MATERIAL AND METHODS

1. Test algae.

Four algal strains were selected for metal ion bioremoval studies based on the previous work carried out by the authors (Ibrahim *et al.*, 2000). The algae were isolated and purified as axenic strains at Mansoura University, Faculty of Science, Phycology lab. They are

1-*Spirulina platensis* (Nordst.) UMANS 11

2-*Chlorella ellipsoida* (Gerneck) UMANS 102

3-*Scenedesmus quadricauda var. longispina* (Chod.; G.M. Smith.) UMANS 125

4-*Nitzschia palea* (Kuts.; W. Smith.) UMANS 201

Previous studies carried out by the authors (Ibrahim *et al.*, 2000 and Azab *et al.*, 2004) indicated that the selected algae maintained high metal ions bioremoval capabilities.

The standard test alga *Pseudokirchneriella subcapitata* NIVA-CHL1 was used for toxicity testing. The alga was obtained from the culture collection of the Norwegian Institute for Water Research (PO Box 173, kjelsas, N-0411,Oslo, Norway). It is perhaps relevant to mention that this alga was worldwide known as *Selenastrum capricornutum*. According to a peer taxonomic study (Nygaard *et al*., 1986), *Selenastrum capricornutum* was given a new generic name *Raphidocelis subcapitata*. However, this generic name has recently been changed to *Pseudokirchneriella subcapitata* NIVA-CHL1.

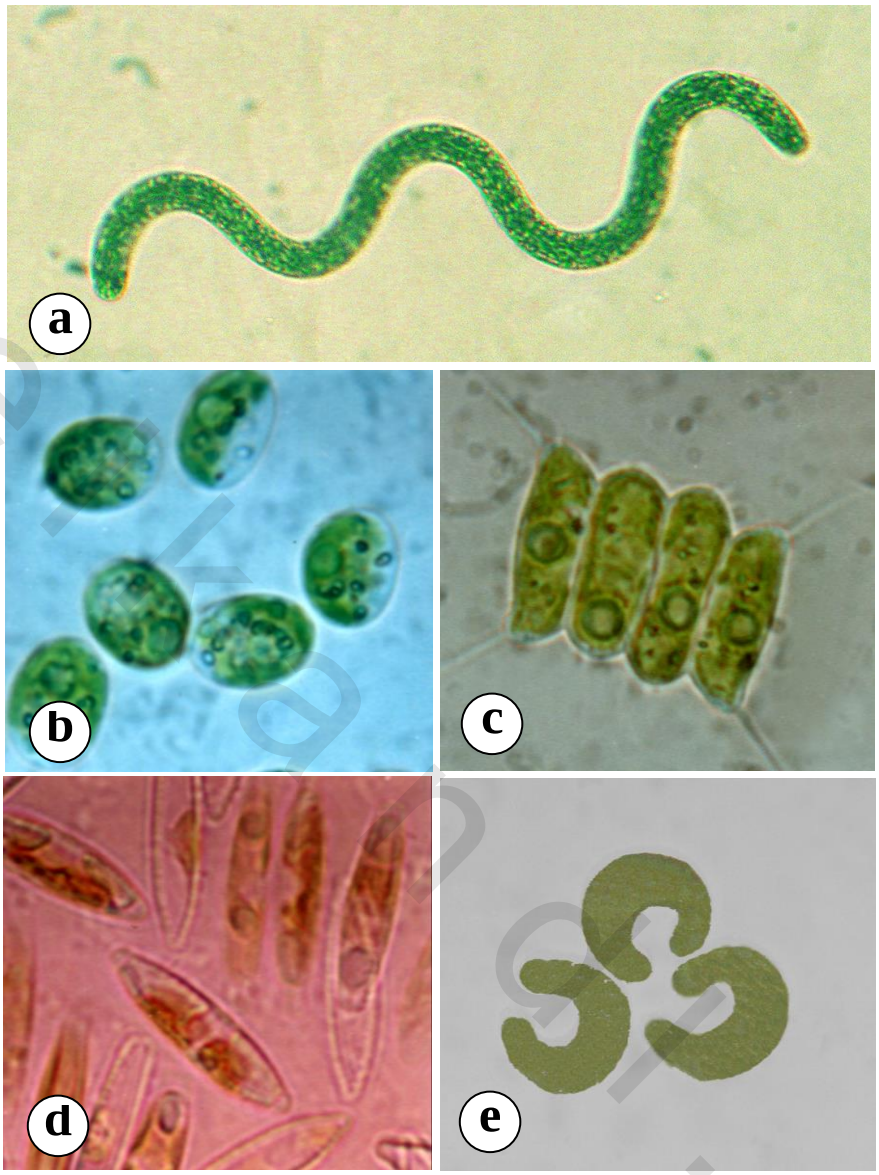

Figure 1: Algal species used for metal ions bioremoval and for toxicity assessment.

a- *Spirulina platensis* (300X),
b- *Chlorella ellipsoida* (450X),
c- *Scenedesmus quadricauda* var. *longispina* (450X)
d- *Nitzschia palea* (450X)
e- *Pseudokirchneriella subcapitata* (520X)

Algal growth media.

Spirulina platensis was grown in Spirulina medium (Table 1). *Chlorella ellipsoida* , *Scenedesmus quadricauda* and *Nitzschia palea* were grown in MBL medium (Table 2). *Pseudokirchneriella subcapitata* was grown in AAM medium (Table 3). Clone cultures of the test algae were grown for 10 days in their respective growth media before use.

Table 1: Composition of the Spirulina Medium (Zarrouk,1966)**.**

Macronutrients (Solution a)	**Quantity (g.)**
1-NaCl	1.0
2-$MgSO_4.7H_2O$	0.2
3-$CaCl_2.2H_2O$	0.04
4-$FeSO_4.7H_2O$	0.01
5-Na-EDTA	0.08
6-K_2HPO_4	0.5
7-$NaNO_3$	2.5
8-K_2SO_4	1.0
9-$NaHCO_3$	16.8
10-Distilled H_2O	1000 ml
11- Add 1.0 ml from stock solution **b** + 1.0 ml from stock solution **c**	

Micronutrient stock solutions .

