

Efficiency of *Dunaliella* sp. and *Aphanocapsa elachista* in removing of copper and nickel from culture media.

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Abstract

The present investigation evaluated the effectiveness of *Dunaliella* sp. and *Aphanocapsa elachista* cells in concentrating copper and nickel in their cells and thereby removing the two metals from solution. *Dunaliella* sp. (unicellular green alga) was isolated from the 1st kilometer of Wadi-Sannur (moisted soil with underground water) at the 10th Kilometer, Eastern-South desert of Beni-Suef Governorate. On the other hand *Aphanocapsa elachista* (blue-green alga) was isolated from polluted agricultural stream receiving domestic wastewater from Wastewater Treatment Station (WWTS) at Beni-Suef City.

The two different taxa were subjected to different concentrations of copper and nickel respectively. It was revealed that *Dunaliella* sp. was the most tolerant species to copper and nickel since the growth of such alga was not affected at higher concentrations of both elements. The highest concentration of the tested substance that does not inhibit growth rate of the alga was demonstrated by copper 10 and nickel 20 ppm. On the other hand *Aphanocapsa elachista* had the lowest tolerance to the two metals (copper 2 and nickel 5 ppm). It was further revealed that *Dunaliella* sp. had higher concentration factor and removal efficiency of nickel (7753 mg L⁻¹ & 78%) and higher concentration factor and removal efficiency of copper (8375 mg L⁻¹ & 68%) when exposed to maximum concentration of nickel (20 ppm) and copper (10 ppm).

Introduction

Due to the increasing awareness of the deleterious ecological effects of toxic heavy metals, a number of studies of metal accumulation from the viewpoint of their removal from aqueous solutions have been launched. Much less has been directed to investigate the tolerance of algal species to heavy metals (Proctor and Woddell, 1975; Craig, 1977; Hill, 1980; Khalill, 1988; Brady *et al.* 1994 and Ibraheem, 1998). Heavy metals are ubiquitous in nature in wide range of concentrations and mixtures. It occurs naturally in at least trace quantities, but their concentrations can be greatly increased by human activities. Some of these are essential for growth, reproduction and/or survival. While others have unknown biological functions and is toxic. The demarcation between toxicity and essentiality is not distinct and usually depends on the active concentration and mixtures of which that are accessible to biological system (Duxbury, 1986). Heavy metals pollute the environment from various diffuse and point sources like, traffic, house hold, industry, energy supply and agriculture (Ernst and Joose, 1983). They are known to be persistent in the soil over long time period, and are known to have ecotoxicological effects on plants and soil microorganisms (McGrowth, 1987 and Smith, 1990). Increasing the contamination of the terrestrial and aquatic environment by a large number of different metal resulting in the appearance of specific microbial flora and plant vegetation (tolerable species) and the disappearance of the sensitive ones, which may affect the soil fertility

due to breaking the complexity and integral role of soil microorganisms (**Obbard *et al.* 1994**). The interaction of heavy metals with particulate materials is important in regulating the concentration of metal ions in water and soil systems. The adaptation mechanisms of plants including algae to toxic concentrations of heavy metals restricted uptake and/or translocation of metals, their compartmentation within the cell and formation of complexes with proteins or peptides (**Grill *et al.* 1985; Tomsett and Thurman, 1988, and Brown & Brinkman, 1992**). Phytoplankton cells exhibit in these systems relatively large surface areas containing various functional groups, such as carboxylic, amino, thio, hydroxo and hydroxy- carboxylic, that can interact coordinatively with heavy metal ions (**Xue *et al.* 1988**). The ability of micro-algae to take up heavy metals from their surrounding waters, suggests that they act as a potential aid in removing metal ions from the aquatic environment (**Bentley-Mowat and Reid, 1977; Ross, 1986**). The present investigation aimed at studying the tolerant ability of two different algal taxa (one unicellular green alga, *Dunaliella sp.* and the second is blue-green alga *Aphanocapsa elachista*) to various concentrations of copper and nickel and ability of these algal taxa in concentrating copper and nickel (concentration factor) and in removing the two metals from the aqueous solution containing these metals.

