

The Eco-Sustainability of Agricultural Drainage Water Bioremediation by *Chlorella Vulgaris*

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Abstract

The aim of this study was to develop a biological treatment using the alga *Chlorella vulgaris* to enable the reuse of drainage water. Water samples were collected from five main drainage sites, including the Om El-Resh drain after it mixed with the Ismailia drain; the Bahr El-Baqar drain (Shader Azzam Bridge); the El-Salam Canal before it mixed with the Bahr Hadous drain; the Bahr Hadous drain at the main station; and the El-Salam Canal after it mixed with the Bahr Hadous drain. Prior to incubation with the green alga *Chlorella vulgaris*, the water samples were analyzed and supplemented with the recommended growth medium (BG-11), including the necessary macronutrients. The results showed that the most effective treatment occurred when the algae were exposed to nitrogen and phosphorus together for 48 hours. The maximum percentage reductions were achieved for nitrogen (97.58%), phosphorus (96.71%), potassium (87.38%), sodium (91.73%), and calcium (90.50%). *Chlorella vulgaris* achieved maximum removal percentages of 86.67% and 84.44% for BOD and COD, respectively. The results revealed the complete disappearance of seven total coliforms and fecal coliforms, with a minimum percentage count of 96.48%.

1. INTRODUCTION

Water resources are critical to Egypt's production, services, and sustainable development. They are a critical factor in the country's production, services, and sustainable development. Currently, wastewater reuse is a valuable additional water resource, and biological treatment is one of the most promising solutions. Algae are increasingly used in wastewater treatment due to their ability to remove nitrogen and phosphorus. Depending on basin pressures, agricultural drainage water (ADW) often contains high concentrations of nitrogen (N) and phosphorus (P), as well as suspended solids, pesticides, and heavy metals.

Microalgae, particularly *Chlorella vulgaris*, have been widely studied for phyco-remediation purposes due to their ability to assimilate nutrients and organic matter, attenuate metals, and produce biomass for use in a circular economy. Recent reviews have established *Chlorella vulgaris* role in open high-rate algal ponds and closed photobioreactors for treating municipal, industrial, and agricultural wastewaters (Abuhasheesh et al., 2025; Fayaz et al., 2024). Seyedeh et al. (2021) implemented mixotrophic microalgae in wastewater treatment processes due to their ability to utilize organic and inorganic carbon, as well as inorganic nitrogen (N) and phosphorus (P), for growth in wastewater.

Microalgae are a critical component of the wastewater treatment process, enhancing the reduction of nitrogen (N), phosphorus (P), and chemical oxygen demand (COD). Recently, many suggestions have been made to overcome the economic constraints of large-scale microalgae production. These include cultivating microalgae at a low cost by recovering nutrients from wastewater and removing pollutants from the environment (Mesa et al., 2025; Park et al., 2011; Pittman et al., 2011). This method is cost-effective and feasible for bio-fixing CO₂ using microalgae in wastewater treatment because microalgae are a renewable biomass source (Almomani et al., 2019). This method has also been considered for use

in biogas production, with anaerobic fermentation serving as the final step in future microalgae-based biorefinery strategies (Mussnug et al., 2010).

Although it is difficult to compare the effects of algal culture on wastewater treatment, some studies have proven that algal formation can support nutrient removal in wastewater (Chawla et al., 2020). Using algal granules to remove phosphorus from synthetic wastewater has proven highly efficient and the resulting P-rich biomass can be used to recover and reuse (Cai et al., 2019). Additionally, *Chlorella vulgaris* has been cultivated successfully in corn steam liquor effluent using a nutrient-free growth medium (El-Sayed et al., 2015).

Algae play an important role in the self-purification of organic pollutants in natural bodies of water. They accumulate and/or use nutrients, such as nitrogen and phosphorus, as well as heavy metals, pesticides, organic and inorganic toxins, and pathogens. These pollutants are then removed from the surrounding water. Due to their bioaccumulation abilities, algae can also be used in wastewater treatment (Mahapatra et al., 2014). For example, *Chlorella vulgaris* can reduce nitrate and phosphorus levels by an average of 40% and 43%, respectively, from different initial nutrient concentrations (Houser et al., 2014).

Recent research from 2024 and early 2025 highlights the effectiveness of *Chlorella vulgaris* as a sustainable, cost-efficient agent for treating agricultural drainage water. Under optimized conditions, studies have shown that *Chlorella vulgaris* can remove approximately 76.5% of total nitrogen and up to 90.35% of total phosphorus. Compared to *Scenedesmus quadricauda*, *Chlorella vulgaris* exhibits higher total nitrogen removal efficiency and is more effective in removing nitrate nitrogen and orthophosphate. Furthermore, *C. vulgaris* accumulates lipids under nutrient-limited conditions. This enables the harvested biomass to be used for biofuel production, supporting the principles of the circular bio-economy. This integrated approach offers environmental and economic benefits, establishing *Chlorella vulgaris* as a versatile and promising option for sustainably treating agricultural drainage water and recovering bio-resources (Goncu et al., 2025; Diaconu et al., 2023). In this context, Marwa et al. (2020) reported that algae-enriched wastewater could be used safely to irrigate low-fertile soils, thereby promoting castor seed oil production and improving soil quality.

The current study evaluates the potential use of the green microalga *Chlorella vulgaris* in the biological treatment of drainage water intended for reuse in irrigation.

2. MATERIALS AND METHODS

2.1. Water sources

Five main drains were selected for the current study: These drains include Om El Resh (after mixing with the Ismailia drainage), Bahr El-Bakar (before mixing with the El-Salam Canal at the Shader Azzam Bridge), the El-Salam Canal (before mixing with the Bahr Hadous), the Bahr Hadous (at the main station), and the El-Salam Canal (after mixing with the Bahr Hadous). Sampling, preservation, and analytical procedures at the Central Laboratory for Environmental Quality Monitoring, National Water Research

Center (NWRC) were conducted according to the American Public Health Association (APHA, 2023) standards.

2.2. Microalga, growth (medium, unit and conditions)

Chlorella vulgaris, a green microalga belonging to the Chlorophyta that is commonly found in Egyptian freshwater habitats with varying salinity levels, was used to evaluate the bioremediation potential for wastewater intended for agricultural reuse. The algal strain was cultivated in large quantities at the Algal Biotechnology Unit of the National Research Centre in Cairo. A comparable growth medium (BG-11), as described by Stainer *et al.* (1971), was used with one modification: urea was used instead of sodium nitrate to minimize the sodium load. The initial nutrient content (ppm) is listed in Table 1.

