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Development of Algal Biofilters for the Treatment of Heavy Metal Pollution From Industrial Wastewater

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ABSTRACT

Ten algal strains; six blue greens, three greens and one diatom were used to build up twenty types of small laboratory-scale biofilters. Algal strains were inoculated on four carriers (sand, silt, cotton and sponge). Biofilters were used to bioremove different heavy metals from highly toxic industrial effluents from the Egyptian industry. Cotton and sponge biofilters proved to be better candidates to remove all tested heavy metals than sand and silt ones. Moreover, toxicity assessment of raw and filter-treated effluents using standard test alga *Pseudokirchneriella subcapitata* showed that the cotton followed by sponge algal filters were the highest efficient biofilters capable to reduce effluent toxicity than sand and silt biofilters. Thus algal biofilters offer an economically feasible technology for efficient removal and recovery of metals from industrial wastewater.

Keywords: Algal biofilters, heavy metals, industrial effluents, toxicity assessment.

INTRODUCTION

The release of heavy metals into the environment by industrial activities is a serious environmental problem because they tend to remain indefinitely, circulating and eventually accumulating throughout the food chain (Periasamy and Namasivayam, 1994 and 1995). Different conventional processes (precipitation, ion exchange, electrochemical processes and/or membrane processes) are usually applied to the treatment of industrial effluents but the application of such processes is often limited because of technical or economic constraints (Veglio, *et al*., 2003).

Biosorption of heavy metals from aqueous solutions is a relatively new technology for the treatment of industrial wastewater (Volesky, 1990). Adsorbent materials (biosorbents) derived from suitable biomass can be used for the effective removal and recovery of heavy metal ions from wastewater streams. The major advantages of the biosorption technology are its effectiveness in reducing the concentration of heavy metal ions to very low levels and the use of inexpensive biosorbent materials. Different biomasses can effectively uptake heavy metals, but recent research has shown that algae are more effective (Kaewsarn, 2002, Ibrahim *et al*., 2005 and Romera *et al*., 2006).

This work aims at evaluation of the efficiency of ten common species of fresh water algae to develop algal biofilters for the treatment of heavy metals pollution from industrial wastewater.

MATERIALS AND METHODS

Twenty types of biofilters were used in bioremoval of different heavy metals from different industrial effluents. According to their carrier, biofilters were classified into 4 classes; sand, silt, cotton and sponge. According to their complex of algal species, biofilters were classified into 5 groups (described below in details). Each class of biofilter carrier was inoculated with 5 groups of algal complexes (Table 1).

Development of algal flat filters:

Cotton, sponge, sand or silt (0.5 cm thick) were placed on bottom of white plastic containers with the dimensions; 17 cm long, 12 cm wide and 10 cm depth. A mixture of the five test algae of each complex was prepared. The total initial algal cell density was 5 x 10^6 cells / ml with 20% contribution of each test alga. The mixture was shaken for one minute and then added to the plastic boxes until the carrier become fully saturated. In this case, different carriers represent beds for the development of flat algal biofilters. The containers were placed in sunlight and always flooded before drying by MBL medium (Nichols, 1973), until a thick algal mat was developed. The thick algal mat along with its carrier is considered a mature algal biofilter.

Relative species composition of mature algal biofilters after 14 days incubation period

The algal biofilters reached maturity after 14 days of incubation. Therefore, a decision was taken to analyze the algal species composition to check for any changes that may have occurred during the incubation period. This was achieved by cutting a volume of 1 cm^3 from each filter. The cut pieces were placed in vials containing 10 ml of algal preservative solution, 1.0% (v/v) Lugol's solution (Prescott, 1978). Cell count was carried out using a standard haemocytometer slide (filamentous forms were sonicated to break them down to small fragments to make them easy to count).

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Table (1): Numbers of different algal biofilters.

