

RECOVERY OF CADMIUM (Cd) BIOSORPTION BY *ULVA LACTUCA* (CHLOROPHYCEAE) AND *PADINA PAVONICA* (PHAEOPHYCEAE) BIOMASS

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ABSTARCT

The current study focused on *Ulva lactuca* green and *Padina pavonica* brown biomass for recovery and detoxification of Cd (0, 2.5, 5 and 10 mg/L) heavy metal from aqueous solution. Fourier transformation infrared (FTIR) spectroscopy technique has been employed to detect vibration frequency changes in algae after Cd recovery. Results showed that C–Br stretch in alkyl halides groups, C–N stretch in aliphatic amines, C–C stretch in aromatics groups, C=O stretch in amides groups, C–H stretch in alkanes groups and O–H stretch & H–bonded in alcohols and phenols groups, and C–C stretch in aromatics groups were mainly appeared in recovery of Cd biosorption in *U. lactuca*. Whereas, in the case of *P. pavonica*, amides groups (C=O stretch), alcohols (O–H stretch), phenols (H–bonded), aromatics (=C–H oop bend), (C–O & C–N stretch) carboxylic acids and amides groups, (C–C) stretch aromatics groups were mainly appeared. Consequently, the current study could suggest that FTIR was a potent analytical tool for investigation vibration frequency changes in recovery of Cd biosorption in marine algae.

Keywords: Cadmium recovery, Fourier transformation infrared (FTIR) spectroscopy technique, marine algae

No of Tables: 2**No of Figures: 2****No of References: 25**

INTRODUCTION

The majority of heavy metals seem to be toxic even at low concentrations and are capable of entering the food chain and they accumulate and cause damage to living organisms (Ayangbenro and Babalola 2017). It has been demonstrated that heavy metals sorption capacity varied according to some parameters e.g. pH value, biomass and heavy metals concentration, heavy metals and algae species, ions type and exposure time (Sandau *et al.*, 1996). Removal of chemical metal pollutants from aqueous solution formed a big assay and could be achieved by different techniques including ion exchange, precipitation, evaporation, membrane separation and others. However, their limitations e.g. highly cost, requirement of large area of lands, skillful operators, a sludge dewatering facility, and multiple basin configurations (Zhou *et al.*, 1999) encouraged scientists to look for alteration tools to resolve this problem. In this regards, employment of plants and algae species as heavy metals biosorbents seems to be a useful and potent tool with low cost. Overall, living organisms developed different mechanisms to elimination and removing of chemicals pollutants from their environment. Many researches indicated different methods that could help in removing of chemical pollutants from environment ecosystems. The majority of recovering metals are discarded as sludge, because methods employed for recovering of heavy metals are uneconomical (Ayangbenro and Babalola 2017).

Researches demonstrated different factors and metabolic processes (physical, chemicals, molecular....etc.) that could involve in plants and algae recovery from heavy metals unfavorable effects (Kikuchi and Tanaka 2012). In this regards, Rebhun and Ben-Amotz (1988) reported manganese (Mn) antagonism effect against Cd stress in the *Dunaliella salina* alga. Whereas, Rodríguez-Serrano *et al.* (2009) reported importance of Calcium and Superoxide dismutase (SOD) effect against Cd in Pea plants. Moreover, Fojtová *et al.* (2002) reported DNA repairing as a useful strategy to protect tobacco plants from Cd stress. While, others used transfer of living organisms from chemical stress to a basic media without metals to recover them from negative metal pollutants (Tripathi and Gaur 2006). Whereas, Wilde and Benemann (1993) reported advantageous of microalgae for removal of heavy metals with low cost. Moreover, Kipigroch *et al.* (2016) reported importance of mixed population of green algae for removing of Cd and Cu from wastewater as an effectiveness tool for sorption on these metal ions. Recently, Jayakumar and Govindaradjane (2017) reviewed green algae as a potential tool for removing Cd from aqueous solutions. Recovery of Cd biosorption using *U. lactuca* green and *P. pavonica* brown algae through FTIR technique did not emphasis in details. Therefore, the current study will highlight vibration frequency changes in algae after Cd recovery.

MATERIALS AND METHODS

Marine algae sampling: *U. lactuca* and *P. pavonica* samples were collected along the Syrian coast of the Mediterranean Sea. Samples were collected from 34°37'734"N latitude and 38°29'766"E longitude at 4 km North Lattakia - Syria. Samples were collected manually with disposable gloves; biomass was washed with seawater and then transported within a flask with 5 L seawater.

