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Quaternary Adsorption Effect of Nickel (II), Antimony (III), Manganese (II) and Copper (II) onto Living Two Green Microalgae

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Abstract: This study aimed to investigate Ni, Sb, Mn and Cu adsorption from aqueous solution by *Chlorella* and *Scenedesmus* algae. The competitive adsorption efficiency of nickel (Ni^{2+}), antimony (Sb^{3+}), manganese (Mn^{2+}) and copper (Cu^{2+}) onto two living microalgae strains was studied from multi-metal aqueous solution for 24h incubation time. After exposure, chlorophyll a-b, total carbohydrate and Atomic force microscopy (AFM) imaging were performed. Then adsorption isotherms models of metal ions were determined based on Langmuir and Freundlich isotherms. The adsorption capacity in multi-metal system was determined 6.47 mgg^{-1} for antimony, 5.96 mgg^{-1} for manganese, 28.57 mgg^{-1} for copperand 10.71 mgg^{-1} for nickel (Cu>Ni>Sb>Mn) by *Chlorella* respectively, whereas, and 10.82 mg g^{-1} for antimony, 7.07 mgg^{-1} for manganese, 27.09 mgg^{-1} for copper and 9.71 mgg^{-1} for nickel(Cu>Sb>Ni>Mn) by *Scenedesmus* cells. According to AFM images, deformation was detected in two algae cell walls treated with heavy metals compared to untreated cells. For this study, Freundlich adsorption model best fitted the data for all metal ions with 1/n value <1. As a result, when the results obtained in the study are revealed that *Chlorella* and *Scenedesmus* cells were an effective adsorbent for removal of the four heavy metals, especially Cu^{2+} ions from aqueous solutions due to its high efficiency of Cu adsorption.

***Keywords*:** Adsorption, AFM, *Chlorella*, heavy metal, *Scenedesmus*.

Canlı İki Yeşil Mikroalg Üzerinde Nikel (II), Antimon (III), Mangan (II) ve Bakır (II)'ın Dörtlü Adsorpsiyon Etkisi

Özet: Bu çalışmada, *Chlorella* ve *Scenedesmus* algleri kullanılarak sulu çözeltilerden Ni, Sb, Mn ve Cu adsorpsiyonunun araştırılması amaçlanmıştır. 24 saat inkübasyon süresi boyunca çoklu metal sulu çözeltiden canlı iki mikro alg suşu üzerinde nikel (Ni^{+2}), antimony (Sb^{+3}), mangan (Mn^{+2}) ve bakırın (Cu^{+2}) yarışmalı adsorpsiyon verimliliği incelenmiştir. Metal uygulaması sonrasında, klorofil a-b, toplam karbonhidrat ve atomik kuvvet mikroskobu (AFM) görüntülemesi analiz edilmiştir. Metal iyonlarının adsorpsiyon izoterm modelleri Langmuir ve Freundlich izotermlerine göre belirlenmiştir. Çoklu metal sisteminin *Scenedesmus* hücreleri tarafından sırasıyla antimon: 10.82 mgg^{-1}, mangan: 7.07 mgg^{-1}, bakır: 27.09 mgg^{-1} ve nikel: 9.71 mgg^{-1} (Cu>Sb>Ni>Mn) olarak belirlenirken *Chlorella* için adsorbsiyon kapasitesi antimon: 6.47 mgg^{-1}, mangan: 5.96 mg g^{-1}, bakır: 28.57 mgg^{-1} ve nikel: 10.71 mgg^{-1} (Cu>Ni>Sb>Mn) olarak belirlenmiştir. AFM görüntülerine göre, ağır metallere maruz bırakılmış iki algin hücre duvarında, maruz bırakılmamış hücrelere kıyasla deformasyon tespit edilmiştir. Bu çalışma için Freundlich adsorbsiyon modeli 1/n değerinin 1'den küçük olmasıyla tüm metal iyonları için uygundur. Sonuç olarak, çalışmada elde edilen sonuçlar değerlendirildiğinde, *Chlorella* ve *Scenedesmus* hücrelerinin, dört ağır metali, özellikle Cu adsorpsiyonunun yüksek verimliliği nedeniyle, Cu^{+2} iyonlarının sulu çözeltilerden uzaklaştırılmasında etkili bir adsorbent olduğunu ortaya koymuştur.

***Anahtar Kelimeler*:** Adsorpsiyon, AFM, *Chlorella*, ağır metal, *Scenedesmus.*

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1. INTRODUCTION

As a result of the integration of the heavy metal contamination into the food chain has also become a serious problem for human health. The biosorption and bioaccumulation of metals by algae, bacteria, fungi and yeast, dead or alive, has been extensively studied in the last two decades because of their potential use in the treatment of sewage loaded with heavy metals. Of the microorganism studied, algae is gaining increasing attention, due to the fact that algae is a rich source in the aquatic environment, relatively cheap to process and able to accumulate high metal content [1-4].

Planktonic algae used to test the effects of different chemicals released into the aquatic environment are very sensitive indicators. Microalgae are very sensitive to changes in their environment. In response to trace levels in the concentration to various organic and inorganic pollutants including heavy metals, changes take place in their overall metabolism. Microalgae are therefore often used as biological sensors for detecting potential toxic effects of heavy metals like copper, nickel, zinc, cadmium etc. [5-7]. These metals are essential constituents but in higher concentrations, it adversely affects the aquatic ecosystem and indirectly affects the human health [8].

In this study, copper, manganese, nickel and antimony, mainly present in the effluent from industry as well as in fresh water, have been tested that gets adsorbed by the two green algae (*Chlorella* and *Scenedesmus*). Two mathematical models have been used in this study: Freundlich and Langmuir. Atomic force microscopy (AFM) was used to study the interaction between heavy metal ions and *Chlorella* and *Scenedesmus* cell walls.

2. MATERIALS AND METHODS

2.1. Microalgae strain and culture medium

Chlorella and *Scenedesmus* cells were obtained from Culture Collection of Algal Biotechnology Department (Ege University, Izmir, Turkey). Stock cultures were cultivated in BG-11 medium [9] with 16:8 h of light–dark cycle under illumination of white fluorescent lamps (20 E m^{-2} s^{-1} ± 20%). An orbital shaker with 100 rpm was used to maintain culture in turbulent conditions at 28 ± 1°C.

