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Full Length Research Paper

Hg, Cd and Pb heavy metal bioremediation by *Dunaliella* alga

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Environmental pollution with heavy and toxic metals, produced by mining, processes of metallurgy and other chemical industries is a worldwide problem to human health and environment because of their high concentrations in the biological food chains and long term presence in the ecosystems. The present study discusses the heavy-metal elimination by the bioremediation capability of *Dunaliella* algae's. As the elimination of low metal-concentrations (lower than 50 mg/l) from water with usual chemical processes; such as precipitation with lime or ion exchange are not economical, this is supposed that such metals could be eliminated by this alga with high yields. Also, in these methods with physicochemical basis the toxic remedies are remained but in bioremediation ones there is no need for such transportation or other additional removal processes. This paper studies the bioremediation of cadmium (Cd), plumb (Pb) and mercury (Hg) at different concentrations and under different conditions by *Dunaliella* alga. It was shown that the toxic effects of Hg are higher on this organism as compared to those of Cd and Pb. This is vivid that *Dunaliella* is highly tolerant to the ascending concentrations of heavy metals and their absorption in aquatic media. This approves the usage of *Dunaliella* as useful equipment for the elimination of heavy metals environment.

Key words: *Dunaliella*, bioremediation, heavy metals, mercury (Hg), cadmium (Cd), plumb (Pb).

INTRODUCTION

As the small nations growing urban, the industrial, manufactural and official leftovers or sewage have been removed into the rivers and these were supposed to be aquatic animals' food or could have induced their growth and development. So the Mississippi river has changed to a canal full of urban and industrial wastes. Finally in 1928, the removal of wastes into rivers of some states of United States was prohibited and since 1965, special rules were established for removing various types of pollutants and urban and industrial wastes (Berlin et al., 1985; Din et al., 2001; Correia et al., 2000; Dixie et al., 1998). In developing countries, inefficient implementation of laws, weak supervisions and unconcentrated industry growth have caused rising pollution of water sources specially rivers. Any pollution in the upstream trilling water could cause health hazards to different consumers such as animals, human and even the industries (Correia et al., 2000; Dixie et al., 1998; Derek, 1999; Dias et al., 2002; Ballantyne et al., 1999; Ballantyne et al., 1999). So it is important to prevent destruction of water resources by the characterization, and measurement of pollutants and more administration of rules. Heavy metals such as plumb, cadmium, mercury, nickel and zinc in breads and aluminum, arsenic, cupper and iron in salt are mentioned as environmental pollutants, which may cause severe poisoning conditions (Derek, 1999; Dias et al., 2002; Ballantyne et al., 1999).

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Heavy metals and their elimination

Metals with atomic masses between 54.63 and 200.59 and special weights of more than 5 g/cm^3 are classified as heavy metals (Dias et al., 2002). These elements are natural components of earths' crust, which are released into environment because of the natural and human activities. In biology, heavy metals are the atoms with toxic effects such as: Al, As, Br, Cr, Co, Cu, Cd, Fe, Hg, Ni, Mn, Pb, Se and Zn. Volcanoes, burning of forests and aeration of stones and minerals are counted as natural polluting events (Fomina et al., 2005; Arshad and Shafaat, 1997; Çabuk et al., 2005; Clesceri et al., 1989). Digging and mining processes of metal melting factories, burning of wastes, usage of fossil fuels in companies, utilization of pesticides and other ones could be mentioned as human activities in polluting environment (Association of Official Analytical Chemists, 1995; Fomina et al., 2005; Arshad and Shafaat, 1997).

Contamination of agricultural soils and pastures with heavy metals could enter into human food chains by polluted agricultural and bestial products utilization. Some of these atoms could be absorbed into human body by respiration (such as plumb) and some by nutrition, which both are related to the nature of element (Din et al., 2001). Nowadays, the elimination of heavy metals from urban, industrial and agricultural wastes has been changed into a serious discussion as some of these toxic metals are precedent to others, while harder rules are approved for their elimination (Çabuk et al., 2005; Clesceri et al., 1989; Dias et al., 2002). Non degradability of heavy metals and their tendency to concentrate in the human body diverse them from other toxic pollutants. Based on WHO reports, many people are exposed to health risks caused by heavy metals in different ways (Din et al., 2001; Faryal and Hameed, 2005; Faryal et al., 2006; Fomina et al., 2005; Fourest et al., 1994).

