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Bioaccumulation and Toxicity of Different Copper Concentrations in *Tetraselmis chuii*

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Özet: ***Bakır'ın farklı yoğunluklarının*** Tetraselmis chuii'***ye toksik etkisi ve birikimi.*** Çevre kirliliğinden korunmak amacıyla son yıllarda ağır metallerin biyolojik yöntemle ortamdan alınması konusu giderek önem kazanmaktadır. Algal biyomas, endüstriyel atık sular veya diğer kaynaklardan ağır metallerin uzaklaştırılması için ekonomik olarak kullanılabilmektedir. Bu çalışmada bir ağır metal olan bakırın alg büyümesi üzerine etkisi araştırılmıştır. *Tetraselmis chuii*'nin, farklı yoğunluklarını içeren ortamlarda bakır biriktirme düzeyleri belirlenmiştir. Kültürler, I, II, III, IV ve V olmak üzere gruplandırılmış ve Cu^{++} yoğunlukları sırasıyla $3.59.10^{-9}$ (kontrol), 5.10^{-9}, 10.10^{-9}, 15.10^{-9} ve 20.10^{-9} nM olarak oluşturulmuştur. *T. chuii* kültürlerinde büyüme ve denemenin 3, 5 ve 7. günlerinde bakır bağlama düzeyleri belirlenmiştir. Denemenin sonunda kültür ortamlarına eklenen Cu^{++} sebebiyle alg büyümesinde bir gerileme gözlenmemiştir. Ortalama spesifik büyüme oranları (μ), beş grup için sırasıyla 0.53, 0.53, 0.31, 0.56 ve 0.61 olarak hesaplanmış ve benzer bulunmuştur ($p>0.05$). Gruplar için belirlenen bakır miktarları sırasıyla 52.93, 384.99, 552.62, 1611.08 ve 1673.96 μg metal g^{-1}/kuru ağ. olarak hesaplanmış, IV. ve V. gruplarda belirtilen bakır miktarları diğer gruplara göre yüksek olmuştur. Bakır birikimi ve biyokonsantrasyon faktörleri gruplar arasında farklı bulunmuştur ($p<0.05$).

Anahtar Kelimeler: Biyokonsantrasyon faktör, bakır birikimi, *Tetraselmis chuii*.

Abstract: The biological removal of heavy metals has received attention increasingly in recent years because of its potential in environment protection. Algal biomass can be utilized economically for the removal of heavy metals from industrial wastewater or other sources. In this research the heavy metal copper and the growth of algae was focused mainly.The ability of *Tetraselmis chuii* to accumulate copper metal in different concentrations was investigated. The cultures which were grouped I, II, III, IV and V, were exposed to Cu^{++} concentrations of $3.59.10^{-9}$(control), 5.10^{-9}, 10.10^{-9},15.10^{-9} and 20.10^{-9} nM respectively to determine the *T. chuii* growth and the binding levels of copper on the days of 3rd, 5th and 7th of the experiment. At the end of the study the growing of microalgae inhibition wasn't observed due to the addition of Cu^{++} in the culture mediums. The growth rates of the groups were found similar ($p>0.05$) and the mean specific growth rates (μ) computed for the five groups were 0.53, 0.53, 0.31, 0.56 and 0.61, respectively. The copper amounts of groups were found to be 52.93, 384.99, 552.62, 1611.08, 1673.96 μg metal g^{-1} dry weight for the group I to V on 7th day. Copper uptook in group V were calculated to be 1874.47±711.8, 2173.03±611.66 and 1673.96±548.77 μg metal g^{-1} dry weight for the days of 3rd, 5th and 7th, respectively. The amounts of copper accumulation and bioconcentration factors in the groups were found different ($p<0.05$). It was determined that the accumulations of copper in group IV and V contained high copper concentrations were found higher than the other groups.

Anahtar Kelimeler: Bioconcentration factor, copper accumulation, *Tetraselmis chuii*.

