Journal of Elementology ISSN 1644-2296

Goher M.E., Abd El-Monem A.M., Abdel-Satar A.M., Ali M.H., Hussian A.M., Napiórkowska-Krzebietke A. 2016. *Biosorption of some toxic metals from aqueous solution using non-living algal cells of Chlorella vulgaris*. J. Elem. 21(3): 703-714. DOI: 10.5601/jelem.2015.20.4.1037

BIOSORPTION OF SOME TOXIC METALS FROM AQUEOUS SOLUTION USING NON-LIVING ALGAL CELLS OF *CHLORELLA VULGARIS*

Mohamed E. Goher[1], Ahmed M. Abd El-Monem[1], Amaal M. Abdel-Satar[1,2], Mohamed H. Ali[1], Abd-Ellatif M. Hussian[1], Agnieszka Napiórkowska-Krzebietke[3]

[1]Fresh Water and Lakes Division
National Institute of Oceanography and Fisheries (NIOF), Cairo, Egypt
[2]Chemistry Department
Hail University, KSA
[3]Department of Hydrobiology
Inland Fisheries Institute, Olsztyn, Poland

ABSTRACT

Dead cells of *Chlorella vulgaris* were used experimentally to remove cadmium (Cd^{2+}), copper (Cu^{2+}) and lead (Pb^{2+}) ions from aqueous solution under various conditions of pH, biosorbent dosage and contact time. *C. vulgaris* was isolated from Lake Manzala (Egypt) and then cultivated outdoors, in growth media, maintaining pH of nearly 7.5. After cultivation, the cells were harvested by coagulation and prepared to make an experiment with different variants of pH, contact time and adsorbent dose. The adsorption specific characteristics of *C. vulgaris* were examined using Fourier Transformation Infrared Spectroscopy (FTR) and Scanning Electron Microscopy (SEM). The FTIR and SEM data showed vibration frequency changes for the peaks corresponding to the surface functional groups and morphological changes of *C. vulgaris*, respectively, after its exposure to metal ion solution. The maximum removal efficiency for copper and lead at ambient room temperature was recorded at pH 5 and 20-minute contact time, while the contact time of 120 minutes at pH 6 created the most suitable conditions for cadmium biosorption. On the other hand, 2 g dm^{-3} of *C. vulgaris* under optimum conditions was sufficient to remove 95.5%, 97.7% and 99.4% of Cd^{2+}, Cu^{2+} and Pb^{2+}, respectively, from a mixed solution of 50 mg dm^{-3} of each metal ion. The removal trend was in order of $Pb^{2+} > Cu^{2+} > Cd^{2+}$. The results suggested that the biomass of *C. vulgaris* is an extremely efficient and environmentally friendly biosorbent for the removal of Cd^{2+}, Cu^{2+} and Pb^{2} from aqueous solutions, which may be applied on a large, industrial scale.

Keywords: lead, cadmium, copper, removal rate, adsorption capacity.

---

dr Mohamed E. Goher, National Institute of Oceanography and Fisheries (NIOF), Inland Water and Aquaculture Division, 101 El Kasr Aini St., Cairo, Egypt, e- mail*: smgoher@yahoo.com*

INTRODUCTION

Metal pollution is one of the most critical environmental problems due to metal accumulation throughout the food chain (Wai Lynn Aung et al. 2012). Heavy metals polluting aqueous solutions have become a serious threat and a great environmental concern as they are non-biodegradable and will persist in nature (Gautam et al. 2015). Singh et al. (2011) observe that Cd, Cu, Ni, Pb and Zn are especially common metals that tend to accumulate in organisms, causing numerous diseases and disorders. Toxic metal ions can cause physical discomfort and sometimes life-threatening illnesses, which can lead to some irreversible damage to the vital body systems.

Several methods for the removal of heavy metals from aquatic (e.g. Duda-Chodak et al. 2012) or other environments (e.g. polluted soils; Wińska-Krysiak et al. 2015) have been developed. Conventional treatment technologies for heavy metal removal are not economic and can generate huge quantities of toxic chemical sludge. Biosorption of heavy metals by some metabolically inactive, non-living microbial or plant biomass is seen as an innovative and alternative technology used to extract these pollutants from aqueous solutions (e.g. Raize et al. 2004, Ahluwalia, Goyal 2007, Saravanan et al. 2011, Deepa, Suresha 2014).

Microalgae are fundamental to most freshwater and marine ecosystems. Recently, the use of microalgae in biotechnology has been rapidly increasing. These organisms were widely used in risk assessments and, following the development of some environmental regulations concerning metals, they serve as sensitive indicators of environmental change as well as a biological sensor for detecting potentially toxic effects of selected heavy metals (Durrieu et al. 2011).

