Published by :
http://www.ijert.org

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 8 Issue 09, September-2019

Phycoremediation of Metal Pollution of Wastewater

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***Abstract*:- Huge interest has recently arisen toward using various kinds of inexpensive and available alternative techniques for wastewater treatment and removal of heavy metals. One of them is the usage of ready biomass of several kinds of algae (macroalgae and microalgae). This study focused on applying the phycoremediation technology as an alternative, eco-friendly technology for wastewater treatment. We examine the potential of *Chlorella vulgaris* and *Scenedesmus quadricauda (microalgae) and the brown* macroalga, *Dictyota dichotoma* and *Turbinaria ornata* in this technology, (pilot-scale laboratory study). We analyzed the physicochemical characteristic of wastewater before and after treatment with algae and determine the removal capacity of algae for contaminated heavy metals. The results obtained showed that the maximum growth rate, growth index and biomass productivity were obtained at 10^6 cell/ml concentration which was recommended to be used in wastewater treatment. Microalgae pollutants removal efficiency was reached to 92.7and 87.5 % for BOD and COD, respectively and 100% for TN and TP. While of *Turbinaria ornata* reached to 44.4, 29.3, 37.8, 84.2, 65.3, 67.5, 67.8, and 67.1% for TDS, salinity, conductivity, turbidity, BOD, COD, TN and TP, respectively, but of *Dictyota dichotoma* reached to 52.3, 39.3, 50.6, 95.3, 72.1, 71.3, 73.5 and 70.4% , respectively. In similar way removal percent of metal pollution by microalgae reached to (98.8, 90.4, 83.9,75.7, 99.8, 99.7 and 99.5%) for Cu, Zn, Cd, Ni, Co, Fe and Pb. While, *Turbinaria ornata* recorded removal percent (55.5, 70.9, 59.8, 57.6, 55.1, 72.6 and 42.1 %) for Cd, Cu, Pb, Fe, Co, Zn and Ni, respectively, and *Dictyota dichotoma* recorded removal percent (34.2, 40.7, 68.3, 72.7, 57.8, 70.5 and 52.1%) for Ni, Zn, Cd, Cu, Pb, Fe, and Co, respectively.**

Keywords— Phycoremediation, algae, biosorption, heavy metals, wastewater.

INTRODUCTION

The rapid increase in population, industrialization, and urbanization has resulted in the disposal of various pollutants into the water bodies. This discharged effluent is of great concern because it has a toxic or carcinogenic effect on human and living species. Heavy metals for instance, which is widely produced and extremely toxic in relatively low dosages and also recalcitrant and persistent in the environment. Therefore, the removal of these toxins from water prior to supplying water for drinking, bathing, etc. is very important and urgent. There is a wide range of treatment technologies (physical, chemical and biological) such as chemical precipitation, coagulation-flocculation, flotation, ion exchange, and membrane filtration, bioremediation, ozonation and more .most of these current conventional methods for water treatment require high energy requirements, high operation, and maintenance, cannot effectively respond to diurnal, seasonal, or long-term variations in the composition of wastewater and produce large volumes of sludge which, make them economically unviable for many regions,**[1]**. This makes researchers investigate a new alternative, eco-friendly technology for wastewater treatment. Phycoremediation is the use of algae for the removal or biotransformation of pollutants from wastewater, [**2**]. It is considered as an eco-solution to environmental protection and sustainable remediation, **[3].**

FU and Wang [4], proved with evidence from the literature survey of 185 articles that biosorption is recognized as an effective and economical method for low concentration heavy metal wastewater treatment as an alternative and can remove heavy metal ions with high efficiency, more than that processes which have been widely used to remove metals from wastewater.

Many investigations proved that microalgae provides a pathway for the removal of nitrogen, phosphorus, carbon dioxide, heavy metals and pathogens present in wastewaters which necessary for their growth. It also, saves and reduces requirements for chemical remediation and minimizes freshwater use for biomass production as part of a wastewater treatment process. Consequently, it is a promising and advantageous process where faster growth rate accompanied by an elimination of water contamination level, **[5]**.

Previous studies registered the remarkable potential of *Chlorella vulgaris* in wastewater treatment. **Keffer and Kleinheinz [6]**, recorded that, it fixed up to74% carbon dioxide when grown in a photo-bioreactor, and in absorbing 45–97% nitrogen, 28–96% phosphorus and in reducing the chemical oxygen demand (COD) by 61–86% from different type of wastewater such as textile, sewage, municipal ,agricultural and recalcitrant, **[7]**.

