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Using microalga *Coelastrella* sp. to remove some nutrients from wastewater *invitro*

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Abstract:

Microalgae have been increasingly used for wastewater treatment due to their capacity to assimilate nutrients. Samples of wastewater were taken from the Erbil wastewater channel near Dhahibha village in northern Iraq. The microalga *Coelastrella* sp. was used in three doses (0.2, 1, and 2g. l⁻¹) in this experiment for 21 days, samples were periodically (every 3 days) analyzed for physicochemical parameters such as pH, EC, Phosphate, Nitrate, and BOD₅, in addition to, Chlorophyll *a* concentration. Results showed that the highest dose 2g.l⁻¹ was the most effective dose for removing nutrients, confirmed by significant differences ($p \leq 0.05$) between all doses. The highest removal percentage was recorded for ammonium pass 96% followed by NO₃ with 95%, while BOD₅ ranged from 88.7 to 93.5%. Decreases in nutrients coincided with an increase in chlorophyll-*a* content with the highest biomass on the 17th day of the experiment reaching 1.43 mg. l⁻¹.

Keywords: Algal bio-indication, Biomonitoring, Microalgae, Nutrient removal, Water quality, wastewater treatment.

Introduction:

Overuse of water in Erbil Governorate as a result of overpopulation, urbanization, industrialization, and water misuse resulted in a deficiency of this valuable resource, prompting the search for an alternate resource, which turned out to be wastewater¹. Domestic and industrial wastewater, as well as runoff rainwater, make up urban wastewater². Appropriate treatment of urban wastewater is required to protect human health and the environment. Erbil's wastewater system is a combination of sanitary sewage and stormwater³. The amount of sewage discharge changes depending on the season; on average, during the dry season, it reaches 77760 m³.day⁻¹, while during the rainy season, it reaches 108000 m³.day⁻¹⁴.

To prevent eutrophication, wastewater contains high quantities of nitrogen and phosphorus, which are routinely removed from the water. Nitrogen is released into the atmosphere by phosphorus precipitation and nitrification-denitrification processes, while phosphorus is wasted to the sludge⁵.

Algae are common and necessary part of all stream ecosystems^{6,7}. They are the main source of energy

for many food web streams, carbon fixing from the atmosphere by photosynthesis and transporting it through the web via consumer pathways. Due to their ecological importance and distinguishing characteristics, algae are suitable as indicators of nutrient pollution and also as assessment endpoints for developing numeric nutrient criteria for water quality management purposes under the Clean Water Act^{8,9}. Using microalgae for wastewater treatment is an old idea, and several researchers have devised methods for taking use of the algal rapid growth and nutrient-removal capabilities. The nutrient removal is due to the algae's assimilation of nutrients, although other nutrient stripping events, such as ammonia volatilization and phosphorus precipitation as a result of the algal high pH, also occur¹⁰. The appropriate nutrient concentration in any system is influenced by a number of factors, including microalgae species, current environmental conditions (light/temperature), the nature of the pilot system, and so on¹¹.

Treatment methods that combine carbon capture and wastewater treatment while emitting little or no carbon can be considered the most sustainable option¹². Photosynthesis allows microalgae to produce biomass from light, CO₂,

nutrients, and water¹³. Microalgae cultivation often generally requires a large number of nutrients that are readily available in home wastewater¹⁴, and might potentially be obtained for free from wastewater. This can significantly lower the cost of microalgal cultivation, while also reducing pollution and conserving freshwater resources. Microalgal biomass, as a result, is more of a resource than a waste. As a result, utilizing microalgal biomass to treat household waste minimizes the need for sludge disposal and enhances the benefits of using microalgae to treat domestic waste. In order to develop an eco-friendlier wastewater treatment technique, this study attempts to analyze the efficacy of using golden microalga-*Coelastrrella* sp. for nutrient removal in wastewater as a biological treatment.

Material method:

Cultivation of microalgae *Coelastrrella* sp.

The microalga, *Coelastrrella* sp., were isolated from Environmental Science and Health Department laboratory according to a previous investigation conducted by¹⁵. After identification microscopically depending on^{16, 17}. The cells were coccoid, and elliptical until citriform. They appeared as unicellular microalgae or in few-celled aggregations. Cell size ranged between 8-10 μm in width and 9-14 μm in length¹⁸. Molecular identification was conducted through algal DNA extraction, amplification, sequencing, and comparing to the GenBank database, it was identified as *Coelastrrella* sp. (MZ801742)¹⁵. The cells of *Coelastrrella* sp. were cultured in BG11 broth medium in distilled water with light-emitting diode (LED) lamps at ambient temperature.

