Indian Journal of Geo Marine Sciences
Vol. 48 (01), January 2019, pp. 151-162

Enhancement the removal capacity of heavy metals from aqueous solution using different aquatic organisms

Emad A. Shalaby[1*], Awad A. Ragab[1], Ghada I. Mahmoud[1], Sanaa M. M. Shanab[2], Walaa S. Abd El Monsef[1] , Osama Abd-El Fattah[1]& Ahmed E. Ghoneim[3]

[1]Biochemistry Department, Faculty of Agriculture, Cairo University, Giza, 12613, Egypt

[2]Botany and Microbiology Department, Faculty of Science, Cairo University, Giza, 12613, Egypt

[3]Botany Department, Faculty of Agriculture, Cairo University, Giza, 12613, Egypt

[E.Mail: dremad2009@yahoo.com]

Received 02 August 2017; revised 16 November 2017

The Eichhornia sp had high ability for bioremoval of Pb and Cd (97.15 and 97.48%) during 15 min with some ultrastructure changes of the leaf such as, ruptured or disappeared plasma membrane, swollen mitochondria and malformation chloroplast and some anatomical studies; thickness of upper epidermis and mesophyll decreased with presences number of raphide crystals in treated leaf but it not found in untreated plants. It was noticed that treated with Pb was more effect on histological leaf than treated with Cd. While, Gelidium pectinatum had highest efficiency for removal of Cd but during 0.5 hr. The bioremoval efficiency of lead by Gelidium pectinatum increased with Epichlorohydrin 1 from 28.84 to 90.18 % during 240 min contact time and from 77.34 in raw (untreated) algae to 99.58% in case of cadmium during the same contact time.

[Keywords: Biosorption, *Eichhorniacrassipes*, Algae, treated biomass, ultrastructure and anatomy studies]

Introduction

The mining wastes usually generate environmental pollutions and problems because of their properties[1]. An advantage of using different living organisms instead of non-living organisms is that they exhibit rapid growth and regenerate a supply of unsaturated metal-removing biomass[2]. Bioremediation has provided an alternative method for treatment of industrial pollutions when compared with physico-chemical methods. Previous articles showed that by hydrolyzing Datura innoxia cell wall ester groups, the -COOH content was increased and this in turn caused an increase in metal removal[3]. Pre-treatment may be the extent that cementing the cell divider structure through a cross-linking reaction using epichlorohydrin[4], or growing the negative charge on the cell wall by sodium hydroxide treatment[5], or opening of the available goals for the adsorption by destructive treatment [6], and enhancing molecule exchange by calcium treatment [7]. In any case, only a set number of studies have so far been focused on the use of pre-treated green development for Lead (II) ejection from wastewater [8].

E. crassipes (water hyacinth), found in huge sums around the fields of water systems and in the new water bodies during that time in tropical and subtropical nations including Egypt [9]. The capability of utilizing E. crassipes living or dead biomass to expel metal particles from arrangements was as of late explored. Little is thought about the sorts and measures of practical gatherings situated on E. crassipes and also proton and Cu^{2+} restricting constants with E. crassipes[10]. Besides, broad examines have been done on the roots parts of water hyacinth while little is thought about leaves and stems. Present study is to explore the physicochemical attributes of the leaves and stems of E. crassipes biomass in their dead express that help concentrate their reactivity towards lead and cadmium assimilation at various pH's and subsequently proposing a component for the bioremoval procedure. The present work likewise meant to research the bioremoval limit of various sea-going life forms (green growth, water hyacinth) and their adjusted biomass against lead and cadmium overwhelming metals.

Materials and Methods

Dictyochloropsis splendida Geitler (green microalga), isolated from Ain Helwan, Cairo, Egypt, during spring season (March 2014), cultured on

Bold's basal medium [10,11] at 20ºC under 16/8 light/dark cycles and light intensity of 40 µE / m²/s. Spirulina platensis (blue-green alga, Setch. et Gard) cultured on Zarrouk medium [12] was obtained from Faculty of Science, Cairo Univ. Each microalgal species were finally dried after harvesting at 85-90 ºC for 15 min.

Macroalgae with its hold fast were collected from Abu Quir beaches. The algae belong to two families: Ulvaceae (Enteromorpha compressa), Gelidiaceae (Gelidium pectinatum). These algae were collected during spring 2013 and 2014. Thalli of these algae were cleaned from any foreign materials by washing with sea water, tap water followed by dist. water.

Water hyacinth (Eichhornia sp) was collected from the River Nile at the El-Zomor canal during spring 2014, cleaned from any foreign materials, washed with tap, dist. water then air dried and ground then stored at -20 ºC until use.

All collected aquatic species (Algae and Eichhornia) were identified by Prof. Sanaa Mahmoud, Botany and Microbiology Department, Fac. of Sci., Cairo Univ., Giza, Egypt, according to Aleem [13].

A batch laboratory method was used for evaluation the metals binding to cells of different aquatic organisms including micro, macroalgae and Eichhornia crassipes with time. The algal cells were washed for several times with deionized water, air dried and used for 1g/100ml dist. water). However, the concentration of metal ion was 10mg/l of lead (II), 10mg/l Cd (II) and each of the metal ion solution was adjusted at pH=5.4, and the aquatic organisms in water contain ions were shacked in shaker water bath at ≥ 100 rpm for 240 min. At specific time intervals (Zero, 15, 30, 60, 120 and 240 min.) the concentration of Cd and Pb samples were determined by (ICAP 6000 series Spectrometer. Thermo scientific- Faculty of Agric., Cairo Univ.) and their levels were calculated from standard curve. The performances of the aquatic organisms were expressed as removal capacity (RC) and removal efficiency (RE) [14]. This parameter was calculated by the following equations:

$$RC = V (Ci – Ct) /m$$

$$RE (\%) = (Ci – Ct) / Ci \times 100$$

where V is the volume of solution, Ci the initial concentration of M, Ct the equilibrium concentration of M and m the mass of biosorbent added (g).

Ten grams of powdered aquatic biomass was taken into a flask together with 150 ml of DMSO and stirred for 24 h at room temperature. Then 20 ml of epichlorohydrin was added to the mixture to undergo the crosslinking reaction at 20 ºC for 2 h according to Luo et al. [15] with some modification. Twenty millilitres of 5 M NaOH solution was added and stirred for 5 h at 50 ºC. After cooling down to room temperature, it was filtered and washed with 70% aqueous 2-propanol followed by 0.5 M HCl and finally with 70% aqueous 2-propanol to neutral pH. The sample was kept to dry in an oven at 60 ºC overnight, hereafter abbreviated as EPC1. In order to reduce the cost, another sample was obtained by the above pre-treatment only with one change of 20% 2-propanol instead of 70% 2-propanol, hereafter abbreviated as EPC2, other treatment was done using distilled water and abbreviated as EPC3.

Ten grams' sample of dried organism was weighed and washed twice with 0.1M HCl and centrifuged at 2500 rpm. The biomass of aqyatic organism was then reacting with 50 ml of 0.1 M potassium hydroxide for 24 hr. after centrifuged, the supernatant was removed.

A modified method of Jeon et al. [16] was used. Ten grams of dried biomass was oxidized in 20 mM solution of $KMNO_4$ at 30 ºC for 30 min. the mixture was separated by centrifugation and washed with distilled water and dried in an oven at 60 ºC (abbreviated as PC).

The total phosphorus in different aquatic organisms were extracted as reported by Soltanpour (17) and determined according to procedures of Olsen and Watanab [18].

