GROWTH RESPONSES OF THE MICROALGA: *Acutodesmus acutiformis* TO TWO ANTIBIOTICS

BY

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SR/2160/RPR/24/6

DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

BENIN CITY

MARCH, 2025

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY, FACULTY OF LIFE SCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (HONOURS) DEGREE (BSC.) IN PLANT BIOLOGY AND BIOTECHNOLOGY.

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CERTIFICATION

This is to certify that this work was carried out by **Oghenegaverere Godsfavour AHWEYEVU(Miss)** with matriculation number **LSC2003143** of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin city. Nigeria.

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DR. MRS. M. A. AKHERE **DATE**

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PROF. E. D. VWIOKO **DATE**

(HEAD OF DEPARTMENT)

DEDICATION

I dedicate this project to God Almighty, the source of all knowledge and wisdom for his guidance, direction and strength throughout this research study

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this project and I am truly grateful for your support. I pray God continue to bless and increase you all.

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ABSTRACT

Antibiotics play a crucial role in medicine, aquaculture, and agriculture, but their persistence in wastewater and runoff poses significant environmental risks, particularly for aquatic ecosystems. This study evaluated the effects of tetracycline and chloramphenicol on the growth of *Acutodesmus acutiformis,* a freshwater green microalga.. The experiment involved culturing *A. acutiformis* with varying concentrations (0, 5%, 10%, 20%,30%,40%,50%) of tetracycline and chloramphenicol under controlled laboratory conditions at the University of Benin. Microalgal growth was monitored spectrophotometrically at 750 nm over 14 days, alongside physicochemical parameters such as temperature, total dissolved solids (TDS), pH, conductivity, turbidity, dissolved oxygen (DO), chemical oxygen demand (COD), and total organic carbon (TOC). Statistical analysis (one-way ANOVA, paired t-test descriptive statistics) revealed significant differences ($p < 0.05$) in growth response and water quality parameters except for tetracycline and for chloramphenicol which had no significant difference with regards the antibiotic concentrations with a percentage yield of ($P<.05$). Tetracycline exhibited a dose-dependent effect, promoting growth at 50% concentration but inhibiting it at higher levels, while chloramphenicol significantly promoted growth possibly due to the presence of proteisynthesis. The growth response of *Acutodesmus acutiformis* was revealed using an interval of 0 -14days. Meanwhile the result of the physicochemical parameters such as temperature, pH, TD and conductivity after been acted by antibiotic concentration of tetracycline and chloramphenicol were revealed in various upward and downward trend, it was observed that both antibiotics influenced algal growth.

CHAPTER ONE

INTRODUCTION

1.1 Antibiotics

Antibiotics are great invention to save millions of human beings from 1928. They are widely used in treatments of bacterial infection and play important roles in many fields such as medical industry and breeding industry, especially in aquaculture and animal husbandry (Kuemmerer, 2009, Valitalo *et al*., 2017). They are important pharmaceutical and personal care products (PPCPs) and some of them are relatively stable and can persist in surface water and even drinking water, raising concerns about their potential dangers (Chaturvedi *et al*., 2021).Among them, sulfonamides are the earliest category of synthetic drugs with a broad antibacterial spectrum, definite efficacy, convenience, and safety and are widely used in aquaculture; however, the removal rate of sulfonamides is low in the conventional wastewater treatment process (Zhang *et al*., 2019). Moreover, as a heavily used group of veterinary antibiotics, sulfonamides have high mobility and low sorption affinity in soil, making them more likely to leach into groundwater (Rath *et al*.,2019). This has led to a rise in the level of this contaminant in the water.

Antibiotics in wastewater and runoffs could find way into the aquatic ecosystem. This may impact either negatively on the algae present. A recent study shown that the growth of *Chlorella vulgaris* was inhibited with an increasing sulfadiazine concentrations(10–270 mg l−1), which may be related to reactive oxygen species damage to the algal photosynthetic system and chlorophyll biosynthesis. However, some microalgae can withstand antibiotics having the potential to remediate these antibiotics, thereby reducing their negative impacts to the aquatic environment. In a study by Xiong *et al*., the effect of sulfamethazine and sulfamethoxazole on *S.*

obliquus microalgae was investigated. The results indicated that *S. obliquus* could resist high doses of sulfamethazine and sulfamethoxazole, substantially impacting the biochemical features of *S. obliquus* (total chlorophyll, carotenoid, carbohydrate, and FAMEs).

Microalgae are primary producers and affect the structure and function of an aquatic ecosystem (Zhang *et al*., 2017). Microalgae have been considered to be sensitive to the ubiquitous microplastics and antibiotics and studies have been focused on the effects of single pollutants (Prata *et al*., 2018; Machado and Soares, 2019). Research on the combined toxicity of these two types of pollutants remains limited. For example, polystyrene (PS) microplastics influenced the removal of levofloxacin by affecting the adsorption, accumulation, and enzymatic breakdown of the antibiotic by Chlorella vulgaris. After three days, the levofloxacin removal rates for the microplastics group (35 items·L−1) and the control group were 23.34% and 46.71%, respectively. However, the combined toxic effects on microalgae have not been thoroughly explored (Wu *et al*., 2022).

1.2 Antibiotics and the environment

Antibiotics signify one of the notable discoveries of the last century that transformed the control of an extensive range of infections in a significant way. Nevertheless, their amplified consumption has exposed bacterial communities and ecologies to a large amount of antibiotic residue. The emergence of antibiotic deposits in the natural environment results from several patterns of antibiotic usage done to fight against bacterial infections and livestock production (Polianciuc *et al*., 2020).

While antibiotics play a significant part in bringing about recovery from a disease and the management of lingering health disorders both in recognized healthcare settings and in patients' homes (Piat *et al*., 2009), a vital factor in the safe utilization of antibiotic products is the appropriate disposal of unwanted antibiotic products (Chisholm *et al*., 2021) and their containers. When carried out appropriately, antibiotic waste handling can protect the atmospheric setting and prevent hazardous antibiotics from getting into the wrong hands (Chisholm *et al*., 2021, Breve *et al.*, 2022). It is necessary to understand what constitutes pharmaceutical products and antibiotic waste at large and their classification. The various sources of antibiotics waste in form of wastewater, runoff to the environments are hospitals, pharmaceutical manufacturing facilities, laboratories, agricultural and veterinary arena to mention but a few.

1.3 Impact of Antibiotic Waste Mismanagement on The Atmosphere

Antibiotics and their by-products are continuously released into the environment. Antibiotics pollution is initiated when partially degraded and undegraded antibiotics are released into the ecosystem. Bioremediation of this pollution is complex; hence, antibiotics with both narrow and broad spectrums have been found worldwide in various environmental samples (Thermo Fisher Scientific, 2023).The potentially harmful levels of antibiotic-resistant bacteria (ARB), antibiotic resistance genes (ARGs), and metal resistance genes (MGEs) present in municipal solid waste (MSW), which includes household, medical, agricultural, and other waste and their direct disposal in landfills or open dump locations without segregation or treatment is a significant problem (Sarpong and Miller, 2015). Landfills offer a favorable habitat for the growth of antimicrobial resistance (AMR) microorganisms, which in turn spread antibiotic resistance genes(ARGs) horizontally into bacterialstrainsin the surrounding environment (Anand *et al*., 2021).The general ecosystem, including air, soil, surface water, groundwater, animals, and public

health, are all negatively impacted by this (Wang *et al*., 2020). Greenhouse gases (carbon dioxide and methane) that trap heat in the atmosphere are produced in landfills by the decomposition of food leftovers and a sizable proportion of MSW. These gases have the most ability to cause global warming. In the worst circumstances, breathing landfill gas emissions repeatedly can result in tachycardia, exhaustion, nausea, vomiting, collapsing, and even death (Aluko *et al*., 2022).

1.4 Impact of Antibiotic Waste Mismanagement on Land and Animals (Terrestrial Environment)

There is proof that antibiotics exist in the terrestrial environment. Antibiotics are widely used for therapeutic, preventative/prophylactic, and growth-promoting purposes in the cattle and poultry sectors and human medicine. Although the practice of using antibiotics to stimulate the growth of livestock, hog-dogs, and poultry, as well as to increase the effectiveness of the feeding process, was outlawed in the EU in 2006, antibiotics are still used in India and China, particularly in the agricultural and livestock industries (Sharma *et al*., 2018).The use of antibiotics in agriculture improves the growth of animals, beekeeping, and fish farming, but it also contaminates the environment since leftover antibiotics and their metabolites are excreted in the waste of poultry animals. Antimicrobials are routinely and repeatedly released into the environment and natural ecosystem because of their use in agriculture, human health care, livestock, and animal welfare. Antibiotics have a substantial toxicity impact on both the non-target population and the target population (Gonzalez and Angeles, 2017). Recent investigations have found as many as 20 distinct antibiotic compound in stool samples from swine, poultry, and animal production facilities, and up to 90% are expelled without being digested (Grenni *et al*., 2018). While antibiotics used in crops and fish aquaculture can build up in the environment and increase the

pollutant concentration, antibiotics given to livestock can also be spread in fields via manure and leach into the soil and groundwater.

1.5 Impact of Antibiotic Waste Mismanagement on The Aquatic Ecosystem

Antibiotics can be found naturally in the environment, as is well known. Nonetheless, it is believed that man-made activities are the leading cause of these contaminants (Aminov, 2010). Utilization for treating and preventing bacterial infections in humans and animals stands out among these activities. Following administration, these medications are not entirely metabolized and absorbed by the human or animal body (Quaik *et al*., 2019). The non-metabolized substances are discarded through the home and hospital effluents. They can also be partially removed in water treatment facilities until they reach natural aquatic ecosystems such as rivers, lakes, seas, and groundwater (Patel *et al*., 2019). Antibiotic residues in surface water can potentially disrupt fundamental bacterial cycles, mechanisms, and processes essential to maintaining the balance of the aquatic ecosystem or that of the agricultural system and ensuring the production of healthy animals (Llor and Bjerrum, 2014).

1.6 Microalgae

Microalgae were one of the first organisms to come into existence in the Earth's ocean more than 3 billion years ago, when the Earth's environment formed. They are also called phytoplankton. Microalgae are microscopic algae rich in chlorophyll that lack lignin or cellulose and contain proteins. Microalgae are mainly found in freshwater and marine systems. They are unicellular species which exist individually or in chains or groups. Phycology or algology is a scientific study of basic knowledge of algae, continuously developed to applied phycology together with application of microalgae in many fields (Lee, 2008). Microalgae are capable of performing

photosynthesis; they produce approximately half of the atmospheric oxygen and use carbon dioxide to grow (Sharma *et al*., 2017).Microalgae species are estimated to be up to 10 million, but only 5% are well-described. It is highly diverse, and it occupies half of primary production on Earth. In addition, many studies demonstrate that besides the abiotic factors, microalgal biodiversity plays an important role in the establishment of productivity on natural ecosystems globally (Hani and Taufik, 2020).

Their diversity can be compared with the diversity of insects (Olasehinde *et al*., 2017; Demirel *et al*., 2017). Unlike heterotrophic microorganisms, which require various organic compounds for growth, unicellular photosynthetic organisms produce biomass from completely oxidized inorganic substances and mineral elements due to the light energy converted during photosynthesis (Vyacheslav *et al*., 2020). Microalgae is divided into 10 divisions, which consists of cyanophyta, chlorophyta, bacillariophyta, euglenophyta, phaeophyta, chrysophyta, crptophyta, pyrrophyta, xanthophyta, and Rhodophyta. These groups differ in terms of photosynthetic pigment compositions, photosynthates (storage polysaccharide), and plastid structures (John and Giordano, 2014; Lee, 2008; Solymosi, 2012).

Microalgae are rich in nutrients and biologically active substances, such as proteins, polysaccharides, lipids, polyunsaturated fatty acids, vitamins, pigments, phycobiliproteins, enzymes,etc. Biologically active substances from microalgae are capable of exhibiting antioxidant, antibacterial, antiviral, antitumor, regenerative, antihypertensive, neuroprotective and immunostimulating effects (Gürlek *et al*., 2019). These compounds are in demand in pharmacology, medicine, cosmetology, the chemical industry, fish farming, the energy industry, agriculture in the production of feed and functional foods (Bhattacharjee, 2016).

1.7 Botanical description of *Acutodesmus acutiformis*

Acutodesmus was previously classified as a subgenus of *Scenedesmus*. However, in the 2000s, molecular evidence showed that *Scenedesmus* was polyphyletic. As a consequence, it was reclassified into multiple genera, including the original subgenera *Scenedesmus, Desmodesmus,* and *Acutodesmus* (Turiel *et al*., 2021). Given that *Tetradesmus* species are closely related to those once placed in *Scenedesmus* subgenus *Acutodesmus, Acutodesmus* was promoted to a full genus, and *Tetradesmus* was treated as a synonym of *Acutodesmus* (Turiel *et al*., 2021).

Acutodesmus acutiformis, a species from the *Scenedesmaceae* family, is a type of freshwater green microalga commonly found in planktonic habitats (Guiry and Guiry, 2024). It has a small, cylindrical shape that is slightly elongated. The cells are usually arranged in a single line or in short chains. Each cell contains chlorophyll a and b, and is encased by a thin cell wall. The species is named "*acutiformis*," meaning "sharp-shaped" in Latin, due to its pointed, tapering ends. *Acutodesmus acutiformis* is commonly found in freshwater environments, such as ponds and lakes, where it can contribute to the phytoplankton community.

The alga primarily reproduces asexually through binary fission, though certain species within the genus may also reproduce sexually under specific circumstances. As with other green algae, it carries out photosynthesis, generating oxygen and organic compounds.

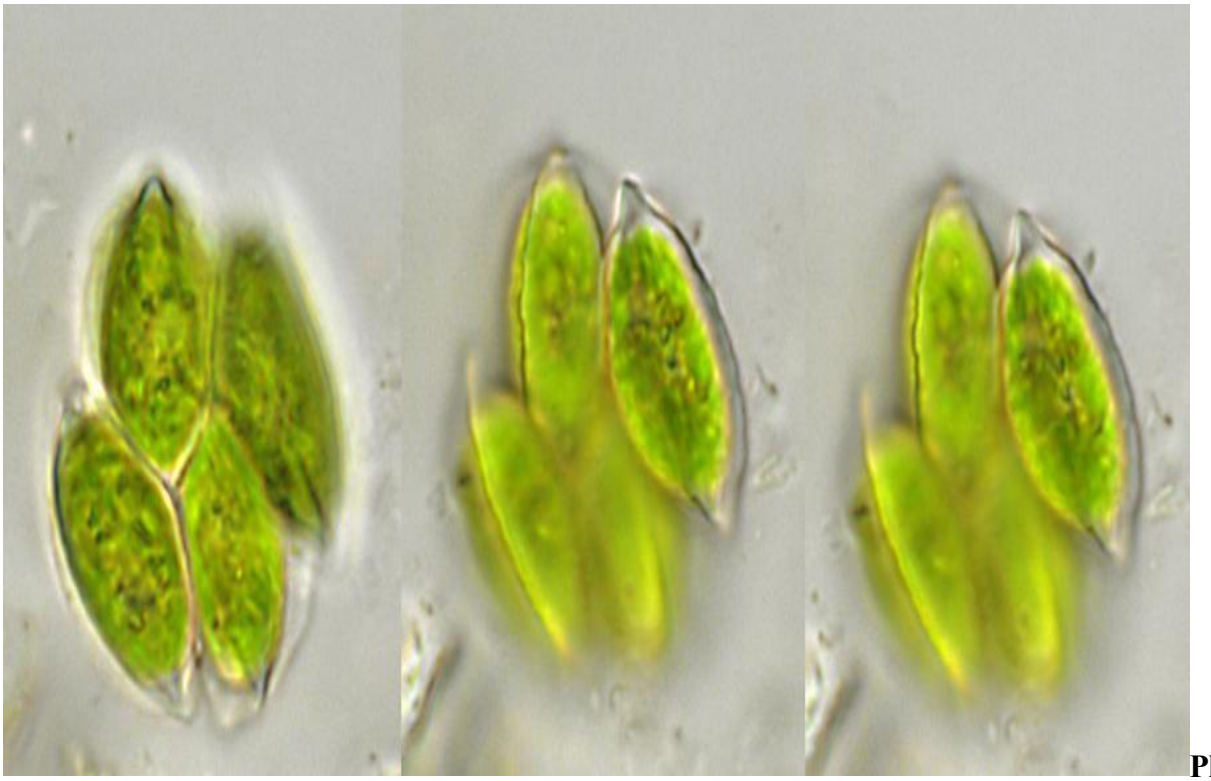

Plate 1: Microscopically viewed species of *Acutodesmus acutiformis*

1.8 Tetracycline and Chloramphenicol antibiotics

1.8. 1 Tetracyclines

Tetracyclines are a class of antibiotics characterized by a core structure featuring a 4-(dimethylamino)-1,10,11,12a-tetrahydroxy-3,12-dioxo-tetrahydrotetracene-2-carboxamide, with some variants incorporating additional groups such as chloro, methyl, hydroxyl, and dimethylamino. Examples include tetracycline, chlortetracycline, oxytetracycline, doxycycline, and minocycline. It has been reported that tetracycline and its derivatives can have an inhibitory effect on the growth of microalgae.