Substratum \ Algal complex	A	B	C	D	E
Sand	1	2	3	4	5
Silt	6	7	8	9	10
Cotton	11	12	13	14	15
Sponge	16	17	18	19	20

Algal complex **A=** *Nostoc ellipsosporum, Zygnema sterile, Chlorella ellipsoida, Scenedesmus quadricauda* and *Navicula cannalis,* **B=** *Oscillatoria foreui, Tolypothrix ceytonica, C. ellipsoida, S. quadricauda* and *N. cannalis.* **C=** *Anabaena oryzae, Cylinderospermus alatosporum, C. elipsoida, S. quadricauda* and *N. cannalis,* **D=** *Calothinx castelli, A. oryzae, C. ellispsoida, S. quadricauda* and *N. cannalis,* **E=** *O. Foreui, C. alatosporum, C. ellipsoida, S. quadricauda* and *N. cannalis.*

Calculation of growth rate of algal biofilters

The average specific growth rate (μ) for a specific period was calculated as the logarithmic increase in biomass from the equation (OECD, 2002).

$$\mu = (\ln N_n - \ln N_0) / (t_n - t_o)$$

where: $\mathbf{N_0}$ = Cell number at the beginning of the test (t_o), and $\mathbf{N_n}$ = Cell number at the t_n time (days).

Bioremoval of different heavy metal from industrial effluents

Two industrial effluents were chosen for this purpose as the most toxic ones based on previous work by the authors (Azab *et al.*, 2004 and Ibrahim *et al.*, 2005). They belong to different industries; Talkha for chemical fertilizers factory as chemical fertilizers industry and Mahalla dyes factory as textile industry. Composite effluent samples were collected, at working hours of the two mention factories in polyethylene containers from their outfalls. Effluent samples were thoroughly mixed; six liters were filtered through GF/C Whatman glass filters. The first one liter filtrate was discarded and five liters were stored at 4°C in dark to be used for chemical and toxicity assessment analysis.

pH of the two GF/C filtered industrial effluents was adjusted at the pH 6.0. pH adjusted effluents were passed through the different types of algal biofilters with flow rate 1 liter/hour.

Toxicity assessment of untreated and treated industrial effluents

The methods described by Porcella (1983), Peltier and Weber (1985) and Greene *et al.* (1989) were adopted to carry out toxicity test of GF/C filtered industrial effluent, pH adjusted effluent and biologically treated effluent.

EC_{50} and SC_{20} express the minimum effluent concentrations inhibiting and stimulating the algal growth by 50% and 20% respectively.

Toxicity reduction (%) = (EC_{50} of biologically treated effluent **-** EC_{50} of pH adjusted effluent / EC_{50} of pH adjusted effluent) x 100 (Abdel-Hamid *et al.*, 1994).

RESULTS

Chemical characteristics of effluents

Some chemical properties of Talkha chemical fertilizers factory and Mahalla dyes factory are given in Table 2. Both effluents were alkaline with pH 10.2 and 8.1 respectively. This is very probably due to the high ammonia content of Talkha chemical fertilizers factory and sodium hydroxide used in textile manufacturing of Mahalla dyes factory. Analysis of heavy metal ions (Table 2) revealed that, the concentration of Cr, Fe, Pb and Cu of Talkha chemical fertilizers factory varied from 0.12 mg l^{-1} to 2.1 mg l^{-1} while those of Cr, Cd, Pb and Ni of Mahalla dyes ranged between 0.25 mg l^{-1} to 0.75 mg l^{-1}.

Table (2): Some chemical properties of the investigated industrial effluents.

Effluent	pH	Cr	Fe	Pb	Cu	Cd	Ni
	mg l-1						
Talkha chemical fertilizers factory	10.2	1.1	1.6	2.1	0.12	-	-
Mahalla dyes factory	8.1	0.75	-	0.7	-	0.25	0.44

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

Mature algal biofilters

Figure (1) illustrates the absolute densities (cells/ml) of test algae grown together for 14 days within silt, sand, sponge and cotton carriers (mature algal biofilters).

The total algal biomass showed different mean growth rate at different carriers with highest growth rate (0.14 day^{-1}) recorded for sponge and cotton carriers, followed by sand (0.13 day^{-1}) and then silt (0.06 day^{-1}).

