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# Biosorption of Cadmium by *Strychnos potatorum* Seed Proteins: Adsorption Kinetics, Proteins Nanoparticles for Enhancing Cd Removal and Relevance to Waste Water Treatment[#]

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**Abstract:** *Strychnos potatorum* seeds (cleaning nuts) widely grown in India have been extensively used by remote village tribals as a natural coagulant for clarification of turbid and metal contaminated water. In the present study the proteins isolated from this seed powder were used in an attempt to understand the role of proteins in Cd(II) adsorption from the aqueous media. As the proteins isolated did not shown any glycoprotein nature or hemagglutinating activity, they might possibly represent the storage proteins in the seed. The present study provided evidence for immobilized proteins as effective biosorbents for adsorption of Cd(II) from aqueous solution. The ability of these seed proteins to bind aqueous cadmium has been investigated. Our initial experiments with the seed meal revealed the presence of some coagulant proteins. These proteins were isolated from the soluble extracts of the seeds by ammonium sulfate fractionation. The (30-70%) fractions containing the bulk of proteins were separated by gel filtration into two peaks A and B. The (30-70%) ammonium sulfate precipitated proteins, as well as those from Peak A and B were separately immobilized to affigel-10. The Cd(II) biosorption efficiency by these proteins was investigated. Several experiments have been done under varying range of pH (2.0-7.0), contact time (5- 600 min), temperatures (4- 40°C) and metal ion concentrations (80 –110 $mg.L^{-1}$). The results show that the optimum conditions for Cd(II) adsorption are almost same for the three proteins used in the study. The Cd(II) removal is pH, contact time, initial metal concentration and temperature dependent .The maximum removal was at pH 5.0, which was achieved at 360 minutes. The # for more details please refer to colloids and surfaces B: Biointerfaces 94(2012)73-79 adsorption value decreased at high temperature (40°C). The equilibrium data fit into Langmuir isotherm than Freundlich model and both the isotherms. Comparing these parameters revealed that the pseudo-second order model was applicable than Lagergren first order model. In summary this is the first report establishing the role of isolated proteins from the seeds in selective removal of the cadmium. Investigations are in progress to test the prepared nanoparticles of these proteins and to enhance the ability of cadmium removal. Our aim of this study in the scope of developing thin films to be used in large scale for removing of heavy metals and purify the contaminated water and also this techniques can be used in group columns for water treatment and purification and right now we standardised the lab scale techniques and we are looking to develop the scale up techniques.

**Key words:**

## INTRODUCTION

The contamination of the environment and surface and ground water with heavy metals has become a worldwide problem. The discharge of metals to the environment is gradually increasing as a result of industrial activities and technological development, posing threat to the environment and public health because of their toxicity, accumulation through food chain and persistence [1]. One of the heavy metals that is toxic

