Bionatura
Latin American Journal of Biotechnology and Life Sciences

Bionatura Issue 2 Vol 8 No 1 2023

Article

Chlorella vulgaris has been used in the biological treatment of some pollutants and heavy metals in municipal wastewater

Ali Hammoud Raheef Al-Shammari [1], Amira Imran Hussein Al-Janabi[2]*
[1]College of Environmental Sciences, Al-Qasim Green University, Iraq.
[2]College of Environmental Sciences, Al-Qasim Green University, Iraq.
[*]Correspondence author: Aliking5666ali@gmail.com
Available from: http://dx.doi.org/10.21931/RB/CSS/2023.08.02.59

ABSTRACT

This study aims to prove the ability of *Chlorella vulgaris* to treat some pollutants such as nitrates, nitrites, phosphates, ammonia, the chemical oxygen requirement and some heavy metals such as cadmium and copper present in the water of municipal wastewater treatment plants**.** Water samples were taken from Al-Rumaitha Central Water Treatment Plant, Al-Muthanna Governorate, Iraq. Contaminated water was added to the algal culture after the algae reached the Stationary phase after 10 days of growth. The tests were performed on the water after two periods, the first after 4 days of the algae reaching the Stationary phase and the second after 8 days before the arrival of the algae to the Death phase.

The results showed a high efficiency of *Chlorella vulgaris* in treating polluted water. The removal percentage after 4 days of exposure period was as follows: BOD5 86.6%, nitrate 62.9%, nitrite 14.7%, ammonia 94.2%, and phosphate 86.2%. As for the heavy elements, they were as follows: copper 98.9%, cadmium 94.75%. After 8 days of the treatment process, the efficiency of the algae was better than the previous days, where the results showed the following removal percentages, BOD_5 88.4%, nitrate 63.7%, nitrite 23%, ammonia 97.9%, phosphate 87.3%. As for heavy metals, the percentages were as follows: copper 99.2%, cadmium 97.9%. The results of this study showed the efficiency of *Chlorella vulgaris* in the treatment process and its high ability to remove pollutants from water, especially nutrients and heavy metals, and green algae is a promising, highly efficient and environmentally friendly biological treatment technology.

Keywords: heavy metals, *Chlorella vulgaris*, wastewater

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 severely impacts ecosystems and public health, affecting society and the environment[1]. One of the main problems of surface and groundwater pollution is the domestic and industrial wastewater thrown directly into rivers because it is often untreated or partially treated due to the lack or inadequacy of treatment plants. This problem exists in many countries, especially Iraq (World Water Assessment Programme (United Nations) & UN-Water., n.d.). Most pollutants come from domestic, industrial or agricultural effluents, and their physical and chemical composition varies according to their source [2]. The most common pollutants are metals, pesticides and nutrients (such as nitrates and phosphates)[3]. On the other hand, the deterioration of water resources due to pollution with heavy metals and other minerals has become a source of concern for organizations concerned with the environment and governments around the world, as the accumulation and stability of heavy metals in the food chain increases the risk of their toxicity even when they are present in low concentrations in water [4].

Consequently, the complexity of effluent formation increases with the increase in human activities, so it is necessary to develop appropriate wastewater treatment processes that must be easy to apply, efficient and environmentally friendly in order to prevent water quality deterioration and protect water resources. Treating at least half the volume of wastewater generated daily will solve the water crisis. Therefore, it is necessary to focus on treatment technologies and methods to partially remove pollutants from wastewater before it is discharged into waterways. 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 [5].

The use of algae in the biological treatment of sewage water is considered one of the best methods used in treatment because it has a great ability to remove carbon, nitrogen and phosphorous from the water contaminated with it, in addition to the fact that it does not require energy or the use of chemicals. The use of algae to remove pollutants from wastewater is called Sanitary or liquid waste before discharging or reusing (phytoremediation)[3,1] Green microalgae (belonging to Chlorophyta) were selected for this study because they are inexpensive, renewable, and available in many parts of the world, and can be grown in both fresh and saltwater under a variety of climatic conditions, as they contain a cell wall with different functional groups [6]

MATERIALS AND METHODS

Preparation and Sterilization of Media:

All equipment and media are sterilized in an autoclave at 121 °C for 15 min at 1.5 j. For the algal formation, modified Chu-10 was used [7]. The stocks for both macro and micro components were prepared by dissolving the weight of the salt in 1 liter of demineralized water; 2.5 ml of each stock solution was taken and bottled up to 1 liter of demineralized water, as shown in Table 1. Then, it was sterilized with an autoclave, except stock solution (K2 HPO4), which was sterilized alone and added finally to get one liter of Chu-10. Its pH was set to 6.4 after sterilizing using (0.01N) sodium hydroxide or hydrochloric acid.

