Biosorption of Cd^{+2} and Pb^{+2} on *Cyperus laevigatus:* application of factorial design analysis

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Abstract: Heavy metals pollution has become one of the most serious problems today and the use of plant biomass for the detoxification of industrial effluents for environmental protection and recovery of valuable metals offers a potential alternative to existing treatment technologies . Considering the fact that Cyperus *laevigatus is* resistant to polluted environmental and has the capability to accumulate heavy metals in its body, plant biomass itself can also be used to remove heavy metals from contaminated water by harnessing its natural properties. In this research the experimental design technique was used to optimize the biosorption of cadmium and lead ions by *Cyperus laevigatus* biomass from aqueous solutions, simulating industrial effluents. The removal of Cd^{+2} and Pb^{+2} was studied, separately, using the factorial design 2^3. The three factors screen were pH , temperature (T) and metal ion concentration (X) at two markedly different levels : pH (2.0 and 6.0) T (20 and 45 ^{0}C) and X (20 and 800 mg l^{-1}) . The most significant effect for Cd^{+2} and Pb^{+2} biosorption was ascribed to T . The interaction effect of X. pH and T.X also have a significant influence on the Pb^{+2} efficiency. A normal distribution was observed between the predicted values (model) and the observed (experimental).

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1. Introduction

Water contamination by heavy metals, released into the environment as a result of different activities, is a very important problem in the current world. Considerable attention has been paid to methods for metal removal from industrial wastewaters because they pose series environmental problem and are dangerous to human health [1].Conventional techniques for removing dissolved heavy metals include chemical precipitation, carbon adsorption, ion exchange, evaporation and membrane processes [2]. However, these techniques have certain disadvantages such as incomplete metal removal, high reagent and energy requirement and generation of toxic sludge that require disposal [3].

Due to the high costs of commercial adsorbents, the search for alternate and innovative treatment techniques has focused attention on the use of biological materials for heavy metals removal and recovery technologies (Biosorption). This technique has gained important credibility during recent years due to its effectiveness in reducing the high concentration of heavy metal ions (from industrial wastewater) to very low levels. It is considered a potentially viable method on both technical and economic grounds, because of its low operating costs [7] and the decontamination efficiency for highly diluted effluents. Additionally, metal can be recovered from the biosorbent and reused. Different types of biomass have been investigated for the biosorption characterstics of Pb^{2+} and Cd^{2+} from aqueous solution [8]. The seaweed *Sargassum* sp was used [9] for removal of lead and cadmium ions from water. Moreover,Vecchio at al. [10] studied the removal of Cu^{2+}, Pb^{2+} and Cd^{2+} ions by biosorption on bacterial cells. Also,Srivastav et al. [11] used the aquatic plants for removal of lead and zinc ions from waste water. The adsorption of lead ions on nonliving *Penicillium chrysogenum* biomass was also investigated [12].

Among the biological materials, *Eichhornia crassipes* (Mart.) Solms (widely distributed aquatic macrophyte) has been reported to have high metal binding capacities and promising results with regard to metal removal from wastewater [13]. It was reported that the ability of *E.crassipes* to accumulate metal ions was found to be in the order of $Pb^{2+} > Cd^{2+} > Cu^{2+} = Zn^{2+}$ [14]. The adsorption capacity of Pb^{2+}, affected by experimental parameters such as pH, contact time and concentration of Pb^{2+} solution, on to *E.crassipes* plant biomass was studied [15]. They found that the uptake percent of Pb^{2+} increased by increasing pH values.

However, some authors have evaluated removal efficiencies of heavy metals by this species as a function of one-factorat-a-time. Few studies employed the factorial design method for evaluating the influence of the operation variables on biosorption processes. The biosorption of Cd^{2+} and Pb^{2+} was optimized using 2^3 factorial designs by Petemele et al.& Barros et al. [16,17]. The biosorption of Cr^{3+} and Cr^{6+} using 2^3 and 2^4 factorial designs, respectively was studied [18, 19]. Factorial design is employed to define the most important process variables affecting the metal removal efficiency [20]. It is also used to reduce the total number of experiments in order to achieve the best overall optimization of the system [21]. The factorial experimental design methodology involves changing all variables from one experiment to the next. The design determines which factors have important effects on the response as well as how the effect of one factor varies with the level of the other factors. The determination of factor interactions could only be attained using statistical designs of experiments [21] , since it cannot be shown when the system optimization is carried out by varying just one factor at the time and fixing the other.

