GROWTH RESPONSE OF TWO FRESHWATER MICROALGAE SPECIES TO HYDROGEN PEROXIDE

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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

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ABSTRACT

Hydrogen peroxide is increasingly found in our environment as a byproduct of both natural processes and industrial activities. Its accumulation in our environment has resulted in hydrogen peroxide pollution, altering aquatic ecosystems and inducing oxidative stress in living organisms. This study evaluated the effects of Hydrogen peroxide on two freshwater microalgae specie; *Scenedesmus* sp. and *Chlamydomonas* sp. for 14 days under various concentrations (control, 1%, 5%, 10%, 15%, 20%, 25%) of Hydrogen peroxide and absorbance was measured using a visible spectrophotometer at 750nm. Physicochemical parameters such as pH, total dissolved solids (TDS), turbidity, dissolved oxygen and conductivity were analyzed using standard methods. Statistical analysis (two-way ANOVA, paired t-test descriptive statistics) was done using Microsoft Excel. Results showed that Hydrogen peroxide had a dose dependent effect on *Scenedesmus* and *Chlamydomonas.* A comparison of the growth response of both microalgae showed that *Scenedesmus* had a higher tolerance to hydrogen peroxide than *Chlamydomonas,* recording peak growth and a higher growth at 25% concentration while *Chlamydomonas* experienced peak growth at 25% concentration but a lower growth compared to *Scenedesmus*. The results revealed significant differences ($p<0.05$) in growth and physicochemical parameters studied across concentrations of Hydrogen peroxide except on the growth of *Scenedesmus* sp.

CHAPTER ONE

INTRODUCTION

Aquatic Ecosystems represent a diverse and vast assemblage of habitats that include freshwater habitats (rivers, streams, springs) to marine habitats (oceans, seas) and transitional zones (estuaries and mangroves). It contains diverse communities of organisms interacting with each other and their physical and chemical environment. Occupying a large proportion of the earth surface, aquatic ecosystems play a vital role in maintaining ecological balance- it's safe to say the world is surrounded by water. They are fundamental to global biogeochemical cycles, including those of carbon, nitrogen and oxygen as well as playing critical roles in nutrient cycling, climate regulation, water purification, energy flow. Furthermore, they provide habitats for millions of organisms (Mitsch and Gosselink, 2015). Aquatic ecosystems harbor immense biological diversity- inhabiting organisms ranging from microscopic phytoplankton and bacteria to zooplankton to small fishes to crustaceans to amphibians to larger fish and all other aquatic animals. They have special adaptations which enable their life in water. Aquatic Biodiversity ensures ecosystem resilience, productivity and resource availability for humans. (UNEP, 2022).

Phytoplankton refers to a category of plankton that are photosynthetic A notable example and the largest member of microscopic phytoplankton is microalgae. Microalgae are microscopic algae; tiny, one-celled living organisms that mostly inhabit freshwater and marine environment). They are photosynthetic organisms and they play a major role in aquatic ecosystems, oxygen production and are indicators of environmental health (Richmond and Hu, 2013). Through the process of photosynthesis, microorganisms harness sunlight and convert this energy to chemical energy which these organisms use to power up an array of their biological processes. They exist mainly as unicellular eukaryotes although we have a few multicellular forms(colonies, filaments).The cellular forms includes species like *Chlorella vulgaris*, *Chlamydomonas*

reinhardtii, Colonial forms like *Volvox* and filamentous forms (*Spirogyra sp*). Microalgae are an exceptionally diverse group of organisms that include green algae(Chlorophyceae), diatoms(Bacillariophyceae), cyanobacteria and dinoflagellates (Mata et *al*., 2010).

Microalgae exhibit several distinctive characteristics that contribute to their ecological adaptability and evolutionary success. Morphologically, they range in shapes from spherical to rods to spirals. The composition of their cell walls vary among species, consisting mainly of polysaccharides and glycoproteins, while in diatoms, silica forms the principal component (Richmond and Hu, 2013). They possess photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) vital for capturing light energy for photosynthesis. The color of micro algae is greatly influenced by the the pigments predominant in it giving it its characteristic coloration (red, green brown) a feature used for classification of algal species. Although a large number of them are photosynthetic, some species feed heterotrophically; utilizing organic and inorganic carbon sources as sources of energy. Some are mixotrophs, combining both modes of nutrition depending on ecological and environmental factors (Khan *et al*., 2018).

Higher organisms depend on microalgae as a vital source of energy giving they are primary producers and form the base of aquatic food chains and webs (Gupta *et al*., 2016). Their presence and abundance also serve as indicators of environmental health and water quality, owing to the fact that they respond to fluctuations in nutrient level or pollutants. Microalgae are important agents in carbon sequestration, a process that helps to reduce climate change by mitigating greenhouse gases. By absorbing carbon from the atmosphere and contributing to carbon fixation, they play a part in the carbon cycle. Some microalgae have the ability to acclimate to changing environmental conditions by altering their chemical compositions. An example is Scenedesmus which increases the production of antioxidant enzymes when face with oxidative stress often

induced by hydrogen peroxide or heavy metals (Oukarroum *et al*., 2012). Certain microalgae are abundant in proteins, lipids, carbohydrates, vitamins, antioxidants and pigments such as astaxanthin. As a result, they are increasingly used as dietary supplements and functional foods (Spolaore *et al*., 2006).

Microalgae are seen as valuable bio fertilizers as they help improve soil fertilization as well as plant growth. They are preferred to agrochemicals as they confer lesser risks to the environment while in turn improving soil fertility and recycling nutrients (Braun and Colla, 2023). With a prevalent use in the cosmetic industry owing to their enormous benefits of compounds that emerge form microalgae like anti aging, skin brightening and UV protection. They are used in sunscreens, moisturizers, lotions, skin sensitizers (Martinez-Riuz *et al*., (2022)

Chlorella was the first algae to be isolated for micro algae culture in 1890 by Beijerinck and was first used for the study of photosynthesis. It is also one of the earliest microalgae that was explored for commercial cultivation and also produced commercially. Microalgae have a wide range of industrial applications due to their simple cell structure, fast growth rates and ability to produce valuable compounds. In the biotechnological field, they have drawn attention for their role in the production of biofuels (including bioethanol, biodiesel and biohydrogen) and bioremediation. Bioactive chemicals, pharmaceuticals and pigments can all be made from microalgae. They are particularly useful in wastewater treatment processes, especially in treating effluents with high nutrient content. Despite being essential components of aquatic ecosystem, certain species can cause algae blooms (HABs). Toxins produced by toxic algae blooms can cause depletion of oxygen in water bodies and harm marine life and other aquatic organisms. (Zhou *et al*., 2025). Microalgae remains a promising prospect in various research fields. They are

globally recognized for their economic significance and its capacity to solve certain worldwide challenges.

Nowadays, it is undoubtedly clear that pollution has become a major concern in our city with water and air pollution being of the greatest concern. A significant part of the general health of the world is the aquatic environment and its condition. In recent years, anthropogenic activities like industrial waste discharge, agricultural run off, oil spills, plastic population and urban sewage have been dumped into our waters and it has seriously affected it. These pollutants alter the health of our waters, reduce the amount of oxygen available for aquatic animals and even unsafe for use by man, disrupt the balance of our aquatic habitats leading to loss of biodiversity. A notable example is eutrophication caused by an overload of nutrients resulting in algal blooms (Smith *et al*., 2003). Heavy metals and organic pollutants introduced into our waters accumulate in aquatic organisms affecting their growth and reproduction. These pollutants negatively impact our aquatic bodies and also the organisms found in such habitats. (Akhere and Eze, 2023). Issues pertaining to water and air pollution spring up everyday and very few things have been invented to help curb these problems; Microalgal technology is one of them which can help mitigate these crises and aid sustainable water quality.

1.1 *Chlamydomomas* sp

Chlamydomonas, a genus of green algae in the class Chlorophyceae consisting of about 500 unicellular flagellate species. It is found inhabiting damp soils, stagnant waters, freshwater, sea water and even in snow hence coining it the name “snow algae”. It is generally found in habitats rich in ammonium salts. The cells have glycoprotein cell walls. The nucleus is enclosed in a cup shaped chloroplast which has a single shaped pyrenoid. Two small contractile vacuoles which

have an excretory function are located near the anterior flagella. The swimming cells of *Chlamydomonas* are phototactic (in response to light).

It is a unicellular, flagellated and motile alga with varying shapes spanning across spherical, oval and even ellipsoidal forms; being wider at the posterior end and pointing at the anterior end terminating in an anterior papilla. Its length varies from 20 to 30µm. It possesses a distinct glycoprotein cell wall and a eukaryotic nucleus suspended within the cytoplasm. It comprises of a cup shaped and parietal chloroplast which have varying shapes in some species containing a single pyrenoid with a starch sheath. Additionally, two small contractile vacuoles are located near the anterior flagella, serving an osmoregulatory and excretory role by eliminating excess water from the cell. (Richmond and Hu, 2013)

It possesses an eye spot that is extremely sensitive to light. It uses its eyespot and flagella for movement and orientation towards light. When in contact with heavy contaminants, the efficiency of the eyespot is impaired and this is often utilized as a biomarker for ecotoxicological studies. One remarkable characteristics of *Chlamydomonas* is that it contains channelrhodopsins or ion channels) found in their eyespots which are directly triggered by light. In *Chlamydomonas reinhardtii* the reddish "eyespot" consists of two highly ordered layers of carotenoid-rich lipid globules inside the chloroplast. The outermost layer is attached to specialized areas of the chloroplast envelope membranes. This "eyespot" is thought to filter and focus the light on a photoreceptor on the plasma membrane overlying the eyespot which then somehow affects the beating of the flagella (Hegemann and Berthold, 2009). On studies on molecular biology, primarily on studies of genetics, flagella motility and biogenesis, it is frequently used as a model organism. They reproduce by asexual reproduction and will only reproduce sexually by triggering factors like stress (Harris, 1989). Asexual reproduction is by zoospores, aplanospores,

hypnospores and a palmella stage while sexual reproduction occurs by isogamy, anisogamy and oogamy.

Ecologically, Chlamydomonas is a primary producer and a important base producer in aquatic food chains and webs linking primary producers to higher tropic levels. It contributes significantly to oxygen production, nutrients recycling. Due to its motile nature, it allows it to easily and rapidly colonize new habitats. Under favorable conditions of nutrients abundance, they grow excessively forming algae blooms which inhibit light penetration in water. However, under balanced quantities, *Chlamydomonas* supports symbiotic microbial communities in aquatic habitats. (Biology discussion n.d.) *Chlamydomonas*-bacterial consortia (an interaction between *Chlamydomonas* and bacterium *Methylobacteria*) have been found to play key roles in biotechnological tasks such as wastewater treatment, biofuel production, biomass valorization, and bioproduct production (Torres *et al.,* 2024).

Among it's species, *Chlamydomonas reinhardtii* is the most abundant and is extensively used for research studies dues to its ease in culturing and ability to manipulate its genetics. *C. reinhardtii* can grow heterotrophically in the dark in the presence of acetate. Notably, *Chlamydomonas reinhardtii* show one of the highest responses and sensitivity to pollutants even at very low concentrations making it an excellent early detecting bioindicator and is preferred for studies on ecotoxicology. It changes forms and exhibits remarkable responses (like antioxidant activity, lipid bioaccumulation, carbon assimilation) when exposed to pollutants and is used as a bio marker for ecotoxicology (Guilia Cheloni *et al*., 2021, Merchant *et al*., 2007). It's fast growth rate with a duplication span of approximately 8h allows it to be easily cultivated in the laboratory. *Chlamydomonas* has shown itself as an organism with exceedingly high biotechnological potential which is demonstrated in it's application in the production of vaccines, antibodies and

even experiments aimed at improving human's gastrointestinal health. It is particularly helpful in bioremediation and hydrogen production (Tejada-Jimenez *et al*., 2023).

1.2 *Scenedesmus* sp.