Cadmium treatment: Marine algae cultivation, cadmium (0, 2.5, 5 and 10 mg/L Cd) treatment and experimental conditions were as described by Saleh (2016). Four days later, samples were transferred from chemical stress to a basic media without Cd metal to recovery them from negative metal pollutant.

Fourier transform infrared spectroscopy (FTIR) technique: A hundred mg of biomass tissues for the both untreated and Cd-treated biomass after recovery were ground and 5mL of 80% acetone were added. Samples were kept at 4°C for 24 h, then they were centrifuged at 1400 g/ 2 min. Filtration of extracts has been carried out using Whatman filter papers (Saleh 2017). These filtrated extracts were used as template for FTIR measurement using NXR FTIR(Thermo, USA) instruments.

RESULTS AND DISCUSSION

FTIR technique has been employed to detect vibration frequency changes in *U. lactuca* green and *P. pavonica* brown marine algae after Cd recovery. The

current study revealed that C–Br stretch in alkyl halides groups, C–N stretch in aliphatic amines, C–C stretch in aromatics groups, C=O stretch in amides groups, C–H stretch in alkanes groups and O–H stretch & H-bonded in alcohols and phenols groups, and C–C stretch in aromatics groups were mainly appeared in recovery of Cd biosorption in *U. lactuca*(Fig. 1). Whereas, in the case of *P. pavonica*, amides groups (C=O stretch), alcohols (O–H stretch), phenols (H-bonded), aromatics (=C-H oop bend), (C-O & C-N stretch) carboxylic acids and amides groups, (C–C) stretch aromatics groups were mainly appeared(Fig.2).

Based upon data presented in Fig. 1 and Fig. 2, estimated peaks and functional groups of untreated and Cd-treated biomass after Cd recovery were summarized in Table 1 for *U. lactuca* and in Table 2 for *P. pavonica*.

Many methods can be used for detecting changes induced by heavy metals stress including physiological, biochemical, molecular and analytical analyses. FTIR among them as qualitative measurement has been widely used for studying chemical functional groups presented on the biomass cell walls reflecting the nature of the possible cell-metal ions interaction in metal biosorption using different biomass (Yun *et al.*, 2001; Adhiya *et al.*, 2002; Tunali *et al.*, 2005). These alterations could be manifested by bands disappearance after saturation of active sites (Yun *et al.*, 2001), decrease (Tunali *et al.*, 2005) or increase (Adhiya *et al.*, 2002) in certain band intensity after metal binding.

In our case study, after Cd recovery, disappearance of C–Cl stretch (848.3 cm^{-1}) in alkyl halides and C–O & C–N stretch in carboxylic acids & amides groups. Moreover, structure changes has been recorded in C–N stretch (1063 cm^{-1}) in aliphatic amines group that could be transferred to R–NO₂ in nitro group in the case of *U. lactuca* biomass (Fig. 1a, b). As for *P. pavonica*, structure changes has been recorded in some peaks. In this regards, reduced prominently in (C–H stretch) alkanes group (2923.3 cm^{-1}) has been recorded combined with intensity increase of C–O & C–N (1059 cm^{-1}) stretch in carboxylic acids & amides groups (Fig. 2a, b).

Saleh (2016) successfully discriminate Cd toxicity between *U. lactuca* and *P. pavonica* based on physiological test [specific growth rate (SGR%), pigments (Chlorophyll a & b, total chlorophyll and total carotenoids) content, osmotic potential and electric conductivity (EC)] after 4 days of Cd (0, 2.5, 5 and 10 mg/L) exposure. The previous study revealed that *P. pavonica* was most tolerant to Cd stress than *U. lactuca*; by showing latest reduction in the above physiological. Recently, Saleh (2017) investigated Cd (0, 2.5, 5 and 10 mg/L) biosorption from aqueous solutions with *U. lactuca* and *P. pavonica* after 4 days of exposure using FTIR technique. The previous study revealed that C–Br stretch in alkyl halides groups, C–N stretch in aliphatic amines and carboxylic acids groups, C–C stretch in aromatics groups (1448 cm^{-1}) seem to play a benefit role via Cd biosorption due to the noticeable shifting in wavelength.

