

# Bioremediation of metal ions from aqueous solutions using Algae *Cladophora* sp.

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## ABSTRACT

The efficiency of *Cladophora* sp. algae, collected from wadi (channel) at Samail, Sultanate of Oman as a biosorbent for the removal of metallic ions from aqueous solutions was investigated. Fourier transform infrared spectrometer analysis (FTIR) showed the presence of carbonyl, ether, amine and alkyl chain stretch functional groups on *Cladophora* sp. algae. The point of zero charge (pHzpc) for the biosorbent was estimated at pH 7.22. pH increased with contact time and biomass showed higher adsorption capacity within first 24 hours. High metal concentration favors the adsorption of metal ions, calcium (Ca<sup>2+</sup>), copper (Cu<sup>2+</sup>) and magnesium (Mg<sup>2+</sup>) on to the surface of *Cladophora* sp. algae. Langmuir's equilibrium isotherm model fitted slightly better with the adsorption data. Copper and magnesium metal ions found to have more affinity towards *Cladophora* sp. biomass than calcium ions.

**Key words :** Water contamination, Bioremediation, Metal ions, Biomass, *Cladophora* sp. algae

## Introduction

Wastewater treatment and reuse was a significant alternate water resource in various parts of the world especially in the middle east, where the availability of water has been under extreme stress conditions. Due to various human and industrial activities, the ground and surface water were getting contaminated with trace heavy metals and other chemical and biological contaminants (Murtuza *et al.*, 2020a); Murtuza *et al.*, 2020b). Researchers developing various cost effective technologies to treat the trace heavy metals present in the wastewater. Nanoparticles can act as effective sorbents for the removal of heavy metal ions at low concentration from wastewater due to their structural properties like high selectivity and adsorption capacity (Mallikarjunaiah *et al.*, 2020). Yang *et al.*, (2019) sys-

tematically reviewed on the applications of various novel nanomaterials for the removal of heavy metal ions from the wastewater. These includes carbon-based nanomaterials, zero-valent metal, metal-oxide based nanomaterials, and nanocomposites. Their efficiency, limitations, and advantages were compared and discussed. The removal of heavy metals from industrial electroplating wastewater using two different nano adsorbents: purified carbon nanotubes (P-CNTs) and polyhydroxybutyrate functionalized carbon nanotubes (PHB-CNTs) has been investigated. Both nano-adsorbents gave optimum contact time, equilibrium time, optimum dosage, and pH of 10 minutes, 70 minutes, 20 mg, and 5.63–5.65 respectively. The heavy metals removal efficiencies by the nano-adsorbents followed the order of PHB-CNTs>P-CNTs based on ion exchange and electrostatic forces mechanism (Bankole *et al.*, 2019).

The use of agricultural waste to remove heavy metals has exhibited many economic advantages and high removal efficiency due to the presence of different functional groups on its surfaces (Hayder *et al.*, 2020). The physicochemical and morphological properties and the sorption capacities of discarded mushroom-stick biochar (DMB) prepared at different pyrolysis temperatures from 300 °C to 800 °C has been investigated. This study provided a promising way not only to recycle discarded mushroom sticks into feasible heavy metal adsorbents, but also for potential utilization of agriculture waste (Wang *et al.*, 2019). The efficiency of the coupling of infiltration-percolation process with adsorption on activated carbon in the removal of heavy metals contained in urban wastewater effluents has been investigated. Several laboratory experiments made it possible to distinguish the optimum quantity of powdered activated carbon necessary to remove a large range of heavy metals. Results showed that the equilibrium of the adsorption was reached very quickly for cadmium ( $\text{Cd}^{2+}$ ), i.e., after 15 min of contact with the activated carbon. On the other hand, the equilibrium of zinc ( $\text{Zn}^{2+}$ ), lead ( $\text{Pb}^{2+}$ ) and copper ( $\text{Cu}^{2+}$ ) was achieved after 45 min. The withdrawal rates were 70.77% for  $\text{Zn}^{2+}$ , 64.75% for  $\text{Pb}^{2+}$ , 67.07% for  $\text{Cu}^{2+}$  and 78.42% for  $\text{Cd}^{2+}$  (Bali and Tlili, 2019).

