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Removal of lead from aqueous solution by EPS producing bacteria

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Abstract
Heavy metal pollution in soil currently becomes a serious environmental issue of worldwide. The present study was carried out to optimize the various environmental conditions for biosorption of lead by investigating as a function of the initial metal concentration (50-250mg L^{-1}), contact time (24-120 hours), and pH (4-8) using EPS producing bacteria (UPI-7) as adsorbent. Biosorption of lead ion from aqueous solution was optimized in a batch system using response surface methodology. The values of R^2 (0.9710) for lead ions indicated the validity of the model. The analysis of variance (ANOVA) of the cubic model demonstrates that the model was highly significant. The response surface method indicated that 50 mg L^{-1} initial lead concentration, pH 6 and a contact time of 72 h were optimal for biosorption of lead in which 61% of the lead was removed from the solution. FTIR spectroscopy spectra analysis indicated that presence of hydroxyl and carboxyl functional groups were involved in lead biosorption process.

Keywords: heavy metal, lead, exopolysaccharide, biosorption

1. Introduction
Due to tremendous increase in heavy metal release into the environment in the past few decades, worldwide attention has been given for metal remediation than any other environmental problems. The disposal of heavy metal is a consequence of industrial activities like mining, chemical manufacturing, painting and coating, nuclear and other industries (Lokendra and Pradeep, 2014) [9].
Non-biodegradable nature of heavy metals, their high aquatic solubility triggers bioaccumulation and bio magnifications which eventually leads irreversible health hazard even in very minimal concentration (1mg L^{-1}).
Lead is being one of the three most hazardous metal, accumulates in the body causing severe damage to the central nervous system, kidney, bone marrow and liver (Tsoi *et al.*, 2016). Recent studies indicate that the rise in mortality and systolic blood pressure are due to elevated lead levels in blood. The main source of lead in sewage is due to the release of effluent from processing industries, i.e., electroplating, paint, pigment, basic steel work, metal finishing and electric batteries (Ansari *et al*., 2011) [2].
The existing technologies like chemical precipitation, reverse osmosis, solvent extraction have many disadvantages which add cost to the treatment process. Now a days there is an increase interest on the use of microorganisms for the removal as well as recovery of metals via biosorption. Bacteria are able to synthesis macromolecules such as polysaccharide, nucleic acid, protein, humic substances and uronic acid usually called as extracellular polysaccharide (EPS). In live bacterial cells besides surface accumulation, heavy metals have possibility to enter in the cytoplasm through the specific carrier system and transport process in bacteria (Upadhyay *et al*., 2017) [5].
EPS are metabolic products of bacteria and they originate from their lysis and they restrain the respective functional compounds like hydroxyl, carboxyl and phosphoric groups. EPS involved in cell aggregation, productive fence for cells to keep at bay harmful substances and accept the accumulation of inorganic ions from the environment (Fomina and Gadd, 2014) [7]. With this background the present work was carried out to discuss the potential of EPS producing bacteria for metal remediation from environment.

2. Materials and Method
2.1. Isolation and characterization of bacteria
The soil samples were collected from the Ukkadam treated sewage water irrigated soil.

The characteristics of soil were analyzed as per the standard methods (Bashour and Sayegh, 2007) [3]. One gram of wet soil sample was enriched in enrichment broth, after 15 days of enrichment serially diluted and spread over the nutrient agar plates and incubated at 30±2^{0}C for 24h to isolate individual colonies. From the individual colonies 12 isolates were screened and tested for its tolerant to lead concentration. The tolerance level was tested up to 500 mg L-1. Based on the screening results, three isolates were taken for further study. The isolates were subjects to cultural, morphological and biochemical screening as per the standards procedure (Holt *et al*., 1994). Based on the EPS production, among the three isolates, one isolate namely UBI-7 was used for adsorption studies. Biomass of EPS producing bacteria (UBI-7) were produced by growing bacterial culture in nutrient broth at 30±2^{0}C for 24h, which was used for further studies.

