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Using Live Microalgae *Tetracystis isobilateralis* R.M. Brown & H.C. Bold (Chlorococcales) Cells in the Removal of Heavy Metals

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Keywords: Tetracystis isobilateralis, removal, heavy metals, microalgae, living cell.

Abstract
Microalgae can bind the materials, especially heavy metals, with toxic effects in their natural environment with the help of various metabolites in their system and can embody them as a reduced material. Starting out with this property of microalgae to eliminate heavy metals, the living microalgae cells *Tetracystis isobilateralis* R.M.Brown & H.C. Bold were tested as the model organism in heavy metal removal in this study. Co (II), Cd (II), Pb (II), Zn (II) and Mn (II) were used as metal ions. The heavy metal salts were added into the habitat of the microalgae in different concentrations and the time-dependent absorption capacity was observed. The results obtained showed that where it is alive, microalgae *Tetracystis isobilateralis* has the capacity that allows it to be used in heavy metal absorption.

Keywords: *Tetracystis* isobilateralis, removal, heavy metals, microalgae, living cell.

Introduction

In the past 150 years, penetration of heavy metals into the aquatic environment increased in parallel with the speed of global industrialization. Many heavy metals, while being a micronutrient for biological systems, can be toxic for many aquatic life forms, even in minimal levels. As a result of human activity, precipitation, absorption and particle settlement occurs, which cause metals to be stored in the deposits of streams and seas. The level of existence and the variety of algae in the water play an important role in the determination of the water quality of seas, freshwater lakes, water basins and rivers. Determining the algal vegetation is simpler and more economic in comparison with many chemical methods used in measuring water quality. Many tangle taxons, small and large, are identified to be a potential heavy metal biosorbant. Today, there are many areas where commercial earning could be obtained through algae cultivation. Some of these areas are wastewater treatment, chemicals production and areas for meeting feed and food demand (Borowitzka and Borowitzka, 1988).

There are many studies about using microalgae in the removal of heavy metals (Bates et al. 1982; Mahan et al., 1989; Garnham et al., 1992; Zhou et al., 1998; Gupta et al., 2001; Schmitt et al., 2001; Klimmek et al., 2001; Rangsayator et al., 2002; Gin et al., 2002; Akhtar et al., 2004; Pena-Castro et al. 2004; El-Sheekh et al., 2004; Han et al., 2007; Nakiboglu and Sevindir, 2006; Katircioglu et al. 2008).

Absorption of heavy metals by tangles occurs through four different mechanisms. The first one is the ion substitution mechanism which is a substitution mechanism between light metals in algae such as K^+, Na^+, Ca^{+2}, Mg^{+2} and heavy metals in the environment. The second one is the coordination and complex formation mechanism which occurs through complex formation on cell surface as a result of the interaction between metals and active groups. The third one is the precipitation mechanism that occurs when the pH of the solution that forms during the cellular metabolic process or when the ionic concentration in wastewater rises to the level of saturation index. The final mechanism occurs in four different ways: the micro-precipitation which is the case of metal intake, chelation (a method used in the removal of toxic effects) which occurs as certain living microalgae such as *Chlorella minister* carry the metals by binding the polysaccharides that they use as reductors and place them inside the cell (Brinza et al., 2007).

The factors affecting heavy metal biosorption can be environmental factors such as pH, temperature, mixing (mechanical and air), flow regime (static or dynamic and laminar or turbulent flow for column reactors) and reactor types (group reactor or stable, fluid, enlarged bed column reactors) as well as biomass factors such as types of radical groups on cell wall, use of living or non-living tissues, exposure of cells to heavy metals during early phase, the size of the algal tissue or parts of the algae used in different growth stages, food and oxygen requirements, cell size and biomass density, tolerance of biomass, selectivity of biomass and thickness at where the binding occurs (Ting et al., 1989).

As a result of the literature review, it is found that no studies have been made on microalgal biotechnological field about the species *Tetracystis isobilateralis* R.M.Brown & H.C. Bold.

Materials and Methods

In this study, the taxon *Tetracystis isobilateralis* R.M.Brown & H.C. Bold. was used, which was sent to Ege University Microalgae Culture Collection and then given the catalog number MACC63. The light microscope image of the microalgae is shown in Figure 1.

