International Journal of Aquatic Science
ISSN: 2008-8019
Vol 13, Issue 01, 2022

Treatment of Municipal Wastewater Contaminated with Heavy Metals and Hydrocarbons by Chlorella vulgaris

Ali Hammoud Raheef Al-Shammari[1], Amira Imran Hussein Al-Janabi[2]

[1,2]College of Environmental Sciences, Al-Qasim Green University, Iraq

Abstract*: The current study was conducted using the green algae Chlorella vulgaris to treat municipal sewage water taken from the central Rumaitha water treatment plant, Muthanna Governorate, Iraq. It aimed to assess the efficiency of algae in improving water quality through algae treatment (Phycoremediation) for some heavy metal pollutants such as lead, cadmium and copper, in addition to the total hydrocarbons present in those waters. The experiment was carried out by adding contaminated water to the algal culture after the algae Chlorella vulgaris reached to the Stationary phase. The treatment process continued for 8 days before the algae reached to the Death phase, in order to obtain the best efficiency period for the algae during the treatment process. The results showed a high efficiency of Chlorella vulgaris in treating municipal wastewater, where the results showed the removal percentage after 4 days of treatment and it was as follows, copper 98.9%, cadmium 94.75%, lead 57.5% and total hydrocarbons 64.5%. While it was noted that the reduction percentage increased after a treatment period of 8 days, the results were as follows: copper 99.2%, cadmium 97.9%, lead 75.3% and total hydrocarbons 72.3%. The results of this study confirmed the efficiency of Chlorella vulgaris in the treatment process and its high ability to remove heavy metal and hydrocarbon pollutants. Thus, algae processing is a promising, highly efficient and environmentally friendly biological treatment technology.

Keywords: Municipal Wastewater, Heavy Metals, Hydrocarbons, Chlorella vulgaris

1. INTRODUCTION:

Water quality is one of the main concerns of the current century, given the increasing scarcity of water resources. Water quality deterioration, either due to human activities (such as pollution and overexploitation of resources) or natural phenomena (such as global warming and extreme weather events), often leads to severe impacts on ecosystems and public health, which in turn affect society and the environment (Pacheco et al., 2020).

The discharge of untreated or insufficiently treated effluents into rivers, lakes, aquifers and coastal waters provides the aquatic environment with a myriad of chemical compounds that can affect aquatic organisms directly by causing dangerous effects on them, or indirectly through by changing some physical and chemical properties of the medium (such as oxygen concentration, pH, nutrient concentration, oxidation and reduction potential) (Menger-Krug et al., 2012; Mohamad et al., 2017). Water pollution with heavy metals is considered one of the most difficult types of pollution, as these heavy metals remain without degradation and are highly susceptible to accumulation in the bodies of living organisms (Briffa et al., 2020).

Petroleum hydrocarbons are also one of the most important environmental pollutants that cause problems for the environment in which they are found, because they are toxic to most living organisms, in addition to the fact that some of them are carcinogenic and have the ability to move in the food chain. The problem of global pollution with hydrocarbons has emerged as a result of the increasing and continuous demand for crude oil (crude oil is a complex compound with hydrocarbons making up 95% of its components, in addition to sulfur, nitrogen, oxygen and minerals such as nickel, vanadium, iron and copper) because it is considered the most important source of energy in the world (Catania et al., 2020).

The techniques used in wastewater treatment vary, some of them are physical or chemical, some depend on the combination of the two methods, and some of the processes depend on biological methods (Bes-Piá et al., 2002). It has been proven that physical and chemical treatment processes alone are ineffective in treating effluents of complex composition (Sutherland & Ralph, 2019). Therefore, a possible solution could be a combination of physical, chemical and biological treatment techniques aimed at developing sustainable treatment processes. The biological treatment system is one of the best types of techniques used in treatment because it is a safe, healthy and low-cost method. As well as the availability of its basic materials in nature because it depends on living organisms (bacteria, fungi and algae) for which water is considered a habitat (Wang et al., 2016).

