

# Synergistic Effect of Chemical and Physical Treatments on *Azolla pinnata* for Cadmium Ions Removal from Synthetic Wastewater Systems

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## Abstract

*Azolla pinnata*, an aquatic fern has been utilized as an effective biofiltering and adsorbent agent to complement many conventional treatment methods for the removal of environmental pollutants. This study is designed to develop an effective regime to treat metal pollutants of industrial and urban waste discharge using a novel strategy involving *Azolla pinnata*. In the present study, cell surface modification by physical treatments that include heating (muffle furnace), and mechanical waves (ultrasonication) and chemical treatments as sulphuric acid and ethanol were employed to enhance the adsorption of metal pollutants. Factors such as biosorbent dose, contact time, initial metal ion concentration, temperature, and solution pH were optimised in batch mode. The point of zero charge of the adsorbent was determined to be at 5.85 pH. The results of surface morphology, elemental analysis, crystallinity, recorded through SEM, FTIR and XRD confirmed the adsorptive properties in both modified and unmodified biomass. The intensity peaks linked to O-H, C-H, C-N, N-H and C=O stretching bands was intense in the treated *A. pinnata* groups indicating the induction of the active groups. Out of the two chemical pre-treatments, the batch adsorption experiment with ethanol found to chelate Cd<sup>+2</sup> metal ions to a higher extent (94.36%)

in contrast to the results obtained from H<sub>2</sub>SO<sub>4</sub> treated biomass. Whereas, the physical treatments exhibited the strong adsorption (83.28 and 96.92±0.55%) for ultrasonicated and muffle furnace pre-treated biomass respectively for the dosage of 0.25g. The adsorption efficiency of physically modified sorbent revealed the cent percent removal of Cd<sup>+2</sup> ions from the aqueous phase with the dosage of 1.0g in 15min of contact time which is due to the incorporation of new binding sites. Moreover, these results proved that the highest rate of cadmium adsorption onto *A. pinnata* is in result of the modifications caused onto surface structure, porosity and the addition of functional groups on the surface of the treated biomass.

**Keywords:** *Azolla pinnata*, phytoremediation, cadmium chelation, surface properties, chemical and physical modifications.

## Introduction

Rapid population explosion coupled with industrialization and urbanization has given way to increased ecosystem degradation (1). The major environmental issue raised throughout the world is the egression of toxic pollutants in water bodies due to the disposal of toxic wastes originated from automobile engines, industrial sectors and domestic operators

Synergistic effect of chemical and physical treatments on *Azolla pinnata* for cadmium ions removal from synthetic wastewater systems

(2). Wastewater, especially discarded from the industries, contain a wide range of heavy metals such as Cd, As, Fe, Cr, Zn, Cu, Mn, Pb, Ni, etc, turns out to be a major environmental concern due to their bioaccumulation properties (3). Being a simple and persistent element, cadmium cannot be broken down into less toxic forms in the environment. Accumulation of such toxicants into the water either in the form of organic or inorganic compounds successively enter the food chain leading to various health hazards (4). The conventional treatment methods for metal removal from aqueous streams such as chemical precipitation, lime coagulation, ion exchange, reverse osmosis and solvent extraction etc. are not economical besides posing toxicity (5,6). Among the existing technologies, adsorption method meets the requirements to maximize the removal of heavy metals, supporting the objective of identifying the most effective water treatment system. Adsorption is widely regarded as the most popular technique for removing heavy metals from systems as it is cheap, easy to use, readily available in a wide variety of adsorbents, highly efficient, easy to operate, reversible, and ability to regenerate (7). As release of hazardous pollutants are inevitable with industrial revolution, employment of novel remedial measures such as bioremediation and phytoremediation using adsorbents may mitigate the toxic effects caused by the pollutants.

Utilization of aquatic plants to hyperaccumulate the pollutants from wastewater and their application in biomonitoring of pollution had been well reported (8). Aquatic macrophytes are considered to be strong indicators of heavy metal pollution in aquatic ecosystems, acting as biological filters by absorbing heavy metals (9,10,11). *Azolla pinnata*, an aquatic pteridophyte commonly known as water fern, has emerged as a promising candidate due to its remarkable characteristics such as rapid growth rate, high biomass production, and efficient nutrient uptake (12). *Azolla*'s high biomass productivity, ability to proliferate in diverse aquat-

ic habitats, and relatively short lifecycle further enhance its suitability for remediation studies (13). Despite such promising attributes, the precise mechanisms underlying *Azolla*-mediated heavy metal removal, as well as its efficacy under varying environmental conditions, remain unclear. Moreover, *Azolla* species possess an exceptional capacity to accumulate heavy metals from surrounding water, attributed to mechanisms such as adsorption, chelation, and bioaccumulation. This study aims to provide a comprehensive overview of the current state of knowledge regarding the role of *Azolla pinnata* in heavy metal remediation and to assess the efficiency of the native, chemically and physically modified *Azolla pinnata* to uptake the metal Cd. Experimental findings of this research seek to elucidate the factors that should be considered in the removal of Cd from wastewaters by native, and modified *A. pinnata*.

## Materials and methods

### *Biomass preparation*

The healthy biomass of *Azolla pinnata* was collected from the experimental site (pollution-free site) and thoroughly rinsed with distilled water to eliminate soil and dust contaminants, followed by desiccation in a shaded environment for a duration of 3 days. Subsequently, it was subjected to oven drying at a controlled temperature of 40°C for a period of 24 hours. The dried material was pulverized using blender and sieved to obtain constant size particles (0.85–1.70 mm) and was used for characterization without any prior chemical treatment and stored in aliquots for further study (14).

### *Health properties by pigmentation analysis*

Transmission, absorption, and reflection, along with many other plant stress and pigment indicators was measured using CI-710s Spectra Vue Leaf Spectrometer (CID Bio-Science, USA). The operating environment of the instrument was maintained at -10°C to 50°C with non-condensing humidity (0% - 90%). The

detector with the wavelength range of 360-1100 nm and the spectrometer with the minimum leaf size of 20mm x 20mm was used to allow full operation in a handheld form factor with a 7inch 1024 x 600 IPS touchscreen display (Integration time of 30 $\mu$ s – 60s). The quantification of chemical concentrations, pigment analysis, and the quantification of physical or optical properties such as film thickness, index of refraction, and extinction coefficient were analysed with a full suite of the built-in indices' software (15).

### **Chemical modification of azolla pinnata biomass**

#### **Sulphuric acid treatment**

10g of biomass of *A. pinnata* was added to 100 ml of 0.1N H<sub>2</sub>SO<sub>4</sub> and the solution was then placed on a rotary shaker at 40 °C, at 105 rpm, for 24 h. The adsorbent was then cooled to room temperature and was separated by filtration using Whatman No.1 Ashless filter paper. Following filtration, the biomass was thoroughly washed with distilled water until pH 6-7 was obtained and was dried in an oven at 70 °C overnight until the constant weight was acquired. The material was sieved and stored in a desiccator prior to further analysis (16).

#### **Ethanol treatment**

*Azolla* biomass of about 10g was exposed to 25% ethanol solvent for 4hrs at 40 °C and was placed in a shaking incubator overnight at 105 rpm. The solution mixture was then subjected to filtration and washed with distilled water until the solution reaches neutral pH. Then the activated biomass was dried overnight at 60°C and stored in air tight container for the further study (17).