Introduction

Industrial wastes, geo-chemical structure and mining of metals create a potential source of heavy metal pollution in the aquatic environment (Gumgum *et al.*, 1994). The toxic metals can be broadly divided into two groups. The first group consists of metals that are essential as nutritional requirements at trace amount for many organisms but are toxic when present in greater amounts. This group includes As, Cr, Co, Cu, Ni, Se, Va and Zn. The second group includes Pb, Hg, Cd, Ur, Ag and Be, all of them are highly poisonous and are not know to have any nutritional value (Inthorn, 2001). Under certain environmental conditions, heavy metals may accumulate to a toxic concentration (Guven *et al.*, 1990), and cause ecological damage (Freedman, 1989). The biological removal of heavy metals has received attention increasingly in recent years because of its potential in environment protection. Algal biomass can be utilized economically for the removal of heavy metals from industrial wastewater or other sources (Becker, 1994; Inthorn, 2001; Richmond, 1986). In recent years, research on toxic heavy metals and algae was focused mainly on the toxicological aspects on living algal populations. The studies also used increasing concentrations of individual and combined heavy metal elements on the growth and propagation of different algal species. Accumulation of the heavy metals by the algae was also studied (Inthorn, 2001). According to Gale *et al.*, It was also shown that the green algae appeared to be more tolerant to metals such as zinc, lead and copper than blue green algae and diatoms in general (Inthorn, 2001) . In the previous work, some algae were found to have accumulated from 12000 to 83000 times the ambient copper concentration in the water (Trollope and Evans, 1976). The microalgae, *Chlorella* sp. and *Scenedesmus* sp., investigated by Stokes *et al.* were able to

bioaccumulate up to 2400 mg Cu/kg on a dry weight basis, representing a 3400 bioaccumulation factor (Stokes and Hutchinson, 1976). *Chlorella vulgaris* have commonly been used in toxicological studies because it is known to response to pollutions (Munoz *et al.*, 1996; Nriagu, 1979; Stokes and Hutchinson, 1976; Kasai and Hatakeyama, 1993; Rai *et al.*, 1993; Rachlin and Grosso, 1991; Megharaj *et al.*, 1991). Since the 1970s, many studies have been conducted metal removal and develop optimal systems and process conditions for applications. Many species of algae were studied to illustrate the metal accumulation properties (Borowitzka and Borowitzka, 1988)). Little is known, however, about mineral concentrations and metal uptake capaticies in *Tetraselmis chuii*, while such information has been increasingly demanded in the assestment of algal food values (Costa and Franca, 1998). It is known that algal biomass can be utilized economically for the removal of heavy metals from industrial wastewater or other sources. One of the advantages of the bioremoval is use of cheap and renewable biomass. The microalgae *Tetraselmis* sp. cultured and used of live food for aquaculture, widely (Liao *et al.*, 1983). Copper is a constituent of many enzymes and is essential for their activities. It is involved in the activity of enzymes such as cytochrome oxidase, superoxide dismutase, lysyl oxidase, dopamine hydroxilase ve tyrosinase. And, considerably higher dietary copper levels (about 600 mg kg^{-1}) did not adversly affect rainbow trout (Lanno *et al.*, 1985).

Our concern in the present study is to document the influence of copper concentrations changes on the toxicity of copper to the green alga *Tetraselmis chuii*. To accomplish this, we elected to use the parameter of population growth by determining the number of cells, the amount of chlorophyll-a, measuring the copper accumulation and bioconcentration factor simultaneously for a period of days.

Materials and Methods

The culture of *Tetraselmis chuii* was obtained from the culture collection of the School of Ocean Science, University of Wales, Bangor. Cultures of *T. chuii* were grown in Conway medium of which copper concentration is 3.59 10^{-9} nM in 2 L polyethylene vessels containing natural seawater taken from the coast of Yumurtalık, North- Eastern Mediterranean, Turkey, in August 2001. The seawater (‰38 salinity) was filtered through 0.45 -µm pore size filters and autoclaved.

Stock copper solution ($CuSO_4$. $5H_2O$) was prepared in bidistilled water and sterilized by passing through Millipore membrane filters (0.22 µm), before supplementing to the culture medium. It was added to each test vessel containing seawater to achievement the required range of copper concentrations of 3.59.10^{-9} (control), 5.10^{-9}, 10.10^{-9}, 15.10^{-9} and 20.10^{-9} nM. They were named as group I (0.23 ppm Cu^{++}), II (0.32 ppm Cu^{++}), III (0.64 ppm Cu^{++}), IV (0.96 ppm Cu^{++}) and V (1.28 ppm Cu^{++}), respectively.

