RESULTS

1- Optimum contact time for metal ions bioremoval from synthetic solutions by immobilized test algae.

The contact time is the time period during which beads of alginate immobilized algae were left or soaked on a given metal ion test solution. Five time periods (1 min., 5 min., 15 min., 30 min. and 60 min.) were chosen to select the optimum time at which maximum efficiency of metal ion bioremoval by a given test alga is attained. The experimental pH was around 7.0.

The metal ion bioremoval efficiency is expressed as the % metal ion concentration removed by a test alga relative to the initial metal ion concentration of a given test solution. In all cases the initial metal ion concentration was 10 mgl^{-1} for the metal ions Ni(Π), Cd(Π) and Pb(Π) and 1.0 mgl^{-1} for Hg(Π).

The basic test procedure involved soaking the algae alginate beads of the four test algae (*Spirulina platensis*, *Chlorella ellipsoida*, *Scenedesmus quadricauda var*. *longispina* and *Nitzschia palea*) in different test metal solutions (Ni(Π), Cd(Π), Pb(Π) and Hg(Π)) at different contact times. Different tests were carried out under identical conditions.

Detailed results showing the effect of different contact periods on metal ion bioremoval efficiency of different test algae, are given in Tables 8, 9, 10 and 11 for the metal ions Ni(Π), Cd(Π), Pb(Π) and Hg(Π) respectively. The results are also graphically presented in Figure 7.

It is clear from Tables 8-11 and Figure 7 that, the efficiency of metal ion bioremoval increases as contact time increases. For almost all cases, the highest metal ion concentrations removed by different test algae was achieved at 60 minutes. The efficiency of metal ion bioremoval ranged between 20.9% and 97% .

Considerable variations existed in efficiency of different immobilized algae to remove metal ions from their aqueous solutions. It is evident that metal ion bioremoval efficiency (Tables 8-11 and Figure 7) was to a large extent, dependent on contact time, metal and algal species.

To reduce the great bulk and the overwhelming nature of data obtained, the results of metal ions bioremoval efficiencies were further statistically analyzed. A simple t-test was used to ascertain whether or not, the differences obtained in metal ion bioremoval efficiencies at different contact times are statistically significant. To do so, the metal ion concentration removed by a given test alga at a given contact time was compared (on basis of t-test) with its preceding one. Concisely, the % metal ion bioremoval efficiencies at 5.0 minutes was compared with that achieved at 1.0 minute contact period and so on. Differences are considered statistically significant

(*) at $P \leq 0.05$, highly significant (**) at $P \leq 0.01$ and very high significant (***) at $P \leq 0.001$.

Table 12 sets the statistical difference in metal ion bioremoval efficiencies of different immobilized test algae at different contact time periods. From statistical point of view, it is clear from Table 12 that considerably high metal ion bioremoval efficiency is obtained at 15 minutes for, almost, all test algae. Although, in some cases their was slightly increase in metal ion concentration removed by immobilized algae at contact times beyond 15 minutes, the differences were always statistically non significant (Table 12).

The period of 15 minutes was, therefore, the shortest time period at which considerably high metal ion bioremoval was achieved. Consequently, 15 minutes was selected as an optimum contact time for metal ion bioremoval.

The metal ions bioremoval efficiencies of different test algae were compared at a fixed contact time of 15 minutes. Beads of the test algae *Spirulina platensis*, *Chlorella ellipsoida*, *Scenedesmus quadricauda var. longispina* and *Nitzschia palea* removed 63.4%, 72.5%, 71.1% and 68.4% from the initial concentration of Ni (Table 8, Figure 7), 61.1%, 60.3%, 59.4% and 60.8% from Cd (Table 9, Figure 7), 59.5%, 68.4%, 67.2% and 68.2% from Pb (Table 10, Figure 7) and 87.7%, 97%, 96.8% and 77.5% from Hg (Table 11, Figure 7) respectively, it is clear that, the metal ions bioremoval efficiencies of different algae were around or higher than 60%.

Table 8: Effect of time on bioremoval of **Nickel**(Π) by different algal beads.

Algal beads	**Initial Ni conc.(mgl^{-1})**	**Amount of Ni bioremoved after time intervals in minutes**				
		1	**5**	**15**	**30**	**60**
Spirulina platensis	10	2.49 (24.9)	4.12 (41.2)	6.34 (63.4)	6.84 (68.4)	7.34 (73.4)
Chlorella ellipsoida	10	3.52 (35.2)	5.12 (51.2)	7.25 (72.5)	7.43 (74.3)	7.88 (78.8)
Scenedesmus quadricauda	10	3.11 (31.1)	4.93 (49.3)	7.11 (71.1)	7.53 (73.5)	7.92 (79.2)
Nitzschia palea	10	2.81 (28.1)	4.1 (41)	6.84 (68.4)	7.11 (71.1)	7.51 (75.1)

Values in parentheses represent the percentage of bioremoval.

Table 9: Effect of time on bioremoval of **Cadmium**(Π) by different algal beads.

Algal beads	**Initial Cd conc.(mgl^{-1})**	**Amount of Cd bioremoved after time intervals in minutes**				
		1	**5**	**15**	**30**	**60**
Spirulina platensis	10	2.15 (21.5)	3.87 (38.7)	6.11 (61.1)	6.98 (69.8)	7.13 (71.3)
Chlorella ellipsoida	10	2.43 (24.3)	3.83 (38.3)	6.03 (60.3)	6.58 (65.8)	6.84 (68.4)
Scenedesmus quadricauda	10	2.13 (21.3)	3.91 (39.1)	5.94 (59.4)	6.82 (68.2)	6.95 (69.5)
Nitzschia palea	10	2.09 (20.9)	2.93 (29.3)	6.08 (60.8)	6.71 (67.1)	7.21 (72.1)

Values in parentheses represent the percentage of bioremoval.

Table 10: Effect of time on bioremoval of **Lead**(Π) by different algal beads.

Algal beads	**Initial Pb conc.(mgl^{-1})**	**Amount of Pb bioremoved after time intervals in minutes**				
		1	**5**	**15**	**30**	**60**
Spirulina platensis	10	2.46 (24.6)	3.78 (37.8)	5.95 (59.5)	7.09 (70.9)	8.88 (88.8)
Chlorella ellipsoida	10	3.12 (31.2)	4.61 (46.1)	6.84 (68.4)	7.88 (78.8)	8.75 (87.5)
Scenedesmus quadricauda	10	2.88 (28.8)	4.11 (41.1)	6.72 (67.2)	7.45 (74.5)	8.11 (81.1)
Nitzschia palea	10	2.13 (21.3)	4.29 (42.9)	6.82 (68.2)	7.31 (73.1)	8.19 (81.9)

Values in parentheses represent the percentage of bioremoval.

Table 11: Effect of time on bioremoval of **Mercury**(Π) by different algal beads.

Algal beads	**Initial Hg conc.(mgl^{-1})**	**Amount of Hg bioremoved after time intervals in minutes**				
		1	**5**	**15**	**30**	**60**
Spirulina platensis	1.00	0.28 (28)	0.377 (37.7)	0.877 (87.7)	0.968 (96.8)	0.963 (96.3)
Chlorella ellipsoida	1.00	0.38 (38)	0.472 (47.2)	0.97 (97)	0.96 (96)	0.957 (95.7)
Scenedesmus quadricauda	1.00	0.28 (28)	0.475 (47.5)	0.968 (96.8)	0.968 (96.8)	0.961 (96.1)
Nitzschia palea	1.00	0.279 (27.9)	0.379 (37.9)	0.775 (77.5)	0.96 (96)	0.968 (96.8)

Values in parentheses represent the percentage of bioremoval.

Fig (7)

Table 12: Statistical differences in metal ions bioremoval efficiency of different immobilized test algae at different contact time periods.

Metal ions	Algal beads / Time (min.)	*Spirulina platensis*		*Chlorella ellipsoida*		*Scenedesmus quadricauda*		*Nitzschia palea*	
		t-value	Significance	t-value	Significance	t-value	Significance	t-value	Significance
Nickel	1	-	-	-	-	-	-	-	-
	5	3.96	*	3.91	*	4.45	*	3.15	*
	15	5.43	**	5.21	**	5.33	**	6.71	**
	30	1.22	NS	0.44	NS	1.02	NS	0.66	NS
	60	1.22	NS	1.10	NS	0.95	NS	0.97	NS
Cadmium	1	-	-	-	-	-	-	-	-
	5	4.21	*	3.42	*	3.36	*	2.05	NS
	15	5.48	**	5.38	**	4.97	**	7.71	**
	30	2.13	NS	1.34	NS	2.15	NS	1.54	NS
	60	0.36	NS	0.63	NS	0.31	NS	1.22	NS
Lead	1	-	-	-	-	-	-	-	-
	5	3.23	*	3.64	*	3.01	*	5.29	*
	15	5.31	**	5.46	**	6.39	**	6.19	**
	30	2.79	*	2.54	NS	1.78	NS	1.20	NS
	60	3.38	*	2.13	NS	1.61	NS	2.15	NS
Mercury	1	-	-	-	-	-	-	-	-
	5	1.64	*	1.12	NS	2.38	**	1.22	NS
	15	6.12	***	6.09	***	6.03	***	4.84	***
	30	1.12	NS	-0.01	NS	0.0	NS	2.16	*
	60	-0.06	NS	-0.03	NS	-0.08	NS	0.09	NS

NS **Non significant** ***Significant ($P \leq 0.05$)** ****Highly significant ($P \leq 0.01$)**
*****Very highly significant ($P \leq 0.001$).**
- No preceding value to compare with.

2- Optimum pH value for metal ions bioremoval from synthetic solutions by immobilized test algae.

Eight pH values (2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0) were chosen to select the optimum pH at which maximum metal ion bioremoval was attained.

The basic test procedure, in this case, involves soaking the algae-alginate beads of test algae in different test metal solutions (Ni(Π), Cd(Π), Pb(Π) and Hg(Π)) at different pH values for fixed contact time of 15 minutes.

Results indicating the effect of pH values on metal ion bioremoval efficiency are shown in Table 13, 14, 15 and 16 for the metal ions Ni(Π), Cd(Π), Pb(Π) and Hg(Π) respectively. The results are also graphically presented in Figure 8.

A significant increase ($P \leq 0.05$) in metal ion bioremoval efficiency with the increase of pH was noticed until pH 5.0, became more or less stable at pH 6.0 and then gradually decreased as pH increased further (Figure 8).

It is clear from Tables 13-16 and Figure 8 that, the efficiency of metal ion bioremoval increased with increasing pH values from 2.0 to 5.0 then decreased with increasing pH values from 6.0 to 9.0. For almost all cases, the highest metal ion concentrations removed by different test algae was achieved at pH 6.0. Generally, the efficiency of metal ions bioremoval ranged between 30.9% and 96%, depending mainly on pH value and then on metal and test algal species.

Results of metal ions bioremoval efficiencies were statistically analyzed using simple t-test (Table 17). Differences were considered statistically significant (*) at $P \leq 0.05$, highly significant (**) at $P \leq 0.01$ and very highly significant (***) at $P \leq 0.001$.

From statistical point of view, it is clear from Table 17 that the highest metal ion bioremoval efficiency is obtained at pH 6.0 for almost all test algae. Although, in some cases their was slightly increase in metal ion concentration removed by immobilized algae at lower pH values, the differences were statistically non significant (Table 17).

Therefore, a pH 6.0 was considered as an optimum pH value for metal ion bioremoval. The metal ion bioremoval efficiencies of different test algae were compared at pH value 6.0. Beads of the test algae *Spirulina platensis, Chlorella ellipsoida, Scenedesmus quadricauda var. longispina* and *Nitzschia palea* removed 65%, 65.1%, 62% and 58.3% (Table 13, Figure 8) from the initial concentration of Ni, 61.2%, 67%, 56.4% and 56.4% (Table 14, Figure 8) from the initial concentration of Cd, 72%, 64.6%, 65.9% and 64.6% (Table 15, Figure 8) from the initial concentration of Pb and 96%, 95%, 96% and 92% (Table 11, Figure 8) from the initial concentration of Hg respectively. Based on these results, it can be concluded that the capacities of different test algae to remove Ni(Π), Cd(Π) and Pb(Π) at pH 6.0 ranged between 56% and 72%. However, the efficiencies of all test algae to remove Hg(Π) at the same pH value were typically above 90% (Table 16).

Tab 13, 14

Tab 15, 16

Fig. 8

Table 17: Statistical differences in metal ions bioremoval efficiency of different immobilized test algae at different pH values.

