EFFECTS OF WATER-SOLUBLE FRACTION OF SPENT AND UNSPENT ENGINE OIL ON FRESHWATER MICROALGAE (*Scenedesmus ecornis* and *Chlorella vulgaris*).

BY:

BENITA EFE AMIATOR (MISS)

LSC2103839

AN UNDERGRADUATE PROJECT SUBMITTED TO THE DEPARTMENT OF ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA; IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR AWARD OF BACHELOR OF SCIENCE (B.Sc) DEGREE IN ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY

OCTOBER, 2025

CERTIFICATION

This is to certify that this research titled **“EFFECTS OF WATER-SOLUBLE FRACTION OF SPENT AND UNSPENT ENGINE OIL ON SCENEDESMUS ECORNIS AND CHLORELLA VULGARIS.”** was carried out by **“BENITA EFE AMIATOR (MISS)”** and presented to the Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin, Benin City; in partial fulfilment of the requirements for the award of Bachelor of Science (B.Sc) in Environmental Management and Toxicology. It was conducted under suitable conditions, was carefully supervised and subsequently approved as having met the requirements for the award of a Bachelor of Science degree in Environmental Management and Toxicology.

DR. J. U. OGBEBOR	**DATE**
(PROJECT SUPERVISOR)	

PROF. (MRS.) E. T AISEN	**DATE**
(HEAD OF DEPARTMENT)	

DECLARATION

I **“BENITA EFE AMIATOR (MISS)”** declare that **“EFFECTS OF WATER-SOLUBLE FRACTION OF SPENT AND UNSPENT ENGINE OIL ON SCENEDESMUS ECORNIS AND CHLORELLA VULGARIS”** is my work and that all sources that I have used or quoted have been acknowledged using complete references and that this work has not been submitted before for any other degree at any other University.

BENITA EFE AMIATOR	**DATE**

DEDICATION

This project report is dedicated to God Almighty for the strength, wisdom, grace and preservation throughout this journey. To my lovely parents Mr. and Mrs. Amiator for their endless support, prayers, sacrifices, and words of encouragement which has been a driving force for me, and to my wonderful siblings, your unwavering belief have been my greatest strength, even during hard days.

ACKNOWLEDGEMENTS

First and foremost, I am deeply grateful to God Almighty for the gift of life, strength, wisdom and guidance that made the successful completion of this project work.

I extend my sincere appreciation to my project supervisor, Dr. J. U. Ogbebor whose guidance, constructive criticism, selfless support and encouragement were instrumental throughout the course of the research. His commitment and patience inspired me greatly.

I also wish to acknowledge the efforts of some of my lecturers for their help and support to me. I am thankful the Head of Department, Professor (Mrs.) E.T Aisen, for her continuous leadership and support throughout the course of the project, Dr. A. F. Eghomwanre (my course adviser), Dr. (Mrs) O. A. Edene, Dr. N. Obayagbona and other lecturers in the Department of Environmental Management and Toxicology for their invaluable contributions to my academic development.

My heartfelt gratitude goes to my parent, Mr. and Mrs. Samuel Amiator for their unwavering support, financial assistance, prayers and motivation throughout my journey in school. To my wonderful siblings, Faithlyn Amiator, King Amiator and Godswill Amiator, I am grateful for your endless support towards my academic journey.

Lastly, to my biggest supporters, Miss Judith, Mr. Sam, Femi, Emmanuella, Nora, Godsown, Edosa and Abimbola, I want to thank you for being the best and for your support in the successful completion of this project.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ABSTRACT

This study was carried out to investigate the effects of the water-soluble fractions of spent and unspent engine oil on two microalgae, *Chlorella vulgaris* and *Scenedesmus ecornis*. The test algae were exposed to varying concentrations (0%, 5%, 10%, 25%, 50%, 75% and 100%) which were set up in triplicates. The growth response was monitored every two days for 14 days using a visible spectrophotometer (Model No. HV-721). The percentage inhibition, dry weight and analysis of variance (ANOVA) were calculated using Microsoft Excel software. Physicochemical parameters such as pH, electrical conductivity and total dissolved solids were measured using the appropriate method. The results revealed that growth of both microalgae decreased progressively with increasing concentration, with spent engine oil exhibiting greater toxicity than unspent engine oil. Growth rate analysis consistently favored *Chlorella vulgaris* in both engine oil. Statistical analysis using ANOVA indicated that it was significant ($p < 0.0001$) in growth response among the various concentrations for both species. The dry weight analysis revealed that in spent engine oil, the highest cumulative dry weights occurred in the control (391.23 mg/L for *Scenedesmus ecornis* and 394.24mg/L for *Chlorella vulgaris*), with marked reduction at 75-100%, with both species recording their lowest biomass at 100% (104.13 mg/L for *Scenedesmus ecornis* and 117.97 mg/L for *Chlorella vulgaris*) while in unspent engine oil, dry weight remained relatively high at 5-10% Percentage inhibition analysis revealed growth stimulation at low concentrations (5-10%), particularly in *Scenedesmus ecornis* exposed to unspent engine oil. In spent engine oil, *Scenedesmus ecornis* exhibited higher sensitivity than *Chlorella vulgaris*, while in unspent engine oil, *Chlorella vulgaris* exhibited greater sensitivity. The total dissolved solids, electrical conductivity, and pH decreased significantly across all concentrations. Spent engine oil is more toxic to microalgae than unspent oil, and the result indicates that *Chlorella vulgaris* is more suited for bioremediation of aquatic environments contaminated with unspent engine oil, while *Scenedesmus ecornis* show stronger potential for the bioremediation of water bodies polluted with spent engine oil.

CHAPTER ONE

INTRODUCTION

All of Earth´s natural systems depend on water to function and remain sustainable. It drives biological and climatic processes, sustains life, forms landscapes, and serves as a vehicle for energy transfer (Xia *et al*., 2021). All living things require water for physiological and metabolic functions. In microbes, plants, and mammals, it facilitates cellular processes, waste elimination, and nutrient delivery. On Earth, water comes in a variety of forms, each having special physical characteristics and functions in the natural world. These include solid, liquid, and gas. The continuous movement and unique properties are essential for the stability, sustainability, and resilience of ecosystems and human societies. Important functions that water bodies offer include habitat formation, nutrient cycling, flood and erosion management, water provisioning, purification, and recreation. When ecosystems are in good ecological condition, these services are maximized, and their loss or degradation has a direct impact on human health, food security, and economies (Zulian *et al*., 2019).

Despite Earth's surface being covered by about 71% water, the vast majority roughly 97.5% is saltwater found in the oceans and is not directly suitable for drinking or irrigation without costly desalination. Less than 1% of Earth's water is easily accessible for human use in rivers, lakes, and shallow aquifers, with the rest being saltwater or locked in ice and deep underground (Musie and Gonfa, 2023). Over half of accessible freshwater runoff is already appropriated for human use, and more than a billion people lack access to clean drinking water (Baggio *et al*., 2021). This limited availability creates significant disparities in access, especially as global population increases and climate change alters precipitation patterns. Water is essential across agriculture, industry, and energy sectors, with irrigation and energy generation being the largest users. It is vital in industrial processes, from manufacturing to cooling systems, and in energy generation, particularly hydropower and cooling for thermal plants (Davies *et al*., 2016). However, water resources worldwide are under growing pressure from overuse, pollution, and the impacts of climate change. These factors are leading to reduced water availability, declining water quality, and increased risks to both human and ecosystem health (Menció and Mas-Pla, 2018). Rapid urbanization, industrialization, and agricultural expansion are causing excessive groundwater withdrawal and surface water use, leading to resource depletion and declining water tables. Rivers, lakes, and groundwater are increasingly contaminated by chemicals, fertilizers, heavy metals, plastics, and pathogens (Li and Wu, 2023).

Pollution introduces harmful substances into the environment, causing significant negative effects on living organisms and ecosystems. It disrupts biodiversity, alters ecosystem functions, and poses direct health risks to both wildlife and humans. Pollution also leads to the loss of ecosystem services such as clean water, fisheries, and recreation, with negative socio-economic consequences for human communities (González *et al*., 2020). Water pollution is a critical global issue, impacting both environmental and human health. It results from the introduction of harmful substances into water bodies, making water unsafe for consumption and damaging ecosystems. Water pollution occurs when contaminants are introduced into water bodies such as rivers, lakes, oceans, aquifers, and groundwater, making the water unsafe for human use and harmful to aquatic life (Thirumalaisamy *et al*., 2023).

Water pollution can originate from various sources including industrial discharge, agricultural runoff, domestic sewage, plastic waste, heavy metals, and oil-based pollutants like petroleum hydrocarbons. One particularly concerning group of water pollutants is petroleum hydrocarbons, originating from sources such as oil spills, industrial discharges, and improper waste disposal. These substances including crude oil, gasoline, diesel, and lubricants pose risks to ecosystems and human health due to their toxicity, and their potential to bioaccumulate (Mishra and Kundu, 2018).

Petroleum hydrocarbons are toxic, mutagenic, and carcinogenic, threatening aquatic life, plants, animals, and humans. These pollutants can disturb aquatic ecosystems, reduce water quality, and impact the safety of seafood and drinking water (Hazaimeh and Ahmed, 2021). When petroleum hydrocarbons enter aquatic environments through oil spills, runoff, leaks, or improper disposal, they become major pollutants that significantly disrupt ecological balance. Algae, especially microalgae and phytoplankton, are among the organisms affected by petroleum hydrocarbon contamination in water, which disrupts algal growth, alters species composition, and impairs essential ecological functions such as primary production and nutrient cycling (Cui *et al*., 2024).

Algae are essential primary producers of biomass across diverse ecosystems including oceans, freshwater bodies, wet surfaces, and soils. They carry out photosynthesis using sunlight, carbon dioxide, and water, producing oxygen and forming the foundation of food chains in water bodies. Algae are a highly diverse group of photosynthetic organisms found in a wide range of environments (Ahmad *et al*., 2024). They include green, red, and brown algae, as well as blue-green algae (cyanobacteria), diatoms, and dinoflagellates. Algae have a wide range of applications across different sectors. They are used in various industries, including food, fuel, cosmetic products, and biofertilizer (Subedi, 2024). They provide food for a variety of aquatic organisms, from microscopic zooplankton to larger fish and shellfish, and help regulate nutrient cycles in aquatic systems, particularly nitrogen and phosphorus. Algae are also consumed directly as food and food supplements, providing proteins, minerals, fiber, and essential fatty acids, and are increasingly recognized for their nutritional and health benefits (Mendes *et al*., 2022).