Experimental design of batch Cultivation

The water sample collection took place in Dhahibah village wastewater channel in Erbil north of Iraq. The wastewater samples were autoclaved for 15 minutes to eliminate bacteria and protozoa. The experiments were carried out in a batch reactor using 2L conical flasks. Each series of experiments began with 1200 mL of wastewater inoculated in flasks with pre-cultured *Coelastrrella* sp. Three different fractions were prepared with different concentrations of air dried *Coelastrrella* sp. to assess the efficiency of nutrient removal by *Coelastrrella* sp. Wastewater with 0.2 g. l⁻¹ *Coelastrrella* sp. (run 1), wastewater with 1 g. l⁻¹ *Coelastrrella* sp. (run 2), wastewater with 2 g. l⁻¹ *Coelastrrella* sp. (run 3). The experiments were carried out using municipal wastewater for 21 days at neutral and constant pH (7.5 \pm 0.3), constant temperature of 25 \pm 1°C, and at constant light intensity (3000 lux)¹⁹.

Analytical Methods

All of the sampling and measurements took place at the same time of day. The dry weight (air dried) of microalgae produced per liter (g. l⁻¹) was used to compute biomass. The following approach was used to calculate the dry cell weight of the microalgal biomass: Before conducting the experiment, untreated wastewater was analyzed for physicochemical parameters. Once a day, 100 mL samples were taken and centrifuged at 3000 rpm. At a 21-day interval, the percentage of NO₃, NO₂, NH₃-N, PO₄-P, BOD₅ elimination, and growth rate were calculated. Samples were taken from flasks every day at the same time and centrifuged to separate algae. The BOD₅ was measured using standard methods at a certified laboratory²⁰. All of the other analyses were used photometrically with a spectrometer (UV-Visible 1240; Tecator, Rodgau, Germany). The result of treatment measured as percentage according to this equation²¹.

% Treatment = $\frac{\text{before treatment value} - \text{after treatment value}}{\text{before treatment value}} \times 100$

Estimation of chlorophyll-*a* concentration

To calculate chlorophyll-*a* concentration, 10 mL of culture was centrifuged at 3000 rpm for 5 minutes. The pellet was mixed with 5 mL of diethyl ether. The mixture was centrifuged at 3000 rpm for 5 minutes, and the absorbance of the supernatant was measured with a UV spectrometer (UV-Visible 1240; Tecator, Rodgau, Germany) at 660 and 643 nm²².

Chlorophyll-*a* = (9.92 * A₆₆₀) - (0.77 * A₆₄₃)

Statistical analysis:

Factorial analysis for parameters was performed by using (SPSS version 25) program and Excel spreadsheets.

The data were subjected to standard analysis of variance (Two-way ANOVA) and the Duncan test was used to compare means at a significant (P \leq 0.05) level²³.

Result and discussion:

The micro-alga, *Coelastrrella* sp. was used for the treatment of wastewaters collected from the Erbil wastewater channel near Dhahibha village. Some physicochemical parameters such as pH, EC, BOD₅, NO₃, NO₂, and PO₄ of wastewater were analyzed before treatment as control and after treatment with algae species for 21 days.

Before treatment (Control), the mean of initial values of pH, EC, BOD₅, PO₄, NH₄, NO₃, and NO₂ were 8.09, 912 $\mu\text{S}/\text{cm}$, 775, 23.7, 42.23, 23.7, and 8.94 mg. l⁻¹, respectively (Figs. 1-6). The pH value of wastewater at all doses (0.2g.l⁻¹, 1g.l⁻¹, and 2g.l⁻¹) was steadily increased by utilizing *Coelastrrella* sp., reaching 8.45, 8.52, and 8.65 (Fig. 1) after 21

days of treatment. The enhanced photosynthetic activity of algae or the chemical composition of water may both contribute to the alkaline tendencies²⁴⁻²⁶. Photosynthesis-induced increases in pH can accelerate the removal of nutrients via ammonia stripping or phosphorous precipitation²⁷ showed that the pH of the wastewater sample treated with *Chlorella minutissima* was altered from 8.01 to 8.82, while the pH of the wastewater sample treated with *Scenedesmus* sp. was changed from 8.01 to 9.09. pH changes can have a variety of effects on algal metabolism and growth, including changing the equilibrium of inorganic carbon species, modifying the availability of nutrients, and impacting cell physiology directly²⁷. High pH values during the treatment period and interpreted the research that the values of the exponent of hydrogen of the medium increase if the ratio of phosphorus absorption into energy that is derived from the process of photosynthesis or respiration as influenced by the absorption of phosphorus by many factors most important of pH, temperature, and light intensity as it was observed that the pH increase by precipitating phosphorus as influenced by the absorption of phosphorus by precipitating phosphorus^{28, 29}. Highest applied dose (2g.l⁻¹) was more effective role for rises pH values than other doses.