2.2. Biomass concentrations and pretreatment

The growth of algae and biomass concentration was monitored by measuring optical density at a wavelength of 660 nm and 730 nm for 30 days. Cells were harvested from the culture by centrifugation. The biomass pellets collected were then washed with distilled water and centrifuged again for the removal of medium.

2.3. Dissolutions

Experiments were performed using synthetic single-metal solutions of Sb, Mn, Cu and Ni prepared from chemical reactants of analytical grade: $C_8H_4K_2O_{12}Sb_2.H_2O$, $MnSO_4.H_2O$, $CuSO_4.5H_2O$ and $NiSO_4.6H_2O$, respectively. Stock solutions of the heavy metals were prepared, from which concentrations 2.5-100 ppm were used in case of algal tolerance experiments. The microalgae *Chlorella* and *Scenedesmus* were used in the experiment of heavy metal removal using the algal concentrations 10 mL. The metal concentration used was 40 mL and the exposure time was 24 h. pH was adjusted to 4-5 for all heavy metalsremoval assay and incubation was performed at the previously mentioned conditions. After preparing solutions with an initial concentration of 2.5, 5, 10, 25, 50 and 100

ppm of Sb, Mn, Ni and Cu at pH 5- 5.5, respectively.

2.4. Analytical procedure

Dry weight biomass (mg L^{-1}) was measured gravimetrically using 10 mL of the sample. A definite volume (10 mL) of algal suspension was filtered through Whatman GF/C filter membrane (47 mm in diameter, 0.22 µm in pore size) and dried overnight in an oven at 80°C. Data were given as mg mL^{-1} algal suspension.

2.5. Chlorophyll a-b (µg L^{-1}) was measured after extraction in 90% aqueous acetone [10]. For determination of pigment concentrations, 10 mL of culture was filtered using GF/C filters. An aliquot of the sample was centrifuged at 12000 rpm for 5 min and supernatant discarded. The pellet was suspended in 10 mL of boiling acetone at 4°C and stored in dark for 24 h. Chlorophyll a-b were thus extracted in acetone and the absorbance of acetone solution was measured at 630 nm, 645nm, 665 nm, and 750 nm using a spectrophotometer with 90% acetone as blank.

2.6. Total carbohydrate contents (mg mL^{-1}) were measured using the phenol-sulfuric acid assay and using glucose as a standard. 1 mL aliquots of the cultures were used to quantify spectrophotometrically the total carbohydrate content by the phenol-sulfuric acid assay [11].

2.7. AFM (Atomic Force Microscope)

At the end of the bioremoval experiments, algal pellets were harvested by centrifugation (1000 rpm) and prepared for AFM (hpAFM-IN) for the detection of heavy metal ions damage on the cell surface. This was performed at the Experimental Science Applications and Research Center Lab., Celal Bayar Univ., Manisa/Turkey.

2.8. Chemical analysis

The effect of co-cations on the adsorption of Sb, Mn, Ni and Cu by *Chlorella* and *Scenedesmus* was studied in quaternary component systems. Experimental design used in the study consisted of system, viz. Sb+Mn+Ni+Cu at 2.5 ppm, Sb+Mn+Ni+Cu at 5 ppm, Sb+Mn+Ni+Cu at 10 ppm, Sb+Mn+Ni+Cu at 25 ppm, Sb+Mn+Ni+Cu at 50 ppm and Sb+Mn+Ni+Cu at 100 ppm. Biosorption tests were carried out in 100 mL Erlenmeyer flasks placed on a magnetic stirring plate. Each flask contained the solution (40 mL) and sufficient biomass (10mL). Periodically, 10 mL of sample was removed for analysis, and the pH was measured. After centrifugation at 4000 rpm to separate the biomass, the samples were analyzed by ICP-MS (Inductively Coupled Plasma–Mass Spectrometer-Agilent 7700) [12].The metal uptake loading capacity qe (mg of metal per g of adsorbent) and efficiency (%) for each sorption system was determined using Eq. 1 and Eq. 2:

$$q(mg\ g^{-1}) = q(mg\ g^{-1}) = \frac{C_i - Ce}{m} xV \qquad (Eq.1)$$

$$(\%) = 100x\frac{C_i - C_e}{C_i} \qquad (Eq.2)$$

Where q is the metal uptake (mg g^{-1} of biomass); C_i and C_e are the metal concentrations before and after adsorption (mg mL^{-1}), respectively; m is the mass of biosorbent used (g) and V is the volume of solution (mL).

2.9. Adsorption isotherm models

The adsorption equilibrium isotherms were evaluated in terms of maximum sorption capacity and sorption affinity. Among the several isotherm equations, two isotherms (Langmuir and Freundlich adsorption isotherms) were investigated, which are widely used to analyses data for water and wastewater treatment applications [13]. Freundlich isotherm model is derived for describing single-component adsorption equilibria on heterogeneous surfaces. Langmuir isotherm represents a single layer and uniform adsorbent without interactions between adsorbed molecules. In the current study, the Freundlich (Eq. 3) and Langmuir (Eq. 4) models were used to determining the concentration of the adsorbed material. If 1/n= 0, the adsorption process is irreversible. If 1< 1/n<0, it is desired. If 1/n>0, it is undesirable [14,15].

$$\log q_e = \log K_F + \left(\frac{1}{n}\right)\log C_e \quad \text{(Eq. 3)}.$$

q_e: the amount of metal adsorbed (mg g^{-1}).

K_F: Adsorption capacity at unit concentration (L g^{-1}).

1/n: Intensity of adsorption (L g^{-1}).

C_e: the equilibrium concentration of metal ion (mg L^{-1}).

In the Langmuir model, q_m and b are Langmuir parameters, which are the maximum adsorption capacity and associated energy, respectively. The equilibrium parameter (R_L) is the basis of the Langmuir isotherm, which is defined by equation, $R_L=1/(1+bC_0)$ [14]. In this equation, C_0 is the initial concentration and R_L is the type of isotherms. $1<R_L<0$ is favourable adsorption, $R_L>1$ is for undesirable adsorption, $R_L=1$ shows linear adsorption and $R_L=0$ demonstrates irreversible adsorption [16]. The R_L value was calculated at 50 ppm of initial metal concentration.

$$\frac{C_e}{q_e} = \frac{1}{q_m b} + \frac{C_e}{q_m} \text{(Eq. 4)}.$$

q_m: Langmuir maximum adsorption capacity (mg g^{-1}).

b: the constant related to free energy of adsorption (L mg^{-1}).

