



## Use of *Chara* sp. Alga in the Treatment of Polluted Water with Lead

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### ABSTRACT

*Chara* sp. is a genus of green algae that was selected and dried for the present investigation. The effective functional groups of the algae for infrared adsorption include COOH, OH, C=O, and C-H. Several samples were taken and tested, including negative groups such as CH<sub>2</sub>, CO<sub>3</sub>, Cl, Br, and P=O, on the surface of algae weighing 2 grams. These samples were exposed to varying concentrations of lead ions, starting at 0, 5, 10, 20, 40, and 100mg L<sup>-1</sup>. After the incubation period, the samples were incubated at a temperature of 298K. Using a number of equations, stabilizers were extracted and lead was quantified. The determination values for all the equations were significant, indicating that any of the six equations could be used. The most effective mathematical description of adsorption was provided by the Langmuir equation, which demonstrated a strong correlation between the measured and calculated adsorption values. This equation had the highest coefficient of determination (R<sup>2</sup>) of 0.71 and the lowest standard error of 0.001, giving it an advantage over the other equations. Data analysis revealed that the highest adsorption capacity (X<sub>m</sub>) on both surfaces of the green algae was 0.93 and 58.48mg kg<sup>-1</sup>, respectively. At the same time, the binding energy (K) on both surfaces was 99.22 and 4.38L mg<sup>-1</sup>, respectively. These results suggest that algae could be used as a low-cost technology for treating lead-contaminated water and reducing its associated risks.

### INTRODUCTION

Bioremediation is used to describe the process of using biological agents to remove toxic pollutants from the environment. Biological therapy is the most effective tool for managing the polluted environment and is a successful technique for cleaning it. It has been used in various locations around the world including Europe, with varying degrees of success (Mousavi *et al.*, 2019). Bioremediation is also known as the use of living organisms and microorganisms primarily to convert environmental pollutants into less toxic forms (Almasi *et al.*, 2017). In this treatment, naturally occurring bacteria, fungi, plants are used to remove hazardous, toxic substances from ecosystems and human health. That is, the microorganisms used in treatment may be primarily present in the contamination zone or may be isolated from other areas and transported to the

contaminated sites, where the transfer of polluting compounds occurs through interactions of organisms as part of their metabolic processes (Piccini *et al.*, 2019). These microorganisms can adapt to different environments, making them useful for addressing environmental hazards (Hammud *et al.*, 2014). One of the major problems in industry-dominated societies is the large use of water with many requirements for clean water, thus several methods have been used to reduce water consumption, but in the long term it is only possible to recycle sewage into high quality water. Currently, alternative treatment techniques suitable for industrial societies and developed countries are being discussed to ensure the provision of high-quality water. The available techniques for treating sewage are struggling to keep pace with increased pollution, especially the rise in organic compounds used in the health and household sectors. Among the best techniques for contaminant treatment are those that involve various organisms (including bacteria, fungi, algae, and plants) to treat and remove toxic pollutants (organic, inorganic, and heavy metals) from polluted water. This process, known as bioremediation, is safe for the environment and inexpensive compared to other technologies (Mahajan *et al.*, 2019). Green algae (Chlorophyta) includes species such as *Chara vulgaris* and *Scenedesmus quadricauda*. *Chara vulgaris* belongs to the Chlorellaceae family and is characterized by small, non-motile, monolithic algae, ranging in diameter from 2 to 12 microns. These spherical algae may form irregular clusters, and their cellulose cell walls are thin. The plastids are mural or side-shaped, containing a single starch center (de Foucault, 2016). This alga is employed in numerous processes, including food production and oxygen release from wastewater (Laffont-Schwob *et al.*, 2015).

## MATERIALS AND METHODS

For the implementation of this treatment, the green algae *Chara* sp., which belongs to the division Chlorophyta, class Charophyceae, order Charales, family Characeae, and genus *Chara* (Kannah *et al.*, 2019), was selected. According to de Foucault (2016), it was identified and obtained from the Tigris River in Mosul City using a plastic net. The algae were then dried, weighed (2 grams), and exposed to lead ions using a sequence of concentrations in the following range: 0, 5, 10, 20, 40, and 100mg L<sup>-1</sup>. The samples were incubated at 25°C. Following the incubation period, stabilizers were removed, and the lead concentration was determined using a quantitative analysis based on adsorption formulas.

