

Cadmium Biosorption Investigation from Aqueous Solutions with *Ulva lactuca* (Chlorophyta) and *Padina pavonica* (Phaeophyta) Seaweeds

Basel Saleh

Department of Molecular Biology and Biotechnology, Atomic Energy Commission of Syria, P.O. Box 6091, Damascus, Syria

*E-Mail: ascientific@aec.org.sy

Received September 24, 2017

Fourier transform infrared spectroscopy (FTIR) analysis has been employed to identify various functional groups involved in cadmium biosorption into *Ulva lactuca* (Chlorophyta) and *Padina pavonica* (Phaeophyta) seaweeds exposed to different cadmium (Cd) (0, 2.5, 5 and 10 mg/L) concentrations for 4 days. Data revealed that Cd stress induced chemical structure alteration into studied seaweeds. Overall, for *U. lactuca*, carboxylic acids (C-O) & amides (C-N stretch) groups and aromatics groups (C-C stretch) showed a shift in wavelength with Cd stress referring that they may play an important role in Cd biosorption process. Whereas, for *P. pavonica*, amides groups (C=O stretch), alcohols (O-H stretch) and phenols (H-bonded) groups showed a slight shift in wavelength indicating that these peaks could involve in Cd biosorption process. Thereby, FTIR analysis seems to be a potent tool for screening chemical structure alteration induced by Cd stress in marine ecosystems.

Key words: Seaweeds, Cadmium, Fourier transform infrared spectroscopy, Functional groups

Heavy metals impact on living organisms could be manifested by morphological, physiological, biochemical and molecular levels (Saleh 2017a). Biochemical analytical methods could help us to understanding binding metals mechanisms. This could be varied according to different factors *e.g.* species, metal ion nature, age and cell walls composition (Ovza and Ovez 2013). For seaweeds, this investigation seems to be simplest because seaweeds biomass was directly in contact with chemical solution and thereby, rapidly respond.

Cadmium (Cd) impact among various heavy metals, on living organisms in particularly on seaweeds, has extensively investigated in many researches (D'Souza *et al.*, 2008; Lee *et al.*, 2011; Ova and Ovez 2013; Saleh 2016a; b; c). Fourier transform infrared spectroscopy (FTIR) is considered as one of fewer potential valuable tools for screening and identification of various functional groups and organic compounds in plants and seaweeds (Lammers *et al.*, 2009; Radhika and Mohaideen 2015). Its utility could be manifested by its simplicity and rapidity, nondestructive methods, direct analysis with no request of special sample preparation with low cost. Their advantages make it one of the most recent analytical techniques applied in characterization of different groups involved in plants and seaweeds and their interaction with wide ranges of heavy metal ions (Murphy *et al.*, 2007; Ferreira *et al.*, 2011; Fakhry 2013; Ghoneim *et al.*, 2014; Radhika and Mohaideen 2015; Saleh 2017b).

To our knowledge, little informations have been published regarding chemical structure alteration in *U. lactuca* (Chlorophyta) and *P. pavonica* (Phaeophyta) seaweeds via Cd stress. Thereby, the current investigation aimed to characterize spectra of untreated compared to Cd-treated seaweeds using FTIR and consequently determine the main functional groups that could involve in Cd biosorption process.

MATERIALS AND METHODS

Seaweeds sampling

U. lactuca and *P. pavonica* samples were collected along the Syrian coast of the Mediterranean Sea. Samples collection was carried out 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.

Application of cadmium stress

Seaweeds cultivation, cadmium (0, 2.5, 5 and 10 mg/L Cd) treatment and experimental conditions were as recently reported by Saleh (2016a).

Fourier transform infrared spectroscopy (FTIR) technique

A hundred mg of seaweed tissues for the both untreated and Cd-treated seaweeds were ground and 5 mL of 80% acetone were added. Tubes were kept at 4°C for 24 h, then they were centrifuged at 1400 g/ 2 min. Extracts were filtrated using Whatman filter papers as reported by Saleh (2015). These filtrated extracts were used as template for FTIR measurement using NXR FT-IR (Thermo, USA) instruments.