The amount of substances (salts, minerals, metals, calcium, and other organic and inorganic compounds) that have been dissolved in the liquid is referred to as total dissolved solids (TDS). Total solids levels that are too high or too low can reduce the efficiency of wastewater treatment plants as well as the operation of process industries that use raw water. TDS concentration levels in wastewater typically ranged from 250 to 850 mg. l⁻¹. TDS variations have the same pattern as EC variations³⁰. Salt ions typically affect microalgal cells via the ion homeostasis mechanism³¹. Conductivity is a general indicator of water quality, especially as the number of dissolved salts changes during wastewater treatment processes; changes in total salt concentration cause a change in conductivity³². As shown in Fig. 2, the EC values decreased to 353, 368, and 373.5 $\mu\text{S}\cdot\text{cm}^{-1}$ which comprised (59.04, 59.64, and 61.2%) of removal percent respectively on the 21st day of treatment. TDS ratio decreased from 668 mg. l⁻¹ to 199.16 mg. l⁻¹ run1, 185.7 mg. l⁻¹ run2, and 173.9 mg. l⁻¹ run3 on the 21st day. Generally, low level of salts may be facilitated photosynthesis by inorganic nutrient uptake and alkalinity tolerance³¹. The maximum reduction of TDS and EC by *Coellastrela* sp. occurred at the highest dose (2g.l⁻¹) (Table 1).

On the 21st day of treatment, the maximal removal percentages of BOD₅ were, 93.54, 90.32, and 88.70 % respectively³³, found that BOD₅ removal was 61.38 % on the 10th day and 78.64 % in natural wastewater, and that percent removal with different algal doses (20g, 40g, 60g, 80g, 120g, and 140g) was 12, 69, 68, 44, 24, and zero, respectively. In contrast to³⁴, for pure wastewater the maximum removal of BOD₅ by *C. vulgaris* was 21.63%, while for 50% diluted wastewater, the maximum removal of BOD₅ was 30.36%. The lowest dose of algae, 0.2g.l⁻¹, is the most effective amount for removing BOD₅ (Fig. 3). As a result, even a small dose of *C. vulgaris* is sufficient to efficiently reduce BOD₅ in wastewater.

The most oxidized form of nitrogen compounds is nitrate. Nitrate determination aids in determining the nature and degree of oxidation in biological processes, as well as enhanced wastewater treatment³⁵. In incubation of *Coelastrealia* sp. in wastewater showed that the minimum and maximum removal of NO₃ was occurred on 3rd day and 21st day respectively. The lowest reduction of NO₃ could be attributed to the nitrification process; algae prefer to assimilate N in the form of ammonia because it is a more passive and energy-efficient method of assimilation than nitrate uptake²⁶, or maybe because some nutritional elements, such as nitrate, are gradually consumed as *Coelastrealia* sp. grows, nitrate concentrations reduce. Results came in accordance with³⁶, which showed that *Monoraphidium* sp. removed NO₃ in wastewater by 51% and 95% in 5 days ;³⁷, determined that *Isochrysis zhanjiangensis* removed 78% active phosphorous and 84.7% nitrate nitrogen in wastewater within 11 days; ³⁸, showed that *Scenedesmus dimorphus*, *Selenastrum minutum*, and *Scenedesmus* sp. removed phosphate between 91% to 99% in mixed municipal and industrial wastewater ;²⁵, who reported that NO₃ and PO₄ removal were 87.6 %, and 90 %, respectively by *Chlorella* sp. in wastewater.

