GROWTH RESPONSE OF TWO FRESHWATER MICROALGAE TO UREA

BY

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SR/2281/RPR/25/23

DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

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

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CERTIFICATION

We certify that this research work was carried out by **Glory Ijeliweme IDEHAI** of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.

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Prof. Beckley Ikhajiagbe Date

Head of Department

DEDICATION

This project work is dedicated to God Almighty for strength, life and making this work possible.

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ABSTRACT

This study investigated the effects of different urea concentrations on the growth of *Chlorella vulgaris* and *Scenedsemus*, two freshwater microalgae species were analyzed under laboratory conditions. The experiment involved subjecting the microalgae to varying concentration of urea (Control, 10%, 20%, 40%, 60%, 80% and 100%). Absorbance was measured using a 721-visible Spectrophotometer at 750nm. Physicochemical parameters such as pH, turbidity, dissolved oxygen, conductivity and total dissolved solids were analyzed. Data was evaluated using descriptive statistics, two way analysis of variance and t-tests analysis and was conducted in Microsoft Excel 2010. Results revealed significant variation in the growth responses of both microalgae. *Chlorella vulgaris* exhibited optimal growth at 20% and 40% urea concentration with lowest growth occurring at higher concentrations. *Scendesmus,* on the other hand had the highest growth at 20% urea concentration with extremely low growth at higher levels. This infers that *Chlorella vulgaris* was more tolerant to higher concentrations of urea and ammonia toxicity than *Scenedesmus.* All physicochemical parameters and growth showed significant differences across concentrations of urea apart from Turbidity.

CHAPTER ONE

1.0 INTRODUCTION

Algae are photosynthetic (autotrophic), Chlorophyll-bearing, non-vascular plants with simple naked reproductive structures. They lack true root, stems and leaves. (Kadiri and Akhere 2021)
Microalgae are microscopic, photosynthetic organisms that contribute substantially to global carbon cycling and are promising feedstocks for renewable fuels, feed and food ingredients, wastewater remediation, and other high-value bioproducts because of their high photosynthetic efficiencies and flexible cultivation options (Chisti, 2007). They can be grown on non-arable land and in wastewater systems, and several reviews highlight their potential for large-scale oil and biomass production for biodiesel and biorefinery uses. Among green microalgae, *Chlorella vulgaris* and *Scenedesmus* are especially well studied: they combine comparatively fast growth rates, broad environmental tolerance, and the capacity to accumulate proteins, neutral lipids (triacylglycerides), and industrial pigments; these traits make them attractive for integrated bioprocesses (biofuels, nutraceuticals, and wastewater treatment) that require both high biomass productivity and useful biochemical composition (Li *et al.*, 2008).

Chlorella vulgaris is a unicellular green alga within the division *Chlorophyta* and class *Trebouxiophyceae*. Its spherical cells, ranging from 2 to 10 μm in diameter, possess a thick cellulose-based wall that provides mechanical strength and protection against osmotic fluctuations (Richmond, 2004). The organism contains chlorophyll *a* and *b*, as well as carotenoids such as violaxanthin and neoxanthin, which enhance its ability to perform photosynthesis under diverse light conditions (Komárek and Fott, 1983). It reproduces asexually through autospores and has a single cup-shaped chloroplast with a prominent pyrenoid that supports carbon fixation and starch synthesis (Becker, 1994). *Chlorella vulgaris* is highly valued for its nutritional and biotechnological applications due to its high protein (up to 60% dry weight) and lipid content, along with a wide range of vitamins and minerals (Becker, 1994; Richmond, 2004).

In cultivation systems, *Chlorella vulgaris* effectively utilizes urea as a nitrogen source, hydrolyzing it via urease into ammonium, which is rapidly assimilated into amino acids through the GS–GOGAT pathway (Huang *et al.*, 2010). The bicarbonate generated during urea breakdown contributes to enhanced photosynthetic carbon fixation, particularly in closed photobioreactors and wastewater media (Sigurdarson *et al.*, 2018). Studies have shown that moderate urea supplementation improves biomass yield and chlorophyll content, while excessive levels lead to ammonia toxicity, damaging photosystems and cellular membranes (Rosa *et al.*, 2023). Variations in urea tolerance among *Chlorella* strains indicate that each strain requires specific optimization for best productivity and biochemical performance (Li *et al.*, 2008).

In comparison, both *Chlorella* and *Scenedesmus* demonstrate robust growth and adaptability, but they differ in morphology, colony formation, and urea assimilation dynamics. *Scenedesmus* tends to form colonies that enhance sedimentation resistance and facilitate wastewater applications, while *Chlorella vulgaris* exhibits a simpler unicellular structure that supports easy harvesting and higher lipid productivity. Together, these genera provide valuable insights into optimizing nitrogen management, particularly urea-based systems, for scalable biomass and biofuel production.

Scenedesmus species are colonial green microalgae belonging to the division *Chlorophyta* and are commonly found in freshwater and wastewater environments. They typically form coenobia composed of 4 to 8 cells arranged in a linear or curved pattern, and each cell is covered by a robust cell wall sometimes bearing spines that provide mechanical protection and aid flotation (Komárek and Fott, 1983). The cells contain chlorophyll *a* and *b*, along with carotenoids such as lutein and β-carotene, which assist in light harvesting and photoprotection (Bold and Wynne, 1985). The cup-shaped chloroplast and central pyrenoid serve as centers for carbon fixation and starch deposition, enabling high photosynthetic performance under variable conditions (Trainor, 1998). *Scenedesmus* exhibits broad physiological adaptability and can grow efficiently under both nutrient-rich and nutrient-limited environments, which explains its wide application in biofuel production and wastewater treatment (Hu *et al.*, 2008). Its ability to assimilate urea as a nitrogen source through active urease enzymes enables improved biomass production while providing bicarbonate that enhances photosynthetic carbon fixation (Sigurdarson *et al.*, 2018).

However, excessively high urea concentrations can lead to ammonia accumulation, oxidative stress, and a decline in chlorophyll content (Rosa *et al.*, 2023).

1.1 Botanical Characteristics of *Chlorella vulgaris* and *Scenedesmus*

Microalgae such as *Chlorella* and *Scenedesmus* are unicellular, photosynthetic microorganisms belonging to the division *Chlorophyta*. They have attracted wide research interest due to their simple structure, rapid growth rates, and adaptability to diverse environmental conditions. Both genera contain chlorophyll *a* and *b* as the primary photosynthetic pigments, along with accessory carotenoids such as lutein and β-carotene, which play essential roles in light harvesting and photoprotection (Komárek and Fott, 1983). These pigments are responsible for their characteristic green coloration and efficient absorption of light in the blue and red regions of the visible spectrum. Microalgae from these genera are vital components of aquatic ecosystems, significantly contributing to global carbon fixation and nutrient cycling. Their metabolic versatility allows them to thrive under autotrophic, mixotrophic, or heterotrophic conditions, making them key models for understanding algal physiology and for developing sustainable biotechnological applications (Richmond, 2004).

Because of their high adaptability, *Chlorella* and *Scenedesmus* are among the most widely used microalgae in environmental and industrial research. They are capable of utilizing different nitrogen sources such as nitrate, ammonium, and urea, giving them a distinct advantage in wastewater treatment and biofuel production (Sigurdarson *et al.*, 2018). The following sections describe in detail their morphology, physiology, and ecological significance.

1.1.1 Botanical Characteristics of *Chlorella vulgaris*

Chlorella vulgaris is a unicellular green alga within the division *Chlorophyta* and class *Trebouxiophyceae*. It is characterized by its spherical, non-motile cells, usually 2–10 μm in diameter, surrounded by a thick cellulose-based wall that provides mechanical strength and resistance to osmotic stress (Richmond, 2004). Each cell contains a single cup-shaped chloroplast with a distinct pyrenoid, which serves as a site for carbon fixation and starch formation (Becker, 1994). The chloroplasts contain chlorophyll *a* and *b*, along with carotenoids

such as violaxanthin, neoxanthin, and lutein that enhance its ability to absorb light and protect the photosystems from oxidative damage (Komárek and Fott, 1983).

Chlorella vulgaris reproduces asexually through autospores and exhibits rapid growth under a wide range of conditions, including autotrophic and mixotrophic regimes. It is widely cultivated for its high nutritional value, containing up to 60% protein, 20–30% lipids, and a rich assortment of vitamins and minerals (Becker, 1994; Richmond, 2004). Because of its ability to efficiently assimilate organic and inorganic nitrogen sources, *Chlorella vulgaris* is frequently used in wastewater treatment and biofuel production (Li *et al.*, 2008).

During urea assimilation, urease catalyzes the conversion of urea into ammonium and bicarbonate, both of which enhance growth and photosynthetic performance (Sigurdarson *et al.*, 2018). The ammonium is rapidly incorporated into amino acids via the GS–GOGAT pathway, while the bicarbonate contributes to carbon fixation. Moderate urea levels have been reported to increase biomass yield and chlorophyll content, but excessive urea concentrations may lead to ammonia toxicity, resulting in reduced photosynthetic efficiency and cell damage (Rosa *et al.*, 2023). *Chlorella vulgaris* exhibits strain-specific responses to urea dosing, suggesting that optimal concentrations must be determined experimentally to maximize biomass and lipid productivity (Huang *et al.*, 2010).

In general, *Chlorella vulgaris* differs from *Scenedesmus* in its unicellular morphology and simpler structure, which make it easier to harvest and cultivate in high-density systems. Its strong adaptability to nutrient and environmental variations, coupled with its balanced biochemical profile, has made it one of the most commercially important microalgae for large-scale biomass, feed, and biofuel production (Becker, 1994; Richmond, 2004).

1.1.2 Botanical Characteristics of *Scenedesmus*

Scenedesmus species are colonial green microalgae commonly found in freshwater and wastewater environments. Their colonies, called coenobia, typically consist of 2, 4, or 8 elongated cells arranged linearly or slightly curved, with some species having terminal spines or thickened cell walls that provide structural rigidity and protection from grazers (Komárek and

Fott, 1983). The cells are non-motile and have a well-defined cell wall made up of a trilaminar structure, which enhances their resistance to environmental stress. Each cell contains a single cup-shaped chloroplast with a central pyrenoid where carbon fixation and starch accumulation occur (Trainor, 1998).

The chloroplasts of *Scenedesmus* contain chlorophyll *a* and *b* and accessory pigments such as lutein and β-carotene, which enhance photosynthetic efficiency and help the cells adapt to varying light intensities (Bold and Wynne, 1985). Under nutrient-limited or high-light conditions, *Scenedesmus* species can accumulate lipids, particularly triacylglycerides, which are valuable for biodiesel production (Mata *et al.*, 2010). Their physiological plasticity allows them to thrive under a wide range of temperatures, pH levels, and nutrient concentrations, making them suitable for large-scale cultivation in open or closed systems (Hu *et al.*, 2008).