Chlorella vulgaris Beyerinck [Beijerinck] (Chlorophyta) is a planktonic unicellular green alga that can be grown in fresh and marine waters, to be later used as food resource or in the cosmetics industry, aquaculture, pharmaceutical industry, etc., owing to its numerous health benefits. Additionally, it can be used for removal of various heavy metal ions from contaminated water (Wai Lynn Aung et al. 2012).

This study aimed to determine the use of inexpensive natural material such as dead algal cells of *C. vulgaris* to remove three ions of toxic metals (Cd, Cu and Pb) from an aqueous system, and to explore the effect of various parameters such as contact time, pH, and biomass dosage on the extraction process.

MATERIAL AND METHODS

Culture condition, cultivation and harvesting

The microorganism used in the present study was *C. vulgaris*. It was isolated from the northern side of Lake Manzala (brackish coastal lake), the largest of the Delta Lakes in Egypt. This lake was chosen because of the *Chlorella* presence and very high contamination with heavy metals (Ali 2008). The amount of these elements discharged into the lake together with treated sewage has been found to exceed the national water quality threshold values.

Isolation and purification were achieved by dilution and the planting technique. Enriched seawater medium according to Sen et al. (2005) was used for algal cultures. The algae were grown in 500 ml flasks containing 250 ml of enriched seawater medium. The culture flasks were aerated by an air pump and incubated at 25 ± 1°C under continuous illumination provided by a daylight fluorescent tube generating light of the intensity of 80 μmol photon m^{-2} s^{-1} (Kuhl, Lorenzen 1964). The pH was adjusted to 7.5 with 1N NaOH or HCl.

Cells *of C. vulgaris* (lag phase) were transferred, adapted in fresh culture medium and then cultivated outdoors in the growth media with ammonium sulfate NH_4 SO_4 – 0.05 g dm^{-3}, urea CO $(NH_2)_2$ – 0.2 g dm^{-3}, T-super P_2O_5 – 0.05 g dm^{-3} made up in distilled water. The pH of the media was maintained at nearly 7.4 by aeration.

The cultures were prepared in two Erlenmeyer flasks (2 dm^3) containing 1500 cm^3 of the culture medium. When the required density of algae was reached, the algae from 2 flasks were tranferred to a 20 dm^3 glass tank and their cultivation continued. Each batch was cultivated for one week.

The cells were harvested by coagulation with sodium hydroxide and filtered through Whatman cellulose nitrate membrane filters with the pore size of 45 μm, after which they were washed with deionized water. After washing, the biomass was dried at 105°C for 48 h, ground in an electric mill and passed through a 100 mesh size sieve. Thus, uniform powdered biomass material was produced and stored in a refrigerator.

Adsorbent characterization

The adsorption specific characteristics of *C. vulgaris* have been examined. The surface area and the pore volume of dry *C. vulgaris* samples were determined by a Quantachrome instrument, model NOVA 3200, with surface area analyzers using N_2 as adsorbate at -196°C (i.e. the liquid nitrogen temperature) – Table 1. Dried *C. vulgaris* surface was submitted (after and before sorption) to diffuse Fourier-transform infrared analysis (FTIR) in order to determine qualitatively the functional groups. To this aim, a JASCO FT/IR-6300 FTIR spectrometer, set in the frequency range from 4000-400 cm^{-1}, was

Table 1

Physical properties of dried *Chlorella vulgaris* samples ($n = 3$)

Physical properties	
Density	0.43 g cm^{-3}
Bulk density	0.813 g cm^{-3}
Surface area	16.2 m^2 g^{-1}
Pore volume	0.0116 cc g^{-1}

applied. Each sample was mixed with KBr crystals and pelletized under the pressure of 150 kg cm^{-2} so as to explore the chemical characteristics of *C. vulgaris* significant for biosorption.

The Fourier Transformation Infrared Spectroscopy (FTIR) analysis was carried out in order to identify different functional groups present in *C. vulgaris*, which were responsible for the adsorption process, and to detect possible interaction between the metals and the functional groups. Scanning electron microscopy (SEM) was carried out on *C. vulgaris* samples to study the surface morphology before and after adsorption, using a JEOL- JSM 6510LV scanning electron microscope. SEM has a high resolution, making higher magnification possible for densely spaced materials.

Adsorption study

The batch equilibrium experiment was performed as a function of pH, contact time and adsorbent dose. 1000 mg dm^{-3} of analytical grade solutions of the metals Cd, Cu and Pb from Sigma-Aldrich were used to prepare mixed synthetic wastewater solutions, containing 50 mg dm^{-3} of each metal, Cd, Cu and Pb, and each stock was diluted with deionized water. Then, batches of 50 cm^3 of such synthetic water were transferred to 150 cm^3 flasks with different amounts of powdered *C. vulgaris.* 0.1 N NaOH and HCl were used to adjust the pH values. The flasks were magnetically agitated at a constant speed of 200 rpm, at ambient temperature. The adsorbent samples were filtered and concentrations of the metal ions in the remaining solutions were determined using a GBC atomic absorption reader (a SavantAA AAS model with a GF 5000 Graphite Furnace).

