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Methodology

The current study focuses on examining the potentiality of *Adenanthera pavonina* seeds in removal of heavy metals from simulated waste water solutions. The methodology adopted in order to achieve the aim of the current study, is described in the current chapter.

4.1 Collection and preparation of the biosorbent, powdered seeds of *Adenanthera pavonina* (PSAP)

The seeds of *Adenanthera pavonina* were collected from the village areas of Bongaigaon district (Assam, India). The seeds were washed thoroughly with distilled water, dried and then crushed using a mechanical crusher. The powdered seeds were air dried and sieved to 250 μm . The powdered biosorbent was washed further till the washings were colorless and free from turbidity. Wet biosorbent was soaked in filter paper in order to remove the excess water. The powdered biosorbent was air dried and preserved in air tight containers to be used as such without any pre-treatment. Plates 4.1-4.3 show the steps for the preparation of the powdered biosorbent from the seeds of *Adenanthera pavonina* (PSAP).



Plate 4.1 Washed and dried seeds of *Adenanthera pavonina*

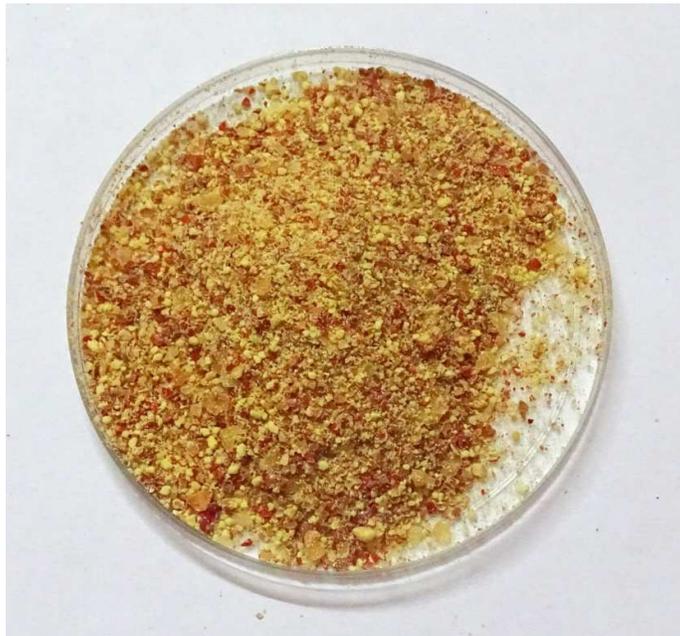


Plate 4.2 Mechanically crushed *Adenanthera pavonina* seeds



Plate 4.3 Powdered and sieved seeds of *Adenanthera pavonina* (PSAP)

4.2 Characterization of powdered seeds of *Adenantha pavonina* (PSAP)

4.2.1 pH of the biosorbent

The pH of the biosorbent was determined by taking 10 g of powdered biosorbent in a beaker and by adding 100 ml of double distilled water to it. The pH of the slurry was then estimated using Systronics μ pH system 361.

4.2.2 Determination of point of zero charge

The point of zero charge (pH_{pzc}) is the pH at which the charge of the positive sites on the surface of the sorbent balances that of the negative sites thereby assigning a zero value to the sorbent surface charge (Fiol and Villaescusa, 2009). In the current study, mass titration (MT) method is used to determine the pH_{pzc} of the powdered *Adenantha pavonina* seeds (PSAP). The method is based on the fact that pH attains a limiting value (pH_{∞}) on continuous addition of sorbent to an aqueous medium. pH_{pzc} is the limiting value (pH_{∞}) at maximum solid content (Zalac and Kallay, 1992). The method was originally suggested by Noh and Shwarz in 1989 and was limited to determination of point of zero charge for oxides, hydroxides and pure samples. Fiol and Villaescusa (2009) used mass titration (MT) to study the pH_{pzc} of vegetable wastes.