Whereas, C=O stretch in amides groups, C–H stretch in alkanes groups and O–H stretch & H-bonded in alcohols and phenols groups slightly shifted indicating that all the previous groups could not involve in Cd biosorption. While, C–Cl stretch in alkyl halides groups and C–C stretch in aromatics groups (1420.5 cm^{-1}) did not show shift in peak referring that these groups have not involved in Cd biosorption. Whereas, for *P. pavonica*, five [(=C–H oop bend (712.8 and 874.6 cm^{-1})) in aromatics groups, C–O & C–N stretch in carboxylic acids and amides groups, C–C stretch in aromatics groups and C–H stretch in alkanes groups]] showed no differences following Cd treatment referring that these peaks have not involved in Cd biosorption. Whereas, C=O stretch in amides groups and O–H stretch & H-bonded in alcohols and phenols groups showed a slight shift in wavelength referring that these peaks could involve in Cd biosorption process. Tripathi and Gaur (2006) reported that under 10 M Cu and 25 M Zn for 96 h of exposure in *Scenedesmus* sp. different parameters did not recovered. Whereas, most of physiological parameters like photosynthesis and respiration were recovered after 6 h of exposure. The previous study suggests that many factors such photosynthesis and respiration and other metabolic processes could involve in algae recovering from Cu and Zn stresses. Whereas, D'Souza *et al.* (2008) reported different functional groups involved in Cd sorption in *Padina tetrastromatica* brown marine algae after Cd treatment based on FTIR analysis. The previous study showed that carbonyl of COOH (1710 cm^{-1}),

bending vibrations (1571 cm^{-1}) and N-H stretching vibrations (3350 cm^{-1}) were mainly functional groups appeared in treated *P. tetrastromatica*. Indeed, C = O stretching of ester carbonyl (1764 cm^{-1}) in control was shifted to 1760 cm^{-1} under Cd treatment. Moreover, a peak of 1577 cm^{-1} in control showed a down shift to 1571 cm^{-1} in treated algae due to N-H bending vibrations. As well as, Cd stress induced a peak of P = O asymmetric stretching vibrations of PO₂-phosphodiester (1220 cm^{-1}).

Arief *et al.* (2008) reported advantageous of different analytical methods such FTIR for studying removal of heavy metal from aqueous solutions by various biosorbents. The previous study revealed that carboxyl, hydroxyl, sulfate and amino groups displayed significant role in biosorption process. Whereas, Romera *et al.* (2007) reported recovery of cadmium, nickel, zinc, copper and lead from aqueous solutions using six different algae (green, red and brown) species. Results showed that the highest sorption metals capacity was recorded at the lowest Biomass concentration of 0.5 g/L and that the sorption capacity was in the following order: $\text{Pb} > \text{Cd} > \text{Cu} > \text{Zn} > \text{Ni}$. Indeed, the previous study showed that the best sorption results were recorded with *Fucus spiralis*. Indeed, Wilke *et al.* (2006) studied biosorption of cadmium, lead, nickel and zinc from aqueous solutions by 30 strains of microalgae. The previous study revealed that the cyanophyceae *Lyngbya taylorii* displayed the highest biosorption capacity for the four mentioned metals using Scanning electron microscopy (SEM)

technique. Moreover, Carmo *et al.* (2013) reported recovery of Cu by *Cladosporium cladosporioides* filamentous fungi. Whereas, Ayangbenro and Babalola (2017) reviewed capacity of various microbial biosorbents (fungi, bacteria, yeasts and algae) from aqueous solution as effectiveness tool. Sulaymon *et al.* (2013) reported different frequency alterations induced by lead, cadmium, copper and arsenic ions in Cyanophyta (*Oscillatoria princeps* 92 %, *Oscillatoria subbrevis* 2 %, and *Oscillatoria formosa* 1 %) and Chlorophyta (*Spirogyra aequinoctialis* 3 %, *Mougeta* sp. 1 %, and others 1 %) algae using FTIR technique. The previous study revealed that the different functional groups after metal stress were hydroxyl and amine groups at $3,414\text{ cm}^{-1}$, alkyl chains at $2,966$ and $2,943\text{ cm}^{-1}$, C=O of the carboxylic groups or ester groups at $1,797$ - $1,647\text{ cm}^{-1}$, C=O of amide groups at $1,647\text{ cm}^{-1}$, COO- of the carboxylate groups at $1,427\text{ cm}^{-1}$, S=O of the sulfonate groups and COO- groups of the fatty acids at $1,300\text{ cm}^{-1}$, and the $1,033\text{ cm}^{-1}$ band assigned to the P-O-C links of the organic phosphate groups. Similarly, Naja *et al.* (2005) reported same functional groups in Pb biosorption by *Rhizopus arrhizus*. Some bands in the fingerprint regions could be attributed to the phosphate groups (Diniz *et al.*, 2008). It has been demonstrated that kinetic of community recovery follow perturbation is variable. In global, physiological chemical stress recovery is more efficient at high microbial diversity and at high functional redundancy. However, function recovery after a constraint does not necessarily

