Bioaccumulation of Cadmium in Marine Diatom: *Thalassiosira allenii* Takano

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Abstract

The present study achieved cadmium bioaccumulation ability using growth kinetics and ICP-OES for Cd measurements in marine diatom *Thalassiosira allenii* Takano. *T. allenii* cells were exposed to different cadmium concentrations (0.5, 1, 2, 5, 10, 25, 50, 75, and 100 mg. Cd L^{-1}) for 4 days. *T. allenii* cells were seen to have bioaccumulation ability both in cells and cellular surfaces. The cadmium uptake of *T. allenii* species followed a linear trend for most of days for ≤ 25 mg L^{-1}Cd concentrations while the cell growth was inhibited at concentrations of >25 mg L^{-1}Cd. *T. allenii* removed much of cadmium by taking it into the cells in ≤ 10 mg. L^{-1} Cd concentrations in the medium. While the adsorption on the cell surfaces is dominant in cells on the first day, the amount of Cd allowed in the cell has become dominant beginning with the second day. The growth rate decreased with increasing Cd concentration and remained stable at about 0.1-0.2 day^{-1}. However up to the 5 mg. L^{-1} Cd concentration, the growth rates are not different from the control and continued for about 0.3-0.4 day^{-1}. When *T. allenii* species is used in the bioremediation systems, this limit value does not affect growth of the species.

Keywords: Bioaccumulation, bioadsorption, cadmium, *Thalassiosira allenii*.

Denizel Diyatomda Kadmiyum Biyoakümülasyonu: *Thalassiosira allenii* Takano

Özet

Bu çalışmada, denizel diatom *Thalassiosira allenii* Takano türünün kadmiyum biyoakümülasyon kabiliyeti, büyüme kinetiği ve ICP-OES ile Cd ölçümleri kullanılarak araştırılmıştır. *T. allenii* hücreleri 4 gün için farklı kadmiyum konsantrasyonlarına (0.5, 1, 2, 5, 10, 25, 50, 75 ve 100 mg. Cd L^{-1}) maruz bırakılmıştır. *T. allenii* hücrelerinin, kadmiyumu hem hücre içinde ve hem de hem de hücre yüzeyinde biyoakümülasyon yeteneğine sahip olduğu görülmüştür. Hücre büyümesi >25mg L^{-1}Cd konsantrasyonlarında inhibe olurken, *T. allenii* türünün kadmiyum alımı, $\leq$25mg L^{-1} Cd konsantrasyonları için çoğu gün boyunca doğrusal bir trend izlemiştir. *T. allenii,* ortamdaki $\leq$10 mgL^{-1} Cd konsantrasyonlarda, kadmiyumun çoğunu hücre içine alarak uzaklaştırmıştır. Hücrelerde ilk günde hücre yüzeyine adsorpsiyon dominant iken, 2. günden itibaren hücre içine alınan Cd miktarı dominant olmuştur. Artan Cd konsantrasyonuyla birlikte büyüme hızı azalmış ve 0,1-0,2 $gün^{-1}$ civarında sabit kalmıştır. 5 mg L^{-1} Cd konsantrasyonuna kadar ise, büyüme hızları kontrolden farklı değildir ve 0,3-0,4 $gün^{-1}$ civarında seyretmiştir. Bu sınır değer, *T. allenii* türü bioremedasyon sistemlerinde kullanıldığında türün gelişimini etkilemeyecektir.

Anahtar Kelimeler: Biyoakümülasyon, biyoadsorpsiyon, kadmiyum, *Thalassiosira allenii*.

Introduction

Metal pollution with increasing intensive anthropogenic activities entries into marine environments has been a major environmental concern for the world. Trace metals (zinc, iron, manganese, copper, cobalt etc.) are essential micronutrients for phytoplankton and other organisms (Frausto da Silva and Williams, 2001), but they are known to have toxic effects on organisms at high concentrations (Allen *et al.*, 1980; Genter, 1996; Walker *et al.*, 2001). Other metals such as lead, silver, cadmium, and mercury are not essential or beneficial, and have been found to be toxic for living organisms at very low concentrations (Torres *et al.*, 1998; Fehrmann and Pohl, 1993; Jennings and Rainbow, 1979; Li, 1980).

