Biosorption of Lead (II) from aqueous solution and Industrial Effluent by using leaves of *Araucaria cookii*: Application of Response Surface Methodology

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Abstract: *The aim of the present study involves the biosorption of Pb (II) by using the leaves Araucaria cookii from aqueous solution and industrial effluent. The batch studies were performed to evaluate the different parameters like pH, contact time, metal ion concentration, adsorbent mass and size variation. Characterization of biosorbent was carried out by Fourier Transform Infrared (FTIR) and Scanning Electron Micrograph (SEM). Experimental results shows that at pH 5 the biosorption efficiency reached a maximum removal of 98.52% with a contact time of 60 min and an initial ion concentration 200 mg/L. Kinetic models are described and fitted good with Pseudo- second order reaction. Langmuir and Freundlich isotherms were also applied and evaluated. The Central Composite experimental Design (CCD) in Response Surface Methodology was used to design the experiment and the optimum conditions. The result showed that the model and experimental data was favourable in the removal of Lead (II) in the industrial waste water and can be used as low cost biosorbent.*
Keywords: *Araucaria cookii, biosorption, Lead (II), Langmuir & Freundlich isotherms, RSM*

I.   Introduction

Natural waters have been found to be contaminated with rapid industrialization which leads to an increased disposal of heavy metals in the environment. Heavy metals are among the conservative pollutants that are not subject to bacterial attack or other break down or degradation process and are a permanent addition to the marine environment [1, 2]. Heavy metal ions such as cobalt, copper, nickel, chromium, lead, and zinc are detected in the waste streams from mining operations, tanneries, electronics, electroplating, batteries and petrochemical industries as well as textile mill products. Major lead pollution is through automobiles and battery manufacturers [3].

Lead is considered as one of the top sixteen toxic pollutants because of its carcinogenic characteristics for humans [4]. Lead is used as electrodes in the process of electrolysis [5]. The permissible limit of lead (II) in drinking water is 0.05 mg/l [6]. The maximum discharge limits for Pb (II) in waste water is 0.05 mg/l [7] and sewage sludge applied to agriculture land is 420 mg/l [8] as set by the Environment protection Agency.

Toxic levels of lead in human beings have been associated with encephalopathy seizures and mental retardation [9]. Lead poisoning causes severe damage to the Kidneys, nervous system, reproductive system, liver and brain [10]. Lead is known to inhibit the activity of three critical enzymes (5- aminolaevulinate dehydrase (ALA-D), coproporphyrinogen oxidase (COPRO-O) and ferrochelatase (FERRO-C) critical in haem synthesis, causing abnormal concentrations of haem precursors in blood and urine [11]. Contamination of water through anthropogenic practices is the primary cause of lead poisoning in fish [12].

The several methods which were used for the treatment of waste water include Precipitation, adsorption with activated carbon, ion exchange, membrane processes, oxidation and reduction [13]. But, technical and economical factors limit sometimes the feasibility of such process [14, 15].

The promising method for heavy metal removal from waste water is biosorption. Biosorption is an economically feasible means for the removal and/or recovery of heavy binding abilities of various biological materials. Recently, there has been an intensive study on the use of seeds, pods, and bark of plants which is called as biomass in removing heavy metals from waste water. In addition, studies have been carried out to ascertain the optimum conditions necessary for efficient removal of these metals from polluted sites [11, 16].

Response surface methodology (RSM) is a collection of mathematical and statistical techniques useful for analyzing the effects of several independent variables on the response [17]. RSM generates an experimental design for model preparation. An experimental design is a specific set of experiments defined by a matrix composed of the different level combinations of the variables studied [18].

In this study, the leaves of *Araucaria cookii* which belong to the family *Araucariaceae* are used as a low cost biosorbent for the removal of Pb (II). The factors studied include pH, contact time, dosage, size variation and metal ion concentration. Experimental data is obtained by using Langmuir and Freundlich isotherms. Kinetic studies of Pseudo first order and Pseudo second order reactions have been carried out.

II. Materials and Methods

2.1 Biomass Preparation

The leaves were collected from the premises of Manasa gangotri, University of Mysore, Mysore, Karnataka, India. The leaves were sun dried for one month and ground approximately to different particle sizes from 100 μm- 400 μm in size. The biosorbent was washed with double distilled water several times to remove the floatable biomass and sun dried for 5 days. The samples were kept in an air tight plastic jar until the usage for the experiment.

2.2 Preparation of Adsorbate

Metal ion solution of Pb (II) was prepared from Lead nitrate (Merck- A.R. grade). About 1.598 g of lead nitrate was weighed and a standard stock solution of concentration 1000 mg/l$^-$ was prepared in double distilled water and further working solutions of lower concentrations were prepared as and when required. The pH of the solution was adjusted using 0.01 M HCl and 0.01 M NaOH. The final concentration of metal ions were analysed by AAS.

2.3 Industrial effluent:

The effluent was collected from a Paper industry in Nanjangud Taluk, Mysore, Karnataka, India. The effluent was collected in a clean plastic container and stored in the refrigerator until further experiments and its Physico - Chemical characteristics of effluent was analysed according to standard methods described by the American Public Health Association (APHA; APHA 1998) [19] and the results are presented in Table 1.