Growth units composed of fully transparent thick Plexiglass columns (100-cm-long, 7-cm-diameter, 5-mm-thick) containing 7.0 L of growth medium x 3 replicates of each treatment (El-Sayed, 2008) containing 2.5 L each. A white fluorescent lamp bank (5x40 W) provided illumination at a light intensity of 120 μE . Aeration was maintained using oil-free compressed air from the bottom of the columns. All experiments were conducted at ambient room temperature.

2.3. Experimental design

As previously mentioned, the current study includes five drainage locations. Each location underwent four different treatments and was compared with the recommended growth medium (BG-11). The treatments were T1: Growing alga with original drain water, T2: Growing alga with original drain water enriched with urea nitrogen, T3: Growing alga with original drain water enriched with phosphorus, T4: Growing alga with original drain water enriched with urea nitrogen and phosphorus. Urea nitrogen and phosphorus were supplied based on the initial concentration of the original growth medium.

All water samples were filtered through white sawdust to remove particles that cause odor and turbidity. Growth was achieved by inoculating pre-filtered water with the appropriate algal inoculum in indoor growth units that contained no external nutrients. Prior to inoculation, previously grown algae were harvested using a cooling centrifuge (RUNDELL, Heidelberg, Germany) and washed three times with distilled water to remove all nutrients from the outer cell membrane.

Based on the results obtained, the water analysis (Table 1) showed that the drainage water had a low nitrogen and phosphorus content. Thus, the drainage water was separately enriched with nitrogen (urea) and phosphorus (phosphoric acid) at concentrations equivalent to those in the BG-11 growth medium. The same quantity of nitrogen received from the original BG-11 growth medium (0.53 g. l^{-1}) was added to the drainage water in the form of urea.

To evaluate the growth potential of the selected alga, *Chlorella vulgaris*, under phosphorus-enriched drainage water conditions, phosphoric acid was added to the cultures based on the original growth medium ($7.0 \text{ g. l}^{-1} \text{ P}$). Using this technique, *Chlorella vulgaris* was grown in nitrogen- and phosphorus-enriched drainage water. Therefore, nitrogen was added as urea at a concentration of 0.53 g. l^{-1} in the

presence of phosphoric acid at a concentration of 7 g.l^{-1} . This step was performed to optimize the growth medium in terms of macronutrients (NPK) and enhance algal growth, thereby increasing the removal rate of the grown algae. Nutrient removal was determined after each experiment.

2.4. Growth measurements and analyses

Throughout the growth period, the dry weight of the incubated algae was determined by precipitating a defined volume onto a pre-weighed, $0.54\text{-}\mu\text{m}$ membrane filter. After drying at 50°C for 60 minutes, the weight difference represented the accumulated dry weight during the sampling period. To analyze the water and measure the nutrients removed by the algae, the remaining water after determining the dry weight was collected and supplemented with 25 ml of deionized water to measure the remaining nutrients, including nitrogen, phosphorus, potassium, sodium, and calcium (APHA, 2023).

3. RESULTS

3.1 Nutritional load of water and growth medium.

As shown in Table 1, all of the examined waters lacked essential nutrients (NPK), with more than 90% deficiency in nitrogen and phosphorus compared to the recommended growth medium (BG-11). However, there was nearly sufficient K^{+} ions, and a hyper load was observed with Ca^{2+} and Na^{+} ions, which is the main reason for salinity. To enhance removal capacity, algal growth should be stimulated by adding the lacking nutrients (P and K) to reach the levels of the BG-11 medium.

Table 1: Initial Content of Different Minerals (ppm) in BG-11 Medium and Examined Waters

Element	N	P	K	Ca	Na
BG-11.	252	7.11	17.91	9.81	7.42
Om El-Resh	21.7	0.79	34	318.07	1150
Shader Azzam	22.7	1.21	27	156.22	575
Salam Canal before mix	24.7	0.85	21	125.91	320
Bahr Hadous	21.6	0.18	12	44.59	130
Salam Canal after mix	21.7	0.35	13	63.03	145

3.2 Growth of *Chlorella* under different water sources and nutrient enrichment

A comparative analysis of dry biomass growth revealed that *C. vulgaris* achieved higher growth rates in the BG-II growth medium than in various wastewater sources. The only exception was the Om El-Resh drain. Despite its high pollution levels, it exhibited different growth behaviors. The reduced growth observed in most wastewater samples was mainly due to limited initial nutrient availability, particularly

nitrogen deficiency. The rate at which algae uptake nutrients depend largely on the concentration and physiological accessibility of these nutrients.

The results indicated that the growth of the green alga *Chlorella vulgaris* under different wastewater sources (Fig. 1) was directly related to the level of contamination and growth potential. This finding also reflected the level of pollution in the studied drains. The Om El-Resh drain was identified as the most polluted site.

The growth of *Chlorella* with different types of crude water was compared to the control among the different treatments. Dry weight was influenced by nutrient concentrations. The highest dry weight was recorded in algae cultivated using wastewater from the Om El-Resh drain (0.71 g l^{-1}), surpassing the control and other water sources (Fig. 1). Samples grown in Shader Azzam and El-Salam before mixing with Bahr Hadous Drain (BHD) followed, as did samples grown in El-Salam after mixing with BHD and BHD itself, resulting in dry weights of 0.62, 0.49, 0.37, and 0.31 g l^{-1} , respectively.

These results suggest that it is feasible to directly cultivate *Chlorella vulgaris* using Om El-Resh wastewater. In contrast, other tested sites that exhibited lower growth than the original growth medium required supplementation with macronutrients to support sufficient growth. However, this may increase the nutrient load and potential contamination.

Despite the differences in inoculation amounts at time zero among the different treatments, the calculated percentage increase reflected the actual increase. Therefore, growth in both the recommended growth medium and the Om El-Reesh drain was recorded as 64.26% and 68.81%, respectively. This can be attributed to the higher contamination load. The lowest percentage increase, 32.96%, was observed in samples from the Bahr Hadous Drain (BHD). These results suggest that the growth variations observed in the El-Salam Canal may be due to the dilution effect caused by its mixture with the BHD, which had the lowest contamination rate. As shown in Fig. 1, Growth and dry weight accumulation of the green alga *Chlorella vulgaris* grown with different wastewater sources enriched by N or/and P.

Comparing the growth of *Chlorella vulgaris* in crude wastewater with those were grown in the examined wastewater enriched with an equivalent level of urea nitrogen as in the recommended BG-II medium showed the positive effect of nitrogen supplementation on algal growth. As previously mentioned, the nitrogen content in untreated wastewater is low, which severely limits algal growth. Of the chosen sites, Om El-Resh exhibited the highest growth performance, likely due to its elevated levels of contamination, salinity, and nitrogen, which created a nutrient-rich environment favorable for algal growth. As shown in Figure (1b); the minimal growth was observed in the BHD and El-Salam (A) samples might attributed to their low nutrient concentrations others than nitrogen and phosphorus.

