EFFECTS OF VINEGAR ON THE GROWTH OF TWO FRESHWATER MICROALGAE (*Chlamydomonas sp. and Scenedesmus sp.*)

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UNIVERSITY OF BENIN

BENIN CITY

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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 REQUIREMENT FOR THE UNIVERSITY OF BENIN, BENIN CITY, AWARD OF BACHELOR OF SCIENCE (B.Sc HONS) DEGREE.

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This is to certify that this project work was carried out by Beatrice Ogheneyoma OROGUN of the Department of Plant Biology and Biotechnology. Faculty of Life Sciences, University of Benin, Benin city, Nigeria.

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(Project Supervisor)	
Prof. Beckley Ikhajiagbe	**Date**
(Head of Department)	

DEDICATION

This work is dedicated to the pursuit of knowledge and to God Almighty for his wisdom, strength and direction.

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ABSTRACT

This study examined the effects of vinegar on the growth of two freshwater microalgae: Scenedesmus sp. and Chlamydomonas sp.. The experiment was setup in triplicates of different concentrations of vinegar (0% (control), 1%, 5%,10%,15%,20% and 25%) in a culture media and monitored for 14 days. The samples were analyzed every two days to monitor growth using a visible spectrophotometer at a wavelength of 750nm as well as other physicochemical parameters such as pH, conductivity, turbidity, total dissolved solids and dissolved oxygen. The results were analyzed using statistical two way analysis of variance ANOVA repeated measures, paired t-test, microsoft excel and descriptive statistics. The result showed that the growth of Scenedesmus sp. and Chlamydomonas sp. were affected by the different concentrations of vinegar with a significant difference ($p<0.05$) across all parameters. The control (0% vinegar) recorded the highest growth, yield and dissolved oxygen while the 10%vinegar concentration produced the lowest values, indicating that increasing acidity inhibited algal growth and photosynthetic activity. The comparative percentage yield between both species showed no significant difference ($p>0.05$), suggesting comparable productivity under mild acidity.

CHAPTER ONE

INTRODUCTION

Aquatic ecosystems are continuously subjected to a wide variety of natural and anthropogenic inputs, ranging from nutrients and trace metals to household and industrial effluents. The productivity, biodiversity, and ecological stability of these systems depend strongly on the activities of primary producers, especially microalgae. Microalgae form the foundation of aquatic food webs, supply oxygen through photosynthesis, and recycle nutrients critical for ecosystem functioning (Barsanti and Gualtieri, 2014). Any chemical disturbance that alters the growth or metabolism of these organisms can have cascading effects on higher trophic levels and overall ecosystem services.

One group of compounds that has received growing attention is organic acids, particularly acetic acid, the main component of vinegar. Vinegar is a weak acid traditionally used for food preservation, cleaning, and medicinal purposes, but in modern times it is also employed in agriculture, industrial processes, and domestic waste management (Budak *et al.,* 2014). Its widespread usage increases the likelihood of its introduction into aquatic environments via wastewater discharge, food processing effluents, or agricultural runoff. When vinegar enters aquatic systems, it may influence microbial communities directly by acting as a nutrient or indirectly by altering pH levels.

The ecological implications of vinegar pollution or supplementation are not well understood, especially concerning photosynthetic microorganisms such as microalgae. Vinegar is not inherently toxic in the same way as synthetic pesticides or heavy metals, but it can influence growth patterns in multiple ways. At low concentrations, acetic acid may serve as an additional carbon source for mixotrophic or heterotrophic microalgae, potentially enhancing

biomass productivity. However, at higher concentrations, its acidic nature can inhibit cell division, disrupt enzyme activity, damage membranes, and interfere with chlorophyll synthesis (Kumar *et al.,* 2020). This duality makes vinegar both a potential enhancer and stressor, depending on dosage and algal species.

Microalgae are microscopic, photosynthetic microorganisms that inhabit aquatic and terrestrial environments and contribute substantially to global primary productivity. They are a diverse group of prokaryotic and eukaryotic organisms capable of converting sunlight, carbon dioxide, and water into organic biomass through photosynthesis. Unlike higher plants, microalgae lack true roots, stems, and leaves but possess chlorophyll pigments and photosystems that allow efficient energy capture and conversion (Richmond and Hu 2013). They constitute an essential component of the Earth's biosphere, performing nearly half of the planet's total photosynthetic activity and producing significant amounts of atmospheric oxygen (Falkowski and Raven 2013).

Taxonomically, microalgae include species distributed across several phyla such as *Chlorophyta* (green algae), *Bacillariophyta* (diatoms), *Cyanobacteria* (blue-green algae), *Chrysophyta* (golden algae), and *Rhodophyta* (red algae). They exhibit remarkable physiological and biochemical diversity, enabling adaptation to extreme habitats ranging from freshwater and marine systems to deserts and snowfields (Barsanti and Gualtieri 2014). Their cellular structures vary from simple unicellular forms such as *Chlamydomonas reinhardtii* to complex colonial and filamentous species like *Scenedesmus obliquus* and Spirogyra spp. Cell walls are typically composed of polysaccharides, glycoproteins, or silica, depending on the species, and contain chloroplasts with pigments such as chlorophyll a, chlorophyll b, β-carotene, and *xanthophylls* that support photosynthesis and provide distinctive coloration (Hu *et al*., 2008).

Physiologically, microalgae can grow under three main nutritional modes: autotrophic, heterotrophic, and mixotrophic. Under autotrophic conditions, they depend solely on light energy and inorganic carbon (CO_2) for photosynthesis. In heterotrophic growth, they utilize organic carbon sources in the absence of light, while mixotrophic growth combines both processes, allowing simultaneous use of light and organic carbon substrates (Perez-Garcia *et al*., 2011). This metabolic flexibility makes microalgae highly resilient to fluctuations in light intensity, nutrient availability, and carbon source. Species such as *Chlamydomonas reinhardtii* and *Scenedesmus obliquus* exemplify this versatility; both can assimilate acetate as an external carbon source, which enhances biomass yield and lipid accumulation under low-light or nutrient-limited conditions (Lauersen *et al.,* 2019; Li *et al*., 2019).

Microalgae possess rapid growth rates and high photosynthetic efficiency, often surpassing terrestrial plants in biomass productivity per unit area (Brennan and Owende 2010). Their ability to assimilate nitrogen, phosphorus, and trace metals from wastewater makes them valuable agents for bioremediation. Additionally, they synthesize a wide range of metabolites including proteins, lipids, pigments, and polysaccharides of industrial significance. These compounds have applications in food, pharmaceuticals, cosmetics, and renewable energy sectors (Spolaore *et al*., 2006). The lipids extracted from microalgae, rich in triacylglycerols, serve as precursors for biodiesel production, while their carbohydrates can be fermented into bioethanol.

Ecologically, microalgae form the foundation of aquatic food webs by serving as primary producers and supporting higher trophic levels. They play vital roles in nutrient cycling, carbon sequestration, and oxygen release, thereby maintaining the balance of aquatic ecosystems (Falkowski and Raven 2013). However, their growth and physiological responses are highly sensitive to environmental parameters such as temperature, pH, salinity, nutrient concentration, and exposure to organic or inorganic compounds. Variations in these

factors can promote or inhibit algal proliferation, influence community structure, and alter metabolic pathways.

In recent years, increasing interest has emerged in understanding how organic compounds like acetic acid (the main component of vinegar) affect microalgal metabolism. Acetic acid is both a naturally occurring intermediate of microbial fermentation and a common anthropogenic pollutant resulting from household and industrial discharges. When introduced into aquatic systems, it can act either as a supplementary carbon source or as a stress-inducing agent depending on its concentration (Wang *et al.,* 2018). At low levels, acetic acid enhances growth by entering the tricarboxylic acid cycle, supporting energy production and biosynthesis. At elevated concentrations, however, it may lower the medium pH, disrupt cellular homeostasis, and inhibit photosystem II activity (Gao *et al*., 2017).

Species such as *Scenedesmus* and *Chlamydomonas* are widely used as model organisms to study these interactions because of their well-characterized genomes, ease of cultivation, and predictable growth behavior under controlled laboratory conditions (Harris 2009). They exhibit rapid physiological responses to external stimuli, including pH fluctuations and organic acid exposure, making them suitable bioindicators of environmental stress. Investigating their growth response to vinegar provides valuable insight into both ecological tolerance and the potential application of organic acids in enhancing biomass productivity.

Consequently, the study of microalgal responses to vinegar integrates ecological, biochemical, and biotechnological perspectives. It helps elucidate how small organic molecules influence carbon metabolism and energy flow in algal systems and offers guidance on managing organic waste to minimize ecological disturbances. It also supports the optimization of culture conditions for sustainable bioresource production while assessing the limits of algal resilience in the presence of anthropogenic organic compounds.

1.1 Vinegar and the Environment

Vinegar is a dilute aqueous solution of acetic acid (CH_3COOH) typically containing between four and eight percent acid by volume. It is one of the oldest and most widely used organic acids in human history, traditionally produced through the fermentation of ethanol by acetic acid bacteria belonging primarily to the genera *Acetobacter* and *Gluconobacter* (Solieri and Giudici 2009). The process occurs aerobically, where ethanol is oxidized to acetic acid in the presence of oxygen, resulting in the distinctive sour taste and pungent odour characteristic of vinegar. Over centuries, vinegar has been employed for culinary, medicinal, cleaning, and agricultural purposes. Its ubiquitous domestic and industrial use, coupled with its water solubility, makes it a frequent component of household effluents and wastewater discharges (Budak *et al*., 2014).

Chemically, acetic acid is a weak monoprotic acid that can dissociate partially in water, releasing hydrogen ions and acetate ions. This partial dissociation enables vinegar to influence the pH and buffering capacity of aquatic environments when released in significant quantities. At low concentrations, it can serve as a biodegradable carbon source for various microorganisms, supporting heterotrophic growth and metabolic activity. However, when introduced in excess, vinegar can acidify water bodies, lower pH levels, and disrupt the natural equilibrium of microbial and algal communities (Russell 1992). The resulting acidification can have far-reaching ecological consequences, altering nutrient availability, enzyme function, and photosynthetic efficiency in primary producers.

From an environmental standpoint, vinegar's influence extends beyond its acidity. Acetic acid plays an important role in the global carbon cycle as an intermediate in the decomposition of organic matter. Many bacteria and fungi produce acetic acid during fermentation or anaerobic respiration, and other microorganisms subsequently oxidize it into carbon dioxide and water. When anthropogenic sources of vinegar enter aquatic ecosystems,

they interact with these natural microbial processes, potentially accelerating or disturbing biogeochemical cycles (Wang et *al*., 2018). In controlled amounts, this can enhance microbial respiration and stimulate primary productivity. However, persistent or concentrated inputs may deplete dissolved oxygen through microbial overconsumption, leading to hypoxia or eutrophic conditions detrimental to aquatic fauna (Jenkins *et al.*, 2011).

The environmental impact of vinegar is influenced by its concentration, frequency of discharge, and the buffering capacity of the receiving water body. For instance, small streams or ponds with limited buffering potential are more susceptible to pH fluctuations than large rivers or lakes. The pKa value of acetic acid (4.76) indicates that even modest quantities can appreciably lower water pH, particularly in systems already burdened by other acidifying pollutants (Rittmann and McCarty 2012). This acidity may inhibit the activity of photosynthetic organisms such as microalgae, whose cellular metabolism and chlorophyll synthesis are sensitive to hydrogen ion concentration. Excess acidity can impair photosystem II, hinder carbon fixation, and induce oxidative stress through the accumulation of reactive oxygen species (Gao *et al.,* 2017).

