lMaterials and methods of objective 1:

Objective 1: Characterization of landfill leachate, groundwater and soil collected from open landfill sites in the Brahmaputra valley.

3. Area of the study:

Samples were collected from landfill sites. The study area is the **municipality garbage dumping site located near the Morabharali river** (*Longitude*: 92.81°E *Latitude*: 26.63°N), having an area of 8 acres in Tezpur town. The time of the collection was **Pre monsoon** (March 2021& March 2022). Because the Concentration of TDS was maximum during pre-monsoon and reduced during post-monsoon which may be due to the dilution of the ions due to precipitation.

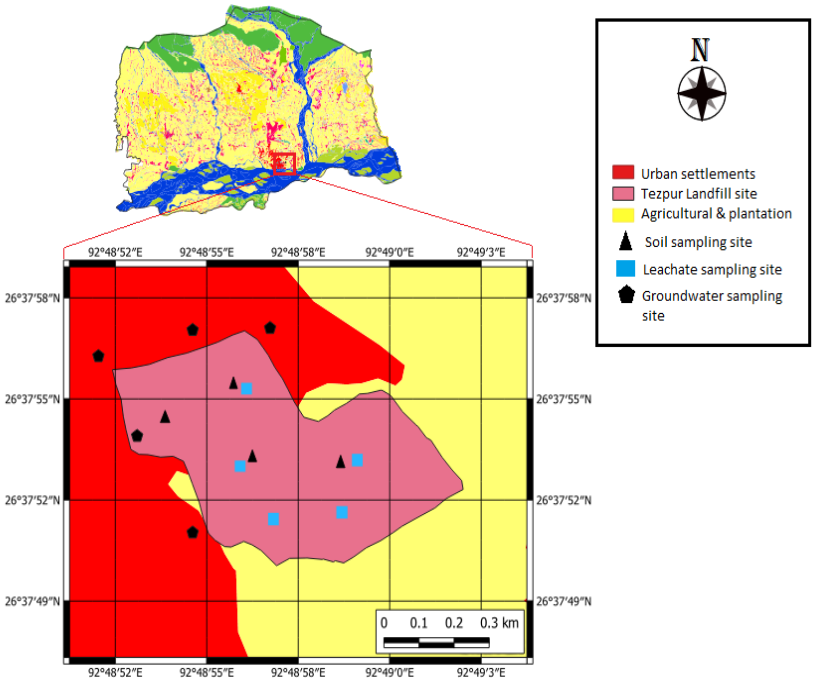

Fig.3.1. Map of study area showing the location of Tezpur dumping site

3.1.Sample collection techniques of landfill leachate, groundwater and soil

3.1.1. Leachate and ground water (APHA, 1995):

Collected representative samples from the landfill sites (3 Replicas). Added HNO_3 immediately after sampling and preserved in fridge. Filtered the samples with syringe filter and checked the concentration of toxic metals by ICP-MS (Thermo Fisher scientific iCAPQnova Series)

3.1.2. Soil (Method EPA 3051A):

Collected soil samples from landfill sites (3 replicas). Digested the soil sample adding HCl and Nitric acid in microwave digester (Acid Digestion). Filtered the soil samples in filtration unit with whatmann filter paper. Checked the concentration of toxic metals by ICP-MS (Thermo Fisher scientific iCAPQnova Series).

3.2. Methodology for environmental and ecological risk assessments:

3.2.1. Quantification of metal pollution in soil

3.2.1.1. Enrichment factor (EF)

Using equation, the enrichment factor (EF) was determined to the amounts of metals that humans have added to soil [6].

$$EF = \frac{(\frac{Co}{Ci})s}{(\frac{Co}{Ci})r} \qquad (1.1)$$

Where$(\frac{Co}{Ci})s$ is the background value of the soil and $(\frac{Co}{Ci})r$ is the concentration of the reference samples

EF values are categorised as follows:

- EF ≤ 1 indicates no enrichment.
- 1 ≤ EF ≤ 2 equals minimal enrichment.
- 2 ≤ EF ≤ 5 = moderate enrichment.
- 5 ≤ EF ≤ 20 = significant enrichment.

3.2.1.2. Enrichment factor percentage (EF %)

Using Eq. [47], enrichment factor percentage (EF%) was determined as follows:

$$EF(\%) = \frac{C-Cmin}{Cmax-Cmin} \times 100 \qquad (1.2)$$

3.2.1.3. Geo-accumulation index (Igeo)

Using Eq. (1.3) (Turekian and Wedepohl, 1961) to determine the level of heavy metal pollution (1.3)

$$Igeo = log2 \times \frac{Cm}{1.5Bm}$$

Where Cm is the concentration of the metal under examination in the soil, Bm is the metal's geochemical background value in the shale, and factor 1.5 is used to account for any fluctuations in the background values caused by lithogenic influences [1]. (1.3)

- $Igeo < 0$ Unpolluted
- $0 < Igeo < 1$ Unpolluted to moderately polluted

- $1 < Igeo < 2$ Moderate pollution
- $2 < Igeo < 3$ Slightly to heavily contaminated
- $3 < Igeo < 4$ Heavily contaminated
- $5 < Igeo$ Extremely contaminated

3.2.1.4. Metal pollution index (MPI)

MPI was calculated by following method described [7]

$$MPI = (C_f^1 \times C_f^2 \times C_f^3 \ldots\ldots C_f^n)^{1/n} \quad (1.4)$$

3.2.1.4. Ecological risk assessment

Ecological risk assessment was performed using Eqs. (1.5) [6]

$$RI = \sum_{i=1}^{m} E_r^i \quad (1.5)$$

$$E_r^i = T_r^i \times C_f^i \quad (1.6)$$

$$C_f^i = C_s^i \times C_n^i \quad (1.7)$$

Where, C_f^i is the contamination factor for the ith metal; C_s^i is the amount of the ith metal in the soil sample; and C_n^i is the amount of the ith metal in the uncontaminated soils. Metals' toxic reaction factor is T_r^i, E_r^istands for Potential Ecological Risk Factor of Individual Metals, RI Stands for Potential ecological risk factor of multiple metals; “n" stands for "Pure or Uncontaminated Soils"; "s" stands for "Soil Sample Under Investigation"; r stands for response factor in T_r^i and risk factor in E_r^i.

To estimate the level of ecological risk, Hakanson classified E_r^i and ERI values as follows:

- $E_r^i < 40 \; and \; ERI < 150$ Represents little ecological danger.
- $40 \leq E_r^i < 80 \; and \; 150 \leq ERI < 300$ Poses a medium risk to the environment.
- $80 \leq E_r^i < 160 \; and \; 300 \leq ERI < 600$ Poses a significant ecological concern.
- $160 \leq E_r^i < 320 \; and \; ERI > 600$ Poses a significant ecological concern.
- $E_r^i \geq 320$ Represents a really powerful ecological risk.

