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COMPARATIVE STUDY OF PHYCOREMEDIATION THROUGH MICRO ALGA FOR REFINERY WASTEWATER TREATMENT

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Abstract

The use of microorganisms like microalgae in bioremediation and phycoremediation are essential methods for treating wastewater. The effectiveness of Scenedesmus sp., Tribonema sp., and Lyngbya sp. in spontaneously refining refinery wastewater was evaluated in this study. The American Public Health Association (APHA) methods were used to carefully analyze parameters like pH, Chemical Oxygen Demand (COD, Total Soluble Solids (TSS), Oil and Grease (O&G), Biological Oxygen Demand (BOD), Phenol, Sulfur (S), Chlorine (Cl), Total Dissolved Solid (TDS) and Sulphates (SO4). Over the course of three weeks in the spring and fall, field testing was done. Outcomes showed that all three types performed admirably, with Scenedesmus sp. standing out in particular in the 3rd week's TSS (6.87), pH (7.26), COD (9.71), BOD (4.36), O&G (0.37), S2 (0.01), Phenol (0.02), TDS (154) and Cl (155) (ppm) tests. Remarkably, TDS dropped to its bottom point of 106 ppm within the first week. In conclusion, this study highlights how these native plants might improve wastewater quality, especially before it is released into the environment or used for irrigation. These results imply that the microalgae have potential applications in wastewater treatment, both alone and in combination.

Key words: Phycoremediation, Micro Alga, Refinery wastewater treatment.

Introduction

Water is one of the most important natural resources for life on Earth, and its quality is one of the most important environmental issues. Water availability and quality have a significant impact on human habitat selection, which is closely linked to an individual's overall quality of life. Freshwater scarcity is a severe problem in arid and semi-arid countries due to the depletion of freshwater supplies brought on by increasing industrialization and population growth (Gani *et al*., 2015; Al-Hussieny *et al*., 2020).

Current wastewater treatment techniques, which are associated with high energy use, come at a high cost. This technique leads several activities in increasing nation-states to relief natural wastewater into freshwater bodies due to the pathogenic content, which has detrimental effects on animal habitats and human health. Long-term exposure, especially from drinking and irrigation, can cause gastrointestinal disorders, cancer, and birth defects in children, among other problems (Singh & Mishra, 1987). Until now, most study projects have concentrated on wastewater from industries including paper mills, tanneries, automobiles, and textiles, leaving refinery wastewater relatively unstudied. The complexity of refinery wastewater must be addressed in light of the rising global demand for energy and the concomitant expansion of refineries.

Despite being essential to modern life, the petroleum industry unintentionally becomes a source of pollution, dumping heavy metals into freshwater environments. Petroleum refineries produce large amounts of wastewater that are heavily contaminated since they are essential to transformation of hydrocarbon derivatives and simple lubricant into lucrative intermediates and final goods (Hodges *et al*., 2017). Due to the high concentration of biodegradable organic materials in this effluent, fish mortality and foul conditions result from oxygen deprivation in water bodies. At the same time, harmful effects are caused by nutrients and heavy metals such as lead, nickel, cadmium, copper, chromium, nitrogen, potassium, and ammonium. Furthermore, inorganic materials like bases and acids (like hydrogen sulfide) upset the balance of the environment, causing corrosion and altering the circumstances that are ideal for water life and animals (Xiong *et al*., 2019). Particularly, the hydrocarbons in simple lubricant and the inherent immiscibility of water make wastewater management more difficult.

A number of on-site methods, including both primary and secondary treatment stages, are used in wastewater treatment protocols. Wastewater has historically been treated using new mud techniques, gas introduction, membrane filtration, hydro cyclones, electrochemical coagulation, water balance ponds, evaporation ponds and water filtration unit (Mohamed *et al*., 2018; Hodges *et al*., 2017). Following the above procedures, natural therapies in postponed development formations are naturally applied. Nevertheless, above mentioned methods are frequently beset by functioning difficulties, like low food-to-microorganism ratios leading to poor sludge settling, the formation of extracellular polymers including lipids, proteins, and carbohydrates that obstruct settling, and biological inhibition caused by toxic chemicals. These techniques are expensive and need expert personnel, especially when it comes to managing sedimentation basins. On the other hand, new wastewater techniques show promise in addressing toxicity issues (Hodges *et al*., 2017).

In the midst of an increasingly industrialized and urbanized future, conventional wastewater treatment techniques like Phycoremediation and Bioremediation become vitally important. Phycoremediation/ Bioremediation (Mustafa and Hayder, 2021) is a wastewater treatment technique that uses microorganisms, most commonly microalgae. Over fifty years have passed since the concept of Phycoremediation was first recognized (Oswald & Gotaas, 1957; Ahmad *et al*., 2013). It has shown to have a wide range of applications, such as the removal of nutrients from municipal wastewater, the treatment of organically rich effluents, the removal of nutrients and xenobiotic compounds using algae-based biosorbents, the treatment of acidic conditions and wastewater, the sequestration of CO2, the transformation and degradation of xenobiotics, and the use of algae-based biosensors for the detection of toxic compounds. Notably, there is a discernible difference between conventional methods and microalgae with respect to the effectiveness of nutrient removal (Danouche *et al*., 2021; Noüe & Lavoie, 1985).

Comparing the nutrient-treatment capacities of different algae species in the watercourse of Attock Refinery Limited (ARL), located in Rawalpindi, MorgaH, Punjab, Pakistan, is the primary objective of this study. For the fall and spring, a wealth of information about the pre- and post-treatment circumstances after the Phycoremediation technique is supplied. The data will then be analyzed and compared in order to determine which specific microalgal species are most effective in improving the feature of plant wastewater through Phycoremediation.

Material and Method

Study area: The study was conducted on the grounds of Attock Refinery Limited, which is sited in Morgah, Rawalpindi. With a history dating back to 1922, ARL is notable for being one of the first companies in Pakistan to refine crude oil. The study's initial phase involved conducting a number of thorough surveys in Rawalpindi's Morgah neighborhood, with a focus on ARL. These surveys were conducted for two reasons: first, to help people get to know the area better, and second, to identify the best places to gather samples. Concurrently, a thorough evaluation of the site's accessibility was carried out, which was crucial for the later acquisition of water and microbiota samples. Seven different sampling locations were ultimately identified after a careful selection procedure; their distribution is shown in (Table 1 Map of sampling site), which emphasizes the purposeful unpredictability of their choice.

Wastewater sampling and analysis: Grab sampling procedures were employed to get wastewater samples from several points within the refinery effluent drain. The aforementioned samples were combined to generate composite images, filtered to remove bigger particles, and then divided into three identical sections for duplication. Following the guidelines set forth by the American Public Health Association (APHA, 2005), a thorough examination of a number of physio-chemical parameters was carried out, includes sulfur (S2), phenols, chlorides (Cl), sulphates (SO4), total soluble solids (TSS), Ph, chemical oxygen demand (COD), oil and grease (O&G) and biological oxygen demand (BOD). In addition, samples of raw wastewater were kept at 4 degrees Celsius until they were utilized in experiments involving the development of algae.

