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POTENTIALITY OF *CHROOCOCCUS MINUTES* (CYANOPHYTA) AND *CHLORELLA VULGARIS* (CHLOROPHYTA) ISOLATED FROM MANZALA LAKE FOR BIOREMEDIATION OF CADMIUM AND LEAD

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Abstract

There are multiple benefits and uses of micro-algae of different species especially their role in the bioremediation of heavy metals. This study which be occurred in phyto-lab. of Limnology Department, Central Laboratory for Aquaculture Research (CLAR), Abbassa, Abou-Hammad, Sharkia, Egypt, aimed to explain the role of two wild algal species; *Chlorella vulgaris* as green alga and *Chroococcus minutus* as Cyanobacteria isolating from El-Ginka location in Manzala lake for bioremediation of heavy metals Cd^{+2} and Pb^{+2} from aqueous solutions through exposing of both algae to different concentrations of Cd^{+2} and Pb^{+2}. Optical density and chlorophyll "a" as indicators of growth rate were measured every three days in all concentrations. In case of *Chroococcus*; it was noticed that the removal percentage of Cd reaches to 54.43 and 48.05% in the two higher concentrations 0.4 and 1.0 ppm, which exposed to the alga till the last day from the experiment. While removal percentage of lead (51.44 and 38.98%) was recorded in the 12^{th} day of the experiment for 10 and 20 ppm and decrease to 30.98 and 29.43% for two concentration; 30 and 40 ppm, respectively. The reduction in the amount of Cd^{+2} by *Chlorella* after 12 days of culturing was 53.26%, 78.33%, 85.87% and 87.95% for 2.0, 4.0, 6.0 and 8.0 ppm; respectively. *Chlorella* could also remove Pb^{+2} 48.23%, 67.08%, 72.94 and 84.98 for 3, 6, 12 and 18ppm, respectively after 12 days from the medium. This means that the heavy metal uptake ability of *Chlorella* can be exploited for metal detoxification and environmental clean-up operations. This study provides a deep insight for exploring potential of

using algal species isolated from polluted sites for bioremediation of heavy metals.

Keywords: Bioremediation, heavy metals, green alga, Cyanobacteria, *Chlorella*, *Chroococcus*.

INTRODUCTION

Pollution of aquatic environments by toxic heavy metals has been taking place because of discharge of untreated effluents from many industrial processes. The environmental injuries brought about by such effluents have received major attention by national and international authorities, and consequently led to directives and regulations aimed at minimizing their impact. In attempts to remove (or, at least, reduce the concentration of) those toxic metals, distinct types of microbial biomass have been scrutinized as alternatives to conventional physicochemical technologies (Vilar *et al*.; 2008); the latter are in fact characterized by a limited effectiveness, and are typically too expensive when the target metal concentration is at the ppm level or below (Fraile *et al*.; 2005). One of the most perspective methods of biological cleaning of the water environment from ions of heavy metals in the polluted ecosystem is introduction of active strains of the microorganisms possessing high cumulative ability (Muhaemin, 2004). The success of use of this method is provided with the correct selection of the optimum micro-organisms bioaccumulators, being characterized high metal-accumulation ability. It is known that active bioaccumulators of ions of heavy metals are microalgae. So, it is shown that such heavy metals as Cu, Pb, Cd, are collected by green and cyanobacterium algae at insignificant their small contents in a reservoir. Lead, as a heavy metal; particularly has become a cosmopolitan environmental pollutant (Sharma and Dubey, 2005). Cadmium, one of the most toxic heavy metals commonly found in contaminated ecosystems, is frequently considered as a nonessential element for living organisms (Tukaj *et al*., 2007).

Bioremediation typically provides an efficient and economical way to reduce environmental toxins using indigenous or introduced microbes that

naturally degrade contaminants. The major advantage of bioremediation is that it is a natural process and can be used at much lower cost than many other treatment technologies. The decrease in growth have also been described for various cyanobacterial strains exposed to abiotic stresses including heavy metals Al-Enazi (2017) and Alharbi (2017) who examined toxicity and bioaccumulation of Lead and Cadmium heavy metals on *Chroococcus minutes* through different concentrations ranged from15 to 50 ppm for Pb^{+2} and from 0.1 to 1.5 ppm for Cd^{+2} and found that Lead was the most hazardous chemical to the tested microalgae, followed by cadmium. The inhibitory effects of the used heavy metals depend on the used concentration. The uptake of an element from the surrounding medium is seldom exactly proportional to the amount present in the medium.

Dewi and Nuravivah (2018) determine the ability of *Chlorella vulgaris* in absorbing Pb (lead) and the effect of the variation of Pb metal concentration ranged from 5 to 15 ppm on the growth of *Chlorella vulgaris* and found that the analysis of the content of Pb in the F test shown that the difference in concentration of water Pb given real influence on the ability of *Chlorella vulgaris* in absorbing Pb and growth. Various studies have been carried out to show the role of algae in the bioremediation of heavy metals. The accumulation of cadmium was studied in an experimental aquatic of the phytoplankton *Chlorella vugaris* as a primary producer (Ruangsoboon and Wongrat, 2006). Oghenemise and Medina (2011) was studies on the bioaccumulation of zinc, iorn, copper, cadmium and aluminium by *Chlorella vugaris*, *Phacus curvicauda*, *Euglena acus* and *Oscillatoria bornettia* for four weeks in the laboratory. There are many species of blue green algae have ability for bioremediation as a bio-accumulator of heavy metal ions as *Chroococcus* sp that has high removal percentage of cadmium and Nickel (Al-Mayaly *et al.*, 2012). The aim of the present study was to evaluating the efficiency of using of some algae to remove heavy metals from sewage and agricultural water.

MATERIALS AND METHODS

Micro-algae organisms and culturing Medium:

In the present experiment, we used some microalgae isolated from the polluted regions from Manzala Lake. The tested microalgae *Chroococcus minutes* (as blue green algae) and *Chlorella vulgaris* (as green algae) were isolated from a heavy metal contaminated region of Manzala Lake "El-Ginka", where Pb and Cd appear as a major contaminants, so their intrinsic capacity for metal uptake was expected to be high enough for eventual bioremediation on larger scale. In Phyto-lab of limnology department, Central Laboratory for aquaculture Research (CLAR), Abbassa, Abou-hammad, Sharkia, Egypt; isolation and purification were done by dilution culture technique (Venkataraman, 1969). Both algal strains grown on BG-11 Medium (Rippka *et al*., 1979) which consists of the following components in g L^{-1} of distilled water: 1.5 $NaNO_3$, 0.04 $K_2HPO_4.3H_2O$, 0.075 $MgSO_4.7H_2O$, 0.02 Na_2CO_3, 0.036 $CaCl_2.7H_2O$, 0.002 EDTA, 0.006 Citric acid, 0.006 Ferric ammonium citrate with addition of 1 ml from trace metal solution for liter; having the following composition in g L^{-1} of distilled water: 2.86 H_3BO_3, 1.81$MnCl_2$, 0.222 $ZnSO_4.7H_2O$, 0.39 $NaMoO_4.2H_2O$, 0.079 $CuSO_4.5H_2O$ and 0.0494 CO $(NO_3).6H_2O$.After shaking and stirring, adjusted the pHof the medium into 7 ± 0.1.

