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Comparing the proficiency of *Chlamydomonas reinhardtii* and *Anabaena* sp. in phycoremediation of contaminated wastewater

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Abstract: **Water pollution is emerging problem of this era mainly due to anthropogenic activities including pollutants released by industrial dumps, sewage leaks, oil spills, heavy metals, animal wastes, chemical wastes, eroded sediments, deforestation, littering, fertilizers, herbicides, pesticides, etc. Microalgae such as *Chlamydomonas reinhardtii* and *Anabaena* sp. can absorb and detoxify various heavy metals and organic pollutants, making it a versatile agent for cleaning contaminated water. Its ability to grow rapidly and thrive in wastewater environments further enhances its suitability for large-scale application. Various parameters such pH, color, odor, EC, TDS, COD, BOD, Nitrate and Heavy metal concentrations of wastewater collected from hospital and nearby industries of Shillong area were analyzed before and after their growth with microalgal isolates. A significant reduction in pH, EC values, TDS, COD and BOD were observed. Nitrate levels of collected wastewaters showed significant differences when compared with control and treatment conditions. The precise quantification of heavy metals (Pb, As, Ni, Fe) using ICP-OES, helped to understand the pollution levels and potential environmental risks associated with the wastewater samples analyzed.**

Index Terms: **Wastewater, phycoremediation, microalgae, heavy metals, pollution.**

I. INTRODUCTION

The escalating global population and industrialization have led to a surge in water demand, placing immense pressure on our limited freshwater resources (Singh et al., 2021). Rivers, groundwater, and lakes, the primary sources of water for agriculture, industry, and human consumption, are increasingly threatened by pollution (Khan et al., 2020). Industrial effluents, agricultural runoff, domestic sewage, and other anthropogenic activities have significantly degraded water quality and quantity (Rani & Nandhini, 2022). To ensure the availability of potable water, effective wastewater treatment is imperative.

Polluted water, laden with unsafe substances, renders it unfit for consumption, domestic use, and other purposes (WHO, 2021). It originates from various sources such as industries, agriculture, and urban areas, can adversely impact aquatic ecosystems and human health. The improper disposal of these effluents into water bodies can lead to the contamination of groundwater and surface water, endangering aquatic life and compromising water quality. Several studies have demonstrated the potential of microalgae-based technologies for treating specific industrial wastewater, including those generated by the chemical fertilizer industry, animal agriculture, and palm oil production. By harnessing the ability of microalgae to assimilate nutrients and pollutants, this approach offers a sustainable and eco-friendly solution for wastewater treatment Rathore et al., 2023).

Bacteria play a crucial role in the biodegradation of organic pollutants and nitrogen cycling within wastewater treatment processes. For instance, in activated sludge systems, bacterial communities break down organic matter, generating biomass and producing treated effluent suitable for discharge or reuse (Metcalf & Eddy, 2022). However, bacterial treatment systems can be limited by factors such as lower nutrient uptake rates compared to algae (Li et al., 2019), specific growth requirements, and susceptibility to inhibition by toxic compounds or high pollutant concentrations. The optimal selection of algae, bacteria, or a combination thereof depends on the specific characteristics of the wastewater and the desired treatment objectives. Key factors to consider include treatment efficiency, cost-effectiveness, and environmental sustainability (Bharagava et al., 2020).

Bioremediation, a sustainable approach, leverages biological agents like bacteria, algae, and fungi to mitigate heavy metal pollution. Among these, algae have demonstrated exceptional potential for heavy metal removal, with studies

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reporting remediation rates ranging from 15.3% to 84.6% (Chugh et al., 2022). Algae offer several advantages over traditional methods: high metal uptake capacity, regenerative ability, year-round applicability, non-toxic byproducts, and cost-effectiveness (Patel et al., 2021). A wide range of heavy metals, including chromium (Cr), zinc (Zn), lead (Pb), copper (Cu), iron (Fe), cadmium (Cd), nickel (Ni), arsenic (As), and mercury (Hg), are released into the environment by industries such as paint and dye manufacturing, textiles, pharmaceuticals, paper, and fine chemicals. Additionally, phenolic compounds are major pollutants in industrial wastewater. Algal growth is influenced by nutrients like nitrogen, phosphorus, and metals. While nitrogen and phosphorus are essential for algal growth, excessive nutrient input can lead to algal blooms. Species like *Chlorella vulgaris* and *Spirulina platensis* are well-suited for wastewater treatment due to their ability to thrive in nutrient-rich environments. In phycoremediation, microalgae detoxify arsenic by converting it from an inorganic to an organic form. This transformation often involves chelators or functional groups like phosphate and nitrate. Various algal species, including Chlorophytes (e.g., *Chlorella salina*, *Ostreococcus tauri*, *Dunaliella salina*, *Dunaliella tertiolecta*) and Heterokontophytes (e.g., *Heterosigma akashiwo*, *Phaeodactylum tricornutum*, *S. costatum*, *Thalassiosira pseudonana*), have been shown to reduce arsenic levels.

Physicochemical parameters are measurable physical and chemical properties that influence the behavior and interactions of a substance or system. In the context of wastewater treatment, these parameters include pH, temperature, dissolved oxygen, turbidity, conductivity, total suspended solids (TSS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), and nutrient concentrations (e.g., nitrogen and phosphorus). pH measures the acidity or alkalinity of the water, influencing microbial activity and chemical reaction rates (Yu et al., 2022). Temperature affects the rate of biological and chemical processes. Dissolved oxygen is crucial for aerobic biological processes. Turbidity indicates the presence of suspended particles, which can interfere with light penetration and affect algal growth. Conductivity reflects the ionic strength of the wastewater, which can influence the performance of certain treatment processes. TSS quantifies the number of suspended solids, which can contribute to water pollution and hinder light penetration. BOD and COD are indicators of organic pollution, representing the amount of oxygen required to biologically and chemically oxidize organic matter, respectively. Nutrient concentrations (e.g., nitrogen and phosphorus) are important for assessing the potential for eutrophication (Wurtsbaugh et al., 2019).