### **Physical modifications of azolla pinnata biomass**

#### **Ultrasonication treatment**

In this type of physical modification, the powders was ultrasonicated at an amplitude of

20 for the time interval of 1hr, followed by washing with distilled water and was oven dried for one day at 70°C. The resultant biosorbents, modified via physical means, were then evaluated for their effectiveness in the biosorption of cadmium metal ions (18).

#### **Muffle furnace treatment**

Thermal treatment was carried out at 300°C with no external air or gas supply for 1 h in a muffle furnace. The yield of char obtained was 52%. The prepared biosorbents were tested directly for the biosorption of cadmium metal ions from the aqueous solutions (18).

#### **Preparation of metal stock solution**

The analytical grade of Cd(NO<sub>3</sub>)<sub>2</sub> · 4H<sub>2</sub>O of 99% assay was used in this study. The standard metal stock solution was prepared using standard procedures, with varying initial concentrations by dissolving Cd(NO<sub>3</sub>)<sub>2</sub> · H<sub>2</sub>O in distilled water. All glassware utilized in the biosorption studies was frequently rinsed with HNO<sub>3</sub> followed by extensive washing with distilled water to mitigate interference from contaminants. The pH of each solution was determined using a digital pH meter and subsequently adjusted using 0.1 M HCl or 0.1 M NaOH solutions.

#### **Biosorption studies and research design**

Biosorption potential of raw chemically, and physically modified *Azolla pinnata* was tested. The effect of experimental factors such as biosorbent dose, metal ion concentration, contact time, temperature and pH was investigated independently for Cd<sup>+2</sup> ion biosorption onto *Azolla pinnata* biomass. Each parameter was analysed stepwise in batch mode while keeping one constant against other suitable variables. To test the biosorption efficiency, known biomass of *Azolla* was exposed to varying concentrations of Cd<sup>+2</sup> ( 15.75ppm and 500 ppm). The batch of test tubes were placed on a rotary shaker with a constant shaking of 100 rpm with pre-defined temperature and exposure time.

Synergistic effect of chemical and physical treatments on *Azolla pinnata* for cadmium ions removal from synthetic wastewater systems

The solution was filtered and the biomass was separated from the filtrate using filter paper. The concentration of Cd<sup>2+</sup> metal ion in the filtrates were analysed by atomic adsorption spectroscopy (AAS). Two controls, including metal-free and biosorbent-free blanks were employed.

Batch adsorption experiments were conducted with different pH (5-9), adsorbent doses (0.25-1.0g), Cd<sup>2+</sup> ion concentrations (15.75-500ppm), temperatures (15-45 °C) at different time intervals (15 ,30,60 and 180mins).

Metal adsorption capacity (qe) is defined as the quantity of metal adsorbed per gram of biosorbent and can be calculated using the following equation Eq. (1) and expressed in mg/g:

$$q_e = (C_i - C_e) \times V/m \dots \dots \dots (1)$$

where m is the mass of biosorbent (g), V is the volume of solution (L), C<sub>i</sub> is the initial metal-ion concentration (mg/L), and C<sub>e</sub> is the equilibrium concentration of metal ions (mg/L). The percentage of metal removed was computed using equation Eq. (2) as follows:

$$\text{Removal efficiency (\%)} = (C_i - C_e)/C_i \times 100 \dots \dots \dots (2)$$

#### **Instrumental characterization of unmodified and modified azolla pinnata biomass**

The adsorbent developed by chemical and physical modification were characterized to understand the surface morphology, surface chemistry, and to analyse the functional sites of the adsorbent. The interaction of cadmium ions with the biosorbent material was detected using analytical tools. The surface morphology, chemical characterization and elemental composition of the adsorbent was analysed using Apreo S LoVac Scanning Electron Microscopy (SEM, ThermoFisher Scientific Corporation). The active sites and functional groups on the adsorbent were assessed by Fourier transform infrared (FTIR) spectrophotometer with QATR-S

single reflection ATR accessory having extended range diamond crystal (Shimadzu Model IR Spirit). The nature and chemical composition of metal on the surface of *Azolla* sorbent was detected through Rigaku Mini Flex Powder X-ray Diffractometer (XRD, Rigaku Corporation) with Cu K $\alpha$  radiation. The generator voltage and current were set at 35 KV and 25 mA respectively. The *A. pinnata* samples were scanned in the 2 $\theta$  ranges 20-80°C in continuous scan mode with a 10°/ min scan speed (19).

#### **Statistical analysis**

The statistical analysis was carried out by using SPSS statistics software version 27. The results were interpreted by One-way Analysis of Variance (ANOVA) to verify the significance using Duncan's Test by the probability less than p<0.05.

#### **Results and discussion**

*Azolla* species was identified and authenticated as *Azolla pinnata* R. Br. (Voucher no.1094) by Central Ayurveda Research Institute, Bangalore, India. The fresh *Azolla* collected from the study site and was subjected to CI-710s Spectra Vue Leaf Spectrometer to detect the health properties of *A. pinnata* before its utilization in various environmental applications. The present investigations showed the existence of chlorophyll *a* and chlorophyll *b* that was approximately in the standard range of ¼ and ¾ with total chlorophyll content of 6.9821±0.3943ugcm<sup>-3</sup> (Table 1). The presence of anthocyanin (0.3695±0.0780), flavanols (0.0643±0.0376) and carotenoid (0.0186±0.0046) indices signified the ability of the plant to protect against biotic and abiotic stresses. The decrease in the chlorophyl content index due to water stress in plant was recorded in previous studies and a strong correlation with the amount of chlorophyll in the plant was observed (20,21). Further research on the pigmentation and its correlation to plant stress properties in aquatic plants is required as the information is scarce.

Table 1. Pigmentation index, health and stress of *A. pinnata*

Sl. no.	Index	Abbrev	Index value
1	Anthocyanin Reflectance Index	ARI	0.3695±0.0780
2	Carotenoid Reflectance Index	CRI	0.0186±0.0046
3	Chlorophyll A (ugcm <sup>-3</sup> )	CPHLA	1.7563±0.1648
4	Chlorophyll B (ugcm <sup>-3</sup> )	CPHLB	5.4326±0.2720
5	Chlorophyll Total (ugcm <sup>-3</sup> )	CPHLT	6.9821±0.3943
6	Chlorophyll Content Index	CCI	1.3390±0.1717
7	Chlorophyll Normalized Difference Vegetation Index	CNDVI	0.2077±0.0205
8	Flavanols Reflectance Index	FRI	0.0643±0.0376
9	Greenness Index	G	2.1098±0.1715
12	Photochemical Reflection Index	PRI	0.2998±0.0224
13	Plant Senescence Reflectance Index	PSRI	0.1049±0.0186
16	Water Band Index	WBI	1.8289±0.0335

**Optimization factors affecting the cadmium biosorption potential of *Azolla pinnata***

Main parameters such as pH, temperature, contact time, biosorbent dosage and heavy metal initial concentration that influence the biosorption of cadmium was investigated. The present biosorption experiments were carried out using varying adsorbent dosages to determine the effect of each factor at different mass of *A. pinnata* biomass. The results depicted in Table 2. show that the percentage of Cd<sup>2+</sup> removal increased from 52.46 to 89.62% with the increase in adsorbent concentration from 0.25 to 1g/25mL in the metal solution (500mg/L). Increased adsorption is due to the more availability of unsaturated active sites on the biomass surface for the attachment of the metal ions (22). The rate of adsorption above the optimal dose of 1g/25ml of 500mg/L was found to be remain unchanged or decreased due to insufficient Cd<sup>2+</sup> ion concentration to fit all the active sites on surface of the adsorbent, which is in consistent with the results of earlier reports (23). However, at a lower adsorbent dosage, the interaction of Cd<sup>2+</sup> ions with adsorbent decreases because of the limited binding sites available for adsorption (24).