In addition to their biofuel potential, *Scenedesmus* species are extensively used in wastewater treatment systems because they efficiently remove nitrogen and phosphorus from contaminated water while producing oxygen and valuable biomass. Their ability to utilize urea as a nitrogen source is attributed to active urease enzymes that hydrolyze urea into ammonium and bicarbonate, providing readily assimilable nitrogen and inorganic carbon to enhance growth (Sigurdarson *et al.*, 2018). However, excessive urea concentrations can cause an accumulation of toxic free ammonia, resulting in membrane damage and inhibited photosynthesis (Rosa *et al.*, 2023). Optimizing urea concentration, pH, and light intensity has therefore been shown to significantly improve biomass productivity and biochemical composition in *Scenedesmus* cultures (Li *et al.*, 2008).

1.3 Ecological role of *Chlorella vulgaris* and *Scenedesmus* in aquatic systems

Chlorella vulgaris and *Scenedesmus* are important primary producers in freshwater and nearshore systems, where they contribute substantially to carbon fixation, oxygen generation and the base of aquatic food webs (Chisti, 2007; Richmond, 2004). Their rapid growth and capacity to take up dissolved nutrients make them effective biological agents for removing excess nitrogen and phosphorus from nutrient-enriched waters, thus mitigating eutrophication and improving water quality when managed in pond or constructed wetland systems (Li *et al.*, 2008).

Because both genera can grow mixotrophically or heterotrophically when organic substrates are available, they can capitalize on a range of water chemistries typical of sewage-impacted or agricultural runoff systems, converting waste nutrients into harvestable biomass (Chisti, 2007; Li *et al.*, 2008).

When present at moderate densities, these microalgae enhance dissolved oxygen through photosynthesis during daylight, supporting higher trophic levels (zooplankton, macroinvertebrates, fish) and promoting ecosystem productivity; however, when uncontrolled blooms occur they may cause diel oxygen swings and shading that stress aquatic fauna (Chisti, 2007). In engineered systems (wastewater ponds, algal turf reactors, photobioreactors), *Scenedesmus* and *Chlorella* are intentionally cultivated to extract dissolved nitrogen (nitrate, ammonium, urea) and phosphorus, enabling nutrient recycling and generation of biomass for feed, fertilizer or bioenergy (Li *et al.*, 2008; Sigurdarson *et al.*, 2018).

The ecological performance of these genera in real waters depends on species/strain traits and environmental drivers. *Scenedesmus*'s colonial form enhances resistance to grazing and improves settling behavior, which can be advantageous in wastewater clarification systems where biomass separation is required (Komárek & Fott, 1983; Trainor, 1998). *Chlorella vulgaris*, by contrast, with its unicellular morphology and small size, forms dense suspensions that are easily grown to high cell densities and take up dissolved nutrients rapidly — traits that make it valuable in high-rate algal ponds and closed reactors aimed at maximizing biomass yield (Becker, 1994; Richmond, 2004).

Urea is a low-cost, high-nitrogen fertilizer that has been adopted increasingly as an N source in algal cultivation because its hydrolysis yields both ammonium and inorganic carbon (bicarbonate), potentially improving photosynthesis and biomass accumulation under some growth regimes (Sigurdarson *et al.*, 2018). Urea hydrolysis, catalyzed by urease, can rapidly raise ammonium (and free ammonia) levels and alter medium pH—effects that can be beneficial at moderate doses but toxic at high concentrations (Hodson and Thompson, 1969; Rosa *et al.*, 2023). Urea's effects are strongly species- and condition-dependent, dose and delivery must be optimized: moderate supplementation can increase biomass and sometimes lipid content,

whereas excessive urea or rapid hydrolysis to free ammonia can impair photosynthesis and cell viability (Rosa *et al.*, 2023).

Urea dynamics are central to how these organisms influence and respond to aquatic nutrient cycles. Urea present in agricultural runoff, municipal wastewater or aquaculture effluents is rapidly hydrolyzed by microbial and algal urease activity into ammonium and bicarbonate, increasing the pool of assimilable nitrogen and inorganic carbon that drives algal productivity (Sigurdarson *et al.*, 2018). Moderate urea availability can therefore enhance algal growth and nutrient removal rates, but rapid urea hydrolysis may also transiently raise free ammonia (NH_3) and shift pH, producing toxicity risks for algae and co-occurring organisms if not buffered or controlled (Hodson & Thompson, 1969; Rosa *et al.*, 2023).

In tropical and sub-tropical regions, including many Nigerian inland waters, warm temperatures and nutrient inputs can favor fast algal growth; thus, *Scenedesmus* and *Chlorella* frequently dominate phytoplankton assemblages in eutrophic conditions and engineered wastewater systems (Li *et al.*, 2008). Their presence is therefore both an indicator of nutrient enrichment and an opportunity for nutrient recovery: when harvested, algal biomass converts dispersed nutrient loads into concentrated organic material that can be valorized (e.g., as feed, fertilizer or feedstock for biofuels), supporting circular-economy approaches to water management (Chisti, 2007; Li *et al.*, 2008).

Overall, *Chlorella vulgaris* and *Scenedesmus* perform dual ecological functions — they mitigate nutrient pollution through assimilation and simultaneously produce biomass with commercial value — but the net ecosystem outcome depends on controlling growth, avoiding uncontrolled blooms, and matching strain selection and management to local environmental and operational conditions (Sigurdarson *et al.*, 2018; Rosa *et al.*, 2023).

1.4 LITERATURE REVIEW

Microalgae are photosynthetic microorganisms that possess the remarkable ability to fix carbon dioxide and convert it into valuable biomass with high efficiency. Chisti Y. (2007) reported that microalgae can be cultivated in both open raceway ponds and closed photobioreactors, which

allow precise control of temperature, light, and nutrient supply. Becker (2013) emphasized that microalgae have faster growth rates and higher photosynthetic efficiency compared with terrestrial plants, making them important biological systems for carbon sequestration and bioresource production. Zhu L. (2016) described microalgae as third-generation biofuel feedstocks due to their superior oil yield per unit area when compared with conventional crops such as soybean and palm oil.

According to Smith and Graham (2010), *Scenedesmus* species are common inhabitants of freshwater ecosystems and are frequently used as indicators of water quality. Their ability to tolerate a wide range of pH, temperature, and light intensities enables them to thrive in eutrophic and polluted waters. The colonial form of *Scenedesmus* provides protection against grazing by zooplankton, while the presence of spines deters predation. Furthermore, the genus demonstrates remarkable physiological flexibility, shifting between photoautotrophic and mixotrophic modes depending on nutrient conditions.

Chlorella, on the other hand, is known for its extraordinary metabolic versatility. According to Lee (2008), it can grow photoautotrophically, heterotrophically, or mixotrophically depending on the carbon source available. This adaptability allows *Chlorella* to colonize diverse habitats ranging from freshwater ponds to wastewater systems and soil crusts. Its robust photosynthetic machinery and efficient nutrient uptake mechanisms make it suitable for large-scale biotechnological applications. Moreover, *Chlorella* species tolerate a wide range of environmental stresses, including variations in salinity, temperature, and light intensity.

The integration of microalgal cultivation into wastewater treatment systems enables the removal of excess nitrogen and phosphorus while simultaneously producing biomass that can be processed into biofuels, food supplements, and bioproducts. Chisti Y. (2007) further explained that photobioreactors designed for microalgal cultivation can be optimized for light distribution, gas exchange, and mixing efficiency, thus achieving high biomass productivity. Becker (2013) also noted that the closed nature of these systems minimizes contamination and allows for continuous operation under well-defined conditions. Zhu L. (2016) indicated that integrating microalgae into industrial carbon capture systems can substantially reduce CO_2 emissions while producing renewable biomass, thereby supporting both environmental protection and sustainable

production goals. Consequently, microalgae are increasingly recognized as key biotechnological resources in the fields of renewable energy, environmental management, and bio-based industry.

Nitrogen is an essential macronutrient required for protein synthesis, pigment formation, and cellular growth. Li Y. *et al.* (2008) stated that nitrate (NO_3^-), ammonium (NH_4^+), and urea are the most commonly used nitrogen sources in microalgal cultivation systems. Each nitrogen source differs in uptake pathway and energy requirement. Sigurdarson J. J. *et al.* (2018) observed that nitrate must be reduced to ammonium before assimilation, a process that consumes significant metabolic energy. In contrast, ammonium can be directly incorporated into amino acids through the glutamine synthetase–glutamate synthase (GS–GOGAT) pathway, making it a more energy-efficient nitrogen source. However, Sigurdarson J. J. *et al.* (2018) warned that at higher pH, the equilibrium between NH_4^+ and NH_3 shifts toward the toxic free-ammonia form, which can impair photosynthetic activity and reduce cell growth.

Rosa S. M. *et al.* (2023) reported that urea provides a dual advantage by supplying both nitrogen and inorganic carbon through its hydrolysis products. When urea is decomposed, it releases ammonium and bicarbonate ions, which can support carbon fixation under CO_2-limited conditions. Li Y. *et al.* (2008) explained that the optimization of nitrogen concentration and timing often improves biomass yield and product quality compared with cultures using a single nitrogen form. Rosa S. M. *et al.* (2023) further highlighted that well-regulated urea feeding strategies enhance photosynthetic activity, whereas unregulated addition may lead to ammonia toxicity. Therefore, the selection and regulation of nitrogen sources are critical for achieving balanced growth and high productivity in microalgal cultures.

Sigurdarson J. J. *et al.* (2018) stated that urea is hydrolyzed by the enzyme urease to produce carbamic acid, which rapidly decomposes into ammonia and carbonic acid. Hodson R. E. and Thompson J. C. (1969) found that urease activity in algae is widespread and extremely efficient, allowing rapid nitrogen conversion. The resulting ammonium is assimilated into amino acids via the GS–GOGAT pathway, while bicarbonate released during hydrolysis enhances photosynthetic carbon fixation. Rosa S. M. *et al.* (2023) observed that although urea metabolism supports growth and photosynthesis, it is strongly influenced by the pH of the medium. At higher pH

levels, the equilibrium between ammonium and free ammonia shifts toward NH_3, which is toxic to algal cells.

Sigurdarson J. J. *et al.* (2018) noted that uncontrolled accumulation of free ammonia can damage cellular membranes, reduce chlorophyll content, and inhibit photosystem II activity, ultimately leading to growth inhibition. To prevent such toxicity, Li Y. *et al.* (2008) recommended maintaining optimal pH and implementing controlled urea dosing schedules. Rosa S. M. *et al.* (2023) also demonstrated that cultures receiving moderate urea concentrations exhibited higher biomass and lipid productivity than those receiving excess ammonium or nitrate. These findings indicate that urea, when properly managed, can be a sustainable nitrogen source that simultaneously supports nitrogen assimilation and carbon fixation.

Furthermore, Becker (2013) explained that the beneficial effect of urea on photosynthesis is partly due to the additional bicarbonate generated during hydrolysis, which serves as a carbon source for the Calvin cycle. Zhu L. (2016) stated that the coupling of urea metabolism and CO_2 fixation can improve carbon-use efficiency, particularly in closed photobioreactors where gas exchange is limited. Chisti Y. (2007) emphasized that this metabolic flexibility makes microalgae suitable for integration into circular bioprocesses that utilize both organic and inorganic waste streams. Li Y. *et al.* (2008) suggested that successful use of urea as a nitrogen source depends on species-specific optimization of nutrient concentration, pH regulation, and dosing frequency.

