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Phycoremediation of Paracetamol and Tetracycline by *Scenedesmus quadricauda*: Removal Efficiency and Physiological Responses

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ABSTRACT: Pharmaceutical contaminants are increasingly detected in aquatic environments because conventional wastewater treatment often fails to remove them, posing risks to microorganisms, algae, and ecosystem stability. This study evaluated the phycoremediation potential of *Scenedesmus quadricauda* to remove paracetamol and tetracycline at 3, 6, and 9 mg/L. Removal efficiency was determined by HPLC after 6 and 9 days, while photosynthetic pigments, reactive oxygen species (ROS), malondialdehyde (MDA), proteins, and carbohydrates were measured to assess physiological stress and cellular adaptation. *S. quadricauda* significantly enhanced pharmaceutical removal compared with controls, with most treatments achieving 100% removal by day 9. Biochemical analyses showed that paracetamol was better tolerated, whereas tetracycline induced greater physiological and oxidative stress, particularly affecting photosynthetic pigments. These findings indicate that *S. quadricauda* is a promising, low-cost, and environmentally friendly biological agent for pharmaceutical wastewater remediation.

1. INTRODUCTION

Antibiotics are widely used antimicrobial agents, but their persistence in wastewater and aquatic environments promotes antibiotic resistance, disrupts ecosystems, and facilitates the spread of antibiotic-resistance genes (ARGs), posing a major public health threat (Serwecińska, 2020; Rajput et al., 2024; Sanchez et al., 2024; Larsson and Flach, 2022). They also impair microbial processes in wastewater treatment plants, including denitrification, methanogenesis, and sulphate reduction (Srivastava et al., 2021; Xiao et al., 2021). Common antibiotic classes include tetracyclines, cephalosporins, sulfonamides, penicillins, lincomycins, macrolides, and quinolones (Patel et al., 2019). Their increasing occurrence and misuse emphasize the need for effective removal before wastewater discharge (Srivastava et al., 2022; Hejna et al., 2022).

Paracetamol, a widely used NSAID, may cause hepatotoxicity after prolonged exposure (Freo et al., 2021), while NSAIDs can also promote the transfer of antibiotic-resistance genes (Wang et al., 2021). Pharmaceuticals are excreted as unmetabolized compounds or transformation products that may generate toxic by-products harmful to ecosystems and human health (Maculewicz et al., 2022). Conventional physicochemical treatments, such as adsorption, ion exchange, precipitation, coagulation, and filtration, are commonly used for pharmaceutical wastewater treatment (Wang and Wang, 2016), whereas advanced oxidation processes (AOPs), including UV/solar irradiation, electrolysis, ozonation, ultrasonication, wet air oxidation, and Fenton oxidation, effectively mineralize pharmaceutical contaminants into carbon dioxide, inorganic ions, and water (Anjali and Shanthakumar, 2019; Miklos et al., 2018).

The inability to effectively treat pharmaceutical compounds in wastewater treatment plants can lead to their leakage and spread through the recycling of treated wastewater for agricultural uses (Zewdu et al., 2026). In this context, biological treatments have played a distinctive role in treating contaminated wastewater using living organisms such as algae and have gained importance due to their ability to break down various pharmaceutical compounds. The use of microalgae in wastewater treatment has

recently gained particular importance due to their unique role in removing pollutants, including pharmaceutical compounds, and this is achieved through their ability to absorb and break down pollutants, they require low energy and their biomass can be recovered through biofuel production; therefore, the aim of this study was to investigate the effectiveness of *Scenedesmus quadricauda* in removing paracetamol and tetracycline from wastewater, with the goal of developing environmentally friendly pollutant management strategies.

2. MATERIALS AND METHODS

2.1 Cultivation and Growth of *Scenedesmus quadricauda*

This study was conducted in the Department of Biology, College of Science, University of Babylon. Chu-10 medium was used to grow *Scenedesmus quadricauda* algae obtained from the Algal Culture Group (UTEX) at the University of Texas (Austin, Texas, USA). Concentrated solutions of all macro and micronutrients were prepared consisting of 1.5 g of sodium nitrate (NaNO_3), 40 g/L of potassium dihydrogen phosphate trihydrate ($\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$), 75 g/L of magnesium sulfate heptahydrate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), 36 g/L of calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), 20 g/L of sodium carbonate (Na_2CO_3), 6 g/L of hexamethylene tetra acetic acid ($\text{C}_6\text{H}_8\text{O}_7$), 1 g/L of sodium EDTA (Na-EDTA), and 6 g/L of ferrous sulfate hexahydrate ($\text{FeH}_8\text{N}_2\text{O}_8\text{S}_2$). The concentrated trace element solution consisted of 2.86 g of boric acid (H_3BO_3), 1.81 g of manganese chloride tetrahydrate ($\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$), 0.222 g of zinc sulfate heptahydrate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$), 0.39 g of sodium molybdate dihydrate ($\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$), 0.079 g of copper sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), and 0.049 g of cobalt nitrate hexahydrate ($\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$). These solutions were dissolved in 1 liter of distilled water to prepare the Chu-10 culture. One milliliter of each pre-prepared chemical was taken and mixed with 1 liter of distilled water. Chu-10 medium was steam sterilized at 121°C for 15 minutes (Okmen et al., 2011). The algae were cultured in the aforementioned medium and incubated under conditions of $286 \mu\text{mol}/\text{m}^2/\text{s}$ light intensity, a 16:8 h light/dark photoperiod, and a temperature of $25 \pm 2^\circ\text{C}$ (Chia et al., 2013) for 15 days (Gour et al., 2014). Propagation was then achieved by transferring the culture to a flask containing 1000 mL of the same medium and incubating for another 15 days. The cultures were also gently shaken daily to prevent clumping and promote growth (Selvan et al., 2013).

2.2 Experimental Setup

To evaluate the role of *Scenedesmus quadricauda* in removing paracetamol and tetracycline used in the current study, three concentrations of each compound (3, 6, and 9 mg/L) were prepared and replicated in three replicates. The experiment included several groups:

(1) *Algal treatments exposed to pharmaceuticals*. Three concentrations of each pharmaceutical compound were prepared at concentrations of (3, 6, and 9 mg/L) and replicated in three replicates in 1-liter glass flasks containing 850 ml of culture medium treated with Chu-10 and inoculated with 150 ml of active algal culture after it reached the stationary phase.

(2) *Algal control group*. A group containing *Scenedesmus quadricauda* growing in 1 liter of culture medium completely free of any antibiotics (for comparison and monitoring of normal growth).

(3) *Abiotic control group*. Control cultures containing the specified antibiotic concentrations but without algal inoculation were used to assess abiotic degradation (autolysis or chemical degradation) independent of biological activity. All treatments were maintained under identical culture conditions for nine days. Samples were collected on days 6 and 9, filtered to remove algal cells, and analyzed by HPLC to determine residual antibiotic concentrations. Removal efficiency was then calculated from the measured antibiotic residues using the corresponding equation.

$$\frac{\text{Concentration before treatment} - \text{concentration after treatment}}{\text{concentration before treatment}} \times 100\%$$

2.3 Physiological and Biochemical Responses of *Scenedesmus* sp. Exposed to Paracetamol and Tetracycline

The growth of *Scenedesmus quadricauda* was monitored daily by measuring optical density (OD) at 750 nm using UV-Vis spectroscopy to determine growth stages, growth rate, and doubling time. Chlorophyll content was measured according to Juneja et al. (2013), total protein by the Bradford (1976) method, and carbohydrate content by DuBois et al. (1956). Oxidative stress was evaluated by measuring reactive oxygen species (ROS) following Erel (2005), while malondialdehyde (MDA), an

indicator of lipid peroxidation and membrane damage, was determined using the thiobarbituric acid-reactive substances method described by Buege and Aust (1978).