Algae produce valuable compounds such as polysaccharides, lipids, pigments, antioxidants, and pharmaceuticals. Algal biorefineries enable the extraction of multiple high-value products, supporting a circular economy and reducing waste (Yasmin *et al*., 2022). Despite their benefits, algae can become problematic in polluted environments. When water bodies receive excess nutrients from fertilizers, sewage, or industrial discharge, algae may grow excessively in what is known as an algal bloom.

Microalgae are increasingly recognized for their dual role in sustainable biofuel production and efficient wastewater treatment. Their rapid growth and high lipid content make them promising candidates for generating renewable biofuels, while their ability to absorb nutrients and pollutants enables effective removal of nitrogen, phosphorus, and even heavy metals from various wastewater streams, including municipal and industrial sources (Khoo *et al*., 2021). Microalgae are widely recognized as sensitive and effective bioindicators in ecotoxicological studies due to their rapid physiological and biochemical responses to pollutants such as heavy metals, antibiotics, microplastics, and emerging contaminants (Cavalletti *et al*., 2022). Their responses include changes in growth rate, photosynthetic efficiency, reactive oxygen species (ROS) production, and alterations in biochemical composition, which can be measured through multiple endpoints to assess toxicity mechanisms and ecological risks in aquatic environments (Sarmento *et al*., 2019; Chen *et al*., 2021).

Algae are highly sensitive to environmental changes, and pollution can have significant effects on their growth, reproduction, and ecological roles. One of the most harmful types of pollution affecting algae is contamination by petroleum hydrocarbons, which are compounds found in crude oil, gasoline, diesel, and lubricants such as engine oil. Petroleum hydrocarbons interfere with these functions by disrupting trace element uptake and altering physiological markers, which can affect nutrient cycling and the overall health of aquatic ecosystems (Cui *et al*., 2024). They can inhibit the growth of algae, leading to a decline in primary biomass, especially at medium to high contamination levels. This reduction is linked to increased production of reactive oxygen species, which stress algal cells and trigger antioxidant responses, ultimately impairing growth and physiological functions (Lin *et al*., 2023). These hydrocarbons can enter water bodies through oil spills, industrial discharges, urban runoff, or improper disposal of used oil.

Oil spills are the accidental or intentional release of petroleum-based substances such as crude oil, gasoline, diesel, or used engine oil into the environment. These events can occur during oil extraction, transportation, storage, or use, and their impacts are both immediate and long-lasting. Oil spills contaminate sediments, reduce the abundance and diversity of marine life, and disrupt entire ecosystems, with effects that are often unpredictable and persistent, especially in anoxic environments where oil degrades slowly (Yim *et al*., 2020). While oil spills are mostly associated with massive tanker accidents or offshore drilling disasters, smaller but more frequent spills involving engine oil are a major and often overlooked source of pollution, especially in urban and industrial areas. Engine oil is essential for reducing friction, wear, and heat in internal combustion engines, directly impacting engine performance, efficiency, and longevity. Its key properties such as viscosity, thermal stability, and resistance to oxidation change over time due to engine operation, load, and environmental conditions. This can lead to decreased lubrication effectiveness and increased engine wear if not monitored or replaced appropriately (Ďurišová *et al*., 2020).

Engine oil, whether fresh or used, is a petroleum-derived lubricant essential for reducing friction and wear in engine components, thereby enhancing engine reliability and fuel efficiency. Engine oil spills from vehicle leaks, improper disposal, or runoff, introduce toxic substances such as heavy metals and polycyclic aromatic hydrocarbons (PAHs) into aquatic ecosystems, causing both immediate and long-term harm. Even small, frequent spills can rapidly increase the bioavailability and toxicity of oil in water, leading to the decline of sensitive organisms like plankton, which are foundational to aquatic food webs (Shah *et al*., 2024).

Unspent engine oil, also called fresh engine oil, is a highly refined lubricant made from base oils blended with chemical additives to optimize engine performance. Its key properties include high viscosity, a clean amber or golden color, and a formulation that resists oxidation, foaming, and corrosion (Nour *et al*., 2021). The oil's primary use is to lubricate moving engine components, thereby reducing friction and heat, while also helping to remove contaminants and maintain engine cleanliness. Its chemical stability and protective qualities make it essential for the longevity and efficiency of internal combustion engines. Unspent engine oil can also serve as a base for producing other lubricants, such as greases, and is sometimes used in industrial applications requiring high-performance lubrication. When compared to used oil, unspent oil retains its full additive package and optimal physical properties, ensuring maximum protection and performance for engines (Isa *et al*., 2023).

Spent engine oil is also known as used or waste lubricant is a complex mixture that contains degraded base oil, depleted additives, and a range of contaminants such as heavy metals (e.g., lead, cadmium, zinc), polycyclic aromatic hydrocarbons (PAHs), and oxidation products. During engine operation, engine oil is exposed to intense heat, friction, pressure, and contamination from fuel combustion by-products, metal particles, dust, and water. As a result, the oil undergoes chemical and physical degradation, losing its effectiveness as a lubricant. Spent engine oil also thickens, darkens, and accumulates soot, metal particles, and fuel residues, reflecting its loss of lubricating (Santana *et al*., 2022). Once engine oil is spent, it is no longer capable of effectively lubricating engine components or protecting them from corrosion and wear. Instead, it becomes a harmful waste product that poses serious environmental and health risks if not handled and disposed of properly. Discarding spent oil into soil and water disrupts microbial communities, impairs plant growth, and contaminates water bodies, leading to bioaccumulation in aquatic organisms and potential harm to human health through the food chain and water supply (Hassan *et al*., 2024).

Spent engine oil is highly toxic to aquatic environments, causing significant harm to water quality, aquatic organisms, and ecosystem health. When released into water bodies, it forms a surface film that blocks sunlight and reduces oxygen exchange, disrupting photosynthesis and threatening the survival of microalgae, plankton, and other foundational species in aquatic food webs (Hassan *et al*., 2024). Spent engine oil is particularly harmful to microalgae and plankton, which are the base of aquatic food chains and organisms may die or suffer growth inhibition when exposed to the toxic components of the oil.

In aquatic environments, oil pollution does not remain confined to the water surface. Some of the components of engine oil dissolve in water, forming what is known as the water-soluble fraction (WSF). This fraction typically contains toxic compounds such as polycyclic aromatic hydrocarbons (PAHs), phenols, and heavy metals. Water soluble fractions are rapidly formed after oil spills and despite their relatively short persistence can cause acute and chronic toxicity in a wide range of organisms (Moreira *et al*., 2021). While several studies have examined the toxic effects of petroleum-based pollutants on aquatic life, there is limited research that directly compares the impact of water-soluble fractions of spent and unspent engine oils on microalgae. This gap is important because spent engine oil contains additional hazardous components that result from its use and degradation, which may make it more toxic than unspent oil. Examining the differences in toxicity between these two forms of engine oil is important for environmental monitoring, pollution management, and developing bioremediation strategies.

AIM AND OBJECTIVE

The study aimed at determining the effect of water-soluble fraction of spent and unspent engine oil on the growth of the microalgae.

The objectives were to:

1. evaluate the growth response of *Chlorella vulgaris* and *Scenedesmus ecornis*.
2. determine the dry weight of the test microalgae in various concentrations of spent and unspent engine oil WSF.
3. examine growth stimulation or inhibition of the microalgae on exposure to WSF of spent and unspent engine oil.
4. assess the bioremediation potential, if any, of the algae to the WSF of spent and unspent engine oil.

CHAPTER TWO

LITERATURE REVIEW

This literature reviews examines research investigating the effects of the water-soluble fraction of petroleum hydrocarbons on microalgae, with a focus on study objectives, methodologies and key findings regarding growth inhibition, photosynthetic activity and cellular response.

Kadiri and Eboigbodin (2012) investigated the effect of water-soluble fractions (WSF) of petroleum products diesel, kerosene and petrol on two microalgae, *Desmodesmus quadricauda* and *Eudorina elegans* under laboratory conditions for 14 days. The algae were exposed to different WSF concentrations (0%, 10%, 25%, 50%, 75%, and 100%), and growth responses were monitored spectrophotometrically using optical density measurements at 680 nm. In general, high WSF concentrations suppressed the growth of both species, while lower concentrations stimulated growth. *Eudorina elegans* showed maximum growth at 10% for all fuel oils except kerosene with peak growth at 25%. Maximum growth of *Desmodesmus quadricauda* was achieved at 10% of diesel, 25% of kerosene, and 50% of petrol. The maximum inhibition of growth in both species was observed at 100% WSF level. *E. elegans* was in general more sensitive to WSF than *D. quadricauda* at all tested concentrations, except 100% WSF where inhibition was greater for *D. quadricauda*. Statistical analysis revealed significant differences among most WSF concentrations tested, except between the control and 10% petrol WSF for *E. elegans*, and between 10% and 25% diesel WSF for both algal species.

The acute microtox toxicity of the water accommodated fraction (WAF) of six commercial soybean biodiesel/petrodiesel blends was investigated by Yasmin *et al.* (2012) at different oil loads. The study quantified five fatty acid methyl esters (FAMEs), C10–C24 *n*-alkanes, four aromatic compounds, methanol, and total organic carbon (TOC). At higher oil loadings, WAF toxicity was significantly greater in blends containing higher proportions of biodiesel. In contrast, at the lowest loading, toxicity declined almost linearly as the biodiesel content of the blend decreased. At intermediate loadings, the WAFs of all blends exhibited comparable toxicity levels. Chemical analyses revealed the presence of FAME autoxidation byproducts in WAFs at high oil loadings. Pure unsaturated FAMEs and *n*-alkanes were nontoxic at their reported aqueous solubility limits; however, 24-h equilibrated WAFs of pure FAMEs were highly toxic for C18:1 and C18:3, but not for C18:2. The authors concluded that acute toxicity at high oil loadings was primarily attributable to autoxidation byproducts of FAMEs, whereas at low oil loadings, toxicity was mainly driven by aromatic constituents of petrodiesel. Although the addition of a synthetic antioxidant to biodiesel did not alter the concentration of autoxidation byproducts in the WAF, it led to a slight reduction in toxicity. Notably, the major autoxidation byproducts detected in WAFs of commercial biodiesel were absent from WAFs of pure unsaturated FAMEs and from a laboratory-transesterified soybean biodiesel, which was nontoxic. These findings suggest that the transesterification process itself may play a more decisive role in determining aquatic toxicity than the original feedstock source.