Table -1 Physico-chemical Characteristics of Paper Industry Effluent

Parameters	Contents (mg/l)
pH	3.0
Conductivity (μs/cm)	1980
Total Dissolved Solids	1531
COD (mg/L)	520
Calcium (mg/L)	110.5
Magnesium (mg/L)	51.6
Chloride (mg/L)	215.2
Sulphate (mg/L)	56.0
Sodium (mg/L)	80.5
Potassium (mg/L)	5.0
Lead (II)	6.0
Zinc (II)	22
Nickel (II)	5

2.4 Characterization of Biosorbent:

A Fourier Transform Infrared (FTIR) spectrum of unloaded and metal bound *A. cookii* samples were recorded by using standard KBr pellet method to obtain the spectrum using Jasco FT / IR- 4100 in wave number range of 400-4000 cm^{-1}. The scanning electron micrograph was used to reveal the surface texture and morphology of the biosorbent by using ZEISS , EVO/LS 15-15-41, smart SEM version 5.05.

2.5 Optimization procedure: Central Composite Design (CCD)

Response surface methodology (RSM) procedure [20, 21] was used for optimising the process variables. The three main steps involved in this process are experimental design, modelling and optimization. RSM model shows relatively few combinations of variables for determination of complex response function [22]. In the present study, the Central Composite Design model has been used for the optimization of lead Biosorption. The five variables of lead ions were pH, contact time, metal ion concentration, biomass and size variation. The independent process variables of RSM in the quantitative form are represented as follows:

$$Y= f (A_1, A_2, A_3 \text{-----------------} A_n) \quad\quad\quad (1)$$

where, Y is the amount of metal adsorbed (mg L^{-1}), f is the response function and A_1, A_2, A_3 ----------------- A_n are independent variables

In the linear manner, if the response varies, the linear function can be given by the following formula:

$$Y= b_0+ b_1A_1+b_2A_2+ \text{------------------} +b_2A_n \quad\quad\quad (2)$$

Where b_0, offset term; A_i first order main effect: A_{ii} second order main effect and A_{ij} interaction effect.

Table. 2 Different parameters chosen for the biosorption as independent variables.

Factor	Name	Units	Minimum	Maximum
A	pH		2	7
B	Biomass	g	0.5	4
C	concentration	mg/g	50	250
D	Time	min	5	90
E	Size variation	Micron	100	400

If there is a curvature in the system, the higher order polynomial quadratic model which is used can be stated as:

$$Y = b_0 + \sum b_i A_i + \sum b_{ii} A_i^2 + \sum b_{ij} A_i A_j \qquad (3)$$

There are five parameters in this study mainly pH, contact time, metal ion concentration, adsorbent dose, and particle size variation and these parameters were studied using the CCD model with two levels minimum and maximum. The model consists of pH (2-10), contact time (5- 90 min), metal ion concentration (50 -250 ppm), adsorbent dose (0.5- 4 g) and particle size from (100- 400 μ) and the independent variables of these parameters are shown in Table 2. The correlation co efficient R^2 value was also calculated to analyse the variance whether this model is good and appropriate.

2.6 Batch Experiments

Metal analysis was carried out in the batch method. The biosorption studies were carried out in the experimental conditions for different pH 1-7, contact time 5-90 min, metal ion concentration 50- 250 mgL^{-1} , adsorbent dose 0.5- 4.0 g and size variation 100- 400 μ. Each batch experiment was carried out by taking 2 g powder of *A. cookii* leaves with 20 ml of 1000 mgL^{-1} in an Erlenmeyer flask. A stirring speed of 200 RPM was kept constant in the rotary shaker with the appropriate time intervals from 5- 90 min. The stirred solution is filtered and the decanted solution was analysed using AAS. The mean value was calculated by repeating the experiment.

The adsorbed metal is based on the soluble metal concentration before and after the batch experiment. The amount of percent removal can be calculated as:

$$q_e \, (mg/g) = V \, (C_0 - C_e)/ m \qquad (4)$$

Where C_0 is the initial concentration (mg/l) and C_e is the equilibrium metal concentration (mg/l), V is the volume of metal solution (L) and m is the mass of the biosorbent.

III.   Results and discussion

3.1 FTIR analysis

The FT- IR spectroscopy method was used to obtain information about the nature of the functional groups responsible for the biosorption of metal ions on the binding sites of the biosorbents [23, 24, 25]
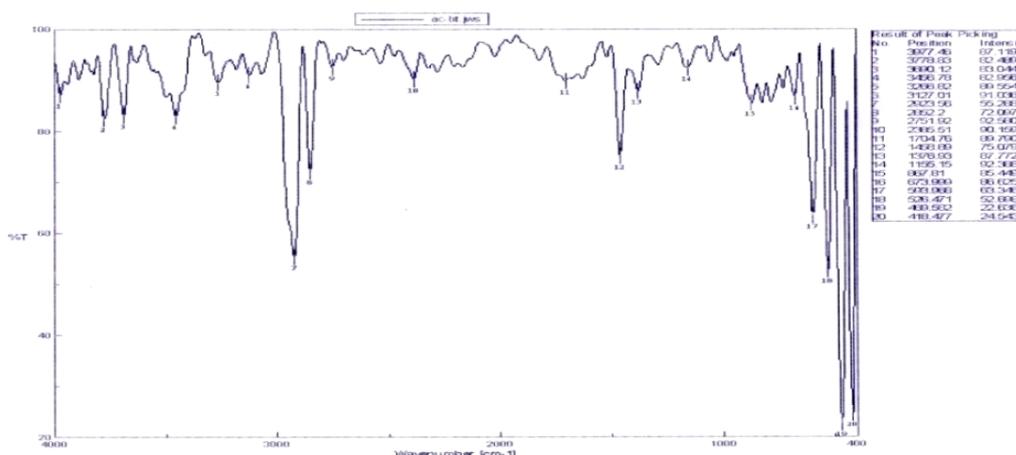

