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CHLORELLA VULGARİS ALGİ KULLANILARAK BAZI NON-ESANSİYEL METALLERİN GİDERİMİ

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ÖZ

Teknolojinin hızla gelişmesi, nüfus yoğunluğunun artması, su kaynaklarının bilinçsiz kullanımı, endüstriyel ve evsel atıkların su kaynaklarına boşaltılması, sucul ekosistemlerin zenginleştirilmesi sonucu denizler ve içsu kaynakları non-esansiyel element (kadmiyum, arsenik, baryum vb.) kirliliğinin etkisi altındadır. Bu nedenle bitkilerle özellikle alglerle yapılan giderim tekniği, yüksek giderim verimi, ucuz bakım ve işletme maliyeti sebebiyle tüm dünyada uygulanmaktadır.

Bu çalışma canlı C. vulgaris (chlorophyta) mikroalgini kullanarak 0.5; 1; 2.5; 5 ve 10 ppm konsantrasyonlu sulu solüsyonlardan kadmiyum (Cd^{+2}), kurşun (Pb^{+2}), kalay (Sn^{+4}), baryum (Ba^{+2}) ve arsenik (As^{+5}) ağır metallerinin giderim kapasitesinin belirlenmesini amaçlamaktadır. Deneyler sırasıyla $CdSO_4 \cdot 8H_2O$, $Pb(NO_3)_2$, $Sn0_2$, $Ba(NO_3)_2$ ve $Na_2HAsO_4.7H_2O$ analitik derecedeki kimyasal reaktiflerden hazırlanan Cd, Pb, Sn, Ba ve As sentetik tek metal çözeltileri kullanılarak gerçekleştirildi. Deneysel verilere dayanarak, C. vulgaris hücreleri kullanılarak non-esansiyel metallerin ortalama adsorpsiyon kapasitesi sırasıyla Sn için 254.939 mg/g, Pb için 254.536 mg/g, Ba için 238.563 mg/g, Cd için 235.288 mg/g ve As için 227.543 mg/g (Sn>Pb>Ba>Cd>As) olarak belirlenmiştir. Bu çalışma, Chlorella hücrelerinin, Sn, Pb, Ba, Cd ve As adsorpsiyonunun yüksek verimliliği nedeniyle sulu çözeltilerden beş non-esansiyel ağır metal iyonunun uzaklaştırılması için etkili bir adsorbent olduğunu ortaya koymuştur.

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

UPTAKE OF SOME NON-ESSENTIAL METALS BY THE ALGA CHLORELLA VULGARIS

ABSTRACT

Seas and inland water resources are under the influence of pollution with non-essential elements (cadmium, arsenic, barium etc as a result of the rapid development of technology, increasing population density, unconscious use of water resources, discharge of industrial and

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domestic wastes to water resources, enrichment of aquatic ecosystems. For this reason, the treatment technique has been applied all over the world with plants, especially with algae, because of the high treatment yield and low maintenance and operation costs.

This study aimed to determine the removal capacity for cadmium (Cd^{2+}), lead (Pb^{2+}), tin (Sn^{4+}), barium (Ba^{2+}) and arsenic (As^{5+}) 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 Cd, Pb, Sn, Ba and As prepared from chemical reactants of analytical grade: $CdSO_4{\cdot}8H_2O$, $Pb(NO_3)_2$, $Sn0_2$, $Ba(NO_3)_2$ and $Na_2HAsO_4.7H_2O$, respectively. Based on the experimental data, the mean adsorption capacity of non-essential metals was determined as 254.939 mg/g for Sn, 254.536 mg/g for Pb, 238.563 mg/g for Ba, 235.288 mg/g for Cd and 227.543 mg/g for As (Sn>Pb>Ba>Cd>As) by C. vulgaris cells respectively. This study revealed that Chlorella cells were an effective adsorbent for removal of the five non-essential heavy metals ions from aqueous solutions due to its high efficiency of Sn, Pb, Ba, Cd and As adsorption.

Keywords: *Non-essential elements, Water pollution, Chlorella vulgaris, Heavy metal uptake.*

1. Introduction

Heavy metals are elements having atomic weights between 63.5 and 200.6, and a specific gravity greater than 5.0. Living beings require follow measures of some substantial trace heavy metals, including cobalt, copper, press, manganese, molybdenum, vanadium, strontium and zinc. Extreme levels of essential metals, be that as it may, can be inconvenient to the life form. Non-essential heavy metals of specific concern to surface water frameworks are cadmium, chromium, mercury, lead, arsenic, and antimony. Heavy metals which are moderately inexhaustible in the world's covering and habitually utilized as a part of modern procedures or farming are poisonous to people. These can make critical modifications to the biochemical cycles of living bodies (Srivastava, 2008). 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).