The objective of this study was to establish how pH, temperature and initial concentration of lead and cadmium ions interacted and ultimately affected their removal efficiency from aqueous solutions by means of *Cyperus laevigatus* biomass. A factorial design 2^3 scheme was used to study the removal of Cd^{+2} and Pb^{+2} , separately, for the benefit of both the remediation of heavy metal pollutants from aquatic environment and the management of *Cyperus laevigatus* harvested from wetlands .

2. Materials and Methods

2.1. Biomass preparation

A biomass of Cyperus laevigatus was used as biosorbent for the biosorption of Cd^{2+} and Pb^{2+}. Samples of the biomass were collected from industrial zone in Riyadh City . They were washed several times using de-ionized water to remove soils , then dried in an oven at 60 ◦C for 48 hrs, chopped and sieved. The particles with an average of 0.5mm were used for the experiments.

2.2. Reagents and equipments

Doubly distilled water was throughout employed. Initial solutions with different concentration of Cd^{2+} and Pb^{2+} were prepared by proper dilution from stock standards (800 g L^{-1} Cd^{2+} and Pb^{2+}). The pH adjustment of the solutions were made with aliquots of 1.0 mol l^{-1} of HNO_3 utilizing a pH/mV hand-held meter (Crison pH meter, PH 25)

2.3. Batch biosorption procedure

Batch experiments were carried out under the following conditions: 0.2 g of Cyperus laevigatus biomass, 30 ml of Cd^{2+} and Pb^{2+} solution, and an agitation speed of 200 rpm. The pH, temperature, and initial Cd^{2+} and Pb^{2+} concentration employed are shown in Table 1. The experiments were carried out with the values of pH (2, 6) that were not influenced by the metal precipitation, as metal hydroxide [22]. The maximum temperature employed in the present study was 45 ^{o} C, as the higher temperature damages the active sites in the biomass [23]. Samples were collected after 6 hours to reach equilibrium for the sorption system [18]. Control samples were made in absence of any metal. Aliquots for analysis were filtered using glass filter provided with Whatman filter paper, and the residual Cd^{2+} and Pb^{2+} concentration was measured by Varian ICP-AES.

Sixteen duplicate experiments were carried out: eight for Cd^{2+} and eight for Pb^{2+}. All possible combinations of variables, called factors in the jargon, were used, and a matrix was established according to their high and low levels, represented by ± 1, respectively.

Table 1 : High and low levels of factors

*Factor	Element			
	Cd^{+2}		Pb^{+2}	
	Low level	High level	Low level	High level
T (°C)	20.0	45.0	20.0	45.0
X (mg/l)	20.0	800.0	20.0	800.0
pH	2.0	6.0	2.0	6.0

*T: Temperature ; X : Initial concentration

The removal efficiency (R) of Cd^{2+} and Pb^{2+} from aqueous solution was defined as:

$$R = \frac{C - C_F}{C} . 100 \qquad (1)$$

Where: C and C_F are, the initial and final concentrations of Cd^{2+} and Pb^{2+}, respectively.

2.4 Statistical design of experiments (full factorial design)

For studying the Cd^{2+} and Pb^{2+} biosorption on *Cyperus laevigatus* biomass, the removal efficiency (R) could depend on the acidity of the medium (pH), initial metallic ion concentration (X), and temperature (T). Other variables such as biosorbent concentration and speed of agitation were kept constant. A full 2^3 factorial design and results for removal efficiency are shown in Table 2. For treatment of data, the Minitab Statistical Software (release 14.1) was employed throughout in order to obtain the effects, coefficients, standard deviation of coefficients and other statistical parameters of the final model.