Micro algae

Microscopic algae are of the oldest inhabitants of oceans and sweet waters. Their existence is not only thousands years before human, but also before any kind of plants and animals. Now, they are playing important and key role in our ecosystems (Fomina et al., 2005). Algae grow in water surfaces where they are exposed to the sun light. Algae are classified into three divisions: red, brown and green algae. Seaweeds or macroalgae, because of their worldwide importance are supposed to produce 5.5 to 6 milliard US dollars per year (FAO). In IRAN, seaweeds are located at the south coasts especially, at Sistan and Balochistan seashores that, according to the botanists, are recognized as chlorophytes phytophites and rodophytes. The phycologists of different countries are working on the advantages and industrial usage of algae. Microalgae are cultured at the industrial level for different applications. Some of these creatures are produced in large scales in order to use them as an intact source of vitamins and minerals for human or animal food, fish breeding and waste-water bioremediation. For instance, *Chlora volgaris* alga is being used as powder in crumby alimentary such as: bread, cake, biscuit, macaroni or along with yoghurt, milk, ice cream and etc. the algae are also used in tablets or capsules of 3 to 10 g per day and could be handled as poultry and bestial food supplement (Faryal and Hameed, 2005). *Dunaliella* is a monocellular microalga, which lives in salty coastal waters, rocky basins and briny lakes. It is known as one of the most important species of salty lakes because of its excessive tolerance against high concentrations of salts.

β-carotene, α-carotene, zacxantin, lutein and other orange or red pigments could be extracted from the *Dunaliella* alga (Faryal et al., 2006).

Methods of heavy metals elimination

Physical and chemical methods such as: chemical precipitation, ion exchange, membrane filtration, coalescence and active carbon absorption are some of the usual purification processes. Any of these methods has its disadvantages including high investment and the exploiting of costs necessity or sludge excretion prohibitions (Khan and Jaffar, 2002; Kim et al., 1995; Konopka et al., 1999; Martins et al., 2006; Faryal and Hameed, 2005; Faryal et al., 2006; Fomina et al., 2005; Fourest et al., 1994; İlhan et al., 2004). On the other hand, it is considered to use low-cost methods for heavy-metal elimination. Studies have shown that the biological methods of heavy metal elimination have proven to be beneficial as compared to the physical and chemical ones (Fomina et al., 2005; Fourest et al., 1994; İlhan et al., 2004; Khan and Jaffar, 2002). Biological elimination of heavy metal ions has recently become an important technique. Many researchers have done the elimination of mercury and other heavy metals by microorganisms but it had problems with the recognition of mechanisms (Kim et al., 1995). It seems that the ability of microorganisms in metal ion exchange is higher than that of industrial resins (Kim et al., 1995; Konopka et al., 1999; Martins et al., 2006; Faryal and Hameed, 2005; Faryal et al., 2006; Fomina et al., 2005). This capability could be presented as physical and chemical processes of absorption on cell walls or related mechanisms to microbial metabolism such as; transportation and precipitation which may be accomplished by secreted components like cellular metabolites and polysaccharides or cell wall elements.

Absorption mechanisms of dead or alive cells are different and uptake yields, reception capacity and the amount of metal concentration vary among the organisms (İlhan et al., 2004; Khan and Jaffar, 2002; Kim et al., 1995; Konopka et al., 1999).

Table 1. Components of Zarrouk medium.