### Calculating the amount of lead adsorption

The amount of lead adsorption was determined using the difference in lead concentration between before and after equilibrium using the formula below:

$$Pb^{++} - ad. = \frac{V(C_{in} - C_{fin})}{W} \dots \dots \dots (1)$$

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$Pb^{++}$  = adsorption of lead ions in units of (mg.kg<sup>-1</sup>);  $C_{in}$  = lead ion concentration (mg. L<sup>-1</sup>) before equilibrium, and  $C_{fin}$  = represents lead ion concentration following equilibrium (mg. L<sup>-1</sup>), while  $V$  = represents volume of added solution (ml), and  $W$  = represents algae weight (gram).

**The equations of adsorption**

1. Equation (I) of Langmuir (**Langmuir, 1918**)

$$\frac{C}{X} = \frac{1}{K X_m} + \frac{C}{X_m} \dots \dots \dots (2)$$

2. Equation (II) of Langmuir (**Langmuir, 1918**)

$$X = \frac{K_1 b_1 C}{(1 + K_1 C)} + \frac{K_2 b_2 C}{(1 + K_2 C)} \dots \dots \dots (3)$$

Where,  $X$  = represents quantity of absorbed Lead (mg.kg<sup>-1</sup>);  $C$  = represents the concentration of the lead in the equilibrium (mg.L<sup>-1</sup>);  $K$  = represents a constant of bond energy (L. mg<sup>-1</sup>), and  $X_m$  = represents the constant which indicates maximum adsorption (mg. kg<sup>-1</sup>).

$K_1$  and  $K_2$  = a constant representing bond energy(L. mg<sup>-1</sup>), while  $b_1$  and  $b_2$  = represent constants.

3. The equation of Freundlich (**Freundlich, 1906**)

$$\text{Log } X = b \text{ Log } C + \text{Log } K \dots \dots \dots (4)$$

Where,  $X$  represents quantity of absorbed lead (mg. kg<sup>-1</sup>);  $C$  = represents the concentration of lead in the equilibrium (mg. L<sup>-1</sup>) and  $K$ ,  $b$  = represent constants.

4. The equation of Temkin (**Temkin, 1934**)

$$X = \alpha + \beta \text{Ln}C \dots \dots \dots (5)$$

Where,  $X$ =quantity of lead absorbed (mg. kg<sup>-1</sup>);  $C$  = represents lead concentration in the equilibrium solution (mg. L<sup>-1</sup>); ( $\beta$  and  $\alpha$ ) = are constants.

5. The equation of Dubinin-Radushkevich (**Dubinin, 1947**)

$$\text{Log}q = \text{log}K_f + n \text{Log}C_e \dots \dots \dots (6)$$

Where,  $C_e$  = concentration of lead in equilibrium solution;  $q$  = quantity of lead adsorbent = distribution coefficient, and  $n$  = constant.