The removal rate of heavy metal ions was calculated from the following formula:

$$R = \left(\frac{C_o - C_t}{C_o} \right) \cdot 100.$$

The adsorption capacity or the removal efficiency of *C. vulgaris* was calculated using the equation:

$$q_e = (C_o - C_t) \cdot \frac{v}{m},$$

where: R – removal percentage of metal (%), C_o – the initial concentration of metal in the solution (mg dm^{-3}), C_t – the equilibrium concentration of metal in the solution (mg dm^{-3}) after t – a period of time; v – the volume of solution in oscillated flask (dm^3), m – the mass of the of powdered *C. vulgaris* in the flask (g) and q_e – the adsorption capacity, mg of metal adsorbed per g of biosorbent (METCALF, EDDY 2003).

Statistical analysis

The effects of pH and adsorbent dosage on the removal of heavy metals from aqueous solution with *C. vulgaris* were tested with non-linear regression. Furthermore, the significance of changes in the removal rate of heavy metals relative to pH and absorbent dosage were tested with the non-parametric Kruskal-Wallis test (because data were not normally distributed). It was assumed that statistically significant changes were at the 0.05 significance level.

RESULTS AND DISCUSSION

The peaks appearing in the FTIR spectrum (Figures 1*a*,*b*) were assigned to various functional groups according to their respective wave numbers (GHONIEM et al. 2014). The peaks corresponding to the functional groups of *Chlorella vulgaris* showed some vibration frequency changes after treatment with Cd, Cu and Pb ions, which may be attributed to possible interactions between the metals and the functional groups (Figure 1*b*). After the exposure of the cells of *C. vulgaris* to metal sorption, the following were observed: the absorption peak at 3413.39 cm^{-1} corresponding to an overlap between the –N-H groups and –OH groups shifted to 3428.81 cm^{-1}; 2937.06 cm^{-1} corresponding to the C-H alkane groups shifted to 2964.05 cm^{-1}. Also, the peak of 1644.02 cm^{-1} corresponding to the –C=C groups shifted to 1644.98 cm^{-1}; 1527.35 cm^{-1} corresponding to the N-O groups shifted to 1479.13 cm^{-1} after the loading of metals. This observation indicates the involvement of these functional groups in the biosorption process. Our findings coincide with the results of metal biosorption with non-living algal cells (MURPHY et al. 2007) or even other adsorbents, e.g. garden grass (ABBAS et al. 2014). It is noteworthy that after the biosorption process some peaks were shifted and other peaks disappeared, and further new peaks were detected suggesting that binding occurred on the surface of *C. vulgaris*.

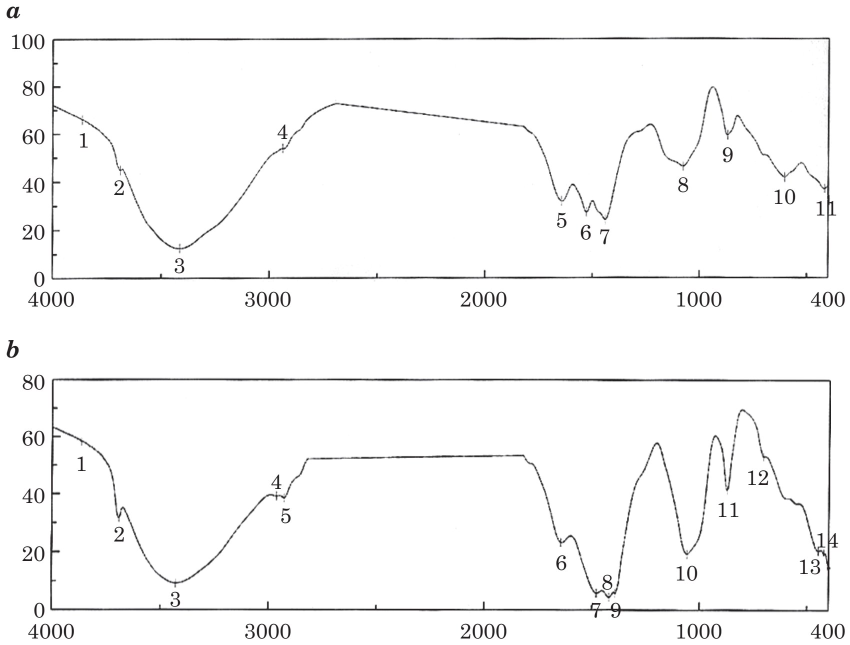

Fig. 1. FTIR spectrum of *Chlorella vulgaris* (*a*) before and (*b*) after biosorption of metals

The present study of *C. vulgaris* surface morphology using the SEM technique showed distinct changes between before and after the adsorption processes (Figures 2*a*,*b*). The cells before adsorption were smooth with well -defined dimensions. After biosorption, morphological changes occurred, probably due to the exposure of a heavy metal to dead algal cells, and they became distended and wrecked with a sinuosus surface. Similar changes were observed in other algae (Raize et al. 2004, Saravanan et al. 2011). It was clear that Cd, Cu and Pb had been sorbed on the active sites of *C. vulgaris* cells.

