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Dynamic removal of Pb(II) by live *Dunaliella salina*: A competitive uptake and isotherm model study

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Abstract

The main aim of this study is modeling of a continuous biosorption system for the removal of lead (II) ions in the aqueous conditions using live *Dunaliella salina* microalgae. The live microalgae can grow in saline water and opens new opportunities in varying the biosorbent properties. The effects of five parameters including pH, optical density of algae as a factor indicating the adsorbent dosage, injection time, contact time and initial concentration of lead ion were optimized by means of Response Surface Methodology (RSM) based on the Central Composite Design (CCD). *Dunaliella salina* algae showed maximum Pb (II) biosorption with 96% efficiency. For the selective lead (II) uptake in the presence of cadmium and nickel ions binary and ternary systems of ions were chosen. The mutual effect of each heavy metal ion in all systems on total uptake percentage was also examined. The ion selectivity was investigated in the presence of diverse heavy metal ions and lead (II) uptake percentage was determined to be 70%. Both Langmuir and Freundlich isotherm models were suitable for describing multicomponent binary and ternary systems depending on competitive ions. Main functional groups and surface properties of the *Dunaliella salina* was identified by Fourier transform infrared spectroscopy, scanning electron microscopy and energy dispersive spectrometry. Hence, effective heavy metal ion uptake, simple design and cost-effective cultivation confirmed live *Dunaliella salina* as suitable microalgae for purifying contaminated water in an economic and safe manner.

Keywords: biosorption; heavy metal ions, *Dunaliella salina,* Response Surface Methodology

1. Introduction

The environmental pollution of heavy metal ions due to industrial development such as mining, tannery, photography, petroleum refining, pesticides, metallurgical and manufacturing activities have become a major global concern. These activities have led to the release of various amounts of toxic metal ions in the food chain of living creatures due to waste disposal in their ecosystem(Kaushik and Raza, 2019). Heavy metal ions are non-biodegradable even at low concentrations. Hence, their persistence has become a global issue in many countries because of their capacity for bioaccumulation in human and animal' tissues by exposure of water or food derived from natural water(Wei et al., 2021; Ziaei et al., 2022).

Lead, cadmium and nickel as heavy metal ions are used in a wide range of commercial procedures(Esvandi et al., 2019). For example, lead ions can damage the brain and central nervous system(Eliescu et al., 2020). Cadmium ion can be found in many products, such as batteries, paints, and plastics. It can cause damage to the lining of the lungs, liver, and kidneys, as well as cancer and the destruction of red blood cells(Abdel et al., 2013; Verma et al., 2021). Also, nickel ions at over permissible levels cause kidney damage, lung cancer, and gastrointestinal disorder(Giraldo et al., 2019).

Therefore, the elimination of heavy metal ions at acceptable level is considered a major issue due to the economic and environmental concerns. To reduce these effects, many traditional methods consist of chemical or electrochemical precipitation, ion exchange systems, membrane separation process, advanced oxidation, coagulation, reverse osmosis and electro dialysis, and solidification have been developed and established to remove heavy metal ions from wastewaters(Gu et al., 2021; Zhang et al., 2022, 2020). Unfortunately, application of these methods have been limited by some factors such as: ineffective performance, deficiency of eco-friendliness and maintenance

costs that make them unattractive and unsatisfactory in wastewater treatment(Anastopoulos and Kyzas, 2015; Qin et al., 2020; Wang and Chen, 2009). In comparison to conventional techniques, numerous studies reports on various biological adsorption methods are considered suitable for treatment of contaminated waters(Kamal and Rafey, 2021; Katheresan et al., 2018; Morosanu et al., 2017). Biosorption by natural materials are considered as an innovative and cost effective methods(Pugazhendhi et al., 2018; Qin et al., 2020). Various natural biosorbents such as rice straw(Shen et al., 2019),fungal biomass(Kumar et al., 2008), cucumber peel(Basu et al., 2017), fruit peels(Turkmen Koc et al., 2020)and yeast(Wang and Chen, 2009), have been examined to eliminate heavy metal ions. Recently, one of the most promising biosorbents, introduced are the algal species(Elleuch et al., 2021; Mohd Udaiyappan et al., 2017). Algae are primary organisms in biological system, widely spread around the world. Algae cell walls are surrounded by polysaccharides, proteins and lipid matrix that provide facilitating functional groups to bind heavy metal ions(Gu and Lan, 2021; Naveed et al., 2019; Suresh Kumar et al., 2015; Yan et al., 2022). Several studies have been reported on biological metal ions uptake including copper, lead, chromium, arsenic and nickel ions by various algae species(Bai and Venkateswarlu, 2019; Giraldo et al., 2019; Lee and Chang, 2011). Different mechanisms such as electrostatic attraction, cation exchange, formation of metal complexes, hydrogen bond, and micro precipitation can occur in the biosorption process(Giraldo et al., 2019). Hence, the biosorption capacity relies on the algal species and charges on metal ions(Dönmez and Aksu, 2002).

Dunaliella salina (D.s) is one type of green microalgae with a very high capacity for binding metal ions due to the presence of functional groups such as amines, hydroxyls, carboxyl, and sulfates, which can cross-link with heavy metal ions depending on temperature, concentration of heavy metal ions and pH(Wang et al., 2017). Moreover, researchers confirmed that *D.s* requires minimal

nutrients and environmental conditions(Anastopoulos and Kyzas, 2015) and can survive even in harsh natural conditions.

Most reports on using microalgae for biosorption of heavy metal ions use biomass as the biosorbent. So for, there is few reports on using live microalgae as a continuously growing biosorbent(Suresh Kumar et al., 2015). The aim of the current research is to investigate the efficiency of live *D.s* algae as an environment-friendly and economic biosorbent for wastewater treatment in a dynamic process. This microalgae has high growth rate in saline water and can produce harvestable biomass. Several studies have been introduced to use *D.s* biomass as an effective metal ion bisorbents from wastewater(Belghith et al., 2016; Elleuch et al., 2021). On the contrary of previous reports, the present research uses a singular step of growing algae directly for the removal of heavy metal ions through a continuous sorption system of contaminated water. In particular, no previous report is seen on continuous sorption of lead ions by living *D.s* and measuring the time of feeding contaminated water, which is an effective parameter in the growth process.

Additionally, industrial wastes often include multicomponent heavy metal ions. The assessment of the only one type of metal ion uptake may not be acceptable(Plazinski, 2013). Based on the literature, no report was found on simultaneous lead, cadmium and nickel ions uptake using live *D.s*. In order to more precisely understand the interactions between the competitive heavy metal ions and environmental complications, biosorption of heavy metal ions in single, binary and ternary solutions were investigated.

In this work, the influence of several important factors such as pH, contact time, optical density as an adsorbent dosage, injection time of lead ion and initial concentration of lead ions are investigated by Response Surface Methodology (RSM). Out of several designs available for the

optimization, the RSM design was chosen as the most efficient design, with less time and fewer test runs for Pb (II) uptake(Isam et al., 2019). *D.s* surface also was characterized by means of scanning electron microscopy (SEM) and energy dispersive spectrometry (EDS). Also, to identify the main functional groups for the interaction of algae and heavy metal ions, Fourier transform infrared (FT-IR) spectroscopy was used.(Lee and Chang, 2011) Hence, this study recommends an effective and dynamic method to use live *D.s* for purification of waters from heavy metal ions with low cost.

2. Materials and method

2.1. Chemical materials

Pb $(NO_3)_2$, Cd $(NO_3)_2 \cdot 4H_2O$, and Ni $(NO_3)_2 \cdot 6H_2O$, were purchased from Merck (Darmstadt, Germany). Sodium hydroxide (0.1 mol L^{-1}) and nitric acid (1%) solutions were prepared for pH adjustment (Riedel-de-Haen, Germany). All the stock solutions were prepared using deionized (DI) water. All chemical reagents used were of analytical grade without further purification.