Despite these potential hazards, vinegar is generally regarded as more environmentally benign compared to synthetic chemical contaminants such as pesticides, heavy metals, and antibiotics. Acetic acid biodegrades readily under aerobic and anaerobic conditions, with a short half-life in water, often less than a few days depending on temperature and microbial activity (European Chemicals Agency 2023). This rapid degradation minimizes its long-term persistence but does not eliminate its potential for acute or chronic ecological effects during exposure. The biodegradability of vinegar makes it a double-edged substance: while not persistent, it can cause transient but significant stress to sensitive aquatic organisms during peak discharge events.

In agricultural and domestic contexts, vinegar has gained popularity as an eco-friendly herbicide and cleaning agent. Organic farmers frequently use vinegar to control weeds because of its acetic acid content, which denatures plant cell membranes and proteins upon contact (Fausey 2003). Similarly, households use vinegar as a natural disinfectant or descaling agent. However, the unregulated disposal of these solutions—especially in rural areas without proper wastewater treatment—can result in the accumulation of acidic effluents in nearby water bodies. When combined with runoff from agricultural soils and household waste, vinegar can modify local aquatic chemistry, influencing microbial balance and algal composition (Budak *et al.,* 2014).

The presence of vinegar in aquatic systems can also have indirect ecological effects. For instance, the acidification it induces can alter nutrient solubility and availability. Phosphorus, for example, becomes more soluble at lower pH levels, potentially promoting algal blooms when sufficient light and nitrogen are present. Conversely, extremely low pH values can precipitate essential micronutrients such as iron and magnesium, limiting algal photosynthesis (Raven *et al*., 2000). Additionally, acetic acid can chelate metal ions, altering their bioavailability and toxicity profiles for aquatic organisms. Thus, vinegar does not act merely as a simple acidifying agent but also as a modulator of chemical interactions in aquatic ecosystems.

Several studies have examined the impact of organic acids, including acetic acid, on microbial and algal physiology. For example, Narendranath (*et al*., 2001) observed that acetic acid accumulation in fermentation broths inhibited yeast metabolism by disturbing intracellular pH and membrane potential. Similar effects have been reported in microalgae, where excessive acid exposure reduces photosynthetic pigment content and induces lipid peroxidation (Li *et al.*, 2019). In contrast, low concentrations of acetic acid can stimulate mixotrophic growth in species capable of assimilating acetate as a carbon source, such as

*Chlamydomonas reinhardtii (*Yang *et al.,* 2015). This concentration-dependent response underscores the complexity of vinegar's ecological role: it can function as both a nutrient and a toxin.

From a sustainability perspective, understanding how vinegar interacts with aquatic environments is increasingly important. Global efforts to reduce chemical pollution have promoted the substitution of synthetic compounds with biodegradable alternatives. However, even natural substances like vinegar can have unintended ecological consequences if used or discharged in large quantities. Assessing these effects is essential for maintaining the delicate balance of aquatic ecosystems, particularly in urbanized regions where domestic wastewater and agricultural runoff converge.

1.2 Botanical Characteristics of *Scenedesmus* and *Chlamydomonas*

Microalgae constitute a broad and diverse group of unicellular photosynthetic organisms that occupy nearly every illuminated aquatic environment on Earth. They exhibit extraordinary structural and physiological diversity, ranging from simple unicellular species to complex colonial or filamentous forms. Two genera of microalgae that have been extensively studied for their ecological, physiological, and biotechnological importance are *Scenedesmus* and *Chlamydomonas*. Both belong to the division *Chlorophyta* (green algae), which is characterized by the presence of chlorophylls a and b, starch as the principal storage product, and cell walls primarily composed of cellulose and glycoproteins (Bold and Wynne 1985; Graham *et al.,* 2009).

1.2.1 Scenedesmus sp.

The genus *Scenedesmus* comprises non-motile, unicellular, or colonial green algae commonly found in freshwater environments such as ponds, lakes, and slow-moving rivers. Taxonomically, *Scenedesmus* belongs to the class *Chlorophyceae*, order *Sphaeropleales*, and family *Scenedesmaceae* (Komárek and Fott 1983). Morphologically, cells of *Scenedesmus* are typically ellipsoidal or cylindrical and often occur in coenobia (colonial units) of four, eight, or occasionally sixteen cells aligned in a single row. Each cell within the colony is enclosed by a firm, sometimes ornamented cell wall containing sporopollenin-like compounds that confer mechanical strength and resistance to environmental stress (Trainor 1998).

Scenedesmus cells possess a single cup-shaped chloroplast that occupies a large portion of the cytoplasm and contains a prominent pyrenoid associated with starch accumulation (Barsanti and Gualtieri 2014). This chloroplast structure is optimized for photosynthetic efficiency, allowing the alga to adapt to fluctuating light intensities. Unlike many other *chlorophytes, Scenedesmus* lacks flagella in its vegetative state and relies entirely on passive movement within the water column. Reproduction occurs primarily by autospores, where the parent cell divides mitotically to produce daughter cells within the original cell wall before release (Lürling 2003).

One of the most notable characteristics of *Scenedesmus* is its morphological plasticity. The species can alternate between unicellular and colonial forms in response to environmental conditions, particularly the presence of grazers such as Daphnia or chemical stressors (Lürling and Van Donk 1997). This induced morphological change, often called “defensive colony formation,” serves as an anti-predator strategy, increasing cell size and reducing palatability. Moreover, *Scenedesmus* can modify its cell wall thickness and external spines to enhance protection against environmental stress and predation. Such phenotypic

flexibility demonstrates a highly adaptive ecological strategy and underscores the genus's evolutionary success in freshwater ecosystems.

From a physiological perspective, *Scenedesmus* species are efficient photosynthetic organisms capable of utilizing various inorganic nitrogen sources such as nitrate, nitrite, and ammonium. They are also known for their tolerance to a wide range of environmental conditions, including variations in pH, salinity, and nutrient availability (Richmond 2004). Under mixotrophic or heterotrophic conditions, *Scenedesmus* can utilize organic carbon compounds like acetate or glucose, which enhances its growth in wastewater and organic-rich environments (Li *et al.,* 2019). This ability to adapt metabolically makes it particularly valuable in studies assessing the ecological impact of organic acids such as vinegar and acetic acid.

Ecologically, *Scenedesmus* contributes significantly to aquatic primary productivity, serving as a vital food source for zooplankton and forming the base of many freshwater food webs. Its capacity for rapid biomass accumulation also makes it a promising candidate for biofuel production, carbon sequestration, and wastewater treatment applications (Chisti 2007; Li *et al.,* 2010). In the context of environmental studies, *Scenedesmus* is frequently used as a model organism to evaluate the toxicity of various substances, including heavy metals, pesticides, and organic acids, due to its sensitivity and well-characterized growth kinetics (OECD 2011).

1.2.2 Chlamydomonas sp.

Chlamydomonas is a genus of unicellular, motile green microalgae that also belongs to the division *Chlorophyta*, class *Chlorophyceae*, order *Chlamydomonadales*, and family *Chlamydomonadaceae* (Harris 2009). More than 500 species of *Chlamydomonas* have been described, inhabiting a wide variety of aquatic and terrestrial environments, including

freshwater, marine, and even snow habitats. Among these, *Chlamydomonas reinhardtii* has emerged as a model organism for photosynthetic and molecular biology research due to its well-mapped genome, ease of cultivation, and versatile metabolic pathways (Merchant *et al.,* 2007).

The vegetative cell of *Chlamydomonas* is typically spherical or oval, measuring 10–15 μm in diameter, and possesses two anterior flagella of equal length that enable active motility through coordinated beating. The flagellar apparatus is a hallmark feature, conferring both phototactic and chemotactic responses that allow the organism to move toward favorable light and nutrient conditions (Kamiya and Witman 1984). Each cell contains a single cup-shaped chloroplast similar to that of *Scenedesmus*, but it is distinguished by the presence of a distinct eyespot (stigma) located within the chloroplast. The eyespot functions as a photoreceptive organelle that mediates phototaxis by detecting changes in light intensity and direction (Hegemann 2008).

The *Chlamydomonas* chloroplast contains a central pyrenoid where ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is concentrated, surrounded by starch sheaths that facilitate carbon fixation and storage. The photosynthetic pigments include chlorophylls a and b as well as carotenoids, providing efficient absorption across the visible light spectrum (Grossman *et al.,* 2007). Under nutrient-sufficient conditions, *Chlamydomonas* reproduces asexually through mitotic division, producing two or four daughter cells within the parental cell wall. Under environmental stress such as nitrogen deprivation, it undergoes sexual reproduction, forming flagellated gametes that fuse to produce a diploid zygote (Harris 2009).

Chlamydomonas is metabolically versatile and capable of phototrophic, heterotrophic, and mixotrophic growth. It can assimilate acetate and other simple organic acids as carbon

sources when light or CO_2 availability is limited, a property that has made it a key organism for bioenergy research (Yang et al. 2015). This mixotrophic capability provides a biological rationale for examining its responses to vinegar and acetic acid, as these compounds can serve both as nutrients and stress agents depending on concentration and environmental context (Li et al., 2019). Additionally, *Chlamydomonas* exhibits remarkable adaptive responses to environmental stress, including changes in lipid metabolism, antioxidant enzyme activity, and gene expression related to photosynthesis and carbon assimilation (Harris 2009; Merchant *et al.,* 2007).

In ecological terms, *Chlamydomonas* occupies an important niche in microbial food webs, linking primary production to higher trophic levels. It contributes to oxygen generation and nutrient recycling, and its motility allows it to colonize new habitats rapidly. Moreover, its sensitivity to environmental fluctuations makes it an excellent indicator species for assessing the ecological impacts of pollutants and environmental stressors (Rosenbaum *et al.,* 2014). Its genomic tractability has further positioned it as a model for studying photosynthetic efficiency, stress tolerance, and carbon metabolism.

1.3 LITERATURE REVIEW

Sharma *et al.* (2020) investigated the metabolic responses of microalgae to organic acids and reported that low concentrations enhanced mixotrophic growth by supplying an additional carbon source, while higher concentrations disrupted cellular homeostasis.

Harris (2009) studied *Chlamydomonas reinhardtii* and observed that it utilizes acetate through the glyoxylate cycle, which facilitates carbon assimilation when photosynthetic activity is limited.

Liu *et al.* (2013) carried out an experiment on *Chlamydomonas reinhardtii* and found out that growth inhibition occurred when acetic acid concentrations exceeded 2 g L^{-1}, attributing this effect to intracellular acidification and damage to chloroplast membranes.

Singh *et al.* (2017) evaluated the response of *Scenedesmus obliquus* to organic acids and reported a threshold-dependent effect where low concentrations of acetic acid stimulated lipid accumulation and biomass yield, while higher concentrations led to pigment degradation and oxidative stress that reduced photosynthetic efficiency.

Wang *et al.* (2021) emphasized that environmental pH plays a significant role in determining the toxicity of acetic acid to microalgae and explained that undissociated acetic acid molecules readily penetrate algal cell membranes, disturbing intracellular pH balance.