Bioremediation/Biosorption process consists of biological matrix as absorbent and involves rapid reversible binding of ions from aqueous solutions onto functional groups, that were present on the surface of the biomass with alone or combination of adsorption, ion exchange, complexation and precipitation mechanisms (Davis *et al.*, 2003). Low cost, high efficiency, less sludge formation, regeneration was some of the advantages in biosorption process compare to conventional treatment methods (Marina *et al.*, 2019). Kratochvil and Volesky (1998) indicated that presence of chelating functional groups over the surface of biological materials leads to greater affinity towards metal ions. Bacteria, cyanobacteria, algae (including microalgae, macro algae, seaweeds), yeasts, fungi, and lichensexhibits considerable good performance for the removal and recovery of metal ions in a cost-effective process (Izabela *et al.*, 2013). Spent mushroom substrate (SMS) of agaricus bisporus cultivation was used as a bioremediating agent for the removal of heavy metals present in industrial wastewater. The performance of SMS bioreactors with different groups

of heavy metals at various concentrations were analyzed. It was reported that 80 to 98% of all contaminants has been removed with 5 kg of SMS at hydraulic retention times of 10 and 100 days (Marina *et al.*, 2019). Igiri *et al.* (2018) through their review studies critically evaluated the bioremediation capacity of microorganisms (bacteria, fungi, biofilm, algae, genetically engineering microbes and immobilized cells) in the context of environmental protection. According to their evaluation, the use of biofilm has showed synergetic effects with many fold increase in the removal of heavy metals as sustainable environmental technology. De-juCao *et al.* (2015) investigated the effects of lead concentration on the growth, bioaccumulation, and anti-oxidative defense system of green algae, *Cladophora*. They concluded that it can be a choice organism for the phytoremediation of lead polluted coastal areas. The presence of metal ions at an elevated level behind the permissible limits will be toxic and create health hazards. Proper treatment of metal ions presents in aqueous medium, in an economical and environment friendly manner was a challenging task. The bioremediation of wastewater using algae showed promising means of sequestering the heavy metals and delivering an improved quality of water for discharge to the environment (Roberts *et al.*, 2018). In the present research work, an attempt was made to investigate the efficiency of locally available algal biomass, *Cladophora sp.* for removal of metal ions, calcium, copper and magnesium present in aqueous solutions.

## Materials and Methods

### Identification and preparation of algae

The algae used in the present study was collected from wadi Samail located at AlDhakhliyah region of Sultanate of Oman. Identification of algae species was carried out using Zeiss-Axiovert 25 Inverted microscope. The algae species identified was *Cladophora sp.*, which was filamentous green algae that consists of 6 main branches in the cell as shown in Fig. 1.

The algae samples were collected along with the water source in a glass vessel. It was washed with distill water to remove the impurities and dried at room temperature for 24 hours. Dried algae were then grinded using a mixer grinder into micro par-



Fig. 1. Microscopic image of *Cladophora sp.*

ticles, which was subsequently used in series of experiments.

### Metal ions stock solution

Metal stock solutions of 2000 mg/L were prepared by dissolving the respective salts, cupric chloride-dihydrate ( $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ ), magnesium chloride-hexahydrate ( $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ ) and calcium chloride ( $\text{CaCl}_2$ ) in distilled water. Stock solution of 0.1N NaOH solution, 0.1 N HCl solution and 0.1M NaCl solution were also prepared and used in the experiments. The chemicals and reagents was analytical grade and used as received without further purification.

### pH and concentration measurements

The pH of the metal solution in contact with the algal biomass was recorded at 24 hours, 3 day and 5 days of contact time using pH meter (JENWAY 3520). Metal ion concentrations were measured using varianspectra 220FS atomic absorption spectrometer by standard procedures.

### Characterization of algae

The Fourier transform infrared spectrometer (FTIR) analysis was carried out before and after the metal biosorption on *Cladophora sp.* The functional groups on the surface of algae and the adsorption of the metal ions on the functional groups over its surface was evaluated.