2.2. Analytical methods

The concentration of lead, cadmium and nickel ions in the solution was determined using a flame atomic absorption spectrophotometer (FAAS) (Analytik Jena AG, 07745 Jena, Germany). The surface morphological features of the *D.s* before and after removal of heavy metal ions, were analyzed using scanning electron microscopy (SEM) and an energy dispersive X-ray Spectroscopy (EDX) (TESCAN-XMU, Czech Republic). Fourier transform infrared (FT-IR) spectrometry (Thermo Nicolet Avatar 370, Pittsfield, USA) was used to determine the functional groups of the *D.s* surface. Ion selectivity study was using inductively coupled plasma-optical emission spectrometry (ICP-OES) (76004555 SPECTRO ARCOS System)

128 *2.3. D.s microalgae growth conditions*

129 The stock microalgae *Dunaliella salina (D.s)* was cultured in a 300 mL Erlenmeyer flask
130 containing 250 mL artificial sea water (ASW) with the following composition (g L^{-1}): NaCl- 23.6,
131 $MgCl_2.6H_2O$- 4.1, $MgSO_4.7H_2O$- 4.9, $CaCl_2$- 1.1, KNO_3- 0.3, KCl- 0.075, Na_2EDTA - 0.012,
132 $Na_2SiO_3.9H_2O$- 0.04, enriched with 1.0 mL of trace elements stock with the following composition
133 (g L^{-1}): H_3BO_3- 0.57, $MnCl_2.4H_2O$- 0.36, $ZnCl_2$- 0.62, $CuCl_2.2H_2O$- 0.27, $Na_2MoO_4.2H_2O$- 0.25,
134 $CoCl_2.6H_2O$- 0.42, $FeSO_4$- 1.36, $MnCl_2.4H_2O$- 0.36, $Na_2C_4H_4O_6$- 1.77 and 10 mL KH_2PO_4- 4.6 (g
135 L^{-1}). This solution was sterilized by autoclaving at 120ºC for 2h. The growth medium pH was set
136 to 6.4. The culture was maintained under continuous fluorescent lamps with 50 µmol m^{-2} s^{-1} light
137 intensity with 16–8 h light–dark cycle at 25 ± 2 °C and for 14 days. To determine the algae growth
138 curve, turbidity of *D.s* culture was measured at 680 nm daily using spectrophotometer. Then stock
139 *D.s* was placed in refrigerator and sub-cultures were prepared from this solution. All experiments
140 were conducted in triplicates and data were expressed as mean values with standard deviations to
141 minimize error.

142 *2.4. Batch biosorption*

143 The metal ion stock solutions of Cd $(NO_3)_2$, Pb $(NO_3)_2$ and Ni $(NO_3)_2$ were prepared in a 100mL
144 volumetric flask with concentration of 1000 (mg L^{-1}) in deionized water. All necessary solutions
145 were diluted from the stock solutions.

146 The effects of several parameters including initial Pb (II) concentration, pH, OD, injection time
147 of Pb (II) ion and contact time on the lead ion uptake using *D.s* were investigated. The range and
148 level designed by CCD method are represented in Table 1. The flasks of algal culture were
149 prepared according to section 2.3. After the end of biosorption, the algae samples were centrifuged
150 at 5000 rpm to separate the biosorbent. For pH adjustment, small amount of HNO_3 (1%) or NaOH

(0.1 mol L^{-1}) were used. The optimum conditions for Pb (II) uptake were selected using these experiments.

To study the influence of heavy metal ions on biosorption and total uptake, Pb (II), Cd (II) and Ni (II) ions with concentration range of 0.00–300.00 mg L^{-1} were examined. For binary systems, six experimental sections were designed as following: the concentration of Cd (II) and Ni (II) were individually varied from 0 to 300 mg L^{-1} in the presence of lead ions (50 mg L^{-1}). The concentration of Pb (II) and Ni (II) ions were individually changed from 0 to 300 mg L^{-1} in the presence of 50 mg L^{-1} of Cd (II). The concentration of Pb (II) and Cd (II) ions were individually changed from 0 to 300 mg L^{-1} in the presence of 50 mg L^{-1} of Ni (II). For ternary biosorption, one experimental section was designed as: the concentration of Cd and Ni ions were simultaneously changed from 0 to 300 mg L^{-1} in the presence of 50 mg L^{-1} of Pb ion.

2.5. Biosorption isotherms and data evaluation

The biosorption isotherms with the initial concentration range of 50–300 mg L^{-1} for Cd, Ni and Pb ions were investigated under optimal conditions. The final heavy metal ion concentrations were analyzed using AAS. The individual and total uptake of heavy metal ion were calculated using Equations (1) and (2), respectively:

$$\%\, M(II)\ uptake = \frac{(C_i - C_f)}{C_i} \times 100 \qquad (1)$$

$$\%\, Total\ uptake = \frac{\sum(C_i - C_f)}{\sum C_i} \times 100 \qquad (2)$$

The biosorption capacity q_e (mg g^{-1}) was obtained to determine the Langmuir and Freundlich models according to the following equation:

$$q_e = \frac{(C_i - C_f)V}{m} \qquad (3)$$

where in the equations (1) to (3), C_i (mg L^{-1}) and C_f (mg L^{-1}) represent the initial and final concentrations of heavy metal ions, respectively; V (L) is the volume of algae sample and m (g) is the mass of biosorbent dosage (*D.s)*.

2.6. CCD experimental design

Response surface methodology with central composite design (RSM-CCD) approach was utilized to design and optimize the biosorption tests. Simultaneously, the effects of five parameters were determined including: pH (A), optical density of algae (biosorbent dosage) (B), initial Pb(II) concentration (C), injection time of lead ions (D), and contact time (E). Table 1 shows the coded levels and lower and higher ranges for each selected parameter.

Table1. Levels of independent parameters based on CCD method for Pb (II) uptake%

parameters	unites	symbols	Levels				
			-α	-1	0	+1	+α
pH	-	A	3	4	5	6	7
Optical density (OD)	-	B	0.1	0.2	0.3	0.4	0.5
Pb(II) concentration	mg L^{-1}	C	10	82.5	155	227.5	300
Injection time	day	D	2	4	6	8	10
Contact time	h	E	24	36	48	60	72

Thirty two experiments were recommended by CCD design. Pb (II) uptake was considered as the response. In this study, half factorial CCD was applied. The CCD for the five independent parameters was based on ten axial points, sixteen factorial points and six replicates as the center

point. The selected parameters were varied at five levels labeled as: (-α, -1, 0, +1, +α). The number of experiments was obtained using equation (4):

$$N_e = \frac{2^P}{2} + 2P + C.P \qquad (4)$$

where N_e the number of experiments, P is the number of independent parameters, and C.P is the number of experiments conducted at the center point. A set of 32-experiment matrix was obtained as shown in section 3.1.1. To minimize systematic bias, the experiments were carried out in a randomized design. The experimental data matrix was employed using Minitab, 17.0.1 and Design-Expert12. 3D contour plots were investigated to study the interaction between selected parameters.

3. Results and discussion

3.1. Experimental design and statistical analysis

3.1.1. Optimization of lead (II) uptake

The effects of five independent variables including: pH (A), optical density of algae (biosorbent dosage) (B), initial Pb(II) concentration (C), injection time of lead ions (D) and contact time (E) on biosorption were carried out with RSM-CCD to obtain the optimum conditions. The CCD proposed matrix of input parameters and lead (II) uptake with the 32 randomized experiments are designed in Table 2. Based on the mathematical relationship among variables and response function using RSM, the coefficients of the equation and quadratic model for lead uptake is shown as Equation 5:

$$\text{Pb (II) uptake } \% = 98.0 - 5.85\,A - 12.8\,B + 0.0080\,C + 0.136\,D + 0.173\,E + 0.094\,A^2 + 63.7\,B^2 + 0.000092\,C^2 - 0.0303\,D^2 - 0.00011\,E^2 + 0.37\,AB + 0.00326\,AC + 0.581\,AD + 0.0330\,AE - 0.0523\,BC - 1.36\,BD - 0.174\,BE - 0.00728\,CD - 0.000841\,CE - 0.01959\,DE \qquad (5)$$

where A, B, C, D and E are the coded values for the selected variables, while AB, AC, AD, AE, BC, BD, BE, CD, CE and DE are the interaction parameters. The quadratic terms are shown as A^2, B^2, C^2, D^2 and E^2. The analysis of variance (ANOVA) for lead (II) uptake is given in Table 3 which consist of F–value, p–value, lack of fit and pure error.

