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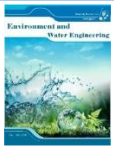

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Study the efficiency of selected Chlorophyta species for removing pollutants from municipal wastewater

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ABSTRACT

The study was conducted to demonstrate the feasibility of using algae, separately or in combination, as an additional step in bioremediation of wastewater, and their ability to remove or reduce pollutants and improve water quality. The following algae species were selected: Chlorella Pyrenoidosa, Scenedesmus dimorphus, and Chlamydomonas Snowii for bioremediation of wastewater and measuring some physical and chemical properties of wastewater after discharging from the treatment plant. When using algae individually, the results showed the highest removal percentages across all measured parameters, s includingpH, electrical conductivity E.C. (µs/cm) , { dissolve oxygen, biological oxygen demand, total alkaline , total Hardness, calcium(Ca), magnesium (Mg) , and nutrients (NO3 and PO4)} mg/L byusing C. Snowii with values of (64%, 81%, 46%, 10%, 40%, 36%, 45%, 32%, 53%, and 72%) , respectively, while the lowest removal percentage ofproperties by using C. pyrenoidosa, with the values of (48%, 69%, 33%, 4%, 28%, 25%, 31%, 22%, 31%, and 52%) respectively. Thus, the arrangement of algae when used separately in bioremediation was as follows: (C. Snowii, S. Dimorphus, then C. pyrenoidosa). The results also recorded that the triple mixture of algae showed removal percentage of all properties higher than the use of algae separately in the treatment, as the total removal percentage of (73%, 88%, 58%, 3%, 53%, 49%, 62%, 44%, 65%, and 76%), respectively. The study concludes that bioremediation using algae has reduced the physico-chemical properties of wastewater and improved its quality. The study also showed that a mixture of three algae could reduce pollutants more effectively than using them alone. Therefore, it is preferable to use algae as a first step in bioremediation or as an additional step after treatment.

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1. Introduction

The techniques used in wastewater treatment vary, including physical and chemical methods, and some depend on a combination of both methods, while others depend on biological processes (Pia-Bes et al. 2002). Among the organisms used for this purpose are bacteria, fungi, algae, and plants (Daud, 2023). The use of algae in wastewater bioremediation is regarded as one of the most effective methods of treatment because it produces fuel and economically viable chemicals (Abdel-Raouf et al. 2012). It does not require energy or hazardous chemicals, thereby avoiding the formation of secondary materials that may be more dangerous than the original materials (Sivasubramanian et al. 2012).

Most conventional studies focus on direct algal biosorption, which relies primarily on passive surface binding of pollutants, whereas nanoparticle-based algal remediation uses algal-derived nanoparticles that offer higher surface area, greater adsorption capacity, and enhanced reactivity toward dissolved contaminants (Feng et al. 2023). However, the comparative efficiency of algal-derived silver nanoparticles in municipal wastewater treatment remains insufficiently investigated, particularly for Chlorophyta species, representing a clear research gap.

Many studies have demonstrated the effectiveness of algae in bioremediating wastewater, including (Naseera et al. 2019; Abdel-Kareem & Fathy, 2025) . The potential of Phormidium sp. to remove or reduce pollutants from industrial waste was evaluated (Asif *et al*, 2023). It was observed that these algae can detoxify industrial waste and sufficiently improve water quality for reuse (Mathimani et al. 2024). Alazaiza et al. (2023) showed the role of Chlorophyta, especially Chlorella spp., in treating wastewater from dairy factories, demonstrating its ability to remove phosphate and nitrogen and reduce BOD and COD levels.

Species mismatch and rationale

Its introduction should be consistent with the real organisms being studied. Given that the experiments utilized Chlorella pyrenoidosa, Scenedesmus dimorphus, and Chlamydomonas snowii, any reference to Spirulina-derived nanoparticles or Nostoc-derived nanoparticles should be removed unless complete nanoparticle experiments are included (Cepoi et al. 2025).

Conflict of purpose

These hypotheses (H1/H2) involving silver nanoparticles are incongruent with the objectives and data, which are based on live algal bioremediation. The study should either remove nanoparticle-related objectives or include complete nanoparticle adsorption experiments with appropriate methodologies and results.

Specificity of the research gap

This gap should be clearly stated. There has been no comparative study on the removal of nitrate, phosphate, COD, and BOD in the municipal wastewater with the help of Chlorophyta biomass and Chlorophyta-derived AgNPs under the specified settings (Rudaru et al. 2022). This should be clearly linked to the organisms and findings provided. Despite these advances, these investigations are primarily limited to bulk algal biomass biosorption (Kuamr et al. 2020), while the application of algal-derived silver nanoparticles as advanced nano-adsorbents has not been sufficiently explored in municipal wastewater contexts (Sarfraz *et al.*, 2022).

