

## DISCUSSION

The idea to conceive this research study originated from a previous heavy metal biological control study carried out by the authors (Ibrahim *et al*, 2000), which addressed a pressing need for a new metals clean up technologies to remove toxic heavy metal ions from industrial wastewater. The important of this study arose from the fact that, the use of biological materials for removing and recovering of heavy metals from contaminated industrial effluents has emerged as a potential alternative method to conventional techniques.

To do so, different algal bioreactors were designed to investigate the consistency of metal bioremoval efficiency of test algae along successive removal-elution cycles, reducing effluent toxicity several times.

### **1. Bioremoval of heavy metal ions from synthetic solutions by immobilized test algae.**

#### **1.1. Optimum contact time for metal ion bioremoval:**

The experimental results (Tables 8-11, Figure 7) indicated that the efficiency of metal ion bioremoval increases significantly (Table 12) as the contact time increases. Considerable variations were noticed in efficiencies of different immobilized test algae to remove metal ions. In general, the metal ions bioremoval efficiencies were, to a large extent, dependent on contact time, algal species and on the metal removed.

The results (Figure 7) showed clearly that all test algae sustained relatively higher efficiencies to remove the heavy metal ions tested at contact time of 15 minutes. Although, in some cases, there was slight increase in metal ion bioremoval beyond 15 minutes, but the differences were always statistically non significant (Table 12). Based on statistical analysis, the contact time of 15 minute was selected as the optimum time for metal ion bioremoval and fixed for all subsequent treatments. In this case, the optimum time may be defined as the shortest time at which the highest metal ions bioremoval was attained.

Choosing 15 minutes as the maximum contact time for metal ions bioremoval seems a proper selection since the test immobilized algae will be kept for relatively short period in test solutions containing heavy metal ions in concentrations largely exceeding their toxic thresholds for many algal species including the test algae (Bartlett *et al.*, 1974; Blaise *et al.*, 1986; Takamura *et al.*, 1989; Outridge and Scheuhammer, 1993 and Lee and Lustigman, 1996).

The selection of a relatively short contact time for metal ion bioremoval may extend the possibility of repeated use of the test algae in several metal bioremoval cycles. The results (Table 37, Figure 21) came to fully support and validate the selection of less algae-metal contact time as the efficiency of immobilized algae to remove heavy metal ions through five successive cycles never fell below 70%. Therefore, the proper selection of algae-metal

contact time seems crucial for ensuring the sustainable applied significance of the test algae for metal ions bioremediation.

In accordance with our results Carrilho *et al.* (2003) indicated that *Chlorella vulgaris* was able to bind Cr(II), Cu(II), Mn(II), Ni(II) and Zn(II) in 15 minute contact time. Also, Ahuja *et al.* (1999) found that the cyanobacterium *Oscillatoria angustissima* adsorbed appreciable amount of Co(II) from the aqueous solution within 15 minutes.

Many algae-metal bioremoval studies have also indicated that the majority of test algae attained maximum metal ions bioremoval within periods slightly fluctuated between 10 and 20 minutes (Singh and Prasad, 2000; Zhao *et al.*, 2001; Cossich *et al.*, 2002 and Nuhoglu *et al.*, 2002). It seems that, the test algae used in present study belong to this category as indicated by the experimental results (Figure 7).

In spite of considerable variations in efficiencies of different algal species to remove heavy metal ions from aqueous solutions, the highest bioremoval efficiencies may be reached within relatively short time periods with nearly upper limits of 30 minutes (Wang *et al.*, 1998 and Hashim and Chu, 2004).

It has been thoroughly reported, and found in this study, that the contact time required for maximum metal ion bioremoval by algae varied considerably from one algal species to another and is highly dependent on metal to remove. In this context, research studies revealed that *Chroococcus parisi* removed 90% of Cd(II) within 1.0 minute (Les and Walker, 1984 and Disyawongs, 2002), *Spirulina platensis* adsorbed 60% of Cu within 6.0

minutes (Zhou *et al.*, 1998), the efficiency of *Laminaria japonica* and *Padina* sp to remove Cd(II) attained 90% within 20 minutes and 35 minutes respectively (Kaewsarn and Yu, 2001 and Yin *et al.*, 2001). *Chlorella vulgaris* and *Chlorella regularis* achieved rapid Cd(II) bioremoval with highest efficiencies attained at 10 minutes and 30 minutes, respectively (Sakaguchi *et al.*, 1979 and Khummongkol *et al.*, 1982). An interesting relevant study was that of Inthorn *et al.* (2002) who tested the efficiency of forty-six strains of green and blue green microalgae to remove Hg(II), Cd(II) and Pb(II) from aqueous solutions within the relatively short time of 10-20 minutes. They found that the highest Hg(II) removal was achieved by *Scenedesmus* sp. (97%) and the lowest by the cyanobacterium *Fischerella* (92%). However, the highest Cd(II) removal was achieved by *Lyngbya heironymusii* (97%) and the lowest by *Scenedesmus acutus* (88%). Pb(II) removal was the highest with *Nostoc punctiforme* (98%) and the lowest with *Chlorella vulgaris* (84%).

### **1.2. Optimum pH value for metal ion bioremoval:**

The results showing the effect of pH on metal ion bioremoval efficiency (Figure 8) indicated that, the efficiency of metal ion bioremoval was significantly increasing with the progressive increase of pH value until pH 5.0. At pH 6.0, the efficiency of metal ion bioremoval became more or less stable, then gradually decreased as pH increased further.

In general, the metal ions bioremoval efficiencies were strongly dependent on the pH value, algal species and the metal removed.

It is clear that, all the test algae sustained higher efficiencies to remove all metal ions tested at pH 6.0. In some cases, there was slight increase in metal ion bioremoval beyond pH 6.0 (Figure 8), however, the differences were statistically non-significant at  $P \leq 0.05$  (Table 17). Therefore, pH 6.0 was selected as the optimum pH for metal ion bioremoval and adjusted for all subsequent treatments.

It may be convenient to mention that all the test algae except *Spirulina platensis* attend optimum growth at pH 7.2 (Table 2), however *S. platensis* grows well at pH ranged between 9.0-9.5 (Table 1).

It has been thoroughly documented that physiological activities of a given organism are highly pH dependant.

In this study the pH 6.0 was selected as an optimum for metal ion biosorption. The experimental results revealed that the capacity of test algae to remove metal ions, either singly or in combination, from aqueous solutions never fell below 70% (Table 37) even after five successive sorption-desorption metal cycles.