6. The equation of polani

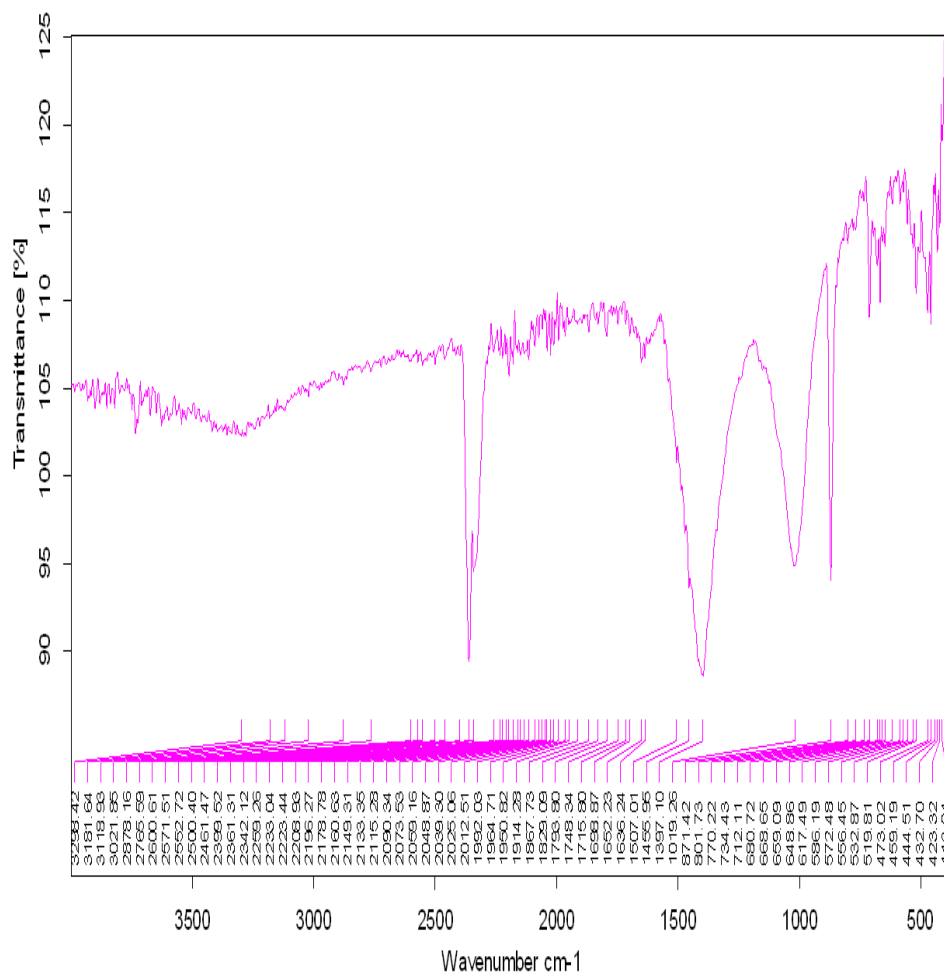
$$\ln q = \ln q_m - K \varepsilon^2 \dots \dots \dots (7)$$

$$\varepsilon = \left[ RT \ln \left( 1 + \frac{1}{C_e} \right) \right] \dots \dots \dots (8)$$

Where,  $\varepsilon$  = represents polani-potential;  $q$  = represents the amount of adsorbed lead on the algae surface ( $\text{mol. g}^{-1}$ );  $K$  = the adsorption power constant ( $\text{mole}^2. \text{Kg}^2$ );  $q_m$  = adsorption capacity.

### Diagnosis of active groups using IR

Fig. (1) shows the effectual groups of algae that are responsible for infrared absorption, along with the presence of negatively charged groups for instance  $\text{CH}_2$ , Br,  $\text{CO}_3$ , (P = O), and Cl on the surface of algae (**Kumar *et al.*, 2018**).

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**Fig. 1.** Infrared spectrum of algae