**Effect of metal ion concentration at varying adsorbent dosages**

The mechanism of metal uptake is dependent on the initial metal ion concentration in the solution. The present study evaluated the highest amount of Cd<sup>2+</sup> ions that could be up taken by the adsorbent at a 0.25g dose at a pH of 6.6 is up to 408.65mg/L when the time of exposure increased from 15min to 24hr. The rate of Cd<sup>2+</sup> adsorption onto *A. pinnata* at different concentrations (15.75 to 500mg/L) of Cd<sup>2+</sup> ions is displayed in Table 2. The removal efficiency increased proportionally with the increase in initial metal concentration from 15.47 to 365.95mg/L of Cd<sup>2+</sup> respectively in 15min of contact time. Similarly, the decrease in the percent removal from 99.24 to 81.58% when concentration of Cd<sup>2+</sup> increased from 20 to 200 mg/L was reported (25). Nonetheless, increasing the Cd<sup>2+</sup> ion concentration from 15.75 to 250 mg/L did not show further increase in the rate of uptake and attained equilibrium (95.82 ± 3 % of adsorption) after 1hr of exposure. Further increase in the initial Cd<sup>2+</sup> concentration (500mg/L) decreased the rate to 89.62%, which is in strong agreement with the recent studies (26,27). This could be due to weak and inconsistent force of contact

Synergistic effect of chemical and physical treatments on *Azolla pinnata* for cadmium ions removal from synthetic wastewater systems

between metal ions and the surface of adsorbent indicating the saturation point, which may lead to diffusion of ions into the aqueous phase or due to limited availability of active sites on the adsorbent (22,28).

Table 2. Factors influencing on the cadmium biosorption onto untreated *Azolla pinnata*.

<b>BD: 0.25g</b>							
<b>Metal Concentration (mg/L)</b>	<b>15min</b>	<b>30min</b>	<b>1hr</b>	<b>3hr</b>	<b>6hr</b>	<b>12hr</b>	<b>24hr</b>
500	262.3	278.9	368.3	375.15	381.05	379.85	385.65
250	163.2	181.02	201.57	210.2	213.1	217.32	222.57
125	89.07	96.46	112.14	115.26	115.4	115.95	117.36
62.5	50.26	51.33	58.9	60.74	60.82	61.63	60.93
31.25	26.95	27.85	30.05	30.27	30.71	31.51	31.19
15.75	14.85	14.92	15.13	15.28	15.12	15.32	15.54
<b>BD: 0.50g</b>							
500	339.1	358.15	416.2	423.95	432.7	434.45	441.65
250	188.37	197.95	221.77	235.8	237.96	238.72	239.55
125	97.89	106.82	116.54	120.9	121.09	121.28	122.15
62.5	52.26	54.64	60.32	60.93	61.35	61.72	62.14
31.25	28.83	29.73	30.74	30.92	31.12	31.04	31.18
15.75	15.12	15.37	15.71	15.57	15.68	15.7	15.73
<b>BD: 0.75g</b>							
500	350.05	374.8	430.5	432.8	436.85	439.7	445.83
250	202.82	210.35	228.2	237.83	239.67	241.03	242.9
125	105.26	111.46	119.7	123.79	123.97	124.25	124.79
62.5	55.11	58.29	61.44	61.56	61.67	62.31	62.48
31.25	29.7	30.85	30.9	30.98	31.13	30.82	31.22
15.75	15.15	15.23	15.42	15.53	15.61	15.67	15.73
<b>BD: 1.0g</b>							
500	365.95	376.3	448.1	451.04	456.6	460.55	461.6
250	215.53	223.58	233.42	239.8	241.21	242.33	244.1
125	113.49	117.13	121.39	123.6	124.07	124.28	124.91
62.5	58.14	60.44	61.26	61.94	61.71	61.58	61.42
31.25	30.6	30.92	30.53	31.03	31.11	31.2	31.15
15.75	15.47	15.52	15.61	15.65	15.58	15.71	15.74

BD- Biosorbent dosage, mg/L- milligram per litre

**Effect of contact time at varying adsorbent dosages**

Contact time is one of the significant factors that possess a major impact on the ad-

sorption rate of the *Azolla* biomass. The rate of adsorption of Cd<sup>+2</sup> ions onto the surface of *A. pinnata* at various contact times (15 min, 30 min, 1hr, 3hr, 6hr, 12hr, and 24hr) with respect to varying metal ion concentration and adsor-

bent dosages is listed in Table 2. The uptake of  $\text{Cd}^{+2}$  ions was found to be increase rapidly during the first 15min-1hr which is due to the abundancy of unsaturated active sites on the biomass surface (demonstrating an adsorption process occurs through surface binding with no energy-mediated reactions). Similar to the present findings, the rate of adsorption of  $\text{Cd}^{+2}$  ions increased from 83.7% to 87.2%, when the contact time raised from 15min to 3hrs at the dosage of 0.25g/100ml (25). After 1hr of biosorption, the removal of metal ions gradually decreased and eventually reached equilibrium due to the attainment of saturation point (29). The time required for the metal ions in a solution to become constant is an equilibrium time of adsorption. The maximum biosorption rate for  $\text{Cd}^{+2}$  uptake was 461.6 mg/g and the removal percentage was 92.32% within 1hr of adsorbent - adsorbate contact time. According to the present investigation, a rapid absorption rate (365.95 - 448.1mg/g) was noted till 1hr, followed by a slower adsorption during the 3 - 6hr ( $453.82 \pm 2.78$  mg/g) and, eventually reached an equilibrium. Due to the rapid increase in metal bio-adsorption rate, the more efficient contact time required for the further batch experiments was considered to be 30mins-1hr.

#### **Effect of temperature at varying adsorbent dosages**

The temperature is the main factor that influences the adsorption process. The adsorption capacities of *A. pinnata* for the  $\text{Cd}^{+2}$  ions at different temperatures with varying adsorbent dosages are shown in Figure 1. A temperature ranges between 5 °C to 45 °C with the differences of 10 °C were studied. It was observed that adsorption increased slightly with increasing temperature from 5 °C to 25 °C. But it was analysed that the percentage of  $\text{Cd}^{+2}$  ion adsorption onto *A. pinnata* was higher between 25 °C and 35 °C up to 99.7%.