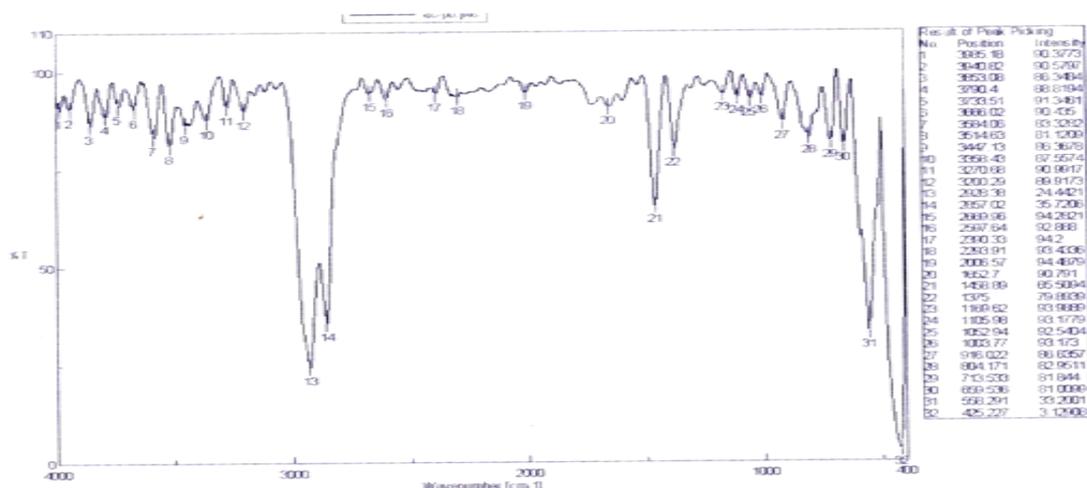**Fig. 1** FTIR spectra of unloaded *A. cookii*

Fig. 2 FTIR spectra of Pb (II) loaded *A.cookii*

The powder of *A.cookii* leaves were sieved to a particle size ranging between 100 to 400 µm sizes. 100 µm particle size were used for adsorption studies and were characterised by FTIR studies of before and after adsorption shown in Fig-1 and Fig-2 respectively. The peak at 3690 and 3456 cm^{-1} is the indicator of O-H (free) and –NH groups. The stretching of –OH groups bound to methyl group radicals presented in figure between 2983 and 2852 cm^{-1}. The peaks located at 2923 and 2751 cm^{-1} are the characteristics of carbonyl group stretching from aldehyde and ketones. The appearance of –OH group and C-O group confirms the presence of carboxylic group in the biosorbent. The peak 1458 and 1376 cm^{-1} are characterising the presence of Nitroso N =O stretching and S=O sulfonyl groups associated with stretching aromatic rings. The peaks observed at 1155, 867, and 673 cm^{-1} are due to C=S (Thiocarbonyl) and S-OR esters stretching. The –OH, -NH, carbonyl, carboxylic groups are important sorption sites [26]. The FTIR spectrum of the *A.cookii* after biosorption of Pb (II) is shown in the Fig 2 reveals that the involvement of Hydroxyl and carboxyl groups in the biosorption.
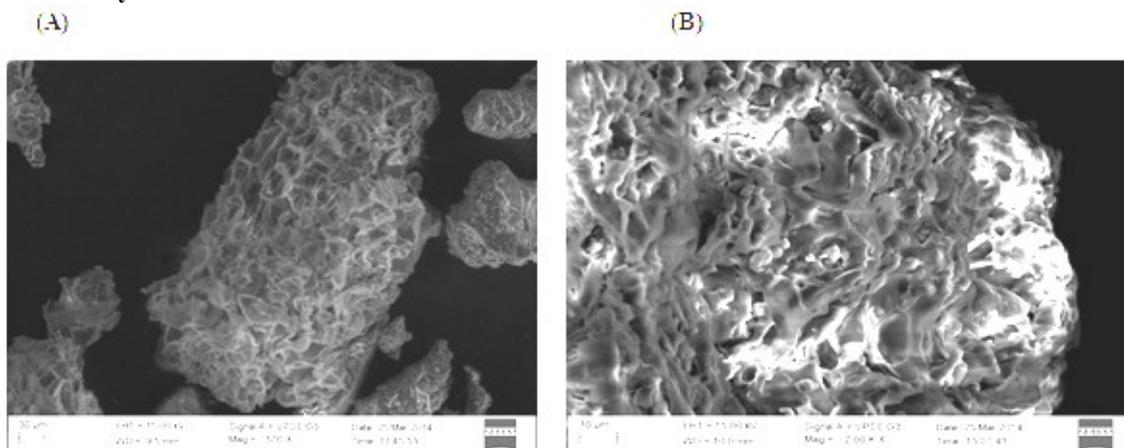
3.2 SEM analysis

Figure 3 (a) shows the SEM pictures of raw *A.cookii* and **Figure 3(b)** shows the SEM pictures of loaded *A.cookii*

Figure 3 (a) and (b) shows the SEM image of *A.cookii* which were used in the experiments. SEM clearly reveals the surface texture and morphology of the biosorbent. An amorphous structure with no definite surface can be seen in native biosorbents (Fig 3a). Fig 3(b) shows progressive changes in the surface particles with native biosorbent which depicts that Pb (II) ion have deposited as aggregates in the biosorbent.