The results obtained are in a good agreement with those of Rangsayatorn *et al.* (2002) who used *Spirulina platensis* to remove Cd(II) from wastewater at different pH values. They found that Cd(II) was hardly sorbed (0.91%) at pH 3.0, but it was rapidly sorbed when the pH was increased to above this value. Cd(II) removal was significantly increased from 72.64% at pH 4.0 to 91.86% at pH 6.0. The maximum uptake was 95.93% at pH 7.0 and it leveled off at pH 8.0. Zhou *et al.* (1998) found that the removal of Cu(II) and

Cd(II) by *Laminaria japonica* and *Sargassum kjellmanianum* increased with increasing pH from 3.0 to 5.0, but further increases above pH 5.0 led to a reduction in sorptive capacity. Gardea-Torresdey *et al.* (1996a) studied the effect of pH on uptake of Cu(II), Pb(II) and Ni(II) ions to the *Synechococcus* biomass. High affinity for all metal ions was noticed as the pH increased from 2.0 to 6.0 with optimum binding occurring at pH 5.0.

As reported in this study and found in others, Hashim and Chu (2004) found that the maximum efficiency of seven marine algal weeds to remove Cd ions was attend at pH 5.0. The cyanobacterium *Lyngbya taylorii* maintained considerable efficiency to remove the metal ions Cd(II), Pb(II), Ni(II) and Zn(II) at pH value ranging between pH 3.0 and pH 7.0, with highest capacity reported at the more slightly acidic range (Klimmek *et al.*, 2001). Sanchez *et al.* (1999) investigated the removal of Cu(II) and Zn(II) by brown alga *Cymodocea nodosa*, they found that the maximum removal was attend at pH 5.0.

One mechanism that explains the highest metal ion biosorption attend at slightly acidic pH range (pH 5.0 - pH 6.0) (Figure 8) may follow the role of carboxyl groups in sequestration of metal ions. In this context, Hashim and Chu (2004) reported that negatively charged groups such as carboxylate present in algal cell walls are believed to be responsible for removing of metal ions from aqueous solutions. Torresdey *et al.* (1998) found that carboxyl groups played an important role in metal binding capacity of algae. The acid dissociation constants for carboxyl groups have been reported to be

around pH 4.0. At pH 5.0 these groups become deprotonated and are able to attract positively charged metal ions (Segel, 1976 and Hunt, 1986). Moreover, Cossich *et al.* (2002) reported that carboxyl and sulphate groups in biopolymers of algal biomasses are the main metal ions sequestering sites, as these groups are acidic, their availability is pH dependent. Both carboxyl and sulphate groups become negatively charged at pH range 3.5-5.5 and thus are able to attract positively charged metal ions.

On basis of this fact, the negatively acidic groups of test algal biomasses may play a prominent role of biosorption of the tested metal ions.

However, the role of extracellular and intracellular accumulation (Muraleedharan *et al.*, 1991 and Khattar *et al.*, 1999), extracellular polymeric substances (Kropfl *et al.*, 2003), transportation into the cellular interior (Chojnacka, 2003), intracellular chelation and precipitation (Greene and Bedell, 1990), metallothioneins (Turner and Robinson, 1995 and Pinto *et al.*, 2003), phytochelatins (Gekeler *et al.*, 1998; Ahner and Morel, 1995; Skowronska *et al.*, 1998 and Skowronska, 2000) and the role of other chemical groups including amine, imidazole, phosphate, sulfhydryl and hydroxyl groups (Gupta *et al.*, 2000 and Hashim and Chu, 2004) in metal bioremoval can not be ruled out.

It has been thoroughly concluded that the mechanisms by which algae remove metal ions are diverse and ranged from a simple physical phenomenon to a highly complicated physiological and biochemical process and all are highly pH dependent.

## **2. Elution of heavy metal ions from algae-alginate beads.**

### **2.1. Metal elution-pH dependence:**

In this study the acidic solution of 0.1 M thiourea (acidified with HCl) was used to elute metal ions from beads of test algae. The same chemical was also reported by others to be highly efficient desorbing agent of many metal ions including those tested here in.

Nakajima (2003) screened thirty species of microorganisms for maximal gold accumulation. He found that the gold adsorbed on the cells was easily desorbed with 0.1 M acidic thiourea solution.

Lau *et al.* (2003) reported that 0.1M acidic thiourea desorbed 100% of metal ions Cu(II), Ni(II) and Zn(II) being adsorbed by the green seaweed *Ulva lactuca*.

It must be, however, taken into consideration that other chemicals including 0.1M H<sub>2</sub>SO<sub>4</sub> (Bux *et al.* 1996), 0.1M HCl (Gardea-Torresdey *et al.*, 1996b), 0.1M HNO<sub>3</sub> (Jalali *et al.*, 2002) and 0.1N EDTA (Aldor *et al.*, 1995) have been thoroughly reported as highly efficient metal ions eluting agent.

Experimental results (Figure 10) showed that the % of metal ions eluted by 0.1 M thiourea decreased as pH increased with highest and lowest elution capacity being recorded at pH 2.0 and pH 6.0, respectively. Selection of pH 3.0 as an optimum for the elution of metal ions tested based on the fact that differences in metal ion elution efficiency at pH 2.0 and pH 3.0 were statistically non significant (Table 22). Compared to pH 2.0, the pH 3.0 is



supposed to be relatively less harmful to the viability and/or vitality of the test algae.

In agreement with our results, Greene *et al.* (1987) found that, binding of metal cations such as Cu(II), Cr(III), Ni(II), Pb(II), Zn(II), Cd(II) and Co(II) to *Chlorella vulgaris* at pH 5.0 is quantitatively reversed by lowering the pH to 2.0.

Ehrlich and Brierley (1990); Gardea-Torresdey *et al.* (1998); Nakajima (2003) and Dziwulska *et al.* (2004) reported that the eluting agent, 0.1 M thiourea attend maximum capacity to desorb metal ions at pH 2.0 to pH 3.0 range.

## **2.2. Metal elution-time dependence:**

It is clear from Figure 11 that the elution capacity of acidic (pH 3.0) 0.1 M thiourea increased significantly ( $P \leq 0.05$ ) as contact time increased from 1.0 to 10 minutes.

The contact time of 5.0 minutes was selected as the optimum time for metal ions elution. This selection is validated on fact that the differences in metal ion elution capacity recorded at 5.0 and 10 minutes were statistically non significant ( $P \leq 0.05$ ) with all metal ions tested (Table 27).

It seems logical that lowering the algae-elutant exposure time would minimize the risks of acidity and toxicity of thiourea on biological performance of the test algae. Geyer *et al.* (1985) reported that thiouria is toxic to growth of *Scenedesmus subspicatus* with  $EC_{50}$  of 4.8-10  $\text{mg l}^{-1}$ .

These results are in agreement with Gardea-Torresdey *et al.* (1998) who using biorecovery system from *Medicago sativa* (alfalfa) biomass for the recovery of gold from aqueous solutions in an environmentally friendly manner. They found that, alfalfa binds gold quickly (within five minutes). Treatment of the column containing immobilized alfalfa with 0.2M acidic thiourea (in 0.2M HCl) for 10 minutes recovered up to 99.1% of the bound gold metal. Also, Bux *et al.* (1996) who found that desorption of metal ions from column of metal-loaded *Azolla filiculoides* using different acids such as, H<sub>2</sub>SO<sub>4</sub> or HCl occurred immediately within 10 minutes.