Though cadmium is one of the most dangerous heavy metals in aquatic ecosystem, it has been found that there exists a metal enzyme characterized in a

marine diatom species called *Thalassiosira weissflogii,* and that cadmium has been used in biological processes (Lane *et al.*, 2005). *T. weissflogii* species have genes for two separate carbonic anhydrase enzyme. Carbonic anhydrase enables a carbon dioxide to be produced by carbonic acid and/or bicarbonate. The enzyme embodies Zn or Cd. Researchers point out that rather than an environmental waste, the cadmium is environmentally necessary element in global sensibility (Lane *et al.*, 2005). The main sources of cadmium in the marine environment are intensive anthropogenic activities, including industrial and urban development.

Phytoplankton is indisputably the major component of marine food chain. In addition, phytoplankton can develop various strategies to handle the presence of elevated environmental metal levels (Wang and Wang, 2009). Disposals to the external environment of the phytochelatins that are inner cell chelators via accumulation and exudation (Rausser, 1995; Lee *et al.*, 1996) enable a metal detoxification for the phytoplankton. Phytochelatins are peptides with small sulfhydryl. Laboratory studies using Cd indicate that there exists a phytochelatin export in a marine diatom *T. weisflogii*, (Lee *et al.*, 1996), and electrochemical analyses applied to coastal surface water demonstrated that it varied with unidentified dissolved thiols' Chl *a* concentrations (Al-Faramati and Van den Berg, 2001). However, phytochelatins' removal rates in existence of metals is 5 times lower (Wei and Ahner, 2005). Metal-ligand complexes are known to be less sensitive to degradation than free ligands (Satroutdinov *et al.*, 2000; Vandevivere *et al.*, 2001). Bioaccumulation by phytoplankton is therefore of great significance to understand marine processes concerning heavy metals accumulation and biomagnifications throughout the food chain. Laboratory trials on heterotrophic protists (Storm *et al.*, 1997) and mixotrophic nanoflagellates (Twiss and Campbell, 1995) which are the main consumers of phytoplankton in natural environment determined that planktonic grazers are the potential main resource of the dissolved organic carbon in marine environment, and resorption of the Cd, which is regenerated as dissolved phase, by plankton is less available than the added inorganic metal types. In other words, the grazing activity serves to increase the trace metal retention time of the seas.

The purpose of the present study is to determine changes in growth rate as a parameter to monitor impact of Cd when the marine diatom *T. aleni* is exposed to Cd concentrations in a broad range and analyze the short term uptake kinetics and adsorption pattern on the cell surface during the exposure time.

Materials and Method

Diatom Culture and Experiment Setup

The marine diatom *T. allenii* was originally isolated from the surface water of inner part of Izmir Bay (Turkey) using the dilution method under conditions described by Şişman Aydın *et al.* (2009). *T. allenii* cells were cultured in batch conditions in natural seawater (salinity of 33‰; and initial pH of 8.2). Seawater was collected from clean zones of Izmir Bay to minimize the influence of anthropogenic activies. It was filtered through 0.2 µm filter cartridge (Sartorius-Sartobran® P) and sterilized at 121°C for 30 min before it was used in the experiment. The diatom stock cultures were maintained in the f/2 enrichment medium (Guillard, 1975).