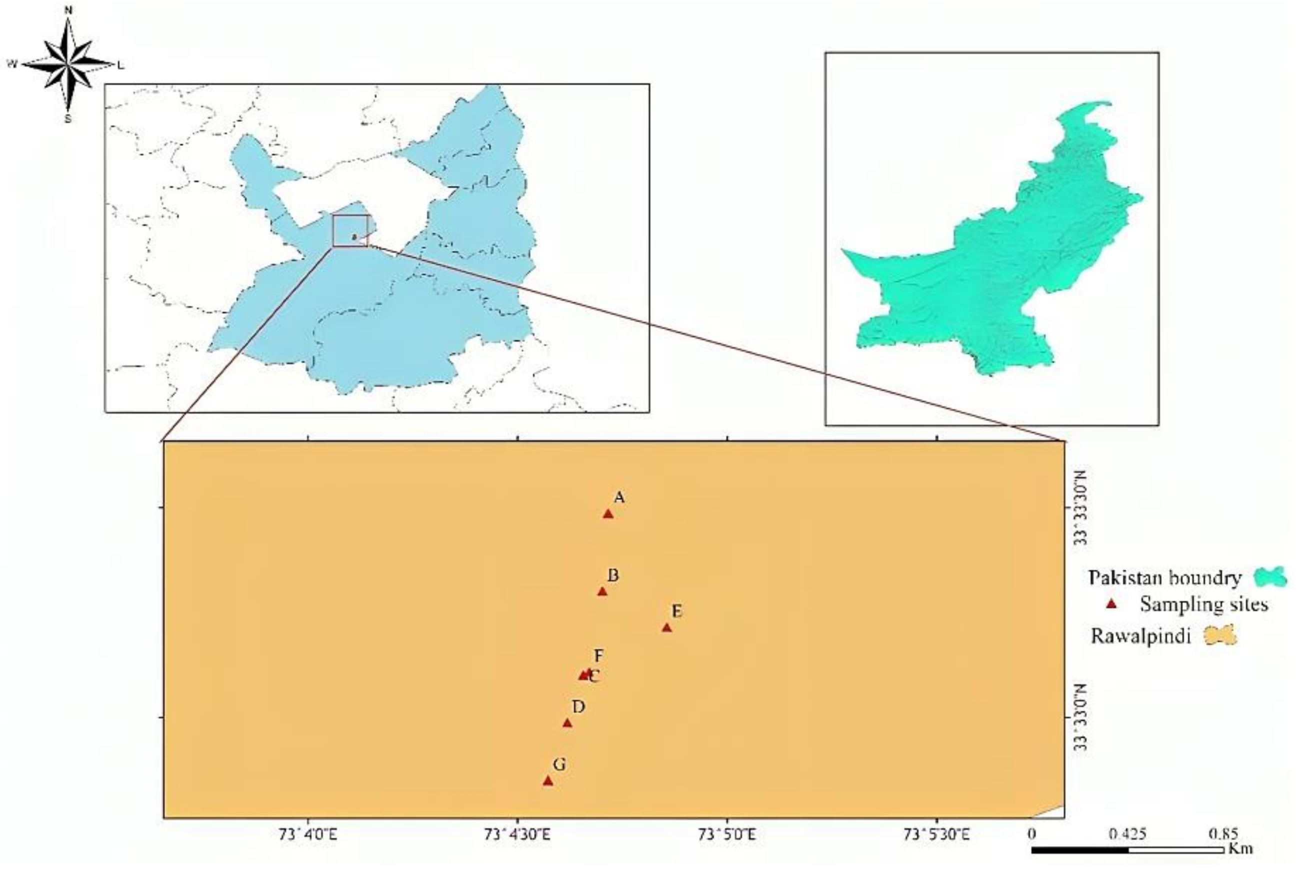
Table 1. Sampling places of different zones of Attock Refinery Limited (ARL).

No.	Sampling sites	Latitude (N)	Longitude (E)	Elevation (m)
1.	A	33°33′29″	73°4′43″	509
2.	B	33°33′18″	73°4′42″	507
3.	C	33°33′6″	73°4′39″	501
4.	D	33°32′59″	73°4′37″	510
5.	E	33°33′12″	73°4′51″	500
6.	F	33°33′7″	73°4′40″	501
7.	G	33°32′51″	73°4′34″	504

Algal Sample collection and research: Researchers meticulously collected epiphytic species, benthic and periphyton from seven separate locations with waster bodies near ARL using tools including pipettes, knives, forceps, and toothbrushes. Falcon tubes were used to preserve the specimens after that. Then, in order to replicate natural climate conditions in a controlled environment, these specimens were transferred to growth chambers using the methods drawn by Neveux *et al*., (2016), (Taylor *et al*., 2000) and Ahmad *et al*., (2013). These growth chambers were used for the isolation and cultivation of the algae species using BG-0 and BG-11 agar-based solutions. Among the eighteen species of algal identification, three were carefully chosen for the current study due to their widespread presence at most sampling sites, as outlined by Ahmad *et al*., (2013).

Method for phycoremediation: The three selected species were cultured under carefully regulated conditions in the lab: 26°C ± 2, 80% relative humidity, 10,000 lux of light, and temperature. They were then moved to the controlled greenhouse environment of the refinery to continue their propagation. After becoming acclimated, the algae species were placed into specific cells inside the apparatus for the experiment. These fiberglass chambers were made with the intention of avoiding any possible chemical interference from the nearby earth. The ideal circumstances were guaranteed by the experimental setup, which included a 1% slope to aid in water circulation and a 4 mm chamber thickness. The experiment was conducted in an ambient climate with temperatures between 25°C and 30°C and a normal timeline of 10 hours of darkness and 14 hours of light between August and April. Wastewater tanks were connected to the treatment chamber intake using PVC pipes to provide continuous wastewater flow, and treated wastewater was collected at the exit using polyethylene drain flex pipes. Natural dirty water was gravity-fed into the cells through PVC pipes and round taps at a constant Hydraulic Loading Rate of 6 gallons per day, when the acclimatization phase concluded and wastewater had entirely replaced tap water. Wastewater discharge rates were adjusted based on preliminary readings. Samples were carefully taken from every batch in sterile one-liter vials after wastewater treatment. A thorough investigation of these samples was conducted in a laboratory environment in order to evaluate the effectiveness of the Phycoremediation procedure. The analytical protocols followed the Standard Methods for the pertinent parameters, as described by Kiran *et al*., (2018) and Bansal & Steel (2018). Notably, these lab tests took place at the International Islamic University, Islamabad (IIUI) laboratory of the Department of Environmental Science, the National Cleaner Production Center (NCPC) Environmental Lab, and the Quality Control Laboratory (QCL) of ARL.

Google Map of Study Area



Statistical analysis

SPSS PASW Statistics-21 was used for the statistical analysis, which included one-way ANOVA and T-tests. Using a one-way ANOVA with a significance level of $p<0.05$ made it easier to evaluate significant differences between different months and how those differences changed throughout the course of the sample year. To identify major variances ($p<0.05$) among the extract data and water indoors particular time, t-tests were employed. The outcomes from individually of the three different arena repeats for each month were clearly shown visually using bar charts. The means derived from triple analytical measures served as the foundation for the results.

Results and Discussion

A rigorous three-week testing session was part of the field assessment in order to analyze the differences in performance based on seasonal changes and evaluate the effectiveness of the three selected species in two separate growth seasons, August 2020 (S1) and April 2021 (S2). With a significance level of $p<0.05$, robust statistical analyses were carried out utilizing the Independent T-test and (ANOVA) for thoroughly examine the entire dataset across all parameters.

Algal type's impact on the pH of wastewater from refineries: The pH data, as shown in (Fig. 1) show that over both growing seasons, all three species significantly reduced their pH values as compared to the control. The largest decrease in wastewater's mean pH happened throughout the 3rd week of S1, mainly due to Lyngbya sp, Tribonema sp., and Scenedesmus sp. (whose pH values were 7.33, 7.24, and 7.26, correspondingly). However, first week had the lowest pH reduction, which was mostly brought on by Tribonema sp. (7.72 in S1 and 7.96 in S2), Lyngbya sp. (7.53 in S1 and 7.48 in S2) and Scenedesmus sp. (7.59 in S1 and 7.76 in S2).

With the exception of Lyngbya sp. in the second week (7.39 in S1 and 7.41 in S2), a comparison analysis shows significant differences in the average pH values for every species over the course of the three weeks in both seasons. The third week of S1 was the most effective for lowering pH levels due to wastewater treatment, with Tribonema sp. performing at its peak (7.24), Scenedesmus sp. (7.26), and Lyngbya sp. (7.33). A comparative analysis revealed that Lyngbya sp. was more likely to efficiently change pH levels in the direction of near neutrality than Scenedesmus sp. and Tribonema sp.

Figure 2 shows total soluble solids (TSS) data that show significant reductions in TSS stages across S1 and S2 because of entirely three types. In stark contrast to the control (28.4 ppm), Lyngbya sp. showed the low TSS value (5.00 ppm) in the 3rd week of S1, next to Tribonema sp. (6.83 ppm) and Scenedesmus sp. (6.87 ppm). The wastewater treated with Scenedesmus sp. in S2 during the third week had the lowest mean TSS value (8.33 ppm) related to the control treatment (32.7 ppm). Tribonema sp. (8.67 ppm) and Lyngbya sp. (17.0 ppm) were next in line. Scenedesmus sp. proved to be the most productive species during both seasons,outperforming the other two in terms of TSS reduction every time.