Jalali *et al.* (2002) found that *Sargassum hystrix* biosorbed Pb rapidly, in less than 30 minutes of contact. Removal of Pb from *Sargassum* biomass was successfully achieved by eluting with 0.1M HNO<sub>3</sub> for 15 min. Pb released to this dilute mineral acid with 95% elution efficiency. Aldor *et al.* (1995) found that 99.5% recovery of Cu was achieved in less than 2 hours from column packed with Cu-loaded *Sargassum kjellmanianum* after 0.11N HCl or 0.1N EDTA were applied as a desorbing agent.

Their findings may be due to the difference in the concentration and type of eluting agents. Stirk and Van Staden (2002) investigated the efficiency of the brown alga *Ecklonia maxima* to remove Cd(II) from wastewater and used different acids for elution. They found that optimum time for recovery ranged from 30 minutes to 2.0 hours depending on the acid and concentration used for desorption.

### **3. Consistency of metal ion bioremoval along successive removal-elution cycles.**

The efficiency of individually immobilized test algae and composite algae-alginate beads (beads immobilizing the four test algae together) to remove different heavy metal ions prepared either singly or in one mixture solution was assessed through five successive bioremoval-elution cycles.

In all cases the highest and the lowest bioremoval efficiencies were recorded after the first and the fifth cycles, respectively (Tables 28-36). The main capacities of individual test algae to remove metal ions fluctuated within narrow ranges between 84% and 96.5% for single metal ion and between 75.4% and 86.56% for metal ions mixture (Table 37).

Slight variation exists in metal ions bioremoval efficiencies of individual test algae were, to some extent, dependent on test algae and on metal ion tested. Statistical analysis (Table 38) indicated that the variations were non significant. This finding may indicate that the four test algae maintain more or less comparable capacities to remove metal ions tested either singly or in combination.

After five successive bioremoval-elution cycles, all the test algae maintained considerably high capacities to remove metal ions as percent of metal ion removed in all cases were typically above 70% (Table 37). This finding may indicate that the test algae can efficiently accumulate the harsh conditions imposed by toxic levels of heavy metal ions. This result may also highlight the applicability of test algae to design specific biofilters for

removal of toxic metal ions from aqueous phases including industrial wastewaters.

The mean efficiencies of 0.1M acidified thiourea to elute metal ions, through five successive bioremoval-elution cycles, varied from 69.84% to 96.1% and from 67.12% to 92.36% in cases of single metal-laden and four metals-laden single algal beads, respectively (Table 41).

These results are in agreement with Rangsayatorn *et al.* (2004) who tested the reusability of immobilized *Spirulina platensis* in five cycles of Cd(II) adsorption and desorption. They reported that, after the first cycle, Cd(II) uptake by alginate and silica immobilized cells was reduced from 94.07% and 92.67% to 70.79% and 66.99%, respectively. The adsorption efficiency of both alginate and silica immobilized cells was still high and ranged from 78.47% to 63.21% with five successive cycles.

Jalali *et al.* (2002) found that *Sargassum hystrix* biosorbed Pb(II) rapidly, in less than 30 min of contact. Removal of Pb(II) from *Sargassum* biomass was successfully achieved by eluting with 0.1M HNO<sub>3</sub> for 15 min. Pb(II) released to this dilute mineral acid with 95% elution efficiency. In repeated use of biomass experiment, the Pb(II) uptake capacity of *Sargassum* biomass was constantly retained (98%) and no significant biomass damage took place after 10 sorption-desorption cycles.

Also with the finding of Akhtar *et al.* (2004) who used *Chlorella sorokiniana* immobilized on luffa sponge as a new biosorption system for the removal of Pb ions from aqueous solutions. The maximum biosorption

(96%) was obtained at a solution pH of about 5.0 for 15 minutes. The immobilized *Chlorella sorokiniana* biomass could be regenerated using 0.1M HCl with up to 99% recovery. The desorbed biomass was used in five biosorption-desorption cycles, and no noticeable loss in the biosorption capacity was recorded.

Schneider *et al.*, (1999) observed that, although there was some loss of Cu(II) sorptive capacity of freshwater macrophyte *Potamogeton lucens* biomass after several loading and elution cycles, the sorptive capacity remained essentially unchanged for up to 100 loading and elution cycles.

*Microcystis aeruginosa* immobilized in a natural polymer was found to be effective in Cu(II) removal from solution for up to 10 cycles of adsorption-desorption, using HCl as desorbent agent for regeneration of biomass for reuse (Pradhan and Rai, 2001).

The efficiency of thiourea to elute Ni(II), Cd(II) and Pb(II) from different algal beads was higher than that achieved for Hg(II)-laden beads (Table 37 and Figure 21). This may be due to that most algal strains prefer to remove Hg by volatilization instead of accumulation. This result is in agreement with De Filippis and Pallaghy (1976) and De Filippis (1978) who reported that the resistance of *Chlorella* to Hg(II) was due to its cells has high capacity to volatilize Hg into the atmosphere. Moreover, Bartless *et al.* (1977) found that *Chlorella* produces glucose dehydrogenase, which reduced Hg ions and volatile metallic Hg at outer cell surface.

Generally, through five successive cycles, the efficiency of test algae to remove different metal ions prepared singly was slightly higher than that achieved when metal ions prepared in mixture solution. The mean bioremoval efficiency in case of single metal ion solution ranged between 84% to 96.5%. whereas, the mean efficiency in case of metal ions mixture solution varied from 75.4% to 86.5% (Table 37 and Figure 21). This finding may be due to the competition of metal ions for binding sites on algal cell surface.

Our result is in agreement with Chong *et al.* (2000) who studied the ability of 11 microalgal species; including *Chlorella vulgaris*, *Chlorella sorokiniana* and *Scenedesmus quadricauda* in removing Ni(II) and Zn(II) from synthetic wastewater. They found that Ni(II) removal was significantly reduced by the presence of Zn(II). Zhou *et al.* (1998) found that, the sorptive capacity of *Laminaria japonica* for Cd(II) decreased from 97% to 39% when the competing cation, Cu(II), was added. Also the sorptive capacity of *Sargassum kjellmanianum* for Cu(II) was 92% when no competing cations were present. This were reduced to 76% when Cd ions were present. Similarly, for Cd(II), the sorptive capacity in the presence of Cu(II) was a fifth of that when no Cu(II) was present. Kuyucak and Volesky (1989a) also found that the presence of co-cations generally caused a decrease in Co(II) uptake by brown marine alga *Ascophyllum nodosum*.

Compared to the metal ion sorption capacity in one-metal system, the binding of three metal ions (Cu, Cd and Pb) by *Chlorella vulgaris* decreased

when other ions were present. Also, *Oscillatoria limnetica* bound less Cu(II) and Pb(II) and *Eudorina elegans* bound less Cu(II) and Cd(II) in a three metal ions system. The antagonistic effect result from the competition of metal ions to algal surface binding sites (Tien, 2002).