Efficiency of algal filters to remove metal ions from industrial effluents

Obvious variations in capacity of algal biofilters to remove different heavy metals were noticed. The variations were to some extent dependent on the nature of physical carrier (sand, silt, cotton and sponge) and on the effluent type (Fig. 2-5).

Compared to sand biofilters, the sponge and cotton biofilters were relatively more efficient to remove different heavy metals from different effluent samples. However, silt biofilters showed the least efficiency to remove metals from the industrial effluents (Fig. 2-5).

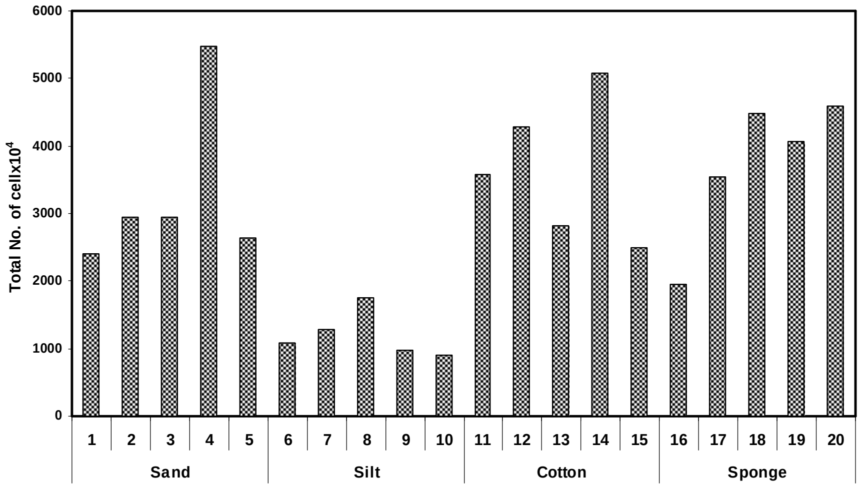

Figure (1): Total algal biomass on different carriers.

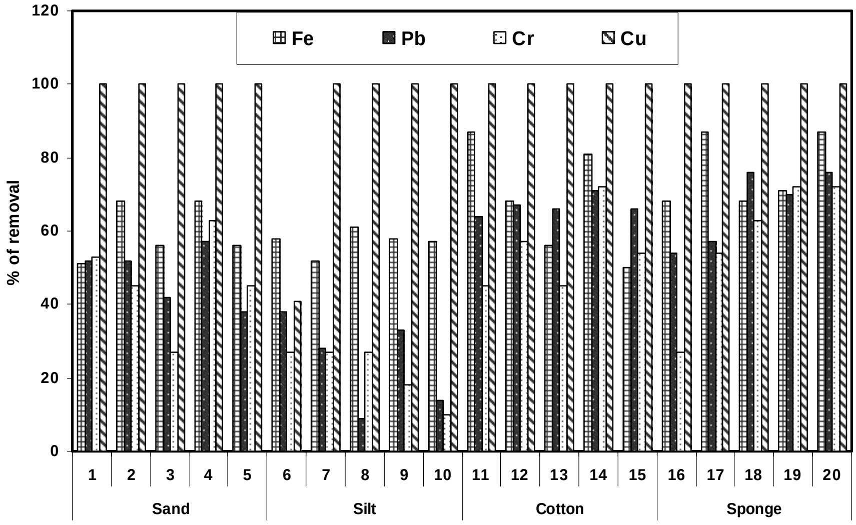

Figure (2): Percentage of bioremoval of different heavy metals from Talkha for chemical fertilizers factory effluent.