3.3 Effect of pH

The pH of the solution is one of the most important parameter from the aqueous solution. 2 g of biosorbent was taken in an Erlenmeyer flask with the pH range of 1- 7 with 20 ml of 1000 mgL^{-1} of Pb (II)

solution. The solution is stirred for half an hour at a speed of 200 rpm in a mechanical shaker, the resultant solution is filtered, decanted and the Pb (II) solution is measured by AAS.
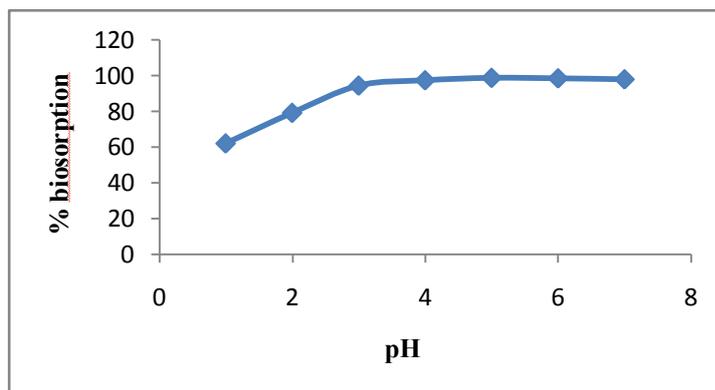

Figure.3 Effect of pH on adsorption of Pb (II) by *A.cookii*

The effect of biosorption of pH on Pb (II) is shown in Fig 3. The result shows that the percentage adsorption of Pb (II) increases from 62.07- 98.52% with increase in pH from 1.0 to 5.0, afterwards the rate of adsorption is stable. This may be due to the hydrogen ions which are strong competing ions and also influence the chemical speciation of functional groups on the surface of the adsorbents. Depending upon the pH, lead can be found in various forms, at pH < 5 approximately 79% of Pb^{2+} and 21 % of $PbNO_3$ will be at equilibrium and when the solution of the pH increases beyond 5, it begins to precipitate forming $[Pb (OH)_2]$ which is insoluble. So when the solution is in the solid lead hydroxide which is to be able to bind the negatively charged groups of the biomass decreases and hence the uptake of the biomass also reduces [27]. Therefore, the biosorbents used for removal of Pb (II) the pH should be maintained at pH 5 or lower, to avoid lead precipitation. Similar findings agree with that reported by using waste biomass of *Corynebacterium glutamicum* and also by using Groundnut hull at pH 5 [28, 29].

3.4 Effect of contact time

The effect of biosorption of Pb (II) on *A. cookii* was studied by taking 200 mg/L^{-1} of Pb (II) solution with 2g/l of 100 μm particle size in a clean conical flask. The resulting mixture was subjected to various time intervals from 5-90 min. It was observed that the biosorption of Pb (II) up to (98.52%) increases with increasing contact time up to 60 min and became constant. These results indicate that the sorption process can be considered very fast because of the largest amount of active adsorption sites within 20 – 30 min. The maximum adsorption attained was at 60 min reaching almost 98.52 % of the removal of Pb (II). The percentage removal of Pb (II) ions with contact time is shown in Fig 4.
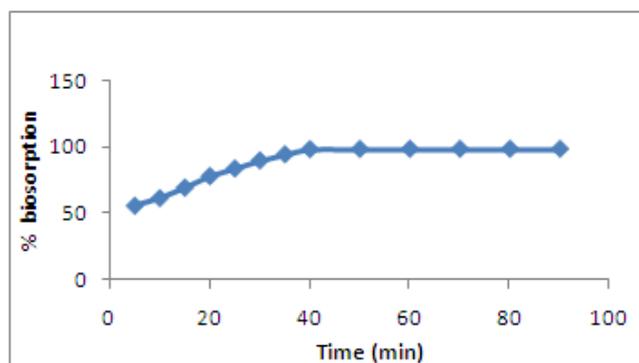

Figure. 4 Effect of contact time on adsorption of Pb (II) by *A.cookii*

The sour sop seed, bamboo dust and commercial activated carbon were used as a biosorbent for the removal of Lead [30, 31].