Total sulfate contents in different aquatic organisms were determined by turbidimeteric method according to APHA method [19].

Total chloride contents in different aquatic organisms were determined by turbidimeteric method according to Kraemer and Stamm [20].

Total hydrolysable carbohydrates were determined using 5% phenol / sulfuric acid reagent [21].

Total nitrogen content was carried out according to Kjeldahel method. The total crude protein was calculated by divide total nitrogen percent by 6.25 [22].

In order to investigate the functional groups involved in biosorption of lead and cadmium in the samples, FT-IR analysis was carried out. Infrared spectra of different aquatic biomass with and without heavy metals (control) were studied. The lyophilized cell pellets were grounded and desorbed at 60 ◦C for 24 h and pressed to obtain IR transparent pellets. The samples were dried and mixed with KBr (1:20; 0.02 g of sample with KBr at a final weight of 0.4 g).

Infrared spectra were obtained using a Fourier transform infrared spectrometer Perkin Elmer FTIR spectra (system 2000, FRAP) USA. The spectra were collected within a scanning range of 400–4000/cm.

To elute the adsorbed metal ions, the adsorbed materials (different aquatic organisms) were washed with Nitric acid 0.1 M with shaking, and the metal ions (lead or cadmium) were determined according to the method mentioned in step (2.2) for ten times.

This work was carried out on aquatic organism biomass which showed the most prominent response of the investigated treatments at TEM lab. Faculty of Agriculture, Cairo University -Research Park FARP). In brief; Slice tissue samples into ~ 1 mm slices. Slice tissue was processed for TEM by fixation in glutaraldehyde and osmium tetroxide, dehydrated in alcohol and embedded in an epoxy resin. Microtome sections prepared at approximately 500-1000 µm thickness with a Leica Ultracut UCT ultramicrotome. Thin sections were stained with tolodin blue (1X) then sections were examined by camera Lica ICC50 HD. Ultra-thin sections prepared at approximately 75-90 nm thickness and were stained with uranyl acetate and lead citrate, then examined by transmission electron microscope JEOL (JEM-1400 TEM) at the candidate magnification. [23-25].

Specimens of water hyacinth leaf were killed and fixed at least for 48 hours in FAA (10 ml Formalin, 5 ml Glacial Acetic Acid, 50 ml ETOH 95% and 35 ml Distilled water). The selected materials were washed in 50% ETOH, dehydrate in a normal butyl alcohol series, embedded in paraffin wax of melting point 56oc, sectioned to a thickness of 20 µm, double stained with safranine- light green, cleared in xylem and mounted in Canda Balsam [26,27]. Sections were microscopically analysed and photomicrographed.

Results and Discussion

Aquatic organisms such as Algae and Eichhornia sp are the most important biosorbents. Figure (1) showed the bioremoval efficiency of heavy metals (Pb and Cd) by two macroalgae (Gelidium pectinatum and Enteromorpha compressa), two microalgae (Spirulina platensis, Dictyochloropsis splendida and one aquatic plant Eichhornia crassipes) during 30 min. The obtained data revealed that Eichhornia sp had high capacity for bioremoval of Pb and Cd (97.15 and 97.48%) during 15 min. while Gelidium sp had highest ability for absorption of Cd (96.80%) but during 30 min.

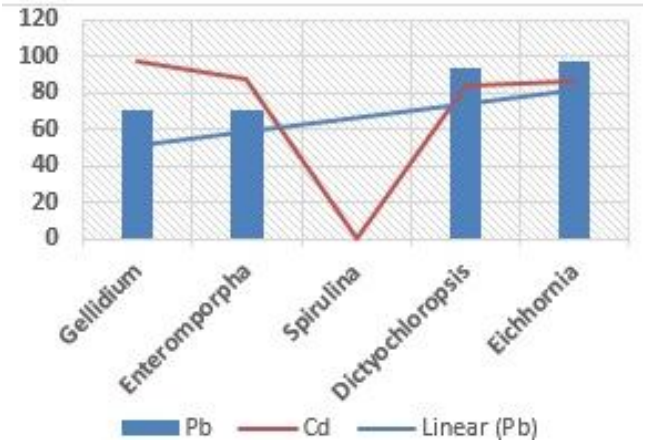

Fig. 1 — Removal efficiency (%) of lead and cadmium using different aquatic organisms during (30 min)

On the other hand, D. splendida had the high ability but for absorption of Cd (96.05%) during 15 min. However, S. platensis had the lowest biosorption ability against both Pb and cd (23.21 and 39.64%) as maximum biosorption during 30 min contact time.

These biosorptive abilities may be due to the active groups in different aquatic organisms especially water hyacinth and algae from phycocolloide, sulfate,chloride, phosphorus and nitrogen. However, the obtained data revealed that the maximum biosorption capacity arrived to 97% for tested organisms occurred after 15 min which was decreased during 240 min of contact with the heavy metals. These results may be due to breaking down of the bonds between active groups on plant cell wall and metals by microorganism such as fungi and bacteria. The present data were in agreement with those obtained by Pandya et al. [28] who reported that, different species of seaweeds have good removal capacity for different metals, Sargassum sp showed the best biosorption ability. Sandau et al. [29], reported that sorption capacities are generally similar in living and dead biomass of a specific type. Dead biomass (heat, acid, and/or otherwise chemically treated) had great biosorption capacity, probably due to the uncovering of masked binding sites. The two principle mechanism involved in biosorption appear to be the ion exchange where ions such as Na+. Mg++ and Ca++ became displaced by heavy metals ions, and the complexion between metal ions and various active groups such as COOH, -NH_2, -SH, -OH, PO4--- , that can interact in a coordinated way with heavy metal ions [30].

Also, Kumar et al. [31] showed that, bioremoval materials primarily weak acidic and basic functional

groups. It follows from the theory of acid-base equilibrium that, in the pH range 2.5-5, the binding of heavy metal cation is determined primarily by the state of dissociation of the weak acidic groups. At pH 5, there are low concentrations of competing hydrogen ions and more ligands are exposed with negative charges, resulting in greater metal sorption. But for pH values from 6 to 10, lower adsorption capacity was observed due to the precipitation and lower polarity of metal at high pH.Biosorption of lead by eight brown, green and red marine algae was investigated by Jalali et al. [32]. They reported that biosorption of Pb was rapidly occurred onto biosorbents and most of the sobbed metals were bound in short time of contact (<30 min).

Raw biosorbents (especially the promising ones from Figure 1) have generally been modified with chemical treatments such as Epichlorohydrin, KOH and $KMNO_4$ to increase their sorption capacity. Data in Figure (2) clearly reported the bioremoval ability of aquatic organisms (Gelidium pectinatum and Eichhornia crassipes) treated with Epichlorohydrin (EPC1, EPC2 and EPC3). The obtained data also indicated that, the chemical treatment (with EPC) improved the sorption capacity of both aquatic organisms against Pb and Cd. The bioremoval efficiency of Pb by G. pectinatum increased with EPC1 70% propanol from 28.84 to 90.18 % during 240 min contact time and from 77.34 in raw algae to 99.58% in case of Cd during the same contact time.