## Results and Discussion

### Determination of point of zero charge (pHzpc)

0.04 g of *Cladophora sp.* biomass was taken in a cen-

trifuge tubes with different volumes of NaCl, HCl and NaOH, combined add up to a total volume of 10 mL. The tubes were kept for incubation and pH was noted at different intervals up to 5 days. A graph was plotted as shown in the Figure 2 between the volumes of HCl and NaOH taken vs. the pH measured at each interval. The point of intersection of curves was recorded as the point of zero charge (pHzpc) on the surface of *Cladophora sp.* (Sharma *et*

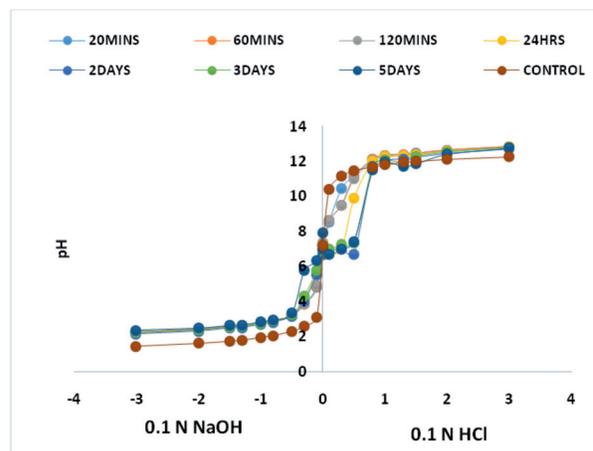


Fig. 2. pH zpc of dried *Cladophora sp.*

*al.*, 2009). 7.22 was the pHzpc on the surface, which indicates that the active sites on the surface of dried *Cladophora sp.* was positively charged at a pH lower than that of pHzpc.

### Infrared Spectra of *Cladophora sp.* before and after metal adsorption

FTIR analysis of *Cladophora sp.* showed the presence of functional groups, hydrogen bonded alcohols, alkyl chains ( $\text{CH}_3$  symmetric and  $\text{CH}_3$  asymmetric stretch), aldehydes, ketones, carboxylic acids, carboxylate ions, amides, fatty acids, ethers, and esters.  $\text{C}=\text{O}$ ,  $-\text{NH}_2$ , alkyl chains,  $\text{C}-\text{O}-\text{C}$  functional groups were present on the surface of the biomass, which

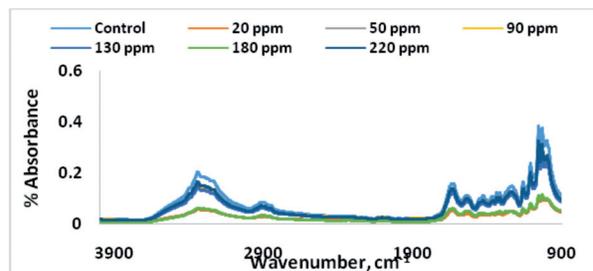


Fig. 3. Adsorption of  $\text{Cu}^{2+}$  on dried *Cladophora sp.* for 24 hours.

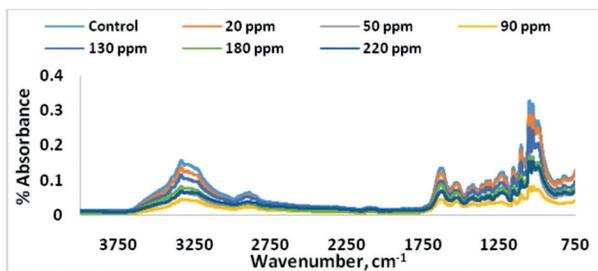


Fig. 4. Adsorption of Cu<sup>2+</sup> on dried *Cladophora sp.* for 3 days.

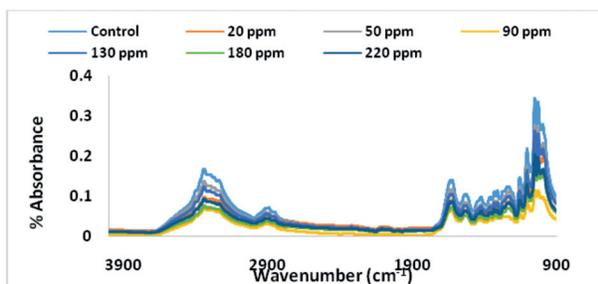


Fig. 5. Adsorption of Cu<sup>2+</sup> on dried *Cladophora sp.* for 5 days.