Compared to single-algae-beads condition, the composite beads proved to be more efficient in metal ion biosorption through five successive bioremoval-elution cycles. The main efficiency of composite beads to remove metal ions from its mixture fluctuated slightly between 92.6% and 94% (Table 37 and Figure 21). One way analysis of variance (ANOVA) reported that, the efficiency of composite beads to remove metal ions from a mixture metal ions solution was significantly higher compared to the singly tested algae (Table 40).

Composite beads were prepared by immobilizing the four test algae in alginate matrices. As these algae belonging to different algal classes (Cyanophyceae, Chlorophyceae and Bacillariophyceae) they are definitely different greatest in the cellular structures and may involve different mechanisms of metal ion bioremoval and different binding sites. As composite beads maintained significantly higher efficiencies to remove metal ions, we may postulate that mechanism maintained by co-immobilized test algae to remove metal ions may act on a synergistic or addition manner.

A strong evidence to what we mentioned above concerning the high capabilities of composite beads in removing heavy metal ions comes from the work of Gupta *et al.*, (2000) who reported that, difference in cell wall

composition among different groups of micro-organisms, viz. algae, bacteria and cyanobacteria and the intragroup differences can thus cause significant differences in the type and amount of metal ion binding to them. Among the photoautotrophs, eukaryotic algal cell walls are mainly cellulosic. According to Crist *et al.* (1981) the potential metal binding groups in these classes of microbes are carbohydrate, amine, imidazole, phosphate, sulphhydryl and hydroxyl groups. Cell wall of cyanobacteria are principally composed of peptidoglycan chains which were found to be a potent binder of most of the metal ions.

Our results indicated that, the efficiency of thiourea to elute metal ions from composite beads was significantly higher compared to single and four metal-laden algal beads (Tables 41 and 44). This obvious result is due to the higher capabilities of composite beads in removing different heavy metal ions than single-algae-beads conditions.

#### **4. The use of co-immobilized test algae to treat heavy metal ions in toxic industrial effluents.**

##### **4.1. Chemical characteristics of industrial effluents:**

Analysis of pH of different effluents (Table 45) showed that, effluents #1, #2 and # 3 were alkaline with pH values of 10.2, 9.5 and 10.24 respectively. This very probably due to the high ammonia content of Talkha chemical fertilizers Factory and sodium hydroxide used in both textile and soap manufacturing of Mahalla dyes Factory and Sandoub oils and soap factory.



The effluent # 4 (Kafr-Ezzayyat salt and soda factory) was slightly acidic with pH value of 4.8. This may be due to the repeated washing of soda basins by acids.

Analysis of heavy metal ions (Table 45) revealed that, the concentration of heavy metals Ni(II), Cd(II), Pb(II) and Hg(II) varied slightly from one effluent to another. Ni(II) varied between 0.80 mg l<sup>-1</sup> and 1.95 mg l<sup>-1</sup>, Cd(II) between 0.37 mg l<sup>-1</sup> and 1.32 mg l<sup>-1</sup>, Pb(II) between 1.20 mg l<sup>-1</sup> and 1.85 mg l<sup>-1</sup> and Hg(II) between 34 µg l<sup>-1</sup> and 59 µg l<sup>-1</sup>.

This is in agreement with the finding of Sponza (2002) who found that high levels of heavy metal ions such as Hg(II), Al(III), Pb(II) and Zn(II) characterize pigment (dye) wastewater such as that in the textile industry. Casas *et al.* (2003) studied the concentration of heavy metal ions (Cd(II), Cr(III), Cu(II), Hg(II), Ni(II), Pb(II) and Zn(II)) in sediments from the River Llobregat, Spain. They reported that the high level content of heavy metal ions originating from different industrial activities.

#### **4.2. Efficiency of composite beads and flat filters to remove heavy metal ions from industrial effluents:**

The capacities of the four test algae, either co-immobilized in alginate matrices or developed on cotton and sponge flat-bed filters, to remove metal ions from four industrial effluents through 10 successive bioremoval-elution cycles were tested and compared. All the work was achieved at the optimum

pH (6.0) and optimum bioremoval time (15 minutes) and elution time (5 minutes).

**Nickel.**

The capacities of algae-cotton filter, algae-sponge filter and algae alginate beads to remove Ni(II) ranged between minimum and maximum values of 75% to 93%, 68% to 93% and 45% to 88% with mean values of 87.2%, 81.1% and 65.9%, respectively (Table 50). In general, the highest and lowest capacity of all biofilters to remove Ni(II) was recorded after the first and tenth bioremoval cycles respectively (Table 46).

These results are in agreement with Tam *et al.* (2001) who used two living *Chlorella* species (*Chlorella vulgaris* and *Chlorella miniata*) to remove Ni(II) from solution containing 30 mg l<sup>-1</sup> in 10 successive cycles. They found that Ni(II) removal percentage of cells was maintained at around 85% in the first five cycles, then declined slightly from the fifth cycle onwards, and finally achieved around 70% removal at the end of the 10<sup>th</sup> cycle.

Akhtar *et al.* (2003a) used *Chlorella sorokiniana* immobilized within luffa sponge discs for the removal of Ni ions from wastewater streams. They found that Microalgal-luffa sponge immobilized discs (MLIDs) removed 97% of Ni(II) within 5 min. The regenerated MLIDs retained 92.9% of the initial binding capacity for Ni(II) up to five cycles of reuse. The decline in efficiency was not more than 7.1%, which shows that the biosorbent has good potential to adsorb metal ion from wastewater.

Zhao *et al.* (2001) found that biomass of red tide alga *Prorocentrum micans* has high uptake capacities for six heavy metal ions ( $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Ag}^{2+}$  and  $\text{Cd}^{2+}$ ) and about 90% of the biosorption occurred within 10 minutes. Hammouda *et al.* (1995) found that, *Scenedesmus* and *Chlorella* in a microalgal wastewater treatment system were able to remove heavy metal ions with the ranges between 52.3% to 100% in a batch system and from 64.2% to 100% in case of the continuous system. Also, Sandau *et al.* (1996b) reported that, *Chlorella vulgaris* was able to bioaccumulate heavy metal cations in low concentrations and to remove them from the cultivation suspension.

### **Cadmium.**

The algae-cotton filter maintained the highest Cd(II) removal efficiency (65%-100%) followed by algae-sponge filter (65%-100%) followed by algae-alginate beads (51%-83%) with mean metal ion bioremoval efficiencies of 86.4%, 82.6% and 68.1%, respectively through ten successive bioremoval-elution cycles (Table 50).