Obayori *et al*. (2014) evaluated the biodegradation of two grades of fresh and used engine oils (SAE 40W and SAE 20W-50) in liquid culture using *Pseudomonas aeruginosa* strain LP5, a hydrocarbon-degrading bacterium isolated from petroleum-contaminated soil based on its ability to utilize pyrene. The strain degraded over 90% of all oil types within 21 days, achieving degradation efficiencies of 95% and 93% for fresh and used SAE 40W, and 96% and 92% for fresh and used SAE 20W-50, respectively. Growth rates were marginally higher in fresh oils, with values of 0.17, 0.13, 0.14, and 0.13 day^{-1} recorded for fresh SAE 40W, used SAE 40W, fresh SAE 20W-50, and used SAE 20W-50, respectively. Initial degradation rates were significantly higher for fresh oils, reaching 177.42 mg/L/day for SAE 40W and 207.14 mg/L/day for SAE 20W-50 during the first 21 days, compared with 73.23 mg/L/day and 74.37 mg/L/day for their used oil counterparts. In used oils, smaller peaks that were absent at day 0 reappeared by day 21, while all hydrocarbon peaks above C20 disappeared. Across all treatments, only medium-chain hydrocarbons (C14, C15, and C17) showed detectable peaks at day 21, though at very low intensities (<10% of day-0 values). The authors concluded that although degradation rates varied with oil type and fresh oils were more readily degraded during the early stages, *P. aeruginosa* LP5 demonstrated strong potential for the biodegradation of both fresh and used engine oils.

The relative acute toxicity of refined petroleum products (diesel, kerosene, and petrol), as well as unused and spent engine oils, and their effects on superoxide dismutase (SOD) activity and lipid peroxidation in tadpoles of the common African toad (*Amietophrynus regularis*) was assessed by Amaeze *et al*. (2014). After 48 hours of exposure, kerosene was the most toxic substance (LC_{50} = 4,930 mg/L), whereas unused engine oil was the least toxic (LC_{50} = 7,777 mg/L). At 96 hours, spent engine oil exhibited the highest toxicity (LC_{50} = 2,915 mg/L), while unused engine oil remained the least toxic (LC_{50} = 7,353 mg/L). Oxidative stress responses were further evaluated using sublethal concentrations equivalent to 1/100 of the 96-hour LC_{50} values. Tadpoles exposed to the test substances showed a significant reduction in SOD activity compared with the control ($P < 0.05$), with the lowest SOD activity observed in petrol-exposed individuals and the highest in those exposed to unused engine oil. Lipid peroxidation, assessed by malondialdehyde (MDA) levels, was significantly elevated in all exposed tadpoles relative to the control. The highest MDA production occurred in tadpoles exposed to unused engine oil, while diesel exposure resulted in the lowest MDA levels. Consistent patterns across toxicity, SOD inhibition, and lipid peroxidation were observed in tadpoles exposed to diesel, kerosene, petrol, and spent engine oil; however, responses to unused engine oil were inconsistent across these parameters.

Ramadass *et al*. (2015) investigated the toxicity and oxidative stress effects of used and unused motor oil on the freshwater microalga *Pseudokirchneriella subcapitata*. The study focused on two key endpoints: algal growth inhibition and oxidative stress responses, assessed through changes in antioxidant enzyme activity. Microalgae were exposed to varying concentrations of whole oil and water-accommodated fractions (WAFs) over a two-week period. The results demonstrated that used motor oil was considerably more toxic than fresh oil. Exposure to 0.20% used oil reduced algal growth, measured as chlorophyll *a* content, by 44%, whereas fresh oil showed no toxic effects even at concentrations as high as 2.8%. Similarly, WAFs derived from used oil caused significant toxicity at concentrations above 50%, while WAFs from fresh oil remained nontoxic even at 100%. Both used oil and its WAF induced elevated antioxidant enzyme activities, even at low concentrations, indicating a stress response by the algae. In contrast, exposure to WAFs of fresh motor oil did not result in any detectable changes in antioxidant enzyme levels. The authors concluded that the release of used motor oil into aquatic environments may compromise ecosystem health by impairing primary producers that form the foundation of aquatic food webs.

The effect of petrol and spent lubricating oil on the major growth traits (such as root length, stem length, leaf area, and biomass), and the changes in epidermal layer of leaf and its mitotic index in Guinea Corn (*Sorghum bicolor* L.) was examined by Komolafe (2015). The plants were treated with 0% (control), 5%, 10%, 15%, and 20% concentrations of petrol and spent oil, each mixed with 3 kg of soil in plastic pots and replicated three times. After 40 days of growth, leaf area declined from 95.83 cm^2 in the control to 89.67, 89.47, and 77.80 cm^2 at 5%, 10%, 15%, and 20% petrol concentrations, respectively. Mean stem lengths also decreased from 32.50 ± 0.5 cm in the control to 22.60 ± 0.65 cm, 21.27 ± 0.75 cm, and 20.83 ± 0.28 cm with increasing pollutant levels. Overall, both leaf area and stem length showed a progressive reduction as pollutant concentration increased.

Similarly, seedling dry weight decreased with rising concentrations of both petrol and spent lubricating oil. Microscopic examination of the leaf upper epidermis revealed disrupted and scattered epidermal cells, along with reduced stomatal size, with damage intensifying at higher treatment levels. Statistical analysis confirmed a significant reduction ($P < 0.05$) in stem length and leaf area across treatments. The study concluded that petroleum pollutants negatively affect the germination, growth, and development of guinea corn, although at low concentrations, petroleum products such as spent oil may supply nutrients that support plant growth and yield.

Wang and Zhang (2015) investigated the ecotoxicological impacts of a petroleum hydrocarbon mixture on the population densities of two algal species, *Platymonas helgolandica* var. *tsingtaoensis* and *Isochrysis galbana*, as well as a rotifer, *Brachionus plicatilis*, using both single-species assays and tailored community experiments. Organisms were exposed to concentrations ranging from 0 to 100 mg L^{-1}, with five to seven treatment levels tested in triplicate over a one-month period. The petroleum hydrocarbons used were derived from the water-accommodated fraction of crude oil collected from the Bohai offshore oil field in China. In single-species toxicity tests, organism densities declined significantly at concentrations exceeding 1.0 mg L^{-1}. In contrast, within the customized community, algal equilibrium densities followed a different trend, increasing with concentration and reaching a maximum at 20.0 mg L^{-1}. The no observed effect concentration (NOEC) determined from the community experiments was 1.0 mg L^{-1}, which differed from the lower NOEC of 0.25 mg L^{-1} obtained from single-species tests. These findings indicate that ecotoxicological responses of plankton within a community context can differ substantially from those observed in single-species assays due to ecological interactions.

The effect of water-soluble fractions (WSFs) of crude oil, diesel fuel and gasoline on *Salvinia nymphellula* (Desv) was investigated by Bamidele and Eshagberi (2015). The study measured several growth indicators, including leaf number, biomass accumulation, relative growth rate, and doubling time. Plants were exposed to WSF concentrations of 25%, 50%, and 100% for crude oil and diesel, and 5%, 10%, and 20% for gasoline. The results showed that exposure to all WSF concentrations for four weeks caused significant reductions ($P < 0.01$) in leaf production, biomass, and relative growth rate in *S. nymphellula*. Conversely, doubling time increased, reflecting inhibited growth. The observed effects were both concentration- and medium-dependent, with gasoline WSF being the most toxic. Leaves of the macrophyte became chlorotic, wilted, and disintegrated within four days at gasoline WSF concentrations above 25%. Although no significant difference ($P < 0.05$) was found between crude oil and diesel WSFs in terms of leaf production, diesel WSF exerted greater toxicity on biomass production and relative growth rate than crude oil WSF. The authors concluded that WSFs of petroleum hydrocarbons are harmful to aquatic macrophytes and emphasized the importance of prompt response to oil spill incidents.

The sensitivity and antioxidant response of *Chlorella* sp. MM3 to spent and unspent engine oil, and its water accommodated fraction was conducted by Ramadas *et al*. (2016). The microalgal strain was exposed to whole oils and WAFs to assess growth inhibition and the activities of key antioxidant enzymes, including peroxidase, superoxide dismutase, and catalase. Algal growth was strongly influenced by both oil type and concentration. Exposure to spent (used) engine oil at 0.04% caused a 50% reduction in growth, as indicated by chlorophyll-a content, whereas the same concentration of unspent oil produced no toxic effect. Likewise, the WAF of spent oil significantly inhibited algal growth at a 10% concentration, while WAF derived from unspent oil remained nontoxic even at 100%. Peroxidase activity increased significantly in response to spent oil at concentrations above 0.06 g L^{-1}, whereas noticeable induction of superoxide dismutase and catalase occurred only at 0.06 g L^{-1}. Antioxidant enzyme activities were also markedly elevated when the microalga was exposed to 75% and 100% WAFs of spent oil. The authors suggested that the toxicity of spent oil to the microalga is likely due to the accumulation of toxic soluble mono- and polyaromatic hydrocarbons, heavy metals, and other contaminants acquired during engine use.

Yakub and Ajijo (2016) evaluated the acute toxicity of the water-soluble fraction (WSF) of diesel fuel by examining its effects on the growth of two marine microalgae, *Isochrysis* and *Chaetoceros*. Pure cultures of both species were exposed, in triplicate, to diesel WSF concentrations of 0% (control), 5%, 10%, 15%, and 20% for a 96-hour period. Cell density was measured at 24-hour intervals, and standard methods were used to determine cell density, growth rate, percentage growth inhibition, and IC_{50} values. While control cultures exhibited exponential growth over the 96 hours, all diesel WSF treatments caused progressive inhibition of algal growth at each sampling interval. Growth inhibition increased significantly ($P < 0.05$) with rising WSF concentrations from 5% to 20% in both species, reaching 100% inhibition in *Isochrysis* and 98% in *Chaetoceros* at 20% WSF after 96 hours. The IC_{50} values of diesel WSF were 7.59% for *Isochrysis* and 8.08% for *Chaetoceros*. No significant difference ($P > 0.05$) was observed in the growth inhibition responses of the two microalgal species.

Soares *et al*. (2017) investigated the impact of the water-soluble fraction (WSF) of petroleum on photosynthesis and chemical defenses in two sympatric brown algae from the Dictyotaceae family, *Dictyota caribaea* and *Stypopodium zonale*. The algae were grown in laboratory incubator chambers for 8 days in flasks containing either WSF or seawater as a control. Chlorophyll fluorescence measurements revealed species-specific responses: *S. zonale* was less tolerant than *D. caribaea*, exhibiting a greater decline in the potential quantum yield of photosystem II (F_v/F_m) and reduced intrinsic photosynthetic capacity as indicated by rapid light curve parameters. Feeding assays with the crab *Pachygrapsus transversus* showed that lipophilic extracts from both algae became less palatable after WSF exposure. Chromatographic analysis of these extracts indicated that WSF-induced changes in secondary metabolite production occurred only in *D. caribaea*. Overall, the study demonstrated that *S. zonale* was more sensitive to WSF toxicity than *D. caribaea*, yet both species enhanced their anti-herbivory defenses, highlighting potential ecological consequences.