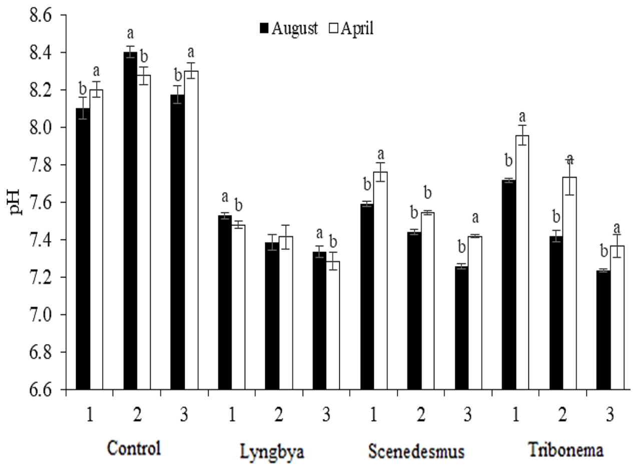


Fig. 1. Comparison of pH reduction by Algal species.

Algal species' impact on the total soluble solids (TSS) of wastewater:

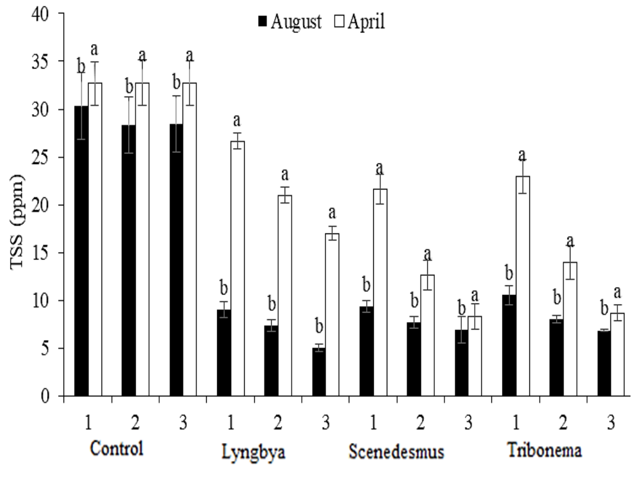


Fig. 2. Comparison of TSS reduction by Algal species.

Algal species' effects on the biological oxygen demand (BOD) of wastewater from refineries:

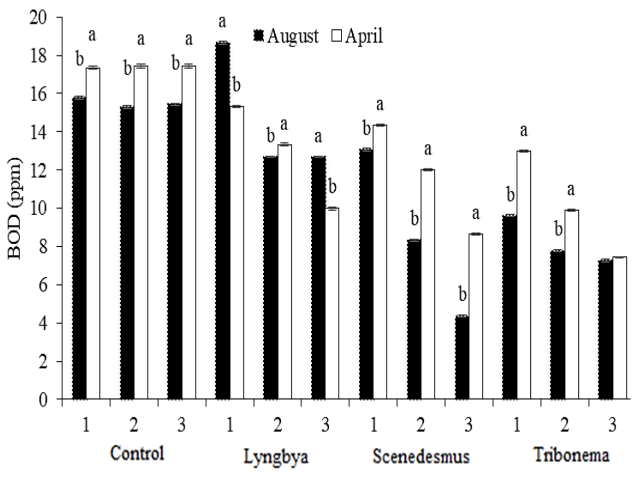


Fig. 3. Comparison of BOD reduction by Algal species.

Figure 3's biological oxygen demand (BOD) data highlights the substantial decreases in BOD stages that entirely three types contributed to during each week of S1 and S2, with the exclusion of Tribonema sp., which showed no discernible variation in week three values in either season. With the exception of Lyngbya sp., which had an unusually high value during the first week of S1 (18.67 ppm), all species notably showed a progressive drop in BOD in comparison to the control treatment. It's possible that an analysis error made by a person led to this unusual value. When comparing the mean BOD value of Scenedesmus sp. in the setting of S1 to that of the control treatment (15.44 ppm), the latter had the lowest value. The next lowest were Lyngbya sp. (12.67 ppm) and Tribonema sp. (7.27 ppm). In contrast to the control treatment (17.43 ppm), Tribonema sp. in S2 during the 3rd week had the lowest mean BOD value (7.43 ppm), next to Scenedesmus sp. (8.67 ppm) and Lyngbya sp. (10.0 ppm). A comparison study reveals that Scenedesmus sp. performed significantly better during both growing seasons.

Algal species' effects on the chemical oxygen demand (COD) of wastewater from refineries:

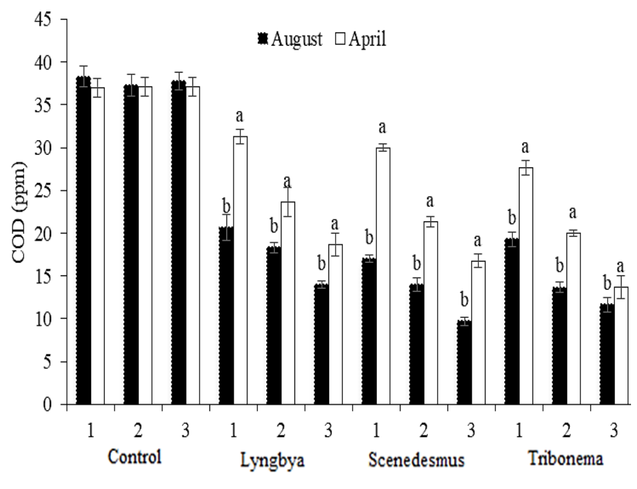


Fig. 4. Comparison of COD reduction by Algal species.

Analyzing the Chemical Oxygen Demand (COD) numbers, as illustrated in (Fig. 4) all three species significantly reduced COD levels during S1 and S2. For the control treatment, however, there was no discernible change in values between the two periods. In particular, Scenedesmus sp. produced the lowest mean COD value (9.71 ppm) in the third week of S1, closely next to Tribonema sp. (11.6 ppm) and Lyngbya sp. (14.0 ppm). These results differed considerably from the regular therapy at 37.7 ppm. In the third week, S2 water treatment with Tribonema sp. had the lowest mean COD (13.6 ppm) compared to 37.1 ppm for the control treatment. Lyngbya sp. (18.6 ppm) and Scenedesmus sp. (16.7 ppm) were the next.

Figure 5's study of the Grease & Oil (G & O) information reveals subtle trends in all three species. Interestingly, Lyngbya sp. behaved consistently, showing no appreciable changes in values over the course of the three weeks for S1 and S2. On the other hand, in both seasons, Scenedesmus sp. revealed unique values during the third week but no discernible change during the first two. In a similar vein, Tribonema sp. showed significant variations in readings during every week of two periods (0.37 ppm in S1 and 0.42 ppm in S2). When it came to treatment efficacy, wastewater treated with Tribonema sp. in the third week of S1 yielded the lowest mean O&G value (0.24 ppm), which, in comparison to the control treatment (0.61 ppm), was considerably different. Lyngbya sp. (0.36 ppm) and Scenedesmus sp. (0.37 ppm) were next. With Lyngbya sp. treatment, the lowest mean O&G value (0.37 ppm) was seen in S2, with Scenedesmus sp. (0.42 ppm) and Tribonema sp. (0.43 ppm) following closely behind. This was significantly different from the third-week control therapy (0.60 ppm). Compared to the other two species, Lyngbya sp. fared quite well in both growing seasons.

Algal species' effects on the oil and grease (O&G) in wastewater from refineries:

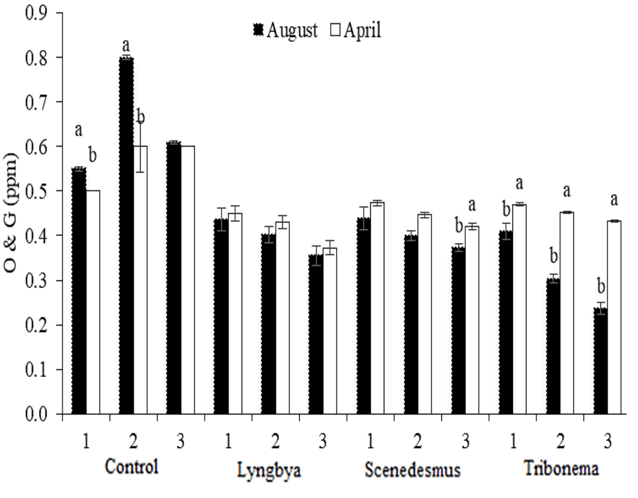


Fig. 5. Comparison of O&G reduction by Algal species.