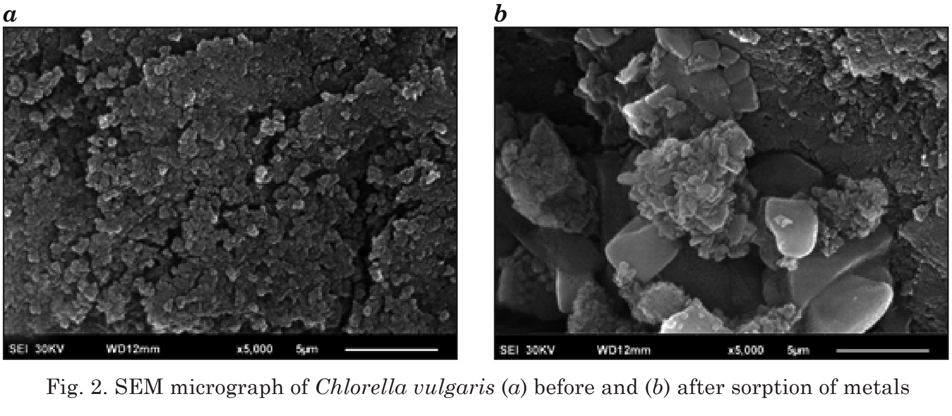

Fig. 2. SEM micrograph of *Chlorella vulgaris* (*a*) before and (*b*) after sorption of metals

Effect of pH

The replicates of 50 cm^3 synthetic solution samples containing 50 mg dm^{-3} of Cu, Cd and Pb were put in flasks at pH values of 2, 3, 4, 5 and 6 at room temperature, respectively. 0.05 g *C. vulgaris* was added in these flasks for the purpose of determining the removal rate of cations after 120 min of oscillation adsorption**.** Similar conditions were set for blank solution.

The pH is one of the most important parameters of the biosorption of heavy metals (Aksu 2001) or other compounds (Shaheen et al. 2012, Mann, Mandal 2014). The biosorption of Cd, Cu and Pb by *C. vulgaris* at different pH values is illustrated in Figure 3. The removal rate was mainly controlled by the pH of a solution (the coefficient of determination $R^2 > 0.9$ for all metals implicating very strong relationships), namely, the removal efficiency of metals ions increased from 22.7% and 29.9% to 87.7% and 96.4% for Cu and Pb respectively, while the pH increased from 2 to 5. For Cd, it increased from 13.7% to 84.7% when the pH rose from 2 to 6. This indicates that the optimal pH for removal Cu^{+2} and Pb^{+2} was 5, and pH 6 was the best for Cd^{+2}. The sorption process decreased with any further increase of pH (> 6). According to Farooq et al. (2010), the decrease of metal biosorption at higher pH values may be related to the precipitation of metals in the form of metal hydroxide due to the reaction of metal ions with OH$^-$ (where the amount of OH$^-$ increases at high pH). On the other hand, when the pH value is low (pH = 2), the H$^+$ concentration is high; H$^+$ will competitively exchange cations on the surface of algae. As the solution's pH increases, the process is reversed, metal ions will start to replace hydrogen ions. Furthermore, when the pH of the adsorbing medium increased from 2 to 6, there was a corresponding increase in the de-protonation of the adsorbent surface, leading to a decrease in H$^+$ ions on the adsorbent surface. This creates more negative charges on the adsorbent surface, which favours the adsorption of positively charge species (Abdus-Salam, Adekola 2005). Similar results were found by

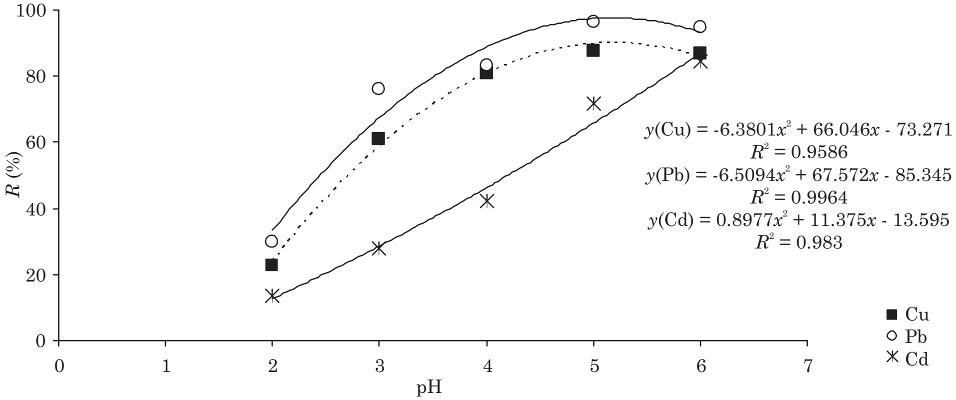

Fig. 3. Effect of pH on removal of heavy metals from aqueous solution using 1 g dm^{-3} *Chlorella vulgaris* at 120 min retention time