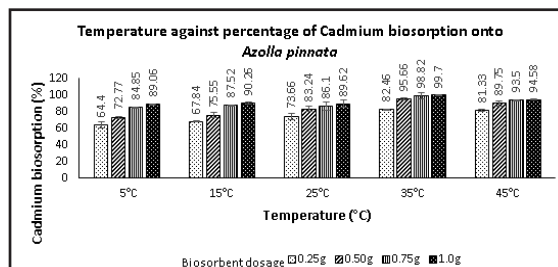


Figure 1. Effect of solution temperature on the removal of  $\text{Cd}^{+2}$  ions (initial conc.= 500mg/L, exposure time = 60min, pH = 7, agitation speed = 100rpm)

Based on the present findings, the adsorption of the  $\text{Cd}^{+2}$  metal ions onto the *A. pinnata* biomass increased with the rise in temperature which indicates the endothermic nature of the adsorption process due to higher diffusion rate and increased transportation of metal ions (30,31). The adsorption decreased approximately 5-10 % when the temperature was further raised to 45 °C. for the same dosages of unmodified *A. pinnata* biomass and  $\text{Cd}^{+2}$  metal ion concentration. Several studies have reported the reduced metal ion uptake by increasing temperature (32). This decrease is due to the tendency of the  $\text{Cd}^{+2}$  ion molecules to migrate from the adsorbent (solid phase) to the metal medium (liquid phase) or due to weak adsorptive forces between the  $\text{Cd}^{+2}$  ions adsorbed and the active sites on the biomass and also between adjacent  $\text{Cd}^{+2}$  molecules adsorbed on the adsorbent surface (33). This indicated that the adsorption process using unmodified biomass is physisorption.

#### **Effect of pH at varying adsorbent dosages**

The point of zero charge ( $\text{pH}_{\text{PZC}}$ ) refers to the pH at which the surface charge density of the adsorbent is electrically neutral exhibiting minimal interaction with metal solution (34). In the present study,  $\text{pH}_{\text{PZC}}$  of the adsorbent was determined to be 5.85 (Figure 2). Consequently, at pH of metal solution below 5.85, the adsorbent surface acquires positive charge enhancing its attraction to anions. Conversely, when

the pH exceeds the  $pH_{PZC}$ , the adsorbent surface becomes negatively charged, creating conditions conducive to the adsorption of cationic species (35).

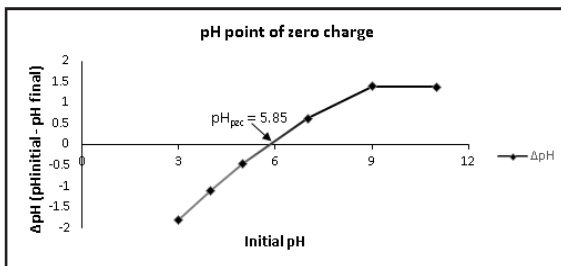


Figure 2. Point of zero charge of untreated *Azolla pinnata* biomass

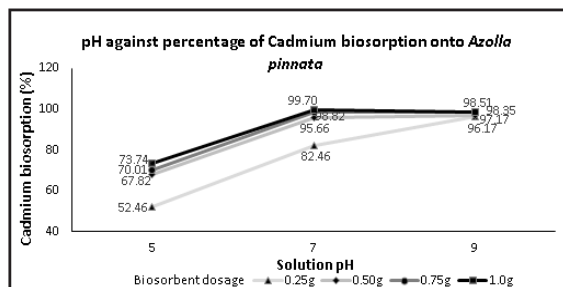


Figure 3. Effect of solution pH on the removal of  $Cd^{+2}$  ions (initial conc. = 500mg/L, exposure time = 60min, temperature = 35°C, agitation speed = 100rpm)

The effects of pH on the biosorption of  $Cd^{+2}$  ions onto *Azolla pinnata* biomass were investigated at a pH ranging from 5 to 9 are shown in Figure 3. The biosorption capacities of adsorbent ( $q_e$ ; mg/L) for  $Cd^{+2}$  cations increased with the increasing pH from pH 5 to pH 7. Under alkaline conditions, the anions on the biosorbent surface increases, so, the biosorption efficiencies increases rapidly from 52.46 to 99.7%. This occurs due to the decrease in competitive adsorption between metal cations ( $Cd^+$ ) and protons ( $H^+$ ) to bind onto the same functional groups/ active sites (ion exchange mechanism) (36). Beyond the optimum pH, metal cations react with hydroxide ions and precipitate as metal hydroxide (precipitation mechanism) which could have cause the diffusion of  $Cd^{+2}$  ion from

the adsorbent surface into the medium above pH 7 and decreases the efficiencies of adsorption gradually to 96.17% (37). But due to the formation of insoluble metallic hydroxide and to ignore the effect of precipitation of  $Cd^{+2}$  ions, further adsorption studies were performed at pH 7.

### Characterization of *Azolla* for adsorptive properties

#### FTIR analysis

FTIR spectroscopy was used as a major tool to detect the characteristic functional groups present on the surface of biomass which may facilitate the binding sites for the attachment of  $Cd^{+2}$  ions. The adsorbent spectra of the showed multiple types of vibrational frequencies in peaks due to the presence of distinct functional groups. The FTIR graphs of the physically and chemically modified *A. pinnata* biomass before and after cadmium adsorption (in the range of 400–4000  $cm^{-1}$ ) are shown in Figure 4.

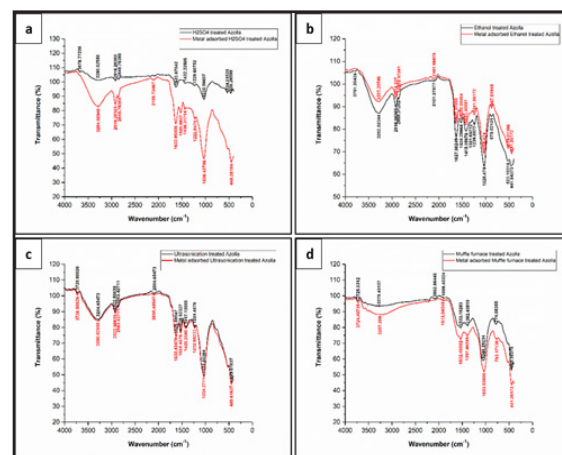


Figure 4. FTIR spectra of  $H_2SO_4$  (a), EtOH (b), ultrasonication (c), and muffle furnace (d) treated *Azolla* biomass before (black line) and after (red line) adsorption of cadmium ions.

The FTIR spectroscopic graph of  $H_2SO_4$  treated *Azolla* biomass before  $Cd^{+2}$  adsorption revealed strong adsorption bands at 3290  $cm^{-1}$ , confirming the presence of N-H and O-H broad stretching. Peaks at 1623  $cm^{-1}$  and 1032  $cm^{-1}$  were linked to N-H and C-N representing amine



bending and stretching respectively. However, the Cd<sup>2+</sup>-loaded H<sub>2</sub>SO<sub>4</sub> treated adsorbent displayed supplementary peaks around 2916 (C-H stretching), 2848 (C-H stretching), 1525 (–COO– anti-symmetric stretching), and 448 (C–H bending) cm<sup>-1</sup> in Figure 4a. Whereas, the ethanol modified biomass displayed the significant peaks at 3282, 2916, 1627, 1415 and 1028 cm<sup>-1</sup> for O-H stretching, C-H alkane stretching, N-H amine bending, O-H carboxylic acid bending, and C-O ether stretching respectively. Only shift in few peaks with no significant changes after Cd load onto EtOH treated biomass surface was noticed (Figure 4b).