Table 2. Designed runs for Pb (II) uptake using *D.s*

Run	A:pH	B:OD	C:Pb(II) mg L^{-1}	D:injection time day	E:contact time h	Pb(II) uptake %
1	4	0.2	227.5	8	60	80.5997
2	4	0.4	227.5	8	36	82.2084
3	4	0.4	227.5	4	60	84.9648
4	6	0.2	227.5	4	60	85.3196
5	6	0.2	82.5	8	60	94.3595
6	5	0.3	155	6	48	88.0008
7	5	0.3	155	6	48	86.42
8	4	0.4	82.5	8	60	90.7251
9	5	0.3	155	10	48	88.2815
10	6	0.4	82.5	8	36	93.122
11	5	0.3	10	6	48	96.318
12	4	0.2	82.5	4	60	90.7057
13	5	0.5	155	6	48	90.0047
14	5	0.3	155	6	72	87.3666
15	5	0.3	155	6	48	87.68
16	3	0.3	155	6	48	85.4408
17	5	0.3	155	2	48	84.7873
18	4	0.4	82.5	4	36	90.1154
19	6	0.4	82.5	4	60	91.5168

20	6	0.4	227.5	8	60	84.3975
21	6	0.4	227.5	4	36	84.7014
22	6	0.2	82.5	4	36	85.5901
23	5	0.1	155	6	48	89.1273
24	5	0.3	300	6	48	81.584
25	5	0.3	155	6	48	85.889
26	4	0.2	82.5	8	36	89.502
27	5	0.3	155	6	48	86.24
28	4	0.2	227.5	4	36	85.2141
29	5	0.3	155	6	48	85.95
30	7	0.3	155	6	48	89.3518
31	5	0.3	155	6	24	86.55
32	6	0.2	227.5	8	36	85.8862

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217

218 **Table 3.** ANOVA table for Pb (II) uptake using *D.s* microalgae

Source	Sum of Squares	DF	Mean Square	F-value	p-value
Model	377.36	20	18.87	19.16	< 0.0001
A-pH	**14.54**	**1**	**14.54**	**14.76**	**0.0027**
B-OD	1.67	1	1.67	1.69	0.2196
C-Pb(II)	**278.89**	**1**	**278.89**	**283.17**	**< 0.0001**
D-injection time	3.89	1	3.89	3.95	0.0724
E-contact time	2.59	1	2.59	2.63	0.1332
AB	0.0218	1	0.0218	0.0221	0.8845
AC	0.8918	1	0.8918	0.9055	0.3617
AD	**21.63**	**1**	**21.63**	**21.96**	**0.0007**
AE	2.51	1	2.51	2.55	0.1386
BC	2.30	1	2.30	2.34	0.1545
BD	1.19	1	1.19	1.21	0.2952
BE	0.6952	1	0.6952	0.7059	0.4187
CD	**17.83**	**1**	**17.83**	**18.10**	**0.0014**

CE	**8.56**	**1**	**8.56**	**8.70**	**0.0132**
DE	3.54	1	3.54	3.59	0.0847
A^2	0.0460	1	0.0460	0.0467	0.8329
B^2	**9.94**	**1**	**9.94**	**10.09**	**0.0088**
C^2	**5.38**	**1**	**5.38**	**5.46**	**0.0394**
D^2	0.9075	1	0.9075	0.9214	0.3577
E^2	0.1434	1	0.1434	0.1456	0.7101
Residual	10.83	11	0.9849		
Lack of Fit	6.67	6	1.11	1.34	0.3842
Pure Error	4.16	5	0.8325		
Cor. total	388.19	31			

In general, if the p-value in the ANOVA is less than 0.05, the variables are significant(Mosleh et al., 2018; Sohbatzadeh et al., 2016). According to the ANOVA results of Table 3, A, C, AD, CD, CE, B^2, C^2 are significant model terms. The F-value of 19.16 implies that the model is significant. There is only a 0.01% chance that an F-value of this large could occur due to noise. The coefficient of determination, R^2 value, for Equation 1 and the standard deviation of the model are 0.9721 and 0.9924 respectively (Table. 4).

The regression model is an appropriate way to describe the correlation between variables and the response function. The closer R^2 to 1, and smaller the standard deviation, implied that the model is significant and could establish suitable relationship between the variables and lead ion uptake. Moreover, the F-value of 1.34 for Lack of Fit implies that Lack of Fit is not significant relative to the pure error. This result demonstrated that Lack of Fit is good. The other numerical parameters of this model are summarized in Table 4.

Table. 4 Statistical variables from (CCD-RSM)

No.	Parameter	Pb(II) uptake
1	Standard deviation	0.9924
2	Mean	87.44

3	C.V. %	1.14
4	R^2	0.9721
5	Adjusted R^2	0.9213
6	Predicted R^2	0.5486
7	Adequate precision	18.1624

233

234 Furthermore, adequate precision which measured the signal to noise ratio (S/N) was 18.162. A
235 ratio (S/N) greater than 4 is desirable and shows a suitable signal. This model can be utilized to
236 navigate the design space(Isam et al., 2019). Based on the F–value results of independent
237 parameters, the main effects of independent parameters on lead (II) uptake followed this sequence:
238 initial concentration of Pb (II) > pH > injection time > contact time > OD. Based on the results of
239 Table 3, this model was significantly affected by the two first orders of independent parameters
240 including pH and initial lead (II) concentration (p–value < 0.05). The OD, contact time and
241 injection time had a little effect on the lead ion uptake. Therefore, they are identified as
242 insignificant factors (p–value > 0.05).

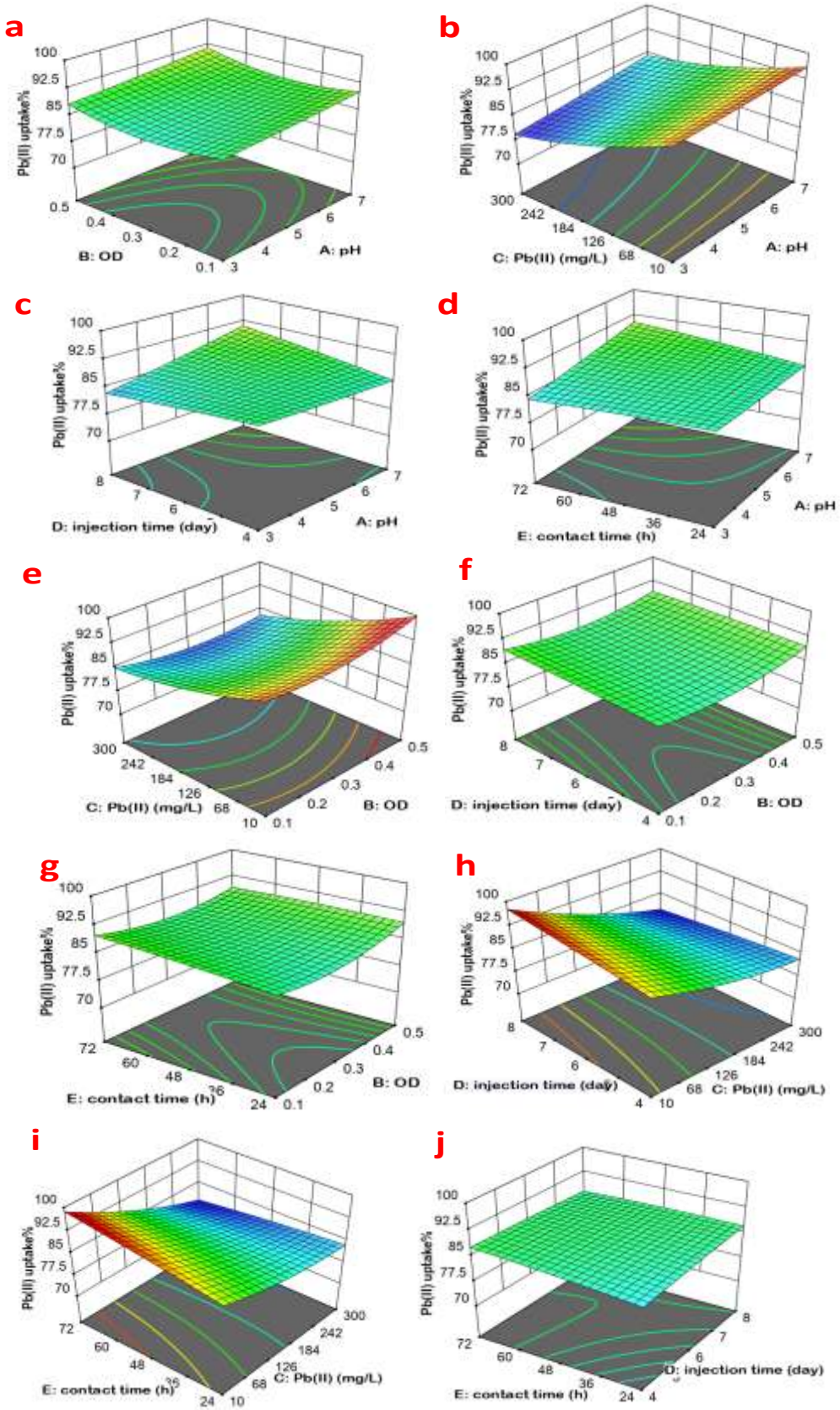
243 *3.1.2. Effect of process parameters on the Pb (II) uptake*