Examination of the Phenol data, as displayed in (Fig. 6) indicates unique patterns for each of the three species. Scenedesmus sp. showed no appreciable change in values between the two seasons, whereas Lyngbya sp. and Tribonema sp. did not show a significant difference in values between S1 and S2. The species in S1 with the lowest mean Phenol values in the third week were Lyngbya sp. (0.010 ppm), Scenedesmus sp. (0.023 ppm), and Tribonema sp. (0.007 ppm). This stood in stark contrast to the control treatment's third-week results of 0.162 ppm. Notably, performance in all treatments improved in the third week compared to the first two. Likewise, the third week's effluent from Tribonema sp. in S2 showed the lowest mean. Lyngbya sp., in contrast to the other two species, typically displayed outstanding performance during both growth seasons.

According to (Fig. 7) which displays the study of Sulphate (SO_4) data, over both growing seasons, the sulphate concentrations in wastewater samples were dramatically reduced by all three species. Scenedesmus sp. was the only species whose values did not significantly change in the second or third weeksWastewater treated with Tribonema sp. in S1 post-treatment showed the lowest mean sulphate value (128 ppm), which was statistically different from the control treatment (406 ppm) in the 3rd week. Lyngbya sp. (141 ppm) and Scenedesmus sp. (162 ppm) followed. In a similar vein, after three weeks, wastewater treated with Scenedesmus sp. in S2 had the lowest result (165 ppm) when relate to the regular management (396 ppm). Lyngbya sp. (223 ppm) and Tribonema sp. (166 ppm) were next in line. Tribonema sp. was the best-performing species out of the three during both growth seasons, with S1 showing better overall performance than S2.

Figure 8's study of the Sulphur (S2) data shows significant differences in mean values for all species during both S1 and S2. In contrast, there is no discernible divergence between Scenedesmus sp. and Lyngbya sp. during the 3rd week of either season (S1 and S2). The dirty water management including Scenedesmus sp and Tribonema sp., Lyngbya sp. in S1 had the lowest mean S2 value (0.01 ppm) during the third week when compared to the control treatment (0.16 ppm). Comparably, in the 3rd week of S2, the wastewater treatments based on Lyngbya sp. and Scenedesmus sp. had the lowest values, at 0.01 ppm, next to Tribonema sp. (0.02 ppm), compared to 0.15 ppm for the control treatment. Surprisingly, compared to S2, S1 showed the greatest drop in sulfur concentration throughout the course of the three weeks across all treatments. Notably, both Lyngbya sp. and Scenedesmus sp. exhibit excellent performance during both growth seasons.

Algal species' effects on chlorides (Cl2) in wastewater from refineries: With the exclusion of Lyngbya sp., that showed no major change in the 3rd week, and Scenedesmus sp., that indicated no major change in the 2nd week, the Chlorides (Cl2) data, as shown in (Fig. 9) clearly reveal substantial differences among all species during both S1 and S2. In comparison to the regular management (365 ppm), wastewater treated with Tribonema sp. during the third week of S1 generated the lowest mean Cl2 value (99 ppm). Following were Scenedesmus sp. (258 ppm) and Lyngbya sp. (114 ppm). Likewise, in S2, Lyngbya sp. and Tribonema sp. wastewater treatments during the 3rd week showed the lowest values, at 133 ppm each, in comparison to the control (353 ppm), followed by Scenedesmus sp. (155 ppm).

Algal species' effects on the total dissolved solids (TDS) in wastewater from refineries: Figure 10's study of Total Dissolved Solid (TDS) data reveals considerable fluctuations in both S1 and S2, with the exception of Lyngbya sp. and Tribonema sp. during the third week of both seasons, when no significant differences were found. Wastewater treated with Tribonema sp. in S1 during the third week had the lowest average TDS value (568 ppm), followed by Scenedesmus sp. (1542 ppm) and Lyngbya sp. (1238 ppm), in sharp contrast to the control (1617 ppm). The dirty water cured by S2 with Tribonema sp. yielded the lowest result (557 ppm) when compared to the control's (1669 ppm), which was followed by Lyngbya sp. (1196 ppm) and Scenedesmus sp. (596 ppm).

Algal species' effects on the phenol in wastewater from refineries:

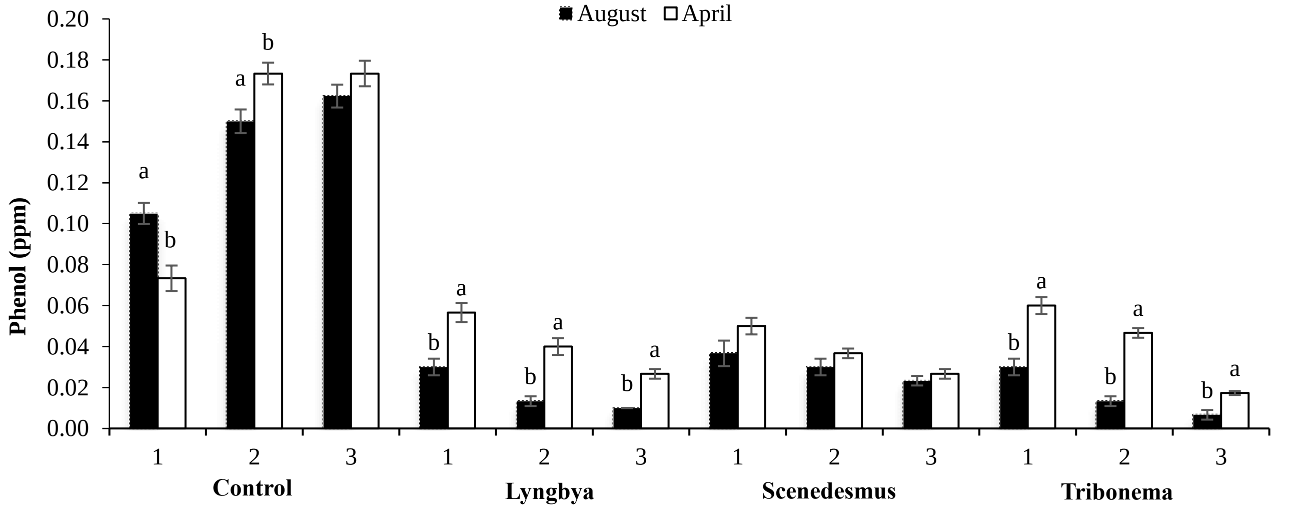


Fig. 6. Effect of algal species on phenol of refinery wastewater.

Effect of algal species on Sulphate (SO_4) of refinery wastewater:

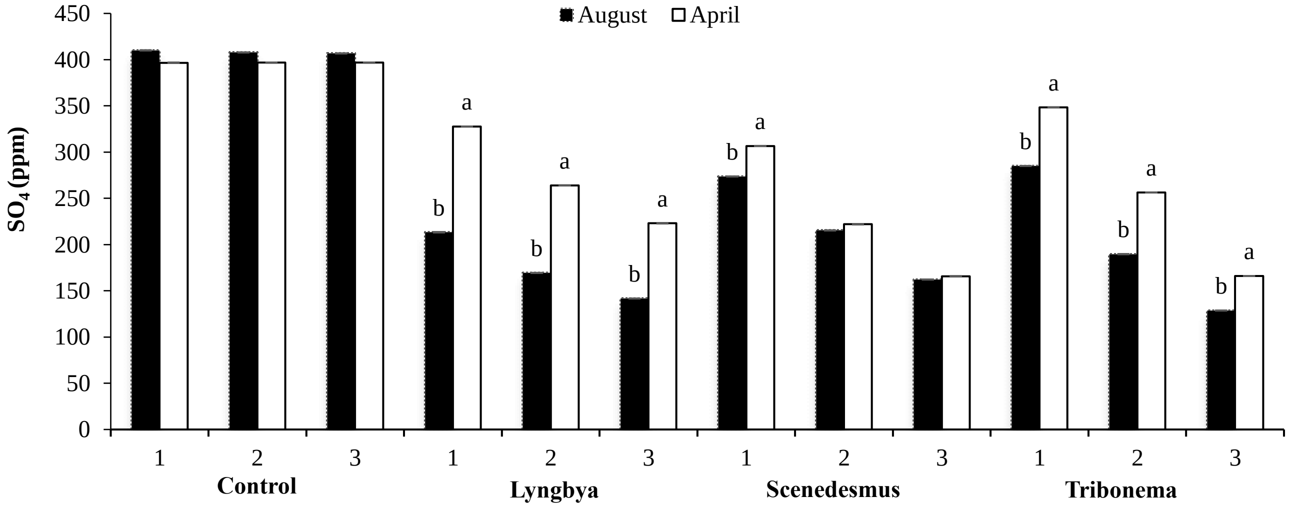


Fig. 7. Comparison of Sulphates reduction by Algal species.