Metal ions	Algal beads / pH value	*Spirulina platensis*		*Chlorella ellipsoida*		*Scenedesmus quadricauda*		*Nitzschia palea*	
		t-value	**Significance**	**t-value**	**Significance**	**t-value**	**Significance**	**t-value**	**Significance**
Nickel	**2**	-	-	-	-	-	-	-	-
	3	3.24	*	3.67	*	3.67	*	3.06	*
	4	3.67	*	1.83	NS	3.42	*	1.06	NS
	5	2.44	*	8.02	**	11.65	***	6.89	**
	6	6.55	***	0.0	NS	0.0	NS	-0.79	NS
	7	-0.61	NS	-0.67	NS	-0.36	NS	0.0	NS
	8	-0.41	NS	-0.21	NS	-0.32	NS	-2.45	NS
	9	-0.98	NS	-0.61	NS	-0.31	NS	-6.12	*
Cadmium	**2**	-	-	-	-	-	-	-	-
	3	5.32	*	1.87	*	1.04	NS	5.08	*
	4	0.30	NS	0.77	NS	1.59	NS	1.41	NS
	5	6.73	**	13.91	***	7.59	**	13.4	***
	6	5.63	**	-0.61	NS	-0.22	NS	-0.41	NS
	7	-3.18	*	-2.14	NS	0.0	NS	-2.69	NS
	8	-1.04	NS	-0.73	NS	-0.85	NS	-0.48	NS
	9	-0.18	NS	-1.22	NS	-2.14	NS	-1.6	NS
Lead	**2**	-	-	-	-	-	-	-	-
	3	1.71	NS	1.91	NS	1.41	NS	0.85	NS
	4	0.55	NS	0.55	NS	-0.31	NS	1.95	NS
	5	3.24	*	2.75	*	4.0	*	6.61	**
	6	2.17	NS	0.41	*	-1.59	NS	-1.7	NS
	7	-1.22	NS	0.0	NS	0.0	NS	-2.5	NS
	8	-0.61	NS	-1.89	NS	-1.34	NS	0.0	NS
	9	0.0	NS	-0.61	NS	-1.22	NS	-1.77	NS
Mercury	**2**	-	-	-	-	-	-	-	-
	3	0.81	NS	1.44	*	0.81	NS	3.96	*
	4	6.94	**	2.41	*	4.49	**	4.28	**
	5	4.53	*	4.49	**	8.57	***	2.85	*
	6	12.65	***	-0.41	NS	0.0	NS	-0.4	NS
	7	-0.41	NS	-0.43	NS	-2.44	*	0.0	NS
	8	-0.43	NS	-1.22	NS	-0.40	NS	-0.41	NS
	9	-1.22	NS	-0.41	NS	-1.22	NS	-1.22	NS

NS Non significant ***Significant ($P \leq 0.05$)** ****Highly significant ($P \leq 0.01$)**
*****Very highly significant ($P \leq 0.001$).**
- No preceding value to compare with.

3- Elution of heavy metal ions from algae alginate beads.

3.1- Optimum pH for elution.

Elution means stripping off metal ions that were removed and retained by algal beads. To select the optimum pH of metal ion elution, the metal-laden beads were transferred to conical flasks each containing 50 ml thiourea solution at different pH values of 2.0, 3.0, 4.0, 5.0 and 6.0.

Concentrations (mgl^{-1}) of different metal ions removed at optimum pH (6.0) and for optimum time (15 minutes) by different algal beads before being eluted with thiourea are graphically presented in Figure 9.

The elution capacity is the % of metal ion eluted from that retained by algal beads. The effect of pH on the capacity of 0.1 M thiourea to elute metal ions are given in Tables 18-21 and graphically presented in Figure 10. The highest and the lowest elution capacity of thiourea were recorded at pH 2.0 and pH 6.0 respectively (Tables 18-21, Figure 10). Considerable decrease in elution capacity was clearly noticed as pH increased from pH 2.0 to pH 6.0.

In all cases, differences in metal ion elution capacities obtained at pH 2.0 and pH 3.0 were statistically non significant ($P \leq 0.05$), however, beyond pH 3.0 the decrease in elution capacity was almost highly significant (Table 22). Based on these results, the pH 3.0 was selected as an optimum pH value for metal ion elution by 0.1 M thiourea.

At pH 3.0, the capacity of thiourea to elute metal ions from different algal beads varies significantly ($P \leq 0.05$) and seem to be dependent on immobilized test alga and on the tested metal. At this pH value the metal ion elution capacity of 0.1 M acidic thiourea ranged between 73.1% - 82.9% for Ni(Π) (Table 18), 84% - 89.4% for Cd(Π) (Table 19), 62.7% - 77% for Pb(Π) (Table 20) and 59.1% - 63.9% % for Hg(Π) (Table 21). Generally, the metal ion elution efficiency of 0.1 M acidic thiourea was almost above 60%.

Fig. 9

Table 18, 19

Table 20, 21

Fig. 10

Table 22: Statistical differences in metal ions elution efficiency of different immobilized test algae at different pH values.

Metal ions	Algal beads / pH values	*Spirulina platensis*		*Chlorella ellipsoida*		*Scenedesmus quadricauda*		*Nitzschia palea*	
		t-value	Significance	t-value	Significance	t-value	Significance	t-value	Significance
Nickel	**2**	-	-	-	-	-	-	-	-
	3	**-0.98**	NS	**-1.35**	NS	**-0.19**	NS	**-1.70**	NS
	4	**-6.31**	**	**-15**	***	**-14.75**	***	**-12**	***
	5	**-4.63**	**	**-1.64**	NS	**2.62**	*	**-0.42**	NS
	6	**1.28**	NS	**0.0**	NS	**-1.64**	NS	**-3.93**	*
Cadmium	**2**	-	-	-	-	-	-	-	-
	3	**-1.05**	NS	**-5.50**	*	**-0.760**	NS	**-1.39**	NS
	4	**-8.0**	***	**-10.7**	***	**-12.59**	***	**-13**	***
	5	**-3.53**	*	**-2.75**	*	**-0.32**	NS	**-1.97**	NS
	6	**-3.29**	*	**-1.96**	NS	**-3.14**	*	**-4.19**	**
Lead	**2**	-	-	-	-	-	-	-	-
	3	**-2.66**	NS	**-3.41**	*	**-1.72**	NS	**-2.27**	NS
	4	**-12**	***	**-14.8**	***	**-10.28**	***	**-7.72**	**
	5	**-5.32**	**	**-3.16**	*	**-6.18**	**	**-2.99**	*
	6	**0.0**	NS	**-0.48**	NS	**0.0**	NS	**0.0**	NS
Mercury	**2**	-	-	-	-	-	-	-	-
	3	**-1.91**	NS	**-1.91**	NS	**-1.27**	NS	**-3.82**	*
	4	**-12**	***	**-11**	***	**-12.75**	***	**-14**	***
	5	**-0.63**	NS	**-4.21**	**	**-0.63**	NS	**-2.6**	*
	6	**-11**	***	**-0.8**	NS	**-3.82**	*	**-6.2**	**

NS **Non significant** ***Significant ($P \leq 0.05$)** ****Highly significant ($P \leq 0.01$)**
*****Very highly significant ($P \leq 0.001$).**
- No preceding value to compare with.

3.2- Optimum time for elution

Metal laden-algal beads were transferred to conical flasks each containing 50 ml of acidic 0.1 M thiourea at the optimum pH for elution (pH 3.0) and left to stand for different time periods (1.0, 2.0, 5.0 and 10 minutes). Detailed results about the effect of time on metal ion elution capacity of thiourea are shown in Tables 23-25.

Sharp increase in capacity of 0.1 M acidic thiourea (pH 3.0) to elute metal ions from algal beads was noticed with the increase of elution time (Figure 11). For all metal ions, the highest elution rate was recorded at 10 minutes and the lowest at 1 minute (Figure 11). Statistically significant increase ($P \leq 0.05$) in capacity of thiourea to elute metal ions from algal beads were obtained as elution time increased from 1.0 to 5.0 minutes, beyond which statistically non significant differences were recorded (Table 27).

Accordingly, the optimum time for metal ion elution was fixed at 5.0 minutes. The optimum metal ion elution capacity of 0.1 M acidic thiourea varied between 59.6% - 62.9% for Ni(Π) (Table 23), 58.4% - 61.1% for Cd(Π) (Table 24), 61.6% - 64.9 % for Pb(Π) (Table 25) and 55% - 57.4% for Hg(Π) (Table 26).

Table 23: Effect of time on elution of **Nickel**(Π) from different algal beads using 0.1M thiourea.

Algal beads	Initial Ni conc.(mgl^{-1})	Conc. (mgl^{-1}) of Ni removed by algal beads	Conc. (mgl^{-1})of Ni eluted at different time intervals (minutes)			
			1	**2**	**5**	**10**
Spirulina platensis	10	6.2	2.5 (40.3)	2.9 (46.7)	3.7 (59.6)	3.8 (61.2)
Chlorella ellipsoida	10	6.1	3.1 (50.8)	3.3 (54)	3.7 (60.6)	3.9 (63.9)
Scenedesmus quadricauda	10	6.2	2.3 (37.0)	2.9 (46.7)	3.8 (61.2)	4 (64.5)
Nitzschia palea	10	5.4	2.43 (45)	2.7 (50)	3.4 (62.9)	3.6 (66.6)

Values in parentheses represent the percentage of metal ion eluted from algal beads.

Table 24: Effect of time on elution of **Cadmium**(Π) from different algal beads using 0.1M thiourea.

Algal beads	Initial Cd conc.(mgl^{-1})	Conc. (mgl^{-1}) of Cd removed by algal beads	Conc. (mgl^{-1})of Cd eluted at different time intervals (minutes)			
			1	**2**	**5**	**10**
Spirulina platensis	10	5.3	1.9 (35.8)	2.1 (39.6)	3.1 (58.4)	3.3 (62.2)
Chlorella ellipsoida	10	7.0	3.3 (47.1)	3.5 (50)	4.2 (60)	4.6 (65.7)
Scenedesmus quadricauda	10	6.3	3.3 (52.3)	3.5 (55.5)	3.8 (60.3)	4 (63.4)
Nitzschia palea	10	6.7	3.3 (49.2)	3.6 (53.7)	4.1 (61.1)	4.4 (65.6)

Values in parentheses represent the percentage of metal ion eluted from algal beads.

Table 25: Effect of time on elution of **Lead**(Π) from different algal beads using 0.1M thiourea.

Algal beads	**Initial Pb conc.(mgl^{-1})**	**Conc. (mgl^{-1}) of Pb removed by algal beads**	**Conc. (mgl^{-1})of Pb eluted at different time intervals (minutes)**			
			1	**2**	**5**	**10**
Spirulina platensis	10	5.7	3.3 (57.8)	3.3 (57.8)	3.7 (64.9)	3.8 (66.6)
Chlorella ellipsoida	10	6.8	3.8 (55.8)	3.8 (55.8)	4.3 (63.2)	4.5 (66.1)
Scenedesmus quadricauda	10	6.7	3.3 (49.2)	3.5 (52.2)	4.2 (62.6)	4.4 (65.6)
Nitzschia palea	10	7.3	3.3 (45.2)	3.7 (50.6)	4.5 (61.6)	4.7 (64.3)

Values in parentheses represent the percentage of metal ion eluted from algal beads.

Table 26: Effect of time on elution of **Mercury**(Π) from different algal beads using 0.1M thiourea.

Algal beads	**Initial Hg conc.(mgl^{-1})**	**Conc. (mgl^{-1}) of Hg removed by algal beads**	**Conc. (mgl^{-1})of Hg eluted at different time intervals (minutes)**			
			1	**2**	**5**	**10**
Spirulina platensis	1.00	0.83	0.24 (29.7)	0.32 (39.3)	0.45 (55)	0.49 (59.8)
Chlorella ellipsoida	1.00	0.94	0.27 (29.3)	0.37 (40)	0.52 (55.9)	0.56 (60.2)
Scenedesmus quadricauda	1.00	0.91	0.26 (29.5)	0.34 (38.3)	0.51 (57)	0.54 (60.3)
Nitzschia palea	1.00	0.89	0.28 (31.5)	0.34 (38.3)	0.51 (57.4)	0.54 (60.7)

Values in parentheses represent the percentage of metal ion eluted from algal beads.

Figure 11

Table 27: Statistical differences in metal ions elution efficiency of different immobilized test algae at different contact time periods.

Metal ions	Algal beads / Time (min.)	*Spirulina platensis*		*Chlorella ellipsoida*		*Scenedesmus quadricauda*		*Nitzschia palea*	
		t-value	Significance	t-value	Significance	t-value	Significance	t-value	Significance
Nickel	1	-	-	-	-	-	-	-	-
	2	3.95	*	3.12	*	5.92	**	3.06	*
	5	7.90	**	4.01	*	8.89	***	5.12	**
	10	0.98	NS	1.25	NS	1.97	NS	0.81	NS
Cadmium	1	-	-	-	-	-	-	-	-
	2	2.31	*	1.74	NS	2.944	*	2.74	*
	5	11.5	***	6.12	***	3.91	**	4.56	**
	10	1.31	NS	3.49	*	1.94	NS	1.74	NS
Lead	1	-	-	-	-	-	-	-	-
	2	0.0	NS	0.0	NS	1.82	NS	3.35	*
	5	4.29	**	4.50	**	6.39	**	6.71	**
	10	1.07	NS	1.80	NS	1.82	NS	1.67	NS
Mercury	1	-	-	-	-	-	-	-	-
	2	5.90	**	3.91	**	5.38	*	4.12	*
	5	9.59	***	9.77	***	11.43	***	11.6	***
	10	2.95	*	2.06	NS	2.01	NS	2.06	NS

NS Non significant ***Significant ($P \leq 0.05$)** ****Highly significant ($P \leq 0.01$)**
*****Very highly significant ($P \leq 0.001$).**
- No preceding value to compare with.