Stock solution b		**Stock solution c**	
1-NH_4NO_3	0.023 g	1-H_3BO_3	2.82 g
2-$K_2Cr_2(SO_4)_2.27H_2O$	0.096 g	2-$MnCl_2.4H_2O$	1.81 g
3-$NiSO_4.7H_2O$	0.044 g	3-$ZnSO_4.7H_2O$	0.222 g
4-$Na_2SO_4.7H_2O$	0.0179 g	4-$CuSO_4.5H_2O$	0.077 g
5-$Ti(SO_4)_3$	0.04 g	5-MoO_3	0.015 g
6-Co $(NO_3)_2.6H_2O$	0.044 g	6-Distilled H_2O	1000 ml
7-Distilled H_2O	1000 ml		

The final pH was adjusted to 9.0 – 9.5

Table 2: Composition of the Woods Hole MBL Medium pH 7.2 (Nichols, 1973).

Stock solutions of macro- and micronutrients were prepared as follows:

Chemical compound	Quantity (g.)	Volume of stock solution (ml distilled water)
Macronutrient stock solutions		
1- $CaCl_2.H_2O$	36.76	1000
2-$MgSO_4.7H_2O$	36.97	1000
3- $NaHCO_3$	12.6	1000
4-K_2HPO_4	8.71	1000
5-$NaNO_3$	85.01	1000
6- $NaSiO_3.9H_2O$	28.42	1000
7-Micronutrient stock solution		1000
a-Na-EDTA	4.36	
b-$FeCl_3.6H_2O$	3.15	
c-$CuSO_4.5H_2O$	0.01	
d-$ZnSO_4.7H_2O$	0.022	
e-$CoCl_2.6H_2O$	0.01	
f-$MnCl_2.4H_2O$	0.18	
g-$NaMoO_4.2H_20$	0.006	

The nutrient medium was prepared by using one ml of each of the stock solutions (from 1 to 7) and making it up to one liter by distilled water. The final pH was adjusted to 7.2.

Table 3: Composition of the Algal Nutrient Medium (AAM) (Miller *et al*., 1978).

Stock solutions of macro- and micronutrients were prepared as follows:

Chemical compound	Quantity (mg)	Volume of stock solution (ml glass distilled water)
Macronutrient stock solutions		
1-$NaNO_3$	12750	500
2-$MgCl_2.2H_2O$	6082	500
3-$CaCl_2.2H_2O$	2205	500
4-$MgSO_4.7H_2O$	7350	500
5-K_2HPO_4	522	500
6-$NaHCO_3$	7500	500
7-Micronutrients stock solution		500
a-H_3BO_3	92.76	
b-$MnCl_2.4H_2O$	207.69	
c-$ZnCl_2$	1.635	
d-$FeCl_3.6H_2O$	79.88	
e-Na_2-EDTA.$2H_2O$	150	
f-$CoCl_2.6H_2O$	0.714	
g-$Na_2MoO_4.2H_2O$	3.63	
h-$CuCl_2.2H_2O$	0.006	

The nutrient medium was prepared by adding one ml of each stock solution to one liter of distilled water. The final pH was adjusted to 7.5.

2. Chemicals.

The chemicals used in preparations of standards and other reagents were obtained from BDH chemicals (Itd Pools England) or AR chemicals (Mallianckrodt Chemical Works, St. Louis, MO 63147 USA), Sigma (St. Louis, MO 63178 USA) and MERCK (61 Darmstadt Germany).

3. Preparation of glassware.

Glassware were washed with liquid detergent, flushed thoroughly with tap water, rinsed with 10% (v/v) HCl solution, neutralized by swirling with a saturated sodium carbonate solution, rinsed several times with tap water followed by distilled water, dried at 50°C and stored in a closed cabinet until needed.

Water.

Glass distilled water was used for preparing standards and chemical reagents used in experimental work and the culture media as well.

4. Standard solution of heavy metals.

Four metal ions namely Nickel (Π), Cadmium (Π), Lead (Π) and Mercury (Π) were selected for this investigation. Stock solution containing 1000 mgl^{-1} prepared for each heavy metal salts (BDH) as follows:

Nickel

Dissolve 4.0495 g of $NiCl_2.6H_2O$ in 1000ml of distilled water.

Cadmium

Dissolve 1.7911 g of $CdCl_2.H_2O$ in 1000ml of distilled water

Lead

Dissolve 1.598 g of $Pb(NO_3)_2$ in1000 ml of distilled water.

Mercury

Dissolve 1.3535 g of $HgCl_2$ in 1000 ml of distilled water.

Stock solutions were used for preparing different test solutions with varying metal ion concentrations .

5. Alginate immobilization and de-immobilization of test algae.

The four test algae (*Spirulina platensis*, *Chlorella ellipsoida*, *Scenedesmus quadricauda var. longispina* and *Nitzschia palea*) were immobilized in alginate matrices. Figure 2 summarizes the experimental procedure of algal immobilization. The method was carried out according to Abdel-Hamid (1991). Test algae were immobilized singly and in a mixture with initial cell density of $5x\ 10^6$ cells/ml alginate. The term composite beads was used to denote beads immobilizing a mixture of all the test algae each with initial cell density of $1.25x\ 10^6$ cells/ml.

Volumetric measurement of the average bead volume was carried out by dropping a number of beads (5, 10, 15, ……..,75 beads) into a measuring cylinder (5 ml capacity with 0.1 ml divisions) containing a known volume of MBL medium at 20ºC. In each case the increase in volume (ml) was divided

by the number of beads added, in order to calculate the mean actual volume of a single bead.