mean that a community will return to its original composition and tolerance. In this regards, Dorigo *et al.* (2010) investigated the structural and functional recovery of bacterial and eukaryote communities in biofilms naturally grown on stones for 9 weeks in a pesticide impacted site, after transferring them from the pesticide-polluted downstream site to a non-contaminated upstream site. The previous study revealed that toxicant-induced changes between non-impacted biofilm (NIB) and impacted biofilm (IB)

communities remained present after translocation throughout the recovery. Indeed, the biofilm transfer from impacted site to non-impacted one did not break up structural differences between NIB and IB, and brought on only weak functional alterations. Whereas, Shariati and Yahyaabadi (2006) reported effect of different Cd concentrations (0, 0.005, 0.05 and 0.5 mg/L Cd) on physiological parameters (growth rate and beta-carotene) in *Dunaliella salina* unicellular green alga.

Table 1. FT-IR transmission frequencies (cm^{-1}), estimated peaks and functional groups of untreated and Cd-treated *U. lactuca* biomass after Cd recovery.

IR frequency (cm-1)	Bond	Functional groups	C	T1	T2	T3
515-690	C-Br stretch	Alkyl halides	613.2	585.9	580.4	582.1
1020-1250	C-N stretch	Aliphatic amines	1063	1063.8	1055.6	1054.8
1400-1500	C-C stretch	Aromatics	1420.5	1420.5	1420.5	1420.5
1400-1500	C-C stretch	Aromatics	1448	1423.5	1423.4	1540.9
1600-1670	C=O stretch	Amides	1635.4	1636.1	1633.1	1637
2850-3000	C-H stretch	Alkanes	2927.6	2926.5	2927.1	2927.1
3200-3500	O-H stretch, H-bonded	Alcohols, phenols	3431.1	3440.5	3440.5	3431.1

Notes. C: Control, T1: 2.5 mg/L Cd, T2: 5 mg/L Cd and T3: 10 mg/L Cd.

Table 2. FT-IR transmission frequencies (cm^{-1}), estimated peaks and functional groups of untreated and Cd-treated *P. pavonici* biomass after Cd recovery.

IR frequency (cm-1)	Bond	Functional groups	C	T1	T2	T3
690-900	=C-H oop bend	Aromatics	712.8	712.8	712.7	712.8
690-900	=C-H oop bend	Aromatics	874.6	874.5	874.8	874.7
1000-1350	C-O; C-N stretch	Carboxylic Acids, Amides	1059.1	1059.3	1059.1	1058.9
1400-1500	C-C stretch	Aromatics	1428.4	1427.5	1428.8	1428.5
1600-1670	C=O stretch	Amides	1641.9	1644.4	1634.6	1638.5
3200-3500	O-H stretch, H-bonded	Alcohols, phenols	3411.9	3414.7	3427.7	3419.5

Notes. C: Control, T1: 2.5 mg/L Cd, T2: 5 mg/L Cd and T3: 10 mg/L Cd.