Scenedesmus is a genus of the green algae belonging to the class Chlorophyceae and family Scenedesmaceae. It is a multi-shaped, single celled, colonial or coenobial form (of four or eight cells) alga with distinct species. The formation of coenobia is dependent on a number of factors. Studies have shown that a higher proportion of unicellular organisms were found at high light intensities and temperatures suggesting that at high growth rates, they prefer non-colonization. Rarely seen in brackish water, it is found mainly in freshwater ponds and lakes. The cell wall is three layered composing of cellulose, sporopollenin and either pectin or mucilage or both (Trainor, 1995). It is non-flagellated and non-motile relying primarily on passive movement within the water column. *Scenedesmus* cells possess a single cup shaped chloroplast that occupies a large portion of the cytoplasm, where starch is stored (Barsanti and Gualteri, 2014).

It is well noted for its high lipid content which constitutes approximately 10 to 50% of its dry biomass and as well as its remarkable morphological plasticity when exposed to certain biochemicals released by grazers. In the presence of consumers like *Daphnia,* to avoid predation the cells will form eight-celled coenobia even in cases of limiting growth conditions, as a defensive mechanism. The species exhibit a number of defensive mechanisms; *Scenedesmus* are spineless in contrast to other species like *Desmodesmus* which have spines. They have thick cell walls and mucilage which may make them digestion resistant. Some chemicals in *Scenedesmus* may be toxic to certain organisms on consumption (Lurling, 1999). Bristles ranging from 10 to 100μm may form a net in some species to discourage predation. These bristles are induced when

kairomones are detected, an infochemical released by *Daphnia* that *Scenedesmus* has evolved to recognize as a predation warning signal (Lurling and Donk, 2000).

Scenedesmus is nutritionally valuable due to its high protein and fatty acid content which allow its incorporation into animal feed. The alga produces such essential chemicals such as beta carotene and astaxanthin that is utilized by the cosmetic industry. *Scenedesmus* is capable of producing many kinds of biofuels including bioethanol, biohydrogen, biomethanol and biodiesel but more extensive research has been done on its use for biodiesel production, According to Prabakaran and Ravindran (2012), the alga produces a high amount of oleic acid making it a worthy feedstock for biodiesel production. Research conducted by Renuka *et al*., (2015) showed that the micro alga has great promise for use as a biofertilizer.

Reproduction in *Scenedesmus* is asexual through the production of autospores or autocolonies. By 2 successive, non-vegetative cell divisions, each *Scenedesmus* cell is capable of producing either a new 4-celled colony or unicellular algae (Egan and Trainor, 1988). Some species are capable of higher number of divisions. The parent cell under favorable conditions then ruptures to release the new colony. Sexual reproduction is rare or absent in some species.

Scenedesmus feed either autotrophically or heterotrophically or both. It records the highest biomass productivity among green algae. Its heterotrophic production of biomass and lipid in optimized conditions is reported to have greater efficiency than autotrophic production (Mandal and Malick, 2009). It has gained interest as a significant producer for various secondary metabolites particularly carotenoids. *Scenedesmus* can effectively grow in a wide temperature range and have been shown to utilize glucose as a carbon source in heterotrophic or mixotrophic growth. Environmentally, it is an important tool for bioremediation and wastewater treatment

owing to its ability to remove heavy metals and effluents by breaking them down. A comparative study on the removal efficacy of ammonia and phosphorus removal from an agrochemical wastewater using *Scenedesmus dimorphus* and *Chlorella vulgaris, Scenedesmus* exhibited higher efficiency in removing ammonia while both removed phosphorus to the same extent (Oliveira *et al*., 2019). Additionally, it can effectively fix CO_2 while showing high tolerance to industrial gases i.e. they aid in carbon sequestration and mitigate global warming (Shahid *et al*., 2020)

1.3. Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) is a colorless liquid at room temperature with a molecular weight of 34.015g/mol. It occurs naturally in trace gaseous quantities within the atmosphere. It is highly unstable decomposing readily oxygen and water with the release of heat. This property makes it a powerful oxidizing agent that can cause spontaneous combustion when it comes in contact with organic material (Akuji and Chambers, 2017).

Hydrogen peroxide is usually found available as 3% aqueous solution in our households and is considered safe unless used inappropriately. Improper use can cause irritation to the eyes, skin and mucous membranes. Ingestion of dilute hydrogen peroxide often result in mild symptoms like the ones aforementioned. Industrial grade concentrations usually up to 70% can cause significant mortality and damage at all types of exposures. Toxicity would only result when hydrogen peroxide comes in contact with the catalase of tissues. Industrial concentrations would cause significant damage like chemical burns and more likely to interact with the catalase in tissues (Akuji and Chambers, 2017).

Hydrogen peroxide serves numerous household and industrial purposes and is primarily used as a cleaning and disinfecting agent. It is widely utilized as bleach and for disinfecting surfaces

including surgical equipment and may be deployed as a vapor-phase sterilant for entire rooms (Rutala and Weber, 2004). It is an environmental friendly bleach and is considered suitable alternative to chlorine-based bleaches because it degrades completely to oxygen and water, leaving no trace or toxicity behind. It demonstrates broad spectrum anti-microbial activity against viruses, bacteria, yeasts and bacterial spores, though its efficacy may be reduced in the presence of peroxidases and catalases found in some microorganisms. Lower concentrations are more efficient against bacterial spores (McDonnel and Russel, 1999). Diluted H_2O_2 mixed with aqueous ammonia, aniline and a coupler has been used to color human hair where it acts as an oxidizing agent. Hydrogen peroxide is also used for teeth whitening; it works by oxidizing colored pigments into the enamel to produce a lighter appearance (Sulieman *et al*., 2004). It may also be employed in acne treatments though benzoyl peroxide is a more common treatment

Beyond its role as a disinfectant, Hydrogen peroxide has many uses ecologically and environmentally. It functions as an oxidant in wastewater treatment systems often in combination with catalysts such as UV, ozone - to degrade persistent organic pollutants or detoxify industrial effluents. It does this by generating oxygen radicals that break down the pollutants (Glaze *et al*., 1987).

Some horticulturists employ the use of hydrogen peroxide in irrigation systems. Its spontaneous decomposition releases oxygen that enhances plant root development and combats root rot. For this purpose, no more than 3% concentrations are used with 0.1% being the most acceptable (Bhattari *et al .,*2005). It is commonly used in waste water and disinfection treatment. It is often used as a feasible algicide to control cyanobacteria blooms because of its rapid decomposition rate leaving no residual trace making it environmentally friendly (Wang et *al*., 2019). It is particularly useful in controlling algal blooms. A downside effect is that it could harm other

species hence treatment is done in extremely low concentrations. It can be used for rebalancing aquatic community composition (Chao Chen *et al.,* 2021).

Due to both natural and anthropogenic activities (textile bleaching, paper production, industrial waste), Hydrogen peroxide is abundant in our environment than its usual composition. This accumulation results to what we call hydrogen peroxide pollution. it is particularly helpful in moderate amounts and confer toxicity in high amounts. Hydrogen peroxide pollution interferes with primary producers which form the foundation of aquatic food webs upsetting ecosystem stability. A loss in primary producers is a decrease in nutrient cycling and oxygen production (Barroin *et al*., 2020)

When Hydrogen peroxide comes in contact with algae cells, it generates reactive oxygen species such as hydroxyl radicals which can cause ultrastructure damage, inhibit photosynthesis, collapse colonial forms when introduced in high concentrations and even induce programmed cell death. In response, microalgae produce antioxidants like lutein, astaxanthin which are highly valuable in the health industry and to the economy. In comparison to cyanobacteria, Green microalgae such as Scenedesmus and Chlamydomonas require hydrogen peroxide in higher concentrations to effect growth inhibition (Santos *et al*., 2021). Hydrogen peroxide is a non-discriminating agent and can harm other harmless non-target species and as a result may cause cytotoxicity in some species. A study carried out by (Mardones *et al*, 2002) on the use of hydrogen peroxide in controlling cyanobacterial blooms. It showed that after hydrogen peroxide cleared out microalgae, cytotoxicity in gill cells of fish was recorded. Hydrogen peroxide also causes change in water quality parameters (turbidity, dissolved oxygen, pH) which was studied during the course of this research.

The effects of hydrogen peroxide on microalgae depends on an array of factors like algal density, species, salinity, environmental conditions like light, presence of other stressors. For instance, water bodies with higher decomposition rate will require more dosages of hydrogen peroxide (Weenick *et al*., 2015). An advantageous effect of the chemical may become disadvantageous with varying concentrations and species. Hydrogen peroxide is at the same time both beneficial and harmful to the aquatic environment hence appropriate control is needed when dealing with the chemical.

1.4 LITERATURE REVIEW

Hernandez (2021) evaluated the impacts of hydrogen peroxide on algae and *Microcystinin* in lake and reagent grade water. The findings showed that hydrogen peroxide reduced the concentrations of cyanobacteria as the doses were increased and was most efficient at low dosages. ($50\mu L\ L^{-1}$). The addition of consequent hydrogen peroxide was significantly indifferent in the concentrations of cyanobacteria.

Wong *et al*., (2003) investigated the role of marine phytoplankton in decomposing hydrogen peroxide on five oceanic and four coastal species of phytoplankton*; Pleurochrysis carterae, Synechococcus sp, Chaetoceros simplex, Thalassiosira oceanica, Skeletonema costatum, Isochrysis galbana, Tetraselmis sp., Dunaliella tertiolecta* and *Amphididnium carterae*. The rates at which all nine species examined broke down hydrogen peroxide ranged from 10^{-4} to 10^{-2} µg Chl-a^{-1} h^{-1}. The most efficient decomposers were found to be *Synechococcus sp* and *S. costatum*. A number of variables like increased salinity, temperature, presence of light and depletion of nutrients all accelerated the breakdown of hydrogen peroxide. The growing phase had minimal impact. The magnitude and pattern of these modifications were specific to each species.

Santos *et al*., (2021) conducted an experiment to study the efficiency of hydrogen peroxide in mitigating cyanobacteria blooms. For 120 hours at high irradiance, temperature, and carbon content, a mesocosm containing precisely 10 mg L^{-1} of hydrogen peroxide was introduced into a semi-arid area dominated by *Plankthothrix*. In 72h, along with enhancing the water quality, Hydrogen peroxide efficiently suppressed cyanobacteria, green algae leading to an increase in transparency and dissolved organic carbon and a decrease in dissolved oxygen and pH. Concentrations of nutrients were not affected. By the end of the 120h, there was a subsequent dominance of green algae. The initial dominated *Plankthothrix* cyanobacterial bloom was suppressed and a rise in *Cyanobacterium* was present. This suggests rebound due to the availability of nutrients.

Zhou *et al*., (2017) carried out a research to track the combined effects of sunlight and hydrogen peroxide in controlling algal blooms. Hydrogen peroxide induced cyanobacterial stress and damage were dose dependent and was quicker and efficient at full light than under shade. However, it could only control bloom for a short time as there was a rebound growth of eukaryotic algae. Addition of same dose had no effect suggesting a need for higher concentrations but this wasn't considered as it could endanger the aquatic body. Following a hydrogen peroxide re-addition showed a slight decrease in the proportion of eukaryotic algae.

Southard (2005) conducted a study to determine the efficacy of hydrogen peroxide as an algicide for *P. parvum*. The specific aim was to investigate if selected concentrations of hydrogen peroxide would lyse *P. parvum* cells in 15- 60 min and access the effect that 15 min to 24h exposures of concentrations of hydrogen peroxide deemed acceptable by fish would have on the algae. The results indicated that at a concentration of 12500 µg/ml, all *P. parvum.* cells were lysed, while concentrations between 250 and 3125 µg/mL inhibited cell motility. After 1 hour of

exposure, hydrogen peroxide at 3125 µg/mL resulted in complete lysis of all cells, whereas no lysis occurred at 781.25µg/mL, with motility inhibited at 500µg/mL. Complete lysis was observed across all hydrogen peroxide concentrations after 24 hours. The findings suggest that concentrations ranging from 62.5 to 500µg/mL for 24 hours would lyse *P. parvum* cells; however, the hydrogen peroxide levels tolerable to fish are significantly lower. Therefore, this chemical is not recommended.