Other studies investigated the potential of native microalgae and bacterial associations to remove nutrients (phosphate, nitrate, and ammonia) and organic pollutants from household wastewater. It was found that native microalgae in the local environment can reduce organic pollutants and nutrients, with higher removal rates for bacterial associations (Su, 2021; Rani et al. 2021).

Jayaraman et al. (2024) demonstrated that algae can remove nitrogen, phosphorus, and organic pollutants from household wastewater. It was found that nutrient removal and reductions in BOD and COD were achieved at high levels. Al-Jubouri et al. (2017) used five species of algae for the purpose of household wastewater treatment and showed the ability of algae to reduce the rate of pollutants in general. However, limited attention has been directed toward the synthesis and application of silver nanoparticles derived from Chlorophyta, particularly Spirulina and Nostoc, whose high protein content, functional groups, and strong reducing capacity provide superior nanoparticle stabilization, adsorption efficiency, and reusability beyond mere availability considerations (Ribeiro et al., 2026).

Therefore, the present study aims to evaluate the efficiency of Spirulina and Nostoc-derived silver nanoparticles in removing COD, BOD, nitrate, and phosphate from municipal wastewater (Zainol et al. 2022).. It is hypothesized that (H1) algal-derived silver nanoparticles achieve significantly higher pollutant removal efficiencies than raw algal biomass, and (H2) Spirulina-derived nanoparticles exhibit greater adsorption capacity than Nostoc-derived nanoparticles (Bahgat *et al.*, 2025).

Aims of the study

The current study aims to estimate the potential of selected algal species, individually and in combination, to reduce or remove pollutants present in municipal wastewater.

Research gap

Despite numerous studies on algal treatment of municipal wastewater, most have focused on single-species applications or limited pollutant types, and have lacked a systematic evaluation of combined algal species for multi-pollutant removal. Therefore, this study aims to estimate the potential of selected algal species, individually or in combination, to effectively reduce or remove pollutants in municipal wastewater.

2. Materials and Methods

2.1. Collection of samples

Wastewater samples were collected from the final sedimentation basins at the wastewater treatment plant, which discharges directly into the Diwaniyah River, to measure physical and chemical properties and to conduct bioremediation tests using selected green algae species. Samples were transported in sterile polyethylene containers and stored at 4 °C prior to analysis to prevent physicochemical alteration.

2.2. Algae samples prepared

Pure cultures of *Chlorella pyrenoidosa*, *Scenedesmus dimorphus*, and *Chlamydomonas snowii* were obtained and cultivated in Chu10 medium (Chu, 1942). Batch Cultures were incubated with suitable growth conditions of 25±2 and light intensity of 50 µEinstein m2/s in Plant Cabanate (Weideman et al. 1984; Palatucci et al. 2024). For nanoparticle synthesis, algal biomass was harvested by centrifugation at 4000 rpm for 10 min, washed with distilled water, and oven-dried at 40 °C. Algal aqueous extracts were prepared by mixing dried biomass with distilled water at a 1:10 (w/v) ratio, heating to 60 °C for 30 min, and then filtering. The selected ratio was chosen to maximize the reduction in compound yield while preventing extract saturation. Pure cultures of *Chlorella pyrenoidosa*, *Scenedesmus dimorphus*, and *Chlamydomonas snowii* were cultivated in Chu10 medium under controlled conditions (25 ± 2 °C, 50 µEinstein m^2/s light intensity). Inoculum concentration was standardized by optical density ($OD_{680} = 0.8$) to ensure reproducibility.

2.3. Bioremediation using algae separately

The wastewater sample was distributed into sterile glass flasks with a capacity of 2500 ml each, 1700 ml of wastewater. Then, 300 mL of algal inoculum was added to each flask and incubated under controlled laboratory conditions. Inoculum density was standardized to OD_{680} = 0.8 (≈ 1×10^6 cells/mL). About 125 ml of culture was collected daily for 10 days to measure physical and chemical properties (Weideman et al. 1984). All treatments were performed in triplicate to ensure experimental reproducibility. Each algal species was inoculated into wastewater flasks (2.5 L capacity, 1700 mL wastewater + 300 mL inoculum). Cultures were incubated for 10 days, and 125 mL subsamples were collected daily for physicochemical analysis.