A stock solution of cadmium was prepared by dissolving $CdCl_2$ in Milli-Q water (18.2 MΩ) to give a final concentration of 10 mg L^{-1} of Cd^{2+}. For the experiment, appropriate volumes of stock solution was added to seawater to obtain final cadmium concentrations of 0.5, 1, 2, 5, 10, 25, 50, 75 and 100 mg Cd L^{-1}. Control cultures without cadmium were also included. The assays were carried out in triplicate in borosilicate glass bottles of 500ml natural seawater, into which one added N, P, Si and vitamins (at f/2 levels), the trace metals (at f/10 levels) a suitable volume of the stock solution of cadmium with *T. allenii* (in mid-exponential growth phase) at an initial cell density of 6.84×10^4 cells ml^{-1}. The bottles were soaked in 10%(v/v) nitric acid for at least 24 h and finally rinsed several times with Milli-Q water (18.2 MΩ) before it was used. Cultures were maintained at a constant temperatures of 11±0.5°C under illumination of 2700 lux, with a dark:light cycle of 12:12h, for 96 hours. Cultures were gently shaken daily to ensure homogeneous exposure to the metal. The measurements were assessed as the amount of Chl *a* by the Turner Designs 10-AU Field Flourometer (Brand and Guillard, 1981). The chlorophyll based specific growth rates were calculated following Guillard (1973).

$$\mu = \frac{1}{(t_2 - t_1)} \times \log_2 \left(\frac{N_2}{N_1} \right)$$

where μ: specific growth rate (day^{-1}), N_1: the Chl *a* measure at the beginning of the exponential growth phase, µg L^{-1}; N_2:Chl *a* measure at the end of the exponential growth phase, µg L^{-1}, t_1: the time period during which N_1 was determined, t_2: the time period during which N_2 was determined.

Determination of Cadmium Removed by the Cells

The cadmium removed by the microalga *T. allenii* was evaluated using a modified method described by Torres *et al.* (1998).

Total Removed Cadmium

Total cadmium in the cells was determined by filtration of 15 ml aliquots from each culture of *T.*

allenii. Each aliquot was filtered through the two superposed 0.45μm filters (Millipore). Filters were separately digested for 24 h with 1 ml of 15 M HNO_3 and 0.5 ml of 72% (w/w) $HClO_4$ until there appeared strong fumes of $HClO_4$ (24h). Cadmium was measured in both filters and the lower filter used as blank.

Intracellular Cadmium

A 15 ml aliquot from each microalgal culture was centrifuged at 6000 g for 10 minutes, the pellet was resuspended for 20 minutes in 15 ml of a solution containing 0.02 M Ethylenediaminetetraacetic acid (EDTA) dissolved in natural seawater, the cells were centrifuged at 6000 g for 10 minutes and the pellet was washed through natural seawater and centrifuged (wash procedure was repeated two times). The EDTA washing removed cadmium adsorbed on the cell surface, thereby allowing only intracellular cadmium to be measured. The washed pellet was digested as in total cadmium determination.

Bioadsorbed Cadmium

The determination of cadmium bioadsorbed on the surface of algal cells was calculated by subtracting the intracellular cadmium concentration from the total removed cadmium concentration (bioadsorbed cadmium = total cadmium−intracellular cadmium).

Cadmium Measurement

Digested samples were brought to a final volume of 5ml with Milli-Q water. Finally, cadmium ions in the samples were measured by ICP-OES. The limit of detection (LOD) was $0.0001 mgL^{-1}$.

