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BAZI ESANSİYEL METALLERİN *CHLORELLA VULGARİS* İLE BİYOLOJİK ARITIMI

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ÖZ

Ağır metal kirliliği bugün en önemli çevre sorunlarından biridir. Esansiyel elementler organizmalar tarafından hayatlarını sürdürmek için metabolizma faaliyetlerinde kullanılan elementlerdir. Bu elementlerin bulunmaması homeostazi dengesizliğine neden olur. Bakır, çinko, kobalt, manganez ve molibden, antropojenik yollarla besin zincirine girerek ciddi sağlık tehlikesi oluşturan esansiyel ağır metallerdir. Bu çalışma canlı C. vulgaris (Chlorophyta) mikroalgini kullanarak 0.5; 1; 2.5; 5 ve 10 ppm konsantrasyonlu sulu solüsyonlardan Cu (II), Co (II), Zn (II), Mo (VI) ve Mn (II) ağır metallerinin giderim kapasitesinin belirlenmesini amaçlamaktadır. Deneyler sırasıyla $CuSO_4.5H_20$, $CoSO_4.7H_20$, $ZnSO_4.H2O$, $Na2MoO_4.2H_20$ ve $MnCl_2.4H_20$ analitik derecedeki kimyasal reaktiflerden hazırlanan Cu, Co, Zn, Mo ve Mn sentetik tek metal çözeltileri kullanılarak gerçekleştirildi. Deneysel verilere dayanarak, Chlorella hücreleri tarafından Cu (II), Co (II), Zn (II), Mo (VI) ve Mn (II) için ortalama adsorpsiyon kapasiteleri, sırasıyla 241.006, 238.120, 237.033, 223.396 ve 54.899 mg/g olarak belirlenmiştir. Elde edilen sonuçlar, alg hücrelerini kullanan bu biyosorpsiyon sistemlerinin, kirli sulu ortamlardan ağır metal iyonlarının uzaklaştırılması için umut verici bir alternatif olduğunu göstermiştir.

Anahtar Kelimeler: *Esansiyel elementler, Su kirliliği, Toksik atıklar, Biyolojik arıtım, Metal giderimi.*

BIOLOGICAL TREATMENT OF SOME ESSENTIAL METALS BY USING *CHLORELLA VULGARIS*

ABSTRACT

Heavy metal pollution is one of the most important environmental problems today. Essential elements are the elements that are used in metabolic activities by organisms in order to sustain their lives. Nonexistence of this elements causes imbalance of the homeostasis. Copper, zinc, cobalt, manganese and molybdenum are essential heavy metals that pose serious health hazards through entry into the food chain by anthropogenic pathways. This study aimed to

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determine the removal capacity for Cu (II), Co (II), Zn (II), Mo (VI) and Mn (II) heavy metals from 0,5; 1; 2,5; 5 ve 10 ppm concentration of aqueous solutions by using live C. vulgaris (Chlorophyta) microalgae. Experiments were performed using synthetic single-metal solutions of Cu, Co, Zn, Mo and Mn prepared from chemical reactants of analytical grade: $CuSO_4 \cdot 5H_2O$, $CoSO_4 \cdot 7H_2O$, $ZnSO_4 \cdot H_2O$, $Na_2MoO_4.2H_2O$ and $MnCl_2.4H_2O$, respectively. Based on the experimental data, the average adsorption capacities for Cu(II), Co(II), Zn(II), Mo(VI) and Mn(II) were 241.006, 238.120, 237.033, 223.396 and 54.899 mg/g by Chlorella cells, respectively. The obtained results showed that these biosorption systems using algal cells represent promising alternative for the removal of heavy metal ions from polluted aqueous environments.

Keywords: *Essential elements, Water pollution, Toxic wastes, Biological treatment, Metal removal.*

1. Introduction

One of the biggest problems of our age is environmental pollution. Both industrial and home pollutants are harmful to nature. Heavy metals are important in these pollutants. Due to the poisonous properties of heavy metals, it is very serious to be given to nature without treatment. Even small quantities that can be taken in the living body can cause poisoning or even death (Erdem, 2014).

It is one of the major pollution sources that discharge industrial wastewaters containing heavy metals and pollute the water without being adequately treated. Among the major sources of heavy metals that can be counted as industrial sources are the wastewater from industries such as metal production, dyes, battery production, metal finishing, mining and mineral processing, coal mining and oil refining. In the removal of heavy metals in the waters, methods such as chemical precipitation, ion exchange, membrane filtration and phytoestraction are ineffective because they are expensive and inadequate for wastewaters with low metal content (Uzun, 2014).