It is perhaps relevant to mention that the calculated (expected average) algal density per bead was determined according to the following equation:

$$\mathbf{d = D/N}$$

where

d = algal cell bead^{-1}, D = algal cells ml^{-1} alginate-algae mixture and

N = bead ml^{-1} mixture.

By knowing the initial concentration (cells ml^{-1}) of the algal culture and considering the dilution due to added alginate, D could be easily determined.

To determine the actual algal density, fifty beads were randomly selected and dissolved separately in 1% sodium hexametaphosphate, cells were counted using standard haemocytometer. The procedure of algal cell de-immobilization was carried out as outlined in Figure 3 (Abdel-Hamid, 1991).

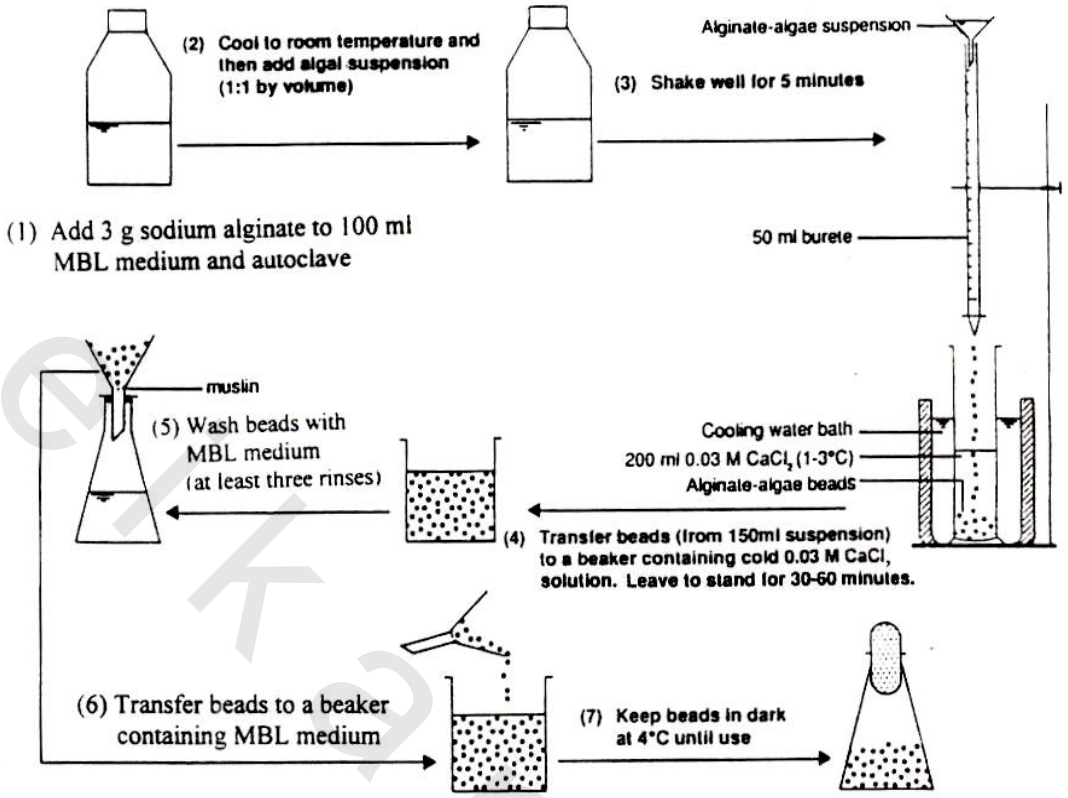

Figure 2: Outline of procedure for immobilization of algae

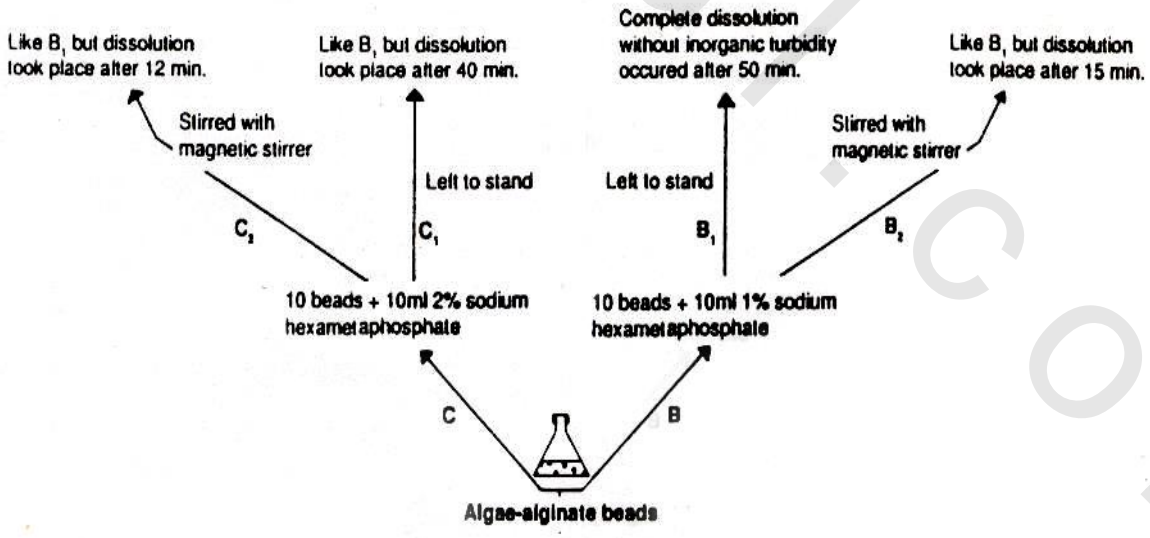

Figure 3: Procedure used for de-immobilization of algae

6. Bioremoval of heavy metal ions.

Test solution containing metal salts 10 mgl^{-1} of Cd(Π), Ni(Π), Pb(Π) and 1.0 mgl^{-1} of Hg(Π) were prepared. Metal ion bioremoval capabilities of immobilized test algae were tested. The basic experimental design for bioremoval testing was given in Table 4.