Table 2 : Experimental factorial design results for Cd^{+2} and Pb^{+2} biosorptin

Factor	Element	
	Cd^{+2}	Pb^{+2}
T X pH	Removal efficiency (%)* Average	Removal efficiency (%)* Average
1 1 1	69.2 68.5 68.8	68.2 68.4 68.3
1 1 -1	85.7 85.5 85.6	44.1 44.4 44.2
1 -1 1	72.8 72.6 72.7	82.4 82.6 82.5
1 -1 -1	45.8 45.6 45.7	79.1 79.4 79.3
-1 1 1	21.3 21.7 21.5	25.4 25.6 25.5
-1 1 -1	10.2 10.6 10.4	9.4 9.2 9.3
-1 -1 1	34.4 34.6 34.5	30.7 30.9 30.8
-1 -1 -1	21.1 21.3 21.2	9.9 9.7 9.8

*Experimental in duplicate

3. Results and Discussion

Metallic ion uptake by a biosorbent in a batch system usually depends on several factors, such as acidity of the medium (pH), initial metallic ion concentration, time of contact between the metallic ion and the biosorbent, speed of shaking, etc. The optimization of all those variables using the univariate procedure is very tedious and the best condition could not be attained, because the interactions among all the factors are neglected. Also, it is not known if the set of other fixed variables were kept at other levels, the results would lead to the same optimization. In addition, the total number of experiments to be carried out in the univariate procedure is much higher when compared with statistical design of experiments.

In this study, the factors screened were pH, initial metallic ion concentration (X), and temperature (T), for removal efficiency of Cd^{2+} and Pb^{2+} by *Cyperus laevigatus* biomass using a batch adsorption system. Main interaction effect, coefficients of the model, standard error of each coefficient and the probability for the full 2^3 factorial designs for Cd^{2+} and Pb^{2+} are presented in Tables 3 &5, respectively.

The codified mathematical model employed for the 2^3 factor design is:

$$R = A_o + A_1T + A_2X + A_3pH + A_4TX + A_5TpH + A_6XpH + A_7TXpH \quad (2)$$

Where: A_o represents the global mean and A_i the other regression coefficients.

Substituting the coefficients A_i in Equation (2) by their values from Tables 3 & 5 we get the following equations:

$$RCd^{2+} = 43.713 + 24.86T - 6.88X + 8.06pH - 5.43TX - 1.23TpH + 2.01XpH + 0.39TXpH \quad (3)$$

$$RPb^{2+} = 45.05 + 23.15T + 1.52X + 4.32pH + 7.47TX - 1.77TpH - 5.75XpH + 0.01TXpH \quad (4)$$

The effects of the main factors (T, X, pH) represent deviations of the average between high and low levels for each one of them. In case of Cd^{2+}, a change in pH and T values from low to high level results in 16.125 % and 49.725 % increase in the removal efficiency (Table 3). If a variation from high to low is made for X , increases of 13.775 % in the removal efficiency are observed, respectively. In case of Pb^{2+}, T, X and pH exert an influence in their low levels, increasing removal efficiency by 46.3 %, 3.05 % and 8.65 %, respectively. It can be concluded that when the effect of a factor is positive an increase in the value of the removal efficiency is observed when the factor changes from low to high level. In contrast, if the effect is negative, a reduction in removal efficiency occurs for the high level of the same factor.

As can be seen from Tables (3&5), some main factors and their interactions were significant at 5% of probability level (p <0.05). On the other hand, some effects were discarded, because they did no exhibit any statistical significance. As such, the resultant models can be represented by:

$$RCd^{2+} = 43.71 + 24.86T - 6.88X + 8.06pH - 5.43TX \quad (5)$$

$$RPb^{2+} = 45.05 + 23.15T + 7.47TX - 5.75XpH \quad (6)$$

In order to better evaluate each factor and its interaction in case of Cd^{2+}, Fig.1A, presented the normal probability plot of standardized effects. The graph of Cd^{2+} could be divided in two regions: the region with percent below 50%, where the factors and their interactions presented negative coefficients (TpH, T.X, X), and the region with percent above 50%, where the factors presented positive coefficients (T, pH, X.pH). All these factors and interactions which were represented as a square were significant figures while the effects represented by a circle were not significant (Fig.1A).