Zhao *et al.* (2018) investigated the effects of vinegar on aquatic environments and found that its introduction caused acidification that lowered pH beyond the tolerance range of many microalgae, resulting in impaired photosynthetic electron transport and altered enzyme activity.

Harris (2009) also reported that *Scenedesmus* species tolerate pH levels as low as 6.0, while *Chlamydomonas reinhardtii* thrives best in neutral to slightly alkaline conditions.

Tafreshi and Shariati (2009) explained that the organic composition of vinegar, mainly acetic acid with traces of phenolic compounds, influences algal physiology and noted that even minor phenolic contents can induce oxidative stress, reducing chlorophyll content and lipid metabolism.

Zhang *et al.* (2020) observed that prolonged acetate exposure in *Chlamydomonas reinhardtii* affected nutrient uptake, particularly nitrogen and phosphorus assimilation, which are essential for growth and protein synthesis, and concluded that acetate modulates the expression of nitrate reductase genes, linking carbon and nitrogen metabolism.

Li *et al.* (2016) studied microalgae exposed to acidic environments and reported that antioxidant defense mechanisms were activated to counter oxidative damage through increased activities of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD). These enzymes maintained cellular integrity by neutralizing reactive oxygen species, explaining the resilience of *Scenedesmus* in polluted habitats.

Johnson and Alric (2013) described *Chlamydomonas reinhardtii* as an excellent model for studying mixotrophy and demonstrated that the species can metabolize acetate while performing photosynthesis.

Siaut *et al.* (2011) observed that excessive organic acid exposure limited photosynthetic oxygen evolution and increased respiratory activity, leading to reduced biomass accumulation.

Singh *et al.* (2017) conducted a laboratory experiment on *Scenedesmus obliquus* to examine its physiological responses to varying concentrations of acetic acid ranging from 0.5 to 5 g L^{-1}. The researchers measured growth rate, chlorophyll content, lipid accumulation, and photosynthetic efficiency under controlled light and temperature conditions. Singh *et al.* (2017) further reported that under mild acetic acid exposure, *Scenedesmus* species accumulated lipids as a stress-adaptive mechanism, enhancing their potential for biodiesel production by redirecting carbon flux toward energy-dense storage molecules.

Zhao *et al.* (2018) investigated the effects of vinegar on aquatic environments and found that its introduction caused acidification that lowered pH beyond the tolerance range of many microalgae, resulting in impaired photosynthetic electron transport and altered enzyme activity.

Kumar *et al.* (2021) assessed the ecological consequences of vinegar or acetic acid discharge into aquatic systems and explained that low levels may promote heterotrophic and

mixotrophic growth, while excessive accumulation causes eutrophication, oxygen depletion, and acidification.

López-García *et al.* (2020) evaluated the use of vinegar in wastewater treatment and reported that controlled addition could serve as a carbon supplement to improve nutrient removal efficiency by algal–bacterial consortia, although excessive organic carbon may favor competing microbes and reduce autotrophic dominance.

Zhang *et al.* (2020) finally reported that long-term exposure to low acetic acid concentrations led to physiological tolerance in both *Scenedesmus* and *Chlamydomonas reinhardtii*. Their adaptive evolution experiments showed enhanced acetate tolerance through membrane modification and stress-gene expression, highlighting the evolutionary resilience of these microalgae in vinegar-contaminated environments.

1.4 AIM AND OBJECTIVES

Aim:

The aim of this study is to determine the effect of vinegar on the growth of microalgae: *Scenedesmus* and *Chlamydomonas*

Objectives:

The objectives of this study are:

- To determine the effect of different concentrations of vinegar on the growth of *Scenedesmus*
- To determine the effect of different concentrations of vinegar on the growth of *Chlamydomonas*
- To determine the bioremediation capability of *scenedesmus* and *Chlamydomonas* if any.

CHAPTER TWO

MATERIALS AND METHOD

2.1 Test Microalgae

The test microalgae used in this experiment were *Scenedesmus obliquus* and *Chlamydomonas reinhardtii*. These two species were selected because they are common freshwater green algae frequently used in environmental and physiological studies.

2.2 Source of Microalgae

The freshwater microalgae; *Scenedesmus* and *Chlamydomonas* were locally sourced from fish ponds at Uteh Community, Benin City.

2.3 Taxonomy of the Test Microalgae

Scenedesmus sp.

Kingdom: Plantae

Division: Chlorophyta

Class: Chlorophyceae

Order: Sphaeropleales

Family: Scenedesmaceae

Genus: *Scenedesmus*

Chlamydomonas sp.

Kingdom: Plantae

Division: Chlorophyta

Class: Chlorophyceae

Order: Volvocales

Family: Chlamydomonadaceae

Genus: *Chlamydomonas*

2.4 Collection of Vinegar

The Vinegar used in this study was purchased from a certified chemical store located in Benin City, Edo State, Nigeria. The reagent was of analytical grade and was stored under appropriate laboratory conditions until use.

2.5 Culture Medium Preparation

The freshwater microalgae were cultivated in a modified Chu number 10 medium. Stock solutions were prepared by dissolving the required salts in 100 mL of distilled water. An iron solution was made by dissolving citric acid in 100 mL of distilled water, followed by the addition of 3.35 g of ferric citrate. The resulting mixture was autoclaved and refrigerated until use. A trace element solution was also prepared by dissolving the appropriate salts in specified quantities (in grams) into 100 mL of distilled water. The solution was autoclaved and stored under sterile conditions. All media were prepared using distilled water, and sterilization was carried out using an autoclave at 121°C for 15 minutes.

Table 1: Composition of the modified Chu medium

SALTS/NUTRIENTS	g/100ml
$Cacl_2 2H_2O$	367
$MgSO_4.7H_2O$	3.69
$NaHCO_3$	1.26
K_2HPO_4	0.87
$NaNO_3$	8.5
Na_2SiO_3	2.84

Table 2: Composition of Vitamin Stock

Component	**g/100ml**
Thaimine (vitamin B_5)	0.004
Biotin (vitamin B_7)	0.004
Cyatocobalamin (vitamin B_{12})	0.004

Table 3: Trace Element Composition of the Modified Chu No. 10 Meduim

Trace Element	**Concentration (mg/l)**
$CuSO_4 \cdot 5H_2O$	19.6
$ZnSO_4 \cdot 7H_2O$	44.0
$CaCl_2 \cdot 6H_2O$	20.0
$MnCl_2 \cdot 4H_2O$	36.0
$NaMoO_4 \cdot 2H_2O$	12.6
H_3BO_3	618.4

Iron Stock	**g/100 ml**
Citric acid ($C_6H_8O_7 \cdot H_2O$)	3.5
Ferric citrate ($FeC_6H_5O_7 \cdot 5H_2O$)	3.5

Table 4: Preparation of different concentration of treatment

Vinegar Concentration (%)	Vinegar Volume (ml)	Culture Volume (ml)	Distilled Water (ml)	Total Volume (ml)
0	0	5	395	400
1	1	5	394	400
5	5	5	390	400
10	10	5	385	400
15	15	5	380	400
20	20	5	375	400
25	25	5	370	400

2.6 Experimental Setup

The experiment was designed to evaluate the growth response of the two microalgae species to different concentrations of vinegar. The cultures were exposed to seven concentrations: 0.0%, 1%, 5%, 10%, 15%, 20%, and 25%. Each concentration was prepared in triplicate to ensure statistical accuracy. The cultures were maintained for **14 days** in transparent containers placed on an east-facing window of the Microbiology Laboratory at the University of Benin. This placement helped avoid the direct effects of intense sunlight while ensuring adequate natural illumination for photosynthetic activity.

2.7 Inoculation Procedure

Using a 5 mL sterile syringe, 5 mL of each algal culture was inoculated into the experimental vessels containing the different concentrations of vinegar. The containers were covered with cotton wool to permit air exchange while preventing contamination and limiting evaporation.

2.8 Measurement and Monitoring of Algal Growth

The growth rate of each culture was monitored at two-day intervals over the 14-day experimental period. Optical density was measured using a UV/Visible spectrophotometer set at an absorbance wavelength of **750 nm**. The recorded absorbance values were used to plot growth curves for each vinegar concentration.

The growth rate (G_r) was calculated using the formula:

$GR = (Ge - Gi) / T \times 100$

Where:

G_e = growth at the end of the experiment,

G_o = growth at the beginning of the experiment,

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

2.9 Physicochemical Analysis

2.9.1 pH Measurement

The pH of the culture media was determined using a **pH/Conductivity/TDS/Salinity/Temperature meter** (Model EZ-9909). The probe was immersed in each culture and allowed to stabilize for a few minutes before readings were taken.

2.9.2 Total Dissolved Solids (TDS)

Total dissolved solids (in ppm) were measured using the same EZ-9909 multiparameter meter. The probe was immersed in each culture and readings were recorded after stabilization.

2.9.3 Electrical Conductivity

Conductivity was measured in microsiemens per centimeter (µS/cm) using the same meter. The probe was allowed to equilibrate in each culture before readings were taken.

2.10 Determination of Physicochemical Parameters

2.10.1 Conductivity Measurement

The electrical conductivity of each algal culture was determined using a conductivity meter. This device measures the ability of the culture medium to conduct electrical current, which reflects the concentration of dissolved ions such as salts or nutrients. The probe was immersed in the culture medium and allowed to stabilize before readings were recorded in microsiemens per centimeter (µS/cm).

2.10.2 Dissolved Oxygen (mg/L)

Dissolved oxygen in the culture medium was measured using a dissolved oxygen (DO) meter. This instrument quantifies the amount of oxygen available for algal respiration and photosynthesis. The probe was gently inserted into the culture and readings were recorded in milligrams per liter (mg/L) after stabilization.

2.10.3 Turbidity (NTU)

Turbidity was measured using a turbidity meter to assess the cloudiness or particulate concentration in each culture. High turbidity values indicate increased algal growth due to the presence of suspended algal cells. The instrument was calibrated before use, and readings were expressed in nephelometric turbidity units (NTU).