244 Three-dimensional (3D) surface plots were used to assess the interactions behavior of two
245 parameters (horizontal plane) on the Pb (II) uptake (vertical axis), while other three parameters are
246 kept at fixed levels (Figure.1 a-j). pH is one of the essential parameters that seriously affects
247 biosorption(Esposito et al., 2002). The interaction of pH with OD, Pb (II) concentration, injection
248 time and contact time on Pb (II) uptake, is depicted in Figure. 1(a-d) respectively. As shown in
249 Figure.1b, interaction of lead ion concentration and pH indicates the significant response on Pb
250 (II) uptake. Uptake efficiency by increasing the solution pH increased, because lowering of
251 hydrogen ion causes the deprotonated of functional groups and more affinity interaction with the
252 lead ions(Kit and Chang, 2020). At pH >7, the precipitation would occurre and lead uptake will

not be feasible. Moreover, functional groups release protons to the aqueous solution depending on distribution of heavy metal ions (alpha values), their acidity constant (pKa) and solubility in the aqueous solution(Zeraatkar et al., 2016). In addition, H^+ ions may compete with lead species.

Also, it was observed that uptake efficiency was reduced by increasing the lead ion concentration. This behavior might be due to the saturated of active functional groups existing on the *D.s* surface at high concentration of lead ion(Kit and Chang, 2020).

Interestingly, as apparent in Figure.1 (h), the lead uptake percentage was increased by increasing the injection time of lead ion from 2 to 10 day in growing process of algae. This important result, confirmed that growing rate of *D.s* due to increasing the fresh biomass with new binding sites on the *D.s* surface.



265 **Figureure.1** (3D-RSM) plots for Pb(II) uptake versus effect of parameters

3.2. Single, binary and ternary biosorption process

In order to investigate the effect of competitive ions on heavy metal ion uptake efficiency, seven experimental sections (A-G) were carried out. Table 5 shows the heavy metal ion uptake by *D.s.*in the single, binary and ternary systems. Generally, in multicomponent biosorption process, three types of behaviors may occur: 1- synergism phenomenon can occur leading to increasing the total uptake in the presence of other heavy metal ions; 2-antagonism behavior happens due to decreasing the total uptake in the presence of other heavy metal ions and, 3- non-interaction may happen(Verma et al., 2021). The simultaneous Cd (II), Ni (II) and Pb (II) uptake from binary heavy metal ion solutions were examined in sections (A-D). In section A and B, initial Pb(II) concentration was fixed (50 mg L^{-1}) while, Cd and Ni as competitive ions were increased (0 –300 mg L^{-1}) respectively. It was observed that the Pb uptake% decreased from 96.19 to 80.99 and from 96.19 to 95.513 with increasing the initial concentration of Cd and Ni ions respectively. Also, Cd and Ni uptake percentage were decreased as compared to single metal ion uptake. This phenomenon shows a competitive effect among Cd (II), Ni (II) and Pb (II) ions. However, lead uptake is higher than Cd and Ni uptake. Similar pattern was observed by Ni (II) in the presence of lead ion in section B. But, the amount of Ni (II) uptake (35.63- 10.80) was lower as compared to Cd (II) uptake (76.22-22.00). It was noticed that with increasing competitive ion concentration, the Pb (II) % uptake was reduced. Therefore, the antagonistic effect occurred in the presence of cadmium and nickel ions on the lead ion uptake.

In sections C and D, the concentration of Cd and Ni ions were fixed (50 mg L^{-1}) and Pb (II) ion was increased from 0 to 300 mg L^{-1}. As shown in these sections, Pb (II) uptake was higher than Cd (II) and Ni (II) uptake percentages. Cd (II) and Ni (II) uptake were similarly decreased. In section E and F, the effect of competition between Cd and Ni ions in the absence of Pb ion has

been investigated. In the presence of fixed level of Cd (II) and increasing Ni (II) concentration, Ni (II) uptake was decreased in the presence of a lower percentage of Cd (II). A similar result occurred in F section with the increasing of Cd ion as well as fixed level of Ni ion. However, the results showed that the uptake percentage of Cd (II) is higher than Ni (II) uptake in all of experimental sections. These results showed higher selectivity of *D.s.* for Cd (II) ion than that of Ni (II). In the last part of the simultaneous biosorption (G), lead uptake in the presence of competitive ions; Cd and Ni ions in ternary systems were investigated. The concentration of lead ion was fixed and the concentration of cadmium and nickel ions increased simultaneously. It can be seen that Pb (II) uptake (96.19- 61.73) is highest over Cd (II) uptake (69.06-19.12), and Ni (II) uptake (28.15-8.5) in ternary systems.

It may be due to the possible interaction between various types of metal ions and *D.s* cell wall as a dynamic sorbent in biosorption systems. Basic factors of biosorption may affect the binding site properties such as surface structure, functional groups, ionic size, ionic charge, ionic weight (the weight of an ion as determined by the sum of the atomic weights of its components), heavy metal ion concentration, standard redox potential of the metal ions, ionic strength and pH. Therefore, it is difficult to explain which common parameters has more influence on the interaction mechanism and selectivity of biosorption in a mixed heavy metal ion system.(Verma et al., 2021)

Table. 5 Comparison of single, binary and ternary heavy metal ion uptake.

	Concentration(mg L^{-1})			**Uptake%**			
Section	**Pb (II)**	**Cd (II)**	**Ni (II)**	**Pb (II)**	**Cd (II)**	**Ni (II)**	**Total**
	50	0	0	96.19	0	0	96.19
	50	50	0	95.91	76.22	0	86.06
	50	100	0	94.99	73.50	0	84.24

A	50	150	0	94.35	68.34	0	81.34
	50	200	0	93.79	60.96	0	77.37
	50	250	0	91.22	43.84	0	67.53
	50	300	0	80.99	22.00	0	51.49
	50	0	0	96.19	0	0	96.19
	50	0	50	98.73	0	35.63	67.180
	50	0	100	97.51	0	28.64	63.07
B	50	0	150	97.10	0	20.18	58.64
	50	0	200	96.86	0	18.42	57.64
	50	0	250	95.97	0	12.15	54.06
	50	0	300	95.51	0	10.80	53.15
	0	50	0	0	80	0	80
	50	50	0	95.91	76.22	0	86.06
	100	50	0	91.32	69.26	0	80.29
C	150	50	0	88.92	60.45	0	74.68
	200	50	0	84.34	55.67	0	70.00
	250	50	0	81.44	49.92	0	65.68
	300	50	0	78.51	35.64	0	57.07
	0	0	50	0	0	55.88	55.88
	50	0	50	98.73	0	35.63	67.18
	100	0	50	95.32	0	28.43	61.87
D	150	0	50	91.92	0	19.91	55.91
	200	0	50	87.34	0	17.11	52.22
	250	0	50	85.44	0	12.10	48.77
	300	0	50	80.51	0	10.60	45.555
	0	50	0	0	80.00	0	80
	0	50	50	0	78.25	52.75	65.50
	0	50	100	0	62.16	49.1	55.63
E	0	50	150	0	51.84	44.97	48.40