Constituents	Concentration (g/l)
EDTA	0.08
CaCl2.2H2O	0.04
NaCl	1.00
NaNO3	2.50
NaHCO3	16.80
FeSO4.7H2O	0.01
MgSO4.7H2O	0.02
K2SO4	1.00
K2HPO4	0.50

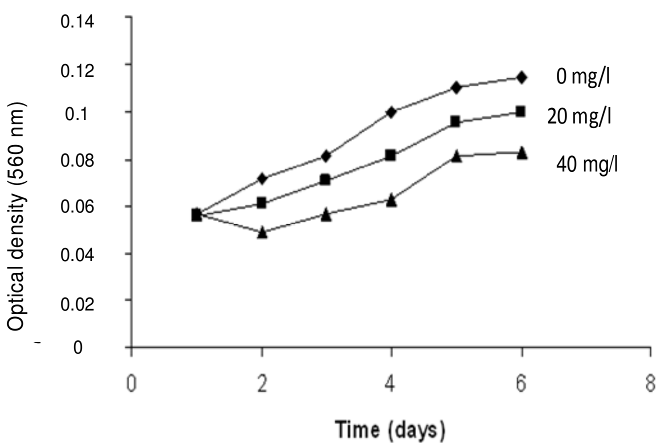

Figure 1. Growth of *Dunaliella* alga in the presence of different concentrations of Pb.

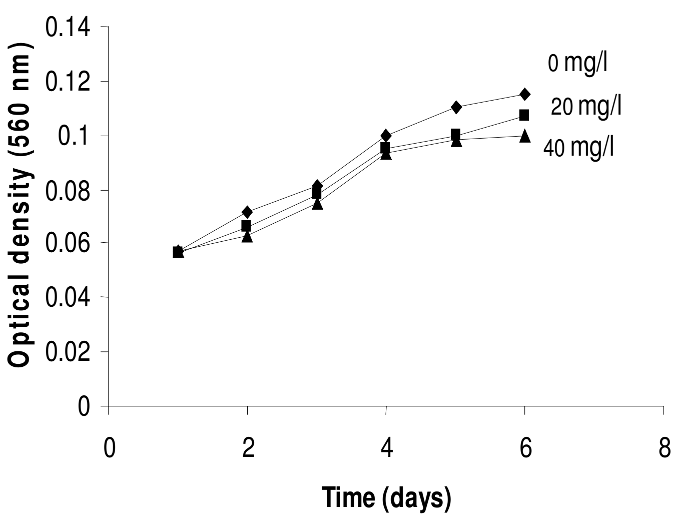

Figure 2. Growth of *Dunaliella* alga in the presence of different concentrations of Cd.

MATERIALS AND METHODS

Dunaliella alga sample was purchased in the University of Tehran, Iran. Firstly, the alga samples were centrifuged at 500 rpm and cultured in Zarrouk broth medium then stored at 20 to 25°C for 7 days in the presence of 40 W fluorescent lamp (Khan and Jaffar, 2002; İlhan et al., 2004; Fourest et al., 1994). Studying the biological processes of absorption, the alive-biomass amount varied between $10^5 \times 10^5$. The concentration of ions is mentioned in Table 1. Required concentrations of all heavy metals have been prepared from their standard solutions performing the absorption experiment. In each bioremediation study, new eluted solution were used and brought about in 250 ml flasks containing 100 ml concentrations of each metal. Flasks were shaken for 48 h at 25°C. At scheduled times of 5, 15, 20, 30, 60, 100, 120 and 150 min for 5, 12 and 24 h, 10 ml of each solution were taken for analysis. Determining the amount of remaining metal, the solutions were filtered and remedies analyzed. Absorbed amount of metal was determined by "atomic absorbtion spectrophotometer". Lung-moyer isothermal model was used representing the quality surface-absorption of each metal as stated:

$$Q = Q_{max}. BC/(1 + BC)$$

Where C is the final concentration of heavy metal (mg/l), Q is removed amount of metal from the environment (mg/105 cells), B is absorbtion constant and Q_{max} is saturation capacity (mg/105 cells).

All amounts are average with ± shown as standard diversion. Statistical analysis of data was achieved by ANOVA and Paired *t*-tests. Based on the analyses, $p < 0.05$ was considered as significant. All curves were drawn in Microsoft Excel program.