The use of algae in the biological treatment of wastewater is one of the best methods used in the treatment because it has a great ability to remove carbon, nitrogen and phosphorous from the water contaminated with it, as well as it does not require energy or the use of chemicals, where the use of algae is called to remove pollutants from wastewater Sanitary or liquid waste before being discharged or reused (phycoremediation) (Emparan et al., 2019; Pacheco et al., 2020). Green microalgae (belonging to Chlorophyta) were selected for this study because they are inexpensive, renewable, available in many parts of the world, and can be grown in both fresh and salt water under a variety of climatic conditions, as they contain a cell wall with different functional groups (Gharbani, 2018). Thus, the adoption of algae in the biological treatment process of municipal or industrial wastewater treatment plants is a promising approach that enhances the treatment of effluents in an environmentally sustainable manner (Bes-Piá et al., 2002). and achieve lucrative economic benefits, as global interest is increasing with regard to the cultivation of microalgae to take advantage of the biomass produced from Microalgae and its use in many industries, for example (food, feed, textiles, pharmaceuticals, bioplastics, biofuels and biofertilizers) (Enamala et al., 2018; Ravindran et al., 2016; Satyanarayana et al., 2011).

2. MATERIALS AND METHODS:

Prohibition of growth medium for algae:

The modified Chu-10 medium was used by (Kassim et al., 1999). Where all the chemicals used in the medium were prepared individually by dissolving a specific weight in one liter of deionized water, all the materials used in the medium are shown in Table 1. Then 2.5 ml of each solution was taken and filled in one liter of deionized water. Then it is autoclaved except for (K2HPO4) solution which is autoclaved alone and finally added to get 1 liter of Chu-10 after which the pH is adjusted to 6.9-7.0 using (0.01 N) NaOH or HCl.

Table 1. The components and concentration of modified Chu-10 medium and the concentration of each component (Kassim et al., 1999).

stock solution		g/l
1	(MgSO4.7H2O)	10
2	(MnCl2.4H2O)	0.02
3	Na2-EDTA	4
4	(Na2CO3)	8
5	NaCl	30
6	CoCl2.6H2O	0.004
7	(NH4)6MO7O24.4H2O	0.028
8	(NaNO3)	8
9	(ZNSO4. 7H2O)	0.224
10	CaCl2	16
11	(CUSO4.5H2O)	0.08
12	(H3BO3)	0.288
13	(FeCl3)	0.32
14	K2Hpo4	4
15	Demineralized water	1L

Growth and propagation of the alga Chlorella vulgaris

Chlorella vulgaris was grown in medium Chu-10, Table 1 (Chu, 1942) and modified by (Kassim et al., 1999). The algae were cultivated using the meal culture method by 100 ml of medium and 10 ml of pure algae, as they were observed in suitable growth conditions of temperature (25 ± 2 ° C) and intensity of illumination (50 µE / cm 2 / s) with a ratio of 8:16 light: Darkening for 7 days, after which the volume is completed to 1000 ml of culture medium. The optical density of algae was calculated using a spectrophotometer at a wavelength of 540 nm to track their growth (Juda et al., 2019).

Treatment of contaminated water in a farm of Chlorella vulgaris

The algae treatment was carried out by taking 1 liter glass beakers and placing 850 ml of household waste water collected in 5 liter polyethylene containers, and 150 ml from the demand farm after it reached the stationary phase (Al-Rubaie, 2003), with three replications taking into account Considering the control treatment that contains 1 liter of waste water only, then the treatments were placed in duplicate in the culture room and a certain size of the farm was taken every four days by 8 days for testing on it.

Analyzes used in the study

Total Heavy Metals Determination

The method described in (APHA, 2005). was followed, where 50 ml of sample water was taken and digested by adding (5) ml of concentrated nitric acid HNO3 and the samples were heated on a hot plate at 80 ºC, then (1) ml of concentrated nitric acid HNO3 was added. A period of time was left on the plate to complete dissolution, then the final volume was completed to (50) ml with distilled water free of ions. The samples were kept in plastic containers until measurement. The concentrations were measured using atomic absorption spectrometry, and the results were expressed in ppm.

International Journal of Aquatic Science
ISSN: 2008-8019
Vol 13, Issue 01, 2022

Determination of total hydrocarbons

The method approved by the United Nations Environmental Protection Program (UNEP, 1989) was used to extract petroleum hydrocarbons from water. For every one liter of sample, 10 ml of Chloride Tetra Carbon (CCl4) was added, then the sample was shaken well using an electric mixer for 30 minutes, and the contents were transferred to a separating funnel and left until settling. The organic layer is separated because it is heavier than water. The organic layer was passed over a column containing glass wool at the bottom, topped with a layer of anhydrous sodium sulphate (Na2SO4) sulphate sodium anhydrous to remove the water remaining in the sample. Spectrofluorometer The concentrations were calculated according to the following equation:

$$C = \frac{X \times D \times 1000}{Q \times L}$$

$$X = \frac{Y - 1.37838}{28.14496}$$

Where:
C = total hydrocarbon concentration
Y = sample reading - solvent reading
D = dilution factor
Q = the quantity of sample injected into the device