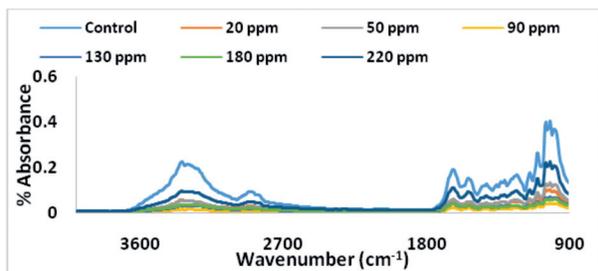


Fig. 6. Adsorption of Ca<sup>2+</sup> on dried *Cladophora sp.* for 24 hours.

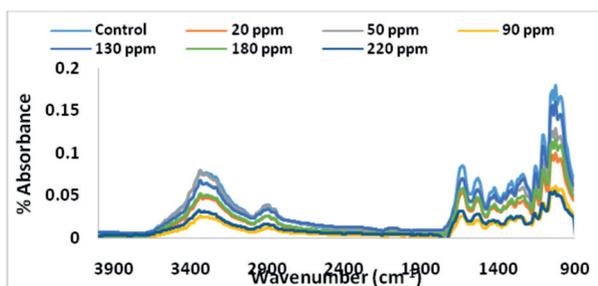


Fig. 7. Adsorption of Ca<sup>2+</sup> on dried *Cladophora sp.* for 3 days.

plays a significant role in attracting the metal ions. Figure 3 to 11 shows the loading of metal ions, copper, calcium and magnesium on functional groups of the algae at various concentrations ranging from 20 to 220 ppm. The peaks were mainly distributed in

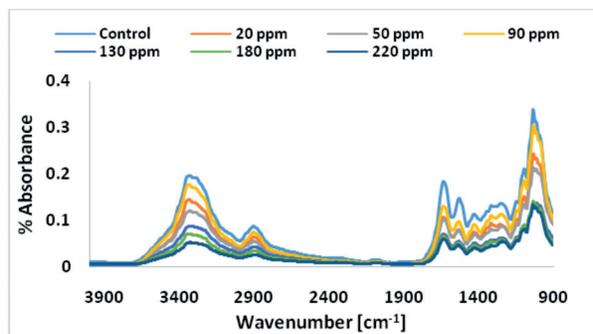


Fig. 8. Adsorption of Ca<sup>2+</sup> on dried *Cladophora sp.* for 5 days.

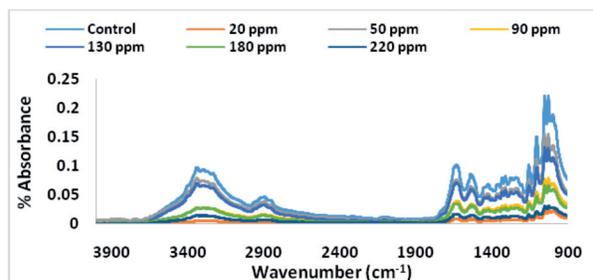


Fig. 9. Adsorption of Mg<sup>2+</sup> on dried *Cladophora sp.* for 24 hours.

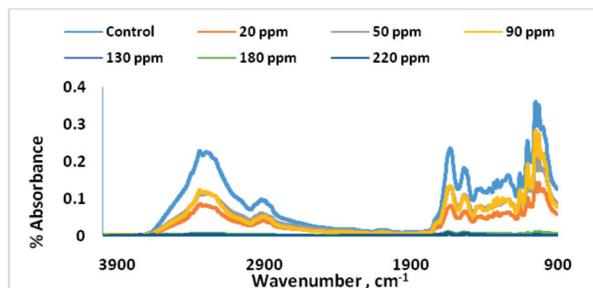


Fig. 10. Adsorption of Mg<sup>2+</sup> on dried *Cladophora sp.* for 3 days.

the range of 3600-2700 cm<sup>-1</sup> and 1900-900 cm<sup>-1</sup> wave numbers, which indicates C=O, NH<sub>2</sub>, alkyl chain stretch, C-O-C functions groups that were present on the surface of *Cladophora sp.* were the main candidates that attract and bind the metal ions.