Results and Discussion

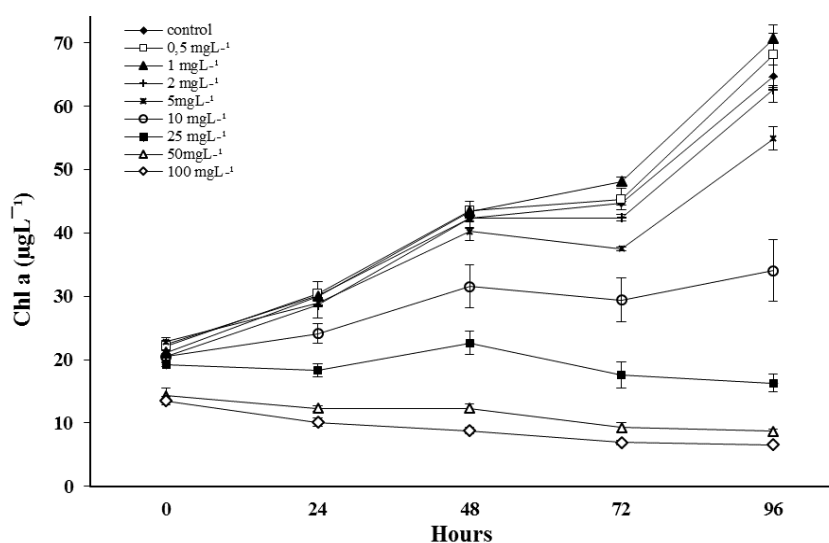Figure 1 exhibits the growth curves obtained for *T. allenii* exposed to the different concentrations of Cd. The statistical analyses showed a significant effect of cadmium concentrations of $> 5 mg L^{-1}$ on Chl *a* content of *T. allenii* under Fisher's least significant difference (LSD) procedure ($P<0.05$). There were no significant differences between control cultures without cadmium and those with 0, 5, 1, 2, and 5 mg L^{-1} ($P>0.05$), which indicates that the cadmium concentration was not inhibitory to *T. allenii* growth. A major decline ($P<0.05$) emerged in Chl *a* (biomass) and specific growth rates in $>5 mg L^{-1}$ Cd concentrations (Figure 2). However, any statistical differences ($P>0.05$) were not observed between the growth rates of high concentrated experiment series which were for $0.1- 0.2 day^{-1}$. When the experimental series ranged in $0.5-5 mg L^{-1}$ Cd are compared to the control group, an increase will be found to be in the growth rate but not statistically significant different (Figure 2). This limit value will not affect diatom *T. allenii*'s growth in treatment systems. In other words, in $\leq 5 mg L^{-1}$ Cd concentrations with a growth rate about $0,4 day^{-1}$ corresponds to a 2 day-hydraulic retention time of the continuous flow systems in a steady state. In $\geq 10 mg L^{-1}$ Cd concentrations however, the treatment system can be kept in a steady state with a retention time for about 5-10 days or its performance improved by dilution.

As a function of exposure time in different Cd concentrations, the total amount of Cd adsorbed by the cell surface is shown in Figure 3. The amount of adsorbed Cd per unit biomass attained a maximum and cells reached the highest amount of Cd for all the concentrations at the end of the first day. The amount of bioadsorption determined in 5 and 10 mg L^{-1} Cd concentrations were found to be statistically significant from the other concentrations ($P<0.05$) on the 1st day. In Figure 3, the decrease in the amount of adsorbed Cd observed following the first day explains that Cd uptake in the cell started as in Figure 4. The observed decrease in 2 mg L^{-1} Cd concentration on the 4th day might have resulted from a biomass which
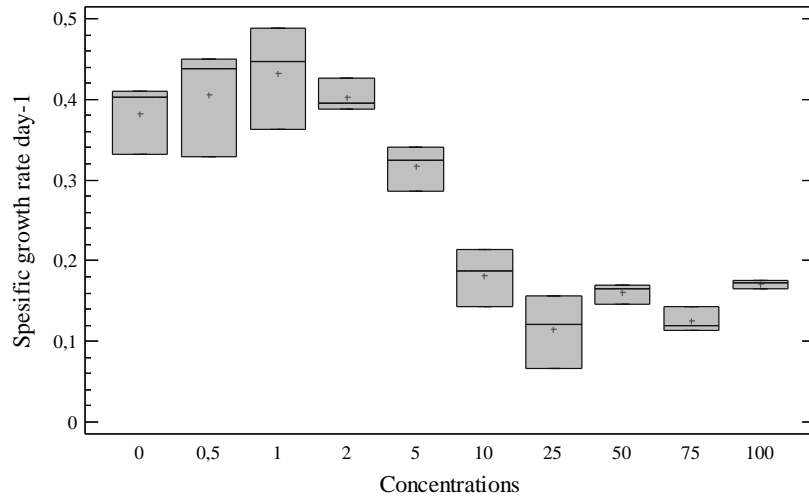

Figure 1. Growth curves of *T. allenii* cells exposed to different cadmium concentrations.