To evaluate the effectiveness and efficiency of algal-based bioremediation, it is essential to assess the physicochemical properties of wastewater before and after treatment. By analyzing parameters such as pH, temperature, dissolved oxygen, turbidity, conductivity, TSS, BOD, COD, and nutrient concentrations, researchers can establish a baseline understanding of the wastewater's initial condition and pollution levels. This baseline information is invaluable for selecting appropriate algal species and optimizing treatment conditions to maximize the remediation process.

II. MATERIALS AND METHODS

A. Microalgae samples and culture condition

Microalgal samples were obtained from the Environment Biotechnology Laboratory of the Biotechnology department of North-eastern Hill University. Microscopic examination confirmed the health and morphology of the cells.

The microalgae were initially cultured in a 500 mL conical flask containing BG-11 culture medium. A 1 L batch of the culture medium was prepared using distilled water. The pH was adjusted to 7.4 to optimize the growth conditions for the chosen strains of *C. reinhardtii* and *Anabaena* sp. The culture was incubated under continuous light conditions at a temperature of 25 ± 2°C. It was manually shaken twice daily to ensure uniform distribution of nutrients and light exposure. After the initial culture period, the culture volume was expanded by transferring it into a new 2 L conical flask and adding further BG-11 medium to reach a total volume of 1L. The culture's growth progress was monitored daily over a period of approximately 2-3 weeks.

B. Wastewater sample collection

Wastewater samples were randomly collected in four distinct places throughout Guwahati and Shillong. The AA wastewater samples were taken from Noonmati, Guwahati; BB from Panikhati, Guwahati; CC wastewater near the NEHU campus in Shillong; and DD wastewater from NEHU. The effluent wastewater samples were filtered through a 0.22-µm pore size membrane. The wastewater samples were kept in white-colored plastic bottles to prevent light penetration which may help in the growth of algae. The wastewater samples were labelled with AA, BB, CC and DD afterwards.

C. Wastewater and algal mixture preparations

The filtrated wastewater samples and cultivated microalgae were mixed in certain amounts for each kind of wastewater. The wastewater samples were diluted with microalgae into different level labelled as 75% (100 ml of microalgae and 300 ml of wastewater) (Bhuyar et al.,2021). The proportions of wastewater were 75% for industrial effluent wastewaters, respectively mixed with microalgae. The total mixture of microalgae and wastewater were 400 ml as measured by using measuring cylinder. The cultures were shaken by hand twice a day. The results were examined every five days for 15 days. All treatments were in a biological incubator and the cultural conditions were the same as the pre-culture conditions. The nutrient removal percentage was calculated according to the following equation:

$$\%\ Nutrient\ removal\ efficiency = (C0 - CF) - C0 * 100$$

where, C0 and CF stand for the initial concentration at the beginning of the experiment (day 0) and final concentration at the end of the experiment (day 15), respectively.

D. Physico-chemical parameters

Monitoring physicochemical parameters is crucial in the bioremediation process of wastewater to ensure optimal conditions for microbial activity and pollutant removal efficiency. Parameters such as pH, temperature, dissolved oxygen, and nutrient concentrations directly influence the metabolic functions and survival of microorganisms involved in bioremediation

1) Determination of pH

The pH meter needs to be adjusted using buffer solutions that span the anticipated pH range to measure pH. Before testing, make sure the electrode of the meter is dry and clean. Stir the wastewater sample to ensure uniformity, then immerse the electrode into the sample without touching the container's sides. Allow the reading to stabilize, typically within seconds to a minute, and record the pH displayed on the meter.

2) Determination of Total Dissolved Solids (TDS)

For the measurement of TDS, the wastewater sample was first filtered using a Whatman filter paper to remove any suspended solids. After preparation, a calibrated TDS meter is used. The meter is rinsed sequentially with distilled water and the sample itself to ensure accurate readings. Subsequently, the TDS meter probe is immersed into the sample, and the reading is recorded once it stabilizes. This process ensures precise measurement of dissolved solids in the wastewater sample.

3) Determination of Electrical Conductivity

To test electrical conductivity (EC) with a combined conductivity-TDS meter such as the model 308, a well-mixed sample of wastewater was prepared, free of air bubbles and at a constant temperature. The meter was turned on, EC mode was selected, and the probe was gently inserted into the sample without touching the container's sides or bottom. After stabilizing the reading, the EC value displayed on the meter screen in µS/cm or mS/cm, depending on the unit, was recorded.

4) Determination of Chemical Oxygen Demand (COD)

In separate conical flasks, 50 mL of wastewater and 50 mL of distilled water were first collected to calculate the Chemical Oxygen Demand (COD) of a water sample. Each flask was then filled with 5 mL of potassium dichromate solution, and they were immersed in a boiling water bath for an hour. Following the heating time, the flasks were allowed to cool before each one was filled with 10 mL of 2M sulfuric acid solution and 5 mL of potassium iodide solution. Sodium thiosulfate was added to the solutions until a light straw color developed. At this point, each flask received two drops of starch solution, and titration was carried out until the blue color disappeared. COD was determined using the formula:

$$\mathrm{COD} = 8 \times \mathrm{C}(\mathrm{A} - \mathrm{B})/\mathrm{S}$$

where 8 represents the equivalent weight of oxygen, A is the volume of titrant used for the blank, B is the volume of titrant used for the sample, C is the concentration of the titrant, and S is the volume of the water sample used.