2.2 Adsorption studies

The standard Pb(II) stock solution (1,000 mg L^{-1}) were prepared by dissolving 1.6 g of lead nitrite (Pb NO_3) in milli-Q water. From the Pb(II) stock solutions, working standards were prepared appropriate dilution with milli-Q water.

Response surface methodology (RSM)

Optimum condition for the biosorption of Lead (Pb) by EPS producing bacteria (UBI-7) was determined by means of Central Composite Design (CCD) under response surface methodology (RSM). The study carried out involved the employment of central composite design to optimize the biosorption process due to its suitability to fit quadratic surface which usually works well for process optimization. The effects of three independent variables, initial metal concentration, contact time and initial pH on the Pb removal(%) by the isolate UBI-7 was investigated by means of central composite design(CCD) (Amini *et al*., 2008) [1].

For statistical calculation, the variables were coded according to Equation:

$$x_i = \frac{X_{i-}\ X_0}{\Delta X} \qquad (1)$$

Where x_i is the dimensionless coded value of the i^{th} independent variable, X_0 is the value of X_i at the centre point and ΔX is the step change value. The behaviour of system is explained by the following empirical second-order polynomial model

$$Y = \sum_{i=1}^{K} \beta_i x_j + \sum_{i=1}^{K} \beta_{ij} x^2{}_i + \sum_{i=1}^{K-1} \sum_{j=2}^{k} \beta_i x_j \sum \beta_{ij} x_i x_j + \in \qquad (2)$$

Where Y is the predicted response, x_i, x_j, . . ., x_k are the input variables, which affect the response Y, $x_2 i$, $x_2\ j$, . . ., $x_2\ k$ are the square effects, $xixj$, $xixk$ and $xjxk$ are the interaction effects, $\beta 0$ is the intercept term, βi (i=1, 2, . . ., k) is the linear effect, βii (i=1, 2, . . ., k) is the squared effect, βij (i=1, 2, . . ., k; j=1, 2, . . ., k) is the interaction effect and ε is a random error.

The Design Expert 6.07 (Stat-Ease Inc., Minneapolis, cMN, USA) software was used for regression and graphical analysis of the obtained data. The optimum values of the selected variables were obtained by solving the regression equation at desired values of the process responses as the optimization criteria. Each of the parameters was coded at five levels: $-\alpha$, -1, 0, +1 and $+\alpha$. The range of variables was decided on the basis of literature for heavy metals biosorption by EPS producing bacteria. The range and level of the variable in coded units from RSM studies are given in Table 1.

Table 1: The experimental range and levels of the independent variables

	Range and levels				
Factor	$-\alpha$	-1	0	1	$+\alpha$
Contact time (*X*1)	24	48	72	96	120
Initial lead ion concentration, mg L-1 (*X*2)	50	100	150	200	250
pH (*X*3)	4	5	6	7	8

2.3 Batch experiment

Batch experiments were conducted with lead solutions prepared from stock solution. A known quantity of metal concentrate solution was taken in 250 ml conical flasks and one ml culture was added. The flasks were stirred at a constant speed of 160 rpm at room temperature. Test samples were collected at regular intervals of time, centrifuged and filtrated for the estimation of Pb (II) concentration. Experiments were carried out over a wide range of operating conditions and the percentage of Pb (II) removal, i.e., R(%) was calculated using the following equation: The amount of heavy metal adsorbed per unit mass of the adsorbent (Q) was calculated by using the following mass balance equation (Rasulov *et al.,* 2013) [11].

$$Q = \frac{V\,(Ci - Cf)}{m} \qquad (3)$$

And the percentage adsorption of metal was calculated as follow:

$$\% = \frac{(Ci - Cf)}{Ci} \times 100 \qquad (4)$$

Where, Q is the metal ion adsorbed in mg g^{-1} of biomass, C_i is the initial concentration of metal ion in solution before sorption (mg L^{-1}), C_f is the final metal ion concentration after the sorption analysis (mg L^{-1}), V is the volume of solution (L) and m is the mass of biosorbent (g).