Algal species' impact on the concentration of sulfur (S2) in refinery effluent:

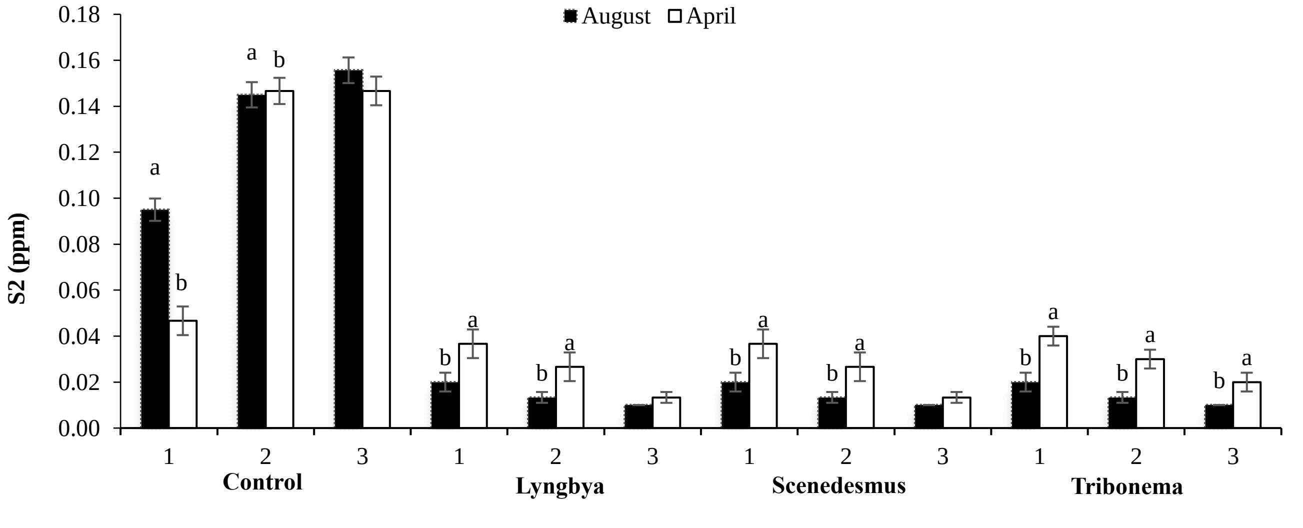


Fig. 8. Comparison of sulphur reduction by Algal species.

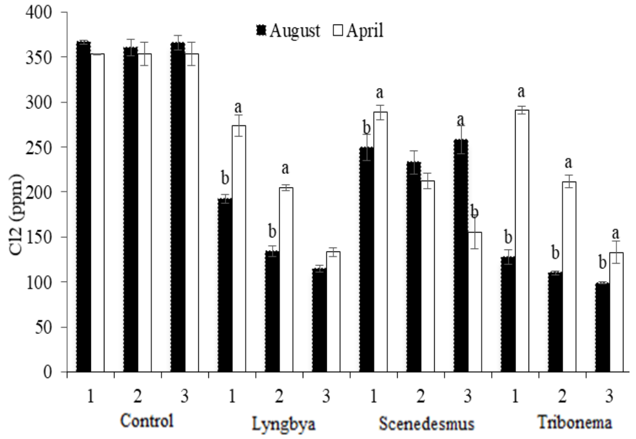


Fig. 9. Comparison of Chlorides reduction by Algal species.

Box plots and scatter graphs were used to better visualize the data and illustrate the results. Scatter plots were specifically created for the following parameters: pH (Fig. 11), Total Suspended Solids (TSS) (Fig. 12), Biological Oxygen Demand (BOD) (Fig. 13), Chemical Oxygen Demand (COD) (Fig. 14), Oil and Grease (O&G) (Fig. 15), Phenol (Fig. 16), Sulfate (SO4) (Fig. 17), Sulfide (S2) (Fig. 18), Chloride (Cl2) (Fig. 19) and Total Dissolvable solids (TDS) (Fig. 20). By providing a more thorough representation of the data distribution, this approach outperforms the traditional use of bar charts to display averages. To ensure openness and enable a comprehensive comprehension of the observed patterns, additional information has been updated with the exact data results for every parameter and sampling point.

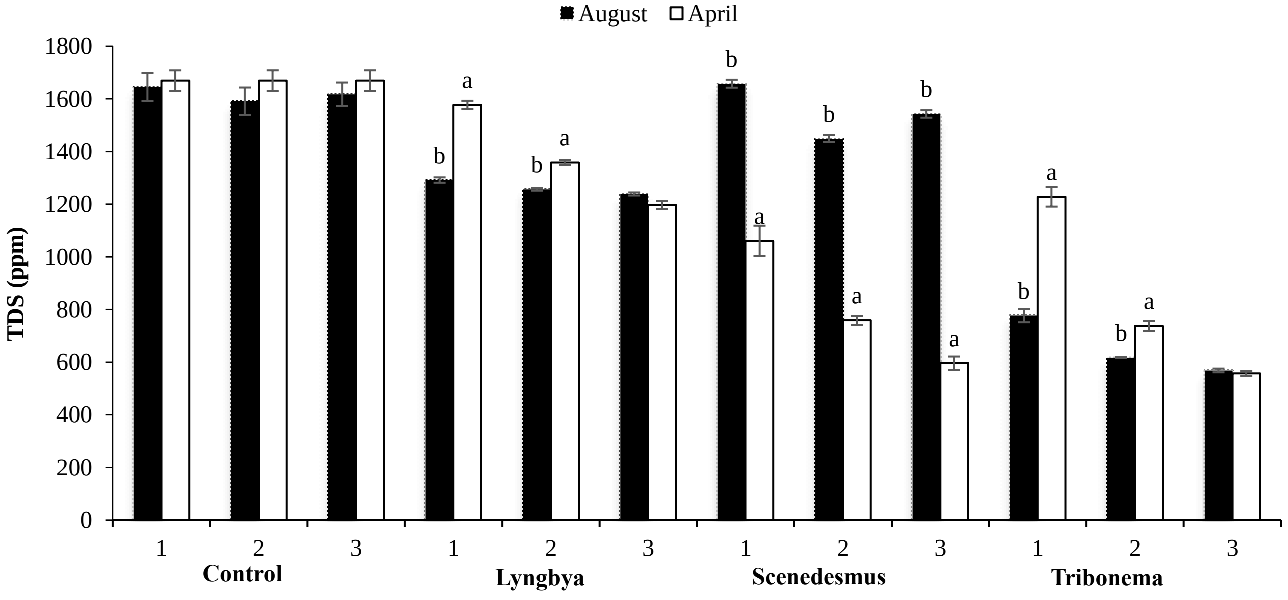


Fig. 10. Comparison of TDS reduction by Algal species.

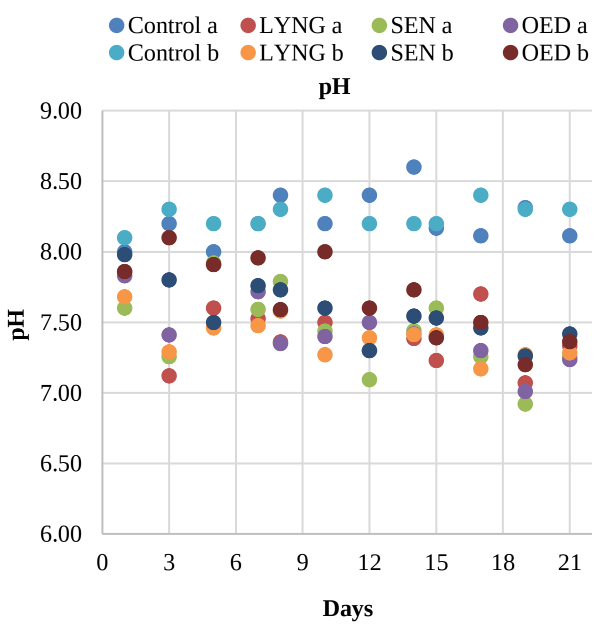


Fig. 11. Scatter plot illustrating result of pH.