Our results are in agreement with Rangsayatorn *et al.* (2004) who used immobilized *Spirulina platensis* to remove Cd(II) through five cycles of adsorption and desorption. After the first cycle, Cd(II) uptake by alginate and silica immobilized cells was reduced from 94.07% and 92.67% to 70.79% and 66.99%, respectively. The adsorption efficiency of both alginate and silica immobilized cells was still high and ranged from 68.47% to 63.21% within five successive cycles.

Perez-Rama *et al.* (2002) found that living cells of the marine flagellate *Tetraselmis suecica* removed 98.1% of added Cd(II). Sternberg and Dorn (2002) found that *Cladophora parriaudii* removed 80-94% of Cd(II) introduced from a synthetic wastewater. Also, Sobhan and Sternberg (1999) studied the ability of *Cladophora* sp. to remove Cd(II) from a synthetic wastewater. They found that Cd(II) removal varied from 86% to 96% from initial Cd(II) concentration.

Hashim and Chu (2004) examined seven species of brown, green, and red seaweeds for their abilities to remove Cd ions from aqueous solution, they found that Cd(II) uptake was fast as 90% or more of the uptake occurring within 30–40 min of contact time.

Yin *et al.* (2001) investigated biosorption and desorption properties of Cd(II) from aqueous solutions by the biomass of marine alga *Laminaria japonica*. They found that more than 90% of the adsorption occurred within 20 minutes and the adsorbed Cd cannot be desorbed by distilled water, but it can be effectively recovered by using acidic or EDTA solutions.

Kaewsarn and Yu (2001) investigated the removal of Cd(II) by marine alga *Padina* sp. from aqueous solutions, and they found that 90% of removal took place within 35 min.

Rangsayatorn *et al.* (2002) examined the efficiency of alginate immobilized cells of *Spirulina platensis* to remove low concentrations of Cd (less than 10 mg l<sup>-1</sup>) from wastewater. They reported that the removal was pH dependent

and the Cd(II) removal was significantly increased from 72.64% to 91.86% by raising pH. The maximum uptake was 95.93%.

**Lead.**

The mean capacity of algae-cotton filter, algae-sponge filter and algae-alginate beads to remove Pb(II) were 78.8%, 70.6% and 76.1%, respectively through ten successive bioremoval-elution cycles (Table 50).

This result is in a kind of agreement with Jalali *et al.*(2002) who found that *Sargassum hystrix* biosorbed Pb(II) rapidly, in less than 30 min of contact. Removal of Pb(II) from *Sargassum* biomass was successfully achieved by eluting with 0.1M HNO<sub>3</sub> for 15 min. Pb(II) released to this dilute mineral acid with 95% elution efficiency. In repeated use of biomass experiment, the Pb(II) uptake capacity of *Sargassum* biomass was constantly retained (98%) and no significant biomass damage took place after 10 sorption-desorption cycles.

Algal biofilm was investigated in an acid stream of southwestern Spain. It was mainly made up of the ulotrichalean alga *Klebsormidium flaccidum*, the diatom *Pinnularia acoricola* and *Euglena mutabilis*. Activity of these organisms creates strong oxidizing conditions that ultimately lead to metal leaching (e.g. Zn, Pb and Cu ions) from the drained substrata (Sabater *et al.*, 2003).

Les and Walker (1984) and Disyawongs (2002) reported that Cu(II), Pb(II), Hg(II) and Cd(II) were removed rapidly by *Chroococcus parisi* and

approximately 90% of the total amount of added metal ion was removed within 1.0 minute. Zhao *et al.* (2001) found that biomass of red tide alga *Prorocentrum micans* has high uptake capacities for six heavy metal ions ( $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Ag}^{2+}$  and  $\text{Cd}^{2+}$ ) and about 90% of the biosorption occurred within 10 min. Also Roy *et al.* (1993) found that *Chlorella minutissima*, adsorbed greater than 90% of the initial Pb(II) and greater than 98% of the initial Co(II) concentrations.

### **Mercury.**

Both algae-cotton filter and algae-sponge filter, showed more or less comparable capacity to remove Hg(II) from different industrial effluents (Table 49 and Figure 35). Their efficiencies ranged between 72% and 100%. However, the algae-alginate beads maintained a relatively inferior capacity to remove Hg(II) with removal efficiency fluctuated from 58.2% to 91.5% (Table 50). Noticeable Hg bioremoval variations are evident among different industrial effluent (Figure 35).

Inthorn *et al.* (2002) tested the efficiency of forty-six strains of green and blue green microalgae to remove Hg(II), Cd(II) and Pb(II) from aqueous solutions within the relatively short time of 10-20 minutes. They found that the highest Hg removal was achieved by *Scenedesmus* sp. (97%) and the lowest by *Fischerella* sp.(92%). However, the highest Cd(II) removal was achieved by *Lyngbya heironymusii* (97%) and the lowest by *Scenedesmus acutus* (88%). Pb(II) removal was the highest with *Nostoc punctiforme* (98%) and the lowest with *Chlorella vulgaris* (84%).

**In general**, even after our 10<sup>th</sup> bioremoval cycle, the efficiencies of algae-cotton filter, algae-sponge filter and algae-alginate beads were typically above 65%, 65% and 45% in removing the metal ions studied respectively (Table 50). Moreover, through ten bioremoval-elution cycles, the efficiencies of cotton and sponge filters to remove different heavy metal ions were generally higher than that achieved by algae-alginate beads (Table 50). The higher capacity of cheap filters than alginate beads to remove different heavy metal ions is a real success and is mainly because of cell immobilization along the surface of the fibrous threads and the reticulated open network of immobilized matrix, together contributing to enhanced surface area and free access of the metal ion to sorption sites.

Although, algae-cotton filters have slightly higher efficiencies to remove different heavy metal ions, algae-sponge filters are more favorable for industrial wastewater treatment applications; since they are more cheaper, durable, non biodegradable, waste byproduct and liable for elastic design.

Our finding is agreement with Akhtar *et al.* (2003b) who found that surface immobilization of *Chlorella sorokiniana* on individual threads of luffa sponge discs providing direct contact of biomass to metal ion solution is better suited for biosorption than enclosed or beaded immobilization in polymeric gel structure.

## **5. Toxicity assessments of untreated and treated industrial effluents using standard algal bioassays.**

The growth of the standard test alga *Pseudokirchneriella subcapitata* (previously known as *Selenastrum capricornutum*) in different raw effluent samples was, mainly, dependant on the toxicity of effluent tested. Toxicity of pH adjusted effluent samples (pH 6.0) was assessed. Standard algal bioassay was also employed to validate the efficiency of co-immobilized test algae to reduce the effluent toxicity through ten successive bioremoval-elution cycles. Growth inhibition of the standard test alga, from dose-response curves, was employed to calculate the effluent toxicity in terms of  $EC_{50}$ .

It should be mentioned that the high the  $EC_{50}$  value, the low the toxicity of a given effluent subsample. In this case the % increase in  $EC_{50}$  was calculated and considered as an analogous of the % toxicity reduction of a treated effluent sample.