Similarly, the FTIR graphs for physically modified biomass showed overlapping peaks with no changes even before and after Cd<sup>2+</sup>-load onto the ultrasonicated biomass surface (Figure 4c). The muffle furnace treated *Azolla* biomass displayed strong bands at 3278 (O-H stretching) and 1048 cm<sup>-1</sup> revealing major changes after Cd<sup>2+</sup> accumulation (Figure 4d). Overall, the FTIR spectroscopic graphs for Cd-loaded physically and chemically modified sorbent showed significant shifting and disappearance of some peaks revealing the strong bonds between the Cd ions and the biomass surface (38). Noticeably, the appearance of some new peaks in the graph evidencing the fact that, the functional groups on the surface of the *A. pinnata* biomass are more affective in the adsorption of Cd<sup>2+</sup> ions. In comparison to the unmodified *A. pinnata*, the strength of the O-H, N-H, C=O and C-H stretching bands on treated *A. pinnata* biomass surface increased as a result of modifications, indicating the increase in the active groups (19)

### XRD analysis

The degree of crystallinity of adsorbent material was determined by X-ray diffraction analysis of *Azolla* biomass before and after treatments and Cd adsorption onto their surface. Figure 5 depicts the high intensity peak at angle of 2θ: 26.8° which disappeared after Cd- load onto acid treated adsorbent. However, few broad peaks obtained for EtOH treated

adsorbent at angles 29.6°, 36.2°, 39.7°, 43.5°, 47.7° and 48.8° indicating the semi-crystalline structure after Cd<sup>2+</sup>-load (Figure 5a,b).

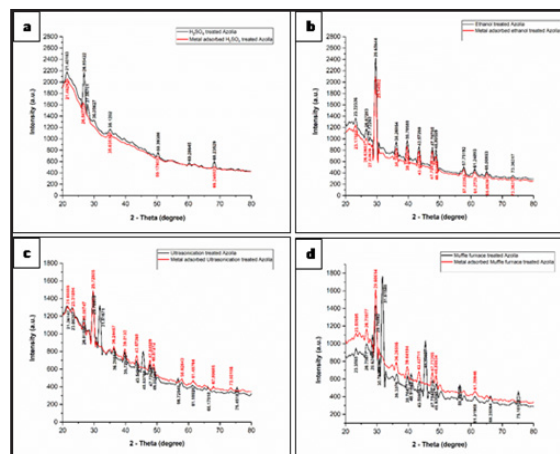


Figure 5. XRD pattern of H<sub>2</sub>SO<sub>4</sub> (a), EtOH (b), ultrasonication (c), and muffle furnace (d) treated *Azolla* biomass before (black line) and after (red line) adsorption of cadmium ions.

The physically modified adsorbents showed the diffraction peaks between 2θ: 30° to 50° for ultrasonicated biomass confirming the crystalline structure. More significant shift in the broad diffraction peak from 2θ: 31.8° to 2θ: 29.7° and disappearance of peaks at angles 2θ: 45.5°, 56.7° and 75.45° was observed that may due to the interaction of Cd<sup>2+</sup> ions with sorbent surface. Similarly, the high intensity peaks at angles 2θ: 28.6°, 31.8°, 45.6°, 56.4° and 75.1° found to be reduced after Cd<sup>2+</sup>-load representing the transition of crystalline to the amorphous structure for furnace modified adsorbent (Figure 5c,d). Although XRD analysis does not directly elucidate the mechanism of the adsorption process between the adsorbent material and Cd<sup>2+</sup> ions, its significance remains paramount due to the high crystallinity that was exhibited by all treated adsorbent structures. The strong bonds between heavy metals and sorbent were evidenced by a reduction in crystallinity, and was indicated by shifts or broadening of diffraction peaks, along with the emergence of new peaks (39). Moreover, variations in crystallinity, wheth-

er increased or decreased, further confirm the interaction of the adsorbent with heavy metal ions after the adsorption of  $\text{Cd}^{+2}$  ions.

### Scanning Electron Microscope analysis

The surface morphological features of blank and  $\text{Cd}^{+2}$ -loaded *Azolla* biomass was demonstrated in Figure 6a, b. The dominance of a complex pore matrices and coarse holes on its preceding structures, which were significant for the adsorption process, can be noticed in the raw (unmodified) biomass surface (Figure 6a) with average pore size up to 253.5nm. In fact, these properties such as rough morphology, porous surface, greater pore size are found to be highly favourable and supportive for metal ion attachment in the adsorption process (31). The adsorption of  $\text{Cd}^{+2}$  ions caused the untreated biomass to form a smoother surface and tight structures throughout the visualization (Figure 6b).

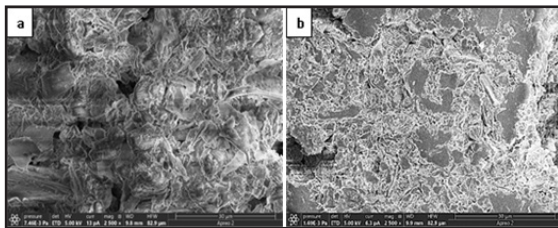


Figure 6. SEM analysis of raw (unmodified) *Azolla* biomass before (a) and after (b) adsorption of cadmium ions.

SEM analysis of treated adsorbents revealed the visualization of minor structural alterations on its surface (Figure 7a, b, c, d). From the SEM images of chemical and physical modified adsorbent, all treated biomass can be described as having porosity with many fragments scattered on its surface and inside the pores before metal adsorption (40). The porous size of treated biomass was slightly increased up to 290.03 nm for  $\text{H}_2\text{SO}_4$  treated biomass and 4-fold increase of pore size up to 1.043  $\mu\text{m}$  was seen in EtOH treated *Azolla* biomass. The porosity on the physical modified biomass surface ranged between 158.7 nm and 217.3 nm due to the re-

duction of particle size after treatments. Unlike these possibilities, the number of pores on the surface of ultrasonicated and muffle furnace treated biomass may increase by facilitating the chelation of  $\text{Cd}^{+2}$  ions to a greater extent. The formation and distribution of the new pores over the entire surface of the material is expected to improve the performance of the biomass as an adsorbent, which may due to the modifications on its surface either physically or chemically (18).

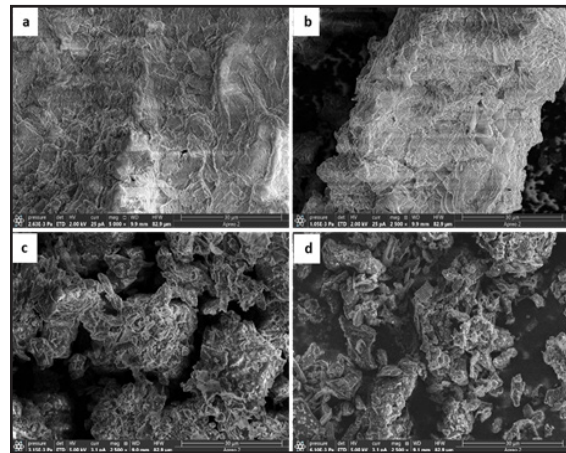


Figure 7. SEM analysis of  $\text{H}_2\text{SO}_4$  (a), EtOH (b), ultrasonication (c), and muffle furnace (d) treated *Azolla* biomass before adsorption of cadmium ions.

Cadmium-loaded *Azolla* biomass was also analysed to observe the morphological changes (Figure 8). The disappearance of previously detected pores, transition of rough to smoother surface, and modulation of small scattered fragments into larger fragments, (41,42) which affirmed the attachment of  $\text{Cd}^{+2}$  ions to the active binding sites on the surface and pores of *A. pinnata* biomass (Figure 8a,b). The surface characterization of physical modified adsorbent was completely varied after  $\text{Cd}^{+2}$  adsorption exhibiting shininess (Figure 8c,d) as described by the earlier report (43).

