent samples were prepared just before recording the FTIR spectra in the range of 400-4000 cm\(^{-1}\) and with a resolution of 2 cm\(^{-1}\). The resulting spectra were the average of 16 scans.

### 3.3.2 Scanning Electron Microscopy (SEM)

The particle size and morphology of the adsorbents are very important in the interpretation of the results. Therefore in this research work these important parameters were determined by SEM. All the adsorbents used in this study were analyzed by using JEOL JSM-6100 scanning microscope. Images were obtained and examined for morphology of these materials. Some of the important aspects which can be obtained from the SEM pictures are as follows:

1. It gives information regarding the external surface, relative roughness and secondary nucleation effects.
2. It also gives information regarding the purity of the phase i.e. whether the phase present is pure or some other phases are also present.
3. The presence of unknown species can also be analyzed by SEM.

### 3.4 Instruments

**Electronic Weighing Balance**

Sartorius Model BL 60S with least measurement 0.0001 gm was used for all type of weighing purposes like weighing metal salts to prepare solutions and weighing of adsorbent doses.

**Ultra Violet and Visible (UV – VIS) Spectrophotometer**

All the metal ions were standardized and determined spectrophotometrically, as we are going to discuss in forthcoming paragraphs. The determination was made with a UV-VIS double beam (HITACHI 330) spectrophotometer to check absorbance of all metal ion solutions to find out their concentration in the solution.
**Chapter 3: Materials and Methods**

**pH Meter**

The solutions of different pH were made with help of pH meter. The pH meter was also used to observe the pH of the influent as well as effluent in the column experiments of this study. A Century model CK710 water analyzer kit was used to adjust as well as verify pH of the solution. The pH meter in this kit with a glass combination electrode was used and it was calibrated at pH 4.0 and pH 7.0 with standard buffer solutions (CDH, AR grade) at experimental temperature 25 ± 1°C, every time before its use.

**Wrist Shaker**

Burrell wrist-action shaker Model: BB was used for the shaking of flasks at desired speed.

### 3.5 Solution Preparation and Metal Ion Estimation

All of the chemical reagents used in this study were of AR grade. Stock solutions of Cu(II), Cr(VI), Ni(II) and Co(II) of concentration 1000 mg L⁻¹ were prepared by dissolving calculated amounts of copper sulphate, potassium dichromate, nickel sulphate and cobalt acetate respectively in distilled water. All working solutions were prepared by diluting the stock solution with distilled water. The pH adjustments were made using sulphuric acid and sodium hydroxide. Detailed information of the procedure is given in the following sections.

**Industrial Effluent**

Since the present study aims at the adsorption of metal ions only, therefore industrial effluents here refer to the metal ion solutions prepared in laboratory at different concentrations.

#### 3.5.1 Calibration Curve

A graph plotted between known metal ion concentration and absorbance at a particular wavelength is known as calibration curve. This curve is used to calculate unknown concentrations. In this study calibration curves were made for all the four metal ions used viz. Cu(II), Cr(VI), Ni(II) and Co(II). Metal ion analysis is done using standard spectrophotometric procedures, detailed account of
preparing stock solutions and analysis methodology for each metal ion is as follows:

### 3.5.2 Estimation of Cr(VI) ions

A stock solution of Cr(VI) ion was prepared by dissolving 2.828 gm of K$_2$Cr$_2$O$_7$ in 1L of distilled water which yielded solution of 1000 mg/L of Cr(VI) ion concentration. Analysis of Cr(VI) ion was carried out using standard analytical procedures [130].

For analysis it needs 10-100 µg of Cr(VI) in 100 mL of final dilution. For this 1 mL of aliquot was diluted to 10 mL with distilled water and then 1 mL from this was taken in 100 mL measuring flask. To this distilled water was added and pH was adjusted to 1 ± 0.3. Then 2 mL of 1, 5-diphenyl carbazide reagent (250 mg/50 mL of acetone) were added to it and volume was made up to 100 mL using distilled water. Absorbance of this solution was checked at a wavelength of 540 nm on a spectrophotometer.