3. Result and Discussions

3.1 characteristics of soil

The characteristics of soil were analyzed as per the standard methods. The pH of soil was 7.88 and the salt content was normal (0.33 dS m^{-1}). The organic carbon content of the soil was found to be in medium status (1.43 per cent). The heavy metals of the soil showed the presence of 38.17 mg/Kg, 26.57 mg/Kg and 1.50 mg/Kg of chromium, lead, and copper concentration respectively. The biological properties of the soil showed the presence of 4.1×10^6, 3.95×10^4 and 3.05×10^2 CFU g^{-1} of bacteria, fungi, and actinomycetes population respectively.

3.2 Screening of metal tolerant bacteria

From this study, a total of 12 isolates were screened and tested for its tolerance to different lead concentration. Among them only 3 isolates showed production of exopolysaccharide (EPS) and tolerance up to 500mg L-1 of Lead. Among the three isolates, UBI-7 recorded higher EPS production compare to other two isolates. All the three isolates were subjected to morphological and cultural characterization and it was tentatively identified as GRAS group of organism. Hence further studies were carried out with the isolate UBI-7.

3.3 Characterization of EPS producing bacteria
The selected bacterial strain were characterised and identified by using standard morphological, physiological and biochemical tests (Table. 2, 3).

Table 2: Morphological and biochemical characteristics of the promising Isolate Ubi7

Characteristics	Results
Colony color	Dull white
Gram staining	+
Shape	**rod**
Pigments	+
Optimum temperature	35 -37°C
Optimum pH range	6.5-7.5
Catalase activity	-
Triple sugar	_
Citrate utilization	_
Methyl red	+
Vogaes Proskaur	-
Nitrate reduction	+
Hydrogen sulfide	+
Catalase test	+
Urease test	+

Table 3: Nutritional characteristics of the promising isolate UBI7

Characteristics	Results
Cellulose degradation	-
Glucose	++
Arbinose	-
Rhamnose	-
Sucrose	++
Mannitol	++
Inositol	+
Xylose	+
Fructose	+
Malate	-
L-asparagine	+
Leucine	+
Tyrosine	+
L-phenylalanine	+

3.4 Metal absorption studies
3.4.1 Effect of contact time and concentration
The adsorption of Pb by EPS producing bacteria were analyzed based on contact time and concentration (Fig.1). It ranged from 0-120 hours and 50-250 mg L^{-1} under shaking at 160rpm. Adsorption percentage of Pb by EPS producing bacteria increased with the increase in contact time from 0-72 hours, after that decreased with increase of time. The highest adsorptions of Pb (61.54%) were recorded at 50 mg L^{-1} in 72 hours equilibration period. Present study is well correlated with the observation of Feng *et al*. (2012) [6] who determined that adsorption of Pb by EPS producing bacteria increases with increase in contact time. The adsorption of metal ions gradually decreases as time progressed. Similar to that Chug *et al*., (2016) [11] maximum EPS production from *Azotobacter beijreinckii* has been reported after 72h incubation. As concentration increases there is a gradual decrease in the percentage removal in EPS producing bacteria in their biosorption capacities (Fig.1). Similar results have been reported by others (Oves, 2013) [10].