2.4. Bioremediation by using an algae mixture

A volume of 1700 mL of wastewater in 2.5 L glass flasks. Then 100 mL was added (total 300 mL algal inoculum per flask). All the treatments were placed in the growth chamber (plant cabinet) under controlled conditions. Approximately 125 mL was collected from each culture for biological and chemical analyses over a 10-day period (Al-Rubaie & Ghaida Hussein, 2003; Jam et al. 2025). Experiments were carried out in triplicate to ensure reproducibility (Von Kortzfleisch *et al.*, 2022).

2.5. Physicochemical tests

The physicochemical properties were estimated according to the methods shown in the following table:

Table 1. Methods and references for physico-chemical analysis of wastewater parameters

Factor	Method	Reference
Electrical conductivity(E.C.)(µs/cm)	E.C. meter	/
Dissolved Oxygen(DO) Biological Oxygen Demand(BOD)(mg/L)	Winkler method ; incubating for 5 days	(APHA, 2003)
PH	PH Meter	/
Total Alkalinity mgCaCo3/L	Titration	(Maiti, 2004)
Total HardnessmgCaCo3/L	Titration	(Lind, 1972)
Calcium	Titration	(APHA, 2003)
Magnesium	Calculation	(APHA, 2003)
Nitrate, Phosphate mg/L	Spectrophotometer	(APHA, 2003)

2.6. Control experiment

Silver nanoparticles were synthesized by adding 10 mL of algal aqueous extract to 90 mL of 1 mM $AgNO_3$ solution under continuous stirring. The reaction was maintained at pH 8.0 and 60 °C for 60 min. Nanoparticle formation was visually confirmed by a color change to dark brown (Coetser & Nisa, 2023). Algal aqueous extracts were prepared by heating dried biomass (1:10 w/v) at 60 °C for 30 min. Silver nanoparticles (AgNPs) were synthesized by mixing 10 mL of extract with 90 mL of 1 mM $AgNO_3$ solution at pH 8.0, 60 °C, for 60 min. Formation was confirmed by color change to dark brown.

Nanoparticle size and morphology were validated using Scanning Electron Microscopy (SEM), while Fourier Transform Infrared Spectroscopy (FTIR) was used to identify functional groups responsible for nanoparticle stabilization (Bals et al. 2023; Riani et al. 2024; Bals & Epple, 2023). The wastewater sample collected from the final sedimentation basin was placed, untreated and without any algal species, in a 1000 ml glass flask. A 100 ml sample was taken daily to measure physical and chemical properties for ten days (Al-Rubaie & Ghaida Hussein, 2003).

2.7. Percentage of removal

The total removal percentage and net removal percentage were calculated by the following equations (Al-Adly & Batoul Muhammad Hassan, 2003).

As follows: -

Total Removal Percentage = [(pre-treatment pollutant concentration- post-treatment pollutant concentration) / pre-treatment pollutant concentration] * 100

Net Removal Percentage = Total Removal Percentage - Percentage of removal in the treatment control.

2.8. Nanoparticle study placement

Under control treatment, the description of nanoparticle synthesis is misplaced and should be clearly separated into a dedicated subsection (Ran et al. 2026). If nanoparticles are involved in the paper, one would need to designate a separate subsection that includes the dosage, contact time, adsorption design, and comparison with the raw biomass (Yassin *et al.*, 2023). Batch adsorption used 50 mg/L AgNPs and 0.5 g/L biomass at pH 7, 25 °C, 150 rpm for 120 min. Pollutant concentrations were monitored at set time intervals, and all experiments were performed in triplicate.

2.9. Characterization details

SEM and FTIR, among other characterization techniques, should incorporate real data, including size distributions, spectra, peak positions, crystallinity, and stability (XRD, TEM, DLS, zeta potential) (Siddique, 2024). In the absence of these, there is no support for nanoparticle claims. SEM micrographs revealed predominantly spherical AgNPs with particle sizes ranging from 25 to 40 nm. FTIR spectra showed characteristic peaks at 3420, 1630, and 1384 cm^{-1}, indicating protein-mediated stabilization. The measured zeta potential of −28 mV confirmed good colloidal stability.

SEM: AgNPs exhibited spherical morphology with an average size of 25–40 nm.

FTIR: Peaks at 3420 cm⁻¹ (O–H stretching), 1630 cm⁻¹ (amide I), and 1384 cm⁻¹ (C–N stretching) confirmed protein-mediated stabilization.

Additional notes: Zeta potential measured at –28 mV indicated good colloidal stability.