	0	50	200	0	45.20	38.83	42.01
	0	50	250	0	31.13	24.90	28.01
	0	50	300	0	28.96	9.3	19.13
	0	0	50	0	0	55.88	55.88
	0	50	50	0	78.25	52.75	65.5
	0	100	50	0	71.26	48.15	59.70
F	0	150	50	0	66.88	35.12	51
	0	200	50	0	57.41	27.45	42.43
	0	250	50	0	43.76	18.34	31.05
	0	300	50	0	16.4	12.15	14.27
	50	0	0	96.19	0	0	96.19
	50	50	50	95.93	69.06	28.15	64.38
	50	100	100	90.07	64.68	24.56	59.77
G	50	150	150	86.01	57.75	18.69	54.15
	50	200	200	79.91	47.73	14.56	47.40
	50	250	250	67.84	26.55	10.12	34.83
	50	300	300	61.73	19.12	8.50	29.78

3.3. Multi component equilibrium isotherms

For the investigation of multicomponent effects on cadmium, nickel and lead ions, it is important to know the biosorption isotherm. Two major mathematical isotherm models namely Langmuir and Freundlich described the distribution of the sorbent surface between *D.s* and liquid phases. The Langmuir isotherm is the most widely models that assume homogeneous adsorption, monolayer coverage on the sorbent with no interaction between the adsorbed ions or molecules. The linearized Langmuir model is expressed in equation (5) (Guo and Wang, 2019; Li et al., 2011).

$$\frac{C_e}{q_e} = \frac{1}{q_m b} + \frac{C_e}{q_m} \qquad (5)$$

316 Where q_e is the adsorbed value of competitive heavy metal ions at equilibrium concentration (mg
317 g^{-1}) on the surface of *D.s*, C_e is the equilibrium concentration of competitive heavy metal ions (mg
318 L^{-1}), q_m is the maximum adsorption capacity on adsorbent (mg g^{-1}), and b represents the Langmuir
319 constant related to affinity of heavy metal ions on the *D.s* and the adsorption energy (L mg^{-1})
320 (Malik et al., 2018).
321 The Freundlich isotherm, unlike the Langmuir isotherm, is considered multilayer adsorption at the
322 surface of *D.s* and heterogeneous adsorption as following equation (6):

323 $$log\ q_e = logk_f + \frac{1}{n}\ logC_e \qquad (6)$$

324 The values of Freundlich constants include n, which is the heterogeneity parameter, related to
325 biosorption intensity of heavy metal ions on the *D.s* and k_f (mg g^{-1}) indicates the maximum
326 biosorption capacity of heavy metal ion. For binary and ternary biosorptions, Langmuir and
327 Freundlich isotherms were applied to determine the effect for the presence of another heavy metal
328 ion on the linear regression coefficients and isotherm constants.
329 In Table 6, the isotherm constant values for (Pb–Cd), (Pb–Ni), (Cd–Ni) and (Pb-Cd-Ni) ions are
330 listed. For (Pb–Cd) and (Pb–Ni) systems, when Pb (II) is the competitive ion, the isotherms best
331 fit with Freundlich isotherm (0.998 and 0.9969) respectively. While, for Cd (II) as a competitive
332 ion, in the (Pb–Cd) and (Cd–Ni) systems, the isotherms best fit with Langmuir under the R^2 values
333 of 0.9644 and 0.9825 respectively. Ni (II) as a competitive ion showed different pattern in binary
334 systems. In (Pb-Ni) binary system, better correlation coefficient has shown for Langmuir isotherm,
335 while in (Cd-Ni) binary system, the best fit has shown for Freundlich isotherm. Ternary systems
336 (Pb- Cd –Ni) have shown the best fit with Langmuir model for both cadmium and nickel as the
337 competitive ions. The applicability of both isotherm models implied homogenous biosorption of

metal ions on *D.s*, as well as heterogeneous surface of *D.s* for the biosorption of different heavy metal ions. Adsorption constants of the isotherms indicated the surface properties and affinity of the functional groups for heavy metal ions. These parameters can be used to compare the biosorption capacity of *D.s* algae for selected heavy metal ions in the binary and ternary systems. All binary and ternary systems show that their q_m and K_f parameters for Langmuir and Frendlich models decrease according to the following category: Pb (II) > Cd (II) > Ni (II).

Table. 6 Biosorption isotherm model constants for binary and ternary systems

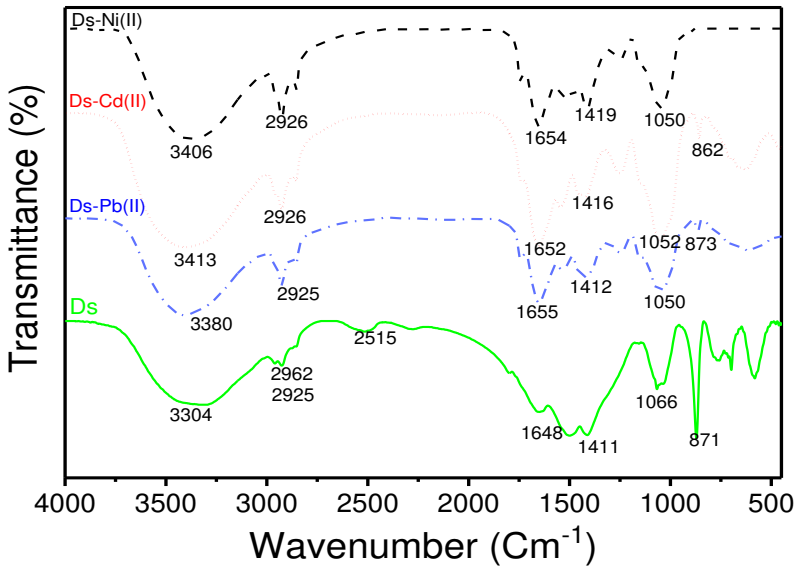
Metal(II)	Langmuir			Freundlich		
	b (Lmg^{-1})	q_m(mg g^{-1})	R^2	1/n	K_f(mg g^{-1})	R^2
Pb (Pb–Cd)	0.06078	476.1905	0.9712	0.4635	57.4645	0.9980
Pb (Pb–Ni)	0.1756	384.6154	0.9621	0.3566	94.7981	0.9969
Cd (Pb–Cd)	0.051802	217.3913	0.9644	0.4491	25.16518	0.827
Cd (Cd–Ni)	0.048832	217.3913	0.9825	0.4264	26.65017	0.8898
Ni (Pb–Ni)	0.050671	58.82353	0.9726	0.2697	13.22818	0.7269
Ni (Cd–Ni)	0.032175	133.3333	0.9006	0.6783	5.261384	0.9876
Cd (Pb-Cd-Ni)	2.184211	120.4819	0.9304	0.3032	31.9595	0.536
Ni (pb-Cd-Ni)	0.068624	46.51163	0.968	0.2747	10.57548	0.6136

3.4. FT- IR analysis

FT- IR spectra before and after heavy metal ions uptake were recorded to identify the functional groups present on the *D.s* surface. The FT- IR spectra are shown in Figure. 2 for the *D.s* surface in four different forms: the *D.s.* algae before biosorption, and after biosorption of lead, cadmium and nickel ions. As can be seen in Figure. 2, some functional group bands have shifted in the biosorption process indicating the involvement in binding to heavy metal ions and *D.s* surface.

The broad band observed between 3700 to 3200 cm^{-1} is attributed to the O-H symmetric stretching vibration and amine groups, on the cell wall of algae(Elleuch et al., 2021). The peaks at 2925 cm^{-1} and in the area around 1400 cm^{-1} were detected in all spectra, can be ascribed to the methyl groups stretching vibrations of aliphatic groups on the microalgae cell wall(Reza et al., 2019). Also, the peak at around 1648- 1650 cm^{-1}, reflects the presence of C=O of amide groups due to the presence of proteins on the cell wall of *D.s*(Sulaymon et al., 2013). The peaks at 1410-1420 cm^{-1} could be assigned to COO^- of the carboxylate groups(Farinella et al., 2008).