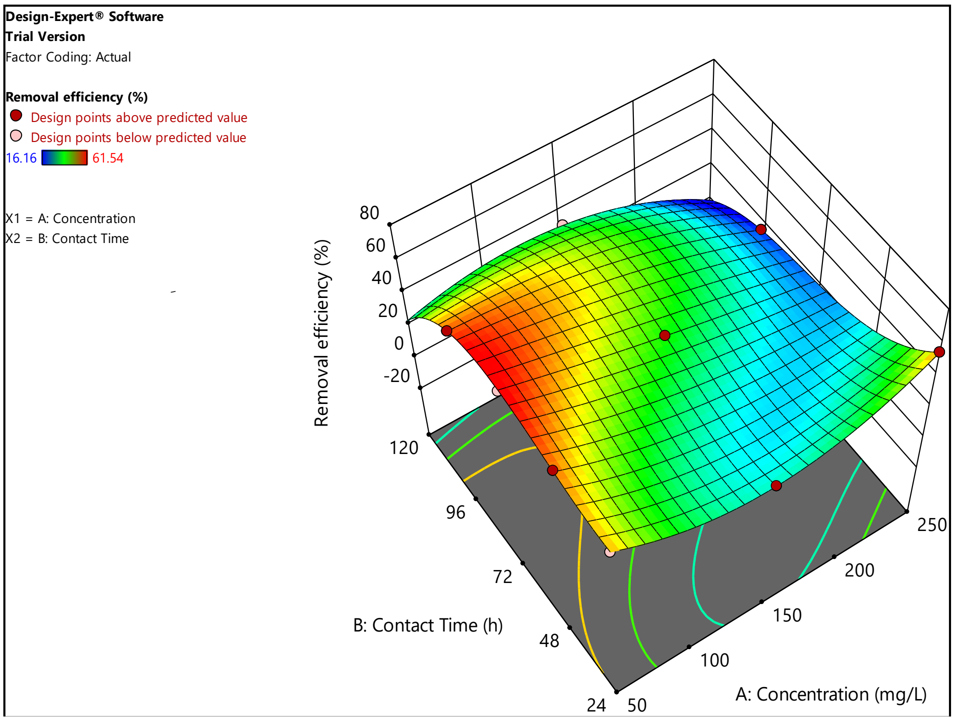

Fig 1: Response surface plots for the effect of contact time and initial lead ion concentration (mg L-1) on the lead removal (%)

Table 4: Analysis of variance for RSM variables fitted to cubic model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3248.46	9	360.94	1637.84	< 0.0001	significant
X_1	110.73	1	110.73	502.48	< 0.0001	
X_2	116.32	1	116.32	527.81	< 0.0001	
X_1X_2	28.97	1	28.97	131.44	< 0.0001	
X^2_1	6.13	1	6.13	27.82	0.0019	
X^2_2	109.38	1	109.38	496.35	< 0.0001	
$X^2_1 X_2$	378.97	1	378.97	1719.67	< 0.0001	
$X_1 X^2_2$	42.24	1	42.24	191.69	< 0.0001	
X^3_1	0.0042	1	0.0042	0.0192	0.8942	
X^3_2	97.31	1	97.31	441.56	< 0.0001	
Residual	1.32	6	0.2204			
Lack of Fit	1.32	1	1.32			
Pure Error	0.0000	5	0.0000			
Cor Total	3249.78	15				

R^2 =0.9710, Adj R^2 =0.9566, Pred R^2 =0.9032, Adeq Precision =22.8943, C.V. %=4.59

3.4.2 Effect of pH

In the batch biosorption experiments, the influence of pH on Pb(II) biosorption was studied using stock solutions. Pb(II) biosorption strongly depends on initial solution pH (Fig.2.) The effect of pH in batch experiment was studied by varying the pH from 4 to 8. Due to the activity of hydrogen ions, at low pH the Pb(II) uptake was observed to be on the higher side in UBI-7. As the pH increases up to 6 the of Pb(II) uptake also increases in UBI-7. At lower pH values, the functional groups transfer H^+ ions which indicates that majority of the binding sites were occupied. When the pH increases, the concentration of H^+ ions decrease and negatively charged biomass surface can interact with the positively charged metal ions. At different pH condition the plenty of carboxyl groups and hydroxyl groups in other polysaccharides are found to play an important role in metal binding (Sethuraman 2010) [12]. Similar to these observations, *Rhodococcus opacus* and *Rhodococcus rhodochrous* are reported to have maximum adsorption of Cr(VI) ions onto both biosorbents was achived at the pH 3 (Dobrowolski *et al.*, 2017) [5].