RESULTS AND DISCUSSION

Interaction of living organisms with heavy metals could be investigated based on morphological, physiological, molecular and biochemical methods. In the current investigation we focusing on functional groups changes in comparative study between untreated and Cd-treated *U. lactuca* and *P. pavonica* seaweeds under different Cd concentrations (0, 2.5, 5 and 10 mg/L Cd) using FTIR analysis.

Our data showed that Cd stress induced some changes in functional groups into *U. lactuca* (Fig. 1) and *P. pavonica* (Fig. 2) seaweeds.

From data presented in Fig. 1 and Fig. 2, estimated peaks and functional groups of untreated and Cd-treated seaweeds were summarized in Table 1 for *U. lactuca* and in Table 2 for *P. pavonica*.

In the case of *U. lactuca*, 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, 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. Inversely to other groups such as 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.

As 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)] out seven peaks showed no differences appeared following Cd treatment indicating 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 indicating that these peaks could involve in Cd biosorption process.

Previously, Murphy *et al.* (2007) reported that carboxyl was the main group involved in brown seaweeds metal sorption. Whereas, D'Souza *et al.* (2008) reported various functional groups involved in Cd sorption in *Padina tetrastratica* brown seaweed exposed to Cd stress using FTIR analysis. The previous study revealed that N–H stretching vibrations (3350 cm^{-1}), bending vibrations (1571 cm^{-1}) and carbonyl of COOH (1710 cm^{-1}) were the major functional groups appeared in treated *P. tetrastratica*. The same study revealed also that C = O stretching of ester carbonyl (1764 cm^{-1}) in control was shifted to 1760 cm^{-1} under Cd treatment. Indeed, a peak of 1577 cm^{-1} in control showed a down shift to 1571 cm^{-1} in treated seaweed 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}).

Lee *et al.* (2011) reported different peaks appeared in *Kappaphycus Alvarezii* seaweed exposed to metal ions (Cu, Cd, Cr, Ni and Zn). In this respect, these peaks were 3420 cm^{-1} (–OH or –NH groups), 2930 and 1385 cm^{-1} (–CH₃ groups), 2350 cm^{-1} (–C≡N groups), 1646 and 1240 cm^{-1} (–COOH groups), 1050 cm^{-1} (–C–O groups), and 1640 and 1240 cm^{-1} (–COO groups) shifted to 1645 and 1235 cm^{-1} combined with appearance of new peak at 1730 cm^{-1} (–C=O groups). Ferreira *et al.* (2011) reported that hydroxyl, amino and amide groups

were mainly associated with heavy metals uptake (Ni, Zn and Pb) into dried *Arthrospira (Spirulina) platensis* and *Chlorella vulgaris* biomass.

Whereas, Fakhry (2013) reported that 3250 , 2550 , 3400 , 1200 , 3000 , 1670 and 1128 cm^{-1} referring to OH, COOH, NH₂, S=O, C–H, C=O and C–O groups, respectively were involved in dye sorption into *P. pavonica*.

Otherwise, Ova and Ovez (2013) reported that carboxylate groups were included in heavy metals absorption (Cd, Ni, Pb, Zn, Fe and Cr) into *Nitzschia closterium* biomass. As well as, Naspu *et al.* (2014) reported functional groups involved in Cd biosorption into *Padina* sp. modified with PAA/HCl (mP–PAA/HCl) using FTIR analysis. The previous study revealed a peak of 3322.78 cm^{-1} yielded by interaction between PAA/HCl and seaweed. As well as, peaks at 1607.52 cm^{-1} (N–H group), 2943.68 cm^{-1} (C–H stretch) and 1040.11 cm^{-1} (C–N group) were involved in biosorption process.

Whereas, Ghoneim *et al.* (2014) characterized Cd adsorption in dried *U. lactuca* by identified the various functional groups involved in this process. The previous investigation indicated the presence of C–H bond of methyl, methylene and methoxy, C–H stretching, C–C stretching, C–O and C–O stretching groups. Moreover, the previous study mentioned some changes in the hydroxyl and amino groups position's due to Cd stress.