### 3.5.3 Estimation of Cu (II) ions

A stock solution of Cu(II) was prepared by dissolving 3.93 gm of CuSO$_4$.5H$_2$O in 1L of distilled water which yielded solution of 1000 mg/L of Cu(II) ion concentration. For analysis of Cu(II) concentration, following procedure has been adopted:

Reagents:

1. 1+1 hydrochloric acid.
2. Hydroxylamine hydrochloride solution: 50 g NH$_2$OH.HCl were dissolved in 450 mL of distilled water.
3. Sodium citrate solution: 300 g Na$_3$C$_6$H$_5$O$_2$.2H$_2$O were dissolved in distilled water and volume was made up to 1000 ml with distilled water.
4. Disodium bathocuproine disulfonate solution: 1g of C$_6$H$_4$N$_2$(CH$_3$)$_2$(C$_6$H$_4$)$_2$(SO$_3$Na)$_2$ was dissolved in distilled water and volume was made up to 1000 mL.
Procedure:

1 mL of aliquot was diluted to 10 mL with distilled water and then 1 mL from this solution was taken in 50 mL measuring flask. To this 1mL of 1+1 HCl solution was added which was followed by the addition of 4 mL of NH$_2$OH.HCl solution, 4 mL of Na$_3$C$_6$H$_5$O$_2$.2H$_2$O solution and 4 mL of C$_6$H$_4$N$_2$(CH$_3$)$_2$(C$_6$H$_4$)$_2$(SO$_3$Na)$_2$ solution respectively. Absorbance of this solution was checked at a wavelength of 484 nm [131].

3.5.4 Estimation of Co(II) ions

A stock solution of Co(II) of concentration 1000 mg/L was prepared by dissolving 4.2 gm of cobalt acetate in 1L of distilled water.

For analysis of Co(II) ion concentration, 1 mL of aliquot was diluted to 10 mL with distilled water and then 1 mL from this solution was taken in 50 mL measuring flask. To this solution 1mL of 10% sodium acetate was added which was followed by the addition of 1 mL of 0.1% nitroso-R-salt, reddish color complex was appeared. Which gives absorbence maxima at 425 nm and therefore spectrophotometric estimation of Co(II) was made at this wavelength [130].

3.5.5 Estimation of Ni(II) ions

Similarly Ni(II) stock solution was prepared by dissolving 4.479 g of nickel sulphate, NiSO$_4$.6H$_2$O in distilled water and making up the volume to 1000 mL in a volumetric flask which yielded solution of 1000 mg/L of Ni(II) concentration.

For analysis of Ni(II) concentration, following procedure has been adopted [130]:

Reagents:
1. HCl solution: 50 mL concentrated HCl was diluted to 1000 mL with distilled water.
2. Sodium citrate solution: 125 g of sodium citrate Na$_3$C$_6$H$_5$O$_2$.2H$_2$O were dissolved in 500 mL distilled water.
3. Iodine solution (0.005N): 20 g potassium iodide, KI was dissolved in 5 mL distilled water. To this solution 6.4 g of the iodine were dissolved and this solution was diluted to 1000 mL.

4. Dimethyl glyoxime solution: 1 g dimethyl glyoxime was dissolved in 100 mL of conc. ammonia solution and followed by the addition of 100 mL distilled water.

Procedure:

1 ml aliquot of the sample containing not more than 2 µg/mL of the solution was placed in 50 mL volumetric flask. To this 20mL of 0.5N HCl were added which were followed by the addition of 10 mL sodium citrate solution, 2mL iodine solution and 4 mL dimethyl glyoxime solution respectively in order. Then volume was made up to 50 mL with distilled water and allowed to stand for 20 min. Absorbance of this solution was checked at a wavelength of 470 nm [130].

3.6 Batch Adsorption Studies

The adsorption experiments have been studied by batch technique in 100 mL measuring flasks. All the batch studies were carried out at a fixed shaking rate. To optimize this adsorption process following studies has been made.