Ezenweani *et al.* [3], recorded that both microalgae removed between 88 and 94% of phosphates and removed 83 and 99% of ammonium and also reduced the concentration of iron by 71% while potassium went down by 70 to 77 %. Furthermore, the performance of *C. Vulgaris* in synthesized wastewater was improved when co-immobilized alginate beads with microalgae growth promotions and *Scenedesmus*, which removed 100% of ammonium during four consecutive cycles of 48h, and 83% for phosphorus after one cycle of 48h. Thus, *Chlorella vulgaris* is considered as one of the best microalgae for bioremediation of wastewater with an impressive potential to completely remove ammonium and sometimes modest potential to eliminate phosphorus present in the medium, **[8]**. The metal uptake capacity of algal biomass depends on the availability of polysaccharide contents such as alginates and fucoidans on the cell surface, **[9]**.

According to **Vigneshwaran *et al.* [10]**, biosorption of metals is a two-step process. First, the metals ions bind and, second, the metal ions accumulate on the binding sites. It was thought that marine brown alga has rich content of extracellular polysaccharide which enables it to exhibit prosperous metal sorption compared to other algal species. Seaweed (macroalgae) collected from the ocean has shown impressive biosorption of metals. Brown algae, especially, contain high amounts of alginate, which are well protected within brown algae's cellular structures, and copious carboxylic groups capable of capturing cations present in solutions, **[11]**. This proved by **Yasser *et al.* [12]** as he founded that the sorption capacity of *Dictyota dichotoma* biomass for the removal of Cd (II) is higher than that of the majority of other adsorbents which reported in the literature. Also, he mentions to the variation of the sorption capacity which depends on the characteristics of the individual adsorbent, the extent of surface/surface modification and the initial concentration of the adsorbate. On the other hand, **Senthilkumar *et al.* [13]** Confirmed, seaweeds have proved to be the most efficient and practical biomass for the removal of heavy metal ions from aqueous solutions. He also thought it is an ideal candidate for heavy metal removal in both batch and column operations because of its compatibility with almost all heavy metal ions, its macroscopic appearance, rigidity, and easy availability. Its polysaccharide content is believed to be responsible for its excellent metal binding capacity.

Accordingly, there is a real need for treatment with algae that offer alternative technology. Using algae for wastewater treatment offers some interesting advantages over conventional wastewater treatment such as in; Cost-effectiveness and safety, Green House Gas emission reduction, Reductions in sludge formation and low energy requirement, Production of algal biomass, Oxygenation of the systems through photosynthesis thereby enabling effective decomposition, Effective reduction of nutrient load and consequent total dissolved solids as these are used up as nutrient sources, Production of high algal biomass which can be used as feed in aquaculture and as bio-fertilizer, Simple operation, and maintenance, Potential for energy and nutrient recovery,**[11]**. This study aimed to investigate the biosorption potential of *Dictyota dichotoma* and *Turbinaria ornata* biomass and evaluate the capacity of two microalgae species, *Chlorella vulgaris* and *Scenedesmus quadricauda* to remove organic and inorganic pollutants and heavy metals from wastewater. Optimum biosorption conditions were determined as a function of pH, biomass dosage, contact time and temperature.

MATERIALS AND METHODS

Algae and wastewater

The wastewater was a composite sample of raw water from Damietta branch-Nile River which collected from different 12 polluted sites along it. The wastewater was collected and its main physicochemical characteristics were analyzed: pH, chemical oxygen demand (COD), biochemical oxygen demand (BOD), Total phosphorus (TP), dissolved oxygen (DO), total nitrogen (TN) and some heavy metals.

Preservation methods were limited to pH control, chemical addition, refrigeration, and freezing, **[14]**. pH value of the samples was measured directly by pH Meter (model, 211 HANNA,.USA) according to the electrometric method, **[15]**. While Turbidity of samples was measured by the Nephelometric Method using (Al1000 Turbidimeter, aqualytic, Germany with measuring 0-200 NTU) according to **APHA [15]**.Where total dissolved solids (TDS), Salinity and Electrical conductivity (EC) were measured by TDS meter (Digital Portable TDS/ Conductivity meter Model. 8033 HANNA, USA) as TDS expressed as ppm (mg/l) and expressed as ds/m. Dissolved oxygen (DO) in the water sample was detected according to Winkler with azide modification method**, APHA [15]**. Biological oxygen demand (BOD) of the samples was determined according to **Adams [16]** where chemical oxygen demand (COD) of the samples was determined by Manual Method: Dichromate Reflux according to **EPA [14].** On the other hand, Nitrite ions and Total Nitrogen (TN) were determined according to **Rump** Method **[17]** and micro-Kjeldahl method but total phosphate (TP) of the samples was detected according to Ascorbic Acid Method **[15]**. However heavy metals analysis of water samples (Cd, Cu, Pb, Fe, Co, Zn, and Ni) were carried out according to **Yasser *et al.* [12].**