3.2.1.5. Human health risk assessment

The USEPA has proposed a method for determining non-carcinogenic and carcinogenic risks following chemical exposure, which involves evaluating the source of pollution, the route of exposure, and the receptors—key components of the dose-response model. In this study, three primary pathways were identified that impact landfill employees and residents: direct soil ingestion, dust inhalation through the mouth and nose, and skin absorption. According to USEPA guidelines, the Average Daily Doses (ADDs) from each exposure route are calculated in

milligrams per kilogram per day (mg/kg/day). For landfill adult male workers, adults, and children in the residential area, the ADDs for direct soil ingestion (ADDing-soil), inhalation of soil particles (ADDinh), and dermal absorption (ADDderm) were determined using specific equations. This assessment allows for a comprehensive evaluation of the health risks posed by exposure to contaminated soils in and around landfill sites.

$$ADD_{ing} = \left(\frac{C_s \times IngR_s \times EF \times ED \times CF}{BW \times AT}\right) \quad (1.8)$$

$$ADD_{inh} = \left(\frac{C_s \times InhR \times EF \times ED}{BW \times AT \times PEF}\right) \quad (1.9)$$

$$ADD_{der} = \left(\frac{C_s \times AF_s \times SA \times ABS \times EF \times ED \times CF}{BW \times AT}\right) \quad (1.10)$$

According to USEPA guidelines, assessing the health risks associated with exposure to heavy metals in soil involves several parameters: C_s represents the concentration of heavy metals in soil (mg kg^{-1}), $IngR_s$ denotes the soil ingestion rate (mg/day), EF stands for exposure frequency (days/year), ED is the exposure duration (years), CF represents the conversion factor (kg/mg), and BW signifies the average body weight (kg). Additionally, parameters such as average time (days), skin adherence factor (mg/cm^2/day), exposed skin area (cm^2), dermal absorption factor (ABS), inhalation rate (*InhR* in m^3/day), particle emission factor (PEF), and mean body weight for children aged 1 to 6 years (15 kg) are crucial in these calculations. These factors are utilized to compute the Average Daily Doses (ADDs) from exposure routes including direct soil ingestion, inhalation of soil particles, and dermal absorption, ensuring a comprehensive evaluation of potential health risks posed by heavy metal exposure through multiple pathways.

Risks to human health were calculated using the hazard quotient (HQ) and equation to determine the non-carcinogenic risk and hazard index were estimated as

$$HQ = \frac{DIM}{RfD} \quad (1.11)$$

HI= $\sum_1^i HQ$

- HQ or HI ≤ 1, there is no negative impact on human health.
- HQ or HI ≥ 1, there may be a non-carcinogenic danger.

To calculate the incremental lifetime cancer risk (ILCR) in equation or the carcinogenic risk CR_i for each heavy metal

CR_i=$DIM \times SF$

Where, daily intake of metals (DIM) was calculated by formula;

$$DIM = \frac{C \times IR \times EF \times ED}{BW \times AT} \quad (1.12)$$

$$ILCR = \sum_1^i CR_i \quad (1.13)$$

Where BW is body weight (kg), AT is the average time, EF is exposure frequency (days per year), ED is exposure duration (years), and C is the metal concentration (mg kg-1) (day). Adults should consume 100 mg of dust per day and 3.45 L of water per day. With an average body weight of 70 kg, an exposure frequency of 350 days per year was employed, along with a lifetime exposure length of 70 years. In accordance with USEPA recommendations,

- CR_i or ILCR is less than 1×10^{-6}, there is very little risk of cancer.
- $1\times 10^{-4} \leq CR_i$ or ILCR $\leq 1\times 10^{-6}$, there is a tolerable or acceptable carcinogenic risk.
- CR_i or ILCR above 1×10^{-4}, it is hazardous to human health.

3.2.2. **Estimation of leachate pollution index**

3.2.2.1. Leachate pollution index

The Leachate Pollution Index (LPI) is a tool developed using the Rand Corporation Delphi method, as described [25], to identify hazards associated with leachate pollution. The index is calculated using Equation (2.1),

$$LPI = \sum_{i=1}^{n} wipi \qquad (2.1)$$

where wi represents the weight assigned to each pollutant variable, pi denotes its sub-index value, and n is the total number of leachate pollutant variables. The weights (wi) for these variables are determined through professional judgement based on their perceived environmental and health impacts. When data for fewer than 18 leachate pollutant variables is available, as is often the case in studies focusing on sites like the Tezpur dumping site, Equation (2.2) is utilized.

$$\text{LPI}= \frac{\sum_{i=1}^{n} wipi}{\sum_{i=1}^{n} wi} \qquad (2.2)$$

This modified equation normalizes the LPI score by dividing the sum of weighted sub-index values by the sum of weights assigned to the variables (wi), ensuring a balanced assessment despite varying data availability. Parameters typically assessed for Tezpur landfills include pH, COD, B, Cr, Mn, Co, Ni, Cu, Zn, Ag, and Cd, reflecting a comprehensive evaluation of potential pollutants affecting the environment and human health in the vicinity.

3.2.3. Indexing approach of Ground water

3.2.3.1. Heavy metal pollution index (HPI) in Ground water

The Heavy Metal Pollution Index (HPI) has become widely recognized globally as a tool for assessing the severity of heavy metal contamination in water bodies. Developed based on the inverse proportionality of suggested standard values for each component, as proposed [2, 4], the HPI is calculated using Equation (3.1).

$$HPI = \frac{\sum_{i=1}^{n} wiQi}{\sum_{i=1}^{n} wi} \qquad (3.1)$$

Here, n represents the number of parameters considered, wi denotes the weight assigned to each parameter, Qi represents the sub-index calculated for the ith parameter using Equation (3.2). The sub-index for ith parameter is calculated.

$$Q_i = \frac{|Mi-Ii|}{Si-Ii} \times 100 \qquad (3.2)$$

In Equation (3.2), Mi refers to the observed concentration of the heavy metal in the water sample, Ii denotes the permissible limit for the heavy metal concentration as per standards, and Si represents the ideal or safe standard value. The sub-index Qi is derived to indicate the deviation of the observed concentration from the permissible limit as a percentage of the permissible range. For drinking water quality as per Indian standards (BIS, 2012), a suggested critical value of 100 signifies a threshold beyond which the contamination level becomes critical, warranting immediate attention and remediation measures to safeguard public health and environmental integrity.

3.2.3.2. Contamination index (CI)

Water quality contamination intensity (CI) was calculated independently for each metric and assesses the total level of pollution. It was derived using the following Eq. and summarises the combined effects of a number of water quality characteristics deemed dangerous for residential water.

$$CI = \sum_{i=1}^{n} \left\{ \frac{C_{ai}}{C_{si}} - 1 \right\} \qquad (3.3)$$

The analytical value and the allowable value of the ith component are represented by *Cai* and *Csi*, respectively.

For the estimation of CI values, heavy metal concentrations that were higher than allowed limits were disregarded. A common indicator for determining the level of metal pollution in water is the degree of contamination (CI). Based on the indicated CI values, three categories were created for the monitoring sites.