Fig. 1B presented the Pareto Chart of standardized effects at p = 0.05. All the standardized effects were in absolute values (to verify which were positives and negatives, see Fig. 1A). All the values that presented an absolute value higher than 1.000 (p = 0.05), which were located at right of the line, were significant. The absolute standardized value of the effect of each factor and its interaction appeared at the right of each bar.

Analyzing the graphs of Fig. 1A and the values of Table 3, it can be inferred that the T was the most important variable of the overall biosorption procedure. The positive value of its coefficient meant that the Cd^{2+} uptake by *Cyperus laevigatus* biomass was favored at high T values (T = 45.0). In order to avoid a disruption of the *Cyperus laevigatus* biomass at T lower than 45.0, this value was fixed for continuing the optimization of this work. The second important factor for overall optimization of the batch system was the pH . Only the achievement of this result emphasizes the merit of using the statistical design of experiments over the conventional univariate process of optimization of the system. This information would not be acquired in a univariate of optimization in biosorption system. The third important factor affect the overall optimization of the batch system was the metallic ion concentration (X). The negative coefficient value justifies that low metallic ion concentration led to high removal efficiency of Cd^{2+} ions . The fourth important factor affect the overall optimization of the batch system was the interaction of two factors T.X which was more significant than the main factors X and pH .

In Table 4 is presented the analysis of variance for the factorial design 2^3 without the insignificant three-way interactions. As can be seen, the main factors and two-way interactions were significant at 5% of probability level (p < 0.05), as discussed above.

(A)

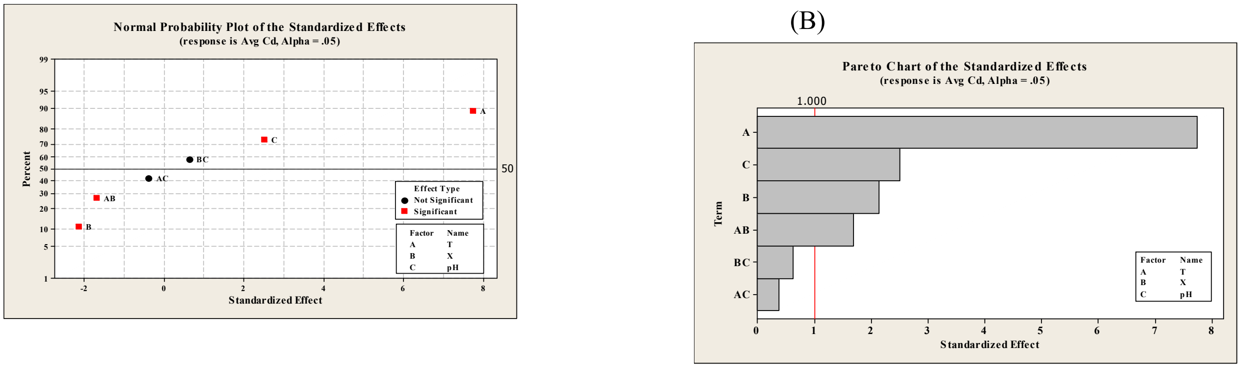

Fig. 1: (A) Cd Normal probability plot of standardized effect at p=0.05. The dotted line at 1.000 divides the negative effects from the positive one. (B) Pareto plot of standardized effect (absolute value) at p=0.05