Preparation of microalgae

Tests were carried out using two species of microalgae, *Chlorella vulgaris* and *Scenedesmus quadricauda*, both supplied by Botany and Microbiology Department, Faculty of Science, Damietta University.

Initial stock cultures of *Chlorella vulgaris* and *Scenedesmus quadricauda* sp. were maintained in the modified Bold Basal medium [18] which containing the following chemicals: $NaNO_3$, $CaCl_2.2H_2O$, $MgSO_4.7H_2O$, K_2HPO_4, KH_2PO_4, NaCl, EDTA, KOH, $FeSO_4.7H_2O$, H_2SO_4, and micronutrients. The culture was inoculated in outdoor conditions for 30 days. Prior to inoculation, microalgae cultures were harvested using a centrifuge at low speed (3500 rpm) for ten minutes and washed three times with sterilized distilled water. This was followed by cell observation and cell concentration count using Neubauer Haemocytometer.

A total of 5 glass vessels (500 ml) were filled with 300 ml of Nile raw water which was used in this experiment as the wastewater. Three wastewater experiment glass vessels (triplicate) were inoculated with microalgae starting with an initial cell concentration of 10^3 cells/ml (population A) based on the standard methods **[15]**, 10^6 cell/ml (population B) according to **Gani *et al.* [19]** and 10^7 cell/ml (population C) **Kothari *et al.* [20]**. The other two glass vessels used as indicator, one of it had not been inoculated with any microalgae (Blank) and the other was control (population D). The glass vessels were covered with sterile cotton plugs and kept under outdoor natural condition during the experimental period. All samples were shaken from time to time to ensure that the microalgae were uniformly homogenized in the wastewater.

Determination of growth rate

The reduction in the pollution load was observed as a function of the increase in the growth rate of microalgae. The growth rate of microalgae was determined according to tow method for insurance. In first one, samples were taken from the culture for cell growth counting in wastewater using Haemocytometer with interval 48 hours according to **Komolafe *et al*. [21]** then the growth of microalgae was determined according to the specific growth rate (μ/day)

$$\text{Specific growth rate } (\mu/\text{day}) = \ln (C_f/C_i) / (T_f-T_i) \qquad \text{Eq. 1}$$

Where

C_f and C_i were defined as the cell concentration ($cell.ml^{-1}$) at time T_f and T_i, respectively.

While in the second method growth was measured every 48 hours using a spectrophotometer at a wavelength of 550 nm.

The data were expressed in agreement with **Eze *et al*.** [22] according to the following relation:

$$Ig = Abs\ 550\ T_{(x)} - Abs\ 550\ T_{(0)} / Abs\ 550\ T_{(0)} \qquad \text{Eq. 2}$$

Where

Ig the growth index
Abs550 $T_{(x)}$ absorbance of the 550-nm wavelength at time x
Abs550 $T_{(0)}$ absorbance of the 550-nm wavelength at time 0

Preparation of macroalgae

The raw biomass of *Dictyota dichotoma* and *Turbinaria ornata* was harvested from the red sea beach (coast of Hurghada north east Egypt). The samples were washed with deionized water to remove extraneous materials and common ions present in seawater. The washed biomass was dried at70°C for 48 h. The dried algae biomass was grinded, sieved and then the particles with an average size less than 0.1mm,0.1mm, 0.5mm, 0.7mm and 1 mm were examined to select the optimum texture which can be used for biosorption experiments. The biosorption experimental parameters (temperature, pH value, retention time and biomass dosage and texture) were optimized for reaching the equilibrium condition.3g/l of the biomass was added in the measuring baker with water and were shaken in an electrical flocculator at 150 rpm for contact time (20-30 mins) at pH 6.5 and temperature of 20◦C.The contents of the measuring baker were filtered through filter paper and the filtrate was analyzed for metal concentration by using the Inductively Coupled Plasma-Mass Spectrometry (ICP-OES 7000) was used for heavy metal analysis with an ultrasonic nebulizer (USN), this nebulizer decrease the instrumental detection limits by 10%, this ICP instrument is Perkin Elmer Optima 3000, USA.