3.2.3.3. Non-carcinogenic health risk

In this study, non-carcinogenic human health risks associated with heavy metal ingestion and dermal exposure from surface and groundwater in the research area were assessed. The focus was on evaluating Hazard Quotients (HQ) and Hazard Index (HI) to gauge potential health risks for adult citizens exposed to heavy metals through drinking water and skin contact. The study employed Chronic Daily Intake (CDI) calculations using formulas outlined by the US Environmental Protection Agency (USEPA, 2004). Equation (3.4) was applied to estimate the CDI of heavy metals through ingestion from food, while Equation (3.5) was used to assess CDI through dermal exposure to contaminated water. These calculations utilized measured concentrations of heavy metals and their corresponding reference values to evaluate exposure risks associated with dietary intake and skin absorption routes. The findings contribute to

understanding the non-cancer health risks posed by heavy metal contamination in both surface and groundwater, emphasizing the importance of effective monitoring and mitigation strategies to safeguard public health.

$$\mathrm{CDI_{oral}(mg\ kg-1\ day-1)} = \frac{C_{hm} \times \mathrm{DI} \times \mathrm{ABS} \times \mathrm{EF} \times \mathrm{ED}}{\mathrm{BW} \times \mathrm{AT}} \quad (3.4)$$

$$\mathrm{CDI_{dermal}(mg\ kg-1\ day-1)} = \frac{C_{hm} \times \mathrm{SA} \times \mathrm{Kp} \times \mathrm{ABS} \times \mathrm{ET} \times \mathrm{EF} \times \mathrm{ED} \times \mathrm{CF}}{\mathrm{BW} \times \mathrm{AT}} \quad (3.5)$$

In this study, non-carcinogenic health risks associated with heavy metals in water were evaluated using Hazard Quotients (HQ) and the Hazard Index (HI) for residents of the research area. The HQ, calculated through Equations (3.6) and (3.7), assesses the ratio of Chronic Daily Intake (CDI) of heavy metals through oral ingestion and dermal absorption to their respective oral Reference Doses (RfD).

$$\mathrm{HQ_{oral}} = \frac{\mathrm{CDI_{oral}}}{\mathrm{RfD_{oral}}} \quad (3.6)$$

$$\mathrm{HQ_{dermal}} = \frac{\mathrm{CDI_{dermal}}}{\mathrm{RfD_{dermal}}} \quad (3.7)$$

Equation (3.8) sums up individual HQ values to determine the HI_{oral}, which represents the cumulative non-carcinogenic health risks from heavy metals ingested orally. Similarly, Equation (3.9) computes the HI_{dermal}, indicating health risks from heavy metals absorbed through the skin.

$$\mathrm{HI_{oral}} = \sum_{i=1}^{n} \mathrm{HQ_{oral}} = \mathrm{HQ_{Cu}} + \mathrm{HQ_{Fe}} + \mathrm{HQ_{Mn}} + \mathrm{HQ_{Zn}} + \mathrm{HQ_{Cr}} + \mathrm{HQ_{Pb}} + \mathrm{HQ_{Cd}} \quad (3.8)$$

$$\mathrm{HI_{dermal}} = \sum_{i=1}^{n} \mathrm{HQ_{dermal}} = \mathrm{HQ_{Cu}} + \mathrm{HQ_{Fe}} + \mathrm{HQ_{Mn}} + \mathrm{HQ_{Zn}} + \mathrm{HQ_{Cr}} + \mathrm{HQ_{Pb}} + \mathrm{HQ_{Cd}} \quad (3.9)$$

According to USEPA guidelines, an HI value exceeding 1.0 suggests potential non-carcinogenic health hazards for residents due to heavy metal exposure via water consumption and skin contact. This threshold value serves as a critical indicator, highlighting the need for monitoring and mitigation measures to protect public health in areas affected by heavy metal contamination in water sources.

3.2.3.4. Carcinogenic health risk

In this study, the Incremental Lifetime Cancer Risk (ILCR) for potential carcinogens such as lead (Pb), chromium (Cr), and cadmium (Cd) was calculated to assess the likelihood of cancer development over a lifetime of exposure. The ILCR was determined by multiplying the Chronic Daily Intake (CDI) of each carcinogen by its respective Cancer Slope Factor (CSF) using Equation (3.10)

$$\mathrm{ILCR} = \mathrm{CDI} \times \mathrm{CSF} \quad (3.10)$$

CSF values were sourced from the California Office of Environmental Health Hazard Assessment (OEHHA, 2019). The total ILCR accounted for both oral and dermal exposures,

while ILCR for surface water considered only dermal exposure. According to the USEPA, the permissible ILCR range for single-element or multi-element carcinogens is between 1.0×10^{-6} and 1.0×10^{-4}, indicating an acceptable level of risk. This evaluation underscores the importance of understanding and mitigating carcinogenic risks associated with heavy metal contamination in the environment.

3.3. Quality Control and Quality Assurance

The current investigation used ICP-MS, or inductively coupled plasma-mass spectrometry (Thermo Fisher scientific iCAPQnova Series), to measure the content of heavy metals [29]. Using approved standard reference material, the estimated values of the researched metals were verified (No. HC073848, CertiPUR Reference Material, INORGANIC VENTURES, Technology VA 24073 USA). Using standard stock, the analytical approach for metals was found to be valid (IV-STOCK-4 M2-MEB656821, Merck CertiPUR®). Standards were produced by adding the proper quantity of a multi-element stock solution. A variety of recognised concentrations were used for standard formulations (i.e., 25, 50, 100, 200, 300 ppb). Each element conducted a calibration run before sample analysis to get the recommended correlation values on the calibration curves. For Cd, Cr, Ni, Pb, Zn, and Cu, the correlation coefficients (R^2) were 0.9942, 0.9842, 0.9749, 0.9893, 0.9988, 0.9997, and 0.9477, respectively. The blanks were run, and frequent washings were carried out to ensure high-quality analysis. The three examination averages for each sample were done. The instrument detection limit was computed using the raw intensity data from the standard and the blank and found out to be less than 0.001 parts per billion (ppb). Cd, Cr, Ni, Pb, Zn, and Cu each had quantification limits of 0.000941, 0.000048, 0.00059, 0.00183, 0.0000671, and 0.000692, respectively.

3.4. Analytical method for chemical analysis of soil, ground water and leachate

3.4.1. pH

10 g (2 mm) air-dry soil was placed in a conical flask. Using a graduated cylinder or a 50-mL volumetric flask, 25 mL of deionised water was added to a 1:2.5 soil solution. After thoroughly stirring the liquid with a glass rod, it was let to stand for nearly an hour. The pH metre (pH 700) was calibrated, and the pH of each sample was recorded.

3.4.2. Electrical conductivity

0.02M KCl solution was prepared by dissolving 1.4912g KCl in 1 L of distilled water. This solution's EC is 2.39 mmhos/cm at 18 degree C and 2.768 mmhos/cm at 25 degrees C. 4g of air-dried soil samples taken into a 100-ml glass. 10ml of distilled water was added using a 50-ml volumetric flask in a 1: 2.5 sample suspension, mixed using a glass rod, and allowed to stand for 30 min. The suspension was allowed to stand till the particles in the sample settle down. The conductivity meter's cell was immersed in standard KCl solution, and the meter's conductivity value was recorded. The cell was washed with DW before being immersed in the test sample suspension, and conductivity was measured.

3.4.3. Bulk density

The empty glass container's weight is taken and entered into the weighing machine (W_1). The glass container is completely filled with dried soil sample, and the bottle's weight and the weighing machine records the soil sample. (W_2). The bottle was completely filled with water, and the amount of water was calculated (V, in cm^3 or ml).