After heavy metal ions uptake, FT-IR spectrum of lead cadmium and nickel ions binding on *D.s* surface has undergone changes and shifts in intensity and wavenumber of some peaks (**Figure.2**).



Figureure.2. FT-IR spectra of the *D.S* after and before Pb (II), Cd (II) and Ni (II) ions uptake

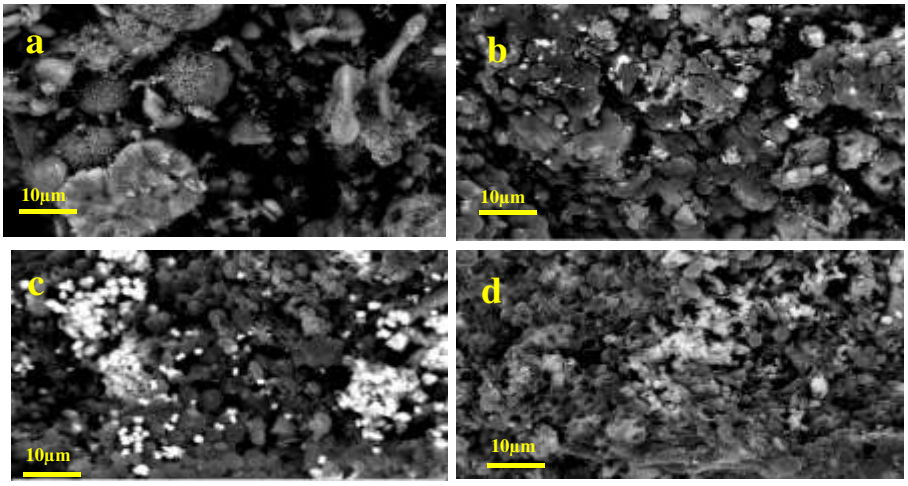
The shifted peak of hydroxyl group indicate *D.s* cell wall binds to heavy metal ions might have that replaced the protons. Furthermore, the peak at 1411 cm^{-1} shifted to the maximum of 1419 cm^{-1} for the *D.s* surface show that binding heavy metal ions to COO^- functional group in the biosorption process. Green microalgal are composed of many carboxyl groups that are responsible

for metal ion biosorption. In addition, peak at around 1648 $cm^{-1}$was observed to shift to 1652-1655 cm^{-1} in all samples indicating that interaction of heavy metal ions with carboxylic groups of proteins for amide component.

As a result, the functional groups including hydroxyl, carboxyl and amide compounds are responsible for the biosorption of heavy metal ions. Moreover, it was found that the *D.s* algae could be efficient biosorbent due to the presence of functional groups participating in the biosorption process(Reza et al., 2019).

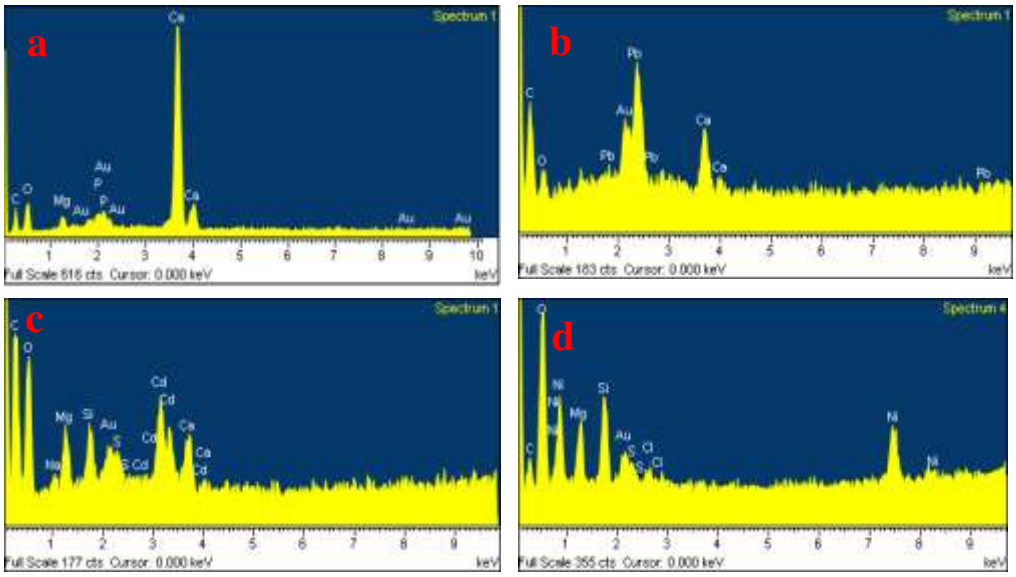
3.5. Characterization of D.s

The morphological characterization and elemental analysis of the *D.s* surface, before and after biosorption of heavy metal ions were obtained by SEM and EDX techniques. The SEM image in Figure. 3. (a) indicates a rough and irregular surface with porous texture of *D.s* as a control sample that provide the interaction with heavy metal ions in aqua solution. In contrast, Figure. 3 (b, c& d) show that *D.s* surface with considerable differences after biosorption of lead (II), cadmium (II) and nickel (II) ions respectively. This significant differences indicates the biosorption of heavy metal ions on *D.s* cell wall.(Verma et al., 2021)



Figureure.3. SEM images before uptake heavy metal ions by *D.s* (a) and after uptake Pb(II)(b), Cd(II)(c), and Ni(II)(d)

EDX spectra of elemental analysis for *D.s* cell wall before and after biosorption of heavy metal ions are also shown in Figure. 4. Heavy metal ions were not found before the biosorption (Figure. 4 (a)) but after biosorption, the peaks of Pb (II), Cd (II) and Ni (II) on the biomass are strong evidence of biosorption by *D.s* cell wall (Figure. 4 (a, b &c). Similar study was reported to remove of Cr ions by *D.s.*(Kaushik and Raza, 2019).

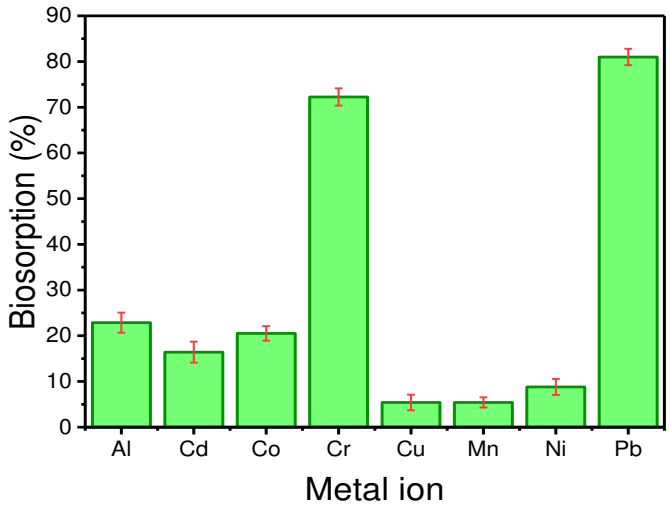


Figureure.4.EDX spectra of *D.s* before (a) and after Pb (II) (b) Cd (II) (c) Ni (II) (d) uptake

3.6. Biosorption selectivity

For adsorption processes, selectivity is a parameter highly related to qualitative analysis. Functional groups are playing an important role for ion selectivity studies(Awual et al., 2020). In this work, to evaluate the effect of heavy metal ions that may compete on biosorption process, several metal ions present in wastewater were investigated in the presence of Pb (II) ion. Therefore, interfering mixed heavy metal ions such as Al^{3+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Mn^{2+} and Ni^{2+}, on Pb (II)uptake by *D.s*. were investigated and the results are showed in Figure.5. The biosorption efficiency in the presence of selected heavy metal ions, demonstrated higher biosorption of lead ion. The biosorption efficiency was 81% for Pb (II) as compared to Cr (III) 72.26% due to competitive effect.

In this experiment, the foreign heavy metal ion concentrations were 20-fold higher than of the Pb (II) concentration. However, the simultaneous removal of these two ions can be important because of multiple heavy metal ions present in wastewaters.



Figureure. 5. Competing heavy metal ion effects on Pb (II) biosorption (Pb(II) concentration: 10 mg L^{-1}, competing ion concentration: 100.0 mg L^{-1}.