Onwusiri *et al*. (2017) studied the effects of spent engine oil on the germination and growth of fluted pumpkin (*Telfairia occidentalis*). The plants were grown in soils contaminated with 20 ml, 40 ml, 60 ml, 80 ml, and 100 ml of spent engine oil, corresponding to 1% to 5% contamination levels, and monitored for eight weeks post-planting. Leaf number, leaf area, and plant height were recorded weekly, while dry matter content was measured at the end of the eighth week. The results showed a decline in mean leaf number, leaf area, and plant height with increasing levels of contamination. The control group (0% contamination) had the highest average leaf number (15.41). Growth parameters plant height, leaf number, leaf area, and dry matter in control plots differed significantly ($P \leq 0.05$) from those in soils contaminated at 4% and 5% levels. These reductions were directly proportional to contamination levels. Additionally, plants grown in contaminated soils exhibited growth retardation and yellowing of leaves. The study concluded that spent engine oil substantially affected the germination and growth of fluted pumpkin.

Eshagberi (2017) examined the toxicity of water-soluble fractions (WSFs) of crude oil, diesel fuel, and gasoline on *Ceratophyllum demersum*. Parameters measured included fresh weight, biomass production, and relative growth rate. WSF concentrations tested were 25%, 50%, and 100% for crude oil and diesel, and 5%, 10%, and 20% for gasoline. The study found that low concentrations

25% WSF of crude oil and diesel and 5% WSF of gasoline significantly increased ($P < 0.05$) fresh weight, biomass production, and relative growth rate of *C. demersum*. Conversely, higher concentrations led to a decrease in growth parameters. Among the petroleum types, high concentrations of gasoline WSF were the most toxic, followed by diesel. The findings indicate that low concentrations of petroleum WSFs can stimulate growth, whereas high concentrations are toxic to this aquatic macrophyte.

Monteiro *et al*. (2019) examined the effects of crude oil water-soluble fractions (WSFs) on freshwater meiobenthos, focusing on nematode assemblages, using community microcosm experiments over a 15-week period. Treatments involved different WSF concentrations high (100%), medium (50%), and low (10%) and effects were assessed at 1, 3, 9, and 15weeks post-contamination to capture both immediate and long-term impacts. The study also compared a single contamination event with a “constant” contamination scenario, in which evaporated water was replenished with medium-concentration oil WSF. Alongside nematodes, the most abundant meiofaunal taxa were rotifers, gastrotrichs, oligochaetes, and tardigrades. Assessment of nematode assemblages included total abundance, diversity indices, feeding-type composition, and age structure. Immediate effects were limited, except for a significant reduction in the index of taxonomic distinctness observed within the first week. More pronounced impacts on total nematode abundance, diversity, and species composition emerged only after 9-15 weeks, suggesting that delayed effects of a single exposure are more substantial than immediate responses. Interestingly, the strongest effects were often observed not in the highest-concentration treatment but in the medium-concentration treatment with regular WSF replenishment, indicating that internal exposure may play a key role. The authors noted that predicting species-specific sensitivity was sometimes unreliable, likely due to both individual species’ responses and shifts in interspecific interactions within polluted communities. They recommended further toxicity testing to elucidate the mechanisms driving the observed long-term sublethal effects on nematode communities.

A comparative assessment of the acute and chronic ecotoxicity of water-soluble fractions (WSFs) of diesel and biodiesel on *Daphnia magna* and *Aliivibrio fischeri* was conducted by Muller *et al*. (2019). Acute toxicity tests using *D. magna* and *A. fischeri*, along with chronic toxicity tests on *D. magna*, were performed to evaluate differences in aquatic ecotoxicity between diesel and biodiesel WSFs. Results showed that diesel WSF was 2.5 to 4 times more toxic than biodiesel WSF in acute tests. Similarly, chronic toxicity tests demonstrated that diesel WSF had greater adverse effects than biodiesel WSF. Diesel WSF caused chronic impacts on the reproduction, longevity, and growth of *D. magna*, with No Observed Effect Concentrations (NOECs) of 12.5%, 12.5%, and 6.25%, respectively. In contrast, biodiesel WSF showed no significant difference from controls for any measured parameter at the tested dilutions (NOEC > 25%). This study was reported as the first to directly compare the chronic toxic effects of diesel and biodiesel WSFs on *D. magna*.

Lemuel *et al*. (2020) investigated the sublethal effects of water-soluble fractions (WSFs) of virgin diesel oil on selected physiological parameters of juvenile *Clarias gariepinus*. The test fish had an average weight of 1.61 ± 1.86 g, a total length of 9.5 ± 10.5 cm, and a standard length of 11.0 ± 12.5 cm. Fish were exposed to sublethal concentrations of 0.58, 0.29, 0.14, 0.07, 0.04 ml/L, and a control (0.00 ml/L). Significant differences ($P < 0.05$) were observed among the different concentration levels. Histopathological examinations focused on the gills, liver, and intestine. In the gills, alterations included inflammation, hyperplasia, lamellar fusion, lamellar aneurysms, epithelial lifting, abrasions, and necrosis. The liver exhibited lesions and inflammation, while the intestine showed sloughing of villi and mucosal damage. The study concluded that exposure to

WSFs of virgin diesel oil in aquatic environments can induce multiple histopathological changes in tissues of juvenile *C. gariepinus*.

Salinas-Whittaker *et al*. (2020) conducted a detailed study on the effects of WSFs from a fuel oil/diesel mixture on the growth and physiology of the microalga *Dunaliella tertiolecta*. To examine the temporal effects of WSF exposure, *D. tertiolecta* was cultivated in WSF for 15 days. The study evaluated three pigments (chlorophyll a, lutein, and β-carotene) and four metabolites (proteins, lipids, fatty acids, and phenols), and used Fourier-transform infrared (FTIR) spectroscopy to assess biomolecular changes in lipid composition and accumulation. Results demonstrated that WSF exposure induced physiological and biochemical responses in the microalga, including alterations in growth rate, pigments, proteins, phenols, and lipids, while fatty acid profiles remained largely unchanged. Significant differences were observed between exposed and control groups for all altered biochemical parameters. By the end of the experiment, most parameters in exposed *D. tertiolecta* returned to control levels, except for lipids. FTIR analysis indicated an increase in unsaturated acyl chains, suggesting hydrocarbon uptake from the WSF. Changes in pigments and phenol content were interpreted as part of an integrated antioxidant response to the stress imposed by the fuel oil/diesel WSF.

The toxicity of the water-soluble fraction (WSF) of diesel and bunker oils to neotropical marine invertebrates was investigated by de Santana (2021). Commercial fuels were obtained for water-soluble fraction (WSF) extraction, analyzed for total petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAHs), and assessed for acute and chronic toxicity. Analyses revealed that WSFs contained varying levels of TPH and PAHs, particularly low molecular weight PAHs, with bunker WSF showing the highest concentrations. Both WSFs caused significant mortality in the brine shrimp *Artemia salina*, reduced reproduction in the copepod *Nitokra* sp., and impaired embryo-larval development in the mussel *Perna perna* and sea urchin *Lytechinus variegatus*. Generally, diesel WSF was more toxic to most tested organisms, whereas bunker WSF was more harmful to *L. variegatus* embryos. Toxic effects were observed at WSF concentrations as low as 3%, a level that could be environmentally relevant following an oil spill, indicating potential short-term impacts on marine biota.

Olaleye and Kadiri (2021) studied the toxicity of water-accommodated fractions (WAFs) of waste engine oil on the growth of selected marine algae. The effects of WAF were assessed on three marine phytoplankton species *Isochrysis galbana*, *Thalassiosira pseudonana*, and *Skeletonema tropicum* over 14 days in laboratory conditions. Algae were exposed to WAF concentrations of 5%, 10%, 15%, 25%, 50%, 75%, and 100%, with growth measured spectrophotometrically at 750 nm. All species showed growth inhibition at concentrations from 10% to 100%, while 5% WAF stimulated algal growth. Similarly, dry weight decreased consistently with increasing WAF concentration, except at 5%. *Isochrysis galbana* was generally more sensitive than *T. pseudonana* and *S. tropicum*. Statistically significant differences ($P < 0.05$) were observed in algal growth across the WAF concentrations tested.

Stark (2022) examined the long-term effects of four hydrocarbon products diesel fuel and three lubricating oils (unused, used, and biodegradable) on sediment macrofaunal communities in a shallow Antarctic embayment over five years. Defaunated sediments were treated with hydrocarbons and deployed in seabed trays, including controls. Diesel initially had the strongest impact, particularly on annelids, but also on amphipods, ostracods, and cumaceans, with pronounced effects at 5 weeks and 1 year. By five years, diesel effects had largely recovered and were less severe than those from lubricating oils, with used oil causing the greatest long-term impact. Hydrocarbons affected diversity early on, especially diesel, but by 2-5 years, diversity in treated sediments was similar to or greater than controls. Total abundance remained lower in hydrocarbon treatments, notably for crustaceans, though annelid abundance was higher in oil treatments at 5 years. Some taxa, including molluscs, polychaete families (capitellids, cirratulids, dorvilleids), oligochaetes, ostracods, cumaceans, and isopods, were more abundant in hydrocarbon-treated sediments at 2-5 years. Amphipods and tanaids were particularly sensitive to hydrocarbons, while annelids were very sensitive to diesel. Biodegradable oil produced community effects similar to standard oil at 5 years, but annelids were more affected at 1-2 years, and unlike other oils, biodegradable oil did not enhance annelid or mollusc abundance at 5 years, except for some polychaetes. The study concluded that hydrocarbon impacts in Antarctica persist beyond five years, though diesel effects recover faster than those from oils.