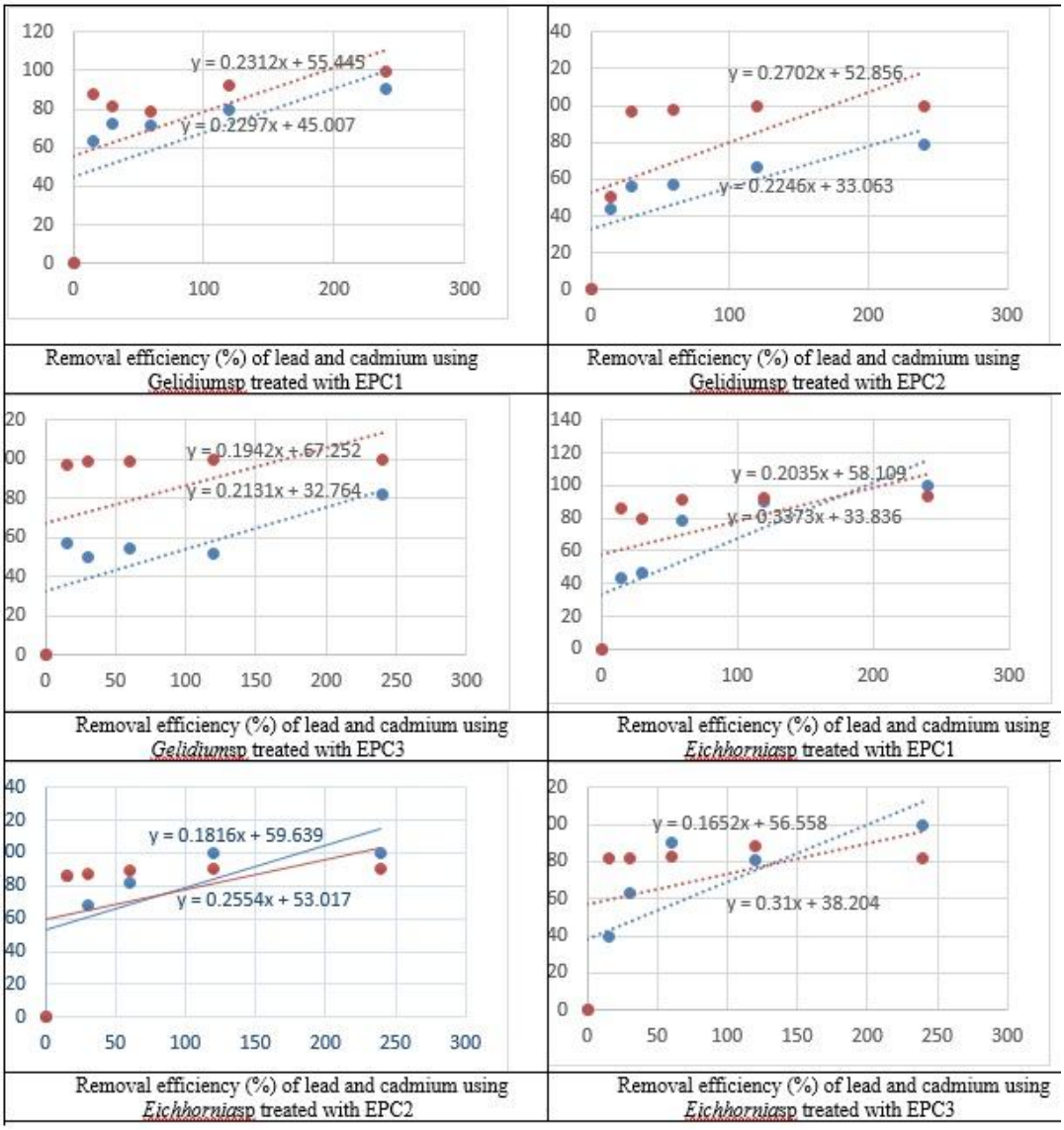

Fig. 2 — Removal efficiency (%) of lead and cadmium using Gellidium and Eichhorniasp treated by EPC compound against time (min.).

The same treatment (EPC1) with E. crassipes (leaves) showed increase of lead sorption from 74.10 in raw cells to 86.48% in chemically modified cells during 15 min contact time [33]. The data of EPC2 treatment showed that, enhancement of Pb sorption capacity of G. pectinatum from 28.84 to

78.41 % during 240 min and Cd sorption ability from 77.34 to 99.58 % at the same contact time (240 min.). Also, the same results were obtained with the treatment of raw organism with EPC3 against Pb and Cd. These data may be due to, the effect of EPC treatment which change chemical properties of aquatic organisms (Gelidium pectinatum and Eichhornia crassipes) such as active groups on cell wall of aquatic organisms.

The obtained data were in agreements with the data obtained by Basha et al. [34] who reported that, pretreatment may be in terms of hardening the cell wall structure through a cross-linking reaction using EPC (EPC 1, 2 and 3) and the results obtained by Shin and Rowell [35] who showed that, chemical treatment (sulfonation) improved the sorption capacity of juniper wood.

Figure 3 presents the results from experiments on adsorption of lead and cadmium onto modified biomass (with potassium hydroxide) of G. pectinatum and E. crassipes. The equilibrium could be achieved after having been shaking more than 3 h. these changes in Pb and Cd uptake may be due to the fact that, initially, all adsorbent sites were vacant and the solute concentration was high. After that incubation time, only a very few increase in the Pb and Cd uptake was observed because there are low surface active sites on the cell wall of tested aquatic organism (algae or water hyacinth). The quick equilibrium time is due to the particle size. The effective surface area is high for small particles. Pre-treatment of raw cell with sodium hydroxide may be in terms of hardening the cell wall structure through increasing the negative charges on the cell surface as reported by Wang et al. [36]. According to experimental data, the maximum uptakes of Pb and Cd were done during 240 min incubation time. The lead has increased in both species (G. pectinatum and E. crassipes) from 28.84 and 12.22 % (in raw cells) to 72.98 and 70.09% (in modified cells) during 240 min respectively. However, the uptake of cadmium using modified cells not significantly increased and was shown to be lowers than metal uptake by raw cells and these results may be due to the particle size of cadmium

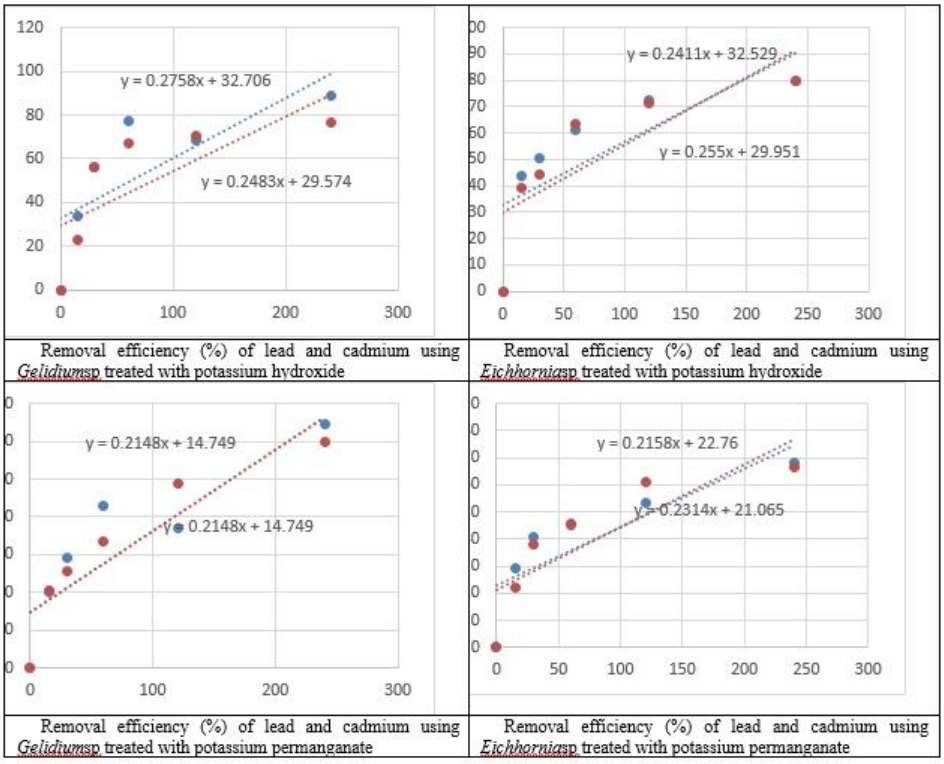

Fig. 3 — Removal efficiency (%) of lead and cadmium using Gellidium and Eichhorniasp treated by potassium hydroxide and potassium permanganate compounds against time (min.).