Biosorbent doses within the range of 5-100 g/L were taken in conical flasks containing 0.03 M KNO_3 solution. The suspensions were agitated for 24 hours on an orbital shaker at 250 rpm until equilibrium pH was obtained. The pH_{pzc} was obtained from the plateau on the curve obtained by converging the equilibrium pH values versus the biosorbent mass (Bourikas *et al.* 2003).

4.2.3 Determination of moisture content

Determination of moisture content in seeds is important as a minute variation in seed moisture content has a significant impact on the storage life of the seeds. The weight (M_1) of a heat resistant dish with cover was taken and to it 0.5 g of *Adenantha pavonina* seeds powder was added. The weight (M_2) of the dish with cover along with the powdered seeds was recorded. The sample was then placed in a hot air oven at a temperature of 130-133⁰C for 1 hour. After 1 hour the dishes were taken out and the covers were replaced and placed in a desiccator for 30 to 45 minutes at room temperature. The dishes are removed and weighed immediately to record the final weight (M_3) after drying. The moisture content of the powdered *Adenantha pavonina* seeds (PSAP) was determined using the following equation (equation 4.1).

$$\% \text{ moisture content} = \frac{M_2 - M_3}{M_2 - M_1} \times 100 \quad (4.1)$$

where, M_1 = Weight of the container with cover (g), M_2 = Weight of the container with its cover and PSAP before drying (g), M_3 = Weight of the container with its cover and PSAP after drying, $M_2 - M_3$ = Moisture loss, $M_2 - M_1$ = Fresh weight of sample.

4.2.4 CHNS analysis

For the better understanding of the composition of organic matter, elemental analysis of carbon, hydrogen, total nitrogen and sulfur are carried out. The CHNS analyzer provides a rapid determination of these four elements in organic matrix. The CHNS analyzer is based on the classical Pregl-Dumas method. The basic principle involves high temperature combustion process (furnace at 1000⁰C) in an oxygen rich environment, where, carbon is converted to carbon dioxide, hydrogen to water, nitrogen to nitrogen gas or oxides of nitrogen and sulfur

to sulfur dioxide. Additional elements if present are also converted to combustion products and removed using a range of adsorbents.

The powdered *Adenanthera pavonina* seeds (PSAP) were analyzed for the carbon, hydrogen, nitrogen and sulfur content using EuroEA Elemental Analyser at Guwahati Biotech Park (GBP), Assam. 5-10mg of dried samples were weighed in silver cartridge and inserted into the pyrolysis reactor. The combustion products (CO_2 , H_2O , NO_x , SO_x) were then carried by a constant flow of carrier gas (helium) over high purity copper reactor where excess oxygen was consumed as CuO and NO_x products were converted to N_2 . The products were then passed through packed GC column where the products were separated and then detected by a TCD detector. The percentages of individual elements were then calculated by the integration software.

4.2.5 Determination of surface area, pore size and pore volume

Surface area, pore size and pore volume are important physical properties that help in determining the utility as well as the quality of the biosorbent. These parameters are determined using surface area and porosity analyzer (BET). Developed in 1938, by Stephen Brunauer, Paul Hugh Emmett, and Edward Teller, the BET theory is based on the amount of gas that is adsorbed on a surface at a given pressure which further determines the surface area of the substance (Brunauer *et al.* 1938). The BET theory is an extension of the Langmuir theory (Langmuir, 1916) which is based on monolayer adsorption, where as the former follows multilayer adsorption based on the following hypotheses.

- i. Adsorption of gas molecules on solid surfaces takes place in layers, infinitely.
- ii. The adsorption layers do not interact.
- iii. Langmuir theory is applicable to each adsorption layer, individually.

The BET equation is expressed by equation 4.2.

$$\frac{p}{v(p_0-p)} = \frac{1}{v_m c} + \frac{c-1}{v_m c} \left(\frac{p}{p_0} \right) \quad (4.2)$$

where, p and p_0 are the equilibrium and saturation pressures of the adsorbate respectively at the temperature of adsorption

The plot of $p/v(p_0-p)$ against p/p_0 from equation 4.2, gives a straight line with intercept $1/v_m c$ and slope is $(c-1)/v_m c$. v_m is the volume of gas required to form a unimolecular layer of adsorbate and c is the BET constant which is expressed by equation 4.3.

$$c = e^{\frac{E_1 - E_L}{RT}} \quad (4.3)$$

where, E_1 is the heat of adsorption for the first adsorption layer and E_L , also known as heat of liquefaction, denotes the heat of adsorption for the higher layers.