Therefore, it could be concluded that the overall nutrient balance is the key factor in determining the potential for algal growth. In particular, nitrogen deficiency led to a significant reduction in dry weight accumulation and, in most cases, resulted in growth retardation.

Furthermore, during autotrophic cultivation, chlorophyll breakdown, lipid accumulation, and a decline in dry-weight biomass are early indicators of nitrogen depletion (Abdel-Raouf *et al.*, 2021; Dziosa and Makowska, 2016). Under heterotrophic or mixotrophic growth conditions, the impact of nitrogen deficiency is less apparent because algae redirect their metabolism toward oil accumulation and away from protein synthesis. Urea functions as both a nitrogen source and a readily metabolizable source of organic nitrogen for algal cells. However, microbial contamination of the algal culture medium can lead to the enzymatic breakdown of urea into ammonia and carbon dioxide via urease or urea amidolyase. These enzymes are absent in algal cells (Zhang *et al.*, 2024; Wang *et al.*, 2019; Chróst *et al.*, 1975).

Adding phosphorus to the *Chlorella vulgaris* growth medium (wastewater) did not result in a significant increase in dry weight, compared to urea supplementation. The growth pattern was similar to that observed in the original wastewater, though there were some variations among the locations. The data indicated that adding urea to drainage water promoted algal growth more effectively than adding phosphorus (Fig. 1). One possible explanation for this phenomenon is the high calcium content in different water types, which leads to phosphate precipitation.

The physiological effects of phosphorus deficiency were recognized early on. The primary impact is an overall reduction in growth, inhibition of cell division, complete breakdown of chlorophyll, and massive accumulation of carotenoids and lipids. This outcome is usually linked to dry weight failure due to blocked CO₂ fixation. To achieve this, supplementing algal cultures with organic carbon supports completion of the photosynthetic process. This is done by facilitating fixation of organic carbon into fatty acids through accessory photosynthetic pigments such as carotenes and carotenoids. This hypothesis may explain the stimulatory effect of urea nitrogen on algal growth, as urea nitrogen serves as a simple organic carbon source. Furthermore, increasing the concentrations of nitrogen, phosphorus, and potassium in *Chlorella vulgaris* cultures has been shown to maximize overall biomass yield by maintaining optimal nutrient ratios (Li *et al.*, 2024; Rahman *et al.*, 2024; Putri *et al.*, 2023).

When the examined wastewater media were enriched with urea nitrogen and phosphorus, *Chlorella vulgaris* exhibited a significant increase in dry weight accumulation in all locations. However, significant variations were observed among the different sampling sites. These variations could be attributed to the initial nutrient composition and balance of the drainage waters, despite the uniform addition of nitrogen and phosphorus. Moreover, supplementing both nutrients to simulate the recommended BG-11 growth medium produced distinct growth responses compared to those observed in untreated drainage water or media enriched with nitrogen or phosphorus alone. Om El-Resh drainage was the most suitable for algal growth (1.89 g. l⁻¹), followed by Shader Azzam (1.72 g. l⁻¹). El-Salam B and El-Salam A had slightly lower biomass yields of 1.54 g. l⁻¹ and 1.53 g. l⁻¹, respectively (Fig. 1c).

An increase in dry weight was observed in correlation with the contamination load of the different drainage sources, primarily due to the balance of available nutrients. Although *Chlorella vulgaris* is typically classified as a freshwater alga, it can survive and complete its life cycle under high nutrient concentrations (Zhang *et al.*, 2024). Al-Hammadi *et al.* (2024) reported that the growth of *Chlorella* sp.

was markedly improved in wastewater environments, largely due to elevated levels of nitrogen, phosphorus, and organic matter (COD). When nitrogen and phosphorus are supplied in sufficient and balanced amounts, the limiting factor may shift to other nutrients, either due to deficiency or excess. This is evident from the interactions among phosphorus, zinc, and carbonate concentrations (Gebara et al., 2023).

3.3 Nutrients removal in association with growth dry weight

A strong relationship exists between growth and nutrient uptake or removal, and research has shown that peak nutrient absorption occurs during the initial growth phase. Furthermore, the disappearance of nutrients from the growth medium is not solely dependent on their accumulation inside algal cells. A considerable amount can be adsorbed onto the cell wall depending on the species of algae, the composition of the cell wall, the growth conditions, and the physical properties of the water. Overall, the growth pattern clarifies the relationship between the quantity of absorbed nutrients and accumulated biomass.

Nitrogen Removal by *Chlorella vulgaris* under Different Wastewater Conditions

The nitrogen concentration in the wastewater fell between approximately 21 and 24 mg.l⁻¹, which were too low to support optimal algal growth. Consequently, although the proportion of nitrogen removed by *C. vulgaris* increased across various wastewater sources, the total amount absorbed remained low due to this initial limitation. For example, the BG-II growth medium had an initial nitrogen concentration of 247 mg. l⁻¹, resulting in high removal efficiency (91.78%). In contrast, Om El-Resh drain water showed a lower removal efficiency of 71.34% due to its comparatively low nitrogen level of 21.7 mg. l⁻¹. This lower removal rate, compared to that of BG-II grown algae, may be due to phosphorus deficiency, which affects nutritional balance and growth. Significant enhancements in nitrogen removal were observed when wastewater samples were supplemented with urea, phosphorus, or both (Table 2). This nutrient supplementation increased the initial nitrogen concentration from 21.7 mg. l⁻¹ to levels comparable to those in the BG-II medium (247 mg. l⁻¹), enabling *C. vulgaris* to absorb substantially more nitrogen.

The results showed a significant increase in nitrogen uptake when the drain wastewater was supplemented with nitrogen, reaching concentrations comparable to those in the BG-II medium. After 48 hours of incubation, the highest percentage of nitrogen removal was recorded in Om El-Resh drain water enriched with both phosphorus and nitrogen (97.58%), followed by nitrogen enrichment alone (81.26%). These values were then compared to those of cultures grown in a standard BG-II medium and in other drain water sources. The lowest nitrogen removal rate (66.11%) occurred in cultures grown in unenriched BHD. By the end of the day 14 incubation period, most treatments showed slight declines in removal efficiency. This could be attributed to severe deficiencies in essential nutrients, particularly phosphorus, which led to retarded algal growth and nutrient removal. Other tested wastewater sources exhibited a similar trend with slight variations, potentially due to the presence of additional nutrients in the wastewater.

The main conclusion of this study is that adding macronutrients (N/P) to drain water improves nitrogen removal by promoting algal growth. The main hypothesis was validated; the enhancement effect of nitrogen and phosphorus depended on the water source's initial nutrient content and contamination level. **Goswami et al. (2024)** demonstrated that *Chlorella pyrenoidosa* can achieve approximately 95% nitrogen removal efficiency when cultivated in wastewater using a two-stage sequential cultivation system. Dahmani et al. (2016) also reported high nitrogen removal capacities with *C. pyrenoidosa* grown under wastewater conditions.