**Effect of contact time and metal concentration**

0.015 mg of dried *Cladophora sp.* was kept in contact with various concentrations of metal solutions (20 ppm, 50 ppm, 90 ppm, 130 ppm, 180 ppm and 220 ppm) in batch experiments with constant volume of 15 mL. All the experimental tests were carried out at room temperature. The amount of metal adsorbed by the biomass, 'q' was evaluated using equation 1.

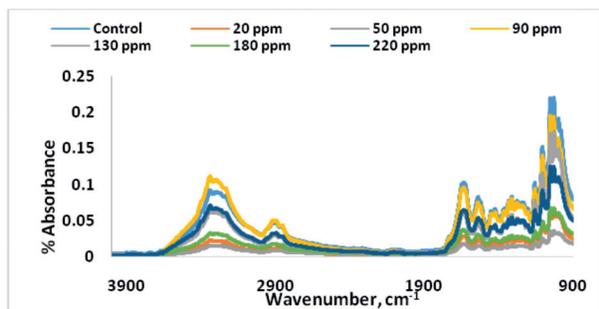


Fig. 11. Adsorption of Mg<sup>2+</sup> on dried *Cladophora* sp. for 5 days.

$$q = \frac{(C_o - C_f)V}{W} \quad \dots (1)$$

q: The amount of Cu<sup>2+</sup>, Ca<sup>2+</sup>, or Mg<sup>2+</sup> adsorbed by the algal biomass, *Cladophora* sp., mg/g.

C<sub>o</sub>: Initial concentration of metal ions in aqueous solution, mg/L.

C<sub>f</sub>: The final concentration of metal ions in aqueous solution, mg/L.

V: The volume of the metal solution, L.

W: The amount of biosorbent, g.

Figure 12–14 shows the metal ions, adsorption on *Cladophora* sp., for various concentrations at contact time of 24 hours, 3 days and 5 days respectively. The adsorption of metal ions showed an increasing trend at each interval of the incubation period.

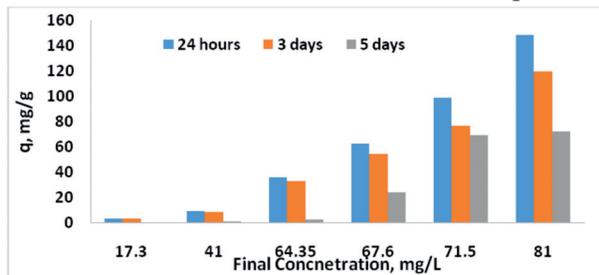


Fig. 12. Adsorption of Cu<sup>2+</sup> on dried *Cladophora* sp.

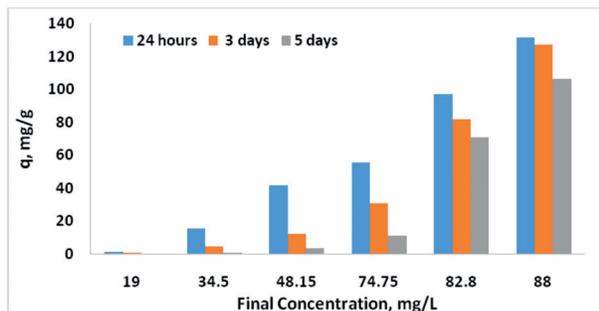


Fig. 13. Adsorption of Ca<sup>2+</sup> on dried *Cladophora* sp.

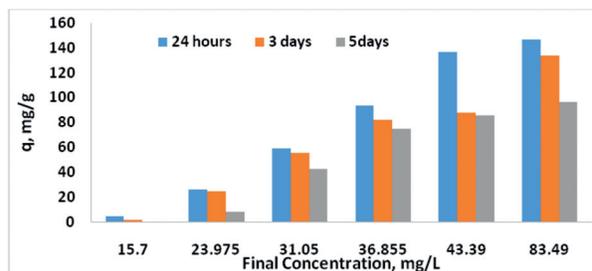


Fig. 14. Adsorption of Mg<sup>2+</sup> on dried *Cladophora* sp.