2.4 HPLC Analysis

Residual concentrations of paracetamol and tetracycline were determined using a SYKAM (Germany) HPLC system. Samples were centrifuged and filtered through 0.22- μ m membrane filters before analysis to remove algal cells and suspended particles. Paracetamol was analyzed on a C18-ODS reverse-phase column (250 $\times$ 4.6 mm) using a mobile phase of 3% formic acid:acetonitrile (70:30, v/v) at 1.0 mL/min with UV detection at 243 nm, following Ahmad and Omar (2018). Tetracycline was analyzed on the same column using a water:methanol (70:30, v/v) mobile phase at 1.2 mL/min, with fluorescence detection (310/420 nm) according to Shama et al. (2016). Concentrations of both pharmaceuticals were quantified by comparing sample peak areas with analytical standards.

2.5 Statistical Analysis

Data analysis was performed using SPSS software (version 26.0, IBM Corporation, Armonk, New York, USA). Results are presented as mean $\pm$ standard deviation. Differences between the treatment and control groups at each concentration and sampling time were assessed using an independent Student's t-test. A p-value < 0.05 was considered statistically significant, while a p-value < 0.01 was considered highly statistically significant.

3. RESULTS AND DISCUSSION

3.1 Removal Efficiency of Paracetamol and Tetracycline by *S. quadricauda*

The results demonstrated that *S. quadricauda* effectively removed tetracycline and paracetamol. However, because HPLC only measured the disappearance of the parent compounds, the exact removal mechanism could not be determined. Removal efficiency depended on both antibiotic concentration and exposure time. At 3 mg/L, *S. quadricauda* achieved 100% removal of both tetracycline and paracetamol after 6 days, with complete removal maintained after 9 days, indicating high efficiency in eliminating low concentrations within a short treatment period (Table 1; Figures 1–15).

Table 1. Effect of *S. quadricauda* on the removal (%) of paracetamol and tetracycline over time (6th and 9th day).

Antibiotic	Period (day)	Conc. before treatment (mg/L)	Control (antibiotic only)		With <i>S. quadricauda</i>		p-value†
			Conc. after treatment (mg/L)	Removal (%)	Conc. after treatment (mg/L)	Removal (%)	
Paracetamol	6	3	2	33.3	0	100	0.0001** (H.S)
		6	3.73	37.8	0	100	0.0001** (H.S)
		9	7.83	13.0	3.32	63.1	0.0001** (H.S)
	9	3	0.87	71.0	0	100	0.017* (S)
		6	1.25	79.1	0	100	0.012* (S)
		9	3.66	59.3	0	100	0.0001** (H.S)
Tetracycline	6	3	1.93	35.6	0	100	0.0001** (H.S)
		6	3.9	35.0	1	83.3	0.0001** (H.S)
		9	6.39	29.0	2.99	66.7	0.004** (H.S)
	9	3	0.35	88.3	0	100	0.017* (S)
		6	1.25	79.1	0	100	0.012* (S)
		9	3	66.6	0	100	0.0001** (H.S)

* t-test; S = significant; H.S = highly significant. † p-values.

At 6 mg/L, *S. quadricauda* achieved 100% removal of paracetamol and 83.3% removal of tetracycline after 6 days, while 100% removal of both compounds was obtained after 9 days, demonstrating that longer exposure enhanced biodegradation. At 9 mg/L, removal efficiencies after 6 days decreased to 63.1% for paracetamol and 66.7% for tetracycline, likely due to physiological stress on the algal cells, but complete removal was also achieved after 9 days, highlighting the importance of treatment duration. Control groups without algae exhibited much lower removal efficiencies, confirming that natural

degradation alone is insufficient. Statistical analysis showed that *S. quadricauda* significantly improved the removal of both pharmaceuticals at all concentrations and exposure times ($p < 0.05$ and $p < 0.001$). The findings support the use of *S. quadricauda* as a sustainable bioremediation agent, with removal occurring through adsorption to the cell surface (Wang et al., 2017b), cellular uptake (Sun et al., 2017), intracellular biodegradation (Ding et al., 2017a), and extracellular polymer-mediated removal (Xiong et al., 2017). Microalgae can further transform pharmaceuticals into less toxic metabolites and non-toxic by-products, reinforcing their value for pharmaceutical wastewater treatment (Garcia-Becerra and Ortiz, 2018).

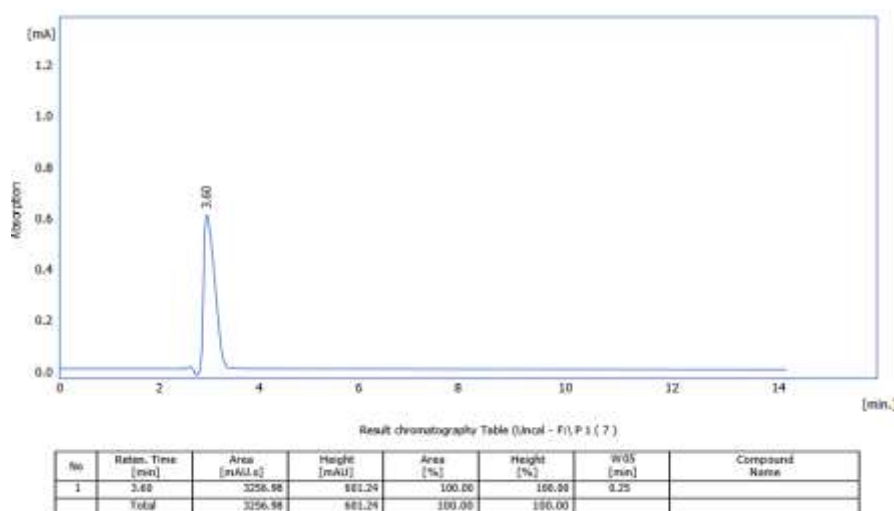


Figure 1. Representative HPLC chromatogram of paracetamol (3 mg/L) on day 6.

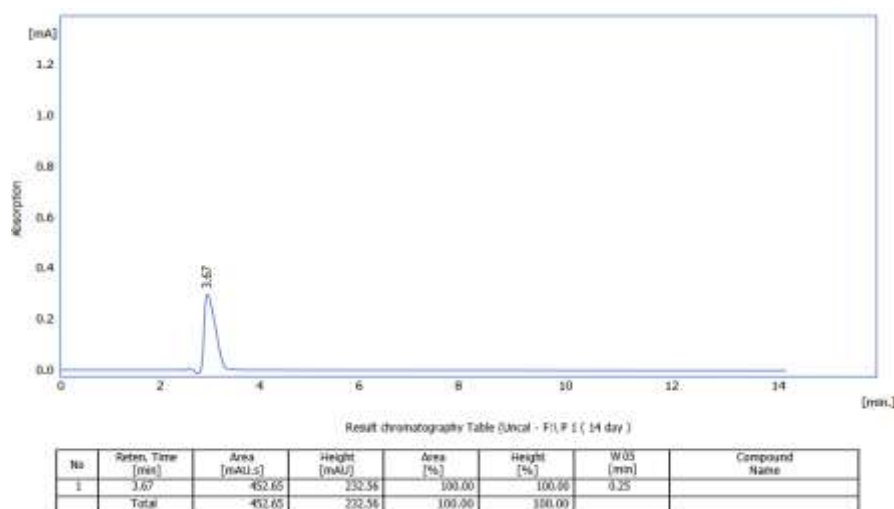


Figure 2. Representative HPLC chromatogram of paracetamol (3 mg/L) on day 9.