The physiological, biochemical and morphological responses of *Nannochloropsis oculata* and *Porphyridium cruentum* to three different petroleum fuels, kerosene, diesel and gasoline were examined by Ezenweani and Kadiri (2023). The effects of water-soluble fractions (WSFs) of three petroleum fuels were evaluated at 0%, 25%, 50%, and 100% concentrations. Algal growth was monitored every two days for 14 days using a 721 visible spectrophotometer. Chlorophyll *a*, morphological changes, and antioxidant enzyme activities were assessed using established methods. Both algae exhibited minimum growth at 100% WSF. In *Nannochloropsis oculata*, growth stimulation occurred, with maximum growth at 25% or 50% WSF depending on the fuel type, while *Porphyridium cruentum* reached maximum growth at 10% WSF for all fuels. ANOVA ($P < 0.05$) indicated significant differences in growth across concentrations, and unpaired t-tests showed significant differences ($P < 0.05$) between the two species for all fuels, with *N. oculata* showing greater tolerance. Morphological observations revealed reduced size in *N. oculata* and severe cell clumping in *P. cruentum*. Antioxidant assays showed that *N. oculata* produced high levels of superoxide dismutase, catalase, and peroxidase, whereas *P. cruentum* generated high superoxide dismutase but lower catalase and peroxidase activities. The study concluded that petroleum fuel pollution affects algae physiologically, morphologically, and biochemically, with clumping and inefficient antioxidant responses impacting overall stress resilience.

Ogbebor and Ekemhankhomhen (2024) assessed the toxicity of petrol WSF on *Chlorella vulgaris* and *Scenedesmus obliquus*. WSFs were prepared at concentrations of 0%, 5%, 10%, 25%, 50%, 75%, and 100%, and 5 mL of test algae were inoculated into each treatment. Growth was monitored at 750 nm every two days over 14 days using a UV/VIS spectrophotometer. Both species showed growth stimulation at lower concentrations (5%-50%) and inhibition at higher concentrations (75%-100%). *Chlorella vulgaris* displayed superior growth compared to *S. obliquus*. The total petroleum hydrocarbon (TPH) concentration in 25% petrol WSF was 5,831.25 µg/L before inoculation, decreasing to 3,849.99 µg/L for *C. vulgaris* and 3,879.50 µg/L for *S. obliquus* by the end of the experiment, suggesting bioremediation potential. Oxidative biomarker activity was not dependent on WSF concentration, and the authors concluded that these microalgae could serve as potential bioremediation agents in hydrocarbon-contaminated waters at low contamination levels.

Bello *et al*. (2024) investigated the inhibitory effects of WSFs of crude oil, diesel, spent engine oil, and their composite mixture on the marine microalga *Skeletonema costatum*. Growth inhibition was assessed 72 hours post-exposure, and IC_{50} values were determined: diesel 1.08% (10.8 g/L) > spent engine oil 2.27% (22.7 g/L) > crude oil 4.57% (45.7 g/L) > composite mixture 5.54% (55.4 g/L). Control cultures increased from an initial cell density of 2×10^4 cells/mL to 33.92×10^4 cells/mL. Inhibition of cellular growth increased with WSF concentration, and growth rates decreased accordingly. The pH across samples ranged from 7.6 to 8.3 over the 72-hour exposure. Diesel WSF exhibited the highest toxicity at 72 hours (IC_{50} = 1.80%, 10.81 g/L), followed by spent engine oil

(IC_{50} = 2.27%, 22.70 g/L) and crude oil (IC_{50} = 4.57%, 45.72 g/L). The composite mixture showed the lowest toxicity, with an IC_{50} of 5.54% (55.36 g/L) in *S. costatum*.

The productivity and bioremediation potential of *Nannochloropsis oculata* and *Porphyridium cruentum* in Water Soluble Fraction (WSF) of petroleum fuels was assessed by Ezenweani and Kadiri (2024). The study examined the growth of *Nannochloropsis oculata* and *Porphyridium cruentum* exposed to 0%, 10%, 20%, 30%, 40%, 50%, 75%, and 100% water-soluble fractions (WSFs) of kerosene, diesel, and gasoline. Algal growth was monitored every two days over a 14-day period using a 721 Visible Spectrophotometer, and productivity was assessed following standard procedures. The bioremediation potential of the algae was evaluated using GC analysis of diesel-range organics in 100% WSFs. Both species exhibited minimal growth at 100% WSF for all fuels. *P. cruentum* showed maximum growth at 10% WSF across all fuels, while *N. oculata* achieved maximum growth at 30% WSF in kerosene and gasoline, and 50% WSF in diesel. The results indicated that *P. cruentum* was strongly inhibited by all fuels, whereas *N. oculata* experienced growth stimulation at lower fuel concentrations. In terms of bioremediation efficiency, *N. oculata* removed 84.58%, 65.51%, and 70.77% of kerosene, diesel, and gasoline, respectively, outperforming *P. cruentum*, which achieved 58.94%, 46.64%, and 56.67% removal. The study concluded that *N. oculata* is a robust and reliable candidate for petroleum hydrocarbon bioremediation and warrants further investigation for sustainable and eco-friendly remediation of petroleum pollution.

Chinweuba *et al.* (2024) examined the comparative toxicities of two petroleum products, Automotive Gas Oil (AGO) and Premium Motor Spirit (PMS), were examined by exposing two species of shrimp: freshwater shrimp *Desmoscaris trispinosa* and brackish water shrimp *Palaemonetes africanus*, to acute concentrations (0.05, 0.1, 1.0, 10.0, and 100 mg/L) of these toxicants for 96 hours. The toxicity was examined in artificially contaminated environmental systems, and the median lethal concentration (LC_{50}) was estimated using the Finney Probity method of analysis. The acute toxicity evaluation yielded 96-hour LC_{50} values for AGO of 0.330 mg/L for freshwater shrimp and 1.986 mg/L for brackish water shrimp, compared to 0.485 mg/L and 2.919 mg/L for PMS in freshwater and brackish water habitats, respectively. These results indicated that AGO was more toxic than PMS, and both petroleum products were more toxic to the freshwater shrimp than to the brackish water shrimp. However, the observed mean LC_{50} values were not significantly different at levels of $P < 0.01$. The findings suggest that petroleum products pose potential risks to the fauna in the shoreline and benthic sediments of the Niger Delta ecological zone. This is important because attention is often focused on visible surface spills of petroleum, while the dissolved aromatic hydrocarbons, which are more bioavailable and potentially toxic to marine organisms, are overlooked.

The effects of the water-soluble fraction of crude oil (WSF) on Indian carp (*Labeo rohita*) with and without treatment with zinc oxide nanoparticles (Nano-ZnO) was examined by Mousaviyon *et al.* (2025). A total of 225 fish were randomly distributed into five groups in triplicate over a 21-day period. Group I served as the control. Groups II and III were exposed to 0.5% and 1% untreated WSF, respectively. Groups IV and V received 5% and 10% WSF treated with Nano-ZnO, while Groups VI and VII were exposed to 5% and 10% WSF without Nano-ZnO. Blood samples could not be collected from fish exposed to untreated WSF due to hemolysis. Exposure to treated WSF caused increases in creatine phosphokinase, alkaline phosphatase, aspartate aminotransferase, lactate dehydrogenase, and gamma-glutamyl transferase activities, whereas alanine aminotransferase activity decreased. Total protein, globulin, and triglyceride levels declined significantly, while albumin and cholesterol increased. Thiol groups and glutathione peroxidase activity were significantly reduced, whereas superoxide dismutase, catalase, total antioxidant capacity, and malondialdehyde levels were elevated. The study concluded that both treated and untreated WSF exposure induced significant biochemical and oxidative stress in *L. rohita*. Although Nano-ZnO treatment reduced hemolysis, it did not fully prevent enzyme and antioxidant imbalances, indicating persistent physiological stress.

Anwar *et al*. (2025) examined the protective effects of dietary *Rosmarinus officinalis* leaves on water quality, growth performance, oxidative stress, stress hormones, and heat shock proteins in juvenile common carp (*Cyprinus carpio*, 40.41 ± 2.28 g). Over a 56-day feeding trial, fish were exposed to 25% and 50% of the LC_{50} (corresponding to 3.57 and 7.14 mg/L) WSF and fed diets containing 0% or 0.1% rosemary. Water quality parameters remained within acceptable ranges to sustain common carp. WSF exposure significantly reduced growth performance and feed utilization, whereas dietary rosemary improved growth. Malondialdehyde (MDA), glutathione peroxidase (GPx), and superoxide dismutase (SOD) levels in serum, liver, and gills increased significantly in WSF-exposed fish, while reactive oxygen species (ROS) and glutathione reductase (GR) decreased ($P < 0.05$). Heat shock proteins (HSP70 and HSP90) and thyroid hormones (T3 and T4) were elevated in serum and gills following WSF exposure, as was cortisol, while acetylcholinesterase (AChE) activity decreased. In the liver, WSF exposure increased T4 and AChE levels, but T3 decreased; cortisol levels in the liver declined, particularly at 50% WSF. Dietary supplementation with rosemary improved growth, feed utilization, oxidative stress markers, HSP expression, and stress hormone balance, especially at the highest WSF concentration. The study concluded that sublethal WSF exposure induces homeostatic stress in juvenile *C. carpio*, affecting growth, heat shock proteins, and stress hormone regulation, while dietary rosemary mitigates these adverse effects.

The comparative toxicities of two petroleum products, Automotive Gas Oil (AGO) and Premium Motor Spirit (PMS), were examined by Chinweuba *et al*. (2024) exposing two species of shrimp: freshwater shrimp *Desmoscaris trispinosa* and brackish water shrimp *Palaemonetes africanus*, to acute concentrations (0.05, 0.1, 1.0, 10.0, and 100 mg/L) of these toxicants for 96 hours. The toxicity was examined in artificially contaminated environmental systems, and the median lethal concentration (LC_{50}) was estimated using the Finney Probity method of analysis. The acute toxicity evaluation yielded 96-hour LC_{50} values for AGO of 0.330 mg/L for freshwater shrimp and 1.986 mg/L for brackish water shrimp, compared to 0.485 mg/L and 2.919 mg/L for PMS in freshwater and brackish water habitats, respectively. These results indicated that AGO was more toxic than PMS, and both petroleum products were more toxic to the freshwater shrimp than to the brackish water shrimp. However, the observed mean LC_{50} values were not significantly different at levels of $P < 0.01$. The findings suggest that petroleum products pose potential risks to the fauna in the shoreline and benthic sediments of the Niger Delta ecological zone. This is important because attention is often focused on visible surface spills of petroleum, while the dissolved aromatic hydrocarbons, which are more bioavailable and potentially toxic to marine organisms, are overlooked

Okwuego *et al*. (2025) investigated the physicochemical properties of water-soluble fractions (WSFs) of Automotive Gas Oil (AGO) and Premium Motor Spirit (PMS). The heavy metal content, total petroleum hydrocarbons (TPH), and polycyclic aromatic hydrocarbons (PAH) content of the WSFs were determined using standard procedures. The WSFs were found to be acidic, with mean pH values of 5.50±0.02 and 6.10±0.02 for AGO and PMS in brackish water, respectively, and 5.30±0.02 and 5.80±0.02 for AGO and PMS in freshwater, respectively. The electrical conductivity (EC) values were 28611.50±0.50 µS/cm for AGO and 28609.00±0.50 µS/cm for PMS in brackish water, and 198.00±0.56 μS/cm for AGO and 196.00±0.56 μS/cm for PMS in freshwater. The total dissolved solids (TDS) content was less than 8.00 mg/L in all samples. Dissolved oxygen (DO) levels were 5.00±0.01 mg/L and 6.00±0.01 mg/L for AGO and PMS in brackish water, and 4.60±0.01 mg/L and 4.40±0.01 mg/L for AGO and PMS in freshwater, respectively. DO was significantly higher in WSFs of PMS compared to AGO in both freshwater and brackish water. In contrast, biochemical oxygen demand (BOD) was significantly higher in WSFs of AGO than in WSFs of PMS in both habitats. The WSFs of AGO and PMS also contained cations and anions such as Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, HCO_3^{-}, SO_4^{2-}, and NO_3^{-}. Heavy metals such as Ni, Mn, Fe, Zn, Cu, Pb, Cr, and Cd were also present. Routine monitoring of the physical and chemical properties of ponds, streams, and rivers in oil-bearing communities was recommended to help mitigate the impacts of petroleum-derived pollutants.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This study was carried out in the University of Benin, Benin City, Edo State, Nigeria. The experiments were conducted in the Limnology and Phycology Laboratory of the Department of Plant Biology and Biotechnology.