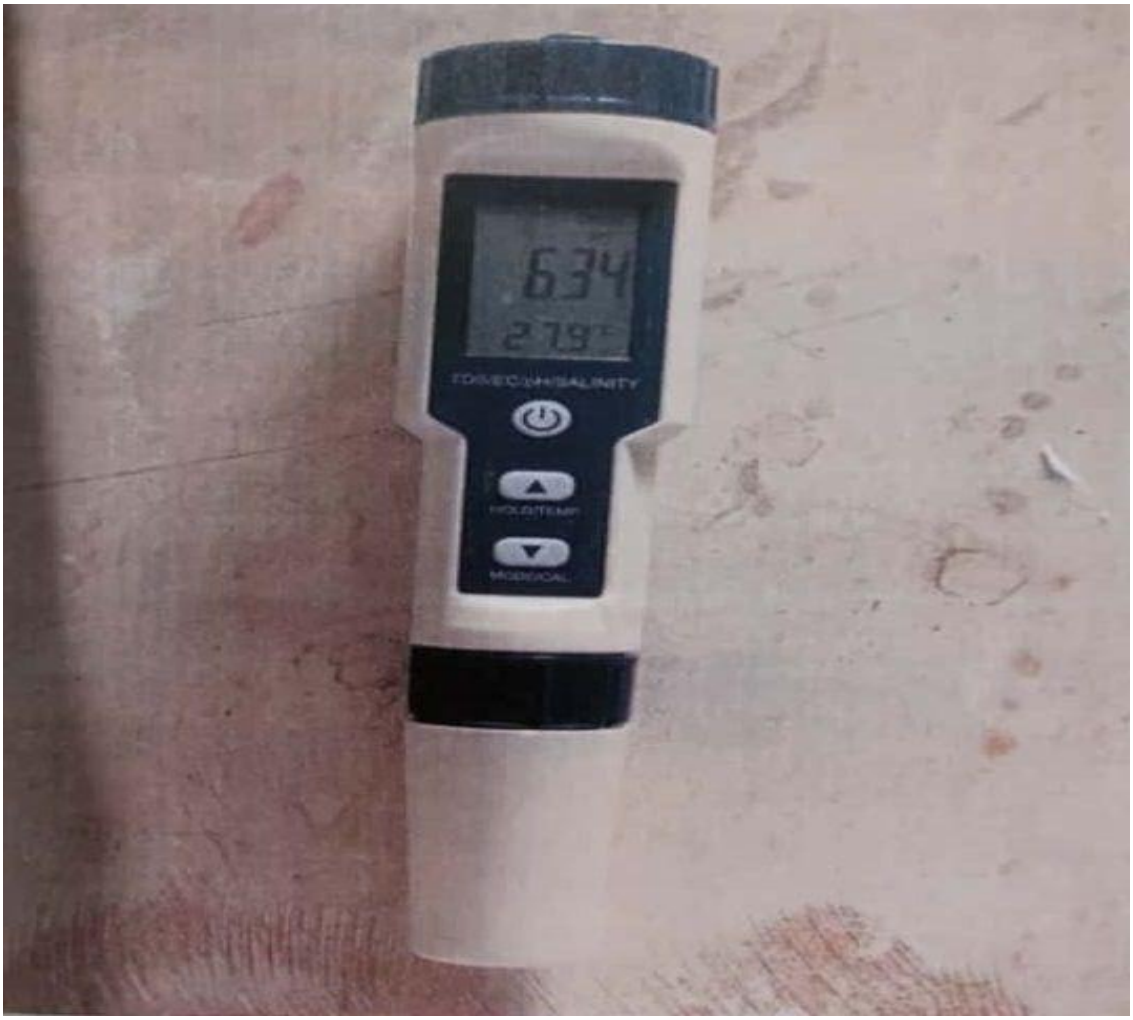

PLATE 1: Conductivity Meter

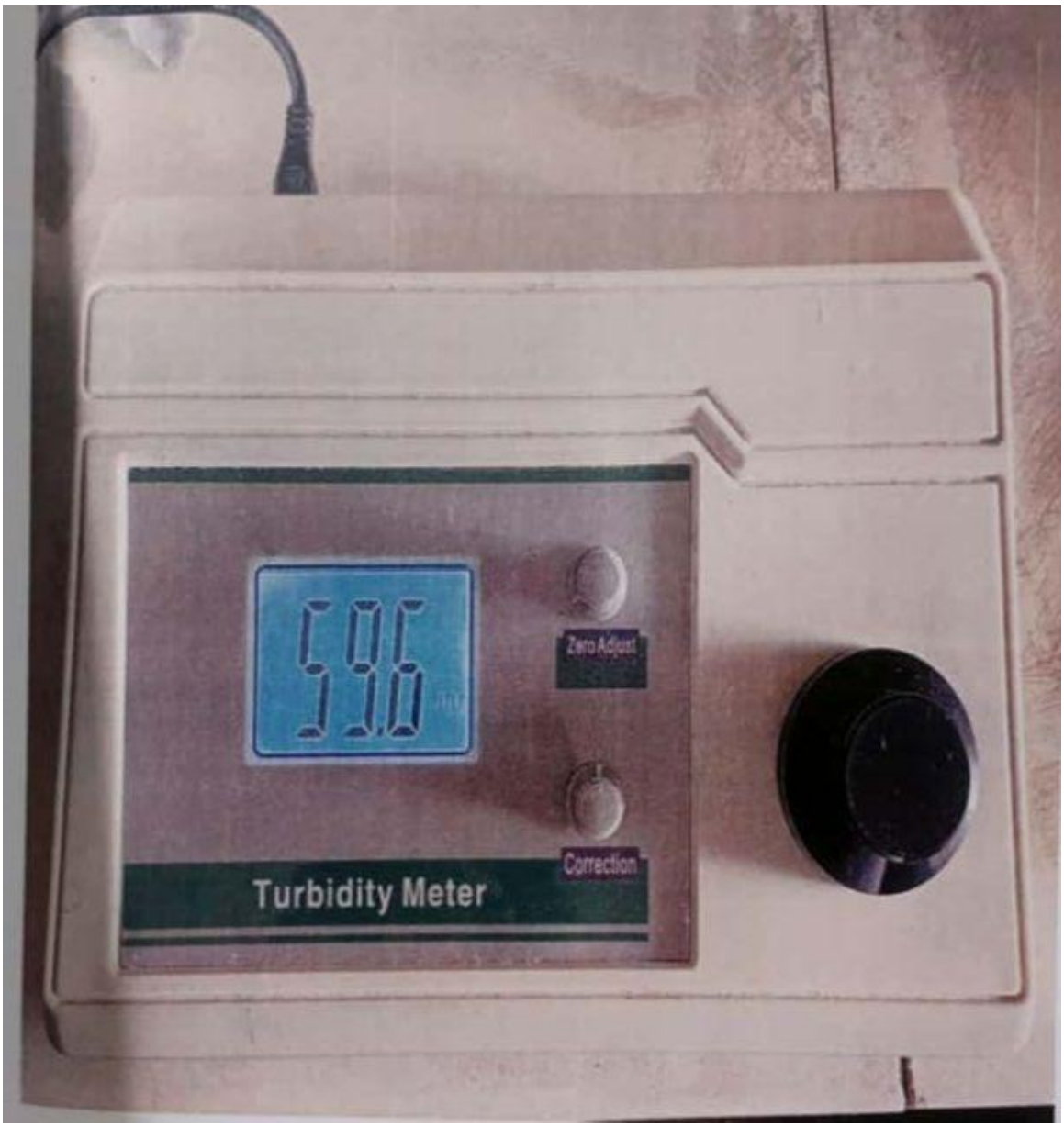

PLATE 2: Turbidity Meter

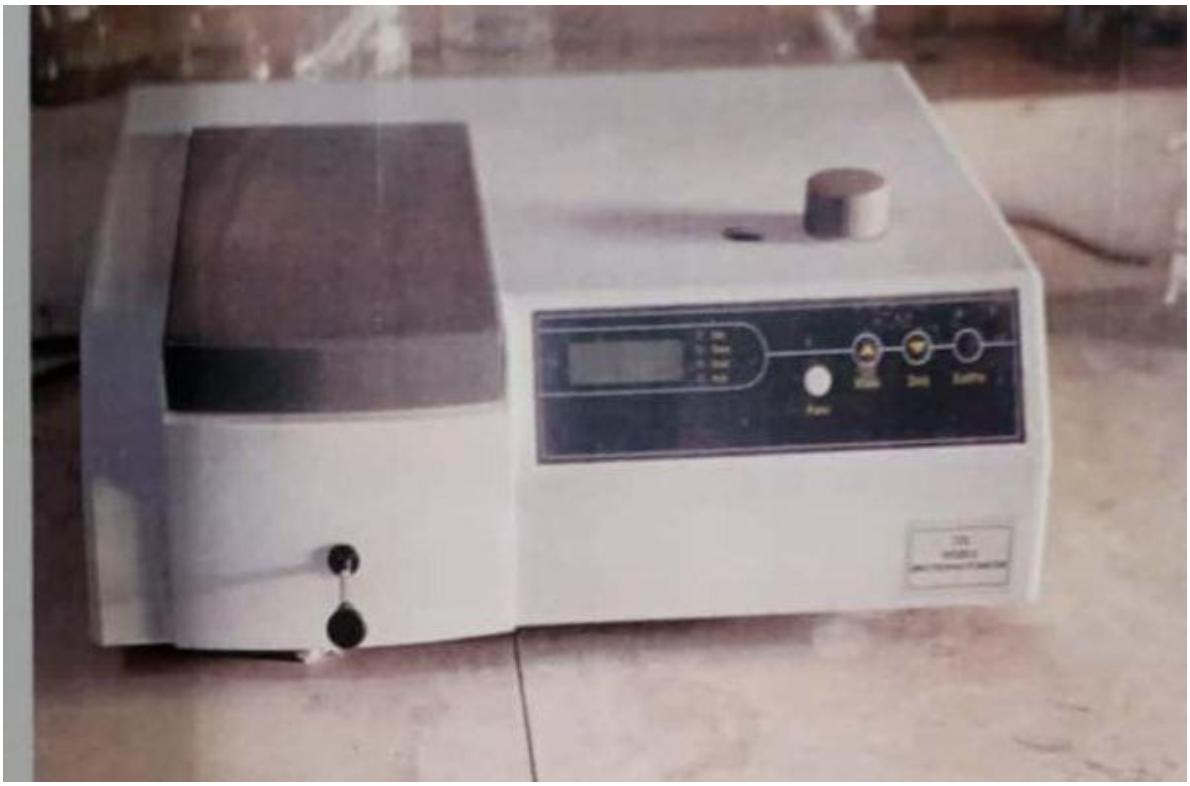

PLATE 3: Visible Spectrophotometer for Monitoring Growth

PLATE 4: Dissolved Oxygen Meter

CHAPTER THREE

RESULTS

Figure 1 shows the effect of different concentrations of vinegar on the growth of *Chlamydomonas* sp.

Statistically, a two-way ANOVA revealed significant differences ($p < 0.05$) in the growth response of *Chlamydomonas* sp. across different concentrations of Vinegar throughout the experiment.

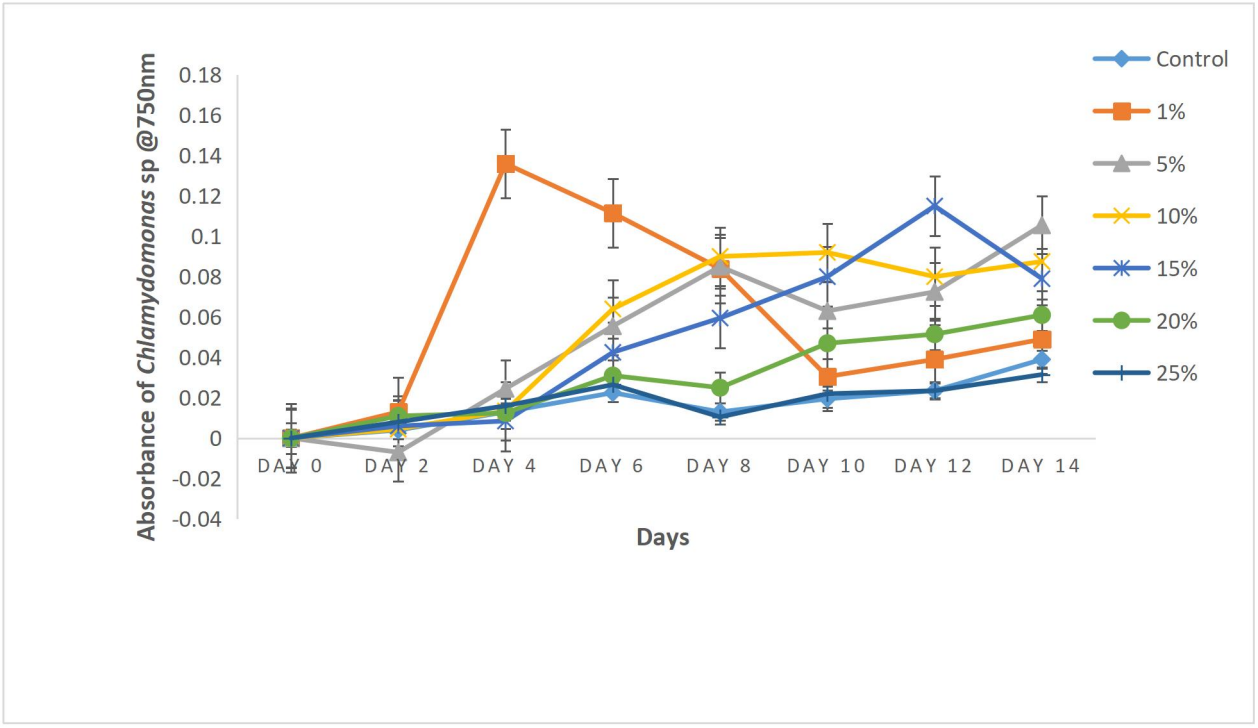

Figure 1: Effect of Vinegar on the growth of *Chlamydomonas* sp.