metal or it needed too long time (more than 240 min) for complete absorption (15). cells modifications in aquatic organism using $KMNO_4$ have been applied to enhance the absorption of Pb and Cd through oxidation reaction with $KMnO_4$. This modification method was favour to increase -COOH group and the Pb uptake capacity is increased in this process.

According to experimental data (Figure 3), the maximum absorption of Pb and Cd were done performed during 240 min incubation time. The Pb removal has increased by both species (G. pectinatum and E. crassipes) from 28.84 and 12.22 % (in raw cells) to 64.54 and 68.19 % (in modified cells by $KMnO_4$) during 240 min respectively. However, the absorption of Cd using modified cells was not significantly increased and was found to be lower than metal uptake by raw cells. These data may be due to the particle size of Cd metal need longer time (more than 240 min) for complete biobsorption [15].

Figure 4 showed the results of elemental analysis, total protein, carbohydrates for promising aquatic organisms as bioadsorbent (E. crassipes and G. pectinatum biomass). The biomass shows a significant content of total protein (41.25 and 17.68 % respectively) and carbohydrates (27.3 and 15.6 % respectively) as well as the elemental analysis (as P, S, N and Cl) is illustrated in Figure 4. This reflected that the biomass tissue has abundant function groups [ACOOH, ANH2, ANHA, AOH, CO, and PO_4-3] that gave a primary anticipation for the biomass capability to react with the cadmium and lead through chelation with those sites [37].

These data confirmed the ability of different aquatic organisms to bind and uptake heavy metal and were in agreement with the data obtained by Awadalla and Pesic [30] who reported that algal biomass has great biosorption capacity, probably due to the uncovering of masked binding sites. The two principle mode of action involved in biosorption

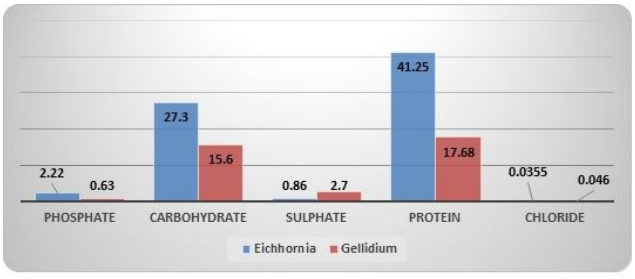

Fig. 4 — Total protein, Phosphate, chloride, sulphate and carbohydrates percentage (%) contents in Eichhornia and Gellidiumsp (as dry weight).

appeared to be: (1) ion exchange wherein ions such as Na, Mg, and Ca became displaced by heavy metal ions, and (2) complexion between metal ions and various active groups such as carboxyl, amino, thiol, hydroxyl, phosphate, and hydroxyl-carboxyl, that can interact in a coordinated way with Pb and Cd ions. These results may be due to the oxidation of -CH groups on plant cell wall and increase the -COOH groups which led to elevation of the acidity and increasing the biomass capability to react with Cd and Pb through chelation and formation of the ionic bonds between negative and positive charges.

Numerous chemical groups have been proposed to be responsible for bioremoval metal binding by different aquatic organisms (-COOH, -OH, -NH2, -O-C-O-, etc); FTIR was used to analyse the functional groups in the raw, chemically modified Gelidium and Eichhornia sp biomass, as well as treated cells with Pb and Cd. In the FT-IR spectral analysis of G. pectinatum we observed characteristic bands corresponding to O-H stretching vibration at 3426 cm-1 shifted to 3426.81 in modified biomass and 3422 and 3427.78 in biomass treated with Pb and Cd respectively. The C-H and C-O stretching were observed at 2927 and 1081 cm-1 respectively. The absorption peaks at 2364 and 2340 were assigned to P-H group. 1050 cm^{-1} assigned to –C-O stretching of alcoholic group. The spectrum showed characteristic difference in the peaks of the biomass after chemically modified. The two peaks at 2340 and 1156 cm^{-1} not detected in modified cell. The H-C-H stretching vibration at 2927 cm^{-1} is shifted to 2928.38, 2929.34 and 2930.3 cm^{-1} in modified biomass, cell treated with Pb and Cd respectively. Similarly, the C-O stretching vibration observed at 1081 resulted in a shift to 1083.8 in modified biomass and 1082.83 in modified biomass treated with both Pb and Cd ions. P-H peak neither shifted from 2364 to 2363 cm-1 and nor detected on both modified biomass treated with Pb or Cd ions (Table 1a). These changes could be attributed to the effective interaction between EPC with the functional groups on the cell wall.

In the FT-IR spectral analysis of E. crassipes we observed characteristic bands corresponding to O-H stretching vibrations at 3424 shifted to 3419 and 3428.81 cm-1 in chemically modified biomass and modified biomass treated with Cd ions. The C-H was observed at 2924 shifted to 2917 and 2928.38 cm-1 in modified biomass and biomass treated with heavy metals (Cd or Pb). A characteristic peak at 1639 cm^{-1} corresponding to C=O was shifted to 1650, 1641.13

and 1642.09 cm^{-1} in modified biomass treated with Pb and Cd respectively. Accordingly, an increase in the peak intensity after chemically modification and appearing of new peaks (at 3000, 2093, 1516-1539 and 1382 cm^{-1}) could be attributed also to the effective interaction of functional groups on the cell walls with EPC as shown in Table (1b). Heavy metal recovery from biosorbents is of major importance in the assessment of competitiveness of biosorption processes. Several desorption agents such as nitric acid, sulfuric acid and EDTA may be used for the selection of the optimal elution conditions for lead and cadmium under study.

Sorption time was optimized as it plays an important role in the sorption-desorption process, being shown that a 30 min sorption period is the best option to ensure metal removal from solution and good recovery from biosorbent. The number of sorption-desorption cycles of Gelidium and Eichhornia was calculated as shown in Table (2). The present data revealed that, 0.1M HNO_3 is the solution that accomplishes the higher lead bioremoval in the first four sorption/desorption cycles (88, 85, 82 and 70%) in *Eichhornia sp* and 88, 85, 80 and 70% respectively for the same organism against cadmium metal. However, the present results also, reported that, 0.1M HNO_3 is the solution that accomplishes the higher lead bioremoval in the first four sorption/desorption cycles (87, 85, 82 and 68%, respectively) in *G. pectinatum* and the same results against cadmium metal. Regarding the damage caused by acid treatment on *G. pectinatum* and *E. crassipes* cells, assessed by the reduction on metal uptake capacity after elution; it was possible to observe that HNO_3 was harmful eluent causing long term negative effects in metal uptake. By the time the experiments were interrupted (10 cycles) and biomass uptake capacity was reduced every cycle. The above results revealed that a compromise situation was desired, making it possible to remove most of the metal from

Table 1a — FT-IR data of the Gelidium pectinatum before and after the cadmium and lead biosorption.