Sigurdarson J. J. *et al.* (2018) proposed combining urea feeding with real-time pH monitoring to minimize the formation of toxic free ammonia. Rosa S. M. *et al.* (2023) observed that gradual urea addition under controlled pH can maximize photosynthetic efficiency and maintain stable biomass productivity. Becker E. W. (2013) recommended adopting mixed nitrogen regimes that include nitrate, ammonium, and urea to balance growth rate, energy efficiency, and product composition. Hodson R. E. and Thompson J. C. (1969) highlighted that differences in urease activity among algal species necessitate strain-specific evaluation of urea metabolism. For instance, species such as *Scenedesmus* and *Chlorella* respond differently to urea depending on their enzyme expression levels and tolerance to ammonia.

Li Y. *et al.* (2008) and Zhu L. (2016) both concluded that fine-tuning nitrogen delivery systems and culture conditions can lead to higher lipid accumulation and overall productivity. Therefore, understanding and optimizing urea metabolism is critical for developing efficient, cost-effective, and environmentally sustainable algal cultivation systems.

1.5 Aim and Objectives

Aim:

To evaluate the effect of urea on the growth, physiology, and biochemical composition of *Scenedesmus* and *Chlorella vulgaris* under controlled laboratory conditions, with the goal of identifying optimal urea concentrations that maximize biomass productivity and nutrient assimilation efficiency. The study also aims to understand the metabolic and physiological responses of both microalgal species to varying nitrogen availability, providing insights into their potential applications in biofuel production, carbon sequestration, and wastewater nutrient recovery. Furthermore, it seeks to establish a comparative understanding of how *Scenedesmus* and *Chlorella vulgaris* adapt to urea-based nitrogen sources in terms of photosynthetic performance, macromolecular composition, and culture stability.

Objectives:

• To assess the effect of different concentrations of urea on the growth rate and biomass yield of *Scenedesmus* and *Chlorella vulgaris.*

• To compare the growth performance and adaptability of the two algal species under identical culture conditions.

• To determine possible concentration-dependent growth inhibition or toxicity resulting from urea supplementation.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Test Microalgae

The test microalgae that were utilized were *Scenedesmus* and *Chlorella vulgaris*

2.2 Source of Microalgae

The research utilized two freshwater algae; Scenedesmus and Chlorella which were locally sourced from fish ponds at Uteh community, Benin City

2.3 Taxonomy of Microalgae

Taxonomy of Scenedesmus sp

Kingdom- Plantae.

Phylum – Chlorophyta (green algae)

Class -Chlorophyceae

Order - Sphaeropleales

Family – Scenedesmaceae

Genus- Scenedesmus

Taxonomy of Chlorella Vulgaris

Kingdom – Plantae

Division – Chlorophyta

Class - Trebouxiophyceae

Order -Chlorellales

Family – Chlorellaceae

Genus – Chlorella

Species – *Chlorella vulgaris*

Culture Vessel

The 500ml of bottles were washed thoroughly with water and detergent. It was dried before acid washing with 1.1M sulphuric acid (H_2SO_4) Solution to remove the contaminants. The work bench was swabbed with cotton wool doused in acetone. To avoid contamination, the bottles were properly covered with cotton wool to prevent other micro-organisms from entering.

Culture Medium

The growth medium used for cultivating both microalgae was Modified Chu number 10 medium. To make this medium, measured salts in specified grams were dissolved in 100ml of distilled water. An Iron solution was made by dissolving 5g of citric acid in 100ml of distilled water, ferric citrate($F_eC_6H_5O_3.5H_2O$) were added to the solution. These medium was autoclaved, refrigerated and the medium were kept sterile.

Plate 1: Culture Vessels containing different concentrations of Urea

Table 1: Composition of the Modified Chu Medium

SALTS/NUTRIENTS	g/100ml
$CaCl_2.2H_2O$	3.67
$MgSO_4.7H_20$	3.69
$NaHCO_3$	1.26
K_2HPO_4	0.87
$NaNO_3$	8.5
Na_2SiO_3	2.84

TRACE ELEMENT	Mg/l
$CaSO_4.5H_20$	19.6
$ZnSO_4.7H_20$	44
$CaCl_2.6H_20$	20
$MnCl_2.4H_20$	36
$NaMO_4.2H_20$	12.6
H_2BO_3	618.4
Iron stock	g/100ml
Citric acid ($C_6H_8O_7.H_20$)	3.5
Ferric citrate($FeC_6H_5O_3.5H_20$)	3.5

Table 2: Trace Element Composition of the Modified Chu No. 10 Medium

Table 3: Composition of Vitamin Stock

Component	g/100ml
Thiamine (Vitamin B1)	0.004
Biotin (Vitamin B7)	0.004
Cyanocobalamin (Vitamin B12)	0.004

Table 4: Preparation of different concentrations of mixture

UREA Concentration (%)	UREA Volume (ml)	Culture Volume (ml)	Distilled Water (ml)	Total Volume (ml)
0	0	5	395	400
10	10	5	385	400
20	20	5	375	400
40	40	5	355	400
60	60	5	335	400
80	80	5	315	400
100	100	5	295	400

Experiment

The microalgae species were cultivated in growth medium and incubated for a duration of fourteen days. Various concentration (0% ,10% ,20%, 40% ,60%, 80% ,100%) were prepared and utilized for the experiment. Each concentration was introduced into separate culture vessels.

Inoculation

Microalgae were taken with 5ml syringe and were inoculated in to the culture bottles. The bottles were covered with cotton wool after every steps to avoid evaporation and contamination.

2.4 Physiochemical Analysis of urea solution.

2.4.1 pH

pH is a measure of how acidic or alkaline solution is, typically ranging from 0 to 14.
The sample were measured with pH tester. The pH meter was calibrated at 25°C using a buffer with a pH range of 4 to7. After calibration the pH meter were inserted in the culture bottles containing the samples. The readings were recorded only when a stable and consistent values was obtained.

2.4.2 Turbidity

Turbidity refer to the cloudiness or haziness of a fluid, particularly water, caused by large numbers of individual particles that are generally invisible to the naked eye. These particles can include sediment, micro-organisms and pollutant, affecting the clarity of the water.
The sample was measured into a small bottle and was properly clean with tissue paper before placing in to a cuvette, and the reading was measured at a stable point.

2.4.3. Dissolved oxygen(mg/L)

Dissolved oxygen(mg/L) refer to the amount of oxygen gas dissolved in water, typically measured in milligrams per liter (mg/L) or as on percentage of saturation.
The dissolved oxygen meter was calibrated and inserted in to the culture for DO measure and readings were taken.

2.4.4. Total dissolved solid(mg/L)

Total dissolved solid(mg/L) refers to the total concentration of dissolved substances in water, including inorganic salts and organic matter, measured in milligrams per litter (mg/L)

The total dissolved solids, conductivity, and temperature were measure using the meter model YL-TDS 2-A, which was designed to measure and the readings were appropriately recorded.

2.4.5. Conductivity ($\mu S/cm^{-1}$)

Conductivity (µS/cm-1) refers to the ability of a material to conduct or transmit heat, electricity, or sound.

The sample was measured using the model YL-TDS 2-A, which is designed to measure Total dissolved Solids, Temperature and conductivity. This involved the probe into solution to obtained the readings.

2.5. Growth Measurement and Monitoring

After inoculation on the first day, the growth was measured using spectrophotometer at an absorbance of 750nm. This was done to monitor and measure the growth of the algae in the culture bottles. The growth was monitored every two days for two weeks.

2.6. Data analysis

Microsoft Excel was used to conduct the data analysis, which include descriptive statistics, inferential (two way) analysis of variance (ANOVA) and paired t-test.

2.7. Percentage Yield

Percentage yield formula shown below:

$$Y = \frac{G_t - G_o}{T} \times 100$$

Where;

G_t = (growth at the end of experiment)

G_o = (growth at the beginning of experiment)

T = time (days at the end of the experiment)


TDS/EC/pH/SALINITY
HOLD/TEMP
MODE/CAL

Plate 2: pH Meter.

Plate 3: Growth Spectrophotometer.


59.4
NTU
Zero Adjust
Correction
Turbidity Meter

Plate 4: Turbidity Meter

Plate 5: Dissolved Oxygen Meter.

CHAPTER THREE

RESULTS

Figure One (1) Shows the effect of urea on the growth of *Chlorella vulgaris*.

Statistically, two-way ANOVA revealed that there were no significant differences ($p>0.05$) in the growth response of *Chlorella vulgaris* across different concentrations of urea through the experiment.

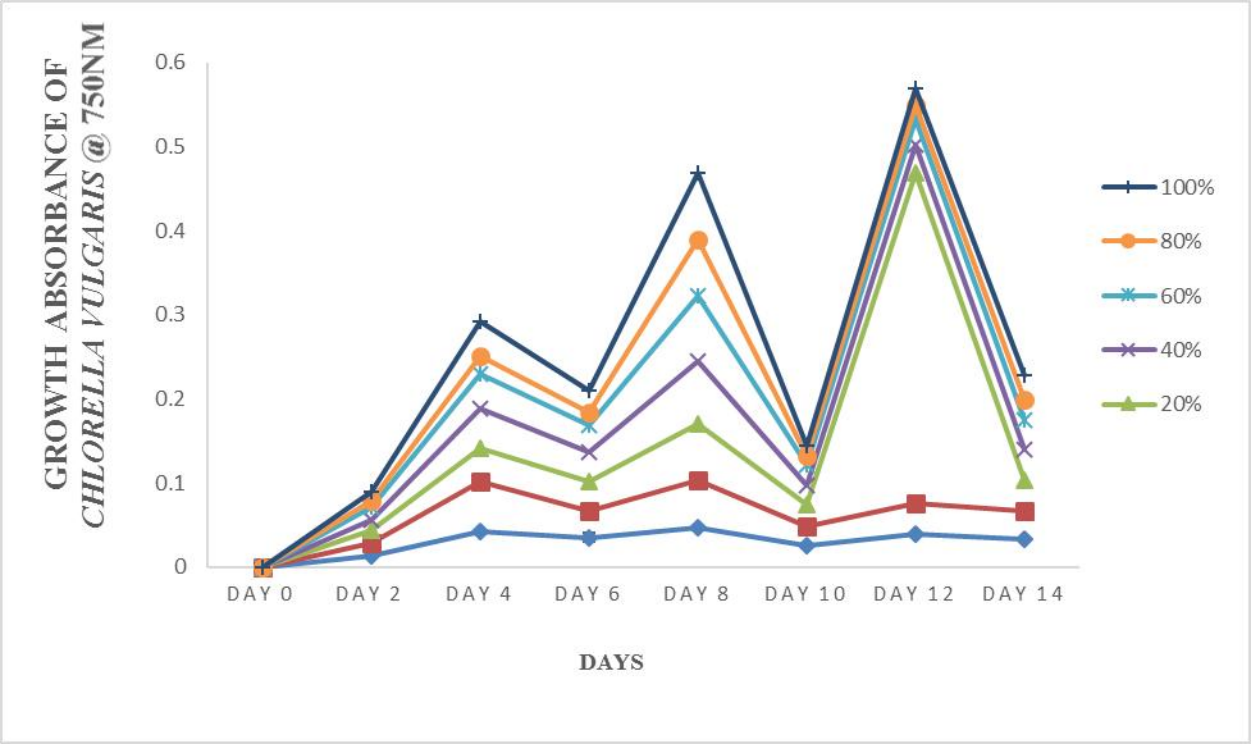

Figure 1: Effect of Urea on the growth of *Chlorella vulgaris*

Figure Two (2) Shows effect of urea on the growth of *Scenedesmus sp.*

Statistically, two-way ANOVA revealed that there were significance differences ($p<0.05$) in the growth response of *Scenedesmus*.