Materials & Methods

Two algal taxa were used in this experiment. Unicellular green alga, *Dunaliella sp.* isolated from the 1st kilometer of Wadi-Sannur (moisted soil with underground water) at the 10th kilometer, Eastern-South desert of Beni-Suef Governorate, Egypt. The other one, is blue-green alga *Aphanocapsa elachista* isolated from polluted agricultural stream receiving domestic wastewater from Wastewater Treatment Station (WWTS) at Beni-Suef City, Egypt. The two algal taxa were identified according to **Prescott (1951 and 1954)**. These algae were grown vigorously on spirulina media, with optimum growth time 7 days for *Dunaliella sp.* and 10 days for *Aphanocapsa elachista*.

Toxicity test of copper & nickel:

A preliminary experiment using a wide range of metal solutions (copper sulphate and nickel sulphate) on the growth of the algal culture was conducted. The actual experiment was then carried out by placing the appropriate volumes of metal solutions into the culture media (spirulina media) making up to 250 ml with re-distilled water and algal cells with a known density of 4×10^3 cells ml⁻¹, using 500 ml conical flasks as culture vessels. The metal concentrations used in this experiment were: copper: 0.5, 5, 10 & 15 ppm while nickel: 5, 10, 15, 20, 30 & 40 ppm for *Dunaliella sp.* and for *Aphanocapsa elachista* were : copper: 0.5, 2, 4, & 6 ppm and nickel: 1, 5, 10 and 15 ppm. The culture media were aerated (to provide CO₂) through the cotton plugs placed at the mouths of the flasks. Experiments were carried out in triplicates for each metal concentration and control. The pH of the medium was adjusted to be 6.5 for *Dunaliella sp.* and 7.5 for *Aphanocapsa elachista* with 1M HCl (NaOH) prior to autoclaving. The culture glasses were incubated under 16 h light (with 4000 Lux of light intensity) 8 h dark cycle at 26 ± 3 °C for 7 days (for *Dunaliella sp.*) and 10 days (for *Aphanocapsa elachista*). The growth rates were determined by cell counts, dry weight and chlorophyll *a* at intervals of 48 h.

Metal uptake studies.

The two algal taxa experimented with (contain 0.0 ppm of copper and nickel in their cells) were exposed to the previously mentioned concentrations (actual toxicity test) of two metals until the mentioned optimum growth time for the two algae. In addition to the calculation of the studied pollutants (Cu^{2+} & Ni^{2+}) before and after growth period in the culture media for determination of removal percentages, these cations were calculated also within the algal cells before and after experimental time to determined the concentration factor. The dry weight of algal cells were determined after being ultracentrifuged at 10000 r.p.m for 15 min. and washed three times with distilled water. The contents of copper and nickel of the two algal cells were determined by mixed acid digestion (conc. nitric acid, conc. perchloric acid at the ratio of 2 : 1 and analysed by means of inductively coupled plasma spectrophotometry (**Grant and Ellis, 1988**). Concentration factors of the two metals, at different metal concentrations were calculated using the following formula (**Trollope and Evans, 1976**).

$$\text{Concentration Factor (C.F)} = \frac{\mu \text{ g metal removed / g of algae}}{\mu \text{ g metal in solution / ml of solution}}$$

Results and Discussion

The first part of the present investigation indicated that *Dunaliella sp.* had a higher tolerance to copper and nickel (tolerated up to 10 and 20 ppm of copper and nickel, respectively). It has been suggested that there may be a detoxifying agent in algal cells that can render it resistant to metal ions (**Gudmund and Jensen, 1976 and Gadd & Griffiths, 1978**).