On the 21st day of treatment, the highest removal percentages of nutrients NO₂, NH₄, and PO₄ were 83.9, 96.6, and 93.1 %, respectively, at a 2g.l⁻¹ dose (Fig. 7). The maximum removal of NO₂ occurred on the 21st day, possibly due to an increase in pH values caused by photosynthesis, which can accelerate the removal of nutrients via ammonia stripping or phosphorous precipitation, which increases phosphate adsorption on microalgal cells³⁹⁻⁴³. In wastewater, NH₃-N, nitrate, nitrite, and simple organic nitrogen such as urea, acetic acid, and amino acids can be assimilated by microalgae^{44, 45}. Because microalgae cells require nitrogen for protein, nucleic acid, and phospholipid synthesis,

microalgae growth is thought to be required for nitrogen removal via uptake, degradation, and sedimentation⁴⁶, also the micro-algal denitrification and nitrification processes⁴⁷.

Ammonia was the most preferable form of nitrogen used by microalgae with removal percent pass 96%. Same results were obtained by⁴⁸.

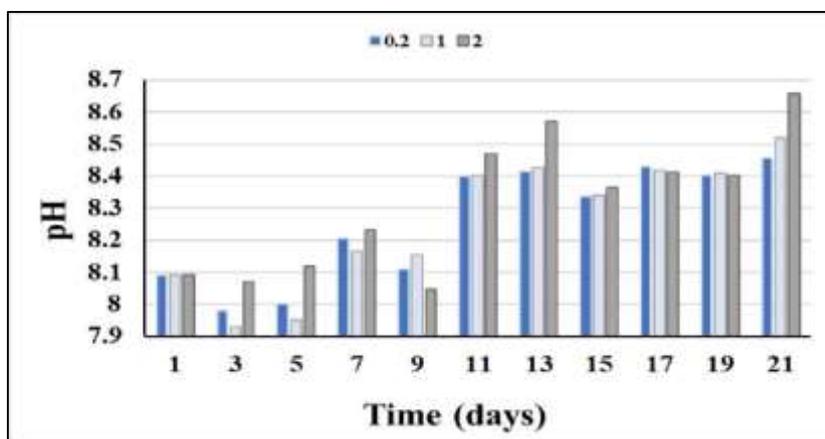


Figure 1. Effect of adding different doses of micro-algae *Coelastrella* sp. (0.2, 1, and 2 g.l⁻¹) on pH values of wastewater.

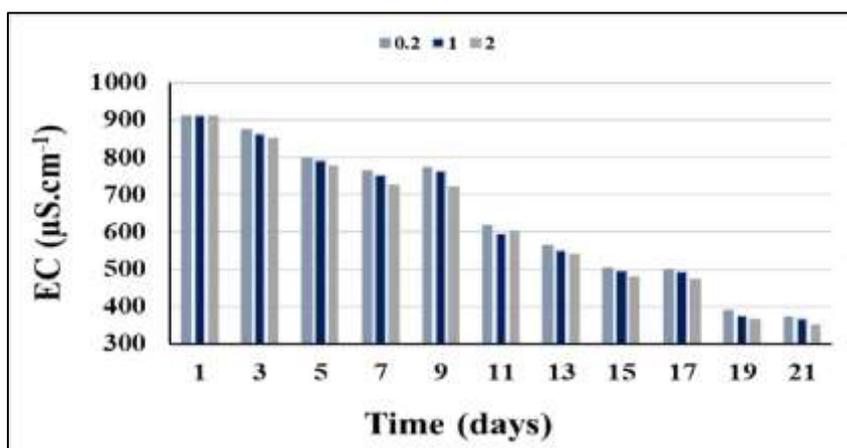


Figure 2. Effect of adding different doses of micro-algae *Coelastrella* sp. (0.2, 1, and 2 g.l⁻¹) on electrical conductivity values of wastewater.

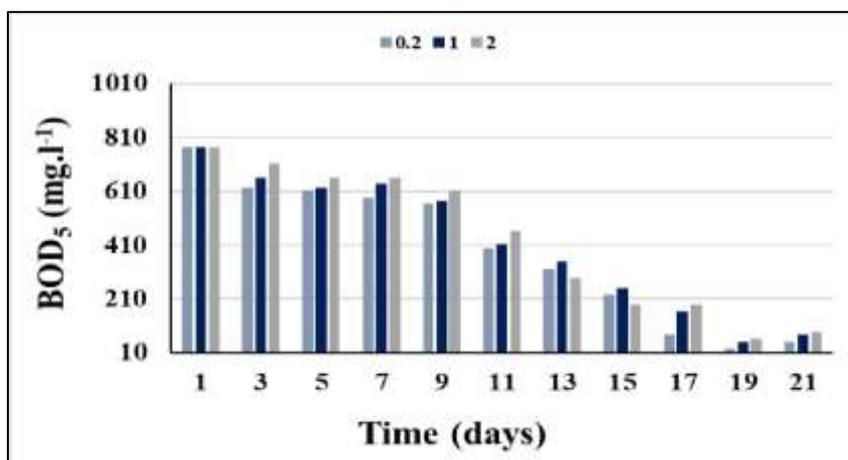


Figure 3. Effect of adding different doses of micro-algae *Coelastrella* sp. (0.2, 1, and 2 g.l⁻¹) on BOD₅ values of wastewater.