Table 4: Constituents of a metal test solution.

Heavy metal ions	**Pb**(Π)	**Cd**(Π)	**Ni**(Π)	**Hg**(Π)
Initial conc. (mgl^{-1})	10	10	10	1
Volume of metal test solution (ml)	50	50	50	50
Volume of algal beads (ml)*	25	25	25	25

***** It has been calculated that one ml of alginate algae suspension gives approximately 15±2 beads, each bead contains an average of 33.3×10^4 cells .

6.1. Test for the optimum contact time for metal ion bioremoval.

The beads of each test alga were soaked in separate test flasks each containing 50 ml of metal ion test solution (Table 4) and left to stand for 1, 5, 15, 30 and 60 minutes. Flasks were shaken by hand. Three replicates were used for each treatment. The algal beads were then picked up. The final metal ion concentrations of the metal test solution were determined using an atomic absorption spectrophotometer (Perkin-Elmer model 2380,USA). Values were then subtracted from the initial metal ion concentration. The

resultant values represented the expected metal ion amount (mgl^{-1}) removed by algal beads.

6.2. Test for the optimum pH for metal ion bioremoval.

The same procedure carried out to determine the best time for metal ion bioremoval was repeated. In this case beads were soaked in metal test solutions (Table 4) at different pH values of 2, 3, 4, 5, 6, 7, 8 and 9. Solutions of 0.1 N HCl and 0.1 N NaOH were used for pH adjustment. The final pH values were checked by pH-meter (Hanna Instrument, 8519, Italy). Beads were left to stand for 15 minutes in metal test solutions, as this period was found to be the optimum contact time for heavy metal ions bioremoval. The concentrations of heavy metal ions left in test solution were determined as described before.

7. Heavy metal analysis.

Metal analysis followed the direct aspiration into an air-acetylene flame using the atomic absorption spectrophotometer (Perkin-Elmer model 2380,USA). The technique employing hollow cathode lamps was adopted for the analysis of cadmium, nickel and lead. A separate unit called mercury hydride system (MHS-10) was connected to the atomic absorption spectrophotometer for the accurate analysis of mercury. In this case nitrogen was used as a purge gas and Sodium Borohydride ($NaBH_4$) was the reductant. The all procedures were carried out according to American Public Health Association (APHA, 1985).

A series of standard solutions was prepared for each of the tested metal, absorbance values of each standard solutions were recorded and employed for plotting the standard curves. A linear regression equation **Y = a + b X** (Where; Y = absorbance, a = slope, b = intercept and X = metal concentration) was calculated for each metal standard curve. The equations were used for the calculation of metal concentrations.

The optimum experimental conditions for the Instrumental metal analysis are listed in Table 5.

Table 5: Instrumental parameters used for the atomic absorption spectrophotometer.

Metal ions	Wave length nm	Lamp Current mA	Air flow rate dm^3min^{-1}	Fuel flow rate $dm^3 min^{-1}$	Burner height cm	Working range mgl^{-1}
Cd^{+2}	228.8	4	21	6	2.1	0 - 10
Hg^{+2}	253.7	6	21	6	2.1	0 -1.0
Ni^{+2}	232.0	13	21	6	2.1	0 - 10
Pb^{+2}	217.0	10	21	6	2.1	0 - 10

8. Elution of heavy metal ions from algal alginate beads.

Elution means stripping off metal ions bound to algal beads. A method adopted by Greene and Darnell (1990) was followed. The metal-laden beads

(beads soaked for 15 minutes in different metal test solution) were placed in conical flasks each containing 50 ml of 0.1 M thiourea.

To select the optimum pH of metal ions elution, aliquots of metal-laden beads (25ml) were transferred to conical flasks, each containing 50 ml of 0.1M thiourea at different pH values 2.0, 3.0, 4.0, 5.0 and 6.0. Solutions of 0.1 N HCl and 0.1 N NaOH were used for pH adjustment. In all cases the beads were left to stand for 10 minutes. Beads were then collected and metal ion concentrations eluted by thiourea were determined by atomic absorption spectrophotometer.

An identically similar procedure was carried out to determine the optimum time of metal ions elution. In this case, the metal-laden beads were transferred to conical flasks, each containing 50 ml of 0.1M thiourea at pH 3.0, which was found to be optimum for the metal ion elution. Flasks were left to stand for 1, 2, 5 and 10 minutes. Beads were then collected and metal ion concentrations eluted by thiourea were determined by atomic absorption spectrophotometer.

9. Bioremoval cycles from experimental standards.

Metal test solutions were prepared separately and in a mixture. The initial concentration of each Ni(Π), Cd(Π), Pb(Π) was 10 mgl^{-1}. Due to its potential toxicity , Hg(Π) was tested only at 1.0 mgl^{-1} concentration level. The metal ion mixture was composed of 3.0 mgl^{-1} Ni(Π), 3.0 mgl^{-1} Cd(Π), 3.0 mgl^{-1} Pb(Π) and 1.0 mgl^{-1} Hg(Π).

Metal ion bioremoval efficiency of individual beads of each test alga and composite beads ; containing a mixture of all test algae, was assessed through a number of bioremoval cycles .

Each cycle involved:

(1) Algal beads were soaked in metal test solution for 15 minutes.

(2) Beads were then collected, placed in a conical flask containing 0.1 M acidic thiourea (pH 3.0) and left for 5 minutes.

(3) Beads were then transferred to conical flask containing MBL medium, and left to stand for approximately 30 minutes.