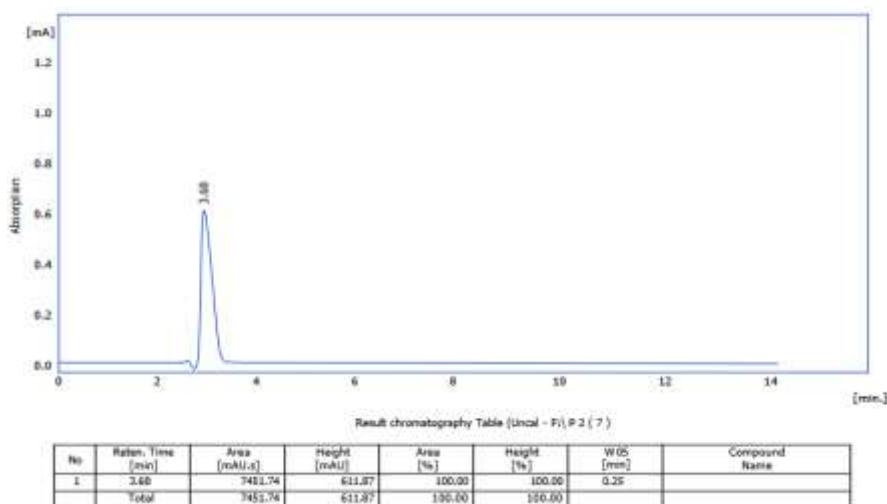


Figure 3. Representative HPLC chromatogram of paracetamol (6 mg/L) on day 6.

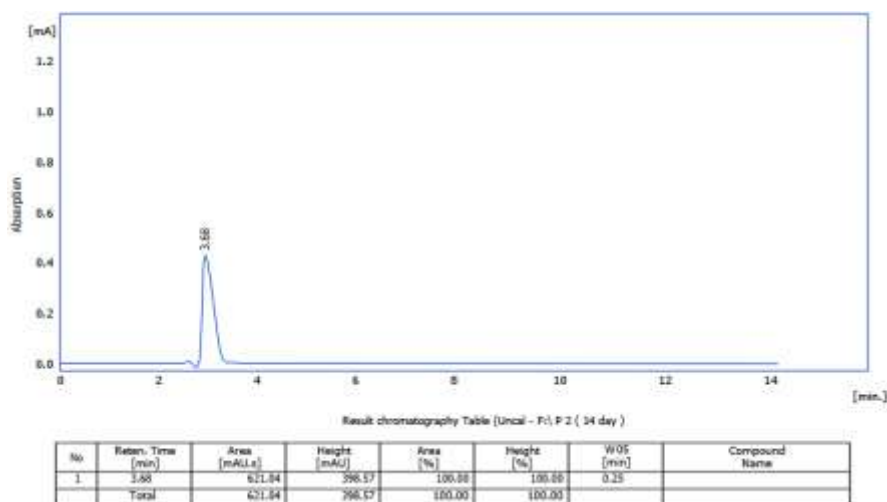


Figure 4. Representative HPLC chromatogram of paracetamol (6 mg/L) on day 9.

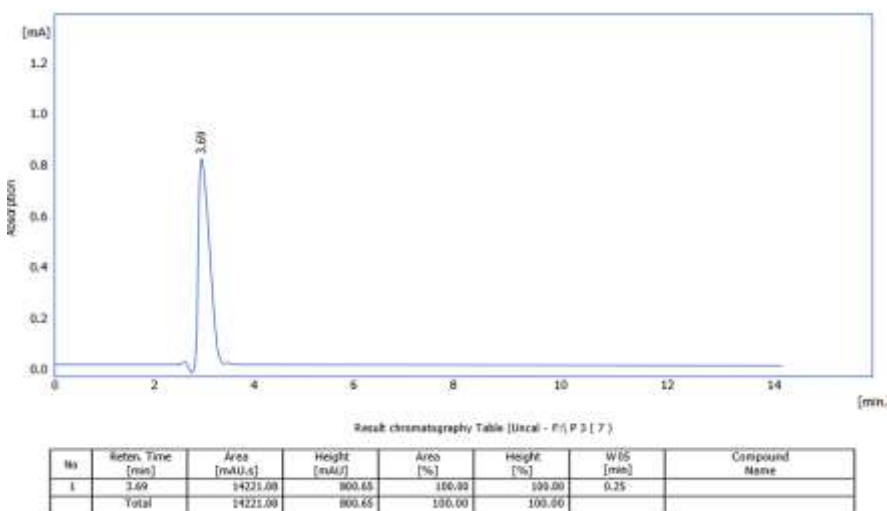


Figure 5. Representative HPLC chromatogram of paracetamol (9 mg/L) on day 6.

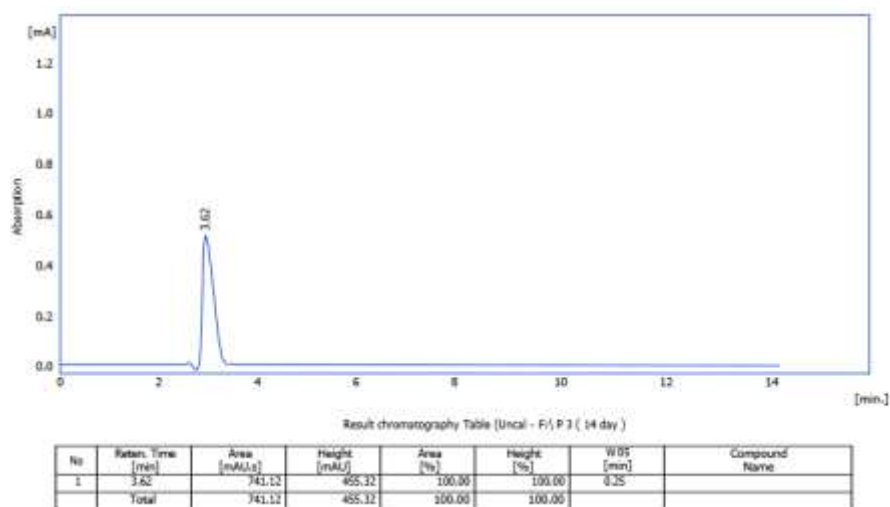


Figure 6. Representative HPLC chromatogram of paracetamol (9 mg/L) on day 9.

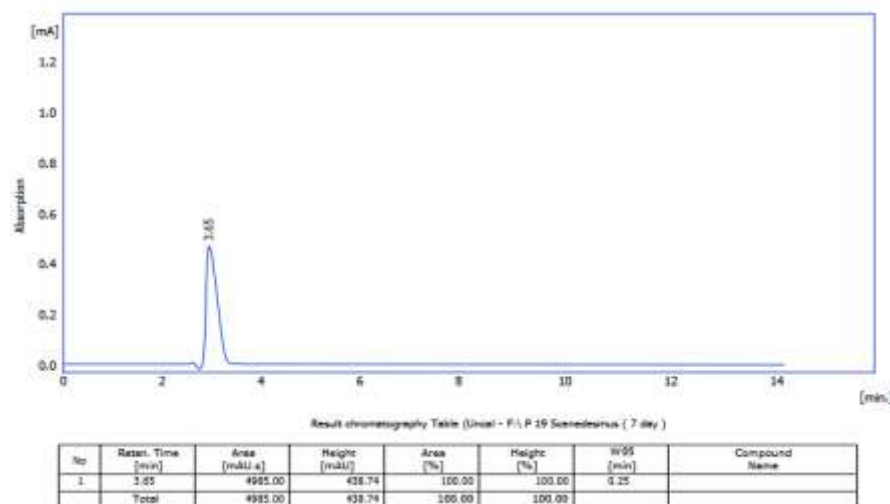


Figure 7. Representative HPLC chromatogram of paracetamol (9 mg/L) on day 6.