After the value of v_m is obtained, multiplication of the number of molecules required to form a unimolecular layer by the average area occupied by each molecule on the surface gives the absolute value of the surface area of the adsorbent (Brunauer *et al.* 1938).

By extending the procedure for calculation of surface area, so that the condensation of gas takes place in the pores, the fine pore structure of the sample can be estimated. The classical method for estimation of pore size distribution is the Barrett-Joyner-Halenda (BJH) method (Barrett *et al.* 1951). The theory takes into account the capillary condensation in the pores using the Kelvin equation, which in turn assumes a hemispherical liquid-vapour meniscus and a well-defined surface tension (Gelb and Gubbins, 1998). The cumulative or differential pore size distribution is obtained by fitting the adsorbed gas volumes versus relative pressures at equilibrium. The pores are completely filled with the adsorbate as the equilibrium adsorbate pressure attains saturation. From the density of the adsorbate, the

volume occupied can be calculated which in turn helps in the estimation of the total pore volume of the sample.

The sample was sent to the Sophisticated Analytical Instrumentation Centre (SAIC), Tezpur University, Assam, and measurements of surface area, pore size and pore volume were carried out using Nova 1000E (Quantachrome, USA). About 2-3mg of sample was inserted into the surface area and porosity analyzer. Prior to gas sorption experiments, the sample was degassed at 77.35 K under nitrogen flow, so as to make it free from any contaminant.

4.2.6 Determination of surface functional groups

For the characterization of the molecules, infrared spectroscopy is applied. This is a non-destructive technique and requires less than 1 mg sample size. It is based on the principle that characteristic of the molecule's structure, bond angle, and length, chemical bond vibrate at specific frequencies. By absorbing radiation at specific wavelengths, interaction between incident radiation and the molecules takes place. FT-IR enables recording energy absorption over a range of frequencies. By assigning individual absorption peaks to individual chemical bonds, qualitative and quantitative identification of molecules in complex systems can be carried out (Swann and Patwardhan, 2011).

The characterization of functional groups of the fresh as well as the metal loaded biosorbent was determined using IR Affinity, Shimadzu, USA at Department of Chemical Engineering, GUIST, Assam. A small amount of sample was taken on a micro-spatula and about 0.25-0.50 teaspoons of KBr pellets were taken in a mortar and mixed thoroughly by grinding with a pestle. A small quantity of this mixture was assembled in a die and pressed at 1000-5000 psi. The sample along with the die was placed in the FTIR sample holder and analyzed.

4.2.7 Study of surface microstructures (FE-SEM) and determination of elemental information (EDX)

Field emission scanning electron microscope (FE-SEM) utilizes high energy beam of electrons to create a raster scan image of the surface of the sample. The interactions of the electrons with the atoms present on the surface of the sample generate signals that contain information regarding the topography and composition of the surface of the sample. Unlike in thermionic emissions where electric current is employed to heat up a filament, in Field Emission, the electric beam employed does not result in heating up of the filament. The filament is placed in a huge electrical potential gradient and the field emission is achieved. The Field Emission Source (FES) is engineered into a sharp point so that the electrical field can be extremely concentrated. This results in lowering of the work function of the material and thereby enabling the electrons to be released from the cathode. The electron gun provides a strong and steady current in the form of a strong beam and the field emitter (FE) source produces electron beams about 1000 times smaller than that in a standard microscope. This results in improved image quality in electron microscopes (Wang, 2000). The beam diameter must be smaller than the feature and must contain high current density in order to resolve the features on the surface of the specimen. This makes it imperative to condense the electron beam. The application of electromagnetic lenses enables the demagnification of electron beam. The crossover diameter in Field Emission Source is smaller and hence condensation of beam is absolutely necessary to have a scan functional for processing the image. This results in high resolution images of FE-SEM (Pawley, 1997).