### **5.1. Effluent #1 (Talkha chemical fertilizer):**

Looking at Table 55 and Figure 40, one can see that the Talkha chemical fertilizer raw effluent is the most toxic one with  $EC_{50}$  as low as 1.0%. Adjustment of pH value of this effluent at pH 6.0 resulted in a significant decrease in its toxicity ( $EC_{50}= 21$ ).

This result is very probably due to the high ammonia content of effluent #1. unionized ammonia ( $NH_3$ ) is toxic at higher pH values, the toxicity increases



with the increasing of pH value and vice versa, adjustment effluent pH at pH 6.0 reduces toxicity by forming ionized  $\text{NH}_4^+$  which may readily be assimilated by the test alga *Pseudokirchneriella subcapitata*.

Our data goes hand to hand with Abdel-Hamid *et al.* (1991) who found that the toxicity of Talkha chemical fertilizer is mainly due to the high ammonia content coupled with the high alkalinity. Also with Mangas-Ramirez *et al.* (2002) who reported that, the toxicity of ammonia is strongly pH dependant, and in most natural waters with high alkalinities, ammonia may reach high toxic levels.

Substantial toxicity reduction were obtained when pH adjusted samples of effluent #1 (Talkha chemical fertilizer) were biologically treated with algae-sponge filter, algae-cotton filter and algae-alginate beads, the toxicity values ( $\text{EC}_{50}$ ) were increased from 21% of pH adjusted sample to 85%, 94% and 80%, respectively (Table 56) at the first bioremoval cycle. Compared to the pH adjusted effluent sample, all algal biofilters showed more or less comparable efficiencies to reduce the toxicity at fourth and seventh bioremoval cycles with % toxicity reduction never fallen below 200% (Table 56, Figure 41). Although the efficiencies of algal biofilters decreased after the tenth cycle, their efficiencies through these cycles were good enough to reduce the effluent toxicity to 95%, 114% and 85% respectively (Table 56, Figure 41).

The treatment of Talkha chemical fertilizer extreme pH value very probably increased the success of our co-immobilized test algae in removing their

toxic heavy metal ions such as; Cd(II), Ni(II), Pb(II) and Hg(II) (Table 45) reducing the effluent toxicity, improving the growth of standard test alga *Pseudokirchneriella subcapitata*. This can be easily noticed from the strong pushes of the toxicity curves towards the wright and increasing the EC<sub>50</sub> of algal treated effluent samples.

This finding was evidenced by comparing the capacities of our biofilters to remove different heavy metal ions which goes hand in hand with those efficiencies to reduce toxicity. Moreover, the highest and the lowest capacities of all biofilters to remove different heavy metal ions were recorded after the first and the tenth bioremoval cycles respectively. Also, the highest and the lowest % toxicity reduction and EC<sub>50</sub> were observed after the first and the tenth bioremoval cycles. Moreover, the algae-cotton filter that proved to be highly efficient than others to remove different heavy metal ions was also more efficient to increase EC<sub>50</sub>.

Our results are in agreement with Bartlett *et al.* (1974) who studied the algistatic effects of Cd(II) on *Selenastrum capricornatum* (*Pseudokirchneriella subcapitata*). The metal growth inhibition started at 50µg l<sup>-1</sup>, with complete inhibition at 80µg l<sup>-1</sup> after four days. Blaise *et al.* (1986) reported that the EC<sub>50</sub> of this metal varies between 30 and 55µg l<sup>-1</sup>.

Also, with Errecalde and Campbell, (2000) who found that the growth of *Selenastrum capricornatum* was inhibited by Cd(II) at concentration 0.2 mM l<sup>-1</sup>

## **5.2. Effluent #2 (Sandoub oils and soap):**

Sandoub oils and soap effluent became completely nontoxic to the growth of *Pseudokirchneriella subcapitata* as the EC<sub>50</sub> values were kept above 100% (Table 56 and Figure 42) after the first bioremoval cycle. At the fourth and seventh bioremoval cycles, the efficiency of all algal biofilters to reduce effluent toxicity were almost above 100% compared to that achieved by pH adjusted effluent samples. After the tenth cycle the capacities of different algal biofilters to reduce the toxicity of this effluent was nearly around 50% of the pH adjusted effluent test samples (Table 56).

This finding is due to the ability of our algal biofilter to remove toxic heavy metal ions such as Ni(II) (Table 46), Cd(II) (Table 47), Pb(II) (Table 48) and Hg(II) (Table 49). The high concentration of these toxic heavy metal ions in raw and pH adjusted effluent (Table 45) very probably inhibited the growth of test alga *Pseudokirchneriella subcapitata*. Removal of different heavy metal ions from this effluent by algal treatment induced noticeable increase in growth of standard test alga which could be considered as increasing in EC<sub>50</sub> and in % of toxicity reduction. Again, the increasing in the efficiency of different algal biofilters to remove heavy metal ions in first bioremoval cycle leads to increase in the value of EC<sub>50</sub>.

The toxic effect of different heavy metal ions to the standard test alga was pointed out by Pardos *et al.* (1998) who studied algal toxicity tests by *Selenastrum capricornutum* for Cd(II) and Zn(II). They found that EC<sub>50</sub> values were 118 µg l<sup>-1</sup> for Cd(II) and 96 µg l<sup>-1</sup> for Zn(II). Wong *et al.* (2001)

exposed cells of *Selenastrum capricornutum* to a stormwater sample containing various concentrations of Pb(II) and Cu(II). They found that, when the concentrations of these heavy metal ions were about 300 and 200  $\mu\text{g l}^{-1}$ , respectively, the growth of *Selenastrum capricornutum* was practically inhibited. The  $\text{EC}_{50}$  values of *Selenastrum capricornutum* were found to be 0.061, 0.076 and 0.015  $\text{mg l}^{-1}$ , respectively for Cu(II), Cd(II) and Zn(II) (Sponza, 2002).

### **5.3. Effluent #3 (Mahalla dyes factory):**

Looking at (Tables 54, 56 and Figures 38, 43), one can see that the pH adjusted industrial effluent of Mahalla dyes factory is toxic with the  $\text{EC}_{50}$  value 32% of the international test alga *Pseudokirchneriella subcapitata*. Our algal biofilters were able to push toxicity curves towards the right increasing of the  $\text{EC}_{50}$  value and decreasing the toxicity especially with first and fourth bioremoval cycles. At the seventh bioremoval cycle, the efficiency of different algal biofilters to reduce the toxicity of this effluent remained above 150% compared to the corresponding toxicity of the pH adjusted sample. Still for the tenth bioremoval cycle, the algae-sponge filter, algae-cotton filter and algae alginate beads were effective to reduce the whole toxicity of the effluent by 87%, 103% and 87%, respectively.