Goher et al (2015), who reported that at pH higher than 3-4, carboxylic groups (– COOH) are de-protonated and negatively charged. Consequently, the attraction of positively charged metal ions would be enhanced (Norton et al. 2004). Ho (2005) recorded that pH values in the range of 4 to 7 were adequate for binding lead, for which pH 4.9 is the optimum value for biosorption with tree fern. Edris et al. (2012) obtained positive results of lead and cadmium biosorption using *C. vulgairs* at pH adjusted to 7, and Fraile et al. (2005) proved a greater heavy metal uptake at a higher pH.

Effect of adsorbent dose

The batch experiments with different adsorbent doses (0.25, 0.5, 1, 1.5, 2, 3 and 4 g dm^{-3} of test solution) were conducted to determine their effect on the removal of Cu^{2+}, Pb^{2+} and Cd^{2+} (Figures 4*a*,*b*). Figure 4*a* shows that the removal efficiency increased from 32.4%, 33.4% and 23.8% to 97.7%, 99.4% and 95.5% for Cu^{2+}, Pb^{2+} and Cd^{2+}, respectively, as the adsorbent dosage increased from 0.25 g dm^{-3} to 2 g dm^{-3}. An additional increase of the amount of adsorbent did not change the removal efficiency. However, the removal efficiency was strongly related to the dosage levels (the coefficient of determination R^2 exceeded 0.9 for all the metals). Generally, it can be concluded that a higher dose of adsorbent significantly increased the removal percentage (Figure 5*a*; test $\text{K-W}_{(6;21)} = 14.7120$; $p = 0.0226$) similarly to pH (Figure 5*b*, test $\text{KW-H}_{(4;15)} = 10.4667$; $p = 0.0333$). This was because more surfaces and functional groups were available on the adsorbent with which the metals could interact (Onyeji, Aboje 2011). Furthermore, Figure 4*b* indicates that

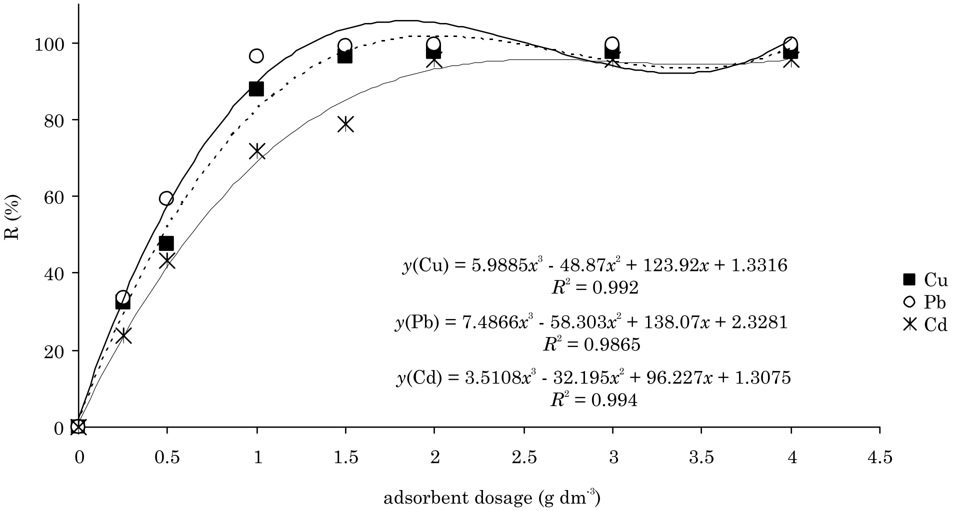

Fig. 4*a*. Effect of adsorbent dosage on the percentage removal of heavy metals with *Chlorella vulgaris* at pH 5 and 120 min retention time