Maximum adsorption took place in the first day and as number of days increase the absorption trends slightly decreased which might be due to the saturation of available sites on the biomass cell surfaces.

### Variation of pH during bioprocess

The role of pH was very significant in biosorption process of heavy metals on biomass, as it effects the protonation of the functional groups on the surface of the biomass (Chang and Lee, 2011; Deng *et al.*, 2006). There was an increasing trend in the pH during biosorption of copper, calcium and magnesium as showed in Figures 15-17. This may be due to ion exchange mechanisms and shift in hydrolysis equilibrium of metal ions (Ying-Zhong Tang *et al.*, 2003). The cell wall of green algae consists of heteropolysaccharides that provides amino, carboxyl and sulfate groups (Margesin and Schinner, 2001). At

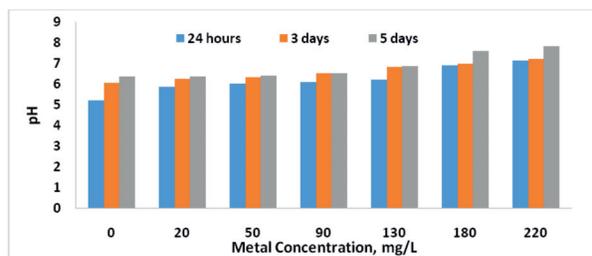


Fig. 15. Variation of pH during Cu<sup>2+</sup> adsorption on *Cladophora* sp.

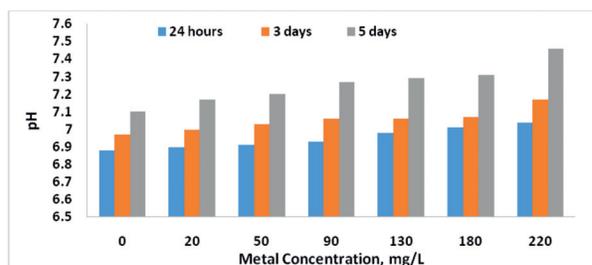


Fig. 16. Variation of pH during Ca<sup>2+</sup> adsorption on *Cladophora* sp.

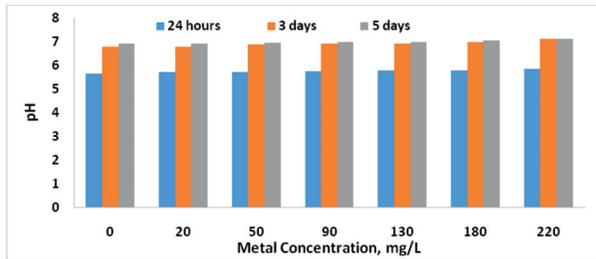


Fig. 17. Variation of pH during Mg<sup>2+</sup> adsorption on *Cladophora sp.*

lower pH, the ligands on the cell wall were protonated and restricts the approach of metal cations due to it repulsive forces. As there was an increase in the pH, more ligands get exposed and carry with them some negative charges with subsequent attraction of metal ions (Aksu and Kutsal, 1998). The effect of pH could be further explained with the relation between H<sub>3</sub>O<sup>+</sup> and metal ions. When the pH was lower, H<sub>3</sub>O<sup>+</sup> resides in the binding sites of the cell walls. As the pH value increase, the effect of H<sub>3</sub>O<sup>+</sup> gradually decreases and allow the metal ions with positively charge to take up the free binding sites (Deng *et al.*, 2006).

**Adsorption equilibrium models**

Adsorption isotherm models were used to represent the equilibrium relationship between the adsorbate adsorbed on the surface of biomass and the adsorbate present in the solution. Langmuir and Freundlich isotherm models were applied to experimental data to find the best fit as shown in Figures 18 and 19.

Langmuir and Freundlich equilibrium models were represented by the equation 2 and 3.

$$\frac{1}{q_e} = \frac{1}{Q_{max}} + \frac{1}{C_e(b * Q_{max})} \quad .. (2)$$

Where 'q<sub>e</sub>' was the quantity of adsorbed metal ions (mg/g) and 'C<sub>e</sub>' was the equilibrium concentration (mg/L). Q<sub>max</sub> represents the maximum adsorption and 'b' was the affinity between the metal ions and the biomass.