3.2 Materials

The materials used in this project includes the following: Chu 10 (culture media), spent engine oil, unspent engine oil, distilled water, beakers, wash bottle, UV-Vis spectrophotometer, pH meter, TDS/EC conductivity meter, conical flask, graduated cylinder, syringe, magnetic stirrer, masking tape, and culture vessels.

3.3 Test Microalgae

The test microalgae were *Chlorella vulgaris* and *Scenedesmus ecornis*.

3.4 Collection of test Microalgae

Wild algal mixtures were collected from different fish ponds located in different areas within Benin City, Edo State.

3.5 Isolation of the Pure Culture

Unialgal cultures were obtained by isolating the desired alga and inoculating it into a suitable growth medium (Chu 10). A microscopic examination was carried out to confirm the species of algae present in the sample.

3.6 Botany of the Test Microalgae

3.6.1 *Chlorella vulgaris*

Description

They are single-celled green microalgal with a cell size 2-10μm in diameter with a spherical shape. This unicellular alga was discovered in 1890 by Martinus Willem Beijerinck as the first microalga with a well-defined nucleus. *Chlorella vulgaris* is a widely studied freshwater microalga recognized for its rich nutritional profile and diverse applications in food, biotechnology, aquaculture and environmental management (Wang *et al*., 2024).

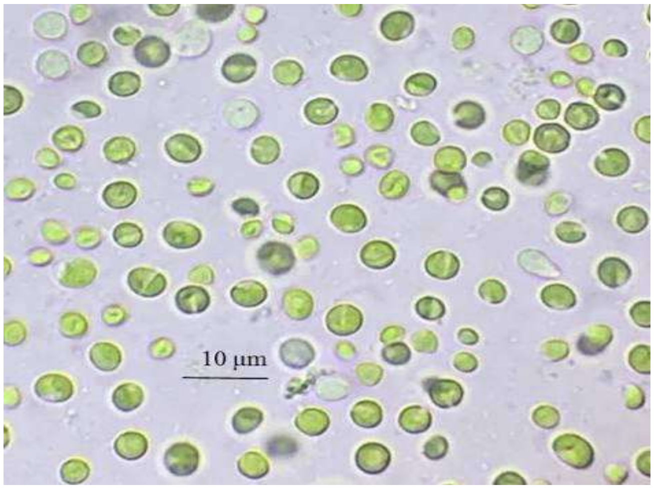

Plate 3.1: A photomicrograph of *Chlorella vulgaris* (Beijerink 1890)

Taxonomic classification of *Chlorella vulgaris*

Division:	Chlorophyta
Kingdom:	Protista
Class:	Trebouxiophyceae
Order:	Chlorellales

Family: Chlorellaceae

Genus: *Chlorella*

Species: *Chlorella vulgaris*

4.6.2 *Scenedesmus ecornis*

Description

Scenedesmus ecornis is a species belonging to the genus *Scenedesmus*, a group of freshwater green microalgae known for its colonial structure of 4 to 32 elliptical cells linked in a row. Unlike some other Scenedesmus species, *S*: *ecornis* typically lacks spines, which makes it smooth-edged and more compact in appearance. It is one of the many species in the genus *Scenedesmus* that are important in ecology, environmental monitoring and biotechnology (Zhao *et al*., 2020).

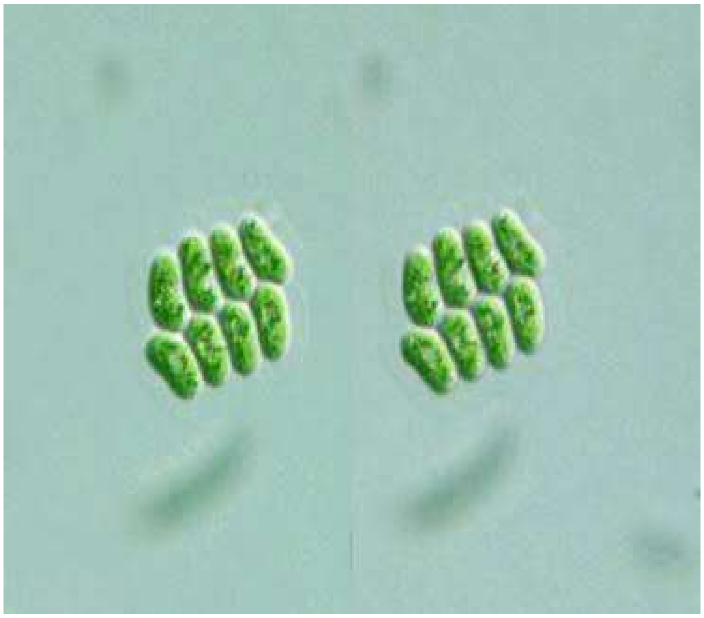

Plate 3.2: A photomicrograph of *Scenedesmus ecornis* (Ehrenberg) Chodat 1926

Taxonomic classification of *Scenedesmus ecornis*

Domain: Eukaryota

Kingdom: Plantae

Phylum: Chlorophyta

Class: Chlorophyceae

Order: Sphaeropleales

Family: Scenedesmaceae

Genus: *Scenedesmus*

Species: *Scenedesmus ecornis*

3.7 Preparation of Culture Media

The microalgae species were grown in an artificial medium, Chu´s modified No 10 medium which was used in combination with the water-soluble fractions of spent and unspent engine oil. The composition of the modified medium is shown in table 3.1.

3.8 Composition of modified Chu 10 culture medium

3.8.1 Macronutrient Stock Solution

A stock solution medium was made by dissolving the salts listed in the amount indicated (in grams) each in 100ml distilled water as shown in table 3.1. The prepared solution was autoclaved and allowed to cool before use.

3.8.2 Trace Element Stock Solution

Trace element stock solution was made by dissolving the salts below in the amount (mg) indicated in 1L of distilled water. The mixture was autoclaved and kept sterile.

3.8.3 Iron Stock Solution

Iron stock solution was prepared by adding 3.35g of citric acid ($C_6H_8O_7.H_2O$) and 3.35g of ferric citrate ($FeC_6H_5O_3$. $5H_2O$) into a 100ml of distilled water and allowed to autoclave.

3.8.4 Vitamin Stock Solution

Vitamin stock solutions provide essential micronutrients for optimal microalgae growth. It was prepared by calculating individual vitamin needs, dissolving them in distilled water using a stir plate, combining them in a volumetric flask, and adjusting the volume.

Table 3.1: Preparation of Chu No. 10 medium

Macronutrients	**g/100ml**
$CaCl_2$ $2H_2O$	3.67
$MgSO_4$ $7H_2O$	3.69
$NaHCO_3$	1.26
K_2HPO_4	0.84
$NaNO_3$	8.50
Na_2SiO_3	2.84
Trace Element Stock	**mg/L**
$CuSO_4$ $5H_2O$	19.6
$ZnSO_4$ $7H_2O$	44.0
$CoCl_2$ $6H_2O$	20.0
$MnCl_2$ $4H_2O$	36.0
$NaMO_4$ $2H_2O$	12.6
H_3BO_3	618.4
Iron Stock Solution	**g/100ml**
Citric Acid ($C_8H_8O_7$. H_2O)	3.35
Ferric Citrate ($FeC_6H_5O_3$. $5H_2O$)	3.35
Vitamin Stock Solution	**g/100ml**
Cyanocobalamin(B_{12})	0.004
Thiamine	0.004
Biotin	0.004

3.9 Culture Vessel

200ml plastics bottles were used for the experiment. The bottles were newly purchased, washed using dilute hydrochloric acid (HCl) and thoroughly rinsed with distilled water. They were then air-dried and labelled according to the different WSF concentrations and the test algae.

3.10 Source of Spent and Unspent Engine Oil

The spent engine oil was collected from an auto-mechanic workshop at Ekosodin road Benin City, Edo State. The unspent engine oil was purchased at the FAGCOOP station on the Ugbowo-Lagos route in Benin City, Edo State.

3.11 Preparation of water-soluble fraction of spent and unspent engine oil

The procedure of Ezenweani and Kadiri (2023) was followed to obtain the 100% stock solution of the WSF of each of the engine oil. For the 1:9 mixture of spent engine oil and distilled water, a 2500ml conical flask with a screw cover was used. The mixture was stirred using a magnetic stirrer hotplate for 24 h. After stirring, the mixture was then transferred into a glass stopper separating funnel and allowed to stand overnight after which the aqueous phase was separated to make the stock solution (100% WSF). The procedure was repeated using unspent engine oil and the WSFs of spent and unspent engine oil were obtained.

Plate 3.3a: Preparation of WSF of unspent engine oil.