1.8. 2 Chloramphenicol

Chloramphenicol, along with thiamphenicol and florfenicol, belongs to the class of antibiotics known as phenicols. These antibiotics typically feature a core structure of 2,2-dichloro-N-[-1-hydroxy-1-(phenyl)propan-2-yl]acetamide, with some variants containing additional functional groups such as hydroxyl, nitro, methylsulfonyl, or fluoro (Krystian and Beata, 2019).

1.9 Literature review

Sharma *et al.*, 2021 carried out a research on the effects of antibiotics on aquatic environment and showed in their result that residual antibiotics in the aquatic environment can harm the non-target microalgae particularly cyanobacteria that share more structural and evolutionary similarities to bacteria), and lead to impaired primary production, which would endanger the entire ecosystem.

Leng *et al*., 2020 reported that the mechanisms of antibiotics removal in microalgae-based systems can be categorized into biodegradation, bioaccumulation, adsorption, photolysis,

hydrolysis, etc., although most studies on microalgae-based antibiotics removal focused much more on the removal performances than on the underlying mechanisms.

Xiong *et al*., 2021 investigated on the potential of microalgae in the bioremediation of antibiotics in the aquatic environment and found out that microalgae have high growth rates and show great potential in the efficient and environmentally-friendly removal of antibiotics.

Bashir and Cho, 2016 carried out a research on the inhibitory effects of Tetracycline (TET) on two green algae. The result showed that Tetracycline inhibited growth of *Dictyosphaerium pulchellum* and *Micractinium pusillum,* within a range of 5–30 mg/L. *Dictyosphaerium* was more sensitive towards tetracycline, with complete growth inhibition in the presence of ≥10 mg/L of this antibiotic. *Micractinium* was more resistant to tetracycline, with a ~50% inhibition at 20 mg/L.

Xiong *et al*., 2019 worked on the toxicity and inhibitory effects of Chloramphenicol (CAP) on different microalgae species in the aquatic environment and reported that Chloramphenicol (CAP) at different concentrations caused 50% inhibition/toxicity to different *Scenedesmus/Desmodesmus* strains (within 0.47–2.28 mg/L)] and *Pseudokirchneriella subcapitata* (at 2.7 mg/L).

Lai *et al*., 2009 evaluated the growth response of microalgae to Thiamphenicol (TAP).They reported that Thiamphenicol at different concentrations caused 50% growth inhibition to *Selenastrum capricornutum* (at 8.9 mg/L) , *Tetraselmis chuii* (at 38 mg/L), the haptophyte *Isochrysis galbana* (at 158 mg/L) and different *Chlorella* strains (within 522–1283 mg/L).

Carusso *et al.*, 2018 investigated the effects of Oxytetracycline (OXY) on two freshwater algae, Oxytetracycline within 0.17–4.5 mg/L, caused 50% growth inhibition/photosynthetic efficiency inhibition/toxicity to *Pseudokirchneriella subcapitata*/*Selenastrum capricornutum.* The result showed that Oxytetracycline (OXY) at concentrations ranging from 0.17 to 4.5 mg/L caused 50% inhibition of growth, photosynthetic efficiency, and toxicity in the freshwater algae *Pseudokirchneriella subcapitata* and *Selenastrum capricornutum.*

Halling-Sorensen, 2000 carried out research on the effects of streptomycin on the growth and photosynthetic efficiency of *Microcystis aeruginosa* and its comparative sensitivity to green microalgae. reported that for *Microcystis aeruginosa,* streptomycin at 0.007 mg/L caused 50% inhibition to *Microcystis* growth , and at 0.034 mg/L, inhibited photosynthetic efficiency by 50% in *Microcystis* culture . In both these studies, the *cyanobacterium* strain was more sensitive to STR than the green microalga strain.

Guo *et al*., 2016 carried out a research to evaluate the toxic effects of Trimethoprim on diatoms, Trimethoprim (TMP) inhibited by 50% the growth of *Phaeodactylum tricornutum* within 5.1–21.6 mg/L and *Navicula pelliculosa* (at 2.13 mg/L (7.36 µmol/L)).

Hena *et al.,* 2021 carried out research on the impact of antibiotics on microalgae, focusing on their role in inducing antibiotic resistance genes and promoting their diffusion in the environment and reported that the biological reactivity, refractory biodegradability, and accumulation characteristics of antibiotics, especially their selective resistance to microorganisms, may induce the production of antibiotic resistance genes, thereby accelerating the diffuse of resistance genes among microalgae in the environment.

Escher *et al*., 2010 investigated the effects of Oseltamivir ethylester (OE) on microalgae. Their study found that OE at a concentration of 6.4 g/L (15.5 mM) inhibited photosynthetic activity by 50% in *Desmodesmus subspicatus.* Additionally, in a separate study, OE at concentrations ranging from 210 to 352 mg/L caused 50% growth inhibition in *Pseudokirchneriella subcapitata.*

Xie *et al*., 2020 treatment method that could convert antibiotics into non-toxic compounds, offering a more effective and environmentally friendly alternative. The result indicated that microalgae has the feature of converting antibiotics into non-toxic carbondioxide and water when compared with other treating methods.

Zhou *et al*., 2022 reported of antibiotics that there is growing attention to the adverse influences of antibiotics in the water environment with antibiotics being widely used for preventing and treating human and animal diseases.

Havelkova *et al.*, 2016 researched on the inhibitory effects of antibiotics on microalgae and they reported Benzylpenicillin (penicillin G) contains a 6-[(2-phenylacetyl)amino] moiety in its penicillin structure and when at 7114 mg/L, it caused 50% toxicity to *Pseudokirchneriella subcapitata* growth.

Vasconcelos *et al*., 2017 carried out research on the inhibitory effects of Nitrofurantoin (NIT) on the growth and photosynthetic activity of Desmodesmus subspicatus and reported that Nitrofurantoin (NIT), within 12–17 mg/L, caused 50% growth inhibition of *Desmodesmus subspicatus*, but in another study, at 500 µg/L, caused 70% inhibition of photosynthetic activity in *Desmodesmus subspicatus* culture.

Duarte *et al*., 2019 carried out a research on the stimulatory effects of pharmaceuticals. *Phaeodactylum tricornutum* was reported to use bezafibrate (60 µg/L) as a carbon source to mixotrophically increase cell densitycompared to a photoautotrophic control.

Liu *et al*., 2016 carried out research on the stimulatory effects of low-concentration antibiotics on cyanobacterial growth, specifically the impact of amoxicillin on *Microcystis aeruginosa* and reported that some antibiotics at low concentrations stimulated cyanobacterial growth. Amoxicillin, at 100–300 ng/L, had a stimulatory effect on *Microcystis aeruginosa* growth.

Xiong *et al*., 2016 evaluated the effects of pharmaceuticals and personal care products on chlorophyll of microalgae. They reported that content and composition of pigments in microalgal cells can change in the presence of PHRs and PCPs. Exposure to carbamazepine (CBZ) at 50 mg/L led to an increase in chlorophyll (by 19%) and carotenoid (by 25%) content in *Chlamydomonas mexicana* cells.

Du *et al*., 2018 discovered in their research involving the effects of Pharmaceuticals on microalga, that the exposure to amoxicillin resulted in a ~22% decrease in chlorophyll a and carotenoid content in *Microcystis aeruginosa* biomass.

Liu *et al.,* 2011 reported that the content of Chlorophyll a in *Selenastrum capricornutum* cells decreased upon treatment with erythromycin (up to 0.3 mg/L), ciprofloxacin (up to 2.5 mg/L) or sulfamethoxazole (up to 2.5 mg/L), with the biggest drop (by up to ~50%) for erythromycin treatment (at 0.3 mg/L)

1.10 Aim and objectives

1.10.1 Aim

The aim of this study is to investigate the effects of the antibiotics Tetracycline and Chloramphenicol on the growth and development of *Acutodesmus acutiformis.*

1.10. 2 Objectives

The Specific Objectives of the study were to:

I. determine the growth response of *Acutodesmus acutiformis* to different concentrations of Tetracycline and Chloramphenicol.

II. determine the growth response of *Acutodesmus acutiformis* to different concentrations of Chloramphenicol

III. evaluate the phytoremediation potential of *Acutodesmus acutiformis.*

CHAPTER TWO

MATERIALS AND METHODS

2.1 Test Microalgae

The test Microalga used in the experiment was *Acutodesmus acutiformis.*

2.2 Source of microalgae

Acutodesmus acutiformis was the microalgae species used in this experiment. The freshwater algae sample was sourced from the Carolina Biological Supply Company, a scientific supplier located in North Carolina, USA.

2.3 Taxonomical classification of *Acutodesmus acutiformis* (Schröder) Tsarenko and John

Kingdom Plantae

Division Chlorophyta

Class Chlorophyceae

Order Sphaeropleales

Family Scenedesmaceae

Genus *Acutodesmus*

Species *acutiformis*

2.4 Culture vessels

The culture containers used in this study were 500 ml glass bottles. They were thoroughly cleaned with detergent and a diluted hydrochloric acid solution to remove any contaminants. The laboratory surfaces were disinfected by wiping them with cotton wool and acetone before placing the bottles on them. To prevent microbial contamination, the culture vessels were covered with cotton wool.

Plate 2: Culture vessels with varying concentrations of the antibiotics (Tetracycline and Chloramphenicol) and the microalga (*Acutodesmus acutiformis*).

2.5 Culture medium

The freshwater microalgae were cultured using CHU modified medium. The composition of CHU modified medium No. 10 is detailed in Table 1. To prepare a stock solution, dissolve the specified amount of salts (in grams) in 100 ml of distilled water. The iron solution was prepared by dissolving 5 grams of citric acid ($C_6H_8O_7 \cdot H_2O$) in 100 ml of distilled water, followed by the addition of 3.5 kg of ferric citrate ($FeC_6H_5O_3 \cdot 5H_2O$)

Table 1: Composition of the modified CHU

Salts/Nutrients	**g/100ml**
CaCl2.2H20	3.67
MgSO4.7H2O	3.69
NaHCO3	1.26
K2HPO4	0.87
NaNO3	8.5
Na2SiO3	2.84

Table 2: Trace elements composition of the modified CHU No.10 medium

TRACE ELEMENTS	**mg/L**
CuSO4.5H2O	19.6
ZnSO4.7H2O	44
CoCl.6H20	20
MnCl2. 4H2O	36
Na2Mo4. 2H2O	12.6
H2BO3	618.4
Iron stock	g/100ml
Citric acid(C6H8O7.H2O)	3.5
Ferric citrate (FeC6H5O3.5H2O)	3.5

Table 3: Composition of vitamin stock

SALTS	mg/L
Thiamine (vitamin B1)	0.004
Biotin (vitamin B7)	0.004
Cyanocobalamin (vitamin B12)	0.004

Table 4: Preparation of experimental mixture with tetracycline

Tetracyclines concentration (%)	Tetracycline volume (ml)	Culture volume (ml)	Distilled water (ml)	Total volume (ml)
0	0	5	295	300
5	5	5	290	300
10	10	5	285	300
20	20	5	275	300
30	30	5	265	300
40	40	5	255	300
50	50	5	245	300

Table 5: Preparation of experimental mixture with chloramphenicol

Chloramphenicol concentration (%)	Chloramphenicol Volume (ml)	Culture volume (ml)	Distilled water (ml)	Total volume (ml)
0	0	5	295	300
5	5	5	290	300
10	10	5	285	300
20	20	5	275	300
30	30	5	265	300
40	40	5	255	300
50	50	5	245	300

2.6 Experiment set up tier

The microalgae were grown in triplicate for 14 days in a solution with different concentrations (0%, 5%, 10%, 20%, 30%, 40%, 50%). Growth responses of the microalgae in each vessel were assessed using a visible spectrophotometer, with absorbance measured at 750 nm.

2.7 Inoculations

five (5) ml syringe was used to inoculate each container with 5 ml of microalgae culture. After inoculation, the vessels were covered with cotton wool to promote airflow, reduce evaporation, and prevent contamination. The culture vessels were then placed in the final-year laboratory of the Department of Plant Biology and Biotechnology at the University of Benin, chosen for its minimal exposure to direct sunlight.

2.8 Growth measurement and monitoring

After inoculation, growth was measured every two days over a two-week period using a spectrophotometer set to an absorbance wavelength of 750 nm.

Plate 3: 721 Spectrophotometer

2.9 Physicochemical analysis of culture

The culture sample was examined for its physical and chemical properties both prior to and following the experiment.

2.9.1 Temperature (°C)

Temperature was measured with the EZ-9909 pH/TDS/salinity/temperature/conductivity meter. The probe was immersed in the culture sample for five minutes before recording the temperature in degrees Celsius (°C), which was noted as 24°C.

2.9.2 Total dissolved solid (mg/L)

The Total Dissolved Solids (TDS) were measured using a pH/TDS/salinity/conductivity/temperature meter, model EZ-9909. The probe was placed in the culture containers for five minutes before recording the reading in parts per million (ppm).

2.9.3 Conductivity (µS/cm)

Conductivity was measured using a pH/TDS/salinity/conductivity/temperature meter, model W2-9909. The probe was immersed in the culture and allowed to stabilize for five minutes before taking the readings.

2.9. 4 pH

The pH values were measured using a pH meter. The meter was first calibrated with a buffer solution (pH = 4-7) at 25°C. The pH meter probe was then placed into the sample in a beaker, and the readings were recorded once the values stabilized.

2.9. 5 Turbidity (NTU)

Turbidity was assessed using a turbidity meter.

Plate 4: Conductivity Meter

Plate 5: Turbidity Meter

2.9.6 Dissolved Oxygen (mg/L)

Dissolved oxygen levels were measured using a DO 200 meter. The meter was immersed in the culture sample for five minutes before recording the reading in mg/L.

Plate 6: Dissolved Oxygen Meter

2.9.7 Chemical Oxygen Demand (COD) mg/L

The Chemical Oxygen Demand (COD) of the culture medium was assessed by mixing 1 ml of the culture sample with 9 ml of distilled water. The resulting solution was then placed in a TOC/COD meter for analysis. COD values were measured before and after the treatment in the experiment.

2.9.8 Total Organic Carbon (TOC) mg/L

The measurement of Total Organic Carbon (TOC) was conducted by first preparing an algal sample treated with antibiotics to prevent microbial interference. A TOC meter was then inserted into the sample solution, which consisted of the algal material and antibiotics. The meter measured the total carbon (TC) content, including both organic and inorganic carbon. The inorganic carbon (IC) content was subsequently determined by removing it from the system. The TOC was calculated by subtracting the inorganic carbon from the total carbon, and the result was recorded for analysis. This method enabled precise quantification of the organic carbon content in the sample.