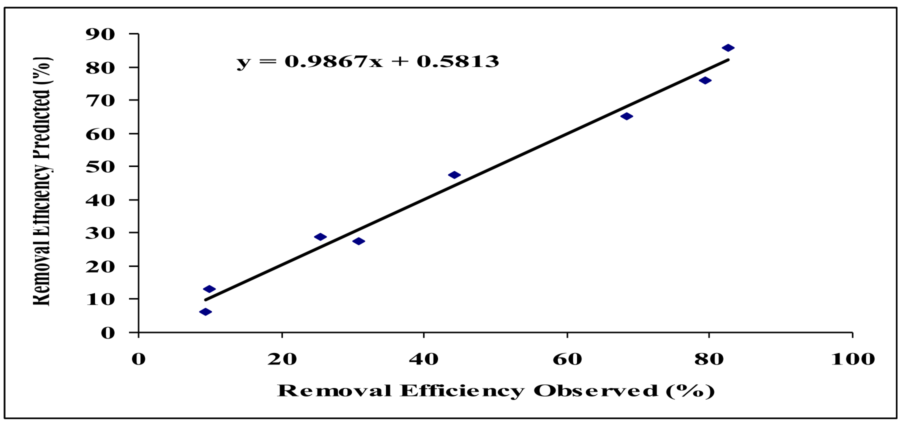

Fig. 2: Normal probability plot for the removal efficiency of Cd^{+2}.

Table 3: Statistical parameters for 2^3 design (Cd^{+2}) .

Term	Effect	Coefficient	S.E of coefficient	*p*
constant		43.713	3.212	0.0047
Main factors				
T	49.735	24.862	3.212	0.0082
X	-13.775	-6.887	3.212	0.0278
pH	16.125	8.063	3.212	0.0241
Interaction of two factors				
T.X	-10.875	-5.438	3.212	0.0340
T.pH	-2.475	-1.238	3.212	0.0766
X.pH	4.025	2.012	3.212	0.0644
Interaction of three factors **T.X.pH**	0.78	0.39	0.388	0.550

The effects and coefficients are given in coded units. *P:* probability and S.E.: standard error of coefficient.

Table 4: Analysis of variance for removal efficiency of Cd^{2+} - full 2^3 factorial design (coded units)

Source	d.f	Seq SS	Adj SS	Adj MS	F	*P*
Main effects	3	5844.68	5844.68	1948.23	23.60	0.0150
2-Way Interactions	3	281.18	281.18	93.73	1.14	0.0583
Residual Error	1	82.56	82.56	82.56		
Total	7	6208.43				

d.f.: degree of freedom. Seq SS: sequential sum of squares, Adj.MS: adjusted sum of squares, *F*= factor F and *p*: probability.

Likewise, the results of Pb^{2+} biosorption (Table 5 and Fig. 3) demonstrated that the T was the most important variable. However, the positive value of its coefficient meant that the Pb^{2+} uptake by *Cyperus laevigatus* biomass was favored at high T values (T=45.0). Accordingly, the T is proved to be a key condition affecting adsorption performance of the studied metals. This is in line with many authors [15, 24, 25, 26]. The second important factor for overall optimization of Pb^{2+} biosorption was the interaction of two factors T.X which was more significant than the main factors X and pH . The positive value of T.X coefficient meant that high metallic ion concentration with high T value would lead to an unexplained increase in the removal efficiency of Pb^{2+} that could not be explained using the univariate procedure of optimization of the system. The third important factor affect the overall optimization of the batch system was the X.pH . The negative value of its coefficient meant that the Pb^{2+} uptake by *Cyperus laevigatus* biomass was favored at low pH values (pH 2.0). In order to avoid a disruption of the *Cyperus laevigatus* biomass at pH higher than 2.0, this value was fixed for continuing the optimization of this work. In Fig 2 B, though the interaction of two factors X.pH was significant, it acquired the least effect on the removal efficiency of Pb^{2+} compared to others. The negative coefficient value of this interaction tells us that both factors should be decreased in order to achieve the highest response . The analysis of variance (Table 6) for the factorial design 2^3, without the insignificant three-way interactions, indicated that the main factors and two-way interactions were significant at 5% of probability level ($p < 0.05$).