The biosorption potential percent of algae for metal ion and the main physicochemical parameters was calculated as follows:

$$\text{Biosorption potential } (\%) = ((Hi-Hf) / Hi) \times 100$$

Where Hi and Hf are the initial and final concentrations before and after treatment, respectively.

Another technique has been used for the investigation and to improve the biosorption of heavy metals by macroalgae according to **Suparna *et al*. [23]**. The difference between the concentrations of heavy metals would be an indicator for biosorption potential of algae. The increase occurred in heavy metals accumulation in algae after usage, will used as indicator for biosorption potential of macroalgae.

RESULT AND DISCUSSION

The nutrient supplements required for microalgae growth depend on wastewater characteristics. The physical and chemical parameters of wastewater used in this study compared to the effluent standard which has been used in the formation of culture media in microalgae growth in previous researches, **Table 1**. The pH value was in acceptable concentration compared to the effluent standard 5 and standard 4 and suitable for microalgae cultivation, **[24]**. The concentration of DO, BOD and COD in this study was 6.53, 23.37 and 62.55 mg/L, respectively, and these concentrations were in the limited range compared to the other standards. The wastewater used also contained 2.8 mg/L of TP and 1.4 mg/L of TN, which was below standard limits. TP was acceptable compared to studies conducted by **Kothari *et al*. [29], Zhang *et al*. [36]** and **Gani *et al*. [19]**, as they founded that TP was 3.4, 1.59 and 3.27 mg/L, respectively. Other parameters were also determined such as TDS which was 40.3 mg/l and it represented acceptable concentration. A gradual reduction in the pollution load was attendant with the increase in algal growth, **Table 2** and **Figure 1**. In the first step in our investigation we found that population B achieved the best growth rate 1.44 - 0.8 (μ/day) from day 2 to the end of the test and also the best growth index (31.1), **Table 2** which was more related to control values (population D with growth rate 1.33 - 0.8 (μ/day) from day 2 to the end of the test and growth index (24.6).

The pollution load of the wastewater after inoculation by algal cells was measured on the 2nd, 6th, 10th, 14th, 18th,

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 8 Issue 09, September-2019

22th, 26th and 30th days of algal growth. Measurement of physicochemical parameters on 30th day showed substantial reduction in the level of BOD, COD, TN and TP concentrations with removal percent 92.7, 87.5,100 and100%, respectively, and obvious increase in DO concentration with percent 89.1%, **Figure 1**. This is Compatible with the results from the investigation of **Adey *et al*. [25]** as he reported that the alga *Chlorella vulgaris*, reduced ammonium by 72%, phosphorus by 82%, and COD by 61%. Similar results were also obtained on sewage water treatment by using phycoremediation **[26]**. In reported papers, **Colak and Kaya [27]** studied the removal potential of nutrients of *Chlorella vulgaris*. They found that the removal efficiency reached (50.2%) for nitrogen and (85.7%) for phosphorus in industrial wastewater treatment and (97.8%) for phosphorus in domestic wastewater and also for BOD and COD which reached 68.4% and 67.2%, respectively. **Lau *et al*. [28]** also recorded that, nutrient removal efficiency reached 86% of nitrogen and 70% of phosphorus. This reduction in nutrient concentration, BOD and COD and the increase in DO concentration were due to the effective growing of microalgae through the photosynthesis process and the complex symbiosis of algae and bacteria in the nutrient-rich environment **[29]**.

Table 2: Specific growth rate (μ/day) of microalgae during test duration

Time	*Specific growth rate (μ/day)*			
	A	*B*	*C*	*D*
Day 2	1.1	1.44	1.09	1.33
Day 6	1.28	1.17	1.17	1.22
Day 10	0.75	0.97	0.69	0.80
Day 14	0.85	0.97	0.75	0.89
Day 18	0.804	1.33	0.93	1.17
Day 22	0.81	1.41	0.75	1.35
Day 26	0.55	1.04	0.45	0.97
Day 30	0.34	0.80	0.20	0.80