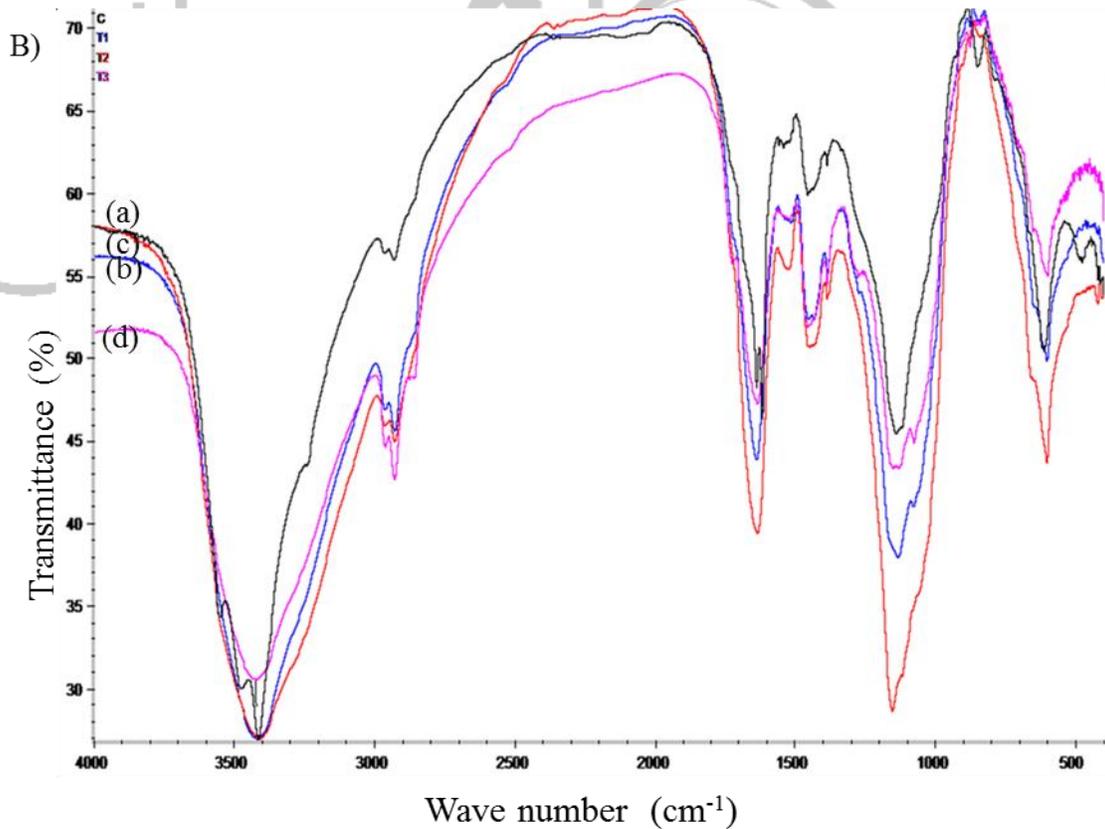
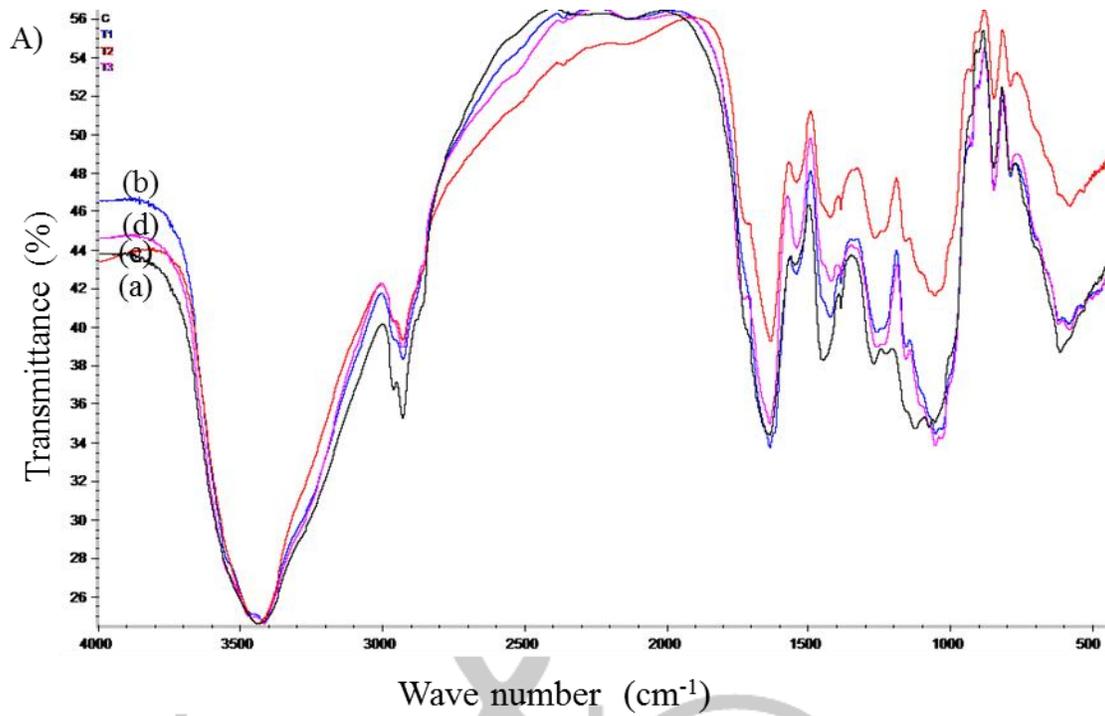