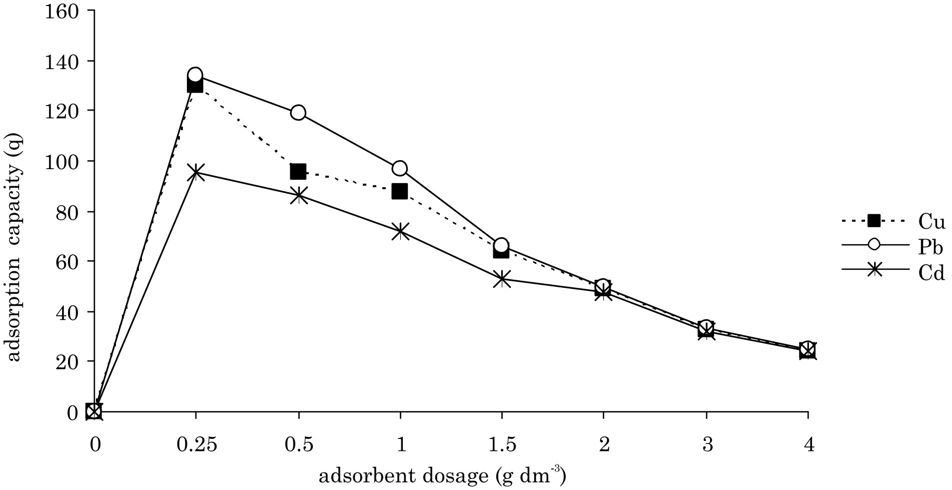

Fig. 4*b*. Effect of adsorbent dosage on the adsorption capacity of heavy metals with *Chlorella vulgaris* at pH 5 and 120 min retention time

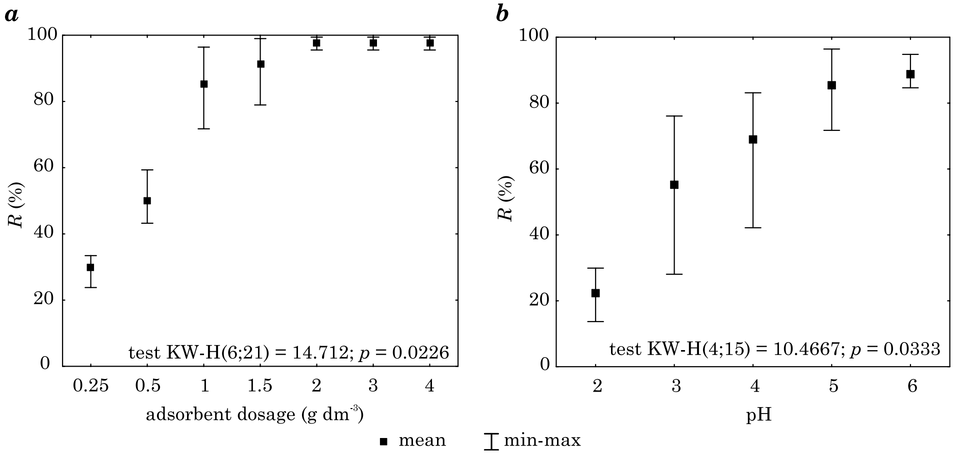

Fig. 5. Changes in the removal rate of heavy metals from aqueous solution with *Chlorella vulgaris* relative to absorbent dosage (*a*) and pH (*b*)

the adsorption capacity decreased from 129.7, 133.8 and 95.2 mg g^{-1} to 24.4, 24.9 and 23.9 mg g^{-1} for Cu^{2+}, Pb^{2+} and Cd^{2+}, respectively, as the adsorbent dose increased from 0.25 g dm^{-3} to 2 g dm^{-3}. The best adsorption capacity was recorded at the lowest adsorbent dosage. Similar findings were given by FRAILE et al. (2005). The data are also in agreement with those of LI (2008), MOUSAVI et al. (2010) and EDRIS et al. (2012). They proved that some equilibrium adsorption capacities decreased along with the adsorbent mass/dose increase. This might be due to the presence of a higher density of solids that could lead to fewer opportunities for contacting all adsorption sites.

Effect of retention time

The variations of metals removal percentage at a different retention oscillation time with *C. vulgaris* are shown in Figure 6. Only 0.1 g of *C. vulgaris* were added to 10 flasks of 50 cm^3 synthetic solution at pH 5. The rapid reduction of the concentration of Cu and Pb in the solution was at the first 2 min (ca. 90%) and up to 20 min, whereby the removal rate reached up to 98% and 99%, respectively, whereas the complete Cd removal was achieved in 120 min. The fast removal at the beginning may be attributed to a larger adsorbent surface area being available for the adsorption of the metals as well as a high number of available adsorptive sites. The adsorption kinetics changed rapidly along with the contact time, after that they decreased. This was probably caused by the decrease in the concentration gradient between the initial concentration and the equilibrium concentration of the solution with the progress of the adsorption process and the metal ion absorption onto the adsorbent surface (LI 2008, LI, CHAMPAGNE 2009).