5) Determination of dissolved oxygen and Biological Oxygen Demand (BOD)

To assess the Biochemical Oxygen Demand (BOD) of water samples, a 250 mL bottle had been filled with water until it overflowed, capturing a representative sample. The reaction started by adding 2 mL of manganese sulfate and alkali iodide solutions. The precipitation was then dissolved by adding 2 mL of sulfuric acid. Then 50 mL of the prepared solution was transferred to a conical flask and titrated with sodium thiosulfate until pale yellow, indicating the initial Dissolved Oxygen (DO) level (DO1). A starch solution was then added, and the titration was repeated until the blue color faded, indicating the endpoint of the day 1 DO measurement. For the calculation of BOD on day 5, water samples were collected and stored in BOD bottles without aeration, kept in darkness. On the fifth day, the DO measurement procedure was repeated for these samples to determine DO5. The BOD was then calculated as BOD = DO1 - DO5, providing insights into the oxygen demand of the organic matter in the water sample over time. This experiment helped assess the pollution level and potential environmental impact of the sample.

6) Estimation of Nitrate in wastewater

For estimation of nitrate concentration in wastewater, 50 mL filtered sample of potassium nitrate (KNO3) was put in a conical flask. To remove chloride interference, an equal volume of silver sulfate solution was added, resulting in the precipitation of silver chloride following slight heating. After filtering off the precipitate, the filtrate was evaporated in a porcelain basin until dry. After cooling, the residue was dissolved in 2 mL of phenol disulphonic acid solution. The solution was then diluted to a final volume of 50 ml. 6 mL of liquid ammonia was added to produce the nitrophenolate complex's characteristic yellow color. Absorbance was then measured at 410 nm with a spectrophotometer.

7) Determination of concentrations of Heavy Metals in wastewater samples

Heavy metals in wastewater were determined using Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES). A 50 mL sample was filtered through Whatman filter paper to remove any suspended particles. The filtrate was transferred to a clean 100 mL beaker, and 4 mL of concentrated nitric acid (HNO3) was added to stabilize the metals and avoid precipitation. Two milliliters of concentrated hydrochloric acid (HCl) were added and carefully mixed. To prevent contamination and loss of volatile chemicals, the acidified sample was cooked on a hot plate at 95°C to 100°C while protected by a suspended watch glass. Digestion continued until the volume was lowered to 5 to 10 mL, ensuring that organic content was broken down and all metals were completely dissolved. Digestion continued until the volume reduced to approximately 5 to 10 ml, ensuring organic matter breakdown and complete metal dissolution. After digestion, the contents were transferred to a 50 ml volumetric flask and diluted with distilled water to reach a final volume of 50 ml. Thorough rinsing of the beaker with distilled water ensured all contents were transferred. This method effectively prepared the wastewater sample for metal analysis, ensuring accurate determination of metal concentrations through subsequent analytical techniques. After sample preparation, the analysis of the wastewater samples was conducted.

III. RESULTS AND DISCUSSION

The cyanobacterial species *Anabaena* sp. and the microalgal species *Chlamydomonas reinhardtii* (Figure 1) used in the study were sourced from the Environmental Biotechnology Laboratory at North-Eastern Hill University (NEHU), Shillong, Meghalaya. These microorganisms were cultured in BG-11 medium and maintained at room temperature (25°C). Subsequently, various physico-chemical parameters of wastewater samples—designated as AA (Noonmati, Guwahati), BB (Panikhaiti, Guwahati), CC (near NEHU Campus, Shillong), and DD (Chemistry Laboratory, NEHU Campus)—were analyzed. The parameters assessed included color, odor, pH, electrical conductivity (EC), total dissolved solids (TDS), nitrate levels, biological oxygen demand (BOD), and chemical oxygen demand (COD), both before and after remediation.

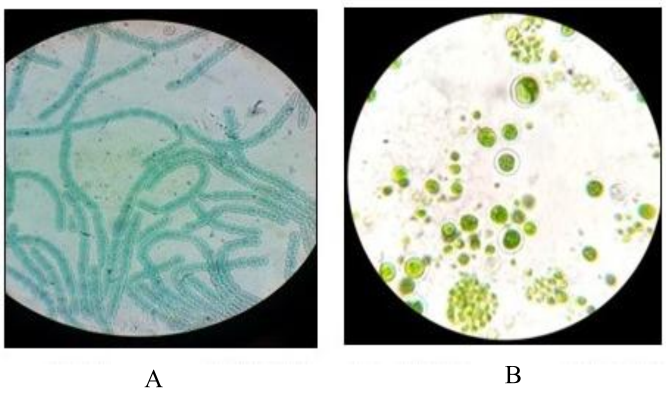


Fig. 1. (A) *Anabaena* sp. (B) *Chlamydomonas reinhardtii*

The remediation process led to significant changes in the color and odor of the wastewater samples. Initially, AA wastewater had a black coloration, but after treatment with *Anabaena* sp. and *Chlamydomonas reinhardtii*, it became translucent. These microorganisms can absorb or transform the chemicals present in wastewater, greatly improving its appearance. BB wastewater, which was initially translucent, became transparent after undergoing the same treatment. Similarly, CC wastewater, which appeared yellow before treatment, turned clear after being treated with these microorganisms. Lastly, DD wastewater, which had a yellowish hue before treatment, became transparent following the application of *Anabaena* sp. and *C. reinhardtii*. The untreated wastewater samples exhibited distinct odors. AA wastewater had an oily rag-like smell, BB wastewater was odorless, CC wastewater emitted a fishy odor, and DD wastewater had a pungent smell. After treatment, however, both *C. reinhardtii* and *Anabaena* sp. proved effective in eliminating unpleasant odors from the wastewater. The biological processes of *C. reinhardtii* and *Anabaena sp.* likely break down or absorb the organic matter and nutrients responsible for the fishy smell, resulting in an odorless effluent.