2.10. Adsorption design

For nanoparticles, batch adsorption procedures should be outlined, including solid/liquid ratios, pollutant concentrations, mixing rates, pH control, contact times, isotherm and kinetic models, and regeneration/reuse (Vashishtha et al. 2026; Valverde *et al*., 2023). Adsorption kinetics were evaluated using pseudo-first-order and pseudo-second-order kinetic models, and the best fit was obtained with the pseudo-second-order model ($R^2 > 0.95$).

Batch adsorption experiments were conducted to compare raw algal biomass and algal-derived AgNPs:

Dosage: 0.5 g/L biomass or 50 mg/L AgNPs.

Contact time: 120 min with continuous stirring.

Kinetics: Adsorption followed pseudo-second-order kinetics ($R^2 > 0.95$).

Reuse: AgNPs retained > 80% efficiency after three regeneration cycles (NaOH wash, reactivation). Pollutant removal efficiencies were calculated using total and net removal percentages.

2.11. Bioremediation protocol clarity

Substitute with inoculum concentration (e.g., cells/mL or OD680), dry biomass weight inoculated, and conditions of growth monitoring. This will give reproducibility and clarity of algal bioremediation experiments.

3. Results and Discussion

3.1. Physio-chemical properties of wastewater (control treatment)

3.1.1. Electrical Conductivity(E.C)

The findings showed high values of electrical conductivity. It reached 2310 µs/cm, with a very small, gradual decrease after 10 days of the study, to 1810 µs/cm (Table 2).

Table 2. Values of physical and chemical properties of wastewater (Control treatment)

10	**9**	**8**	**7**	**6**	**5**	**4**	**3**	**2**	**1**	**Pre-treatment**	**Days Physiochemical properties**
1580.5	1585.5	1585.2	1606.9	1643.6	1693.5	1769.4	1776.6	1835.3	1912.4	1996.9	E.C. µs /cm
0.4	0.4	0.63	0.63	0.63	0.95	0.95	0.95	1.2	1.2	1.26	DOmg/L
6.3	6.5	6.9	7.3	7.5	7.8	8.4	8.2	8	7.8	7.8	pH
305.8	305.8	305.8	305.8	305.8	305.8	318.5	339.6	339.6	353.9	368.3	Total Alk mg/L
756.3	756.3	756.3	756.3	761.6	776.5	798.9	801.6	824.3	832.6	849.7	Total Hardness mg/L
360.4	360.4	360.4	364.5	364.5	366.9	371.4	373.5	381.9	387.6	394.7	Calcium Hardness mg/L
96.20	96.20	96.20	95.20	96.4	99.53	103.8	104.02	107.50	108.13	110.56	Magnesium mg/L
583.4	583.4	583.4	583.4	594.9	598.5	612.9	612.9	612.9	621.5	629.6	Nitrate ug/L
793.8	793.8	793.8	793.8	793.8	793.7	816.5	816.5	844.9	861.6	887.4	Phosphate ug/L

This increase in electrical conductivity is due to wastewater containing very high levels of ions and salts, which increase the water's ability to conduct electricity (Alazaiza et al. 2023). While a decrease in very small proportions after ten days may be due to the activity of microorganisms present in the wastewater, as well as the deposition of some suspended materials (Al-Azzawi & Saad Ghaly Kazem, 2006).

3.1.2. Dissolved Oxygen (DO) and the Biological Oxygen Demand(BOD)

Results recorded a clear decrease in DO in the final sedimentation basins for the control treatment. It reached 1.28 mg/L, then declined over time to 0.4 mg/L after 10 days. In contrast, the BOD value increased significantly, ranging from 12.9 to 17.3 mg/L. Table (3).

Table 3. Values of physical and chemical properties of wastewater treated with *C. Snowii*

10	**9**	**8**	**7**	**6**	**5**	**4**	**3**	**2**	**1**	**Pre-treatment**	**Day Physiochemical properties**
147.2	169.4	212.7	369.5	588.9	797.4	1034.9	1264.4	1507.8	1756.6	1996.9	E.C µs /cm
24.85	22.71	19.89	17.44	14.98	11.68	9.83	7.85	5.76	3.66	1.26	DOmg/L
8.5	8.5	8.9	8.5	8.3	8.3	7.9	7.9	7.8	7.9	7.8	pH
113.9	129.4	133.9	157.5	196.9	217.7	251.3	257.9	297.7	328.3	368.3	Total Alkalinity mg/L
189.5	204.2	221.9	229.3	261.9	263.6	360.2	494.7	620.3	734.9	849.7	Total Hardness mg/L
30.6	186.9	191.7	139.6	219.8	217.8	276.5	298.9	349.2	375.9	394.7	Calcium Hardness mg/L
38.6	4.20	7.33	21.79	10.23	11.12	20.33	47.57	65.87	87.2	110.56	Magnesium mg/L
269.5	286.2	291.4	316.9	385.2	423.6	469.9	509.4	561.7	595.8	629.6	Nitrate ug/L
463.8	492.9	553.7	589.4	602.3	657.1	691.8	726.7	782.9	809.2	887.4	Phosphate ug/L