3.4.4. Soil organic carbon

In a 500 ml conical flask, 1.0 g of soil was collected. Ten millilitres of $K_2Cr_2O_7$ solution and twenty millilitres of concentrated H_2SO_4 were heated until a few bubbles emerged. It was left to cool for a while. Following that, 200ml of distilled water added. There was 10 mL orthophosphoric acid and 1.5 mL diphenylamine indicator added. The solution was titrated to a vivid green colour with standard 0.5M $FeSO_4$ solution.

3.4.5. Available nitrogen

A 5g soil sample was obtained and placed in a 1000 ml round bottom volumetric flask. It was mixed with 100ml 0.32% $KMnO_4$, 100ml 2.5% NaOH, 20ml distilled water, and 1ml liquid paraffin. For distillation, the digestive tube was well-fitted in the distillation unit. In a 250ml conical flask, 20 ml of N/50 N H_2SO_4 was mixed with a few drops of methyl red indicator. The flask was attached to the ammonia exhaust pipe. Distilled the contents of the tube for 100 ml, then collected the distilled ammonia in a N/50 N H_2SO_4 solution in the conical flask. Following distillation, the ammonia solution was recovered and titrated with 0.8% NaOH to achieve the original blue color. Simultaneously, a blank without soil was performed using the same process. The burette reading was taken down.

3.4.6. Available phosphorus

A 250 ml conical flask was filled with a 2g sample. The sample received 20ml of extracting 0.5 M $NaHCO_3$ solution (pH 8.5) and 1g of phosphorus free charcoal. The flask containing the sample was shaken for 30 minutes at 20 rpm in the mechanical shaker. The sample solutions were filtered after 30 minutes with Whatman no. 42 filter paper. 5ml of filtrate was mixed with 4 to 5 drops of 2,4 dinitrophenol, 4N NH_4OH, 4 N HCl, (5%) Stannous chloride, and 5 ml ammonium molybdate, and the volumes were brought up to 25 ml with DW. The solution was agitated, and a spectrophotometer reading at 660 nm was taken.

3.4.7. Available potassium

1 gram of air-dried soil was mixed with 10 millilitres of pH 7 normal ammonium acetate and stirred for 5 minutes. The filtered extract is analyzed using an atomic absorption spectrometer in emission mode at 776 nm to assess the quantity of potassium that is easily accessible.

3.5. Germination Studies

3.5.1. Pot germination

Both the sample and the control soil were collected in pots. *Solanum lycopersicum* (tomato), *Brassica nigra* (mustard), *Spinacia oleraceae* (spinach), and *Coriandrum sativum* (coriander) seeds were used in the germination test. In separate pots of the control soil sample and the soil sample from the dump, 20 undamaged seeds of each kind were taken and planted. Each day, distilled water was added to the pots. The 15-day germination test was ended after the initial period of time. Seed germination was determined by visual seedlings emergence and distilled water was added at alternate intervals for seedling growth. The germination index %, number of seeds germinated, length of root, length of shoot, leaf count, relative root growth, relative seed germination, and number of leaves was calculated.

3.5.2. Petri dish germination

Seed material of *Solanum lycopersicum* (tomato), *Brassica nigra* (mustard), *Spinacia oleraceae* (spinach), *Coriandrum sativum* (coriander) seeds were taken. Five millilitres of waste leachate samples were introduced to a 90 mm Petri dish with twenty unharmed seeds of almost equal sizes filter paper. Seeds were evenly distributed. Only deionized water was introduced to Petri dishes as a control sample. The Petri plates had been covered and kept warm while being incubatory. After 72 hours, the number of seeds that grew into plants, the length of their roots, shoots, and leaves, as well as their RRG %, RSG %, and GI %, were all measured.

3.6. Heavy metal determination

Determination of heavy metals after pot experiment nitric acid and hydrochloric acid were combined in a 3:1 ratio and added to a conical flask holding 0.2 g of soil, for leachate samples, 2ml of leachate was poured into a conical flask and 3ml of nitric acid and 1ml hydrochloric acid was added to it, for plants samples, dried in a hot air oven for 24 hours and grinded in a mortar and pestle, 0.2 g of grinded samples were taken, 2 mL deionized water, 2 mL with 35% H_2O_2 and 4 mL with 65% HNO_3 were added to it .All of the samples were allowed to heat on a hot plate to allow the acids to dissolve and extract the appropriate components. The heavy metal concentration of the samples was determined using ICP-MS after the digested solution was filtered using syringe filter paper and diluted ten times.

Materials and methods of objective 2:

Objective 2: Characterization and application of PAni biocomposites (doped and undoped) for removal of toxic heavy metal from landfill leachate.

3.7. Synthesis mechanism of conductive biocomposite

3.7.1. Reagents

The synthesis of conductive polymer-based biocomposites utilized several chemicals and materials. Monomer aniline (purity 99%; Aldrich, India), dopant para-toluene sulfonic acid (pTSA) (purity 99%; Aldrich, India), and ammonium peroxodisulfate $(NH_4)_2S_2O_8$ (APS) (Merck, India) were used as key chemical reagents. Ethanol (purity 99.9%; Merck, India) served as the solvent in the experimental procedures. Sawdust and sugarcane bagasse, collected locally from a wood processing mill and a sugarcane juice vendor respectively, were employed as the biomass substrates. These materials were thoroughly washed with water to remove impurities, followed by a final cleaning process to ensure their purity before use in the synthesis of the biocomposites.

3.7.2. Synthesis procedure of polyaniline biocomposite

Conductive PAni-based biocomposites were synthesized by polymerizing monomer aniline in the presence of sugarcane bagasse and sawdust separately. Initially, a calculated quantity of sugarcane bagasse or sawdust was added to an aqueous solution of monomer aniline and dopant pTSA, followed by stirring for 1 hour. Subsequently, APS oxidant was added dropwise to the aniline/dopant solution, with the reaction proceeding under constant stirring for 12 hours at a temperature maintained at 0-5°C using an ice bath. The molar ratio of monomer aniline to dopant pTSA was maintained at 1:1, while the ratio of monomer to oxidant was 1:1.1. The formation of green-colored conductive PAni-coated sawdust/sugarcane bagasse indicated successful polymerization of PAni. The resulting powder was filtered and washed multiple times with distilled water until the washings were colorless, followed by a final wash with methanol to remove residual monomers, oligomers, and other organic impurities. The PAni biocomposite was then dried in a vacuum oven at 60°C for 12 hours and stored in a tightly closed container. For a portion of the biocomposite, the doped PAni was converted to its undoped state by immersing it in 1 M ammonia solution for 2 hours, followed by filtering, washing, and drying in a vacuum oven at 60°C for 12 hours. This process ensured the PAni-coated sawdust/sugarcane bagasse was in both conductive (doped) and non-conductive (undoped) states [11].