3. RESULTS

3.1. Analysis results of the chlorophyll a-b (µg L^{-1})

Control chlorophyll-a values of *Chlorella* and *Scenedesmus* cells were determined 0.5149 and 0.3602 µg L^{-1}, respectively. After metals treatment, the average values of chlorophyll-a detected were 0.5438 and 0.3174 µg L^{-1}, respectively. Compare to the control without 10 ppm combined heavy metal exposure, chlorophyll-a biosynthesis showed a stimulatory effect in both organisms.

For *Chlorella* cells, control chlorophyll-b value was determined to be 1.6146 µg L^{-1} which decreased to 0.8117 µg L^{-1} at the end of the 24 h test period. Similar to these results, for *Scenedesmus* cells, the initial chlorophyll-b value, which was found to be 0.5246 µg L^{-1}, decreased to 0.2791 µg L^{-1} at the end of the experiment. Except for metals applied at a concentration of 10 ppm in *Chlorella*, the co-administration of all heavy metals in both of the relevant organism cells negatively affected the chlorophyll-b biosynthesis.

These results show that the formation of photosynthetic pigments, in particular chlorophyll-b, synchronized with the growth of the microalgal cells, making it an indicator for evaluating the removal efficiency of the heavy metal ions. The two algae intolerated the toxicity of all combined heavy metals even at higher concentrations (80–100 ppm), moreover the lower concentration (5–10 ppm) (Table 1). The combined application of the four low and high concentrations of heavy metals tested resulted in a decrease in the chlorophyll-a content while the chlorophyll-b content increased of both green algae at 10 ppm metal solutions.

3.2. Analysis results of the carbohydrate (mg mL^{-1})

As a result of the application of co-metals together with the organisms, the carbohydrate value of *Chlorella* cells was recorded as 0.9562 mg mL^{-1}. At the end of the study, this value was determined as average 0.8531 mg mL^{-1}. These values have been found to fall especially in mixtures with high concentration of metals (>10 ppm) (Figure 1). Contrary to these results, *Scenedesmus* cells showed an increase from 0.0209 mg mL^{-1} to 0.0646 mg mL^{-1}, especially at 2.5 ppm heavy metal concentration. While lower concentration of four metal ions (2.5-5 mg L^{-1}) enhanced the algal growth (total carbohydrate), elevated concentrations (10–100 mg L^{-1}) were inhibitory to the growth especially in *Chlorella* cells. This situation, which is observed in relation to carbohydrate synthesis, can be said to

be effective of carbohydrate synthesis on the growth and survival of two algal species used in the study. This suggests that the photosynthetic apparatus yield is closely related to the yield of carbohydrate and nitrogen metabolism. The arrangement between carbohydrate and N-metabolism is associated with heavy metal tolerance. It may result in the metabolic inhibitor effect of heavy metals on both components (carbohydrate and chlorophyll) [17].

Table 1. Effect of co-application of metals to chlorophyll-a and b and total carbohydrate of *Chlorella* and *Scenedesmus*.

Metal concentrations (ppm)	*Chlorella*		*Scenedesmus* sp.		*Chlorella*	*Scenedesmus*
	Chlorophyll-a (µg L^{-1})	Chlorophyll-b (µg L^{-1})	Chlorophyll-a (µg L^{-1})	Chlorophyll-b (µg L^{-1})	Total Carbohydrate (mg mL^{-1})	Total Carbohydrate (mg mL^{-1})
Control	**0.5149**	**1.6146**	**0.3602**	**0.5246**	**0.9562**	**0.0209**
2.5	0.5239	0.8802	0.3812	0.4894	0.9871	0.2529
5	0.6695	0.6656	0.2472	0.2610	0.9900	0.0159
10	0.2227	0.9836	0.2491	0.2059	0.9042	0.0438
25	0.6229	0.3238	0.2910	0.1879	0.8073	0.0380
50	0.6546	0.4958	0.3630	0.2552	0.6468	0.0316
100	0.5695	0.5211	0.3731	0.2753	0.7835	0.0056
Avarage values	**0.5438**	**0.8117**	**0.3174**	**0.2791**	**0.8531**	**0.0646**

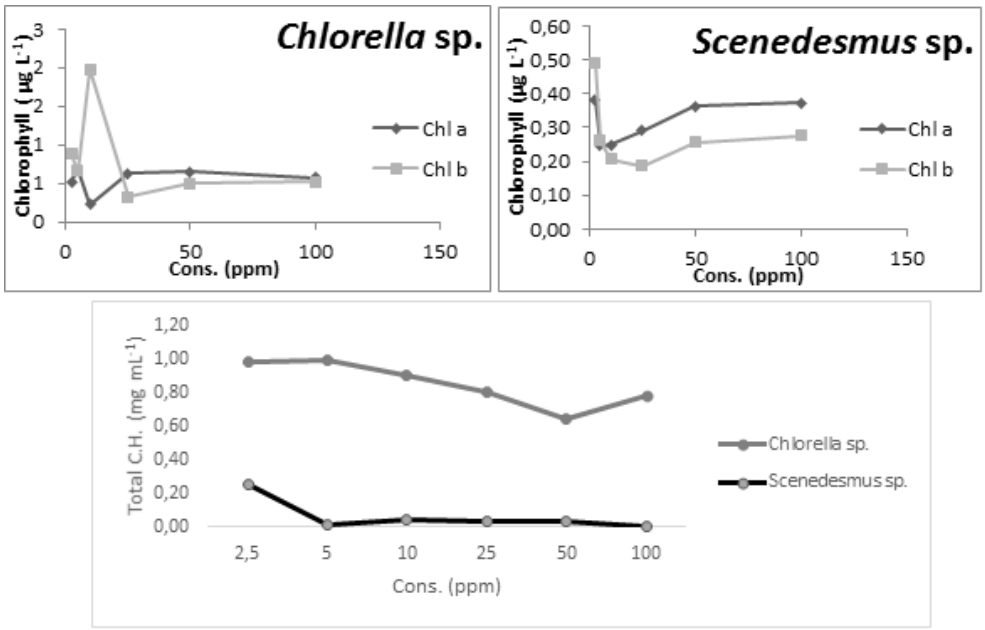

Figure 1. Effect of co-application of metals to chlorophyll-a and b and total carbohydrate of *Chlorella* and *Scenedesmus*.