Treatment of domestic wastewater in algae farms under study:

The algae treatment was carried out by taking 1-liter glass beakers and placing 850 ml of the collected household wastewater in 5-liter polyethylene containers and 150 ml from the demand farm after it reached the Stationary phase [8] And by three replicates, taking into account the control treatment that contains 1 liter of wastewater only, then the treatments were placed in their replicates in the culture room. A certain size of the farm was taken every four days for testing.

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

Table 1. The components and concentration of modified Chu-10 medium and the concentration of each component [6].

Analyzes procedure.

Nitrite

The concentration of nitrite in the sample was measured by following the method described by the American Health Organization [7] by taking 10 ml of the filtered sample and diluting it to 50 ml with distilled water, adding 1 ml of Sulphanil Amid solution, then adding 1ml of N-1-naphthylethelen solution diamine dihydrochloride Then the absorbance was measured at a wavelength of 543 nm using an absorbance measuring device and the result was expressed as mg/L.

Nitrate

The nitrate concentration was measured using the cadmium column reduction method described by the American Health Association 8, where the nitrate was reduced to nitrite. The absorbance was measured at a wavelength of 543 nm after adding 2 ml of a color reagent to 50 ml of the sample that was passed through the cadmium column, and the results were expressed in mg/L.

Phosphate

The method of tin chloride described by the American Public Health Association[7] was used to measure the phosphate concentration by adding 4 ml of ammonium molybdate solution and 10 drops of tin chloride solution to 100 ml of the sample. Then, the absorbance was measured at a wavelength of 690 nm using an absorbance measuring device and through the where results are expressed in mg/L.

Ammonia:

Ammonia was measured using a multi-meter type HANNA\ 211 after adding each of the following reagents, 93715A-0 HI and 93715B-0 HI, to the solution. The sample was placed in the device and waited for three minutes and thirty seconds. The result was expressed in mg/L.

Determination of heavy metals

The method described in [7] 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 ° C, then (1) ml of concentrated nitric acid HNO3 was added and left for a while. The plate was completed to complete dissolution. Then, the final volume was completed to (50) ml with distilled, deionized water. The samples were kept in plastic containers until measurement. The concentrations were measured using atomic absorption spectrometry, and the final product was expressed in mg/liter.

Biological oxygen demand BOD5

BOD5 was measured according to the American Health Organization 7 method. This was done by measuring the value of dissolved oxygen, as Winkler's (Azide Modification) method was used to determine the amount of dissolved oxygen after fixing it in the field and then scaling it with a sodium thiosulfate solution (0.025 N). for the first bottle. Then, the opaque Winkler vial was filled with 250 ml of the sample to be measured and left for 5 days in the incubator. The dissolved oxygen was measured as in the previously described method, and then the value of BOD5 was measured according to the following equation:

Biological oxygen demand BOD5 (mg/L) = initial and final dissolved oxygen. (1)

RESULTS

Characteristics of Municipal Wastewater:

The characteristics of municipal wastewater were measured before treatment, as listed in Table 2. It was found that there is a high percentage of nutrients in the water, such as nitrates, nitrites and phosphates, and a high level of heavy elements without the permissible limits. This is evidence that the water used is completely polluted and unsuitable for various uses, such as humans or agriculture.

Type of water Measured factors	Municipal wastewater
Nitrite mg/l	19.33
Nitrate mg/l	8.83
Phosphate mg/l	11
ammonia mg/l	16.2
Copper ppm	0.401
Cadmium ppm	0.069
BOD5 mg/l	283.6

Table 2. Characteristics of Municipal Wastewater

Algae growth in wastewater:

After Chlorella vulgaris has reached the stage of stability on day 10 of growth in the Chu-10 culture medium, its components are shown in Table 1. 150 ml of algal culture was injected into 850 ml of municipal wastewater under ideal temperature, light intensity and oxygen. The growth of algae was monitored by measuring the absorbance to identify the density of algal cells using a spectrophotometer at a wavelength of 540 nm Figure 1.