Phosphorus Removal by *Chlorella vulgaris* under Different Wastewater Conditions

The impact of phosphorus supplementation on the growth of *Chlorella vulgaris* in drain water was clearly demonstrated through growth analysis. As shown in Table (2); all sites except Shader Azzam exhibited only minor differences in maximum growth rates compared to the recommended growth medium.

Table 2. Nutrient absorption (ppm), dry weight (g.l⁻¹) and overall growth (OG) of *Chlorella vulgaris* grown in different wastewater sources enriched by nitrogen or/and phosphorous

Element		N	P	K	Na	Ca
A	BG-II	226.0	5.0	10.01	353	10.28
B	C.W	21.7	2.12	24.87	948.68	277.62
	C.W +N	247.0	2.15	28.06	1024.9	280.62
	C.W +P	21.7	5.95	26.32	940.35	253.86
	C.W +N+P	247.0	6.71	30.58	1048.8	285.53
C	C.W	22.7	2.60	18.24	469.02	25.12
	C.W +N	247.0	3.30	18.00	483.60	22.14
	C.W +P	22.7	6.03	18.03	459.24	33.96
	C.W +N+P	247.0	6.48	19.12	505.70	17.04
D	C.W	22.7	2.06	13.86	65.21	105.35
	C.W +N	247.0	2.34	15.01	57.88	108.53
	C.W +P	24.7	5.88	13.35	71.83	98.92
	C.W +N+P	247.0	6.62	15.66	52.66	110.04
E	C.W	21.6	0.24	8.47	101.69	36.30
	C.W +N	247.0	0.33	8.99	104.24	37.88
	C.W +P	21.6	4.29	8.15	100.13	34.61
	C.W +N+P	247.0	5.89	9.36	105.89	38.83
F	C.W	21.7	0.77	9.97	116.55	51.07
	C.W +N	247.0	0.88	10.23	117.91	53.02
	C.W +P	21.7	5.91	9.89	112.94	49.17
	C.W +N+P	247.0	6.49	10.34	120.01	53.70

C.W= Crude water; A= BG-11; B=OM El-Resh; C= Shader Azzam; D= Salam Canal before mix; E= Salam Canal and F= Salam Canal after mix.

Phosphorous uptake kinetics and removal efficiency of *C. vulgaris* under different growth conditions, including the BG-II growth medium and various nutrient-enriched wastewater sources were evaluated. Data revealed that during the initial growth phase, *C. vulgaris* exhibited a phosphorus uptake rate of approximately $0.113 \text{ mg.l}^{-1}.\text{h}^{-1}$ in the BG-II medium is achieving a removal efficiency of 77.43%. However, by the 14th day of incubation, the uptake rate had significantly declined to $0.015 \text{ mg.P.l}^{-1}.\text{h}^{-1}$, with a

removal efficiency of 71.86%. This decline suggests that phosphorus uptake slowed due to nutrient depletion or the algae's physiological acclimation.

The phosphorus removal efficiency in the wastewater medium was generally around 70%, which aligns with the typical values reported in the literature for microalgal phosphorus uptake under similar conditions. However, significantly higher removal rates were observed during the early growth phase when the drain wastewater was enriched with urea as a nitrogen source and phosphorus.

The highest removal rate was recorded in Om El-Resh drain wastewater at $0.141 \text{ mg.P.l}^{-1}.\text{h}^{-1}$ and 96.71% removal efficiency. El-Salam B followed with $0.139 \text{ mg.P.l}^{-1}.\text{h}^{-1}$ and 95.44% removal efficiency; then, Shader Azzam with $0.137 \text{ mg.P.l}^{-1}.\text{h}^{-1}$ and 94.04% removal efficiency; then, El-Salam A with 0.136 mg P/L/h and 93.43% removal efficiency; and finally, Bahr Hadous Drain (BHD) with $0.125 \text{ mg.P.l}^{-1}.\text{h}^{-1}$ and 86.00% removal efficiency.

These results suggest that enriching wastewater with nitrogen and phosphorus simultaneously significantly increases algal growth and phosphorus uptake rates. In contrast, phosphorus- or urea-enriched drain treatments exhibited lower removal efficiencies. For instance, Bahr Hadous Drain (BHD) wastewater achieved a removal rate of only 62.00% at $0.09 \text{ mg.P.l}^{-1}.\text{h}^{-1}$. The decline in phosphorus removal observed over the day 14 incubation period with established algal growth dynamics, wherein phosphorus uptake is highest during the early exponential growth phase, when nutrient demand peaks.

Potassium removal by *Chlorella vulgaris* of under different wastewater conditions

The potassium removal capacity of *C. vulgaris* was evaluated in relation to the recommended BG-II growth medium during early (48 hours) and full (14 days) incubation periods. The results showed only minor variations in potassium uptake or removal percentages between cultures grown in BG-II and cultures grown in different enriched and unenriched wastewater samples. These results suggest that potassium bio-removal by *C. vulgaris* remains consistent despite varying potassium concentrations in the tested media.

However, the efficiency of potassium removal strongly depends on its physiological function in algal metabolism, its concentration within the medium, and its balance with other macronutrients, especially nitrogen (N) and phosphorus (P). Despite differences in potassium levels between the drain water samples and the BG-II medium, *C. vulgaris* exhibited higher net potassium uptake across all treatments, highlighting the alga's robust absorption capacity.

Among the tested nutrient enrichment strategies, the combination of urea (N) and phosphorus (P) resulted in the greatest potassium removal efficiency. Specifically, Om El-Resh wastewater enriched with both nitrogen and phosphorus had the highest percentage of potassium removal, at 87.38%, corresponding to an absorption rate of $0.619 \text{ mg.K.l}^{-1}.\text{h}^{-1}$. This elevated removal rate is attributed to the higher initial potassium concentration in this wastewater, which provides more substrate for uptake. Conversely, the lowest removal percentages and uptake rates were recorded in Bahr Hadous Drain (BHD)

(79.58%, or 0.199 mg.K. l⁻¹.h⁻¹) and in El-Salam A (80.31%, or 0.218 mg K/L/h), likely due to their lower initial potassium content. The lowest removal efficiency (71.33% or 0.178 mg.K.l⁻¹.h⁻¹) occurred in treatments involving algae grown solely in untreated wastewater. These results demonstrate that nutrient enrichment enhances potassium uptake.