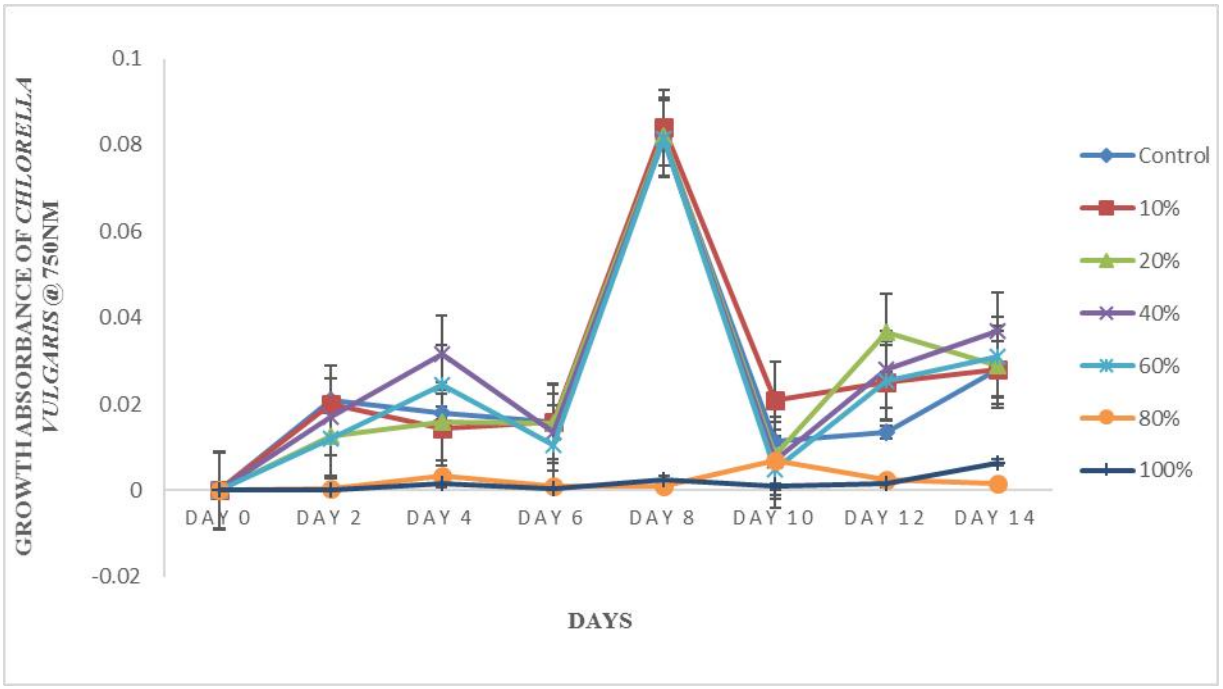

Figure 2: Effect of Urea on the growth of *Scenedesmus* sp.

Figure Three (3) Shows the comparative percentage yield of *Chlorella vulgaris and Scenedesmus sp.*

The results of paired sample -t - test on the effect of different concentrations of urea on the yield of *Chlorella vulgaris and Scenedesmus sp* revealed that there were significant difference ($p<0.05$) in yield.

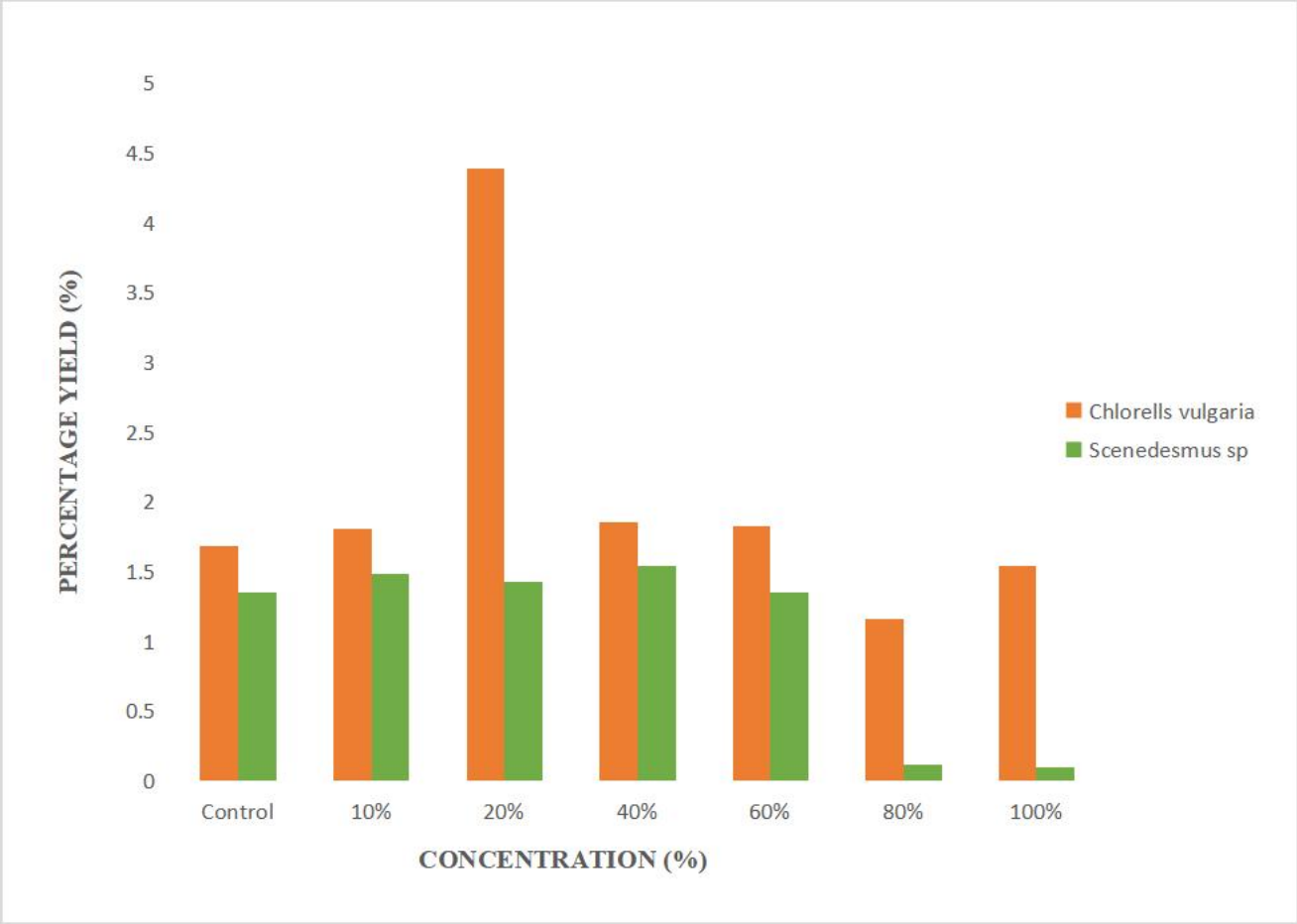

Figure 3: Comparative Percentage Yield of *Chlorella vulgaris* and *Scenedesmus* sp.

Figure Four (4) Shows the turbidity of different concentrations of urea on the growth of *Chlorella vulgaris.*

Statistically, two-way ANOVA revealed that there were no significant differences ($p>0.05$) between urea turbidity levels across each day of *Chlorella vulgaris* growth.

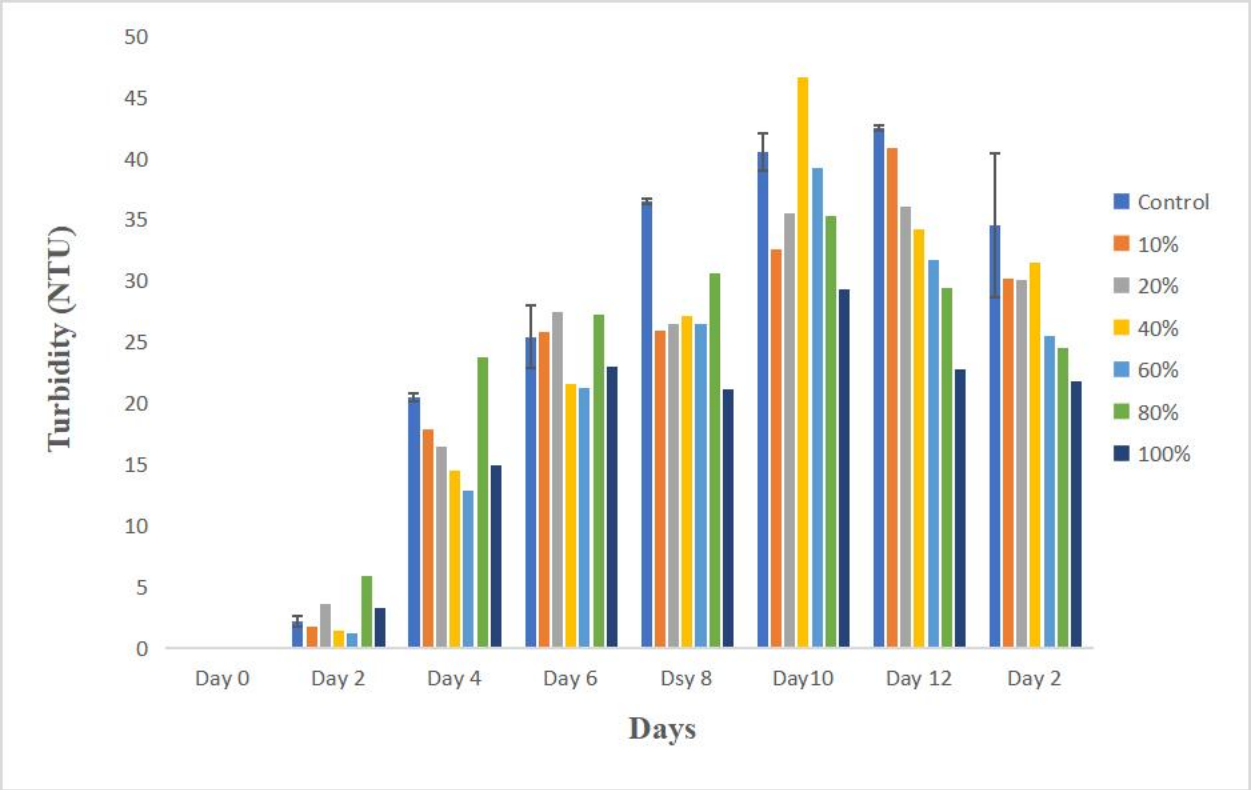

Figure 4: Turbidity of different concentration of Urea on the growth of *Chlorella vulgaris*

Figure Five (5) Shows the turbidity of different concentrations of urea on the growth of *Scenedesmus sp.*

Statistically, two-way ANOVA revealed that there were no significant difference ($p>0.05$) between urea turbidity levels across each day of *Scenedesmus sp* growth.