Figure 2 shows the effect of different concentrations of vinegar on the growth of Scenedesmus sp.

Statistically, two-way ANOVA revealed no significant differences ($p>0.05$) in the growth response of *Scenedesmus* sp. across different concentrations of Vinegar throughout the experiment.

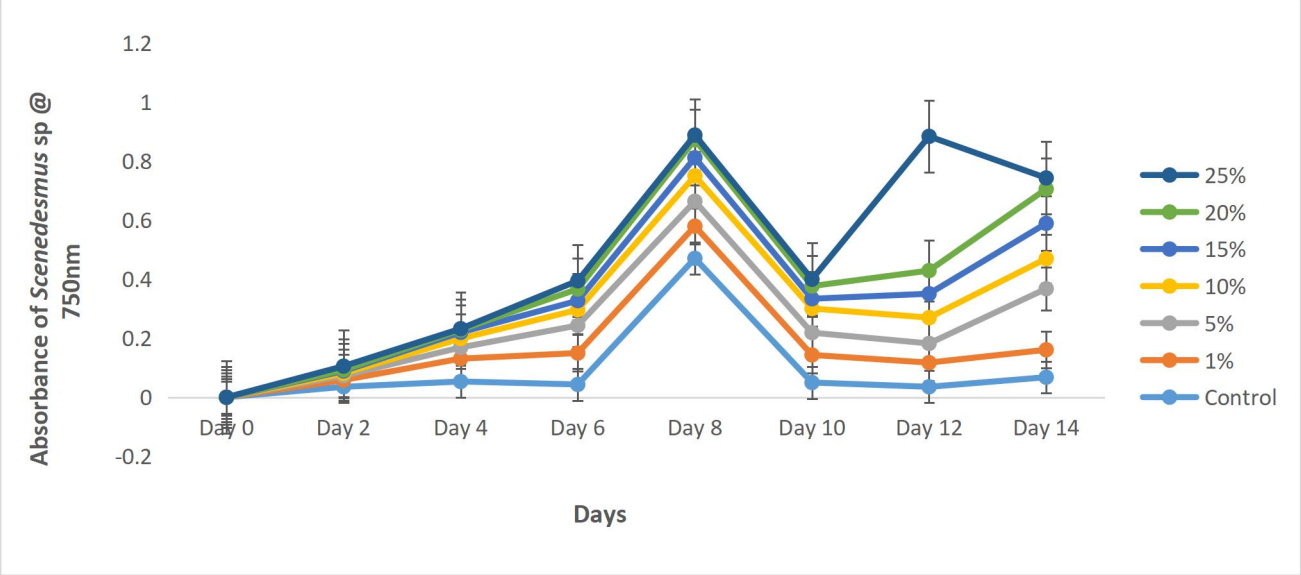

Figure 2: Effect of Vinegar on the growth of *Scenedesmus* sp.

Figure 3 shows the comparative percentage yield of *Chlamydomonas* and *Scenedesmus* sp.

The results of paired samples t-test on the effect of different concentrations of Vinegar on the yield of *Chlamydomonas* sp and *Scenedesmus* sp revealed no significant differences ($p>0.05$) in yield.

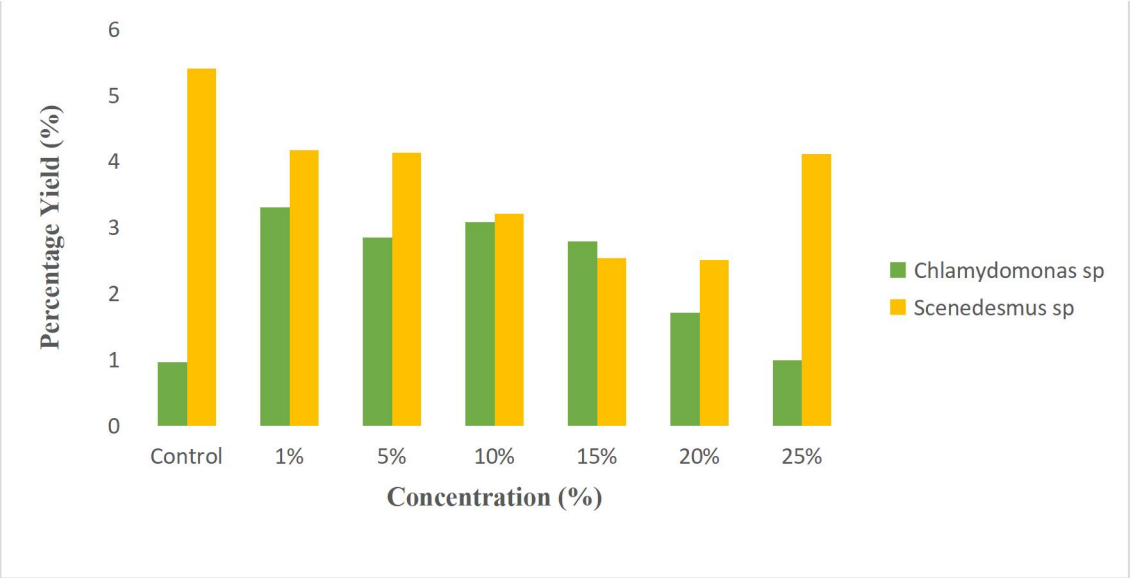

Figure 3: Comparative Percentage Yield of *Chlamydomonas* sp and *Scenedesmus* sp.

Figure 4 shows the effect of different concentrations of vinegar on the turbidity of the culture medium of *Chlamydomonas* sp.

Statistically, two-way ANOVA revealed significant differences ($p<0.05$) between Vinegar turbidity levels across each day of *Chlamydomonas* sp growth.

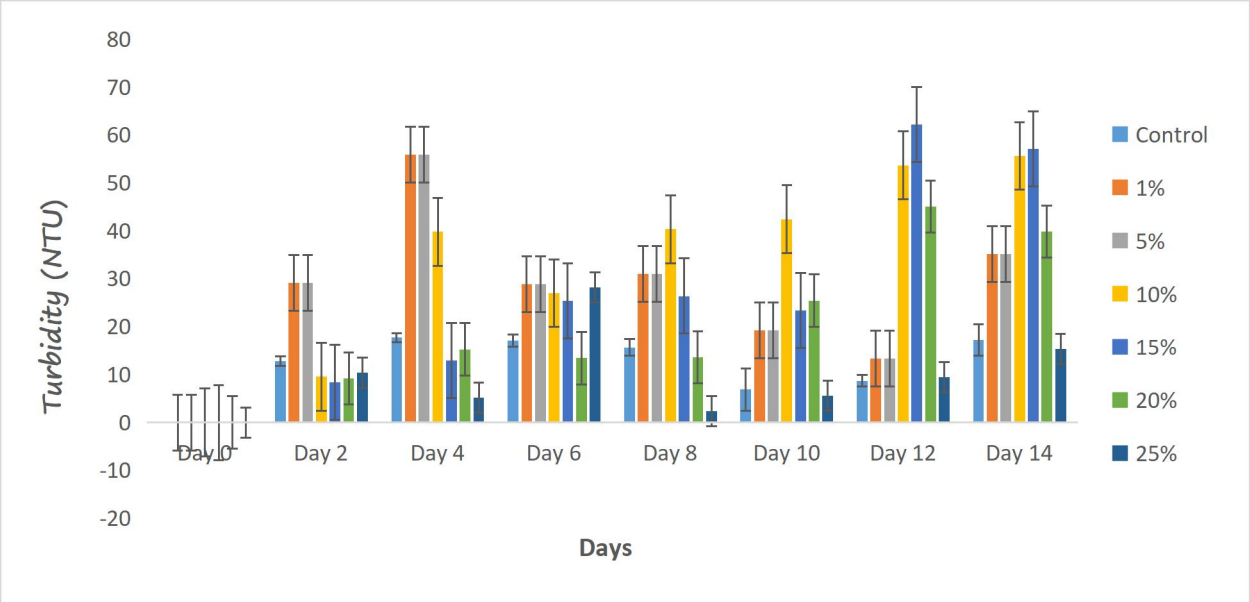

Figure 4: Turbidity of different concentrations of Vinegar on the growth of *Chlamydomonas* sp.

Figure 5 shows the effect of different concentrations of vinegar on the turbidity of *Scenedesmus* sp.

Statistically, two-way ANOVA revealed significant differences ($p<0.05$) between Vinegar turbidity levels across each day of *Scenedesmus* sp growth.

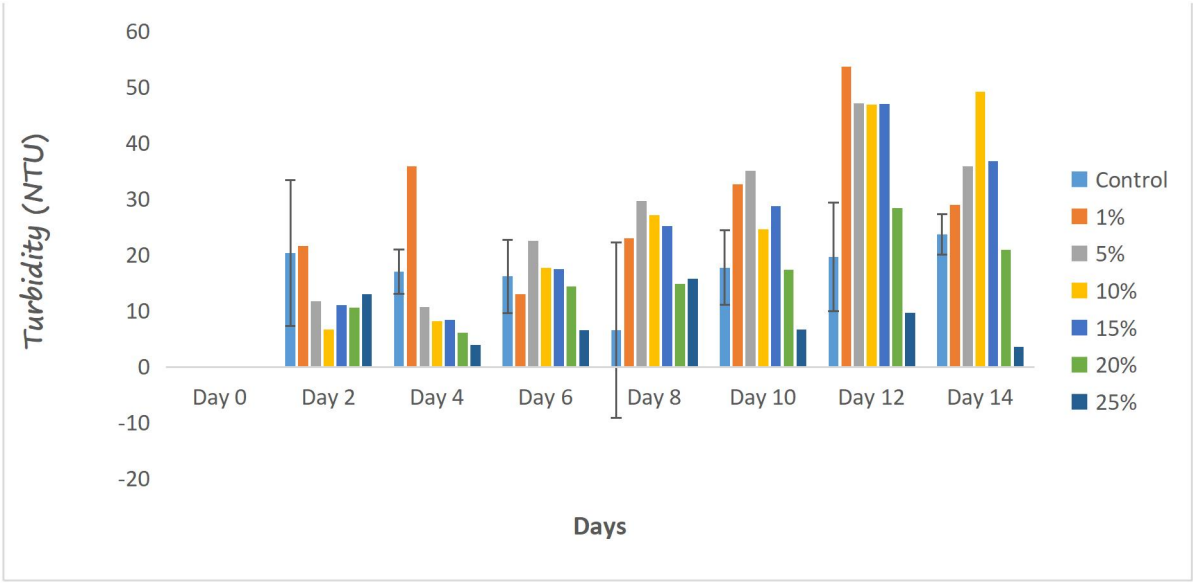

Figure 5: Turbidity of different concentrations of Vinegar on the growth of *Scenedesmus* sp.