4- Consistency of metal ions bioremoval along successive removal-elution cycles.

The efficiency of different immobilized test algae to remove metal ions, prepared either singly or in a mixture, was followed through five successive metal bioremoval cycles. The contact time of metal ion bioremoval was fixed at 15 minutes in all treatments and the hydrogen ion activity of all metal ion test solution was adjusted at pH 6.0 (optimum pH).

The experimental procedure was previously given in details (see material and methods). This investigation seems practically important to validate the applied significance of the test algae for metal bioremediation.

For simplicity, the terms single metal ion solution and mixture metal ions solution will be used to denote metal test solutions containing single metal ion per se and a mixture of the four metal ions respectively.

It may be convenient to mention that, the metal ion bioremoval efficiency is expressed as the % metal ion concentration removed by a given test alga relative to the initial metal ion concentration.

The research experiments yielded a great bulk of data. Full detailed data showing the efficiency of alginate-immobilized cells of *Spirulina platensis, Chlorella ellipsoida, Scenedesmus quadricauda var. longispina* and *Nitzschia palea* to remove different metal ions from single metal ion solution in five successive cycles are listed in Tables 28, 29, 30 and 31 and presented in Figures 12, 13, 14 and 15 respectively.

Results of the capacity of the same test algae (with the same order of sequence) to remove the tested metal ions prepared in mixture are given in Tables 32, 33, 34, 35 and Figures 16, 17, 18, 19 respectively.

The efficiency of composite algal beads (beads immobilizing the four test algae together) to remove different heavy metal ions from a mixture, was also investigated and results were shown in Table 36 and Figure 20.

Basic statistics summarizing the overall efficiency of test immobilized algae to remove heavy metal ions, prepared either singly or in combination, through five successive bioremoval cycles are listed in Table 37 and presented in Figure 21.

The efficiency of immobilized cells of *Spirulina platensis* to remove metal ions prepared singly varied in the different cycles from 89.3% to 95.2% for Ni(Π), 89.3% to 95.2% for Cd(Π), 77.9% to 96.6% for Pb(Π) and 79.8% to 91.6% for Hg(Π) (Table 37 Figure 21).

Spirulina platensis maintained, more or less, comparable efficiencies to remove metal ions from a mixture. The % metal ion concentration removed in this case ranged between 83% - 90%, 71% - 87.6%, 77% - 83.6% and 74% - 86% for metal ions Ni(Π), Cd(Π), Pb(Π) and Hg(Π) respectively (Table 37 Figure 21).

In case of single metal ion solution, the metal ion bioremoval efficiency through five successive cycles of *Chlorella ellipsoida* fluctuated between

89.3% and 93.3% for Ni(Π), 76.8% and 94.2% for Cd(Π), 92.7% and 98.6% for Pb(Π) and 75% and 97.8% for Hg(Π) (Table 37 Figure 21).

The mean % metal ion removed by *Chlorella ellipsoida* from metal ions mixture solutions varied slightly between the lowest value of 76.6% and the highest one of 84.6 % recorded for Hg(Π) and Ni(Π) respectively (Table 37 Figure 21).

Immobilized *Scenedesmus quadricauda* showed higher capacities to remove metal ions from their single than mixture solution, the mean values ranged between 84.36 % and 94.2 % for single and 75.4% and 85.38% for mixture test solutions (Table 37 Figure 21).

Through all the bioremoval cycles, the lowest and highest efficiency of *Nitzschia palea* to remove metal ions singly were 88.4% and 94% for Ni(Π), 86.5% and 89.8% for Cd(Π), 90.4% and 94.8 % for Pb(Π) and 78% and 90.2% for Hg(Π). Compared to single metal ion removal and like other test algae, the efficiency of this pinnate diatom to remove metal ions from a mixture was relatively low (Table 37 Figure 21).

On an average, the composite beads removed 93.7%, 92.6%, 93.6% and 92.6% from the initial concentration of Ni(Π), Cd(Π), Pb(Π) and Hg(Π) respectively (Table 37 Figure 21).

In general, the efficiency of test alga to remove the metal ions tested was slightly decreased with the increase of bioremoval cycles with highest and lowest values being recorded at the first and fifth cycle respectively.

However, even after five successive metal bioremoval cycles, all test alga maintained outstanding efficiency as the % metal ion removal never fallen below 75% (Table 37).

Concisely, the mean efficiency of different test alga to remove metal ions prepared singly in aqueous solution ranged between 84% and 96.5%. Similarly the mean efficiency of algae to remove metal ions prepared in mixture varied from 75.4% to 86.56%. the differences in metal ion bioremoval efficiencies were dependent on algal species and on metal tested (Table 37).

Compared to separate algal beads, the composite alginate beads (beads immobilizing the four algal species together) maintained considerable higher efficiencies to remove metal ions from their mixtures. The mean metal ion bioremoval efficiency of composite beads ranged between 92.6% to 94% (Table 37 Figure 21).

One way analysis of variance (ANOVA) reported non-significant differences ($P \leq 0.05$) in efficiencies of different test algae to remove metal ions either from the single metal ion (Table 38) and mixture metal ions (Table 39) solutions. However, 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).

Table 28

Table 29

Table 30

Table 31

Figure 12-13

Figure 14-15

Table 32

Table 33

Table 34

Table 35

Figure 16-17

Figure 18-19

Table 36

Figure 20

Table 37: Summary statistics of metal ions bioremoval efficiencies through five successive cycles of different alginate-immobilized test algae at contact time of 15 min. and pH 6.0.

Beads of test algae	**Metal ions**	**% metal ions concentration removed from single-metal synthetic solution through five successive cycles.**				**% metal ions concentration removed from metals mixture solution through five successive cycles.**			
		Min.	**Max.**	**Mean**	**S.D.**	**Min.**	**Max.**	**Mean**	**S.D.**
Spirulina platensis	**Ni**	89.3	95.2	92.14	±2.44	83	90	86.56	±2.85
	Cd	89.3	95.2	92.14	±2.44	71	87.6	82.74	±6.68
	Pb	77.9	96.9	87.94	±8.09	77	83.6	80.9	±2.78
	Hg	79.8	91.6	84.92	±5.08	74	86	81	±4.79
Chlorella ellipsoida	**Ni**	89.8	93.3	91.38	±1.45	82	87	84.24	±2.02
	Cd	76.8	94.2	87.34	±6.95	69.6	88.3	80.96	±7.83
	Pb	92.7	98.6	96.5	±2.38	68	86.6	76.64	±8.70
	Hg	75	97.8	88.48	±8.47	65	86	76.8	±8.58
Scenedesmus quadricauda	**Ni**	90.2	94.2	92.04	±1.61	82.3	88.6	85.3	±2.43
	Cd	89.6	95	92.42	±1.98	79.3	89.6	85.38	±3.80
	Pb	91.7	96	94.18	±1.64	80.6	90.3	84.82	±3.96
	Hg	77.5	92	84.36	±5.33	66	85	75.4	±6.80
Nitzschia palea	**Ni**	88.4	94	90.24	±2.2	78	88.6	84.44	±4.38
	Cd	86.5	89.8	88.88	±2.22	80	84.3	82.18	±2.0
	Pb	90.4	94.8	92.3	±1.80	82	89.6	84.96	±2.88
	Hg	78	90.2	84	±5.0	72	83	77	±4.24
Composite beads	**Ni**	-	-	-	-	92.3	97.3	93.96	±2.23
	Cd	-	-	-	-	89.6	95	92.56	±2.16
	Pb	-	-	-	-	90.3	96.3	93.5	±2.17
	Hg	-	-	-	-	85	95	92.6	±4.27

Figure 21

Table 38: One-Way ANOVA of the efficiency of test algae to remove metal ions **singly** through five successive cycles.

Groups	Count	Sum	Average	Variance
Spirulina platensis	20	1770.2	88.51	33.78516
Chlorella ellipsoida	20	1800.4	90.02	43.228
Scenedesmus quadricauda	20	1815	90.75	22.94474
Nitzschia palea	20	1778.1	88.905	18.01839

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	63.169	3	21.0564	0.7139	**0.546685**	2.724
Within Groups	2241.55	76	29.4940			
Total	2304.71	79				

P-value > 0.05 Non significant **P-value ≤ 0.05 Significant**

Table 39: One-Way ANOVA of the efficiency of test algae to remove metal ions in a mixture through five successive cycles.

Groups	Count	Sum	Average	Variance
Spirulina platensis	20	1655.8	82.79	23.06411
Chlorella ellipsoida	20	1593.2	79.66	55.78147
Scenedesmus quadricauda	20	1654.5	82.725	36.23776
Nitzschia palea	20	1651.9	82.595	20.99103

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	139.3225	3	46.4408	0.0652	**0.97812**	2.724
Within Groups	2585.413	76	34.0185			
Total	2724.736	79				

P-value > 0.05 Non significant **P-value ≤ 0.05 Significant**

Table 40: One-Way ANOVA for the percentage of metal ion bioremoval of different algal species and **composite** beads through five successive cycles.

Groups	Count	Sum	Average	Variance
Spirulina platensis	20	1685.1	84.255	116.3742
Chlorella ellipsoida	20	1767.7	88.385	39.77292
Scenedesmus quadricauda	20	1712.2	85.61	111.5262
Nitzschia palea	20	1736	86.8	85.72526
Composite beads	20	1856.9	92.845	20.53208

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	878.2634	4	219.5659	2.9359	**0.024537**	2.4674
Within Groups	7104.682	95	74.78613			
Total	7982.946	99				

P-value > 0.05 Non significant **P-value ≤ 0.05 Significant**

5- Elution of metal ions removed by algal beads through five bioremoval-elution cycles.

Acidified 0.1 M thiourea solution, at pH 3.0, was used to elute metal ions removed by algal beads.

It is perhaps relevant to mention that the elution efficiency is expressed as the % of metal ion concentration eluted by thiourea relative to the metal ion concentration removed by a beads of a given alga.

Detailed results showing the capacity of thiourea to elute metal ions from single metal-laden beads of *Spirulina platensis, Chlorella ellipsoida, Scenedesmus quadricauda var. longispina* and *Nitzschia palea* are included in the bioremoval-elution Tables 28, 29, 30, 31 and simply illustrated in Figures 22, 23, 24, 25 respectively. Full data about the metal ions eluted from four metals-laden beads of *Spirulina platensis, Chlorella ellipsoida, Scenedesmus quadricauda var. longispina* and *Nitzschia palea* are included in mixed metal ions bioremoval-elution Tables 32, 33, 34, 35 and graphically presented in Figures 26, 27, 28, 29 respectively. Metal ion elution capacity of thiourea from four metals-laden composite beads are listed in Tables 36 and given in Figure 30.

Summary statistics of data revealing the efficiency of thiourea to elute metal ions from different algal beads are given in Table 41. On an average basis, the mean efficiencies of thiourea to elute metal ions 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. The mean efficiency of thiourea to elute metal ions from composite algal beads ranged from 73% to 95% (Table 41), through the five bioremoval-elution cycles.

The results indicated that the efficiency of thiourea to elute metal ion was significantly ($P \leq 0.05$) in case of composite beads followed by single metal ion laden beads followed by four metal ions laden beads (Figure 31).

One way analysis of variance (ANOVA) reported non-significant differences ($P \leq 0.05$)in efficiencies of thiourea to elute metal ions from different single metal-laden algal beads (Table 42) and four metal-laden algal beads (Table 43). However, the efficiency of thiourea to elute metal ions from composite beads was significantly higher compared to single and four metal-laden algal beads (Table 44).

Figure 22-23

Figure 24-25

Figure 26-27

Figure 28-29

Figure 30

Table 41: Summary statistics of thiourea efficiency to elute metal ions through five successive bioremoval-elution cycles from algal beads (single metal-laden and four metal-laden beads) at contact time of 15 min. and pH 6.0.