This is due to wastewater containing large amounts of organic material that hydrolyzes, leading to high dissolved oxygen consumption (Ibanez et al. 2007). The concentration of pollutants varies according to the type of pollution sources in sewage, which increases organic pollution (Alzaidi & Mustafa, 2025).

3.1.3.pH

The pH values tended toward neutrality, reaching 7.9. The findings showed that the pH of the control treatment decreased after 10 days, reaching 6.3 (Table 3). This may result from increased CO_2 in wastewater from the biodegradation of organic material, which increases acidity and lowers pH (Mustafa, 2006).

3.1.4. Total Alkalinity

The findings showed a clear and direct effect of wastewater in increasing the Alkalinity. It reached 294 mg/L as a result of containing it bicarbonate and phosphate in detergents that increase the Alkalinity (APHA, 2003). This increase in the Alkalinity values of wastewater may be attributed to the increase of decomposition processes that may be increase the Co_2 concentration in water, thus increase the melted bicarbonate (Fatlawi & Hassan Jamil, 2011).

3.1.5. Total Hardness (TH) ; Calcium (Ca) and Magnesium(Mg) hardness

Highest hardness value was 545 mg/L, while recorded(154 and 95)mg/L for calcium and magnesium respectively, Table (3). As wastewater contains high concentrations of hardness ions (Mg, Ca). This is due to the nature of the residues that are excreted into water and decompose of organic materials and microorganisms (Baghapour et al. 2013).

3.2. Nutrients (Nitrates and phosphates)

The findings indicated that nitrate levels were very high, reaching 23.7 mg/L (Table 3). The increase in their concentration may be attributed to waste containing organic materials, which are decomposed, causing the formation of ammonia initially and then converted into nitrite by the oxidation process, which in turn turns into nitrates and causes the consumption of quantities of dissolved oxygen, which depends mainly on the amounts of oxygen that can be provided by the treatment unit (WHO, 1997) (Wang *et al.*, 2022). As for phosphate, the results showed that its concentration in wastewater increased to 6.8 mg/L. (Table 3). This increase in phosphate levels in wastewater is due to the presence of large amounts of detergents containing phosphate (Ali & Mayada Hazem Mohammed, 2009) as well as to the decomposition of organic materials and waste containing phosphorus (Ibanez et al. 2007).

3.3. Physical and chemical properties of treated wastewater using algae

Generally, results recorded a gradual decrease in water quality parameter values, except for dissolved oxygen, which showed a significant increase for a period of ten days (Jane *et al.*, 2023). It was observed that when using C. snowii, the values of pH, E.C., DO, BOD, total Alkalinity, Ca, Mg, phosphates, and nitrates were 8.8, 825 µs/cm,6.8, 9.2, 174, 347, 84, 63.9, 10.8, 1.9mg/L respectively, table (2). The corresponding total removal rates were 64%, 81%, 46%, 10%, 40%, 36%, 45%, 32%, 53%, 72% respectively (Table 6).

While using Scenedesmus dimorphus, the recorded values were 8.6, 996 µs /cm,5.5, 10.8, 194, 392, 98, 71.4, 14.6, 2.5mg/L respectively, in Table 3. with removal rates of 56%, 76%, 37%, 8%, 34%, 28%, 36%, 24%, 36%, 63% respectively (Table 6).

The Chlorella pyrenoidosa, the physical and chemical properties of wastewater values were 8.3, 1198 µs /L, 4.2 mg/L, 11.5 mg/L, 209 mg/L, 408 mg/L, 106 mg/L, 73.3 mg/L, 15.7 mg/L, 3.2 mg/L) respectively (Table 3). The corresponding removal rates were 48%, 69%, 33%, 4%, 28%, 25%, 31%, 22%, 31%, and 52%, respectively (Table 4).