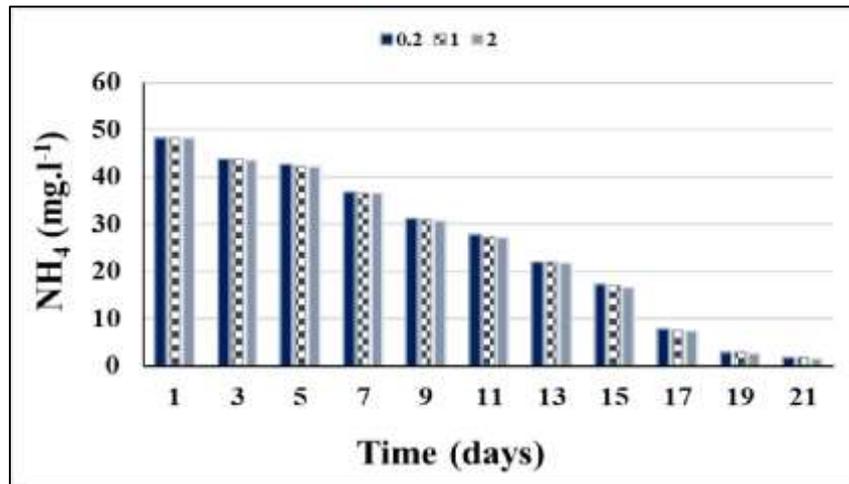


Figure 4. Effect of adding different doses of micro-algae *Coelastrella* sp. (0.2, 1, and 2 g.l⁻¹) on ammonium content values of wastewater.

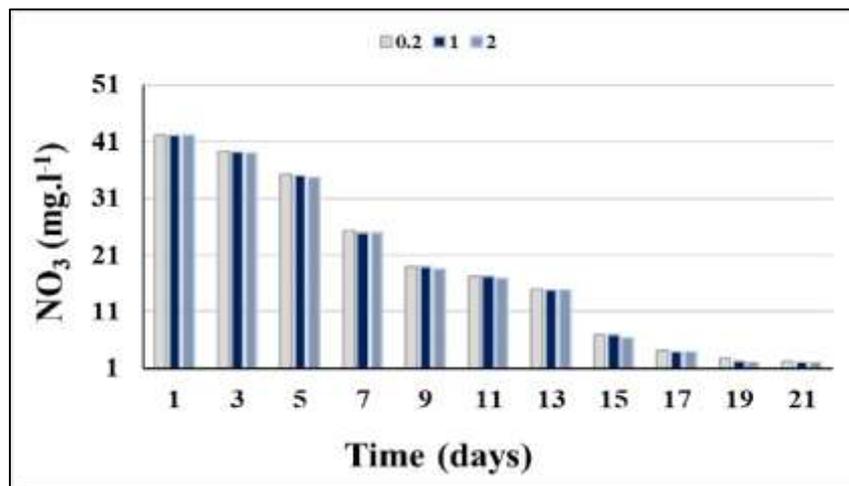


Figure 5. Effect of adding different doses of micro-algae *Coelastrella* sp. (0.2, 1, and 2 g.l⁻¹) on nitrate content values of wastewater.