Wavenumbers (cm^{-1}) of Raw cells	Modified cells	Differences in raw cells				Bond	Functional group
		Lead		Cadmium			
		Raw+HM	Modified+HM	Raw+HM	Modified+HM		
3424	+2.81	-2.01	0.0	+3.78	0.0	O-H, N-H	Alcohol and phenols, amide
2927	+1.38	+1.38	+2.34	+1.38	+3.3	H-C-H	Alkyl chain
2525	0.0	-0.64	-0.64	-1.6	-0.64		
2364	-1.0	-0.66	ND	-2.59	ND	P-H	Phosphorus
2340	ND	ND	ND	-2.7	ND		
1799	-1.67	0.0	0.0	-0.7	+1.22	C=O	Carboxylate and carboxylic acids
1417	-1.67	0.0	0.0	-0.7	+1.22		
1156	ND	-1.81	-5.67	-5.67	-6.63	C=O	Carboxylate and carboxylic acids
1081	+2.8	0.0	+1.83	+1.83	+1.83	C-O	Alcohol, carboxylic acid, esters and ethers

Table 1b — FT-IR data of the Eichhornia crassipes before and after the cadmium and lead biosorption.

Wavenumbers (cm^{-1}) of Raw cells	Modified cells	Differences in raw cells				Bond	Functional group
		Lead		Cadmium			
		Raw+HM	Modified+HM	Raw+HM	Modified+HM		
3424	-0.5	0.0	0.0	+1.92	+4.81	O-H, N-H	Alcohol and phenols, amide
n.p	3000	-	-	-	-	H-C-H	Alkyl chain
2924	-7.0	+4.38	+4.38	+4.38	+3.41		
n.p	2093	2134	2080			-C=C-	Alkene
1639	+11.77	+2.13	-3.3	+3.09	+4.05	C=O	Amide I
n.p	1528	1539	1516	1520	1516	C-N	Amide II
1431	ND	0.0	-3.99	-1.92	-3.03	C=O	Carboxylate and carboxylic acids
n.p	-	-	1382	-	1383	C-H rock	Alkanes
1319	+66.6	+1.04	+1.04	+1.04	+1.04	C-O	Alcohols, ester, ether, carboxylic acid
1243	ND	+1.83	+11.47	0.0	+6.65	C=O	Carboxylate and carboxylic acids
1038	-14.02	+116	+18.8	+14.94	+15.91	C-O	Alcohol, carboxylic acid, esters and ethers

Table 2 — Heavy metals concentration (mg/g) and bioremoval capacity (mg/g) in filtrate after 10 Times recovery using Eichhornia sp and Gelidium sp (Washing by HNO3 0.1N)

Times for bioremoval	*Eichhornia crassipes*				*Gelidium pectinatum*			
	Lead		Cadmium		Lead		Cadmium	
	Metal conc.	RC	Metal conc.	RC	Metal conc.	RC	Metal conc.	RC
Zero time (metal solution)	7.52	0	6.95	0	6.32	0	7.32	0
1	0.83	6.69	0.73	6.22	0.78	5.54	0.83	6.49
2	1.23	6.29	1.20	5.75	1.35	4.97	1.10	6.22
3	1.63	5.89	1.52	5.43	1.73	4.59	1.46	5.86
4	2.2	5.32	2.48	4.47	2.13	4.19	2.30	5.02
5	2.65	4.87	2.74	4.21	2.75	3.57	2.98	4.34
6	3.33	4.19	3.64	3.31	3.38	2.94	3.23	4.09
7	4.02	3.50	4.25	2.70	3.75	2.57	3.63	3.69
8	4.32	3.20	4.84	2.11	4.25	2.07	4.36	2.96
9	5.32	2.20	5.32	1.63	4.83	1.49	4.86	2.46
10	5.98	1.54	5.72	1.23	5.42	0.91	5.37	1.95

solution while keeping recovery at acceptable values. It was still possible to recover more than 40% of bound metals (lead and cadmium) with eluent tested (0.1M HNO_3) after 10 times for using of aquatic organisms. These data were in agreement with the results mentioned by Ferraz et al. [38] who reported that, the ability of metal recovery depend on choice of eluent and elution conditions, as various eluants presenting different desorption mechanisms may be used. Lowering pH (e.g. use acid) causes metal desorption, resulting from competition between protons and metal ions for binding sites. Ghimire et al. [39] reported that the adsorbent prepared from the brown alga *Laminaria japonica* revealed to be simple, cost effective and promising for the adsorption and recovery of metals ions from aqueous medium. Effect of Pb and Cd on water hyacinth leaves (*Eichhornia crassipes*) as promising organism compared with control was examined by transmission electron microscopy (TEM). The ultrastructure of chloroplast, mitochondria and cell membrane is shown in (Fig. 5). It is obvious that control cells contained abundant cytoplasm, the plasma membrane was tightly stuck with the cell wall and intact without any visible damage, the chloroplast membrane was integral, the mitochondria kept normal shape and contained plentiful cristae, and the cell wall showed a normal uniform colour (Fig.5, A). On the other hand, treated leaves with Pb and Cd as 10 ppm for 4 hours in liquid media revealed ruptured or disappeared plasma membrane, swollen mitochondria and malformation of chloroplast. The swollen of mitochondria was attributed to decreased or disappeared cristae and the inner membrane was strewed with completely

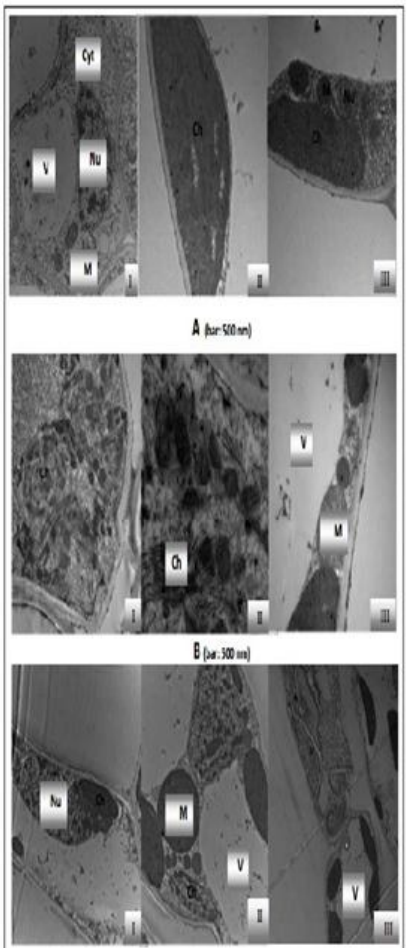

Fig. 5 — TEM electron micrographs of transversal sections of leaf tissue of Eichhorniacrassipes. (A) Control plant, (B and C) treated plant with Pb and Cd by 10 pm respectively, (B; II and C; I) Showed that the swolen mitochondria. While, malformation of chloroplast was illustrated in (B; I, I and C; I). Finally, (B; I and C; I, I) showed the plasmolysis. Ch (Chloroplast), M (Mitochondria), Nu (Nucleus) and V (Vacuole).