Figure 6 shows the effect of different concentrations of vinegar on the conductivity of *Chlamydomonas* sp.

Statistically, two-way ANOVA revealed significant differences ($p<0.05$) between Vinegar conductivity levels across each day of *Chlamydomonas* sp growth.

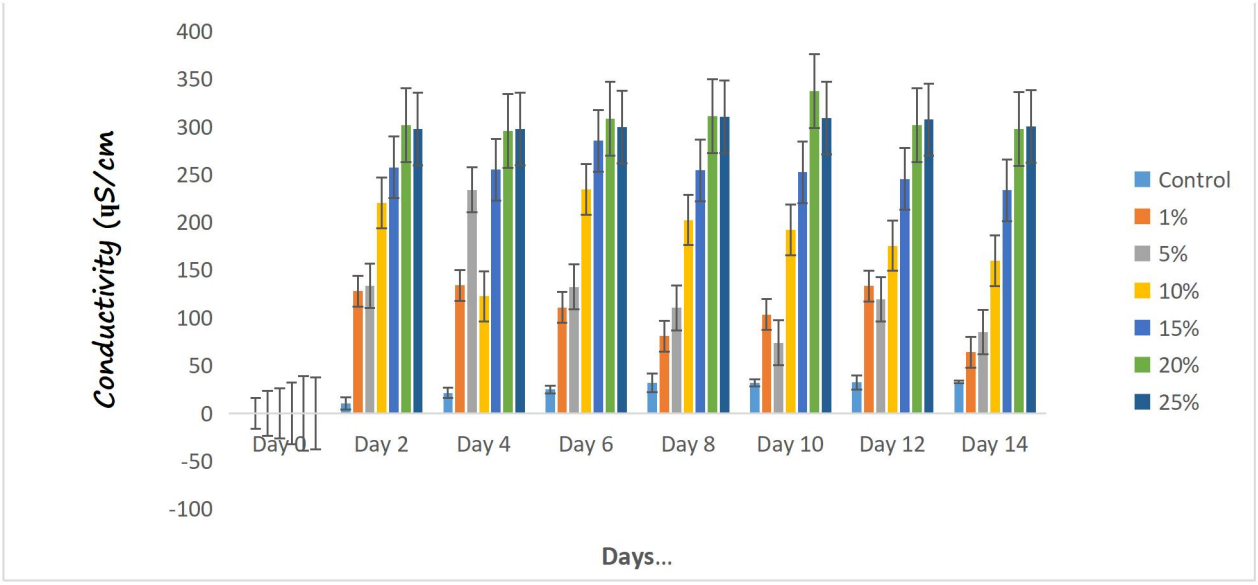

Figure 6: Conductivity of different concentration of Vinegar on the growth of *Chlamydomonas* sp.

Figure 7 shows the effect of different concentrations of vinegar on the conductivity of the growth medium of *Scenedesmus* sp.

Statistically, two-way ANOVA revealed significant differences ($p<0.05$) between Vinegar conductivity levels across each day of *Scenedesmus* sp growth.

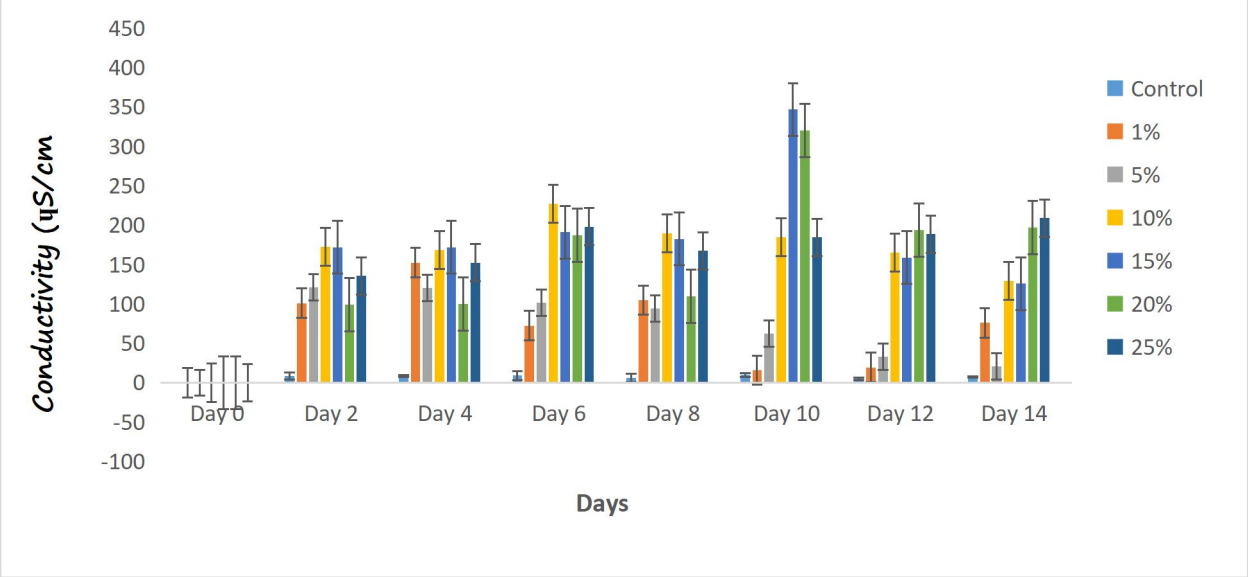

Figure 7: Conductivity of different concentration of Vinegar on the growth of *Scenedesmus* sp.

Figure 8 shows the effect of different concentrations of vinegar on the total dissolved solids level of the growth medium of *Chlamydomonas* sp.

Statistically, two-way ANOVA revealed significant differences ($p<0.05$) between Vinegar total dissolved solid levels across each day of *Chlamydomonas* sp growth.

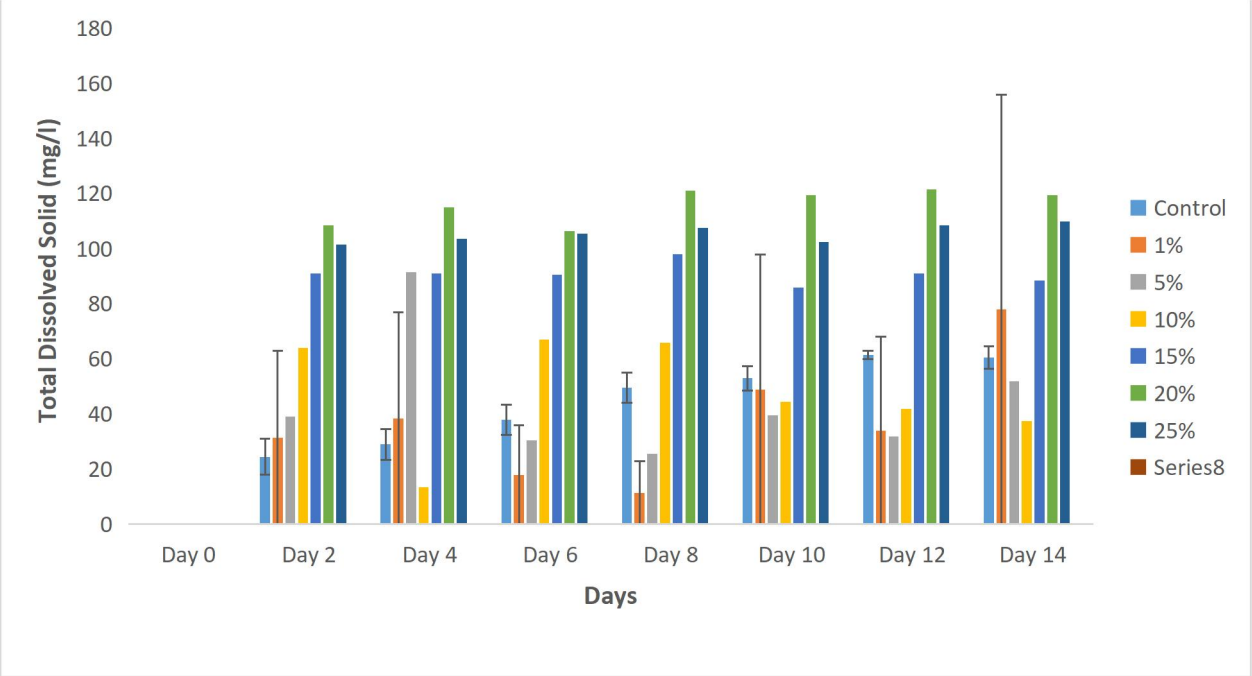

Figure 8: Total Dissolved Solid of different concentration of Vinegar on the growth of *Chlamydomonas* sp.

Figure 9 shows the effect of different concentrations of vinegar on the total dissolved solids level of the growth medium of *Scenedesmus* sp.

Statistically, two-way ANOVA revealed significant differences ($p<0.05$) between Vinegar total dissolved solid levels across each day of *Scenedesmus* sp growth.

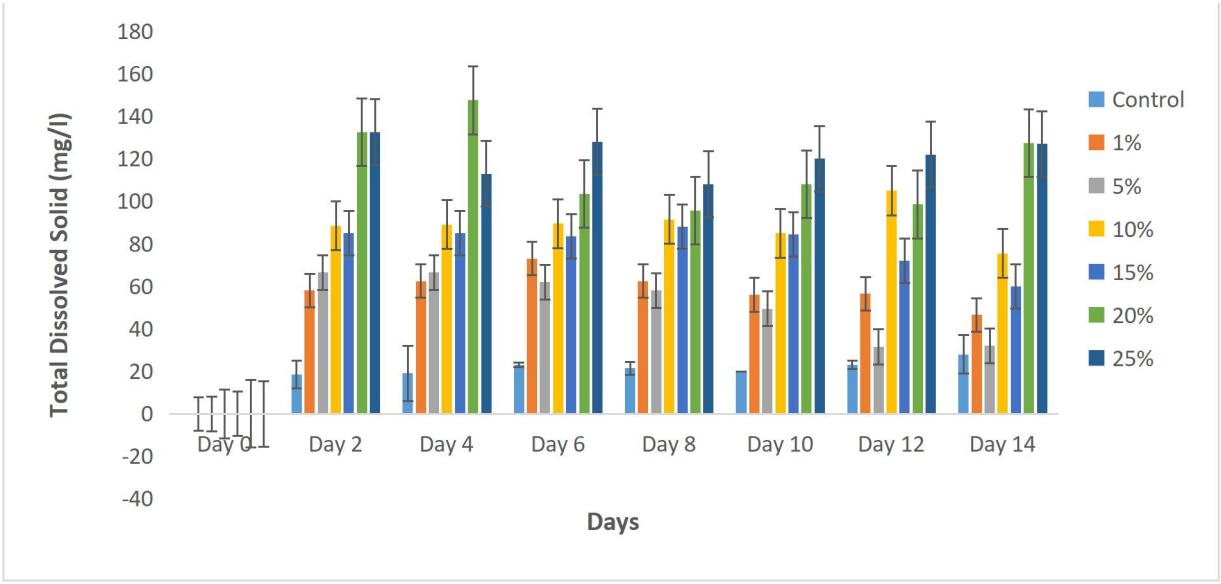

Figure 9: Total Dissolved Solid of different concentration of Vinegar on the growth of *Scenedesmus* sp.