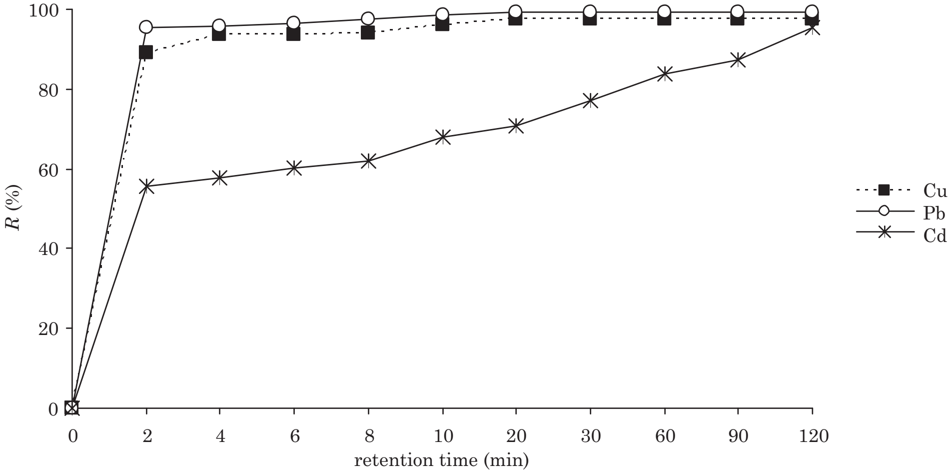

Fig. 6. Effect of the retention time on the removal of heavy metals from aqueous solution by using 2 g dm^{-3} sorbent dosage of *Chlorella vulgaris* at pH 5

CONCLUSION

The FTIR spectrum and differences in the surface morphology of biosorbent indicated that some binding took place after the biosorption process on the surface of *C. vulgaris*. The retention time and pH were the main factors in the removal of heavy metal ions from aqueous solution by non-living algal cells. Under the optimum conditions, a *C. vulgaris* dose of 2 g dm^{-3} was sufficient to give the maximum removal percentage. Biosorption affinity for the metal ions by *C. vulgaris* was in the order of $Pb^{2+} > Cu^{2+} > Cd^{2+}$. It could be concluded that dead cells of *C. vulgaris* are effective, low-cost and environmentally friendly materials and can be used efficiently for the elimination of heavy metal ions such as Cd^{2+}, Cu^{2+} and Pb^{2+} from aqueous solutions.

REFERENCES

Abbas H., Sulaymon A.H., Ahmed A., Mohammed A.A., Tariq J., Al-Musawi T.J. 2014. *Biosorption of cadmium ions onto garden grass.* J. Engin., 04(01): 16-25.

Abdus-Salam N., Adekola F.A. 2005. *The influence of pH and adsorbent concentration on adsorption of lead and zinc on a natural goethite.* Afr. J. Sci. Technol., 6: 55-66.

Ahluwalia S.S., Goyal D. 2007. *Microbial and plant derived biomass for removal of heavy metals from wastewater.* Biores. Technol., 98: 2243-2257.

Aksu Z. 2001. *Equilibrium and kinetic modelling of cadmium (II) biosorption by C. vulgaris in a batch system: effect of temperature.* Separ. Purif. Tech., 21: 285-94.

Ali M.H.H. 2008. *Assessment of some water quality characteristics and determination of some heavy metals in Lake Manzala, Egipt.* Egypt. J. Aquat. Biol. Fish., 12(2): 133-154.

Deepa C.N., Suresha S. 2014. *Biosorption of lead (II) from aqueous solution and industrial effluent by using leaves of Araucaria cookii: Application of response surface methodology.* J. Environ. Sci., Toxicol. Food Technol. (IOSR-JESTFT), 8(7): 67-79.

Duda-Chodak A., Tarko T., Milotta K. 2012. *Applicability of different kinds of yeast biomass to lead removal from water.* J. Elem., 17(1): 7-18. DOI: 10.5601/jelem.2012.17.1.01

Durrieu C., Guedri H., Fremion F., Volatier L. 2011. *Unicellular algae used as biosensors for chemical detection in the Mediterranean lagoon and coastal waters.* Res. Microbiol., 162: 908-914.

Edris G., Alhamed Y., Alzahrani A. 2012. *Cadmium and lead biosorption by Chlorella vulgaris.* Water Technol. Conf., IWTC 16 2012, Istanbul, Turkey, 1-12.

Farooq U., Kozinski J., Ain Khan M., Athar M. 2010. *Biosorption of heavy metal ions using wheat based biosorbents – A review of the recent literature.* Biores. Technol., 101: 5043-5053.

Fraile A., Penche S., González F., Blázquez M.L., Muñoz J., Ballester A. 2005. *Biosorption of copper, zinc, cadmium and nickel by Chlorella vulgaris.* Chem. Ecol., 21(1): 61-75. DOI: 10.1080/02757540512331334933

Gautam R.K., Soni S., Chattopadhayaya M. C., 2015. *Functionalized magnatic nanoparticles for enviromental remediation*, In: *Handbook of research on diverse applications of nanotechnology in biomedicine, chemistry, and engineering.* Soni S., Salhotra A., Suar M. (Eds). USA, IGI Global, 518-550.