Table 1. pH levels of wastewater samples before and after remediation with *Anabaena* and *Chlamydomonas reinhardtii*

Wastewater samples	**pH (Before treatment)**	**pH (After treatment with *Chlamydomonas reinhardtii*)**	**pH (After treatment with *Anabaena*)**
AA	7.1	6.5	6.8
BB	8	7.5	7.5
CC	7.48	6.8	7.3
DD	6.5	6.3	6.1

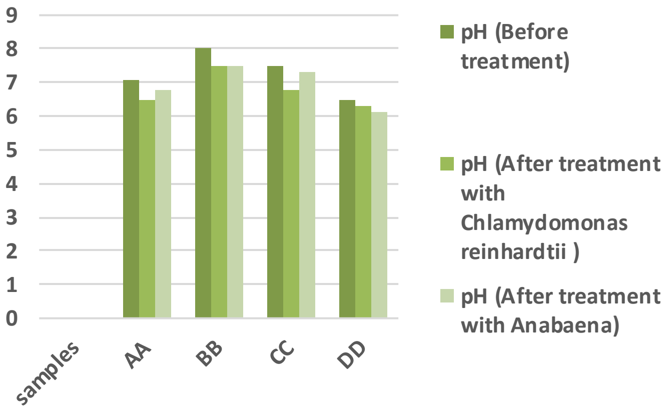


Fig. 2. Variation of pH Levels in wastewater samples before and after treatment with *Chlamydomonas reinhardtii* and *Anabaena* sp.

The pH levels of wastewater samples exhibited notable changes following treatment with *Anabaena* sp. and *C. reinhardtii* (Table 1, Figure 2). For AA wastewater, the initial pH of 7.1 decreased to 6.8 after treatment with *Anabaena* sp., and further to 6.5 when treated with *C. reinhardtii*, suggesting that *C. reinhardtii* had a stronger impact on reducing pH. Similarly, the pH of BB wastewater, initially at 8.0, slightly decreased to 7.5 following treatment with both microorganisms. In the case of CC wastewater, which had an untreated pH of 7.48, *C. reinhardtii* caused a more significant pH reduction compared to *Anabaena* sp. For DD wastewater, with an initial pH of 6.5, *Anabaena* sp. lowers pH more than *C. reinhardtii*. These observations align with a study by Tatarova et al., (2021) where *C. reinhardtii* and *Chlorella vulgaris* treatments reduced pH from 7.0 to 6.5 and 6.6, respectively. This reduction is attributed to the microorganism ability to metabolize organic matter and produce organic acids. The effect of *C. reinhardtii* on pH is moderated by its photosynthetic uptake of CO2, which offsets the production of organic acids, leading to a more balanced pH shift. Overall, the results indicated that *C. reinhardtii* generally induces a more pronounced reduction in pH compared to *Anabaena* sp., highlighting its potential effectiveness in wastewater treatment applications focused on pH adjustment

Table 2. pH levels of wastewater samples before and after remediation with *Anabaena* and *Chlamydomonas reinhardtii*

Wastewater samples	**EC value (Before treatment) (µS/cm)**	**EC value (After treatment with *C. reinhardtii*) (µS/cm)**	**EC value (After treatment with *Anabaena*) (µS/cm)**
AA	270.6	149.9	110.1
BB	340.7	258.3	298.6
CC	342.8	260.7	243.9
DD	362.9	272.6	306.9

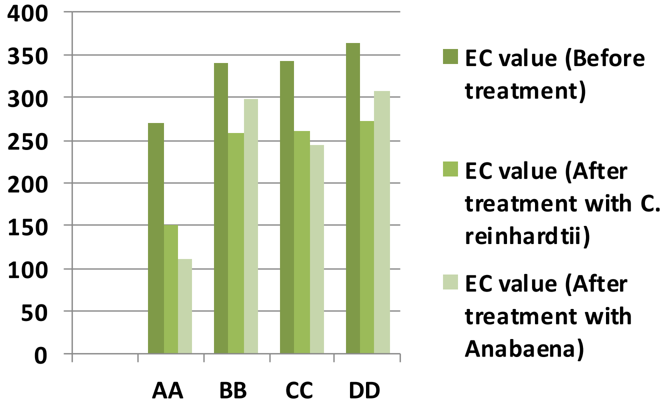


Fig. 3. Variation of pH Levels in wastewater samples before and after treatment with *Chlamydomonas reinhardtii* and *Anabaena* sp.

The electrical conductivity (EC) values of wastewater samples decreased consistently after treatment with both *Chlamydomonas reinhardtii* and *Anabaena* sp., highlighting a reduction in ionic content (Table 2, Figure 3). For AA wastewater, the EC value dropped from 270.6 µS/cm to 149.9 µS/cm with *C. reinhardtii* treatment and further to 110.1 µS/cm with *Anabaena*. Similarly, BB wastewater showed an initial EC of 340.7 µS/cm, which decreased to 258.3 µS/cm with *C. reinhardtii* and 298.6 µS/cm with *Anabaena* sp. Fisheries lake wastewater had a starting EC of 342.8 µS/cm, which decreased to 243.9 µS/cm and 260.7 µS/cm with *C. reinhardtii* and *Anabaena* sp., respectively. For DD wastewater, the untreated EC of 362.9 µS/cm dropped to 272.6 µS/cm with *C. reinhardtii* and 306.9 µS/cm with *Anabaena*. Overall, *C. reinhardtii* was generally more effective at reducing EC values, except in BB and DD wastewater, where *Anabaena* sp. showed slightly better performance. These findings are consistent with a study by La Bella et al., (2023), which reported EC reductions to 5.35 µS/cm and 3.41 µS/cm after treating urban wastewater with *Chlorella vulgaris*. This suggests that while microorganisms effectively remove dissolved ions, their efficiency varies depending on the type of wastewater, likely due to differences in nutrient composition and specific metabolic mechanisms.