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to the humans and widely studied by many researchers is cadmium. The major sources of cadmium(II) release into the environment by waste streams are electroplating, smelting, alloy manufacturing, pigments, plastic, battery, mining and refining processes .Cadmium is non-essential for metabolic processes and is one of the most toxic metals [2, 3]. Cadmium contamination in human was first reported in Japan in the 1950s where the municipal sewage sludge was used as a fertilizer through the rice crop [4]. It has an extremely long biological half-life (>20 years) and is listed by the U.S. Environmental Protection Agency (US-EPA) as one of the 126 priority contaminants and as a known carcinogen by the International Agency for Research on Cancer [5]. Cadmium is a highly toxic element and considered as a carcinogen. It can enter the human body by eating food, drinkingwater, breathing or smoking.Most of the cadmium that enters the body goes to kidney and liver and can remain there for many years and can cause serious damage to kidney and bones [6]. Adverse health and toxic effects due to cadmium exposure were well documented. Diseases such as renal damage [7], hypertension [8], anemia [9], and itai-itai [10] are associated with excess bioavailability of cadmium to humans.Due to the increasing value of some metals such as cadmium, as well as due to a greater awareness of the ecological effects of toxic metals released into the environment, studies of metal accumulation have been carried out on the removal and recovery of metals. Among those methods, using natural materials of biological origin has been focused in recent years [11–13]. A number of methods such as the coagulation, chemical precipitation, ion exchange, evaporation, membrane processing, electrolytic and adsorption technologies are used for removal of toxic metals from industrial waste waters and other effluents [14]. However, these methods have some disadvantages and other limitations which include: low efficiency, intense energy requirement and often are not feasible to reduce the cadmium concentration to the level required by environmental legislation. Furthermore, production of toxic chemical sludge as a secondary contaminant is an additional problem that needs further treatment. Thus, there is a need for the development of economic, effective and safe, method for removal of cadmium from aqueous solutions [15]. However, there are some disadvantages for traditional physicochemical methods to treat cadmium polluted wastewater, such as expensive cost, low efficiency, labor-intensive operation, and lack of selectivity in the treating process [16]. Biosorption utilizes the ability of biological materials to accumulate heavy metals from waste streams by either metabolically mediated or purely physic chemical pathways of uptake [17]. A waid variety of agro-forestry and biological materials are proposed as adsorbents such as wheat shell [18], brown algae [19], olive stone [20], orange peel [21], red mud [22], brown seaweed [23], coconut copra meal [24], olive pomace [25], macrofungus [26], tree fern [27], eucalyptus bark [28], Rosa gruss an teplitz [29], Cupressus sempervirens, Eucalyptus longifolia and Pinus halepensis[30], and papaya wood [31] for removal of toxic metals from aqueous solutions. A commercial product by name “NATFLOC” a “natural polyelectrolyte” has been developed with the seeds of S. Potatorum by the GCC. NATFLOC is recommended by the GCC for turbidity removal of water in a wide range of turbidity levels upto 3000 NTU (Nephelometric Turbidity Unit). It is being used as a secondary flocculent in conjunction with alum for cost reduction of turbid natural water treatment. The raw seed powder is used by indigenous tribals for cleaning the turbid and metal contaminated natural waters. However, the biochemical characterization of the coagulant seed proteins for removal of heavy metals in water has not been scientifically investigated. Therefore, in this study S. potatorum seed proteins were isolated to study their ability for the removal of cadmium from aqueous solution.

## MATERIALS AND METHODS

All the chemicals and reagents used in the present study were of analytical grade. All the glassware used were washed with 10% (v/v) HNO3 and subsequently rinsed several times with de-ionized distilled water to remove any possible interference by other materials. Cd(II) stock solution (1000 mg L-1)was prepared by dissolving 1.6306 g of CdCl2 (qualigens fine chemicals, Mumbai; minimum assay 99%) in 200 mL of (Millipore) Milli-Q water and the final volume made up to 1000 mL with Milli-Q water. Different concentrations of Cd(II) were prepared after diluting the stock solution appropriately. Standard solution of Cd(II) (1000 mg L-1) for atomic spectrophotometer was procured from Sisco Research Laboratories (India). pH of the solutions was adjusted with 1 M NaOH or 1 M HCl. Affigel-10 was procured from Bio-Rad Laboratories, USA. S. potatorum L. seeds were obtained from the divisional forest, flying squad division, Rajahmundry, East Godavari district, Andhra Pradesh India. Seeds were dried at 40°C for 2 days in hot air oven. Seeds were made into powder in Cyclotech 1093 sample mill (Tecator AB, Höganäs, Sweden). 10 g of the seed powder was used for every batch of protein extraction

**Proteins Extraction from the Seed Powder:** S. potatorum seeds were ground to a fine powder and defatted with acetone and hexane. From the dried seed powder, total proteins were extracted overnight at 4°C using 25 mM Tris–HCl buffer pH 7.4 containing 150 mM NaCl (TBS), 1 mM phenylmethylsulfonyl fluoride (PMSF) was added as a protease inhibitor. The suspension was clarified by centrifugation at 10,000 rpm for 20 min. The clear supernatant containing the soluble proteins was subjected to 0–30, 30–70% ammonium sulfate fractionation. Protein concentration in the extracts as well as the ammonium sulfate precipitated proteins was determined using Lowry's Method [32], Bradford method [33] and bicinchoninic acid (BCA) method [34] employing Bovine Serum Albumin (BSA) as standard.

**Gel Filtration:** Seralose 4B (Sisco Research Laboratory, India: equivalent to Sepharose 4B supplied by Sigma, USA) matrix was used for separation of the 30–70% ammonium sulfate precipitated protein. The column (75 mL gel) was equilibrated with 25 mM TBS. The precipitated protein was dissolved and dialyzed against TBS and applied onto the gel in batches to separate the proteins. Protein in column fractions was monitored by measuring the absorbance at 280 nm. SDS-PAGE analysis was carried out according to Laemmli [35]. The protein bands were visualized by silver staining as described by Blum *et al*. [36]. 2.5.