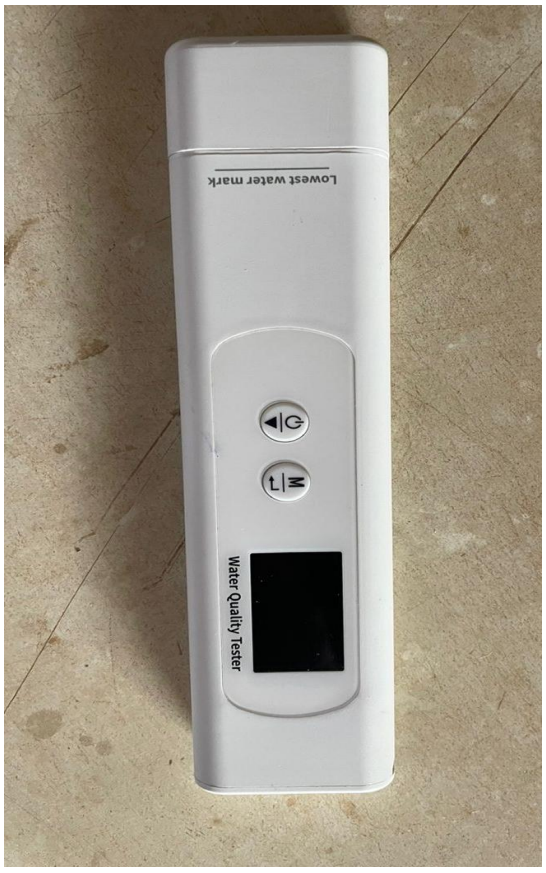

Plate 7: TOC/COD Meter

CHAPTER THREE

RESULTS

Figure 1 Shows growth response of *te,* to Tetracycline. The Stistical analysis showed one-way ANOVA revealed that there were significant differences ($p<0.05$) in the growth response of *Acutodesmus acutiformis* across different concentrations of Tetracycline throughout the experiment

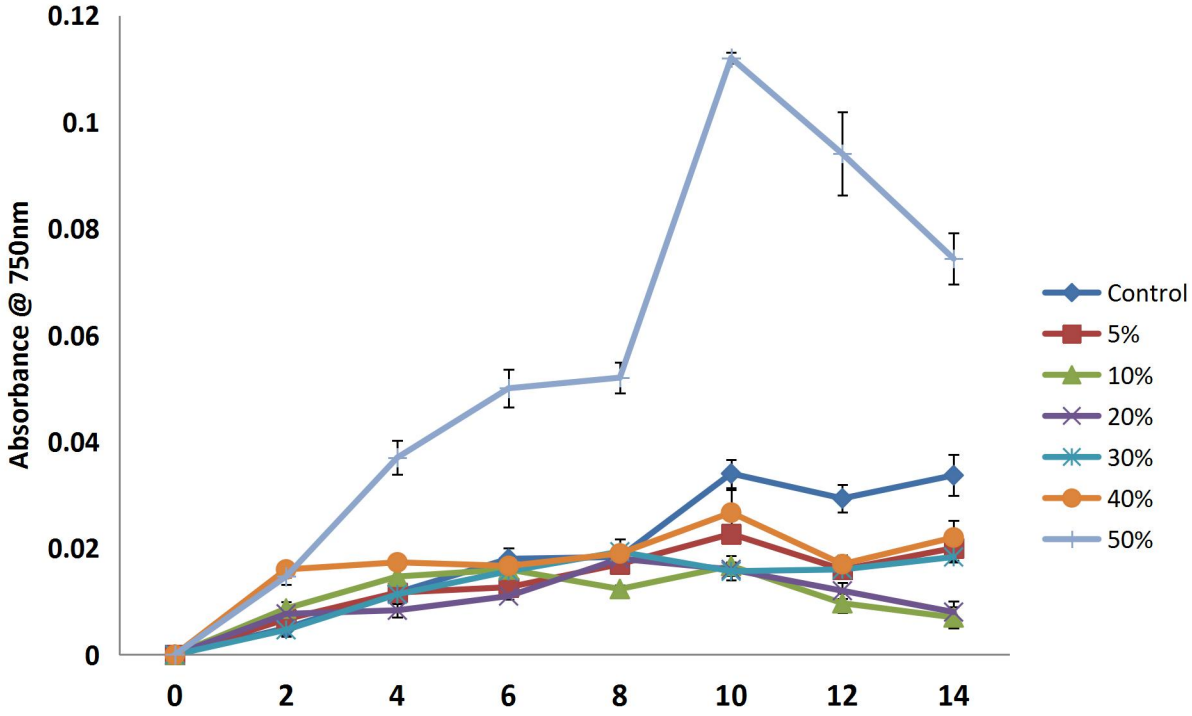

Figure 1: Effect of Tetracycline on the growth of *Acutodesmus acutiformis*

Figure two shows the growth response of Acutodesmus acutiformis to Chloramphenicol. Statistically, one-way ANOVA revealed that there were significant differences ($p<0.05$) in the growth response of *Acutodesmus acutiformis* across different concentrations of Chloraphenicol throughout the experiment.

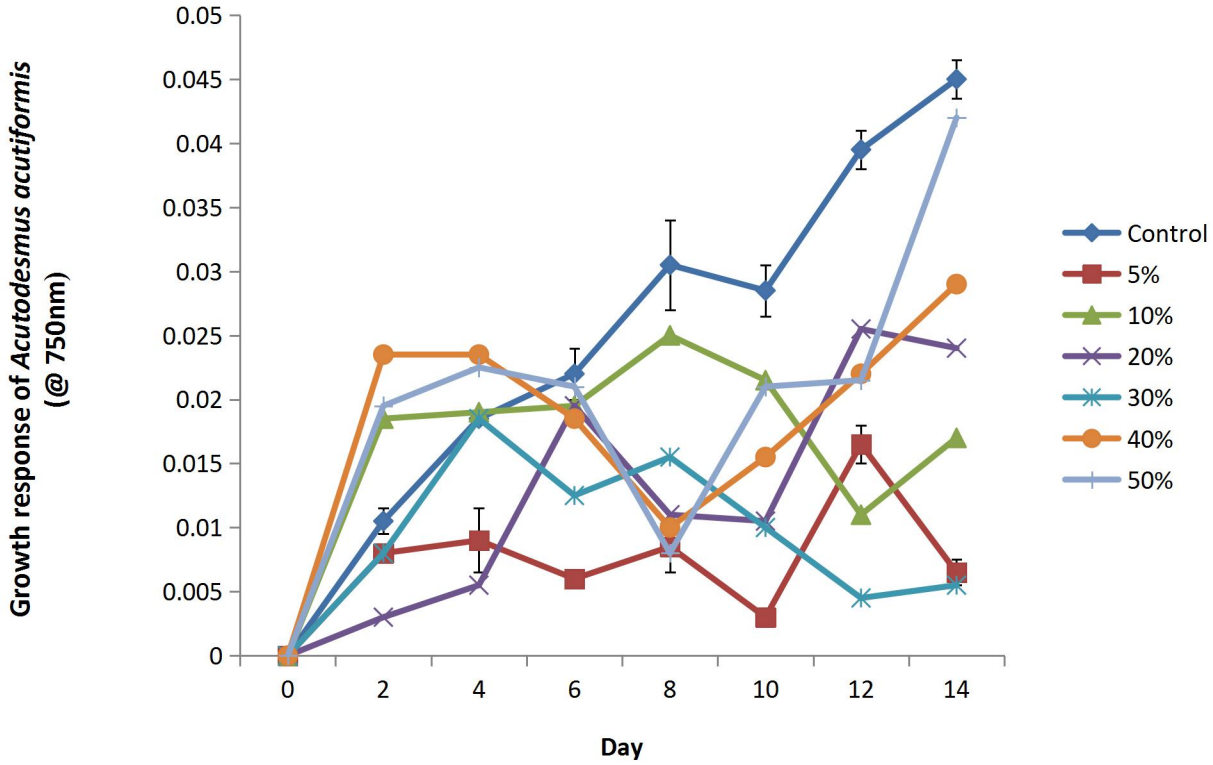

Figure 2: Effect of Chloramphenicol on the growth of *Acutodesmus actutiformis*

Figure 3 shows the percentage yield *Acutodesmus acutiformis* under the influence of Tetracycline and Chloramphenicol. The satistical analysis shown indicated the results of a paired samples t-test on the effect of different concentrations of Tetracycline and Chloraphenicol on the yield of *Acutodesmus acutiformis* showed that there were no significant differences $p > 0.05$ in yield.

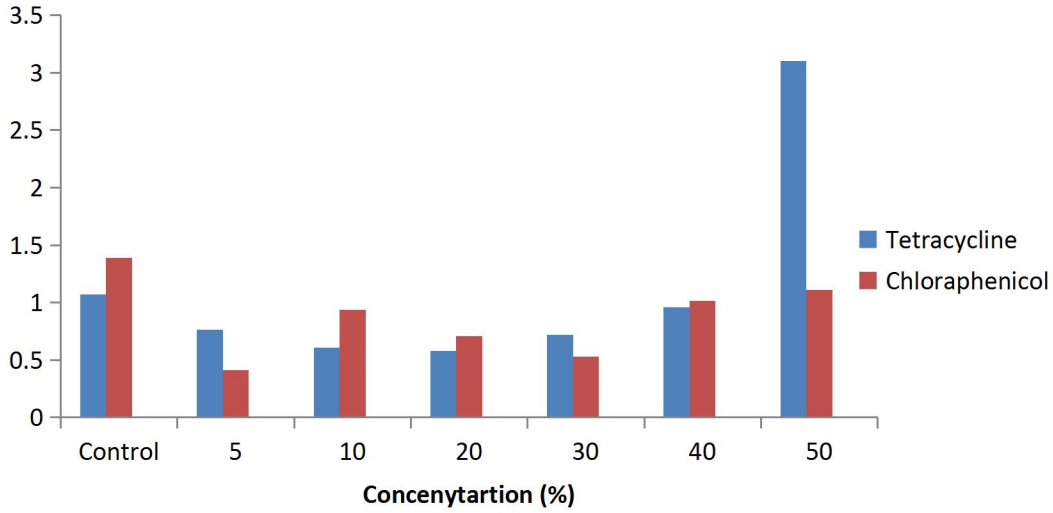

Figure 3: Percentage Yield of *Acutodesmus acutiformis* under the influence Tetracycline and Chloramphenicol on *Acutodesmus acutiformis*

Figure 4 shows temperature of different concentrations of Tetracycline on the growth of *Acutodesmus acutiformis.* Statistically, the one-way ANOVA showed that there were significant differences $p< 0.05$ between tetracycline temperature levels across each day of *Acutodesus acutiformis* growth.

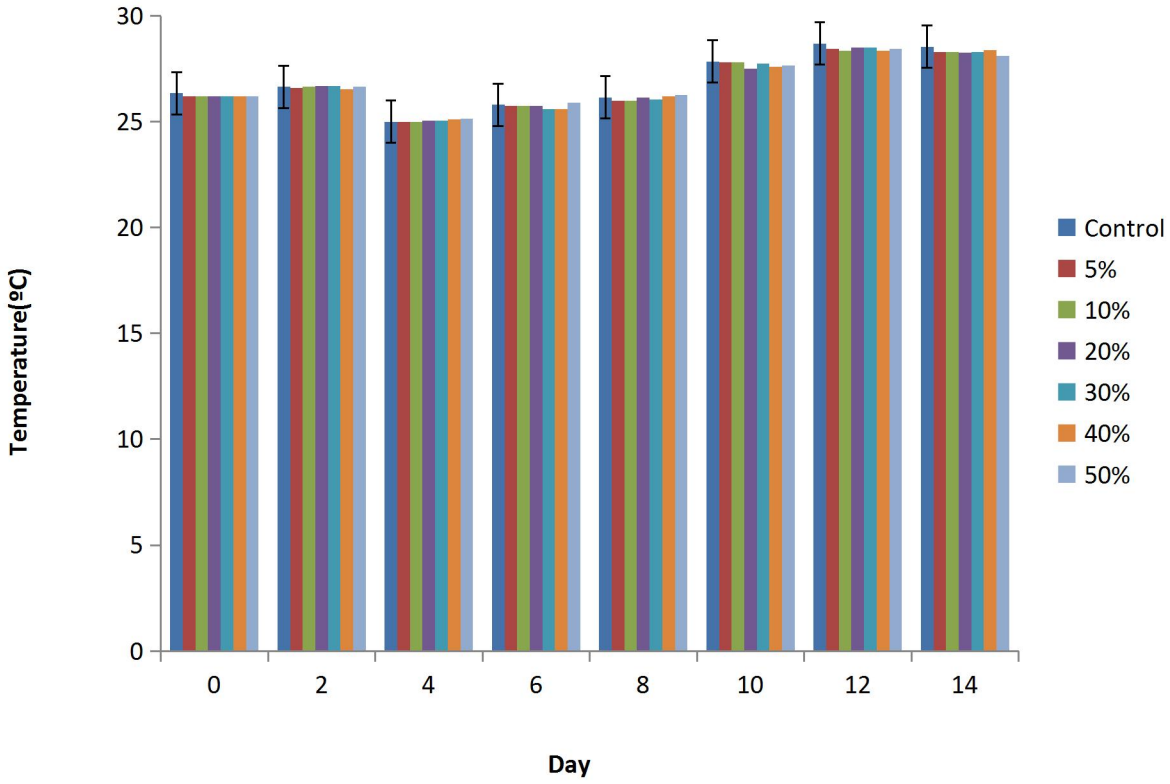

Figure 4: Temperature of different concentrations of Tetracycline on the growth of *Acutodesmus acutiformis*

Figure 5 shows the growth response to the temperature of different concentrations of Chloraphenicol. Statistically, the one-way ANOVA showed that there was no significant differences $p > 0.05$ between chloraphenicol temperature levels across each day of *Acutodesus acutiformis* growth.

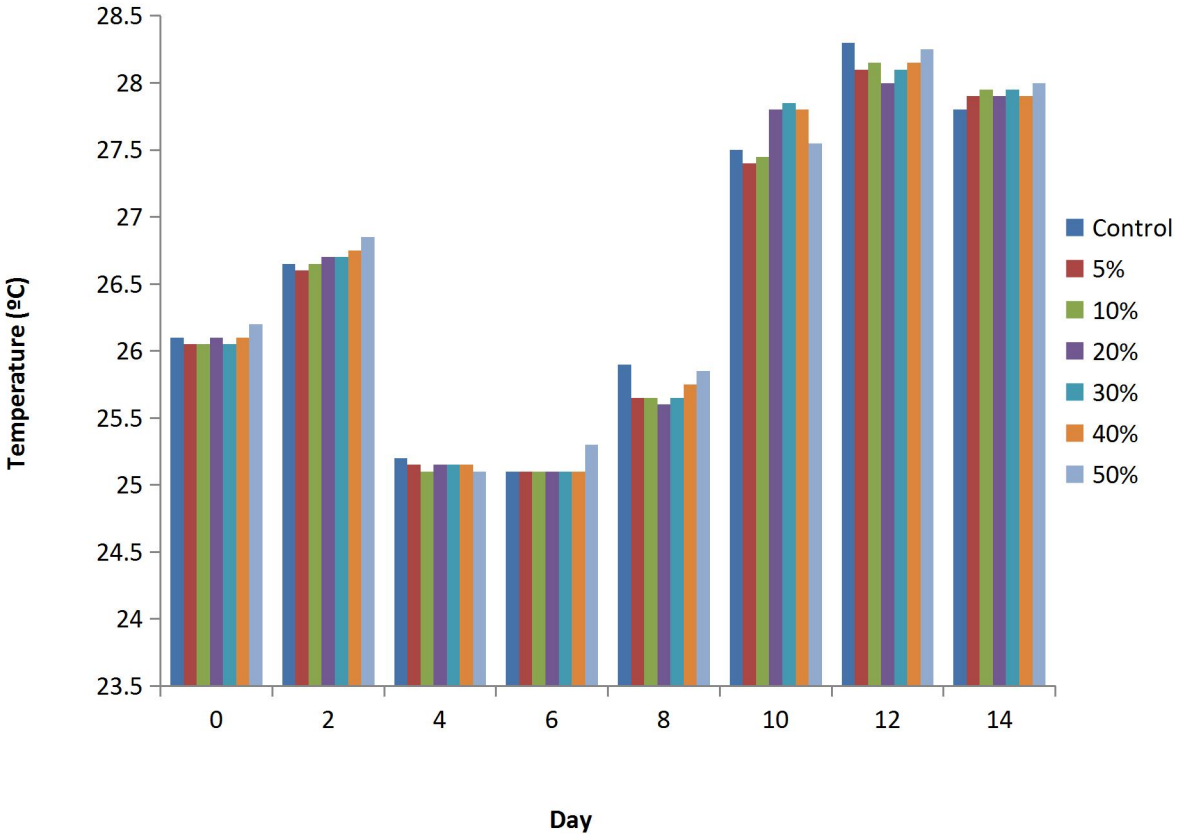

Figure 5: Temperature of different concentrations of Chloramphenicol on the growth of *Acutodesmus acutiformis*

Figure 6 shows the total dissolved solid of different concentrations of Tetracycline on the growth of *Acutodesmus acutiformis*. Statistically, one-way ANOVA showed that there were significant differences ($p < 0.05$) between tetracycline total dissolved solid levels across each day of *Acutodesus acutiformis* growth.