Beads of test algae	**Metal ions**	**% metal ions concentration eluted from single metal-laden beads through five successive cycles.**				**% metal ions concentration eluted from four metal-laden beads through five successive cycles.**			
		Min.	**Max.**	**Mean**	**S.D.**	**Min.**	**Max.**	**Mean**	**S.D.**
Spirulina platensis	**Ni**	92.7	98.9	95.32	±2.75	62.2	95.9	85.24	±13.9
	Cd	89.2	93.6	91.06	±1.72	85.6	92	89.86	±2.69
	Pb	87	95.2	91.72	±3.39	88.2	89.5	88	±1.86
	Hg	65.3	77.1	69.84	±4.63	62.5	69.7	67.12	±2.79
Chlorella ellipsoida	**Ni**	88.1	95.5	92.58	±3.47	85.5	95.9	90.48	±3.73
	Cd	89.6	94.2	92.24	±1.84	55.5	98.6	88.24	±18.3
	Pb	82.7	94.8	91.82	±5.16	90.1	95	91.74	±2.94
	Hg	73.7	84.5	79.08	±4.06	70.4	77.1	74.64	±2.65
Scenedesmus quadricauda	**Ni**	88.1	97.5	96.08	±1.73	87	93.2	90.02	±2.22
	Cd	88.8	97.2	92.74	±3.11	58.9	95.6	84.98	±15.1
	Pb	86.9	95.4	90.58	±3.42	73.8	95.6	88.14	±8.58
	Hg	63.6	80.6	69.98	±6.49	62.1	78.7	69.1	±6.62
Nitzschia palea	**Ni**	87.8	95.7	90.52	±3.07	80.8	97.7	87.7	±7.67
	Cd	90.4	95.6	92.86	±2.22	86.1	93.3	90.92	±3.01
	Pb	86.2	100	92.08	±6.27	88.2	98.4	92.36	±4.83
	Hg	64.1	80.7	74.28	±6.20	64.9	72	67.76	±2.91
Composite beads	**Ni**	-	-	-	-	89.8	97.1	93.72	±3.24
	Cd	-	-	-	-	93.6	98.1	95	±1.87
	Pb	-	-	-	-	86.1	100	94.7	±5.17
	Hg	-	-	-	-	70	78.6	73	±4.11

Figure 31

Table 42: One-Way ANOVA of the efficiency of thiourea to elute metal ions **singly** removed by algal beads through five successive cycles.

Groups	Count	Sum	Average	Variance
Spirulina platensis	20	1749.7	87.485	97.26239
Chlorella ellipsoida	20	1736.46	86.823	135.6072
Scenedesmus quadricauda	20	1695.9	84.795	166.7394
Nitzschia palea	20	1706.4	85.32	122.5164

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	95.04494	3	31.68165	0.2427	**0.866265**	2.7249
Within Groups	9920.384	76	130.5314			
Total	10015.43	79				

P-value > 0.05 Non significant **P-value ≤ 0.05 Significant**

Table 43: One-Way ANOVA of the efficiency of thiourea to elute metal ions in a **mixture** removed by algal beads through five successive cycles.

Groups	Count	Sum	Average	Variance
Spirulina platensis	20	1651.1	82.555	131.4237
Chlorella ellipsoida	20	1716.5	85.825	139.7799
Scenedesmus quadricauda	20	1657.3	82.865	141.2834
Nitzschia palea	20	1688.7	84.435	133.975

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	137.41	3	45.80333	0.3352	**0.799861**	2.7249
Within Groups	10382.78	76	136.6155			
Total	10520.19	79				

P-value > 0.05 Non significant **P-value ≤ 0.05 Significant**

Table 44: One-Way ANOVA for the percentage of metal ions elution from different algal species and **composite** beads through five successive cycles.

Groups	Count	Sum	Average	Variance
Spirulina platensis	20	1685.1	84.255	116.3742
Chlorella ellipsoida	20	1767.7	88.385	39.77292
Scenedesmus quadricauda	20	1712.2	85.61	111.5262
Nitzschia palea	20	1736	86.8	85.72526
Composite beads	20	1856.9	92.845	20.53208

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	878.2634	4	219.5659	2.9359	**0.024537**	2.4674
Within Groups	7104.682	95	74.78613			
Total	7982.946	99				

P-value > 0.05 Non significant **P-value ≤ 0.05 Significant**

6- <u>Capacity of test algae to remove metal ions from industrial wastewaters.</u>

Four industrial effluents were collected (chemical fertilizers, oils and soap, dyes and salt and soda). These four industries were shown in the pervious work of the authors to produce the most toxic effluents among Egyption industries. Details about the industries and the collection procedure were previously given in detail (see materials and methods). The end-of-pipe effluent composite samples were obtained from different four installations with different manufacturing activities. We will use the effluent codes #1, #2, #3 and #4 to designate effluents collected from Talkha chemical fertilizers factory, Sandoub oils and soap factory, Mahalla dyes factory and Kafr-Ezzayyat salt and soda factory, respectively. 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 industrial effluents were tested and compared.

6.1- Chemical characteristics of effluents.

Some chemical properties of the four industrial effluents are given in Table 45. Effluents #1, #2 and # 3 were alkaline with pH values of 10.2, 9.5 and 10.24 respectively. The effluent # 4 was slightly acidic with pH value of 4.8. Concentrations of the heavy metal ions Ni(Π), Cd(Π), Pb(Π) and Hg(Π) varied slightly from one effluent to another. Ni(Π) varied between 0.8 mgl^{-1} and 1.95 mgl^{-1}, Cd(Π) between 0.37 mgl^{-1} and 1.32 mgl^{-1}, Pb(Π) between 1.2 mgl^{-1} and 1.85 $mgl^{-1}$and Hg(Π) between 34 μgl^{-1} and 59 μgl^{-1} (Table 45).

Table 45: Some chemical properties of the investigated industrial effluents.

Effluent	pH	Ni (mgl^{-1})	Cd (mgl^{-1})	Pb (mgl^{-1})	Hg (μgl^{-1})
Talkha chemical fertilizers factory	**10.21**	**0.80**	**0.62**	**1.85**	**34**
Sandoub oils and soap factory	**9.50**	**1.14**	**1.27**	**1.76**	**49**
Mahalla dyes factory	**10.24**	**1.95**	**1.32**	**1.50**	**59**
Kafr-Ezzayyat salt and soda factory	**4.81**	**0.93**	**0.37**	**1.20**	**43**

6.2- Efficiency of composite beads and flat-bed algal filters to remove metal ions from industrial effluents.

As mentioned before, aliquots of industrial effluents were filtered through GF/C filters. The filtered samples were adjusted at pH 6.0, the value which was found to be optimum for metal ion bioremoval by algae. The contact time for metal ion bioremoval was also adjusted at 15 minutes, the period at which maximum metal ion bioremoval was recorded before.

The capacity of the four co-immobilized test algae to remove Ni(Π), Cd(Π), Pb(Π) and Hg(Π) from industrial effluents were tested through 10 successive bioremoval-elution cycles. The test algae were either co-immobilized into alginate matrices or developed on flat-bed filters of cotton and sponge (cotton and sponge proved to be the best carriers in establishing the algal flat filters).

Metal analyses were carried out for effluent samples collected after the first, fourth, seventh and tenth bioremoval-elution cycles.

Full data about alginate immobilized test algae and flat-bed algal filters to remove Ni(Π), Cd(Π), Pb(Π) and Hg(Π) are given in details in Tables 46, 47, 48 and 49 and graphically represented in Figures 32, 33, 34 and 35 respectively. Table 50 lists the summary statistics of algal capacity to remove metal ions from different industrial effluents.

Obvious variations in capacity of algal biofilters to remove Ni(Π) were noticed. The variations were to some extent dependent on the nature of physical carrier (alginate, cotton and sponge) on the effluent type and on the number of bioremoval cycle (Table 46, Figure 32).

Compared to alginate and cotton biofilter, the sponge biofilter was relatively more efficient to remove Ni from different effluent samples. However, the algae-alginate beads showed the least efficiency to remove Ni from different industrial effluents (Figure 32).

The capacities of algae-cotton filter, algae-sponge filter and algae alginate beads to remove Ni(Π) 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(Π) was recorded after the first and tenth bioremoval cycles respectively (Table 46).

The immobilized algae showed, more or less, comparable results to remove Cd(Π) from wastewaters (Table 47, Figure 33). The algae-cotton filter maintained the highest Cd(Π) 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 (Table 50).

The efficiency of immobilized algae to remove Pb(Π) (Table 48, Figure 34) displayed obvious variations that were mainly dependent on nature of immobilizing carrier, bioremoval cycle and the effluent. The algae-cotton filter and algae-alginate beads maintained the highest (65.3% - 90%) and the lowest (55.3% -76.6%) capacities to remove Pb(Π) with algae-sponge filter in between (65%-87.5%), the mean capacity of these filters to remove Pb(Π) were 78.8%, 76.1% and 70.6% respectively (Table 50).

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

In general even after 10th bioremoval cycles, the efficiencies of algae-cotton filter, algae-sponge filter and algae-alginate beads were typically above 65%, 65% and 45% respectively (Table 50) in removing the metal ions studied.

Table 46: Efficiency of sponge and cotton algal flat filters and alginate-immobilized algae to remove **Nickel**(Π) from different industrial effluents through ten bioremoval-elution cycles (each cycle was done using a new raw effluent).

Effluent	Biofilter	Conc. (mgl^{-1}) of Ni in raw effluent	Conc. (mgl^{-1}) of Ni after different bioremoval-elution cycles			
			First cycle	Fourth cycle	Seventh cycle	Tenth cycle
Talkha for chemical fertilizers (Effluent # 1)	Algae-sponge filter	**0.80**	**0.10 (88)**	**0.10 (88)**	**0.14 (83)**	**0.14 (83)**
	Algae-cotton filter	**0.80**	**0.07 (91)**	**0.07 (91)**	**0.10 (88)**	**0.10 (88)**
	Algae-alginate beads	**0.80**	**0.21 (74)**	**0.24 (70)**	**0.41 (49)**	**0.41 (49)**
Sandoub for oils and soap factory (Effluent # 2)	Algae-sponge filter	**1.14**	**0.14 (88)**	**0.21 (82)**	**0.36 (68)**	**0.36 (68)**
	Algae-cotton filter	**1.14**	**0.14 (88)**	**0.14 (88)**	**0.14 (88)**	**0.16 (86)**
	Algae-alginate beads	**1.14**	**0.21 (82)**	**0.36 (68)**	**0.52 (54)**	**0.63 (45)**
Mahalla dyes factory (Effluent # 3)	Algae-sponge filter	**1.95**	**0.14 (93)**	**0.29 (85)**	**0.29 (85)**	**0.33 (83)**
	Algae-cotton filter	**1.95**	**0.14 (93)**	**0.14 (93)**	**0.23 (88)**	**0.23 (88)**
	Algae-alginate beads	**1.95**	**0.23 (88)**	**0.43 (78)**	**0.53 (72)**	**0.64 (67)**
Kafr-Ezzayyat salt and soda factory (Effluent # 4)	Algae-sponge filter	**0.93**	**0.14 (85)**	**0.23 (75)**	**0.23 (75)**	**0.29 (69)**
	Algae-cotton filter	**0.93**	**0.10 (89)**	**0.14 (85)**	**0.21 (77)**	**0.23 (75)**
	Algae-alginate beads	**0.93**	**0.29 (69)**	**0.29 (69)**	**0.35 (62)**	**0.39 (58)**

Values in parentheses represent the percentage of bioremoval.

Figure 32

Table 47: Efficiency of sponge and cotton algal flat filters and alginate-immobilized algae to remove **Cadmium**(Π) from different industrial effluents through ten bioremoval-elution cycles (each cycle was done using a new raw effluent).

Effluent	Biofilter	Conc. (mgl^{-1}) of Cd in raw effluent	Conc. (mgl^{-1}) of Cd after different bioremoval-elution cycles			
			First cycle	Fourth cycle	Seventh cycle	Tenth cycle
Talkha chemical fertilizers (Effluent # 1)	Algae-sponge filter	**0.62**	**0.10 (84)**	**0.13 (79)**	**0.15 (76)**	**0.15 (76)**
	Algae-cotton filter	**0.62**	**0.07 (89)**	**0.10 (84)**	**0.13 (79)**	**0.13 (79)**
	Algae-alginate beads	**0.62**	**0.15 (76)**	**0.17 (72.5)**	**0.21 (66)**	**0.25 (59.6)**
Sandoub oils and soap factory (Effluent # 2)	Algae-sponge filter	**1.27**	**0.13 (90)**	**0.18 (86)**	**0.18 (86)**	**0.23 (83)**
	Algae-cotton filter	**1.27**	**0.13 (90)**	**0.13 (90)**	**0.18 (86)**	**0.18 (86)**
	Algae-alginate beads	**1.27**	**0.23 (83)**	**0.23 (83)**	**0.53 (58.2)**	**0.62 (51.2)**
Mahalla dyes factory (Effluent # 3)	Algae-sponge filter	**1.32**	**0.15 (89)**	**0.18 (86)**	**0.18 (86)**	**0.23 (83)**
	Algae-cotton filter	**1.32**	**0.13 (90)**	**0.13 (90)**	**0.13 (90)**	**0.23 (83)**
	Algae-alginate beads	**1.32**	**0.23 (83)**	**0.33 (75)**	**0.52 (60.6)**	**0.60 (54.5)**
Kafr-Ezzayyat salt and soda factory (Effluent # 4)	Algae-sponge filter	**0.37**	**0.0 (100)**	**0.07 (81)**	**0.10 (73)**	**0.13 (65)**
	Algae-cotton filter	**0.37**	**0.0 (100)**	**0.0 (100)**	**0.07 (81)**	**0.13 (65)**
	Algae-alginate beads	**0.37**	**0.10 (73)**	**0.13 (65)**	**0.13 (65)**	**0.15 (59.4)**

Values in parentheses represent the percentage of bioremoval.

Figure 33

Table 48: Efficiency of sponge and cotton algal flat filters and alginate-immobilized algae to remove **Lead**(Π) from different industrial effluents through ten bioremoval-elution cycles (each cycle was done using a new raw effluent).