Cd^{+2} and Pb^{+2} stock solution:

Analytical grades reagents were used for heavy metals solution. Preparation of stock metal solutions for both heavy metals (Cd^{+2} and Pb^{+2}) of 1000 ppm was occurred by dissolving 1.63 g of cadmium chloride $CdCl_2$, and 1.598 g of lead nitrate $Pb(NO_3)_2$ in 1,000 mL of distilled water individually.Test solutions of desired concentration were obtained by dilution of the stock solutions according to Dinesh Kumar *et al.* (2013).

Design of experiment:

To observe the bioremediation role of *Chroococcus minutus* and *Chlorella vulgaris* on Cd^{+2} and Pb^{+2}; A series of 250 ml of Erlenmeyer flasks containing of 100 mL of sterilized media was inoculated with a pure culture of the both organisms separately with certain cell density of the algal suspension with exponential growth phase harvested from the stock cultures.It was added about 50ml of axenic culture to1000 ml of the medium containing heavy metal ions. Algal media were exposed to different concentrations of both metals cadmium and lead individually as shown in Table (1) and Photo (1). In addition to algae cultured in the BG-11 medium without heavy metals served as controls.

Photo 1. Design of experiment showing the effect of different concentrations of Pb^{+2} and Cd^{+2} on both algae.

Temperature, pH, salinity, and light intensity were maintained to be stable. Each treatment was performed in triplicate. Inoculated flasks were incubated for 12 days for cyanobacterium and chlorophyta algae under

illuminated light cycle of 12:12. The cultures were hand shaken once or twice a day to avoid sticking.

Table 1. Different concentrations of two heavy metals (Pb^{+2}&Cd^{+2}) using for showing the bioremediation rate of two both algae.

Metal ion	*Chroococcus minutes*					*Chlorella vulgaris*				
	Concentrations used (ppm)					Concentrations used (ppm)				
Cd^{+2}	0.0	0.1	0.2	0.4	1.0	0.0	2.0	4.0	6.0	8.0
Pb^{+2}	0.0	10.0	20.0	30.0	40.0	0.0	3.0	6.0	12	18

Growth Monitoring:

1. Optical density.

A spectrophotometer was used to measure the optical density at 650 nm (OD650 nm) to express biomass and initial OD650 nm. (Muhaemin, 2004 and Wetherell, 1961).

2. Chlorophyll a.

The sample of microalga suspension was centrifuged for 10 minutes at 6000 rpm. The supernatants were discarded and the chlorophyll "a" content in the biomass was extracted using the standard acetone extraction method described in **APHA (1999)**. After extraction; for the spectrophotometric determination of chlorophyll, the absorbance of light green supernatant was measured at two wavelengths, 665 (A_{665}) and 750 nm (A_{750}), using the UV/Visible spectrophotometer (model Thermo, Electro Corporation, Nicollet evolution 100). The spectrophotometer was blanked with acetone. The chlorophyll content of the sample was calculated using the following formula**:**

$$Chlorophyll\ a = \frac{11.9 * (A665 - A750) * VAcet.}{Vsample * l} \quad (mg/L)$$

Where: $V_{Acet.}$is the acetone volume (mL), V_{sample} is the sample volume (mL) and *l* is the width of cuvette (cm)

Remediating rate for heavy metals.

At the end of experiment 10 mL sample was taken and centrifuged at 5000 rpm for 15 minutes then the supernatant was filtered. The filtrate was analyzed for residual metal in the solution by using atomic absorption spectrophotometer (Model Thermo Electron Corporation, S. Series AA Spectrometer with Gravities furnace, UK). The removal rate of heavy metal ions was calculated from the following formula:

$$R= \left(\frac{Ci- Cf}{Ci}\right)*100$$

Where: R Removal percentage of metal (%), C_i The initial concentration of metal in the solution, C_f The equilibrium (final) concentration of metal in the solution after incubation.

Statistical analysis:

For comparison of means, two ways ANOVA test and post hoc Duncan test were used. Results of the test were considered significant if the calculated *P* values were ≤0.05. All statistics were run on the computer using the SAS program (SAS, 2000).

RESULTS AND DISCUSSION

It seems that the microalgae species showed different respond to heavy metals. Further research has to be developed to determine the percentage of heavy metal that accumulated on the microalgae. In this study, *C. minutes* grow over a wide concentration range of Pb^{2+}(0- 40 mg L^{-1}) and this appears through effect of optical density values; since, there is a stimulation effect of lead on growth specially in third day of incubation even than control Fig. (1; A). But with increasing of Pb^{2+} concentration resulted in a significant decrease ($p<0.0^{\circ}$) in the growth of *C. minutes* over time Table (2). Progressive inhibition of cell growth was observed at a concentration of 30-40 ppm Pb^{2+}; since, values of optical density in both concentrations were 0.288±0.021 and 0.263±0.012 for 30 and 40ppm, respectively; after 12 days of incubation

The effects of different concentrations of Pb^{+2} on chlorophyll a contents of *C. minutus* are shown in Table (2) and Fig. (1; B).The concentration of chlorophyll-a varied according to Pb^{2+} concentrations since, there were significant decreases ($P \leq 0.05$) of chlorophyll a contents in the microalga when the metal concentration was increased. The lowest chlorophyll accountants were found in case of exposed to 40 ppm of Pb^{+2}. According to Fig. (1; B), it is appeared that the inhibition effect of Pb^{+2} concentrations on the chlorophyll a contents of *C. minutus* is in the following arrangement: 40>30> 20>10 ppm. *C. minutus* showed a large capacity to tolerate Pb^{2+}. Pb^{2+} does not have redox capacity, but it can cause oxidative stress indirectly, may be the reason for the growth and tolerance of the algal cells over the different concentrations. The reduced growth shown by *Chroococcus minutus* at higher concentrations may be due to some alteration in the uptake of magnesium and iron by Pb^{2+}, resulting in an inhibition of chlorophyll synthesis (Nacorda *et al*., 2007 and Miranda *et al.*, 2013).Also, it was found that the decline in chlorophyll content might be caused by increasing chlorophylls activity, by disorders of chloroplast membrane and by inactivation of electron transport in photosystem I.