Sodium Removal by *Chlorella vulgaris* Under Different Wastewater Conditions

The absorption and removal of sodium (Na⁺) by *C. vulgaris* showed minimal variation from the recommended BG-II growth medium during the initial 48-hour incubation period and throughout the full 14-day experimental duration. These results suggest that *C. vulgaris* maintains a consistent capacity for sodium uptake, regardless of medium composition, provided that other growth conditions remain similar. However, the dynamics of sodium removal were markedly influenced by the nitrogen content of the growth medium and the initial sodium concentration in the tested drainage waters. Notably, sodium removal efficiency increased with rising salinity and nitrogen availability, as well as under combined nitrogen and phosphorus (NP) enrichment conditions. The net amount of sodium absorbed by *C. vulgaris* exceeded that in the BG-II medium when the algae were exposed to drainage waters with high sodium levels, such as those in the Om El-Resh drain. BG-II control cultures exhibited an 89.51% sodium removal percentage.

During the early growth stage (48 hours), *C. vulgaris* achieved the highest percentages of sodium removal in Om El-Resh drain wastewater across all treatments. The percentages ranged from 82.72% (phosphorus enrichment only with an absorption rate of 19.82 mg Na/L/h) to 91.73% (combined phosphorus and urea enrichment with an absorption rate of 21.98 mg. Na.l⁻¹.h⁻¹). This increased removal is consistent with the elevated initial sodium levels in the wastewater and the stimulatory effects of nutrient enrichment on algal growth and ion uptake. In contrast, lower removal percentages were observed at other sites. For instance, phosphorus enrichment alone yielded 78.21% sodium removal at a rate of 2.12 mg.Na. l⁻¹.h⁻¹ at Bahr Hadous Drain (BHD). By the end of the day 14 incubation period, sodium removal declined slightly across all treatments.

Calcium Removal by *Chlorella vulgaris* under Different Wastewater Conditions

During the early growth period, *Chlorella vulgaris* efficiently removed calcium, with BG-II control cultures achieving an 80.34% removal rate. The highest removal rate (90.50%) occurred in El-Salam A cultures enriched with phosphorus and urea at an absorption rate of 6.0 mg.Ca.l⁻¹.h⁻¹. Conversely, the lowest removal rate (78.84%) occurred in El-Salam A cultures enriched with phosphorus alone at a rate of 1.04 mg.Ca. l⁻¹.h⁻¹. These variations are primarily due to differences in nutrient balance and specific nutrient enrichment. Combined nitrogen and phosphorus supplementation consistently enhanced calcium removal relative to adding a single nutrient. The calcium removal rate decreased toward the end of the cultivation period for all treatments, reflecting typical algal growth dynamics whereby nutrient uptake is most pronounced during the early exponential phase.

Biological oxygen demand (BOD) and chemical oxygen demand (COD).

Figure 2 shows that *Chlorella vulgaris* exhibited high BOD removal efficiency during the early growth phase (48 hours) when phosphorus and urea were added to the wastewater. The highest BOD removal (86.67%) occurred in the Om el-Resh Drain. The next highest removal rate (78.18%) occurred in the Shader Azzam Drain. This was followed by the El Salam B Drain (70%), the El Salam A Drain (64%), and the Bahr Hadous Drain (60%). In contrast, the lowest BOD removal rate (35%) was observed in Bahr Hadous (BHD) wastewater without nutrient enrichment. Similarly, the highest COD removal rate (84.44%) occurred in Om El-Resh wastewater with combined phosphorus and urea enrichment. Decreases were observed in Shader Azzam (84.29%), El Salam B (74.29%), El Salam A (71.11%), and Bahr Hadous (67.5%). The lowest COD removal rate recorded was 60% in El Salam A and BHD wastewater without enrichment. Notably, no significant changes in BOD removal were observed as algal growth progressed toward the late incubation phase (14 days). This suggests that most organic matter degradation occurs during the early exponential growth period when metabolic activity is highest. As shown in Fig. 2 and Fig. 3 initial, maximum absorption rate of BOD (mg/L) by *Chlorella vulgaris* as affected by wastewater sources and nutrient enrichment.

These observations support the conclusion that the pollution ratio—or the balance of organic and inorganic nutrient loads—markedly influences algal growth and associated metabolic processes, such as nutrient absorption and organic matter degradation. Nutrient enrichment, particularly of nitrogen and phosphorus, increases algal biomass productivity. This enhances the enzymatic and metabolic capacity to decompose organic pollutants, thereby improving the removal efficiencies of BOD and COD. Biotic factors, including the microbial consortia that coexist with *Chlorella vulgaris*, contribute to the biodegradation of complex organic compounds. These factors may also interact with algal uptake mechanisms to optimize wastewater treatment outcomes.

3.4 Coliform Bacteria Under Different Wastewater Conditions by *Chlorella vulgaris*

Significant increases in total coliform removal by *C. vulgaris* were achieved by separately or together enriching the wastewater with phosphorus or nitrogen. Reductions of up to 96.48% were observed, along with nearly complete elimination. A similar inhibitory effect was observed for fecal coliforms. Nitrogen enrichment alone was as effective as treatments containing phosphorus. These results underscore the importance of nutrient supplementation in maximizing the antimicrobial effectiveness of algae in wastewater systems. The pronounced decrease in bacterial load is due to several interrelated mechanisms. First, the robust growth of algae, stimulated by added nutrients, acidifies the medium. These circumstances are unfavorable for bacterial survival. Second, *C. vulgaris* secretes extracellular bioactive compounds, particularly polysaccharides, which exhibit antimicrobial properties and inhibit bacterial proliferation. Third, nutrient enrichment alters the osmotic potential of the growth medium, which further impacts microbial viability. This poses an additional challenge to microbial viability.

4. Discussion

The study highlights the efficacy of *Chlorella vulgaris* in removing nitrogen from wastewater, as demonstrated by previous research. This was observed in nutrient-rich conditions similar to those in standard algal growth media, such as BG-II. *C. vulgaris* achieved nitrogen removal efficiencies of nearly 92%, comparable to the 61–90% range reported for urban and municipal wastewater treatment (Park et al., 2017; Smith et al., 2025). Conversely, the limited nitrogen removal observed in untreated drainage water reflects its nutrient-deficient nature. For instance, the Om El-Resh drain had nitrogen levels of approximately 21.7 mg/L. These levels restrict algal metabolism and growth. (Zhou et al., 2023). Supplying nitrogen to wastewater in the form of urea and phosphoric acid can significantly increase nitrogen removal rates, raising nutrient bioavailability and supporting higher biomass yields (Chen et al., 2019).