**Immobilization of Proteins to Affigel-10 (Bio-Rad Laboratories, N Hydroxysuccinamide Esters of a Derivatised Cross-Linked Agarose Bead Support):** The two distinct peaks (peak A) and (peak B) proteins eluted from the gel above as were separately pooled and concentrated. These and the 30–70% ammonium sulfate precipitated proteins were separately immobilized to 2 mL of affigel-10 each following the manufacturer's instructions.

**Proteins Interaction with Cadmium Metal Ions:** Many experiments were done to evaluate if the proteins immobilized above can specifically interact with metal ions such as cadmium. The three gels prepared above were separately packed into columns (2 mL of each gel) and equilibrated with 25 mM phosphate buffer pH 7.4. The columns were washed with de-ionized Milli-Q (Millipore) water. To each of the gels, 3 mL of (80 mg L-1) cadmium metal ion (pH 5.0) was added and rotated for six hours at 4°C to ensure optimal binding. The effect of different pH solutions on the equilibrium adsorption of Cd(II) ions was investigated (pH 2 to 7.0). Since pH 5.0 was found to be optimal for binding, the other three separate experiments viz., (i) to analyze the binding efficiency at different time intervals (5, 10, 20, 30, 60, 180, 360, 480 and 600 min), (ii) to study the effect of different concentrations of the metal ions (80, 90, 100 and 110 mg L-1) and (iii) to study the efficiency of binding at different temperatures (4, 24 and 40°C) were conducted at pH 5.0. In all these experiments the gels were processed in the same way. After loading the sample, the unbound solution was collected; the gel was washed with de-ionized Milli-Q water for removing the excess metal ions. The bound metal ions were eluted using 0.15 M HCl. The metal concentrations were measured using flame atomic absorption spectrometer (GBC 932 plus, Australia). The wavelength used for analysis of the metal in this study was 228.8 nm. The instrument was calibrated within the linear range of analysis and the correlation coefficient (R2) of 0.996–1.000 was obtained for the calibration curve. To check the reproducibility, all the experiments were repeated thrice and each experiment in turn was carried out in triplicates. The mount of adsorption: and Sorption efficiency were calculated.

All the data presented are the average of the triplicates experiments and the standard errors are calculated using MS excel 7 and presented for n = 3. The instrument was periodically checked throughout the analysis with known standards. The equilibrium metal uptake qe (mg g-1) and the sorption efficiency (%) have been calculated according to the mass balance equations [37].

**Desorption of the Adsorbed Cd(ll):** Cd(II) was first bound on the affigels. The gels were washed to remove unbound metal ion. After extensive washing with deionized distilled (milli-Q) water, the Cd (II) bound on the gel was eluted using 0.15 M HCl. In all cases 3 mL fractions were collected. The Cd(II) concentration in the solutions was measured using flame atomic absorption spectrometer (GBC 932 plus, Australia).

## RESULTS

**Extraction and Isolation of Total Proteins:** To understand and identify the components in the seeds that imparts the property for metal binding. In the present study a detailed analysis of the proteins extracted from the seeds of S. potatorum, was carried out. Toward achieving these goals, the seed extracts were subjected to ammonium sulfate fractionation 0–30 and 30–70%. The 30–70%

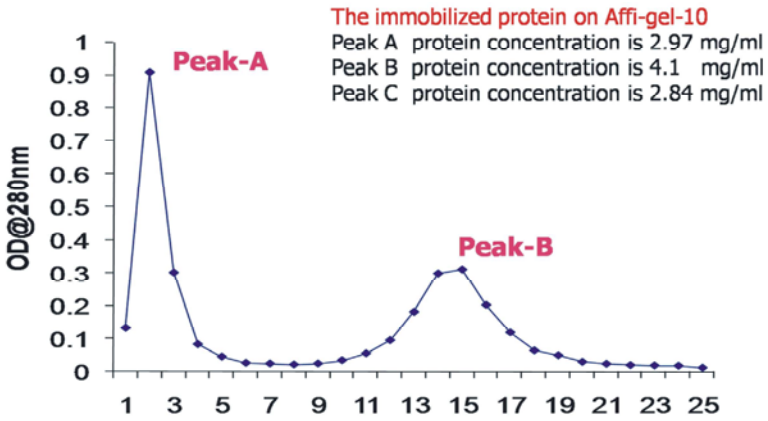


Fig. 1: Gel filtration of the 30-70% ammonium sulfate precipitated proteins on Seralose 4B. After collecting the void volume fraction in a measuring cylinder (25 ml). fractions of 3ml were collected and the absorbance monitored at 280 nm.