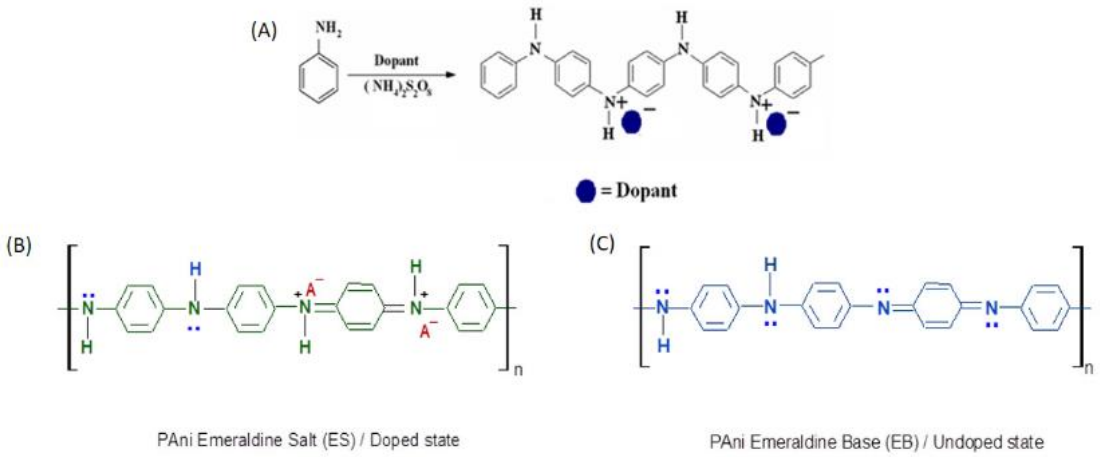

Fig.3.2. A) Reaction scheme for synthesis of PAni; B) Schematic diagram of doped PAni; C) Schematic diagram of undoped PAni.