3.3. Analysis results of the heavy metal uptake (mg g^{-1})and efficiency (%)

Samples were quantified by Coupled Plasma Mass Spectroscopy (ICP–MS). According to the results for *Chlorella*, the lowest and highest percentages of metal removal are identify as 9,10 (50 ppm) – 53,08 (2,5 ppm) for Antimony; 2,09 (50 ppm) – 84,23 (50 ppm) for Nickel; 6,24 (10 ppm) – 56,25 (2,5 ppm) for Manganese and 12,83 (25 ppm) – 77,27 (2,5 ppm) for Copper, respectively (Table 2). Besides, the average removal values for antimony, manganese, nickel and copper heavy metals on *Chlorella* cells were determined as 6.47; 5.96; 10,71 and 28, 57 mg g^{-1} , respectively (Cu> Ni> Sb> Mn).

For *Scenedesmus* cells, the lowest and highest percentages of metal removal are determined as 1,34 (50 ppm) – 59,98 (5 ppm) for Antimony;

12,99 (5 ppm) – 42,57 (10 ppm) for Nickel; 2,88 (5 ppm) – 31,43 (2,5 ppm) for Manganese and 4,74 (5 ppm) – 33,02 (2,5 ppm) for Copper, respectively. The average removal values for antimony, manganese, nickel and copper heavy metals on *Scenedesmus* cells were determined as 10,82; 7,07; 9,71 ve 27,09 mg g^{-1}, respectively (Cu> Sb> Ni> Mn). The obtained results showed that Mn^{2+} was the most toxic of the four metal ions to the test algae even at low concentration (<10 mg L^{-1}) (Figure 2).

Table 2. Metal ion binding capacities and percent of metal ion removal of *Chlorella* and *Scenedesmus*. (Data represent mean values).

		Chlorella		*Scenedesmus*	
	Concentrations	**Metal Removal (mg g^{-1})**	**Metal Removal Efficiency (%)**	**Metal Removal (mg g^{-1})**	**Metal Removal Efficiency (%)**
Antimony	2.5 ppm	3.2162	53.0875	7.5612	39.98
	5 ppm	3.4267	37.7083	3.922	59.9867
	10 ppm	3.0894	27.6345	8.2936	15.9464
	25 ppm	3.7742	10.8282	3.6903	3.3916
	50 ppm	6.6045	9.1057	3.034	1.34
	100 ppm	18.6861	12.1123	38.4175	7.977
Nickel	2.5 ppm	4.6816	83.5175	6.2459	15.21
	5 ppm	5.0616	81.9567	1.3452	12.9933
	10 ppm	8.9037	84.2382	2.7333	42.5764
	25 ppm	3.3496	62.7047	5.322	13.9144
	50 ppm	13.4364	2.0906	9.4095	21.3748
	100 ppm	28.838	57.5585	33.2009	16.9464
Manganese	2.5 ppm	3.4081	56.255	5.9456	31.4375
	5 ppm	3.0858	33.9567	0.817	2.88
	10 ppm	4.964	38.8864	4.1357	7.9518
	25 ppm	3.2268	11.3131	6.6823	29.1902
	50 ppm	4.307	6.2493	3.7726	7.5153
	100 ppm	16.7574	10.531	21.0411	4.2358
Copper	2.5 ppm	5.0598	77.275	2.8766	33.025
	5 ppm	7.4478	55.6983	3.6861	4.7417
	10 ppm	3.0894	62.5336	22.1437	5.2555
	25 ppm	16.2314	12.8311	3.6903	27.8043
	50 ppm	48.0176	19.7364	46.0071	6.3745
	100 ppm	91.5523	18.0995	84.1461	6.675

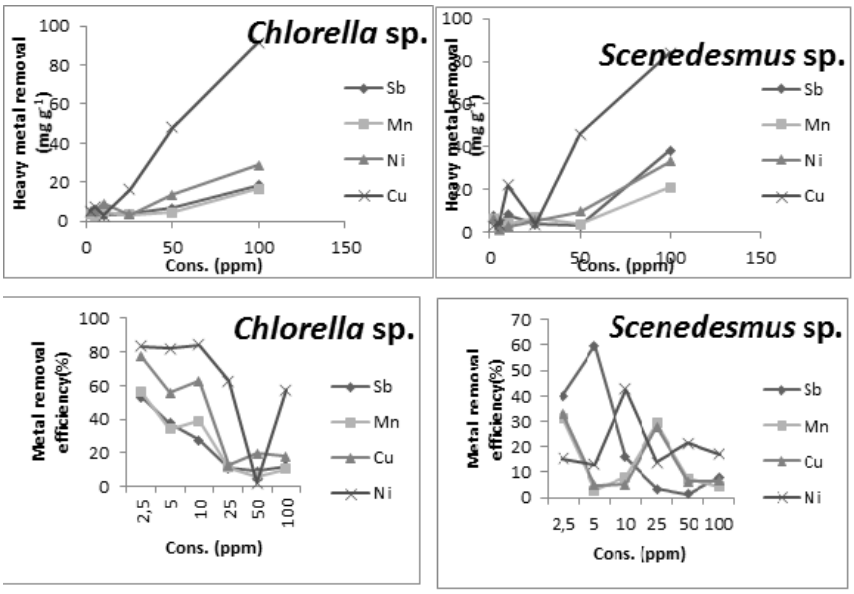

Figure 2. Metal ion binding capacities and percent of metal ion removal of *Chlorella* and *Scenedesmus*

3.4. Statistical results

The results of Langmuir and Freundlich isotherms are presented in Table 3.

Table 3. Langmuir and Freundlich isotherm models for antimony, manganese, nickel and copper ions adsorption by *Chlorella* and *Spirulina* cells.