Zhang *et al*. (2022) examined the effects of hydrogen peroxide on *Scenedesmus obliquus*, utilizing various concentrations (0 – 10 mg L-1) to assess alterations in its morphological, physiological, and proteomic characteristics. Results showed effective inhibition in cell growth and photosynthetic activity, oxidative stress, a decrease in metabolic activity and in terms of proteomic analysis, 251 differentially expressed proteins were identified.

Ting Li *et al*., (2021) carried out an experiment on *Chlorella vulgaris* to investigate the effects of carbon dioxide and hydrogen peroxide concentrations on algal growth and reactive oxygen species. Higher concentrations of carbon dioxide were seen to reduce the ROS level specifically hydrogen peroxide. The algae continued to thrive at elevated concentrations of CO2 and was unaffected by hydrogen peroxide.

Vavailala *et al*., (2015) conducted a research on *Chlamydomonas reinhardtii* to examine the morphological and cellular responses of the algae to oxidative (Hydrogen peroxide) and osmotic (sodium chloride) stress. Results showed that Hydrogen peroxide had a greater effect on the alga causing oxidative stress, caspase activation and creating oligonucleosomal DNA, all indicating apoptotic death which did not happen with NaCl. This shows that C. reinhardtii respond to various stress agents in different ways, leading to different death types in the same organism.

Piel (2025) carried out a study to determine the effects of hydrogen peroxide on cyanobacterial blooms. He recorded that the effects of hydrogen peroxide on cyanobacteria *Microcystin* varied with nutrient availaibilty. He also found out the effectiveness of hydrogen peroxide was greater at high light intensities and treatment with hydrogen peroxide in water bodies altered phytoplankton and zooplankton composition.

Rach *et al*., (2005) evaluated the efficacy of formalin and hydrogen peroxide to increase survival of channel catfish *Ictalurus punctatus* infected with external saprolegniasis. Results showed that both formalin and hydrogen peroxide were effective increasing survival by 20 – 100% depending on the control concentrations.

Latifi *et al*., (2009) investigated oxidative stress control in cyanobacteria and microalgae, focusing on their adaptive defensive mechanisms. They discovered that exposure to hydrogen peroxide disrupts photosystem ii, reduces chlorophyll concentration and triggers lipid peroxidation. However prolonged exposures often overwhelm these defense systems, leading to decreased photosynthetic efficiency and eventual cell death.

Imlay (2008) studied the cellular defenses of microorganisms against reactive oxygen species (ROS), specifically superoxide and Hydrogen peroxide, he found that it induces oxidative stress that ca damage cell membranes, nucleic acid, proteins. The study revealed that when antioxidant enzymes are insufficient. ROS accumulates and disrupts cellular metabolism and leads to growth inhibition in microalgae. The finding established Hydrogen peroxide as a potential ecological stressor capable of altering microalgal physiology and community balance.

Zhang *et al*., (2021) investigated the use of *Azospirillium brasilliense*, a bacterium that produces indole-3-acetic acid (IAA), to inprove the growth of microalgae. Results showed that bioagumentation with the bacterium efficiently increased algal biomass accumulation and enhanced nutrient uptake.

Johnson and Alric (2013) described *Chlamydomonas reinhardtii* as an excellent model for analyzing mixotrophic nutrition and demonstrated that the species can metabolize inorganic compounds while performing photosynthesis.

AIMS AND OBJECTIVES OF THE STUDY

The aim of this study was to evaluate the effects of hydrogen peroxide on the growth of *Scenedesmus* and *Chlamydomonas*.

The objectives of this study were to:

i. Determine the growth responses of *Scenedesmus* to different concentrations of hydrogen peroxide.

ii. Determine the growth responses of *Chlamydomonas* to varying concentrations of hydrogen peroxide.

iii. Evaluate the bioremediation potential of *Scenedesmus* and *Chlamydomonas* if any.

CHAPTER TWO
MATERIALS AND METHODS

2.1 TEST MICROALGAE

The test microalgae used in the experiment were *Scenedesmus* and *Chlamydomonas.*

2.2 SOURCE OF MICROALGAE

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

2.3 TAXONOMY OF ALGAE

Taxonomy of *Scenedesmus* sp.

Kingdom: Plantae

Division: Chlorophyta

Class: Chlorophyceae

Order: Sphaeropleas

Family: Scenedesmaceae

Genus: Scenedesmus

Taxonomy of *Chlamydomonas* sp.

Kingdom: Plantae

Division: Chlorophyta

Class: Chlorophyceae

Order: Volvocales

Family: Chlamydomonadaceae

Genus: Chlamydomonas

2.4 Collection of Hydrogen Peroxide

The chemical; Hydrogen peroxide used in the study was purchased from a chemical store in Benin city, Edo state, Nigeria.

2.5 Culture Media

2.5.1 Freshwater Test Microalgae Culture Medium

The artificial media used to culture the freshwater test microalgae was CHU number 10 Modified medium. The medium's composition is displayed in table 2.1 below. To create a stock solution, the specified salts were dissolved in 100 milliliters of distilled water. 3.35g of ferric citrate was added after 5g of citric acid was dissolved in 100ml of distilled water to create an iron solution. After that, the mixture was autoclaved and placed in the refrigerator. By dissolving the salt below in the amounts (mg) specified in 1L of distilled water, a trace element solution was created. The fluid was kept sterile by autoclaving it.

2.5.2 Culture Vessel

The experiment employed the use of forty-two 500ml bottles as the containers for culture. Before these bottles were used for the experiment, they were thoroughly washed with detergent and rinsed with water followed by acid washing with dilute sulphuric acid (H_2SO_4) to kill any unwanted microorganisms and then rinsed with water again. The work table to be used was cleaned with acetone by wiping it with a cotton wool swabbed with acetone. The bottles were turned over on the work table and allowed to dry and drip out excess water. The bottles were now placed facing up and their mouths sealed with cotton wool in preparation for culturing.

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

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

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 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 treatment

Hydrogen Peroxide Concentration (%)	Hydrogen Peroxide 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 Experiment

The test microalgae species were grown in triplicate for 14 days in a growth medium that had seven concentrations: 0%, 1%, 5%, 10%, 15%, 20%, and 25%. After every reading day, the cultures were put in the east-facing window of the University of Benin's microbiology lab. Avoiding the effects of direct sunlight was the purpose of this stance.

2.7 Inoculation

Each vessel was inoculated with 5 ml of the microalgae cultures using 5 ml syringes. Cotton wool was quickly placed over the experiment bottles to promote airflow, limit evaporation, and avoid contamination.

2.8 Measurement and Monitoring of Algal Growth

The growth potential was measured in a UV/Visible spectrophotometer at an absorbance of 750nm. It was measured at a two-day interval for 14 days. Growth curves of hydrogen peroxide concentrations were plotted.

Plate 1: Culture vessels with varying concentrations of Hydrogen peroxide and microalgae *Chlamydomonas* (left) and *Scenedesmus* (right)

Plate 2: Culture vessels containing growth medium

Plate 3: 721 Visible Spectrophotometer.

2.9 Data Analysis

Descriptive statistics, inferential analysis including two-way analysis of variance(ANOVA), repeated measures and paired t-test were performed using Microsoft Excel.

2.10 Percentage Yield

Percentage yield was calculated at the end of experiment day 14 using the formula shown below:

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

G_t = (growth at the end of experiment)

G_o = growth at the beginning of experiment)

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

2.11 PHYSICOCHEMICAL ANALYSIS

2.11.1 pH: pH is the measure of how acidic or basic a solution is, using a scale of 1 to 14. The pH value of the cultures was recorded using a pH/conductivity/TDS/Salinity/ Temperature meter model EZ-9909. The probe was submerged into the culture and allowed to be stable for few minutes before taking reading.

2.11.2 Total Dissolved Solids (mg/L): Total Dissolved Solids is the measure of all organic and inorganic substances dissolved in the liquid. A pH/conductivity/TDS/Salinity/ Temperature meter model EZ-9909 was used to determine the amount of total dissolved solids. The probe was immersed into the culture and allowed to be stable for few minutes before taking readings in milligram per Litre (mg/L).

2.11.3. Conductivity (μScm^{-1}): Conductivity is the ability of the mixture to conduct an electrical current, which depends on the concentrations of dissolved ions from salts and minerals. A pH/TDS/Salinity/conductivity/Temperature meter model EZ-9909 was used to test conductivity. The probe was immersed into the culture container and allowed to stabilize for few minutes before taking readings.

2.11.4. Turbidity (NTU): Turbidity is the measure of water clarity which is affected by the amounts of suspended particles in the mixture. Turbidity was measured using a turbidity meter. It was done by putting a little quantity of the culture sample into the corvette and putting back into the meter and allowing to stabilize before taking a reading.

2.11.5 Dissolved Oxygen (mg/L): Dissolved oxygen is the amount of free oxygen present in the mixture that is essential for aquatic life. A dissolved oxygen meter model DO 200 was used to measure the levels of dissolved oxygen in each culture sample by inserting the meter into the sample and allowing it to stabilize before raking readings.

Plate 4: Turbidity Meter.

Plate 5: Dissolved oxygen meter.

Plate 6: Multi-parameter (pH/TDS/Salinity/Conductivity/Temperature) meter

CHAPTER THREE

RESULTS

Figure one(1) shows the effect of different concentrations of Hydrogen peroxide on the growth of *Scenedesmus sp*

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

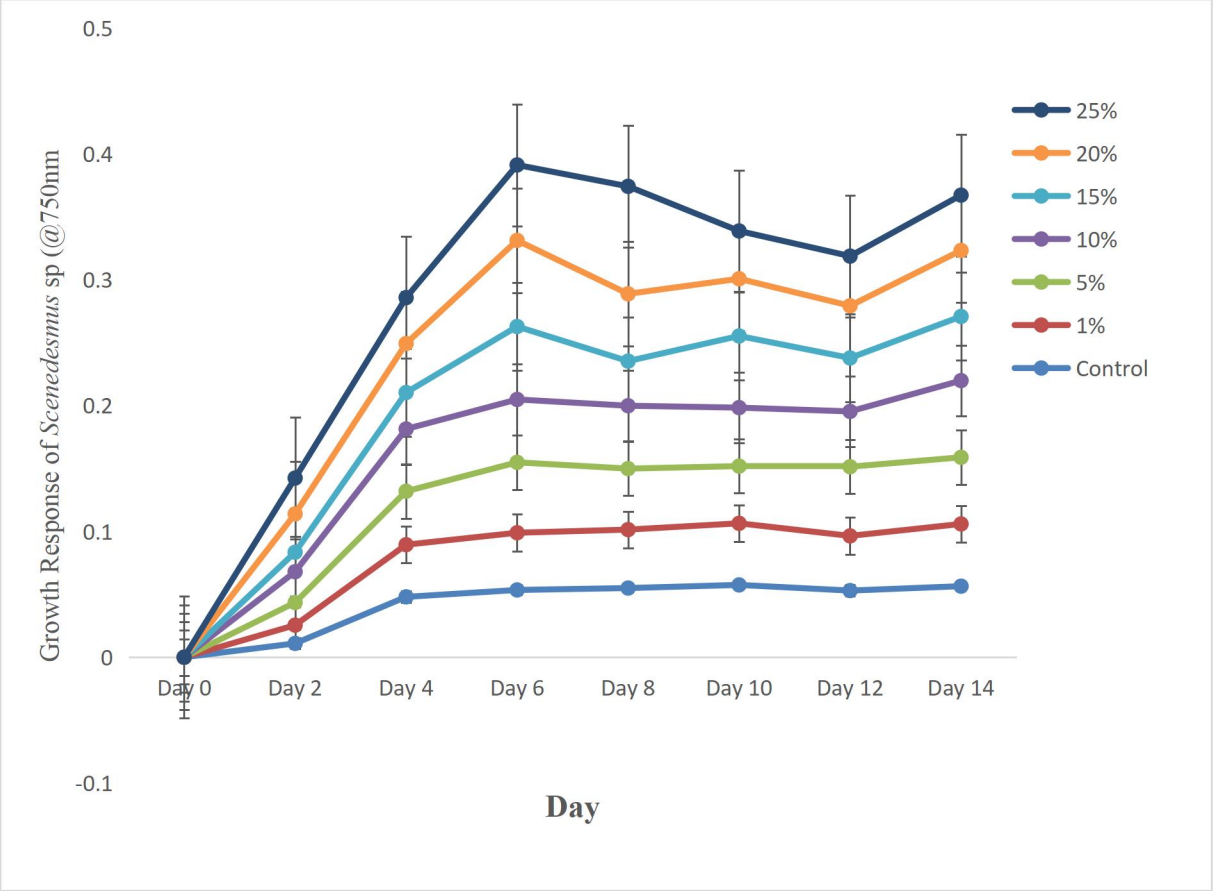

Figure 1: Effect of Hydrogen Peroxide on the growth of *Scenedesmus* sp.