Figure 10 shows the effect of different concentrations of vinegar on the pH of the growth medium of *Chlamydomonas* sp.

Statistically, two-way ANOVA revealed significant differences ($p<0.05$) between Vinegar pH levels across each day of *Chlamydomonas* sp growth.

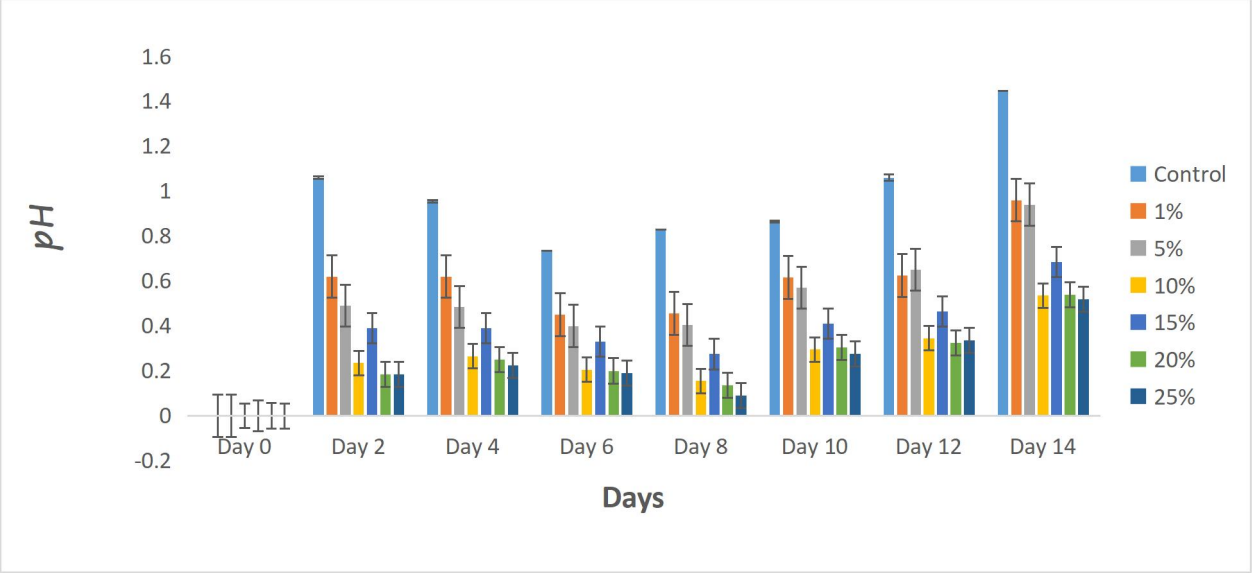

Figure 10: pH of different concentration of Vinegar on the growth of *Chlamydomonas* sp.

Figure 11shows the effect of different concentrations of vinegar on the pH of the growth medium of *Scenedesmus* sp.

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

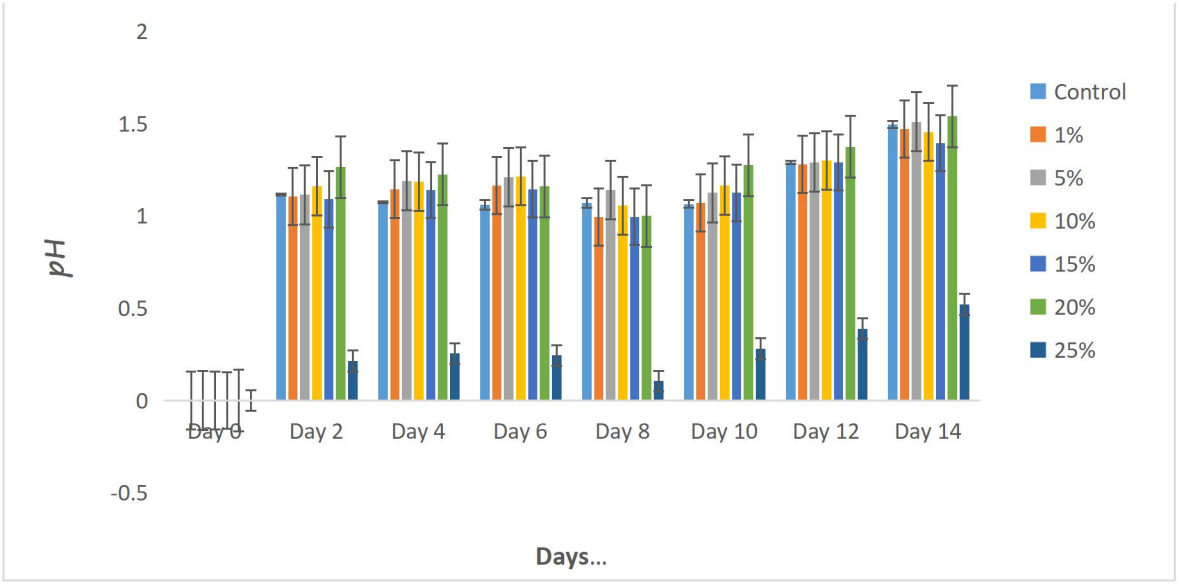

Figure 11: pH of different concentration of Vinegar on the growth of *Scenedesmus* sp.

Figure 12 shows the effect of different concentrations of vinegar on the dissolved oxygen levels of the growth medium of *Chlamydomonas sp*.

Statistically, two-way ANOVA revealed significant differences ($p<0.05$) between Vinegar dissolved oxygen levels across each day of *Chlamydomonas* sp growth.

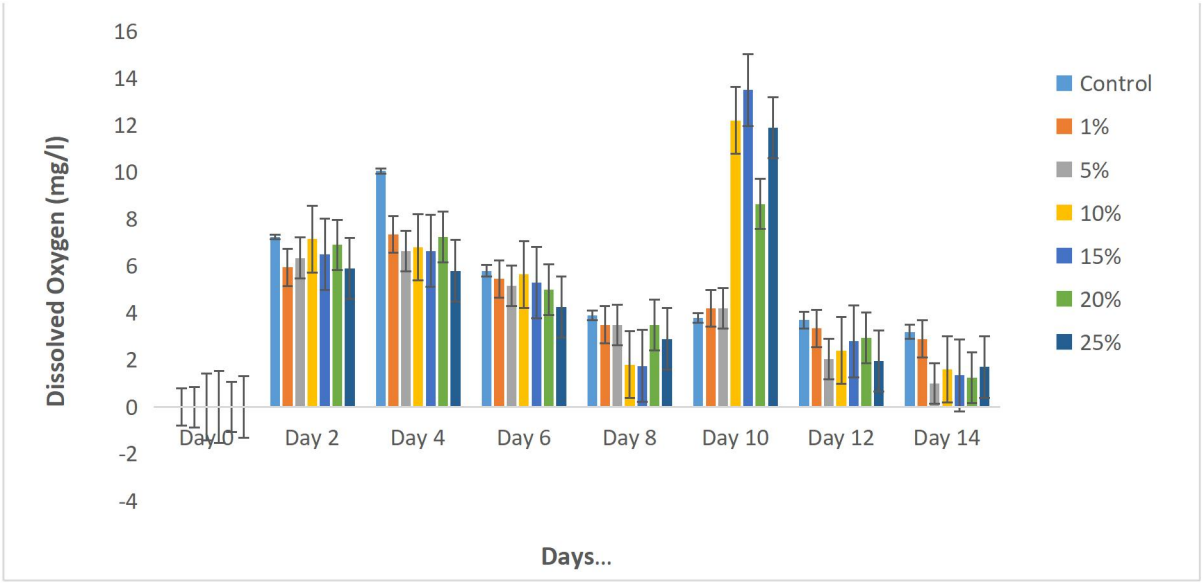

Figure 12: Dissolved oxygen of different concentration of Vinegar on the growth of *Chlamydomonas* sp.

Figure 13 shows the effect of different concentrations of vinegar on the dissolved oxygen levels of the growth medium of *Scenedesmus sp*.

Statistically, two-way ANOVA revealed significant differences ($p<0.05$) between Vinegar dissolved oxygen levels across each day of *Scenedesmus* sp growth.

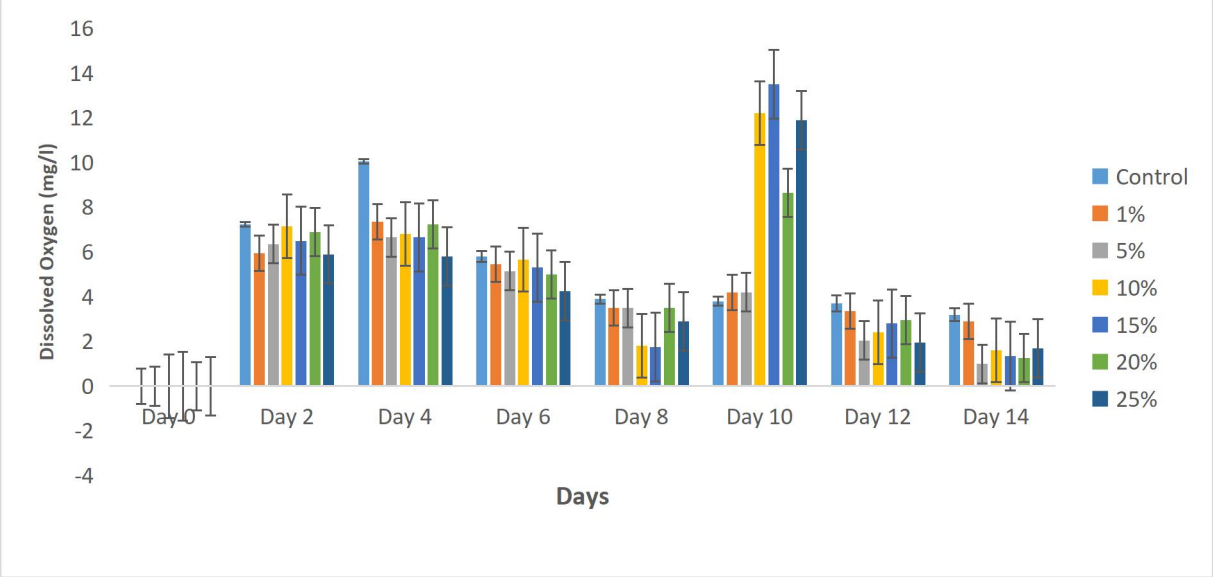

Figure 13: Dissolved oxygen of different concentration of Vinegar on the growth of *Scenedesmus* sp.

CHAPTER FOUR

DISCUSSION

This study aimed at evaluating the effect of **vinegar** on the growth and physiological parameters of *Chlamydomonas sp.* and *Scenedesmus sp.*, both freshwater microalgae species, under laboratory conditions for 14 days. The parameters studied include growth, turbidity, conductivity, total dissolved solids (TDS), pH, and dissolved oxygen (DO).