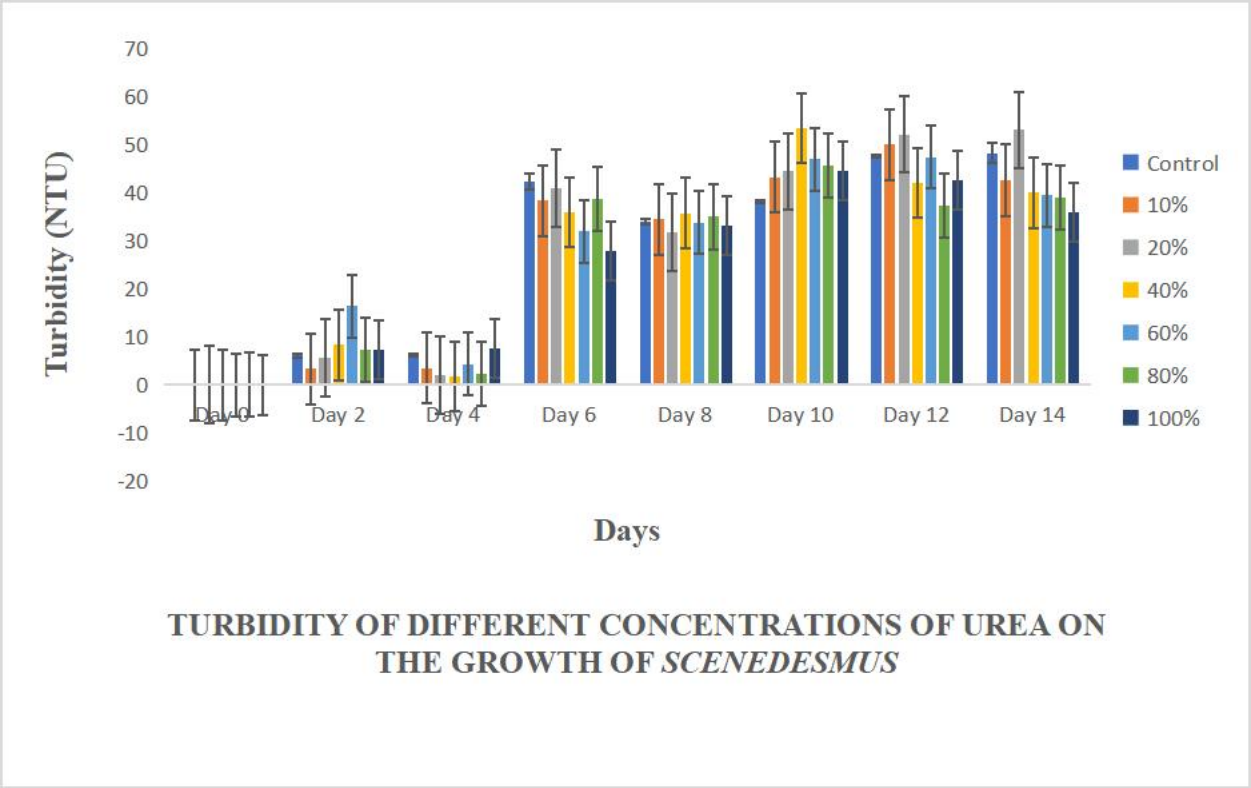

Figure 5: Turbidity of different concentration of Urea on the growth of *Scenedesmus* sp.

Figure Six (6) Shows the conductivity of different concentrations of urea on the growth of *Chlorella vulgaris.*

Statistically, two-way ANOVA revealed that there were significant difference ($p<0.05$) between urea conductivity level across each *Chlorella vulgaris sp* growth.

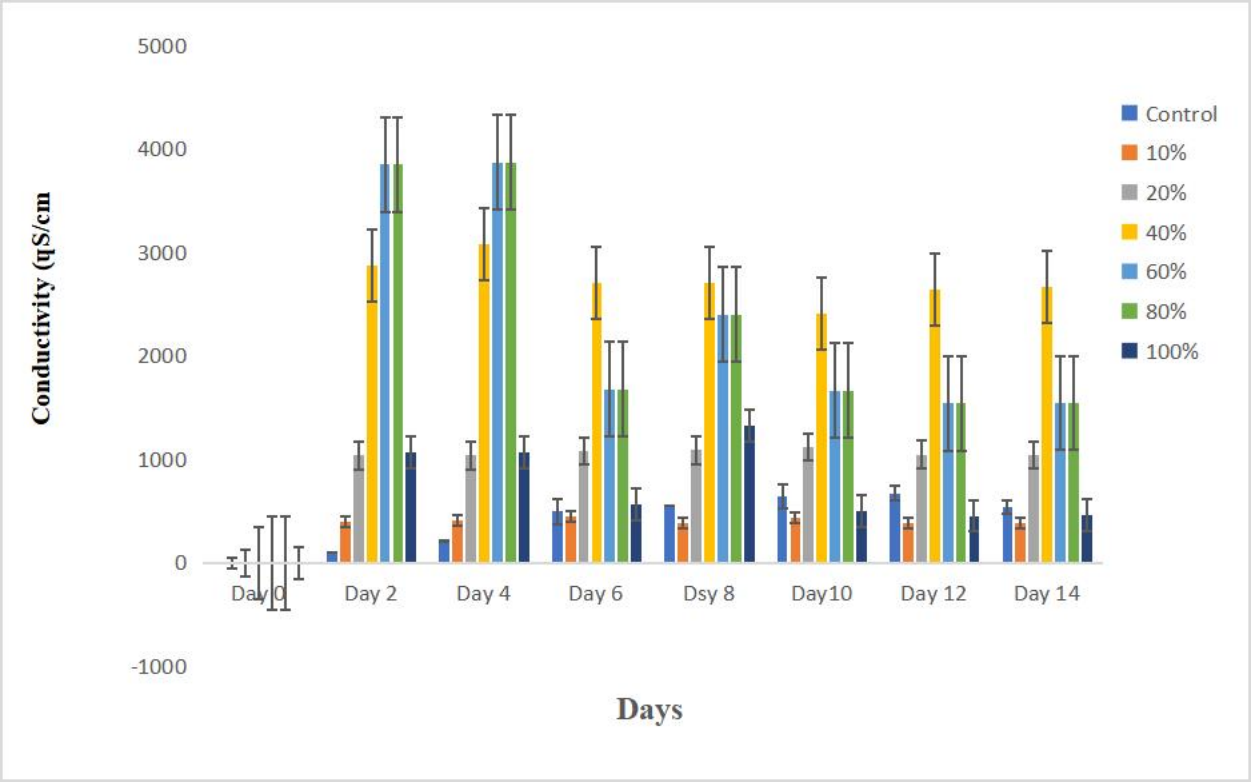

Figure 6: Conductivity of different concentration of Urea on the growth of *Chlorella vulgaris*

Figure Seven (7) Shows the conductivity of different concentrations of urea on the growth of *Scenedesmus.*

Statistically, two-way ANOVA revealed that there were significant difference ($p<0.05$) between urea conductivity level across each day of *Scenedesmus sp* growth.

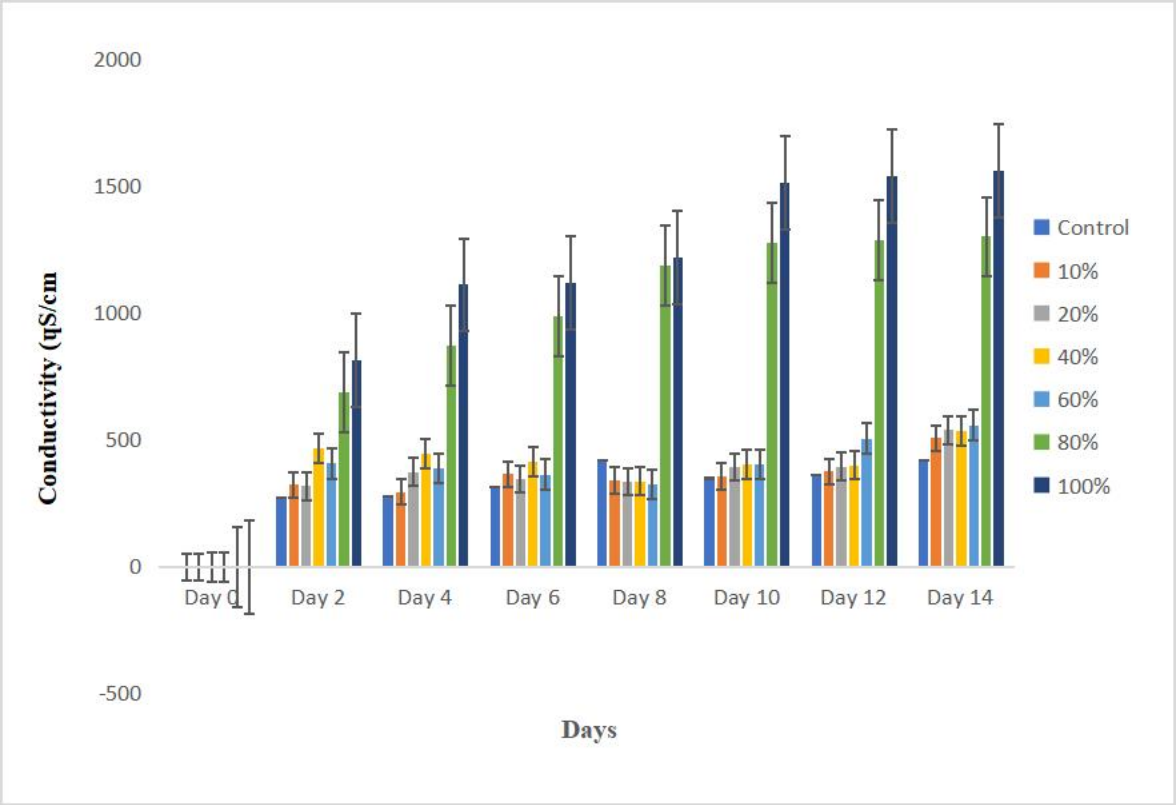

Figure 7: Conductivity of different concentration of Urea on the growth of *Scenedesmus*

Figure Eight (8) Shows the total dissolved solid of different concentrations of urea on the growth of *Chlorella vulgaris*.

Statistically, two-way ANOVA revealed that there were significant difference ($p<0.05$) between urea total dissolved solid level across each day of *Chlorella vulgaris sp* growth.