Figure two (2) shows the effects of different concentrations of Hydrogen peroxide on the growth of *Chlamydomonas* sp.

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

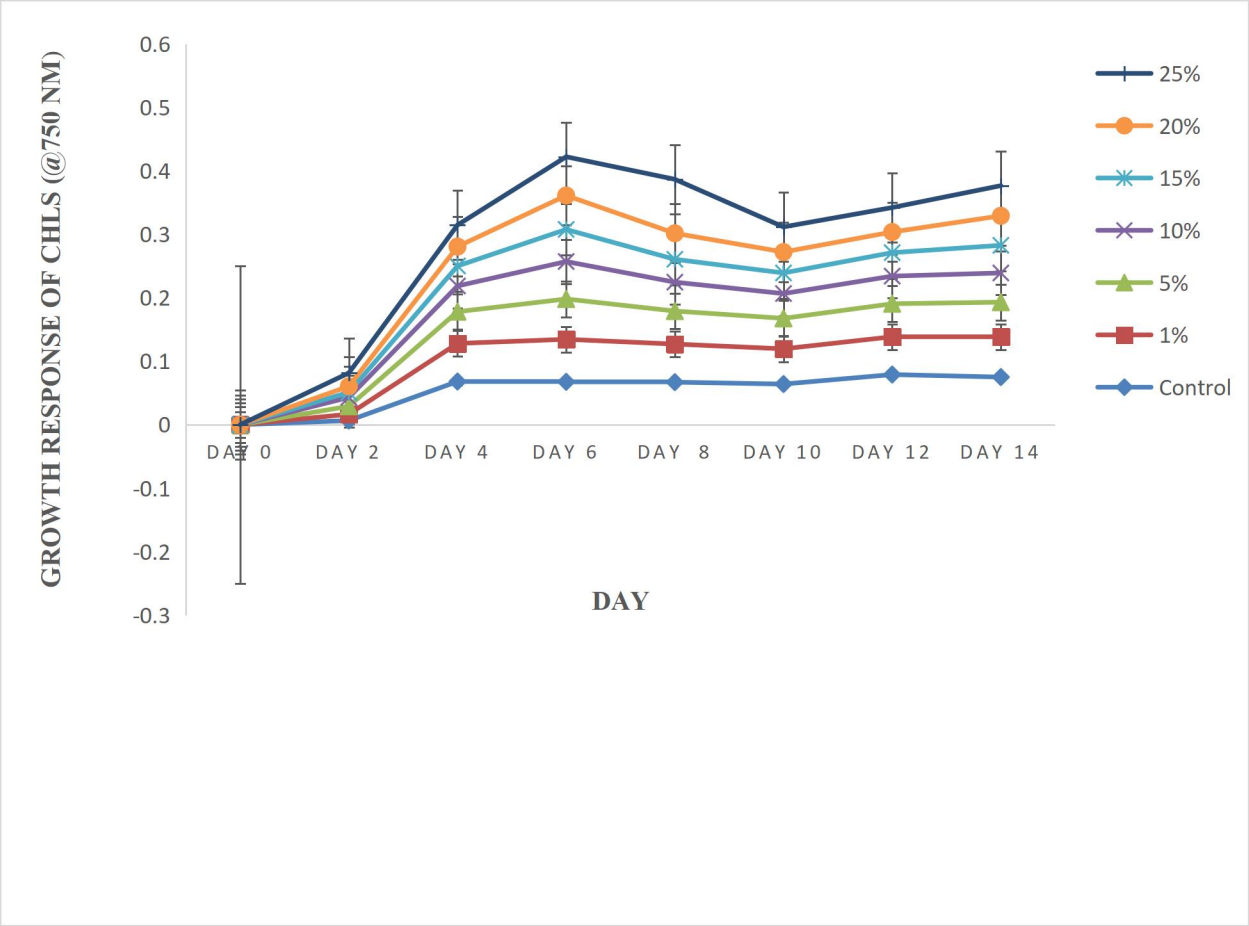

Figure 2: Effect of Hydrogen Peroxide on the growth of *Chlamydomonas* sp.

Figure three (3) shows the comparative effect of different concentrations of hydrogen peroxide on the yield of *Scenedesmus* sp and *Chlamydomonas* sp.

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

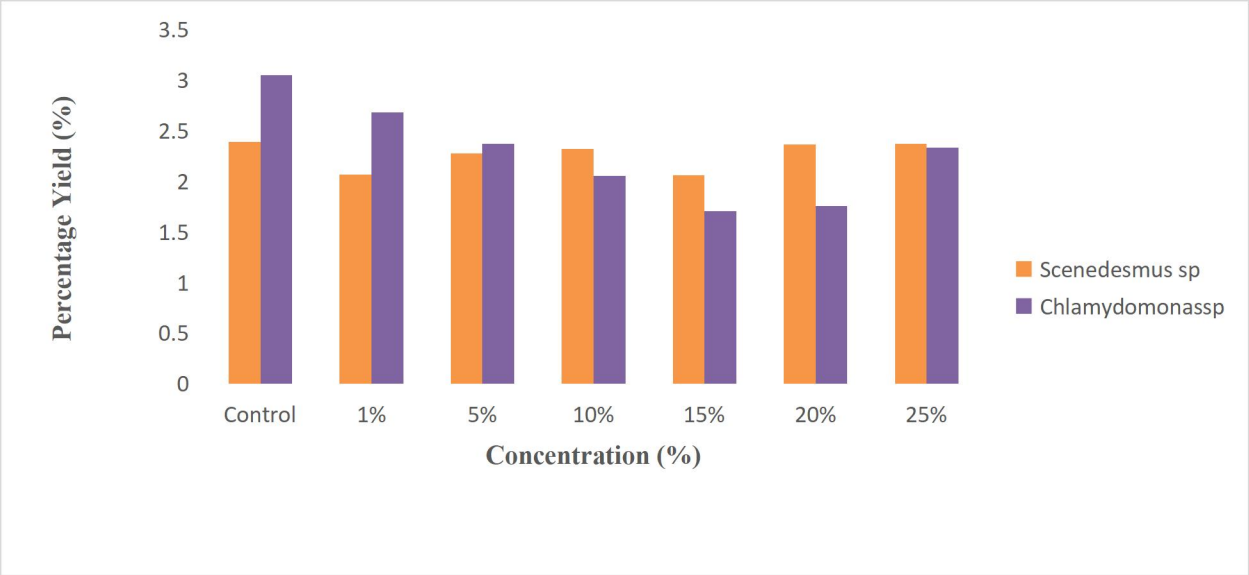

Figure 3: Comparative Percentage Yield of *Scenedesmus* sp and *Chlamydomonas* sp

Figure four (4) reveals the effect of different concentrations of Hydrogen peroxide on the turbidity of *Scenedesmus* sp.

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

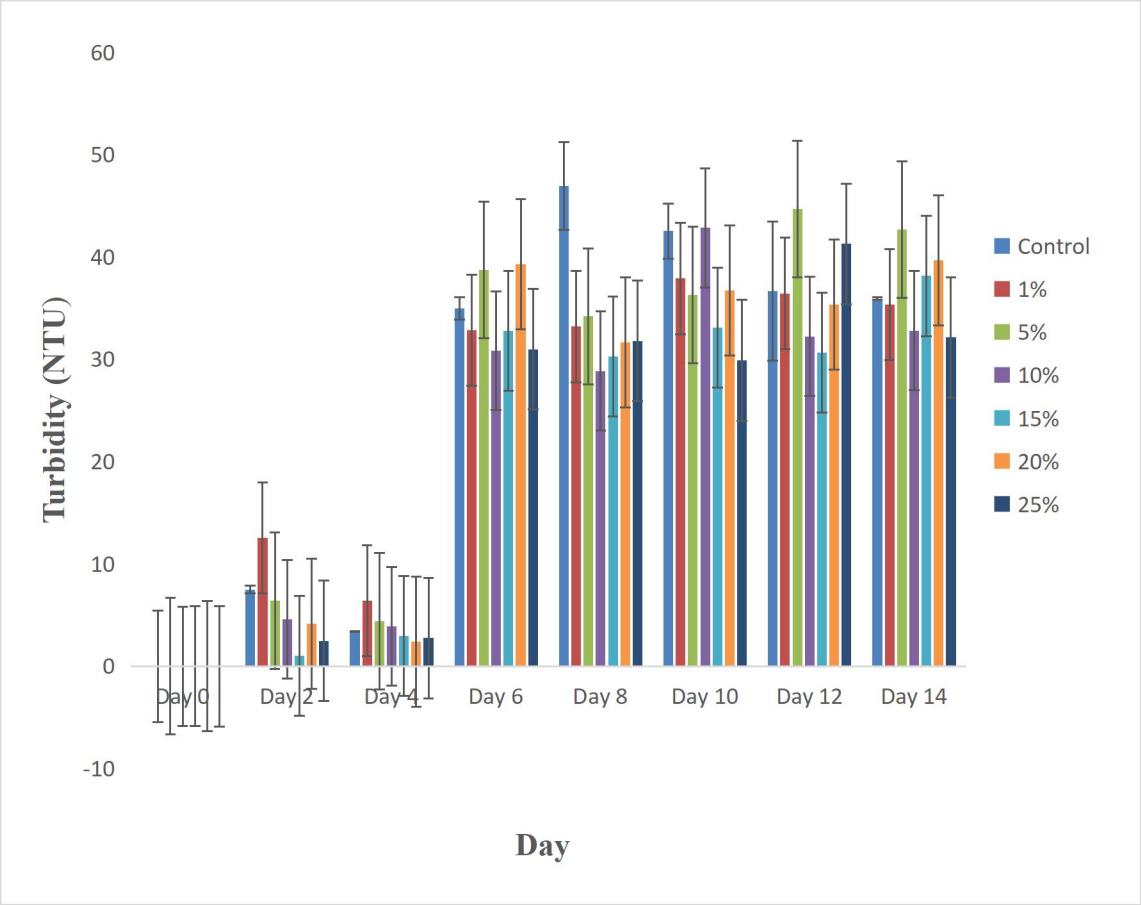

Figure 4: Turbidity of different concentrations of Hydrogen Peroxide on the growth of *Scenedesmus* sp.