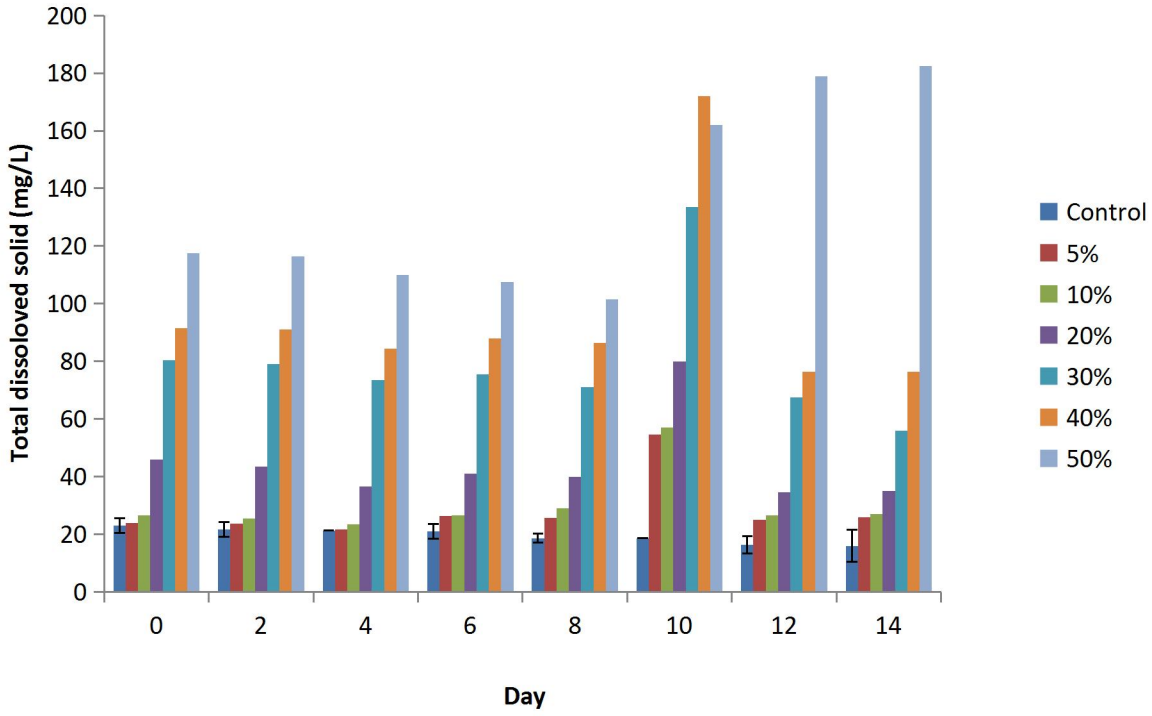

Figure 6: Total dissolved solid of different concentrations of Tetracycline on the growth of *Acutodesmus acutiformis*

Figure 7 shows the total dissolved solid of different concentrations of Chloraphenicol on the growth *Acutodesmus acutiformis* Statistically, one-way ANOVA showed that there were significant differences ($p < 0.05$) between chloraphenicol total dissolved solid levels across each day of *Acutodesus acutiformis* growth.

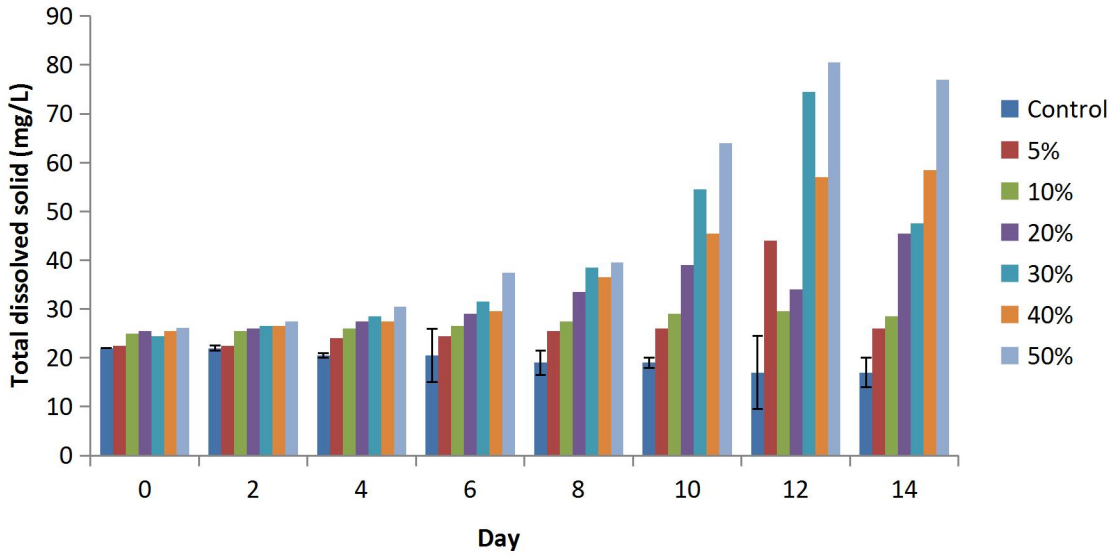

Figure 7: Total dissolved solid of different concentrations of Chloramphenicol on the growth of *Acutodesmus acutiformis*

Figure 8 shows the pH of different concentrations of Tetracycline on the growth of *Acutodesmus acutiformis.* Statistically, one-way ANOVA showed that there were significant differences ($p < 0.05$) between tetracycline pH levels across each day of *Acutodesus acutiformis* growth.

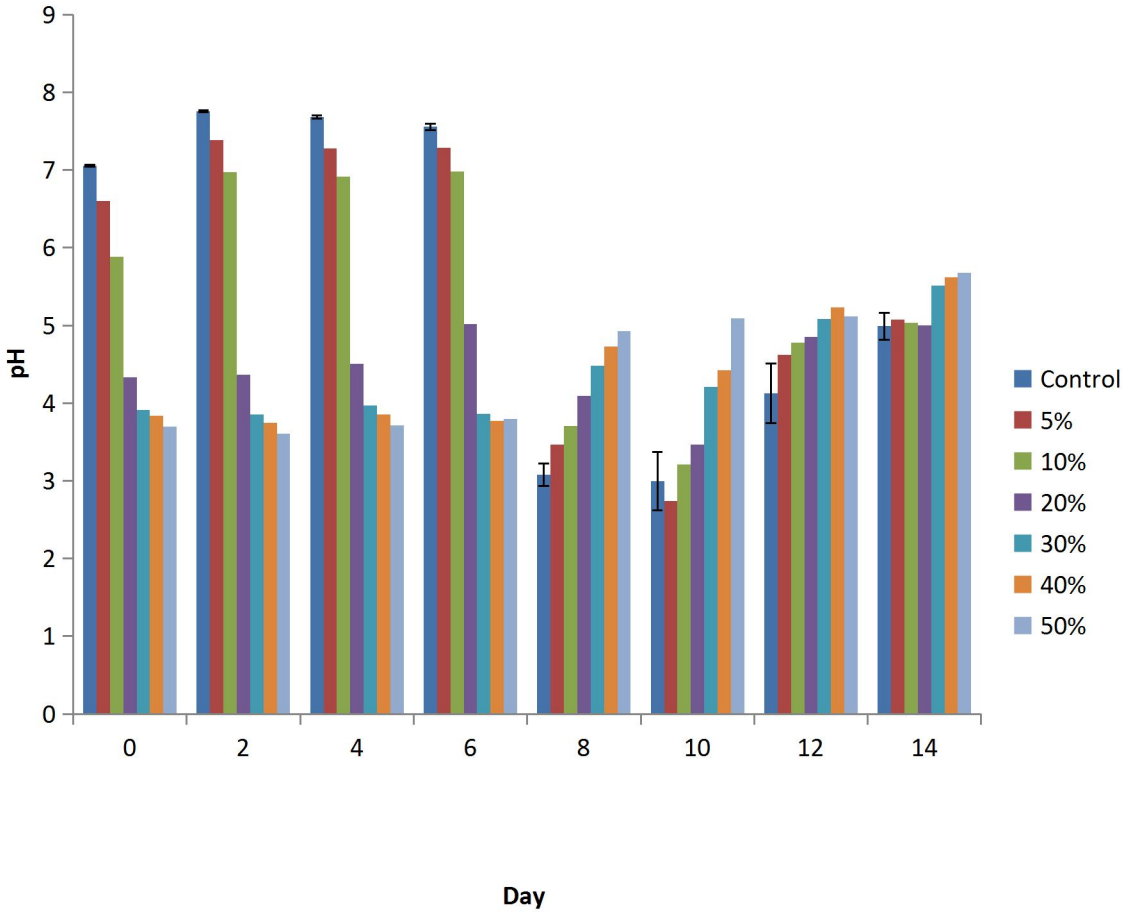

Figure 8: pH of different concentrations of Tetracycline l on the growth of*Acutodesmus acutiformis*

Figure 9 shows the different concentrations of Chloramphenicol on the growth of *Acutodesmus acutiformis.* Statistically, there were significant differences ($p < 0.05$) between chloraphenicol pH levels across each day of *Acutodesus acutiformis* growth.

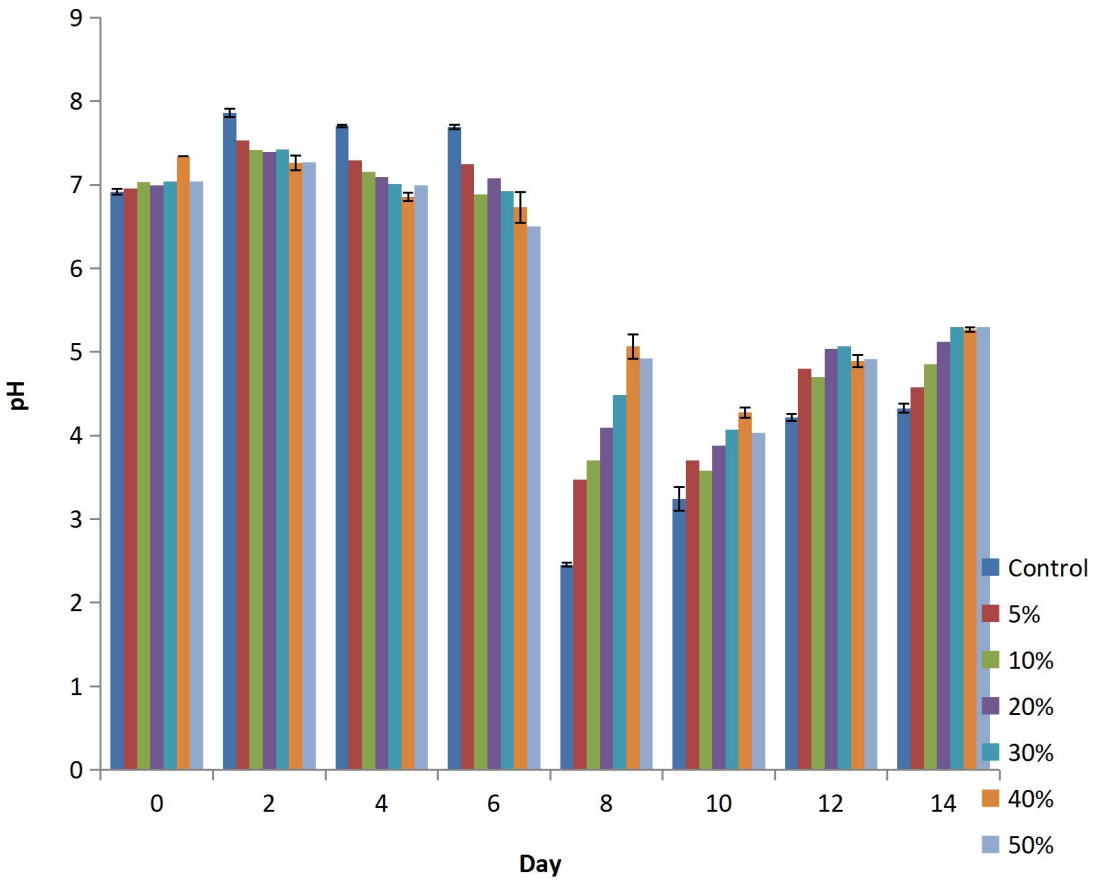

Figure 9: pH of different concentrations of Chloramphenicol on the growth of *Acutodesmus acutiformis*

Figure 10 shows the conductivity of different concentrations of Tetracycline on the growth of *Acutodesmus acutiformis*. The statistical analysis showed that there were significant differences ($p < 0.05$) in tetracycline conductivity levels across each day of *Acutodesus acutiformis* growth.

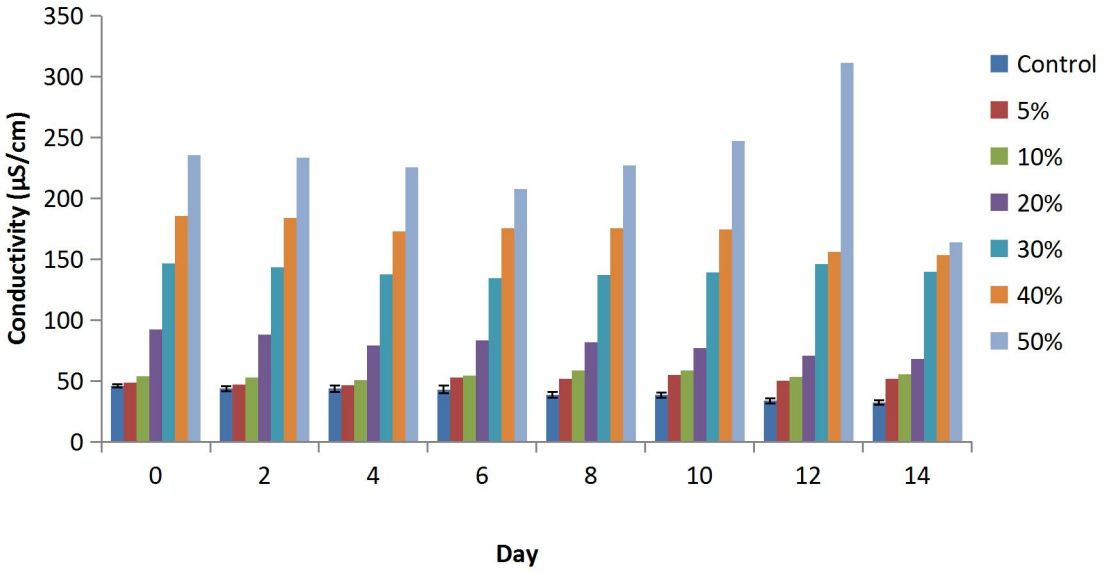

Figure 10: Conductivity of different concentrations of Tetracycline on the growth of *Acutodesmus acutiformis*

Figure 11 shows the conductivity of different concentrations of Chloraphenicol on the growth of *Acutodesmus acutiformis*. Statistically, there were significant differences ($p < 0.05$) in chloraphenicol conductivity levels across each day of *Acutodesus acutiformis* growth.