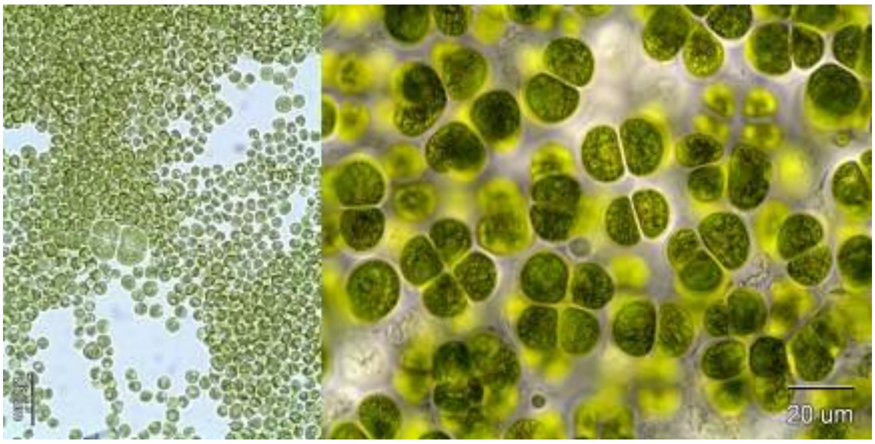

Figure 1. *Tetracystis isobilateralis* cells and aplanospores.

In the determination of absorption capacity of the algae, the metal ions Co (II), Cd (II), Pb (II), Zn (II) and Mn (II) were used. The cells cultivated in OTM (Our Tetracystis Medium) with pH 8.7 and at room temperature were put in Erlenmeyer flasks with a volume of 500 ml (30.1×10^6 ml/cell, dry weight 0.462 gr/l) after reaching the stable phase. The nutrients in OTM are shown in Table 1. The pH value of the cultivation medium was set to 6.5 with 1N HCL. The stock solutions were prepared with salts $CdCl_2.2$, 5H2O, $CoCl_2.6H_2O$, $PbCl_2$, $ZnCl_2$ and $MnCl_2.4H_2O$. Three different concentrations (5, 20 and 40 ppm) of each metal ions from each of these stocks separately were added in the cultivation medium. Samples were taken from the cultures at minutes 0, 10, 30 and at hours 1, 2, 12, 24. These samples were filtered in Whatman GF/C filters and analyses were carried out on the fluid part filtered. In order to determine the amounts of absorption, the analyses were carried out in the central laboratories in Çanakkale Onsekiz Mart University using the device ICP-AES and the amounts of absorption of heavy metals were determined in 3 repetitions.

Table 1. OTM nutrient composition (mg/l)

Nutrients	Amount(mg/l)
$Na_2MoO_4.2H_2O$	0.008
$NaNO_3$	500
NaCl	25
KH_2PO_4	262.5
K_2HPO_4	112,5
$MgSO_4.7H_2O$	75
$CaCl_2.2H_2O$	25
$FeCl_3.6H_2O$	0.194
$ZnCl_2$	0.01
$MnCl_2.4H_2O$	0.082
$CoCl_2.6H_2O$	0.004
Soil extract	20ml
pH	8.7

Results

Tetracystis isobilateralis species was cultivated in the medium where it showed maximum growth (OTM) which we determined before using cultivation studies and the heavy metal absorption of this type of microalgae was studied.

At certain amounts, the heavy metal ions in natural water springs, well waters and wastewaters have a toxic effect for the aquatic species. Due to their toxic effects, the heavy metals threaten the ecosystem and directly or indirectly human health. It is therefore required to make analysis on the waters from natural springs before making them available for the living and to reduce the contamination level of the wastewaters from contaminated sources below the predetermined contamination levels before releasing them to the environment. Metals such as copper, lead, zinc and chromium can be separated through precipitation following neutralization from the aquatic medium. Due to some disadvantages of the conventional methods used in the removal of heavy metals out of aquatic mediums, there has been research on alternative methods in recent years. One of these new methods is the biosorption method. In this method, the removal is achieved through taking the metal ion into the microorganism system as a result of various mechanisms occurring between the living or non-living microorganisms and the metal ions. The non-living microorganisms are more widely used because they don't require a continuous supply of nutrients and they are more workable. The biosorption method is an advantageous method since it is economical and has the capacity to effectively eliminate the metals even in waters with pretty diluted heavy metal content (Aslan et al., 2007).

Microalgae are the prevailing groups of living among those which can carry out the absorption function. The absorption is affected by physical, chemical and biological properties such as surface properties and volumetric dimensions of the microorganism, temperature, pH and initial metal ion concentration of the solution, mixing speed and microorganism concentration (İleri, 2000). The fundamental reasons for using living microorganisms in removing heavy metals from aquatic medium are that the metal ions with low concentrations can be easily absorbed by the cells and they can be produced fast in a cultivated medium (İleri et al., 1994).