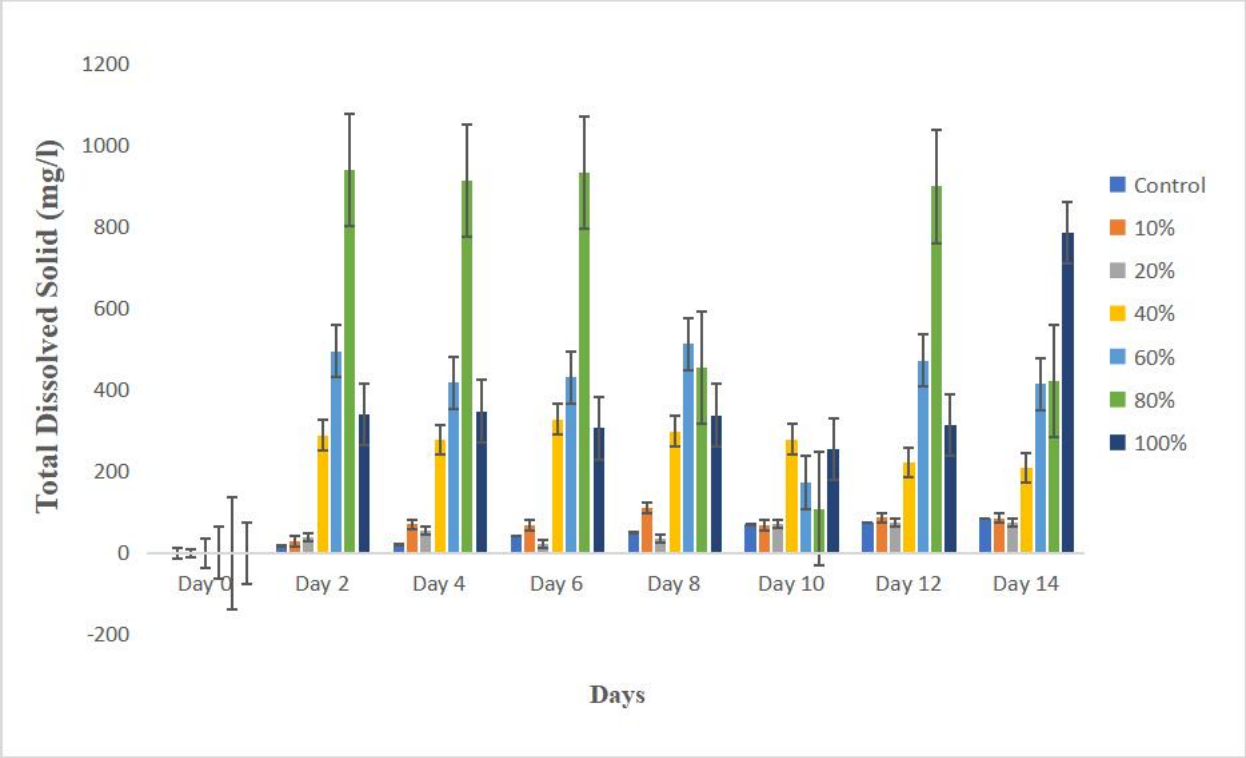

Figure 8: Total dissolved solid of different concentration of Ureaon the growth of *Chlorella vulgaris*.

Figure Nine (9) Shows the total dissolved solid of different concentrations of urea on the growth of *Scenedesmus sp*

Statistically, two-way ANOVA revealed that there were significant difference ($p<0.05$) between urea total dissolved solid level across each day of *Scenedesmus sp* growth.

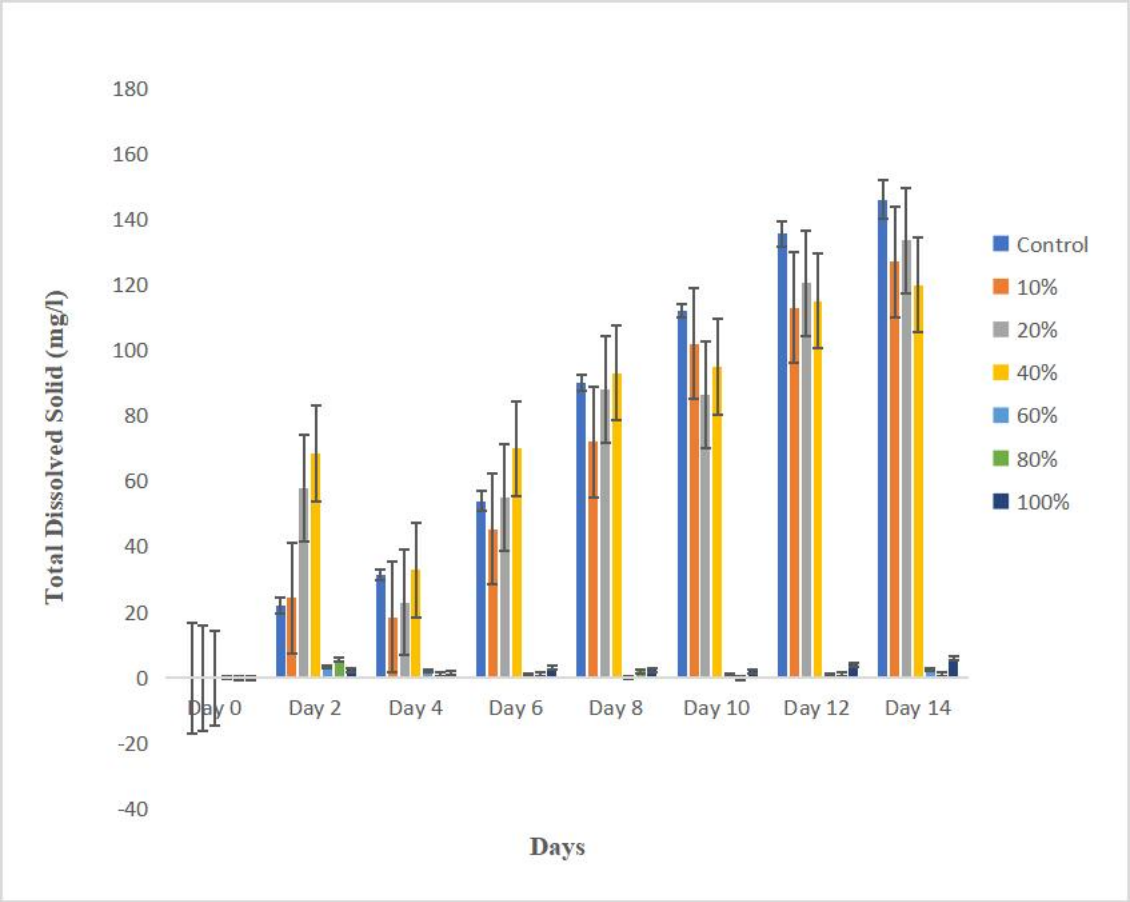

Figure 9: Total dissolved solid of different concentration of Urea on the growth of *Scenedesmus* sp.

Figure Ten (10) Shows the pH of different concentrations of urea on the growth of *Chlorella vulgaris.*

Statistically, two-way ANOVA revealed that there were significant difference ($p< 0.05$) between urea pH level across each day of *Chlorella vulgaris* growth.

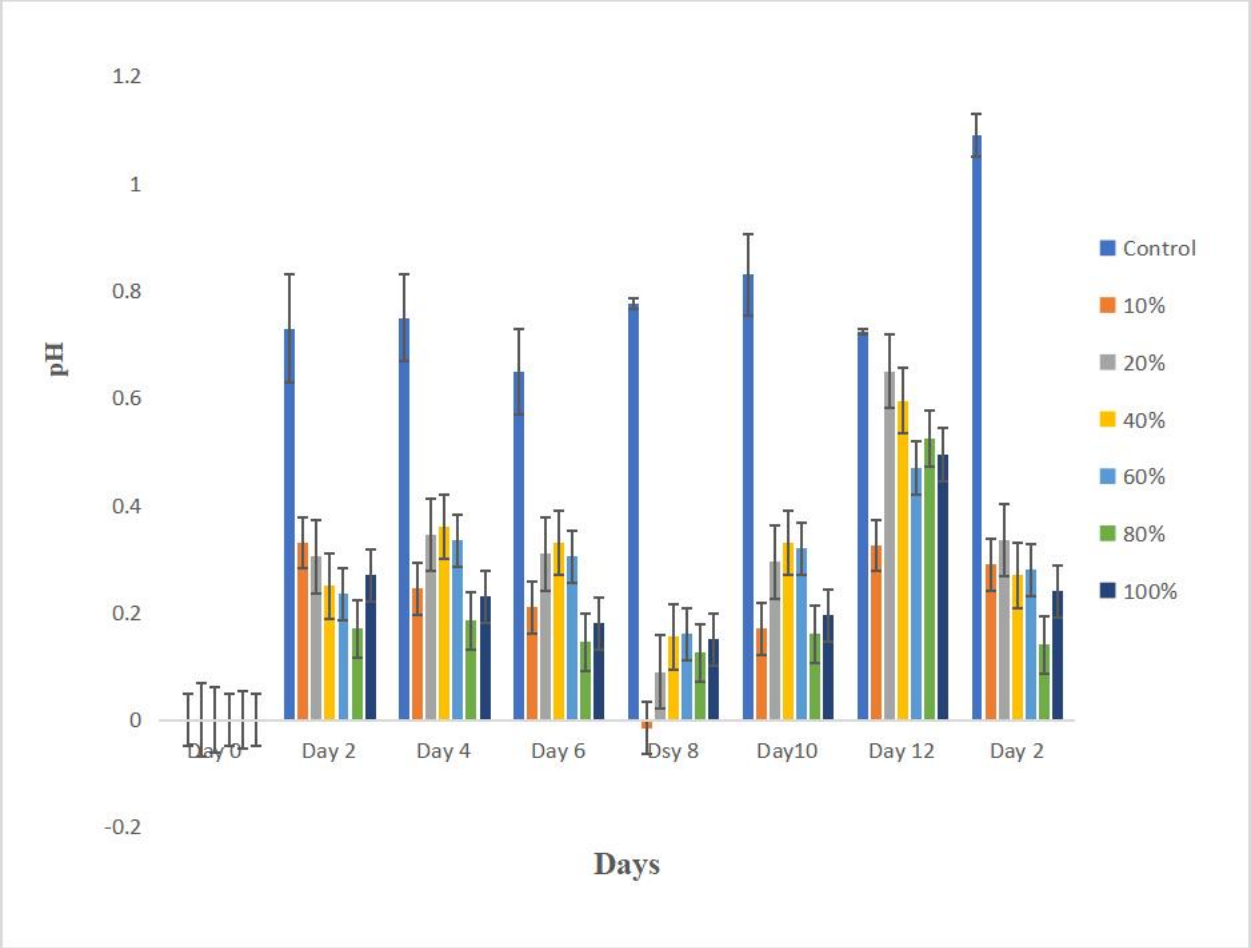

Figure 10: pH of different concentration of Urea on the growth of *Chlorella vulgaris*

Figure Eleven (11) Shows the pH of different concentrations of urea on the growth of *Scenedesmus sp.*

Statistically, two-way ANOVA revealed that there were significant difference ($p< 0.05$) between urea pH level across each day of *Scenedesmus sp* growth.]