The accumulation of metals by algae, bacteria, fungi and yeast has been extensively studied in the last two decades. Of the microorganism studied, algae are gaining increasing attention, due to the fact that algae, particularly marine algae, are a rich source in the oceanic environment, relatively cheap to process and able to accumulate high metal content (Wilde, 1993). 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 non-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. Algal Culture

The algal species used in this study obtained from the Culture Collection of Microalgae at the University of Ege, Izmir, Turkey maintained as pure unialgal isolates on nutritive media (Blue-Green-11 medium for green algae) and incubated at temperature 28±1°C, light intensity of 30mE/m^2/s, photoperiod 16–8 h and regularly subcultured until use (Rippka, 1988). The same conditions were used in tolerance and bioremoval experiments but with using shaking (110M/min) and the culture media were lacking EDTA.

Stock solutions of the heavy metals $CdSO_4 \cdot 8H_2O$, $Pb(NO_3)_2$, $Sn0_2$, $Ba(NO_3)_2$ and $Na_2HAsO_4.7H_2O$ (500 mg/100 ml) were prepared, from which concentrations 0. 5, 1, 2.5, 5 and 10 ppm (mg/L) were used in case of algal tolerance experiments. In biosorption experiment concentration of 40 mL of heavy metals and 10 mL of algal biomass was used and the exposure time was 10 days.

2.2. Heavy metal removal (biosorption)

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. The microalga *C. vulgaris* was used in the experiment of heavy metal removal using the algal concentrations 10 mL. pH was adjusted to 7.0 and incubation was performed at the previous mentioned conditions. At the end of exposure time, decantation was performed and the supernatant was used for the determination of heavy metal removal using Agilent 7700 Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) for heavy metal removal determination (at Experimental Science Research And Applicatıon Center, Manisa/Turkey).

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. Measurements of algal growth

Algal tolerance to different heavy metal concentrations was achieved by the determination of algal growth as chlorophyll-a and b. Chlorophyll content was determined according to Metzner et al. (1965) where, 10 mL of culture sample was ground in a GF/C filters together with acetone and calcium carbonate. 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 and 665 nm in a 1cm quartz cell against a blank of 80% aqueous acetone.

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. Total carbohydrate contents (mg/mL)

Total carbohydrate content of 1 mL aliquots of the cultures in different algae were determined spectrophotometrically at 490 nm, using phenol-sulfuric acid assay and using glucose as a standard according to Dubois et al. (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

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 non-essential metal application. Similar to these results, in the amount of chl-b of living cells was found to increase in all essential metal applications except tin application (Figure 3.1).

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

Metals	Cd	Pb	Sn	Ba	As
Chl-a µg/L (10 ppm cons.)	2.2266	1.0685	0.8339	2.1834	0.7877
Chl-b µg/L (10 ppm cons.)	1.9173	0.3107	0.1735	1.8624	1.1527

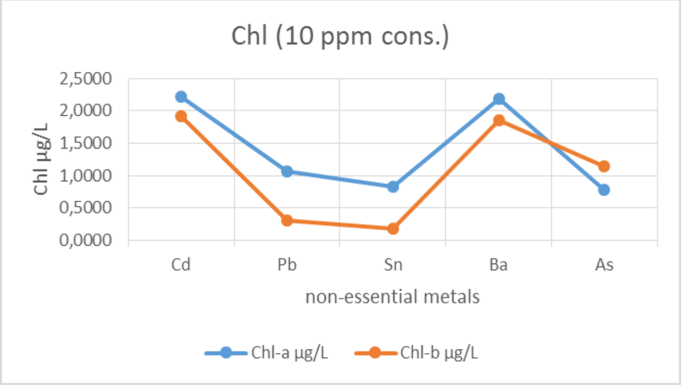

Figure 3.1. Effect of non-essential heavy metals on growth of *C. vulgaris* after 10 d expressed as µg chlorophyll-a and b /L.

C. vulgaris cultures treated with tin heavy metal (10 ppm) displayed chlorosis because a significant loss in total chl-b content was observed between the 5 days and 7 days of cultivation. Heavy metals had an stimulatory effect in chl-a and b at a concentration of 10 ppm except Sn. Heavy metals showed a stimulating influence on the content of chl-a and b 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

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 non-essential metals on living cells is lowest, is given in the table 3.2. The decrease in the carbohydrate value of the living cells has been observed in all heavy metal applications (Figure 3.2).

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

Total CH. mg/mL (10 ppm cons.)					
non-essential metals	**Cd**	**Pb**	**Sn**	**Ba**	**As**
	0.5686	0.6406	0.5037	0.5785	0.4492

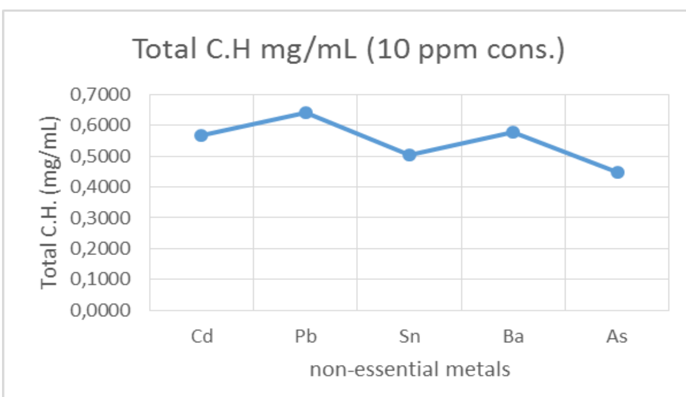

Figure 3.2. Effect of non-essential heavy metals on growth of *C. vulgaris* after 10 d expressed as mg carbohydrate /mL.