Table 3. TDS levels before and after remediation with *Anabaena* and *Chlamydomonas reinhardtii*

Waste water samples	**TDS (Before treatment) (ppm)**	**TDS (After treatment with *Chlamydomonas reinhardtii*) (ppm)**	**TDS (After treatment with *Anabaena*) (ppm)**
AA	567.3	138.9	180.2
BB	176.8	146.7	153.7
CC	177.1	138.1	124.8
DD	186.2	134.9	157.4

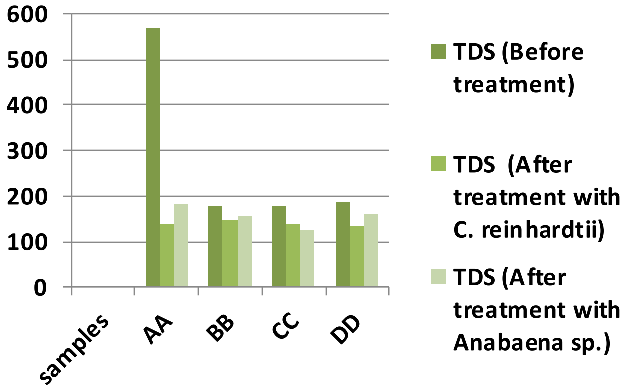


Fig. 4. Variation of TDS Levels in wastewater samples before and after treatment with *Chlamydomonas reinhardtii* and *Anabaena* sp.

The total dissolved solids (TDS) levels in wastewater significantly decreased (Table 3, Figure 4) after treatment with *Chlamydomonas reinhardtii* and *Anabaena* sp., as shown in Table 3. In AA wastewater, TDS dropped from 567.3 ppm to 138.9 ppm with *C. reinhardtii* and to 180.2 ppm with *Anabaena* sp., showing *C. reinhardtii* was more effective. For BB wastewater, TDS decreased from 176.8 ppm to 146.7 ppm with *C. reinhardtii* and 153.7 ppm with *Anabaena* sp., likely due to differences in wastewater composition. In CC wastewater, *Anabaena* performed better, reducing TDS to 124.8 ppm compared to 138.1 ppm with *C. reinhardtii*, reflecting its adaptability to nutrient-rich environments. For DD wastewater, *C. reinhardtii* was more effective, reducing TDS to 134.9 ppm compared to 157.4 ppm with *Anabaena* sp. The variation in TDS reduction efficiency between the two algae depends on the composition of the wastewater and the specific metabolic pathways of the algae. *C. reinhardtii* is generally more effective in chemically contaminated environments, while *Anabaena* performs better in nutrient-rich waters like CC wastewater (Flouty & Estephane, 2012). These findings highlight the complementary roles of the two algae in wastewater treatment.

Table 4. Nitrate levels before and after remediation with *Anabaena* and *Chlamydomonas reinhardtii*

Waste water samples	**Nitrate (Before treatment) (mg/L)**	**Nitrate (After treatment with *Chlamydomonas reinhardtii)* (mg/L)**	**Nitrate (After treatment with *Anabaena)* (mg/L)**
AA	2	1.5	1.53
BB	2.4	1.44	1.51
CC	1.9	1	1.38
DD	2.5	1.56	1.45

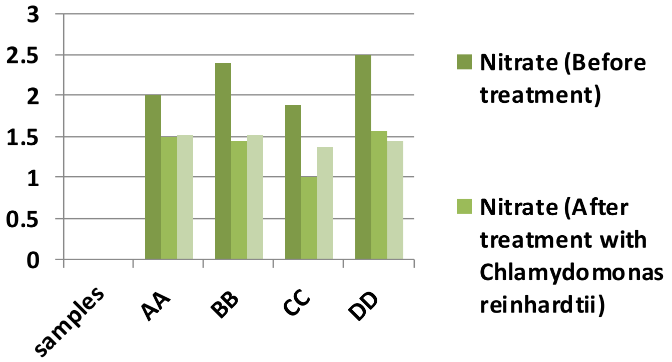


Fig. 5. Variation of nitrate levels in wastewater samples before and after treatment with *Chlamydomonas reinhardtii* and *Anabaena* sp.

Nitrate levels showed significant reductions before and after remediation with *Chlamydomonas reinhardtii* and *Anabaena* sp. (Table 4, Figure 5). In AA wastewater, nitrate concentrations decreased from 2 mg/L to 1.5 mg/L with *C. reinhardtii* and to 1.53 mg/L with *Anabaena* sp., showing both algae effectively utilized nitrate as a nutrient source. BB wastewater's nitrate levels dropped from 2.4 mg/L to 1.44 mg/L with *C. reinhardtii* and to 1.51 mg/L with *Anabaena* sp., demonstrating the efficiency of both algae in nitrate absorption and metabolism. In CC wastewater, nitrate reduced significantly from 1.9 mg/L to 1 mg/L with *C. reinhardtii* and to 1.38 mg/L with *Anabaena* sp. This suggests that *Anabaena* sp. was highly effective in assimilating nitrate, likely due to its competitive uptake abilities. Similarly, DD wastewater showed a reduction from 2.5 mg/L to 1.56 mg/L with *C. reinhardtii* and to 1.45 mg/L with *Anabaena* sp., indicating favorable conditions for nitrate utilization by both algae. These findings align with a study by Kothari et al., (2012), which demonstrated the ability of microalgae like *Chlorella pyrenoidosa* to reduce nitrogen levels in wastewater. The reduction in nitrate concentrations highlights the potential of *C. reinhardtii* and *Anabaena* sp. for effective wastewater treatment by utilizing nitrate as a nutrient source, thereby improving water quality.

Table 5. BOD levels before and after remediation with *Anabaena* and *Chlamydomonas reinhardtii*

Waste water samples	**BOD (Before Treatment) (mg/L)**	**BOD (After treatment with *Chlamydomonas reinhardtii) (mg/L)***	**BOD (After treatment with *Anabaena) (mg/L)***
AA	0.76	0.36	0.56
BB	1.88	0.4	0.32
CC	1	0.32	1
DD	0.88	0.72	0.72