Figure 2. Spesific growth rates (day^{-1}) of *T.allenii* cells exposed to different cadmium concentrations (mg L^{-1}).
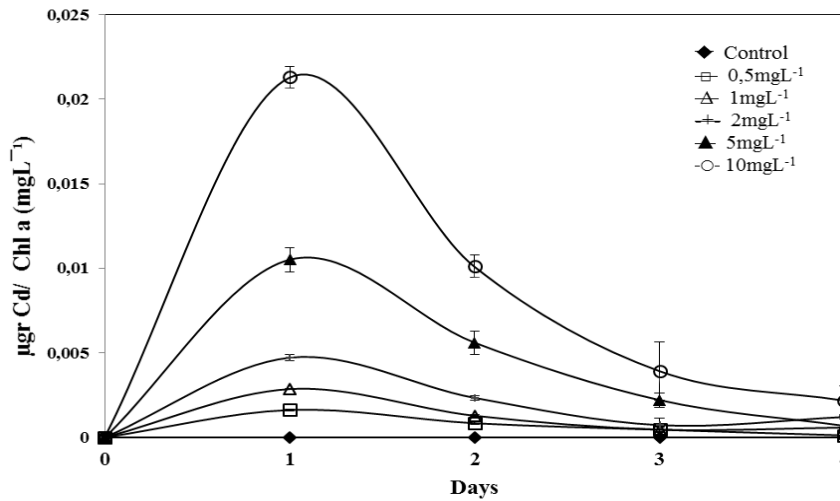

Figure 3. Cadmium bioadsorbed by *T. allenii* cell surface exposed to different Cd concentrations in the medium as a function of time of exposure.
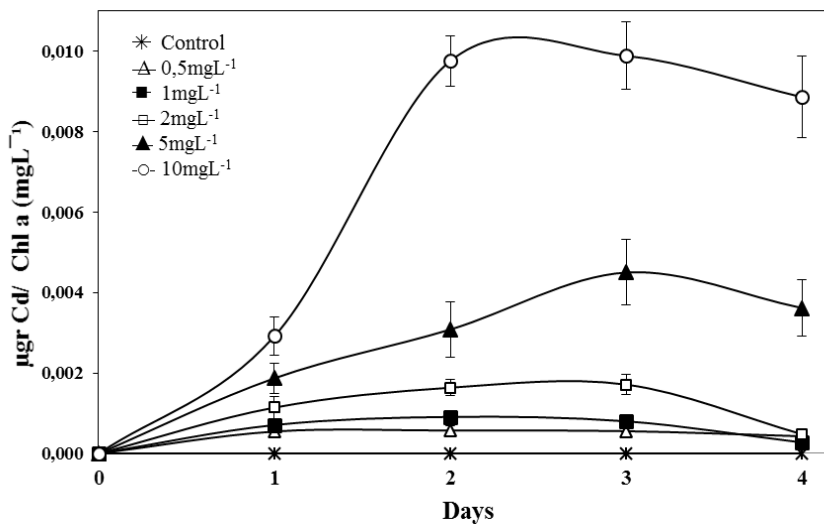

Figure 4. Cadmium removed by uptake of the metal into *T. allenii* cells (intracellular) as a function of time of exposure.

increased with a higher growth rate (Figure 4.). The decrease can have been caused by the environment and inner cell concentration difference (Torres *et al.*, 1998) and detoxification of the Cd (Rausser, 1995; Lee *et al.*, 1996). As with all the Cd concentrations, there was not a drastic change in the intracellular Cd concentrations per biomass after 2nd day.