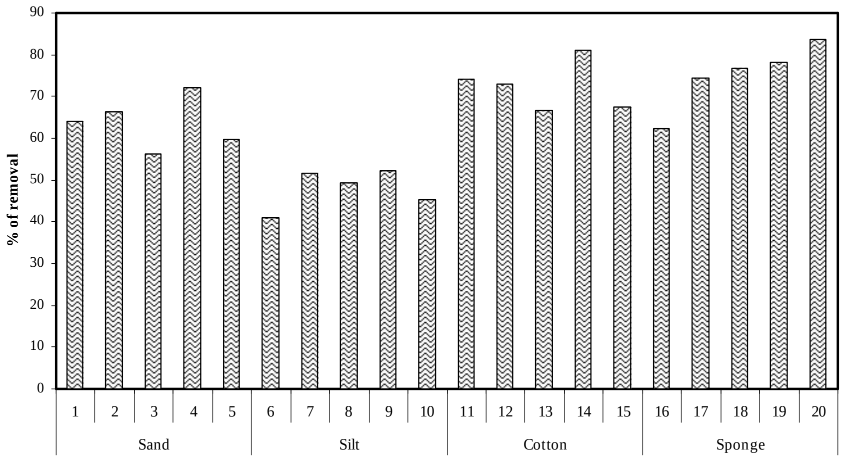

Figure (3): Mean bioremoval effeciency of different heavy metals from Talkha for chemical fertilizers factory effluent.

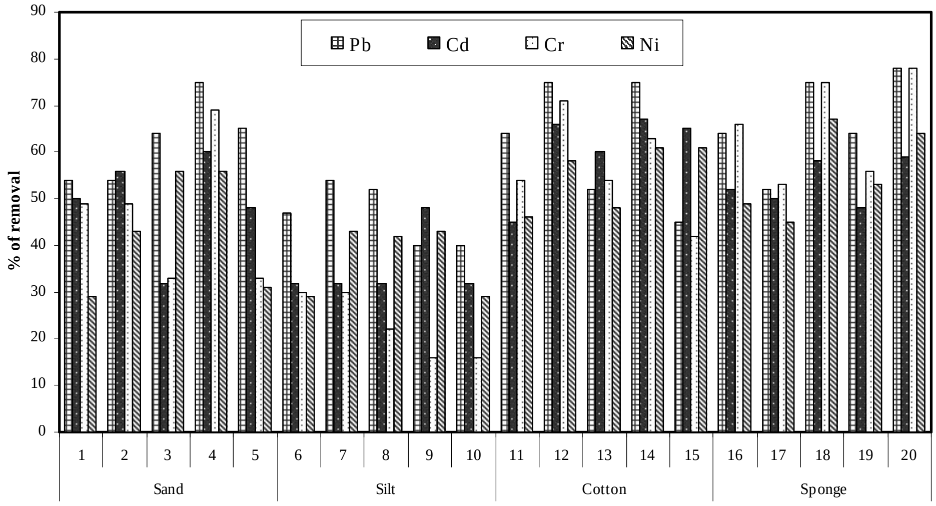

Figure (4): Percentage of bioremoval of different heavy metals from Mahalla dyes factory effluent.

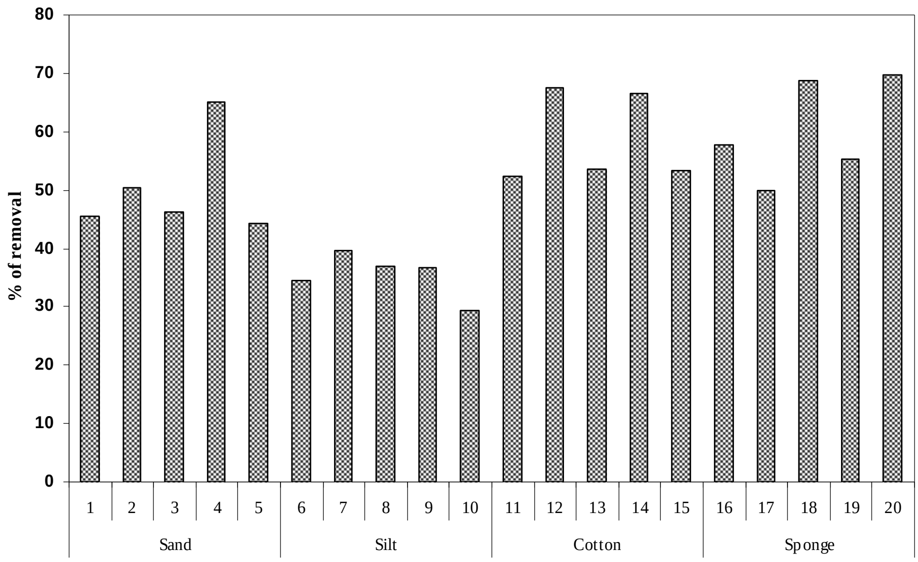

Figure (5): Mean bioremoval effeciency of different heavy metals from Mahalla dyes factory effluent.