Figure five (5) shows the effect of the different concentrations of Hydrogen peroxide on the turbidity of *Chlamydomonas* sp

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

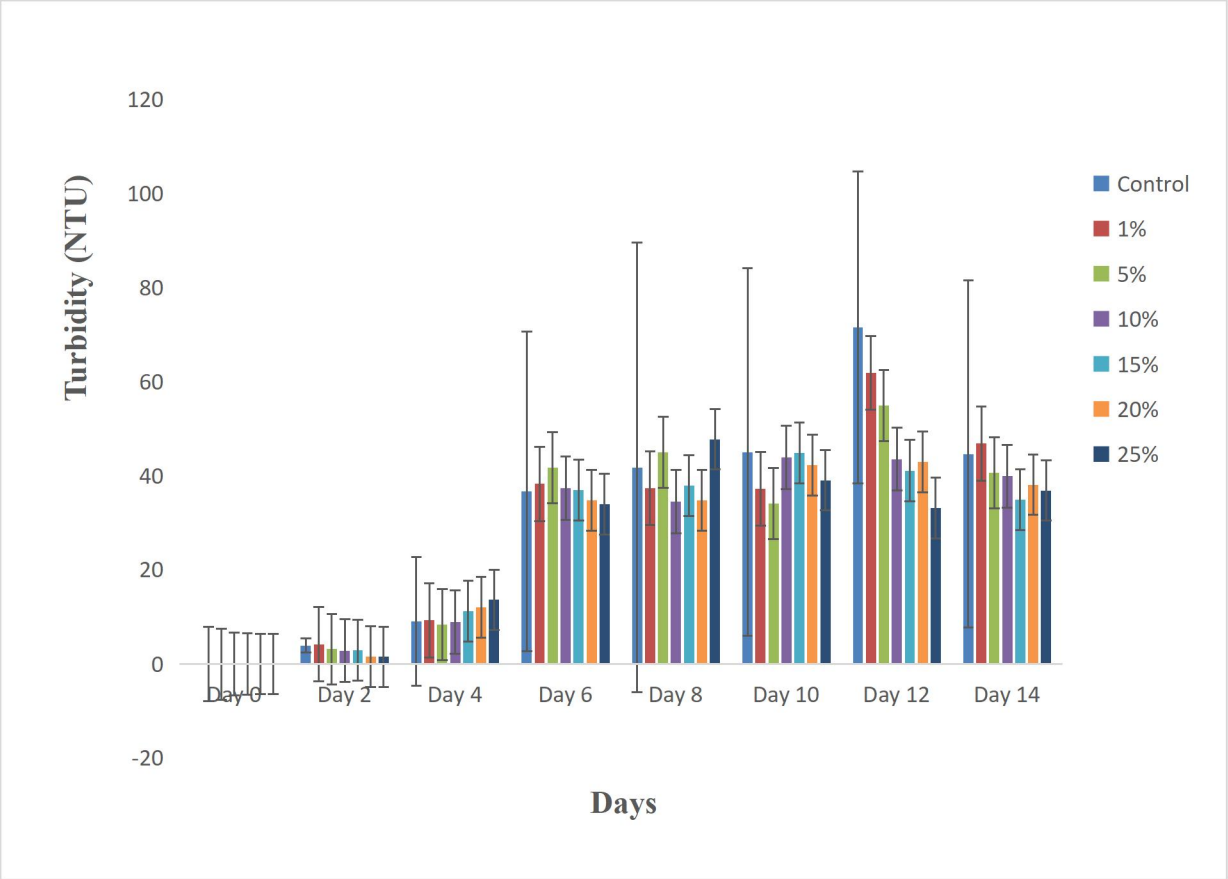

Figure 5: Turbidity of different concentration of Hydrogen Peroxide on the growth of *Chlamydomonas* sp.

Figure six (6) reveals the effect of the different concentrations of Hydrogen peroxide on the conductivity of *Scenedesmus* sp.

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

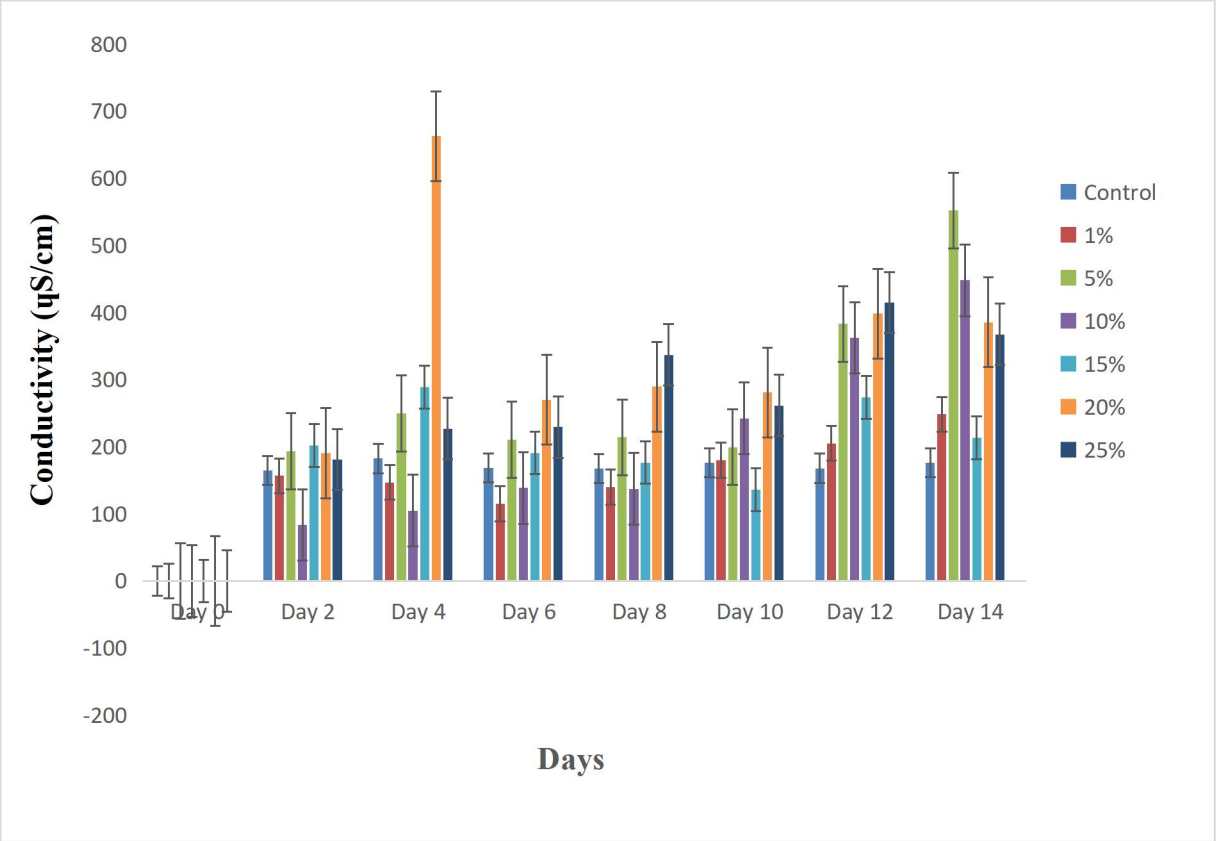

Figure 6: Conductivity of different concentration of Hydrogen Peroxide on the growth of *Scenedesmus* sp.

Figure seven (7) shoes the effect of the different concentrations of Hydrogen peroxide on the conducrtivity of *Chlamydomonas* sp.

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

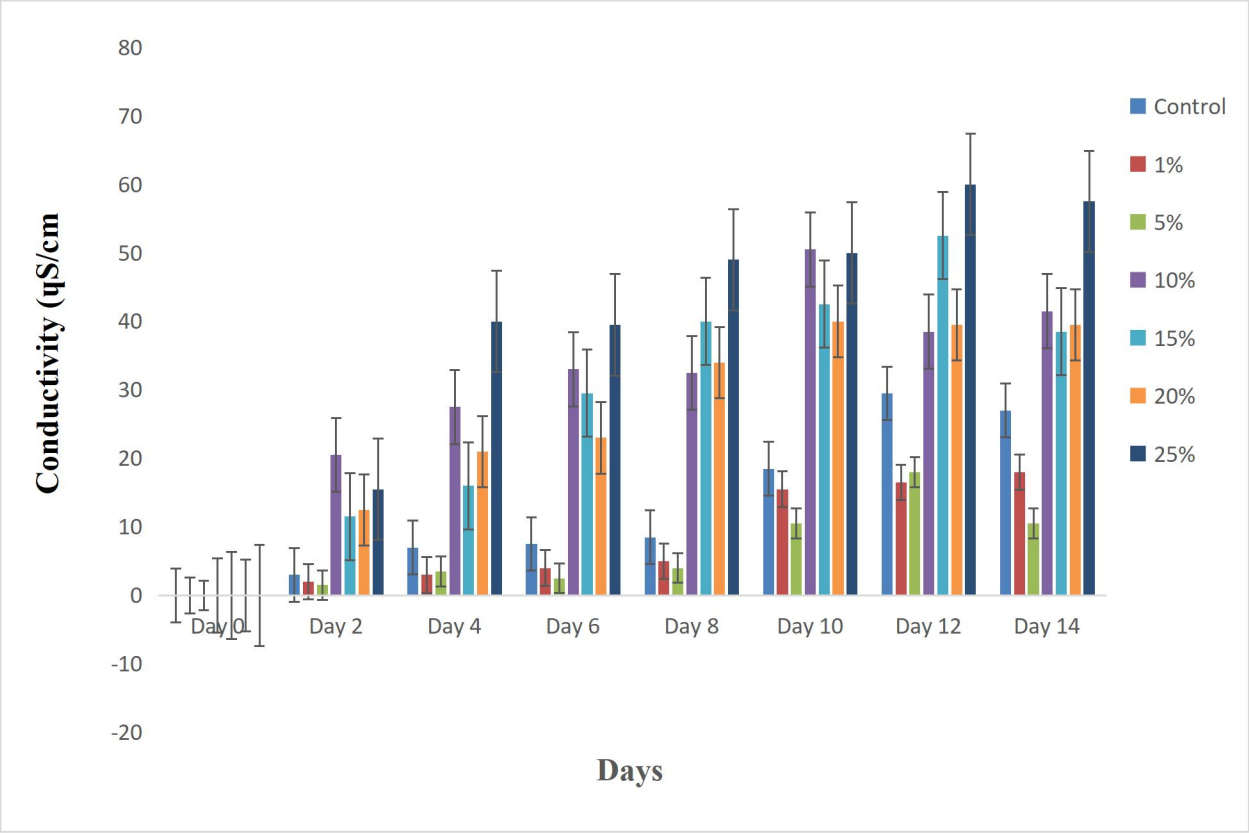

Figure 7: Conductivity of different concentration of Hydrogen Peroxide on the growth of *Chlamydomonas* sp.

Figure eight (8) reveals the effect of the different concentrations of Hydrogen peroxide on the total dissolved solids level of *Scenedesmus* sp.

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

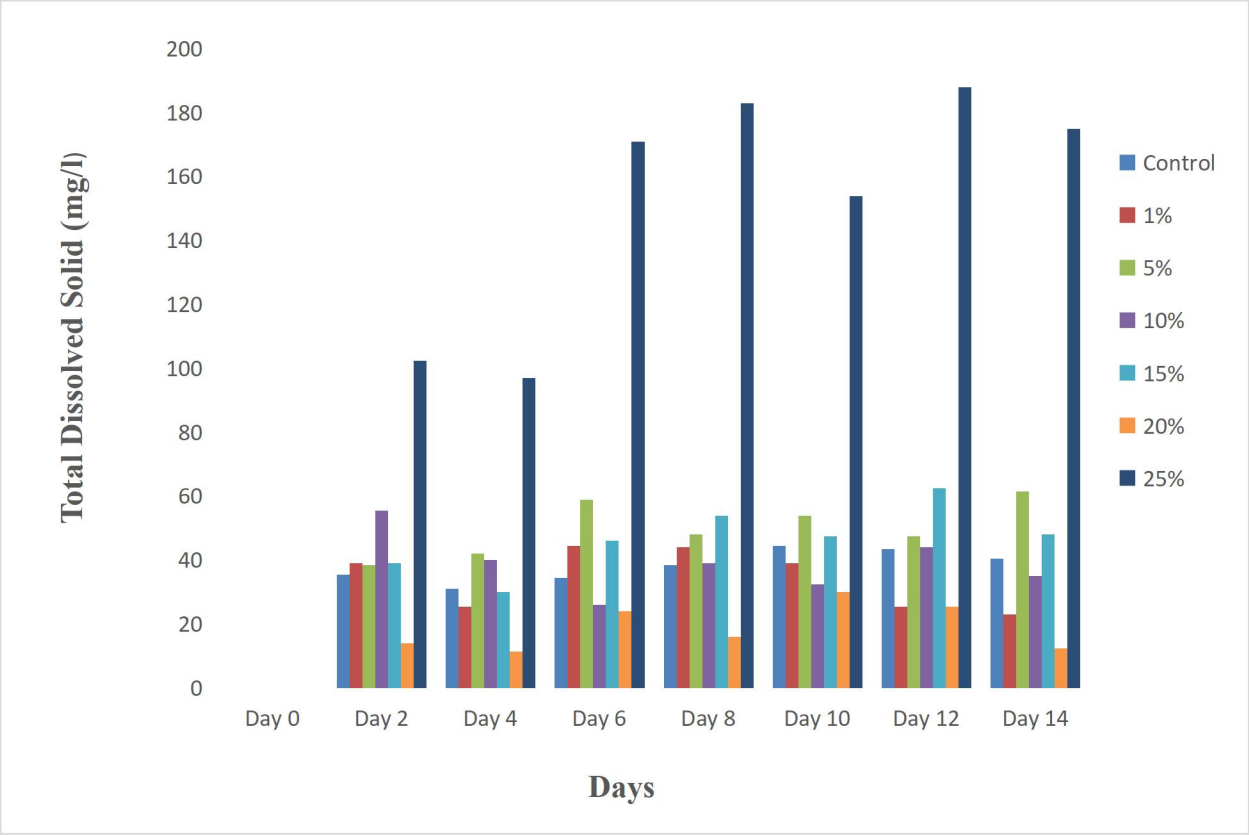

Figure 8: Total Dissolved solid of different concentrations of Hydrogen Peroxide on the growth of *Scenedesmus* sp.