Optimal conditions realized from the optimization experiment (observed values) were verified by comparing with calculated data from the model (predicted values). Figs. 2 and 4 present the normal probability plot of predicted removal efficiency for $Cd^{2+}$and Pb^{2+}, respectively. In both cases, it was observed how closely the set of observed values with the predicted ones, with correlation coefficients (R) of 0.98 and 0.96 for $Cd^{2+}$and Pb^{2+}, respectively.

Temperature has an influence on the biosorption of metal ions, but to a limited extent under a certain range of temperature, which indicates that ion exchange mechanism exists in biosorption to some extent [33]. In the present investigation, temperature has a significant effect on the biosorption efficiency of Cd^{2+} onto *Cyperus laevigatus* biomass . However it was found that temperature (5-40 ^{0}C) had minor effect on the sorption level of Cd, Cu or Co by *Saccharomyces cerevisiae* [34]. Moreover, higher removal efficiency of Pb^{2+} was detected at high temperature condition (45 ^{0}C). This revealed the endothermic nature of Pb^{2+} biosorption onto the studied plant biomass [35]. The adsorption of Cd^{2+} and Pb^{2+} onto carboxymethlylated lignin from sugarcane bagasse and *Ulva lactuca* biomass, respectively, was studied [16, 33]. They reported contrary opinion for our results . They found that the decrease in the biosorption of both ions with the rise in temperature may be due to either the damage of active binding sites in the biomass [23] or increasing tendency to desorb metal ions from the interface to the solution [36].

(A) (B)

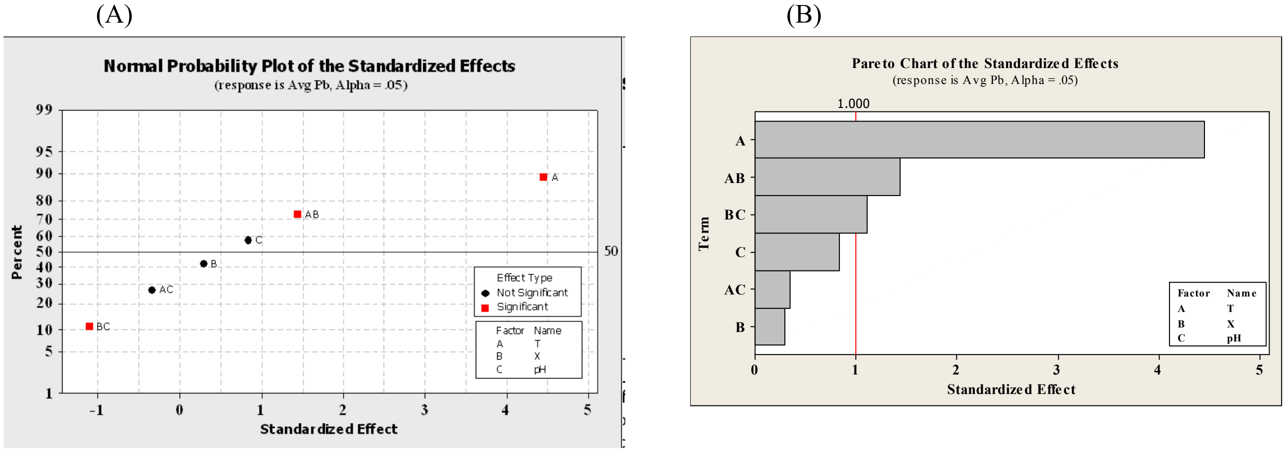

Fig. 3: (A) Pb Normal probability plot of standardized effect at p=0.05 . The dotted line at 1.000 divides the negative effects from the positive one. (B) Pareto plot of standardized effect (absolute value) at p=0.05