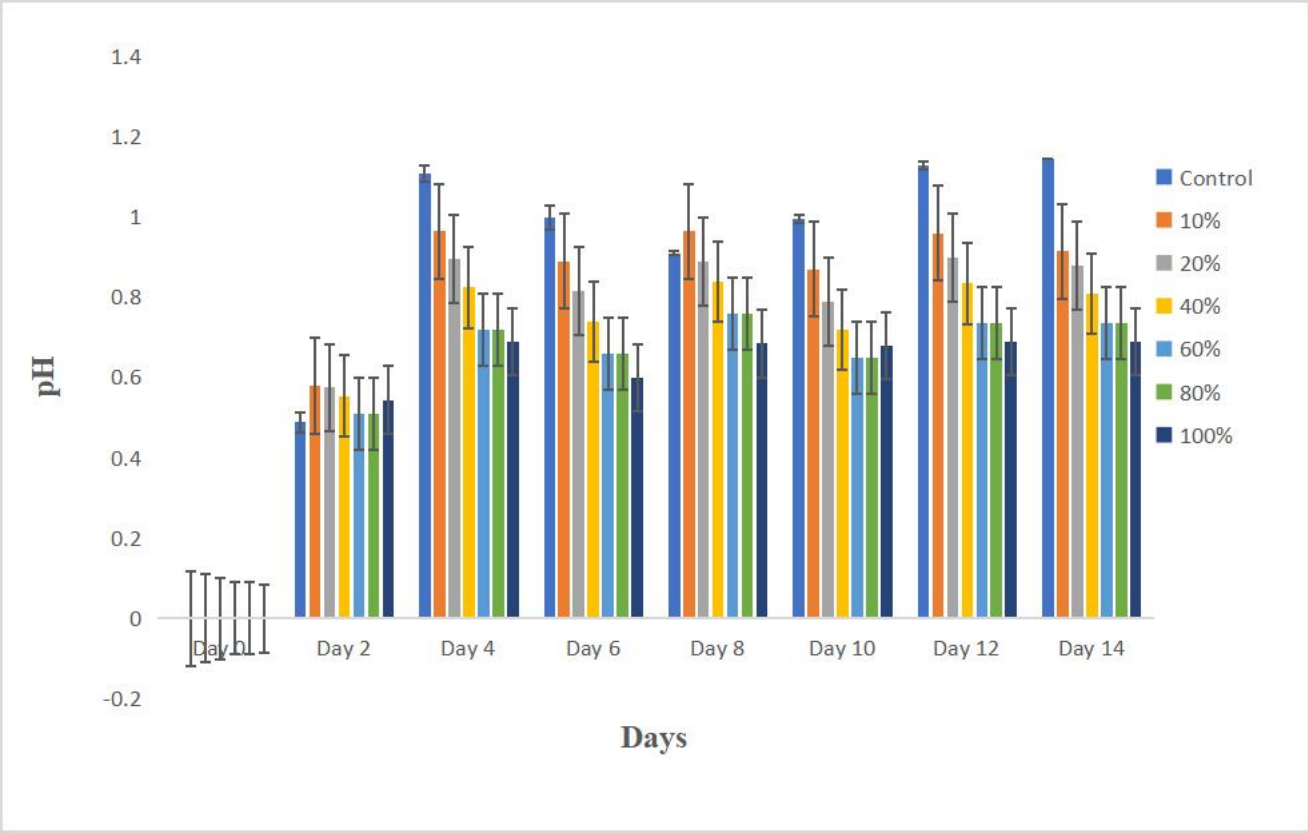

Figure 11: pH of different concentration of Urea on the growth of *Scenedesmus* sp.

Figure Twelve (12) Shows the Dissolved oxygen of different concentrations of urea on the growth of *Chlorella vulgaris*.

Statistically, two-way ANOVA revealed that there were significant difference ($p<0.05$) between urea dissolved oxygen levels across each day of chlorella vulgaris growth.

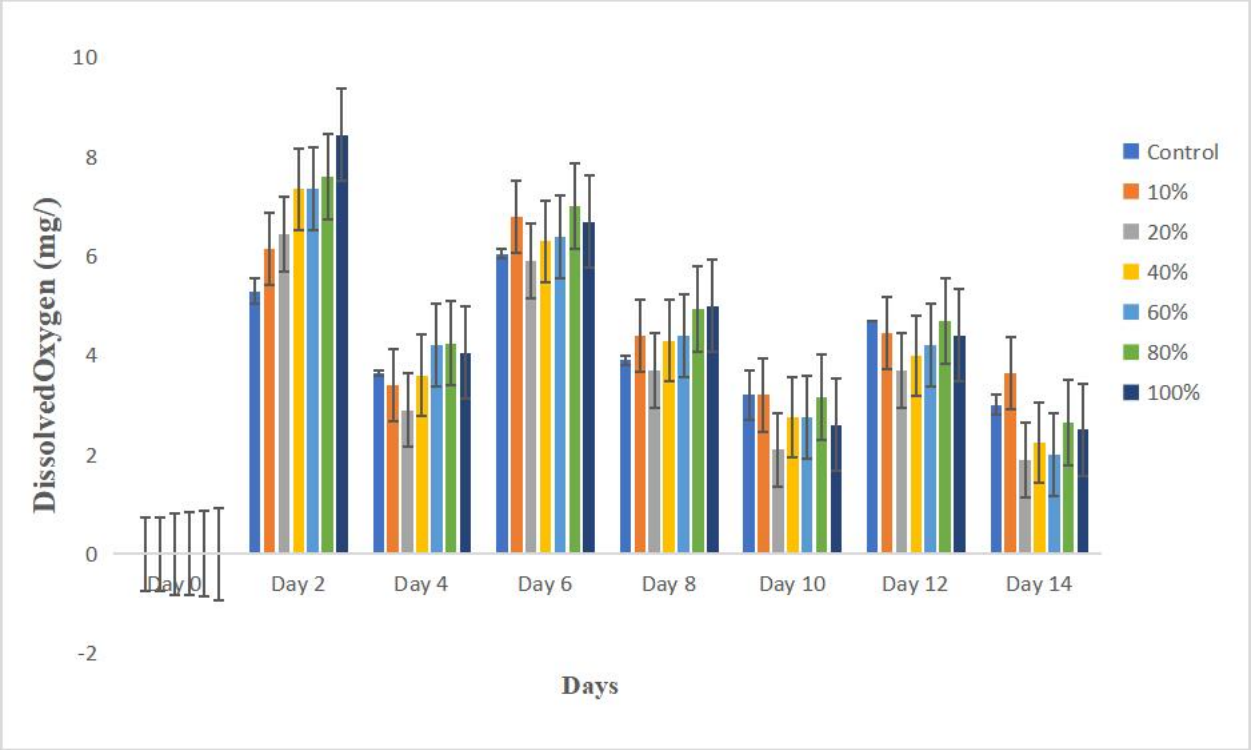

Figure 12: Dissolved oxygen of different concentration of Urea on the growth of *Chlorella vulgaris*.

Figure Thirteen (13) Shows the Dissolved oxygen of different concentrations of urea on the growth of *Scenedesmus sp*.

Statistically, two-way ANOVA revealed that there were significant difference ($p < 0.05$) between urea dissolved oxygen levels across each day of *Scenedesmus growth*.

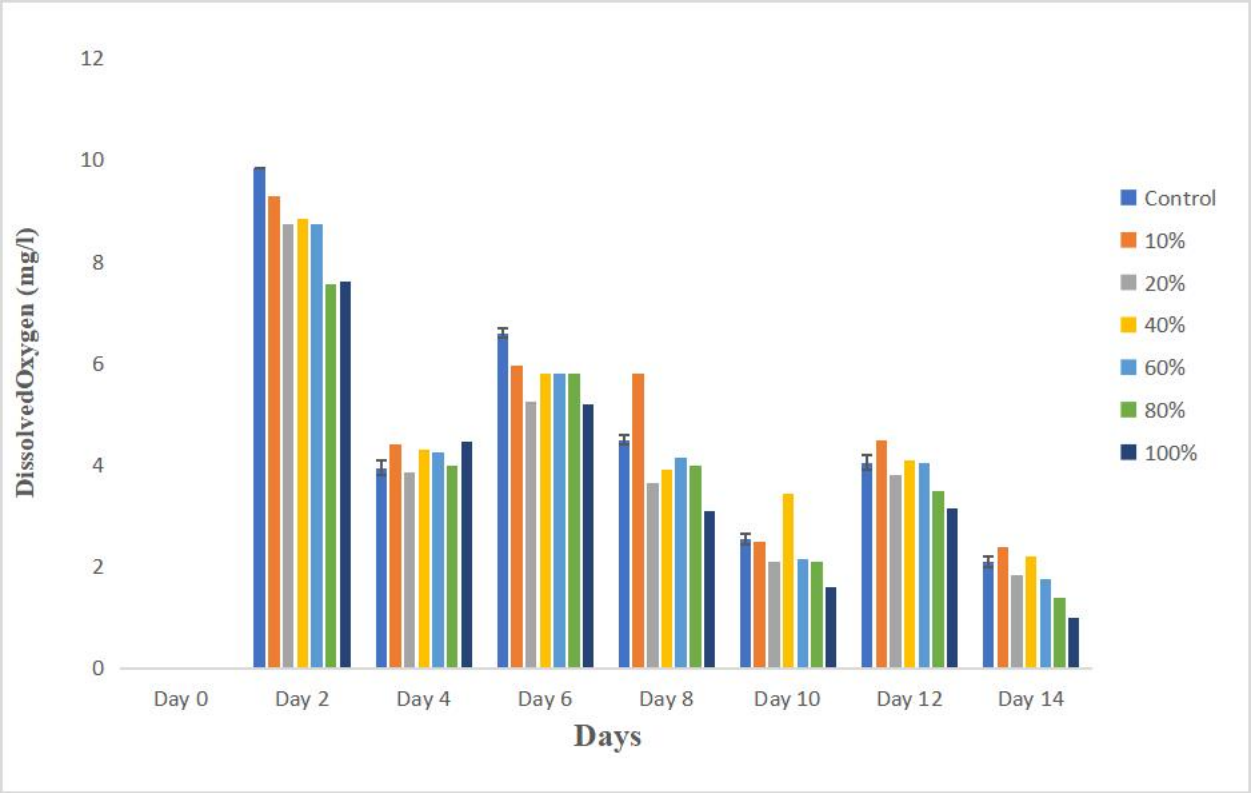

Figure 13: Dissolved oxygen of different concentration of Urea on the growth of *Scenedesmus* sp.

CHAPTER FOUR

DISCUSSION

The study investigated the effect of different urea concentrations on the growth of *Chlorella vulgaris* and *Scenedesmus*; freshwater microalgae species over a fourteen-day period under laboratory conditions. Some physicochemical parameters such as pH, Turbidity, conductivity, total dissolved solids and dissolved oxygen were examined.