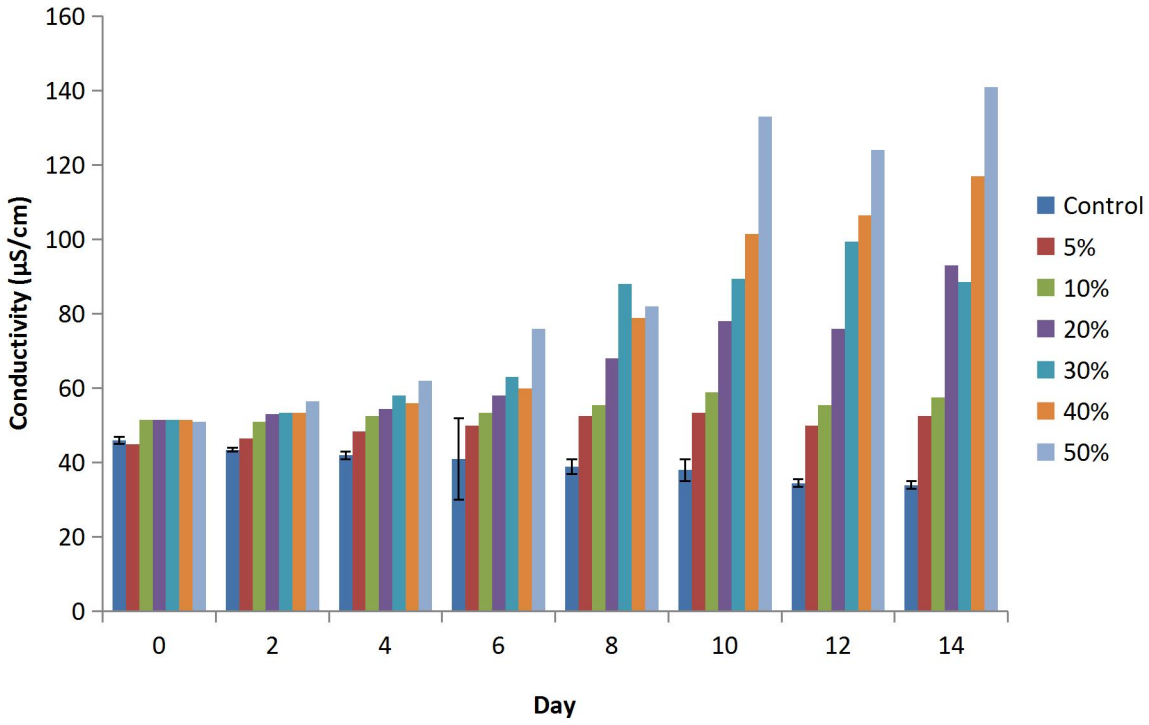

Figure 11: Conductivity of different concentrations of Chloramphenicol on the growth of *Acutodesmus acutiformis*

Figure 12 shows turbidity of different concentrations of Tetracycline on the growth of *Acutodesmus acutiformis*. Statistically, one-way ANOVA showed that there were significant differences ($p < 0.05$) in tetracycline turbidity levels across each day of *Acutodesus acutiformis* growth

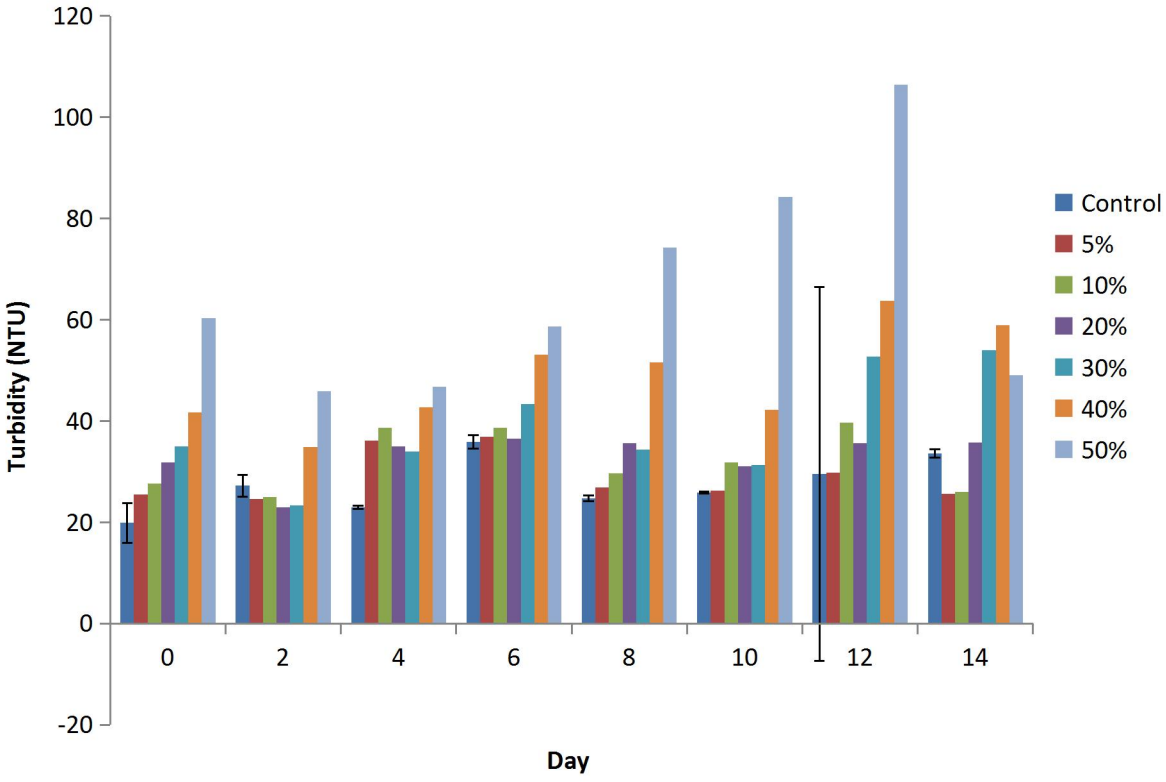

Figure 12: Turbidity of different concentrations of Tetracycline on the growth of *Acutodesmus acutiformis*

Figure 13 shows the turbidity of different concentrations of Chloraphenicol on the growth of *Acutodesmus acutiformis.* Statistically, the one-way ANOVA showed that there were significant differences ($p < 0.05$) in chloraphenicol turbidity levels across each day of *Acutodesus acutiformis* growth.

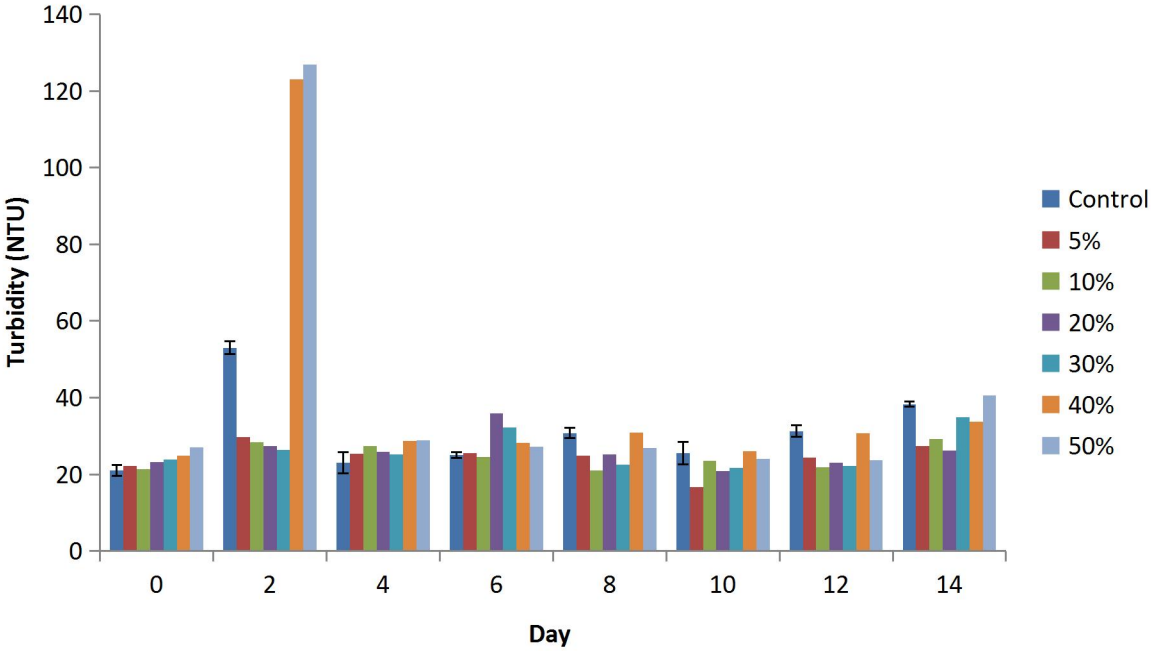

Figure 13: Turbidity of different concentrations of Chloramphenicol on the growth of *Acutodesmus acutiformis*

Figure 14 shows the dissolved oxygen of different concentrations of Tetracycline on the growth of *Acutodesmus acutiformis*. Statistically, the one-way ANOVA showed that there were significant differences ($p < 0.05$) in tetracycline dissolved oxygen levels across each day of *Acutodesus acutiformis* growth.

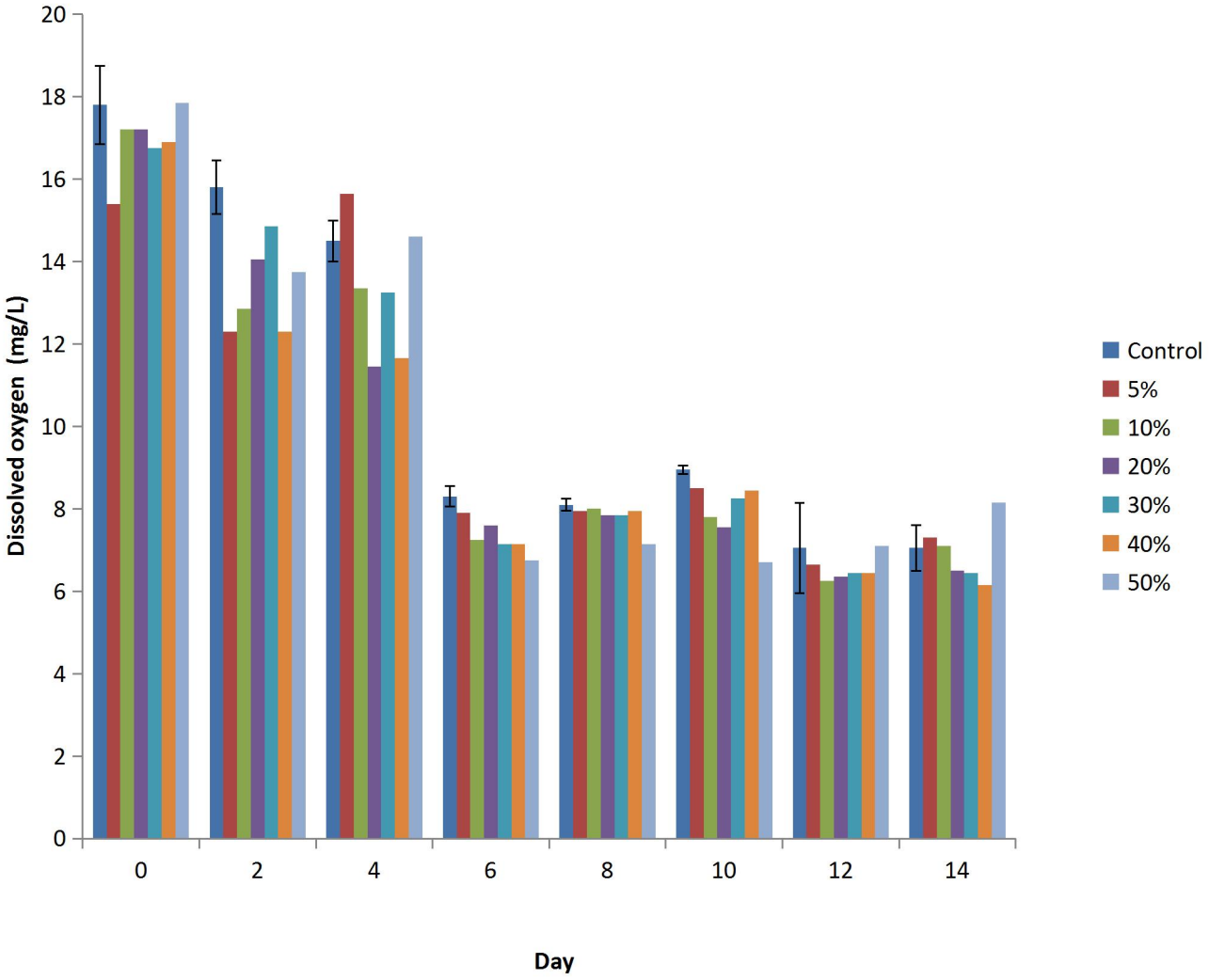

Figure14: Dissolved oxygen of different concentrations of Tetracycline on the growth of *Acutodesmus acutiformi*

Figure 15 shows the dissolved oxygen of different concentrations of Chloraphenicol on the growth of *Acutodesmus acutiformis*. Statistically, the one-way ANOVA showed that there were significant differences ($p < 0.05$) in chloraphenicol dissolved oxygen levels across each day of *Acutodesus acutiformis* growth

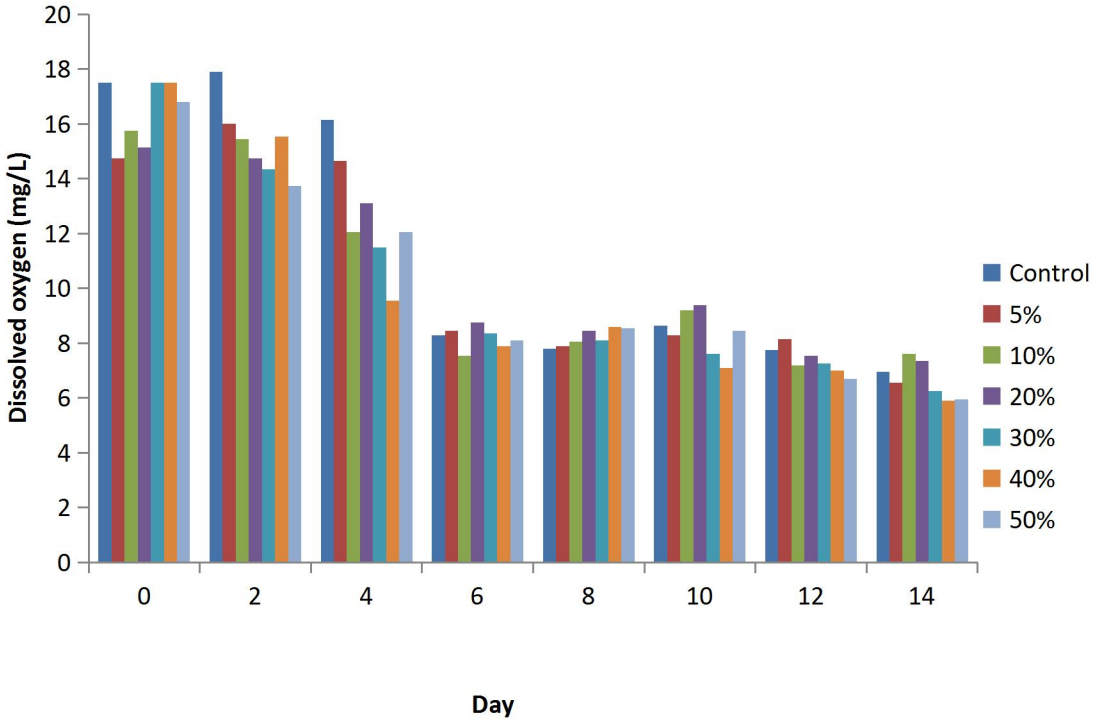

Figure 15: Dissolved oxygen of different concentrations of Chloramphenicol on the growth of *Acutodesmus acutiformis*