To validate the superior nitrogen removal performance of *C. vulgaris*, refer to comparative studies between this species and others, such as *Scenedesmus quadricauda*. *C. vulgaris* appears to have adaptive nutrient uptake mechanisms and is resilient under varying environmental conditions (Wang et al., 2020). The main pathway responsible for nitrogen detoxification and biomass production assimilates inorganic nitrogen species, primarily ammonium and nitrate, into cellular constituents, such as amino acids and proteins. In the raw concentrate (RC), phosphorus degradation occurred within the first two days, with a drastic decrease from 178 mg.l⁻¹ to 57.7, 58.2, 55.3, and 47.3 mg.l⁻¹ observed with initial algal inocula of 0.02, 0.05, 0.1, and 0.2 g.l⁻¹, respectively (Ma et al., 2014).

The phosphorus concentration increased slightly until the end of cultivation and remained at a similar level. The corresponding final phosphorus removal efficiencies were 65.4%, 69.6%, 65.3%, and 63.5%, respectively. Arya and Anand (2013) reported that the algae *Oedogonium* and *Chara* removed 100% of the phosphate by the sixth day. However, their removal capacity decreased over time. Algal degradation begins after the seventh day, which may cause a decrease in the removal rate. Phosphorus deficiency markedly affects growth under saline conditions and high acidity since cells shift to respiratory metabolism. Thus, potassium deficiency damages cell structure, particularly under saline conditions (Wenna, 2014).

This study shows that *Chlorella vulgaris* is highly effective at bioremediating nitrogen and phosphorus from wastewater. This is especially true when nutrient enrichment is used to offset initial deficiencies. Supplying nitrogen and phosphorus at levels comparable to those in standard growth media significantly enhances algal growth, nutrient uptake rates, and removal efficiencies. These conditions achieve nitrogen removal of up to around 92% and phosphorus removal.

Conversely, the lowest potassium removal percentages were observed in drain samples with lower initial potassium content, such as those from Bahr Hadous Drain (BHD) and El-Salam A. Algal cultures grown in unenriched wastewater exhibited reduced removal efficiency, highlighting the importance of nutrient supplementation to maximize the capacity of algal remediation. The decline in potassium removal by the end of the 14-day period coincided with the expected transition to the stationary or senescent phase, characterized by reduced metabolic and uptake activity (Zhou et al., 2023). These dynamics are common

in microalgal cultures, where nutrient assimilation peaks during exponential growth, then decreases as nutrients become scarce or biomass metabolism adapts. Furthermore, *Chlorella vulgaris*'s ability to utilize atmospheric CO₂ ensures carbon availability, even in wastewater with limited dissolved inorganic carbon. This supports sustained growth, nutrient assimilation, and potassium uptake (Li et al., 2021).

In general, sodium ions are adsorbed onto the surfaces of algal cells and extracellular polymeric substances (EPS). The latter contain functional groups that can bind to ions. This process facilitates the initial passive capture of sodium ions (Wang et al., 2025). Meanwhile, active transport systems regulate sodium uptake to maintain ionic balance and osmotic homeostasis within the cells, thereby protecting them in saline conditions. This physiological adaptation contrasts with higher plants, where sodium is typically nonessential or detrimental. Algal growth media commonly contain sodium salts, such as NaCl, NaHCO₃, and Na₂CO₃, reflecting sodium's integral role in algal metabolism. One such role is as a carbon source via bicarbonate (Zhou et al., 2023; Li et al., 2021).

Despite the slight decrease in sodium removal efficiency later in the incubation period, which is consistent with the transition of algae towards stationary or senescent phases, *Chlorella vulgaris* maintained substantial sodium uptake. This finding underscores the potential of *C. vulgaris* for salinity bioremediation. In *C. vulgaris*, calcium primarily plays a structural role rather than a metabolic one. It is involved in structural processes, as opposed to metabolic energy processes, which are characteristic of higher plants (Hepler, 2005). This structural role includes maintaining cell wall integrity, ensuring membrane stability, and activating signaling pathways necessary for algal growth and development. Optimizing algal growth and metabolic activity through balanced nutrient availability, particularly of nitrogen and phosphorus (NPK), is critical to enhancing the removal of organic matter. Variations in the C:N:P ratio, along with environmental factors such as light and pH, influence enzyme activity and biomass productivity, thereby affecting BOD and COD reduction efficiency. Your findings concur with these observations, demonstrating superior removal efficiencies under combined nutrient enrichment compared to unenriched conditions.

In summary, the absorption of calcium by *Chlorella vulgaris* varies widely depending on the availability of nutrients and growth conditions. The highest removal efficiencies occur during the early growth phases under combined nitrogen and phosphorus enrichment. These results highlight the importance of achieving the proper nutrient balance to optimize calcium bio-removal and illuminate the physiological and structural functions of calcium in microalgae wastewater treatment.

Chlorella vulgaris efficiently removes BOD and COD through multiple biological mechanisms. Microalgae can absorb dissolved organic compounds directly and produce extracellular enzymes that break down complex organics. Additionally, microalgae produce extracellular polymeric substances (EPS), which enhance the adsorption and immobilization of organic pollutants and facilitate their removal from the aqueous phase. The symbiotic relationship between *C. vulgaris* and heterotrophic bacteria in mixed microbial consortia promotes biodegradation through complementary metabolic pathways. These findings underscore the importance of tailoring nutrient supplementation to wastewater composition to

maximize treatment. They align with those of Abdel-Raouf et al. (2012), who reported that phosphorus supplementation intensifies algal-mediated reductions in bacteriological contaminants, primarily through enhanced algal biomass and associated biochemical excretions. Furthermore, *C. vulgaris* significantly reduces organic pollutants, achieving high levels of BOD and COD removal. In some cases, it removes over 90% of both BOD and COD, reflecting the efficient degradation of organic matter (Pooja et al., 2022). This reflects the efficient degradation of organic matter.

This species exhibits robust growth in wastewater with varying nutrient loads, promoting biomass production of approximately $0.6\text{--}0.7\text{ g.l}^{-1}$ and enhancing nutrient uptake and the breakdown of organic pollutants (Pooja et al., 2022; Li et al., 2021). Nutrient supplementation, particularly the addition of combined nitrogen and phosphorus, promotes *C. vulgaris* growth. This leads to synergistic effects on the overall treatment of wastewater and increases nutrient assimilation rates. (Goncu et al., 2025). Moreover, *C. vulgaris* demonstrates resilience in the face of varying wastewater conditions, ensuring efficient removal over prolonged cultivation periods. It is therefore well-suited to tertiary treatment stages in wastewater management. Its ability to absorb both inorganic nutrients and organic contaminants, along with its production of bioactive compounds and extracellular polymeric substances (EPS), aids in pollutant sorption and biotransformation. This improves removal efficiencies (Amany and Amany, 2024).