Table 1 illustrate the uptake of copper and nickel by the two algal cells as reflected by the percentage removal of the two metals in the culture solutions and their concentration factors, subjected to different concentrations of copper and nickel. In general it seemed to be a positive relationship between the two parameters, percentage removal and concentration factors. Higher removal efficiencies and concentration factors were observed with the two algae. The above results indicated that *Dunaliella sp.* had higher removal efficiencies of the two metals from the culture solution as compared with that of *Aphanocapsa elachista*.

Xue et al.(1988) suggest that, the kinetics of metal uptake may actually comprise of a two-stage mechanism involving initially passive adsorption, which is very rapid and occurs a short time after the microorganisms come into contact with the metal. This is then followed by a slow, possibly active metabolic uptake. The first step involves nonspecific binding of metal to cell surface, slime layers, extracellular matrices, etc., whereas the second one involves metabolism-dependent intracellular uptake. Exchange of metal cations for constituents on the cell wall has also been proposed as a possible method by which algae could remove metal ions from solution.

The data illustrate in Fig.1 & 2 shown a linear removal percentage and concentration factors of the two metals (copper & nickel) by the two studied algal taxa. A similar work was conducted by **Vymazal (1987)**, who concluded that metal uptake was linear over a certain range, but as the quantity of biomass in relation to the available metal is increased, proportional accumulation diminishes hyperbolically as “ weight dilution effect” occurs.

Table (1) Removal percentage and concentration factors of copper and nickel achieved by *Dunaliella sp.* and *Aphanocapsa elachista* cells cultured in different concentrations of Cu^{2+} and Ni^{2+} (each value is a mean of three replicates).

Metal conc.	<i>Dunaliella sp.</i>		Metal conc.	<i>Aphanocapsa elachista</i>	
	Removal %	C. F		Removal %	C.F
Cu^{2+} ppm			Cu^{2+} ppm		
0.5	42%	4000	0.5	40%	3600
5	50%	4600	2	55%	6875
10	68%	8375	4	20%	2343
15	20%	6666	6	8%	1111
Ni^{2+} ppm			Ni^{2+} ppm		
5	50%	4600	1	46%	4000
10	63%	6200	5	57%	5000
15	78%	7753	10	35%	3750
20	85%	10666	15	8.7%	1333
30	30%	3866			
40	5%	1250			

C.F = concentration factor.

Tolerance to metal toxicity may be achieved by algae in various ways. For instance bioaccumulation may be reduced by decrease in the permeability, active accumulation and absorption surfaces, while active excretion could also play a role (Albergoni *et al.* 1980). Algae may express intracellular chelators in the presence of metal ions. A copper-tolerant strain of *Scenedesmus* has been reported to produce a methallothionein type protein, while *Chlorella pyrenoidosa* and *Dunaliella* produced metallothionein type proteins when exposed to high cadmium concentrations (Gadd, 1990).

Albergoni *et al.* (1980) determined that *Euglena gracilis* was capable of producing two glycoproteins ;the large one (of 100 k Daltons) accumulated cadmium, while a smaller one (6.5 to 8.0 k Daltons) accumulated copper and different from the large glycoprotein in apparently being excreted extracellularly into the medium. Reports of possible exo-chelators in natural freshwater bodies by blue-green algae (*Aphaniclezomenon sp.*) have been conducted by (Dehann *et al.* 1981).

In higher plants, the increases of protein contents induced by cadmium, nickel or lead suggest that the plants operate a metabolic mechanism of channeling heavy metals within their cells (Ewais, 1997). A similar conclusion was made by Grill *et al.* 1985; Leblová *et al.* 1986; Robinson *et al.* 1987 and Razak, 1989.