Moreover, Radhika and Mohaideen (2015) applied FTIR technique to screening different functional groups in two *U. lactuca* and *Gracilaria corticata* seaweeds. Indeed, Ponnaniakamideen *et al.* (2014) identified the different groups presented in different organic solvents characterized *Padina tetrastratica* biomass. The previous study indicated the presence of OH, N–H and C–N stretching, –C–H bending vibrations, –C=C–stretching vibrations and –O–H stretching vibrations groups.

Other study (Sulaymon *et al.*, 2013) however, investigated various functional groups involved in Pb, Cd, Cu, and As ions biosorption with dried different green (Chlorophyta) and blue–green (Cyanophyta) seaweeds species, using FTIR technique. The previous study revealed that interaction of seaweeds with metal ions from metal solution induced different functional

groups such as: amine groups (3414 cm^{-1}), alkyl (2966 and 2943 cm^{-1}), carboxylic groups of C=O or ester groups (1797 cm^{-1}), amide groups of C=O (1647 cm^{-1}), carboxylate groups of COO⁻ (1427 cm^{-1}), sulfonate groups of S=O and fatty acids COO⁻ groups (1300 cm^{-1}), and organic phosphate groups of P–O–C (1033 cm^{-1}).

Moreover, Dekhil *et al.* (2011) applied the same technique to investigate different functional groups appeared into dried *Caulerpa racemosa* green seaweed exposed to Pb and Cd ions. The previous study revealed

the appearance of O–H bending group (1420 cm^{-1}) for the two ions, amide group slightly shifted to N–H stretching (3410 to 3396 cm^{-1}) with Cd and appearance of new peak (3340 cm^{-1}) corresponding to N–H stretching group with Pb. Indeed, the same study revealed that some groups such as C–O stretching group in the amide group did not involve in metals sorption. While, other one like C–O in amino group could exhibited an essential role in metals sorption process.