Comparing these results with the capabilities of different algal biofilters to remove heavy metal ions through ten successive bioremoval cycles (Tables 46-50). We can easily notice that increasing the efficiencies of algal biofilters to remove heavy metal ions through ten cycles leads to increase

the growth of standard test alga *Pseudokirchneriella subcapitata*, increasing EC<sub>50</sub> and % toxicity reduction values.

This finding is in agreement with Chiaudani and Vighi (1978) who reported that, the green alga *Selenastrum capricornutum* was extreme sensitive to heavy metal ions and could be used as an excellent test organism for evaluation of extent of toxicity.

#### **5.4. Effluent # 4 (Kafr-Ezzayyat salt and soda factory):**

Treatment of Kafr-Ezzayyat salt and soda effluent with different algal biofilters minimized the effluent toxicity to zero level (Table 56, Figure 44) after the first and the fourth bioremoval cycles. After the seventh and tenth bioremoval cycles, all algal biofilters maintained good enough efficiency to reduce toxicities and increasing EC<sub>50</sub> values compared to pH adjusted effluent samples.

The effluent of Kafr-Ezzayyat salt and soda was considered as the least toxic one with EC<sub>50</sub> 56% of pH adjusted samples (Table 56), added to that low concentration levels of heavy metal ions such as Pb and Cd compared to other studied industrial effluents.

According to the considerable bulk of data obtained, it should be mentioned that the efficiency of algal-cotton filter to remove different heavy metal ion (Table 50) and to reduce effluent toxicities are higher than that achieved by algae-sponge filter and algae-alginate beads. These results may be due to cellulosic structure of cotton-filter which may add potential metal ion

binding groups with those of the algal biomass increasing their abilities to remove heavy metal ions reducing effluent toxicity.

This is in agreement with the finding of Hashim and Chu (2004) who found that the charged groups such as carboxylate and hydroxyl present in the biopolymers are believed to be responsible for the sequestration of metal ions.

## **6. Effect of industrial effluents on viability of test algae grown within filters and alginate beads.**

In this part of investigation, the capability of four test algae (*Spirulina platensis*, *Chlorella ellipsoidea*, *Scenedesmus quadricauda* var. *longispina* and *Nitzschia palea*) to thrive for ten days within filters and alginate beads at 50% and 100% concentration levels of pH adjusted effluents that were really strongly toxic to the growth of the standard test alga *Pseudokirchneriella subcapitata* were tested and compared with that growth at standard growth medium. This research is necessary to confirm whether or not the mature biofilters of our test algae can maintain durable applied significance for biological treatment of whole toxic industrial effluents.

According to the considerable bulk of data obtained, the effect of different industrial effluents on growth of our test algae composing different biofilters will be debated separately.

### **6.1. Effect of Talkha chemical fertilizers effluent on growth of test algae within algal biofilters:**

Compared to control biofilters, higher concentration levels (50% and 100%) of this pH adjusted effluent induced significant increase in total algal biomass of different algal biofilters (Table 58, 62 and Figure 46). Both of *Chlorella ellipsoidea* and *Spirulina platensis* showed relatively higher biomass production followed by *Scenedesmus quadricauda*. The pinnate diatom *Nitzschia palea* was unable to grow within sponge and cotton substrata treated with 50% and 100% effluent concentrations (Figure 46). The whole pH adjusted effluent concentration increased the algal growth rate by 25.4%, 24.6% and 15.9% at sponge, cotton and alginate carriers respectively (Table 66).

The significant increase in total algal biomass of our algal biofilters may be due to the ionization of free ammonia ( $\text{NH}_3$ ) to ionized  $\text{NH}_4^+$  at pH 6.0. This finding is in agreement with Manning *et al.*, 1996; Andersen and Buckley, 1998 and Mangas-Ramirez *et al.*, 2002 who found that, toxic nonionized (free) ammonia change to non toxic ionized  $\text{NH}_4^+$  with decreasing pH of the medium.

The ability of our algal species *Chlorella ellipsoidea* and *Spirulina platensis* to grow in abnormal high concentrations; 0.80, 0.62, 1.85  $\text{mg l}^{-1}$  and 34  $\mu\text{g l}^{-1}$  of Ni(II), Cd(II), Pb(II) and Hg(II) respectively included in this effluent (Table 45) could be explained by the finding of Chong *et al.* (2000) who compared the growth of Ni(II) and Zn(II) treated cells of *Chlorella vulgaris*,

*Chlorella sorokiniana* and *Scenedesmus quadricauda* with the control cells. They found that, at the end of 1 week incubation, the cell numbers of Ni-treated or (Ni+Zn)-treated cells were the same as that in the control for all species. Wong and Pak (1992) demonstrated that *Chlorella* sp. is tolerant to Cu(II) and Ni(II), and its growth was stimulated at low concentration of Ni(II).

Albertano (1989) examined 45 species and strains of *Chlorella* and found that *Chlorella emersonii* was much more tolerant to mercury. He stated that the tolerance of *Chlorella emersonii* was due to the peculiar structure of the cell wall which is double layered, with the outer trilaminar layer containing sporopollenin. Audholia *et al.* (1993) reported that, *Phormidium uncinatum* (filamentous cyanobacteria) was grown well at high concentration ( $50 \text{ mg l}^{-1}$ ) of Zn(II), Cd(II) and Pb(II).

The tolerance of our cyanobacterium *Spirulina platensis* may be explained by the finding of Bender *et al.* (1995) who found that, the tolerance of cyanobacterial mats to high metal ion concentrations (Pb, Ni and Cd) may be due to their ability to precipitate insoluble metal salts outside the mat cells as either sulfides, oxides or hydroxides.

## **6.2. Effect of Sandoub oils and soap effluent on growth of test algae within algal biofilters:**

Both effluent concentrations (50% and 100%) induced significant growth inhibition in total algal biomass and the biomass of each individual test algae (Table 59, 63 and Figure 47).



On an average, the % growth rate inhibition of total algal biomass developed within Ca-alginate, cotton and sponge carriers were 36.4%, 46.3% and 60.1% at 50% effluent and 56.5%, 72.8% and 89.6% with 100% effluent respectively (Table 67).

Heavy metal analysis (Table 45) of this effluent revealed the presence of Ni(II), Cd(II), Pb(II) and Hg(II) in concentrations 1.14, 1.27, 1.76 mg l<sup>-1</sup> and 49 µg l<sup>-1</sup> respectively. The growth of co-immobilized test algae were greatly inhibited by such high concentration of these heavy metal ions.

Our results are in agreement with Costa and Franca (1998) who found that Cd(II) affected *Spirulina* sp. cell growth and diminished cell productivity above 1.2 mg l<sup>-1</sup>. Wong and Chang (1991) found that high levels of heavy metal ions (Zn, Cd and Fe) seemed to inhibit algal growth of *Chlorella pyrenoidosa*. Kropfl *et al.* (2003) found that, the total biomass production of algal biofilms grown on polycarbonate substrates for 2 weeks were decreased by 14% compared to the control biofilms in presence of nickel ions in concentration of 10<sup>-5</sup> mol l<sup>-1</sup>.