The temperature of the culture room dedicated for this microalgae production was kept at 24±1°C and the cultures were in continual light provided by 420 µmols^{-1}m^{-2}(LI-250 light meter). Under these conditions the pH was maintained between 7 and 8. Cells were counted using microscop with two replicates daily and growth curves were formed using the cell numbers. Growth rates were determined by using the equation K = log (N_1 / N_0) (3.322 / t) (Guillard, 1975). For chlorophyll-a analysis, a 5-ml aliquot of the microalgae solution was filtered through a 0.45 µm filter paper. The filter paper and its contents were placed in a test tubes, 5 ml of 90% methanol was added and the tube was heated in a water bath at 60-70 °C for 2 min. Following centrifugation at 3500 rpm, the supernatant was with-drawn by using a Pasteur pipette and transferred to a 1cm cuvette of the spectrophotometer. The absorption measurement was done at 665 nm and the chlorophyll-a content was calculated using the equation Chl. –a (µg/ml) = 13.9.D_{665} (Talling & Driver, 1974).

The microalgae samples were taken from cultures and separated from the mediums by centrifugation on the days of 3rd, 5th and 7th. For metal analysis, specimens were oven-dried to constant weight at 70 °C and digested in concentrated HNO_3 and $HClO_4$ on a hot plate (Jones & Case, 1990). All specimens were weighed both before and after drying. The digested samples were then diluted to 50 mL with 2.5% of HNO_3. The copper concentrations of these digest were determined by using UNICAM-929 atomic absorption spectrophotometer (AAS). Data were organised based on triplicate experiments and were presented as µg metal/g dry weight. The bioconcentration factors were calculated using the following formula (Sadiq, 1992):

BF= Metal concentration in algal cultures / Metal concentration in culture medium

The study was planned according to randomised complete block design. All data were analysed using the SPSSX 9.05 statistical package. The differences between mean values of each treatment (with triplicate samples) were determined at a probability level of p=0.05 using the program of Duncans multiple range test.

Results

Figure 1 gives the cell numbers in the experimental groups of *T. chuii.* The significant differences were determined between the cell concentrations of the experimental groups contained different amounts of copper ($p<0.05$). On the sixth and seventh days of the study, the cell concentration of the control group that contained 3.59. 10^{-9} nM Cu^{++} was found higher than the other groups. However group II and V of which copper concentrations were 5.10^{-9} and 20. 10^{-9} nM Cu^{++} followed the group control.

The growth rates of *T.* chuii that were incubated in different copper concentrations were calculated by using cell numbers (Table 1). While the cell numbers of 190x10^4 was determined for the control group on the 7th day of the experiment, 127x10^4 and 126x10^4 cells were determined for group II and V, respectively.

The mean specific growth rates (µ) computed for the five groups were 0.53, 0.53, 0.31, 0.56 and 0.61, respectively.

And, the growth rates of the groups were found similar ($p>0.05$).

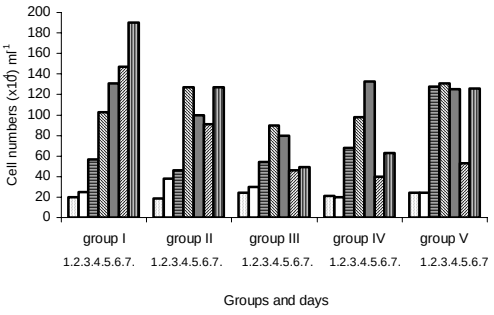

Figure 1. The cell numbers of *Tetraselmis chuii* groups for seven days.

The chlorophyll-a concentrations were evaluated for the 2, 3, 5 and 7th days. While the chl.-a concentrations of the cultures in which was added different amounts of copper were similar on the second day, the control and the group V that contains $3.59.10^{-9}$ and 20.10^{-9} nM Cu^{++} of chl.-a concentrations were found higher than the other groups on the 5th and 7th days ($p<0.05$) (Table 2).