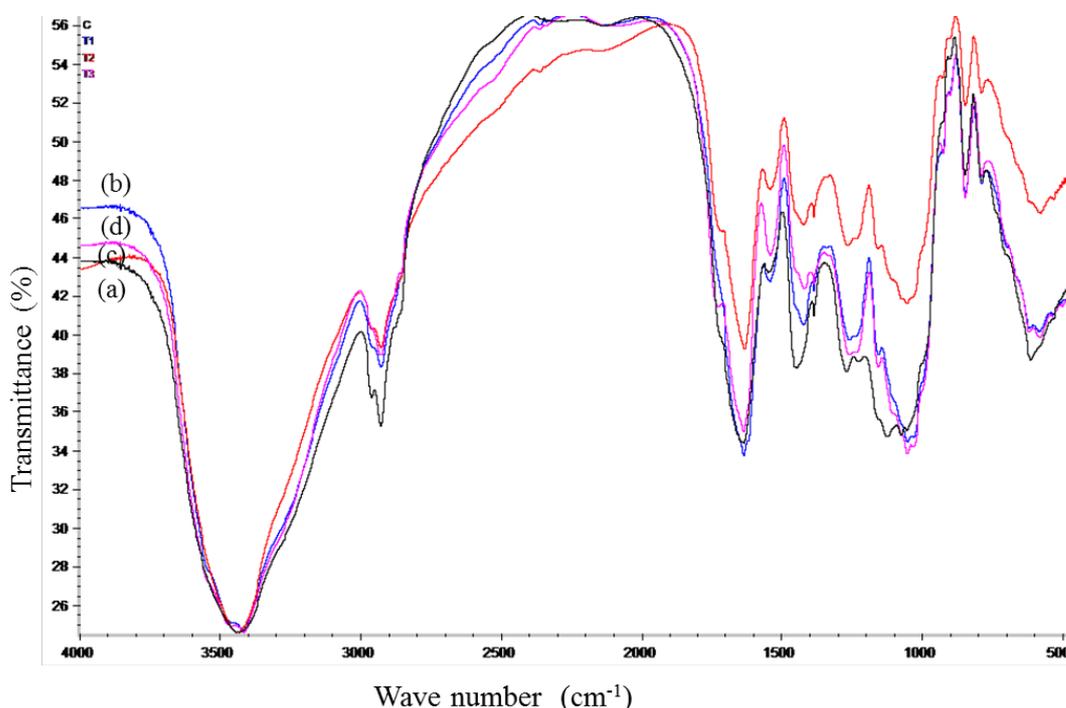


Figure 1. FT-IR spectra of untreated *U. lactuca* (a), 2.5 mg/L (b), 5 mg/L (c) and 10 mg/L (d) Cd after 4 days exposure.

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

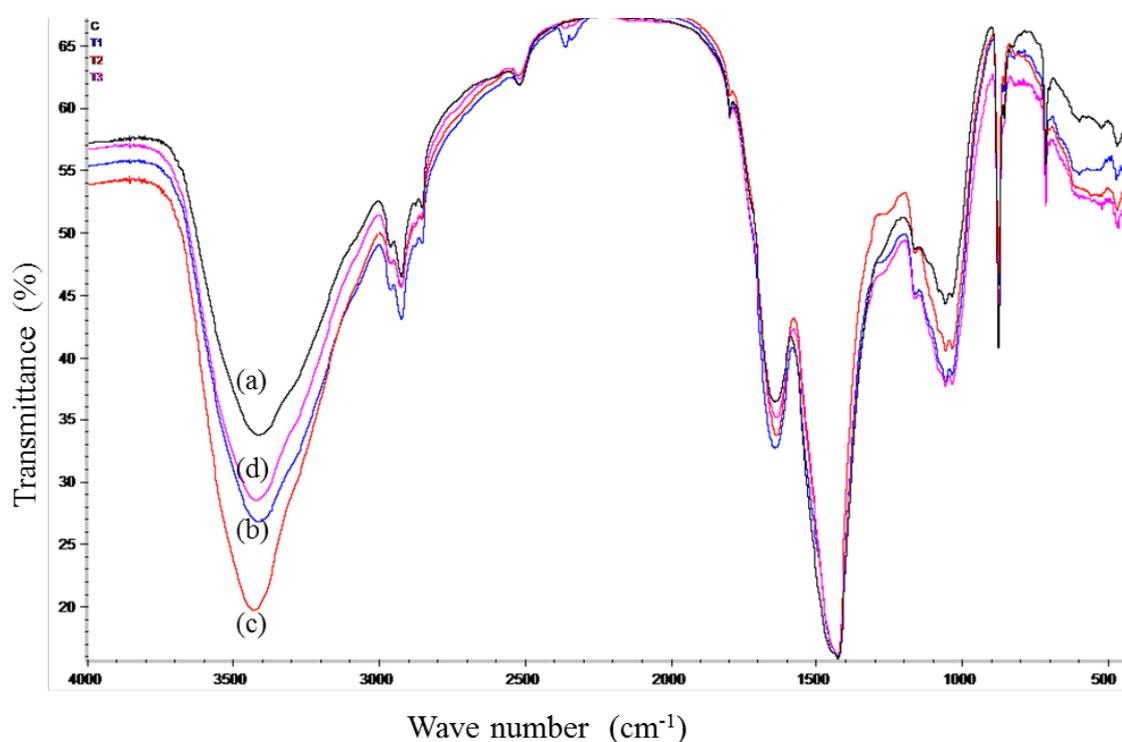
IR frequency (cm^{-1})	Bond	Functional groups	C	T1	T2	T3
515-690	C–Br stretch	Alkyl halides	613.2	585.9	580.4	582.1
550-850	C–Cl stretch	Alkyl halides	848.3	848.3	848.4	848.4
1020-1250	C–N stretch	Aliphatic amines	1063	1063.8	1055.6	1054.8
1000-1350	C–O; C–N stretch	Carboxylic Acids, Amides	1125.1	1258.4	1265	1259.7
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. pavonica* seaweed.