Energy Dispersive X-ray Spectroscopy (EDX or EDS) is a powerful, non- destructive and simple technique utilized for the identification of the elemental composition of a sample. The equipment is attached to the SEM so as to acquire the elemental information of the specimen to be investigated. EDX works on the principle of detecting x-rays generated by the

sample placed under electron beam. The atoms in the sample are excited by the electron beam which results in the production of x-rays to discharge excess energy. The beam can sweep over a particular area, a specific point and even a particle of the sample under investigation and generate x-rays of energy characteristic of the atoms that produce them, forming peaks in the spectrum (Russ, 2013). It must be noted that individual element may generate multiple peaks and sometimes, peaks of different elements may overlap to a certain degree. Thus precise control of the electron beam is of ultimate importance.

Surface topography of PSAP was studied by utilizing field emission scanning electron microscope at ultra-high resolution (Zeiss, Sigma VP) equipped with energy dispersive spectroscopy (FESEM-EDX) operated at 3.0 kV at Central Instrumentation Facility (CIF), IASST, Assam. In the present study, since PSAP is a non-conducting sample, it is studied by sputtering a thin coat of gold so that the electrical charging of the sample is prevented on being bombarded by the electron beam. Electron micrographs are taken of the following specimen.

- i. Untreated PSAP
- ii. PSAP after biosorption of Pb(II) in single ion solution
- iii. PSAP after biosorption of Cu(II) in single ion solution
- iv. PSAP after biosorption of Cd(II) in single ion solution
- v. PSAP after biosorption of Pb(II), Cu(II) and Cd(II) from multi metal system.

4.3 Preparation of metal ion solutions

Stock solutions of Pb(II), Cu(II) and Cd(II) of concentration 1000 mg/L were prepared by dissolving appropriate amounts of $\text{Pb}(\text{NO}_3)_2$, $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ and $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (Analytical grade) in 1000 ml of double distilled water. The solutions are

prepared using standard volumetric flasks. The concentration of the simulated waste water ranged from 10 mg/L to 70 mg/L, prepared by dilution of the stock solutions. Fresh dilutions were carried out for each experiment.

4.4 Batch adsorption studies

Batch adsorption equilibrium experiments were carried out to investigate the adsorption of metal ions on the powdered seeds of *Adenanthera pavonina* (PSAP). 100 mL of metal ion solution of definite concentration with a fixed dose of biosorbent was agitated at 120 rpm for a predetermined interval of time at a fixed pH adjusted by adding 0.1N HNO₃ or 0.1N NaOH. Adsorption involves establishing a rapid equilibrium between the metal ions adsorbed on the active sites of the biosorbent (q_e) and the unadsorbed metal ions in solution. After agitation, the mixture was filtered using Whatman 41 filter paper. The concentration of metal ions in the filtrate was examined using Atomic Absorption Spectrophotometer (AAS) (PerkinElmer AAnalyst 200) at Department of Chemistry, Gauhati University, Assam.

Table 4.1 Metal salts used for preparation of stock solution

Metal ion	Salts	Manufacturer	Strength (mg/L)	Grams in 1000mL
Pb(II)	Pb(NO ₃) ₂	Fisher Scientific	1000	1.59
Cu(II)	Cu(NO ₃) ₂ .3H ₂ O	Fisher Scientific	1000	3.80
Cd(II)	Cd(NO ₃) ₂ .4H ₂ O	Fisher Scientific	1000	2.74

The percent removal of heavy metal was calculated using the following equation:

$$\% \text{ Removal of metal ion} = \frac{(C_0 - C_i)}{C_0} \times 100 \quad (4.4)$$

where, C_0 is the initial concentration of heavy metal, C_i is the final concentration of heavy metal. The adsorption capacity, q_e (mg/g) after equilibrium was calculated from the following equation.

$$q_e = \frac{(C_0 - C_e)}{W} \times V \quad (4.5)$$

where, V is the volume of the solution in litres, W is the mass of adsorbent in grams and C_e is equilibrium concentration. Fresh dilutions were carried out for each sorption experiment.