The mean efficiency of different algal biofilters to remove Fe, Pb, Cr and Cu from Talkha for chemical fertilizers effluent ranged between 47% to 100%, 22% to 88%, 55% to 100% and 58% to 100% for sand, silt, cotton and sponge biofilters respectively (Table 3).

The mean efficiency of different algal biofilters to remove Cd, Pb, Cr and Ni from Mahalla dyes factory effluent ranged between 43% to 62.4%, 23% to 47%, 55% to 62% and 53% to 67% for sand, silt, cotton and sponge biofilters respectively (Table 4).

In general, the highest capacity of all biofilters to remove metals was recorded for cotton and sponge biofilters and the lowest capacity was recorded for silt ones.

Table (3): Summary of the efficiency of algal biofilters to remove metal ions from Talkha for chemical fertilizers effluent.

Biofilter / Metal ions		Sand filter	Silt filter	Cotton filter	Sponge filter
		% metal ion removed			
Fe(II)	Min.	51	52	50	68
	Max.	68	61	87	87
	Mean	59.8	57.2	68.4	76.2
Pb(II)	Min.	38	9	64	54
	Max.	57	38	71	76
	Mean	48.2	24.4	66.8	66.6
Cr(II)	Min.	27	10	45	27
	Max.	63	27	72	72
	Mean	46.6	21.8	54.6	57.6
Cu(II)	Min.	100	41	100	100
	Max.	100	100	100	100
	Mean	100	88.2	100	100

Table (4): Summary of the efficiency of algal biofilters to remove metal ions from Mahalla dyes factory effluent.

Metal ions \ Biofilter		Sand filter	Silt filter	Cotton filter	Sponge filter
		% metal ion removed			
Pb(Π)	Min.	54	40	45	52
	Max.	75	54	75	78
	Mean	62.4	46.6	62.2	66.6
Cd(Π)	Min.	32	32	45	48
	Max.	60	48	67	59
	Mean	49.2	35.2	60.6	53.4
Cr(Π)	Min.	33	16	42	53
	Max.	69	30	71	78
	Mean	46.6	22.8	56.8	65.6
Ni(Π)	Min.	29	29	46	45
	Max.	56	43	61	67
	Mean	43	37.2	54.8	55.6

DISCUSSION

Results of this investigation are in agreement with the finding of Akhtar *et al.* (2004) who found that, immobilized *Chlorella sorokiniana* on luffa sponge as a new biosorption system was able to remove 96% of Pb ions from aqueous solutions after 15 minutes at pH 5.0 and with Ibrahim *et al.* (2005) who used three types of algal biofilters built up from *Spirulina platensis, Chlorella ellipsoida, Scenedesmus quadricauda* and *Nitzschia palea* to remove Ni, Cd, Pb and Hg from four industrial effluents in Egypt. They found that the efficiencies of the three algal biofilters ranged from 58% to 100% in removing metal ions.

Also, Sobhan and Sternberg (1999) studied the ability of *Cladophora* to remove Cd from synthetic wastewater. They found that Cd^{2+} removal varied from 86% to 96%, with high degrees of removal observed in the first 48hrs.

The microalgal based wastewater treatment system, predominated mainly by *Scenedesmus* and *Chlorella* was able to remove heavy metals with the ranges between 52.3% to 100% in a batch system and from 64.2% to 100% in case of the continuous system (Hammouda *et al*., 1995). 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(Π) uptake was fast as 90% or more within 30–40 min of contact time.

Our results are in agreement with Rangsayatorn *et al*. (2004) who used immobilized *Spirulina platensis* to remove Cd(Π) through five cycles of adsorption and desorption. After the first cycle, Cd(Π) 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.