The effects of Cd^{+2} on *Chroococcus minutus* indicate that metal depresses the growth rate that is reflected on optical density and chlorophyll a Table (3) and Fig. 3 (A and B). Data shows the effects of Cd by using different concentrations (0.1, 0.2, 0.4and 1.0 ppm.) on *Chroococcus minutus* stimulate the growth by increasing in optical density especially after 3 days in comparison by control; but with the end of incubation period, the growth rate decreased. When the dose of metal increased the alga growth decreased.

Table 2. The effect of different concentrations of Pb^{2+}(ppm) on optical density and chlorophyll a contents of *Chroococcus minutus* during incubation period 12 days.

Pb^{+2} Conc. (ppm)	*Chroococcus* growth parameters									
	Optical density (O.D)					Chlorophyll *a* (mg L^{-1})				
	0(day)	3rd	6th	9th	12th	0(day)	3rd	6th	9th	12th
0.0	0.053 ± 0.0037^{Da}	0.079 ± 0.0058^{Db}	0.267 ± 0.0197^{Ca}	0.524 ± 0.0386^{Ba}	0.828 ± 0.0613^{Aa}	1.75 ± 0.13^{Ea}	3.16 ± 0.235^{Dab}	5.67 ± 0.421^{Ca}	9.65 ± 0.713^{Ba}	13.04 ± 0.964^{Aa}
10	0.053 ± 0.0037^{Da}	0.104 ± 0.0078^{Ca}	0.137 ± 0.0101^{Cb}	0.286 ± 0.0214^{Bb}	0.413 ± 0.0308^{Ab}	1.75 ± 0.13^{Ea}	3.74 ± 0.276^{Da}	4.70 ± 0.347^{Ca}	6.59 ± 0.487^{Bb}	9.08 ± 0.671^{Ab}
20	0.053 ± 0.0037^{Da}	0.073 ± 0.0052^{Db}	0.118 ± 0.0087^{Cb}	0.205 ± 0.0151^{Bc}	0.295 ± 0.0221^{Ac}	1.75 ± 0.13^{Ea}	2.92 ± 0.217^{Db}	4.62 ± 0.341^{Cab}	5.22 ± 0.385^{Bbc}	6.71 ± 0.496^{Ac}
30	0.053 ± 0.0037^{Da}	0.064 ± 0.0049^{Db}	0.108 ± 0.0078^{Cb}	0.191 ± 0.0142^{Bc}	0.288 ± 0.0212^{Ac}	1.75 ± 0.13^{Ea}	2.42 ± 0.183^{Db}	3.27 ± 0.242^{Cc}	4.65 ± 0.343^{Bc}	5.32 ± 0.393^{Acd}
40	0.053 ± 0.0037^{Da}	0.063 ± 0.003^{Db}	0.106 ± 0.005^{Cb}	0.158 ± 0.0119^{Bc}	0.263 ± 0.0125^{Ac}	1.75 ± 0.13^{Da}	2.78 ± 0.135^{Cb}	3.58 ± 0.265^{Bbc}	3.87 ± 0.286^{Bc}	4.59 ± 0.587^{Ad}

a-d Numbers with different superscript letters in the same column differ significantly ($p<0.05$).
A, B, C and D Values-having different script at the same are significantly ($P<0.05$) different row.

Table 3. The effect of different concentrations of Cd^{+2} (ppm) on optical density and chlorophyll a contents of *Chroococcus minutus* during incubation period 12 days.

Cd^{+2} Conc. (ppm)	*Chroococcus* growth parameters									
	Optical density (O.D)					Chlorophyll *a* (mg L^{-1})				
	0(day)	3rd	6th	9th	12th	0(day)	3rd	6th	9th	12th
0.0	0.041 ± 0.003^{Ea}	0.087 ± 0.0066^{Db}	0.361 ± 0.026^{Ca}	0.575 ± 0.0424^{Ba}	0.783 ± 0.0576^{Aa}	1.44 ± 0.106^{Da}	2.91 ± 0.215^{Db}	6.804 ± 0.503^{Ca}	10.59 ± 0.783^{Ba}	12.32 ± 0.910^{Aa}
0.1	0.041 ± 0.003^{Da}	0.13 ± 0.0095^{Ca}	0.127 ± 0.009^{Cb}	0.239 ± 0.0177^{Bb}	0.453 ± 0.033^{Ab}	1.44 ± 0.106^{Da}	4.14 ± 0.306^{Ca}	3.87 ± 0.286^{Cb}	6.01 ± 0.443^{Bb}	9.65 ± 0.712^{Ab}
0.2	0.041 ± 0.003^{Ca}	0.057 ± 0.0043^{Cc}	0.114 ± 0.008^{Bb}	0.154 ± 0.0116^{Bc}	0.29 ± 0.0215^{Ac}	1.44 ± 0.106^{Ea}	2.46 ± 0.183^{Db}	3.28 ± 0.243^{Cb}	4.406 ± 0.326^{Bc}	5.73 ± 0.423^{Ac}
0.4	0.041 ± 0.003^{Ba}	0.034 ± 0.0026^{Bd}	0.037 ± 0.003^{Bc}	0.045 ± 0.003^{Bd}	0.077 ± 0.0058^{Ad}	1.44 ± 0.106^{Ba}	1.19 ± 0.088^{Cc}	1.36 ± 0.1^{Bc}	1.583 ± 0.117^{Bd}	2.71 ± 0.2^{Ad}
1.0	0.041 ± 0.003^{Ba}	0.028 ± 0.001^{Cd}	0.032 ± 0.0015^{Cc}	0.047 ± 0.002^{Bd}	0.081 ± 0.006^{Ad}	1.44 ± 0.106^{Ba}	0.929 ± 0.068^{Cc}	1.14 ± 0.054^{Cc}	1.648 ± 0.078^{Bd}	3.06 ± 0.145^{Ad}

a-d Numbers with different superscript letters in the same column differ significantly ($p<0.05$).
A, B, C and D Values-having different script at the same are significantly ($P<0.05$) different row.