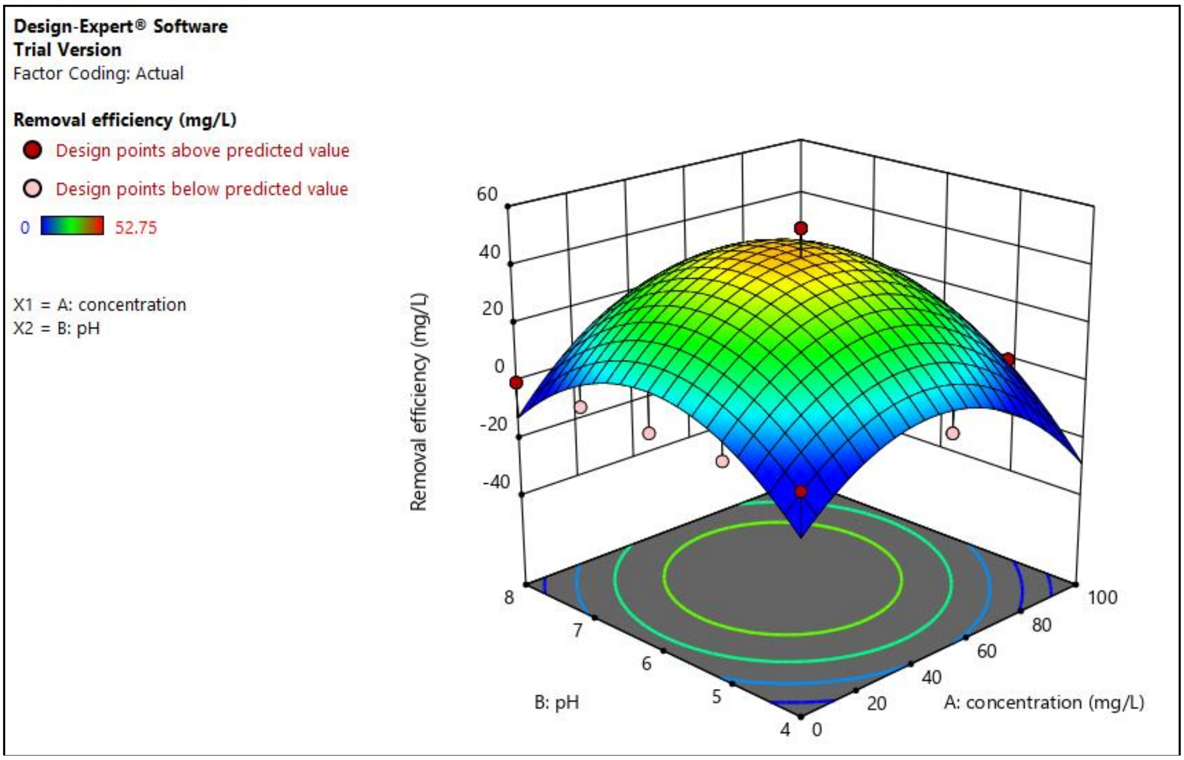

Fig 2: Response surface plots for the effect of (a) initial solution pH and initial lead ion concentration (mg L^{-1}) on the lead removal (%).

Fitting the model

The statistical significance of the cubic model was evaluated by the analysis of variance (ANOVA) as presented in Table 5. The results showed that this regression was statistically significant at *F* value of 1637.84 and values of prob > *F* (<0.0001). The fit of the model was checked by the determination of coefficient (R^2). In this case, the value of the determined co efficient (R^2 =0.9710) indicated that only 0.29% of the total variable was not explained by the model. The closer the R^2 is to 1, the stronger the model and the better it predicts the response. The value of adjusted determination coefficient (adjusted R^2 = 0.9566) is also high, showing a high significance of the cubic model. The value of predicted R^2 is also high to support for a high significance of the model. The predicted R^2 obtained 0.9032, indicating that the model does not explain only10.68% of the total variations. This also revealed that predicted R^2 of 0.9632 is in reasonable agreement with the adjusted R^2 of 0.9566. At the same time, relatively low value of the coefficient of variation (CV=4.59) indicates a good precision and reliability of the experiments (Singh, Chadetrik *et al*. 2010). The regression equation after the analysis of variances (ANOVA) gave the level of lead ion removal as a function of the initial lead ion concentration, initial solution pH and contact time. By applying multiple regression analysis on the experimental data, the experimental results of the CCD design were fitted with a second-order full polynomial equation. The empirical relationship between lead ion removal (*Y*) and the independent variables in coded units obtained by the application of RSM is given by

$$\text{Pb removal (\%) (Y)} = 39.51 - 20.53x_1 + 20.74x_2 - 6.76\, x_1 x_2 + 2.09x_2 - 8.78\, X^2{}_2 - 21.78\, X^2{}_1 X_2 + 15.20\, X_1 X^2{}_2 - 0.1559\, X^3{}_1 - 19.68\, X^3{}_2 \quad (5)$$