(4) Steps 2 and 3 were successively repeated three times, to ensure complete elution.

(5) Finally, beads were collected, washed thoroughly with MBL medium and transferred to the original test solution at which they were presoaked.

(6) The steps from 1 to 4 were identically carried out as mentioned before.

Steps from 1 to 6 represent a single bioremoval cycle.

The whole procedure was identically repeated for five times.

Algal beads were collected at the end of each cycle, thoroughly washed with MBL medium, transferred to a conical flask containing the growth media and left overnight under conditions favorable for algal growth. The second bioremoval cycle was carried out in the next day.

Bleaching of algal beads from a dark green to a faint yellowish coloration was noticed after five experimental standard bioremoval cycles. Therefore, no metal ion bioremoval was assessed beyond .

10. Metal ions bioremoval from complex industrial effluents.

The efficiency of test algae to remove metal ions from complex industrial wastewaters was further investigated. Four industrial effluents were chosen for this purpose as the most toxic ones based on previous work by the authors (Ibrahim *et al.*, 2000 and Azab *et al.*, 2004). They were sampled from different industrial installations in Egypt (Figure 4). Information about those industries and their effluents are summarized in Table 6.

10.1. Effluent sampling and preparation for analysis:

Composite effluent samples were collected, at working hours of the industries, in polyethylene containers from their outfalls. The containers were placed in an ice box and driven to laboratory at Botany Department, Faculty of Science, Mansoura University .

Upon arrival, 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.

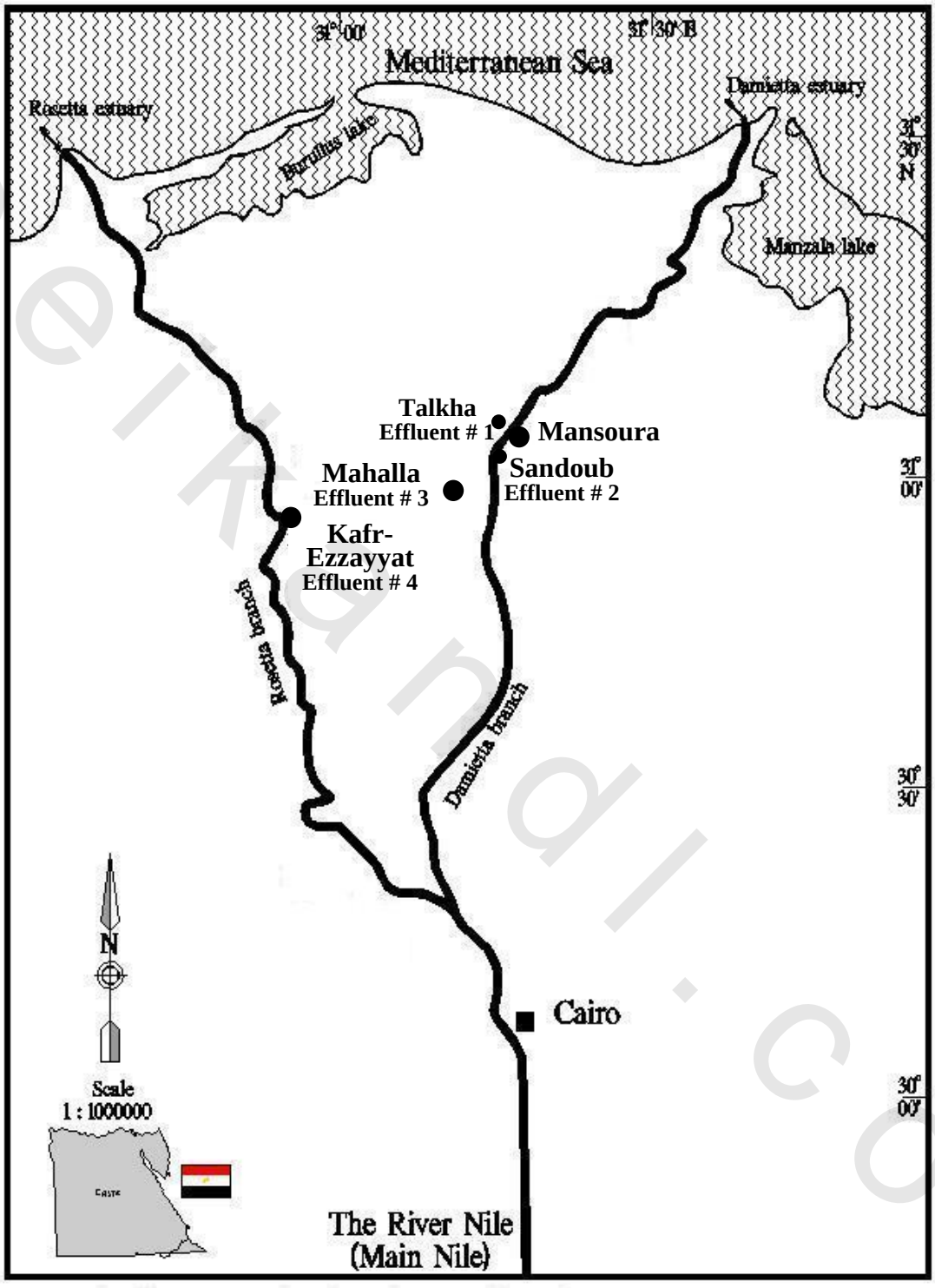

Figure 4: A map showing the sampling sites of the industrial effluents**.**

Table 6: Investigated industries, their geographical locations and effluents**.**

Factory name	Location	Effluent code	Type of industry	Remarks
Talkha chemical fertilizers factory	2 km West El-Mansoura (Talkha)	Effluent #1	Chemical fertilizers	This factory has multiple outfalls discharging the effluents to a main stabilization canal at the beginning of which a composite sample was collected
Sandoub oils and soap factory	4 km South El-Mansoura (Sandoub)	Effluent #2	Detergents	This factory has one outfall discharging to an agricultural drain
Mahalla dyes factory	30 km South, West El-Mansoura (Mahalla)	Effluent #3	Textile	This factory has one outfall discharging to an agricultural drain
Kafr-Ezzayyat salt and soda factory	120 km North, West Cairo (Kafr-Ezzayyat)	Effluent #4	Chemicals	This effluent is discharged directly to Rosetta branch of the River Nile

10.2. Chemical analysis.

Effluent pH values were measured using pH-meter (Hanna Instrument, 8519, Italy). Analysis of the heavy metal ions Ni(II), Cd(II), Pb(II) and Hg(II) were carried out using atomic absorption spectrophotometer according to APHA (American Public Health Association; 1985).