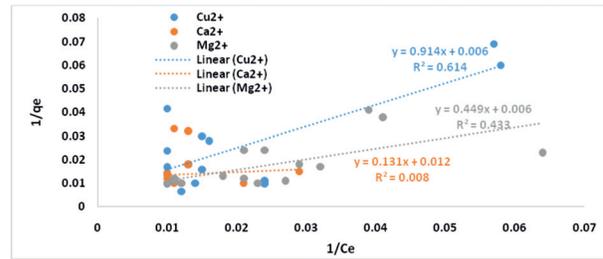


Fig. 18. Langmuir adsorption isotherms.

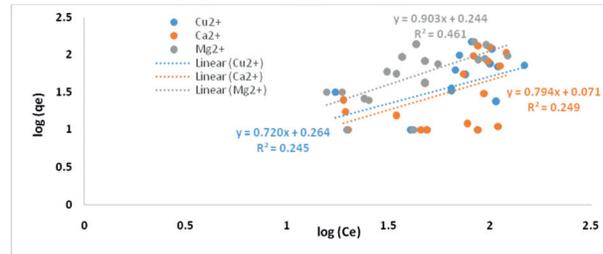


Fig. 19. Freundlich adsorption isotherms.

$$\log q_e = \log K + \frac{1}{n} \log C_e \quad .. (3)$$

Where K and n were the Freundlich equilibrium constants.

The constants obtained from both the models were showed in Table 1. Langmuir model seems to be slightly better fit for the metal ions adsorption on *Cladophora sp.* with high correlation relation coefficient (R<sup>2</sup>). In Langmuir equilibrium model, the maximum adsorption capacity (Q<sub>max</sub>) was 151.52 mg/g and the affinity of bisorbent(b) toward biomass was 0.092. In Freundlich equilibrium model, the higher adsorption volume (K) was 1.84 mg/g and higher adsorption strength of biomass (n) was 1.39. Copper and magnesium metal ions obtained higher values, which indicates its strong affinity towards *Cladophora sp.*

**Conclusion**

The adsorption of metal ions on *Cladophora sp.* with variation in metal concentration was investigated. pHzpc of biomass was 7.22 with various functional groups on the surface that attract the metals ions.

**Table 1.** Langmuir and Freundlich constants for metal ions

Metal Ion	Langmuir Constants			Freundlich Constants		
	Q <sub>max</sub>	b	R <sup>2</sup>	K	n	R <sup>2</sup>
Cu <sup>2+</sup>	151.52	0.007	0.615	1.84	1.39	0.245
Ca <sup>2+</sup>	82.64	0.092	0.008	1.18	1.26	0.249
Mg <sup>2+</sup>	151.52	0.015	0.433	1.76	1.11	0.462

Higher pH and high concentration of metal ions were favorable in adsorption process. Langmuir model showed slightly better fit for the adsorption of metal ions on biomass with copper and magnesium metals ions showing higher affinity towards the biomass.