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
2850-3000	C-H stretch	Alkanes	2923.3	2923.1	2923.2	2924.8
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.

**Figure 2.** FT-IR spectra of untreated *P. pavonica* (a), 2.5 mg/L (b), 5 mg/L (c) and 10 mg/L (d) Cd after 4 days exposure.

Other recent investigation (Saleh 2016a) focused on physiological impact of Cd stress into *U. lactuca* green and *P. pavonica* brown seaweeds for 4 days. The previous investigation revealed that Cd treatment reduces studied physiological parameters (specific growth rate, chlorophyll *a* & *b*, total chlorophyll and osmotic potential values) into the both seaweeds. In this respect, this decline was more noticeable in *U. lactuca* compared to *P. pavonica*. Indeed, carotenoids content decreased into *U. lactuca* and inversely increased into *P. pavonica*. These compounds could be considered as

an antioxidant due to their important role as a defenses mechanism against reactive oxygen species (ROS) induction by heavy metals exposure; suggesting that *P. pavonica* could declare more tolerant to Cd treatment compared to *U. lactuca*.

Moreover, Saleh (2016b) reported DNA changes induced by Cd in *U. lactuca* for 4 days based on Random Amplified Polymorphic DNA (RAPD) marker. The previous study revealed that genomic template stability (GTS%) value increased with Cd concentration increased. Similar recent findings were also reported by

Saleh (2016c) in *P. pavonica* treated with similar Cd concentrations using also the same marker.

More recently, Saleh (2017b) reported biosorption of four metal ions (Pb, Cu, Cd and Zn) after 5 days exposure into *U. lactuca* seaweed using FTIR analysis. The previous study revealed that O-H, N-H, -C-O, P-O-C, S-O, C-C, -C-C, -C-OH, C-H and C-I groups, were the main functional groups involved in metal ions biosorption process. Moreover, Saleh (2017a) reviewed physiological and molecular impacts of various heavy metal pollutants in plants and seaweeds. The previous review revealed the importance of some plants and seaweeds as a cheap potent source for heavy metals bioremediation.

CONCLUSION

In the current study, FTIR technique has been employed to monitoring and identifying the functional groups in two seaweeds (*U. lactuca* and *P. pavonica*) under different Cd concentrations. From data presented herein, for *U. lactuca*, C-O & C-N stretch in carboxylic acids and amides groups and C-C stretch in aromatics groups seem to play an important role in Cd biosorption. Whereas, C=O stretch in amides groups, O-H stretch in alcohols groups and H-bonded in phenols groups showed a slight shift in wavelength indicating that these peaks could involve in Cd biosorption process in *P. pavonica*.