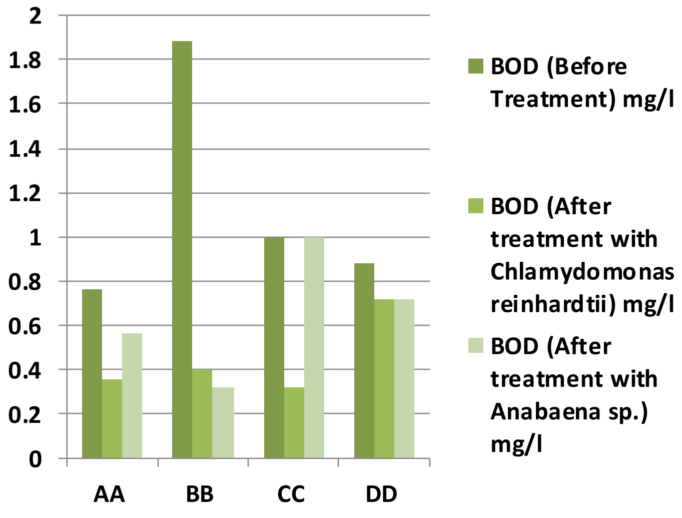


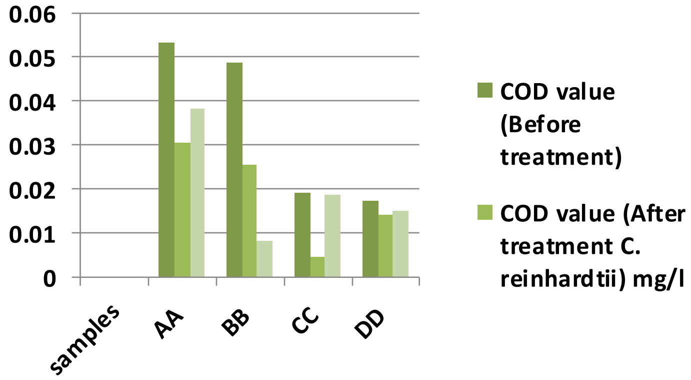
Fig. 6. Variation of BOD levels in wastewater samples before and after treatment with *Chlamydomonas reinhardtii* and *Anabaena* sp.

The analysis of dissolved oxygen (DO) and Biochemical Oxygen Demand (BOD) levels in wastewater samples highlights the effectiveness of *Chlamydomonas reinhardtii* and *Anabaena* sp. in treating organic pollutants (Figure 6). Before incubation, DO levels in AA, BB, CC, and DD wastewater were 7.12 mg/L, 4.44 mg/L, 4.3 mg/L, and 7.04 mg/L, respectively. After 5 days, these values decreased to 6.36 mg/L (AA), 2.56 mg/L (BB), 3.4 mg/L (CC), and 6.16 mg/L (DD). Initial BOD levels were 0.76 mg/L (AA), 1.88 mg/L (BB), 1.0 mg/L (CC), and 0.88 mg/L (DD) (Table 5). Treatment results showed varying BOD reductions across wastewater types. In AA wastewater, *Anabaena* sp. reduced BOD to 0.56 mg/L, while *C. reinhardtii* achieved a further reduction to 0.36 mg/L, demonstrating its superior pollutant removal efficiency. BB wastewater, with an initial BOD of 1.88 mg/L, showed reductions to 0.4 mg/L and 0.32 mg/L following treatment with *C. reinhardtii* and *Anabaena* sp., respectively, highlighting their efficiency in handling higher organic loads. For CC wastewater, *C. reinhardtii* significantly reduced BOD from 1 mg/L to 0.32 mg/L, while *Anabaena* sp. had no effect on the BOD. In DD wastewater, *C. reinhardtii* and *Anabaena* sp. both reduced BOD modestly to 0.72 mg/L, showing their consistent performance in low-pollutant scenarios. DO reduction patterns further illustrate the metabolic activity of the algae. The most substantial DO decrease with *C. reinhardtii* occurred in DD wastewater, where levels dropped from 2.36 mg/L to 1.64 mg/L. Conversely, *Anabaena* sp. caused the largest DO reduction in AA wastewater, from 2.28 mg/L to 1.72 mg/L. These findings align with studies by Ahmad et al., (2018), Maizatul et al., (2017), and Kothari et al., (2012), indicating that the effectiveness of algae in reducing BOD is influenced by the type and concentration of organic pollutants. Simpler organic compounds and nutrient availability enhance degradation efficiency, as seen in the high BOD reductions for BB and CC wastewater. Overall, *C. reinhardtii* demonstrates strong potential for reducing BOD across diverse wastewater types, while *Anabaena* sp. is particularly effective in environments with simpler organic pollutants.

Table 6. COD levels before and after remediation with *Anabaena* and *Chlamvdomonas reinhardtii*

Waste water samples	COD value (Before treatment) (mg/L)	COD value (After treatment *C. reinhardtii*) (mg/L)	COD value (After treatment *Anabaena*) (mg/L)
AA	0.0534	0.0304	0.0384
BB	0.04864	0.0256	0.00816
CC	0.0192	0.0048	0.0187
DD	0.0176	0.0144	0.015

Fig. 7. Variation of COD levels in wastewater samples before and after treatment with *Chlamydomonas reinhardtii* and *Anabaena* sp.



Chlamydomonas reinhardtii and *Anabaena* sp. were both effective in reducing the Chemical Oxygen Demand (COD) across different wastewater types (Figure 7), indicating their ability to break down and metabolize organic pollutants. In AA wastewater, the COD decreased from 0.0534 mg/L to 0.0304 mg/L after treatment with *C. reinhardtii* and to 0.0384 mg/L after treatment with *Anabaena* sp., showing a significant reduction with *C. reinhardtii*. In BB wastewater, *C. reinhardtii* reduced COD from 0.04864 mg/L to 0.0256 mg/L, and *Anabaena* sp. achieved an even greater reduction, lowering it to 0.00816 mg/L (Table 6). This demonstrates *Anabaena*'s superior ability to lower organic pollutants in BB wastewater. In CC wastewater, the COD was initially 0.0192 mg/L, and after treatment, it decreased to 0.0048 mg/L with *C. reinhardtii* and only slightly reduced to 0.0187 mg/L with *Anabaena* sp., highlighting *C. reinhardtii*'s greater efficiency in reducing COD in this type of wastewater. For DD wastewater, the COD was 0.0176 mg/L before treatment, decreased to 0.0144 mg/L with *C. reinhardtii*, but increased to

0.150 mg/L with *Anabaena* sp., suggesting a less favorable response to *Anabaena* sp. in this case Overall, these results show that both algae have the capacity to lower COD levels, with *C. reinhardtii* generally being more effective in reducing organic pollutants, while *Anabaena* sp. performs particularly well in certain wastewater types, such as BB. The variations in COD reduction across different wastewaters may be influenced by the composition of the effluents, suggesting that the algae's metabolic pathways are more suited to specific types of organic compounds. These findings are in line with previous study by Maizatul et al., (2017), which also reported the potential of algae in reducing organic contamination in wastewater.