Effluent	**Biofilter**	**Conc. (mgl^{-1}) of Pb in raw effluent**	**Conc. (mgl^{-1}) of Pb after different bioremoval-elution cycles**			
			First cycle	**Fourth cycle**	**Seventh cycle**	**Tenth cycle**
Talkha chemical fertilizers (Effluent # 1)	Algae-sponge filter	**1.85**	**0.28 (85)**	**0.28 (85)**	**0.56 (70)**	**0.56 (70)**
	Algae-cotton filter	**1.85**	**0.28 (85)**	**0.36 (80.5)**	**0.36 (80.5)**	**0.44 (76.2)**
	Algae-alginate beads	**1.85**	**0.44 (76.2)**	**0.44 (76.2)**	**0.56 (70)**	**0.64 (65.4)**
Sandoub oils and soap factory (Effluent # 2)	Algae-sponge filter	**1.76**	**0.36 (79.5)**	**0.44 (75)**	**0.52 (70)**	**0.60 (65.9)**
	Algae-cotton filter	**1.76**	**0.26 (85.2)**	**0.36 (79.5)**	**0.52 (70)**	**0.6 (65.9)**
	Algae-alginate beads	**1.76**	**0.44 (7 5)**	**0.44 (75)**	**0.52 (70)**	**0.60 (65.9)**
Mahalla dyes factory (Effluent # 3)	Algae-sponge filter	**1.50**	**0.23 (84.6)**	**0.36 (76)**	**0.45 (70)**	**0.52 (65.3)**
	Algae-cotton filter	**1.50**	**0.22 (85.3)**	**0.26 (82.6)**	**0.37 (75.3)**	**0.52 (65.3)**
	Algae-alginate beads	**1.50**	**0.36 (76)**	**0.36 (76)**	**0.52 (65.3)**	**0.61 (55.3)**
Kafr-Ezzayyat Salt and Soda Factory (Effluent # 4)	Algae-sponge filter	**1.20**	**0.15 (87.5)**	**0.18 (85)**	**0.26 (78.3)**	**0.36 (70)**
	Algae-cotton filter	**1.20**	**0.12 (90)**	**0.18 (85)**	**0.24 (80)**	**0.30 (75)**
	Algae-alginate beads	**1.20**	**0.28 (76.6)**	**0.28 (76.6)**	**0.52 (65.6)**	**0.52 (65.6)**

Values in parentheses represent the percentage of bioremoval.

Figure 34

Table 49: Efficiency of sponge and cotton algal flat filters and alginate-immobilized algae to remove **Mercury**(Π) from different industrial effluents through ten bioremoval-elution cycles (each cycle was done using a new raw effluent).

Effluent	Biofilter	Conc. (μgl^{-1}) of Hg in raw effluent	Conc. (μgl^{-1}) of Hg after different bioremoval-elution cycles			
			First cycle	Fourth cycle	Seventh cycle	Tenth cycle
Talkha chemical fertilizers (Effluent # 1)	Algae-sponge filter	**34**	**0 (100)**	**5 (85.2)**	**9 (73.5)**	**9 (73.5)**
	Algae-cotton filter	**34**	**0 (100)**	**0 (100)**	**5 (85.2)**	**7**
	Algae-alginate beads	**34**	**9 (73.5)**	**9 (73.5)**	**12 (64.7)**	**12 (64.7)**
Sandoub oils and soap factory (Effluent # 2)	Algae-sponge filter	**49**	**0 (100)**	**7 (85.7)**	**8 (83.7)**	**7 (85.7)**
	Algae-cotton filter	**49**	**0 (100)**	**0 (100)**	**5 (89.7)**	**11 (77.5)**
	Algae-alginate beads	**49**	**11 (77.5)**	**12 (75.5)**	**15 (69.3)**	**15 (69.3)**
Mahalla dyes factory (Effluent # 3)	Algae-sponge filter	**59**	**0 (100)**	**4 (93)**	**9 (84.7)**	**15 (74.5)**
	Algae-cotton filter	**59**	**0 (100)**	**4 (93)**	**8 (86.4)**	**10 (83)**
	Algae-alginate beads	**59**	**5 (91.5)**	**8 (86.4)**	**15 (74.5)**	**15 (74.5)**
Kafr-Ezzayyat salt and soda factory (Effluent # 4)	Algae-sponge filter	**43**	**0 (100)**	**7 (83.7)**	**7 (83.7)**	**12 (72)**
	Algae-cotton filter	**43**	**0 (100)**	**0 (100)**	**7 (83.7)**	**12 (72)**
	Algae-alginate beads	**43**	**7 (83.7)**	**15 (65.1)**	**18 (58.2)**	**18 (58.2)**

Values in parentheses represent the percentage of bioremoval.

Figure 35

Table 50: Summary statistics of the efficiency of immobilized algal beads and flat algal biofilters to remove metal ions from different industrial effluents through ten bioremoval-elution cycles.

Metal ions \ Biofilter		Algae-sponge filter	Algae-cotton filter	Algae-alginate beads
		% metal ion removed *		
Nickel(Π)	Min.	68	75	45
	Max.	93	93	88
	Mean	81.1	87.2	65.9
Cadmium(Π)	Min.	65	65	52
	Max.	100	100	83
	Mean	82.6	86.4	68.1
Lead(Π)	Min.	65.3	65.3	55.3
	Max.	87.5	90	76.6
	Mean	76.1	78.8	70.6
Mercury(Π)	Min.	72	72	58.2
	Max.	100	100	91.5
	Mean	86.2	90.6	72.5

***** Calculations are based on data from the 1^{st}, 4^{th}, 7^{th} and 10^{th} bioremoval-elution cycles.

7- Toxicity assessment of the four industrial effluents using standard algal bioassays.

Toxicity of raw (GF/C filtered) effluent samples was assessed using a standard algal biotest procedure with the green Chlorococcal alga *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) as a standard test alga. Toxicity of effluent sub-samples adjusted to pH 6.0 was also determined. Toxicity values were expressed in quantitative terms of EC_{50} which is the medium effective effluent concentration (%v/v) inhibiting the growth of the test algae by 50% compared to control culture. Raw data showing the effects of raw and pH adjusted samples of effluents #1, #2, #3 and#4 on growth of standard test alga are listed in Tables 51, 52, 53 and 54. Dose-response curves illustrating the relative growth (% of the control count, considering control = 100%) of *Pseudokirchneriella subcapitata* at different effluent doses are given in Figures 36, 37, 38 and 39 respectively. Values of EC_{50} were derived from dose-response curves (Figures 36-39). Results are given in Table 55 and graphically presented in Figure 40.

GF/C filtered effluent samples adjusted to pH 6.0 brought about substantial decrease in their toxicities (Tables 51-54, Figures 36-39). For instance the EC_{50} of raw and pH adjusted samples of effluent #1 were 1% and 21% respectively (Table 55). The same is held true for the rest industrial effluents (Table 55, Figure 40).

Although data are clearly presented in either Tables or Figures; yet, there are some elegant findings that must be highlighted. These findings can be simply pointed as follows.

7.1- Algal growth stimulation.

Both raw and pH adjusted samples of effluent #1 (Talkha chemical fertilizers, Figure 36) induced marginal algal growth stimulation at very low concentration (below 0.1% v/v), beyond which all concentration levels were growth inhibitory. Biologically treated samples of the same effluent exhibited marked algal growth stimulation at concentration levels below 40% (v/v) and 30% after the first and the tenth bioremoval cycles respectively (Figure 36).

Effluent #2 (Sandoub oils and soap, Figure 37) and effluent #3 (Mahalla dyes factory, Figure 38) maintained more or less comparable algal growth stimulation effects at concentration level typically below 10% (v/v), beyond which marked algal growth inhibition was clearly noticed.

All concentrations of raw sample of effluent #4 (Kafr-Ezzayyat salt and soda, Figure 39) showed algal growth inhibition. Marginal growth stimulation was only noticed at low concentration levels (< 1.0%) of pH adjusted and biologically treated effluent subsamples (Figure 39).

In general the intensity of algal growth at lower effluent concentration varied from one effluent to another. Algal growth stimulation was to a large extent, dependent on the nature of the tested effluent sample whether raw or pH adjusted or biologically treated with different algal biofilters. In all cases, relatively higher growth stimulation was noticed (Figures 36-39) for all the biologically treated effluent samples particularly at the earlier bioremoval cycles (1^{st} and 4^{th} cycles).

7.2- Algal growth inhibition

Growth inhibition of *Pseudokirchneriella subcapitata* was employed to calculate the effluent toxicity in terms of EC_{50} (Table 55) from dose-response curves (Figures 36-39). It was generally noticed 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 (Tables 55 and Figure 40).

Compared to other effluents, the effluent #1 (Talkha chemical fertilizers) was relatively the most toxic as raw effluent concentration higher than 0.1 % exhibited strong growth inhibition of the test algae (Figure 36) with EC_{50} of 1.0 % (Table 55). Adjustment of pH of effluent #1 to pH 6.0 resulted in a marked decrease in its toxicity (EC_{50} = 21) (Table 55 and Figure 40).

The effluent #3 (Mahalla dyes factory) was ranked as the second toxic on growth of test alga with EC_{50} values of 20% and 32% calculated before and after pH adjusted (pH=6.0) respectively (Table 55).

Compared to effluents of Talkha chemical fertilizers and Mahalla dyes factory, the effluent of Sandoub oils and soap was relatively low toxic on growth of *Pseudokirchneriella subcapitata*, with EC_{50} of 24% and 41% of raw and pH adjusted samples respectively (Table 55).

As was noticed with other effluents, the adjustment of effluent pH to 6.0 induced very high significant ($P \leq 0.001$) reduction in toxicity of raw effluent (Figure 40).

Based on its algal growth toxicity, the effluent #4 (Kafr-Ezzayyat salt and soda) was considered the least toxic one with EC_{50} of 43% and 56% of raw and pH adjusted samples respectively (Table 55). Reduction in whole effluent toxicity due to pH adjustment was very high significant ($P \leq 0.001$) (Figure 40).

7.3. Bioremediation of highly toxic effluent doses with algal biofilters.

The co-immobilized test algae proved to be highly efficient to remove heavy metal ions from the four tested toxic industrial effluents (Tables 46-49). This finding indicates that the whole effluent toxicity may be substantially reduced when biologically treated with algal biofilters.

The term algal biofilters embraces all the four test algae co-immobilized into either alginate beads or sponge or cotton substrates. Standard algal bioassay was employed to validate the efficiency of algal biofilter to reduce the whole effluent toxicity.

In this case, the effects of raw, pH adjusted (pH 6.0) and algal biofiltered effluent samples on growth of the standard test alga *Pseudokirchneriella subcapitata* were assessed and compared.

As previously mentioned, the efficiencies of different algal biofilters to remove heavy metal ions from industrial wastewaters was assessed through ten successive biosorption-desorption cycles. Toxicity assessment was only carried out on four biologically treated effluent sub-samples collected after the first, fourth, seventh and tenth bioremoval cycles.

Table 56 includes toxicity values (EC_{50}) of the four industrial effluents before and after being biologically treated with different algal biofilters at different metals bioremoval cycles. Values of the corresponding percentage of toxicity reduction due to biological treatments are given in parentheses and also included in Table 56.

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.

Toxicity reduction values (EC_{50}) of pH adjusted and biologically treated effluents #1, #2, #3 and #4 are given in Figures 41, 42, 43 and 44 respectively. These Figures make it easy to contrast and compare the efficiencies of different algal biofilters to reduce the toxicity of different effluent samples collected after different metal bioremoval cycles.

Substantial toxicity reduction were obtained when pH adjusted subsamples of effluent #1 were biologically treated for only one time with algae-sponge filters (EC_{50} = 85%), algae-cotton filters (EC_{50} = 94%) and algae alginate beads (EC_{50}= 80%). The calculated % toxicity reduction, in this case, were 305%, 348% and 280% respectively (Table 56, Figure 41).

All algal biofilters showed more or less comparable efficiencies to reduce the toxicity of Talkha chemical fertilizers effluent (Effluent #1) at the first, fourth and seventh bioremoval cycles with % toxicity reduction never fell below 200% compared to that achieved only by the adjustment of effluent hydrogen ion activity at pH 6 (Table 56).

Although the efficiency of algal biofilter declined sharply after the tenth bioremoval cycle (Table 56, Figure 41), yet the algae-sponge filter, algae-cotton filter and algae-alginate beads maintained considerably higher capacities to reduce the effluent toxicity to 95%, 114% and 85% respectively compared to the pH adjusted subsamples of effluent #1.

Treatments with different algal biofilters brought about substantial reduction in whole toxicity of Sandoub oils and soap effluent (Table 56, Figure 42). After the first bioremoval cycle the effluent became relatively nontoxic to the growth of test algae as the EC_{50} values were kept above 100% effluent concentration (Figure 42, Table 56). At the fourth bioremoval cycle, the capacity of all algal biofilters to reduce effluent toxicity were almost above 100% compared to that achieved by pH adjustment of raw effluent samples. More or less, comparable results were also noticed after the seventh bioremoval cycles. However, after the tenth cycle the capacity of algal biofilter to reduce the toxicity of effluent #2 was nearly around 50% of the original pH adjusted effluent test samples (Table 56).