damaged cristae which caused swollen of the mitochondria (Fig. 5, B; II and C; I). Likewise, chloroplast of the treated leaf showed many features of malformation of chloroplast structure including the rupture of chloroplast membrane or covered by fragmented membrane with lose structure of grana lamelae and disarrangement of thylakoids that overlap each other (Fig.5, B; I,I and C; I) Finally, liquid media of Pb and Cd have clearing effects on cell membrane compared with control by a discontinuous plasma membrane or disappearance of condensed cytoplasm which caused plasmolysis (Fig.5 B; II and C; I, I). This plasmolysis was due to effect of heavy metal by dilation of organelles and increasing of vacuole size with depression on organelles toward cell wall plasmolysis causing an adversely effects on the plant cell. It could be noted that Pb showed more pronounced effects than Cadmium on malformation of chloroplast and plasmolysis. In contrast, Cd showed more abnormal mitochondria shape than Pb which was seen more swollen. It was oblivious from results mention before that Pb and Cd caused adversely interrupted photosynthesis by causing disturbance of chloroplast ultrastructure and negative effects on most of metabolic processes in cell by causing an injury of mitochondria structure. The present data concerning the ultrastructure's of the water hyacinth leaf which was affected by heavy metal was in accordance with Lage-Pinto et al. [40] who found that structural changes, including thylakoid disorganization, in the chloroplasts of samples of *E. crassipes* originating from industrial areas containing of heavy metals. The presence of 3 and 6mg Cd induced the most sever modifications in cell ultra-structure detected by TEM analyses of leaves of *Hordeumvulgare*, which included changes occurred in the shape the chloroplast ultrastructure Grana were slightly disorganized, granal and stromal thylakoids were swollen The shape of chloroplasts was almost spherical. Vassilev et al. [41] and Hakmaoui et al. [42] reported that the effect of Cd by (133.5 µm) on leaf of Salix purpurea and Phragmitesaustralis induced distortion of thylakoids, reduction of grana stacks, Chloroplasts showed swollen but organized thylakoids with thylakoid-free stroma areas. Liu et al. [43] on Sedum alfredi, they showed that TEM studies on Sedum alfredi leaves of Pb stress obviously affected the cell membrane and chloroplast and mitochondrial structure. Moreover, Pb stress (of 0.2 mM) caused plasmolysis. After the structure of chloroplasts and mitochondria was destroyed, the photosynthesis was inhibited. The presence of Cr at 50 mg l^{-1} induced the most severe modifications in cell ultra-structure detected by TEM analyses of leaves of *Eichhorniasp*, which included changes in nuclear shape and envelop integrity together with modifications in the shape of leaf chloroplasts, resulting in the structural disarrangement of thylakoids and stroma in comparison with control plants. Mangabeira et al. [44] and Vitoria et al. [45] reported that TEM images of chloroplasts from the water hyacinth leaves as affected by Pb showed rearrangement and increase of the stroma to a side of the chloroplast. Effect of (Pb 90 mg/kg) on tomato leaves was studied by Zhao et al. [46] They showed that the ultra-structure of leaves and calyx include malformation of chloroplast by the lose structure of grana lamelae in the chloroplast, the declined or disappeared cristae in mitochondria, and the ruptured or disappeared plasma membrane, the ultra-structure of calyx cells was also damaged and malformed, intensely condensed cytoplasm induced the departure of cytoplasm completely from the cell wall. The swollen chloroplast was covered by fragmented membrane and the thylakoid overlapped each other. The thylakoids were integrated closely to form grana lamellae. The mitochondria were swollen and the inner membrane was strewed with plentiful cristae.

Anatomy of leaf

Data presented in Table (3) and Fig. (6) Stated that treatments of Pb or Cd play an important role for the affecting on the internal structure of water hyacinth leaf as considered these matters used as a heavy metal. It's obvious from Table (3) that, treated with Pb was more effect on leaf than treated with Cd and that confirmed from the histological measurements on leaf¸ as shown that thickness of upper epidermis decreased for treatment with Pb than control by 6% whereas treatment with Cd showed a slightly increase for upper epidermis by 3.6% over the control . On the other hand thickness of lower epidermis was similar to control when treated with Pb but increase by 15.7% over the control when treated with Cd. Effect of both treatments was more clear on mesophyll which consists of palisade and with Pb than Cd. It's obvious from table (3) that thickness of upper palisade for both treatments were decreased than control and maximum decrease obtained by treated with Pb which showed a percent by 5.7% below the control whereas

Table 3 — Measurements and counts in µm of some histological characters in transverse sections through the median part of leaf blade of water hyacinth affected by Heavy metals (Pb and Cd). (Means of three sections from three specimens)

Histological characters	Control	Pb	+	% to Control	Cd	+	% to Control
Thickness of upper epidermis.	30.1	28.3	-	6	31.2	+	3.6
Thickness of lower epidermis.	21.0	21.0	=	--------	24.3	+	15.7
Thickness of upper palisade	239.2	225.6	-	5.7	227.6	-	4.8
Thickness of lower palisade	82.4	65.2	-	20.8	80.4	-	2.4
No. of upper Palisade layers	5.7	5.1	-	10.5	5.4	-	5.3
No. of lower Palisade layers	3.3	2.8	-	15.1	2.8	-	15.1
Thickness of Spongy tissue	1200	696.8	-	41.9	457.6	-	61.9
Thickness of mesophyll	1521.6	987.6	-	35.1	765.6	-	49.7

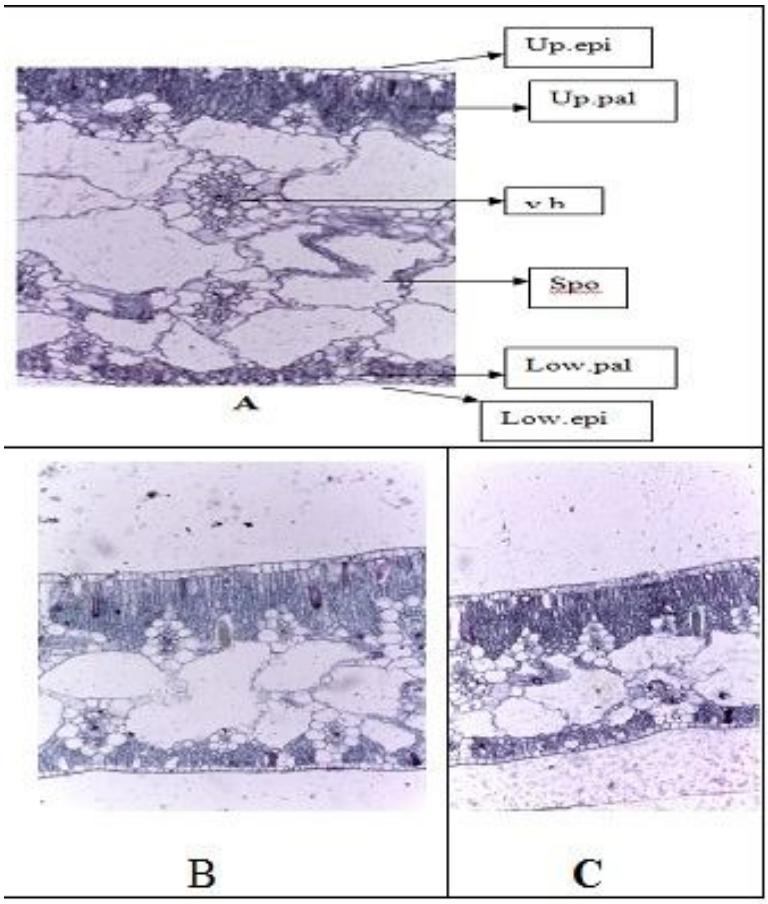

Fig. 6 — Transverse section of water hyacinth leaf A= control B= Pb treatment C= Cd treatment (x= 100) Up. ep= upper epidermis up. pal= upper palisade v.b=vascular bundle Spo= spongy tissue low pal=lower palisade l0w.epi=lower epidermis.