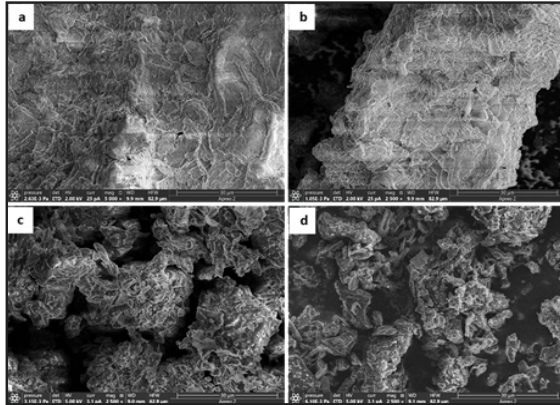


Figure 8. SEM analysis of H<sub>2</sub>SO<sub>4</sub> (a), EtOH (b), ultrasonication (c), and muffle furnace (d) treated *Azolla* biomass after adsorption with cadmium ions.

### Biosorption efficiency of chemically and physically modified *A. pinnata* biomass

The adsorption experiments were conducted using different chemical and physical modified *A. pinnata* sorbents. The results obtained from the optimization studies for some factors such as initial Cd<sup>2+</sup> ion concentration= 500mg/L, temperature = 35°C, pH=7.0 were maintained during the experimentation. The contact time is found to be saturated at 60min. But the exposure time of 15min with agitation speed of 100rpm were considered for further studies with treated adsorbents to understand the short time of exposure required for its applicability at pilot scale.

Table 3. The percentage of metal recovered from treated and untreated *A. pinnata* adsorbents

Biosorbent dosage	Untreated	Chemical treatments		Physical treatments	
	Raw	H <sub>2</sub> SO <sub>4</sub>	EtOH	Ultrasonication	Muffle furnace
0.25g	71.43±1.36 <sup>c</sup>	64.72±0.92 <sup>d</sup>	94.36±1.58 <sup>b</sup>	83.28±2.62 <sup>c</sup>	96.92±0.50 <sup>c</sup>
0.50g	86.49±0.52 <sup>b</sup>	77.95±1.88 <sup>c</sup>	99.21±0.60 <sup>a</sup>	91.12±1.22 <sup>b</sup>	98.82±1.17 <sup>b</sup>
0.75g	90.76±4.65 <sup>ab</sup>	87.22±3.55 <sup>b</sup>	100.22±0.87 <sup>a</sup>	96.47±2.81 <sup>a</sup>	100.78±0.91 <sup>a</sup>
1.0g	91.80±1.64 <sup>a</sup>	91.12±2.84 <sup>a</sup>	100.36±0.68 <sup>a</sup>	99.44±2.88 <sup>a</sup>	100.74±0.61 <sup>a</sup>
<b>p value</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>0.001</b>

Values are expressed as mean ± SD (%) for triplicates, SD: Standard deviation, H<sub>2</sub>SO<sub>4</sub>: Sulphuric acid treated adsorbent, EtOH: Ethanol treated adsorbent. \*p value significant (p≤0.05) accordance to Duncan's multiple range test in different treatments are expressed in increasing order as a-d.

Based on the earlier reports, the analytical investigations have revealed that the modifications of biomass surface result in notable changes in their adsorptive properties such as hydrophobic properties, water absorption characteristics, ion exchange capacity, resilience against microbial degradation, and thermal stability. Therefore, in the present study some methods such as physical treatments which include heating (muffle furnace), and mechanical waves (ultrasonication) and chemical treatments as acid solutions (sulphuric acid) and organic compounds (ethanol) were employed for the modification of cell surface.

The cadmium removal efficiency of different chemical and physical treated and untreated *A. pinnata* adsorbents is displayed in Table 3. The results obtained from these adsorption studies showed the metal recovery of up to 71.43% and later increased to 91.80% when the dose of untreated biomass increased from 0.25 – 1.0g. The chemical treatment with ethanol has showed faster adsorption kinetics as well as higher biosorption for Cd<sup>2+</sup> ions than the virgin *A. pinnata* biomass. In contrast, H<sub>2</sub>SO<sub>4</sub> pre-treatment showed the significant decrease in the Cd<sup>2+</sup> biosorption onto *Azolla* biomass of about 64.72% which is lesser than its untreated

Synergistic effect of chemical and physical treatments on *Azolla pinnata* for cadmium ions removal from synthetic wastewater systems

counterpart. This may occur due to the formation of large amount of oxygen functionalities in narrow pore openings and closure of some micropores and mesopores which in turn result in decrease of surface volume and surface area on biosorbent (44). Whereas, the physical treatments exhibited the strong adsorption that are reported to be 83.28 and 96.92±0.55% for ultrasonicated and muffle furnace pre-treated biomass (BD: 0.25g) respectively. Further reached 99.44 to 100% (maximum up to 500ppm) revealing the cent percent removal of Cd<sup>+2</sup> ions from the aqueous phase with the dosage of 1.0g in 15min of contact time which is due to the incorporation of binding sites. The introduction of various cell surface modifications such as phosphorylation and carboxylation of hydroxyl group, amination of hydroxyl and carboxyl group, carboxylation of amine groups, saponification of carboxylate ester groups, oxidation, etc. has led to an increased biosorptive efficiency (45). Thus, it can be concluded that that the functional groups on the biomass facilitated the occurrence of a polar and hydrophilic surface, which enabled the binding or absorption of cationic metal ions.

### Conclusion

In conclusion, the modifications undertaken to enhance the adsorptive properties on biomass surface has witnessed *Azolla pinnata* as an effective adsorbent for cadmium remediation from synthetic wastewater, which entails a balance between heightened adsorption potential, prolonged stability, production costs and environmental implications. The present research findings demonstrate the utilization of organic solvents and heat in chemical and physical treatments as a novel exploration in phytoremediation, respectively. As the current investigation focuses solely on cadmium as the target metal, modifications involving chemical agents (such as sulphuric acid and ethanol) and physical techniques (such as ultrasonication and muffle furnace treatment) may not uniformly enhance adsorption for all contaminants. Consequently, ongoing research aims to explore more efficient

chemical and physical methodologies for surface modifications and metal desorption techniques to mitigate environmental and human health risks. Hence, a meticulous assessment of the optimistic treatment approaches is essential in making well-informed decisions regarding the adoption of these technologies.

### Acknowledgement

The laboratory facilities and support for this study was provided by CHRIST (Deemed to be University) which the authors gratefully acknowledge and would like to extend their sincere gratitude to Centre for research for providing necessary facilities (MRP DSC - 1936).