Fig. 1. FT-IR spectra of untreated *U. lactuca* biomass(a), 2.5 mg/L (b), 5 mg/L (c) and 10 mg/L (d) Cd after 4 days exposure. A) after Cd stress (adopted by Saleh 2017) and B) after Cd recovery

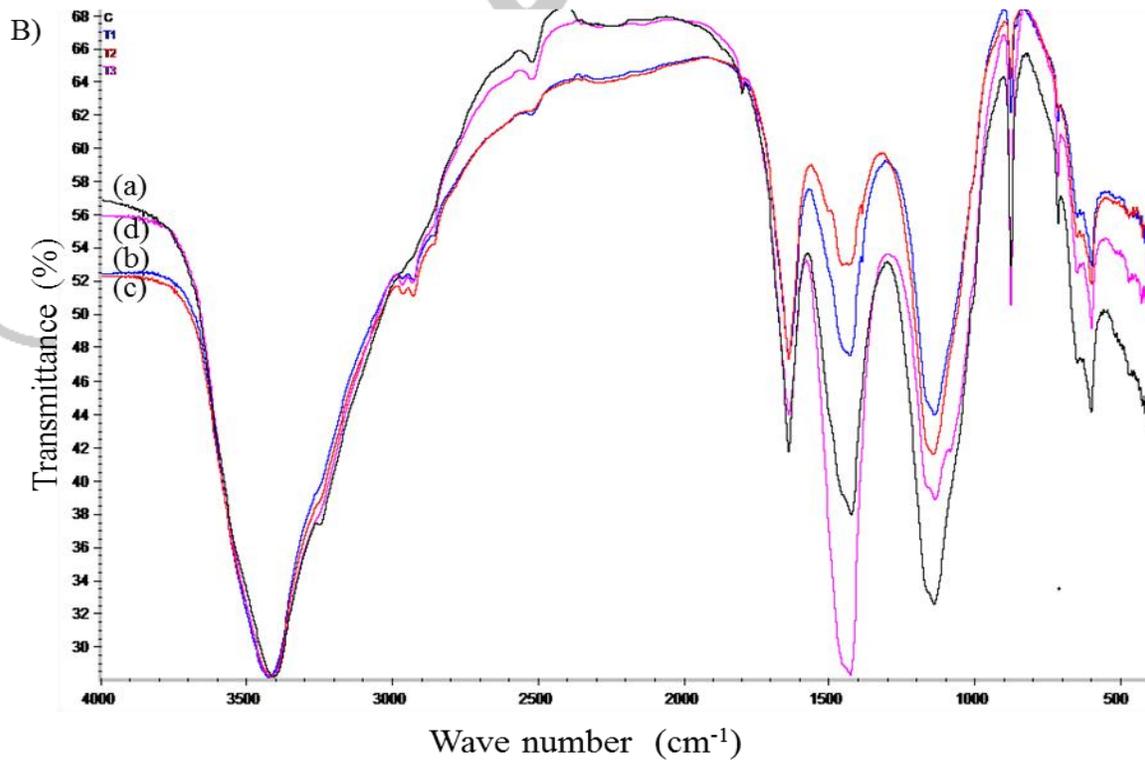
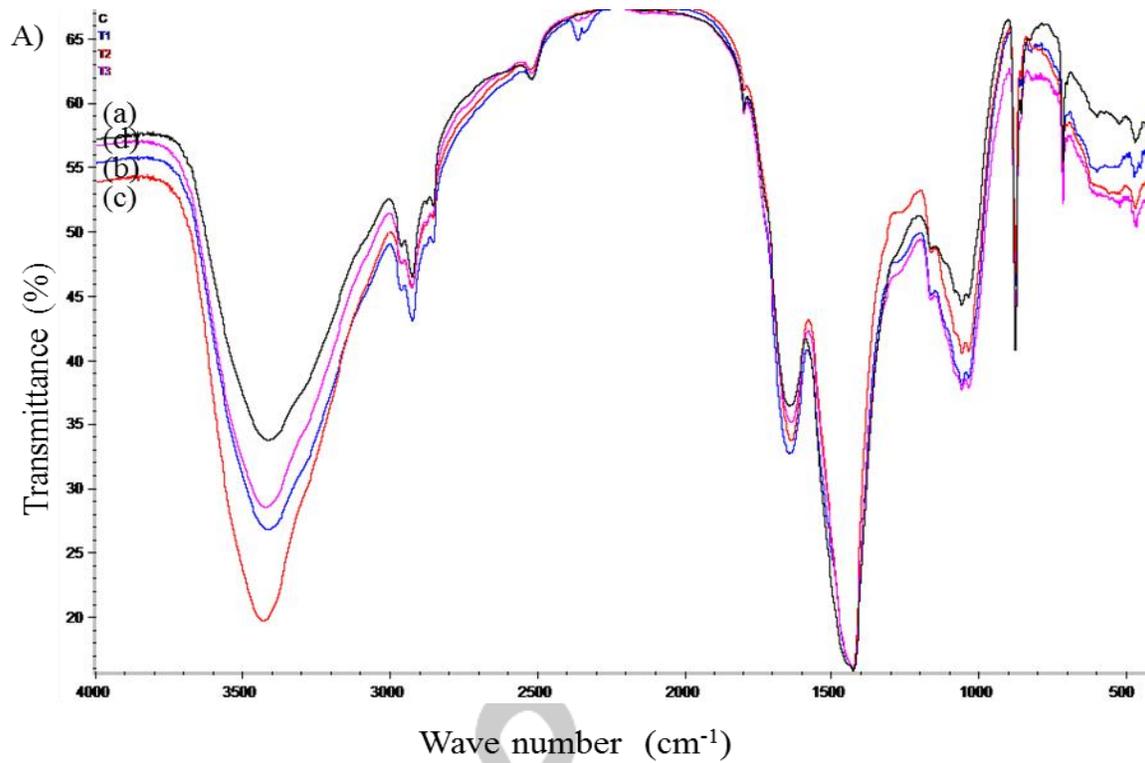