## References

- Aksu, Z. and Kutsal, T. 1998. Determination of kinetic parameters in the biosorption of copper(II) on *Cladophora* sp., in a packed bed column reactor. *Process Biochemistry*. 33 (1): 7-13.
- Bali, M. and Tlili, H. 2019. Removal of heavy metals from wastewater using infiltration-percolation process and adsorption on activated carbon. *I. J. Environ. Sci. Tech.* 16 : 249-258.
- Bankole, M.T., Abdulkareem, A.S., Mohammed, I. A., Ochigbo, S.S., Tijani, J.O., Abubakre, O.K. and Roos, W.D. 2019. Selected heavy metal removal from electroplating wastewater by purified and polyhydroxybutyrate functionalized carbon nanotubes adsorbents. *Scientific Reports*. 9: 4475.
- Chang, S. and Lee, Y. 2011. The biosorption of heavy metals from aqueous solution by *Spirogyra* and *Cladophora filamentous* macroalgae. *Bioresource Tech.* 102 : 5297-5304.
- Davis, T. A., Volesky, B. and Mucci, A. 2003. A review of the biochemistry of heavy metal biosorption by brown algae. *Water Research*. 37 : 4311-4330.
- De-ju Cao, Xiao-dong Shi, HaoLi, Pan-pan Xie, Hui-min Zhang, Juan-wei Deng, and Yue-gan Liang. 2015. Effects of lead on tolerance, bioaccumulation, and antioxidative defense system of green algae, *Cladophora*. *Ecotoxicology and Environ. Safety*. 112: 231-237.
- Deng, L. Su, Y. Su, H. Wang, X. and Zhu, X. 2006. Biosorption of Copper (II) and Lead (II) from aqueous solutions by nonliving green algae *Cladophora fascicularis*: Equilibrium, kinetics and environmental effects. *Adsorption*. 12 : 267-277.
- Hayder, A.A., Kadhom, M.A. and Alminshid, A.H. 2020. Removal of heavy metals from wastewater using agricultural byproducts. *J. Water. Supply*. 69 (2): 99-112.
- Izabela, M. Katarzyna, C. and Anna, W. 2013. State of the Art for the Biosorption Process. *Appl Biochem Biotechnol*. 170 : 1389-1416.
- Igiri, B.E., Okoduwa, S.I.R., Idoko, G.O., Akabuogu, E.P. Adeyi, A.O. and Ejiogu, I.K. 2018. Toxicity and bioremediation of heavy metals contaminated ecosystem from tannery wastewater: A review. *J. Toxicology*. 16.
- Kratochvil, D. and Volesky, B. 1998. Biosorption of Cu from ferruginous wastewater by algal biomass. *Water Research*. 32 : 2760-2768.
- Mallikarjunaiah, S. Pattabhiramaiah, M. and Metikurki, B. 2020. Application of nanotechnology in the bioremediation of heavy metals and wastewater management. *Nanotech. Food. Agri. Environ.* 297-321.
- Marina, C.B., Ana, G.M., Eliseo, P.V.G. and Fernando, A.E. 2019. Bioremediation of wastewater to remove heavy metals using the spent mushroom substrate of *agaricusbisporus*. *Water*. 11 : 454.
- Margesin, R. and Schinner, F. 2001. Biodegradation and bioremediation of hydrocarbons in extreme environments. *Appl Microbiol Biotechnol*. 56 : 650-663.
- Murtuza Ali Syed, Mohammed Al Sawafi, Feroz Shaik, Mohammed Nayeemuddin. 2020a. Polyurethane Green Composites: Synthesize, Characterization and Treatment of Boron Present in the Oil Produced Water. *Int. J. Eng. Res. and Tech.* 13: 1866-1873.
- Murtuza Ali Syed, Amit Kumar Mauriya, Feroz Shaik. 2020b. Investigation of epoxy resin/nano-TiO<sub>2</sub> composites in photocatalytic degradation of organics present in oil-produced water. *Int. J. Env. Ana. Chem.* DOI: 10.1080/03067319.2020.1784889
- Roberts, D.A. Shiels, L. Tickle, J. Nys, R. and Paul, N.A. 2018. Bioremediation of aluminum from the wastewater of a conventional water treatment plant using the freshwater macroalga *oedogonium*. *Water*. 10: 626.
- Sharma, Y. Uma. and Upadhyay, S. 2009. Removal of cationic Dye from wastewater by adsorption on activated carbon developed from coconut coir. *Energy & Fuels*. 23 : 2983-2988.
- Wang, X. Li, X. Liu, G. He, Y. Chen, C. Liu, X. Li, G. Gu, Y. and Zhao, Y. 2019. Mixed heavy metal removal from wastewater by using discarded mushroom-stick biochar: adsorption properties and mechanisms. *Environ. Sci: Processes & Impacts*. 3.
- Yang, Y. Hou, B. Wang, J. Tian, B. Bi, J. Wang, N. Li, X. and Huang, X. 2019. Nanomaterials for the removal of heavy metals from wastewater. *Nanomaterials (Basel)*. 9(3): 424.
- Ying-Zhong Tang, Karina, Y.H. Gin. and Aziz, M.A. 2003. The relationship between pH and heavy metal ion sorption by algal biomass. *Adsorption Science and Technology*. 21 : 525-537.