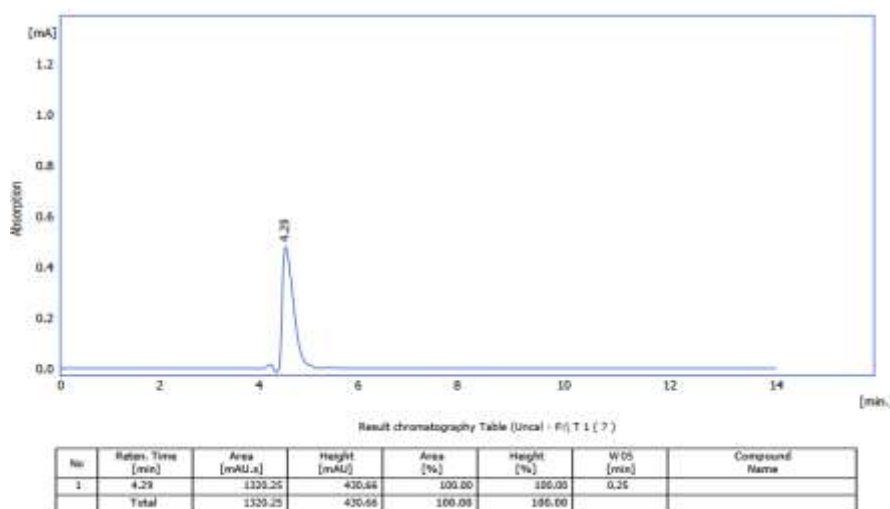


Figure 8. Representative HPLC chromatogram of tetracycline (3 mg/L) on day 6.

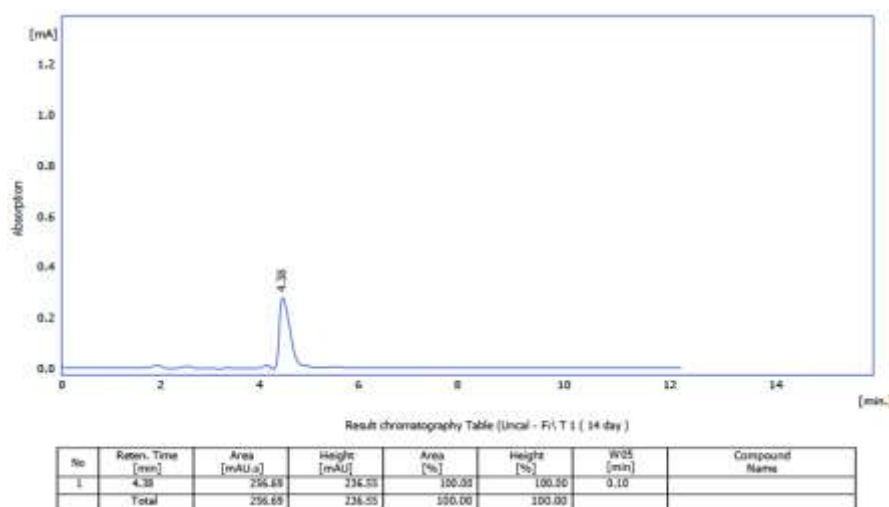


Figure 9. Representative HPLC chromatogram of tetracycline (3 mg/L) on day 9.

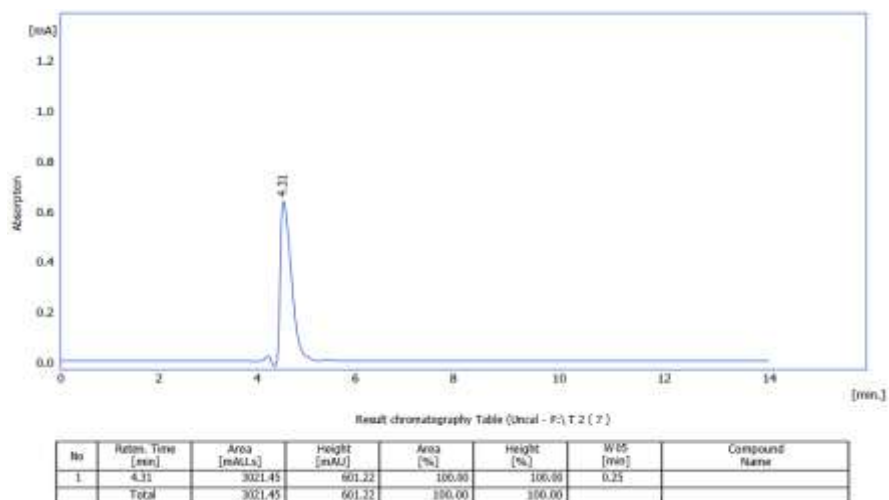


Figure 10. Representative HPLC chromatogram of tetracycline (6 mg/L) on day 6.

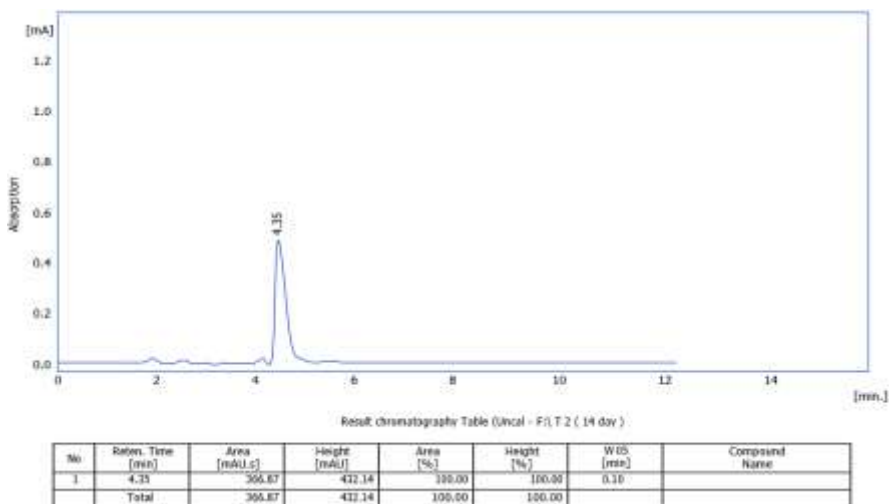


Figure 11. Representative HPLC chromatogram of tetracycline (6 mg/L) on day 9.