	Adsorption Isotherm Constants	Sb	Mn	Ni	Cu
Chlorella	**Freundlich**				
	1/n (L g^{-1})	0.825	0.813	0.777	0.86
	K_F (L g^{-1})	1.251	0.81	1.285	1.162
	R^2	0.9963	0.9416	0.9007	0.9499
	Langmuir				
	q_m (mg g^{-1})	29.644	26.832	31.414	72.832
	R_L (L mg^{-1})	1.666	0.555	2.503	1.860
	R^2	0.0113	0.7101	0.021	0.1586
Scenedesmus	**Freundlich**				
	1/n (L g^{-1})	0.375	0.179	0.251	0.102
	K_f (L g^{-1})	0.941	0.350	0.875	0.177
	R^2	0.0009	0.5689	0.8972	0.9709
	Langmuir				
	q_m (mg g^{-1})	30.1207	26.812	28.8546	66.153
	R_L (L mg^{-1})	0.083	0.355	0.117	0.015
	R^2	0.4293	0.2096	0.1529	0.6937

We concluded that maximum adsorption capacity of copper calculated from Langmuir isotherm was around 72.832 and 66.153 mg g^{-1} for *Chlorella* and *Scenedesmus*, respectively. Compared to *Scenedesmus* (q_m = 26.812, 30.1207, 28.8546 and 66.153 mg g^{-1} for Mn^{2+}, Sb^{3+}, Ni^{2+} and Cu^{2+}, respectively), *Chlorella* behaved as a better biosorbent because of higher equilibrium sorption capacity (q_m = 26.812, 29.644, 31.414 and 72.832 mg g^{-1}, respectively).

The experimental data showed that for *Chlorella* and *Scenedesmus* cells, Langmuir isotherm model was applied to experimental equilibrium data of metal ions adsorption (R_L) for Mn, Sb, Ni and Cu were found between 0.555-2.503 and 0.015-0.355, respectively (Figure 3-6). For *Chlorella* and *Scenedesmus* cells, 1/n value was determined between 0.777-0.860 and 0.102-0.375, respectively.

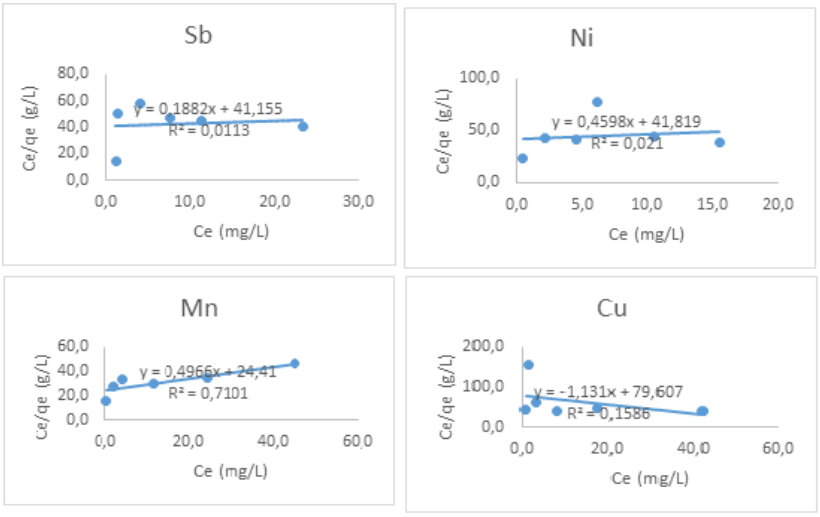

Figure 3. Langmuir adsorption isotherm of *Chlorella* on Sb, Mn, Ni and Cu.

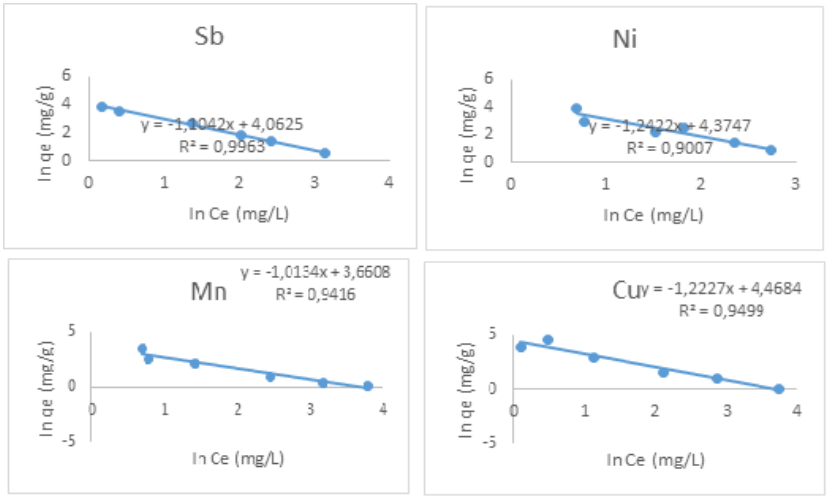

Figure 4. Freundlich adsorption isotherm of *Chlorella* on Sb, Mn, Ni and Cu.

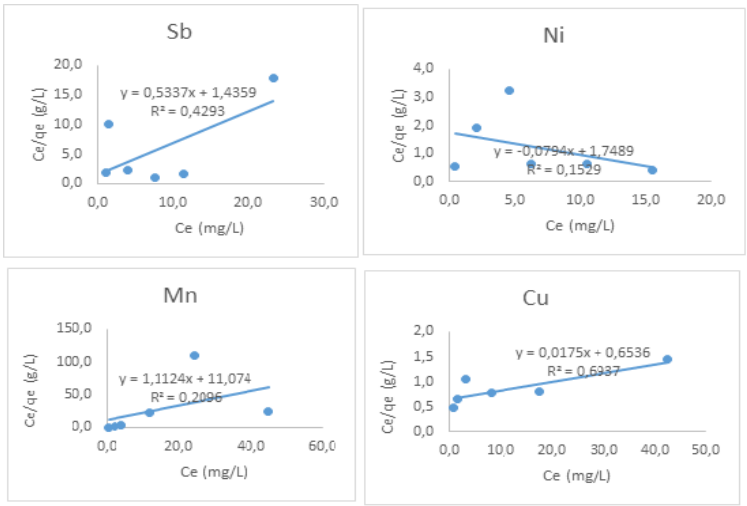

Figure 5. Langmuir adsorption isotherm of *Scenedesmus* on Sb, Mn, Ni and Cu.