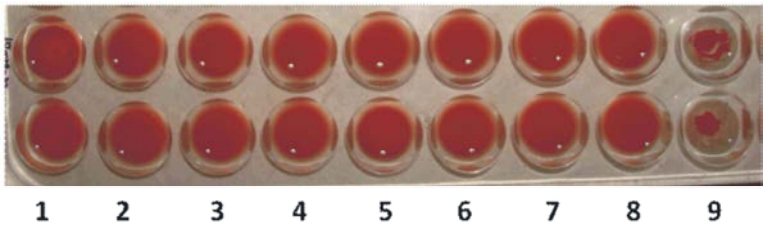


Fig. 2: Hemagglutination Activity for the proteins isolated from seed powder of S. potatorum 1 to 8 wells in different concentrations of the proteins and well 9 are positive control.

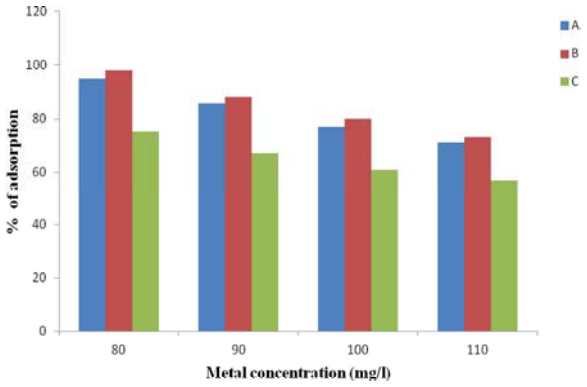


Fig. 3: Effect of different concentrations of $Cd^{+2}$ ($Cd^{+2}$ concentration = 80, 90, 100, 110 adsorption by proteins isolated from the seeds of *Strychnos potatorum* at temperature = $4^0$C, contact time = 360 minutes, PH 5. (Solid squares: Cadmium adsorption by protein from peak A in gel filtration. Solid circle: Cadmium adsorption by proteins from the peak B in gel filtration. Solid triangle: Cadmium adsorption by 30-70% ammonium sulfate precipitated proteins). Error bars show standard deviation, n= 3.

fraction that contained most proteins was further processed by gel filtration Fig. 1. As the proteins isolated neither showing glycoprotein natures nor hemagglutination activity Fig. 2., the proteins isolated might represent storage proteins in the seed material and so we further investigated if these proteins have the ability to bind metal ions. For this purpose the 30–70% ammonium sulfate precipitated fraction, the peak A and peak B separated on gel filtration, were all separately immobilized to affigel at a concentration of 2.97 mg mL-1, 4.1 mg mL-1 and 2.84 mg mL-1, respectively. In all figures solid squares: cadmium adsorption by protein of the peak A in gel filtration. Solid circle: cadmium adsorption by proteins from the peak B in gel filtration. Solid triangle: cadmium adsorption by 30–70% ammonium sulfate precipitated proteins, the error bars show the standard deviation for the data of triplicate experiments done.

**Effect of Initial Cd(ll) Concentration:** The rate of adsorption is a function of the initial concentration of metal ions, which makes it an important factor to be considered for effective biosorption [38]. The effect of metal concentration is shown in Fig. 3. The percentage of cadmium ions adsorption at different metal concentrations

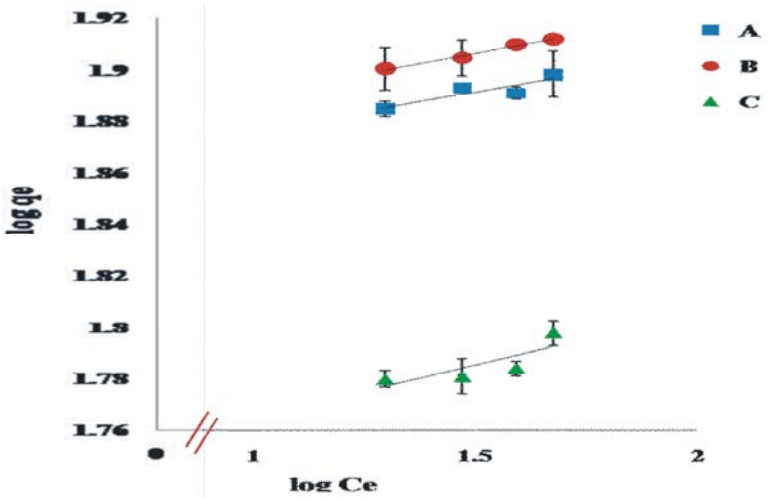


Fig. 4: Linearized Freundlich isotherm plot for adsorption of $Cd^{+2}$ by Strychnos potatorum seed proteins. (Solid squares: Cadmium adsorption by protein from peak A in gel filtration. Solid circle: Cadmium adsorption by proteins from the peak B in gel filtration. Solid triangle: Cadmium adsorption by 30-70% ammonium sulfate precipitated proteins). Error bars show standard deviation, n= 3.