Fig. 2. FT-IR spectra of untreated *P. pavonica* biomass(a), 2.5 mg/L (b), 5 mg/L (c) and 10 mg/L (d) Cd after 4 days exposure. A) after Cd stress (adopted by Saleh 2017) and B) after Cd recovery

CONCLUSION

Recovery of Cd biosorption by *U. lactuca* and *P. pavonica* biomass after Cd (0, 2.5, 5 and 10 mg/L) recovery has been screened using FTIR technique. The current investigation revealed that after Cd recovery, vibration frequency changes have been recorded. In this regards, in the case of *U. lactuca* biomass, disappearance of C-Cl stretch (848,3 cm⁻¹) in alkyl halides and C-O & C-N stretch in carboxylic acids & amides groups. Moreover, structure changes has been recorded in C-N stretch (1063 cm⁻¹) in aliphatic amines group that could be transferred to R-NO₂ in nitro group. As for *P. pavonica*, structure changes has been recorded in some peaks. Reduction prominently in (C-H stretch) alkanes group (2923.3 cm⁻¹) has been recorded combined with increase intensity of C-O & C-N (1059 cm⁻¹) stretch in carboxylic acids & amides groups. Thereby, FTIR could be used as a useful tool to monitoring and identifying the vibration frequency changes in the two mentioned algae.

ACKNOWLEDGEMENT

I thank Professor Othman Ibrahim (Director General of AECS) and Professor Mir Ali Nizar (Head of Molecular Biology and Biotechnology Department) for their support and to the Plant Biotechnology Group for technical assistance.

REFERENCES

Adhiya J., Cai X.H., Sayre R.T. & Traina S.2002. Binding of aqueous cadmium by

the lyophilized biomass of *Chlamydomonas reinhardtii*. *Colloids and Surfaces A: Physicochemical and Engineering Aspects***210**:1–11.

Arief V.O., Trilestari K., Sunarso J., Indraswati N. & Ismadji S. 2008. Recent progress on biosorption of heavy metals from liquids using low cost biosorbents: Characterization, biosorption parameters and mechanism studies. *CLEAN Soil, Air, Water* **36**:937-962.

Ayangbenro A.S. & Babalola O.O. 2017. A new strategy for heavy metal polluted environments: A review of microbial biosorbents. *International Journal of Environmental Research and Public Health***14**: 94-109.

Carmo J.R., Pimenta C.J., Silva J.F. & Souza S.M.C. 2013. Recovery of copper (II) absorbed in biomass of *Cladosporium cladosporioides*. *Scientia Agricola***70**: 147-151.

Diniz V., Weber M.E., Volesky B. & Naja G. 2008. Column biosorption of lanthanum and europium by *Sargassum*. *Water Research***47**:363–371.