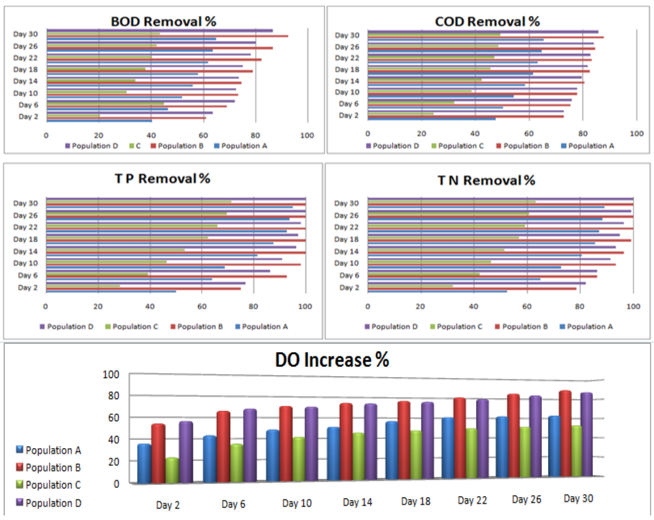

Figure 1: Treatment of water with the different population of microalgae.

In **Figure 2**, the pH value decreased gradually from 8.2 in the beginning of the investigation to 6.8 at the end of treatment. This due to the removal of various salts or metallic ions and also due to decomposition of organic matter which increased by microbial activities, **[26]**. Opposite observation was reported by **Ezenweani *et al*. [3]** as he founded that pH increase in case of usage of macroalgae and he up that to algal photosynthesis where CO_2 and H^+ ions removed when the algae are carbon limited.

Treatment with macroalgae

There were very limited studies that focused on the usage of macroalgae in TDS, Salinity, Conductivity, Turbidity, BOD and COD removal from wastewater while the majority focused on the removal of heavy metals, **[30]**. In this study, macroalgae achieved close results to microalgae in the wastewater treatment, **Figure 3.** The biosorption potential percent of *Turbinaria ornata* was 44.4, 29.3, 37.8, 84.2, 65.3, 67.5, 67.1, and 67.8% for TDS, Salinity, Conductivity, Turbidity, BOD, COD, TP and TN, respectively. While the biosorption potential percent of *Dictyota dichotoma* was 52.3, 39.3, 50.6, 95.3, 70.4, 71.3, 73.5 and 72.1% for TDS, salinity, conductivity, turbidity, BOD, COD, TP and TN, respectively. Simultaneously DO concentration increase with both algae, as it was 59.6% for *Turbinaria ornata* and 65.4% for *Dictyota dichotoma*.

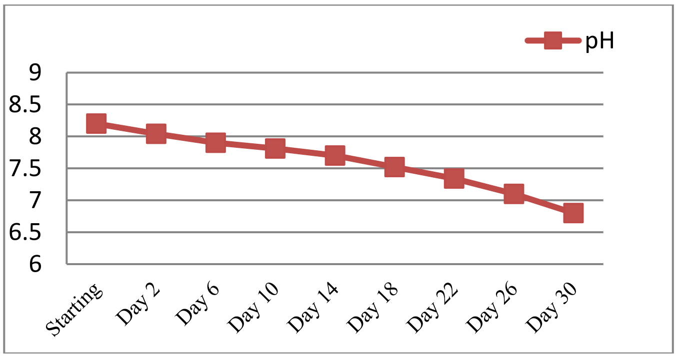

Figure 2: pH values during the treatment

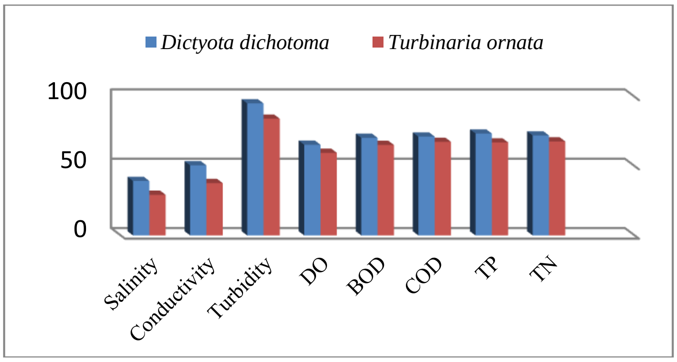

Figure 3: Biosorption potential percent of macroalgae

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
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Treatment of heavy metals