Table 3 shows mean copper concentrations (µg metal/g d.w.) that *T. chuii* accumulated on the days of 3rd, 5th and 7th after the inoculation. The accumulations of the copper in the *T. chuii* cells in the groups were found different ($p<0.05$). It was determined that the accumulation of copper in groups IV and V were found higher than the other groups (Table 3).

The metal uptake capacity of *T. chuii* was also estimated based on bioconcentration factors (BF), which indicate a constant proportion between the internal and external metal concentrations. The graph of bioconcentration factors calculated for 3rd, 5th and 7th day of *T. chuii* in the different Cu^{++} concentrations is shown in Figure 2. While the Cu^{++} concentrations were increasing, it was observed that BF values were increasing staidly on 3rd day of experiment. BF values for group I to V were found 14.3, 103.7, 108.5, 150.5 and 179 on the 3rd day, respectively. For the groups (III through V) having higher Cu^{++} ion concentrations was determined smaller bioconcentration factors than that of the mediums having lower Cu^{++} ion concentrations on the 5th day of experimental period. Bioconcentration factor calculated for group IV was lower on the 5th day than the others (Fig. 2). The similar result was determined on the 7th day of the study. When we compared the results obtained for the other days, we did not realize any similarity all the experimental mediums.

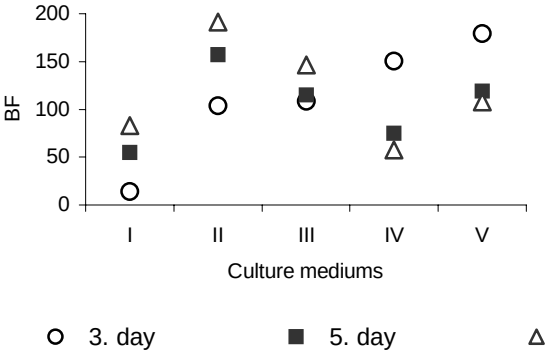

Figure 2. Bioconcentration factors (BF) of *Tetraselmus chuii* versus copper concentrations in the culture medium.

Discussion

Metal concentrations in cells vary considerably both with species and environment (Eisler, 1981). Copper concentrations, for example, were shown to be 65 and 652 µg g^{-1} respectively, in the marine microalgae *Dunaliella tertiolecta* and *Tetraselmis suecica* (Fabregas & Herrero, 1986). In the medium contained 0.20 and 1.00 mg L^{-1} Cu^{++}, *Pavlova viridis* accumulated 125 ±5.0 and 599 ±18 µg.g^{-1} dw respectively (Chen *et al.*, 1998). In the experimental mediums, the highest copper concentrations were found on the 5th day of incubation, while the lowest copper concentrations were always found in 7th day of incubation, expect control group.

Table 1. Cell numbers and growth rates of *T. chuii* cultures.

Days	Group I		Group II		Group III		Group IV		Group V	
	cell ml^{-1}	µ	cell ml^{-1}	µ	cell ml^{-1}	µ	cell ml^{-1}	µ	cell ml^{-1}	µ
1	$20x10^4$	-	$19x10^4$	-	$24x10^4$	-	$21x10^4$	-	$24 x10^4$	-
2	$25x10^4$	0.32	$38x10^4$	1	$30x10^4$	0.32	$20x10^4$	-0.07	$24 x10^4$	0
3	$57x10^4$	1.18	$46x10^4$	0.27	$54x10^4$	0.84	$68x10^4$	1.76	$128x10^4$	2.41
4	$103x10^4$	0.85	$127x10^4$	1.46	$90x10^4$	0.73	$98x10^4$	0.52	$131x10^4$	0.03
5	$131x10^4$	0.34	$100x10^4$	-0.34	$80x10^4$	-0.16	$133x10^4$	0.44	$125x10^4$	-0.06
6	$147x10^4$	0.16	$91 x10^4$	-0.13	$46x10^4$	-0.79	$40 x10^4$	-1.73	$53 x10^4$	-1.23
7	$190x10^4$	0.37	$127x10^4$	0.48	$49x10^4$	0	$63 x10^4$	0.65	$126x10^4$	1.24

Table 2. The chlorophyll-a concentrations of *T. chuii* cultures.