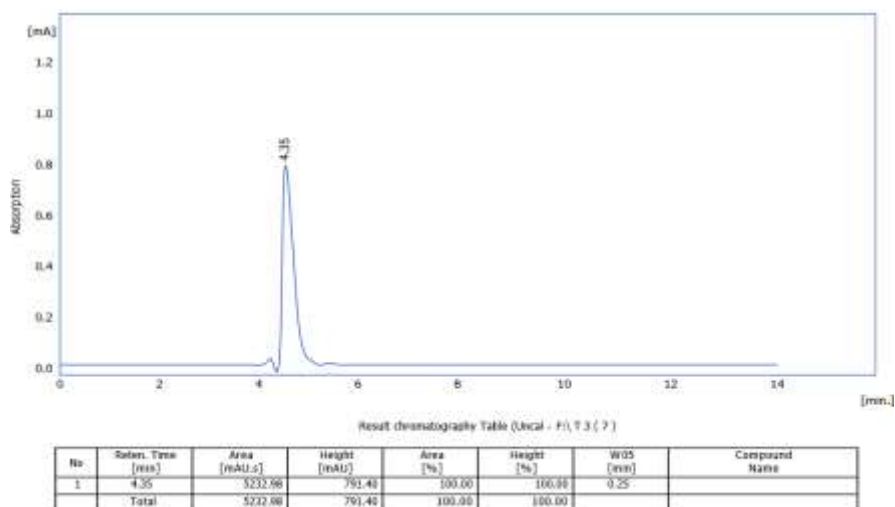


Figure 12. Representative HPLC chromatogram of tetracycline (9 mg/L) on day 6.

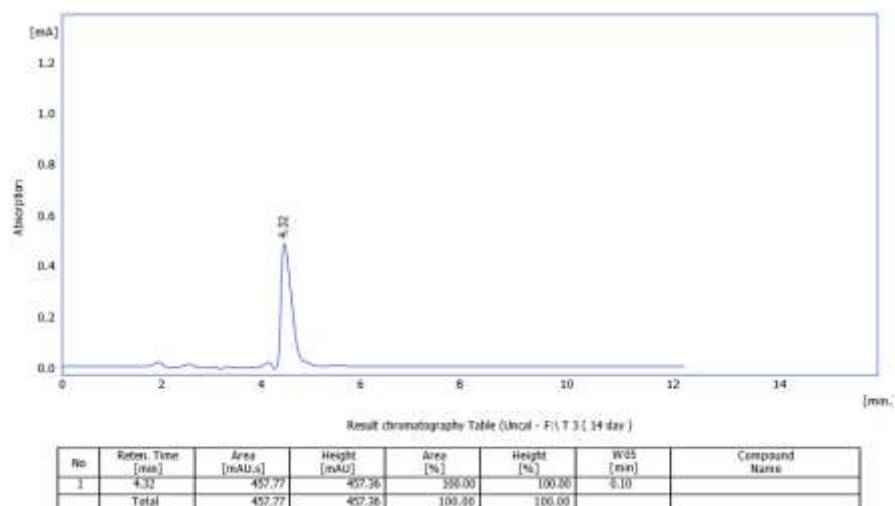


Figure 13. Representative HPLC chromatogram of tetracycline (9 mg/L) on day 9.

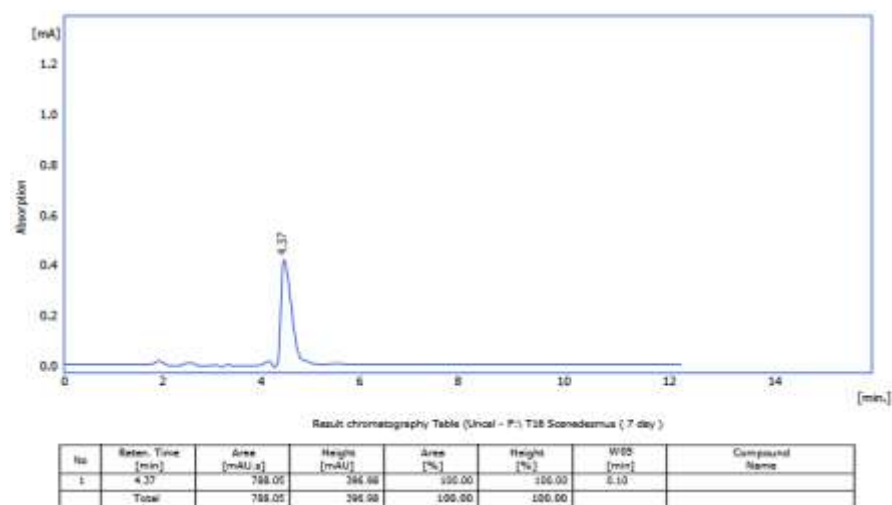


Figure 14. Representative HPLC chromatogram of tetracycline (6 mg/L) on day 6.

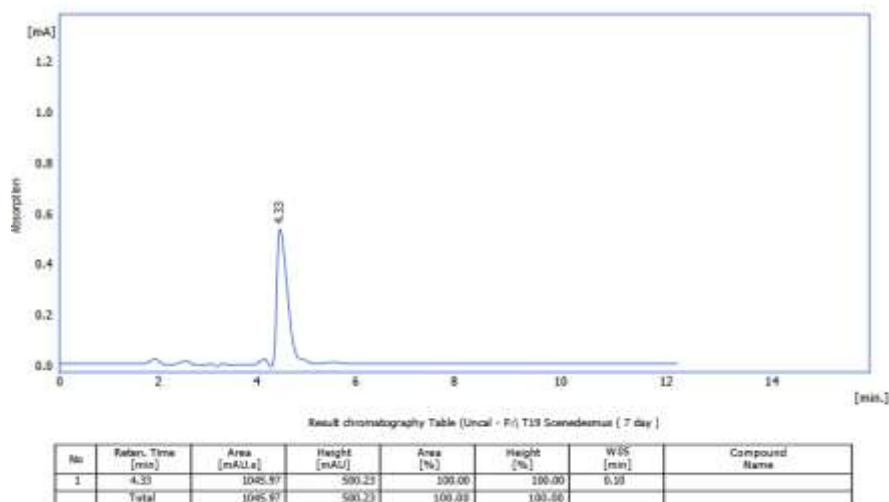


Figure 15. Representative HPLC chromatogram of tetracycline with *Scenedesmus quadricauda* (9 mg/L) on day 6.

3.2 Effects of Tetracycline and Paracetamol on *S. quadricauda*

3.2.1 Effects on Growth Rate and Doubling Time

The results (Figure 16 and Figure 17) showed that both paracetamol and tetracycline significantly inhibited the growth of *S. quadricauda* compared with the uncontaminated control, which exhibited the highest optical density throughout the experiment. Exposure to all tested concentrations (3, 6, and 9 mg/L) reduced algal growth, with optical density declining markedly after prolonged exposure. Paracetamol caused progressive inhibition of cell division and biomass accumulation, primarily through oxidative stress induced by excessive reactive oxygen species that damage nucleic acids, pigments, proteins, and cell membranes, thereby reducing photosynthetic efficiency (Miazek and Brozek-Pluska, 2019). Tetracycline also significantly suppressed algal growth, with the 9 mg/L concentration producing the strongest inhibitory effect. Its toxicity is attributed to interference with protein synthesis by targeting 70S ribosomes in chloroplasts, reducing chlorophyll production, photosynthetic activity, cell proliferation, and energy production. In addition, tetracycline-induced oxidative stress further contributes to growth inhibition and cellular damage (Xu et al., 2019).

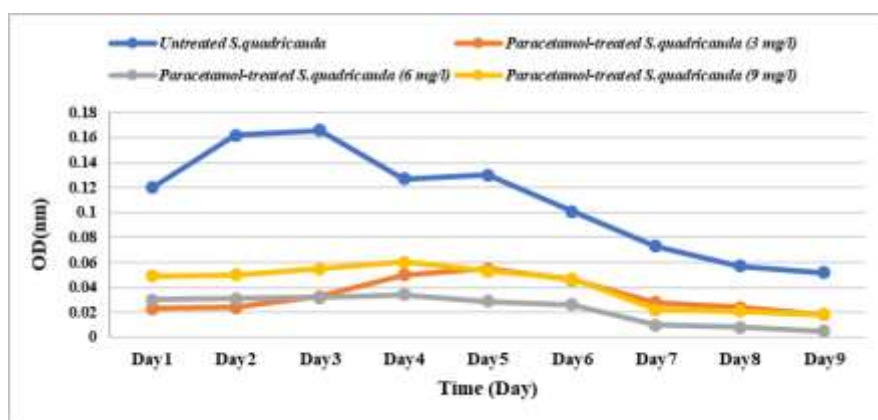


Figure 16. Growth rate of *S. quadricauda* under different concentrations of paracetamol.