It was generally noticed concerning toxicity assessment that the values of EC_{50} of pH adjusted effluent subsamples were significantly ($P \leq 0.05$) higher compared to the corresponding values of raw effluent samples (Fig. 6-9).

Compared to Mahalla dyes factory effluent, the Talkha chemical fertilizers effluent was relatively the most toxic as raw effluent concentration; exhibited strong growth inhibition of the test algae (Fig. 6-9) with EC_{50} of 0.8 %. Adjustment of pH of Talkha effluent resulted in a marked decrease in its toxicity (EC_{50} = 12%).

The Mahalla dyes factory effluent was ranked as the second toxic on growth of test alga with EC_{50} values of 15% and 32% calculated before and after pH adjusted respectively (Fig. 9).

Biological treatment of Talkha chemical fertilizers and Mahalla dyes effluents with different algal biofilters reduced its toxicity as EC_{50} values were significantly ($P \leq 0.05$) increased from 12% to 90% and 32% to 71% respectively (Fig. 2-5) (higher EC_{50} means less toxicity).

Our results are in agreement with Bartlett *et al*. (1974) who studied the algistatic effects of Cd(Π) on *Selenastrum capricornatum* (*Pseudokirchneriella subcapitata*). The metal growth inhibition started at 50 $\mu g\ l^{-1}$, with complete inhibition at $80 \mu g l^{-1}$ after four days. Blaise *et al*. (1986) reported that the EC_{50} of this metal varies between 30 and 55 $\mu g\ l^{-1}$.

Also, with Errecalde and Campbell, (2000) who found that the growth of *Selenastrum capricornatum* was inhibited by Cd(Π) at concentration 0.2 mMl^{-1}.

This finding is due to the ability of algal biofilters to remove toxic heavy metal ions such as Ni, Cd, Cr, Fe, Pb and Cu. The high concentration of these toxic heavy metal ions in raw and pH adjusted effluent (Table 2) 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_{50} and in percentage of toxicity reduction.

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(Π) and Zn(Π). They found that EC_{50} values were 118 $\mu g\ l^{-1}$ for Cd(Π) and 96 $\mu g\ l^{-1}$ for Zn(Π). Wong *et al*. (2001) exposed cells of *Selenastrum capricornutum* to a stormwater sample containing various concentrations of Pb(Π) and Cu(Π). They found that, when the concentrations of these heavy metal ions were about 300 and 200 $\mu g\ l^{-1}$ respectively, the growth of *Selenastrum capricornutum* was practically inhibited. The EC_{50} values of *Selenastrum capricornutum* were found to be 0.061, 0.076 and 0.015 $mg\ l^{-1}$, respectively for Cu(Π), Cd(Π) and Zn(Π) (Sponza, 2002).

The present study highlights the role of algal technology representing a good hope for clean environment through treatment of heavy metal pollution in industrial wastewater using algal biofilters.