The amount of cadmium accumulated within *T. allenii* cells after 1 day of exposure was higher than that adsorbed onto the surface for all the cadmium concentrations (Figure 4 and 5) which also applies to *P. tricornutum* cells (Torres *et al.*, 1998). After 2 days of cadmium exposure, difference between the amounts in total intracellular cadmium showed (P>0.05) a decreasing trend in all the concentrations (Figure 4). While the biomass increase rate was higher than uptake rate in ≤2mg L^{-1} Cd concentrations, the biomass growth rate was equal to uptake rate in 5 and 10 mgL^{-1} Cd concentrations. Intracellular Cd levels per observed biomass remaining constant, statistical decrease in 2 mg L^{-1} Cd experiment series on 4th day, phtyochelatin production and exudation to external environment of the *T. allenii* type when exposed to Cd stress (just like in other *Thalassiosira* genus members) may show that detoxification mechanism actually works. In other words, the Cd taken in inner cell via uptake creates a complex with phytochelatins and is returned to the environment by exudation. Blockage of the microgel formation from the phytochelatin-Cd complexes via UV-B radiation on surface waters and transferring them to a less available form for phytoplankton in nano size and cells consumed with grazing in natural environment contribute to the refractory organic carbon pool by returning them into fecal pellet (Orellana and Verdugo, 2003; Lee *et al.*, 1996). The, *T. allenii* species in works just as a natural treatment facility does in a marine environment due to the dissolved Cd becoming a fraction which cannot be further disintegrated and taken by phytoplankton.

The results of the study, point out that *T. allenii* cells are able to bioaccumulate cadmium intracellularly and adsorb it onto the cell surface. Figure 5 presents, the surface-adsorbed and the cell-absorbed amounts via uptake and the changes in their sums for 4 days as a function of Cd. The total Cd and intracellular Cd accumulation increased with cadmium, which is consistent with results from previous studies (Torres *et al.*, 1998; Perez-Rama *et al.*, 2010). At lower cadmium concentrations <25 mg L^{-1}, the uptake of cadmium followed a linear trend for all days of culture. While adsorption was dominant in
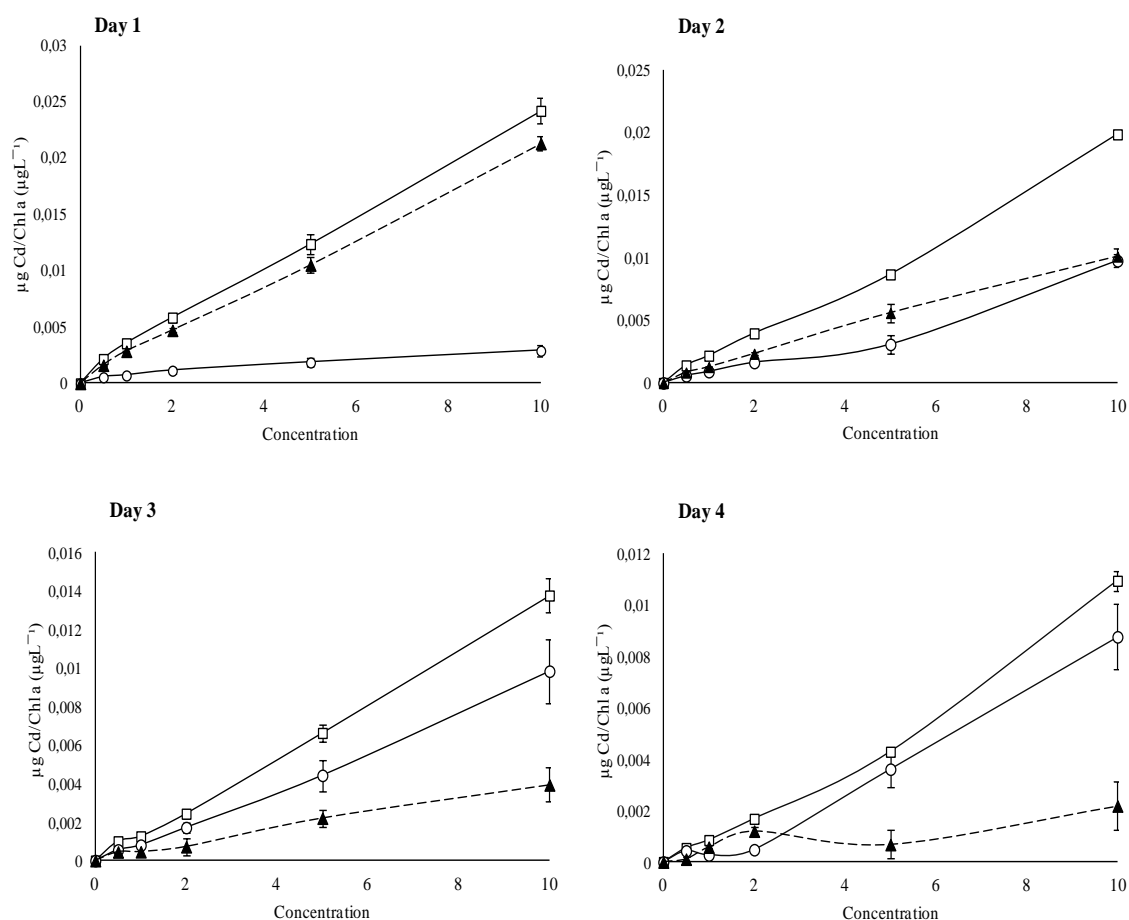