Dorigo U., Berard A., Rimet F. & Montuelle B.2010. In situ assessment of periphyton recovery in a river contaminated by pesticides. *Aquatic toxicology***98**:396-406.

D'Souza L., Devi P., Divya Shridhar M.P. & Naik C.G.2008. Use of fourier transform infrared (FTIR) spectroscopy to study cadmium-induced changes in *Padina*

tetrastromatica (Hauck). *Analytical Chemistry Insights***3**: 135–143.

Fojtová M., Fulnecková J., Fajkus J. & Kovarík A. 2002. Recovery of tobacco cells from cadmium stress is accompanied by DNA repair and increased telomerase activity. *Journal of Experimental Botany***53**: 2151-2158.

Jayakumar V. & Govindaradjane S. 2017. Biosorption of cadmium by green algae – A review. *Journal of Advanced Chemical Sciences***3**: 480–484.

Kikuchi T. & Tanaka S. 2012. Biological removal and recovery of toxic heavy metals in water environment. *Critical Reviews in Environmental Science and Technology***42**: 1007–1057.

Kipigroch K., Janosz-Rajczyk M. & Skowron-Grabowska B. 2016. The use of algae in the removal of Cd and Cu in the process of wastewater recovery. *Desalination and Water Treatment* **57**:1508-1514.

Naja G., Mustin C., Berthelin J. & Volesky B. 2005. Lead biosorption study with *Rhizopus arrhizus* using a metal-based titration technique. *The Journal of Colloid and Interface Science* **292**:537–543.

Rebhun S. & Ben-Amotz A. 1988. Antagonistic effect of manganese to cadmium toxicity in the alga *Dunaliella salina*. *Marine Ecology Progress Series* **42**: 97-104.

Rodríguez-Serrano M., Romero-Puertas M.C., Pazmiño D.M., Testillano P.S., Risueño M.C., Del Río L.A. & Sandalio L.M. 2009. Cellular response of pea plants to cadmium toxicity: Cross talk between reactive oxygen species, nitric oxide, and calcium. *Plant Physiology* **150**: 229–243.

Romera E., González F., Ballester A., Blázquez M.L. & Muñoz J.A. 2007. Comparative study of biosorption of heavy metals using different types of algae. *Bioresource Technology***98**: 3344-53.

Saleh B. 2016. Cadmium stress assessment in *Ulva lactuca* (Chlorophyta) and *Padina pavonica* (Phaeophyta) marine algae. *Journal of BioInnovation* **5**: 835-849.

Saleh B. 2017. Cadmium biosorption investigation from aqueous solutions with *Ulva lactuca* (Chlorophyta) and *Padina pavonica* (Phaeophyta) seaweeds. *The Journal of Stress Physiology & Biochemistry* **13**: 81-87.

Sandau E., Sandau P., Pulz O. & Zimmermann M. 1996. Heavy metal sorption by marine algae and algal by-products. *Engineering in Life Sciences***16**: 103-119.

Shariati M. & Yahyaabadi S. 2006. The effects of different concentrations of cadmium on the growth rate and beta-carotene synthesis in unicellular green algae *Dunaliella salina*. *Iranian Journal of Science & Technology, Transaction A***30**: 57-63.

Sulaymon A.H., Mohammed A.A. & Al-Musawi T.J. 2013. Competitive biosorption

of lead, cadmium, copper, and arsenic ions using algae. *Environmental Science and Pollution Research* **20**:3011–3023.

Tripathi B.N., & Gaur J.P. 2006. Physiological behavior of *Scenedesmus* sp. during exposure to elevated levels of Cu and Zn and after withdrawal of metal stress. *Protoplasma* **229**: 1–9.

Tunali S., Kiran I.& Akar T. 2005. Chromium (VI) biosorption characteristics of *Neurospora crassa* fungal biomass. *Minerals Engineering* **18**: 681-689.

Yun M.S., Reddy N.A. & Condon J. J.2001. Radio properties of infrared-selected galaxies in the IRAS 2 Jy sample. *The Astrophysical Journal* **554**: 803-822.

Wilde E.W. & Benemann J.R. 1993. Bioremoval of heavy metals by the use of microalgae. *Biotechnology Advances* **11**:781-812.

Wilke A., Buchholz R. & Bunke G. 2006. Selective biosorption of heavy metals by algae. *Environmental Biotechnology* **2**: 47-56.

Zhou P., Huang J., Alfred W.F.&Wei S. 1999. Heavy metals removal from wastewater in fluidized bed reactor. *Water Research* **33**: 1918–1924.