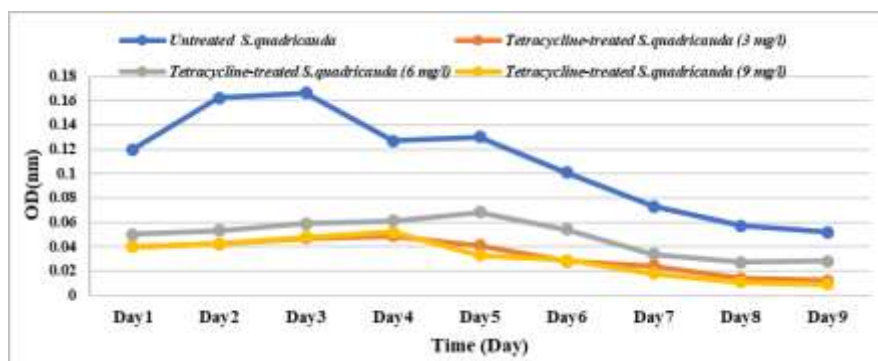


Figure 17. Growth rate of *S. quadricauda* under different concentrations of tetracycline.

Doubling time is a reliable indicator of microalgal growth, with shorter doubling times reflecting faster growth. Under contaminant-free conditions, *S. quadricauda* exhibited the shortest doubling time, particularly on day 9, indicating optimal growth due to efficient photosynthesis, cell division, and nutrient uptake. Exposure to 3 mg/L paracetamol caused only a slight increase in doubling time, suggesting mild physiological stress, whereas 6 mg/L markedly increased doubling time by day 9, indicating strong growth inhibition associated with oxidative stress. Similarly, tetracycline increased doubling time in a time- and concentration-dependent manner, with 9 mg/L producing the greatest inhibitory effect and the highest doubling time on day 9, reflecting substantial suppression of cell proliferation (Figure 18 and Figure 19). Low pharmaceutical concentrations have limited effects on algal growth because antioxidant defense systems and detoxification pathways remain active (Xiong et al., 2016; Yan et al., 2020). In contrast, high paracetamol concentrations inhibit growth by promoting excessive reactive oxygen species production, disrupting chlorophyll biosynthesis, reducing photosynthetic efficiency, and inducing lipid peroxidation, thereby decreasing the energy available for cell proliferation and biomass production and prolonging doubling time (Wang et al., 2021).

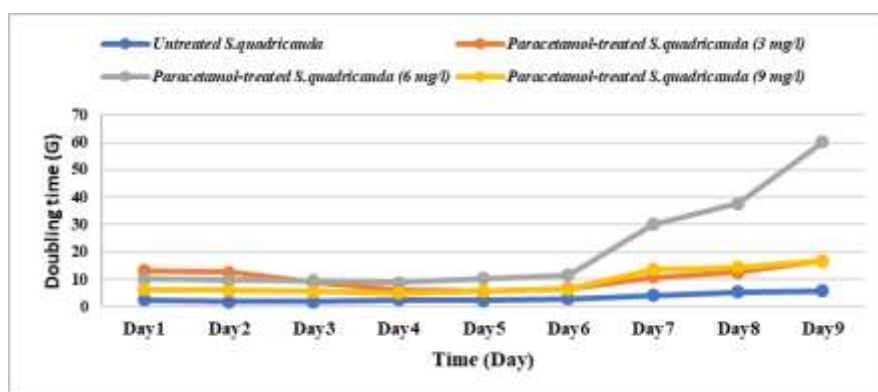


Figure 18. Doubling time of *S. quadricauda* under different concentrations of paracetamol.

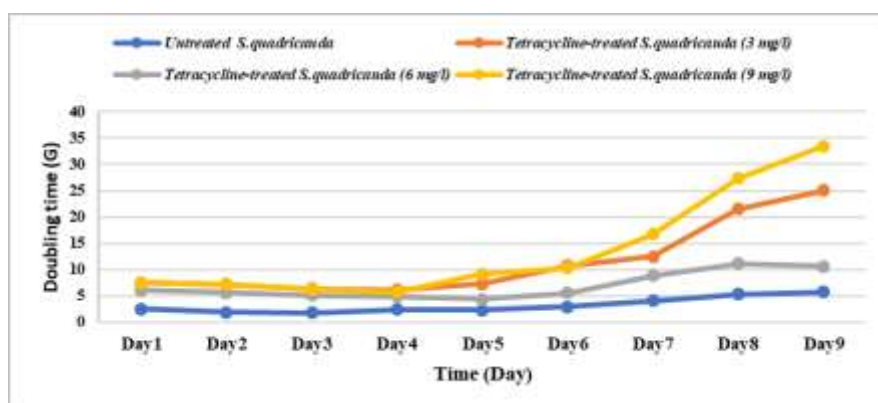


Figure 19. Doubling time of *S. quadricauda* under different concentrations of tetracycline.

3.2.2 Effects on Photosynthetic Pigments and Biochemical Parameters in *S. quadricauda*

Exposure of *S. quadricauda* to paracetamol and tetracycline caused significant changes in photosynthetic pigments, indicating that both pharmaceuticals affect cellular metabolism through oxidative stress and alterations to the photosynthetic apparatus. The control culture contained 7.7 mmole/ml total chlorophyll. Paracetamol slightly increased chlorophyll at 3 mg/L (8.05 mmole/ml), suggesting a hormetic response, but reduced it at 6 and 9 mg/L, indicating impaired chlorophyll biosynthesis and photosynthesis. In contrast, tetracycline initially reduced total chlorophyll at 3 mg/L, followed by recovery and a significant increase at 6 and 9 mg/L, suggesting activation of adaptive physiological mechanisms that help maintain photosynthetic efficiency under stress (**Figure 20**). This response is consistent with the resilience of *Scenedesmus* species in pharmaceutical-contaminated environments (Hejna et al., 2022). Similarly, chlorophyll a content was affected by both contaminants (**Figure 21**). Compared with the control (4.06 µg/ml), paracetamol slightly increased chlorophyll a at 3 mg/L before decreasing it at higher concentrations, whereas tetracycline caused a marked reduction at 3 mg/L followed by gradual recovery at 6 and 9 mg/L. The stimulation observed at low paracetamol concentrations reflects a hormetic response (Agathokleous et al., 2019), while the reductions at higher concentrations indicate inhibition of chlorophyll biosynthesis and oxidative damage to chloroplast membranes. Tetracyclines also interfere with photosystem II and electron transport, reducing chlorophyll accumulation (Wang et al., 2017a). Because chlorophyll pigments are key indicators of cellular physiological status and stress tolerance, these changes demonstrate the impact of pharmaceutical exposure on microalgal photosynthetic activity (da Silva Rodrigues et al., 2020; Hejna et al., 2022).

Chlorophyll b responded differently from chlorophyll a (**Figure 22**). Compared with the control (3.64 mmole/ml), paracetamol caused only minor changes across all concentrations, whereas tetracycline increased chlorophyll b, peaking at 6 mg/L before a slight decline at 9 mg/L. This increase likely represents an adaptive response that enhances light absorption and compensates for reduced photosynthetic efficiency under stress (Taiz et al., 2022).