Figure 16 shows the chemical oxygen demand of Tetracycline on the growth of *Acutodesmus acutiformis.* Statistically, the one-way ANOVA showed that there were significant differences ($p < 0.05$) in tetracycline chemical oxygen demand levels across each day of *Acutodesus acutiformis* growth

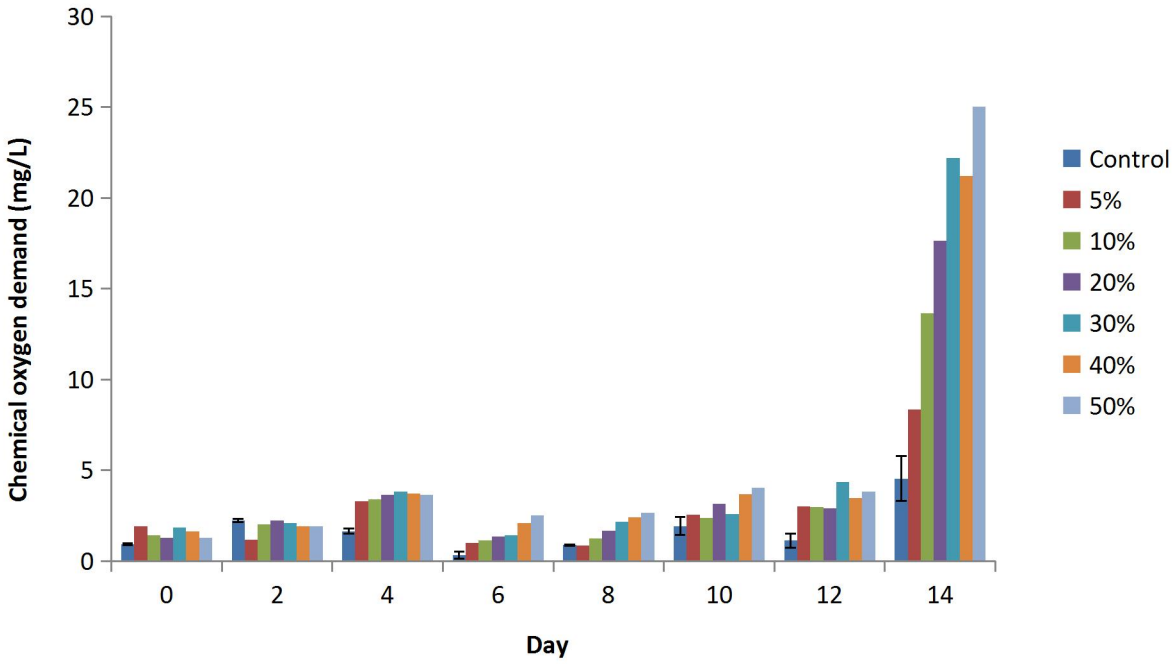

Figure 16: Chemical oxygen demand of different concentrations of Tetracycline on the growth of *Acutodesmus acutiformis*

Figure 17 shows chemical oxygen demand of different concentrations of Chloraphenicol on the growth of *Acutodesmus acutiformis.* Statistically, the one-way ANOVA showed that there were significant differences ($p < 0.05$) in chloraphenicol chemical oxygen demand levels across each day of *Acutodesus acutiformis* growth

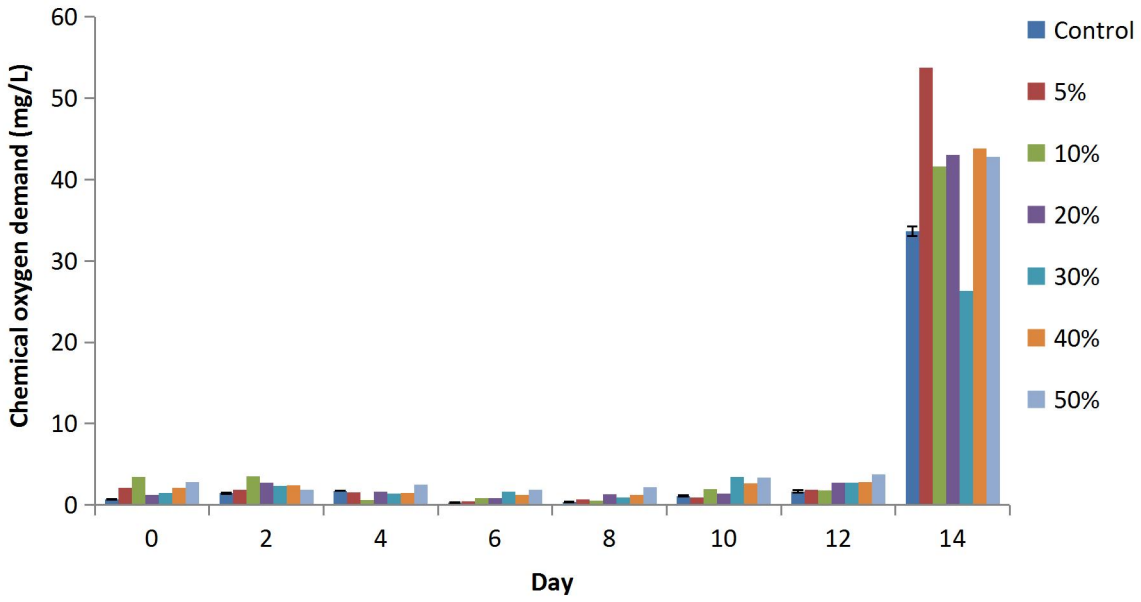

Figure 17: Chemical oxygen demand of different concentrations of Chloramphenicol on the growth of *Acutodesmus acutiformis*

Figure 18 shows the total organic carbon of different concentrations of Tetracycline on the growth of *Acutodesmus acutiformis*. Statistically, the one-way ANOVA showed that there were significant differences ($p < 0.05$) in tetracycline total organic carbon levels across each day of *Acutodesus acutiformis* growth

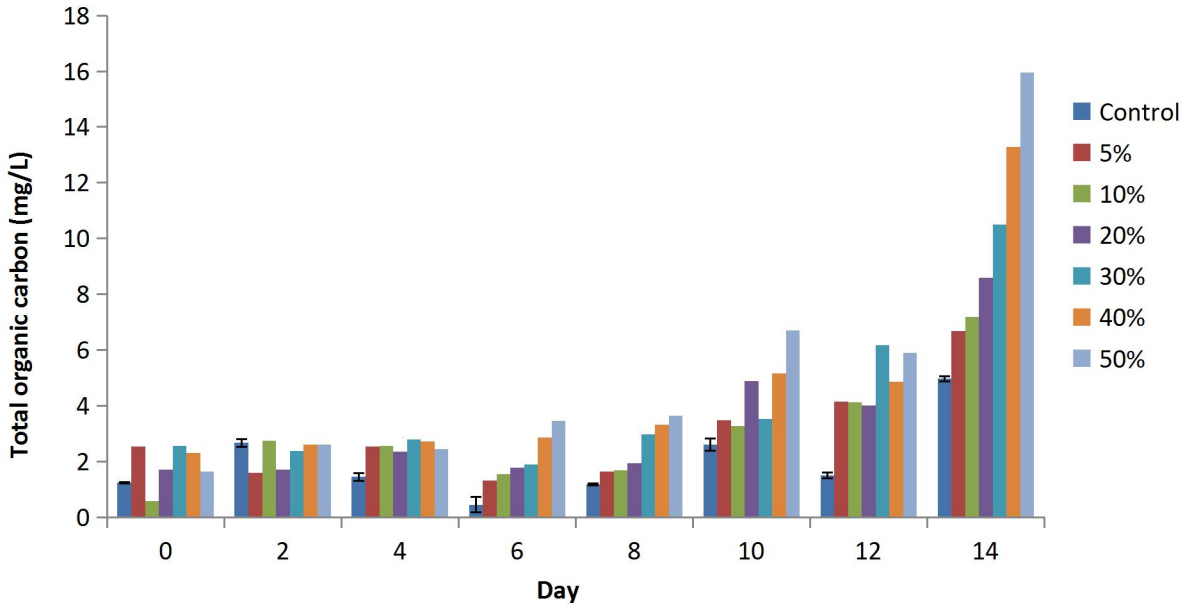

Figure 18: Total organic carbon of different concentrations of Tetracycline on the growth of *Acutodesmus acutiformis*

Figure 19 shows the total organic carbon of different concentrations of Chloraphenicol on the growth of *Acutodesmus acutiformis*. Statistically, one-way ANOVA showed that there were significant differences ($p < 0.05$) in chloraphenicol total organic carbon levels across each day of *Acutodesus acutiformis* growth.

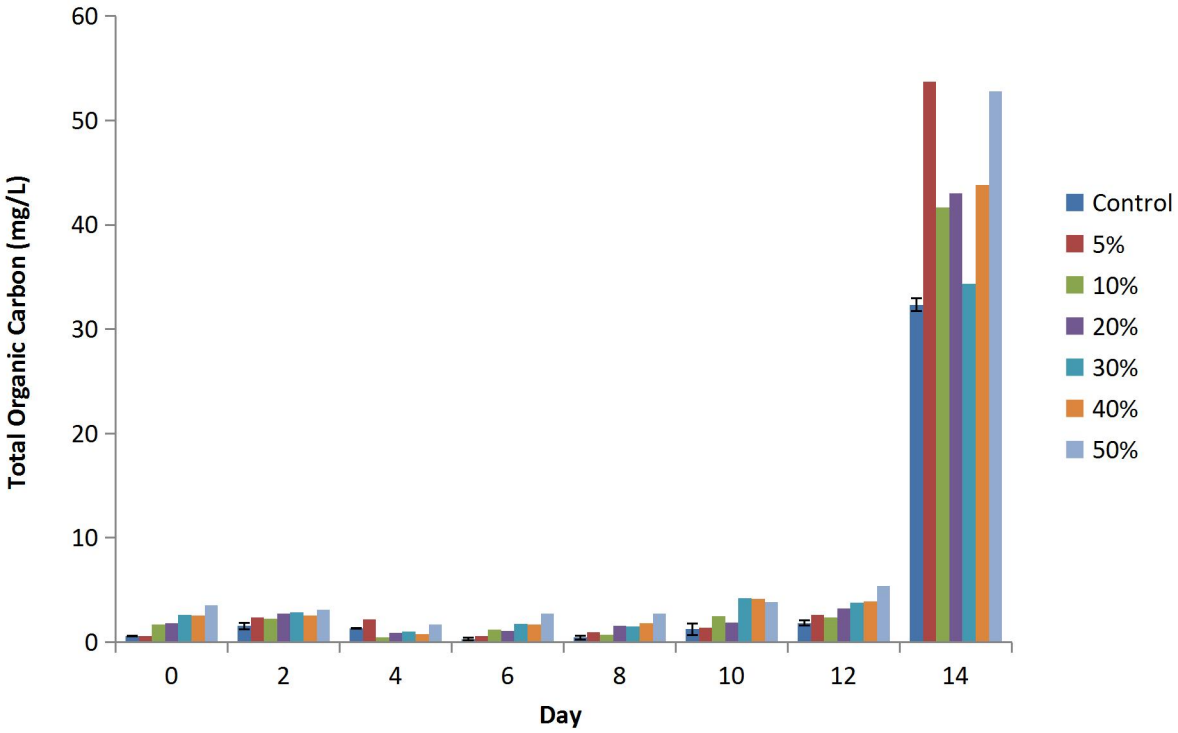

Figure 19: Total organic carbon of different concentrations of Chloramphenicol onthe growth of *Acutodesmus acutiformis*

CHAPTER FOUR

DISCUSSION

This study aimed to evaluate the effect of two antibiotics: Tetracycline and Chloramphenicol, on the growth i of *Acutodesmus acutiformis,* a freshwater microalga species.

The notable variations in the growth of *Acutodesmus acutiformis* at varying concentrations of both Tetracycline and Chloramphenicol (Figures 1 and 2) suggest that these antibiotics influence algal growth at different levels.

The findings for tetracycline revealed that the 50% treatment of *Acutodesmus acutiformis* yielded the greatest growth. This aligns with the research of Yernar *et al*. (2023), which highlighted that tetracycline influence algal populations by restricting the development of various microalgal species at elevated levels. Similarly, Tang *et al.* (2022) explored the influence of tetracycline on the freshwater microalga *Chlorella pyrenoidosa*. Their results support this observation, showing that tetracycline significantly reduced algal expansion, reduced pigment concentration, and lowered photosynthetic performance in a dose-dependent fashion. Specifically, when concentrations increased, growth inhibition, chlorophyll decline, and decreased efficiency in photosynthesis reached 49.68%, 62.54%, and 48.08%, respectively. These results suggest that excessive tetracycline levels pose a serious threat to microalgal ecosystems.

Temperature plays a vital role in microalgal metabolism by regulating enzyme function, nutrient absorption, and photosynthetic activity. Favorable temperature conditions enhance enzymatic efficiency and cellular processes, whereas deviations may induce metabolic strain.

The growth pattern of *Acutodesmus acutiformis* varies with temperature across different tetracycline concentrations. Results demonstrated an increasing growth trend daily, with peaks on days 10, 12, and 14. Studies on comparable microalgae, such as *Scenedesmus acutus,* have

recorded optimum growth at 30°C, with substantial biomass accumulation at both 25°C and 30°C (Abomohra *et al*., 2013). The findings in this research align with these observations, as peak growth occurred between 26°C and 28°C. However, temperatures surpassing 30°C were linked to reduced biomass and slower growth rates (Abomohra *et al*., 2013). Similarly, Ishikawa-Ishiwata *et al*. (2023) explored Desmodesmus species, a thermotolerant green alga, and noted that specific growth rates were highest between 20°C and 35°C but dropped considerably at 40°C. The temperature range observed in this study corresponds to these findings.

Rico *et al*. (2023) investigated *Scenedesmus obliquus* under various antibiotic concentrations and temperature conditions. Their research showed that Photosystem II efficiency remained relatively stable between 20°C and 30°C, while growth rates based on chlorophyll-a content were negatively impacted at elevated temperatures. These findings highlight how temperature can intensify the inhibitory effects of antibiotics on microalgal development.

Total dissolved solids (TDS), comprising inorganic salts and minor organic matter in water, significantly influence microalgal growth. In the cultivation of *Acutodesmus acutiformis,* varying concentrations of tetracycline affected total dissolved solid levels, which in turn impacted algal development. Notably, optimal growth occurred at 50% tetracycline treatment, particularly on days 12 and 14, suggesting a complex interplay between antibiotic concentration and total dissolved solid (TDS) that potentially affects nutrient availability and cellular processes.

Similarly, different concentrations of chloramphenicol altered the total dissolved solid (TDS), influencing the growth response of *Acutodesmus acutiformis.* The highest yield was observed at 50% treatment, while the lowest occurred in the control group (0%).

The pH of the culture medium significantly influenced the growth response of *Acutodesmus acutiformis* under antibiotic exposure. Antibiotic-induced stress triggered metabolic shifts that altered pH levels and, consequently, growth patterns. In cultures treated with tetracycline, pH gradually declined, particularly on days 8 and 10, correlating with reduced algal growth. This trend aligned with Frascaroli *et al*. (2024), who observed similar pH reductions in *Auxenochlorella protothecoides*, *Tetradesmus obliquus*, and *Chlamydomonas acidophila* exposed to antibiotics. Their findings suggested that antibiotic stress shifted algal metabolism towards heterotrophic activity, increasing CO_2 production and lowering pH. These pH fluctuations not only affected growth but also influenced the algae's ability to remove antibiotics from wastewater.

The growth response of *Acutodesmus acutiformis* to pH levels in varying concentrations of chloramphenicol initially increased from day 1 but later declined, particularly on day 10. The initial rise in growth suggested that *Acutodesmus acutiformis* exhibited some level of tolerance or partial adaptation to the antibiotic stress during the early phase. However, the subsequent decline indicated that prolonged exposure to chloramphenicol and pH fluctuations negatively impacted cellular metabolism. These observations were consistent with the findings of Miazek and Brozek-Pluska (2019), who reviewed the effects of pharmaceuticals and personal care products on microalgal growth and metabolism. Their study reported that antibiotics inhibited microalgal growth by inducing oxidative stress and altering enzyme activities, both of which were sensitive to pH variations. They emphasized that pH played a crucial role in modulating the toxicity of antibiotics to microalgae.