3.5 Effect of Adsorbent dose

In order to find out the effect of dosage for the removal of Pb (II) from aqueous solution were investigated by varying the dosage from 0.5 to 4.0 g/L^{-1}. The Fig- 5 depicts that the percentage removal increases with increasing in biosorbent dosage of 98.52%. It is expected that on increasing biosorbent dose will

have more surface area and adsorption sites. Afterwards, there was a negligible increase in the percentage removal beyond the dose of 2 g/l. This is due to the agglomeration of particles which causes the stable in adsorption rate. Based on this result, a dose of 2 g/l was considered sufficient for the optimal removal of metal ions. Similar results were reported for the removal of Pb (II) by groundnut hull and by using Rice Husk and its ash [29, 32].
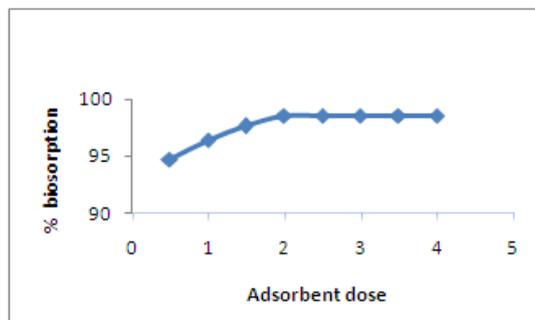

Figure.5 Effect of adsorbent dose on adsorption of Pb (II) by *A.cookii*

3.6 Effect of Metal ion Concentration

Initial concentration of Pb (II) was varied from 50- 250 mg/L^{-1} and the quantity of biosorbent was kept constant at 2 g/l, pH 6 maintained with contact time 60 min. Fig- 6 shows that the percentage of biosorption decreases from 99.5% to 78.82% with increase in the metal ion concentration. It was observed that with the high concentration of metal ions there is a competition between metal ions to adsorb on the active site of adsorbent because less number of active site is available as compared to the metal ions. So there is a decrease in the adsorption efficiency with the increasing of metal ion concentration. Similar results were reported by using Nordmann fir cones to remove Lead (II) and Zinc (II) and Rice straws for removal of Pb (II) [33, 34].
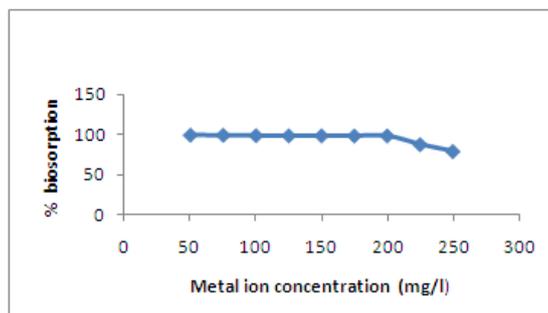

Figure.6 Effect of metal ion concentration of Pb (II) by *A.cookii*

3.7 Effect of particle size

In order to study the effect of particle size on the removal of Pb (II) ion from an aqueous solution, various sizes of 100 μm to 400 μm were introduced. Fig-7 shows that as the particle size increases from 100 μm to 400 μm, the rate of adsorption decreases from 98.52% to 70.0%. This is because the surface area per volume is higher for smaller particles with increase in the binding sites and contact surfaces, which shows that there is more rapid sorption and high mass transfer compared to the larger particles when they are used. The maximum uptake for the metal ion was obtained at 100 μm of 98.52% and found to be the best among other particle sizes.
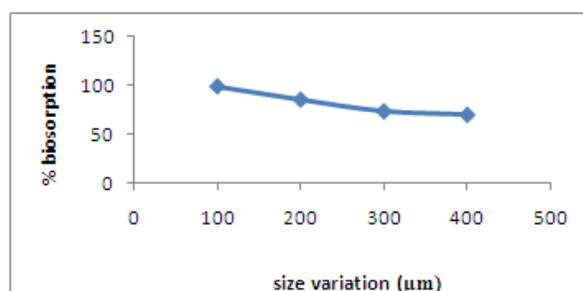

Figure.7 Effect of particle size of Pb (II) by *A.cookii*

3.8 Biosorption Kinetics / Kinetic studies

The Pb (II) biosorption on *A. cookii* leaves was investigated using Pseudo- first order and Pseudo-second order kinetic models.

The simple Pseudo first- order equation was first suggested by Lagergren [35] and the model integrated in linearized form is expressed as

$$\log (q_e - q_t) = \log q_e - k_1 t /2.303 \qquad (5)$$

Where k_1 is the adsorption rate constant (min^{-1}), q_e metal biosorbed at equilibrium (mg g^{-1}), q_t metal biosorbed at any time t (mg g^{-1}). Fig. 8 shows the plot t against log ($q_e - q_t$) for the metal biosorption with straight line which shows that the data fitted with poor correlation co-efficient indicating that pseudo first order equation did not apply for the removal of Pb (II) from A.cookii.
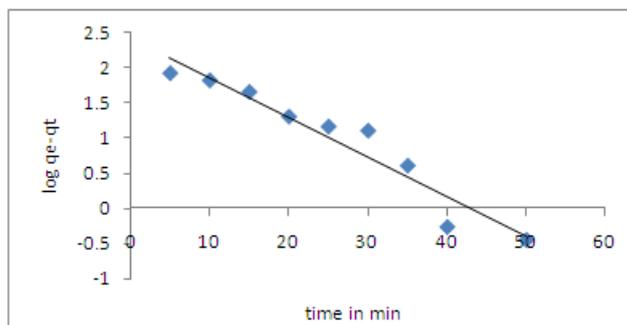

Figure.8 Pseudo first – order biosorption Kinetics for Pb (II) by *A.cookii*

Table -3 Pseudo- first order and Pseudo –second order kinetics for Pb (II) biosorption by *A.cookii*

Metal	Pseudo first order			Pseudo second order			
Pb (II)	q_e (mg g^{-1})	K_1 (min^{-1})	R^2	q_e mg g^{-1}	K_2(g mg^{-1}min)	R^2	h
	1.247	0.004	0.976	1.820	0. 130	0.996	0.430

The rate expression of Pseudo-second order reaction in linearized form which many scientists use has been utilized as follows:

$$t / q_t = 1/ k_2 q_e^2 + 1 / q_e\ t \qquad (6)$$

Where, k_2 is equilibrium rate constant for second order model (g mg^{-1} min^{-1}), q_e is the equilibrium capacity and q_t is the biosorption capacity at any time t. The plotting of time, t against t/qt shows straight line as shown in Fig 9.