3. RESULTS AND DISCUSSION

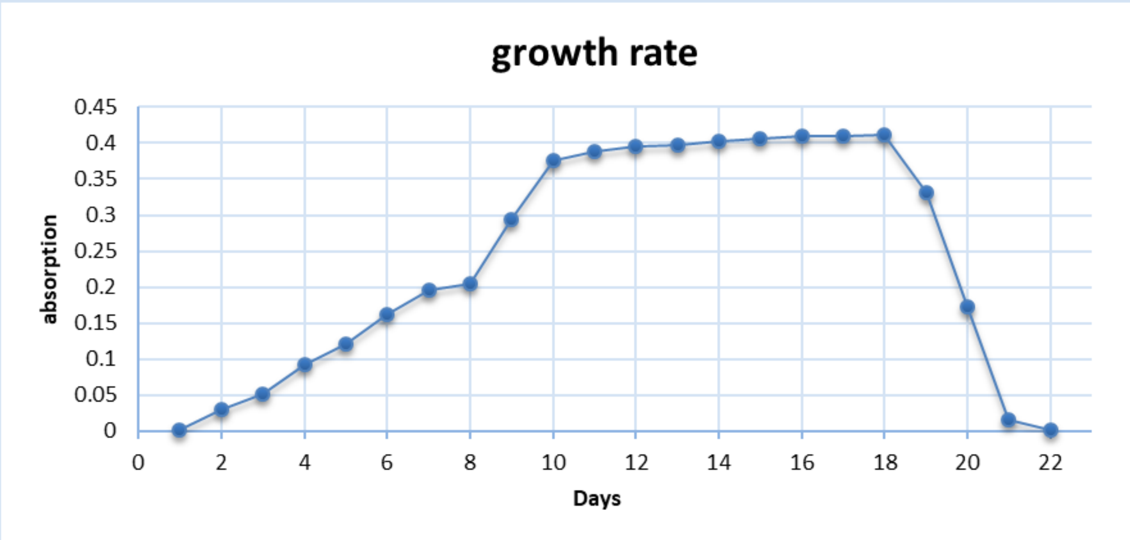
Characteristics of municipal wastewater: The characteristics of municipal wastewater were measured before treatment, which are listed in Table 2. It was found that there was a high percentage of heavy metals, such as lead, cadmium and copper, below the permissible limits, in addition to the high concentration of total hydrocarbons. This is evidence that the water used is completely polluted and is not suitable for various uses, such as human or agricultural.

Type of water / Measured factors	Municipal wastewater
Total hydrocarbons mg/l	**5.38**
Lead ppm	**0.261**
Copper ppm	**0.401**
Cadmium ppm	**0.069**

Table 2. Characteristics of Municipal Wastewater

Algae growth in wastewater: After Chlorella vulgaris reached stabilization on day 10 of growth in Chu-10 culture medium whose components are shown in Table 1. 150 ml of algal culture was injected into 850 ml of municipal wastewater, under ideal conditions. From the temperature, light intensity and oxygen, the algae growth was monitored by measuring the absorbance to identify the density of algae cells using a spectrophotometer at a wavelength of 540 nm. Figure 1. Although water contains high concentrations of nutrients that can inhibit algal growth (Wrigley & Toerien, 1990). In addition, the presence of toxic heavy metals, organic compounds and hydrocarbons in wastewater is another important inhibiting factor for microalgae (Chinnasamy et al., 2010). However, Chlorella vulgaris was able to grow perfectly and maintained a slightly increased rate on days 12 to 18 when it reached its highest growth rate. On day 19 he started dying and continued to decline until day 22 when his death was fully confirmed.

Fig 1. Growth rate of algae *Chlorella vulgaris*



Heavy Metal Reduction

Cadmium: Cadmium is a highly toxic rare, non-essential heavy metal well known for its detrimental effect on cellular enzyme systems and its tendency to bioaccumulate in living organisms (Irfan et al., 2013). The International Agency for Research on Cancer classified cadmium and its compounds as carcinogenic to humans (Henson & Chedrese, 2004). The results of the current study indicated the high efficiency of Chlorella vulgaris in reducing heavy metals, including cadmium, with a reduction rate of 94.75% recorded on day 4 of the treatment process and continued to increase until it reached 97.9% on day 8 of the experiment, and this is evidence of the efficiency Algae in the treatment of heavy metals in municipal wastewater. Previous studies showed the high efficiency of algae in reducing cadmium values, in a study conducted on the alga Chlorella minutissima, where it proved an efficiency in removing cadmium by 84% after planting it in wastewater (Yang et al., 2015). Brahmbhatt et al. (2012) indicated that Oscillatoria sp was highly efficient in reducing cadmium in return for low chlorophyll and protein content. Al-Husseini and Al-Mayali (2015) showed the role of Westiellopsis prolifica in reducing the element cadmium when exposing the alga to different concentrations of the elements, where the removal rate was 73%. While in another study, Cladophora fracta was used, the percentage of cadmium removal was 78% (Bulgariu & Bulgariu, 2016).