Figure 1 and 2 revealed the effects of Urea on the growth of *Chlorella vulgaris* and *Scenedesmus* sp respectively. In figure 1, the graph showed that *Chlorella vulgaris* exhibited increased growth with higher concentration of urea with fluctuations over the fourteen-day period with the least growth occurring at 0% concentrations. This finding indicates that urea positively influenced the growth of *Chlorella vulgaris* especially at higher concentrations, promoting greater biomass production. However, excessive urea led to growth inhibition due to ammonia toxicity within the culture medium. Similar findings were recorded by El-Sayed *et* al., (2025) who observed a positive growth between urea levels, with the highest growth at full nitrogen concentration. Figure 2 shows the growth response of *Scenedesmus* to different concentrations of Urea. The growth increased gradually with a peak at day 8, especially in culture medium of 40 – 60% concentration of urea. The lowest growth was seen at 0% and 100% concentrations, indicating that *Scenedesmus* required moderate concentrations for optimal growth. This aligns with Rincon *et al.,* (2025) who reported moderate urea concentrations improved growth while excessive concentrations inhibited photosynthetic activity.

Figure 3 show the comparative yield of *Chlorella vulgaris and Scenedesmus sp.* at different urea concentrations. *Chlorella vulgaris* recorded the highest yield at 20% Urea, come after 40%, while growth decreased at higher levels (80% and 100%). *Scenedesmus sp* showed a lower yield across the concentration .This indicate that a moderate urea levels enhanced growth due to optimal nitrogen availability. Therefore higher concentration likely caused ammonia toxicity that suppressed growth. Similar observation were reported by Kumbhar *et al .,*(2023) who find that moderate urea promote algal productivity and inhibit photosynthesis.

Figure 4 shows that *Chlorella vulgaris* revealed the highest turbidity at 20% and 40% concentration of urea, showing the optimal nitrogen levels that support active growth and photosynthesis. Turbidity reduced at 80% and 100%, indicating too much urea caused nutrient stress or ammonia toxicity. This were confirmed by Li *et al*.,(2022), who stated that moderate urea enhanced algal growth while high concentrations inhibit cell activity.

Figure 5 shows that *Scenedesmus sp. o*bserved the highest turbidity at 20% and 40% urea concentrations. This implies that moderate concentrations promoted optimal nutrient uptake and growth; however excess concentrations caused nutrient inhibitions to growth, hence lesser cell biomass conferring decreased turbidity. This result is similar to Liang and Kun *et al*., (2021) who observed that urea as a nitrogen source increased the growth rate of *Scenedesmus dimorphus* significantly and high concentrations inhibited growth.

The conductivity results (Figure 6) shows that *Chlorella vulgaris* recorded highest growth at urea concentrations of 60% and 80%, but subsequently over the fourteen-day period, 40% had optimal growth. The increase in conductivity in Chlorella vulgaris at moderate urea concentrations is due to its ability to actively metabolize urea, breaking it down to ammonium and carbonate ions, thus increasing the ionic strength of the medium and as such increasing electrical conductivity. Similar finding was recorded by Vincze *et al.,* (2021), who reported that moderate showed the highest conductivity values.

Figure 7 shows that the conductivity of the culture medium increased steadily across all concentration from day 0 to day 14. 100% concentration urea recorded the highest growth, however there was a slow rise in growth over the days in comparison to moderate concentration to urea. This reflects enhanced metabolic activity and nutrient uptake. Growth became slower in higher concentrations due to ammonia toxicity; as a result *Scenedesmus* could not effectively utilize urea for optimal growth. Kebeish *et al*., (2014) had a different finding where moderate urea concentrations recorded the highest growth.

Figure 8 and 9 revealed the effect of different concentrations of urea on the total dissolved solids of *Scenedesmus* and *Chlorella vulgaris.* In Figure 8, *Chlorella vulgaris* peaked early and then declined after day 8, indicating that it rapidly utilized available nutrients in the culture, increasing its TDS Levels and experienced a drop after exhausting available nutrients. Higher

concentrations of Urea (100%) had lower TDS values owing to ammonium toxicity and reduced biomass activity. This result is in line with Li *et al*., (2011) study that stated that the microalgae efficiently utilized nitrogen at moderate levels and showed lower TDS as biomass increased. *Scenedesmus,* on the other hand could not withstand high concentrations of Urea (60-100%) and hence recorded extremely low TDS values. It grew best at moderate concentrations and increased progressively over the culture period. Rai *et al*., (2015) had a different observation in his work where *Scenedesmus* isolates experienced stable TDS values even at high nitrogen conferring urea toxicity.

Figure 10 shows how different concentrations of Urea impacted the growth of *Chlorella vulgaris*. The control concentration maintained the highest pH. Moderate concentrations maintained a stable pH across the growth period. Higher concentrations recorded low pH owing to urea toxicity (because of high ammonia content) with a gradual decline towards latter days. The drop in pH was due to urea hydrolysis which released carbonate and ammonium ions, which acidified the medium. Photosynthetic activity within the medium subsequently increased pH values. Abdel Raouf *et al*., (2012) reported pH fluctuation in algal cultures treated with nitrogenous sources which concurs with my findings. Figure 11 showed a more stable upward trend across all concentrations increasing gradually from Day 0 to 14. The control concentration had the highest pH and moderate concentrations (20-60%) maintained higher pH values, indicating efficient photosynthetic activity unlike higher concentrations which showed lowered pH conferring slight inhibitions and toxicity.

Figure 12 and 13 reveals the effect of different concentration of urea on the dissolved oxygen of *Chlorella vulgaris* and *Scenedesmus*. *Chlorella vulgaris* showed fluctuating patterns in dissolved oxygen levels. 100% concentration of urea initially recorded the highest DO values: it however experienced a decline over the fourteen-day period and as such moderate concentrations recorded the highest DO values. Increasing dissolved oxygen levels can be attributed to active photosynthetic oxygen generation showing healthy growth. This totally agrees with Li *et al*¸ (2011) study who reported highest DO and growth rate at moderate concentration and photoinhibitions in the presence of excessive urea. Figure 13, on the other hand showed a steady decline from day 0 to 14 across all concentrations with the control efficiently having the highest

dissolved oxygen level. The decline in DO corresponds with Rai *et al.,* (2015) observation on nutrient exhaustion and reduced light penetration in *Scenedesmus* at subsequent growth periods.

CONCLUSION

This study demonstrated the effect of different concentration of urea on the growth of *Chlorella vulgaris* and *Scenedesmus.* The results show that urea concentration affected the growth of both microalgae. Urea was analyzed and found to contain high levels of some physiochemical substances that cause ammonia toxicity and suppressed growth. However *Chlorella vulgaris* and *Scenedesmus* exhibited optimal growth at moderate concentration of urea. Growth inhibition happened at high concentration of urea. However, *Chlorella vulgaris* had a higher tolerance to urea than *Scenedesmus* and thus maintained a higher yield .Urea inhibited the growth of both microalgae and hence cannot be used for bioremediation purposes.

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APPENDIX I

NOVA: Growth Response of *Chlorella vulgaris* to Urea

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.080799	6	0.013466	4.462421	0.000436	2.180564
Columns	0.14602	7	0.02086	6.912446	7.8E-07	2.092381
Interaction	0.520437	42	0.012391	4.106157	1.35E-09	1.493427
Within	0.337988	112	0.003018			
Total	1.085244	167				

ANOVA: Growth Response of *Scenedesmus* sp. to Urea

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.001239	6	0.000207	7.870992	4.39E-07	2.180564
Columns	0.090186	7	0.012884	491.0287	6.27E-81	2.092381
Interaction	0.003027	42	7.21E-05	2.746457	1.26E-05	1.493427
Within	0.002939	112	2.62E-05			
Total	0.09739	167				

ANOVA: Turbidity for *Chlorella vulgaris*

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	775.7878	6	129.298	8.025457	3.25E-07	2.180564
Columns	29110.42	7	4158.632	258.1241	5.29E-66	2.092381
Interaction	1158.173	42	27.57554	1.7116	0.013596	1.493427
Within	1804.43	112	16.11098			
Total	32848.81	167				

ANOVA: Turbidity for *Scenedesmus*

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	226.6283	6	37.77139	26.32119	2.34E-19	2.180564
Columns	56536.65	7	8076.664	5628.265	1.7E-139	2.092381
Interaction	2242.85	42	53.40119	37.2129	4.57E-49	1.493427
Within	160.7221	112	1.435018			
Total	59166.85	167				

APPENDIX II

ANOVA: pH for *Chlorella vulgaris*

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	6.228375	6	1.038063	55.36158	2.91E-31	2.180564
Columns	3.579857	7	0.511408	27.27424	1.56E-21	2.092381
Interaction	1.753635	42	0.041753	2.226767	0.000455	1.493427
Within	2.100067	112	0.018751			
Total	13.66193	167				

ANOVA: pH for *Scenedesmus*

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	1.654212	6	0.275702	75.57176	4.5E-37	2.180564
Columns	12.85302	7	1.836146	503.2998	1.64E-81	2.092381
Interaction	0.772931	42	0.018403	5.044418	4.12E-12	1.493427
Within	0.4086	112	0.003648			
Total	15.68876	167				

ANOVA: Conductivity for *Chlorella vulgaris*

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	1.21E+08	6	20242060	105.5561	9.64E-44	2.180564
Columns	43318127	7	6188304	32.2701	3.97E-24	2.092381
Interaction	28202473	42	671487.5	3.5016	7.18E-08	1.493427
Within	21477781	112	191765.9			
Total	2.14E+08	167				

ANOVA: Conductivity for *Scenedesmus*

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	16672548	6	2778758	105047.6	1.6E-207	2.180564
Columns	8287135	7	1183876	44755.01	7.2E-190	2.092381
Interaction	3926198	42	93480.89	3533.931	1.5E-157	1.493427
Within	2962.667	112	26.45238			
Total	28888843	167				

ANOVA: Total Dissolved Solid for *Chlorella vulgaris*

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	6099531	6	1016588	1656.388	7E-107	2.180564
Columns	1838518	7	262645.4	427.9438	1.06E-77	2.092381
Interaction	3335998	42	79428.52	129.4176	8.91E-78	1.493427
Within	68738.67	112	613.7381			
Total	11342785	167				

ANOVA: Total Dissolved Solid for *Scenedesmus*

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	6340842	6	1056807	23217.42	7.8E-171	2.180564
Columns	885362.5	7	126480.4	2778.698	2.1E-122	2.092381
Interaction	1522153	42	36241.73	796.2091	2.1E-121	1.493427
Within	5098	112	45.51786			
Total	8753456	167				

ANOVA: Dissolved Oxygen for *Chlorella vulgaris*

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	10.95206	6	1.825344	7.357354	1.2E-06	2.180564
Columns	713.2582	7	101.894	410.7009	9.67E-77	2.092381
Interaction	22.4154	42	0.5337	2.151167	0.000762	1.493427
Within	27.78696	112	0.248098			
Total	774.4127	167				

ANOVA: Dissolved Oxygen for *Scenedesmus*

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	20.50119	6	3.416865	47.20669	1.85E-28	2.180564
Columns	1023.848	7	146.2639	2020.752	1E-114	2.092381
Interaction	18.55119	42	0.441695	6.102365	1.02E-14	1.493427
Within	8.106667	112	0.072381			
Total	1071.007	167				