Where Y = Removal percentage, x_1, x_2 and x_3 are variables, contact time (x_1) in g/l, initial lead ion concentration (x_2) in mg L-1 and initial solution pH (x_3).

Table 5: Sigificance of regression coefficients for the removal of lead

Factor	Coefficient Estimate	Standard Error	F-value	p-value
Intercept	39.51	0.2677	1637.84	< 0.0001
X_1	-20.53	0.9160	502.48	< 0.0001
X_2	20.74	0.9029	527.81	< 0.0001
X_1X_2	-6.76	0.5898	131.44	< 0.0001
$X^2{}_1$	2.09	0.3953	27.82	0.0019
$X^2{}_2$	-8.78	0.3940	496.35	< 0.0001
$X^2{}_1 X_2$	-21.78	0.5252	1719.67	< 0.0001
$X_1 X^2{}_2$	15.20	1.10	191.69	< 0.0001
$X^3{}_1$	-0.1559	1.12	0.0192	0.8942
$X^3{}_2$	-19.68	0.9364	441.56	< 0.0001

The combined effect of contact time and initial Lead concentration on Pb removal at constant biomass dosage was depicted in fig 2 form of 3D surface plot. Clearly the initial pH exerted stronger influence than initial metal concentration, which could also be deduced from the coefficient of factors in equation (5). By setting the three factors from$-\alpha$ *to*$+\alpha$ level, the maximum Pb removal of 61.54% was obtained at contact time 72h, initial concentration 50mg L-1 as the software Design Export suggested.

3.4.3 FTIR spectral analysis

The IR spectra of UBI-7 are given in Fig.3 and Fig.4. The FTIR spectra of Pb(II) loaded and unloaded biosorbent was in the range of 400-4000 cm^{-1}. IR spectra clearly showed the difference between before and after Pb(II) metal ion in biosorbent. The band corresponding to alkane (C-H) was detected at 2946.7cm^{-1} before adsorption in EPS producing bacteria (UBI-7) and after adsorption of Pb, it was changed to 2929.34 cm^{-1}. The alcohol group (O-H) which was not there during before, adsorption of Pb appeared after Pb adsorption. The band corresponding to 2125.17 cm^{-1} (-C=C) before adsorption changed to 2130.96 cm^{-1} after adsorption. The band at the wave length of 1241.93 cm^{-1} showed that presence of alkyl halide group before adsorption changed to 1236.15 cm^{-1} after adsorption. These changes in band group might be due to Lead adsorption. The isolate UBI-7 had strong and broad peak due to OH stretching shows the presence of hydroxyl groups of EPS may be due to functional groups like alcohol, alkane and alyl halides. The strong intensity of alkene functional group with (=C-H) band was detected at 1646.91 cm^{-1} before adsorption, and after adsorption of Pb it was changed to 1644.98 cm^{-1}(Fig.3 and Fig.4). Singh *et al.*, (2010) reported that the adsorption peaks at 3200-3500cm^{-1} indicates the presence of carboxylic acid (1658cm^{-1}) and amino groups (1548cm^{-1}).