Figure nine (9) reveals the effect of the different concentrations of Hydrogen peroxide on the total dissolved solids level of *Chlamydomonas* sp.

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

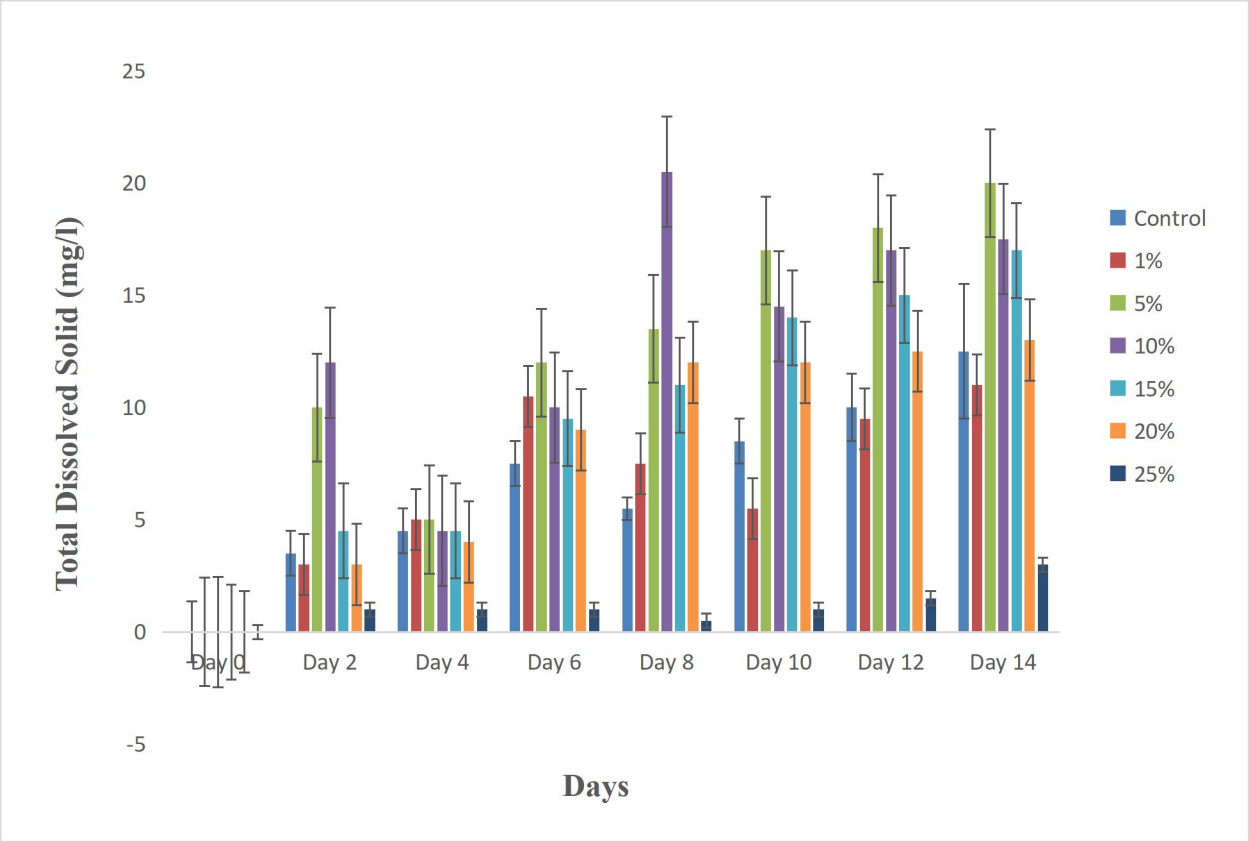

Figure 9: Total dissolved solid of different concentration of Hydrogen Peroxide on the growth of *Chlamydomonas* sp.

Figure ten (10) shows the effect of the different concentrations of hydrogen peroxide on the pH of *Scenedesmus* sp.

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

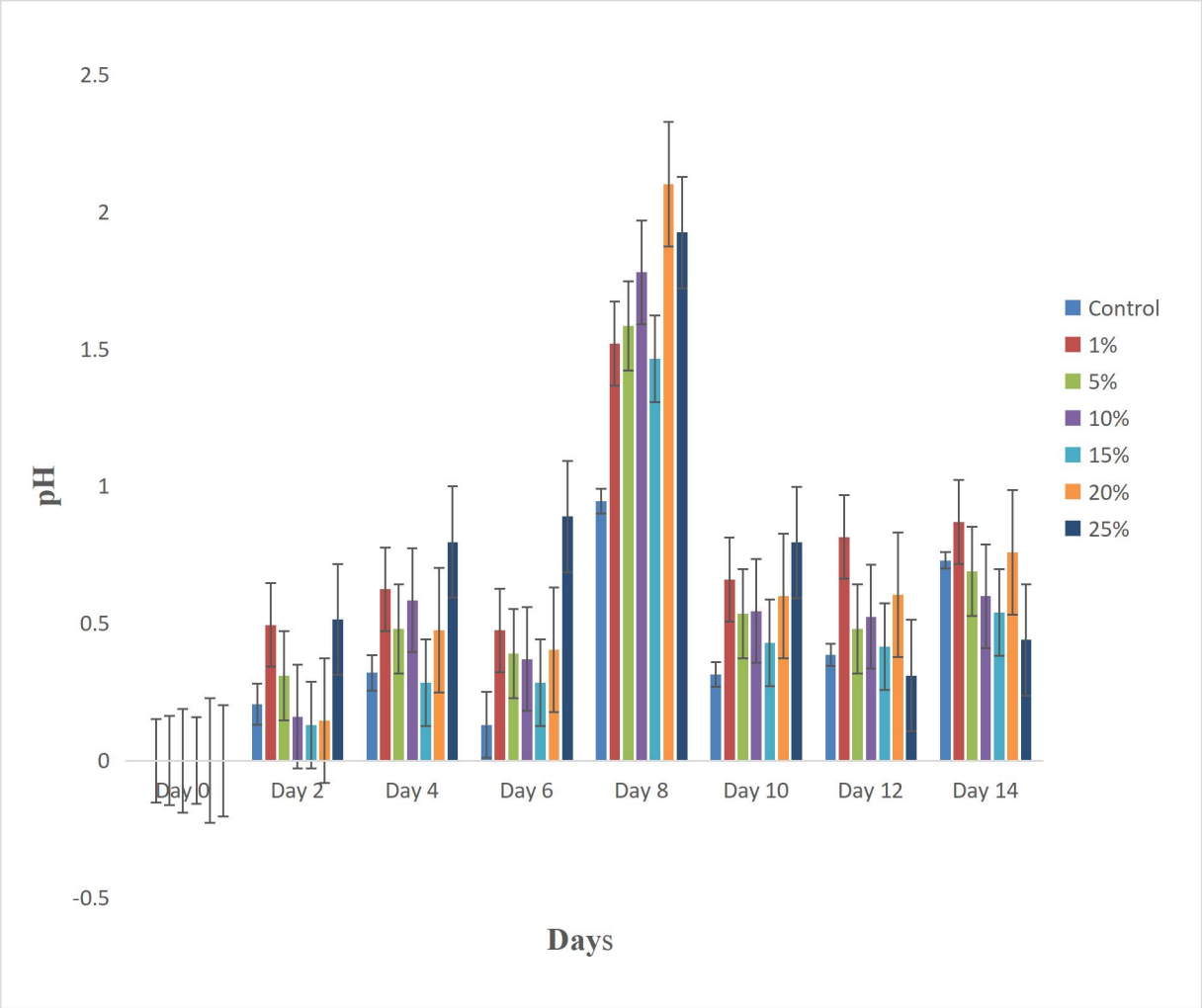

Figure 10: pH of different concentrations of Hydrogen Peroxide on the growth of *Scenedesmus* sp.

Figure eleven (11) reveals the effect of the different concentrations of hydrogen peroxide on the pH of *Chlamydomonas* sp.

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

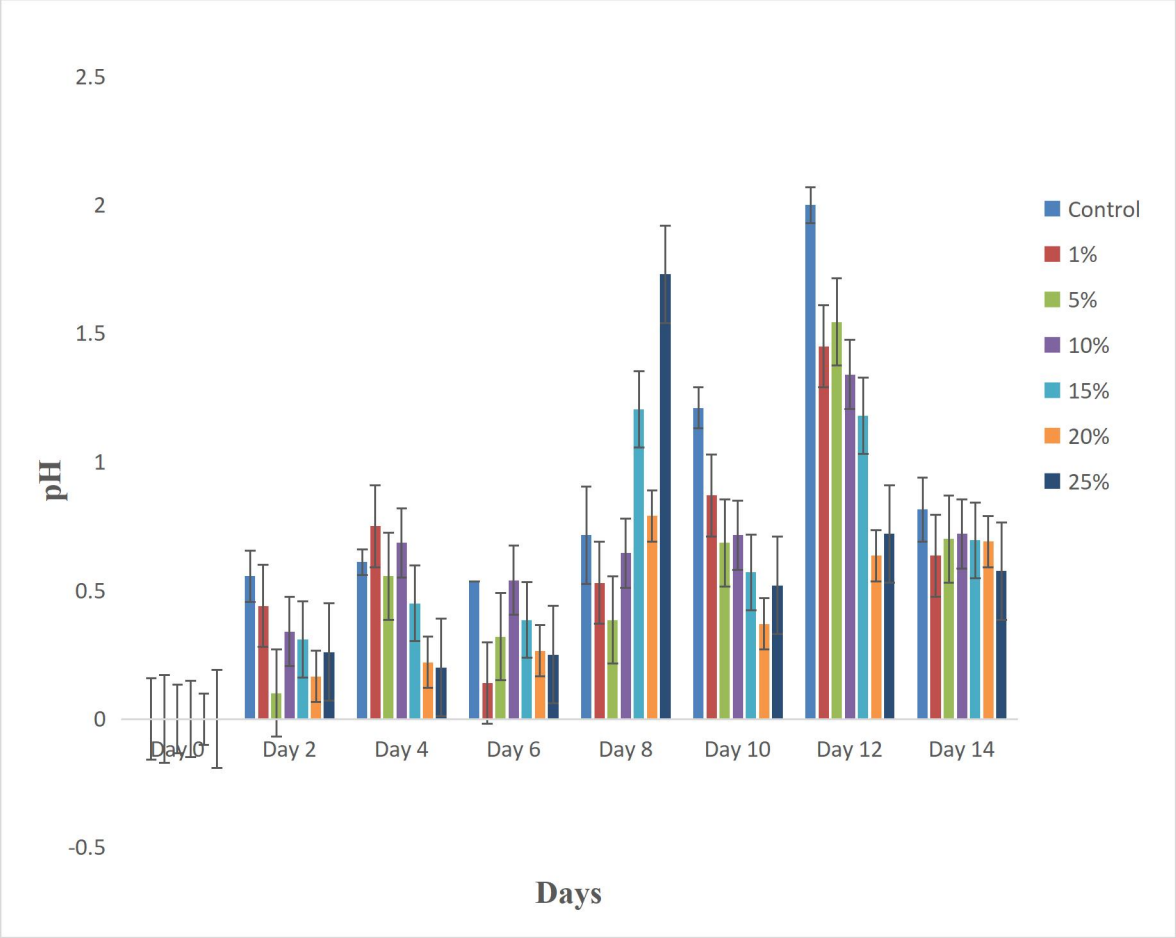

Figure 11: pH of different concentration of Hydrogen Peroxide on the growth of *Chlamydomonas* sp.