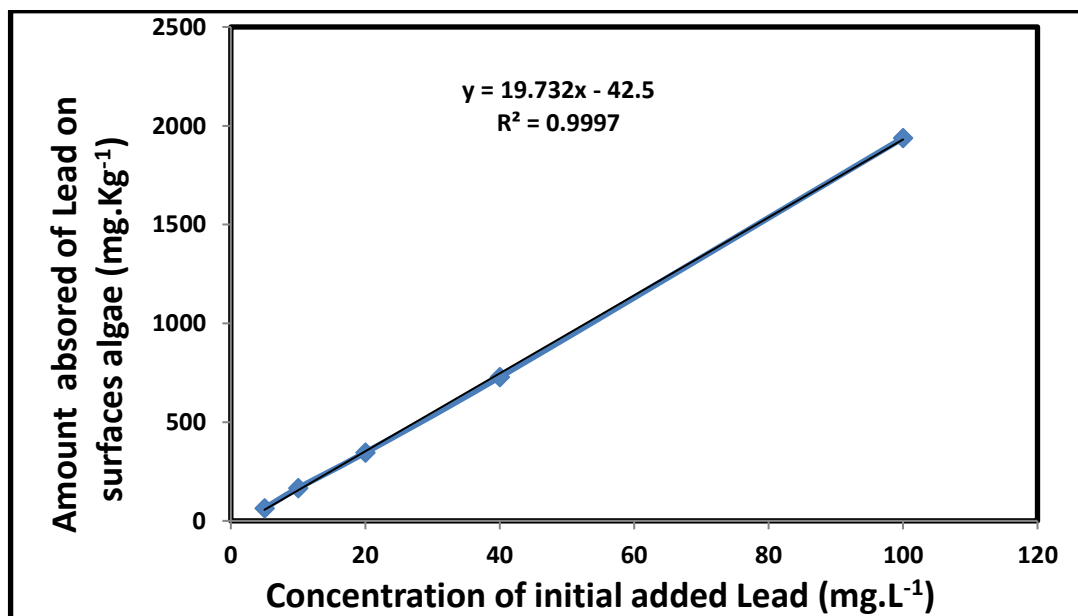
## RESULTS AND DISCUSSION

It was found that the lead ions were completely absorbed from the surface of the dried algae at a temperature of 25°C, at concentrations of 0, 5, 10, 20, 40, and 100mg L<sup>-1</sup>, with other variables kept constant. These experiments were performed in a single batch using a lead solution. The amount of adsorbed material and the adsorption efficiency (percentage of adsorption) were compared with the initial concentration, as shown in Table (1).

**Table 1.** Percentage change of adsorption with initial concentrations at (25°C) of lead

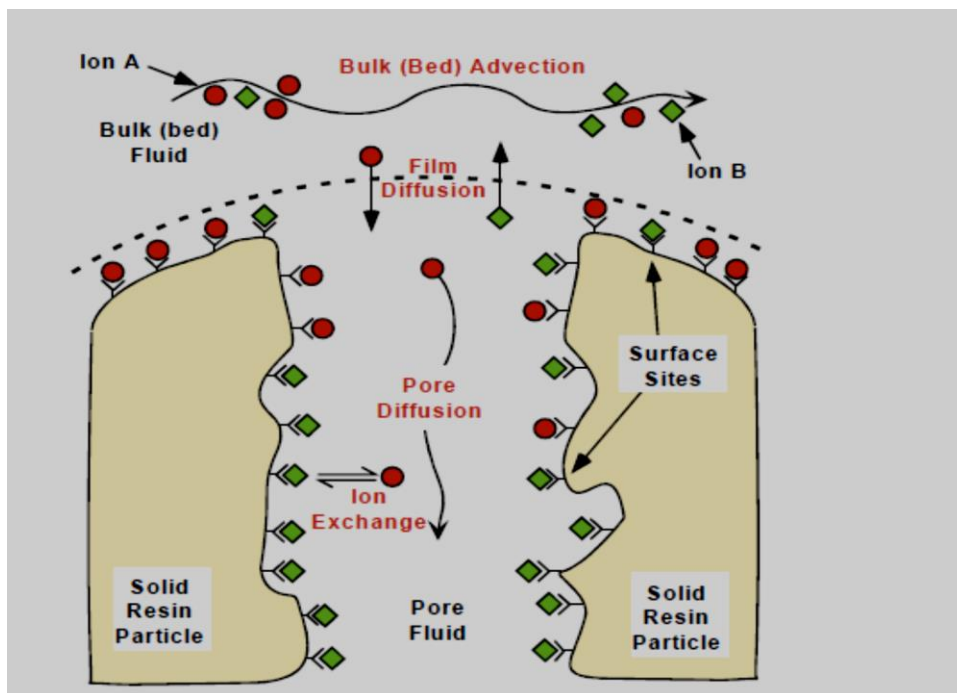
% Adsorption	Adsorbed amount (mg. Kg <sup>-1</sup> )	Initial added Lead concentrations (mg. L <sup>-1</sup> )
-	-31.00	0
63.40	63.40	5
82.60	165.20	10
86.45	345.80	20
91.03	728.20	40
96.90	1938.00	100
84.08	648.12	Average

The findings presented in Table (1) show that the investigated ion demonstrates a consistent trend in the removal of the water solution from the algae's surface. As shown in Fig. (2), it is noticed that as the initial concentration of lead increases, the quantity of adsorbed material also increases, reflecting the extent of biological reclamation (bioremediation).