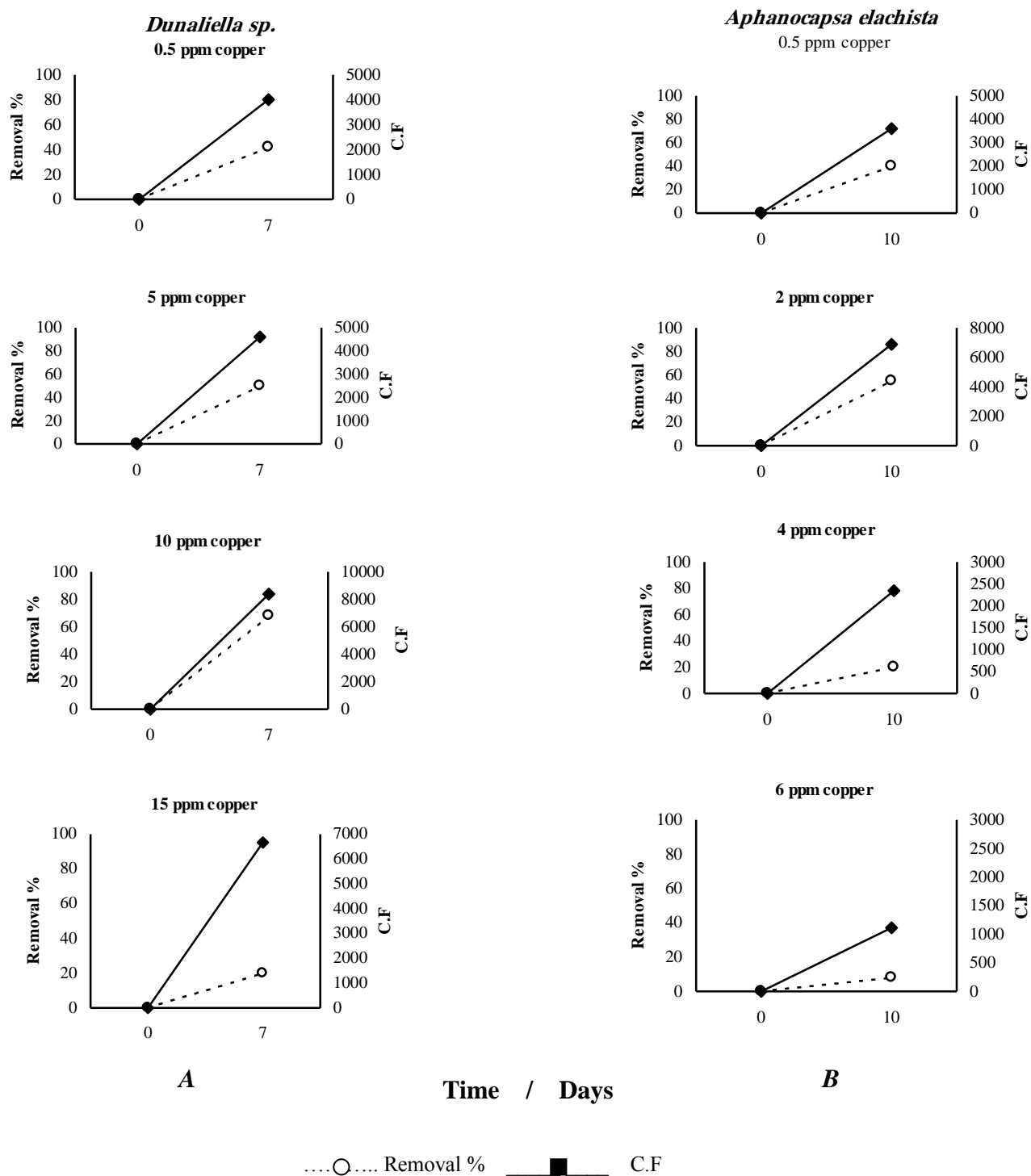


Fig. (1) Removal percentage and concentration factor(C.F) of copper achieved by *Dunaliella sp.* (A) and *Aphanocapsa elachista* (B) cells from culture media at different concentrations of copper, after 7th and 10th days respectively.