The biological molecules to be effectively used in metal biosorption have a wide spectrum. In particular, various bacteria, yeasts and fungi species can be used, including the algae in the microorganism group (Sağlam and Cihangir, 1995).
The time-dependent amounts of absorption of the metal ions cadmium (Cd^{+2}), cobalt (Co^{+2}), manganese (Mn^{+2}), lead (Pb^{+2}) and zinc (Zn^{+2}) in the living culture are given in Figure 2.

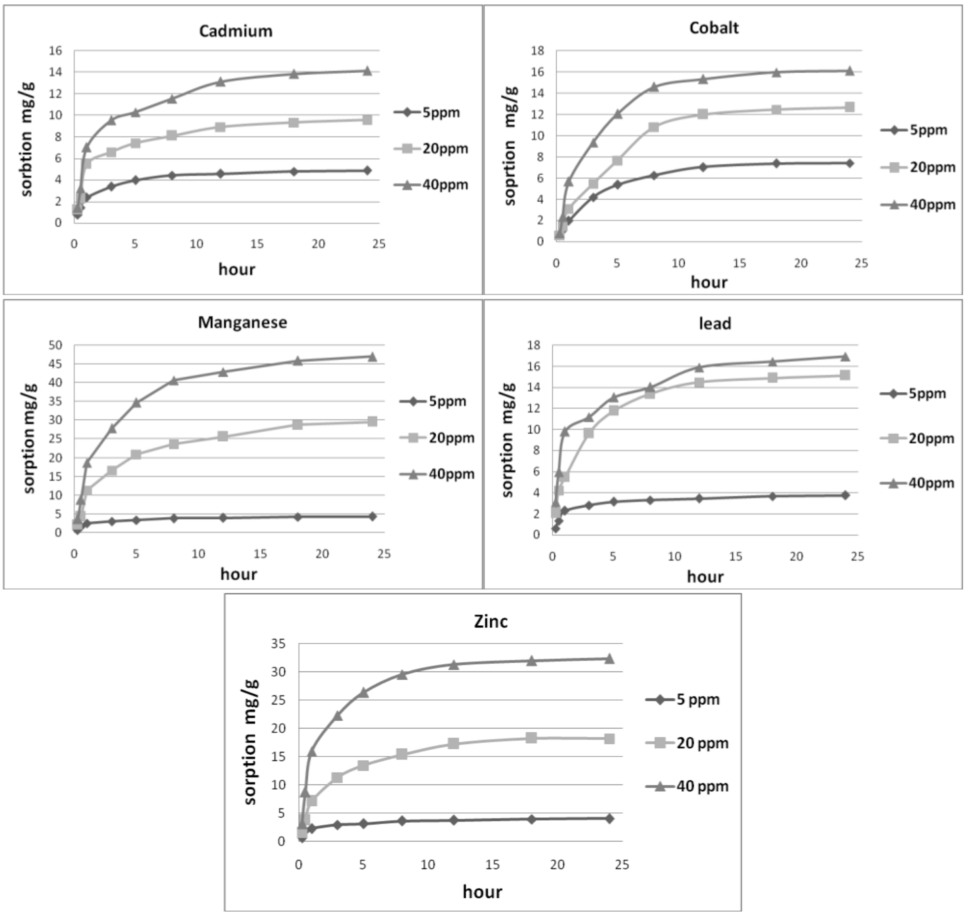

Figure 2. Removal diagram of metals cadmium,cobalt, manganese, lead, zinc.

Discussion

In the study made, it is observed that the removal of heavy metals by the algae concerned in OTM changes depending on the metal density in the medium and time. When the diagrams which show the time-dependent removal of heavy metals are examined, it can be seen that the heavy metal with the highest removal amount is manganese and zinc, lead, cobalt and cadmium follows it in order. All these data show that the taxon *T. isobilateralis* can be used in the refinement of heavy metals in wastewaters through biosorption. The study results show that the taxon *T. isobilateralis* can be used in microalgal biotechnological studies due to its heavy metal absorption capacity. Considering the increasing world population, inadequacy of cultivated areas or failure to utilize them effectively and low quality agricultural products, this microalgae type which can be presented as the alternative of a few nutritional algae types can be used in the biological removal of heavy metals released into the water sources through natural and human activity.

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