Protein and carbohydrate contents were also affected (**Figure 23** and **Figure 24**). The control algae contained 1.44 mg/ml protein, while paracetamol reduced protein levels, indicating inhibition of protein synthesis or enhanced protein degradation under oxidative stress (Zhong et al., 2021; Gill and Tuteja, 2010). In contrast, tetracycline maintained protein concentrations close to the control, suggesting activation of compensatory metabolic mechanisms, consistent with previous observations in freshwater microalgae exposed to antibiotics (Li et al., 2020). Carbohydrate content generally declined after exposure to both pharmaceuticals, reflecting the utilization of stored carbohydrates to meet the increased energy demand required for stress adaptation and antioxidant defense. Partial recovery in some treatments indicates physiological adaptation during prolonged exposure (Hejna et al., 2022; Gill and Tuteja, 2010; Ding et al., 2017b). Exposure of *Scenedesmus quadricauda* to paracetamol and tetracycline altered oxidative stress biomarkers in a concentration-dependent manner (**Figure 25**). Compared with the control, both pharmaceuticals increased reactive oxygen species (ROS) at 3 mg/L, indicating oxidative stress. At 6 mg/L, ROS remained elevated with paracetamol but decreased with tetracycline, suggesting activation of antioxidant defenses. At 9 mg/L, ROS declined slightly with paracetamol but increased again with tetracycline, reflecting renewed oxidative stress. These findings indicate that oxidative stress is a primary toxicity mechanism caused by disruption of electron transport in chloroplasts and mitochondria, while antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and peroxidase may partially counteract ROS at moderate concentrations (Gill and Tuteja, 2010; Wang et al., 2017a; Zhong et al., 2021). Similar ROS induction and antioxidant disruption by antibiotics have been reported previously (Roy et al., 2021; Aderemi et al., 2021; Qian et al., 2008; Liu and Xiong, 2009).

Malondialdehyde (MDA), a biomarker of membrane lipid peroxidation, generally decreased following exposure to both pharmaceuticals compared with the control (**Figure 26**). This reduction suggests that *S. quadricauda* activated effective antioxidant defense mechanisms that limited lipid peroxidation despite increased ROS production, or that pharmaceutical exposure reduced metabolic activity and membrane lipid turnover (Gill and Tuteja, 2010; Zhong et al., 2021). Previous studies have shown that MDA responses vary with algal species, pollutant concentration, and exposure duration. While some antibiotics increase SOD, CAT, and MDA during prolonged exposure, indicating oxidative stress (Li et al., 2020), algal sensitivity differs among species and antibiotic types (Zhou et al., 2020; Han et al., 2020). Similar effects of antibiotics on antioxidant systems and photosynthetic machinery have also been reported by Wang et al. (2017a).

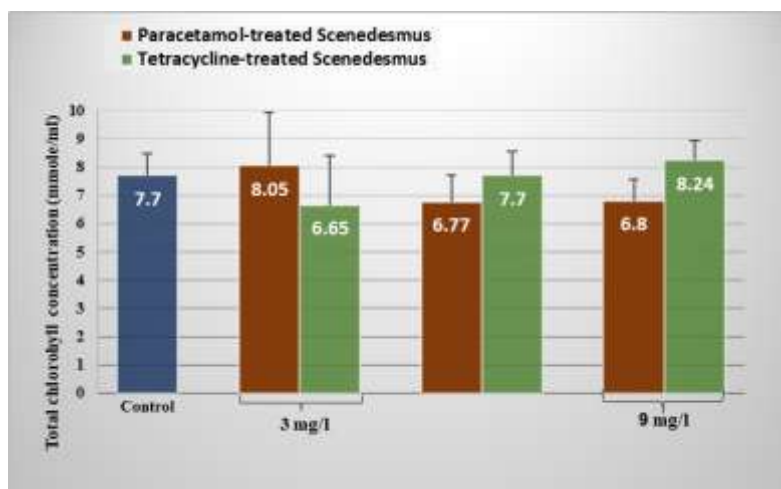


Figure 20. Changes in total chlorophyll concentration in *Scenedesmus quadricauda*