5. The economic feasibility and practical applications of

5- The economic feasibility and practical applications of *Chlorella vulgaris*

Using *C. vulgaris* to treat agricultural drainage water offers environmental, economic, and practical benefits. The phycoremediation treatment costs for *C. vulgaris* range from 0.05 to 0.12 USD.m⁻³, which is significantly lower than the 0.20–0.40 USD.m⁻³ cost of conventional chemical treatments. This is primarily because the algae are self-sustaining and require minimal energy and chemicals to grow (Pooja et al., 2022).

Biomass and biofuel production: Yields of around $1.2\text{--}1.5\text{ g.l}^{-1}$ ($1.2\text{--}1.5\text{ tons.1,000 m}^{-3}$) of biomass with 20–30% lipid content could produce 200–300 kg of biodiesel per 1,000 m³. Increasing production to 100 million m³ per year could yield 20,000–30,000 tons of biodiesel annually, supporting renewable energy use and offsetting treatment costs (Kanaga et al., 2022).

Economic Feasibility: Combining savings from water treatment with revenue from biomass valorization, such as biofuels, animal feed, and fertilizers, could render algal treatment cost-neutral or profitable when fully integrated. These findings corroborate the previously stated benefits and confirm the economic, practical, and environmental viability of using *Chlorella vulgaris* for agricultural drainage treatment. This approach aligns well with sustainable water and renewable energy goals (Yousif et al., 2022).

6. Conclusion

Adding urea nitrogen and phosphoric acid to wastewater significantly increases the growth of *Chlorella vulgaris* and improves the efficiency of nutrient removal. Combining the algae treatment with nitrogen and phosphorus enrichment after 48 hours produced the greatest reductions in nitrogen (97.58%), phosphorus (96.71%), potassium (87.38%), sodium (91.73%), and calcium (90.5%), as well as exceeding 84% of both the biological oxygen demand (BOD) and chemical oxygen demand (COD). This resulted in the near-complete elimination of total and fecal coliforms (at least 96.48%). Increasing nitrogen (N) and phosphorus (P) levels in nutrient-poor wastewater markedly improves the bioremediation process by *C. vulgaris*, consistent with scientific literature. These results support using *C. vulgaris* to efficiently remove nutrients and reduce pathogens in wastewater treatment, particularly under nutrient-enriched conditions. However, calcium removal varies widely with nutrient availability, emphasizing the importance of balanced nutrients during early growth phases. Effective BOD and COD removal requires a balanced supply of nitrogen, phosphorus, and potassium; phosphorus plays a key role in controlling pathogens. These findings suggest that *C. vulgaris* is a promising, eco-friendly biotechnological solution for wastewater remediation and the safe reuse of water. These findings confirm that algal-based wastewater treatment is a sustainable and feasible option for reuse in Egyptian agriculture.

Declarations

Author Contribution

Abo El-Khair B. El-Sayed: Conceptualization, methodology, supervision, writing and review of the manuscript. Marwa M. Reda: Conceptualization, methodology, investigation, biochemical analysis, writing and review. Hisham El-Kassas: Conceptualization and methodology. Maryam M. Mostafa: Conceptualization, investigation, writing—original draft, preparation, and review. All authors read and approved the final manuscript

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Figures

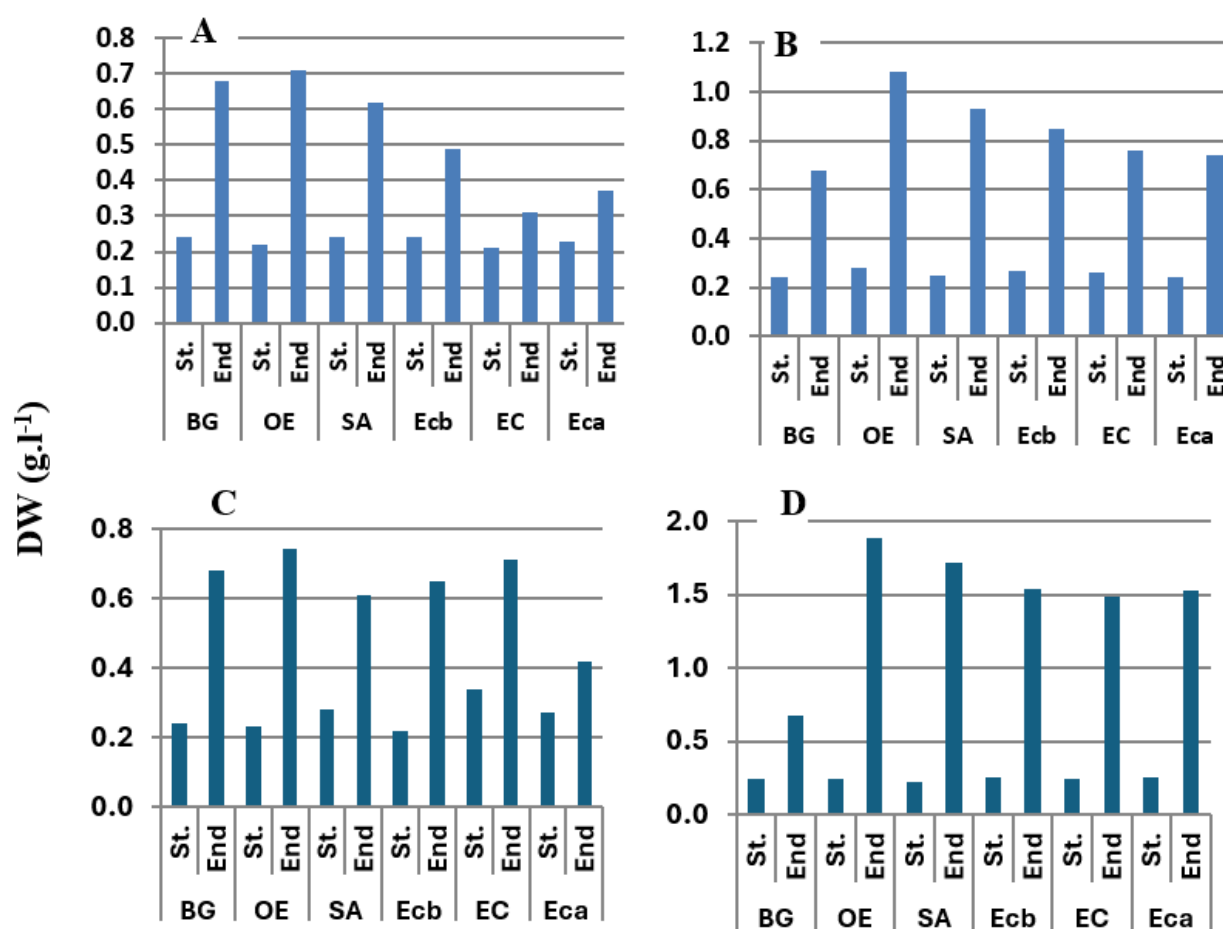


Figure 1

Growth and dry weight accumulation of the green alga *Chlorella vulgaris* grown with different wastewater sources enriched by N or/and P.