Chlorella and *Scenedesmus* are the most used microalgae for metal removal due to its efficient biotransformation ability, **[32]**. **Abedi *et al.* [33]** considered it a preferred option for wastewater treatment because of its rapid growth and efficient metal removal. On the other hand, marine macroalgae considered excellent biosorbents for metal uptake (Fe, Co, Cu, Mn, Ni, Zn, Cd, and Pb) especially brown algae, **[34].**

Treatment with microalgae

A gradual reduction was observed in the heavy metal concentration in wastewater from the beginning to the end of the test. This reduction was accompaniment to the present of algae **Table 3**. Best removal percent was achieved for Co, Fe and Pb with close percent 99.8, 99.7, 99.5%, respectively, **Figure 4** with minimum and maximum metal uptake 0.1765-0.8982 mg/l, 0.6706-1.2809 mg/l and 0.6817-1.3911 mg/l, respectively, **Table 3**. Cu removal percent followed that with percent 98.8% and that with minimum and maximum metal uptake 0.7501 and 1.3313mg/l. Zn removal percent reached 90.4% with minimum and maximum metal uptake 0.7398 and 1.1447 mg/l. The lowest removal was for Cd and Ni with percent 83.9 and 75.7 % respectively and with minimum and maximum metal uptake 0.1282-0.3057 mg/l. and 0.0224-0.0742 mg/l, respectively. **Sengar *et al.* [26]**, reported similar results as he recorded complete removal of Fe, Zn, Cu and he confirmed that due to the utilization of this element by microalgae (trace elements essential to algal growth).

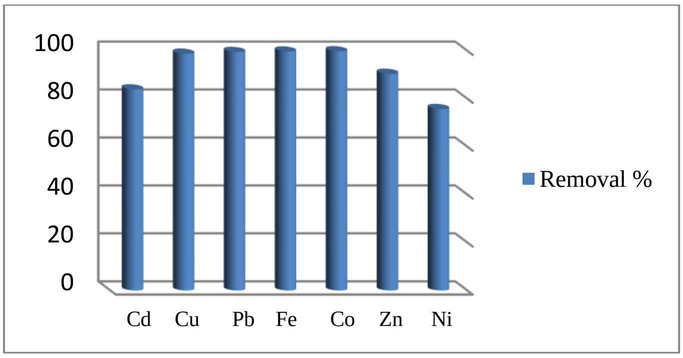

Figure 4: Heavy metal removal % by microalgae

Treatment with macroalgae

Turbinaria ornata and *Dictyota dichotoma* achieved different percent of heavy metal removal, **Figure 5**. Although this percent was lower than the percent achieved by microalgae but, it still valuable and considerable. *Turbinaria ornata* recorded removal percent of 55.5, 70.9, 59.8, 57.6, 55.1, 72.6 % for Cd, Cu, Pb, Fe, Co and Zn, respectively, with mean metal uptake of 0.202, 0.955, 0.835, 0.739, 0.495 and 0.918 mg/l, respectively. However, the lowest removal percent of Ni with percent 42.1 % and mean metal uptake 0.041 mg/l. In a similar way, *Dictyota dichotoma* remove Ni with the lowest removal percent 34.2% and mean metal uptake 0.033 mg/l. Zn is the second-order following Ni as it removed by percent 40.7 and with mean metal uptake 0.346 mg/l. Cd, Cu, Pb, Fe, and Co were removed with acceptable higher percent 68.3, 72.7, 57.8, 70.5, 52.1%, respectively, and that with mean metal uptake of 0.248, 0.979, 0.8075, 0.905 and 0.404 mg/l. **Mazen [35]** explains this removal potential to the cell wall which has a lot of active chemical functional groups that offer a selective interaction and binding with metals such as phenolic, hydroxyl, amine, carboxylic acid.