The biosorption rate, h (mg/g min) at t=0 is shown as:

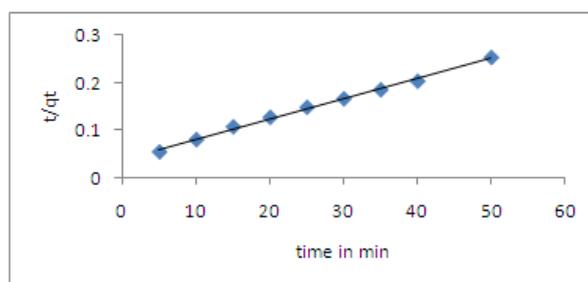$$h= K_2\ q_e^2 \qquad (7)$$

Figure.9 Pseudo second– order biosorption Kinetics for Pb (II) by *A.cookii*

In the present system, Pseudo-second order shows the applicability of correlation coefficient (R^2) 0.996 and the values of equilibrium rate constant k_2 0.130 gmg^{-1}min and equilibrium capacity q_e 1.820 mg g^{-1}were shown from the slope and intercept of the lines. Table-3 and Fig-8 shows that the poor description of data in Pseudo first-order equation. In the Pseudo second-order equation, the data fit well and a straight line was obtained, which suggests that the adsorption capacity depend only on this kinetic model. The rate limiting step in the adsorption mechanism involves chemisorptions which purely depend on physico-chemical interactions between adsorbent and solution in the metal removal [36].

3.9 Biosorption equilibrium

In the literatures several models has been used to describe the data of biosorption isotherms. In the present work, Langmuir [37] and Freundlich [38] models were used and Langmuir mathematical transformation of isotherm equation is shown as:

$$q= q_{max} \, bC_e/1+bC_e \qquad (8)$$

Where q (mg/g) is the amount of metal adsorbed per unit mass of adsorbent and Ce (mg/ L) is the equilibrium concentration of adsorbate in solution after adsorption. q_{max} and b are Langmuir adsorption coefficient and Langmuir constant.

Figure 10 shows the graph plotted C_e/q_e vs C_e where intercept and slope can be calculated. The q_{max} value is 37.03; b is 0.0267 and correlation coefficient (R^2) as 0.966 respectively as shown in Table 4.
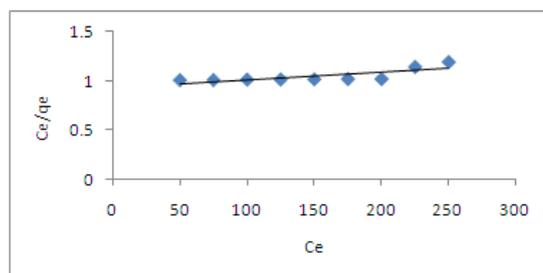

Figure.10 Langmuir adsorption isotherms of Pb (II) by *A.cookii*

Table -4 Langmuir and Freundlich isotherms for Pb (II) biosorption by *A.cookii*

Metal	Langmuir			Freundlich		
Pb (II)	q_{max}	b	R^2	K_f	1/n	R^2
	37.03	0.0267	0.966	29.51	1.054	0.991

Freundlich isotherm equation is shown as:

$$q= K_f \, (C_e) \, 1/n \qquad (9)$$

Where K_f is the Freundlich constant indicative of the relative adsorption capacity of the adsorbent (mg/g) and the constant 1/n indicates the tendency of the adsorbate to be adsorbed [39, 40].
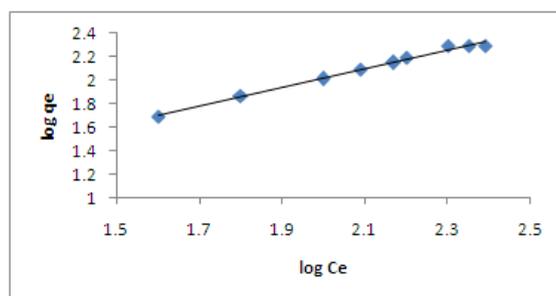

Figure.11 Freundlich adsorption isotherms of Pb (II) by *A.cookii*

3.10 Central Composite Design Model

In the present study Response Surface Method (RSM) is used to optimize different parameters which include pH, biomass, metal ion concentration, contact time and size variation as shown in Table 1. The data obtained were fitted to a second-order polynomial equation. Subsequent regression analyses, analyses of variance (ANOVA) and response surfaces were performed using the Design Expert Software (Version 8.0.7.1). The experimental data with multiple regression analysis was obtained from the following regression equation for the biosorption of Lead:

% Biosorption for lead $=75.26 + 4.20*A + 1.69*B - 4.71*C + 19.09*D -14.21*E -11.84*A^2-1.68*B^2-13.82*C^2- 7.62*D^2 + 4.97*E^2 + 0.0781 *AB +1* AC + 0.188 * AD - 0.187 * AE + 0.143 * BC + 0.238 * BD - 0.238 * BE - 0.625 * CD + 0.625 * CE + 1.17 * DE$

Where A, B, C, D and E are the code values for the independent variables, AB, AC, AD, AE, BC, BD, BE, CD, CE, A2, B2, C2, D2, and E2 are the significant model terms for the biosorption of Lead.