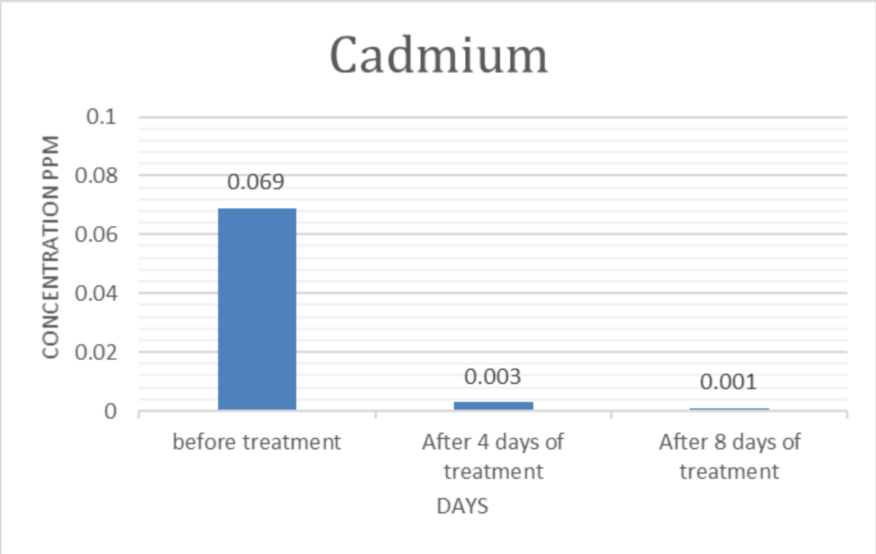


Fig 2. Cadmium before and after treatment

Copper:

Copper is an essential element for living organisms, as it is considered a micronutrient for cellular metabolism as it is an essential component of metabolic enzymes (Monteiro et al., 2009). Despite its usefulness in many daily operations, excessive exposure to copper can lead to serious damage to plants, animals and humans. When exposed to high concentrations that exceed normal levels, copper is highly toxic to the vital processes inside the cell (Trumbo et al., 2001). The results of the current study indicated the high efficiency of copper reduction by the alga Chlorella vulgaris. Figure 3 shows the reduction values of copper in municipal wastewater, where the alga reduced 98.9% on day 4, the process of copper reduction continued to increase to 99.2%. Through the previous results, it is clear that Chlorella vulgaris has a high ability to reduce heavy metals. The role of algae in the reduction was recorded in many studies, as Ajayan et al. (2011) recorded the role of the algae Oscillatoria quadripunctulata in the reduction of many heavy elements, and a reduction of copper was recorded by 50%. And Abdul-Jabbar (2008) showed the efficiency of blue-green algae such as Nostoc linckia and Oscillatoria limosa in removing copper from waste water in Nasiriyah power plant. Copper is one of the essential elements important for algae growth as it enters into metabolic reactions such as respiration and photosynthesis when it is present in trace quantities (Al-Saadi et al., 2002). In another study to remove the element of copper by the blue-green alga Spirulina sp. It was found that it is able to remove 95% of the element (Chojnacka et al., 2005). It was also found that the alga Oscillatoria anguistissima is able to absorb the element copper with high efficiency, and it was found that it increases in absorption, the higher the concentration of the element, as it removed about 68.4 µg/ml of the element copper (Ahuja et al., 1997).