Figure twelve (12) reveals the effect of the different concentrations of Hydrogen peroxide on the dissolved oxygen levels of *Scenedesmus* sp.

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

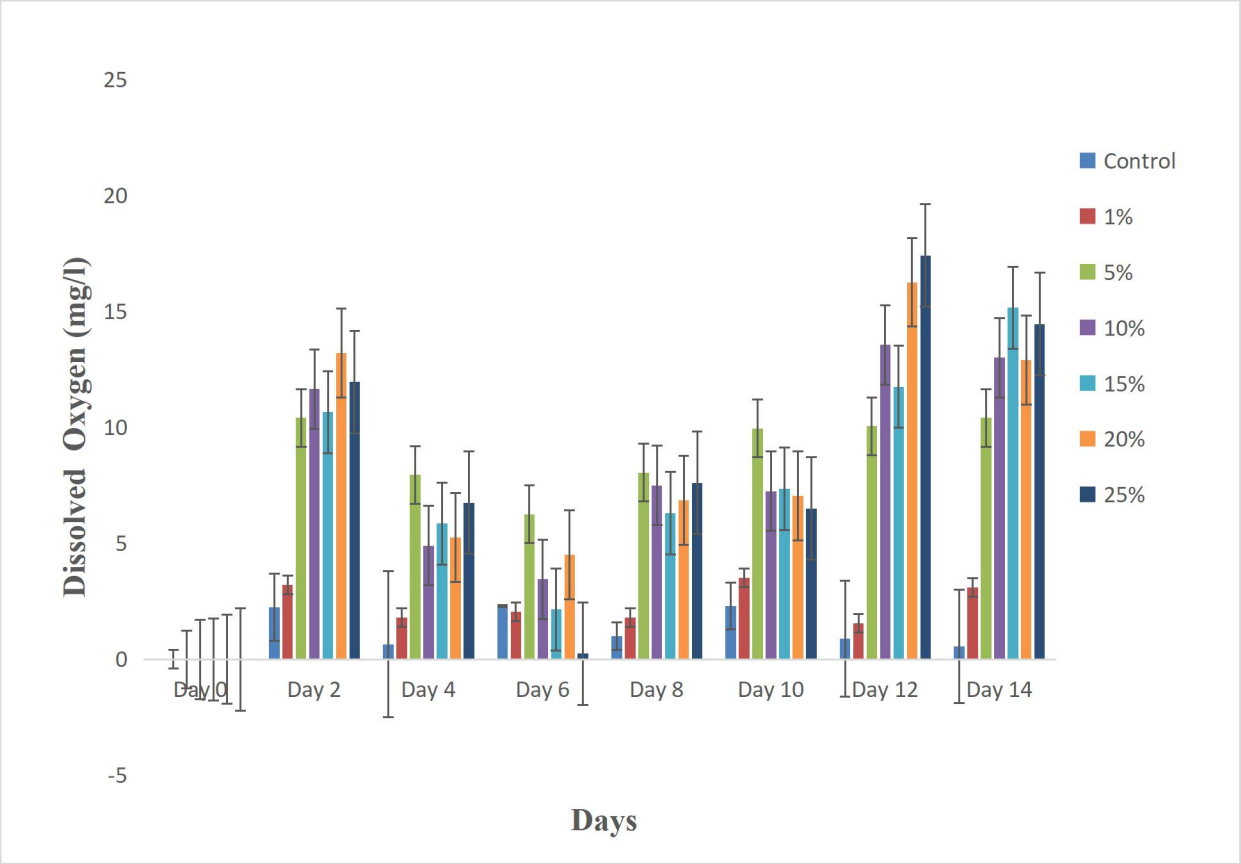

Figure 12: Dissolved Oxygen of different concentrations of Hydrogen Peroxide on the growth of *Scenedesmus* sp.

Figure thirteen (13) reveals the effect of the different concentrations of Hydrogen peroxide on the dissolved oxygen levels on *Chlamydomonas* sp.

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

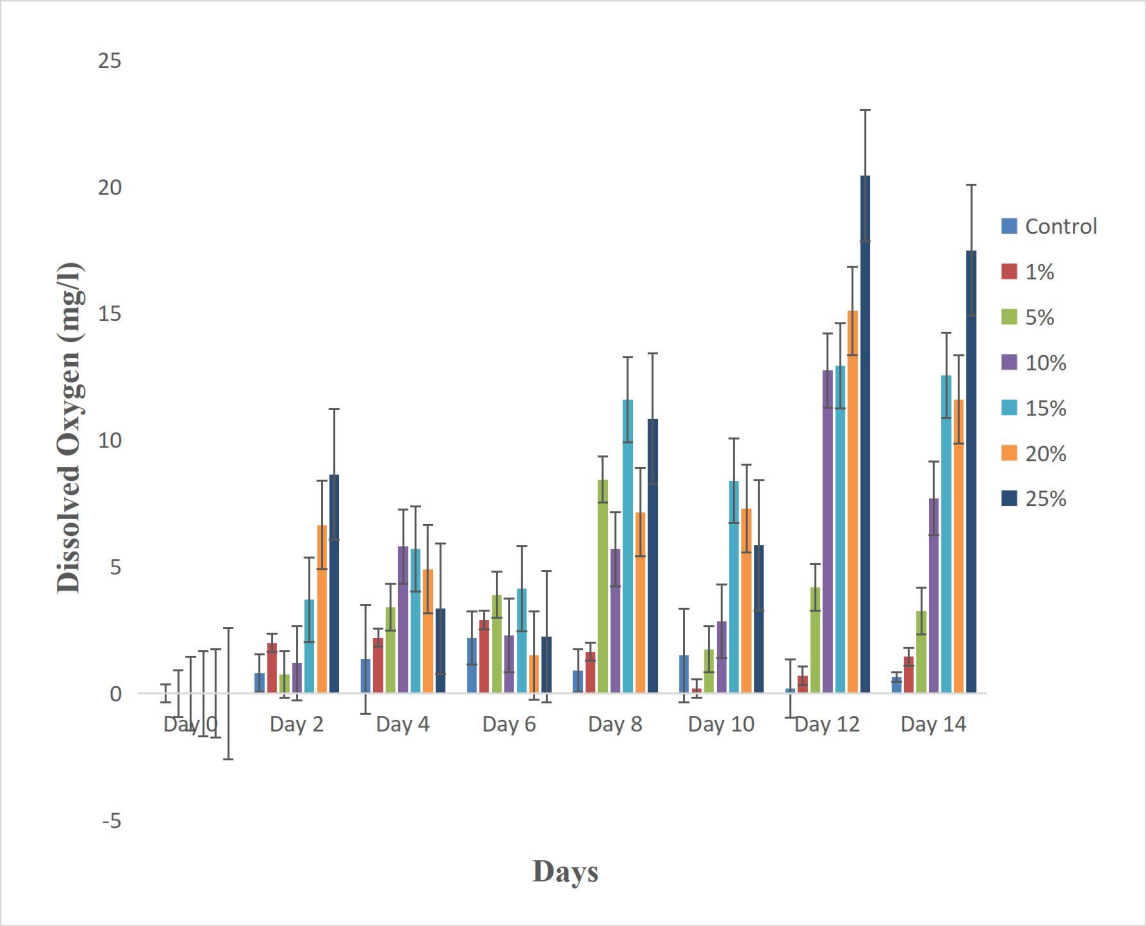

Figure 13: Dissolved Oxygen of different concentration of Hydrogen Peroxide on the growth of *Chlamydomonas* sp.

CHAPTER FOUR

DISCUSSION

This study aimed to evaluate the effect of Hydrogen peroxide on the growth of *Scenedesmus* sp and *Chlamydomonas* sp, which are both freshwater microalgae species, over a period of 14 days under laboratory conditions. Some physicochemical parameters such as growth, turbidity, conductivity, total dissolved solids, pH, and dissolved oxygen were analyzed.

Figure 1 and 2 showed the effects of hydrogen peroxide on the growth of *Scenedesmus* sp. and *Chlamydomonas* sp. respectively. The growth pattern of *Scenedesmus* sp. revealed a steady moderate growth across the concentrations 0 to 10% H_20_2 while notably higher growth occurred at 15 to 25% H_2O_2 . This indicates that Hydrogen peroxide has a stimulatory rather than inhibitory effect on *Scenedesmus* within the concentrations tested. The findings revealed that the growth of *Scenedesmus* increased with increasing concentrations of Hydrogen peroxide, with the highest growth being at 25% concentration of H_2O_2 . This increase in growth at higher concentrations (15-25%) could be attributed to hormetic effect, where low to moderate oxidative stress activates defensive and metabolic pathways that enhance growth and reproduction. In this case, hydrogen peroxide may have acted as a signaling molecule, stimulating antioxidant enzymes such as catalase and peroxidase to neutralize oxidative stress and promote cellular activity. This is in line with Zhang *et al*., (2022) study who examined the effects of hydrogen peroxide (0- 10mg/L) on *Scenedesmus obliquis* growth and reported that that there was an increase in antioxidant enzyme activity and lowered growth and photosynthetic activity in a dose-dependent fashion, as the cells responded to oxidative stress. Also, the decomposition of hydrogen peroxide potentially enhanced photosynthetic oxygen supply for the culture.

In contrast, *Chlamydomonas* sp. (figure 2) showed a comparatively different pattern. While it also experienced growth across concentrations, the rate of increase was not as pronounced as *Scenedesmus.* There was a decline from day 6 in growth and a slight increase from day 10 for concentrations 10 to 25%. This indicates that *Chlamydomonas* activated antioxidant and defensive mechanisms when exposed to H_2O_2 but had a low threshold and hence started experiencing oxidative stress when it exceeded its threshold hence limiting further growth acceleration. This response can be attributed to its thinner cell wall and unicellular morphology

in contrast to *Scenedesmus* which has a robust cell wall and a colony forming nature, inferring specie-specificity of green algae to Hydrogen peroxide. This finding is in line with Kim *et al*., (2018) who reported that *Chlamydomonas reinhardtii* had fluctuating growth under oxidative stress characterized by initial suppression of growth and later recovery, owing to its morphology and physiological characteristics. This agrees with Michaela Drabkova *et al*., (2007) that Hydrogen peroxide has selective effects on algae species.