Ghoniem M.M., El-Desoky H.S., El-Moselhy K.M., Amer A., Abo-El-Naga E.H., Mohamedein L.I. and Al-Prol A.E. 2014. *Removal of cadmium from aqueous solution using green marine algae, Ulva lactuca.* Egypt. J. Aquat. Res., 40(3): 235-242.

Goher M.E., Hassan A.M.A., Abdel-Moniem I.A.I., Fahmy A.H.A., Abdo, M.H., El-sayed S.M.M. 2015. *Removal of aluminum, iron and manganese ions from industrial wastes using granular activated carbon and Amberlite IR-120H, Egypt.* Egypt. J. Aquat. Res., 41(2): 155-146.

Ho Y.S. 2005. *Effect of pH on lead removal from water using tree fern as the sorbent.* Biores. Technol., 96: 1292-1296.

Kuhl A., Lorenzen H. 1964. *Handling and culturing of Chlorella* In: *Methods of cell physiology.* Preston D.M. (ed.). Academic Press, London, 1: 159-187.

Li C. 2008. *Batch and bench-scale fixed-bed column evaluations of heavy metal removals from aqueous solutions and synthetic landfill leachate using low-cost natural adsorbents* (M.Sc. thesis in Engineering). Queen's University Kingston, Ontario, Canada, 1-332.

Li C., Champagne P. 2009. *Fixed-bed column study for the removal of cadmium (II) and nickel*

(II) ions from aqueous solutions using peat and mollusk shells. J. Hazard. Mater., 171(1-3): 872-878. DOI: 10.1016/j.jhazmat.2009.06.084

MANN S., MANDAL A. 2014. *Removal of fluoride from drinking water using sawdust.* Int. J. Engin. Res. Appl., 4(7): 116-123.

METCALF & EDDY 2003. *Wastewater engineering: Treatment and reuse.* In: The McGraw Hill series in civil and environmental engineering (4th edition), McGraw-Hill, New York, pp. 1149 and 1819.

MOUSAVI H.Z., HOSSEYNIFAR A., JAHED V., DEHGHANI S.A.M. 2010**.** *Removal of lead from aqueous solution using waste tire rubber ash as an adsorbent.* Braz. J. Chem. Eng., 27(1): 79-87.

MURPHY V., HUGHES H., MCLOUGHLIN P. 2007. *Cu(II) binding by dried biomass of red, green and brown macroalgae.* Water Res., 41: 731-740.

NORTON K., BASKARAN T., MCKENZIE T. 2004. *Biosorption of zinc from aqueous solutions using biosolids.* Adv. Environ. Res., 8(3-4): 629– 635.

ONYEJI L.I., ABOJE A.A. 2011. *Removal of heavy metals from dye effluent using activated carbon produced from coconut shell.* Int. J. Engin. Sci. Technol. (IJEST), 3(12): 8238-8246.

RAIZE O., ARGAMAN Y., YANNAI S. 2004. *Mechanisms of biosorption of different heavy metals by brown marine macroalgae.* Biotechnol. Bioeng., 87: 451-458.

SARAVANAN A., BRINDHA V., KRISHNAN S. 2011. *Studies on the structural changes of the biomass Sargassum sp. on metal adsorption.* J. Adv. Bioinf., 2: 193-196.

SEN B., ALP M.T., KOCER M.A.T. 2005. *Studies on growth of marine microalgae in batch cultures.* I. *Chlorella vulgaris (Chlorophyta).* Asian J. Plant Sci., 4(6): 636-638.

SHAHEEN S.M., DERBALAH A.S., MOGHANM F.S. 2012. *Removal of heavy metals from aqueous solution by zeolite in competitive sorption system.* Int. J. Environ. Sci. Develop., 3(4): 362-367.

SINGH R, GAUTAM N, MISHRA A, GUPTA R. 2011. *Heavy metals and living systems: An overview.* Ind. J. Pharmacol., 43(3): 246-253. DOI:10.4103/0253-7613.81505

WAI LYNN AUNG W.L., KYAW NYEIN AYE K. N., NWAY NAY HLAING N.N. 2012. *Biosorption of Lead (Pb^{2+}) by using Chlorella vulgaris.* Int. Conf. on Chemical engineering and its applications (ICCEA), September 8-9, Bangkok (Thailand).

WIŃSKA-KRYSIAK M., KOROPACKA K., GAWROŃSKI S. 2015. *Determination of the tolerance of sunflower to lead-induced stress.* J. Elem., 20(2): 491-502. DOI: 10.5601/jelem.2014.19.4.721