RESULTS AND DISCUSSION

First toxic effects of all tested heavy metals on *Dunaliella* were studied. Investigating the resistance of *Dunaliella* to each of the metals, live cells were cultured in solutions containing each metal ion. Growth curve of *Dunaliella* alga in the presence of various concentrations of Pb is represented in Figure 1. Results have demonstrated that the inhibitory effects of Pb increased as the concentration of this element was elevated in the culture medium. As stated in Figure 1, 40 mg/l Pb caused death in many of the algal cells on the first day. *Dunaliella* alga growth curve, in the presence of different concentrations of Cd is represented in Figure 2. The inhibitory effects of Cd on the algal cell growth increased with the elevation of Cd concentration. As shown in Figure 2, 20 and 40 mg/l Cd resulted in a significant amount of growth inhibition as compared to the control group. The effect of Cd, especially on the second day, was much less toxic on the algal cell growth as compared to that of Pb. Figure 3 represents the growth curve of *Dunaliella* alga in presence of different concentrations of Hg. Results have demonstrated that as the Hg concentration increases, the inhibitory effect of this element increases too. At 20 and 40 mg/l Hg, the growth inhibition could be observed in comparison to the control groups. Toxic effects of Hg, upon this alga, are lesser than those of Pb in contrast to Cd (Figure 3). Results of toxic effects of heavy metals

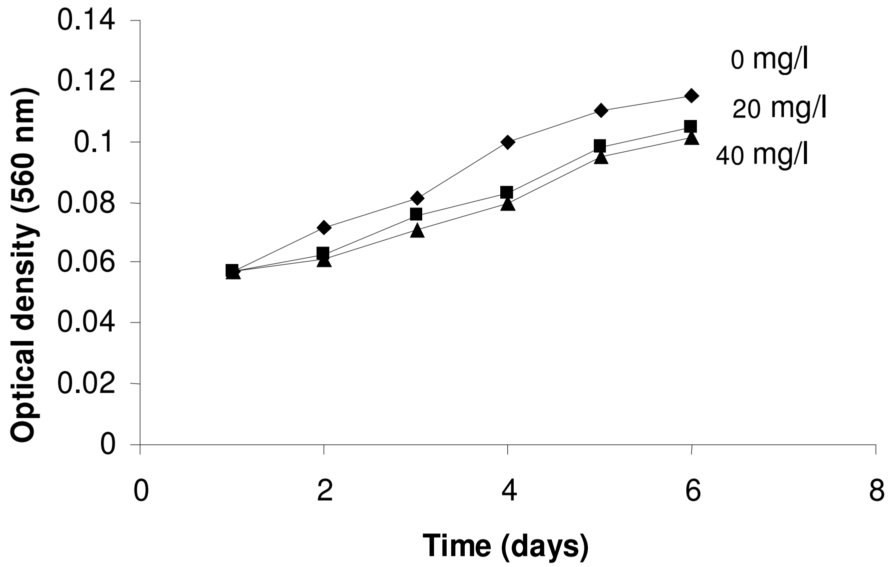

Figure 3. Growth of *Dunaliella* alga in the presence of different concentrations of Hg.