Varying removal efficiencies were observed for BOD, TDS, COD, and nitrate concentrations across different wastewater types treated with *Chlamydomonas reinhardtii* and *Anabaena* sp. In terms of TDS, *C. reinhardtii* demonstrated higher removal efficiencies, especially in AA wastewater (75.5%) compared to *Anabaena* sp. (68.2%). For COD, *Anabaena* sp. showed superior efficiency in BB wastewater (83.2%), while *C. reinhardtii* was more effective in CC wastewater (75.0%). BOD removal was most significant in BB wastewater, with *C. reinhardtii* reducing it by 78.7%, while *Anabaena* sp. achieved 83.0% removal. Regarding nitrate reduction, *C. reinhardtii* had the highest efficiency in CC wastewater (47.4%), whereas *Anabaena* sp. was more efficient in DD wastewater (42.0%). Overall, *C. reinhardtii* generally outperformed *Anabaena* sp. in most wastewater types, particularly in reducing BOD and TDS, whereas *Anabaena* sp. excelled in COD reduction in BB wastewater. These results demonstrate the potential of both algae for wastewater treatment, with *C. reinhardtii* being particularly effective in reducing organic pollutants and *Anabaena* sp. excelling in nitrate removal in certain wastewater types.

The study for physico-chemical parameters before and after remediation was followed by the determination of the efficacy of *Chlamydomonas reinhardtii* in remediation of heavy metals (Pb, As, Ni, Fe) from the collected wastewater samples as *C. reinhardtii* displayed promising removal efficiencies across all the four parameters of BOD, TDS, COD, and nitrate concentrations (Table 7).

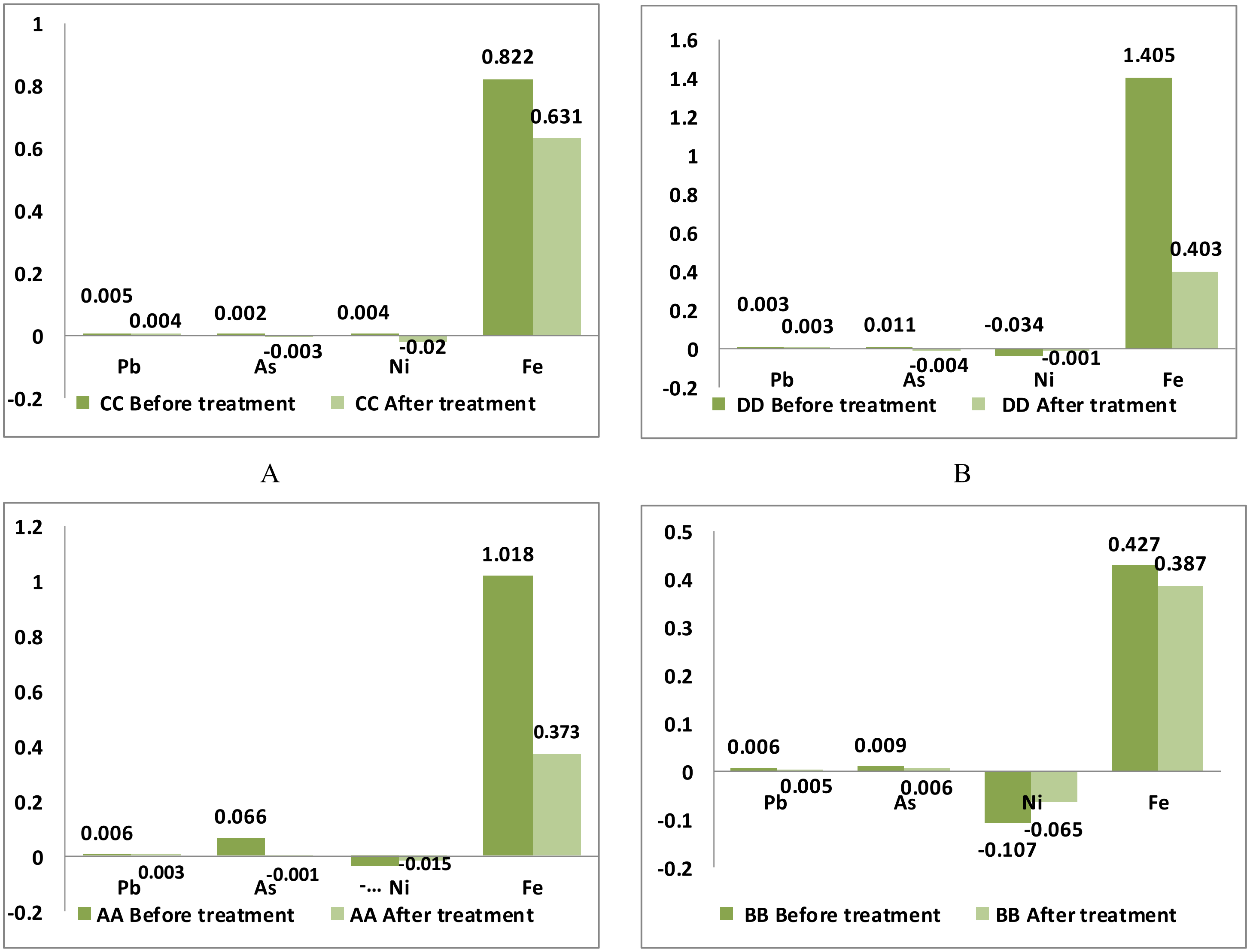
The treatment of wastewater from various sources resulted in significant reductions of heavy metals, including lead (Pb), arsenic (As), nickel (Ni), and iron (Fe). In the oil refinery effluent, lead decreased from 0.006 mg/L to 0.003 mg/L, arsenic from 0.066 mg/L to -0.001 mg/L, and iron from 1.018 mg/L to 0.373 mg/L. Nickel, showed a slight decrease from -0.034 mg/L to -0.015 mg/L. In BB wastewater, treatment with *Chlamydomonas reinhardtii* reduced lead from 0.006 mg/L to 0.005 mg/L, arsenic from 0.009 mg/L to 0.006 mg/L, nickel from -0.107 mg/L to -0.065 mg/L, and iron from 0.427 mg/L to 0.387 mg/L. Similar reductions were observed in CC wastewater, where lead decreased from 0.005 mg/L to 0.004 mg/L, arsenic from 0.002 mg/L to -0.003 mg/L, nickel from 0.004 mg/L to -0.02 mg/L, and iron from 0.822 mg/L to 0.631 mg/L. In DD wastewater, lead remained unchanged at 0.003 mg/L, arsenic decreased from 0.011 mg/L to -0.004 (interpreted as 0.004 mg/L), nickel from 0.034 mg/L to 0.001 mg/L, and iron significantly decreased from 1.405 mg/L to 0.403 mg/L (Table 8, Figure 8). These results indicate that the treatment process, involving biological agents like *C. reinhardtii*, where concentrations reduced below the method detection limit in some cases. So, the alga effectively reduced the concentrations of these heavy metals across different wastewater samples, improving the water quality.