ACKNOWLEDGEMENT

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

REFERENCES

- Dekhil, A.B., Hannachi, Y., Ghorbel, A. and Boubaker, T. (2011) Removal of lead and cadmium ions from aqueous solutions using dried marine green macroalga (*Caulerpa racemosa*). *Int. J. Environ. Res.*, **5**: 725-732.
- D'Souza, L., Devi, P., Divya Shridhar, M.P. and Naik, C.G. (2008) Use of fourier transform infrared (FTIR) spectroscopy to study cadmium-induced changes in *Padina tetrastromatica* (Hauck). *Anal. Chem. Insights.*, **3**: 135-143.
- Fakhry, E.M. (2013) *Padina pavonica* for the removal of dye from polluted water. *Am. J. Plant. Sci.*, **4**: 1983-1989.
- Ferreira, L.S., Rodriguesa, M.S., Monteiro de Carvalho, J.C., Lodi, A., Finocchio, E., Perego, P. and Converti, A. (2011) Adsorption of Ni²⁺, Zn²⁺ and Pb²⁺ onto dry biomass of *Arthrospira (Spirulina) platensis* and *Chlorella vulgaris*. I. Single metal systems. *Chem. Eng. J.*, **173**: 326- 333.
- Ghoneim, M.M., El-Desoky, H.S., El-Moselhy, K.M., Amer, A., El-Naga, E.H.A., Mohamedein, L.I. and Al-Prol, A.E. (2014) Removal of cadmium from aqueous solution using marine green algae, *Ulva lactuca*. *Egypt. J. Aquat. Res.*, **40**: 235-242.
- Ponnanikajamideen, M., Malini, M., Malarkodi, C. and Rajeshkumar, S. (2014) Bioactivity and phytochemical constituents of marine brown seaweed (*Padina tetrastromatica*) extract from various organic solvents. *Inter. J. of Pharm. Ther.*, **5**: 108-112.
- Lammers, K., Arbuckle-Keil, G. and Dighton, J. (2009) FT-IR study of the changes in carbohydrate chemistry of three New Jersey pine barrens leaf litters during simulated control burning. *Soil. Biol. Biochem.*, **41**: 340-7.
- Lee, K.O., Ramli, N., Said, M., Ahmad, M., Yasir, S.Md. and Ariff, A. (2011) Competitive metal sorption and desorption onto *Kappaphycus Alvarezii*, seaweed waste biomass. *Malays. J. Anal. Sci.*, **15**: 252 - 257.
- Muntean, C.M., Halmagyi, A., Puia, M.D. and Pavel, I. (2009) FT-Raman signatures of genomic DNA from plant tissues. *Spectroscopy.*, **23**: 59-70.
- Murphy, V., Hughes, H. and McLoughlin, P. (2007) Cu (II) Bin- ding by dried biomass of red, green and brown macro- algae. *Water. Res.*, **41**: 731-740.
- Naspu, A.S.M., Hasan, S., Awatif, N.S. and Kamal, M.L. (2014) The feasibility study of brown marine algae toward cadmium ions as a low cost biosorbent. *J. Med. Bioeng.*, **3**: 227-230.
- Ova, D. and Övez, B. (2013) Characterization of heavy metal biosorption from aqueous solutions with

- Nitzschia closterium biomass. Digital Proceeding Of THE ICOEST'2013 - Cappadocia C. Ozdemir, S. Şahinkaya, E. Kalıpcı, M.K. Oden (editors). Nevsehir, Turkey, June 18 – 21.
- Radhika, D. and Mohaideen, A. (2015) Fourier transform infrared analysis of *Ulva lactuca* and *Gracilaria corticata* and their effect on antibacterial activity. *Asian. J. Pharm. Clin. Res.*, **8**: 209-212.
- Saleh, B. (2015) Physiological response of the green algae *Ulva lactuca* (Chlorophyta) to heavy metals stress. *J. Stress. Physiol. Biochem.*, **11**: 38-51.
- Saleh, B. (2016a) Cadmium stress assessment in *Ulva lactuca* (Chlorophyta) and *Padina pavonica* (Phaeophyta) marine algae. *J. Bio. Innov.*, **5**: 835-849.
- Saleh, B. (2016b) Genetic variation in *Ulva lactuca* (Chlorophyceae) marine algae under cadmium stress. *J. Bio. Innov.*, **5**: 673-685.
- Saleh, B. (2016c) DNA alteration induced by cadmium (Cd) metal in *Padina pavonica* (Phaeophyta) marine brown algae. *Plant. Cell. Biotech. Mol. Biol.*, **17 (7-8)**: (In press).
- Saleh, B. (2017a) Chemical pollutants impact in plants: physiological and molecular study-a review. *J. Bio. Innov.*, **6**: 176-193.
- Saleh, B. (2017b) Analytical methods employment for heavy metals pollution investigation in *Ulva lactuca* (Chlorophyceae) seaweed. *J. Bio. Innov.*, (accepted).
- Sulaymon, A.H., Mohammed, A.A. and Al-Musawi, T.J. (2013) Competitive biosorption of lead, cadmium, copper, and arsenic ions using algae. *Environ. Sci. Pollut. Res.*, **20**: 3011–3023.