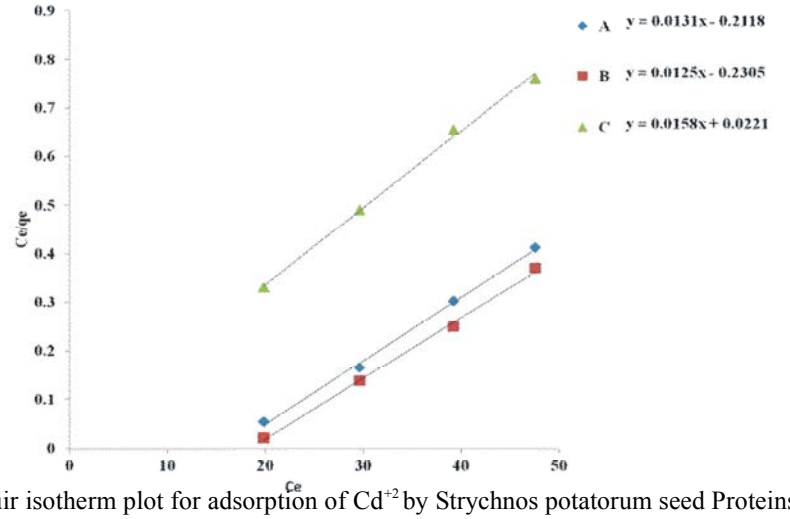


Fig. 5: Linearized Langmuir isotherm plot for adsorption of $Cd^{+2}$ by Strychnos potatorum seed Proteins. (Solid squares: Cadmium adsorption by protein from peak A in gel filtration. Solid circle: Cadmium adsorption by proteins from the peak B in gel filtration. Solid triangle: Cadmium adsorption by 30-70% ammonium sulfate precipitated proteins). Error bars show standard deviation, n= 3.

using proteins isolated from the seeds of S. Potatorum decreased with increase in metal ion concentration and showed little decrease in percentage (%) of adsorption at higher concentration. This may be due to saturation of active adsorptiosites on proteins.

**Adsorption Isotherm:** The capacity of cadmium adsorption by S. potatorum seed proteins at different concentrations of cadmium on a fixed amount of adsorbents was evaluated using the Freundlich and Langmuir adsorption isotherms [39]. In the Freundlich plot of log qe versus log Ce in Fig.4 represents a measure of non-linearity involved. The values of the constants are given in Table 1 which suggests that the cadmium adsorption by S. potatorum seed proteins is not following the Freundlich isotherm model. The Langmuir isotherm model [40] assumes monolayer coverage and constant binding energy between surface and adsorbate suggesting that the adsorbate surface has fixed number of binding sites and each can hold only one metal ion at a time. The monolayer forms when the equilibrium is attained. In the Langmuir isotherm a plot of Ce/qe versus Ce gives straight line as shown in Fig. 5. The values of qmax and b and the other parameters are given in Table 2.

Table 1: The Freundlich isotherm model constants for Cd(II) uptake by *Strychnos potatorum* seed proteins.

| | peak- A proteins | peak- B protein | 30-70 % proteins |
|---|---|---|---|
| Slope | 0.029 | 0.03 | 0.038 |
| Intercept | 1.841 | 1.854 | 1.726 |
| $R^2$ | 0.794 | 0.984 | 0.653 |
| $k_f$ (mg $g^{-1}$) | 69.34 | 71.45 | 57.81 |
| n | 34.483 | 33.33 | 26.316 |

Table 2: The Langmuir isotherm model constants for Cd (II) uptake by *Strychnos potatorum* seed proteins.

| | Peak- A proteins | Peak- B protein | 30-70 % proteins |
|---|---|---|---|
| Slope | 0.013 | 0.012 | 0.015 |
| Intercept | 0.211 | 0.230 | 0.022 |
| $R^2$ | 0.997 | 0.997 | 0.996 |
| $q_{max}$ (mg $g^{-1}$) | 76.92 | 83.33 | 66.67 |
| b (l$mg^{-1}$) | 61.61x$10^{-3}$ | 52.2x$10^{-3}$ | 0.682 |

The qmax was calculated from the slope of the plot, and also these calculated qmax is matching with the theoretical qe of Cd(II) with all of the three protein samples that were used in this study. According to the correlation coefficient it can be observed that the Cd(II) adsorption experimental data was better fitted to the Langmuir model than that of the Freundlich isotherm model.