Biological treatment of Mahalla dyes effluent (effluent #3) with different algal biofilters exterminated its toxicity as EC_{50} values were typically >100% until the fourth bioremoval cycle (Figure 43, Table 56). The efficiency of different algal biofilters to reduce the toxicity of effluent #3 remained above 150% compared to the corresponding toxicity of the original sample (pH= 6.0), even after the seventh bioremoval cycle. At the end of the tenth bioremoval cycle, the algae-sponge filter, algae-cotton filter and algae- alginate beads were still effective and reduced the whole toxicity of the effluent by 87%, 103% and 87%, respectively.

Treatments of Kafr-Ezzayyat salt and soda effluent (Effluent #4) with different algal biofilters minimized the effluent toxicity to level, at which no EC_{50} values (Figure 44) were obtained even at 100% of effluent particularly after the first and fourth bioremoval cycle (Table 56). After the seventh bioremoval cycle all algal biofilters maintained relatively low toxicity reduction capacities ranged between 60% and 78% (Table 56, Figure 44). After the tenth treatment cycle, the capacities of algal biofilters to reduce the original effluent toxicity fell sharply to 16%, 51% and 33% with sponge, cotton and alginate algal biofilters respectively (Table 56).

Table 51

Figure 36

Table 52

Figure 37

Table 53

Figure 38

Table 54

Figure 39

Table 55: Toxicity expressed as **EC_{50}** of GF/C filtered effluent samples before and after pH adjustment.

Effluent	EC_{50} (% effluent v/v)			
	Effluent # 1	**Effluent # 2**	**Effluent # 3**	**Effluent # 4**
EC_{50} of raw Effluent	1	24	10	43
Original pH	10.21	9.50	10.24	4.81
EC_{50} of pH adjusted Effluent (pH=6.0)	21	41	32	56

EC_{50} = % Concentration of the effluent decreasing the control count by 50%.

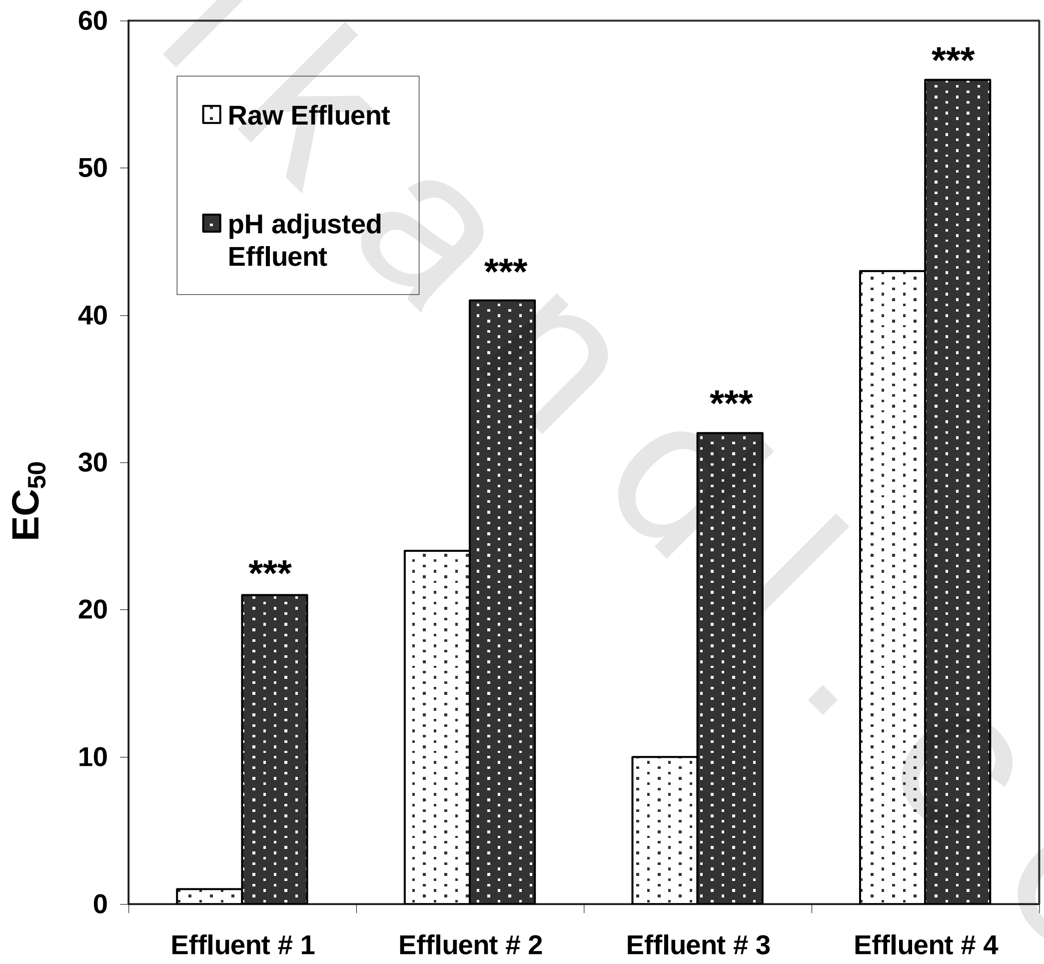

Figure 40: Toxicity of GF/C filtered effluent samples before and after pH adjustment.

***** Toxicity reduction by pH adjustment was very highly significant compared to GF/C filtered effluent samples.**

Table 56: Efficiency of co-immobilized test algae to increase **EC_{50} (**reduce effluent toxicity) through 10 successive bioremoval cycles. Values of % toxicity reduction are given in parentheses.

Bioremoval Cycles		Biological treatment	EC_{50} (% effluent v/v)			
			Effluent # 1	**Effluent # 2**	**Effluent # 3**	**Effluent # 4**
Bioremoval Cycles	First cycle	**Algae-sponge filter**	85 (305)	>100	>100	>100
	First cycle	**Algae-cotton filter**	94 (348)	>100	>100	>100
	First cycle	**Algae-alginate beads**	80 (280)	>100	>100	>100
	Fourth cycle	**Algae-sponge filter**	85 (305)	90 (119)	>100	>100
	Fourth cycle	**Algae-cotton filter**	90 (328)	94 (129)	>100	>100
	Fourth cycle	**Algae-alginate beads**	80 (281)	85 (107)	>100	>100
	Seventh cycle	**Algae-sponge filter**	62 (195)	80 (95)	90 (181)	95 (69)
	Seventh cycle	**Algae-cotton filter**	72 (242)	85 (107)	95 (197)	100 (78)
	Seventh cycle	**Algae-alginate beads**	65 (209)	83 (102)	85 (165)	90 (60)
	Tenth cycle	**Algae-sponge filter**	41 (95)	66 (61)	60 (87)	65 (16)
	Tenth cycle	**Algae-cotton filter**	45 (114)	63 (53)	65 (103)	85 (51)
	Tenth cycle	**Algae-alginate beads**	39 (85)	60 (46)	60 (87)	75 (33)
EC_{50} of filtered effluent samples (pH=6)			21	41	32	56

EC_{50} = the effluent concentration (% v/v) inhibiting algal growth by 50% compared to control.

Figure 41

Figure 42

Figure 43

Figure 44

8- Effect of industrial effluents on viability of test algae grown within filters and alginate beads.

Experimental results of standard algal bioassays revealed that the toxicity (EC_{50}) of different effluent samples, adjusted at pH 6.0, ranged between 21% and 56% (Table 55). A decision was taken to test for the capability of the test algae (*Spirulina platensis, Chlorella ellipsoida*, *Scenedesmus quadricauda var*. *longispina* and *Nitzschia palea*) to thrive at 50% and 100% concentration level that were really strongly toxic to the growth of the standard test alga *Pseudokirchneriella subcapitata*.

The objective of this particular investigation was to test for the ability of test algae to tolerate the effluents toxicity and whether or not they can grow and build up biomass at toxic effluent concentration levels. This research is necessary to validate whether or not the mature biofilters of the test algae can maintain durable applied significance for bioremediation of whole toxic industrial wastewaters.

8.1- Mature algal biofilters.

Table 57 and Fig. 45 illustrate the absolute densities (cells/ml = cells/cm^3) of different test algae grown together for 14 days within sponge and cotton substrata (mature algal biofilters). The initial cell density of algal inocula used to irrigate the sponge and cotton filters or to prepare algal alginate beads was 5x 10^6 cells/ml with 25% density contribution of each of the four different test algae. Relative algal densities are given in parenthesis (Table 57).

After 14 days, the sponge co-immobilized test algae *Spirulina platensis, Chlorella ellipsoida, Scenedesmus quadricauda var. longispina* and *Nitzschia palea* built up different biomasses with relative cell densities of 40.7%, 36.2%, 15.1%, and 8% respectively. The four test algae maintained more or less comparable growth patterns with cotton substrata (Table 57).

Both *Spirulina platensis* and *Chlorella ellipsoida* proved to be relatively more efficient to grow within sponge and cotton filters. Both algae maintained similar mean specific growth rates (μ) of 0.2 day^{-1} followed by *Scenedesmus quadricauda* (μ = 0.14 day^{-1}). *Nitzschia palea* showed the least growth efficiency within sponge and cotton filters with mean growth rate of 0.09 day^{-1} only (Table 57).

8.2- Control algal growth (0.0% effluent) within artificial substrata.

Based on total algal biomass, the co-immobilized test algae of control cultures maintained slow steadily increasing growth pattern throughout the incubation period that lasted 10 days (Figures 46-49). The total algal biomass showed different mean growth rate at different carriers with highest rate (0.2 day^{-1}) recorded for alginate-immobilized algae, followed by sponge (0.105 day^{-1}) and cotton (0.09 day^{-1}) substrate (Table 66).

Individually, both *Chlorella ellipsoida* and *Spirulina platensis* of control cultures showed closely similar growth patterns (Figures 46-49). The mean growth specific rates of *Chlorella ellipsoida* grown within sponge, cotton and alginate carriers were 0.13 day^{-1}, 0.14 day^{-1} and 0.26 day^{-1} respectively (Table 66). The corresponding mean growth specific rates of *Spirulina platensis* were 0.1 day^{-1}, 0.07 day^{-1} and 0.24 day^{-1} respectively (Table 66). Based on these results it seems that growth of *Chlorella ellipsoida* within the support carriers was relatively higher than that of *Spirulina platensis*. However, the biomass of both algae formed approximately 90% of total immobilized algal biomass calculated at the end of incubation period (see Table 58).

The control growth of *Scenedesmus quadricauda* maintained low growth rates when compared with either *Chlorella ellipsoida* or *Spirulina platensis*. This alga showed mean specific growth rates of 0.05day^{-1}, 0.05day^{-1} and 0.11 day^{-1} when grew for ten days within the artificial carriers of sponge, cotton and Ca-alginate, respectively (Table 66).

The pinnate diatom *Nitzschia palea* did not succeed to grow within either sponge or cotton filters as its growth was steadily declining throughout the incubation period (Figure 45), with negative mean specific growth rates of -0.1 day^{-1} and -0.23 day^{-1} were recorded for control grown within sponge and cotton filters respectively. This pinnate diatom showed a very slow growth rate (0.04 day^{-1}) within Ca-alginate matrices (Table 66).

Based on these results, it is evident that the total bulk of immobilized algal biomass is mainly composed of *Chlorella ellipsoida* and *Spirulina platensis* (≈90%) followed by *Scenedesmus quadricauda* (≈9.5%) with almost negligible biomass contribution of the pinnate diatom *Nitzschia palea*. For this reason, the effect of different effluent treatments on growth of *Spirulina platensis, Chlorella ellipsoida* and *Scenedesmus quadricauda* will only be highlighted.

8.3- Effect of toxic effluent doses on growth of test algae of mature biofilters.

The abilities of the test algae of both mature sponge and cotton filter to tolerate and/or to grow at highly toxic doses of the four tested industrial wastewaters, were tested and compared. It should be mentioned that the efficiency of alginate co-immobilized test algae to thrive at 50% and 100% of a given industrial effluent was tested immediately after algae-alginate beads preparations. The idea to include alginate-immobilized test algae aimed primarily at testing the ability of fresh preparation of co-immobilized test algae with equal biomasses proportions (25%) to tolerate and/or grow at higher effluent doses that were highly toxic to the standard test alga *Pseudokirchneriella subcapitata.*

Bi-daily growth of different test algae (co-immobilized into different artificial substrata) at 0.0% (control = standard growth medium), 50% and 100% effluent concentrations was estimated for ten days. The pH of all test solutions was kept at pH 6.0. Absolute cell densities of control cultures and those treated with 50% and 100% of effluents #1, #2, #3 and #4 are listed in Tables 58, 59, 60 and 61 respectively.

The differences in algal biomass of control and treated cultures were statistically evaluated through simple t-test. Differences are considered significant at $p \leq 0.05$. The results of t-test analysis are given in Tables 62, 63, 64 and 65 for effluents #1, #2, #3 and #4 respectively.