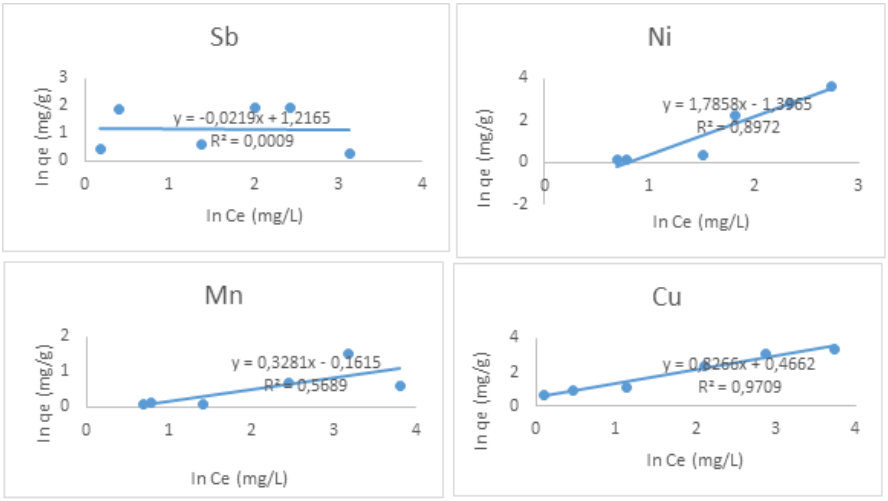

Figure 6. Freundlich adsorption isotherm of *Scenedesmus* on Sb, Mn, Ni and Cu.

3.5. AFM Images

Atomic force microscopy (AFM), Scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR) analyses are the most useful methods for the surface characterization and elemental analysis of materials [18,19]. AFM analyses were therefore applied to study the surface morphologies of the biosorbents after the biosorptions of each metal. The AFM images of *Chlorella* cells were determined after the treatment with the metal mixture at the concentration of 10 ppm, which is the heaviest metal adsorption. According to the findings, differences were determined between untreated surface topology of both organisms and the cell surfaces in which heavy metals are caused (Figure 7-8). Deformation was detected in cells treated-heavy metals as compared with untreated cells. The AFM images of *Chlorella* cells exposed to Cu, Ni, Sb and Mn ions presented in Figure 8 show that the surfaces of such cells were relatively dynamic and rough compared to the unexposed cells, the surfaces of which were more uniform and smooth. This may have been the result of heavy metal binding to the cell wall surface.

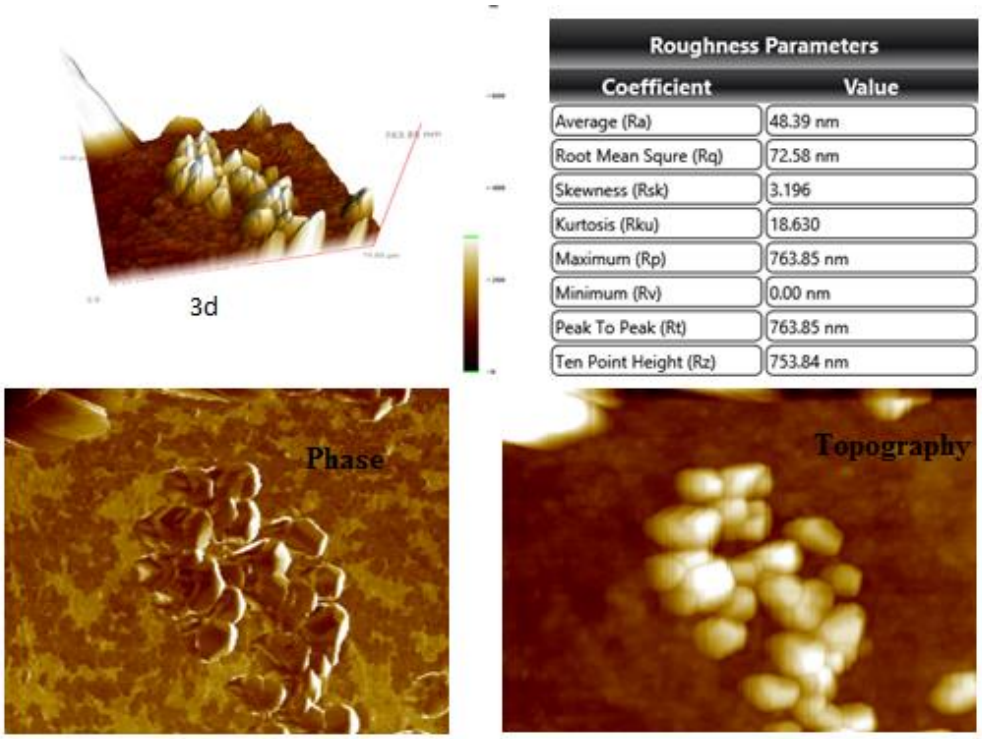

Figure 7.AFM images of untreated *Chlorella* cells.

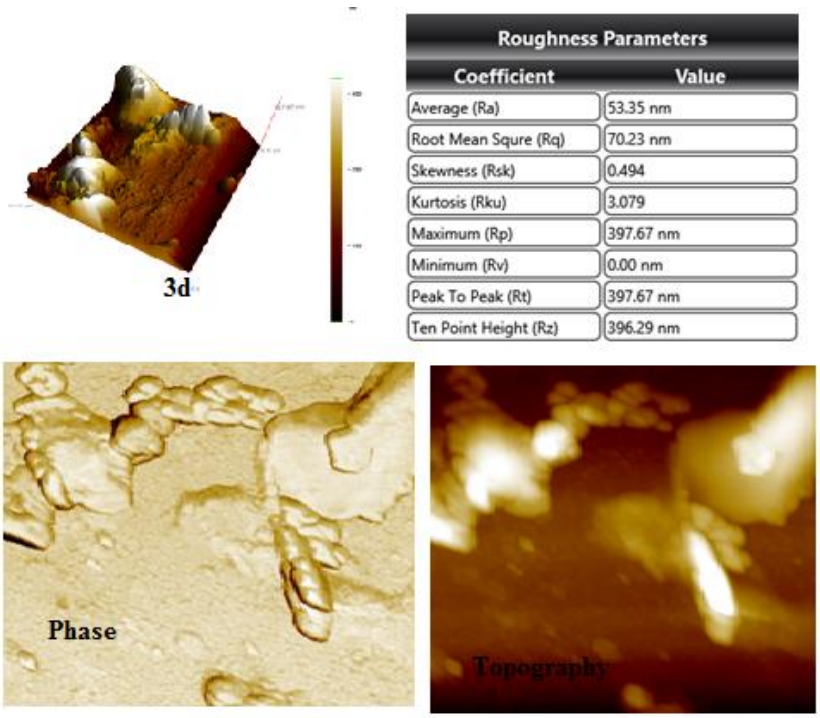

Figure 8. AFM images of treated *Chlorella* cells at10 ppm heavy metal solution.