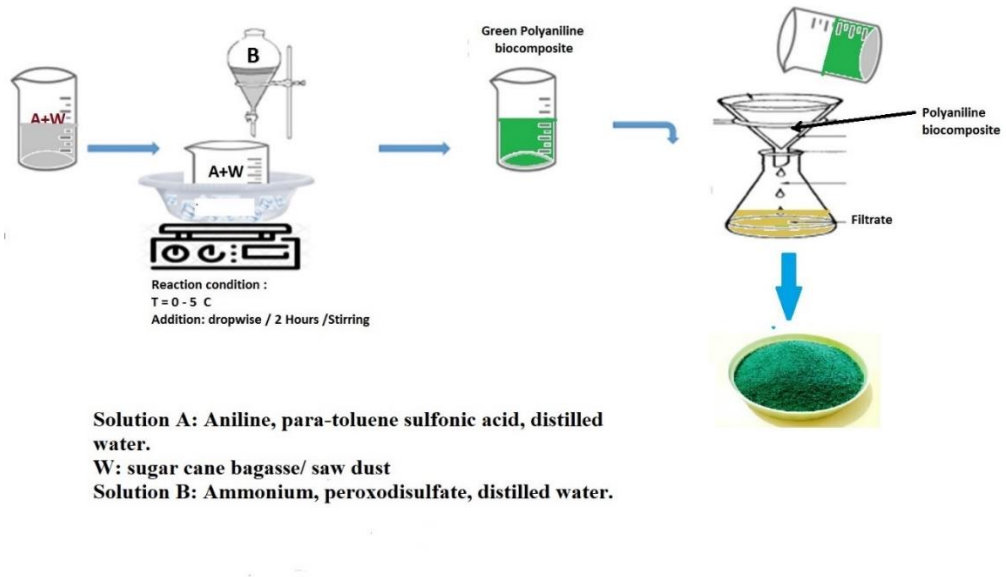

Fig.3.3. Synthesis mechanism of PAni biocomposites

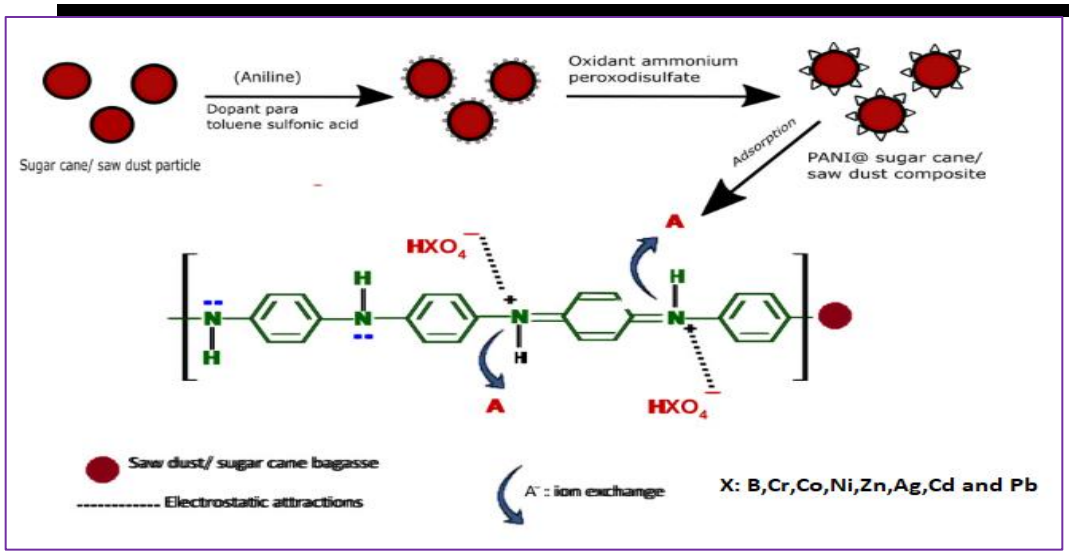

Fig.3.4. Steps for preparation and removal of heavy metal by PAni bio composites

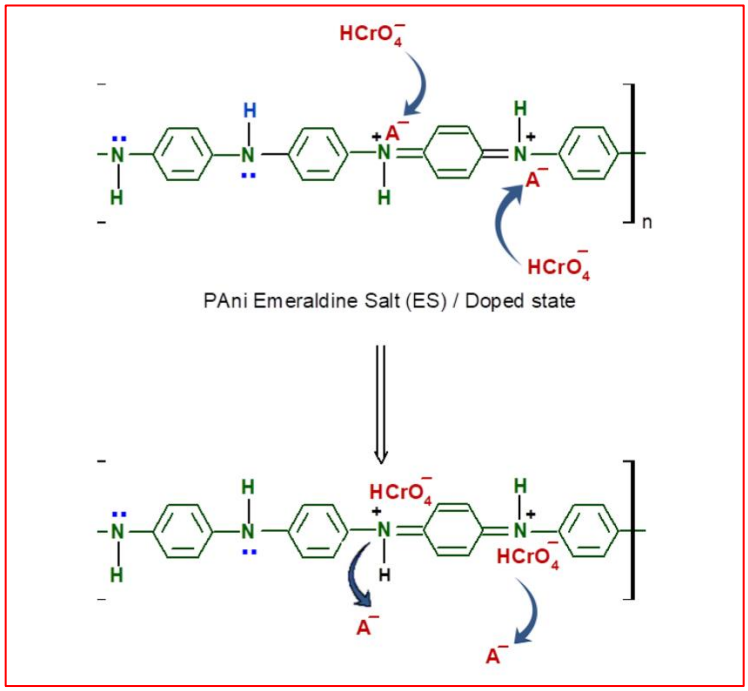

Fig.3.5. Metal removal by doped PAni bio-composites

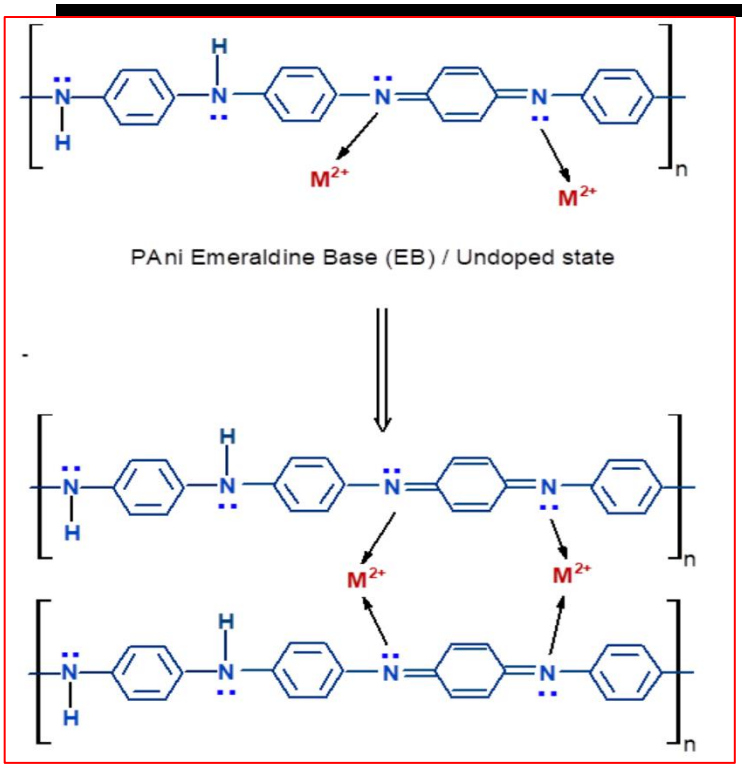

Fig. 3.6. Metal removal by undoped PAni bio-composites

3.8. Parameters selected for characterization of biosorbents

The crystal structure and particle size of the PAni biocomposite materials were determined using powder X-ray diffraction (XRD) with Cu Kα radiation. The average crystallite size was calculated from the diffraction peaks using Debye–Scherrer's formula.

$$d = \frac{0.9\lambda}{\beta \mathrm{Cos}\theta}$$

This formula relates the crystallite size d to the X-ray wavelength λ, the broadening of the diffraction peak at half maximum β\betaβ, and the diffraction angle θ. The morphological and elemental composition of the synthesized biodegradable and conductive materials were examined using scanning electron microscopy (SEM) (Jeol JSM-6360) coupled with an energy-dispersive X-ray (EDX) analyzer. Particle size and shape were further confirmed by analyzing the nanoparticles (NPs) with transmission electron microscopy (TEM) (Philips CM200). Functional group characterization was performed using a Perkin Elmer Spectrum 100 FTIR spectrometer, providing insight into the chemical functionalities present in the PAni biocomposites.

3.8.1. Surface morphology and surface elemental composition study

To analyse the morphological changes occurring in a biosorbent prior and after metal treatment, biosorbent samples were imaged in a JEOL-JSM-6390 LV Scanning Electron Microscope (JEOL USA, Peabody, MA, USA) under Sophisticated Analytical Instrumentation Centre (SAIC) of Tezpur University. SEM uses high-energy beam electrons generated in a vacuum from a tungsten filament lamp. The surface of the sample is coated with nonconducting material and is scanned with focused electron beams that interact with the atoms of the sample at various depths. This results in exciting of atoms that emit a secondary electron beam which gets detected by the detector to produce an image. An energy-dispersive X-ray (EDX) unit, operating with a step-up voltage of 15 kV and 20 kV was utilized for determining the elemental composition of the biosorbents. SEM-EDX instrument helps in identifying and quantifying the elemental compositions of the given sample [11].

3.8.2. Surface functional group study by FTIR

FTIR analysis of all the biosorbent samples was performed to identify the presence of varied functional groups in the biosorbent samples. The analysis was carried out with a spectrometer (Perkin Elmer Spectrum100) operating at a wavelength of 400 to 4000 cm^{-1}. FTIR spectra were obtained by the KBr pellets method by mixing100 mg of dry KBr salt with 1mg dry biosorbent (treated and untreated) which is then pressed in a muller by applying a force of 1ton for 1 minute. The pellet thus formed was used for the IR spectra study[11].

Materials and methods for objective 3:

Objective 3: Assessment of the adsorption kinetics and equilibrium studies for the potential removal of toxic metals using polyaniline (PAni) biocomposite.

3.9. Reagents and Instruments

All the reagents used for this investigation are of analytical grade with purity of 98.5 % and these are purchased from the Marck life science Private limited. The instruments utilized for the analysis are performed at sophisticated analytical instrumentation facility (SAIC), Tezpur University. Stock solutions containing 1000 ppm were made for two metals Cd and Pb by dissolving 1.8274g of $CdCl_2$ and 1.5984g of $Pb(NO_3)^2$ in 1000 mL of water. To achieve the desired solutions with concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm and 50 ppm the stock solution was diluted using a serial dilution method. The pH levels were adjusted to desired values of 2, 4, 6.5, 8 and 10, in the working solution by adding either a solution of 0.1 N HCl or a solution of 0.1 N NaOH and were measured using a Hanna multiparameter meter. The prepared solutions are ready for the batch experiments. The glassware used in the experiments were washed drastically and shocked overnight in 20% HNO_3 then it was washed under tap water and dried. The glassware was rinse with distilled water before using for batch experiments.


Selection of bio adsorbent for this study (sugarcane bagasse and Wooden sawdust)


Chemically modified the bio adsorbent with PAni

Desired concentration of metal solution for checking the parameters (contact time, dosage, pH and initial ion conc.)

Shake using mechanical rotatory shaker for the desired time

Filter the sample using syringe filter of pore size 0.2 µm and stored in air tight container

The filtered solution is stored and analysis is done in thermos scientific iCE 3000 series AAS (Atomic absorbance spectroscopy) for the quantification of uptake capacity of heavy metals by the bio adsorbent.

Fig 3.7: Schematic representation of process conducted for the batch experiments.

3.10. Batch adsorption experiments

Batch experiments were systematically conducted to optimize various adsorbent parameters such as contact time, adsorbent dose, initial metal ion concentration, and pH for different biosorbents prepared from PAni biocomposites. Specifically, 0.5 g of the adsorbent, accurately weighed using an electronic balance (Denver/TP214DE), was added to 250 mL Erlenmeyer flasks containing 50 mL of each metallic aqueous solution (Cd and Pb). The mixtures were then shaken at a steady speed using a mechanical flask shaker for a fixed duration at constant room temperature. Samples of 10 mL were withdrawn at 10-minute intervals, filtered immediately using a syringe filter (Whatman Puradisc, 0.45 µm pore size), and transferred to airtight, sterile containers. The study investigated the effects of adsorbent dose (0.1-0.5 g), contact time (6-24 hours), pH (2-10), and initial metal ion concentration (10-50 mg L^{-1}). To ensure accuracy, all experiments were performed in triplicates, with only the average values considered for data interpretation. Two control experiments were conducted: one without adsorbent to check for potential metal adsorption by the flask walls and one without metallic ions (using distilled water) to assess any leaching from the adsorbents. The concentrations of metallic ions in the solutions before and after equilibrium were measured using Inductively Coupled Plasma-Mass Spectroscopy (Thermofisher Scientific, Model: iCAP RQ (C2))

The concentration of metal ions was determined by Inductive Coupled Plasma Mass Spectroscopy (Thermo Fisher scientific iCAPQnova Series ICP-MS).

$$RE(\%) = \frac{(Co-Ce)}{Co} \times 100 \qquad (4.1)$$

The removal efficiency (RE) and adsorption capacity (Qe) in mg g-1of contaminants was calculated by the equation (Benafqir et al. 2019; El Ouardi et al. 2019):

$$Qe = \frac{(Co-Ce)}{m} \times V \qquad (4.2)$$

Where, Co is the initial concentration of adsorbate in mg L^{-1}, Ce is the final concentration of adsorbate in mg L^{-1}, m is the mass of the PAni biocomposite in g, and V is the volume of the adsorbate solution in L [10].