Figures 46, 47, 48 and 49 illustrated the growth curves of different test algae grown at well defined growth standard medium (control) and those treated with 50% and 100% of effluents #1, #2, #3 and #4 respectively.

Mean specific growth rates (μ day^{-1}) of different test alga grown for 10 days within artificial carriers of sponge, cotton and Ca-alginate at standard growth medium and at 50% and 100% of effluents #1, #2, #3 and #4 are listed in Tables 66, 67, 68 and 69 respectively.

It should be mentioned that the concentration levels 50% and 100% of the effluent #1 at pH 6.0 stimulated significantly the growth of test algae compared to control. Contrarily, the same concentration level of effluents #2 ,#3 and #4 were inhibitory for the growth of test algae. Therefore, the percent stimulation of main specific algal growth rate (%Sμt) for each treatment of effluent #1 were calculated and included in Table 66. Similarly the percent inhibition of specific algal growth rate (%Iμt) due to different treatments of effluents #2 ,#3 and #4 are showen in Table 67, 68 and 69 respectively.

8.3.A- Effect of effluent #1 (Talkha chemical fertilizers) on growth of test algae within algal filters.

In contrast with other effluents, higher concentration levels (50% and 100%) of this effluent induced noticeable increase in total algal biomass of different artificial biofilters (Table 58 and Figure 46).

Compared to control biofilters, the growth of artificially co-immobilized test algae at 50% and 100% concentration level was relatively slightly high (Figure 46).

Chlorella ellipsoida and *Spirulina platensis* showed comparable growth patterns at 50% and 100% effluent (Figure 46). In all cases both algae showed relatively higher biomass production followed by *Scenedesmus quadricauda*. The pinnate diatom *Nitzschia palea* was unable to grow with sponge and cotton substrata treated with 50% and 100% concentration (Figure 46).

Compared to control case the percent stimulation of growth rate of total algal biomass developed within sponge, cotton and alginate matrices at 50% effluent were 9.09%, 14.7% and 9.2% (Table 66) respectively. Tested as a whole, the pH adjusted effluent induced markedly high growth stimulation of total algal biomass of different biofilters (Table 66). Compared to control, 100% pH adjusted effluent concentration increased the algal growth rate by 25.4%, 24.6% and 15.9% at sponge, cotton and Ca-alginate carriers (Table 66). The main specific growth rates of the algae *Chlorella ellipsoida*, *Spirulina platensis* and *Scenedesmus quadricauda* were also increased in all cases (Table 66)

8.3.B- Effect of effluent #2 (Sandoub oils and soap) on growth of test algae within algal filters.

Both 50% and 100% of effluent #2 (Figure 47) induced marked growth inhibition manifested by significant reduction in total algal biomass and the biomass of each individual test algae (Table 59).

Growth potentialities of individual test algae grown within the artificial carriers were almost negligible (Figure 47) as their growth rates were extremely low compared to those of control cultures (Table 67). In all effluent treatments the total algal biomass was to a large extent a product of *Chlorella ellipsoida* and *Spirulina platensis* biomass. The biomass contribution of *Scenedesmus quadricauda* was relatively very low and that of *Nitzschia palea* was almost nil (Table 59).

The % inhibition in growth rate due to effluent treatments of *Chlorella ellipsoida* (20.3% - 76.5%) and *Spirulina platensis* (22.8% - 100%) were to high extent dependent on effluent concentration and to a little extent on the artificial carriers within which they have been grown (Table 67).

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% at whole effluent treatments respectively (Table 67).

8.3.C- Effect of effluent #3 (Mahalla dyes factory) on growth of test algae within algal filters.

Compared to control filter, the concentrations 50% and 100% of effluent #3 caused significant growth inhibition of all test algae (Figure 48).

Growth inhibition lead to obvious reduction in total algal biomass at the end of incubation period (Table 60) particularly at 100% effluent concentration.

The pinnate diatom *Nitzschia palea* was the most sensitive to effluent toxicity as its growth rate was steadily declining throughout the incubation period at both 50% (μ= 0.84) and 100% (μ= 1.07) concentration levels of the effluent #3 (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 chlorococcal alga within sponge filter was inhibited by 69.5% and 241.1% at 50% and 100% doses respectively (Table 68). Comparable results were obtained in cases of cotton filter (Table 68).

Chlorella ellipsoida and *Spirulina platensis* were relatively more tolerant to the toxicity of effluent #3 (Figure 48).

Spirulina platensis in the biofilter showed different growth rates ranged between 0.06 day^{-1} and 0.18 day^{-1} depending on both effluent concentrations and on the artificial carrier (Table 68).

Growth inhibition of this cyanophyte fluctuated between 8.5% and 36% and was mainly concentration dependent.

Growth of *Chlorella ellipsoida* (Figure 48) within effluent treated filters was more or less comparable to that of *Spirulina platensis* (Table 68). However the inhibition in *Chlorella ellipsoida* growth rate was relatively higher as it ranged between 14.9% and 59.01% depending mainly on effluent concentration level (Table 68).

It should be mentioned that there was non significant difference between growth rates of treated and control filters for *Chlorella ellipsoida* and *Spirulina platensis* (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 start cell count (Table 60). These finding indicates that *Chlorella ellipsoida* and *Spirulina platensis* are efficient candidate algae for the bioremediation of the effluent #3.

8.3.D- Effect of effluent #4 (Kafr-Ezzayyat salt and soda) on growth of test algae within algal filters.

The growth of *Nitzschia palea* within effluent treated Ca-alginate, cotton and sponge filters was again entirely exterminated after 4 days (Figure 49). Growth inhibition of *Scenedesmus quadricauda* within artificial biofilters ranged between 24.7% (for 50%) and 241.1% (for 100%). Growth inhibition was largely effluent dose-dependent (Table 68).

Chlorella ellipsoida followed by *Spirulina platensis* were the most tolerant algae capable of growing within artificial carriers treated with 50% and 100% effluent concentrations (Fig. 49). Growth rates of effluent treated filters of *Chlorella ellipsoida* ranged between 0.18 day^{-1} and 0.6 day^{-1} and those of *Spirulina platensis* between 0.15 day^{-1} and 0.02 day^{-1} (Table 69) depending to a little extent on type of artificial carrier but mainly on effluent dose with lowest growth rate obtained at highest effluent dose.

It should be mentioned that both algae, *Chlorella ellipsoida* and *Spirulina platensis* , were able to build up biomass of different artificial carriers treated with either 50% or 100% effluent doses (Figure 49). The cell count of both algae, treated for 10 days with different effluent doses, was significantly higher ($P \leq 0.05$) than the start cell count (Table 61). These finding indicates that *Chlorella ellipsoida* and *Spirulina platensis* are efficient candidates for metal ion bioremoval of effluent #4.

Table 57: Absolute cell densities and specific growth rates of different test algae grown for 14 days on sponge and cotton substrates under favorable growth conditions.

Algal Species	Initial cell density * (millions/ml)	Cell density after 14 days of incubation (millions/ml)		Specific growth rate (µ/days)	
		Sponge filter	Cotton filter	Sponge filter	Cotton filter
Spirulina platensis	**1.25 (25%)**	**23 (40.7%)**	**21.5 (40.2%)**	**0.21**	**0.20**
Chlorella ellipsoida	**1.25 (25%)**	**20.5 (36.2%)**	**19.5 (36.4%)**	**0.2**	**0.2**
Scendesmus quadricauda	**1.25 (25%)**	**8.5 (15.1%)**	**7.5 (14.1%)**	**0.14**	**0.14**
Nitzschia palea	**1.25 (25%)**	**4.5 (8%)**	**5 (9.3%)**	**0.09**	**0.09**
Total number	**5**	**56.5**	**53.5**	**0.17**	**0.17**

Relative cell densities (% total number) are given in parentheses.

* Algal cells with the same proportions were used for preparation of algae-alginate beads.

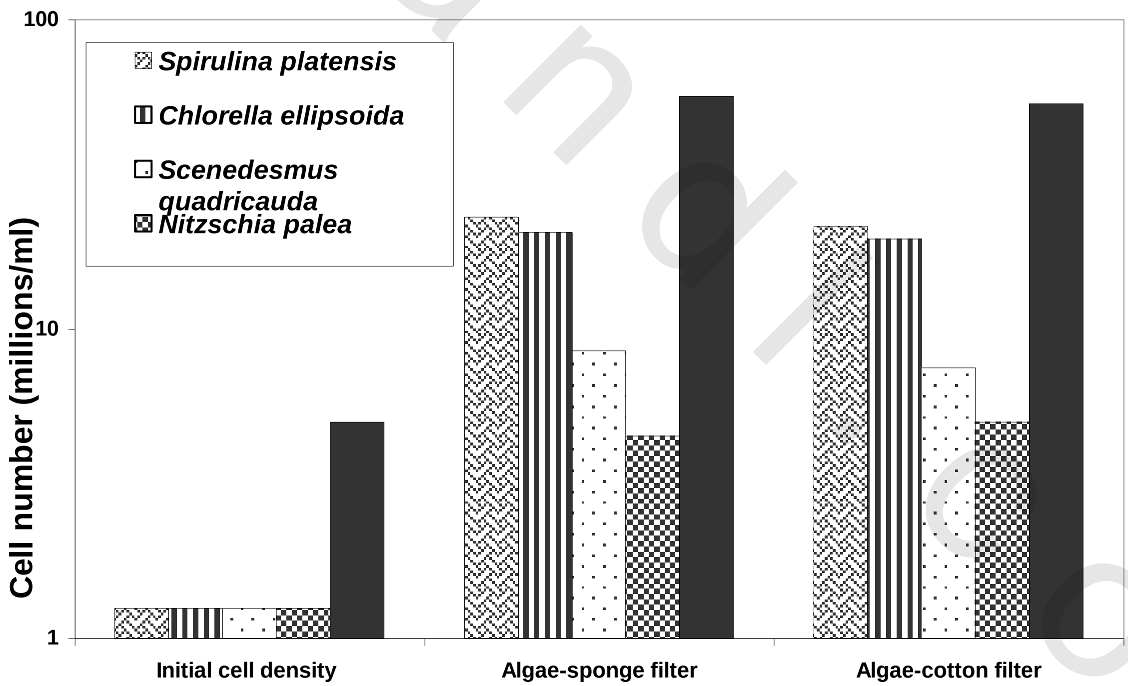

Figure 45: Absolute cell densities of different test algae grown for 14 days on sponge and cotton substrates under favorable growth conditions.

Table 58: Growth (Cell count = millions/ml**)** of test algae grown for different time periods within sponge, cotton and alginate carriers at 50% and 100% concentrations (v/v) of effluent #1 **(Talkha chemical fertilizers)**.

Time (Days)	**Biofilter / Algal Species**	**Algae-sponge filter**			**Algae-cotton filter**			**Algae-alginate beads**		
		Control	**50%**	**100 %**	**Control**	**50%**	**100%**	**Control**	**50%**	**100%**
2	***Spirulina platensis***	27	26	28	25	24	26	3	4	5
	Chlorella ellipsoida	26	25	26	24.5	23	29	4.5	4	5
	Scenedesmus quadricauda	11.5	10	11	7.5	8	12	2	2	3
	Nitzschia palea	5	3.5	1.5	5	1.5	1.5	1.5	2.5	3
	Total number	69.5	64.5	66.5	62	56.5	68.5	11	12.5	16
4	***Spirulina platensis***	47	46	48	37.5	40	48	4.5	6	7
	Chlorella ellipsoida	35	38	42	32	33	37	6	6	8
	Scenedesmus quadricauda	12.5	13	15	12	12	18	3	4	5
	Nitzschia palea	5	4	1.5	4.5	0.5	0.5	2.5	3	4
	Total number	99.5	101	106.5	86	85.5	103.5	16	19	24
6	***Spirulina platensis***	52	56	59	40.5	50	58	7.5	8	9
	Chlorella ellipsoida	40	44	48	46.5	50	54	9.5	10	12
	Scenedesmus quadricauda	10.5	14	19	12	15	22	4	6	8
	Nitzschia palea	5	1.5	0.5	4.5	0	0.5	2.5	3	4
	Total number	107	115.5	126.5	103	115	134.5	23.5	27	33
8	***Spirulina platensis***	60	63	70	44.5	60	70	10	12	14
	Chlorella ellipsoida	57.5	60	76	62	71	80	12	13	15
	Scenedesmus quadricauda	12	16	20	13	16	24	4	6	8
	Nitzschia palea	3.5	0	0	3	0	0	2	4	5
	Total number	133	139	166	122	147	174	28	35	42
10	***Spirulina platensis***	65.5	68	84	46.5	65	76	14.5	16	18
	Chlorella ellipsoida	81	96	128	85	90	97	17	18	21
	Scenedesmus quadricauda	14.5	16	20	12.5	16	26	4	8	10
	Nitzschia palea	1.5	0	0	0.5	0	0	2	4	6
	Total number	162	180	232	144	171	199	37.5	46	55

Figure 46

Table 59: Growth (Cell count = millions/ml) of test algae grown for different time periods within sponge, cotton and alginate carriers at 50% and 100% concentrations (v/v) of effluent #2 **(Sandoub oils and soap)**.