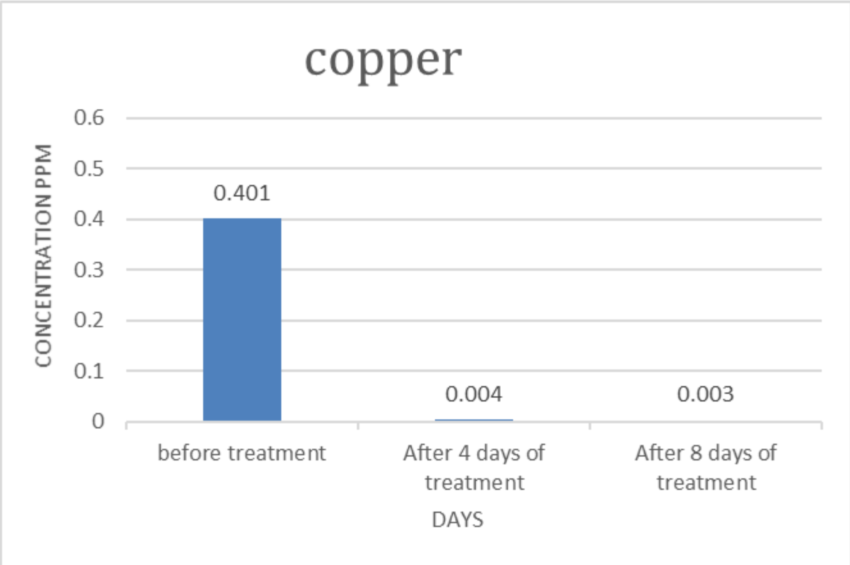


Fig 3. copper before and after treatment

Lead:

It is one of the very toxic heavy metals because it not only accumulates in individuals, but has the ability to enter the food chain and disrupt the health system of humans and influence on animals and phytoplankton. and teeth and can affect the kidneys, nervous system and brain (D. Singh et al., 2012). The results of the current study indicated the efficiency of Chlorella vulgaris in reducing lead from polluted wastewater. Figure 4 shows the reduction percentage of lead from municipal wastewater. On day 4 of treatment, the algae recorded a reduction rate of 57.5%, while this percentage increased to reach 75.3% on day 8 of treatment, which is a good result, but it is less than the rest of the other heavy metals. The high efficiency of algae in reducing lead element was recorded in several studies, such as the study of Ajayan et al. (2011), which showed that the algae Oscillatoria quadripunctulata possesses a high ability to reduce heavy elements and recorded a lead reduction rate of 100%. And between Al-Husseini and Al-Mayahi (2015), the efficiency of Westiellopsis prolifica in reducing lead element, with the highest reduction rates recorded on the last day of the experiment. In another study conducted by Pithophora odeogonia and Spirogyra neglecta, it confirmed the high reduction rate of lead, which was 97% and 89%, respectively, in the two algae (A. Singh et al., 2007).

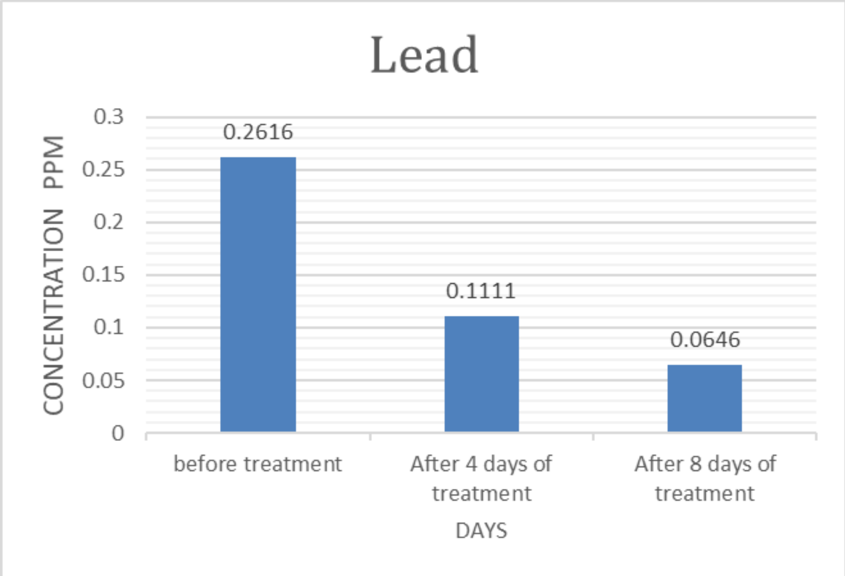


Fig 4. Lead before and after treatment

Total hydrocarbons

Petroleum hydrocarbons are one of the most dangerous pollutants in the aquatic environment, as oil spills are one of the main factors that have long-term adverse effects on marine life and marine biodiversity (Demirel et al., 2017; Jung et al., 2017; Kalhor et al., 2016). Some types of algae can oxidize and decompose many types of hydrocarbons into less harmful components, indicating their ability to process crude oil (El-Sheekh et al., 2013; He et al., 2013). In the current experiment, we examined the possibility of Chlorella vulgaris algae in the treatment of total hydrocarbons in municipal wastewater, where Chlorella vulgaris is a kind of green microalgae with a high potential to treat the aquatic environment polluted with crude oil (El-Sheekh et al., 2013; Kalhor et al., 2016). In the current study, the efficiency of Chlorella vulgaris in treating hydrocarbon pollutants from wastewater was proven. The results showed a reduction rate of 64.5% on day 4 of treatment, and this percentage increased to reach 72.3% on day 8 of treatment. Figure 5 shows the reduction ratio of total hydrocarbons during the treatment period.