The results recorded similar to inhibitory effects of heavy metal Cd^{+2} on chl *a* content noticed in investigations particularly at higher doses. Data in Table (3) showed thegrowth rate by measuring the content of chlorophyll a of *Chroococcus minutus*, after 3 days was relatively high in treatment Cd^{+2} 0.1ppm in comparison with the other doses used, but after that the results obtained were inhibitorier with all doses used. This result agree with general assumption thus this measurecan be used as indicator of stress, as observed by Al-Mayaly *et al* (2012) who found Cd had slight inhibitory effects on algal growth at low concentration (0.05 mg/L), while it severely inhibited algal growth at higher concentrations (>1.0 mg/L) causing a decrease of the cellular volume, the growth rate and of the level of photosynthetic pigments. Based on growth data Table (4); Fig. 3; (A & B), a decrease in the chlorophyll content and optical density of algal cells was observed when cells were grown in culture media containing Pb^{+2}. Pb^{+2} have reduced the growth of cells when metal concentration was increased. The highest one was in the treatment of control and the lowest in 18 ppm at the end of incubation period; 1.405±0.1 and 1.021±0.048, respectively. An increase was occurred through third day at Pb^{+2}3ppm than control, this means that at certain concentrations of Pb^{+2} is required.

According to Hala *et al.,* (2012) microalgae generally have a protective mechanism against toxic metals to keep to his own life. If so high metal concentrations, accumulation may inhibit cell growth because the organism protection system is no longer able to offset the effects of toxic metals. Decreasing the concentration of Pb is also affected due to nutritional factors and increases the number of cells in the culture medium.Higher initial accumulation of Pb may cause serious damages to the algal cell and this might be responsible for the lower tolerance of *Chlorella* to Pb^{+2} that reflected on optical density and chlorophyll content (Muhaemin, 2004). This might be due to the fact that Pb induces the activity of the enzyme peroxidase that is involved in the degradation of indoleacetic acid (IAA), the hormone which stimulates plant

growth and multiplication (Lamai *et al*., 2005). The lower *Chlorella* growth was on Pb^{+2} expose than in presence of Cd^{+2}, this may be due to differing concentrations used for both metals as well as the difference in optical density of stock culture of alga since it was 1.251 in case of lead while it was 1.789 in case of cadmium.

Table 4. The effect of different concentrations of Pb^{+2}(ppm) on optical density and chlorophyll a contents of *C. vulgaris* during incubation period 12 days

Pb^{+2} Conc. (ppm)	*Chlorella* growth parameters									
	Optical density (O.D)					Chlorophyll *a* (mg L^{-1})				
	0(day)	3rd	6th	9th	12th	0(day)	3rd	6th	9th	12th
0.0	0.079 ± 0.061^{Da}	0.276 ± 0.0209^{Cb}	0.637 ± 0.0473^{Ba}	0.808 ± 0.0598^{Ba}	1.405 ± 0.103^{Aa}	0.011 ± 0.001^{Ea}	0.381 ± 0.0282^{Dab}	1.496 ± 0.0696^{Ca}	2.163 ± 0.1085^{Ba}	3.589 ± 0.265^{Aa}
3.0	0.079 ± 0.061^{Da}	0.346 ± 0.0255^{Ca}	0.437 ± 0.0325^{Cb}	0.781 ± 0.0578^{Ba}	1.265 ± 0.0932^{Aab}	0.011 ± 0.001^{Da}	0.412 ± 0.0305^{Ca}	0.839 ± 0.0619^{Bb}	1.222 ± 0.0903^{Bb}	1.866 ± 0.137^{Ab}
6.0	0.079 ± 0.061^{Ea}	0.229 ± 0.0168^{Db}	0.428 ± 0.0316^{Cb}	0.754 ± 0.056^{Ba}	1.083 ± 0.0799^{Ab}	0.011 ± 0.001^{Ea}	0.328 ± 0.0241^{Dabc}	0.645 ± 0.0476^{Cc}	1.02 ± 0.0755^{Bbc}	1.657 ± 0.122^{Abc}
12	0.079 ± 0.061^{Da}	0.215 ± 0.0159^{Cb}	0.322 ± 0.0241^{Cb}	0.674 ± 0.0499^{Ba}	1.121 ± 0.0828^{Aab}	0.011 ± 0.001^{Ea}	0.306 ± 0.0226^{Dbc}	0.508 ± 0.0377^{Ccd}	1.01 ± 0.0755^{Bbc}	1.622 ± 0.12^{Abc}
18	0.079 ± 0.061^{Ea}	0.214 ± 0.01^{Db}	0.4 ± 0.019^{Cb}	0.624 ± 0.08^{Ba}	1.021 ± 0.048^{Ab}	0.011 ± 0.001^{Ea}	0.267 ± 0.022^{Dc}	0.401 ± 0.02^{Cd}	0.823 ± 0.039^{Bc}	1.164 ± 0.198^{Ac}

a-d Numbers with different superscript letters in the same column differ significantly ($p<0.05$).
A, B, C and D Values-having different script at the same are significantly ($P<0.05$) different row.

Optical density as a measure of growth (Table. 5). There were significant differences in the optical density of *C. vulgaris* under high Cd^{+2} concentrations and it decreased in response to increasing cadmium doses, as shown in Fig. 4, A. The inhibited growth was mainly occurred under high cadmium concentration (6 ppm and 8 ppm). Under low cadmium supplement, there was barely no inhibited even slightly promotion after 12 days, and the effect was not obvious. The result indicted that *C. vulgaris* can be well tolerated with 2–4 ppm Cd^{+2}, although the growth is inhibited under high concentration.Cd^{+2}as heavy metals had adverse effects on the growth of *C. vulgaris* (Cheng *et al*.; 2016), same result also was found in this study, the inhibited of growth is mainly under

high cadmium concentration. Effects of cadmium stress on Chl *a* of *C. vulgaris* are presented in Table (5) and Fig.4; B. Cadmium had an adverse influence on Chl *a* production by *C. vulgaris*. The Chl *a* content significantly decreased ($p<0.05$), while the Cd^{+2} concentrations was increased from 0 (control) to 8 ppm and this is in agreement with results of Cheng *et al*. (2016). Such a decrease was attributed to the disruption of thylakoid membranes by cadmium ions, resulting to the degradation of pigments, (Masojidek *et al*.; 2000).