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The AFM images of *Scenedesmus* cells were determined after the treatment with the metal mixture at the concentration of 5 ppm, which is the heaviest metal adsorption. Similar to *Chlorella* cells, *Scendesmus* cells treated-heavy metals showed deformation relative as compared with untreated cells revealed that *Scenedesmus* cells were cylindrical, have spines found in the terminal cells, and smooth before exposure to heavy metal (Figures 9-10).

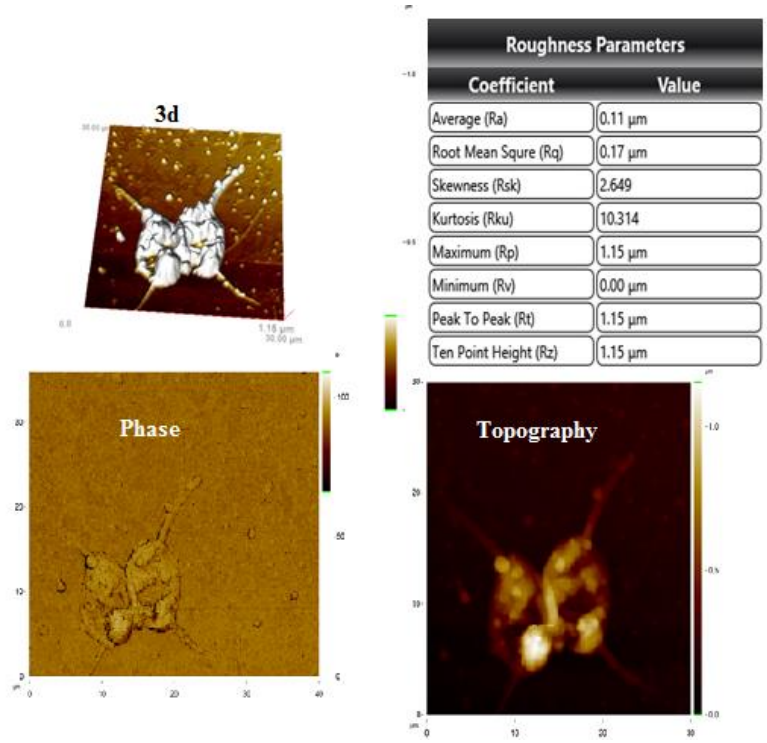

Figure 9. AFM images of untreated *Scenedesmus* cells.

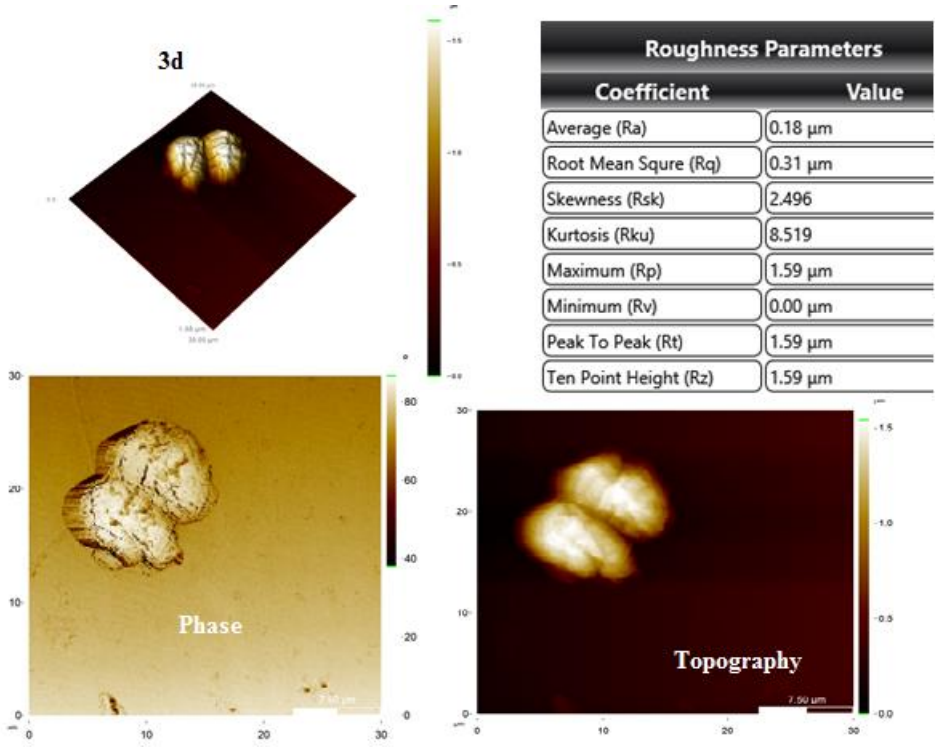

Figure 10. AFM images of treated *Scenedesmus* cells at5 ppm heavy metal solution.

4. DISCUSSION

High metal concentrations cause significant toxic effects on enzyme systems that control biochemical and physiological processes such as photosynthesis, respiration and synthesis of biological molecules. Studies have been carried out that inhibit photosynthesis by inhibiting ion distribution and enzyme activity, especially in cyanobacteria and green algae in high-structure plants [20-22]. *Chlorella and Scenedesmus* cultures treated with heavy metals displayed chlorosis because a significant loss in chlorophyll-b content was observed within the 24 hours of experiment. Heavy metals had the greatest inhibitory effect at a concentration of chlorophyll-a and b in *Scenedesmus cells.*

Heavy metals were characterized by an inhibitory influence on the total carbohydrate content in a concentration-dependent manner. Therefore, the significant decrease in the level of total carbohydrate has been obtained under the high concentration of all heavy metals in *Chlorella* cells. The inhibitory effect of heavy metals on the total carbohydrate content was also arranged in the following order 50>100 >25>10 ppm. The inhibitory effect of heavy metals on the content of total carbohydrates in *C. vulgaris* cells were suppressed by the coapplication of heavy metals. Rachlin and Grosso (1993) [23] found that C*hlorella* cells growth was inhibited under the influence of Cd, Cu and Co. They also reported that the effects of metals on the cell growth is Cd> Cu> Co, respectively.