Furthermore, a review by Eguchi *et al.* (2012) highlighted that low, sublethal concentrations of antibiotics can lead to stimulation of growth and photosynthesis in microalgae, which could

result in fluctuations in parameters such as conductivity due to changes in metabolic activity and nutrient uptake.

The observed growth pattern of *Acutodesmus acutiformis* under conductivity of varying concentrations of chloramphenicol—initial growth reduction followed by an increase, peaking on day 14 at 50% treatment—suggests a complex interaction between the antibiotic and the microalga's physiological processes. The subsequent increase in growth observed in *Acutodesmus acutiformis* after the initial decline may be attributed to the microalga's adaptive responses to prolonged antibiotic exposure. Microalgae can develop mechanisms to mitigate antibiotic-induced stress, such as activating efflux pumps, modifying antibiotic targets, or enhancing repair systems. These adaptive responses could lead to a recovery and eventual increase in growth rates over time. This result is consistent with research by Andrade *et al*. (2017), who examined the impact of chloramphenicol on various microalgal strains. Their findings revealed that all tested strains exhibited lower growth rates in the presence of chloramphenicol compared to control cultures without the antibiotic. This supports the observation of an initial growth reduction in *Acutodesmus acutiformis* upon chloramphenicol exposure.

The result agreed with the study by Yang and Yee (2022) in which a method was developed to rapidly assess microalgal concentration using turbidity measurements. They established linear relationships between turbidity and cell concentration for various microalgal species, highlighting the utility of turbidity as an indicator of algal growth. However, they also noted that factors such as cell size, shape, and the presence of suspended particles can affect turbidity readings, potentially leading to fluctuations in measured values.

The observed growth pattern of under turbidity of varying concentrations of chloramphenicol—exhibiting maximum growth at 50% treatment on day 2, followed by a uniform reduction from days 4 to 14—suggests an initial adaptive response to the antibiotic, succeeded by inhibitory effects over prolonged exposure. A research by Andrade *et al*. (2017) examined the impact of chloramphenicol on various microalgal strains. Their findings revealed that all tested strains exhibited lower growth rates impacted by turbidity in the presence of chloramphenicol compared to control cultures without the antibiotic. This supports the observation of an initial growth reduction in *Acutodesmus acutiformis* upon chloramphenicol exposure.

In cultures with different tetracycline concentrations, the highest growth yield was recorded in the control group (0% tetracycline). However, a decline was observed from the first day, with the lowest growth occurring on day 12. This pattern suggested that tetracycline negatively impacted *Acutodesmus acutiformis,* likely by affecting its photosynthetic capacity and oxygen generation. Since photosynthesis is the primary contributor to dissolved oxygen (D O) in microalgal environments, the reduction in growth indicated that tetracycline interfered with the photosynthetic system, leading to decreased oxygen release.

Similarly, in cultures containing varying chloramphenicol levels, maximum dissolved oxygen (DO) levels were linked to the highest growth yield, which was observed on day 2 in the control group (0% chloramphenicol). However, a gradual decline in growth and DO levels began from day 6, reaching their lowest values by day 14. This suggested that prolonged chloramphenicol exposure impaired microalgal metabolism and oxygen production.

Tetracycline and chloramphenicol exhibited similar effects on the growth of *Acutodesmus acutiformis* concerning total organic carbon (TOC). The peak growth yield at 50%

concentrations of both antibiotics on day 14 indicates that the microalga may have adapted to their presence, possibly utilizing them as alternative carbon sources. This adaptation may involve the breakdown of these antibiotics through biodegradation, resulting in an increase in TOC within the culture medium. The microalga could then assimilate this organic carbon to sustain its growth. Zhou *et al.* (2022) supported this finding, reporting that microalgae have the ability to metabolize antibiotics, leading to their degradation and potential use as carbon sources.

CONCLUSION

The growth responses of *Acutodesmus acutiformis* to two antibiotics, tetracycline and chloramphenicol, were examined in this study using different concentrations. At the end of the experiment, it was observed that both antibiotics significantly influenced algal growth, with varying effects across concentrations. These changes suggest a potential ecological impact in aquatic environments where such antibiotics persist. Additionally, the study highlights the need for further research into the long-term effects of sub-lethal antibiotic exposure on algal communities and their potential for bioaccumulation.

REFERENCES

Aluko, O. O., Obafemi, T. H., Obiajunwa, P. O., Obiajunwa, C. J., Obisanya, O. A. *et al.* (2022). Solid waste management and health hazards associated with residence around open dumpsites in heterogeneous urban settlements in Southwest Nigeria. *International Journal of Environmental Health Research,* **32**: 1313 - 1328.

Aminov, R. I. (2010). A brief history of the antibiotic era: Lessons learned and challenges for the future. *Frontiers in Microbiology*, **1:** 134.

Anand, U., Reddy, B., Singh, V. K., Singh, A. K., Kesari, K. K. *et al.* (2021). Potential environmental and human health risks caused by antibiotic-resistant bacteria, antibiotic resistance genes and emerging contaminants from municipal solid waste landfill. *Antibiotics*, **10:** 374.

Bashir, K. M. I. and Cho M. G. (2016). The effect of kanamycin and tetracycline on growth and photosynthetic activity of two chlorophyte algae. *Biomedical Research International*, **8:** 45 - 54.

Bashir, K. M. I. and Cho, M. G. (2016). The effect of kanamycin and tetracycline on growth and photosynthetic activity of two chlorophyte algae. *Biomedical Research International*, **56:** 56 - 92.

Bhattacharjee, M. (2016). Pharmaceutically valuable bioactive compounds of algae. *Asian Journal of Pharmaceutical and Clinical Research*, **7:** 43 – 47.

Borecka, M., Białk-Bielinska, A., Halinski, Ł. P., Pazdro, K., Stepnowski, P. *et al*. (2016). The influence of salinity on the toxicity of selected sulfonamides and trimethoprim towards the green algae Chlorella vulgaris. *Journal of Hazardous Materials,* **308:** 179 – 186.

Breve, F., LeQuang, J. A. and Batastini, L. (2022). Controlled substance waste: Concerns, controversies, solutions. *Cureus*, **14**: e22564.

Cahill, N., O'Connor, L., Mahon, B., Varley, A., McGrath, E. *et al.* (2019). Hospital effluent: a reservoir for carbapenemase-producing Enterobacterales? *Science of the Total Environment*, **672:** 618 - 624.

Campa-Cordova, A. I., Luna-Gonzalez, A., Ascencio, F., Cortes-Jacinto, E. and Caceres-Martinez, C. J. (2006). Effects of chloramphenicol, erythromycin, and furazolidone on growth of *Isochrysis galbana* and *Chaetoceros gracilis. Aquaculture*, **260:** 145 – 150.

Carusso, S., Juarez, A. B., Moretton, j. and Magdalena, A. (2018). Effects of three veterinary antibiotics and their binary mixtures on two green alga species. *Chemosphere*, **194:** 821 – 827.

Chaturvedi, P., Shukla, P., Giri, B. S., Chowdhary, P., Chandra, R. *et al*. (2021). Prevalence and hazardous impact of pharmaceutical and personal care products and antibiotics in environment: a review on emerging contaminants. *Environmental Research,* **194:** 110664.

Chisholm, J. M., Zaman, I, R., Negm, A. M., Said, N., Abdel-daiem, M. M. *et al.* (2021). Sustainable waste management of medical waste in African developing countries: a narrative review. *Waste Management Research,* **39:** 1149 - 1163.

De Liguoro, M., Leva, V. D., Bona, M. D., Merlanti, R., Caporale G. *et al*. (2012). Sublethal effects of trimethoprim on four freshwater organisms. *Ecotoxicology and Environmental Safety,* **82:** 114 – 121.

de Orte, M. R., Carballeira C., Viana, I. G. and Carballeira, A. (2013). Assessing the toxicity of chemical compounds associated with marine land-based fish farms: the use of mini-scale microalgal toxicity tests. *Chemical Ecology*, **29(6):** 554 - 563.

Dolganyuk, V., Belova, D., Babich, O., Prosekov, A., Ivanova, S. *et al*. (2020). A review: Microalgae: A Promising Source of Valuable Bioproducts. *Biomolecules*. **10:** 1153.

Du, Y., Wang, J., Li, H., Mao, S., Wang, D. *et al.* (2018). The dual function of the algal treatment: Antibiotic elimination combined with CO2 fixation. *Chemosphere*, **211:** 192 – 201.

Duarte, B., Prata, D., Matos, A. R., Cabrita, M. T., Cacador, I. *et al.* (2019). Ecotoxicity of the lipid-lowering drug bezafibrate on the bioenergetics and lipid metabolism of the diatom *Phaeodactylum tricornutum. Science of the Total Environment*, **650:** 2085 – 2094.

Eguchi, K., Nagase, H., Ozawa, M., Endoh, Y. S., Goto, K. *et al.* (2004). Evaluation of antimicrobial agents for veterinary use in the ecotoxicity test using microalgae. *Chemosphere*, **57:** 1733 – 1738.

Eisentraeger, A., Dott, W., Klein, J. and Hahn, S. (2003). Comparative studies on algal toxicity testing using fluorometric. microplate and Erlenmeyer flask growth-inhibition assays. *Ecotoxicology and Environmental Safety,* **54:** 346 – 354.

Escapa, C., Coimbra, R. N., Paniagua S., Garcia A. I. and Otero M. (2016). Comparative assessment of diclofenac removal from water by different microalgae strains. *Algal Research,* **18:** 127 – 134.

Escapa, C., Coimbra, R. N., Paniagua, S., Garcia, A. I. and Otero M. (2017). Paracetamol and salicylic acid removal from contaminated water by microalgae. *Journal of Environmental Management*, **203:** 799 – 806.

Escher, B. I., Bramaz, N., Lienert, J., Neuwoehner, J. and Straub, J. O. (2010). Mixture toxicity of the antiviral drug and its active metabolite oseltamivir acid. *Aquatic Toxicology,* **296:** 194 – 202.

Ferrari, B., Mons, R., Vollat, B., Fraysse, B., Paxeus, N. *et al*. (2004). Environmental risk assessment of six human pharmaceuticals: Are the current environmental risk assessment procedures sufficient for the protection of the aquatic environment? *Environmental Toxicology and Chemistry,* **23:** 1344 – 1354.

Ferreira, C. S. G., Nunes, B. A., Henriques-Almeida, J. M. M. and Guilhermino, L. (2007). Acute toxicity of oxytetracycline and florfenicol to the microalgae *Tetraselmis chuii* and to the crustacean *Artemia parthenogenetica. Ecotoxicology and Environmental Safety,* **67:** 452 – 458.

Fu, L., Huang, T., Wang, S., Wang, X., Su, L. *et al*. (2017). Toxicity of 13 different antibiotics towards freshwater green algae *Pseudokirchneriella subcapitata* and their modes of action. *Chemosphere*, **168:** 217 – 222.

Gonzalez, R. M. and Angeles, H. J. C. (2017). Antibiotic and synthetic growth promoters in animal diets: Review of impact and analytical methods. *Food Control,* **72:** 255 - 267.

Gonzalez-Pleiter, M., Onzalo, S., Rodea-Palomares, I., Leganes, F., Rosal, R. *et al*. Toxicity of five antibiotics and their mixtures towards photosynthetic aquatic organisms:.

Implications for environmental risk assessment. *Water Research,* **47:** 2050 – 2064.

Halling-Sorensen, B. (2000). Algal toxicity of antibacterial agents used in intensive farming. *Chemosphere*, **40:** 731 – 739.

Yang, W., Tang, Z., Zhou, F., Zhang, W. and Song, L. (2023). Toxicity studies of tetracycline on *Microcystis aeruginosa* and *Selenastrum capricornutum. Environmental Toxicology and Pharmacology,* 35: 320 – 324.

Ye, J., Du, Y., Wang, L., Qian, J., Chen, J. *et al.* (2017).Toxin release of cyanobacterium *Microcystis aeruginosa* after exposure to typical tetracycline antibiotic contaminants. *Toxins*, **9:** 53.

Dias, E., Oliveira, M., Jones-Dias, D., Vasconcelos, V., Ferreira, E. *et al.* (2015). Assessing the antibiotic susceptibility of freshwater Cyanobacteria spp. *Frontier in Microbiology,* **6:** 799.

Grenni, P., Ancona, V. and Barra, C. A. (2018). Ecological effects of antibiotics on natural ecosystems: a review. *Microchemical Journal,* **136:** 25 - 39.

Guo, J., Selby, K. and Boxall, A. B. A. (2016). Comparing the sensitivity of chlorophytes, cyanobacteria, and diatoms to major-use antibiotics. *Environmental Toxicology and Chemistry,* **35:** 2587 – 2596.

Gürlek, C., Yarkent, C., Köse, A., Oral, I., Öncel, S. S. *et al.* (2019). *Evaluation of Several Microalgal Extracts as Bioactive Metabolites as Potential Pharmaceutical Compounds.* Springer Nature, Cham, Switzerland. pp. 267 – 272.

Halling-Sorensen, B. (2000). Algal toxicity of antibacterial agents used in intensive farming. *Chemosphere*, **40:** 731 – 739.

Han, P., Jia, S., Sun, Y., Tan, Z., Zhong, C. *et al.* (2014). Metabolomic approach to optimizing and evaluating antibiotic treatment in the axenic culture of cyanobacterium *Nostoc flagelliforme. World Journal of Microbiology and Biotechnology,* **30:** 2407–2418.

Harrass, M. C., Kindig, A. C.. and Taub, F. B. (1985). Responses of blue-green and green algae to streptomycin in unialgal and paired culture. *Aquatic Toxicology,* **6:** 1–11.

Havelkova, B., Beklova, M., Kovacova, V., Hlavkova, D. and Pikula J. (2016). Ecotoxicity of selected antibiotics for organisms of aquatic and terrestrial ecosystems. *Neuroendocrinology Letters,* **37:** 38 – 44.

Holten-Lutzhoft, H. C., Halling-Sorensen, B. and Jorgensen S. E. (1999). Algal toxicity of antibacterial agents applied in danish fish farming. *Archives of Environmental Contamination and Toxicology*, **36:** 1 – 6.

Isidori, M., Lavorgna, M., Nardelli, A., Pascarella, L. and Parrella, A. (2010). Toxic and genotoxic evaluation of six antibiotics on non-target organisms. *Science of the Total Environment,* **346:** 87 - 98.

Khan, M. I., Jin, H. S. and Jong, D.K. (2018). The promising future of microalgae: Current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. *Microbial Cell Factories,* 1 - 21.

Klobuchar, R. S., Brozovic, A. and Stambuk, A. (2013). Ecotoxicological assessment of nitrofurantoin in fish cell lines, unicellular algae *Desmodesmus subspicatus,* and bacterial strains of *Salmonella typhimurium*. *Fresenius Environmental Bulletin.*

Kolar, B., Arnus, L., Jeretin, B., Gutmaher, A., Drone, D. *et al*. (2014). The toxic effect of oxytetracycline and trimethoprim in the aquatic environment. *Chemosphere*, **115:** 75 – 80.

Kovácik, J., Babula, P., Peterková, V. and Hedbavny, J. (2017). Long-term impact of cadmium shows little damage in *Scenedesmus acutiformis* cultures. *Algal Research,* **25:** 184 - 190.

Kołodziejska, M., Maszkowska, J., Białk-Bielinska, A., Steudte S., Kumirska J. *et al.* (2013). Aquatic toxicity of four veterinary drugs commonly applied in fish farming and animal husbandry. *Chemosphere*, **92:** 1253 – 1259.

Krystian Miazek and Beata Brozek-Pluska. (2019). A Review. Effect of pharmaceuticals and personal care product on microalgal Growth, metabolism and microalgae-Based bioremediation processes. *International Journal of Molecular Science*s, **20:** 24 - 92.