The wavelength and slit widths maintained during operation of AAS for estimation of Pb(II), Cu(II) and Cd(II) is shown in table 4.2.

4.4.1 Effect of contact time

0.5 g biosorbent was weighed in each conical flask containing 100 mL of metal ion solution of concentrations ranging from 10 mg/L to 70 mg/L with pH adjusted at 4,5 and 6 for Pb(II), Cu(II) and Cd(II) respectively. The mixtures were agitated at 120 rpm on an orbital shaker for a definite duration of contact time ranging from 5 to 60 minutes at room temperature. At the end of each time interval, the mixtures were filtered and the filtrates were analyzed using AAS.

Table 4.2 Operating conditions of AAS for quantification of different heavy metals along with their characteristic wavelengths

Metal	Wavelength (nm)	Slit width (nm)	Flame composition
Pb(II)	283.3	0.7	Air- C ₂ H ₂ AAS
Cu(II)	324.8	0.7	Air- C ₂ H ₂ AAS
Cd(II)	228.8	0.7	Air- C ₂ H ₂ AAS

4.4.2 Effect of initial metal ion concentration

100 mL of metal ion solution of different initial concentrations (10 mg/L to 70 mg/L) with pH values of 4,5 and 6 for Pb(II), Cu(II) and Cd(II) respectively were agitated with 0.5 g of adsorbent at an agitation speed of 120 rpm. The contact time for the batch adsorption process was maintained at 30 minutes for Pb(II) and Cd(II), where as for Cu(II) the contact time was maintained at 40 minutes. The samples were filtered after each contact period and the filtrates were analyzed using AAS.

4.4.3 Effect of biosorbent dose

A biosorbent dose of range 1 g/L to 8 g/L were taken in 100 mL of metal ion solutions of a fixed concentration of 10 mg/L at pH values of 4,5 and 6 for Pb(II), Cu(II) and Cd(II) respectively and agitated at a constant speed of 120 rpm for a contact period of 30 minutes for Pb(II) and Cd(II) and 40 minutes for Cu(II) at room temperature. The unadsorbed metal ions in the adsorbate were estimated using AAS.

4.4.4 Effect of initial pH

100 mL of metal ion solution of concentration varying from 10 mg/L to 70 mg/L was agitated with 0.5 g of biosorbent for 30 minutes for Pb(II) and Cd(II) and 40 minutes for Cu(II). The initial pH of the experimental solutions was maintained from 1 to 7. No attempt was made to maintain the pH during adsorption.

4.5 Competitive biosorption

The studies of several authors have reported the removal of individual heavy metals using biosorbents. Heavy metals do not exist as single species in waste water. Rather, they coexist with other inorganic species. Industrial waste water usually contains more than one

metal. Thus it becomes important to study metal removal in a multi-component system. For this purpose, the removal of Pb(II), Cu(II) and Cd(II) are studied in their binary and ternary solutions.

4.5.1 Removal of heavy metals from binary solutions

The removal of heavy metals from binary solutions was studied in the following stages.

- i. Maintaining a fixed initial concentration of Pb(II) and investigating its removal at pH 4 with increasing concentrations of Cu(II).
- ii. Maintaining a fixed initial concentration of Pb(II) and investigating its removal at pH 4 with increasing concentrations of Cd(II).
- iii. Maintaining a fixed initial concentration of Cu(II) and investigating its removal at pH 5 with increasing concentrations of Pb(II).
- iv. Maintaining a fixed initial concentration of Cu(II) and investigating its removal at pH 5 with increasing concentrations of Cd(II).
- v. Maintaining a fixed initial concentration of Cd(II) and investigating its removal at pH 6 with increasing concentrations of Pb(II).
- vi. Maintaining a fixed initial concentration of Cd(II) and investigating its removal at pH 6 with increasing concentrations of Cu(II).

In each stage, the initial concentration of competing metal ion was maintained within the range 20-60 mg L⁻¹, while the concentration was kept constant at 20, 40, and 60 mg L⁻¹ for the dominant metal ion. The adsorbent dose and contact duration were maintained at 5 g/L and 60 minutes, respectively.