Certain natural materials of biological origin, such as bacteria, fungi, yeast and algae possess metal-sequestering properties and therefore could be used to rapidly decrease the concentration of heavy metal ions with high efficiency. These biosorbents are ideal candidates for the treatment of high-volume and lowconcentration complex wastewaters contaminated with heavy metal ions (Kurniawan, 2006; Mack, 2007).

Living and dead biomass of algae cells can be used to decrease environmental heavy metal pollution. Several algae were tested for their ability to adsorb heavy metals (Zhou, 1998; Mehta, 2001; Moreno, 2005, Vijayaraghavan, 2006; Gokhale, 2008; Cabrita, 2014).

The present study considers the adsorption properties of green microalgae *C. vulgaris* alga strain. In this study, *C. vulgaris* cells were cultured under suitable laboratory conditions under laboratory conditions. These cultured cells were investigated for their adsorption properties after treatment with essential metals. On the other hand, information on the carbohydrate and chlorophyll levels of the relevant organisms has been given.

2. Materials and Methods

2.1. Materials and adsorbent

C. vulgaris strains were obtained from the Culture Collection of Microalgae at the University of Ege, Izmir, Turkey. The heavy-metal test solutions containing Cu(II), Co(II), Zn(II), Mo(VI) and Mn(II) ions were prepared from $CuSO_4{\cdot}5H_2O$, $CoSO_4{\cdot}7H_2O$, $ZnSO_4{\cdot}H_2O$, $Na_2MoO_4.2H_2O$ and $MnCl_2.4H_2O$ in the concentration range of 0.5-10 ppm (mg/L).

2.2. Algae cultivation and ICP-MS analysis

A standard initial inoculum of the isolated algae was inoculated to culture flasks (500 mL each) that contained 200 mL of sterile BG-11 (Rippka, 1979) and incubated at 28±1^{o}C under 16 h light (20 E m^{-2} s^{-1}±20%)/ 8 h dark regimen, with aeration (1.2 L min^{-1}) and magnetic stirring (110 rpm). The required pH was regulated with 0.1 M NaOH and 0.1 M HCl solutions. Continuous stirring of the culture was achieved using a 110 rpm magnetic plate. At the end of the incubation period (80–90 days) cultures were filtered and washed several times by distilled water. The algal stock was stored at 4^{o}C in dark until use. At least three replicates for each sample and controls were used. *Chlorella* cells 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 10 days. pH was adjusted to 5-6 for Cu and Zn, and 5-7 for Co and Mn, 7-8 for Mo (Schenck, 1988; Geoffrey, 1991; Aslan, 2007; Rezaei, 2011). Similar results have also been reported by Meitei and Prasad (2014) and further increase of pH causes reduction of metal adsorption due to metalhydroxide ions formation.

The heavy metal content in the supernatant was measured by ICP-MS. The efficiency of the removal was calculated using the following equation:

$$\text{Removal efficiency} = 100*(C_0 - C_e)/C_0$$

C_0 and C_e are the metal concentrations initially and in the equilibrium (mg/L), respectively (Ji, 2011).

The metal uptake per gram of adsorbent, q (mg/g), was calculated using the equation:

$$q\ (mg/g) = (C_0-C_e)*V/m$$

Where V is the volume of the solution (L) and m is the mass of biosorbent (g) (Ji, 2011).

2.3. Determinations of chlorophyll-a and b content

Chlorophyll-a and b content were estimated in acetone extract according to Parsons and Strickland (1963). 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. Pigment content in the filtered extract were determined by the absorbance at 630, 645, 665 and 750 nm in a 1cm quartz cell against a blank of 90% aqueous acetone (Gonzales, 1997).

2.4. Determination of dry weight

A definite volume (10 mL) of algal suspension was filtered through weighted glass fiber (Whatman GF/C). The cells, after being precipitated on the filter study, were washed twice with distilled water and dried overnight in an oven at 105°C. Data were given as mg/mL algal suspension.

2.5. Determination of total carbohydrate contents (mg/mL) were mesured using the phenol-sulfuric acid assay and using glucose as a standard. 1 mL aliquots of the cultures were used to quantified spectrophotometrically the total carbohydrate content by the phenol-sulfuric acid assay (Dubois, 1959).

2.6.Statistical analysis

All experiments were conducted as triplets and all the results found with ICP-MS were provided as a mean values.

3. Result and Discussion

3.1. Chlorophyll-a and b analysis results

The chl-a and b values of *C. vulgaris* cells were initially recorded as 0.6812 and 0.2441 μg/L. The chl-a and b values at concentration of 10 ppm, which is the maximum metal uptake concentration of application on living cells, are given in the following tables (Table 3.1). The mean value of chl-a has been increased in the final essential metal application. In the amount of chl-b of living cells was found to decrease in all essential metal applications except molybdenum application (Figure 3.1).

Table 3.1. Effect of essential heavy metals on chlorophyll-a and b content of *C. vulgaris*.