Table 7. Removal efficiencies of TDS, COD, BOD and nitrate concentrations by *Anabaena* and *Chlamydomonas reinhardtii*

Parameter	**Wastewater type**	***Chlamydomonas reinhardtii* removal efficiency (%)**	***Anabaena* sp. removal efficiency (%)**
TDS	AA	75.5	68.2
	BB	17	13.1
	CC	22	29.6
	DD	27.6	15.6
COD	AA	43	28.1
	BB	47.3	83.2
	CC	75	2.3
	DD	18.2	14.2
BOD	AA	52.6	26.3
	BB	78.7	83
	CC	68	0
	DD	18.2	18.2
Nitrate	AA	25	23.5
	BB	40	37.9
	CC	47.4	27.4
	DD	37.6	42

Table 8. Heavy metal concentrations of wastewater samples before after treatment with *Chlamydomonas reinhardtii*

Waste water samples	**Lead (Pb)**		**Arsenic (As)**		**Nickel (Ni)**		**Iron (Fe)**	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
AA	0.006	0.003	0.066	-0.001	-0.034	-0.015	1.018	0.373
BB	0.006	0.005	0.009	0.006	-0.107	-0.065	0.427	0.387
CC	0.005	0.004	0.002	-0.003	0.004	-0.02	0.822	0.631
DD	0.003	0.003	0.011	-0.004	-0.034	-0.001	1.405	0.403



A

B

C

D

Fig. 7. (A-D) Variation of Heavy Metal concentrations before and after treatment with *Chlamydomonas reinhartii*

The analysis of heavy metal removal efficiency across various wastewater samples reveals considerable variability in treatment effectiveness (Table 8). In AA wastewater, *Chlamydomonas reinhardtii* was most effective in removing arsenic, with a removal efficiency of approximately 98.5%, followed by iron at 63.3% (Zhou et al., 2022). Lead and nickel saw moderate reductions, with efficiencies of 50% and 55.9%, respectively, suggesting that these metals were reduced, but need further improvements in their treatment methods (Rahman et al., 2021). In BB wastewater, nickel removal was the most efficient at 39.25%, while arsenic showed a lower removal efficiency of 33.33%. Lead and iron had much lower removal efficiencies, at 16.67% and 9.37%, respectively, indicating that the treatment was less effective for these metals (Liu et al., 2023). For CC wastewater, arsenic removal was again highly effective at 95%, while nickel also showed strong removal at 90% (Martínez-Ruiz et al., 2022). However, lead and iron exhibited significantly lower removal efficiencies of 20% and 23.2%, suggesting moderate success in reducing these contaminants (Wang et al., 2021). In DD wastewater, arsenic removal was again outstanding at 98.4%, with nickel following closely at 97.1% (Alves et al., 2024). Iron showed a 71.3% reduction, indicating a decent removal, but not as effectively as arsenic or nickel. Lead, however, showed no change, suggesting that the treatment method was ineffective for lead removal in this case.

Overall, the results demonstrate that *C. reinhardtii* is highly effective in removing arsenic across all wastewater types, but less effective in removing lead, particularly in DD wastewater. Iron and nickel removal efficiencies varied, with these wastewater samples showing more successful treatment than others.

IV. CONCLUSION

The study highlights the effective use of *Chlamydomonas reinhardtii* and *Anabaena* sp. for wastewater remediation, showcasing their abilities to significantly improve water quality by addressing key physico-chemical parameters. Both species demonstrated effectiveness in reducing color, odor, pH, electrical conductivity (EC), total dissolved solids (TDS), nitrates, biochemical oxygen demand (BOD), and chemical oxygen demand (COD) across various wastewater types. *Chlamydomonas reinhardtii* outperformed *Anabaena* sp. in reducing TDS, BOD, and COD, while *Anabaena* sp. showed greater efficiency in nitrate reduction under specific conditions. Heavy metal analysis further confirmed the potential of *Chlamydomonas reinhardtii*, especially for removing arsenic and nickel, achieving removal efficiencies of up to 98.5% and 97.1%, respectively. However, its efficacy varied for other metals like lead and iron, suggesting that treatment optimization is needed for broader applicability. Overall, the findings emphasize the potential of these algae as cost-effective and sustainable solutions for wastewater treatment, with *Chlamydomonas reinhardtii* showing potential for broad-spectrum remediation.

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