Table 5. The effect of different concentrations of Cd^{+2}(ppm) on optical density and chlorophyll a contents of *C. vulgaris* during incubation period 12 days

Cd^{+2} Conc. (ppm)	***Chlorella* growth parameters**									
	Optical density (O.D)					**Chlorophyll *a* (mg L^{-1})**				
	0(day)	**3rd**	**6th**	**9th**	**12th**	**0(day)**	**3rd**	**6th**	**9th**	**12th**
0.0	0.138 ± 0.0104^{Da}	0.5 ± 0.064^{Ca}	0.959 ± 0.071B^{Ca}	1.187 ± 0.085^{Ba}	1.70 ± 0.13^{Aa}	0.407 ± 0.0195^{Ca}	1.28 ± 0.094^{Ba}	1.49 ± 0.11^{Ba}	1.71 ± 0.126^{Ba}	3.54 ± 0.262^{Aa}
2.0	0.138 ± 0.0104^{Ca}	0.29 ± 0.021^{Cb}	0.611 ± 0.045^{Bb}	0.833 ± 0.062^{Ab}	0.899 ± 0.067^{Ab}	0.407 ± 0.0195^{Ca}	0.429 ± 0.032^{Cb}	0.561 ± 0.042^{Cb}	0.913 ± 0.068^{Bb}	2.91 ± 0.215^{Aab}
4.0	0.138 ± 0.0104^{Ca}	0.23 ± 0.017^{Cbc}	0.488 ± 0.036^{Bb}	0.612 ± 0.045^{Bc}	0.86 ± 0.063^{Ab}	0.407 ± 0.0195^{Ca}	0.365 ± 0.014^{Cbc}	0.454 ± 0.034^{Cbc}	0.765 ± 0.0564^{Bbc}	2.71 ± 0.199^{Ab}
6.0	0.138 ± 0.0104^{Da}	0.194 ± 0.014^{Dc}	0.327 ± 0.024^{Cc}	0.512 ± 0.037^{Bcd}	0.718 ± 0.053^{Abc}	0.407 ± 0.0195^{Ca}	0.224 ± 0.032^{Dcd}	0.301 ± 0.022^{Dcd}	0.636 ± 0.0473^{Bcd}	1.52 ± 0.112^{Ac}
8.0	0.138 ± 0.0104^{Ca}	0.172 ± 0.013^{Cc}	0.274 ± 0.013^{Bc}	0.329 ± 0.015^{Bd}	0.5 ± 0.024^{Ac}	0.407 ± 0.0195^{Ba}	0.144 ± 0.094^{Cd}	0.208 ± 0.0095^{Cd}	0.422 ± 0.02^{Bd}	1.23 ± 0.058^{Ac}

a-d Numbers with different superscript letters in the same column differ significantly ($p<0.05$).
A, B, C and D Values-having different script at the same are significantly ($P<0.05$) different row.

The accumulation capabilities of *Chroococcus minutus* and *Chlorella vulgaris* algae for Cd metal was shown as Fig. 5 (a, b) and Fig 6 (a, b); respectively. The highest accumulation of Cd ion was observed by *Chroococcus minutus* was 0.48 mg/l at 1 ppm concentration for the duration of 12 days, whereas the lowest value was 0.02 mg/l at 0.1 ppm concentration for the duration of 3 days. In *Chlorella vulgaris*, highest cadmium accumulation

was 7.036 mg/l at 8 ppm concentration was observed for 12 days duration, while the lowest value was 0.266 mg/l at 3 ppm concentration for the duration of 3 days. The degree of Pb removal by both algae *Chlorella vulgaris* and *Chroococcus minutus*, under various initial Pb concentrations, for a period of 12 days, is represented in Fig. 7 (a, b) and Fig. 8 (a, b), respectively. For alga *Chlorella vulgaris*, removal increased with increasing initial metal concentration, but this wasn't occurred in case of *Chroococcus minutes.* The maximum extent of Cd removal was achieved by *Chlorella vulgaris* was 15.296 mg/l for the duration of 12 days, at 18 ppm initial concentration tested, whereas the minimum was 0.3615 mg/l at 3 ppm initial concentration for the duration of 3 days. In case of *Chroococcus minutes*, the maximum level of removal was 11.772 mg/l for the duration of 12 days, at 40 ppm initial concentration, whereas the lowest value was 1.286 mg/l at 10 ppm concentration for the duration of 3 days.

As shown in Table (6), it was cleared from the obtained results, that the removal rate of Pb^{2+} ions was increased in the range of 10 ppm in case of *Chroococcus* microalga. The efficiency of heavy metal removal depends on two reasons; lower concentrations of Pb^{2+} ions can provide a positive force which enhances the adsorption process, or the greater number of Pb^{2+} ions can lead to competition for binding sites available in the biomass (Bankar *et al*., 2009). Increasing concentrations of Pb^{2+} above 10 mg/l leading to declining in the removal percentage gradually which can be attributed to the saturation of all binding sites on the surface of the biomass of algae (Kiran *et al*., 2007). Also, it was found that *Chroococcus* sp has a higher removal percentage of Cadmium at end of the experimental period Table (6), these results may relate to blocking the functional groups in the outer membrane of bio-sorbents in the first days as well as by the partial destruction of functional groups during the immobilization process (Wilke *et al*., 2006).

Data from Table (6), shows that the highest concentrations of Pb absorbed contained on the concentration of Pb^{+2} 18 ppm (84.98±6.27), this means that the more the concentration of each metal were absorbed by the *Chlorella vulgaris*.

This is in accordance with the results of research by (Aunurohim, 2013), that an increase in metal remediation ability is directly proportional to the increase in concentration. There are many things that cause absorption mechanism of Pb by *Chlorella vulgaris* occurred in the different treatments such as the cellulose in the cell walls. Cellulose is potentially large enough to be used as a metal ion catcher because of the OH groups in its structure. The presence of the OH group causes metal ions Pb absorption mechanism. Interactions between both (the cellulose in the cell walls with Pb ion) is an extracellular detoxification mechanism or mechanisms of tolerance. Detoxification is the process of conversion of heavy metals into non-toxic form.

According (Lehniger *et al*., 1993), *Chlorella* sp, cells of phytoplankton metabolic processes can synthesize metal chelating protein fitokelatin to respond to the negative effects of heavy metals. The protein can bind to heavy metals because it has sulfhydryl groups (-SH) and will accumulate in the vacuole, through enzymatic processes. This can also reduce levels of Pb in the culture medium. In addition, the mechanism of absorption of Pb by *Chlorella vulgaris* can also be due to the alginate to the wall sel (Aunurohim, 2013).