Metals	**Cu**	**Zn**	**Co**	**Mn**	**Mo**
Chl-a µg/L (10 ppm cons.)	0.7500	0.6846	0.8404	0.7683	2.9670
Chl-b µg/L (10 ppm cons.)	0.0971	0.1142	0.0320	0.1666	2.7291

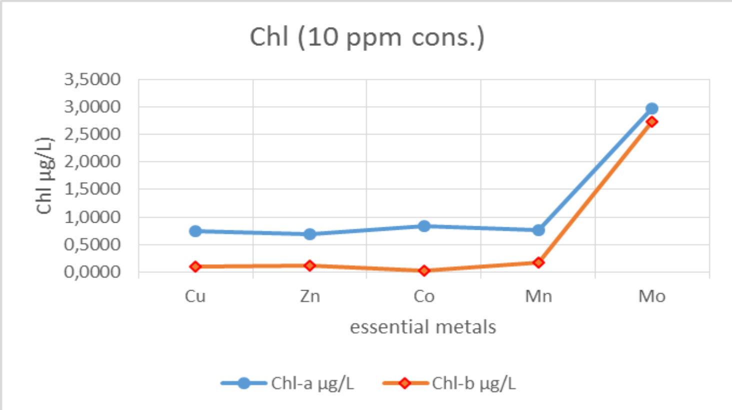

Figure 3.1. Effect of essential heavy metals on chlorophyll-a and b.

C. vulgaris cultures treated with cobalt heavy metal (10 ppm) displayed chlorosis because a significant loss in total chl-b content was observed between the 2 days and 4 days of cultivation. Heavy metals had an inhibitory effect in chl-b at a concentration of 10 ppm except Mo. Heavy metals showed a stimulating influence on the content of chl-a in the cells of *C. vulgaris*. These results show that the formation of photosynthetic pigments of chl-a and b, synchronized with the growth of the microalgal cells, making it an indicator for evaluating the removal efficiency of the heavy metal ions. The alga intolerated the toxicity of all heavy metals even at 10 ppm concentrations.

3.2. Total carbonhydrate analysis results

The total carbohydrate values of *C. vulgaris* cells were initially recorded as 0.7035 mg/mL. The change in the total carbohydrate values at 10 ppm concentration, the concentration range at which the metal removal from the application of the essential metals on living cells is highest, is given in the table 3.2. The increase in the carbohydrate value of the living cells has been observed in zinc and copper metal applications while a decrease in the carbohydrate value of the organism was determined in other metal applications (Figure 3.2).

Table 3.2. Effect of essential heavy metals on total carbohydrate content of *C. vulgaris*.

Total CH. mg/mL (10 ppm cons.)					
essential metals	**Cu**	**Zn**	**Co**	**Mn**	**Mo**
	0.7402	0.9370	0.6677	0.4475	0.6940

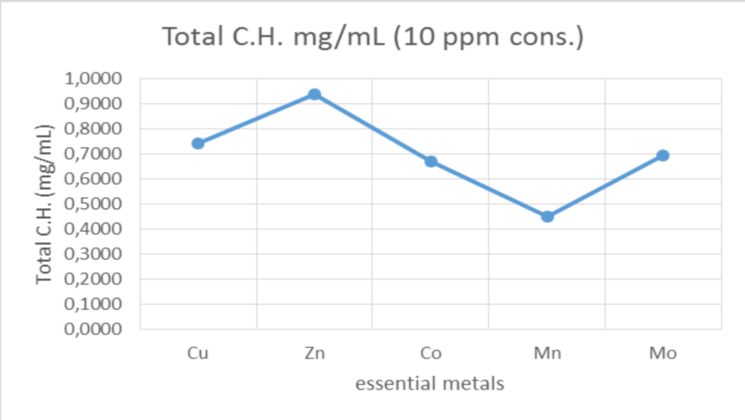

Figure 3.2. Effect of essential heavy metals on total carbohydrate.

As seen in the analysis results obtained in the study, a strong decrease was observed in the total carbohydrate values of *C. vulgaris* cells from 0.7035 mg/mL to 0.4475 (63.61%) in manganese application. It has been found that, it has a high toxic effect on *C. vulgaris* cells in terms of carbohydrate values even at low concentrations of Mn (ppm <20).

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 *C. vulgaris*. 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) (Moiseenko, 2001).

3.3. ICP-MS analysis results

3.3.1. Analysis results of the heavy metal uptake (mg/g) and efficiency (%)

The metal sorption efficiency by *C. vulgaris* was greatly affected with the initial concentrations of metals in the aqueous solution. Metal adsorption increased with increasing metal concentration in aqueous solution. The metal concentrations slightly decreased in the control treatment without algae.