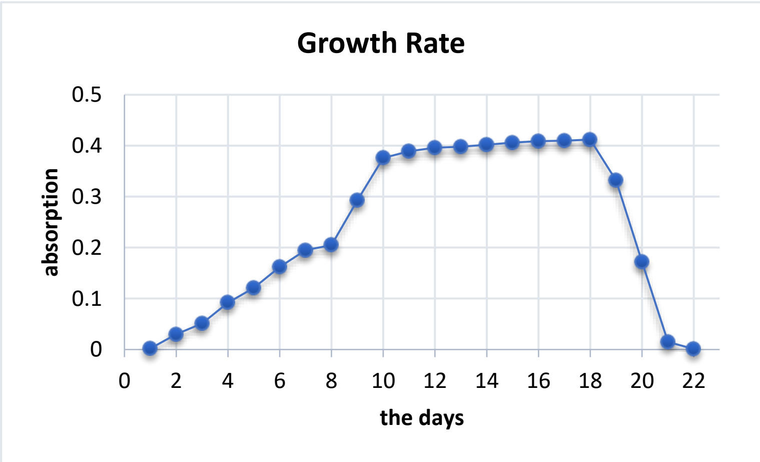

Figure 1. The growth rate of algae Chlorella vulgaris

Reduction of the Biological Oxygen Demand (BOD5)

The value of BOD5 is defined as the amount of oxygen microorganisms consume in the aerobic oxidation of organic materials. Thus, it is considered a measure of the amount of organic matter oxidized by microorganisms[11]. As oxygen is consumed in the process of organic decomposition, its concentrations decrease, thus affecting some aquatic organisms that are less efficient in consuming more oxygen to completely decompose some pollutants[12]. The results indicate the efficiency of Chlorella vulgaris in reducing BOD5 values throughout the experiment period for municipal wastewater.

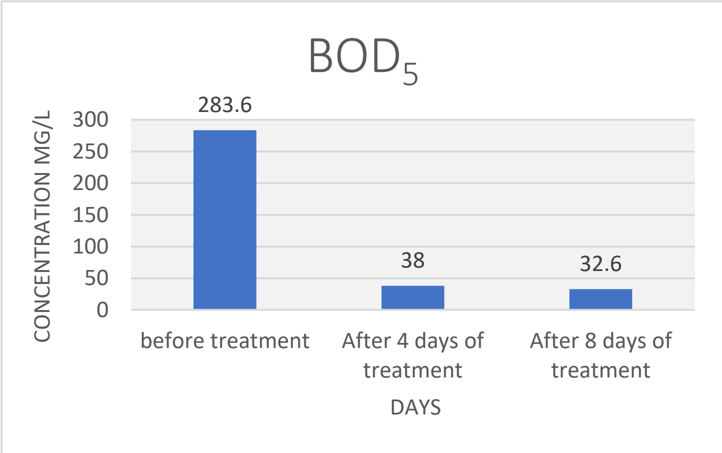

Figure 2. BOD5 before and after treatment

Nutrient removal
Nitrite:

Nitrogen is present in the environment in several oxidative states, and it converts from one form to another depending on oxygen availability. The nitrification process biologically oxidizes ammonia to nitrate by autotrophic bacteria. These types of bacteria are often very sensitive to PH values, heat, heavy metals and chemical compounds [16]. Nitrate is one of the unstable forms of nitrogen, which converts to ammonia and nitrate, and this means that it is the form that has the highest oxidation capacity compared to the rest of the nitrogen forms [17]. The results of the current experiment indicated in Figure 3 that the alga Chlorella vulgaris was inefficient in the treatment process, or rather, the reduction rates were slight, where the percentage of nitrite reduction on day 4 was 14.7%. This percentage rose to reach the highest removal level of 23% on day 8, which does not achieve an efficiency High compared to the removal of other nutrients.