The statistical significance of the second –order polynomial equation is evaluated by the F-test of analysis of variance (ANOVA) as shown in Table 4. Probability> F value indicates the adequacy of any model. Model having prob > F values less than 0.0001 means that the experimental data obtained can be experimentally

explained with 99% accuracy by the model generated by RSM [41] whereas a low p-value (<0.05) indicates that the model is considered to be statistically significant[42]. The F-value of 34.65 and p- value of <0.0001 shows that the model is statistical significance. The relationship between the independent variables and response can be explained by the regression model. The model is best suited by determination of correlation R^2 91.6% (0.916) value which is close to 1[43].

In this model A, B, C, D and E represents the pH, Biomass, Concentration, Contact time and Size variation respectively. Table 5 gives comparison between the actual values and predicted values obtained from the experimentation by establishing the validity of the model and also these two sets of values shows that they are in a close agreement with each other.

Table 5: ANOVA for Response Surface Method

Source	Degree of freedom	Sum of squares	Mean square	F value	P value
Model	10	4813.195	481.3195	34.65524	<0.0001
Residual	32	444.4414	13.88879		
Total	42	5257.636			
A	1	70.16426	70.16426	5.051861	0.0316*
B	1	9.995664	9.995664	0.719693	0.4025
C	1	110.4359	110.4359	7.951439	0.0082**
D	1	2952.928	2952.928	212.6122	<0.0001**
E	1	812.9224	812.9224	58.53081	<0.0001**
A^2	1	259.337	259.337	18.67239	0.0001**
B^2	1	5.572332	5.572332	0.401211	0.5310
C^2	1	251.4326	251.4326	18.10327	0.0002**
D^2	1	657.0442	657.0442	47.30751	<0.0001**
E^2	1	26.02666	26.02666	1.873932	0.1806+

Table-6 The actual and predicted values

Standard Order	A	B	C	D	E	Actual value (%)	Predicted value (%)
1	5	2	200	60	200	85.3	84.13638
2	5	2	175	60	100	98.57	99.7997
3	5	2	200	50	100	98.45	95.62593
4	5	0.5	200	60	100	94.7	94.93589
5	5	2	225	60	100	87.57	92.53321
6	5	2	200	60	100	98.52	98.03043
7	5	3.5	200	60	100	98.52	98.32101
8	5	2	200	60	100	98.52	98.03043
9	5	2	50	60	100	99.5	94.72583
10	5	2	200	30	100	89.9	84.76862
11	5	2	75	60	100	99.33	99.59659
12	5	2	200	60	100	98.52	98.03043
13	5	2	200	35	100	94.47	88.23898
14	10	2	200	60	100	94.92	92.18604
15	5	2	200	20	100	78.25	76.31582
16	8	2	200	60	100	96.19	98.96298
17	5	1	200	60	100	96.4	96.24137
18	2	2	200	60	100	79.07	83.78035
19	5	2	200	60	300	73.5	74.66562
20	5	3	200	60	100	98.52	98.65931
21	5	2	200	60	100	98.52	98.03043
22	4	2	200	60	100	97.17	94.76013
23	3	2	200	60	100	94.22	90.0101
24	5	2	150	60	100	98.66	99.8409
25	5	2	250	60	100	78.82	85.30799
26	5	2.5	200	60	100	98.52	98.51402
27	5	4	200	60	100	98.52	93.14738
28	5	2	200	5	100	56.25	59.85644
29	5	2	200	25	100	83.92	80.79423
30	5	2	200	80	100	98.52	98.72365
31	7	2	200	60	100	97.72	100.1319
32	5	2	200	70	100	98.52	98.41885
33	5	2	200	60	100	98.52	98.03043
34	5	2	200	90	100	98.25	99.82101
35	5	2	125	60	100	98.72	99.1541
36	5	2	200	15	100	69.73	71.53339
37	6	2	200	60	100	98.25	91.20552
38	9	2	200	60	100	95.75	96.31437
39	5	2	200	5	100	70.00	69.61213
40	5	2	200	10	100	61.88	65.84693
41	5	1.5	200	60	100	97.64	97.27288
42	5	3.5	200	60	100	98.52	96.79116
43	5	2	100	60	100	98.9	99.7394

3.10.1 Response surface plots

The 3D response surface plots are helpful in order to understand the effect of interaction of any two factors at fixed levels. The response plots are used to determine the interaction of variables at optimum level of each variable. Graphs have been plotted keeping center points constant and varying two factors at a time. Fig 12 - 14 shows the response curve plots with effect of solution concentration for the removal of Lead (II).