### Conflict of Interest

The authors declare no conflict of interest

### References

1. Kobilov, E., Batirov, K., & Ozdamirova, E. (2023). Urban Ecosystems of Uzbekistan and Ways of Their Ecologization. BIO Web of Conferences. <https://doi.org/10.1051/bioconf/20236303002>.
2. Lin, L., Yang, H., & Xu, X. (2022). Effects of water pollution on human health and disease heterogeneity: a review. *Frontiers in environmental science*, 10:880246. <https://doi.org/10.3389/fenvs.2022.880246>.
3. Kumar, P., Gacem, A., Ahmad, M. T., Yadav, V. K., Singh, S., Yadav, K. K., ... & Cabral-Pinto, M. (2022). Environmental and human health implications of metal (loid) s: Source identification, contamination, toxicity, and sustainable clean-up technologies. *Frontiers in Environmental Science*, 10:949581. <https://doi.org/10.3389/fenvs.2022.949581>.
4. Vinanthi Rajalakshmi, K. S., Liu, W. C., Balamuralikrishnan, B., Meyyazhagan, A., Sattanathan, G., Pappuswamy, M., ... & Lee, J. W. (2023). Cadmium as an Endocrine Disruptor That Hinders the Repro-

- ductive and Developmental Pathways in Freshwater Fish: A Review. *Fishes*, 8:589. <https://doi.org/10.3390/fishes8120589>.
5. Razzak, S. A., Faruque, M. O., Alsheikh, Z., Alsheikhmohamad, L., Alkuroud, D., Alfayez, A., ... & Hossain, M. M. (2022). A comprehensive review on conventional and biological-driven heavy metals removal from industrial wastewater. *Environmental Advances*, 7:100168. <https://doi.org/10.1016/j.envadv.2022.100168>.
  6. Karim, A., Raji, Z., Karam, A., & Khalloufi, S. (2023). Valorization of Fibrous Plant-Based Food Waste as Biosorbents for Remediation of Heavy Metals from Wastewater—A Review. *Molecules*, 28:4205. <https://doi.org/10.3390/molecules28104205>.
  7. Türkmen, D., Bakhshpour, M., Akgönüllü, S., Aşır, S., & Denizli, A. (2022). Heavy metal ions removal from wastewater using cryogels: A review. *Frontiers in Sustainability*, 3:765592. <https://doi.org/10.3389/frsus.2022.765592>.
  8. Ali, S., Abbas, Z., Rizwan, M., Zaheer, I. E., Yavaş, İ., Ünay, A., ... & Kalderis, D. (2020). Application of floating aquatic plants in phytoremediation of heavy metals polluted water: A review. *Sustainability*, 12(5):1927. <https://doi.org/10.3390/su12051927>.
  9. Kristanti, R. A., & Hadibarata, T. (2023). Phytoremediation of contaminated water using aquatic plants, its mechanism and enhancement. *Current Opinion in Environmental Science & Health*, 100451. <https://doi.org/10.1016/j.coesh.2023.100451>.
  10. Sarojini, G., Kannan, P., Rajamohan, N., & Rajasimman, M. (2023). Bio-fabrication of porous magnetic Chitosan/Fe<sub>3</sub>O<sub>4</sub> nanocomposite using *Azolla pinnata* for removal of chromium—Parametric effects, surface characterization and kinetics. *Environmental Research*, 218:114822. <http://dx.doi.org/10.1016/j.envres.2022.114822>.
  11. Sharma, K., Saxena, P., & Kumari, A. (2023). Phytoremediation of Heavy Metals by *Azolla filiculoides* Lam. From Fly Ash Polluted Water Bodies. *Water, Air, & Soil Pollution*, 234(7):419. <http://dx.doi.org/10.1007/s11270-023-06423-4>.
  12. Kumar, S., Thakur, N., Singh, A. K., Gudade, B. A., Ghimire, D., & Das, S. (2022). Aquatic macrophytes for environmental pollution control. In *Phytoremediation Technology for the Removal of Heavy Metals and Other Contaminants from Soil and Water* (pp. 291-308). Elsevier. <https://doi.org/10.1016/B978-0-323-85763-5.00023-4>.
  13. Sachdeva, S., & Sharma, A. (2012). *Azolla*: role in phytoremediation of heavy metals. *Int J Eng Sci*, 1: 9698. <http://dx.doi.org/10.13140/RG.2.1.1276.0407>.
  14. Bhatt, N., Chandra, R., Kumar, S., Singh, K., & Pratap, N. (2020). Nutritive analysis of *Azolla pinnata* and its cultivation during winter season. *Int. J. Curr. Microbiol. App. Sci*, 9:2012-2018. <https://doi.org/10.20546/ijcmas.2020.903.233>.
  15. Kupčinskienė, A., Brazaitytė, A., Rasiukevičiūtė, N., Valiuškaitė, A., Morkeliūnė, A., & Vaštakaitė-Kairienė, V. (2023). Vegetation Indices for Early Grey Mould Detection in Lettuce Grown under Different Lighting Conditions. *Plants*, 12:4042. <https://doi.org/10.3390/plants12234042>.
  16. Li, Z., Teng, T. T., Alkarkhi, A. F., Rafatullah, M., & Low, L. W. (2013). Chemical modification of *imperata cylindrica* leaf powder for heavy metal ion adsorption. *Water, Air, & Soil Pollution*, 224:1-14. <https://doi.org/10.1007/s11270-013-1505-5>.
  17. Argun, M. E., & Dursun, S. (2006). Removal of heavy metal ions using chemically modified adsorbents. *J. Int. Environ. Appl. Sci*, 1:27-40. <https://www.researchgate.net/publication/266888800>.

Synergistic effect of chemical and physical treatments on *Azolla pinnata* for cadmium ions removal from synthetic wastewater systems

18. Aswani, M. T., & Kumar, M. P. (2019). A novel water hyacinth based biosorbent for 2, 4-dichlorophenoxyacetic acid (2, 4-D) removal from aqueous solution. *Desalination. Water Treat.*, 165:163-176. <https://doi.org/10.5004/dwt.2019.24581>.
19. Rajalakshmi, K. S. V., & Paari, K. A. (2023). A comprehensive study on the assessment of chemically modified *Azolla pinnata* as a potential cadmium sequestering agent. *Int. J. Exp. Res. Rev*, 36:01-19. <https://doi.org/10.52756/ijerr.2023.v36.001>.
20. Ghasemi M, Arzani K, Yadollahi A, Ghaseemi S, Khorrami SS (2011). Estimate of leaf chlorophyll and nitrogen content in Asian pear (*Pyrus serotina* Rehd.) by CCM-200. *Not. Sci. Biol.*, 3:91-94. <https://doi.org/10.15835/nsb315623>.
21. Khaleghi, E., Arzani, K., Moallemi, N., & Barzegar, M. (2012). Evaluation of chlorophyll content and chlorophyll fluorescence parameters and relationships between chlorophyll a, b and chlorophyll content index under water stress in *Olea europaea* cv. Dezful. *International Journal of Agricultural and Biosystems Engineering*, 6:636-639. <https://doi.org/10.5281/zenodo.1082211>.
22. Yousaf, A., Athar, M., Salman, M., Farooq, U., & Chawla, F. S. (2017). Biosorption characteristics of *Pennisetum glaucum* for the removal of Pb (II), Ni (II) and Cd (II) ions from aqueous medium. *Green Chemistry Letters and Reviews*, 10:462-470. <https://doi.org/10.1080/17518253.2017.1402093>.
23. Manzoor, Q., Nadeem, R., Iqbal, M., Saeed, R., Ansari, T.M. (2013). Organic acids pretreatment effect on *Rosa bourbonia* phyto-biomass for removal of Pb (II) and Cu (II) from aqueous media. *Biore-sour. Technol.* 132:446-452. <https://doi.org/10.1016/j.biortech.2013.01.156>.
24. Fawzy, M., Nasr, M., Abdel-Gaber, A., & Fadly, S. (2016). Biosorption of Cr (VI) from aqueous solution using agricultural wastes, with artificial intelligence approach. *Separation Science and Technology*, 51:416-426. <https://doi.org/10.1080/01496395.2015.1115068>.
25. Kumar, P., & Kumar, P. (2019). Removal of cadmium (Cd-II) from aqueous solution using gas industry-based adsorbent. *SN Applied Sciences*, 1:1-8. <https://doi.org/10.1007/s42452-019-0377-8>.
26. Ding, Z., Hu, X., Wan, Y., Wang, S., & Gao, B. (2016). Removal of lead, copper, cadmium, zinc, and nickel from aqueous solutions by alkali-modified biochar: Batch and column tests. *Journal of Industrial and Engineering chemistry*, 33:239-245. <https://doi.org/10.1016/j.jiec.2015.10.007>.
27. Sazali, N., Harun, Z., & Sazali, N. (2020). A review on batch and column adsorption of various adsorbent towards the removal of heavy metal. *Journal of Advanced Research in Fluid Mechanics and Thermal Sciences*, 67:66-88. <https://akademiabaru.com/submit/index.php/arfmts/article/view/2854>.
28. Nassar, N.N. (2010). Rapid removal and recovery of Pb (II) from wastewater by magnetic nanoadsorbents. *J. Hazard. Mater*, 184:538–546. <https://doi.org/10.1016/j.jhazmat.2010.08.069>.
29. Taha, A. A., Shreadah, M. A., Heiba, H. F., & Ahmed, A. M. (2017). Validity of Egyptian Na-montmorillonite for adsorption of Pb<sup>2+</sup>, Cd<sup>2+</sup> and Ni<sup>2+</sup> under acidic conditions: characterization, isotherm, kinetics, thermodynamics and application study. *Asia-Pacific Journal of Chemical Engineering*, 12:292-306. <https://doi.org/10.1002/apj.2072>.
30. Deosarkar, S. D., & Hangirgekar, S. P. (2012). Cu (II) adsorption from aqueous