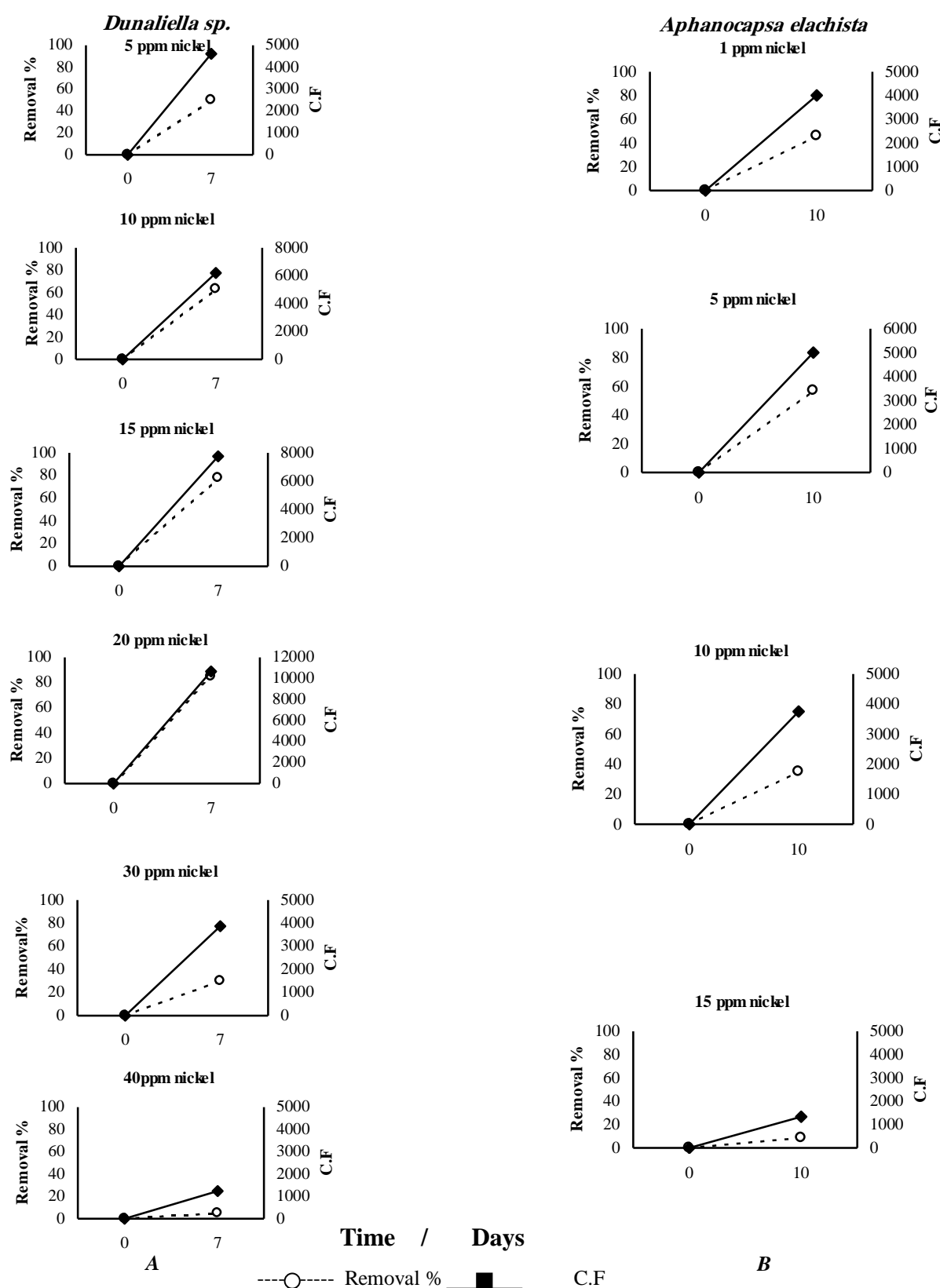


Fig. (2) Removal percentage and concentration factor (C.F) of nickel achieved by *Dunaliella sp.* (A) and *Aphanocapsa elachista* (B) cells from culture media at different concentrations of nickel, after 7th and 10th days respectively.

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