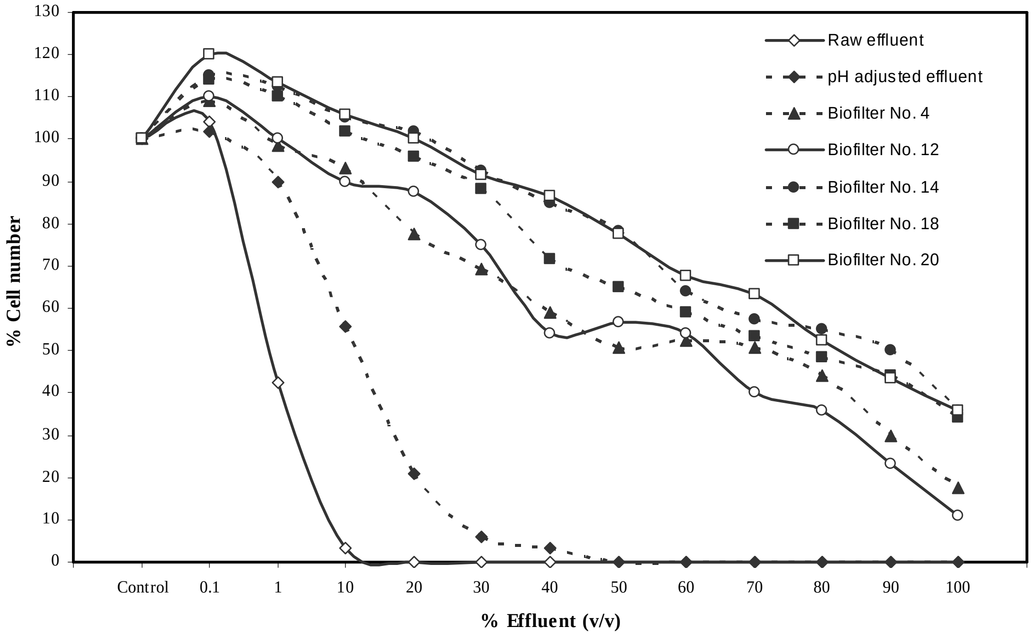

Figure (6): Dose-response curves of *Pseudokirchneriella subcapitata* grown for 5 days at different concentrations of Talkha chemical fertilizers factory effluent.

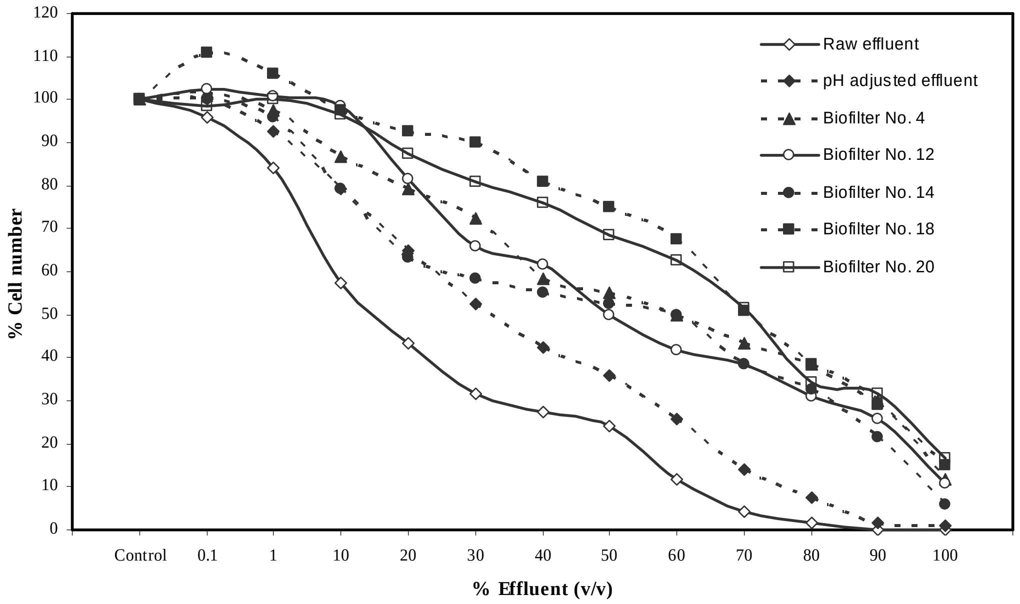

Figure (7): Dose-response curves of *Pseudokirchneriella subcapitata* grown for 5 days at different concentrations of Mahalla dyes factory effluent.