The influence of various experimental variables on adsorption was calculated using Equations (1) and (2) respectively.

3.10.1. Contact time

A 0.3g of bio-composite is weighed using electronic balance then transferred to conical flask (250 mL) having 30 mL of metal ion conc. solution and the sample were shake using mechanical rotary shaker for a total duration of 24 hrs. At an interval of 6 hrs, a 5 mL of content is taken out and filtered using syringe filter and stored in an air-tight and

sterile container for further analysis. The experiment is carried out in a batch wise manner for undoped (UD), doped (dope) PAni modified bio-composite of wooden sawdust and sugarcane bagasse respectively [9].

3.11. Absorbent dosage

Absorbent dosages were varied at three different quantities (0.1, 0.25 & 0.5 g) and were weighed using electronic balance and transferred to conical flask (250 mL) containing 10 mL of metallic concentration solution 10 ppm. The solutions were prepared simultaneously and were shake in mechanical rotary shaker for 24hrs. After 24 hrs, 5 mL of sample is taken out and filter with the help of syringe filter and this samples are stored in an air-tight and sterile container for further analysis. The procedure is repeated for dope sugarcane, undoped sugarcane, dope sawdust and undoped sawdust simultaneously [9].

3.12. Initial metal concentration

For determining the initial metal ion 0.3 g of absorbent is weighed using electronic balance and transferred into a conical flask (250 mL) containing 30 mL of different metal ion conc. (10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm) respectively. The content is then shake in mechanical rotary shaker for 24hrs. At interval of every 6hrs, a 5mL of sample is taken out and immediately filtered using syringe filter and stored in an air-tight and sterile container. Simultaneously for different adsorbent dope/UD PAni modified sawdust and sugarcane bagasse are also being carried out [9].

3.13. pH of the solution

A 0.3 g of absorbent is measured using electronic balance and transferred into a conical flask (250 mL) containing 30 mL of different pH (2, 4, 6.5, 8, 10 pH) adjusted metal solution of 10 ppm concentration. The pH of the metal concentration solution is being adjusted with our desired pH level using 0.5 N HCl or 0.5 N NaOH. The sample is being shake using rotary shaker for a duration of 24 hrs and then 5mL of sample is taken out and filter using syringe filter. The filtrate is stored in an air tight packed and sterile container for further analysis. The same process is being repeated for UD/dope PAni modified sawdust and sugarcane bagasse [9].

3.14. Adsorption models

The Adsorption kinetic allow us to comprehend the mechanism of metal adsorption and identify rate-controlling steps. Adsorption kinetics studies provide valuable insights into the mechanism of adsorption and can forecast equilibrium time, which is the time it takes for the adsorbent to remove adsorbate from aqueous solution during the biosorption reaction. The adsorption process is influenced by two variables: equilibrium and kinetics [8].The study of adsorption kinetics not only provides important insights into the mechanism of adsorption but also helps in predicting the equilibrium time which is the time required by the adsorbent to remove adsorbate from the aqueous solution in biosorption reaction. The mechanism of metal adsorbed onto

the adsorbent surface, several kinetic models were applied for determining the rate controlling step: 1) Lagergren's pseudo first-order model, (2) Pseudo second-order model.

3.11.1.1 Lagergren's pseudo first-order model:

The pseudo-first-order model is appropriate for reactions that follow a higher-order true rate law but exhibit first-order behavior, thus termed pseudo-first-order reactions. This kinetic model suggests that the rate at which active sorption sites are occupied is directly proportional to the number of unoccupied sites, and it is based on the solid's capacity. The model is generally expressed by the following equation:

$$\frac{d_{q_t}}{d_t} = (q_e - q_t)k_t \qquad (4.3)$$

q_e is the amount of metal ion sorbed at equilibrium (mg g^{-1}), $q_t$is the same and at time t, K_1 is the Lagergren rate constant (min^{-1}).

$$q_t = q_e\ (1 - e^{-k1t}) \qquad (4.4)$$

The linear form of the pseudo-first-order model is expressed

$$Log(q_e\text{-}q_t) = Log q_e - \frac{k_1 t}{2.303} \qquad (4.5)$$

The rate constants K_1(min^{-1}) can be determined from the slope of the plot of log (qe – qt) versus t.

3.11.1.2. Lagergren's Pseudo-second-order model

The pseudo-second-order kinetic model is grounded in the sorption capacity of the solid phase and effectively predicts behaviour over the entire study range. It assumes that the rate-controlling step is chemisorption, involving valence forces through sharing or exchange of electrons between adsorbent and adsorbate. The rate constant of the Pseudo second-order equation can be determined from the plot t/qt versus t. This model is useful for identifying the rate-limiting step, which is surface adsorption, implying that chemisorption is involved. The resulting removal of the adsorbate from the solution is due to physicochemical interactions between the two phases. The pseudo-second-order model is typically represented in its linear form and is expressed by the following kinetic equation for chemisorption.

$$\frac{t}{q_t} = \frac{1}{K_2 q_e^2} + \frac{1}{q_e} t \qquad (4.6)$$

where K_2 (g mg^{-1} min^{-1}) is the rate constant of second order. q_e is the amount of metal ion sorbed at equilibrium (mg g^{-1}), q_t is the same and at time t.

3.11.2. Adsorption isotherm models:

Adsorption isotherm asserts the relationship between the mass of adsorbate to be adsorbed per unit weight of adsorbent at equilibrium concerning the concentration of adsorbate present in the

liquid phase (aqueous solution) and grants imperative design data for adsorption systems. The efficacy of the process of adsorption was determined by adsorption isotherm studies. The isotherm models enforced to the adsorption of heavy metals by biosorbents were (1) Langmuir, (2) Freundlich isotherm models.

3.11.2.1. Langmuir isotherm

The Langmuir isotherm presumes monolayer adsorption facilitated by finite sorption sites of uniform strategies onto an adsorbent surface thereby, allowing no transmigration of adsorbate in the plane surface. The model assumes adsorption onto the adsorbent surface in form of a monolayer onto finite binding sites which is identical at equilibrium. The Langmuir isotherm relationship is of a hyperbolic form which can be linearized as shown in:

$$\frac{C_e}{q_e} = \frac{1}{bq_{max}} + \frac{1}{q_{max}} C_e \qquad (4.7)$$

Where, C_e is metal ion concentration in the solution at equilibrium (mg g^{-1});q_e is the metal ion concentration on the adsorbent at equilibrium (mg g^{-1}); q_{max} is the maximum sorption capacity of the adsorbent. b is the Langmuir constant which is the coefficient related to the affinity between the sorbent and sorbate. The higher the value of b, the higher is the affinity of the sorbent for the sorbate.