The mean adsorption capabilities of *C. vulgaris* were different for Cu, Zn, Co, Mn and Mo (241.006; 237.033; 238.120; 54.899 and 223.396 mg/g, respectively) at 28°C and pH 5-8 (Table 3.3). Furthermore, the removal efficiencies for Cu, Zn, Co, Mn and Mo were observed from 87–95%, 87–94%, 91–96%, 6-56% and 84–87%, respectively (Table 3.4). The highest removal efficiency was observed for Cu and Zn from aqueous solution at 10 ppm metal concentrations. The results summarized that *C. vulgaris* is a suitable candidate for removal of selected essential heavy metals from the aqueous solutions (Figure 3.3-3.4).

Table 3.3. Essential heavy metals uptake values of *C. vulgaris* (mg/g).

ppm	Cu	Zn	Co	Mn	Mo
0.5	30.067	29.463	32.013	19.060	28.188
1	58.456	62.282	64.765	12.752	57.181
2.5	157.584	158.725	161.879	19.262	147.047
5	321.275	312.013	314.564	20.336	295.235
10	637.651	622.685	617.383	203.087	589.329
mean	**241.006**	**237.033**	**238.120**	**54.899**	**223.396**

Table 3.4. Essential heavy metals removal efficiency of *C. vulgaris* (%).

ppm	Cu	Zn	Co	Mn	Mo
0.5	89.60	87.80	95.40	56.80	84.00
1	87.10	92.80	96.50	19.00	85.20
2.5	93.92	94.60	96.48	11.48	87.64

5	95.74	92.98	93.74	6.06	87.98
10	95.01	92.78	91.99	30.26	87.81
mean	**92.27**	**92.19**	**94.82**	**24.70**	**86.52**

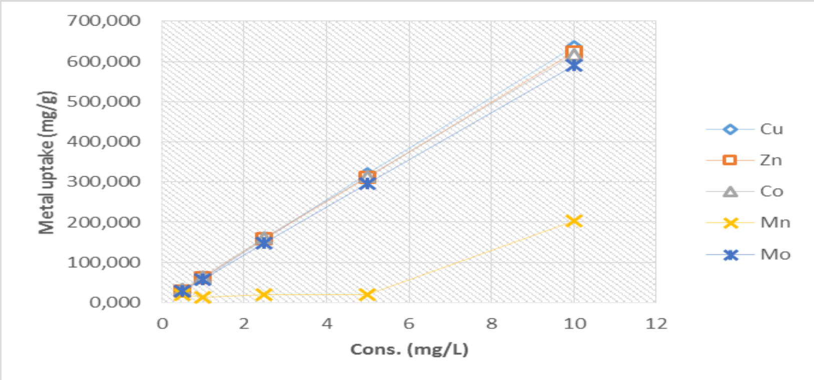

Figure 3.3. Essential heavy metals uptake values of *C. vulgaris* (mg/g).

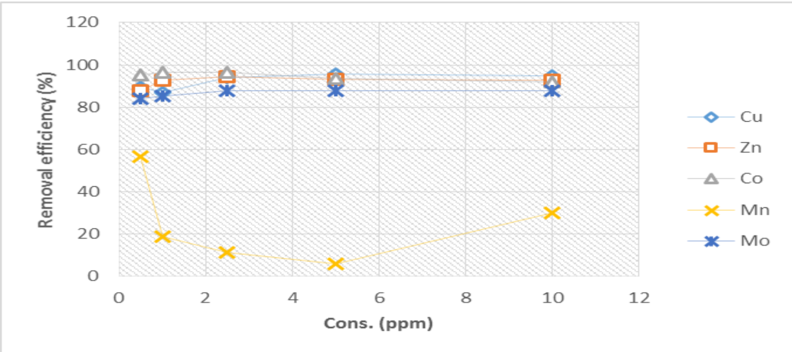

Figure 3.4. Essential heavy metals removal efficiency of *C. vulgaris* (%).

In this experiment we used freshwater algae *C. vulgaris* which grow very well under the experimental condition mentioned above with no yellow and dead part apparently appeared after 10 days culturing in the aqueous solution containing the selected heavy metals. This study results showed that *C. vulgaris* metal sorption reached to high levels at low and high concentrations of Cu, Zn, Co and Mo aqueous solution but for Mn the metal sorption was highest at low metal concentrations in aqueous solutions.

4. Conclusion

In conclusion the *C. vulgaris* is very stable in the removal of essential toxic heavy metals from aqueous solutions and can be used to develop a high capacity biosorbents for the removal of Cu, Zn, Co, Mn and Mo. The results indicated that *C. vulgaris* are eco-environment friendly for the treatment of domestic and industrial wastwater because of their easy availability, wide distribution, easy cultivation and has low cost.

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