Time (Days)	Biofilter / Algal Species	Algae-sponge filter			Algae-cotton filter			Algae-alginate beads		
		Control	50%	100 %	Control	50%	100%	Control	50%	100%
2	*Spirulina platensis*	27	23	21	25	24	21	3	3	2
	Chlorella ellipsoida	26	26	26	24.5	23	24	4.5	5	4
	Scenedesmus quadricauda	11.5	10	8	7.5	8	8	2	2	1
	Nitzschia palea	5	3.5	2	5	1.5	1.5	1.5	1.5	1
	Total number	69.5	62.5	57	62	56.5	54.5	11	11.5	8
4	*Spirulina platensis*	47	26	22	37.5	25	21	4.5	3	2
	Chlorella ellipsoida	35	27	24	32	26	26	6	6	4
	Scenedesmus quadricauda	12.5	11	9	12	9	8	3	2	2
	Nitzschia palea	5	1.5	0.5	4.5	1.5	1.5	2.5	2.5	1
	Total number	99.5	65.5	55.5	86	61.5	56.5	16	13.5	9
6	*Spirulina platensis*	52	34	25	40.5	35	29	7.5	5	3
	Chlorella ellipsoida	40	29	25	46.5	28	26	9.5	7	5
	Scenedesmus quadricauda	10.5	12	8	12	10	8	4	3	2
	Nitzschia palea	5	1.5	0.5	4.5	1.5	0.5	2.5	2	1
	Total number	107	76.5	58.5	103	74.5	63.5	23.5	17	11
8	*Spirulina platensis*	60	40	26	44.5	44	36	10	6	2
	Chlorella ellipsoida	57.5	36	26	62	38	30	12	8	6
	Scenedesmus quadricauda	12	12	8	13	8	8	4	3	2
	Nitzschia palea	3.5	0.5	0	3	0	0	2	2	1
	Total number	133	88.5	60	122	90	74	28	19	11
10	*Spirulina platensis*	65.5	32	23	46.5	39	27	14.5	4	2
	Chlorella ellipsoida	81	46	32	85	44	35	17	10	7
	Scenedesmus quadricauda	14.5	8	8	12.5	8	8	4	2	2
	Nitzschia palea	1.5	0	0	0.5	0	0	2	2	1
	Total number	162	86	63	144	91	70	37.5	18	12

Figure 47

Table 60: Growth (Cell count = millions/ml) of test algae grown for different time periods within sponge, cotton and alginate carriers at 50% and 100% concentrations (v/v) of effluent #3 **(Mahalla dyes factory)**.

Time (Days)	Biofilter / Algal Species	Algae-sponge filter			Algae-cotton filter			Algae-alginate beads		
		Control	50%	100 %	Control	50%	100%	Control	50%	100%
2	*Spirulina platensis*	27	25	23	25	23	21	3	2	1.5
	Chlorella ellipsoida	26	31	22	24.5	25	20	4.5	5	4
	Scenedesmus quadricauda	11.5	9.5	8	7.5	8	8	2	1.5	1.5
	Nitzschia palea	5	3	1.5	5	1.5	0.5	1.5	2	2
	Total number	69.5	68.5	54.5	62	57.5	49.5	11	10.5	9
4	*Spirulina platensis*	47	26.5	24	37.5	30	28	4.5	3	3
	Chlorella ellipsoida	35	36	26	32	27	23	6	6	5
	Scenedesmus quadricauda	12.5	8	8	12	8	8	3	2	2
	Nitzschia palea	5	1.5	1.5	4.5	1.5	0.5	2.5	2	2
	Total number	99.5	72	59.5	86	66.5	59	16	13	12
6	*Spirulina platensis*	52	41	32	40.5	36	32	7.5	5.5	3.5
	Chlorella ellipsoida	40	37	28.5	46.5	36	25	9.5	8	5.5
	Scenedesmus quadricauda	10.5	8	8	12	8	4	4	3	2
	Nitzschia palea	5	1.5	0.5	4.5	1.5	0.5	2.5	2	1.5
	Total number	107	87.5	69	103	81.5	61.5	23.5	18.5	12.5
8	*Spirulina platensis*	60	51	46	44.5	40	36	10	6.5	4
	Chlorella ellipsoida	57.5	43	30	62	46	34	12	11	6
	Scenedesmus quadricauda	12	8	8	13	8	4	4	3	2
	Nitzschia palea	3.5	0	0	3	0	0	2	1.5	1.5
	Total number	133	102	84	122	94	74	28	22	13.5
10	*Spirulina platensis*	65.5	60	51	46.5	41	40	14.5	8	6
	Chlorella ellipsoida	81	52	36	85	58	46	17	11.5	6
	Scenedesmus quadricauda	14.5	10	4	12.5	8	4	4	2	2
	Nitzschia palea	1.5	0	0	0.5	0	0	2	1	0.5
	Total number	162	122	91	144	107	90	37.5	22.5	14.5

Figure 48

Table 61: Growth (Cell count = millions/ml) of test algae grown for different time periods within sponge, cotton and alginate carriers at 50% and 100% concentrations (v/v) of effluent #4 **(Kafr-Ezzayyat salt and soda)**.

Time (Days)	Biofilter / Algal Species	Algae-sponge filter			Algae-cotton filter			Algae-alginate beads		
		Control	50%	100 %	Control	50%	100%	Control	50 %	100%
2	*Spirulina platensis*	27	21.5	21	25	22	21	3	2	2
	Chlorella ellipsoida	26	24	25	24.5	20	19	4.5	3.5	3
	Scenedesmus quadricauda	11.5	8	8	7.5	6	6	2	2	2
	Nitzschia palea	5	2.5	1.5	5	3.5	1.5	1.5	1.5	1
	Total number	69.5	56	55.5	62	51.5	47.5	11	9	8
4	*Spirulina platensis*	47	36.5	32	37.5	25	22	4.5	2.5	2.5
	Chlorella ellipsoida	35	28	28	32	25	22	6	4.5	3
	Scenedesmus quadricauda	12.5	8	8	12	8	6	3	2	2
	Nitzschia palea	5	1.5	1.5	4.5	2.5	1.5	2.5	2	1.5
	Total number	99.5	74	69.5	86	60.5	51.5	16	11	9
6	*Spirulina platensis*	52	40	38	40.5	30	25	7.5	3	3
	Chlorella ellipsoida	40	30	28	46.5	32	26	9.5	5	3.5
	Scenedesmus quadricauda	10.5	8	6	12	8	6	4	3	3
	Nitzschia palea	5	1.5	0.5	4.5	1.5	1.5	2.5	2	1.5
	Total number	107	79.5	72.5	103	71.5	58.5	23.5	13	11
8	*Spirulina platensis*	60	44	36	44.5	31.5	26.5	10	4.5	3
	Chlorella ellipsoida	57.5	36	32	62	42	36	12	6	4.5
	Scenedesmus quadricauda	12	6	4	13	9	6	4	3	3
	Nitzschia palea	3.5	0	0	3	0.5	0	2	2	1
	Total number	133	86	72	122	83	68.5	28	15.5	11.5
10	*Spirulina platensis*	65.5	43.5	40	46.5	38	28	14.5	6	5
	Chlorella ellipsoida	81	46	40	85	53	47	17	8	7
	Scenedesmus quadricauda	14.5	6	4	12.5	8	6	4	3	3
	Nitzschia palea	1.5	0	0	0.5	0	0	2	2	1
	Total number	162	95.5	84	144	99	81	37.5	19	16

Figure 49

Table 62: Statistical differences in mean growth (expressed as cell count ml^{-1}) of different test algae grown within sponge, cotton and alginate carriers for 10 days at standard growth media and those grown at 50% and 100% concentrations of **Talkha chemical fertilizers** effluent.

Algal Species	t-test	Algae-sponge filter		Algae-cotton filter		Algae-alginate beads	
		50%	100%	50%	100%	50%	100%
Spirulina platensis	**t-value**	+0.15	+0.645	+1.089	+1.746	+0.437	+0.86
	Variance	NS	NS	NS	NS	NS	NS
Chlorella ellipsoida	**t-value**	+0.771	+0.789	+0.209	+0.559	+0.119	+0.573
	Variance	NS	NS	NS	NS	NS	NS
Scenedesmus quadricauda	**t-value**	+1.234	+2.551	+1.093	+3.366	+1.643	+2.607
	Variance	NS	*	NS	**	NS	*
Nitzschia palea	**t-value**	-2.13	-4.296	-3.55	-3.464	+3.394	+4.234
	Variance	NS	**	**	**	**	**
Total number	**t-value**	+0.233	+0.785	+0.464	+1.85	+0.627	+1.3
	Variance	NS	NS	NS	NS	NS	NS

NS Non significant *Significant ($P \leq 0.05$) **High significant ($P \leq 0.01$)
*****Very high significant ($P \leq 0.001$).**
Positive and negative t-values indicate increase and decrease in growth of a given test algae compared to control, respectively.

Table 63: Statistical differences in mean growth (expressed as cell count ml^{-1}) of different test algae grown within sponge, cotton and alginate carriers for 10 days at standard growth media and those grown at 50% and 100% concentrations of **Sandoub oils and soap** effluent.

Algal Species	t-test	Algae-sponge filter		Algae-cotton filter		Algae-alginate beads	
		50%	100%	50%	100%	50%	100%
Spirulina platensis	**t-value**	-2.648	-4.012	-0.995	-2.548	-1.735	-2.773
	Variance	*	**	NS	*	NS	*
Chlorella ellipsoida	**t-value**	-1.447	-2.165	-1.575	-1.976	-1.088	-1.977
	Variance	NS	NS	NS	NS	NS	NS
Scenedesmus quadricauda	**t-value**	-1.6	-5.733	-2.261	-3.425	-2.132	-3.577
	Variance	NS	***	*	**	NS	**
Nitzschia palea	**t-value**	-2.861	-4.427	-2.888	-3.152	-0.408	-5.879
	Variance	*	**	*	*	NS	**
Total number	**t-value**	-2.325	-3.525	-1.812	-2.709	-1.528	-2.774
	Variance	*	**	NS	*	NS	*

NS Non significant *Significant ($P \leq 0.05$) **High significant ($P \leq 0.01$)
*****Very high significant ($P \leq 0.001$).**

Table 64: Statistical differences in mean growth (expressed as cell count ml^{-1}) of different test algae grown within sponge, cotton and alginate carriers for 10 days at standard growth media and those grown at 50% and 100% concentrations of **Mahalla dyes** effluent.

Algal Species	t-test	Algae-sponge filter		Algae-cotton filter		Algae-alginate beads	
		50%	100%	50%	100%	50%	100%
Spirulina platensis	t-value	-1.009	-1.724	-0.947	-1.477	-1.245	-1.979
	Variance	NS	NS	NS	NS	NS	NS
Chlorella ellipsoida	t-value	-0.78	-1.938	-0.929	-1.723	-0.581	-1.991
	Variance	NS	NS	NS	NS	NS	NS
Scenedesmus quadricauda	t-value	-4.409	-4.811	-3.425	-4.158	-0.054	-3.368
	Variance	**	**	**	**	NS	**
Nitzschia palea	t-value	-3.15	-4.296	-2.882	-3.852	-1.46	-1.809
	Variance	*	**	*	**	NS	NS
Total number	t-value	-1.284	-2.484	-1.318	-2.317	-1.131	-2.309
	Variance	NS	*	NS	*	NS	*

NS Non significant *Significant (P ≤ 0.05) **High significant (P ≤ 0.01)
*****Very high significant (P ≤ 0.001).**
Positive and negative t-values indicate increase and decrease in growth of a given test algae compared to control, respectively.

Table 65: Statistical differences in mean growth (expressed as cell count ml^{-1}) of different test algae grown within sponge, cotton and alginate carriers for 10 days at standard growth media and those grown at 50% and 100% concentrations of **Kafr-Ezzayyat salt and soda** effluent.

Algal Species	t-test	Algae-sponge filter		Algae-cotton filter		Algae-alginate beads	
		50%	100%	50%	100%	50%	100%
Spirulina platensis	t-value	-1.688	-2.269	-2.026	-3.564	-1.979	-2.276
	Variance	NS	NS	NS	**	NS	NS
Chlorella ellipsoida	t-value	-1.443	-1.716	-1.261	-1.666	-1.867	-2.381
	Variance	NS	NS	NS	NS	NS	*
Scenedesmus quadricauda	t-value	-6.603	-5.567	-3.252	-5.44	-1.705	-1.71
	Variance	***	***	*	***	NS	NS
Nitzschia palea	t-value	-3.443	-4.294	-1.824	-2.882	-0.942	-4.024
	Variance	**	**	NS	*	NS	**
Total number	t-value	-2.118	-2.667	-1.842	-2.726	-1.961	-2.502
	Variance	NS	*	NS	*	NS	*

NS Non significant *Significant (P ≤ 0.05) **High significant (P ≤ 0.01)
*****Very high significant (P ≤ 0.001).**

Tab 66, 67

Tabl 68, 69