**Fig. 2.** The correlation between the initial lead concentration in the equilibrium solution and the amount of lead absorbed by the algae at 25°C

In this regard, a number of studies in the discipline of bio-absorption of algae have established the type of interaction that occurs between cations of some metals (potassium, sodium, calcium, and magnesium) associated with elements present in solutions of water as the mechanism responsible for this reaction (Ahmed & Hameed, 2018), as depicted in Fig. (3). This process illustrates the development of algae molecules in order to adsorb lead ions on the algae adsorption sites (Hamm, 2004).



**Fig. 3.** Outline of mass transfer mechanisms responsible for ionic adsorption process (A & B) in algae (Hamm, 2004)

Two main factors determine the biological adsorption process: electronegativity and the volume of adsorbed ions, which is influenced by the atomic diameter of the adsorbent ion (Naja & Volesky, 2006; Sulaymon *et al.*, 2013; Javanbakht *et al.*, 2014). Calcium, magnesium, sodium, and potassium are among the base elements commonly found in algal biomass. As a result, when the biological mass of algae adsorbs heavy metals (such as lead, Pb) transported into the water, the water's reaction increases, and light alkaline ions are produced (Sulaymon *et al.*, 2013). Alkaline ions such as calcium, magnesium, sodium, and potassium were found to be removed from the equilibrium solution by the algal mass, which biologically adsorbs the heavy metals.

Researchers have noted that upon implementing the method of dry algae-filled columns, the quantity of adsorbed heavy metal depends on the equilibrium state of adsorption which is

thermally adsorbed and the rate of mass transfer. In addition to these two parameters, other factors, including pH, the nature of the adsorbent and adsorbate, the effect of temperature, and Traubel's rule, along with hydrodynamic factors such as column thickness and contact duration (**Wang *et al.*, 2011**) are included.

However, as the initial concentration increases, the efficiency of algae removal decreases. This is due to the increased number of isotherm sites available for adsorption at higher initial concentrations, which leads to more material remaining in solution and a reduction in the removal percentage. These ions, in contrast, compete for a limited number of available active adsorption sites on the algae's surface, lowering the adsorption efficiency. As a result, there is a reduction in the bonding ability between the active adsorption sites and the metal ions, caused by the increased competition for surface sites.

There is a presumption that the ability of algae to remove heavy metals can be attributed to various mechanisms by which heavy metals are adsorbed onto algae. As a result, the elimination of toxicity and the resistance mechanisms to heavy metals depend on the type of metal, and algae have shown some capacity to bind metals through extracellular compounds (**Sulaymon *et al.*, 2013**). Metals and their ions, as is well known, can combine with functional groups (OH, SH, COOH, NH<sub>4</sub>, PO<sub>4</sub>), converting them into structures that cannot penetrate the cell (**Utomo *et al.*, 2016**; **Bwapwa *et al.*, 2017**). Algae also use exclusion mechanisms to resist the toxic effects of metals (**Al-Qahtani, 2012**; **Farhan *et al.*, 2013**; **Shartooh *et al.*, 2014**). In the presence of high mineral concentrations, many algae species possess the ability to remove intracellular toxicity (**Bwapwa *et al.*, 2017**).

At sub-lethal concentrations, certain peptide or proteolytic compounds known as phytochelating compounds, which are rich in the amino acid cysteine, can bind metal ions in their molecular forms, resulting in mineral complexes (**Narula *et al.*, 2015**; **Herburger *et al.*, 2016**). From another perspective, the accumulation of certain properties in microorganisms may provide solutions by using organisms that can adapt to tolerate high concentrations of pollutants, which would, in turn, help clean the environment. Therefore, it is crucial to seriously consider using these organisms in biological treatments.

While many algae species are sensitive to pollutants, some organisms require pure isolates, which can be obtained through algae cultivation techniques (**Al-Hussainy & Al-Mayaly, 2015**; **Wang *et al.*, 2016**). Some algae species are already used to produce products that remove heavy metals from wastewater (**Athbi *et al.*, 2015**; **Mehrabadi *et al.*, 2015**). Thus, bio-absorption plays an essential role in addressing water pollution caused by heavy metals released by various industrial processes. Therefore, it is important to explore strains that can perform this task effectively.