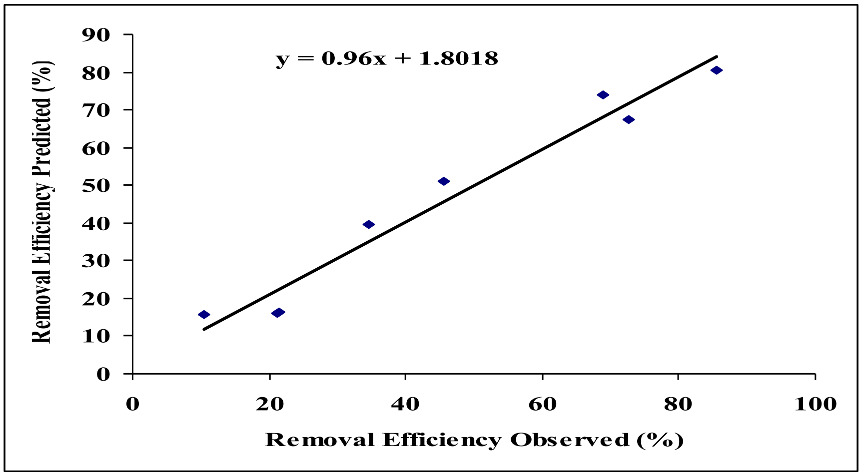

Fig.4: Normal probability plot for the removal efficiency of pb^{+2}.

Table 5: Statistical parameters for 2^3 design (pb^{+2}) .

Term	Effect	Coefficient	S.E of coefficient	*p*
constant		45.050	5.200	0.0073
Main factors				
T	46.300	23.150	5.200	0.0141
X	3.050	1.525	5.200	0.0818
pH	8.650	4.325	5.200	0.0558
Interaction of two factors				
T.X	14.950	7.475	5.200	0.0387
T.pH	-3.550	-1.775	5.200	0.0791
X.pH	-11.500	-5.750	5.200	0.0468
Interaction of three factors				
T.X.pH	0.02	0.01	0.001	0.932

The effects and coefficients are given in coded units. *P:* probability and S.E.: standard error of coefficient.

Table 6: Analysis of variance for removal efficiency of pb^{2+} - full 2^3 factorial design (coded units)

Source	d.f	Seq SS	Adj SS	Adj MS	F	*P*
Main effects	3	4455.6	4455.6	1485.2	6.87	0.0272
2-Way Interactions	3	736.7	736.7	245.6	1.14	0.0583
Residual Error	1	216.3	216.3	216.3		
Total	7	5408.7				

d.f.: degree of freedom. Seq SS: sequential sum of squares, Adj.MS: adjusted sum of squares, *F*= factor F and *p*: probability.

Many studies concerning sorption of heavy metals by different biomaterials indicated that pH and temperature influence removal efficiency. It was found that the pH is one of the most important environmental factors in biosorption of heavy metal ions [27]. The pH value of solution strongly influences not only the site dissociation of the biomass ' surface, but also the solution chemistry of the heavy metals: hydrolysis, complexation by organic and/or inorganic ligands, redox reactions, precipitation, the speciation and the biosorption availability of heavy metals. It was demonstrated that the suitable pH ranges for the various metal ions were slightly different [28]. The results of the present research indicated that the highest removal efficiency for Cd^{2+} were attended at the higher pH value (pH=6.0) and at lower pH value (pH=2.0) for

Pb^{+2} . These results were concomitant with the findings of Southichak et al. [29]. They studied the effect of pH upon heavy metal adsorption by reed biomass in a wide range of pH and concluded that the maximum sorption was observed near neutral condition (pH = 6) for Cu^{2+}, Ni^{2+}, Cd^{2+} and Zn^{2+}, while that for Pb^{2+} was from the acidic range (pH 2-4). The adsorption of Pb^{2+} at lower pH was also observed in other biomaterials such as the biomass *Zoogloea ramiiger* [30] and fungus *Mucor rouxii* [31]. On the other hand ,Abdel-Halim et al.[15] studied the adsorption capacity of $Pb^{2+,}$ affected by Experimental parameters such as pH, contact time and concentration of Pb^{2+} solution, on to *Eichhornia speciosa* plant biomass. They found that the uptake percent of $Pb^{2+,}$ increased by increasing pH values. Moreover,Sekhar et al. [24] reported that Pb^{2+} removal by different organs of *Hemidsmus indicus* was unaffected by pH change.