11. Preparation of batteries of alginate immobilized algae and flat algal filters:

11.1. Alginate immobilized algal battery.

Ten days old cultures of the four algal species (*Spirulina platensis, Chlorella ellipsoida, Scenedesmus quadricauda var. longispina* and *Nitzschia palea*) were used for preparing the algal beads. Aliquots of different cultures with equal cell density (1.25×10^{6})were mixed and transferred to a conical flask, where 3% alginate emulsion was added on a ratio of 1:1 by volume. Algal immobilization was carried out as outlined in Figure 1. The obtained composite beads were packed onto a glass column (Figure 5). The glass column with algal beads inside, represents the algal battery or bioreactor used to investigate the efficiency of test algae in mixture to remove heavy metal ions from the complex industrial effluent.

11.2. Algal immobilization on flat filters.

Physically rigid supports including cotton and sponge substrata were used to develop flat algal biofilters. Pieces of 0.5 cm thick cotton or sponge were placed on bottom of white plastic containers with the dimensions; 17 cm long,

12 cm wide and 10 cm depth (Figure 6). A mixture of the four test algae (*Spirulina platensis, Chlorella ellipsoida, Scenedesmus quadricauda var. longispina* and *Nitzschia palea*) was prepared. The total initial algal cell density was 5 x 10^{6} cells/ml with 25% contribution of each test alga. The mixture was shaken for one minute and then added to the plastic boxes until the cotton and sponge become fully saturated. In this case, cotton and sponge represent beds for the development of flat algal biofilters.

The containers were incubated in a culture room at 25±1 °C and continuous illumination produced by cool white fluorescent tubes giving an intensity of approximately 3700 Lux. The light intensity was adjusted by Luxmeter (LX-101 Lux meter, USA).

Flat-algal filters were daily watered with MBL and Spirulina media in a ratio of 1:1 by volume (Tables 1 and 2) until a thick algal mat was developed. The thick algal mat along with its substratum is considered a mature algal biofilter.

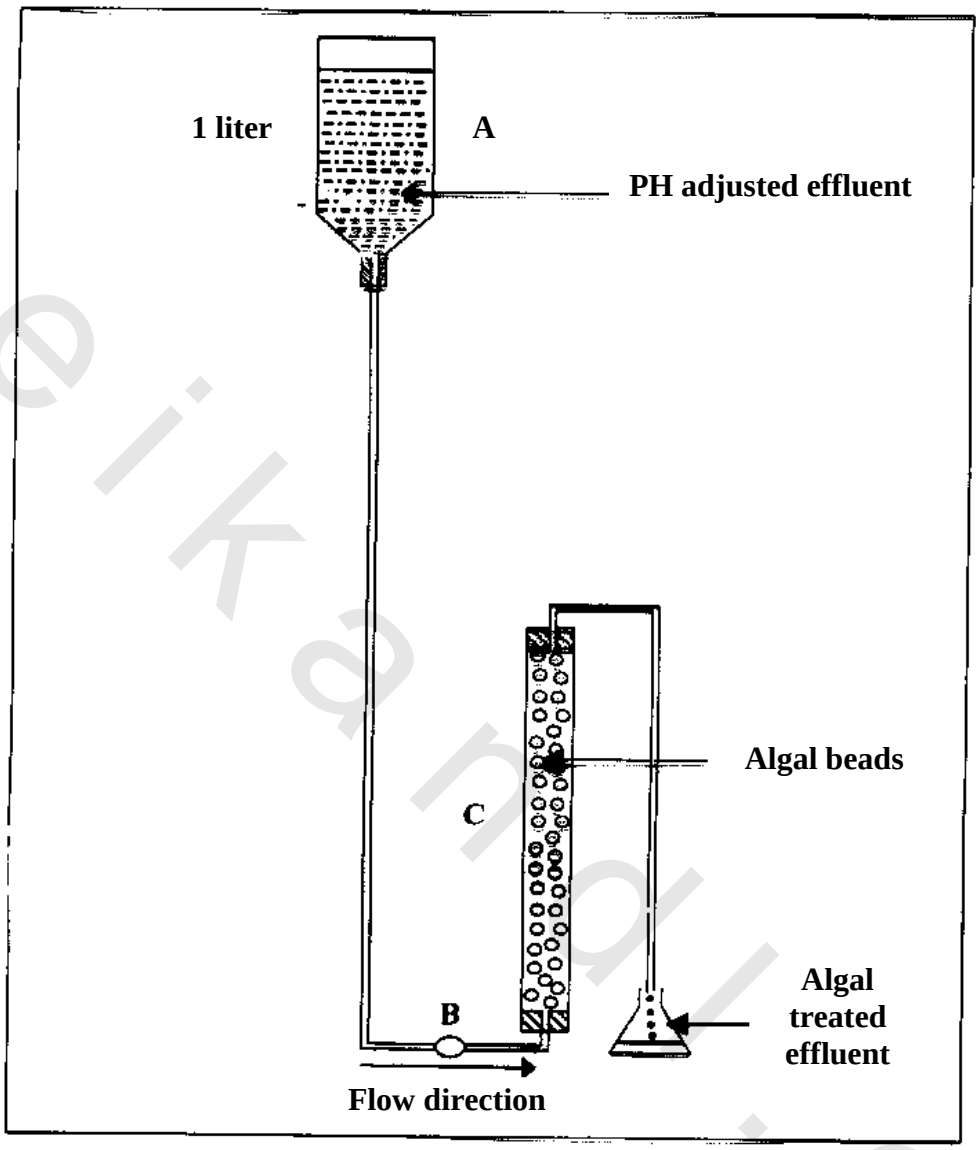

Figure 5: Immobilized algal battery

A-Effluent reservoir: (1.0 liter capacity) used for pH adjusted effluent.