The effectiveness of microalgae has been proven in the treatment of hydrocarbon pollutants in many previous studies, especially those belonging to the genera Selenastrum, Scenedemus or Chlorella, where they showed their efficiency in the decomposition of total hydrocarbons (Ghosal et al., 2016; Lei et al., 2007). While in another study, Chlorella vulgaris showed a high potential for treating water contaminated with crude oil. Where the efficiency of removal was between 88% and 94% (Xaaldi Kalhor et al., 2017). While (Das & Deka, 2019) it was shown that Chlorella vulgaris is able to break down 98% of petroleum hydrocarbon pollutants from the water on day 14 of treatment.

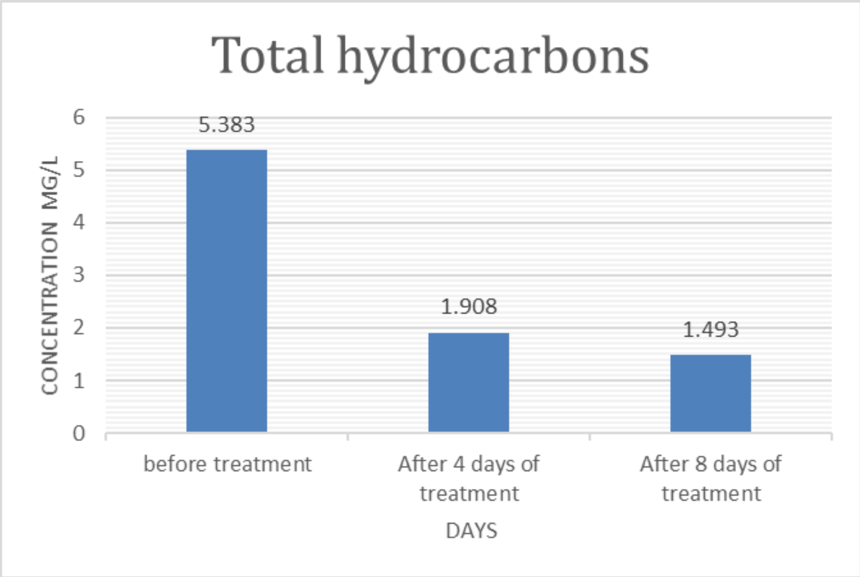


Fig 5. Total hydrocarbons before and after treatment

4. REFERENCES

Abdel-Jabbar, A. S. (2008). Estimation of the efficiency of the algae Nostoc linckia (Roth.) Bron. & Thar. and Oscillatoria limosa (Roth.) CA Ag in removing some heavy metals from waste water in Nasiriyah power station. Journal of Basrah Researches (Sciences), 34(2B).

APHA (American public health association). (2005). Standard methods for examination of water and wastewater.21the Ed. Washington, D.C., U.S.A.

Ahuja, P., Gupta, R., & Saxena, R. K. (1997). Oscillatoria anguistissima: a promising Cu2+ biosorbent. Current Microbiology, 35(3), 151–154.

Ajayan, K. v, Selvaraju, M., & Thirugnanamoorthy, K. (2011). Growth and heavy metals accumulation potential of microalgae grown in sewage wastewater and petrochemical effluents. Pakistan Journal of Biological Sciences, 14(16), 805.

Al-Hussainy, A. K., & Al-Mayaly, I. K. (2015). Bioremoval of Lead and Cadmium by the Alga westillopsis prolifica. Iraqi Journal of Science, 56(4B).

Al-Rubaie, G. H. (2003). The use of algae in treating household wastewater.

Al-Saadi, H. A., Al-Lami, A. A., Hassan, F. A., & Al-Dulymi, A. A. (2002). Heavy metals in water, suspended particles, sediments and aquatic plants of Habbaniya Lake, Iraq. International Journal of Environmental Studies, 59(5), 589–598.