Figure 5. Cadmium removed in the different cell fractions as a function of the concentration of cadmium in the medium for each day of exposure:-□-, total Cd*;*-○-, intracellular Cd; -▲- adsorbed Cd.

cells on the 1st day, the amount adsorped onto the cell surface became equal to the Cd taken in the cell. However on the 3rd and 4th days, the amount of Cd absorbed into the cell became dominant. In other words, it clear that if *T. allenii* type is used in treatment systems, a 3 or 4 day hydraulic retention time is needed for an active Cd removal.

T. allenii accumulated cadmium mainly within the cell (P<0.05) at cadmium concentrations in the medium ≤10 mg L^{-1}. Similar patterns were previously obtained for other heavy metals and other microalgal species (Garnham *et al.*, 1992; Torres *et al.*, 1998; Wang and Wang, 2008; Perez-Rama *et al.*, 2010), the highest amount of cadmium was located on the cell surfaces for all cadmium concentrations assayed on the 1st day. However, in the series containing <2 mg L^{-1} Cd, intercellular Cd's higher than those adsorbed on cell surface can be caused by the growth strategy of *T. allenii* species to adjust itself to Cd. The previous study reveals that the species increases its nutrient uptake quota by increasing its growth rate and biovolume for adaptation to nutrient pulse periods within range of 12-96 hours (Şişman Aydın, 2012). The more cadmium concentration in the medium, the more adsorbed-intracellular Cd difference increased (P<0.05), which means that much of the cadmium was removed by its uptake into the cell at all cadmium concentrations (≤10 mg L^{-1}). This result is in accordance with Perez-Rama *et al.* (2002) who found that the amounts of Cd taken up by intracellular were quite higher than those in adsorbed on the surface of *Tetraselmis suecica* cells.

The accumulation of cadmium in cells by microalgal cells is a two-step process. The first is a rapid energy-independent phase in which cadmium is adsorbed on the cell surface (biosorption) and the second that cadmium is taken up into the cells (bioaccumulation) (Torres *et al.*, 1998). The results of the current study represent the both mechanisms very well (Figure5). On the other hand, the non-living biomass is more preferred than living cells in the metal removal processes. However, the metal is not uptaken into cells by non-living biomass, since metals are adsorbed only on the algal surface (Fehrmann and Pohl, 1993). Previous studies (Matsunaga *et al.*, 1999; Perez-Rama *et al.*, 2002) and the present one using *T. allenii* indicate that intracellular Cd levels are higher than the bio adsorbed, which indicates that living biomass of *T. allenii* cells would be more effective for Cd removal than non-living biomass.

T. allenii is a marine diatom whose cells are capable of bioaccumulating and bioadsorbing cadmium, thus allowing it to be removed from the medium. These cells showed a high ability to remove cadmium and a significant tolerance to cadmium amount. It is known that microalgae cells could act by means of the bioaccumulation process when the metals are at low concentrations. These results indicate that *T. allenii* species can be used in bioremedetion processes of cadmium-contaminated marine environments.

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