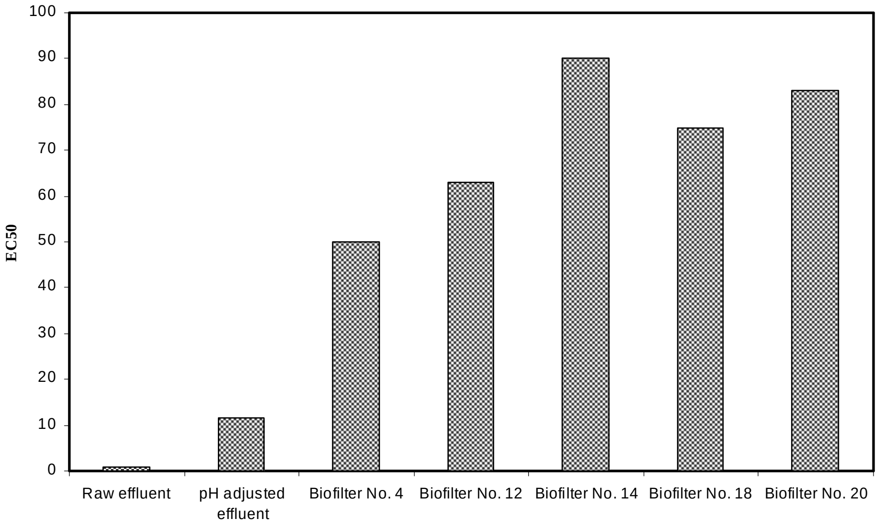

Figure (8): Efficiency of algal filters to reduce toxicity of Talkha for chemical fertilizers factory effluent.

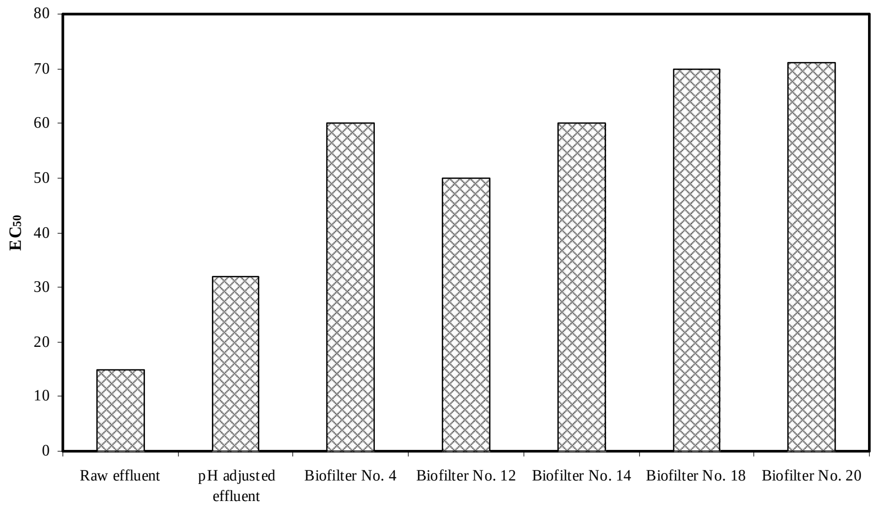

Figure (9): Efficiency of algal filters to reduce toxicity of Mahalla dyes factory effluent.

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تطوير المرشحات الطحلبية لمعالجة التلوث بالمعادن الثقيلة في المخلفات الصناعية السائلة

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الملخص العربـــى

تم إستخدام عشرة من السلالات الطحلبية، ستة من الطحالب الخضراء المزرقة وثلاثة من الطحالب الخضراء وسلاله واحدة من الطحالب الدياتوميه فى بناء عشرين نوعاً من المرشحات الطحلبية الحيوية. تم تنمية جميع السلالات الطحلبية علي أربعة من الحوامل هى الرمل والطين والقطن والأسفنج. استخدمت هذه المرشحات الطحلبية في إزالة بعض العناصر الثقيلة من أكثر المخلفات الصناعية سمية في مصر. أوضحت الدراسة زيادة كفاءة المرشحات الطحلبية القطنيه والأسفنجية عن المرشحات الطحلبية الرملية والطينية في إزالة جميع العناصر الثقيلة. اثبت اختبار السمية باستخدام طحلب سيدوكيرشنييلا سيبكبيتاتا للمخلفات الصناعية الخام والمعالجة بالمرشحات الطحلبية ان المرشح الطحلبى القطنى يليه المرشح الطحلبى الأسفنجى كان اعلى كفاءة فى إختزال سمية المخلفات الصناعية عن المرشحات الطحلبية الطينية والرملية. وعليه فإن المرشحات الطحلبية سوف توفر تكنولوجيا بيئية ملائمة لمعالجة العناصر الثقيلة من المخلفات الصناعية إن شاء الله تعالى.