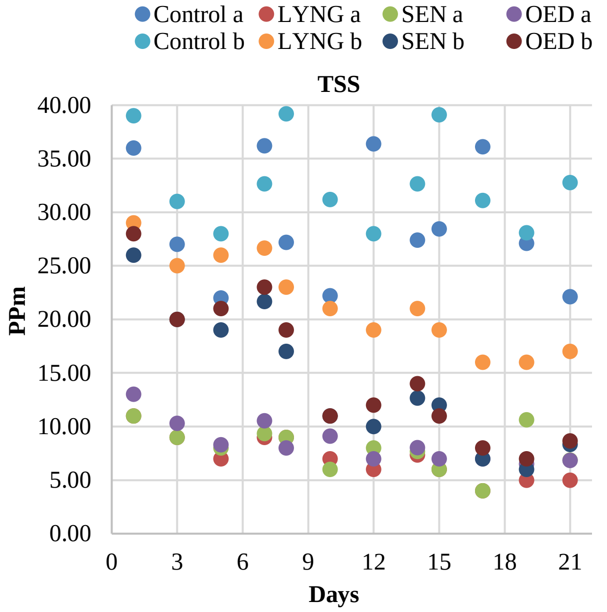
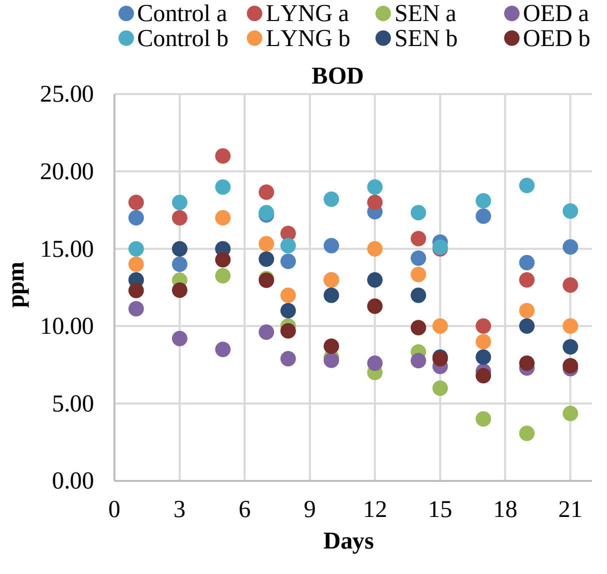


Fig. 12. Scatter plot illustrating Scatter plot illustrating result of TSS.



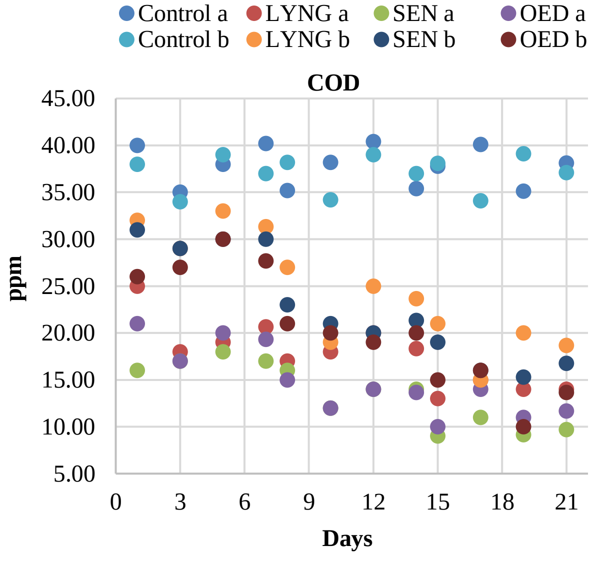
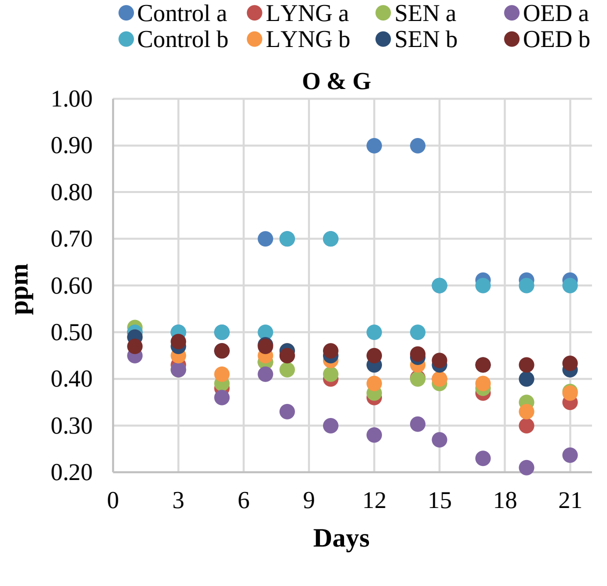


Fig. 13. Scatter plot illustrating result of BOD.

Fig. 14. Scatter plot illustrating result of COD.



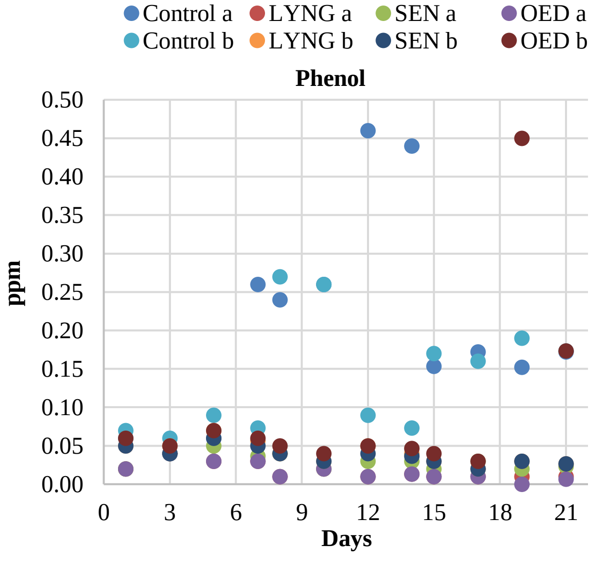


Fig. 15. Scatter plot illustrating result of O & G.

Fig. 16. Scatter plot illustrating result of phenol.

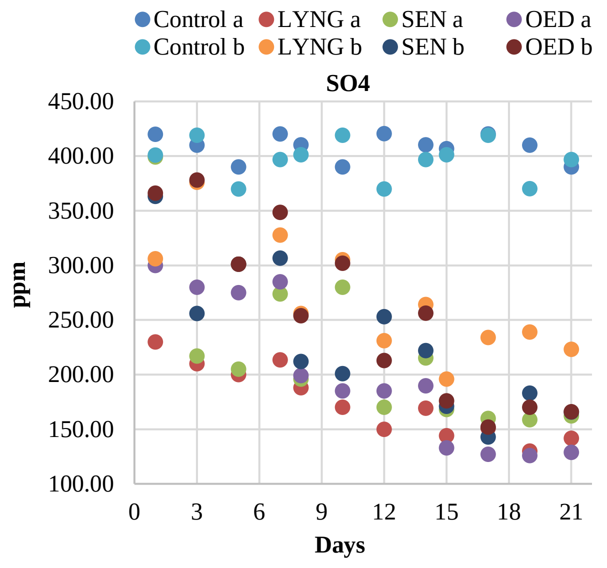


Fig. 17. Scatter plot illustrating result of SO4.