4.5.2 Removal of metal ions from ternary solutions

A 12 run Plackett-Burman experimental design with different combination levels of experimental parameters was applied to examine the removal of metal ions in a three metal ion solution. The high (+1) and low (-1) levels of the experimental variables viz. pH, adsorbent dose, contact time and initial metal ion concentration, were set based on the results from previous experiments and the Plackett-Burman design matrix was obtained (Table 4.3). The significance and the influence of these metals on each other's removal was interpreted using ANOVA and Student's t-test.

4.6 Biosorption of heavy metals from industrial waste water on PSAP

In environment, the pollutants like heavy metals do not exist exclusively as single component, but coexists with other inorganic and organic chemical species (Kiran *et al.* 2016). The waste water discharged into the environment, unlike simulated experimental

Table 4.3 Levels of parameters tested in Plackett- Burman design

Cd	Cu	Pb	pH	Dose	Time
10	10	10	4	0.5	30
25	25	25	4	1	35
25	25	10	7	1	30
25	10	10	4	1	35
25	10	25	7	0.5	35
10	25	10	4	0.5	35
10	25	25	4	1	30
10	25	25	7	0.5	35
10	10	25	7	1	30
25	25	10	7	0.5	30
10	10	10	7	1	35
25	10	25	4	0.5	30

solutions, also varies in terms of pH, temperature and electrical conductivity. Although strict guidelines on the operation of industries are enforced by the Central Pollution Control Board (CPCB), the status of environment is far from satisfactory. Majority of industries have facilities for treatment of industrial waste water. However for small scale industries, adopting measures for treatment of effluent incur huge investment which is not conducive to the meagre profit margins of these industries (Rajaram and Das, 2008). As a result, the pollution load on water bodies is immensely enhanced especially due to the presence of toxic heavy metals (Kikot *et al.* 2010). Thus, it becomes important to study the biosorption of heavy metals in waste water discharged into the environment.

In the current study, the industrial effluent is collected from the waste water outlet of an industrial complex in Amingaon, Kamrup District, Assam. The complex includes manufacturing units for plastic molded furniture, cosmetics, steel furniture, mosquito repellent, ayurvedic medicine and pharmaceuticals, and food processing units.

4.6.1 Collection and storage of sample for water quality analysis of industrial effluent

Grab samples of waste water were collected from the outlet of an industrial complex in Amingaon. Polyethylene sampling bottles of 2 litres capacity were used for sampling. Prior to sampling, the bottles were washed with detergent, followed by dilute HNO₃ and tap water and finally rinsed with distilled water. The bottles were then kept under sterilized conditions. After the samples were collected, parameters like pH and EC were estimated immediately, whereas temperature are recorded in situ. For estimation of DO, BOD bottles were used and the water samples were fixed immediately after collection using appropriate reagents suggested by the modified Wrinkler's method. The samples were then transferred to laboratory and refrigerated at 4⁰C, for further analysis.

4.6.2 Analysis of parameters for water quality

The collected samples were tested for the conservative parameters like Ca^{2+} , Mg^{2+} , Na^+ , K^+ , SO_4^{2-} , PO_4^{3-} , Total Solids (TS), Total Dissolved Solids (TDS), Total Hardness (TH), Turbidity, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD) within one week of sampling following procedures as specified by APHA (2005). Table 4.4 exhibits the analytical procedures adopted for the determination of water quality of the collected sample.