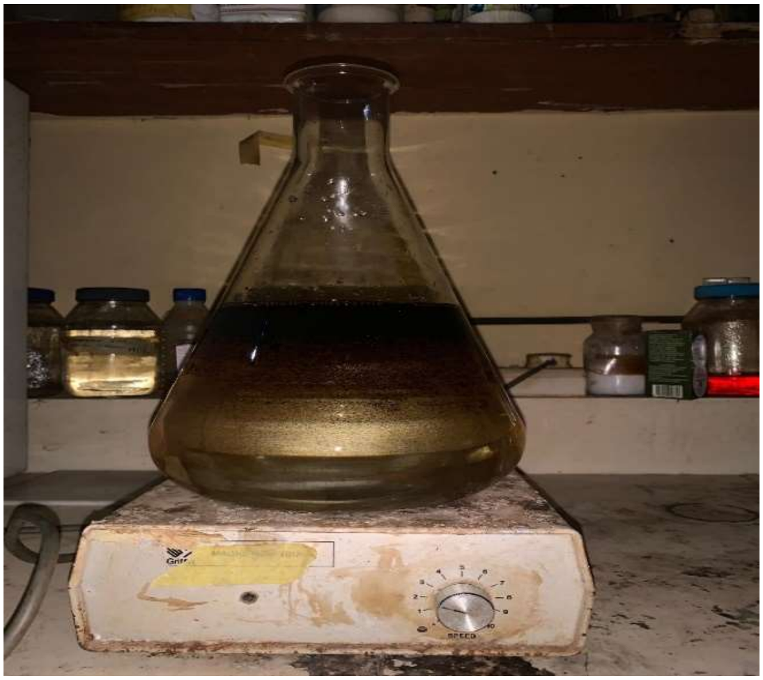

Plate 3.3b: Preparation of WSF of spent engine oil.

3.12 Preparation of different concentrations of treatments

Different concentrations of the water-soluble fractions (WSF) of the engine oil samples were prepared by diluting measured volumes of the stock solution with Chu-10 growth medium to obtain the required treatment levels of 0%, 5%, 10%, 25%, 50%, 75% and 100%. For each concentration, the appropriate volume of stock solution was added to a corresponding volume of Chu-10 medium to make up a total working volume of 900 ml per treatment as shown in Table 3.2.

.

Table 3.2 Preparation of different concentration of treatments

Concentrations (%)	Volume of Stock (WSF) Added (ml)	Volume of Growth Medium (Chu 10) Added (ml)
0	0	900
5	45	855
10	90	810
25	225	675
50	450	450
75	675	225
100	900	0

3.13 Experimental Set up

The experiment was designed with seven treatments (0% - control, 5%, 10%, 25%, 50%, 75%, and 100% WSFs). WSF fractions of spent and unspent engine oil with each in separate triplicate bottles were labelled properly. These treatments were obtained through serial dilution of the WSF with growth medium using the measurements as shown in the table 3.2.

3.14 Inoculation

Five (5) milliliters of the unialgal cultures were used to inoculate each vessel containing the different treatments and repeated for each triplicate. A new syringe was used for the different microalgae to prevent contamination. After inoculation, the vessels were capped slightly to allow effective respiration and also prevent contamination. The culture vessels were then placed in a north facing window of the laboratory to ensure the algae were exposed to sufficient sunlight.

3.15 Growth Response of Microalgae

Growth response test of algae was determined using an E. TRACK INSTRUMENTS ENGLAND STANDARD VIS SPECTROPHOTOMETER (Model No: V721). The spectrophotometer was switched on and allowed to stay for about 20 minutes to stabilize the source and detector. The wavelength of the machine was set at 750nm and doubled checked before every reading. The spectrophotometer was zeroed before every reading with distilled water poured into the cuvette and the reset button was pressed. After zeroing the equipment, the cuvette was filled with approximately four milliliters (4mls) of the mixture and transferred into the cell holder of the spectrophotometer. The lid of the equipment was then closed and a digital reading was shown which was then recorded. This was done for all replicates of the different concentrations i.e. 0%, 5%, 10%, 25%, 50%, 75% and 100%. The cuvette was rinsed with distilled water after each reading and wiped with tissue to clean off any smears on the cuvette before taking any reading. After reading was completed, the culture bottles were taken back to a north-facing window and arranged according to the order of concentration. The order of placement of triplicates was altered for every day that reading was taken to ensure uniform exposure to light.

Plate 3.4a: ***Scenedesmus ecornis*** **and** ***Chlorella vulgaris*** **at Day 0 in culture media of Spent Engine Oil**

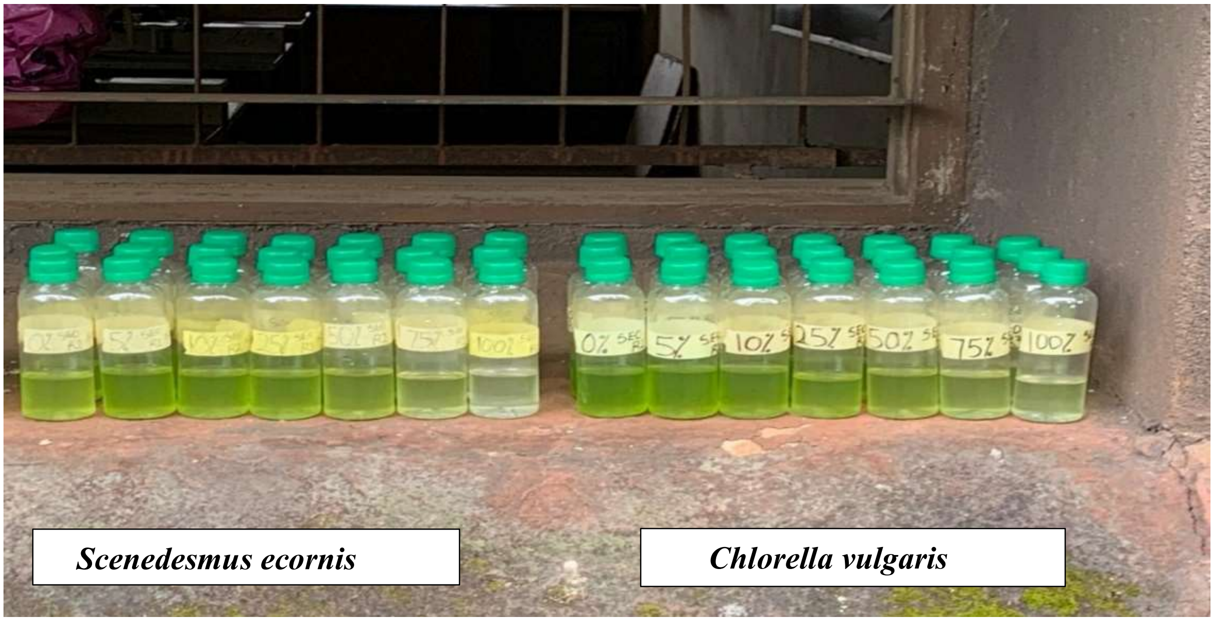

Plate 3.4b: ***Scenedesmus ecornis*** **and** ***Chlorella vulgaris*** **at Day 14 in culture media of Spent Engine Oil**

Plate 3.5a: ***Scenedesmus ecornis*** **and** ***Chlorella vulgaris*** **at Day 0 in culture media of Unpent Engine Oil**

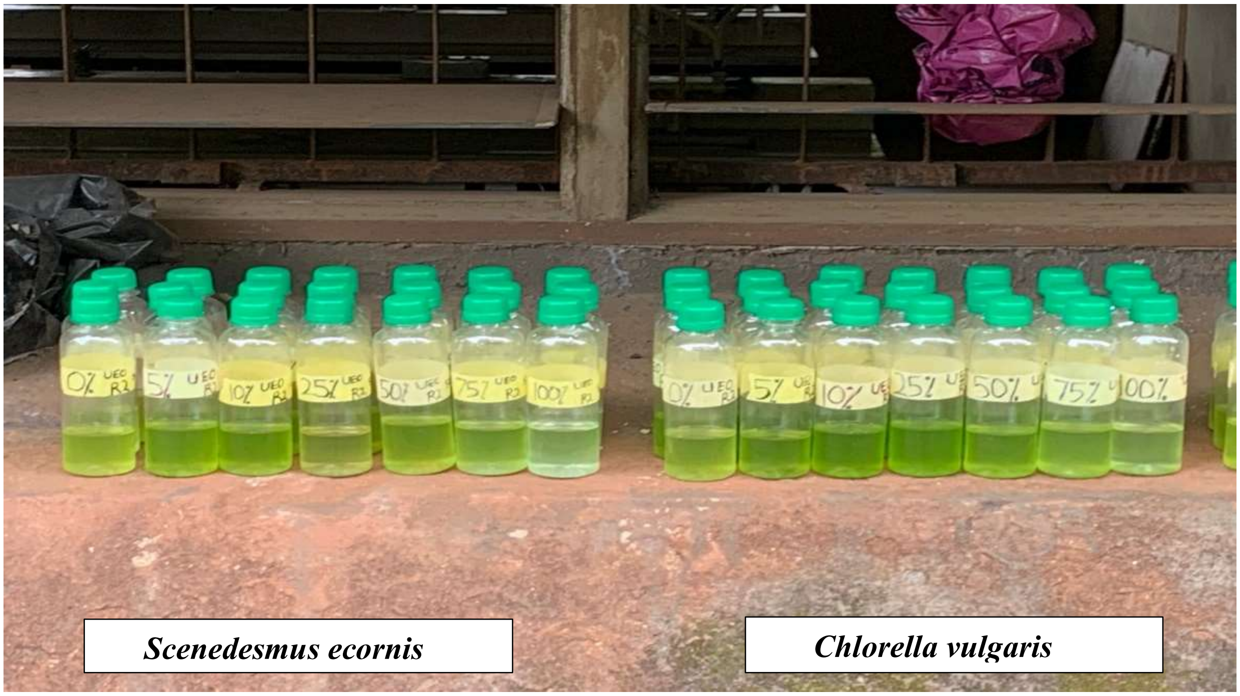

Plate 3.5b: ***Scenedesmus ecornis*** **and** ***Chlorella vulgaris*** **at Day 14 in culture media of Unpent Engine Oil**

3.15.1 Percentage Inhibition

The formula below was used for calculating the percentage inhibition (Rivilli, 2011).

$$Percentage\ Inhibition = 100 - \left(\frac{Measured\ biomass}{Theoretical\ biomass}\ X\ \frac{100}{1}\right) \ldots\ldots\ldots\ldots\ldots\ldots. (i)$$

3.15.2 Biomass´- Dry Weight (mgL^{-1})

The dry weight of the test algae was values gotten from growth response were estimated very two days by computation using the formula described by Horvatic *et al*. (2003)

$$\text{Dry Weight} = 3.31 + 179.45 \text{ x Absorbance at 750 nm} + 617.45 + (\text{Absorbance at 750 nm})^2 \ldots\ldots(ii)$$

3.15.3 Statistical Analysis

The statistical analysis was carried out using Microsoft Excel spread sheet package. Calculations of mean, standard error and analysis of variance (ANOVA) were performed to identify statistically significant differences in microalgal growth in response to treatment concentrations.