**Effect of pH on Metal Biosorption:** The effect of pH solution on the biosorption of cadmium ions using proteins isolated from S. potatorum seed powder with different pH solutions was studied and the results are shown in the Fig. 6. There was a gradual increase in cadmium ion adsorption with the increase of the pH from 2.0 to 7.0. The maximum cadmium uptake was obtained at pH 5.0 for all proteins used in this study; the increase in the biosorption of cadmium with increase in pH can be explained by the fact that at low pH, the biosorbent surface became more positively charged thus reducing attraction between the biomass and metal ions. These bonded active sites thereafter become saturated and therefore are inaccessible to other cations [41]. At higher pH, the biosorbent surface is more negatively charged, thus attracting more cadmium ions. However, with further increase in pH the formation of anionic hydroxide complexes decreases the concentration of free cadmium ions; thereby the biosorption capacity of cadmium ions also decreases [42]. The results obtained in the present study can be correlated with the cited references above.

**The Effect of Time:** The effect of contact time on the adsorption of Cd(II) at 80 mg L-1 and pH 5, is shown in the Fig. 7. The cadmium adsorption increased with increasing the contact time, the maximum removal of cadmium occurred at 180 min, after which there were no significant changes. The equilibrium was reached at 360 min for the Cd(II) adsorption by proteins isolated from the seeds of S. potatorum as we can observe from the figure that the adsorption started fast and increased rapidly till 180 min. Following this, the adsorption rate was uniform as there was no significant change in adsorption with the increasing time. The initial fast adsorption is due to the availability of more active sites and more functional groups which participate in the cadmium uptake till equilibrium is attained and thereafter, there was no further adsorption. Therefore, there is no significant change in the cadmium concentration in the solution.

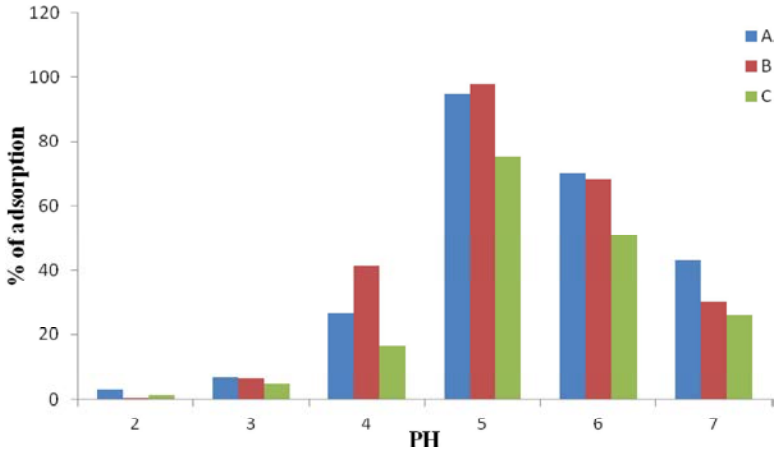


Fig. 6: Effect of PH on $Cd^{+2}$ adsorption by proteins isolated from the seeds of *strychnos potatorum* .initial metal concentration = 80 mg/l,temperature $4^0$C, contact time = 360minutes. (Solid squares: Cadmium adsorption by protein from peak A in gel filtration. Solid circle: Cadmium adsorption by proteins from the peak B in gel filtration. Solid triangle: Cadmium adsorption by 30-70% ammonium sulfate precipitated proteins). Error bars show standard deviation, n= 3.