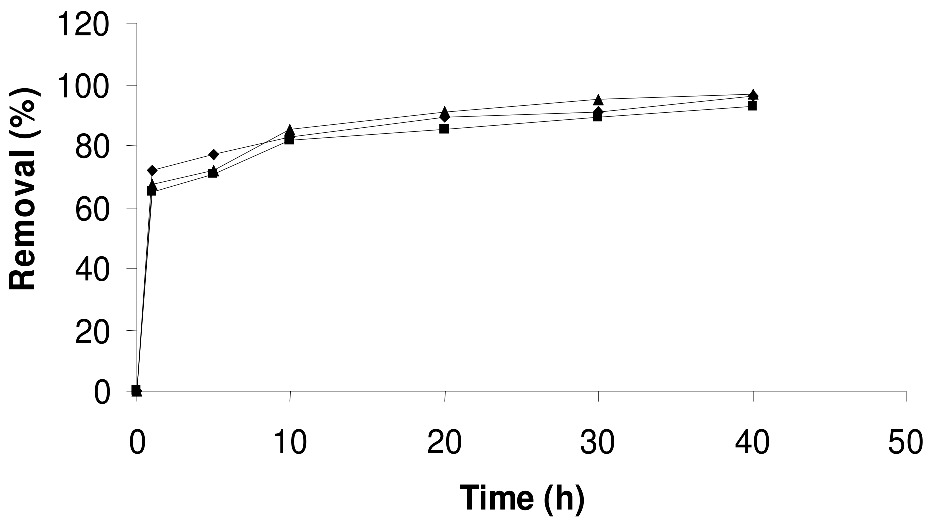

Figure 4. Time dependency of heavy-metal absorption capacity of *Dunaliella* (30 mg/l concentration for each heavy metal ions: Cd, ▲ Pb and ♦ Hg).

have proven the high tolerance of *Dunaliella* to excessive concentrations of heavy metals. Figure 4 represents the relationship between time and Pb, Hg and Cd bioremediation, in the presence of *Dunaliella* alga. It is reported that picking up of the metal ions happens in two phases: rapid and slow phases. During the rapid phase, the ions are absorbed at the surface of microorganism. While, in the next stage, these ions may pass through the cell membrane and penetrate the cytoplasm.

The present study represents the rapid phase of absorption since beginning till 1 h as 67, 65 and 72% for Hg, Pb and Cd, respectively. After 1 h, every experiment showed a constant rate of metal absorption process till 40 h (Martins et al., 2006; Faryal and Hameed, 2005; Faryal et al., 2006; Fomina et al., 2005; Fourestet al., 1994; İlhan et al., 2004; Khan and Jaffar, 2002; Kim et al., 1995; Konopka et al., 1999). Effects of the amount of *Dunaliella* on the absorption rate of tested metals is demonstrated in Figure 5. Utilized biomass concentration of alga varied between 10^5 to 5×10^5 cell/ml. Results have demonstrated that the amount of alga saliently affected the metal absorption rate. As the algal biomass increases the heavy metal-ions absorbs rate increases too. Impact of starting concentration of Pb ions upon

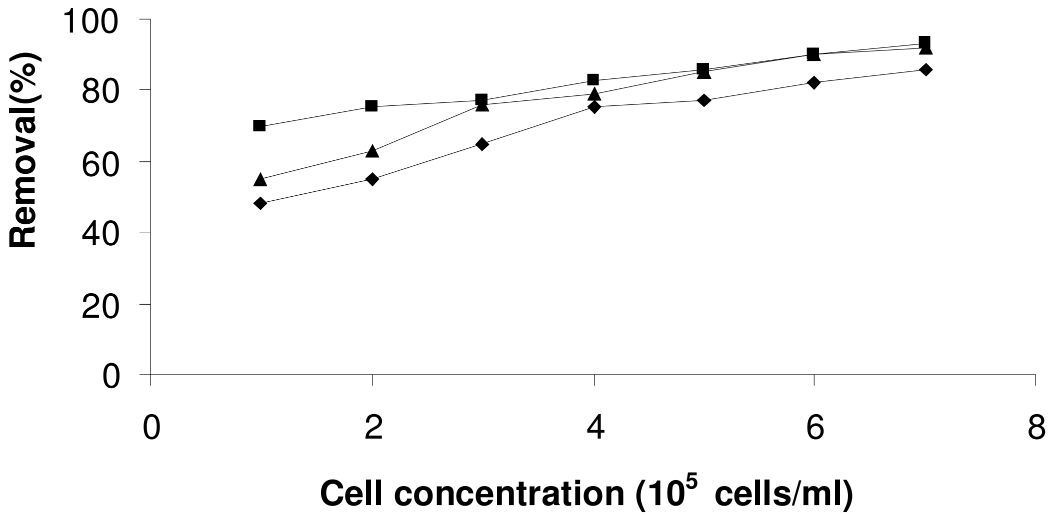

Figure 5. Effect of alga concentration on the heavy metal ion elimination (30 mg/l concentration of each heavy metal ions: Cd, ▲ Pb and ♦ Hg).