treated with Cd at the same trait recorded 4.8% below the control. The same trend noticed at the trait thickness of lower palisade reform plants treated with Pb more than Cd. As shown in Table (3) it's cleared that thickness of lower palisade was decrease for both treatments by 20.8% and 2.8% for Pb and Cd below the control. So this result was harmony with result mentioned before that treated with Pb was more effect on palisade than Cd, although both treatments occurred a decrease on this trait but more clear by Pb treatment than Cd. Number of upper and lower palisade layers by 10.5% and 5.3% for Pb and Cd below the control respectively, whereas for number of lower palisade layers it showed the same decrease for both treatments by 15.1% below the control, knowing that the decrease was more for Pb than Cd. According to thickness of spongy tissue, it's noticed that both treatments recorded a values below the control by

41.9% and 61.9% for Pb and Cd respectively. Although both treatments were decreased below the control but this only trait which exceeded in thickness of spongy than plants which treatment with Cd and that logic where thickness of mesophyll was more thicker than plants treated with Cd and both of them were below the control by 35.1 and 49.7% for Pb and Cd treatment respectively. Vascular bundle was decrease than control for both treatment and this decrease was more pronounced for plants treated with Cd more than plants treated with Pb. Related to dimensions of large bundle both treatments were decrease by 3.6% and 8.7% for length and 30.6% and 28.6% for width below control for Pb and Cd treatments respectively. Thickness of phloem also was decrease than control by 12% and 13.7% for Pb and Cd treatments respectively. At the same time diameter of meta xylem vessels exhibited a decrease by 27.9% and 32.5% below the control for Pb and Cd treatments respectively. It was noticed that in Fig. (6) Presences number of raphide crystals in both of treatments Pb and Cd but it not found in control plants, these raphide crystals as reaction of heavy metals stress on the hyacinth plants. It's obvious from data mentioned before that Pb was more effect than Cd on the internal structure of leaf and that logic where SEM on leaf confirmed the effect Pb on malformation chloroplast was more pronounced than Cd at the same trait and that led to the decrease of measurements and number of palisade for Pb treatment more than treated with Cd. On the other hand the increase of thickness of mesophyll for plants treated with Pb related to the increase of thickness of spongy tissue than plants which treated with Cd although both of them decrease than control. The presence of raphide crystals in contaminated plants by Cd and Pb was confirmed by Mahmood et al. [47] and Vitoria et al. [45] on *Eichhorniasp* plants. The present results concerning the anatomical structures of the leaf of hyacinth plants are in accordance with those reported by Mahmood et al. [47] and Vitoria et al. [45] on *Eichhorina sp* plants as well as by Kasim [48] on Sorghum bicolor (L.), Sridhar et al. [49] on *Hordeumvulgare* and Salah and Omar [50] on Zea mays. On the contrary, Gomes et al. [51] on *Brachiaria decumbens* plants stated that Cd and Pb increased the some anatomical.

Conclusions

From the current work it can be concluded that the modified forms of E. crassipes and G. pectinatum can be effectively used for lead and cadmium bioremoval from aqueous solutions, and can be used more than eight times to remove more than 50% from heavy metals concentration. Furthermore, Pb was more effect than Cd on the internal structure of leaf and that logic where SEM on leaf confirmed the effect Pb on malformation chloroplast was more pronounced than Cadmium at the same trait and that led to the decrease of measurements and number of palisade for Pb treatment more than treated with Cd.

Acknowledgment

Authors would like to thank *the University of Cairo, Faculty of Agriculture* for all the supports

References

1 Younger P.L., (2003), Groundwater Management in Mining Areas, Proc. 2nd Image-Train Advanced Study Course, June 23-27, Pécs, Hungary.
2 Nharingo, T. and Moyo, M. (2016). Application of Opuntiaficus-indica in bioremediation of wastewaters. A critical review. J. Envior. Mang., 166: 55-72.
3 Drake L , Lin S, Rayson GD (1996). Chemical Modification and Metal Binding Studies of Daturainnoxia, Environ. Sci. and Techn., 30: 110-114.
4 Kim Y H , Park J Y , Yoo Y J , Kwak J W (1999). Removal of lead using xanthated marine brown alga, Undariapinnatifida. Process Biochem. 34: 647–652.
5 Gurisik E, Arica M Y, Bektas S, Genc, O (2004). Comparison of the heavy metal biosorption capacity of active, heat-inactivated and NaOH-treated phanero–chaetechrysosporiumbiosorbents. Eng. Life Sci. 4 (1), 86–89.
6 Yang J, Volesky B (1999). Modeling uranium–proton ion exchange in biosorption. Environ. Sci. Technol. 33: 4079–4085.
7 Kratochvil D, Pimentel P, Volesky B (1998). Removal of trivalent and hexavalent chromium by seaweed biosorbent. Environ. Sci. Technol. 32, 2693–2698.
8 Gong R , Ding Y , Liu H , Chen Q , Liu Z , (2005). Lead biosorption and desorption by intact and pretreated Spirulina maxima biomass. Chemosphere 58, 125–130.
9 Schneider I A H , Rubio J , Misra M , Smith R W. (1995). Eichhorniacrassipes as biosorbent for heavy metal ions. Miner. Eng. 9, 979–988.
10 Soltan M E, Rashed M N, (2003). Laboratory study on the survival of water hyacinth under several conditions of heavy metal concentrations. Adv. Environ. Res. 7, 321–334.
11 Bischoff, H.W. and Bold, H.C. (1963). Phycological studies IV. Some soil algae from Enchanted Rock and related algal species. University of Texas Publication 6318: [1]-95.
12 Zarrouk C. (1966). Contribution a l etude d; unecyanophycee. Influence de divers facteurs physiques et chimiques sur la croissance et la photosynthese de Spirulina maxima (setch. Et Gardner) Geilter. Ph. D. thesis, University of Paris, France.
13 Aleem AA (1993). (Ed.) The marine algae of the Alexandria, Egypt, 154p.
14 Chan H W, Lau T C, Ang P O, Wu M, Wong P K (2004). Biosorption of di (2-ethylhexyl) phthalate by seaweed biomass. J. Appl. Phycol., 16: 263-274.
15 Luo F, Liu Y, Li Z, Xuan Z, Ma J (2006). Biosorption of lead ion by chemically-modified biomass of marine brown algae Laminaria japonica. Chemosphere 64: 1122–1127.