3.6.1 Effect of Initial Metal ion Concentration

Batch experimental studies were carried out with known weight of adsorbents and 50 mL working solution of different concentration range (50-500 mgL⁻¹) in the 100 mL measuring flasks. These flasks were shaken on a wrist shaker (Burrell wrist-action shaker, Model: BB, Burrell Corp, Pittsburgh, P.A.) at 200rpm for 1 h. After this the flask were kept in a dessicator for 23 h with occasional shaking. This time was sufficient to achieve equilibrium. After an equilibration the adsorbent was separated by filtration through Whatman’s paper no. 40 and aqueous phase concentration of metal ion is analyzed by using standard spectrophotometric methods mentioned above.

3.6.2 Effect of Adsorbent Dose

It was of interest to optimize the amount of adsorbent to be used for the metal ion adsorption studies. Therefore, to study the effect of adsorbent dose,
above procedure was repeated for three different adsorbent doses viz., 0.5, 1.0 and 1.5 g.

3.6.3 Effect of Initial pH of the Metal ion Solution

For pH study 1 g of each adsorbents was shaken for 1 h at 200 rpm with 50 mL of 500 mg/L metal solution with its initial solution pH varied between 1 to 10 and kept for 24 h in a dessicator. After the equilibration period the adsorbent was separated by filtration through Whatman’s paper no. 40 and aqueous-phase concentration of metal ion is analyzed by using standard spectrophotometric methods mentioned in the previous sections.

3.7 Continuous Column Studies

The details of the experimental apparatus and the accessories used in carrying out the experiment were:

Storage Tank Cl tank, painted, fitted with a globe valve, capacity – 100 liters
Pump Centrifugal pump
Peristaltic pump MCLINS Peristaltic pump PP20, calibrated for three different flow rates.
Column Borosil glass, length 50 cm. diameter 1.75 inches – 2 B40 borosil glass joints, a sieve to support adsorbent bed at the bottom of the column and sampling point below it.
Miscellaneous Rubber and silicon tubing and glass fittings like tee, reducer glass apparatus like pipettes, measuring flasks, beakers, iron stand, clamps, borosil Funnel, glass beads
Metal ions Ni (II), Cu (II)
3.7.1 Set up for column experiment

As shown in the Fig. 3.1 a glass column was used in the experimental set up for up-flow fixed bed column experiment which was packed with the appropriate amount of the adsorbent under study (CDA and GAC) with the help of equal number of glass beads to give them a fixed height. Metal ions solution were run from the lower end of the column with the help of a peristaltic pump at three different flow rates for each metal ions viz. Ni (II) and Cu (II) and adsorbents used.

3.7.2 Experimental Procedure

First of all calibration of the peristaltic pump was done to set the required flow rate. A silicon tube of 0.5 cm diameter was used in the peristaltic pump and with the help of a stop watch and measuring cylinder it was calibrated. After the calibration three different flow rates viz. 20, 32.5 and 43.5 mL/min were selected for the execution of the experimental set up. A glass column (specification are given in section 3.7) was then packed with a fixed weight of the adsorbent i.e., 70 g for every experiment and for each adsorbent used in this study. Adsorbents were packed between the two wire meshes and with the help of the glass beads and it was then vibrated gently to obtain uniform distribution of the adsorbent throughout the column height used (32 cm). An overhead feed tank was used to supply metal ion solution which was pumped up-flow with the help of a peristaltic pump through the column which was mounted in vertical position (Fig. 3.1). A uniform concentration of 200 mg/L was used for each metal ion in this experiment.

At the start of each run the pump was turned on and the adjusted flow was allowed to continue through the columns to drive off the entrapped air and to check and fix any leakage in the setup. The initial start up time was noted. After the start of the experiment samples from the feed and effluent were taken and analyzed for the respective metal ion concentrations. This experiment was repeated for three different flow rates viz. 20 mL/min, 32.5 mL/min and 43.5 mL/min and for two different metal ions viz. Ni (II) and Cu (II) on two adsorbents, one of which is GAC and the other is a biomass i.e. CDA to compare their efficiencies.
Fig. 3.1 Set up of continuous column study apparatus.