Turbidity is a measure of the cloudiness of the culture medium caused by the presence of suspended algal cells. In algal studies, it is often used as an indirect indicator of growth or cell density- higher turbidity means more algal cells in suspension, while lower turbidity indicates reduced growth or cell death. In figure 4, for *Scenedesmus* sp. turbidity increased progressively with higher hydrogen peroxide concentrations throughout the 14-day period. The culture medium became more turbid at the higher concentrations, implying that Hydrogen peroxide acted as a stimulant rather than a toxicant. This observation can be explained by the ability of the algae to tolerate oxidative stress, triggering antioxidant defense mechanisms which protect the cells and may even stimulate faster metabolism and production. The released oxygen from the breakdown of hydrogen peroxide could have also enhanced photosynthetic activity, thereby increasing turbidity through higher cell multiplication. A similar finding was reported by Fan *et al*., (2019) who observed increased biomass and turbidity in green microalgae following mild oxidative treatment, indicating that hydrogen peroxide can promote growth when applied at a tolerable range. However, this result differs from that of Zhang *et al*., (2022) who reported reduced turbidity and growth of *Scenedesmus obliquis* at higher hydrogen peroxide concentrations due to oxidative damage to photosynthetic pigments.

The turbidity pattern of *Chlamydomonas* sp showed fluctuations, there was an increase for some certain concentrations and days while on other days there was a decrease. This suggests that *Chlamydomonas* is more sensitive to hydrogen peroxide stress and could not maintain consistent growth across treatments. The fluctuating turbidity was due to temporary oxidative stress effects such as membrane damage or pigment degradation, which reduce cell density until the organism recovers. This is in line with the findings of Kim *et al*., (2018) who reported that *Chlamydomonas reinhardtii* had fluctuating growth under oxidative stress with initial suppression followed by partial recovery as antioxidant defenses were activated.

Conductivity is an important water quality parameter that measures the ability of a solution to conduct electric current. It reflects the total concentration of dissolved ions present in the culture medium. In Figure 6, the conductivity of *Scenedesmus* sp showed a consistent upward trend with increasing hydrogen peroxide concentrations. The steady rise in conductivity suggests continuous leakage of ions as the cells adjusted to hydrogen peroxide exposure. The ions accumulated in the medium leading to increased conductivity readings. The ability of *Scenedesmus* to maintain a stable increase implies that its cell membranes remained functionally intact, and the stress was within a tolerable limit. Similarly, Zhang *et al*., (2022) made the same observations. For *Chlamydomonas* sp (figure 7), the conductivity for *Chlamydomonas* fluctuated throughout the exposure period. This trend indicates that *Chlamydomonas* was more sensitive to hydrogen peroxide stress and underwent alternating phases of membrane damage (rise in conductivity) and recovery (decrease in conductivity). This result aligns with Kim *et al.,* (2018) who reported that *Chlamydomonas reinhardtii* exhibited irregular ionic and metabolic responses under oxidative stress conditions, attributed to transient changes in membrane stability. This difference highlights the stronger stress tolerance of *Scenedesmus,* likely due to its colonial structure and thicker cell wall, which reduce oxidative damage and allow controlled ion exchange.

Total Dissolved solids (TDS) is an important indicator of the ionic and organic content of the culture medium. Figure 8 shows the effect of Hydrogen peroxide on the total dissolved solids of the *Scenedesmus* sp growth medium. It observed a gradual and steady increase in TDS values. This observation is linked to mild oxidative stress, induced by hydrogen peroxide, causing slight alterations in membrane permeability, allowing limited leakage of soluble metabolites, hence a gradual increase. Higher concentrations induced higher oxidative stress hence more alteration in membrane permeability. The gradual release of solutes combined with continued metabolic activity, enriched the culture with dissolved substances, elevating TDS values. This aligns with Zhang *et al.,* (2022) study reporting increased dissolved solids and conductivity in *Scenedesmus obliquis* under oxidative stress. Figure 9 showed a non-linear response in its TDS values. It rises from the control concentration to peak at 10% concentration of Hydrogen peroxide (optimal metabolic response) and lower values at higher concentrations with lowest being 25%. That is, it showed increased TDS values at mild oxidative stress and lower values at severe oxidative stress.

This finding agrees with Murata *et al.,* (2007) who also observed that environmental stress induces an initial stimulatory response followed by some level of inhibition at higher intensities.

pH is the level of acidity or alkalinity of the growth medium. pH greater than 7 indicate alkalinity and lesser than 7 implies acidity. Both species (Figure 10 and 11) showed a steady increase in pH from day 0 to day 8 and a decline toward day 14. Figure 10 had higher pH at higher hydrogen peroxide concentrations at day 0 to day 8, suggesting that oxidative stress initially enhanced photosynthetic CO_2 uptake, reducing carbonic acid levels. The drop after day 8 reflects a decline in photosynthesis and accumulation of metabolic acids from stress respiration as oxidative damage intensified. *Chlamydomonas,* on the other hand had higher pH at low concentrations and higher concentrations (15-25%) had reduced pH. After day 10, all treatments showed a downward trend. This is as a result of the low sensitivity of *Chlamydomonas* to oxidative stress, hence early inhibition. Murata *et al.,* (2007) also described that moderate stress can activate photoprotective mechanisms that temporarily raise pH before photoinhibiton occurs.

Dissolved oxygen (DO) is an important indicator of photosynthetic and respiratory activities in algal cultures. It represents the balance between oxygen generation and oxygen consumption. *Scenedesmus* (Figure 12) had a fluctuating pattern in dissolved oxygen levels. There was a sharp increase on Day 2 then a decline by Day 4, then a gradual increase up until Day 14. Higher concentrations (15-25%) recorded the highest dissolved oxygen levels. From this, we can infer that oxidative stress simulated photosynthetic activity and oxygen activation which is linked to its antioxidant defense mechanisms. There were temporary dips at Day 4 and 6 due to cumulative oxidative stress but *Scenedesmus* adapted quickly. The observed pattern corresponded with Barone *et al*., (2020) who reported that certain microalgae can sustain dissolved oxygen production under oxidative stress due to their physiological characteristics. *Chlamydomonas* experienced a gradual increase in dissolved oxygen levels signifying photosynthetic activity. However, as exposure continued, the inhibitory effect of hydrogen peroxide became pronounced especially at higher concentrations (15-25%) from day 8 to 10. There was a recovery trend in lower concentrations (1-10%) however higher concentration remain significantly reduced. This implies a concentration dependent inhibitory effect on *Chlamydomonas* sp.

The percentage yield of *Scenedesmus* sp. and *Chlamydomonas* sp. under different hydrogen peroxide concentration (Figure 3) revealed no statistically significant differences between the

two species. The graph reveals that both algal species recorded the highest yield under the control concentrations with *Chlamydomonas* sp being slightly higher. However as hydrogen peroxide concentrations increased, the percentage yield of both species decreased progressively indicating oxidative stress. However, *Scenedesmus* sp. maintained a higher yield than *Chlamydomonas* sp. across most concentrations , showing that *Scenedesmus* sp had greater tolerance to hydrogen peroxide and greater antioxidant defense mechanisms Although, both microalgae exhibited variation in yield across concentrations, their overall productivity remained comparable. Overall, the result demonstrates that hydrogen peroxide negatively impacts algal growth in a concentration dependent manner with *Scenedesmus* sp showing better resilience.

CONCLUSION

This study evaluated the growth responses of *Scenedesmus* and *Chlamydomonas* to concentrations of Hydrogen peroxide. The study showed that both *Scenedesmus* and *Chlamydomonas* responded variably to different concentrations of Hydrogen peroxide. *Scenedesmus* sp recorded growth at all concentrations (Control, 1%, 5%, 10%, 15%, 20%, 25%) of hydrogen peroxide with increased growth at higher concentrations. *Chlamydomonas,* on the other hand also recorded growth at various concentrations of Hydrogen peroxide but had lesser tolerance to higher concentrations of Hydrogen peroxide and exhibited mild inhibitions. The results indicate that Hydrogen peroxide affects algal growth in a concentration dependent manner rather than being strictly toxic. Both microalgae have limited bioremediation potential and can only be used in environments containing low levels of Hydrogen peroxide.

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APPENDIX

ANOVA: Growth Response of *Scenedesmus* sp to Hydrogen Peroxide

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.00212	6	0.000353	0.003178	1	2.171309
Columns	71.7395	8	8.967437	80.67167	7.21E-46	2.012654
Interaction	0.008254	48	0.000172	0.001547	1	1.45825
Within	14.00612	126	0.11116			
Total	85.75599	188				

ANOVA: Growth Response of *Chlamydomonas* sp to Hydrogen Peroxide

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.011016	6	0.001836	125.288	2.62E-47	2.180564
Columns	0.069782	7	0.009969	680.2439	1.24E-88	2.092381
Interaction	0.010615	42	0.000253	17.24543	8.25E-33	1.493427
Within	0.001641	112	1.47E-05			
Total	0.093054	167				

ANOVA: Turbidity for *Scenedesmus* sp

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	473.1015	6	78.85025	3.444739	0.003686	2.180564
Columns	43120.72	7	6160.103	269.1178	5.89E-67	2.092381
Interaction	1244.555	42	29.63226	1.294548	0.143902	1.493427
Within	2563.686	112	22.89005			
Total	47402.06	167				

ANOVA: Turbidity for *Chlamydomonas* sp

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	886.8692	6	147.8115	4.671025	0.000282	2.180564
Columns	57717.73	7	8245.39	260.5644	3.23E-66	2.092381
Interaction	2977.353	42	70.88935	2.24019	0.000416	1.493427
Within	3544.167	112	31.64435			
Total	65126.12	167				

ANOVA: pH for *Scenedesmus* sp

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	1.676173	6	0.279362	25.3133	8.08E-19	2.180564
Columns	32.76821	7	4.681173	424.1659	1.7E-77	2.092381
Interaction	3.134714	42	0.074636	6.762849	3.14E-16	1.493427
Within	1.236053	112	0.011036			
Total	38.81515	167				

ANOVA: pH for *Chlamydomonas* sp

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	1.696133	6	0.282689	14.51991	3.35E-12	2.180564
Columns	23.08077	7	3.297253	169.3587	1.61E-56	2.092381
Interaction	8.087533	42	0.19256	9.890587	2.1E-22	1.493427
Within	2.180533	112	0.019469			
Total	35.04497	167				

ANOVA: Conductivity for *Scenedesmus* sp

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	568332.5	6	94722.08	100.6197	9.2E-43	2.180564
Columns	1622915	7	231845	246.2802	6.24E-65	2.092381
Interaction	831912.7	42	19807.45	21.04071	7.61E-37	1.493427
Within	105435.3	112	941.3869			
Total	3128595	167				

ANOVA: Conductivity for *Chlamydomonas* sp

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	22534.56	6	3755.76	252.1853	1.27E-62	2.180564
Columns	22008.99	7	3144.142	211.1174	1.93E-61	2.092381
Interaction	6350.298	42	151.1976	10.15235	7.37E-23	1.493427
Within	1668	112	14.89286			
Total	52561.85	167				

ANOVA: Total Dissolved Solid for *Scenedesmus* sp

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	223298.7	6	37216.45	336.3114	3.47E-69	2.180564
Columns	68489.6	7	9784.228	88.41645	1.22E-42	2.092381
Interaction	54862.32	42	1306.246	11.80406	1.48E-25	1.493427
Within	12394	112	110.6607			
Total	359044.6	167				

ANOVA: Total Dissolved Solid for *Chlamydomonas* sp

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	1421.071	6	236.8452	92.10648	5.66E-41	2.180564
Columns	4724.661	7	674.9515	262.4812	2.19E-66	2.092381
Interaction	1312.548	42	31.25113	12.15322	4.32E-26	1.493427
Within	288	112	2.571429			
Total	7746.28	167				

ANOVA: Dissolved Oxygen for *Scenedesmus* sp

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	1235.057	6	205.8428	31.00599	1.04E-21	2.180564
Columns	1705.427	7	243.6324	36.69821	3.22E-26	2.092381
Interaction	895.0392	42	21.31046	3.209982	5.16E-07	1.493427
Within	743.5467	112	6.63881			
Total	4579.07	167				

ANOVA: Dissolved Oxygen for *Chlamydomonas* sp

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	1423.413	6	237.2355	52.26853	3.09E-30	2.180564
Columns	1277.555	7	182.5079	40.21074	9.27E-28	2.092381
Interaction	1336.297	42	31.8166	7.009939	8.97E-17	1.493427
Within	508.3438	112	4.538784			
Total	4545.609	167				