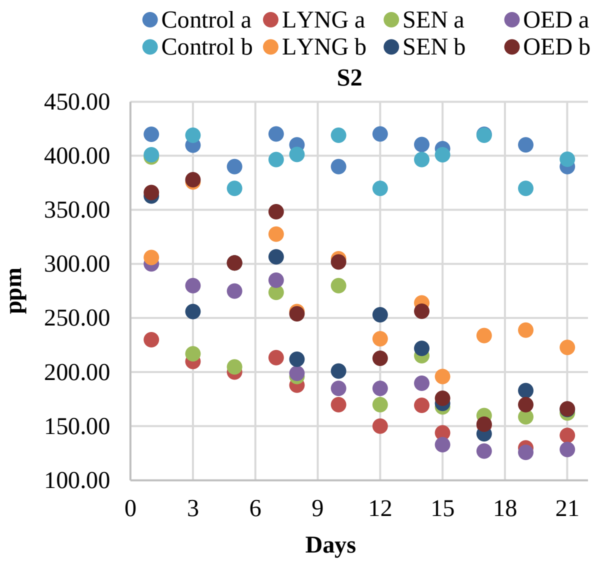


Fig. 18. Scatter plot illustrating result of S2.

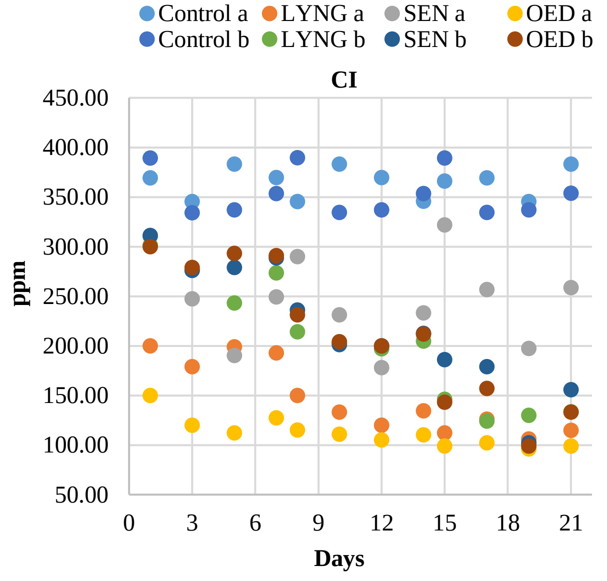


Fig. 19. Scatter plot illustrating result of CI.

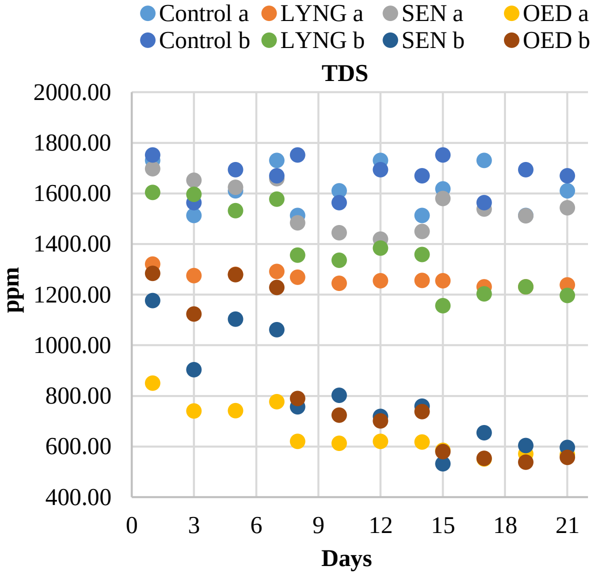


Fig. 20. Scatter plot illustrating result of TSD.

Discussion

Because biological pollution management is an environmentally sustainable strategy, it has become increasingly popular in wastewater treatment. In this context, one well-known natural remedy method for dealing with these issues is called phytocoremediation. Prominent research (Al-Hussieny *et al*., 2020 & Hodges *et al*., 2017) has demonstrated the use of algae species in the treatment of refinery effluents. The results of these investigations are strikingly similar to those of our own study, in which Lyngbya sp. was found to significantly lower the levels of phenol (0.01 ppm) and oil and grease (0.36 ppm) in refinery wastewater. The results of this investigation, which show progressive drops in COD, BOD, TDS, and TSS, highlight the beneficial function of photosynthetic bacteria in wastewater.

COD and BOD values in Scenedesmus obliquus were lower (4.36 ppm and 9.71 ppm, respectively), in line with research published by Rajasulochana *et al*., (2009). It is possible to credit the lower COD & BOD levels to the elimination of soluble organic molecules in the treatment process, which helps S. obliquus effectively degrade pollutants. Scenedesmus obliquus's vital role in phycoremediation is further demonstrated by its ability to reduce grease & oil (0.37 ppm) and phenol (0.02 ppm) while maintaining motion in dirty refinery water. Cao *et al*., (2013) provide more validation for these findings.

All treated water samples showed a decrease in pH, despite the algae species' quick development and photosynthesis. The effective reduction of ammonia and nitrate concentration in refinery wastewater is indicated by this pH decline. Water pH is lowered by biological reactions that are sparked by the uptake of ammonium and other nitrogen molecules, as reported by Ajayan *et al*., (2015); Kumar *et al*., (2012) and Ugya *et al*., (2017) (2019).

Water study showed very little nitrate-N and ammonium-N. These substances are then absorbed by algae species, which promotes biological processes that lessen their presence even more. As a result, ammonium-N and nitrate-N are found to be non-significant pollutants, allaying worries about their effects.

Particularly, higher rates of photosynthesis and algal growth result in greater carbon dioxide consumption, which is reflected in the fall in total soluble solids (TSS) and total dissolved solids (TDS). TSS and TDS in treated water samples are decreased by algal species' absorption of dissolved particles in conjunction with evaporation (Sharma and Sharma, 2013; Ugya *et al*., 2019). Significantly, the capacity of these algae species to amiably adhere to the root's aids in the reduction of their water content. (de Silva and others, 2018; Osti and others, 2018; Ugya & others, 2019).

The consumption of dissolved oxygen and carbon dioxide by BOD is demonstrated by its direct and indirect connections to biodegradable compounds. This demonstrates that activated algal processes can effectively reduce wastewater's BOD and COD levels (Kshirsagar, 2013). Similarly, other studies have confirmed that COD decreases in tandem with BOD as a result of increased photosynthetic activity and algal development (Valderrama *et al*., 2002; Colak & Kaya, 1988; Kshirsagar, 2013; Zhang *et al*., 2008;).

Lower grease and oil values indicate ability of algae species to absorb and degrade substances, which is correlated with higher algal biomass as a result of active photosynthesis. The remediation of oil and grease in refinery wastewater is indirectly improved by these phenomena (Amare *et al*., 2018; Liu *et al*., 2010 and Ugya *et al*., 2019). We note in this examination of the Greece and oil information that the Lyngbya group showed a little greater concentration of O&G than the Tribonema group during the third week of August. But our claim that Lyngbya outperformed is supported by a thorough analysis of data collected over several weeks and during both growing seasons.

Notably, Lyngbya exhibited stable behavior over the course of the three weeks in both seasons, with no discernible changes in O&G values. This consistency, which suggests dependability and predictability in O&G removal, is a crucial component in evaluating a species' effectiveness in wastewater treatment. On the other hand, even though the statistics from the third week seem inconsistent, Lyngbya has proven to be a stable and reliable solution over a long period of time, making it a great option for treating wastewater. Based on this thorough evaluation, we have come to the conclusion that Lyngbya performs admirably, with particular emphasis on its usefulness in wastewater treatment situations.

A number of research (Suyamud *et al*., 2018; Ugya *et al*., 2019; Mustapha *et al*., 2018) have reported a drop in chloride levels and phenol. These findings suggest that algal development encourages the formation of bacteria that facilitates the breakdown of phenol and phenolic compounds. Furthermore, the small quantity of chloride found in refinery wastewater can be attributed to its absorption through the growth of algal biomass.

Furthermore, the wastewater treated by algae showed notable decreases in the amount of sulfur and sulfate. Both the fast development of algae and their absorption by other algae species could be responsible for this decrease. Like chloride, these metals are needed in trace levels and can be found in very small concentrations in wastewater from refineries.

Apart from the fact that these processes are cyclical, it's important to remember that eliminating algae species might not be necessary. Rather, the system becomes a water purification plant or cycle scheme when processed water removal is added before wastewater is introduced.