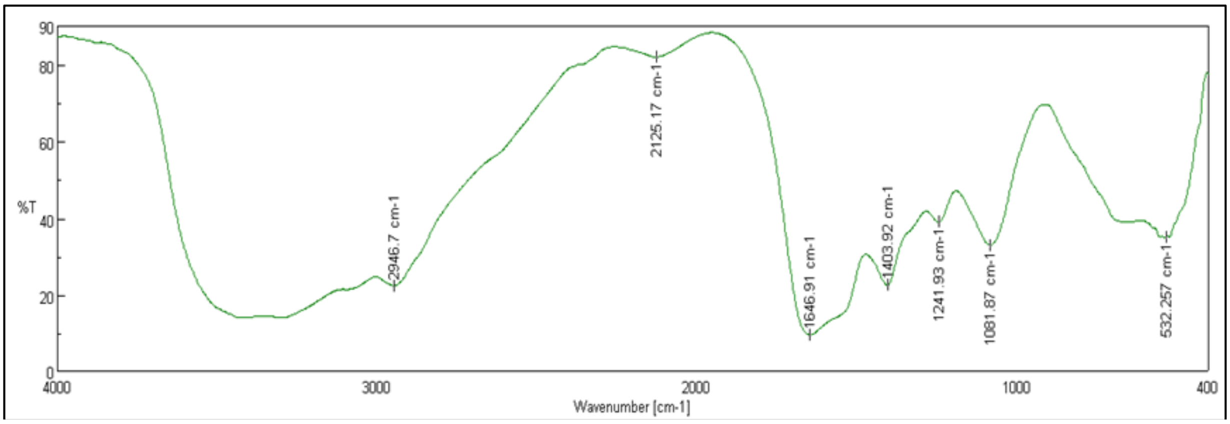

Fig 3: FTIR analysis of EPS producing bacteria *(Azotobacter sp.)* before adsorption

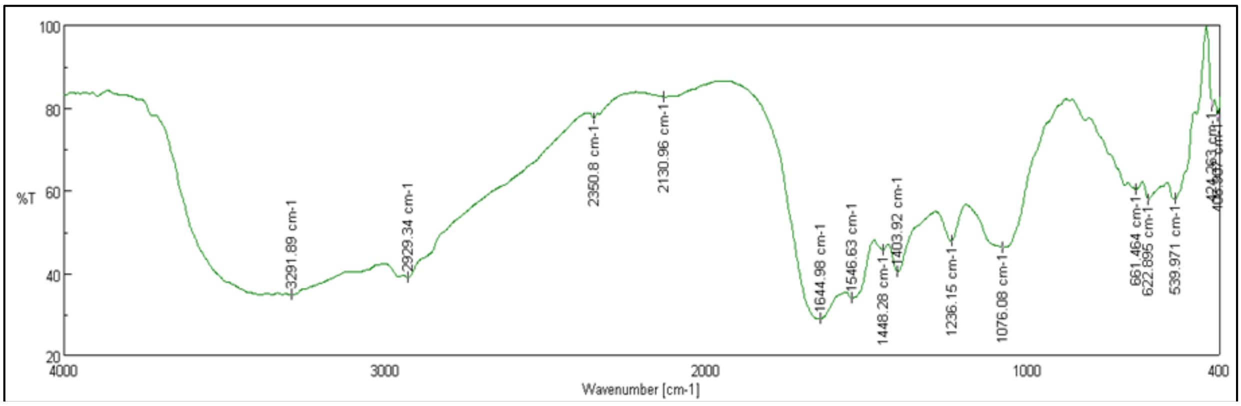

Fig 4: FTIR analysis of EPS producing bacteria *(Azotobacter sp.*) after adsorption

4. Conclusion
Biosorption offers an economically feasible technology for efficient removal and recovery of metals from aqueous solution. The current study was performed as a function of initial metal concentration, contact time and pH. From the study, it was concluded that EPS producing bacteria (UBI-7) serve as a good absorbing medium for lead ion aqueous solution and wastewater containing lead ions. On the basis of RSM approach with central composite design (CCD) and fitness of polynomial equation, optimal conditions for lead adsorption were found to be pH 6 metal concentration 50 mg L-1 which resulted in 61% lead removal by EPS producing bacteria (UBI-7). Besides this, the FTIR analysis also confirmed the involvement of hydroxyl groups and carboxyl groups in lead biosorption by EPS producing bacteria (UBI-7). Further work focussed on genetic identification and conformation of EPS producing bacteria (UBI-7) would be useful for improving its EPS producing potential by genetic modification.

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