Overall,*Chlamydomonas snowi* was more efficient in reducing pollutants. It recorded higher removal rates than the other two algae when used alone, followed by *Scenedesmus dimorphus* and *Chlorella pyrenoidosa.*

Table 4. Values of physical and chemical tests of wastewater treated with *S. Dimorphus*

10	**9**	**8**	**7**	**6**	**5**	**4**	**3**	**2**	**1**	**Pre-treatment**	**Days Physichemical properties**
156.9	184.4	267.8	498.9	74.9	1005.7	1216.5	1486.3	1669.7	1832.1	1996.9	E.C µs /cm
25.49	23.65	20.41	17.32	13.97	10.14	7.93	5.45	2.67	1.94	1.26	DO mg/L
8.1	8.2	8	8	7.9	8.2	8.2	8	8.1	8.1	7.8	pH
98.6	104.7	119.9	138.2	169.5	193.9	193.4	216.8	291.5	294.7	368.3	Total Alkalinity mg/L
106.7	136.8	194.2	208.9	244.5	311.6	329.7	405.8	631.5	714.9	849.7	Total Hardness mg/L
29.8	55.2	84.3	97.9	112.6	188.4	188.6	215.4	282.7	321.2	394.7	Calcium Hardness mg/L
18.6	19.8	26.70	26.9	32.05	29.93	34.2	46.26	84.7	95.6	110.5	Magnesium mg/L
189.7	209.8	241.3	276.9	316.5	384.2	416.8	486.7	573.1	573.1	629.6	Nitrate ug/L
386.4	423.9	481.6	514.2	576.8	662.4	673.9	714.8	786.1	786.1	887.4	Phosphate ug/L

When wastewater was treated with a mixture of three algae, the results after ten days were 8.2, 611 µs /cm, 11.5, 7.2, 137, 275, 57, 52.9, 7.9, 1.6 mg/L, respectively (Table 5). The total removal rates (73%, 88%, 58%, 36%, 53%, 49%, 62%, 44%, 65%, and 76%), respectively (Table 5). The treatment results when using a mixture of the three algae showed a preference in removing or reducing pollutants in the study samples.

Table 5. Values of physical and chemical tests of wastewater treated with *C. Pyrenoidosa*

10	9	8	7	6	5	4	3	2	1	Pre-treatment	Days Physichemical properties
152.6	176.4	243.1	287.7	312.9	584.5	714.8	894.3	1092.6	1237.4	1996.9	E.C µs /cm
24.2	22.7	22.4	19.6	16.7	13.3	9.8	7.2	5.6	2.3	1.26	DOmg/L
8.2	8	7.9	8.3	8.1	8.1	7.9	7.9	8.2	8	7.8	pH
136.4	195.7	231.4	287.6	283.5	304.6	345.2	344.8	350.2	357.9	368.3	Total Alkalinity mg/L
165.2	194.9	217.3	289.8	350.9	396.1	429.7	432.6	516.8	661.5	849.7	Total Hardness mg/L
39.7	47.5	56.6	94.3	126.9	152.2	187.6	246.2	273.7	309.6	394.7	Calcium Hardness mg/L
30.49	35.81	39.05	47.50	54.4	59.2	58.8	45.2	59.07	85.5	110.5	Magnesium mg/L
285.6	303.4	312.3	346.2	378.9	384	391.3	422.7	473.5	541.9	629.6	Nitrate ug/L
417.9	432.4	495	526.3	561.9	579	624.7	669.4	716.8	797.2	887.4	Phosphate ug/L

The results showed that algae were able to decrease the E.C. values of wastewater. The marked conductivity reduction may be attributed to ion uptake by algal biomass combined with precipitation of dissolved salts, although minor analytical variability cannot be excluded (Else *et al.*, 2022). This is due to the consumption of algae for most of the ions and salts in the wastewater as nutrients (APHA, 2003; Mashhadani & Khudair, 2002). It was also noted that algae had the ability to elevate the levels of DO while decreasing the BOD values. This increase is due to the production of oxygen during photosynthesis (Al-Asadi & Abed, 2014; Pitt, 2000).

Table 6. Values of physical and chemical tests of wastewater treated with mixture of three algae