4.6.3 Collection and storage of waste water sample for heavy metal analysis

For collection of water samples for heavy metal analysis, polyethylene bottles of volume 2 litres were washed with 25% aqua regia and the rinsed using distilled water. The bottles were then filled with 1% HNO_3 and stored separately in plastic bags. At the time of

Table 4.4 Methods used for water quality analysis

Parameter	Method/ Procedure used
pH	Digital pH meter (Systronics pH system 361)
EC	Systronics Conductivity meter 306
Turbidity	Spectrophotometry (Digital Spectrophotometer, Electronics India)
DO	Winkler's Iodometric method
BOD	Titrimetric method (Equitron Cooling BOD Incubator- Classic Series)
COD	Closed reflux method (ECO 8 Thermoreactor)
TDS	Gravimetric method
Free CO_2 , Alkalinity	Titrimetric method
TH, Ca^{2+} , Mg^{2+}	EDTA Titrimetric method
Na^+ , K^+ (dissolved)	Flame Photometer (Systronics 128)
NO_3^- , PO_4^{3-}	Colorimetry (UV-Vis Spectrophotometer (Agilent Technologies, Cary 100 series)
S^{2-}	Iodometric
Cl^-	Argentometric method
F^-	SPADNS Method (UV-Vis Spectrophotometer (Agilent Technologies, Cary 100 series)

sampling, the acid was removed and the bottles were rinsed thrice and the water samples were collected (Mohamed *et al.* 2012). The entire sample was acidified with 5 mL concentrated HNO₃ per litre of sample.

4.6.4 Digestion of water samples for Pb(II), Cu(II) and Cd(II) analysis

For analysis of dissolved metals, the waste water sample was filtered using a 0.45 µm pore size filter paper (Whatman, Grade NC45 Membrane Filter). 100 mL of sample was transferred to a beaker and 5 mL of concentrated HNO₃ was added to it. The beaker was covered with a watch glass and the solution was then brought to a slow boil on a hot plate so as to achieve the lowest possible volume of about 15-20 mL, just prior to the onset of precipitation or salting out. In order to obtain a gentle refluxing, 5 mL of concentrated HNO₃ was added whenever necessary and the sample was heated continuously until complete digestion was obtained as indicated by a clear solution. Throughout the digestion, total drying up of the sample was avoided. After a final addition of 1 to 2 mL of HNO₃, the beaker walls as well as the watch glass were washed down with distilled water and filtered. The filtrate was then transferred to a 100 mL volumetric flask, cooled and diluted to the mark. Portions of this digested solution was then used for the analysis of initial concentrations of Pb(II), Cu(II) and Cd(II) in untreated industrial waste water using AAS.

4.6.5 Biosorption of Pb(II), Cu(II) and Cd(II) from industrial waste water using PSAP

100 mL of digested sample was taken in a conical flask and the pH was adjusted to 4.0. Adsorbent dose of 5 g/L was maintained and the mixture was rotated on an orbital shaker for 60 minutes at 120 rpm. The mixture was then filtered and the filtrate then analyzed for the final concentration of unadsorbed Pb(II), Cu(II) and Cd(II) ions in industrial effluent using AAS. Further calculations for percentage removal of metal ion and adsorbent capacity, q_e , was executed by applying equation 4.4 and equation 4.5, respectively.

4.7 Instrumentation

The following instruments are required during the biosorption experiments:

Instrument	Application	Model
Mechanical shaker	To agitate the adsorbent / aqueous metal ion mixtures	Tarsons Sphinx Orbital Shaker
pH meter	To measure the pH of the solution	Systronics pH system 361
Analytical balance	To weigh	
CHNS analyzer	To determine percentage composition of carbon, hydrogen, nitrogen, sulphur content of the biomass	Euro EA Elemental Analyser
Surface area and porosity analyzer (BET)	To measure surface area, pore size, and pore volume of the biomass.	NOVA 1000E (Quantachrome, USA)
FT-IR spectrophotometry	To study the functional groups present in the biomass and to identify the possible functional groups responsible for metal binding.	IR Affinity Shimadzu, USA
Field Emission Scanning electron microscopy (FE-SEM)	To evaluate the surface morphology of the biomass before and after adsorption.	Zeiss Sigma, VP
Energy dispersive X-ray (EDX) analyzer	To understand the elemental information for the surface of the fresh as well as metal loaded biomass	Zeiss Sigma, VP
Atomic Absorption Spectrometer (AAS)	To measure residual metal ion concentrations. The analysis on each sample is conducted in triplicates.	PerkinElmer AAnalyst 200