4. Conclusion

Herein, we suggest to use *D.s* as an economic biosorbent that provide continuous and effective Pb ions uptake. The morphology and structure properties of *D.s* were characterized by SEM, EDX and FT-IR. The influence of vital parameters, including pH, OD of microalgae, contact time, injection time and Pb (II) concentration were successfully modeled and optimized by RSM-CCD method. The results of this study illustrated that the lead uptake is strongly influenced by pH and lead ion concentration. The lead uptake efficiency was 96% at RSM optimum conditions. Biosorption of Cd (II), Ni (II) and Pb (II) have been investigated for single, binary and ternary systems. The biosorption capacity is highest for Pb (II) as compare to Cd (II) and Ni (II) for all systems. Competitive isotherm models indicated that Pb (II) follows the Freundlich isotherm, while the Langmuir isotherm is governed by Cd (II). Additionally, Ni ion follows both Langmuir

and Freundlich isotherms in the presence of Pb and Cd ions respectively. Moreover, *D.s* revealed impressive biosorption of lead ion (80%) in the presence of commonly available heavy metal ions in wastewater at high levels.

Research findings, lead to the recommendation of a novel model for using live microalgae as an eco-friendly and green approach that avoids the usage of any chemical materials that may cause serious damage to the environment. More notably, these results can be scaled up using live *D.s* to establish cost-effectiveness biosorption for the bioremediation of heavy metal contaminations in wastewater.

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Author contribution:

Somayyeh Ziaei: Data collection, sample analysis, Writing - original draft, and data interpretation.

Hossein Ahmadzadeh: Conceptualization, data analysis, Funding acquisition, Project Supervision, data validation.

Zarrin Es'haghi: Conceptualization, Data analysis, Writing - review & editing.

Conflicts of Interest

The authors declare no conflicts of interest.

References

Abdel, A.M., Ammar, N.S., Ghafar, H.H.A., Ali, R.K., 2013. Biosorption of cadmium and lead from aqueous solution by fresh water alga Anabaena sphaerica biomass. J. Adv. Res. 4,

367–374. https://doi.org/10.1016/j.jare.2012.07.004

Anastopoulos, I., Kyzas, G.Z., 2015. Progress in batch biosorption of heavy metals onto algae. J. Mol. Liq. 209, 77–86. https://doi.org/10.1016/j.molliq.2015.05.023

Awual, M.R., Hasan, M.M., Iqbal, J., Islam, A., Islam, M.A., Asiri, A.M., Rahman, M.M., 2020. Naked-eye lead(II) capturing from contaminated water using innovative large-pore facial composite materials. Microchem. J. 154, 104585. https://doi.org/10.1016/j.microc.2019.104585

Bai, M.T., Venkateswarlu, P., 2019. Optimization studies for lead biosorption on Sargassum tenerrimum (Brown Algae) using experimental design : Response Surface Methodology. Mater. Today Proc. 18, 4290–4298. https://doi.org/10.1016/j.matpr.2019.07.387

Basu, M., Guha, A.K., Ray, L., 2017. Adsorption of Lead on Cucumber Peel. J. Clean. Prod. 151, 603–615. https://doi.org/10.1016/j.jclepro.2017.03.028

Belghith, T., Athmouni, K., Bellassoued, K., El Feki, A., Ayadi, H., 2016. Physiological and biochemical response of Dunaliella salina to cadmium pollution. J. Appl. Phycol. 28, 991–999. https://doi.org/10.1007/s10811-015-0630-5

Dönmez, G., Aksu, Z., 2002. Removal of chromium(VI) from saline wastewaters by Dunaliella species. Process Biochem. 38, 751–762. https://doi.org/10.1016/S0032-9592(02)00204-2

Eliescu, A., Georgescu, A.A., Nicolescu, C.M., Bumbac, M., Cioateră, N., Mureșeanu, M., Buruleanu, L.C., Claudia, L., Buruleanu, L.C., 2020. Biosorption of Pb(II) from Aqueous Solution Using Mushroom (Pleurotus ostreatus) Biomass and Spent Mushroom Substrate. Anal. Lett. 53, 2292–2319. https://doi.org/10.1080/00032719.2020.1740722

461 Elleuch, J., Ben Amor, F., Chaaben, Z., Frikha, F., Michaud, P., Fendri, I., Abdelkafi, S., Ben, F.,
462 Chaaben, Z., Frikha, F., Michaud, P., Fendri, I., Abdelka, S., 2021. Zinc biosorption by
463 Dunaliella sp . AL-1 : Mechanism and effects on cell metabolism. Sci. Total Environ. 773,
464 145024. https://doi.org/10.1016/j.scitotenv.2021.145024

465 Esposito, A., Pagnanelli, F., Vegliò, F., 2002. pH-related equilibria models for biosorption in
466 single metal systems. Chem. Eng. Sci. 57, 307–313. https://doi.org/10.1016/S0009-
467 2509(01)00399-2

468 Esvandi, Z., Foroutan, R., Mirjalili, M., Sorial, G.A., Ramavandi, B., 2019. Physicochemical
469 Behavior of Penaeuse semisulcatuse Chitin for Pb and Cd Removal from Aqueous
470 Environment. J. Polym. Environ. 27, 263–274. https://doi.org/10.1007/s10924-018-1345-x

471 Farinella, N. V, Matos, G.D., Lehmann, E.L., Arruda, M.A.Z., 2008. Grape bagasse as an
472 alternative natural adsorbent of cadmium and lead for effluent treatment. J. Hazard. Mater.
473 154, 1007–1012. https://doi.org/10.1016/j.jhazmat.2007.11.005

474 Giraldo, L., Guar, J.R., Rodr, P., Moreno-piraja, J.C., 2019. Simple and Competitive Adsorption
475 Study of Nickel (II) and Chromium (III) on the Surface of the Brown Algae Durvillaea
476 antarctica Biomass. ACS omega 19, 18147-18158.
477 https://doi.org/10.1021/acsomega.9b02061

478 Gu, S., Boase, E.M., Lan, C.Q., 2021. Enhanced Pb (II) removal by green alga Neochloris
479 oleoabundans cultivated in high dissolved inorganic carbon cultures. Chem. Eng. J. 416,
480 128983. https://doi.org/10.1016/j.cej.2021.128983

481 Gu, S., Lan, C.Q., 2021. Biosorption of heavy metal ions by green alga Neochloris
482 oleoabundans : Effects of metal ion properties and cell wall structure. J. Hazard. Mater. 418,

126336. https://doi.org/10.1016/j.jhazmat.2021.126336

Guo, X., Wang, J., 2019. Comparison of linearization methods for modeling the Langmuir adsorption isotherm. J. Mol. Liq. 296, 111850. https://doi.org/10.1016/j.molliq.2019.111850

Isam, M., Baloo, L., Kutty, S.R.M., Yavari, S., 2019. Optimisation and Modelling of Pb (II) and Cu (II) Biosorption onto Red Algae (Gracilaria changii) by Using Response Surface Methodology. Water 11, 2325. https://doi.org/10.3390/w11112325

Kamal, B., Rafey, A., 2021. A mini review of treatment methods for lead removal from wastewater. Int. J. Environ. Anal. Chem. 1–16. https://doi.org/10.1080/03067319.2021.1934833

Katheresan, V., Kansedo, J., Lau, S.Y., 2018. Efficiency of various recent wastewater dye removal methods: A review. J. Environ. Chem. Eng. 6, 4676–4697. https://doi.org/10.1016/j.jece.2018.06.060

Kaushik, G., Raza, K., 2019. Potential of novel Dunaliella salina from sambhar salt lake , India , for bioremediation of hexavalent chromium from aqueous effluents : An optimized green approach. Ecotoxicol. Environ. Saf. 180, 430–438. https://doi.org/10.1016/j.ecoenv.2019.05.039

Kit, Y., Chang, J., 2020. Bioremediation of heavy metals using microalgae : Recent advances and mechanisms. Bioresour. Technol. 303, 122886. https://doi.org/10.1016/j.biortech.2020.122886

Kumar, R., Bishnoi, N.R., Garima, Bishnoi, K., 2008. Biosorption of chromium(VI) from aqueous solution and electroplating wastewater using fungal biomass. Chem. Eng. J. 135,