C. vulgaris can remove and accumulate Cd^{2+} because its exterior surface contains proteins and carbohydrates that are capable of reacting with metal ions. The low removal of Cd^{2+} can also be related to the low cell density used to remove the initial metal concentration in the medium. Additionally, cadmium is not an essential element to the survival of microalgae; therefore, a higher cell density is required for a greater effectiveness of removal (Monteiro *et al*., 2011). *C. vulgaris* exhibits great efficiency to combat Cd^{+2} stresses with the maximum accumulation factor. Metal tolerance efficiency and higher uptake rates make it a significant algal species for bioremediation purpose, more specifically for Cadmium.These results revealed that *Chlorella vulgaris* is not only a tolerant species but also a hyper-accumulator of the two selected metals ($Cd^{+2}$and Pb^{+2}) and it can be used for bioremediation of even highly polluted or disturbed area (Renu *et al*., 2017 and Dewi and Nuravivah, (2018).

Table 6. Percentage of metal removal from the culture media by *Chroococcus minutus* and *Chlorella vulgaris* exposed to different concentrations (ppm) of Pb^{2+} and Cd^{2+} after incubation period 12 days.

Metal Conc. (ppm)		*Chroococcus minutus*	Metal Conc. (ppm)		*Chlorella vulgaris*
		Removal %			**Removal %**
Cd^{+2}	**0.1**	79.88±5.9[a]	**Cd^{+2}**	**2**	53.26±3.93[b]
	0.2	71.97±5.32[a]		**4**	78.33±5.78[a]
	0.4	54.43±4.02[b]		**6**	85.87±6.34[a]
	1.0	48.05±3.55[b]		**8**	87.95±6.49[a]
Pb^{+2}	**10**	51.44±3.8[a]	**Pb^{+2}**	**3**	48.23±3.67[c]
	20	38.98±2.88[b]		**6**	67.08±4.96[b]
	30	30.98±2.29[c]		**12**	72.94±5.88[b]
	40	29.43±2.17[c]		**18**	84.98±6.27[a]

a-c Numbers with different superscript letters in the same column differ significantly ($p<0.05$).

CONCLUSION

As a result of present study, *C. vulgaris* and *Chroococcus minutes* as a wild algal species were selected for metal removal of Pd and Cd those are toxic to aquatic ecosystem. Algae were proved to be a good raw material for bio-removal of heavy metals. The cell growth is slightly effected by the use of heavy metals in culture medium. An integrated process would be developed at large scale by incorporating *Chlorella vulgaris* and *Chroococcus minutes* for waste water treatment.

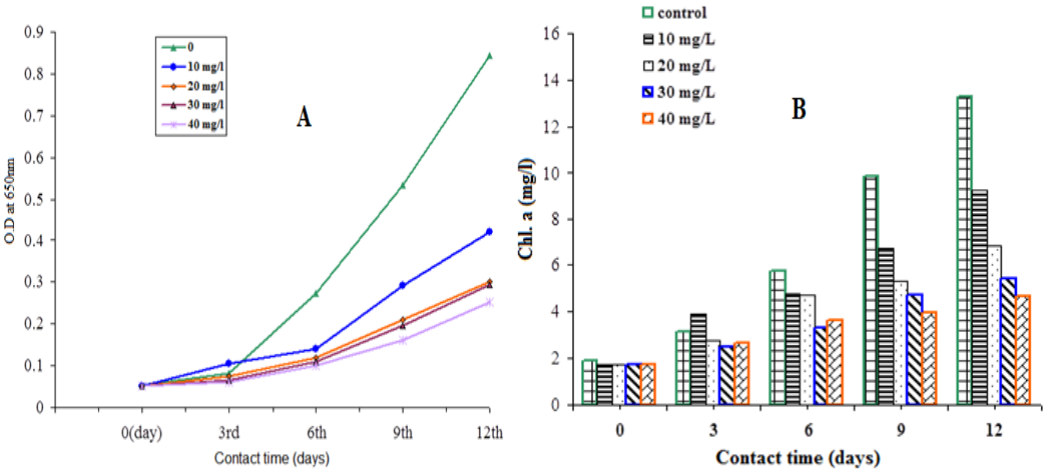

Figure 1 (A and B). Optical density (A), chlorophyll a content (B) indicating the growth of*Chroococcus* sp under different concentrations of pb^{+2}.

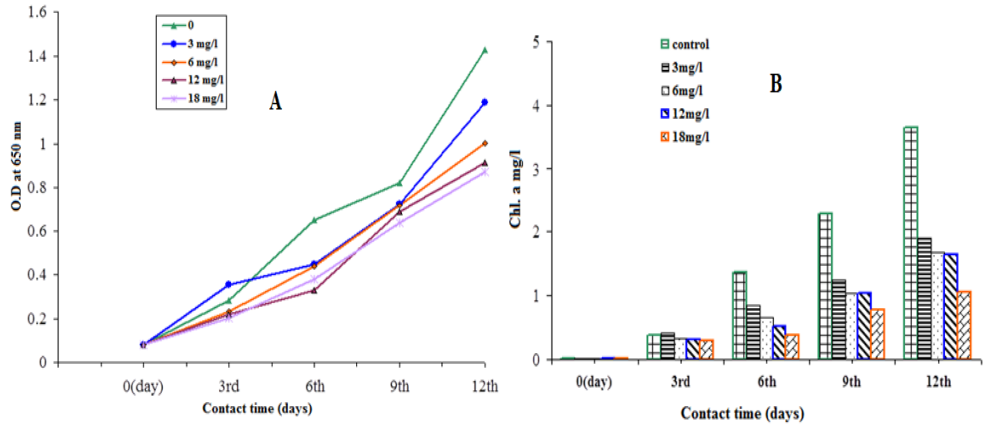

Figure 2 (A and B). Optical density (A), chlorophyll a content (B) indicating the growth of *Chlorella* sp under different concentrations of pb^{+2}.