Another vital characteristic of the Langmuir isotherms is the separation factor or equilibrium parameter, R_L that is expressed by

$$R_L = \frac{1}{1+bC_0} \qquad (4.8)$$

The value of R_L indicates whether the Langmuir isotherm to be favorable ($0 < R_L < 1$), unfavorable ($R_L > 1$), linear ($R_L = 1$) or irreversible ($R_L = 0$).

3.11.2.2. Freundlich isotherm

The Freundlich adsorption isotherm assumes a multilayer adsorption state and was first used to describe gas phase adsorption and solute adsorption. The Freundlich isotherm provides an empirical equation principally excellent in fitting highly heterogeneous sorbent data obtained from the sorbent systems. This isotherm provides an understanding related to the surface heterogeneity and the exponential distribution of the active sites and their energies. The relationship can be expressed in the linearized logarithmic form as shown in

$$\log q_e = \log K_f + \frac{1}{n} \log C_e \qquad (4.9)$$

Where, K_f and n are the Freundlich constants signifying adsorption capacity and intensity. A plot of log q_e against logC_e gives a linear curve from where, K_F(Freundlich isotherm constant) and n (adsorption intensity) are calculated from the intercept and slope of the plot, respectively. The value of n indicates the degree of nonlinearity between solution concentration and adsorption. If n=1, then adsorption is linear; if n < 1 , then adsorption is Chemisorption ; if n > 1 , then

adsorption is a physisorption. The value of n between 1 and 10 indicates favorable adsorption because the higher the value of n, the stronger is the interaction between the biosorbent and metal ions. The slope ranging between 0 and 1 is a measure of adsorption intensity or surface heterogeneity. The literature suggests that n becomes more heterogeneous as its value gets closer to zero.

3.15. Regeneration studies

A 1g of bio-composite is weighed using electronic balance then transferred it to a conical flask (250 mL) containing 100 mL metal ion conc. solution of 10 ppm. The sample is kept for absorption and shake at a steady speed using mechanical rotary shaker for 180 min (i.e. 3 hrs). The sample is taken out after absorption studies under optimal conditions and filtered using Whatman No. 42 filter paper, the desorption of Pb (II) & Cd (II) from UD/ dope PAni modified sawdust & sugarcane bagasse composite were carried out using 1 N NaOH or 1 N HCl as a desorbing solution and repeatedly rinse with distilled water [10]. The elute is again filtered using syringe filter having pore size of 0.25 µm. The regenerated composite is dried using hot air oven at 40-50 °C overnight. The obtained PAni modified UD/dope sawdust & sugarcane bagasse were reused for the further adsorption cycle. This procedure was repeated for five cycles to see its efficiency, reusability, cost effectiveness and environmental friendliness.

3.16. Statistical Analysis

Statistical analysis is conducted to determine whether there are any significant variations either within or between the groups. Analysis of variance (ANOVA) is undertaken to determine whether the study supports the null hypothesis or alternative hypothesis. For this study, I have chosen two-way or one-way analysis of variance for all of the operation parameters: including contact time, dosage, pH, and initial ion concentration depending upon how many dependent and independent factors are there with the help of Microsoft Excel 2019. Apart from this I have also conducted DMRT (Ducan multiple ranging test) a post hoc method after analysis of variance test to identify which specific means are different from each other while comparing multiple groups. This test rank the means and groups them based on their statistical difference with Greek letters. DMRT controls the overall Type I error rate (the probability of incorrectly rejecting a true null hypothesis) more effectively compared to multiple t-tests. It provides a clear and interpretable way to present which groups are significantly different. DMRT is performed using the IBM SPSS (statistical package for the social science) statistics 26 version.

References:

1. Birch, G. (2013). Use of Sedimentary-Metal Indicators in Assessment of Estuarine System Health. In *Treatise on Geomorphology* (Vol. 14). Elsevier Ltd. https://doi.org/10.1016/B978-0-12-374739-6.00392-4
2. Dash, S., Borah, S. S., & Kalamdhad, A. (2019). A modified indexing approach for assessment of heavy metal contamination in Deepor Beel, India. *Ecological Indicators*, *106*(March), 105444. https://doi.org/10.1016/j.ecolind.2019.105444
3. Kumar, D., & Alappat, B. J. (2005). Evaluating leachate contamination potential of landfill sites using leachate pollution index. *Clean Technologies and Environmental Policy*, *7*(3), 190–197. https://doi.org/10.1007/s10098-004-0269-4
4. Mohan, S. V., & Nithila, P. (1996). Journal of Environmental Science and Health. Part A : Environmental Science and Engineering and Toxicology : Toxic / Hazardous Substances and Environmental Engineering Estimation of heavy metals in drinking water and development of heavy metal pollution. *Journal of Enviromental Science Health*, *2*(October 2013), 283–289.
5. Sharma, S., Nagpal, A. K., & Kaur, I. (2018). Heavy metal contamination in soil, food crops and associated health risks for residents of Ropar wetland, Punjab, India and its environs. *Food Chemistry*, *255*(February), 15–22. https://doi.org/10.1016/j.foodchem.2018.02.037
6. Turekian, K. K., Haven, N., Hans, K., & Universitat, W. M. Der. (1961). KARL K. TUREKIAN Dept. Geology, Yale University, New Haven, Conn. KARL HANS WEDEPOHL Mineralogische-Institut der Universitat, Gottingen, Germany Distribution of the Elements in Some Major Units of the Earth's Crust. *America*, *February*, 175–192.
7. Usero, J., Morillo, J., & Gracia, I. (2005). Heavy metal concentrations in molluscs from the Atlantic coast of southern Spain. *Chemosphere*, *59*(8), 1175–1181. https://doi.org/10.1016/j.chemosphere.2004.11.089
8. Naiya, T. K., Bhattacharya, A. K., & Das, S. K. (2008). Adsorption of Pb(II) by sawdust and neem bark from aqueous solutions. *Environmental Progress*, *27*(3), 313–328. https://doi.org/10.1002/ep.10280.
9. Mishra, D., Singh, S. K., Adhikari, A., & Chatterjee, S. (2022). Polyaniline and polypyrrole impregnated polyethersulfone based composite polymer beads for defluoridation application. *Journal of Environmental ChemicalEngineering*, *10*(5). https://doi.org/10.1016/j.jece.2022.108283
10. Hsini, A., Essekri, A., Aarab, N., Laabd, M., Ait Addi, A., Lakhmiri, R., & Albourine, A. (2020). Elaboration of novel polyaniline@Almond shell biocomposite for effective removal of hexavalent chromium ions and Orange G dye from aqueous solutions. *Environmental Science and Pollution Research*, *27*(13), 15245–15258. https://doi.org/10.1007/s11356-020-08039-1

11. AShalini, K. (2017). Synthesis, Characterization of Polyaniline and Polyaniline Encapsulated Cellulose isolated from Sugarcane Bagasse and its study on Electronic Properties. *DJ Journal of Engineering Chemistry and Fuel*, *2*(1).