According to the results of the analysis, it was determined that four heavy metals tested in low and high concentration were inhibited on total chlorophyll-b contents of two green algae. This may be due to the high toxicity of high amounts of manganese and copper on the processes responsible for the chlorophyll-b biosynthesis. Decreases in the amount of chlorophyll in Mn effect onto *Chlorella* and *Scenedesmus* cells may result in degradation of the chlorophyll biosynthesis process, inhibition of the enzymes involved in chlorophyll biosynthesis, or degradation of chlorophyll. Previous studies have reported that heavy metals inhibit chlorophyll pigment biosynthesis and enzymes that work in this process (Senturk and Yildiz 2016) [24].

It has been found that low and high concentrations of heavy metal solutions in the study, especially *Chlorella* cells, give rise to stimulative effect on chlorophyll-a content. This suggests that *Chlorella* cells on chlorophyll-a synthesis are resistant to low and high heavy metals. The bioaccumulation of antimony, manganese, copper and nickel in the culture medium increased in parallel with the increase in concentration. We can say that the metal removal is positively correlated with the increase in the concentration of each metal up to the high enough amount to prevent growth.

Some of the methods of protection from metal toxicity of algae control metal pick-up, give back the exports of imported metals and hold the metals taken in an immobile form in the cell. The main purpose of these methods is to protect sensitive targets such as DNA and protein (Soldo et al., 2005) [25]. At the end of the 24 h incubation period, the percentages of removal on the antimony, manganese, copper and nickel of *Chlorella* biomass were 25.07%, 26.19%, 41.02% and 62.01%, respectively (The average adsorption capacity was determined to be 6.47, 5.96, 28.57 and 10.71 mg g^{-1}, respectively). Antimony and copper accumulation in *Chlorella* cells were observed to be higher at high antimony and copper concentrations (25, 50 and 100 ppm) than the lower concentrations (2.5, 5 and 10 ppm).Antimony and copper accumulation in *Chlorella* has also continuously increased at every concentration and time without showing a recession except the heavy metal at the concentration of 10 ppm. In the adsorption efficiency of the metals, synergistic and antagonistic effects are observed depending on the specific properties of these metals, the number and type of metal found in the media [26,27]. In other words, a metal can affect the amount of accumulation of another metal

synergistically and antagonistically (Eg. antagonistic effect of Cd and Zn in some algal species or synergistic effect of Ni^{2+} and Cu^{2+} in membrane transport of metals). Lopez-Suarez et al. (2000) [28] found that *C. vulgaris* strongly binds heavy metals such as Mn, Cr, Ni, Zn and Cu and they reported that the strongest bonds were observed for Cr and Cu. The results of these reports were found to be consistent with the findings obtained in our study.

However, the percentages of heavy metal removal by *Scenedesmus* cells were 21.43%, 13.86%, 13.97% and 20.50%, respectively (The average adsorption capacity was determined to be 10.82, 7.07, 27.09 and 9.71 mg g^{-1}). Antimony and Manganese can be attributed to the toxic effect on *Chlorella* cells and *Scenedesmus* cells, respectively. The same results were observed in the accumulation of copper on *Scenedesmus* cells. These results suggest that manganese and copper contained in the environment is taken up by two algae and stored in cell components of heavy metals or in specific metal binding proteins. Knauer et al. (1997) [29] reported that the accumulation of Cu on *Scenedesmus subspicatus* increased depending on the concentration. Fayed et al. (1983) [30] found that Cu, Zn, Cd and Pb were rapidly absorbed by *Scenedesmus obliguus*, inhibited the growth of algae [31], especially by copper, and inhibited cell division [32]. These results are consistent with the findings of our study.

The experimental data showed that for *Chlorella* and *Scenedesmus* cells, Langmuir isotherm model was applied to experimental equilibrium data of metal ions adsorption (R_L) for Mn, Sb, Ni and Cu were found between 0.555-2.503 and 0.015-0.355, respectively (Figure 3-6). For *Chlorella* and *Scenedesmus* cells, 1/n value was determined between 0.777-0.860 and 0.102-0.375, respectively. According to Kadirvelu and Namasivayam (2000) [33], n values between 1 and 10 indicate a useful adsorption representative. For a good adsorbent, $0.2<1/n<0.8$ and a smaller value of 1/n indicate better adsorption and formation of rather a strong bond between the adsorbate and adsorbent [34]. Based on the study results, Mn, Sb, Ni and Cu adsorption by *Chlorella* algae complies with Freundlich adsorption isotherm model, whereas *Scenedesmus* algae follows both Freundlich and Langmuir model. The algae biomass was effectively used as a sorbent for removal of metal ions from aqueous solutions. Sorption isotherms were well fitted to the Freundlich model, for four co-effective metal systems. In both adsorption models, the biomass showed a greater affinity for Cu. The removal efficiency decreased with increasing metal concentration, pointing out a passive adsorption process involving the active sites on the surface of the biomasses.

5. CONCLUSION

High concentration of heavy metals, which are important contaminants of the water environment, is a consequence of human activities. Subsequent pollution toxicant affects the biochemistry, community and population levels of living things. Marine and freshwater algae, known as biological monitors of anthropogenic pollution, have a significant potential due to their ability to accumulate heavy metals in the environments and especially microalgae are beneficial in the remediation of various areas contaminated with inorganic nutrients and heavy metals [35]. For this reason, we are benefiting from the use of these two algal species as biosorbents in the heavy metal removal of water. Results from this study highlight the feasibility of *Chlorella* and *Scenedesmus* biomass as alternative low-cost biosorbents for the removal of Cu, Ni, Sb and Mn ions from aqueous solution. The heavy metal uptake ability of particularly *Chlorella* can be exploited for metal detoxification and environmental clean-up operations.

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