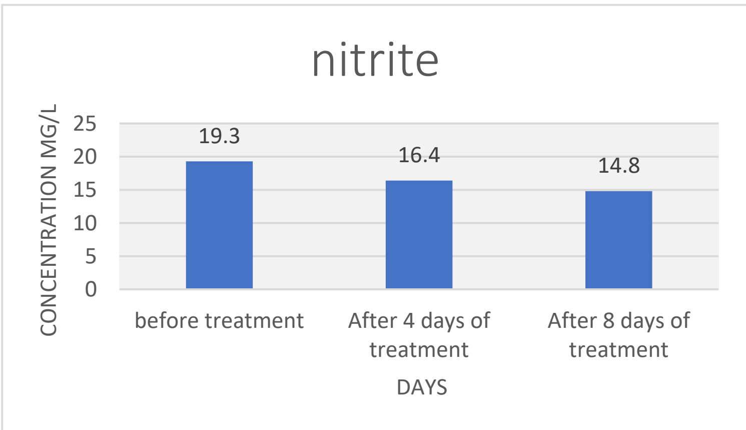

Figure 3. Nitrite before and after treatment

Nitrate:
The other form of nitrogen is nitrate, which is more stable and is in the oxidized state. High nitrate values in water are a dangerous phenomenon for aquatic plants that lead to nutritional enrichment and significant harm to public health if the percentage exceeds 10 mg/l, causing Methemoglobin anemia and fish poisoning [17].

The current study showed high efficiency of nitrate reduction in wastewater by the alga Chlorella vulgaris Figure 4, where the reduction value reached 62.9% on day 4, and the percentage increased slightly on day 8 to 63.7%.

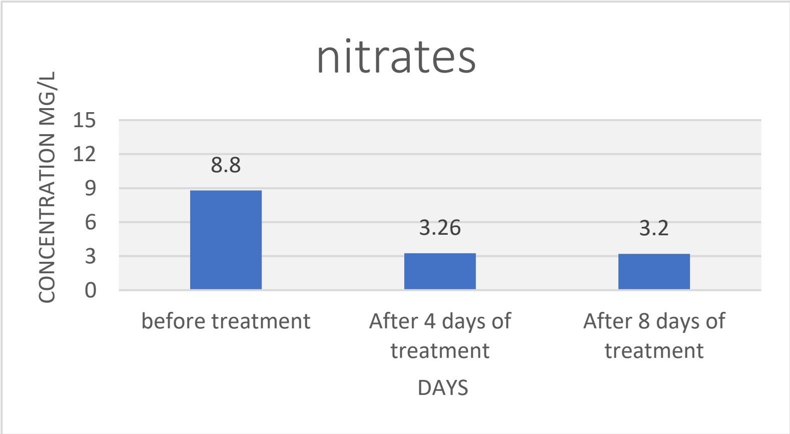

Figure 4. Nitrate before and after treatment

Ammonia: Ammonia is one of the most common nitrogenous compounds in wastewater. Domestic wastewater annually contains about 20 million tons of ammonia, equivalent to 19% of the annual industrial production of ammonia, and it is expected that the percentage will increase to 35 million tons annually by the middle of the twenty-first century [20]. High ammonia levels in water sources can lead to problems such as toxic algal blooms via eutrophication, fish mortality and decreased drinking water quality[21]. In particular, ammonia is toxic to fish. It poses a significant threat to aquatic ecosystems and human health, causing undesirable odors and severely burning the eyes, skin and respiratory tract at concentrations as low as 100-50 ppm[22]. The current study showed a very high efficiency in removing ammonia from wastewater using Chlorella vulgaris. Ammonia was removed by 94.2% on day 4 of the treatment process, and this percentage increased to 97.9% in ammonia reduction on day 8 of the treatment process, as shown in Figure 5.