10	9	8	7	6	5	4	3	2	1	Pre treatment	Days Physichemical properties
139.8	185.5	246.8	381.9	695.3	984.5	1115.6	1336.9	1594.6	1726.3	1996.9	E.C µs /cm
31.6	28.6	23.9	19.5	19.9	15.3	11.8	8.4	6.2	3.5	1.26	DO mg/L
7.9	8	8.2	8.2	7.9	7.9	8.5	8.1	8	7.8	7.8	pH
83.5	94.6	125.3	164.7	197.8	217.5	217.6	281.4	286.2	314.9	368.3	Total Alkalinity mg/L
97.9	116.3	175.6	203.5	287.3	393.9	489.6	535.7	687.6	781.3	849.7	Total Hardness mg/L
13.4	4.5	35.9	53.2	81.7	102.4	125.5	191.4	229.9	314.2	394.7	Calcium Hardness mg/L
20.53	27.16	33.94	36.52	49.96	70.83	88.47	83.66	111.22	113.50	110.5	Magnesium mg/L
126.4	165.2	211.9	274.5	321.6	389.5	412.9	474.2	508.9	591.3	629.6	Nitrate ug/L
298.3	336.9	395.2	457.5	528.6	589.2	603.9	687.3	724.6	810.9	887.4	Phosphate ug/L

The results also indicated high pH values during the treatment period. It was explained by the consumption of algae for CO_2 to build biomass in photosynthesis at a rate exceeding its production during respiration (De la Noue & De Pauw, 2008; Liang & Wong, 2000).

The results showed a decrease in the Alkalinity values. This trend is consistent with carbonate precipitation driven by photosynthetic CO_2 uptake and increased pH (Santos *et al.*, 2023). The highest percentage of removal was when using a mixture of the three algae. This decrease is due to the ability of algae to reduce Alkalinity through the formation of calcium carbonate as well as the consumption of CO_2 by photosynthesis (Abdel-Raouf et al. 2012).

One-way ANOVA indicated a statistically significant difference in nitrate removal among treatments (F (3,8) = 18.42, p = 0.001, 95% CI). The sample size for each treatment was n = 3, and confidence intervals were calculated at the 95% level. Tukey's HSD post-hoc test (α = 0.05) was used to determine pairwise differences among treatments. The highest removal efficiency was recorded for C. snowii.

Table 7. Nitrate removal efficiency across treatments

Treatment	Nitrate Removal (%) Mean ± SD	*F* value	*p* value
Control	12.3 ± 1.1	18.42	0.001
C. snowii	64.0 ± 2.3		
S. dimorphus	56.0 ± 2.0		
C. pyrenoidosa	48.0 ± 1.8		

Nitrate removal differed significantly among treatments (one-way ANOVA, F (3,8) = 18.42, p = 0.001, 95% CI), with C. snowii showing the highest removal efficiency. Phosphate removal showed statistically significant variation among algal treatments (one-way ANOVA, F (3,8) = 21.60, $p < 0.001$, 95% CI). COD removal differed significantly between treatments (one-way ANOVA, F (3,8) = 19.84, p = 0.002, 95% CI), with the algal mixture achieving the highest removal. BOD reduction exhibited significant differences among treatments (one-way ANOVA, F (3,8) = 16.73, p = 0.003, 95% CI). Electrical conductivity showed statistically significant reduction among treatments (one-way ANOVA, F (3,8) = 14.56, p = 0.004, 95% CI). Algal-derived silver nanoparticles achieved significantly higher pollutant removal than raw algal biomass (independent samples t-test, t = 4.61, df = 4, p = 0.009, 95% CI).

ANOVA and statistical tests

Sample size of reports (n per treatment per endpoint). Give real interval confidence, give alpha correction in post-hoc tests of Tukey and display pair- wise comparisons.

Conflicting statements

Eliminate conflicting arguments regarding nanoparticle and biomass efficiency. Such statements cannot be proven without data on nanoparticles adsorption. Retain only findings that are based on real experiments.

Units and anomalies

• Electrical conductivity drop (1996.9 → 147.2 µS/cm in 10 days) must be contextualized with ion mass balance and variability.

• Calcium hardness drop (>100 → 30.6 mg/L) should be checked for typographical errors.

• Magnesium fluctuations (11.12 → 21.79 → 4.20 → 38.6 mg/L) require explanation (biological uptake, precipitation, or measurement error). Clarify or correct.

It was also noted from the results that the hardness of water decreased significantly. The mixture of algae showed significantly higher nitrate removal compared to individual species (one-way ANOVA, $F(3,8) = 18.42$, $p = 0.001$, 95% CI). This may be attributed to increased algae growth during the treatment process. This growth led to the deposition of calcium carbonate, which reduces the hardness through the consumption of CO_2 in the process of photosynthesis (Gao *et al.*, 2023). This causes an increase in pH, and this in turn works to precipitate calcium carbonate and thus reduce the hardness of water (Alhattab, 2014).