Conclusion

The study demonstrates how algae can effectively reduce chemical and biological pollutants found in wastewater from refineries. Algae's potential is highlighted by their easy adaptation to wastewater conditions, which is a result of nutrient availability reflected in their rapid development. The treated wastewater was declared suitable for irrigation since all three algal species provided metrics that were fine by the National Environmental Quality Standards (Ministry of Environment, Local Government, and Rural Development, Islamabad, Pakistan, 2000). This study confirms that phycoremediation is effective in continuously reducing pollutant concentrations throughout the year to levels that are within allowable bounds.

This demonstrates that algal-based treatment is a viable, long-lasting, and ecological way to clean effluent from refineries. Scenedesmus sp. performed particularly well among the species under study, effectively cleaning up both organic and inorganic pollutants in refinery effluents. These findings support the deliberate use of algae-mediated therapy for environmentally friendly wastewater treatment in refineries.

References

Ahmad, F., A. Khan and A. Yasar. 2013. Comparative phycoremediation of sewage water by various species of algae. *Proc. Pak. Acad. Sci*., 50: 131-139.

Ajayan, K.V., M. Selvaraju, P. Unnikannan and P. Sruthi. 2015. Phycoremediation of tannery wastewater using microalgae *Scenedesmus* species. *Int. J. Phytoremediation*, 17: 907-916.

Al-Hussieny, A.A., S.G. Imran and Z.A. Jabur. 2020. The use of local blue-green algae in the bioremediation of hydrocarbon pollutants in wastewater from oil refineries. *Plant Arch.*, 20: 797-802.

Amare, E., F. Kebede and W. Mulat. 2018. Wastewater treatment by Lemna minor and Azolla filiculoides in tropical semi-arid regions of Ethiopia. *Ecol. Eng.*, 120: 464-473.

Bansal, A., O. Shinde and S. Sarkar. 2018. Industrial wastewater treatment using phycoremediation technologies and co-production of value-added products. *J. Bioremediat Biodegrad.*, 9: 1-10.

Cao, X., Y. Xiong and J. Lund. 2013. The effects of micro-algae characteristics on the bioremediation rate of Deepwater Horizon crude oil. *J. Emerg. Invest June*, 17.

Colak, O. and Z. Kaya. 1988. A study on the possibilities of biological wastewater treatment using algae. *Doga Biyolji Serisi.*, 12: 18-29.

da Silva, A.A., J.A. de Oliveira, F.V. de Campos, C. Ribeiro, F. dos S. Farnese and A.C. Costa. 2018. Phytoremediation potential of Salvinia molesta for arsenite contaminated water: role of antioxidant enzymes. *Theor. Exp. Plant Physiol.*, 30: 275-286.

Danouche, M., N. El Ghachtouli and H. El Arroussi. 2021.

Phycoremediation mechanisms of heavy metals using living green microalgae: physicochemical and molecular approaches for enhancing selectivity and removal capacity. *Heliyon* 7.

Gani, P., N.M. Sunar, H. Matias-Peralta, U.K. Parjo, A.R.A. Razak and others. 2015. Phycoremediation of wastewaters and potential hydrocarbon from microalgae: a review. *Adv. Environ. Biol.*, 9: 1-9.

Hodges, A., Z. Fica, J. Wanlass, J. VanDarlin and R. Sims. 2017. Nutrient and suspended solids removal from petrochemical wastewater via microalgal biofilm cultivation. *Chemosphere*, 174: 46-48.

Kiran, M.G., K. Pakshirajan and G. Das. 2018. Metallic wastewater treatment by sulfate reduction using anaerobic rotating biological contactor reactor under high metal loading conditions. *Frontiers of Environmental Science & Engineering*, 12: 1-11.

Kshirsagar, A.D. 2013. Bioremediation of wastewater by using microalgae: an experimental study. *Int. J. Life Sci. Biotechnol. Pharma Res.*, 2: 339-346.

Kumar, P.S., S.S. Kumar, A. Kumari, S. Bharati, S. Ansari and others. 2012. Phytoremediation as an alternative for treatment of paper industry effluents by using water hyacinth (*Eichhornia crassipes*)-a polishing treatment. *Int. J. Res. Chem. Environ. I.J.R.C.E.*, 2: 95-99.

Lavoie, A. and J. De la Noüe. 1985. Hyperconcentrated cultures of Scenedesmus obliquus: a new approach for wastewater biological tertiary treatment? *Water Res*., 19: 1437-1442.

Liu, W., Y. Luo, Y. Teng and Z. Li. 2010. Phytoremediation of oilfield sludge after prepared bed bioremediation treatment. *Int. J. Phytoremediation*, 12: 268-278.

Mohamed, R., A. Al-Gheethi, A. Wurochekke, A. Maizatul, H. Matias-Peralta and A. Mohd Kassim. 2018. Nutrients removal from artificial bathroom greywater using *Botryococcus* sp. strain. *IOP Conference Series: Earth and Environmental Science*. IOP Publishing. pp. 012026.

Mustafa, H.M. and G. Hayder. 2021. Recent studies on applications of aquatic weed plants in phytoremediation of wastewater: A review article. *Ain Shams Eng. J.*, 12: 355-365.

Mustapha, H.I., H.J.J.A. Van Bruggen and P.N. Lens. 2018. Vertical subsurface flow constructed wetlands for the removal of petroleum contaminants from secondary refinery effluent at the Kaduna refining plant (Kaduna, Nigeria). *Environ. Sci. Pollut. Res.*, 25: 30451-30462.

Neveux, N., M. Magnusson, L. Mata, A. Whelan, R. De Nys and N.A. Paul. 2016. The treatment of municipal wastewater by the macroalga *Oedogonium* sp. and its potential for the production of biocrude. *Algal Res.*, 13: 284-292.

Osti, J.A., M.N. Henares and A.F. Camargo. 2018. The efficiency of free-floating and emergent aquatic macrophytes in constructed wetlands for the treatment of a fishpond effluent. *Aquac. Res.*, 49: 3468-3476.

Oswald, W.J. and H.B. Gotaas. 1957. Photosynthesis in sewage treatment. *Trans. Am. Soc. Civ. Eng.*, 122: 73-97.

Rajasulochana, P., R. Dhamotharan, S. Murugesan and A. Rama Chandra Murthy. 2009. Bioremediation of oil refinery effluent by using Scenedesmus obliquus. *J. Am. Sci.*, 5: 17-22.

Sharma, N. and S. Sharma. 2013. Comparative Study on Phytoremediation of Synthetic and Industrial Effluent.

Singh, K. and L. Mishra. 1987. Effects of fertilizer factory effluent on soil and crop productivity. *Water. Air. Soil Pollut.*, 33: 309-320.

Suyamud, B., P. Thiravetyan, B. Panyapinyopol and D. Inthorn. 2018. Dracaena sanderiana endophytic bacteria interactions: Effect of endophyte inoculation on bisphenol A removal. *Ecotoxicol. Environ. Saf.*, 157: 318-326.

Taylor, S.E., L.C. Klein, B.P. Lewis, T.L. Gruenewald, R.A. Gurung and J.A. Updegraff. 2000. Biobehavioral responses to stress in females: Tend-and-befriend, not fight-or-flight. *Psychol. Rev.*, 107: 411-429.

Ugya, A., T. Imam and A. Hassan. 2017. Phytoremediation of textile wastewater using Azolla pinnata; a case study. *World J. Pharm. Res.*, 6.

Ugya, A.Y., X. Hua and J. Ma. 2019. Phytoremediation as a tool for the remediation of wastewater resulting from dyeing activities. *Appl. Ecol. Environ. Res.*, 17: 3723-3735.

Valderrama, L.T., C.M.D. Campo, C.M. Rodriguez, L.E. de-Bashan and Y. Bashan. 2002. Treatment of recalcitrant wastewater from ethanol and citric acid production using the microalga *Chlorella vulgaris* and the macrophyte *Lemna minuscula*. *Water Res.*, 36: 4185-4192.

Xiong, J.Q., B.H. Jeon, S.P. Govindwar, M.B. Kurade, S.M. Patil, J.H. Park and K.H. Kim. 2019. Plant and microalgae consortium for an enhanced biodegradation of sulfamethazine. *Environ. Sci. Pollut. Res.*, 26: 34552-34561.

Zhang, H., L. Hu, K. Hu, Y. He, S. Wang and J. Luo. 2008. Hydrogen sulfide promotes wheat seed germination and alleviates oxidative damage against copper stress. *J. Integr. Plant Biol.*, 50: 1518-1529.

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