Bes-Piá, A., Mendoza-Roca, J. A., Alcaina-Miranda, M. I., Iborra-Clar, A., & Iborra-Clar, M. I. (2002). Reuse of wastewater of the textile industry after its treatment with a combination of physico-chemical treatment and membrane technologies. Desalination, 149(1–3), 169–174.

Brahmbhatt, N. H., Patel, R. v, & Jasrai, R. T. (2012). Bioremediation potential of Spirogyra sps & Oscillatoria sps for cadmium. Asian Journal of Biochemical and Pharmaceutical Research, 2(2), 102–107.

Briffa, J., Sinagra, E., & Blundell, R. (2020). Heavy metal pollution in the environment and their toxicological effects on humans. Heliyon, 6(9), e04691.

Bulgariu, D., & Bulgariu, L. (2016). Potential use of alkaline treated algae waste biomass as sustainable biosorbent for clean recovery of cadmium (II) from aqueous media: batch and column studies. Journal of Cleaner Production, 112, 4525–4533.

Catania, V., Lopresti, F., Cappello, S., Scaffaro, R., & Quatrini, P. (2020). Innovative, ecofriendly biosorbent-biodegrading biofilms for bioremediation of oil-contaminated water. New Biotechnology, 58, 25–31.

Chinnasamy, S., Bhatnagar, A., Hunt, R. W., Claxton, R., Marlowe, M., & Das, K. C. (2010). Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. Google Patents.

Chojnacka, K., Chojnacki, A., & Gorecka, H. (2005). Biosorption of Cr3+, Cd2+ and Cu2+ ions by blue–green algae Spirulina sp.: kinetics, equilibrium and the mechanism of the process. Chemosphere, 59(1), 75–84.

Chu, S. P. (1942). The influence of the mineral composition of the medium on the growth of planktonic algae: part I. Methods and culture media. The Journal of Ecology, 284–325.

Das, B., & Deka, S. (2019). A cost-effective and environmentally sustainable process for phycoremediation of oil field formation water for its safe disposal and reuse. Scientific Reports, 9(1), 1–15.

Demirel, Y. T., Yati, I., Donmez, R., & Sonmez, H. B. (2017). Clean-up of oily liquids, fuels and organic solvents from the contaminated water fields using poly (propylene glycol) based organogels. Chemical Engineering Journal, 312, 126–135.

El-Sheekh, M. M., Hamouda, R. A., & Nizam, A. A. (2013). Biodegradation of crude oil by Scenedesmus obliquus and Chlorella vulgaris growing under heterotrophic conditions. International Biodeterioration & Biodegradation, 82, 67–72.

Emparan, Q., Harun, R., & Danquah, M. K. (2019). Role of phycoremediation for nutrient removal from wastewaters: a review. Appl. Ecol. Environ. Res, 17(1), 889–915.

Enamala, M. K., Enamala, S., Chavali, M., Donepudi, J., Yadavalli, R., Kolapalli, B., Aradhyula, T. V., Velpuri, J., & Kuppam, C. (2018). Production of biofuels from microalgae-A review on cultivation, harvesting, lipid extraction, and numerous applications of microalgae. Renewable and Sustainable Energy Reviews, 94, 49–68.

Gharbani, P. (2018). Modeling and optimization of reactive yellow 145 dye removal process onto synthesized MnOX-CeO2 using response surface methodology. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 548, 191–197.

Ghosal, D., Ghosh, S., Dutta, T. K., & Ahn, Y. (2016). Current state of knowledge in microbial degradation of polycyclic aromatic hydrocarbons (PAHs): a review. Frontiers in Microbiology, 1369.

He, P. J., Mao, B., Shen, C. M., Shao, L. M., Lee, D. J., & Chang, J. S. (2013). Cultivation of Chlorella vulgaris on wastewater containing high levels of ammonia for biodiesel production. Bioresource Technology, 129, 177–181.

Henson, M. C., & Chedrese, P. J. (2004). Endocrine disruption by cadmium, a common environmental toxicant with paradoxical effects on reproduction. Experimental Biology and Medicine, 229(5), 383–392.

Irfan, M., Hayat, S., Ahmad, A., & Alyemeni, M. N. (2013). Soil cadmium enrichment: Allocation and plant physiological manifestations. Saudi Journal of Biological Sciences, 20(1), 1–10.

Juda, S. A., Salah, M. M., & Salman, J. M. (2019). Efficiency of green algae Chlorella vulgaris in remediation of polycyclic aromatic hydrocarbon (anthracene) from culture media. Baghdad Science Journal, 16(3), 543–549.