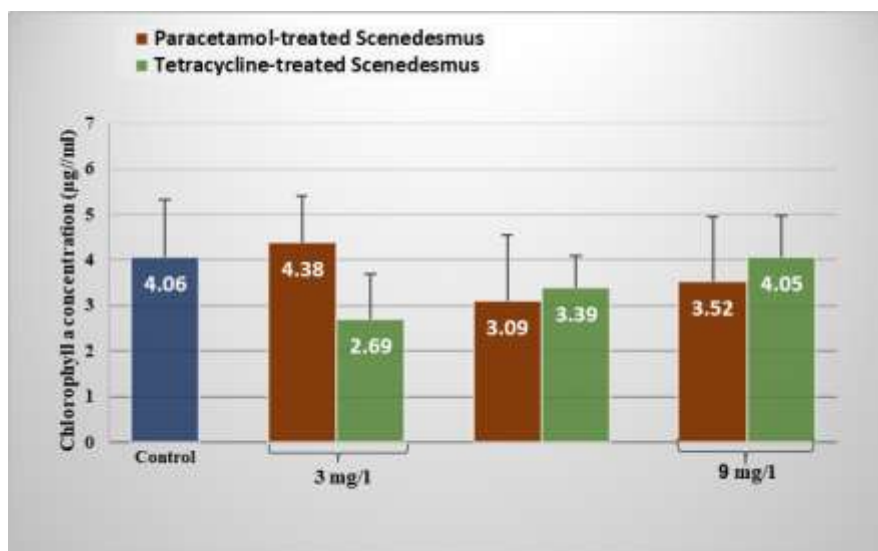


Figure 21. Changes in chlorophyll a concentration in *Scenedesmus quadricauda*

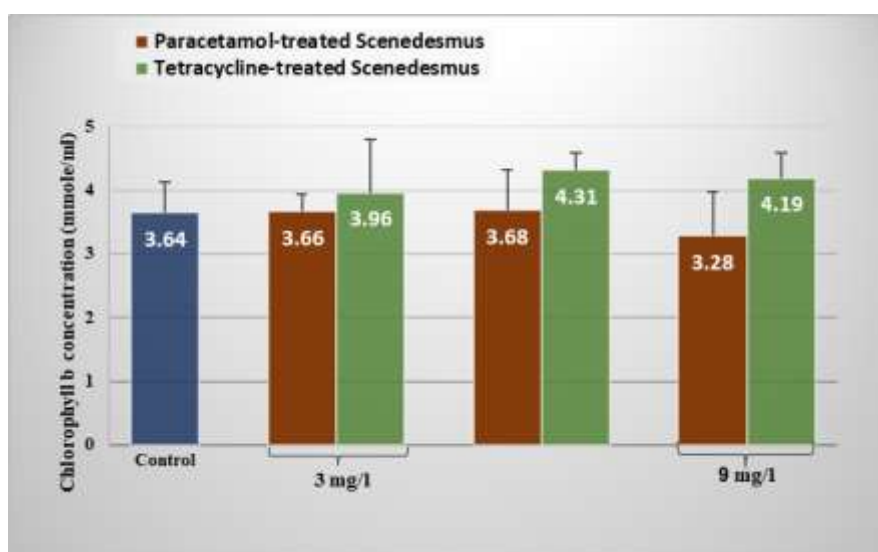


Figure 22. Changes in chlorophyll b concentration in *Scenedesmus quadricauda*

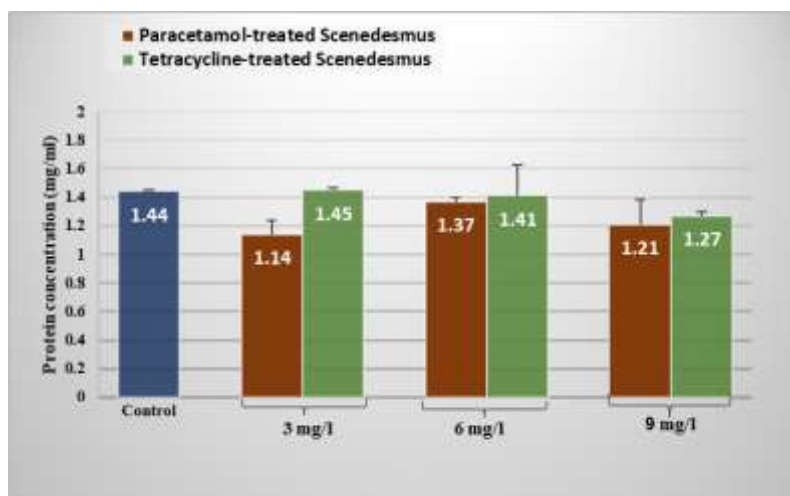


Figure 23. Changes in protein concentration in *Scenedesmus quadricauda*

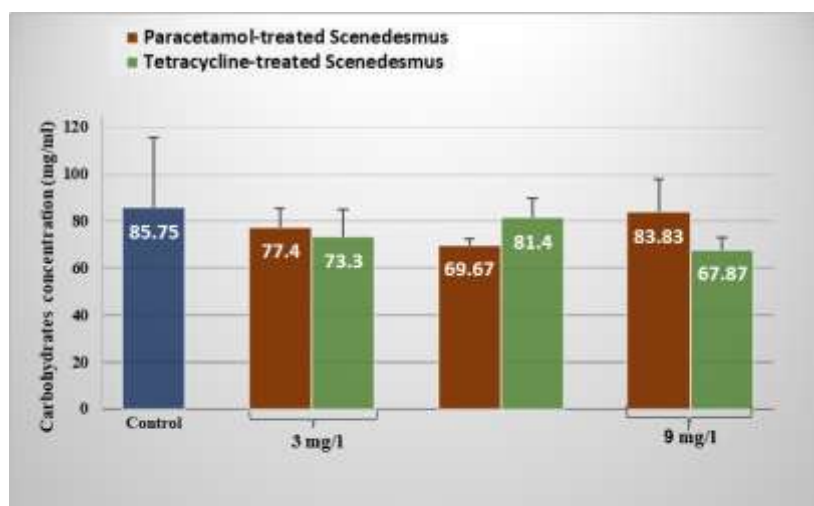


Figure 24. Changes in carbohydrates concentration in *Scenedesmus quadricauda*

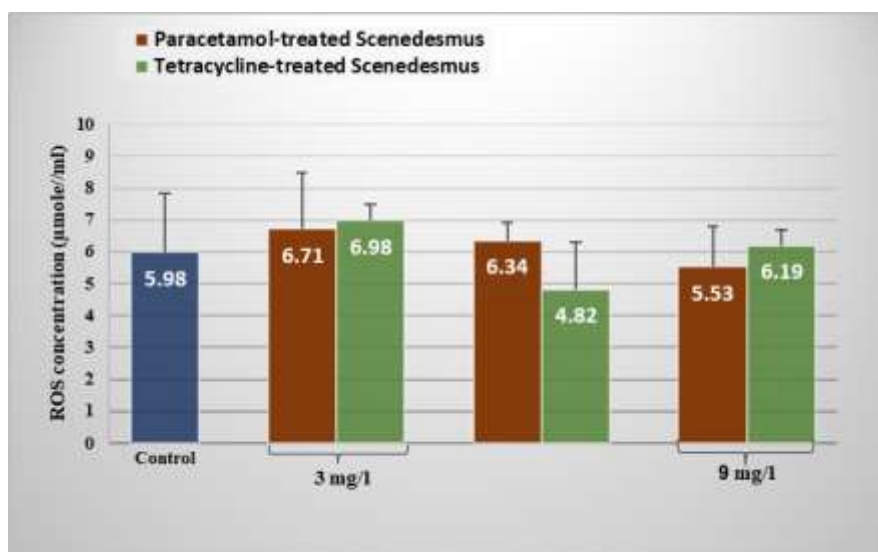


Figure 25. Changes in ROS concentration in *Scenedesmus quadricauda*.

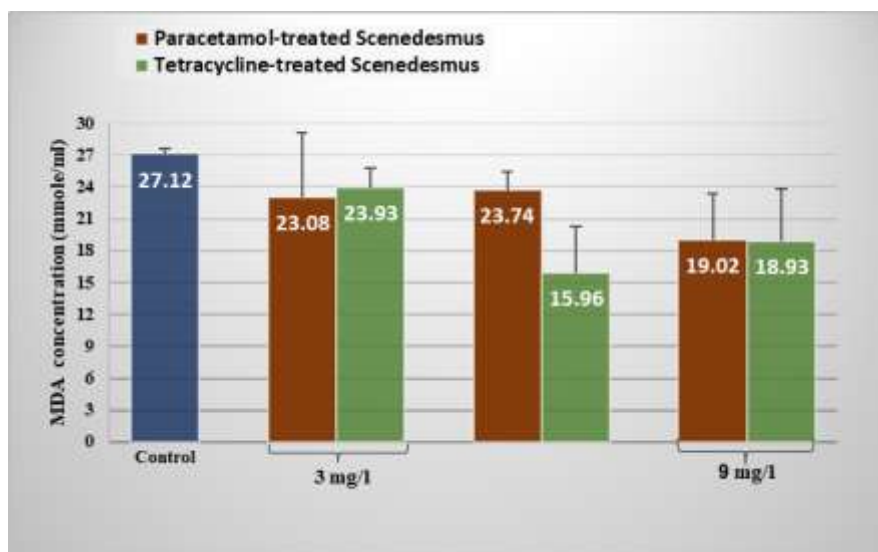


Figure 26. Changes in MDA concentration in *Scenedesmus quadricauda*

4. CONCLUSION

The results demonstrated that *Scenedesmus quadricauda* has strong potential for the bioremediation of tetracycline and paracetamol from pharmaceutical wastewater, achieving significant reductions in both compounds. However, because only the parent compounds were quantified by HPLC, the exact removal mechanisms could not be conclusively identified. Biochemical analyses showed that tetracycline and paracetamol induced different stress responses, with tetracycline causing more pronounced physiological effects, whereas paracetamol was better tolerated. Despite these stress responses, *S. quadricauda* maintained high removal efficiency, highlighting its potential as an effective, low-cost, and environmentally friendly option for pharmaceutical wastewater treatment. Further research should identify degradation metabolites, evaluate post-treatment toxicity, and assess the alga's performance under real wastewater conditions.

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AUTHOR CONTRIBUTIONS

E.A. and N.F.K. contributed to methodology and writing original draft preparation. N.F.K. and E.A. were responsible for preparing drawings and adding references. N.F.K. and J.M.S. contributed to writing, review, and editing final draft. J.M.S. supervision and project administration. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

No conflicts of interest.

ETHICS STATEMENT

Ethical approval was not required for this study.

DATA AVAILABILITY STATEMENT

Data in this study are available from the corresponding author as requested for reasonable uses.

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