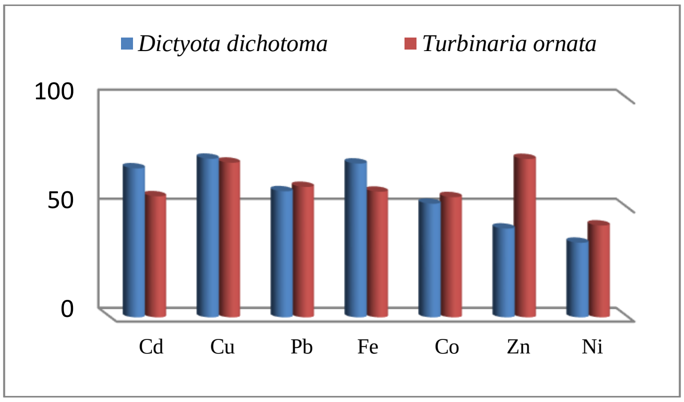

Figure 5: Heavy metal removal % by macroalgae

CONCLUSION

Phycoremediation had become a focus of attention among researchers worldwide. This study demonstrates the ability of macro and microalgae for the removal of pollution and treating wastewater. We found that *Chlorella vulgaris* and *Scenedesmus quadricauda* as microalgae have a high potential of nutrient and metal uptake which can be used for wastewater treatment. In the same way, *Turbinaria ornata* and *Dictyota dichotoma* as macroalgae can be used as an efficient absorbent. Overall, results from this study highlighted the possibility and the need for the usage of algae as an efficient, alternative, eco-friendly and low-cost technique to remediate wastewater and it can also be used as energy source due to the ability to convert the biomass generated from treatment into different forms of bio-energy (biogas, biodiesel, and bio-hydrogen), **[32]**.

ACKNOWLEDGEMENTS

The authors would like to express deepest appreciation to all those who provided us the possibility to complete this research. Thank the support of the staff members and colleagues of the Environmental Sciences Department, Faculty of Science, Damietta University and special thanks go also to Botany and Microbiology Department, Faculty of Science, Damietta University for providing the equipment and research facilities.

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 8 Issue 09, September-2019

Table 1 : Characteristics of the raw domestic wastewater used as the growth media.

Physiochemical parameters (mg/l)	**Present study 2019**	**Gani *et al.* 2016**	**Kothari *et al.* 2012**		**Effluent standard, mg/L (Environmental Quality Act, 1974)**	
		Standard 1	Standard 2	Standard 3	Standard 4	Standard 5
pH	8.2	6.93	6.2	7.3	5.5 – 9.0	6.0 – 9.0
DO	6.53	14.76	-	-	10	5
BOD	23.37	44	-	-	50	20
COD	62.55	76.10	-	-	100	50
TP	2.8	3.27	21	3.4	-	-
TN	1.4	15.79	67.35	15.16	-	-
TDS	40.3	49	90	40	-	-

Table 3: Heavy metal removal % by microalgae

Parameters	***Pretreated water***	***Post treated water***									***MIN***	***MAX***		***STD***	**Metal uptake (mg/l)**
		Day 2	***Day 6***	***Day 10***	***Day 14***	***Day 18***	***Day 22***	***Day 26***	***Day 30***	***Removal %***				***MIN***	***MAX***
Cd	**0.364**	**0.2358**	**0.1924**	**0.1055**	**0.1009**	**0.0978**	**0.0965**	**0.0999**	**0.0583**	**83.9**	**0.0583**	**0.2358**	**0.059029**	**0.1282**	**0.3057**
Cu	**1.347**	**0.5969**	**0.3027**	**0.259**	**0.1278**	**0.1047**	**0.0797**	**0.0659**	**0.0157**	**98.8**	**0.0157**	**0.5969**	**0.189733**	**0.7501**	**1.3313**
Pb	**1.397**	**0.7153**	**0.3113**	**0.290**	**0.2297**	**0.1275**	**0.0928**	**0.0324**	**0.0059**	**99.5**	**0.0059**	**0.7153**	**0.228088**	**0.6817**	**1.3911**
Fe	**1.284**	**0.6134**	**0.551**	**0.4031**	**0.2061**	**0.1102**	**0.0787**	**0.0106**	**0.0031**	**99.7**	**0.0031**	**0.6134**	**0.243521**	**0.6706**	**1.2809**
Co	**0.9**	**0.7235**	**0.490**	**0.4186**	**0.0919**	**0.0859**	**0.045**	**0.0099**	**0.0018**	**99.8**	**0.0018**	**0.7235**	**0.27287**	**0.1765**	**0.8982**
Zn	**1.265**	**0.5252**	**0.3408**	**0.2257**	**0.2247**	**0.2218**	**0.1787**	**0.1685**	**0.1203**	**90.4**	**0.1203**	**0.5252**	**0.127923**	**0.7398**	**1.1447**
Ni	**0.098**	**0.0756**	**0.069**	**0.0524**	**0.0478**	**0.0475**	**0.044**	**0.0339**	**0.0238**	**75.7**	**0.0238**	**0.0756**	**0.016947**	**0.0224**	**0.0742**