202–208. https://doi.org/10.1016/j.cej.2007.03.004

Lee, Y., Chang, S., 2011. The biosorption of heavy metals from aqueous solution by Spirogyra and Cladophora filamentous macroalgae. Bioresour. Technol. 102, 5297–5304. https://doi.org/10.1016/j.biortech.2010.12.103

Li, Y., Zhang, P., Du, Q., Peng, X., Liu, T., Wang, Z., Xia, Y., Zhang, W., Wang, K., Zhu, H., Wu, D., 2011. Adsorption of fluoride from aqueous solution by graphene. J. Colloid Interface Sci. 363, 348–354. https://doi.org/10.1016/j.jcis.2011.07.032

Malik, H., Qureshi, U.A., Muqeet, M., Mahar, R.B., Ahmed, F., Khatri, Z., 2018. Removal of lead from aqueous solution using polyacrylonitrile/magnetite nanofibers. Environ. Sci. Pollut. Res. 25, 3557–3564. https://doi.org/10.1007/s11356-017-0706-7

Mohd Udaiyappan, A.F., Abu Hasan, H., Takriff, M.S., Sheikh Abdullah, S.R., 2017. A review of the potentials, challenges and current status of microalgae biomass applications in industrial wastewater treatment. J. Water Process Eng. 20, 8–21. https://doi.org/10.1016/j.jwpe.2017.09.006

Morosanu, I., Teodosiu, C., Paduraru, C., Ibanescu, D., Tofan, L., 2017. Biosorption of lead ions from aqueous ef fl uents by rapeseed biomass. N. Biotechnol. 39, 110–124. https://doi.org/10.1016/j.nbt.2016.08.002

Mosleh, S., Rahimi, M.R., Ghaedi, M., Dashtian, K., Hajati, S., 2018. Sonochemical-assisted synthesis of CuO/Cu2O/Cu nanoparticles as efficient photocatalyst for simultaneous degradation of pollutant dyes in rotating packed bed reactor: LED illumination and central composite design optimization. Ultrason. Sonochem. 40, 601–610. https://doi.org/10.1016/j.ultsonch.2017.08.007

526 Naveed, S., Li, C., Lu, X., Chen, S., Yin, B., Zhang, C., Ge, Y., 2019. Microalgal extracellular
527 polymeric substances and their interactions with metal(loid)s: A review. Crit. Rev. Environ.
528 Sci. Technol. 49, 1769–1802. https://doi.org/10.1080/10643389.2019.1583052

529 Plazinski, W., 2013. Binding of heavy metals by algal biosorbents. Theoretical models of
530 kinetics, equilibria and thermodynamics. Adv. Colloid Interface Sci. 197–198, 58–67.
531 https://doi.org/10.1016/j.cis.2013.04.002

532 Pugazhendhi, A., Boovaragamoorthy, G.M., Ranganathan, K., Naushad, M., Kaliannan, T.,
533 2018. New insight into effective biosorption of lead from aqueous solution using Ralstonia
534 solanacearum: Characterization and mechanism studies. J. Clean. Prod. 174, 1234–1239.
535 https://doi.org/10.1016/j.jclepro.2017.11.061

536 Qin, H., Hu, T., Zhai, Y., Lu, N., Aliyeva, J., 2020. The improved methods of heavy metals
537 removal by biosorbents: A review. Environ. Pollut. 258, 113777.
538 https://doi.org/https://doi.org/10.1016/j.envpol.2019.113777

539 Reza, A., Ali, M., Sohbatzadeh, H., Mofras, M., 2019. Groundwater for Sustainable
540 Development La (III) and Ce (III) biosorption on sulfur functionalized marine brown
541 algae Cystoseira indica by xanthation method : Response surface methodology , isotherm
542 and kinetic study. Groundw. Sustain. Dev. 8, 144–155.
543 https://doi.org/10.1016/j.gsd.2018.10.005

544 Shen, Z., Hou, D., Jin, F., Shi, J., Fan, X., Tsang, D.C.W., Alessi, D.S., 2019. Effect of
545 production temperature on lead removal mechanisms by rice straw biochars. Sci. Total
546 Environ. 655, 751–758. https://doi.org/10.1016/j.scitotenv.2018.11.282

547 Sohbatzadeh, H., Keshtkar, A.R., Safdari, J., Fatemi, F., 2016. U(VI) biosorption by bi-

functionalized Pseudomonas putida @ chitosan bead: Modeling and optimization using RSM. Int. J. Biol. Macromol. 89, 647–658. https://doi.org/10.1016/j.ijbiomac.2016.05.017

Sulaymon, A.H., Mohammed, A.A., Al-Musawi, T.J., 2013. Competitive biosorption of lead, cadmium, copper, and arsenic ions using algae. Environ. Sci. Pollut. Res. 20, 3011–3023. https://doi.org/10.1007/s11356-012-1208-2

Suresh Kumar, K., Dahms, H.U., Won, E.J., Lee, J.S., Shin, K.H., 2015. Microalgae - A promising tool for heavy metal remediation. Ecotoxicol. Environ. Saf. 113, 329–352. https://doi.org/10.1016/j.ecoenv.2014.12.019

Turkmen Koc, S.N., Kipcak, A.S., Moroydor Derun, E., Tugrul, N., 2020. Removal of zinc from wastewater using orange, pineapple and pomegranate peels. Int. J. Environ. Sci. Technol. https://doi.org/10.1007/s13762-020-03025-z

Verma, A., Agarwal, M., Sharma, S., Singh, N., 2021. Competitive removal of cadmium and lead ions from synthetic wastewater using Kappaphycus striatum. Environ. Nanotechnology, Monit. Manag. 15, 100449. https://doi.org/10.1016/j.enmm.2021.100449

Wang, J., Chen, C., 2009. Biosorbents for heavy metals removal and their future. Biotechnol. Adv. 27, 195–226. https://doi.org/10.1016/j.biotechadv.2008.11.002

Wang, Y., Zhang, C., Zheng, Y., Ge, Y., 2017. Bioaccumulation kinetics of arsenite and arsenate in Dunaliella salina under different phosphate regimes. Environ. Sci. Pollut. Res. 24, 21213–21221. https://doi.org/10.1007/s11356-017-9758-y

Wei, Jinshan, Duan, L., Wei, Jian, Hoffmann, E., Song, Y., Meng, X., 2021. Lead removal from water using organic acrylic amine fiber (AAF) and inorganic-organic P-AAF, fixed bed

filtration and surface-induced precipitation. J. Environ. Sci. 101, 135–144. https://doi.org/https://doi.org/10.1016/j.jes.2020.08.009

Yan, C., Qu, Z., Wang, J., Cao, L., Han, Q., 2022. Microalgal bioremediation of heavy metal pollution in water : Recent advances , challenges , and prospects. Chemosphere 286, 131870. https://doi.org/10.1016/j.chemosphere.2021.131870

Zeraatkar, A.K., Ahmadzadeh, H., Talebi, A.F., Moheimani, N.R., McHenry, M.P., 2016. Potential use of algae for heavy metal bioremediation, a critical review. J. Environ. Manage. 181, 817–831. https://doi.org/10.1016/j.jenvman.2016.06.059

Zhang, J., Wei, S., Liu, Z., Tang, H., Meng, X., Zhu, W., 2022. Release of Pb adsorbed on graphene oxide surfaces under conditions of Shewanella putrefaciens metabolism. J. Environ. Sci. 118, 67–75. https://doi.org/10.1016/j.jes.2021.08.041

Zhang, Q., Zhang, S., Zhao, Z., Liu, M., Yin, X., 2020. Highly effective lead (II) removal by sustainable alkaline activated b - lactoglobulin nano fi brils from whey protein. J. Clean. Prod. 255, 120297. https://doi.org/10.1016/j.jclepro.2020.120297

Ziaei, S., Ahmadzadeh, H., Es'haghi, Z., 2022. Artemia Cysts as dynamic biosorbent for efficient and fast uptake of lead ions from contaminated environments. Int. J. Environ. Sci. Technol. 19, 6467–6480. https://doi.org/10.1007/S13762-021-03844-8

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