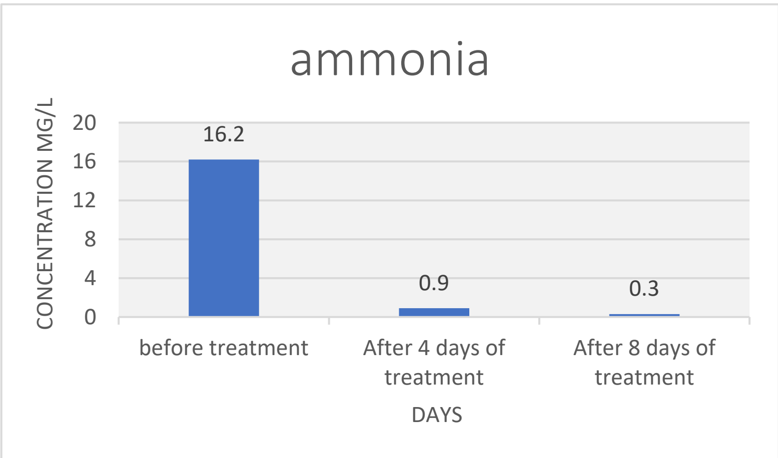

Figure 5. Ammonia before and after treatment

Phosphate removal

Phosphorous is a nutrient necessary for algae's growth processes and cellular functions, and the preferred form is orthophosphate (Po4-3). Phosphorous is also a determinant of alga growth because of its easy bonding with other ions such as iron and carbonates[26]. The process of taking phosphorous is active because it is an important source of energy, as it is used by algal cells to produce phospholipid, adenosine triphosphate (ATP) and nucleic acids, and it is consumed in the form of inorganic orthophosphate [19]. In the current study, the alga Chlorella vulgaris played a distinctive role in phosphate reduction, as the algae can withdraw phosphate quickly and in quantities more than they need for growth and then store it in cells in the form of polyphosphates [27]. The results in Figure 6 showed a reduction rate of 86.2% on day 4 after exposure, and the percentage increased after increasing the exposure period to reach 87.3% on day 8 of exposure.

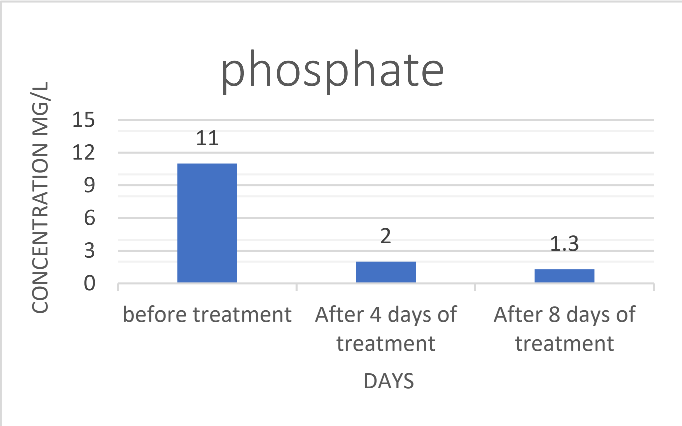

Figure 6. Phosphate before and after treatment

Reduction of the Heavy Metal

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 [29]. The International Agency for Research on Cancer classified cadmium and its compounds as carcinogenic to humans [30]. The results of the current study indicated in Figure 7 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. They continued to increase until they reached 97.9% on day 8 of the experiment, which is evidence of Algae's efficiency in the treatment of heavy metals in municipal wastewater.

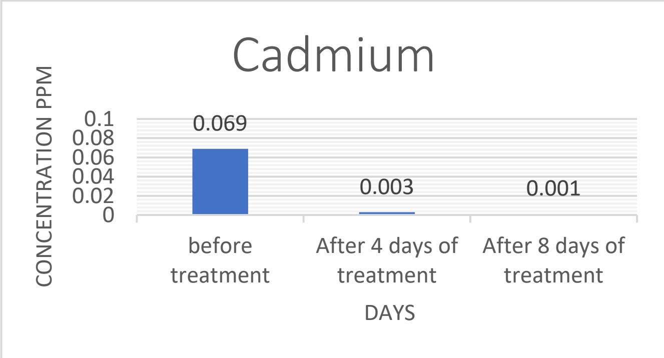

Figure 7. Cadmium before and after treatment

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 [34]. Despite its usefulness in many daily operations, excessive exposure to copper can seriously damage plants, animals and humans. When exposed to high concentrations that exceed normal levels, copper is highly toxic to the vital processes inside the cell. The current study's results indicated the high copper reduction efficiency of the alga Chlorella vulgaris. Figure 8 shows the reduction values of copper in municipal wastewater, where the alga reduced by 98.9% on day 4, where the process of copper reduction continued to increase to 99.2%.