	Chlorophyll-a values (mg L^{-1})				
Days	Group I	Group II	Group III	Group IV	Group V
2th day	0.17	0.17	0.18	0.13	0.15
3th day	0.34	0.26	0.21	0.38	1.25
5th day	1.00	1.34	0.68	0.58	1.72
7th day	1.37	1.02	0.67	0.53	1.50

Table 3. Accumulation of copper in *Tetraselmis chuii* from experimental groups (sample size = 3) (µg metal g^{-1} dry weight)

	Groups				
Harvest days	I	II	III	IV	V
3rd day	44.21± 11.61	391.78±103.67	575.69±161.19	1866.28±192.40	1874.47±711.8
5th day	61.56± 16.33	485.65±184.24	616.07±116.12	1940.10±128.08	2173.03±611.66
7th day	52.93± 21.33	384.99±120.81	552.62±174.69	1611.08±205.18	1673.96±548.77

From an examination of the results it appeared that there were comparatively important differences between the Cu^{++} concentrations in *T. chuii* cells measured at the end of the seven days. It was found that the rate of the cell division was higher in group I. However, it wasn't observed that the inhibition of cell division due to Cu^{++} added in the other mediums. The growth rates of the cultures were found similar. As a result of this study, it can be said that *T. chuii* is able to bind the copper in the culture media. Lopez-Suarez *et al.* (2000) were carried out to determine the ability of *Chlorella vulgaris* to accumulate heavy metals in solution of which Cu^{++} concentration was 0.639 mg L^{-1}. It was found that the binding efficiency was remarkable for Cr and Cu at pH 8 and, Cu has a binding capacity independently of the pH value showing at pH 6 and 8 a100 % binding percentage. Rai *et al.*, (1993) compared the growth, photosyntesis and the other parameters of an acid tolerant starin and wild-type strain of *C. vulgaris* exposed to Cu, 2 mg L^{-1} of $CuCl_2$. $2H_2O$ at different pH values (pH 6.8, 5.0, 4.0, 3.5). It was found that the chl.-a concentration was higher than the other groups at pH 6.8 in wild strain. Pistocchi *et al.* (2000) were carried out the study at which several diatom and dinoflagellate species grown in batch cultures in f/2 medium were exposed to two different concentrations of Cu^{++} and Cd. As a result of that study, it was concluded that the species able to tolerate the highest Cu^{++} concentrations (in order of 0.2-0.5 mg L^{-1}) were diatoms, while some diatoms and all the dinoflagellates were inhibited by concentrations between 0.01 and 0.05 mg Cu L^{-1}.

It can be said that copper accumulated in the cells wouldn't cause any detrimental effect. Although increasing metal concentrations in the culture mediums, *T. chuii* grew well. The capacity of *Tetraselmis chuii* for copper accumulation has been demonstrated to be flexible in response to external copper concentration. On the third day of the investigation where the culture medium containing 5.10^{-9},10.10^{-9},15.10^{-9} and 20.10^{-9} nM Cu, copper accumulation in the algal cultures raised 9, 13, 42, 43 times, respectively, compared with the control. On the basis of this study, it may be concluded that *T. chuii* cells that able to uptake relatively high concentrations Cu^{++} appears to be promising to remove copper from the solutions. Microalgae have been considered to have better advantages over the traditional methods in elimination of heavy metals from aquatic systems (Sandau *et al.*, 1996a). Further investigations may be done when the alga thought to be as an agent for copper removal in waste-water treatment.

Heavy metals from polluted aqueous systems may be removed by phytoplanktonic algae. It is concluded that this method, including the seperation of the metal saturated algae from the medium, is an economic method for removing heavy metals from waste waters, resulting in high-quality reuusable effluent water and valuable algal biomass, which could be used for different purposes for example biofertilizer, living food or biogas (Becker, 1994; Richmond, 1986; Vonshak, 1990). As a result, *T. chuii* cells removed copper from the medium and harvested can be evaluated for reusing.

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