Figure 1 shows the effect of different vinegar concentrations on the growth of *Chlamydomonas sp.*. The results indicated that *Chlamydomonas sp.* experienced its highest growth at low vinegar concentrations but declined sharply as concentration increased. This decline at higher concentrations suggests that elevated acidity from vinegar inhibited cell division and photosynthetic activity, possibly due to acetic-acid–induced oxidative and osmotic stress. These agrees with Zuo *et al.,* (2012) and Kim *et al.,* (2017) who reported that acetic acid can disrupt proton gradients, destabilize cellular membranes, and induce programmed cell death in *Chlamydomonas reinhardtii* under similar stress conditions.

Figure 2 shows the growth pattern of *Scenedesmus sp.* across vinegar concentrations, implying relative stability in growth. *Scenedesmus sp.* maintained moderate to high growth across all concentrations, with the highest growth observed at 25% vinegar. This indicates higher acid tolerance and adaptability compared to *Chlamydomonas sp.* These studies agree with Zhao *et al.,* (2015) and Ho *et al.,* (2016) who reported that it is due to its colonial structure and thicker cell wall which confer protection under acidic conditions.

Figure 3 presents the comparative percentage yield of Chlamydomonas sp. and Scenedesmus sp.. Percentage yield expresses the overall biomass accumulation of the algal culture at the end of the experiment. Scenedesmus sp. had higher average yield values

(≈5.6%–2.8%) than Chlamydomonas sp. (≈3.8%–1.5%). This confirms that vinegar affected both species, but Scenedesmus sp. retained higher productivity and better tolerance. These findings are consistent with Li et al., (2019) and (2013), who reported that multi-celled or colonial green microalgae are generally more resistant to acidic environments than unicellular types.

Figures 4 and 5 shows turbidity changes for both species. Turbidity reflects the cloudiness of the culture, which is directly proportional to algal biomass in suspension. For *Chlamydomonas sp.*, turbidity increased at low to moderate vinegar concentrations (5–10%) but declined at higher levels. This study agrees with Abou-Shanab *et al.,*(2011) who reported that the reduction at elevated concentrations suggests cell lysis or sedimentation due to acid stress, which lowered cell density in suspension.

Scenedesmus sp. showed a similar but less pronounced trend, maintaining higher turbidity at moderate to high concentrations (10–25%). The continued turbidity increase implies sustained cell multiplication and possible adaptation to acidic stress. The colony-forming ability of *Scenedesmus sp.* may have allowed it to withstand proton influx and maintain structural integrity, thus keeping the culture visibly dense.

Figures 6 and 7 illustrate conductivity variations. Conductivity measures the ability of the culture medium to conduct electricity, which depends on the concentration 0f dissolved ions such as nitrates, phosphate and other nutrients. Conductivity increased progressively with vinegar concentration in both species. This study agrees with Wang *et al.,* (2020) who reported that the rise in conductivity is likely due to ion release from acetic acid dissociation and possible leakage of intracellular ions caused by membrane destabilization.

For *Chlamydomonas sp.*, this increase was irregular, indicating alternating phases of stress and temporary recovery. In contrast, *Scenedesmus sp.* showed a more stable upward trend, suggesting better ionic regulation and membrane integrity under acidic exposure (Becker, 2013; Zhao *et al.,* 2015).

Figures 8 and 9 shows the TDS responses. TDS represents the total of inorganic and organic substances dissolved in the culture medium, including minerals, salts and nutrients. Both species recorded higher TDS with increasing vinegar concentration, but the pattern differed. For *Chlamydomonas sp.*, TDS rose sharply at lower concentrations (5–10%) and plateaued afterward, implying limited solute accumulation as metabolic activity declined at high acidity. *Scenedesmus sp.* maintained a more linear TDS increase across all treatments, suggesting steady solute release and active metabolism even under acidic stress (Wang *et al.,* 2020; Zhao *et al.,* 2015).

This pattern indicates that *Scenedesmus sp.* tolerated cell-membrane permeability changes better than *Chlamydomonas sp.*, which suffered greater inhibition and leakage at lower thresholds.

Figures 10 and 11 shows the pH variation for both species. pH is a key parameter influencing algal metabolism, enzyme activity and photosynthesis. As expected, pH decreased steadily with increasing vinegar concentration, confirming the acidifying effect of vinegar. *Chlamydomonas sp.* cultures showed a sharper decline, particularly at higher concentrations, suggesting poor buffering capacity and faster acid accumulation. These agrees with Choi *et al., (2014) and Li et al.,* (2019) study which reported that the drop in pH can negatively affect photosynthesis, enzyme function, and nutrient assimilation.

In contrast, *Scenedesmus sp.* maintained slightly higher pH values throughout the exposure, implying better regulation and utilization of acidic conditions for growth. This study is consistent with Ho *et al*., (2016) who studied that Its physiological traits may have allowed effective proton extrusion and carbonic acid conversion, thereby moderating acid stress

Figures 12 and 13 represent the DO levels. Dissolved oxygen measures the amount of oxygen present in tge culture medium, which is largely produced during photosynthesis. Both species exhibited declining DO levels as vinegar concentration increased, but *Chlamydomonas sp.* showed sharper reductions. These agrees with Orosa *et al.,* (2001) and Dragone *et al.,* (2011) which reeported that impaired photosynthetic oxygen evolution due to acid-induced chloroplast damage and reduced metabolic activity.

For *Scenedesmus sp.*, DO decline was less pronounced, indicating sustained photosynthesis and oxygen production under acidic stress. This tolerance may be linked to its higher photosynthetic efficiency and cellular resistance to pH-induced oxidative stress.

Overall, vinegar induced measurable physiological and biochemical changes in both species. *Chlamydomonas sp.* was more susceptible to acid stress, exhibiting sharp growth reduction, fluctuating turbidity, and greater metabolic inhibition at high vinegar concentrations. *Scenedesmus sp.*, however, demonstrated greater resilience—maintaining growth, turbidity, and DO levels at moderate to high vinegar concentrations. These differences align with previous findings that species-specific structural and metabolic adaptations influence acid tolerance in green microalgae (Ho *et al.,* 2016; Dragone *et al.,* 2011).

CONCLUSION

This study demonstrated that vinegar significantly affected the growth and water-quality parameters of *Chlamydomonas sp*. and *Scenedesmus sp*.. *Chlamydomonas sp*. exhibited growth inhibition and reduced physiological activity at higher vinegar concentrations, while *Scenedesmus sp*. showed greater tolerance and maintained higher biomass production. The observed changes in turbidity, conductivity, TDS, pH, and DO confirm that increasing vinegar concentration imposes acidic stress on both species, indicating that low or neutral vinegar concentrations favour algal growth and enhance bioremediation capability while higher acidity suppresses metabolic activity. However, *Scenedesmus sp*. proved more adaptable and physiologically stable under acidic conditions than Chlamydomonas *sp*..

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APPENDIX

ANOVA: Growth Response of *Chlamydomonas* to Vinegar

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.056558	6	0.009426	33.37051	8.07E-23	2.180564
Columns	0.105407	7	0.015058	53.30777	8.91E-33	2.092381
Interaction	0.087815	42	0.002091	7.401795	1.29E-17	1.493427
Within	0.031637	112	0.000282			
Total	0.281418	167				

ANOVA: Growth Response of *Scenedesmus* to Vinegar

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.031236	6	0.005206	0.567261	0.755606	2.180564
Columns	0.269144	7	0.038449	4.189458	0.000391	2.092381
Interaction	0.396783	42	0.009447	1.02938	0.43965	1.493427
Within	1.027889	112	0.009178			
Total	1.725052	167				

ANOVA: Turbidity for *Chlamydomonas*

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	15658.76	6	2609.794	32.68965	1.66E-22	2.180564
Columns	25260.99	7	3608.713	45.20187	8.55E-30	2.092381
Interaction	20057.44	42	477.5581	5.981778	1.97E-14	1.493427
Within	8941.573	112	79.83548			
Total	69918.76	167				

ANOVA: Turbidity for *Scenedesmus*

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	6370.19	6	1061.698	2835.246	7.7E-120	2.180564
Columns	17812.01	7	2544.573	6795.235	4.5E-144	2.092381
Interaction	8384.154	42	199.6227	533.0888	1.1E-111	1.493427
Within	41.94	112	0.374464			
Total	32608.29	167				

ANOVA: Conductivity for *Chlamydomonas*

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	1234957	6	205826.2	3739.058	1.6E-126	2.180564
Columns	673776.6	7	96253.8	1748.555	3.1E-111	2.092381
Interaction	260891.1	42	6211.692	112.8422	1.53E-74	1.493427
Within	6165.333	112	55.04762			
Total	2175790	167				

ANOVA: Conductivity for *Scenedesmus*

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	559104.5	6	93184.09	1094.215	6.06E-97	2.180564
Columns	340274.2	7	48610.6	570.8101	1.77E-84	2.092381
Interaction	333835.6	42	7948.466	93.33489	4.4E-70	1.493427
Within	9538	112	85.16071			
Total	1242752	167				

ANOVA: Total Dissolved Solid for *Chlamydomonas*

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	129728.1	6	21621.35	761.6665	2.57E-88	2.180564
Columns	93072.37	7	13296.05	468.3869	8.06E-80	2.092381
Interaction	45706.83	42	1088.258	38.33662	1E-49	1.493427
Within	3179.333	112	28.3869			
Total	271686.7	167				

ANOVA: Total Dissolved Solid for *Scenedesmus*

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	147603.5	6	24600.58	181.0054	3.14E-55	2.180564
Columns	118226.3	7	16889.47	124.2688	9E-50	2.092381
Interaction	35390.24	42	842.6247	6.19984	6.03E-15	1.493427
Within	15222	112	135.9107			
Total	316442	167				

ANOVA: pH for *Chlamydomonas*

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	7.388173	6	1.231362	142.7666	4.42E-50	2.180564
Columns	8.421228	7	1.203033	139.482	2.9E-52	2.092381
Interaction	1.716351	42	0.040866	4.73803	2.6E-11	1.493427
Within	0.966	112	0.008625			
Total	18.49175	167				

ANOVA: pH for *Scenedesmus*

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	12.36705	6	2.061175	632.47	6.42E-84	2.180564
Columns	23.87546	7	3.41078	1046.595	6.6E-99	2.092381
Interaction	1.99981	42	0.047615	14.61048	1.46E-29	1.493427
Within	0.365	112	0.003259			
Total	38.60731	167				

ANOVA: Dissolved Oxygen for *Chlamydomonas*

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	54.40786	6	9.067976	74.89774	6.7E-37	2.180564
Columns	431.878	7	61.69686	509.5906	8.39E-82	2.092381
Interaction	123.514	42	2.940811	24.28988	7.7E-40	1.493427
Within	13.56	112	0.121071			
Total	623.3599	167				

ANOVA: Dissolved Oxygen for *Scenedesmus*

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	25.36917	6	4.228194	33.1005	1.07E-22	2.180564
Columns	1266.061	7	180.8659	1415.912	3.7E-106	2.092381
Interaction	395.5765	42	9.418489	73.73281	1.37E-64	1.493427
Within	14.30667	112	0.127738			
Total	1701.314	167				