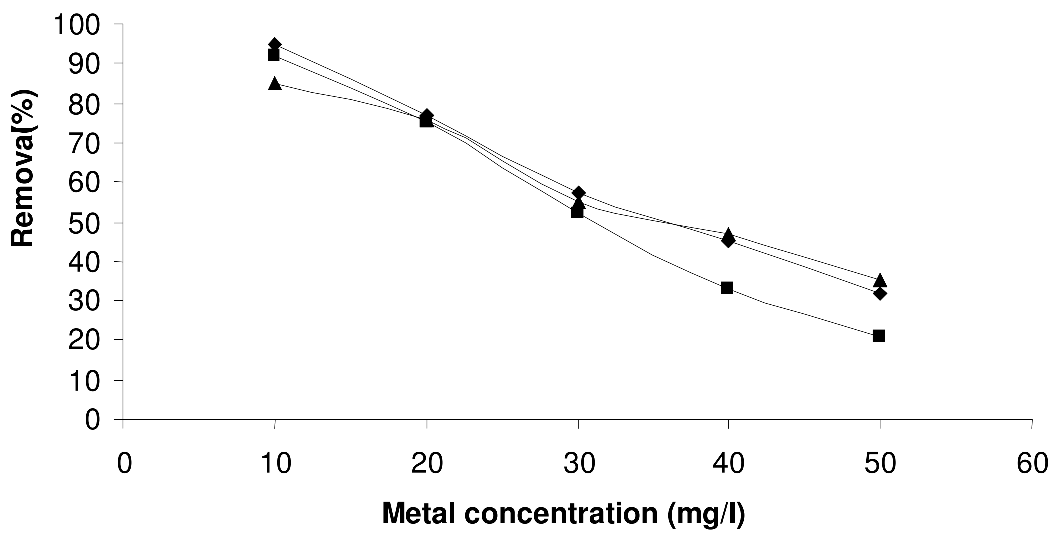

Figure 6. Heavy metal uptake at different concentrations of Pb, Hg and Cd ions. Algal cell count was 5 × 105 cells/ml (concentrations of each heavy metal ions: Cd, ▲ Pb and ♦ Hg).

bioremediation are visualized in Figure 6. Initial concentrations of all heavy-metal ions highly affect the absorption rate in equilibrium phase. It is identified that at the concentration of 10 mg/l Pb ions, the ion bioremediation rate increases higher than 80%. But at more than 30 mg/l concentration of all ions, uptake rate decreases. This kind of drop might be caused by the saturation of absorption sites of the microorganism (Figure 6). Bioremediation technology uses microorganisms to reduce, eliminate, contain, or transform to benign products contaminants present in soils, sediments, water, and air (Faryal et al., 2006). Bioremediation can be a cost efficient and reliable method for removing hazardous waste from the heavily contaminated sites (Dias et al., 2002; Khan and Jaffar, 2002; Faryal and Hameed, 2005). A key factor to the remediation of metals is that metals are non-biodegradable, but can be transformed through sorption, methylation, and complexation, and changes in valence state. These transformations affect the mobility and bioavailability of metals. At low concentrations, metals can serve as important components in life processes, often serving important functions in enzyme productivity. However, above certain threshold concentrations, metals can become toxic to many species (Konopka et al., 1999). Fortunately, microorganisms can affect the reactivity and mobility of metals. Microorganisms that affect the reactivity and mobility of metals can be used to detoxify heavy metals and prevent further metal contamination (Al-Homaidan, 2006; Vilensky et al., 2002). So, for the first time, we used *Dunaliella* alga for the bioremediation purpose of Pb, Hg and Cd.

The present results of this study demonstrate that *Dunaliella* tolerates high concentrations of heavy metals; it also has a great ability to absorb metals from aquatic environments and can be useful for bioremediation of these metals from ecosystems (Çabuk et al., 2005; Vilensky et al., 2002). So, it is concluded that the amount of Cd, Pb and Hg metal ion absorption did not increase in aqueous solution with the increase of time. Rather, the amount adsorbed remained fairly constant with time during the competitive sorption. This was attributed to the fact that all the metal ions will have to be struggling for the same number of adsorption sites at the same time.

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