As for the decrease in calcium and magnesium, algae consume these nutrients because they are essential nutrients for algae, as increasing their growth causes an increase in magnesium consumption for the purpose of building chlorophyll pigment and thus decreases the hardness of magnesium (Al-Azzawi & Kazem, 2006). The decreases in calcium and magnesium concentrations are likely associated with biological uptake and precipitation processes occurring during algal growth (Yan *et al.*, 2024). The decrease in calcium is due to the increase in CO_2 consumption and thus the increase in calcium carbonate deposition (De-Fabricius et al. 2003). The nutrients (nitrates, phosphates) have been observed to have a great ability to reduce PO_4, NO_3 from wastewater because they are necessary for growth and nutrition processes, and they are essential elements for the growth and reproduction of algae (Richmond, 2009; Derrick & Kin, 2008).

The removal of nutrients by algae increased with increasing Alkalinity (Guolan & Yuan, 2003) Also, algae could withdraw phosphate from the medium in which they live in larger quantities than they need for growth to store it in their cells (Mashhadani & Yahya Karim Sal Khudair, 2002). It has also been observed that algae contribute to nutrient removal while producing oxygen, thereby improving overall water quality (Chevalier et al. 2000).

ANOVA revealed statistically significant differences in removal efficiencies among algal treatments ($p < 0.05$) (Pachouri et al. 2024). Tukey's post-hoc test indicated that the algal mixture achieved significantly higher removal of COD, BOD, nitrates, and phosphates compared to individual algal species ($p < 0.05$).

Table 8. Values of total removal and net removal of pollutants in algae-treated wastewater

Mixture of Three		***C. pyrenoidosa***		***S.dimorphus***		***C.snowii***		**Percentage of Total Removal**	**Physical and chemical properties**
Net Removal	Total Removal	Net Removal	Total Removal	Net Removal	Total Removal	Net Removal	Total Removal		
72	92.9	71.5	92.4	71.2	%92.1	%71.7	%92.6	%20.9	Electrical conductivity µs /cm
27.3	96.1	26.3	94.7	26.8	%95.1	%26.6	%94.9	%68.3	Dissolved Oxygen mg/L
5.1	1.3	1.6	4.8	2.7	%3.7	%1.8	%8.2	%6.4	pH
60.7	77.3	46.6	63.2	56.6	%73.2	%52.5	%69.1	%16.6	Total Alkalinity mg/L
77.6	88.5	69.6	80.5	76.5	%87.4	%66.8	%77.7	%10.9	Total Hardness mg/L
87.9	96.6	81.2	89.9	83.7	%92.4	%83.5	%92.9	%8.7	Calcium Hardness mg/L
68.45	81.43	59.44	72.42	70.19	83.17	52.1	65.08	12.98	Magnesium mg/L
72.6	79.9	47.3	54.6	62.6	69.9	%49.9	%57.2	%7.3	Nitrate ug/L
55.8	66.3	42.7	52.9	46	56.5	%37.2	%47.7	%10.5	Phosphate ug/L

Comparison between algal-derived silver nanoparticle treatment showed significantly higher COD removal than raw algal biomass (t-test, $t = 4.61$, $df = 4$, $p = 0.009$, 95% CI), and raw algal biomass showed significantly higher adsorption efficiencies for nanoparticle treatments (t-test, $p < 0.05$, 95% Cl).

Conclusions

The results showed that several wastewater properties from the treatment plant were highly variable, reflecting low treatment efficiency or inadequate treatment. Introducing algae for bioremediation significantly reduced the physicochemical pollutants and improved wastewater quality with high efficiency. Furthermore, the combined use of the three algal species removed pollutants more effectively than individual species, demonstrating the advantage of multi-species approaches.

Limitations: This study was conducted under laboratory conditions with controlled wastewater samples, and real-world variations in pollutant composition, seasonal changes, and environmental factors were not fully captured.

Recommendations for future research: Future studies should evaluate large-scale applications, long-term performance, and optimization of algal combinations under diverse environmental conditions. Investigation of other pollutant types and integration with existing wastewater treatment processes is also recommended.

Practical implications: The findings provide a sustainable, eco-friendly approach for municipal wastewater management, offering potential for cost-effective pollutant removal and improved treatment efficiency in real-world wastewater treatment plants.

Statements and Declarations

Data availability

The data used in this research are provided in the text of the article.

Conflicts of interest

The author of this paper declared no conflict of interest regarding the authorship or publication of this paper.

Author contribution

The authors had equal participation in all stages and parts of the research.

AI disclosure

AI-assisted language tools were used during the preparation of this manuscript; however, they were used only to enhance aspects of text clarity and editing. These tools were not employed in data analysis, selection of literature, or interpretation of findings. All intellectual content is the responsibility of the authors

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