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The goal of this research was to explore the potential of using algae isolated from the local environment to remove heavy metals from contaminated water and soils. This modern, inexpensive technology can protect the environment from pollution, as confirmed by numerous international studies (Ali, 2011; Anastopoulos & Kyzas, 2015; Gupta *et al.*, 2015; Cechinel *et al.*, 2016; Albayati & Kalash, 2018).

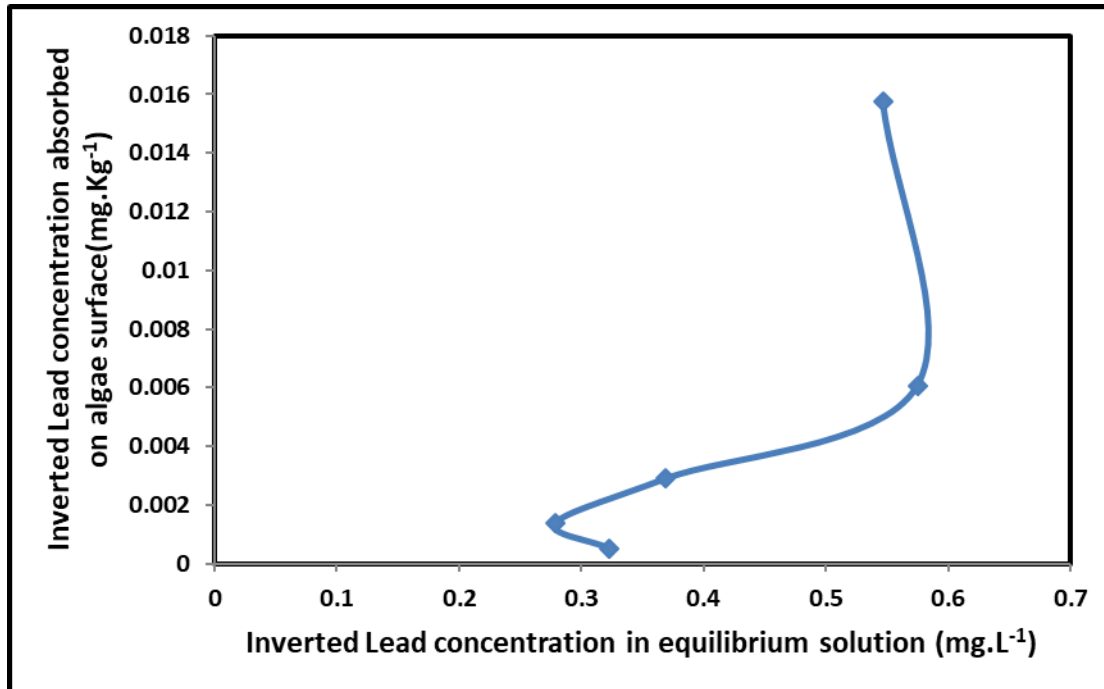
**Description of the adsorption equation mathematically**

The thermodynamically symmetric equilibrium was used to obtain biosorption constants reflecting soluble surface features and its degree of attracting lead ions as a treatment technique for lead removal from wastewater (Hammud *et al.*, 2014; Molazadeh *et al.*, 2015).

Table (2) displays the values of  $R^2$  and SE which stands for standard error for adsorption by conducting (both single and two surfaces Langmuir, Dubinin, Temkin, Polani, and Freundlich) equations to describe lead adsorption. For all equations, the equation was significant, indicating that it is possible to use the previous equations. On the other hand, the two-surface Langmuir equation, is considered as the most efficient method of adsorption mathematically. Furthermore, when compared to the other equations, this one has a higher  $R^2$  and a lower SE.