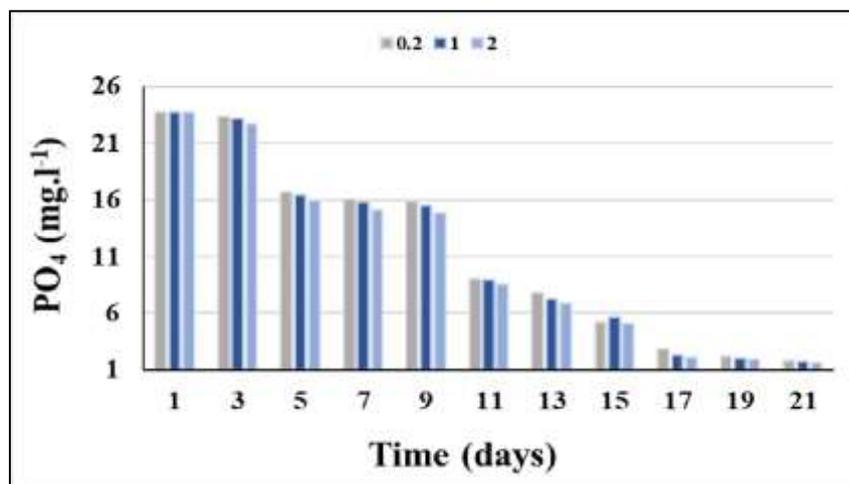


Figure 6. Effect of adding different doses of micro-algae *Coelastrella* sp. (0.2, 1, and 2 g.l⁻¹) on phosphate content values of wastewater.

Table 1. Effect of adding different doses (0.2, 1, and 2 g. l⁻¹) of micro-algae *Coelastrella* sp. on some tested wastewater variables, data represented as (Mean± S.E).

Parameters	D1(0.2g. l ⁻¹)	D2(1g. l ⁻¹)	D3(2g. l ⁻¹)
pH	8.25±0.008 ^b	8.25±0.008 ^b	8.31±0.008 ^b
EC (μS.cm ⁻¹)	643.7±0.82 ^a	632.7±0.82 ^b	619.36±0.82 ^c
TDS (mg. l ⁻¹)	409.9±0.24 ^a	405.7±0.24 ^b	398.49±0.24 ^c
K (mg. l ⁻¹)	5.610±0.13 ^a	5.480±0.13 ^b	5.38±0.13 ^c
NH ₄ (mg. l ⁻¹)	25.67±0.1 ^a	25.47±0.1 ^b	25.31±0.1 ^c
NO ₃ (mg. l ⁻¹)	19.07±0.006 ^a	18.86±0.006 ^b	18.65±0.006 ^c
NO ₂ (mg. l ⁻¹)	4.680±0.009 ^a	4.12±0.009 ^c	4.2±0.009 ^b
PO ₄ (mg. l ⁻¹)	11.32±0.025 ^a	11.13±0.025 ^b	10.79±0.025 ^c
BOD ₅ (mg. l ⁻¹)	386.9±1.5 ^c	415.9±1.5 ^b	427.27±1.5 ^a
Chlorophyll <i>a</i> (mg. l ⁻¹)	0.631±0.003 ^c	0.77±0.003 ^b	0.981±0.003 ^a

Note: Values in each column with different letters are significantly different at P<0.05. Values in rows with same letters are not significantly different.