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 0.5481 (70.91%) in all non-essential metals 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 related heavy metals (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. Heavy metals removal analysis

According to Stokes (1983) algae appearing in polluted sites are considered to be either metal tolerant or metal resistant species. Several green algal species are tolerant or resistant to Cu^{2+}, Cd^{2+}, Pb^{2+} and Zn^{2+} (Hording, 1976; Say, 1977; Whitton, 1980; Foster, 1982). Bioremoval is defined as the accumulation and concentration of pollutants from aqueous solutions by the use of biological material, thus allowing the recovery and environmentally acceptable disposal of the pollutants (Volesky, 1990; Gadd, 1990).

The tolerant green microalga *C. vulgaris* showed a high efficiency of heavy metal (Cd, Pb, Sn, Ba and As) biosorption. The mean adsorption capabilities of *C. vulgaris* were different for Cd, Pb, Sn, Ba and As (235.288, 254.536, 254.930, 238.563 and 227.543 mg/g, respectively) at 28°C (Table 3.3). Furthermore, the removal efficiencies for Cd, Pb, Sn, Ba and As were observed from 91–95%, 99%, 99%, 92-96% and 87–93%, respectively (Table 3.4). The highest removal efficiency was observed for Sn and Pb 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. Non-essential heavy metals uptake values of *C. vulgaris* (mg/g).

ppm	Cd	Pb	Sn	Ba	As
0.5	31.275	33.490	33.490	32.215	31.208
1	64.362	66.980	67.047	63.289	62.282
2.5	155.570	167.315	167.718	159.195	152.685
5	308.255	335.034	335.436	312.013	302.416

10	616.980	669.866	671.007	626.107	589.128
mean	**235.288**	**254.536**	**254.939**	**238.563**	**227.543**

Table 3.4. Non-essential heavy metals removal efficiency of *C. vulgaris* (%).

ppm	Cd	Pb	Sn	Ba	As
0.5	93.20	99.80	99.80	96.00	93.00
1	95.90	99.80	99.90	94.30	92.80
2.5	92.72	99.72	99.96	94.88	91.00
5	91.86	99.84	99.96	92.98	90.12
10	91.93	99.81	99.98	93.29	87.78
mean	**93.12**	**99.79**	**99.92**	**94.29**	**90.94**

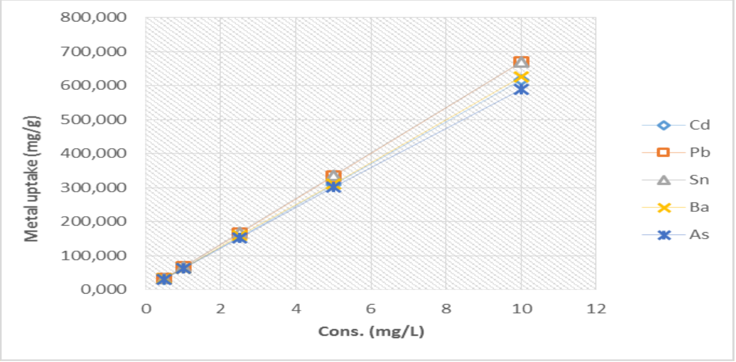

Figure 3.3. Non-essential heavy metals uptake values of *C. vulgaris* (mg/g).

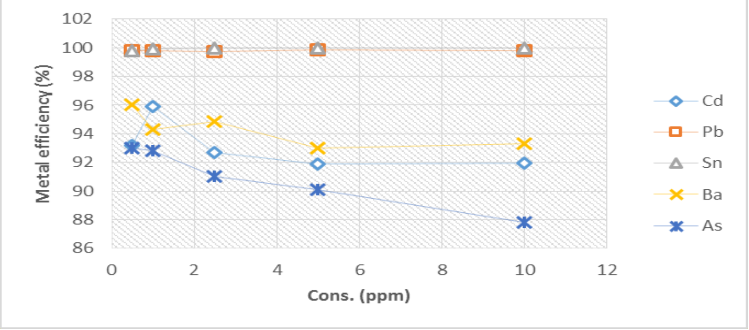

Figure 3.4. Non-essential heavy metals removal efficiency of *C. vulgaris* (%).

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 Cd, Pb, Sn, Ba and As aqueous solution.

4. Conclusion

In conclusion the *C. vulgaris* is very stable in the removal of non-essential toxic heavy metals from aqueous solutions and can be used to develop a high capacity biosorbents for the removal of Cd, Pb, Sn, Ba and As. 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.

5. Funding

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