At lower pH, the adsorption of many heavy metals usually took place with low removal efficiency. This occurred because there was a high concentration of proton in the solution and this proton competed with the metal ions informing a bond with active sites on the surface the biomaterials. These bonded active sites thereafter became saturated and was inaccessible to other cations [32, 22]. The biosorption characteristics of Cd^{2+}ions from aqueous solution using the green alga (*Ulva lactuca*) biomass were investigated as a function of pH, biomass dosage, contact time, and temperature [26]. They found that the maximum biosorption of Cd^{2+}ions was found at pH 5, 20 ^{o}C, 60 min and 20 mg l^{-1} of biosorbent.

In the present research, the metal ion concentration has no effect on the biosorption efficiency of Pb^{2+} onto *Cyperus laevigatus* biomass. However, the results showed that the biosorption of Cd^{2+} increased by decreasing its initial concentration in the solution (10 mg l^{-1}). The sorption of Cd^{2+}, Ni^{2+} and Zn^{2+} by Ca-treated *Sargassum* sp. biomass was compared under low and high ionic strength conditions and an exponential decrease in the removal efficiency of the sorption system with increasing metal concentration was reported [37].

Since there is no literature report on the adsorption of heavy metals by *Cyperus laevigatus* biomass using factorial design, the results obtained were compared with those of many different types of biomaterials. The effects of pH (4.0 and 5.5), initial metal concentration (5.0 and 10 .0 g l^{-1}) and biomass concentration (0.4 and 0.7 g l^{-1}) on biosorption of Cd^{2+} using *Aspergillus niger* was studied [17]. The biosorption process studied was modeled based on 2^3 factorial designs. The most important factor was the biomass concentration. An increase in the removal efficiency occurred with an increase in biomass concentration and pH. However, the removal efficiency decreased with an increase in initial metal concentration .Although the biosorbent mass was constant in the present experiment, pH showed the same tendency in both cases. Moreover, the interaction effects X.pH have significant influence on Cd^{2+} removal efficiency. The biosorption of Cd^{2+} and pb^{2+} onto sugarcane bagasse using 2^3 factorial designs was studied by Brasil et al. [19]. Three operating factors were analysed: temperature (30-50 ^{o}C), initial metal concentration (0.1 and 1.0 mol dm^{-3}) and pH (5 and 6). The fixed parameters were time of exposition (8 h) and initial biosorbent concentration (0.2 g l^{-1}). The authors concluded that temperature is the most important factor in the single system (pb^{2+}), while initial metal concentration was the most important variable for the binary system (Cd^{2+} and pb^{2+}). In the single system the adsorption increases with increasing temperature and in the binary one the adsorption decreasing with increasing initial metal concentration. This is coincide with the results of the present study showed that temperature was the most important variable as it acquired significant influence on the adsorption of pb^{2+} and Cd^{+2}.

Conclusion

The factorial experiment design method is undoubtedly good technique for studying the influence of major process parameters on response factors by significantly reducing the number of experiments and henceforth, saving time, energy and money. The use of factorial design offers good and fast screening procedure and mathematically computes the significance of several factors in one experiment that predicts where the optimum is likely to be located. Besides, it allows the identification of the most important parameters for biosorption of metallic ions under tested conditions. In the present research, the most significant effect for Cd^{2+} and pb^{2+} biosorption were ascribed to T . The interaction effects of T.X also has a significant influence on the Cd^{2+} and pb^{2+} removal efficiency .

The normal probability plot between the predicted values (model) and the observed (experimental) clearly demonstrate how closely the set of observed values with the predicted ones, with high correlation coefficients. In addition, the biosorption studies of Cd^{2+} and pb^{2+} onto *Cyperus laevigatus* biomass showed that this biosorbent was a powerful and low-cost biosorbent for these metallic ions removal from aqueous solution opening the possibility of this biosorbent to be employed in the treatment of industrial effluents and agricultural waste waters before being delivered into the environment. It is worthwhile to advise the metal industry sponsors to apply such experimental designs to maintain high efficiency and profit biosprbtion process.

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