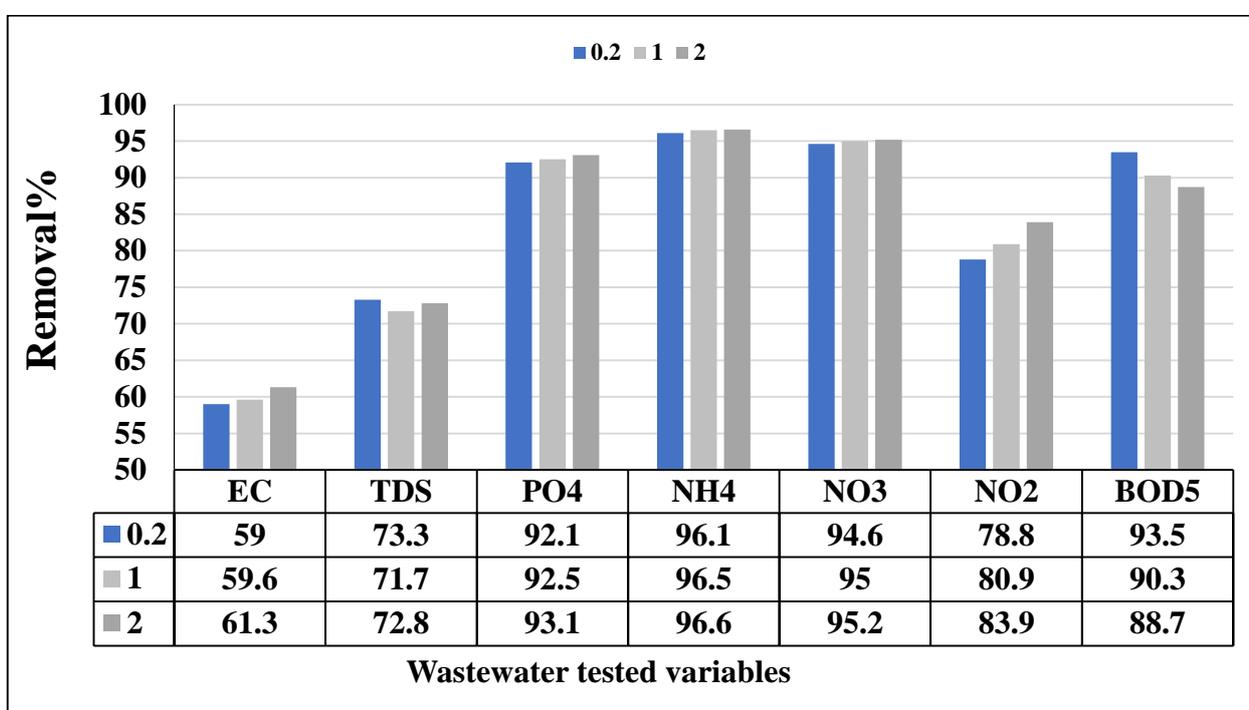


Figure 7. Percent removal from wastewater tested variables after adding three doses of micro-algae *Coelastrella* sp. (2, 1, and 0.2g. l⁻¹).

Algae prefer ammonia to nitrate, and nitrate consumption does not occur until the ammonium has been nearly completely consumed. Previous research revealed a ranking of tolerance to high levels of ammonium/ammonia (39-1.2 mM), with the order of tolerance being: Chlorophyceae > Cyanophyceae, Dinophyceae, Bacillariophyceae, and Raphidophyceae⁴⁸. These findings also demonstrate that ammonical nitrogen removal is preferable to nitrite and nitrate nitrogen removal. In comparison to nitrite, the remaining NH₄-N concentration vanished quickly. After 9 days, the ammonium depletion from synthetic wastewater by *C. vulgaris* and *Scenedesmus rubescens*, respectively was comparable to experiments

conducted by⁴⁹, who described ammonia elimination between (79 and 100 %) after 188.25 hrs. (about 8 days) , and⁵⁰, who recorded ammonium removal efficiencies of 90% from agro-industrial wastewater after 9 days. In the current study, microalgae-*Coelastrella* sp. removed more than 93% of PO₄ from wastewater, which is a greater removal ratio than that reported by⁵¹, who found that *Chlamydomonas* sp. removed 33% of PO₄ from wastewater.

Because of its critical "light-harvesting" role in photosynthesis, chlorophyll is abundant in nature and critical to the survival of both the plant and animal kingdoms⁵². With increasing doses of algae and treatment durations, nitrogen removal rises,

followed by increases in algal biomass via chlorophyll *a* concentration. During the experiment, the amount of chlorophyll-*a* in the *Coelastrella* sp. treatments rose. For all doses, the starting value of chlorophyll *a* was 0.30 mg. l⁻¹, 0.31 mg. l⁻¹, and 0.54 mg. l⁻¹ respectively. Then, on the 17th day, all doses 0.98 mg. l⁻¹, 1.08 mg. l⁻¹, and 1.43 mg. l⁻¹ reached their maximum value (Fig. 8). On the 21st day of the experiment, all treatments observe a considerable decline in biomass and chlorophyll-*a*,

indicating that the algae had reached the stage of death, and the number of algal cells decreased to 0.86 mg. l⁻¹, 1.008 mg. l⁻¹, and 1.25 mg. l⁻¹, accordingly. The maximum chlorophyll-*a* concentration was reported at the end of the treatment period, which could be due to algae's high nutrient consumption for metabolism and growth⁵³, with increases in pH values⁵⁴. As the algal cell increases, the rate of nutrient increase, so algal biomass simply increases.