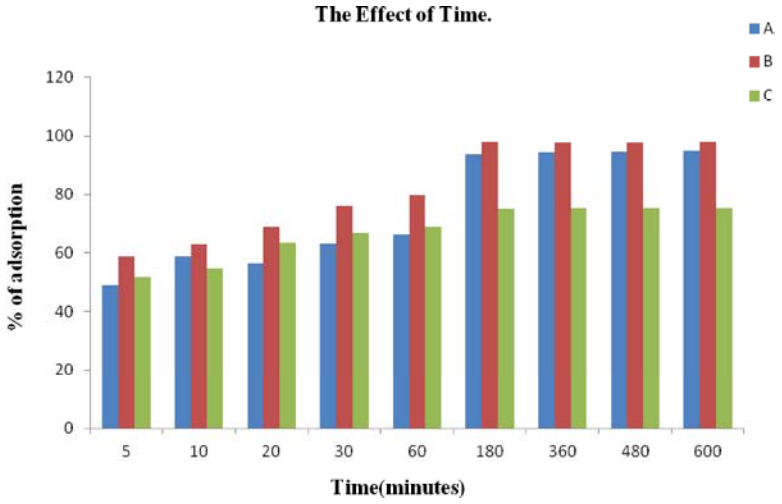


Fig. 7: Effect of Time on $Cd^{+2}$ adsorption by proteins isolated from the seeds of *strychnos potatorum* .initial metal concentration = 80 mg/l,tempreatur= $4^0$C, PH 5, contact time 5, 10, 20, 30, 60, 120, 180, 240,360 and600 minutes. (Solid squares: Cadmium adsorption by protein from peak A in gel filtration. Solid circle: Cadmium adsorption by proteins from the peak B in gel filtration. Solid triangle: Cadmium adsorption by 30-70% ammonium sulfate precipitated proteins). Error bars show standard deviation, n= 3.

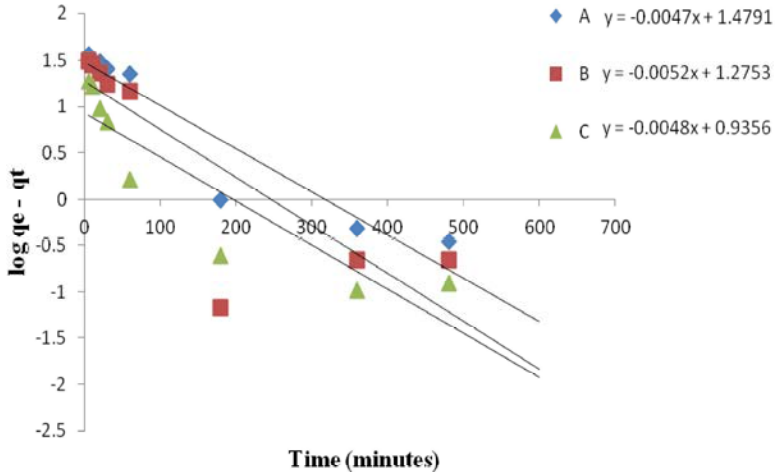


Fig. 8: Pseudo first order kinetic model for adsorption of Cd(ll) by Strychnos potatorum seed proteins. (Solid squares: Cadmium adsorption by protein from peak A in gel filtration. Solid circle: Cadmium adsorption by proteins from the peak B in gel filtration. Solid triangle: Cadmium adsorption by 30-70% ammonium sulfate precipitated proteins). Error bars show standard deviation, n= 3.

**Adsorption Kinetics:** It is very important to understand the kinetics of solute uptake to select the optimum operating conditions for full-scale batch process. A number of models have been developed to describe the kinetics of the sorption process. In this study we used two different models to investigate the mechanism of Cd(II) biosorption to the S. potatorum seed powder proteins. These kinetic models included pseudo first-order Lagergren model and pseudo secondorder model. The pseudo first order model derived by Lagergren [43] is one of the most widely used models for the biosorption of solutes from a liquid solution [44, 45]. The rate constant Ks1 for the pseudo first order and also the value of qe were calculated from the plot of (qe - qt) versus the time Fig. 8. As they are presented in Table 3. The constants determined from the model indicate that the kinetic of adsorption does not fit with this model since the correlation coefficient is low and the experimental qe value differs from the estimated qe, for the total incubation time, whereas the pseudo first order could be applicable for the short time less than 100 min only after that linearity of the graph missed, the possibility of this kinetic change may