3.10.2 Removal of Pb (II) with effect of pH and initial metal concentration by *A. cookii*

The biosorption percentage of Pb (II) by leaves of *A. cookii* was studied by the selected range of pH and initial metal concentration. Fig 12. depicts that the maximum biosorption occurred at pH 6 which is in the acidic range and the results coincide by using *Borasus flabellifer* Coir Powder and the metal ion concentration was at 175 mg/L [44].
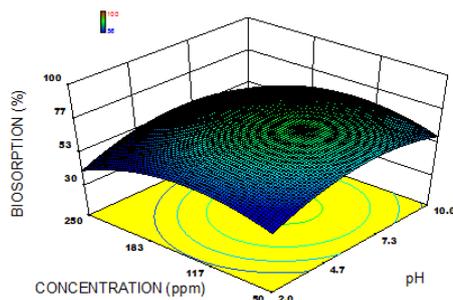
Figure. 12 RSM 3D plot showing the effect of pH and Concentration for Pb removal by *A. cookii*

3.10.3 Removal of Pb (II) with effect initial metal concentration and Biomass by *A. cookii*

The RSM 3D plot shows the combined effect of concentration and biomass for the removal of Pb (II). The results indicate that the maximum adsorption was at adsorbent dose of 2.25 g/l at the initial metal ion concentration of 175 mg/l as shown in Fig 13.
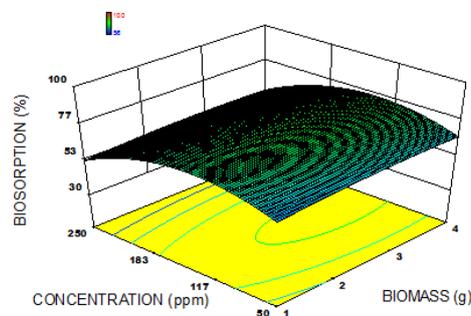
Figure. 13 RSM 3D plot showing the effect of concentration and biomass for Pb(II) removal *A.cookii*

3.10.4 Removal of Pb (II) with effect initial metal concentration and Contact time by *A. cookii*

The biosorption of Pb (II) shows the effect of concentration and contact time. Fig.14 depicts the maximum removal of Lead in the optimized condition was at 32.5 min at the concentration of 175 mg/l. There was no change after this point and the optimum time was considered as 32.5 min and the percentage removal was 91.6%.
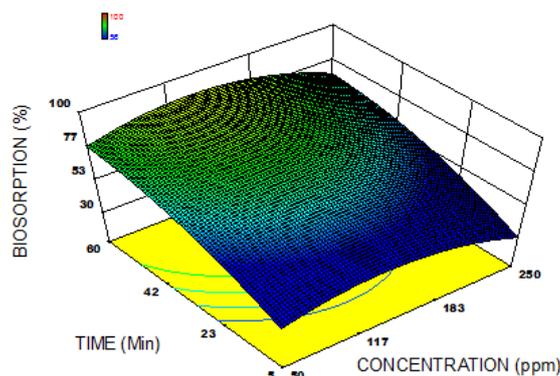
Figure. 14 RSM 3D plot showing the effect of Concentration and time for Pb removal *A. cookii*

The second order polynomial models with the set of metal ion concentration in the number of combinations were maintained at zero levels. Response surfaces were performed using the Design Expert Software (Version 8.0.7.1). The maximum adsorption for optimal set of Lead onto *A. cookii* is pH 6, metal ion concentration 175 mg/L, particle size 250 μm, contact time 32.5 min and adsorbent dose 2.25 mg L^{-1}. By using the optimum conditions for the removal of Lead was achieved at 91.6%.

3.11 Comparison of maximum biosorption capacity with other biosorbents
Table 7 shows the comparison between other biosorbents including *A.cookii* for Pb (II) biosorption. The comparison of biosorption capacity shows that the leaves of *A.cookii* and   *Ficus religiosa* (peepal) leaves has higher q $_{max}$ value of the same range with those of various biomasses in literature. But it is difficult for the direct comparison due to various experimental conditions which is used in the studies.

Table- 7 Comparison of maximum biosorption capacity for Pb (II) with other plant biomass

Biosorbents	q_{max}	Reference
Ficus religiosa (peepal) leaves.	37.45 mg/g	Qaiser et al., 2007 [45]
Biological Activated Dates Stems	27.03 mg/g	Hynda yazid et al., 2008 [46]
Coir	18.9 mg/g	K. Conrad etal., 2007 [47]
Nordmann Fir	29.3 mg/g	Yusuf Kaya et al., 2009 [33]
Groundnut hull	31.54 mg/g	Qaiser et al., 2009 [48]
Araucaria cookii	37.03 mg/g	Present study

* q_{max} is equilibrium and maximum adsorption capacity (mg/g)

IV.   Conclusion
The leaves of *A.cookii* were evaluated as one of the possible biosorbent for the removal of Lead (II) from aqueous solution and also from Industrial effluent. The Lead (II) biosorption was influenced by pH, contact time, initial metal ion concentration, adsorbent dose and size variation. The maximum biosorption was at pH 5.0, contact time 60 min with the percentage removal of 98.52%. Lead biosorption fit best in Pseudo-second order and equilibrium data by Langmuir and Freundlich isotherms with R^2 value of 0.996. The full factorial Central Composite Design (CCD) in Response Surface Methodology was used to determine the optimum conditions for the maximum percentage removal of Lead (II). The optimum conditions was at pH 6, metal ion concentration 175 mg/L, particle size 250 μm, contact time 32.5 min and adsorbent dose 2.25 mg L^{-1} and correlation coefficient of 0.916 with the percentage removal of 91.6% Hence this method can be successfully employed to study the importance of individual, cumulative and interactive effects of different test variables of biosorption.

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