16 Jeon C , Park J Y , Yoo Y J (2002). Characteristics of metal removal using carboxylatedalginic acid. Water Res. 36 (7), 1814–1824.
17 Soltanpour P N. (1985). Use of amm. bicarbonate DTPA soil test to evaluate elemental availability and toxicity. Commun. Soil Sci. Plant Anal., 16(3): 323-338.
18 Olsen S R, Watanab F S (1965).Test of an ascorbic acid method for determine phosphorus in water and NaHCO3 extract from soil. Sci. Am. Proc., 29: 661.
19 APHA (1998). Standard methods for the examination of water and wastewater, 20th ed., American Public Health Association Washington, DC.
20 Kraemer E O, Stamm A J (1924). Mohr's Method for the Determination of Silver and Halogens in other than Neutral Solutions, J. Am. Chem. Soc.; 46(12); 2707-2709.
21 Dubios M, Gilles K A, Hamilton J K, Rebers P A, Smith F (1956). Anal. Chem., 28: 350.
22 AOAC, (1990). Official Methods of Analysis of Official Analytical Chemistry. Pub. By the Association of Analytical Chemistry, Inc., Arlington, West Virginia, USA.
23 Bozzola, J.J. and Hunter, E. (1993). Practical Electron Microscopy: A Beginner's Illustrated Guide, Second Edition. Cambridge, NY: Cambridge University Press.
24 Russell, L.D. (1999). Electron Microscopy, Second Edition. Sudbury, MA: Jones and Bartlett Publishers.
25 John K. (2007). Electron microscopy methods and protocols, Second Edition. Totowa. New Jersey. Human Press.
26 Willey, R.L. (1971). Microtechniques: A Laboratory Guid. Macmillan Publishing Co., Inc., New Yourk.
27 Nassar, M. A. and K. F. El-Sahhar. (1998). Botanical preparation and microscopy (Microtechnique). Academic Bookshop, Dokki, Giza, Egypt, 219 pp. (In Arabic).
28 Pandya, K. Y., Patel, R. V.; Jasrai, R. t. and Brahmbhat. N. H. (2017). Preliminary Study on Potential of Seaweeds in Decolonization Efficacy of Synthetic Dyes Effluent. Int. j. plants, Animals and Env. Sci. 7 (1): 59-69.
29 SandauE, Sandau P, Pulz O. (1996). Heavy-metal sorption by micro algae. Acta Biotech., 16: 227-235.
30 Awadalla F T , Pesic B (1992). Biosorption of cobalt with the AMTTm metal removing agent. Hdrometallurgy, 28: 65-80.
31 Kumar P Y, King P, Prasad V S R K (2007). Adsorption of zinc from aqueous solution using marine green algae-Ulva fasciata sp. Chemical Eng. J. 129: 161-166.
32 Jalali R, Ghafourian H, Asef Y Davarpanah S J, Sepehr S (2002). Removal and recovery of lead using nonliving biomass of marine algae J. Hazardous Mat., B92: 253–262.
33 Abd El Monsef, WS.; Ragab, AA. and Shalaby, EA. (2014). Bioremediation of heavy metals by chemically-modified biomass of algae and Eichhornia sp. Sky Journal of Microbiology Research, 2(7): 051 – 058.
34 Basha, S.; Murthy, Z.V.P. and Jha, B. (2008). Biosorption of hexavalent chromium by chemically modified seaweed, Cystoriaindica. Chem. Eng. J. 137: 480-488.
35 Shin E W, Rowell R M (2005). Cadmium ion sorption onto lignocellulosicbiosorbent modified by sulfonation: the origin of sorption capacity improvement. Chemosphere, 60: 1054-1061.
36 Wang, Z.; Li, J.; Barford, J. P.; Hellgradt, K.; McKay, G. (2016). A comparison of chemical treatment methods for the preparation of rice husk cellulosic fibers. International Journal of Environmental & Agriculture Research, 2(1): 67-76.
37 Komy Z R, Gabar R M, Shoriet A A, Mohammed R M (2006). Characterisation of acidic sites of Pseudomonas biomass capable of binding protons and cadmium and removal of cadmium via biosorption. World J Microb. and Biotechn., 22, (9): 975-982.
38 Ferraz AI, Tavares T, Teixeira JA (2004). Cr(III) removal and recovery from Saccharomyces cerevisiae. Chemical Eng. J 105: 11–20.
39 GhimireKN, Inoue K, Ohto K, Hayashida T (2008). Adsorption study of metal ions onto crosslinked seaweed Laminaria japonica, Bioresour. Technol. 99 : 32–37.
40 Lage-Pinto, F., Oliveira, J.G., Da Cunha, M., Souza, C.M.M., Rezende, C.E, Azevedo, R.A., Vitória, A.P. (2008). Chlorophyll a fluorescence and ultrastructural changes in chloroplast of water hyacinth as indicators of environmental stress. Environ. Exp. Bot., 64, 307-31.
41 Vassilev, A., Ivan, I., Elena, C. and Vassil K. (1995). Effect of cadmium stress on growth and photosynthesis of young barley (H. vulgare L.) plants. 2. Structural and functional changes in the photosynthetic apparatus. Bulg. J. Plant Physiol., 21(4): 12–21.
42 Hakmaoui A., Mohammed, A., Karoly, B. and Matilde, B. (2007). Copper and cadmium tolerance, uptake and effect on chloroplast ultrastructure. Studies on Salix purpurea and Phragmitesaustralis. Z. Naturforsch. 62., 417-426.
43 Liu. D., Li, T. Q., Li., Yang , X. E., Islam, E., Jin, X. F. and Mahmood, Q. (2008). Effect of Pb on Leaf Antioxidant Enzyme Activities and Ultrastructure of the Two Ecotypes of Sedum alfrediiHance. Russian Journal of Plant Physiology., 55(1): 68–76.
44 Mangabeira, P. A., Aluane S. F., Alex-Alan F. A., Vale´ria F. F., Emerson L., Va^nia L. S., Alberto J.S., Arno H. O., Marie F. G., Frederique B. and Delmira C. S. (2011). Compartmentalization and ultrastructural alterations induced by chromium in aquatic macrophytes. Biometals., 24: 1017–1026
45 Vitoria, A. P., Frederico, L.P., Leonardo B.C.S., Maura, C., Jurandi, G. O., Carlos, E.R., Cristina, M. M.S. and Ricardo, A.A. (2011). Structural and ecophysiological alterations of the water Hyacinth [Eichhorniacrassipes (Mart.) Solms] due to anthropogenic stress in Brazilian rivers. Brazilian Archives of Biology and Technology., 54 (5): 1059-1068.
46 Zhao., S., Xuzhu Y. and Jici Z. (2011). Lead-induced changes in plant morphology, cell ultrastructure, growth and yields of tomato. African Journal of Biotechnology, 10(50): 10116-10124.
47 Mahmood, Q., M., Zheng, P., Siddiqi, M.R., Islam, E.U., Azim, M.R. and Hayat, Y. (2005). Anatomical studies on water hyacinth (Eichhorniacrassipes (Mart.) (Solms) under the influence of textile wastewater. Journal of Zhejiang University Science., 6B(10):991-998
48 Kasim, W.A. (2006). Changes induced by copper and cadmium stress in the anatomy and grain yield of Sorghum bicolor (L.) Moench. International Journal of Agriculture and Biology., 8(1): 123-128.
49 Sridhar, B.B.M., Fengxiang, X.H., Susan, V.D., David, L.M. and Yi, S. (2007). Effect of Zn and Cd accumulation on structural and physiological characteristics of barley plants. Brailian Journal of Plant Physiology. 19(1): 15-22.
50 Salah, M.H.G. and Omar, A. A. (2013). Effect of copper and cadmium on germination and anatomical structure of leaf and root seedling in maize (Zea mays L). Australian Journal of Basic and Applied Sciences., 7(1): 548-555.
51 Gomes, M.P., Teresa, C.L.M., Mariana, O.G.N., Evaristo, M.C. and Ângela, M.S. (2011). Ecophysiological and anatomical changes due to uptake and accumulation of heavy metal in Brachiariadecumbens. Sci. Agric., 68 (5):566-573.