- solution by *Punica granatum L.* husk. Journal of Chemical and Pharmaceutical Research, 4:4651-4656. <http://jocpr.com/vol4-iss10-2012/JCPR-2012-4-10-4651-4656.pdf>.
31. Alharbi, R. M., Sholkamy, E. N., Alsamhary, K. I., Abdel-Raouf, N., & Ibraheem, I. B. M. (2023). Optimization Study of the Capacity of *Chlorella vulgaris* as a Potential Bio-Remediator for the Bio-Adsorption of Arsenic (III) from Aquatic Environments. *Toxics*, 11:439. <https://doi.org/10.3390/toxics11050439>.
  32. Gupta, V. K., Rastogi, A., & Nayak, A. (2010). Biosorption of nickel onto treated alga (*Oedogonium hatei*): application of isotherm and kinetic models. *Journal of colloid and interface science*, 342:533-539. <https://doi.org/10.1016/j.jcis.2009.10.074>.
  33. Funtua, M. A., & Ugbe, F. A. (2015). Adsorption of heavy metals from aqueous waste water using unmodified and ethylenediaminetetraacetic acid (EDTA) modified maize cobs. *Int. J. Curr. Res. Biosci. Plant Biol. Int. J. Curr. Res. Biosci. Plant Biol.*, 2: 98-103.
  34. Borba, L. L., Cuba, R. M. F., Terán, F. J. C., Castro, M. N., & Mendes, T. A. (2019). Use of adsorbent biochar from Pequi (*Caryocar Brasiliense*) husks for the removal of commercial formulation of glyphosate from aqueous media. *Brazilian Archives of Biology and Technology*, 62. <https://doi.org/10.1590/1678-4324-2019180450>.
  35. Tsegaye, F., Tadesse, A. M., Teju, E., & Aschalew, M. (2020). Preparation and sorption property study of Fe<sub>3</sub>O<sub>4</sub>/Al<sub>2</sub>O<sub>3</sub>/ZrO<sub>2</sub> composite for the removal of cadmium, lead and chromium ions from aqueous solutions. *Bulletin of the Chemical Society of Ethiopia*, 34:105-121. <https://doi.org/10.4314/bcse.v34i1.10>.
  36. Božić, D., Stanković, V., Gorgievski, M., Bogdanović, G., & Kovačević, R. (2009). Adsorption of heavy metal ions by sawdust of deciduous trees. *Journal of hazardous materials*, 171:684-692. <https://doi.org/10.1016/j.jhazmat.2009.06.055>.
  37. Yao, Z. Y., Qi, J. H., & Wang, L. H. (2010). Equilibrium, kinetic and thermodynamic studies on the biosorption of Cu (II) onto chestnut shell. *Journal of Hazardous Materials*, 174:137-143. <https://doi.org/10.1016/j.jhazmat.2009.09.027>.
  38. Balarak, D., Al-Musawi, T. J., Mohamed, I. A., & Abasizadeh, H. (2020). The eradication of reactive black 5 dye liquid wastes using *Azolla filiculoides* aquatic fern as a good and an economical biosorption agent. *SN Applied Sciences*, 2:1-11. <https://link.springer.com/article/10.1007/s42452-020-2841-x>.
  39. Maponya, T. C., Makgopa, K., Somo, T. R., & Modibane, K. D. (2022). Highlighting the Importance of Characterization Techniques Employed in Adsorption Using Metal–Organic Frameworks for Water Treatment. *Polymers*, 14:3613. <https://doi.org/10.3390/polym14173613>.
  40. Oladipo, B., Govender-Opitz, E., & Ojumu, T. V. (2021). Kinetics, thermodynamics, and mechanism of Cu (II) ion sorption by biogenic iron precipitate: using the lens of wastewater treatment to diagnose a typical biohydrometallurgical problem. *ACS omega*, 6:27984-27993. <https://doi.org/10.1021/acsomega.1c03855>.
  41. Kavand, M., Asasian, N., Soleimani, M., Kaghazchi, T., & Bardestani, R. (2017). Film-pore-[concentration-dependent] surface diffusion model for heavy metal ions adsorption: single and multi-component systems. *Process Safety and Environmental Protection*, 107:486-497. <https://doi.org/10.1016/j.psep.2017.03.017>.

42. Charazińska, S., Burszta-Adamiak, E., & Lochyński, P. (2022). The efficiency of removing heavy metal ions from industrial electropolishing wastewater using natural materials. *Scientific Reports*, 12:17766. <https://doi.org/10.1038/s41598-022-22466-9>.
43. Huang, F., Guo, C. L., Lu, G. N., Yi, X. Y., Zhu, L. D., & Dang, Z. (2014). Bioaccumulation characterization of cadmium by growing *Bacillus cereus* RC-1 and its mechanism. *Chemosphere*, 109:134-142. <https://doi.org/10.1016/j.chemosphere.2014.01.066>.
44. El-Hendawy, A. N. A. (2003). Influence of HNO<sub>3</sub> oxidation on the structure and adsorptive properties of corncob-based activated carbon. *Carbon*, 41: 713-722. [https://doi.org/10.1016/S0008-6223\(03\)00029-0](https://doi.org/10.1016/S0008-6223(03)00029-0).
45. Gupta, V. K., Nayak, A., & Agarwal, S. (2015). Bioadsorbents for remediation of heavy metals: current status and their future prospects. *Environmental engineering research*, 20:1-18. <https://doi.org/10.4491/eer.2015.018>.