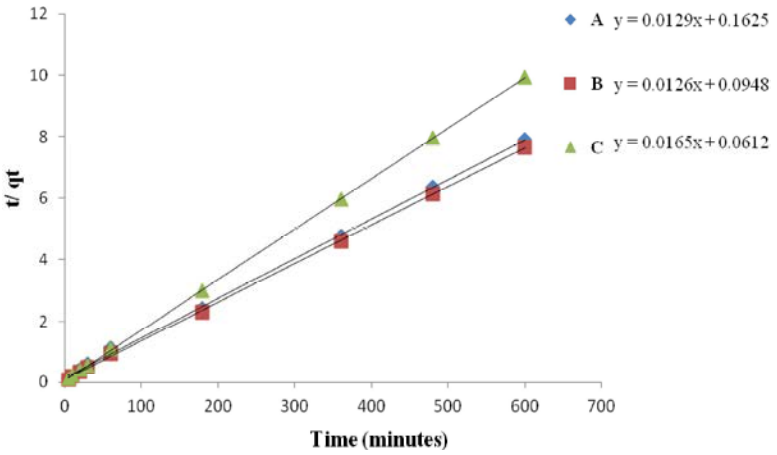


Fig. 9: Pseudo second order kinetic model for adsorption of Cd(ll) by Strychnos potatorum seed proteins. (Solid squares: Cadmium adsorption by protein from peak A in gel filtration. Solid circle: Cadmium adsorption by proteins from the peak B in gel filtration. Solid triangle: Cadmium adsorption by 30-70% ammonium sulfate precipitated proteins). Error bars show standard deviation, n= 3.

Table 3: The Pseudo first order model Kinetic constants for Cd(II) uptake by *Strychnos potatorum* seed proteins

| | Peak - A protein | Peak - B protein | 30- 70% proteins |
|---|---|---|---|
| Slope | 0.004 | 0.005 | 0.004 |
| Intercept | 1.479 | 1.275 | 0.935 |
| $R^2$ | 0.898 | 0.691 | 0.820 |
| qe (mg $g^{-1}$) | 30.130 | 18.837 | 8.61 |
| K1($min^{-1}$) | $9.212x10^{-3}$ | 0.0115 | $9.212x10^{-3}$ |

Table 4: The pseudo second order kinetic constants for Cd (II) uptake by *Strychnos potatorum* seed proteins

| | Peak- A proteins | Peak- B protein | 30-70% proteins |
|---|---|---|---|
| Slope | 0.0129 | 0.012 | 0.016 |
| Intercept | 0.162 | 0.094 | 0.061 |
| $R^2$ | 0.999 | 0.999 | 1 |
| qe (mg $g^{-1}$) | 77.52 | 83.33 | 62.5 |
| K2 (g $mg^{-1}$ $min^{-1}$) | $1.02x10^{-3}$ | $1.532x10^{-3}$ | $4.196x10^{-3}$ |

due to the structural change of the proteins exposed to cadmium for long time. The pseudo second order model [44] assumes that biosorption follows a second order mechanism, so that the rate of occupation of biosorption sites is proportional to the square of the number of occupied sites. The pseudo-second order rate constant k2 and the value of qe were calculated from the plot of t/qe versus t Fig. 9 and presented in Table 4. The value of correlation coefficient (R2) of Cd(II) for the pseudo second-order kinetic model on to S. potatorum seed powder are very high (0.999), (0.999), (1) for Peak-A, Peak-B and 30–70% ammonium sulfate precipitated proteins respectively. The value of theoretical qe is closer to the first and second order correlation coefficients (R2) and qe values we can observe that in the first order the correlation coefficients are lower than (R2) in the second order model, also the theoretical qe values in the second order are closer to the experimental qe values than in the first order model. From all that we can conclude that the Cd(II) biosorption onto S. potatorum seed proteins follows the pseudo second order kinetic model.

## CONCLUSION

S. potatorum seed powder was used as a natural coagulant to clarify turbid water. In the present study the proteins isolated from this seed powder were used in an attempt to understand the role of proteins in Cd(II) adsorption from the aqueous media. The present study provided evidence that the immobilized proteins are effective biosorbents for adsorption of Cd(II) from aqueous solution. The Cd(II) adsorption was dependent on pH, contact time, initial metal concentration and temperature. The optimal Cd(II) adsorption conditions were almost same for all three proteins used in this study. The maximum cadmium adsorption was at pH 5.0 and the equilibrium was attained at 360 min. The adsorption value decreased at high temperature (40°C). All adsorption data were best described by pseudo second order kinetic model. It is clearly shown that the adsorption equilibria for experimental data were fitted to Langmuir isotherm model. In summary this is the first report establishing the role of isolated proteins from the seeds in selective removal of the cadmium. Our future studies are focused on preparing the nanoparticles for these proteins and to assess their abilities for increased cadmium removal.

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