B- Valve: to control the flow rate.

C- Glass column: (56 cm long, 3 cm wide) used for packing algal beads.

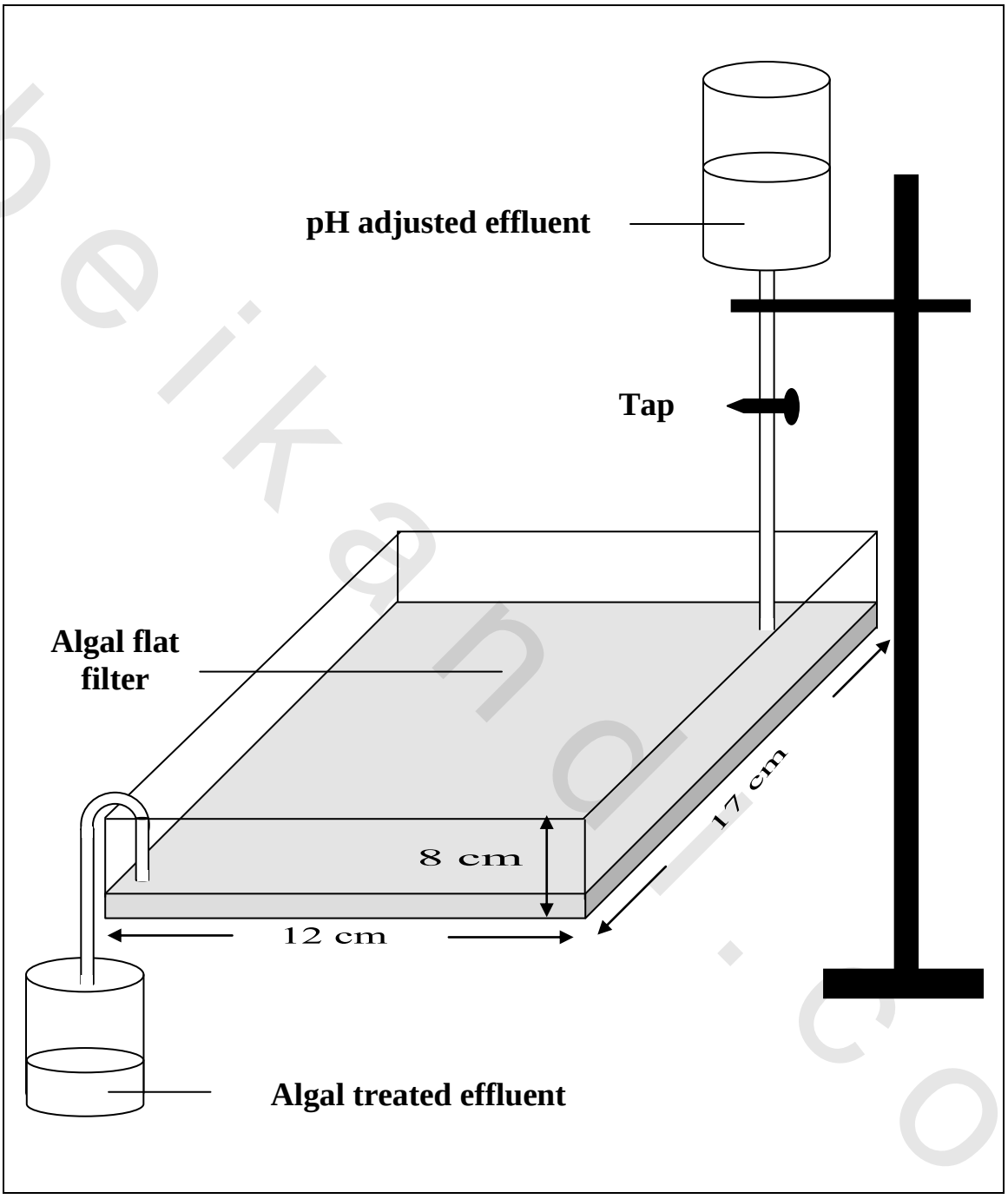

Figure 6: Algal flat filter.

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 on vials containing 10 ml of algal preservative solution, 1.0% (v/v) Lugol's solution (200 ml water, 10 g Iodine, 200 g Potassium Iodide and 20 ml Glacial Acetic Acid; Prescott, 1978). The algal mats were then sonicated with Ultrasonic Homogenizer (Model:cp100, USA) at 20 KHz for 15-60 second (Cronberg, 1982). The time needed for sonication has to be determined exactly, since too long treatment could destroy the cells. Cell count was carried out using a standard haemocytometer.

12. Bioremoval-elution cycles of heavy metal ions from industrial effluents.

Based on the experimental results, pH 6.0 was found to be optimum for algal metal ion bioremoval by the immobilized test algae. Consequently, the hydrogen ion activity of the four GF/C filtered industrial effluents (Table 6) were adjusted at the pH 6.0 using 0.1N HCl and 0.1N NaOH and checked by pH-meter.