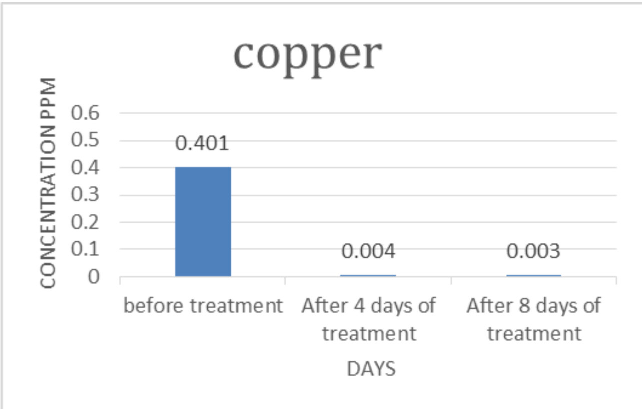

Figure 8. Copper before and after treatment

DISCUSSION

Water contains high concentrations of nutrients that can inhibit algae growth[9]. In addition, toxic heavy metals and organic compounds in wastewater are another important inhibiting factor for microalgae[10]. Chlorella 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, it started dying and continued to decline until day 22, when its death was fully confirmed.

The reduction of BOD5 values occurs when the dissolved organic compounds and their derivatives are reduced from the wastewater during treatment [13]. The results in Figure 2 showed that the reduction percentage reached 86.6% on day 4 of the treatment process, then this percentage increased to 88.4% on day 8. This high reduction ratio indicates the efficiency of Chlorella vulgaris in the treatment process. These results agree with many other algae treatment studies, as reported by [14], which indicated the efficiency of blue-green algae such as Spirulina platensis in reducing BOD5 values when treating wastewater from a dairy plant. The study of [15] showed that microalgae play a distinguished role in domestic water treatment, where the percentage of reducing BOD5 values was high.

The insignificant efficacy is due to the possibility of the algae being satisfied with nitrates and ammonia as major nutrients for growth and nutrition and the absence of nitrites for its basic operations. The study of 18 indicated the low percentage of nitrite reduction by algae, and the reason may be due to the high concentration of ammonia, which leads to the inhibition of nitrite removal. Ammonia is the preferred form of microalgae.[19] noted that heavy metals may inhibit the nitrification and bio-oxidation processes when they are present in the flows more than the treatment system can handle.

The high efficiency of nitrate reduction percentages agrees with many studies, where a study of the green algae Chlorella vulgaris indicated the high possibility of reducing nitrogenous compounds such as nitrite and ammonia from polluted water [18]. Green algae such as Scenedesmus sp and Chlorella sp. and blue-green algae such as Nostoc sp. proved highly efficient in reducing nutrients and organic load when used in wastewater treatment after primary treatment [19].

This high percentage of removal is because Chlorella vulgaris prefers ammonia as a nitrogen source, which is widely available in wastewater due to the decomposition of organic materials such as amino acids and urea by microorganisms[23]. The results of this study are consistent with many previous studies that showed the efficiency of algae in removing ammonia. A study conducted by [24] to treat domestic sewage water with algae proved a high removal efficiency during the trial period. A study on the alga Spirulina maxima showed that the percentage of ammonia removal was 92% on the seventh day after treating the algae with pig litter water [23]. As 25 indicated, the amount of removal depends on the density of cells and the volume of the medium when treating household wastewater with Scenedesmus bicellular alga, where the removal of ammonia reached 90% within three days.

These results agree with several studies, such as the study of [28], which recorded high levels of phosphorous reduction when treating industrial, pharmaceutical water and tanning waste with Chroococcus turgidus. Moreover, [15] indicated that microalgae play a distinguished role in domestic water treatment, and the percentage of phosphorous reduction was (80–100%) after only three days of treatment.

Previous studies showed the high efficiency of algae in reducing cadmium values in a study conducted on the alga Chlorella minutissima, where it proved efficient in removing cadmium by 84% after planting it in wastewater [31]. Thirty-two indicated that Oscillatoria sp was highly efficient in reducing cadmium in return for low chlorophyll and protein content. Furthermore, [33] showed the role of Westiellopsis prolifica in reducing the element cadmium when exposing the alga to different concentrations of the elements.

CONCLUSIONS

The previous results show that Chlorella vulgaris has a high ability to reduce heavy metals. The role of algae in reduction was recorded in many studies, as [35] recorded the role of algae Oscillatoria quadripunctulata in the reduction of many heavy elements, and a reduction of copper was recorded by 50%. Researchers [36] showed the efficiency of blue-green algae such as Nostoc linckia and Oscillatoria limosa in removing copper from wastewater in Nasiriyah power plant [24] in reducing copper from domestic wastewater. Copper is one of the essential elements important for the growth of algae, as it enters metabolic reactions such as respiration and photosynthesis when it is present in trace quantities [37]