A= crude wastewater, B= Nitrogen enriched crude wastewater, phosphorus enriched crude wastewater,

D= Nitrogen and phosphorus enriched crude wastewater, St. = Start, OE=OM El-Resh; SA= Shader Azzam; Ecb= Salam Canal before mix; EC= Salam Canal and Eca= Salam Canal after mix

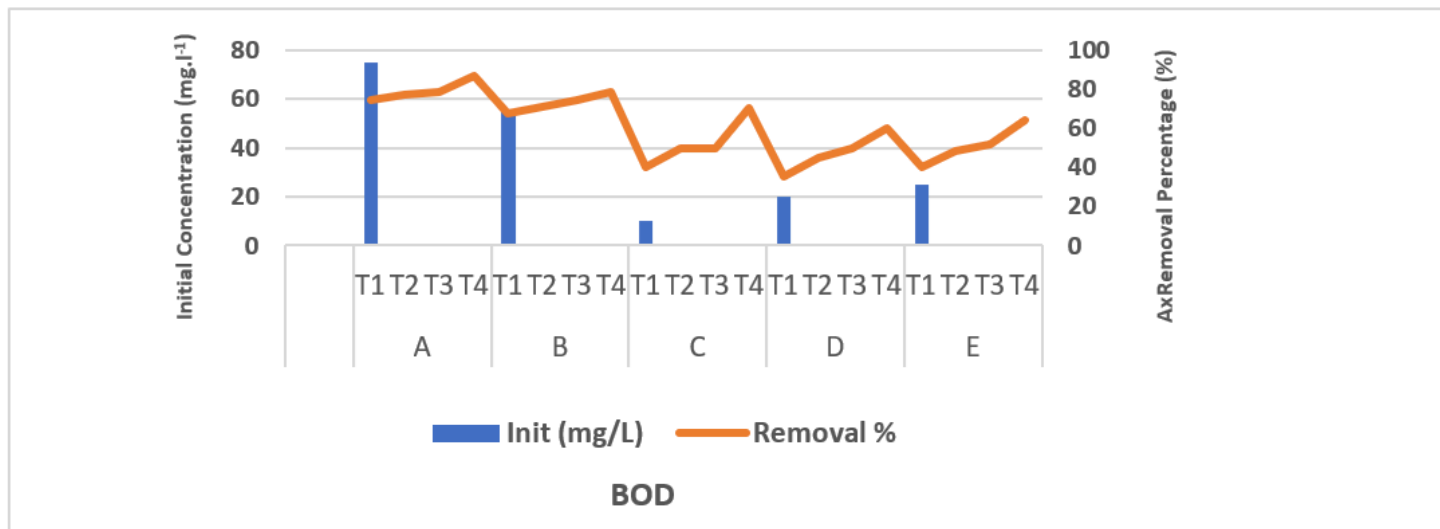


Figure 2

Initial, maximum absorption rate of BOD (mg/L) by *Chlorella vulgaris* as affected by wastewater sources and nutrient enrichment.

T1= Growth with crude drain water, **T2**= Growth under urea nitrogen-enriched drain water, **T3**= Growth under phosphorous-enriched drain water, **T4**= Growth under nitrogen- and phosphorous-enriched drain water, **Init.** = initial cell count **Abs. %** = Rate of absorption percentage, **A**= Om El-Resh; **B**= Shader Azzam; **C**= El-Salam with Bahr Hadous Drain before mixing; **D**= Bahr Hadous Drain and **E**= El-Salam with Bahr Hadous Drain after mixing

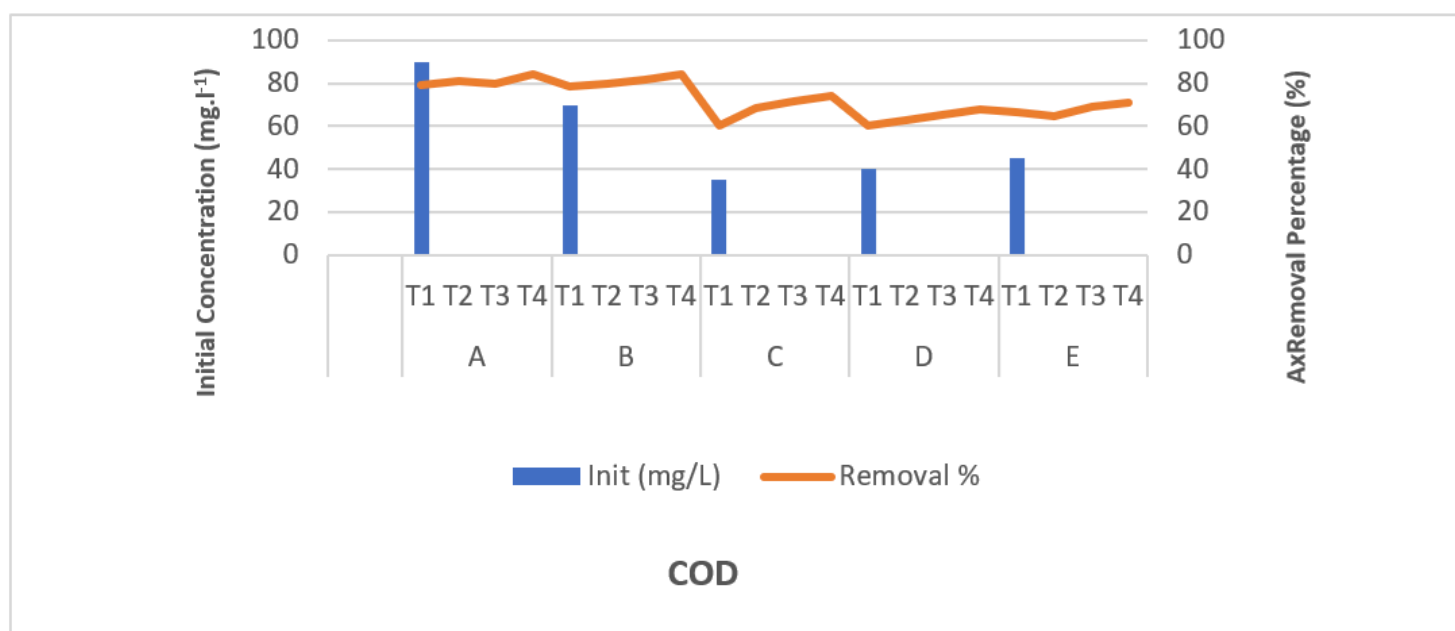


Figure 3

Initial, maximum absorption rate of COD (mg/L) by *Chlorella vulgaris* as affected by wastewater sources and nutrient enrichment.