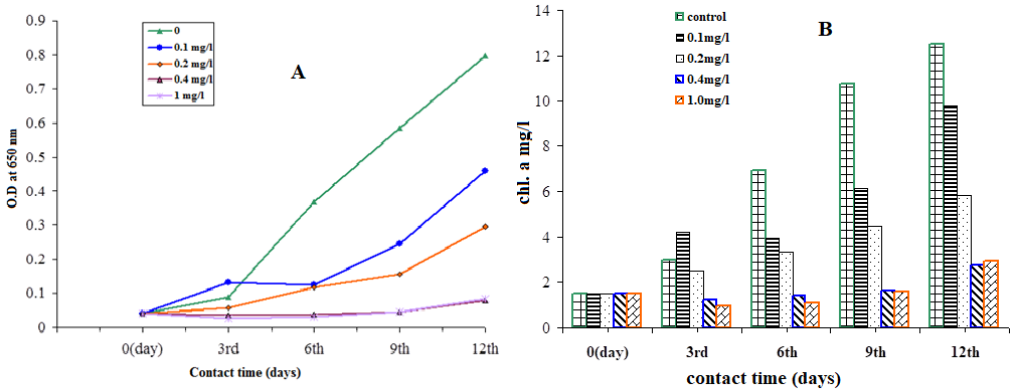

Figure 3 (A and B). Optical density (A), chlorophyll a content (B) indicating the growth of *Chroococcus* sp under different concentrations of Cd^{+2}

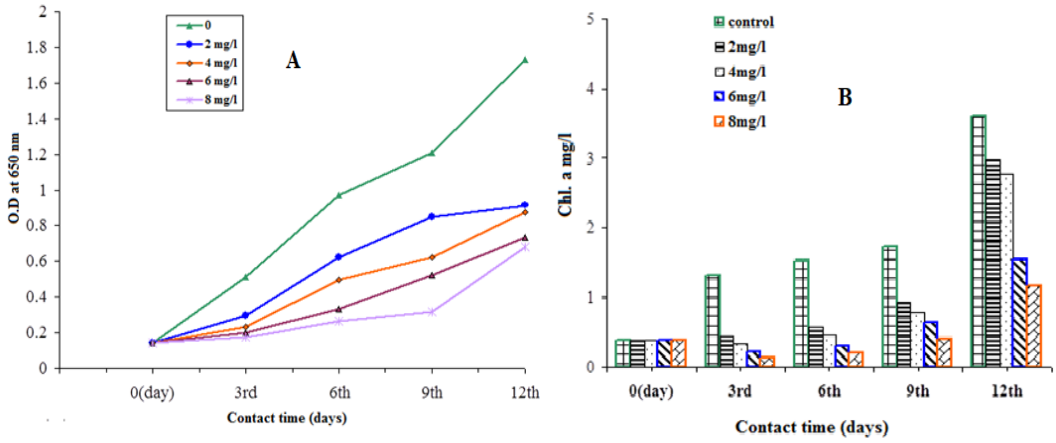

Figure 4. (A and B). Optical density (A), chlorophyll a content (B) indicating the growth of *Chlorella* sp under different concentrations of Cd^{+2}.

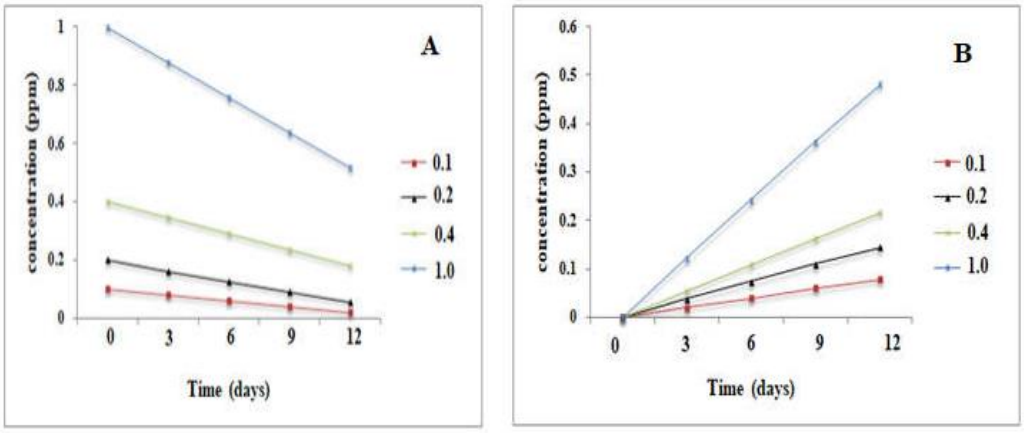

Figure 5 (A and B). Uptake accumulation of Cd ions at various concentrations (ppm) estimated (mg/l) after 3, 6, 9 and 12 days of exposure by *Chroococcus minutus*.

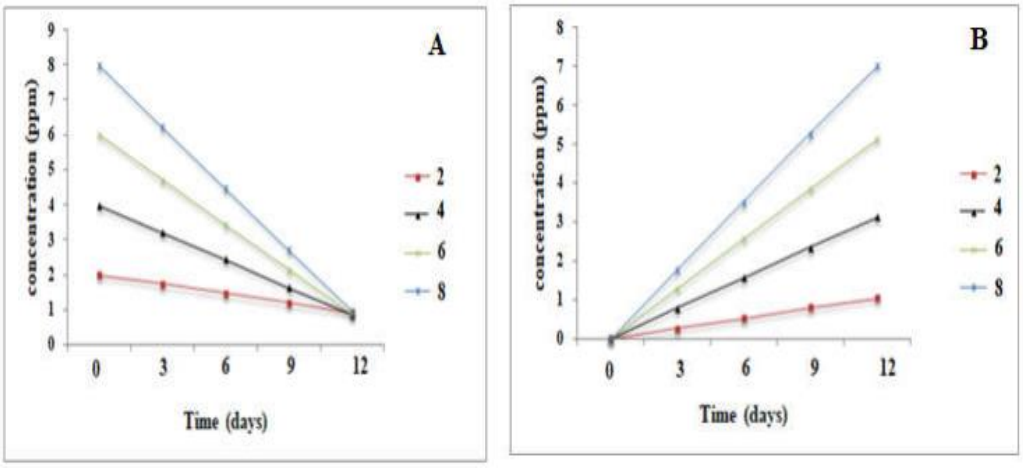

Figure 6 (A and B). Uptake accumulation of Cd ions at various concentrations (ppm) estimated (mg/l) after 3, 6, 9 and 12 days of exposure by *Chlorella vulgaris*.