References

1. Pacheco, D., Rocha, A. C., Pereira, L.; Verdelhos, T. Microalgae water bioremediation: trends and hot topics. *Appl. Scie. J*. **2020**, 10*(5)*, 1886.
2. Wollmann, F.; Dietze, S.; Ackermann, J.; Bley, T.; Walther, T.; Steingroewer, J.; Krujatz, F. Microalgae wastewater treatment: Biological and technological approaches. *Engineering in Life Sciences J*. **2019**, *19(12)*, 860–871.
3. Emparan, Q.; Harun, R.; Danquah, M. K. (2019). Role of phytoremediation for nutrient removal from wastewaters: a review. *Appl. Ecol. Environ. Res J*. **2019**, *17(1)*, 889–915.
4. Peng, Q., Chen, W., Wu, L.; Bai, L. The Uptake, Accumulation, and Toxic Effects of Cadmium in Barnyardgrass (Echinochloa crus-galli). *Polish Journal of Environmental Studies* **2017**, 26(3).
5. Gharbani, P. Modeling and optimizing reactive yellow 145 dye removal process onto synthesized MnOX-CeO2 using response surface methodology. Colloids and Surfaces A: Physicochemical and Engineering Aspects, **2018**, 548, 191–197.
6. Kassim, T. I., Al-Saadi, H.; Salman, N. A. Production of some phyto-and zooplankton and their use as live food for fish larva. *Iraqi J. Agric. Proc*. **1999**, *2*, 188–201.
7. APHA. *Standard Methods for Examining Water and Wastewater*, 23rd Edition (R. B. B. A. D. E. editors EW Rice, Ed.). American Public Health Association, American Water Works Association, Water Environment Federation, **2017**.
8. APHA, L. C. S. *Standard Methods for examining water and waste water*, 17th ed.; APHA. AWWA, WPCE, Washington DC, **1989.**
9. Wrigley, T. J.; Toerien, D. F. Limnological aspects of small sewage ponds. *Water Research journal* **1990**, *24(1)*, 83–90.
10. Chinnasamy, S., Bhatnagar, A., Hunt, R. W., Claxton, R., Marlowe, M., Das, K. C. Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. Google Patents, **2010**.
11. Best, G. A.; Ross, S. L. Clyde river purification board Eastkilbricle. Glasgow, **1997.**
12. Durmishi, B. H., Ismaili, M., Shabani, A., Jusufi, S., Fejzuli, X., Kostovska, M.; Abduli, S. The physical, physical-chemical and chemical parameters determination of river water Shkumbini (Pena)(part A). Ohrid, Republic of Macedonia, 2008, 27(31), 1–11.
13. Kotteswari, M., Murugesan, S., Kamaleswari, J.; Veeralakshmi, M. Bio management of dairy effluent by using cyanobacterium. I*ndian Hydrobiology J*. **2012**, 10*(1)*, 109–116.