Kuemmerer, K. (2009). Antibiotics in the aquatic environment :a review. *Chemosphere*, **75 (4):** 417 - 434.

Lai, H. T., Hou, J. H., Su, C. I. and Chen, C.L. (2009). Effects of chloramphenicol, florfenicol, and thiamphenicol on growth of algae *Chlorella pyrenoidosa, Isochrysis galbana,* and *Tetraselmis chui*. *Ecotoxicology and Environmental Safety,* **72:** 329 – 334.

Lee, R. E. (2008). *Phycology*. Cambridge University Press. pp. 1 - 500.

Llor, C. and Bjerrum, L. (2014). Antimicrobial resistance: Risk associated with antibiotic overuse and initiatives to reduce the problem. *Therapeutic Advances in Drug Safety,* **5:** 229 - 241.

Lopez-Serna, R., Garcia, D., Bola do, S., Jimenez, J. J., Lai, F. Y. *et al.* (2019). Photobioreactors based on microalgae-bacteria and purple phototrophic bacteria consortia: a promising technology to reduce the load of veterinary drugs from piggery wastewater. *Science of the Total Environment,* **692:** 259 - 266.

Lu, L., Wu, Y., Ding, H. and Zhang, W. (2015). The combined and second exposure effect of copper (II) and chlortetracycline on fresh water algae, *Chlorella pyrenoidosa* and *Microcystis aeruginosa. Environmental Toxicology and Pharmacology,* **40:** 140 – 148.

Machado, M. D. and Soares, E. V. (2019). Impact of erythromycin on a nontarget organism: cellular effects on the freshwater microalga *Pseudokirchneriella subcapitata*. *Aquatic Toxicology,* **208:** 179 – 186.

Magdalena, A., Saenz, M. E., Juarez, A. B. and Moretton, J. (2015). Effects of six antibiotics and their binary mixtures on growth of *Pseudokirchneriella subcapitata*. *Ecotoxicology Environmental Safety,* **113:** 72 – 78.

Michael, I., Rizzo, L., McArdell, C.S., Manaia, C.M., Merlin, C. *et al.* (2013). Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: a review. *Water Research,* **47(3):** 957 - 995.

Olasehinde, T. A., Olaniran, A. O. and Okoh, A. I. (2017). Therapeutic potentials of microalgae in the treatment of Alzheimer's disease. *Molecules*, **22:** 480.

Demirel, Z., Yılmaz, F. F., Ozdemir, G. and Dalay, M. C. Influence of media and temperature on the growth and the biological activities of *Desmodesmus protuberans* (F.E. Fritsch and M.F. Rich). *Aquatic Sciences,* **18:** 1195 – 1203.

Patel, M., Kumar, R., Kishor, K., Mlsna, T., Pittman, J. C. U. *et al.* (2019). Pharmaceuticals of emerging concern in aquatic systems: Chemistry, occurrence, effects, and removal methods. *Chemical Reviews*, **119:** 3510 - 3673.

Perales-Vela, H. V., Garcia, R. V., Gomez-Juarez, E. A., Salcedo-Alvarez, M. O. and Canizares-Villanueva, R. O. (2016). Streptomycin affects the growth and photochemical activity of the alga *Chlorella vulgaris. Ecotoxicology and Environmental Safety,* **132:** 311 – 317.

Piat, M., Sabetti, J. and Bloom, D. (2009). The importance of medication in consumer definitions of recovery from serious mental illness: A qualitative study. *Issues Mental Health Nursing,* **30:** 482 - 490.

Pomati, F., Netting, A. G., Calamari, D. and Neilan, B.A. (2004). Effects of erythromycin, tetracycline and ibuprofen on. the growth of *Synechocystis* sp. and *Lemna minor. Aquatic Toxicology,* **67:** 387 – 396.

Prata, J. C., Lavorante, B. R. B., Montenegro, M. C. B. and Guilhermino, L. (2018). Influence of microplastics on the toxicity of the pharmaceuticals procainamide and doxycycline on the marine microalgae *Tetraselmis chuii. Aquatic Toxicology,* **197:** 143 – 152.

Quaik, S., Embrandiri, A., Balasubramani, R., Hossain, K., Ismail, N., *et al.* (2019). Veterinary antibiotics in animal manure and manure laden soil: Scenario and challenges in Asian countries. *Journal of King Saud University of Science*, **32:** 1300 - 1305

Raja, R., Hemaiswarya, S., Venkatesan, G., and Isabel, S. C. (2014). Biomass from microalgae: an overview. *Journal of Oceanography and Marine Research*, 1 - 7.

Raven J. A. and Giordano, M. (2014). Algae. *Current Biology*, 591 - 595.

Sara, T., Antonio, G. J., Cintia, G., Gabriel, A. F., Lorenzo, C. *et al.* (2021). A Polyphasic Characterisation of Tetradesmus almeriensis (Chlorophyta: Scenedesmaceae)". *Processes*, **9 (11):** 2006.

Sarpong, E. M. and Miller, G. E. (2015). Narrow- and broad-spectrum antibiotic use among U.S. children. *Health Services Research,* **50:** 830 - 846.

Seoane, M., Rioboo, C., Herrera, C. and Cid, A. (2014). Toxicity induced by three antibiotics commonly used in aquaculture on the marine microalga *Tetraselmis suecica* (Kylin) Butch. *Marine Environmental Research,* **101:** 1 – 7.

Sharma, G. K., Dev, R. and Daya D. (2017). Microalgae: potential source of third generation biofuels. *Indian Farming*, **67(11):** 15 – 17.

Sharma, A., Gupta, A. K. and Ganguly, R. (2018). Impact of open dumping of municipal solid waste on soil properties in mountainous region. *Journal of Rock Mechanics and Geotechnical Engineering,* **10:** 725 - 739.

Solymosi, K. (2012). Plastid structure, diversification, and interconversion in algae. *Current Chemical Biology,* 167 - 186.

Susanti, H. and Taufik rahman, T. (2020). Microalgae Biodiversity and Applications. *2nd International conference on Universal Well Being*. pp. 63 — 67.

Thermo Fisher Scientific. [Internet]. Norristown, P. A. Thermo Fisher Scientific: Antibiotic Overuse and Resistance. [Online] [Available at: https://www.thermofisher.com/procalcitonin/wo/en/antibiotic-stewardship/antibiotic overuse-resistance] [Accessed: 15/12/2024].

Valitalo, P.,Kruglova, A., Mikola, A., Vahala, R. (2017). Toxicological impacts of antibiotics on aquatic micro-organisms: a mini-review. *International Journal of Hygiene and Environmental Health,* **220 (3):** 558 - 569.

Van der Grinten, E., Pikkemaat, M. G., Van den Brandhof, E. J., Stroomberg, G.J. and Kraak, M. H. S. Comparing the sensitivity of algal, cyanobacterial and bacterial bioassays to different groups of antibiotics. *Chemosphere*, **80:** 1–6.

Vasconcelos, E. C., Dale, C. R. and Oliveira, C. M. R. (2017). Influence of select antibiotics on *Vibrio fischeri* and *Desmodesmus subspicatus* at μg L−1 concentrations. *Environmental Management,* **60:** 157 – 164.

Verlicchi, P., Al Aukidy, M., Galletti, A. and Petrovic, M. D. (2012). Barcelona Hospital effluent: investigation of the concentrations and distribution of pharmaceuticals and environmental risk assessment. *Science of the Total Environment*, **430:** 109 - 118.

Wang, J. Y., An, X. L., Huang, F. Y. and Su J. Q. (2020). Antibiotic resistome in a landfill leachate treatment plant and effluent-receiving river. *Chemosphere*, **242:** 125207.

Wu, X., Wu, H., Zhang, A., Sekou, K., Li, Z. *et al.* (2022). Influence of polystyrene microplastics on levofloxacin removal by microalgae from freshwater aquaculture wastewater. *Journal Environmental Management*, **301:** 113865.

Xiong, J. Q., Govindwar, S., Kurade, M. B., Paeng, K. J., Roh, H. S. *et al.* (2019). Toxicity of sulfamethazine and sulfamethoxazole and their removal by a green microalga, *Scenedesmus obliquus*. *Chemosphere*, **218:** 551.

Xiong, J. Q., Kurade, M. B., Abou-Shanab, R. A. I., Ji, M. K., Choi, J. *et al*. (2016). Biodegradation of carbamazepine using freshwater microalgae *Chlamydomonas mexicana* and *Scenedesmus obliquus* and the determination of its metabolic fate. *Bioresource Technology*, **205:** 183 – 190.

Xiong, Q., Hu, L. X., Liu, Y. S., Wang, T. T. and Ying, G.G. (2019). New insight into the toxic effects of chloramphenicol and roxithromycin to algae using FTIR spectroscopy. *Aquatic Toxicology,* **207:** 197 – 207.

Yang, L. H., Ying, G. G., Su, H. C., Stauber, J. L., Adam's, M. S. *et al.* (2008). Growth-inhibiting effects of twelve antibacterial agents and their mixtures on the freshwater microalga *Pseudokirchneriella subcapitata. Environmental Toxicology and Chemistry,* **27:** 1201 – 1208.

Ye, J., Du, Y., Wang, L., Qian, J., Chen, J. *et al*. (2017). Toxin release of cyanobacterium *Microcystis aeruginosa* after exposure to typical tetracycline antibiotic contaminants. *Toxins*. **9:** 53.

Zhang, C., Chen, X., Wang, J. and Tan, L. (2017). Toxic effects of microplastic on marine microalgae *Skeletonema costatum*: interactions between microplastic and algae. *Environmental Pollution*, **220:** 1282 – 1288.

Zhang, S., Song, H. L., Cao, X., Li, H., Guo, J. H. *et al*. (2019). Inhibition of methanogens decreased sulfadiazine removal and increased antibiotic resistance gene development in microbial fuel cells. *Bioresource Technology*, **281:** 188 –194.

APPENDIX

ANOVA
Growth response of *Acutodesmus acutiformis* to Tetracycline

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.058028	6	0.009671	165.2205	3.1E-53	2.180564
Columns	0.017984	7	0.002569	43.89016	2.82E-29	2.092381
Interaction	0.020602	42	0.000491	8.379994	1.32E-19	1.493427
Within	0.006556	112	5.85E-05			
Total	0.10317	167				

ANOVA Growth response of *Acutodesmus* to Chloramphenicol

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.004919	6	0.00082	151.8479	2.09E-51	2.180564
Columns	0.007379	7	0.001054	195.2433	1.1E-59	2.092381
Interaction	0.006416	42	0.000153	28.29663	4.33E-43	1.493427
Within	0.000605	112	5.4E-06			
Total	0.019318	167				

ANOVA
Temperature for Tetracycline

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	23.46405	6	3.910675	0.932805	0.47447	2.180564
Columns	172.059	7	24.57986	5.862982	8.18E-06	2.092381
Interaction	178.9931	42	4.26174	1.016544	0.459049	1.493427
Within	469.5467	112	4.192381			
Total	844.0628	167				

ANOVA
Temperature for Chloramphenicol

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.282262	6	0.047044	4.621832	0.000312	2.180564
Columns	220.428	7	31.48972	3093.727	5.2E-125	2.092381
Interaction	1.008214	42	0.024005	2.358396	0.000185	1.493427
Within	1.14	112	0.010179			
Total	222.8585	167				

ANOVA Total dissolved solid for Tetracycline

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	265193.2	6	44198.87	1910.319	2.6E-110	2.180564
Columns	31423.69	7	4489.099	194.0233	1.52E-59	2.092381
Interaction	32895.14	42	783.2177	33.85145	5.56E-47	1.493427
Within	2591.333	112	23.1369			
Total	332103.4	167				

ANOVA Total dissolved solid for Chloramphenicol

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	12497.06	6	2082.844	174.8714	1.79E-54	2.180564
Columns	10580.86	7	1511.552	126.9069	3.19E-50	2.092381
Interaction	10325.59	42	245.8474	20.64086	1.89E-36	1.493427
Within	1334	112	11.91071			
Total	34737.52	167				

ANOVA pH for Tetracycline

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	53.2639	6	8.877317	211.815	1.04E-58	2.180564
Columns	60.43564	7	8.633663	206.0013	6.88E-61	2.092381
Interaction	183.7001	42	4.373811	104.3602	1.06E-72	1.493427
Within	4.694	112	0.041911			
Total	302.0936	167				

ANOVA pH for Chloramphenicol

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	3.519387	6	0.586564	12.75768	6.17E-11	2.180564
Columns	329.4643	7	47.06633	1023.684	2.24E-98	2.092381
Interaction	25.29994	42	0.602379	13.10165	1.73E-27	1.493427
Within	5.149467	112	0.045977			

Total	363.4331	167				

ANOVA Conductivity for Tetracycline

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	767243.9	6	127874	208.3062	2.46E-58	2.180564
Columns	7071.28	7	1010.183	1.645584	0.129899	2.092381
Interaction	33982.1	42	809.0975	1.318017	0.128071	1.493427
Within	68754	112	613.875			
Total	877051.3	167				

ANOVA Conductivity for Chloramphenicol

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	40712.48	6	6785.413	84.25346	3.37E-39	2.180564
Columns	27865.6	7	3980.799	49.42899	2.15E-31	2.092381
Interaction	25469.9	42	606.4263	7.529905	6.95E-18	1.493427
Within	9020	112	80.53571			

Total	103068	167

ANOVA
Turbidity for
Tetracycline

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	24833.06	6	4138.843	82.30254	9.76E-39	2.180564
Columns	4940.511	7	705.7873	14.03486	5.78E-13	2.092381
Interaction	7877.03	42	187.5483	3.729474	1.58E-08	1.493427
Within	5632.273	112	50.28815			
Total	43282.87	167				

ANOVA
Turbidity for
Chloramphenicol

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	7649.501	6	1274.917	213.0695	7.7E-59	2.180564
Columns	21282.12	7	3040.302	508.1083	9.82E-82	2.092381
Interaction	31867.62	42	758.7528	126.806	2.7E-	1.493427

Within	670.16	112	5.983571			

ANOVA
Dissolved oxygen for Tetracycline

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	24.881	6	4.146834	5.478006	5.29E-05	2.180564
Columns	2351.011	7	335.8588	443.6725	1.51E-78	2.092381
Interaction	70.71779	42	1.683757	2.224258	0.000463	1.493427
Within	84.78367	112	0.756997			
Total	2531.394	167				

ANOVA
Dissolved oxygen for Chloramphenicol

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	46.3579	6	7.726316	10.92386	1.49E-09	2.180564
Columns	2209.095	7	315.585	446.1902	1.11E-78	2.092381
Interaction	135.0209	42	3.214783	4.545224	8.5E-11	1.493427

Within	79.21627	112	0.707288			
Total	2469.69	167				

ANOVA
Chemical oxygen demand for Tetracycline

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	284.2593	6	47.37656	62.53599	1.74E-33	2.180564
Columns	3347.505	7	478.215	631.233	7.36E-87	2.092381
Interaction	838.2736	42	19.95889	26.34529	1.46E-41	1.493427
Within	84.84993	112	0.757589			
Total	4554.888	167				

ANOVA
Chemical oxygen demand for Chloramphenicol

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	306.9159	6	51.15264	41.98803	1.76E-26	2.180564

Columns	25280.71	7	3611.53	2964.481	5.6E-124	2.092381
Interaction	1806.283	42	43.00675	35.30157	6.66E-48	1.493427
Within	136.4459	112	1.218267			
Total	27530.35	167				

ANOVA Total organic carbon for Tetracycline

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	186.4115	6	31.06859	259.466	2.89E-63	2.180564
Columns	1019.413	7	145.6304	1216.217	1.7E-102	2.092381
Interaction	246.0027	42	5.857207	48.91585	3.46E-55	1.493427
Within	13.41093	112	0.11974			
Total	1465.238	167				

ANOVA Total organic carbon for Chloramphenicol

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	320.1399	6	53.35664	46.85905	2.48E-	2.180564

Columns	28312.52	7	4044.646	3552.103	2.4E-128	2.092381
Interaction	1603.156	42	38.17039	33.52213	9.1E-47	1.493427
Within	127.5302	112	1.138663			
Total	30363.35	167				