Testing the capabilities of algal batteries and flat algal filters were proceeded as follows;

(1) 200 ml of each industrial effluent was added separately to glass columns, each contains 100 ml composite beads. Equal effluent volumes were also added to plastic boxes each contains 100 cm^3 of each mature flat algal filter (sponge and cotton). Beads, filters and effluents were left in contact for 15 minutes, the time previously calculated to be the optimum for metal ion bioremoval.

(2) After 15 minutes the biologically treated effluents were drained off to clean dry glass vessels .

(3) 100 ml 0.1M thiourea at pH 3.0 were transferred to each algal battery and filter for metal ion elution. Elution time was adjusted at 5 minutes in all cases.

(4) Algal batteries and filters were then thoroughly washed using MBL medium. Equal volumes (100 ml) of this medium were poured to each battery and filter and left for 30 minutes. The medium was then discharged.

(5) The biologically treated effluents (Step 2) were transferred to their respective batteries and filters.

(6) Steps from 1 to 4 were identically repeated.

(7) Steps from 1 to 6 were considered as one cycle with two similar runs for metal ion bioremoval from industrial effluents.

(8) The metal ion removal cycle was repeated 10 times for the same algal battery and algal filter. However, each new cycle begins with the original effluent sample (200 ml GF/C filtrated effluent with pH 6.0)

Samples of the biologically treated effluents from cycles 1, 4, 7 and 10 were subjected for metal ion analysis [Ni(Π), Cd(Π), Pb(Π) and Hg(Π)].

13. Toxicity assessment of untreated and treated industrial effluents using a standard algal biotest.

The effluents were first filtrated through GF/C filters. The toxicity of two subsamples of each filtrated effluent was assessed using *Pseudokirchneriella subcapitata* NIVA-CHL1. The pH of one subsample was adjusted at pH 6.0. The methods described by Porcella, 1983; Peltier and Weber, 1985 and Greene *et al*., 1989 were adopted to carry out toxicity testing. The basic experimental design is given in Table 7.

Table 7: Composition of test solutions for the toxicity assessment of industrial effluents.

ml effluent	**0** [a]	**0.01**	**0.1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
ml distilled water	**10**	**9.99**	**9.9**	**9**	**8**	**7**	**6**	**5**	**4**	**3**	**2**	**1**	**0**
ml algal inoculum suspended in double strength AAM medium	**1** [b]	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**

[a] Control

[b] AAM nutrient medium (Miller *et al*., 1978). Twenty percent of this medium is sufficient to insure adequate algal nutrient during the test duration (Porcella, 1983).

Three replicates clean culture flasks (30 ml) were used for each effluent concentration. A volume of 11 ml of the test solution was added to each flask. Test flasks were inoculated with a five day old culture of *Pseudokirchneriella subcapitata* NIVA-CHL1 giving $5x10^6$ cells l^{-1} as initial algal density. Flasks were incubated for five days at 25 ± 1 °C under continuous illumination (approximately 3700 Lux adjusted by Luxmeter 101, USA) provided by cool white fluorescent tubes. Cell count was determined at the end of incubation period using a standard haemocytometer.

The above procedure was identically applied to assess the toxicity of the biologically treated effluents released from the bioremoval cycles 1, 4, 7 and 10.

Calculation of EC_{50}, SC_{20} and % toxicity reduction.

EC_{50} and SC_{20} express the minimum effluent concentrations inhibiting and stimulating the algal growth by 50% and 20% respectively. Values of the cell count were plotted (as relative percents of their controls, control = 100 %) against the corresponding effluent concentration. This allowed the calculation of EC_{50} and SC_{20} by the straight-line graphical interpolation method (Walsh *et al*., 1987). % toxicity reduction was calculated according to the following equation (Abdel-Hamid *et al.*, 1994).

% Toxicity reduction = (EC_{50} of biologically treated effluent - EC_{50} of pH adjusted effluent) / EC_{50} of pH adjusted effluent x 100.

14. Effects of industrial effluents on the viability of test algae grown within filters and alginate matrices.

The effect of two concentration levels 50% and 100% of each effluent at pH 6.0 on growth of the individual algal species growing in a mixture within algal beads and biofilters was investigated. The selection of this concentration levels was based on the toxicity values (EC_{50}) calculated for the different effluents. This was carried out as follows:

(1) Raw effluent samples were filtrated through GF/C filter. Two concentration levels 50% and 100% were prepared. Dilution was done with glass distilled water. The pH of the effluent test solutions adjusted at pH 6.0.

(2) Mature algal mats were immersed in 50% and 100% effluent samples and were incubated for two days at conditions favorable for algal growth. Control algal filters were immersed in respective algal growth media and incubated at similar growth conditions. Typical procedure was applied to algal beads.

(3) After two days effluents were drained off and a volume of 1 cm^3 was taken from each filter for algal analysis. Similarly, 15 algal-alginate beads (=1.0 ml) were picked up and dissolved in 1% sodium hexametaphosphate for qualitative and quantitative algal analysis. Algal filters and beads were then resoaked in new 50% and 100% effluent test solution (pH 6).

(4) The step 3 was repeated four times at two days interval.

Calculation of growth rate (OECD, 2002).

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

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

where:

N_0 = Cell number at the beginning of the test (t_o)

N_n = Cell number at the t_n time (days)

The percentage inhibition of specific growth rate (% Iμt) at each test effluent concentration was calculated according to the formula

$$\% \, I\mu t = (\mu c - \mu t) / \mu c \times 100$$

where:

μc = Mean control specific growth rate.

μt = Mean specific growth rate for the effluent concentration (50% and 100%).

The percentage stimulation of specific growth rate (% Sμt) at each effluent concentration was calculated according to the formula.

$$\% S\mu t = (\mu t - \mu c) / \mu t x 100$$

where:

μc = Mean control specific growth rate

μt = Mean specific growth rate for the effluent concentration (50% and 100%).

15. Statistical analyses.

Statistical analyses for all results were applied according to t-test and ANOVA analysis.