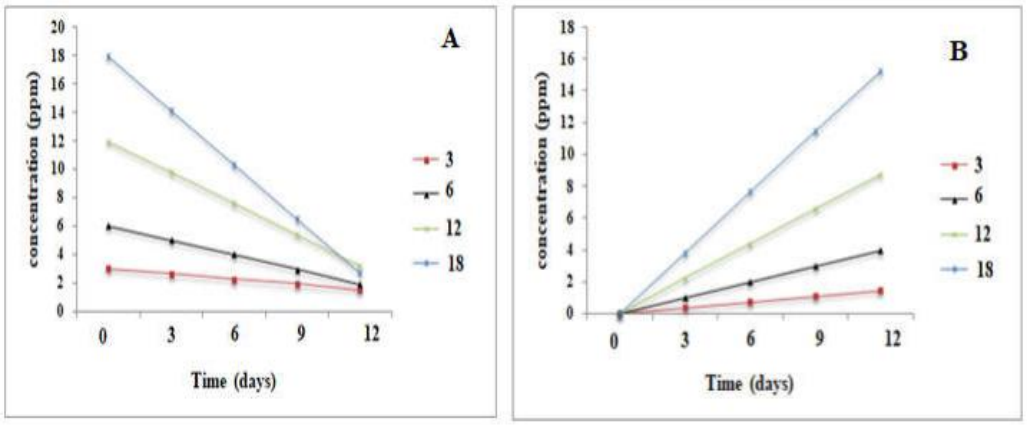

Figure 7 (A and B). Uptake accumulation of Pb ions at various concentrations (ppm) estimated (mg/l) after 3, 6, 9 and 12 days of exposure by *Chlorella vulgaris*.

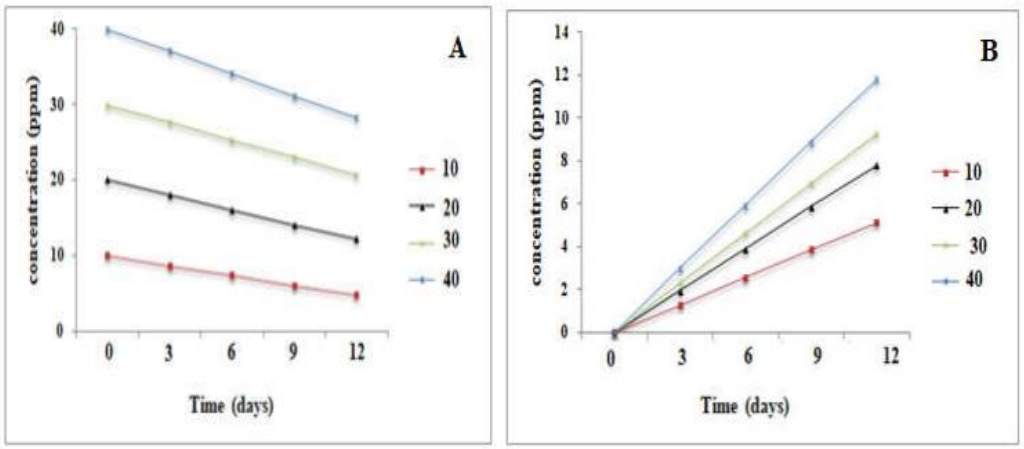

Figure 8 (A and B). Uptake accumulation of Pb ions at various concentrations (ppm) estimated (mg/l) after 3, 6, 9 and 12 days of exposure by *Chroococcus minutus*.

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قدرة طحلبى الكروكوكس والكلوريللا المعزولين من بحيرة المنزلة على المعالجة الحيوية للكادميوم والرصاص

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الملخـــص العربـــى

نظرا لوجود العديد من الفوائد والاستخدامات للطحالب الدقيقة من الأنواع المختلفة وخاصة دورها في المعالجة الحيوية للمعادن الثقيلة. وقد أجريت هذه الدراسة في معمل الطحالب. قسم بحوث المياه والتربة ، المعمل المركزي لبحوث الثروة السمكية (CLAR) بالعباسة – أبو حماد – الشرقية ، مصر بهدف تقييم كفاءة نوعين من الطحالب البرية ؛ *Chlorella vulgaris* كأحد الطحالب الخضراء و*Chroococcus minutus* كمثال للطحالب الخضراء المزرقة المعزولة من منطقة الجنكة ببحيرة المنزلة للمعالجة الحيوية للمعادن الثقيلة الكادميوم (Cd^{+2}) و الرصاص (Pb^{+2}) من المحاليل المائية من خلال تعريض الطحالب لتركيزات مختلفة من Cd^{+2} و Pb^{+2} . وكانت أهم النتائج أن الانخفاض في كمية الكادميوم Cd^{+2} بواسطة *Chlorella* بعد ١٢ يومًا من بدء التجربة هى ٥٣,٢٦٪ و ٧٨,٣٣٪ و ٨٥,٨٧٪ و ٨٧,٩٥٪ للتركيزات ٢,٠ و ٤,٠ و ٦,٠ و ٨,٠ جزء في المليون ؛ على التوالي. كما اتضح أيضا قدرة *Chlorella* على إزالة الرصاص Pb^{+2} بمعدل ٤٨,٢٣ % و ٦٧.٠٨٪ و ٧٢,٩٤ و ٨٤,٩٨ للتركيزات ٣ و ٦ و ١٢ و ١٨جزء من المليون ، على التوالي بعد ١٢ يومًا من بدء التجربة. وهذا يعني أن قدرة امتصاص المعادن الثقيلة بواسطة *Chlorella* يمكن استغلالها لإزالة السموم من المعادن و عمليات التنظيف البيئي. في حالة *Chroococcus* ؛ لوحظ أن نسبة إزالة الكادميوم Cd^{+2} تصل إلى ٥٤,٤٣ و ٤٨,٠٥٪ في التركيزات الأعلى وهى ٠,٤ و ١,٠ جزء في المليون ، والتي تعرض لها الطحلب حتى اليوم الأخير من التجربة. بينما سجلت نسبة إزالة الرصاص Pb^{+2} (٥١,٤٤ و ٣٨,٩٨٪) في نهاية التجربة للتركيزات ١٠ و ٢٠ جزءًا في المليون وانخفض إلى ٣٠,٩٨ و ٢٩,٤٣٪ للتركيزات: ٣٠ و ٤٠ جزء في المليون على التوالي.

تقدم هذه الدراسة نظرة عميقة لاستكشاف إمكانية استخدام أنواع الطحالب المعزولة من المواقع الملوثة للمعالجة الحيوية للمعادن الثقيلة حيث تختلف قدرة الطحالب على إزالة العناصر بإختلاف العنصر والفترة الزمنية وتركيز العنصر وعليه فإن الدراسة تلقى الضوء على ضرورة اختيار الطحلب لإزالة عناصر معينة وتوصى الدراسة بضرورة العمل على استخدام الطحالب فى إزالة الملوثات على نطاق واسع للتغلب على مشاكل ندرة المياه فى مصر.