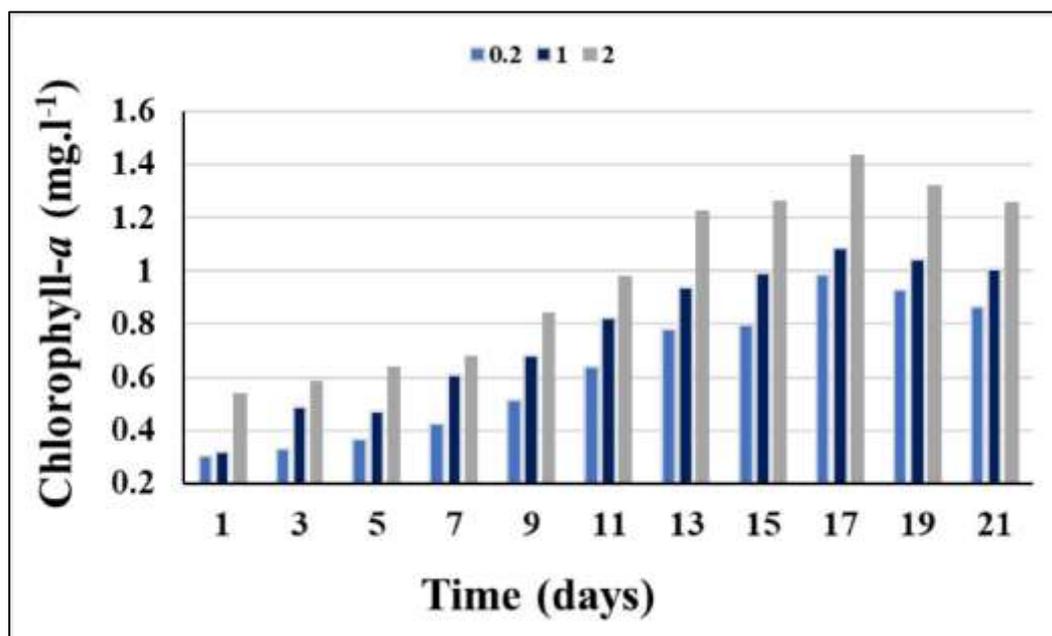


Figure 8. Effect of adding different doses of micro-algae *Coelastrella* sp. (0.2, 1, and 2 g. l⁻¹) on chlorophyll content values of wastewater.

Conclusion:

Finally, algae are increasingly being used to assess the health and ecological quality of aquatic ecosystems because they are relatively inexpensive and easy to measure, and they are sensitive to nutrient pollution, making them a potentially beneficial indicator of ecosystem change. Therefore, algae participate to a significant reduction in the concentrations of all nutrients, accompanied by an increase in their biomass. Also, ammonium nitrogen is a more preferable form of nitrogen for algae and have higher removable percentage than other form of nitrogen. Considerable removal of BOD₅ and dissolved salts (EC value) are observed.

Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.

- Ethical Clearance: The project was approved by the local ethical committee in Environmental Science and Health Department, College of Science, Salahaddin University, Erbil, Iraq.

Authors' contributions statement:

Q, M. Q. contributed to collecting the samples, laboratory analyzing of samples and writing the manuscript. S, Y. A. contributed to the research design, statistical analysis, editing the final revision of the manuscript.

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استخدام الطحلب *Coelastrella* sp. للتخلص من بعض المغذيات من مياه الصرف الصحي

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الخلاصة:

تم استخدام الطحالب الدقيقة بشكل متزايد في معالجة مياه الصرف الصحي نظرًا لقدرتها على استيعاب العناصر الغذائية. تم أخذ عينات من مياه الصرف الصحي من قناة الصرف الصحي لمدينة أربيل قرب قرية ذهبية. *Coelastrella* sp. تم استخدامه في ثلاث جرعات (0.2، 1، و 2 غم. لتر⁻¹) في هذه التجربة لمدة 21 يومًا، تم تحليل العينات بشكل دوري (كل 3 أيام) من أجل تقدير خصائص الفيزيائية والكيميائية للمياه مثل الأس الهيدروجيني، والتوصيل الكهربائي، والفوسفات، والنترات، والمتطلب الحيوي للأوكسجين BOD₅ باستخدام الطرق القياسية، فضلًا عن تحديد كمية الكلوروفيل a. أظهرت النتائج أن أعلى جرعة (2 غم. لتر⁻¹) كانت الأكثر فاعلية في إزالة أعلى معدل للعناصر الغذائية، وأكدت أحصائيا الفروقات المعنوية (p≤0.05) بين جميع الجرعات. تم تسجيل أعلى نسبة إزالة الأمونيوم بنسبة تجاوزت 96% بلبه NO₃ بنسبة 95%، بينما تراوح نسبة إزالة BOD₅ من 88.7 إلى 93.5%. تزامن الانخفاض في المغذيات مع زيادة محتوى الكلوروفيل مع أعلى كتلة حيوية في اليوم السابع عشر من التجربة 1.43 ملغم. لتر⁻¹.

الكلمات المفتاحية: المؤشرات الحيوية للطحالب، الرصد الحيوي، الطحالب الدقيقة، إزالة المغذيات، جودة المياه، معالجة مياه الصرف الصحي.