In all effluent concentrations the total algal biomass was to a large extent a product of *Chlorella ellipsoida* and *Spirulina platensis* biomass. Whereas, the biomass contribution of *Scenedesmus quadricauda* was relatively very low and that of *Nitzschia palea* was almost nil (Table 59).

This result may be due to resistance of *Chlorella ellipsoida* and *Spirulina platensis* towards concentrations of heavy metal ions in this effluent than *Scenedesmus quadricauda* and *Nitzschia palea*.

Our results are in agreement with Rangsayatorn *et al.* (2002) who studied the effect of six cadmium concentrations on the growth of *Spirulina platensis* for 96 h. The cell growth was slightly affected at 4 and 8 mg l<sup>-1</sup> of Cd(II). They reported that *S. platensis* had a high tolerance to Cd(II) with an EC<sub>50</sub> higher than that of some species such as *Anabaena inaequalis*, *Nitzschia clasterium*, *Navicula incerta* and *Chlorococcum* sp. Dickman (1998) working on benthic diatoms found that the most polluted site with heavy metal ions (Cr, Cu, Ni, Pb and Zn) exhibited the highest toxicity levels and the lowest diversity. Bentley-Mowat and Reid (1977) found that concentrations of Pb(II) and Cu(II) as high as 5 x 10<sup>-4</sup>M were able to inhibit the growth of the diatom *Phaeodactylum tricornutum*.

### **6.3. Effect of Mahalla dyes effluent on growth of test algae within algal biofilters:**

Compared to control filters, the concentrations 50% and 100% of this effluent caused significant growth inhibition of all test algae (Table 60, 64 and Figure 48).

The pinnate diatom *Nitzschia palea* was the most sensitive to effluent toxicity as its growth rate was declining throughout the incubation period at both 50% ( $\mu = 0.84$ ) and 100% ( $\mu = 1.07$ ) concentration levels of this effluent (Table 68). In general, this diatom was unable to grow within artificial carrier beyond the day 4 of incubation period (Figure 48).

*Scenedesmus quadricauda* was also highly sensitive and was obviously unable to build up biomass within the artificial carriers. Compared to control, the specific growth rate of this chlorococcalean alga within sponge filter was inhibited by 69.5% and 241.1% at 50% and 100% concentrations respectively (Table 68). Comparable results were obtained in cases of cotton filter (Table 68).

*Chlorella ellipsoidea* and *Spirulina platensis* were relatively more tolerant to the toxicity of this effluent (Figure 48). The growth rates of treated and control filters for *Chlorella ellipsoidea* and *Spirulina platensis* was non significant (Table 64). These algae were able to build up biomass within the artificial carriers treated with either 50% or 100% effluent doses (Figure 48). The cell count of both algae, at the end of incubation period, was significantly higher ( $P \leq 0.05$ ) than the starting cell count (Table 60). This finding indicates that *Chlorella ellipsoidea* and *Spirulina platensis* are efficient candidate algae for metal ion bioremediation of the Mahalla dyes effluent.

Heavy metal analysis of this effluent (Table 45) showed the presence of Ni(II), Cd(II), Pb(II) and Hg(II) with concentrations 1.95, 1.32, 1.5  $\text{mg l}^{-1}$  and 59  $\mu\text{g l}^{-1}$  respectively. This shows that different algal species may have different capabilities to tolerate heavy metal ions.

Our results are in agreement with Terry and Stone (2002) who reported that tolerance of algae to heavy metal ions differed among different algal species.

Mallicka and Mohn (2003) found that the test metal ions (Cu(II), Cr(III), Ni(II), Cd(II), and Zn(II)) substantially inhibited photosystem II (PS II) of the green microalga *Scenedesmus obliquus*.

A reduction in growth rate of the green algae *Kirchneriella lunaris* and *Scenedesmus obliquus* by Cd(II), Co(II), Mn(II) and Ni(II) was observed by Issa (1995) and Issa *et al.* (1998). Bentley-Mowat and Reid (1977); Thomas and Seibert (1977) and Goering *et al.* (1979) reported that diatoms are the most sensitive organisms to heavy metal ions due to inhibition of the uptake of silicic acid. The high resistance of our *Chlorella ellipsoida* and *Spirulina platensis* may be due to the variety of mechanisms for heavy metal ion tolerance they possess.

Inthorn *et al.* (1996) stated that Cd(II) had no effect on growth of cyanobacteria lower than 2.0 mg l<sup>-1</sup>. Maclean *et al.* (1972) found that the tolerance of cyanobacteria results from binding of Cd(II) by low molecular weight proteins excreted by the cells.

Bartless *et al.* (1977) reported that *Chlorella* produces glucose dehydrogenase which reduced Hg ions to volatile metallic Hg at outer cell surface. De Filippis and Pallaghy (1976) and De Filippis (1978) found that resistance of *Chlorella* to Hg was due to its cells has high capacity to volatilize Hg into the atmosphere.

#### **6.4. Effect of Kafr-Ezzayyat salt and soda effluent on growth of test algae within algal biofilters:**

The growth of *Nitzschia palea* within effluent treated Ca-alginate, cotton and sponge filters was fully inhibited after 4 days (Figure 49). Growth inhibition of *Scenedesmus quadricauda* within artificial biofilters ranged between 24.7% for 50% and 241.1% for 100%.

*Chlorella ellipsoidea* followed by *Spirulina platensis* were the most tolerant algae capable of growing within artificial carriers treated with 50% and 100% effluent concentrations (Figure 49).

It should be mentioned that both algae were able to build up biomass in various algal filters treated with different effluent doses. This finding again indicates that *Chlorella ellipsoidea* and *Spirulina platensis* are efficient candidates for metal ions bioremoval of Kafr-Ezzayyat salt and soda effluent.

Heavy metal analysis of Kafr-Ezzayyat salt and soda effluent (Table 45) revealed the presence of Ni(II), Cd(II), Pb(II) and Hg(II) with concentrations 0.93, 0.37, 1.2 mg l<sup>-1</sup> and 43 µg l<sup>-1</sup> respectively.

Again *Chlorella ellipsoidea* and *Spirulina platensis* were the most tolerant towards heavy metal ions of this effluent. This is in agreement with the finding of Rangsayatorn *et al.* (2002) who found that living cells of *Spirulina platensis* had a high tolerance to Cd(II), so it can be effectively applied to phytoremediate wastewater. Wong and Pak (1992) demonstrated

that *Chlorella* sp. is tolerant to Cu(II) and Ni(II), and its growth was stimulated at low concentration of Ni(II). Trevors *et al.* (1986) found that the EC<sub>50</sub> of diatom *Nitzschia closterium* was 0.48 mg l<sup>-1</sup> for Cd(II). Stauber and Florence (1989) found that 10 µg Cu l<sup>-1</sup> gives a 50% reduction in cell division rate for *Nitzschia closterium*.