**Table 2.** The determination factor ( $R^2$ ) and standard error (SE) for lead absorption experiments

<b>R2</b>	<b>SE</b>	<b>Equation</b>
0.60	0.005	single and two surfaces Langmuir
1.00	0.000	single surface      two surfaces
0.41	0.001	two surfaces      Langmuir
0.71	0.001	Average
0.72	0.35	Freundlich
0.72	0.35	Dubinin
0.42	669.56	Temkin
0.72	0.81	Polani



**Fig. 4.** The dominance of two surface Langmuir equation of biological removal of lead from the surface of algae

This aligns with the findings of **Gupta and Rastogi (2008)**, **Montazer-Rahmati et al. (2011)**, **Reddy et al. (2011)**, **Bayo (2012)** and **Yin et al. (2012)**. The biological adsorption or biosorption process in microorganisms occurs in two phases. The first phase involves the rapid adsorption of components onto the surface of the cell wall, leading to quick uptake (**Gupta & Rastogi, 2008; Shartooh et al., 2014**). This is followed by a slower phase, where ions transition through the membrane into the cytoplasm of the cell. The surface cells of these organisms are composed of polysaccharides, proteins, and lipids, which contain many active groups that interact with the ionic species of heavy metals.

As a result, biosorption-based absorption processes are influenced by several factors, such as the structural properties of both the absorbed matter and the biosorbent. Key factors in the adsorption process include the surface characteristics (topography), composition of proteins, surface area, the amount of adsorbent material, the degree of reaction in the medium, contact duration, and temperature.

### Maximum lead adsorption and bonding energy on the surface of algae

The equation of Langmuir is based on the supposition that forces among molecules decrease quickly with the factor of distance and on the certainty of coverage composed of one layer of M-ion upon surface of absorbed material. Adsorption is thought to take place on specialized and uniform absorption sites. Therefore, it is concluded that the algae mass which is used in the adsorption is strongly under the condition of this equation in the adsorption process based on the formula for the strong coefficient of determination obtained from this equation. The maximum adsorption capacity of lead is  $0.93 \text{ mg} \cdot \text{kg}^{-1}$  on the first surface of green algae. According to Table (3), the binding capacity (K) of lead was  $99.22 \text{ L} \cdot \text{mg}^{-1}$  on the first surface of green algae.

**Table 3.** The results of the equation of Langmuir for absorption of lead on the algae surface for the two surfaces

Maximum adsorption capacity(xm2)	Bonding energy (K2)	maximum adsorption capacity(xm1)	Bonding energy (K1)
mg. kg <sup>-1</sup>	L. mg <sup>-1</sup>	mg. kg <sup>-1</sup>	L. mg <sup>-1</sup>
58.48	4.38	0.93	99.22

According to Table (3), the highest adsorption capacity of green algae on the second surface reached  $58.48 \text{ mg} \cdot \text{kg}^{-1}$ , while the binding capacity on the second surface was  $4.38 \text{ mg} \cdot \text{L}^{-1}$ . The presence of multiple efficient substances in the structure of the algae confirms the existence of two surfaces involved in the biological removal process of heavy metals from water solutions. This finding is consistent with what was pointed out by **Gupta and Rastogi (2008)**. For two different types of green algae, the adsorption capacity values (from the Langmuir equation for the two surfaces of the lead element) ranged from  $14.9$  to  $160.3 \text{ mg} \cdot \text{kg}^{-1}$ , while the binding energy values ranged from  $0.02$  to  $0.024$ .

Additionally, the algae had the highest adsorption capacities for lead, cadmium, and nickel, with values of  $19.36$ ,  $16.17$ , and  $116.35 \text{ mg} \cdot \text{kg}^{-1}$ , respectively. The Langmuir and Freundlich equations, which describe the mathematical process of biological adsorption, were both successful in modeling these results. These findings align with the work of **Al-Khazragy et al. (2005)**, **Al-Hares (2017)** and **Kumar et al. (2018)**, who found that the Langmuir equation better explained the process of heavy metal adsorption by algae compared to the Freundlich equation.

## CONCLUSION

The algae found in the Tigris River, which flows through the city of Mosul, can be utilized to treat water contaminated with lead. A laboratory experiment demonstrated that using inexpensive, naturally available materials resulted in the removal of lead ions from contaminated

sewage water, with an efficiency range of 0.93 to 58.48%. This method is considered one of the most cost-effective treatment techniques.

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