Jung, D., Kim, J.-A., Park, M.-S., Yim, U. H., & Choi, K. (2017). Human health and ecological assessment programs for Hebei Spirit oil spill accident of 2007: Status, lessons, and future challenges. Chemosphere, 173, 180–189.

Kalhor, A. X., Nassab, A. D. M., Abedi, E., Bahrami, A., & Movafeghi, A. (2016). Biodiesel production in crude oil contaminated environment using Chlorella vulgaris. Bioresource Technology, 222, 190–194.

Kassim, T. I., Al-Saadi, H., & Salman, N. A. (1999). Production of some phyto-and zooplankton and their use as live food for fish larva. Iraqi J. Agric. Proc, 2, 188–201.

Lei, A.-P., Hu, Z.-L., Wong, Y.-S., & Tam, N. F.-Y. (2007). Removal of fluoranthene and pyrene by different microalgal species. Bioresource Technology, 98(2), 273–280.

Menger-Krug, E., Niederste-Hollenberg, J., Hillenbrand, T., & Hiessl, H. (2012). Integration of microalgae systems at municipal wastewater treatment plants: implications for energy and emission balances. Environmental Science & Technology, 46(21), 11505–11514.

Mohamad, S., Fares, A., Judd, S., Bhosale, R., Kumar, A., Gosh, U., & Khreisheh, M. (2017). Advanced wastewater treatment using microalgae: effect of temperature on removal of nutrients and organic carbon. IOP Conference Series: Earth and Environmental Science, 67(1), 012032.

Monteiro, S. M., dos Santos, N. M. S., Calejo, M., Fontainhas-Fernandes, A., & Sousa, M. (2009). Copper toxicity in gills of the teleost fish, Oreochromis niloticus: effects in apoptosis induction and cell proliferation. Aquatic Toxicology, 94(3), 219–228.

Pacheco, D., Rocha, A. C., Pereira, L., & Verdelhos, T. (2020). Microalgae water bioremediation: trends and hot topics. Applied Sciences, 10(5), 1886.

Ravindran, B., Gupta, S. K., Cho, W.-M., Kim, J. K., Lee, S. R., Jeong, K.-H., Lee, D. J., & Choi, H.-C. (2016). Microalgae potential and multiple roles—current progress and future prospects—an overview. Sustainability, 8(12), 1215.

Satyanarayana, K. G., Mariano, A. B., & Vargas, J. V. C. (2011). A review on microalgae, a versatile source for sustainable energy and materials. International Journal of Energy Research, 35(4), 291–311.

Singh, A., Mehta, S. K., & Gaur, J. P. (2007). Removal of heavy metals from aqueous solution by common freshwater filamentous algae. World Journal of Microbiology and Biotechnology, 23(8), 1115–1120.

Singh, D., Tiwari, A., & Gupta, R. (2012). Phytoremediation of lead from wastewater using aquatic plants. Journal of Agricultural Technology, 8(1), 1–11.

Sutherland, D. L., & Ralph, P. J. (2019). Microalgal bioremediation of emerging contaminants-Opportunities and challenges. Water Research, 164, 114921.

Trumbo, P., Yates, A. A., Schlicker, S., & Poos, M. (2001). Dietary reference intakes. Journal of the American Dietetic Association, 101(3), 294.

UNEP. United Nation Environmental program. (1989). Comparative toxicity test of water accommodated fraction of oils and oil dispersant's to marine organisms. Reference methods for marine pollution No. 45 : 21 p.

Wang, Y., Ho, S.-H., Cheng, C.-L., Guo, W.-Q., Nagarajan, D., Ren, N.-Q., Lee, D.-J., & Chang, J.-S. (2016). Perspectives on the feasibility of using microalgae for industrial wastewater treatment. Bioresource Technology, 222, 485–497.

Wrigley, T. J., & Toerien, D. F. (1990). Limnological aspects of small sewage ponds. Water Research, 24(1), 83–90.

Xaaldi Kalhor, A., Movafeghi, A., Mohammadi-Nassab, A. D., Abedi, E., & Bahrami, A. (2017). Potential of the green alga Chlorella vulgaris for biodegradation of crude oil hydrocarbons. Marine Pollution Bulletin, 123(1–2), 286–290. https://doi.org/10.1016/j.marpolbul.2017.08.045

Yang, J., Cao, J., Xing, G., & Yuan, H. (2015). Lipid production combined with biosorption and bioaccumulation of cadmium, copper, manganese and zinc by oleaginous microalgae Chlorella minutissima UTEX2341. Bioresource Technology, 175, 537–544.