14. Kotteswari, M., Murugesan, S.; Ranjith Kumar, R. Phytoremediation of dairy effluent by using the microalgae Nostoc sp. *Int. J. Environ. Res. Dev*. **2012**, *2(1)*, 35–43.
15. Khambete, A. K. . Treatment of Municipal Sewage with Microalgae-A Laboratory based study. *International Journal of Scientific Engineering and Technology* **2016**, 5(8), 415–417.
16. Hockenbury, M. R.; Grady Jr, C. P. L. Inhibition of nitrification-effects of selected organic compounds. *Journal (Water Pollution Control Federation* **1977**, 768–777.
17. Maiti, S. K. *Handbook of Methods in Environmental Studies, 1: Water and Wastewater Analysis*. ABD publishers, **2004**,
18. Tang, E. P. Y., Vincent, W. F., Proulx, D., Lessard, P.; Noüe, J. de la. Polar cyanobacteria versus green algae for tertiary wastewater treatment in cool climates. *Journal of Applied Phycology*, **1997**, *9(4)*, 371–381.
19. Becker, E. W. *Microalgae: biotechnology and microbiology***,** Volume 10. Cambridge University Press, **1994.**
20. Othman Ghazi Najeeb Alani , Yassen Taha Abdul-Rahaman, and Thafer Thabit Mohammed. Effect of vêo® premium and vitamin C supplementation on lipid profile before and during pregnancy in some local Iraqi ewes during heat stress. *Iraqi Journal of Science* **2021**, 62(4), 2122-2130.
21. Adam, M. R. Othman, M. H. D., Samah, R. A., Puteh, M. H., Ismail, A. F., Mustafa, A., Rahman, M. A.; Jaafar, J. Current trends and future prospects of ammonia removal in wastewater: A comprehensive review on adsorptive membrane development. *Separation and Purification Technology J*. **2019**, *213*, 114–132.
22. Vikrant, K., Kumar, V., Kim, K.-H.; Kukkar, D. Metal–organic frameworks (MOFs): potential and challenges for capture and abatement of ammonia. *Journal of Materials Chemistry* 2017, A, 5(44), 22877–22896.
23. Farhan, S. M., Abdulateef, S. M. Al-Enzy, A. F. M, Mohammed, Th. T., Saeid, Z. J. M., Al-Khalani, F. M. H. & Abdulateef, F. M. Effect of heat stress on blood alkalinity of broiler chicks and its reflection in improving the productive performance. Indian Journal of Ecology. 2020, 47: 107-109.
24. Al-Rubaie, G. H. (2003). The use of algae in treating household wastewater [Master Thesis]. Al-Mustansiriya University.
25. Vikrant, K., Kumar, V., Kim, K.-H., & Kukkar, D. Metal–organic frameworks (MOFs): potential and challenges for capture and abatement of ammonia. *Journal of Materials Chemistry* **2017**, 5(44), 22877–22896.
26. Yaakob, Z., Kamarudin, K. F., Rajkumar, R., Takriff, M. S., & Badar, S. N. The current methods for the biomass production of the microalgae from wastewaters: An overview. *World Applied Sciences Journal* **2014**, 31(10), 1744–1758.
27. Goldman, C. R., & Horne, A. J. (1983). Limnology-Megraw Hillint. B. Co., USA.
28. Vignesh, M., Shivasankar, S., Rao, P. H., Kumar, R. R., & Sivasubramanian, V. (2006). Phytoremediation of effluent from tannery and pharmaceutical industries–a lab study. Ind Hydrobiol, 9(1), 51–60.
29. Irfan, M., Hayat, S., Ahmad, A., & Alyemeni, M. N Soil cadmium enrichment: Allocation and plant physiological manifestations. *Saudi Journal of Biological Sciences*, 2013, *20(1)*, 1–10.
30. 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.

31. 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.
32. Brahmbhatt, N. H., Patel, R. V.; Jasrai, R. T. Bioremediation potential of Spirogyra sps. and Oscillatoria sps for cadmium. Asian *Journal of Biochemical and Pharmaceutical Research* **2012**, *2(2),* 102–107.
33. Al-Hussainy, A. K.; Al-Mayaly, I. K. Bioremoval of Lead and Cadmium by the Alga westillopsis prolifica. *Iraqi Journal of Science*, **2015**, *56(4B).*
34. M. Ajeel, A.; A. Mehdi, L. . Effect Of Eruca Sativa Seeds Powder As Feed Supplementation On Some Physiological Traits Of Male Lambs. Journal of Life Science and Applied Research. 2020, 1, 20-30.
35. Ajayan, K. V, Selvaraju, M.; Thirugnanamoorthy, K. Growth and heavy metals accumulation potential of microalgae grown in sewage wastewater and petrochemical effluents. *Pakistan Journal of Biological Sciences* **2011**, *14(16)*, 805.
36. Abdel-Jabbar, A. S. Estimation of the efficiency of the algae Nostoc linckia (Roth.) Bron., Thar. and Oscillatoria limosa (Roth.) CA Ag in removing some heavy metals from wastewater in the Nasiriyah power station. *Journal of Basrah Researches Sciences*, **2008**, 34(2B).
37. Al-Saadi, H. A., Al-Lami, A. A., Hassan, F. A; Al-Dulymi, A. A. Heavy metals in water, suspended particles, sediments and aquatic plants of Habbaniya Lake, Iraq. *International Journal of Environmental Studies* **2002**, 59(5), 589–598.

Received: May 15, 2023/ Accepted: June 10, 2023 / Published: June 15, 2023
Citation: Al-Shammari, A.H.; Al-Janabi, A. H. *Chlorella vulgaris* has been used in the biological treatment of some pollutants and heavy metals in municipal wastewater**.** Revista Bionatura 2023;8 (2) 63. http://dx.doi.org/10.21931/RB/CSS/2023.08.02.59