3.16 Physicochemical parameters

3.16.1 Hydrogen Ion Concentration (pH)

This was determined using a hand-held pH meter which was dipped into the cultures after being transferred to a 50ml beaker and the appropriate values were taken.

3.16.2 Total Dissolved Solids (TDS) (mg/L)

This was done using a HACH COISO TDS/Conductivity meter. The samples were shaken vigorously and 20ml of aliquot were taken and transferred to a 50ml beaker containing the cultures. The readings were then recorded when the values steadied.

3.16.3 Electrical Conductivity (μS/cm)

Conductivity values were obtained using a HACH COISO TDS/Conductivity meter. The culture vessel was shaken thoroughly and 20ml of aliquot were taken and transferred to a 50ml beaker after which the readings were taken when the values became steadied.

CHAPTER FOUR
RESULTS

4.1a: Growth response of *Scenedesmus ecornis* in WSF of spent engine oil

Figure 4.1a shows the growth response of *Scenedesmus ecornis* in WSF of spent engine oil. All treatments showed a prolonged lag phase from day 0-6. Rapid growth was observed after day 6 which peaks around day 10 for many treatments. Most treatments showed a sharp exponential growth response between day 8 and day 10 with the exception of 100% WSF treatment which recorded a noticeble decline between day 8 and day. Overall, low to moderate concentrations 0%, 5%, 10%, 25% and 50% recorded maximum growth, while more depressed growth was seen in 75% and 100%.

Using ANOVA statistically significant differences were recorded for the concentrations and experimental days ($p < 0.001$).

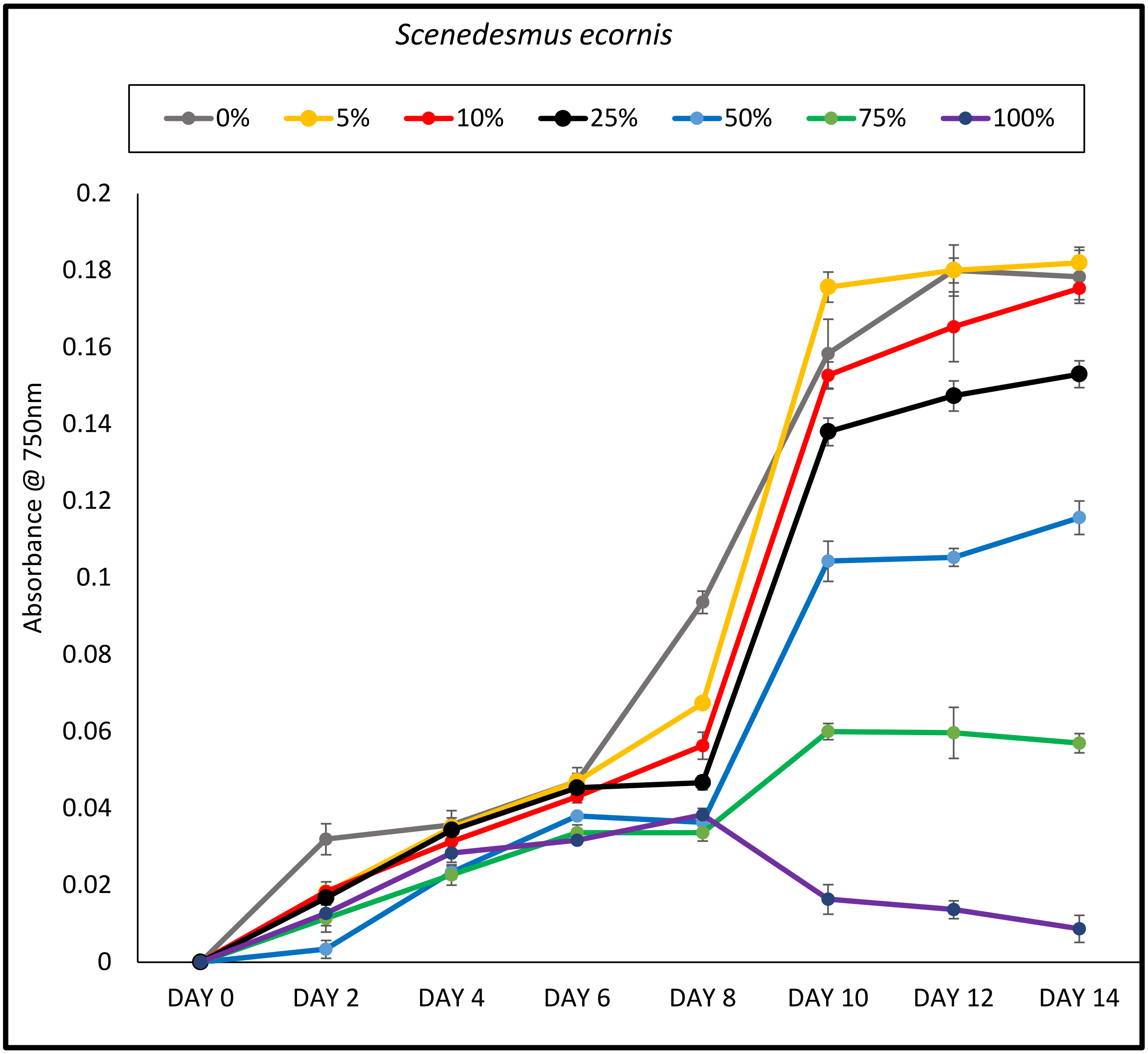

Figure 4.1a: Growth response of *Scenedesmus ecornis* to WSF of Spent engine oil.

4.1b: Growth response of *Chlorella vulgaris* in WSF of spent engine oil

Figure 4.1b shows the growth response of *Chlorella vulgaris* in varying concentrations of the WSF of spent engine oil. The growth pattern in different concentrations showed a short lag phase from day 0-6. From day 8 onwards the growth response for most treatments showed an increasing trend till day 10, and mostly stabilized by day 12-14. The lowest concentrations (0%, 5%, 10%, and 25%) recorded the highest growth rate, 50% showed moderate growth, while 75% and 100% showed the least growth throughout.

Using ANOVA the difference between the different concentrations were statistically significant across all days ($p < 0.001$).

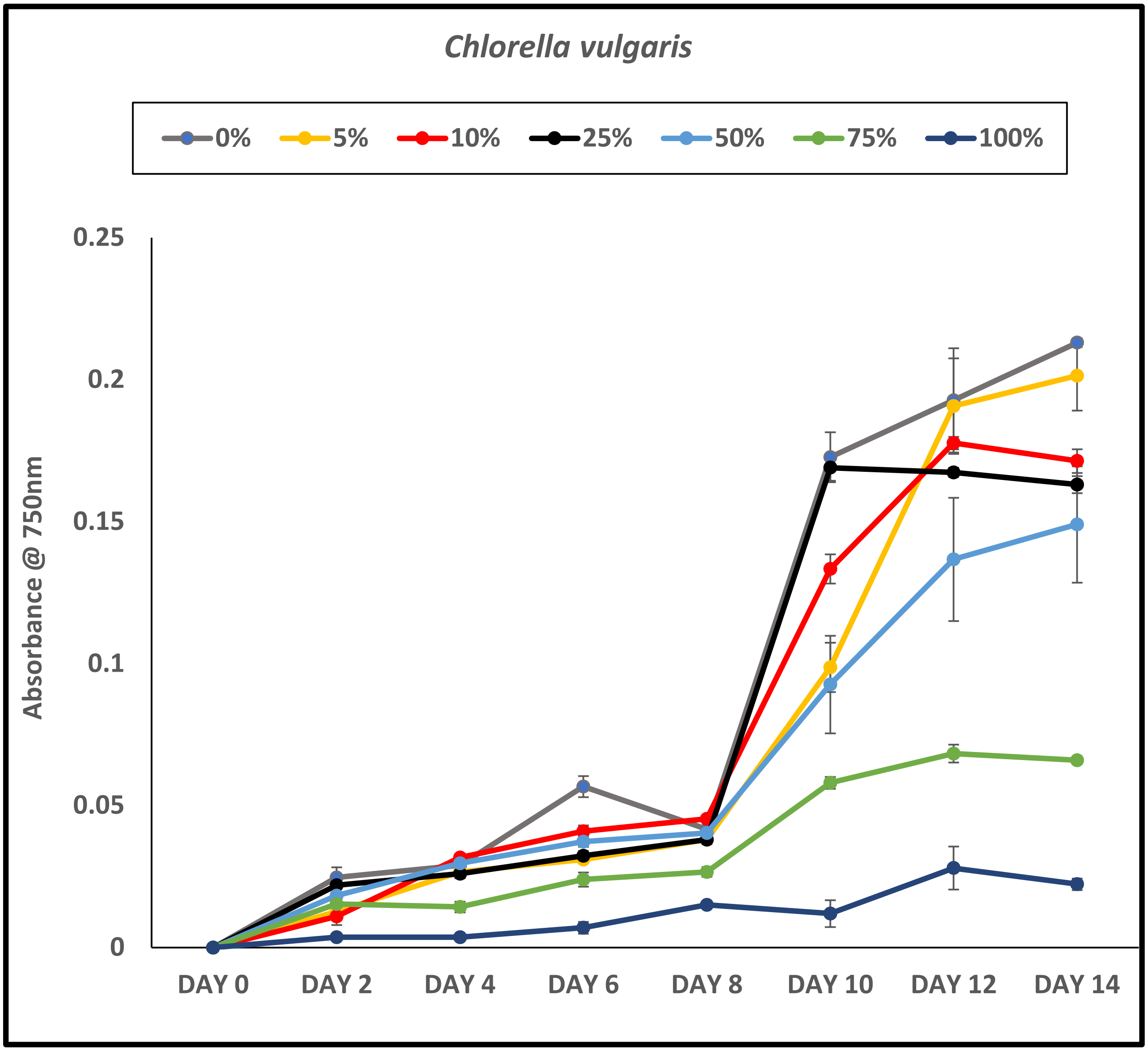

Figure 4.1b: Growth response of *Chlorella vulgaris* to WSF of Spent engine oil.

4.2a: Growth response of *Scenedesmus ecornis* in WSF of unspent engine oil

Figure 4.2a shows the growth response of *Scenedesmus ecornis* to various concentrations of the WSF of unspent engine oil. There was a lag phase for all concentrations from day 0-6 before significant growth began. From day 8-10, there was an exponential growth especially in the lower concentration treatments. The maximum growth was recorded in 5% and 10%, followed closely by 0% (control) and 25% treatments. Growth at 50% and 75% were remarkably comparable, while 100% noticeably showed the least growth.

As measured by ANOVA, differences between concentrations recorded were statistically significant in all days ($p < 0.001$).

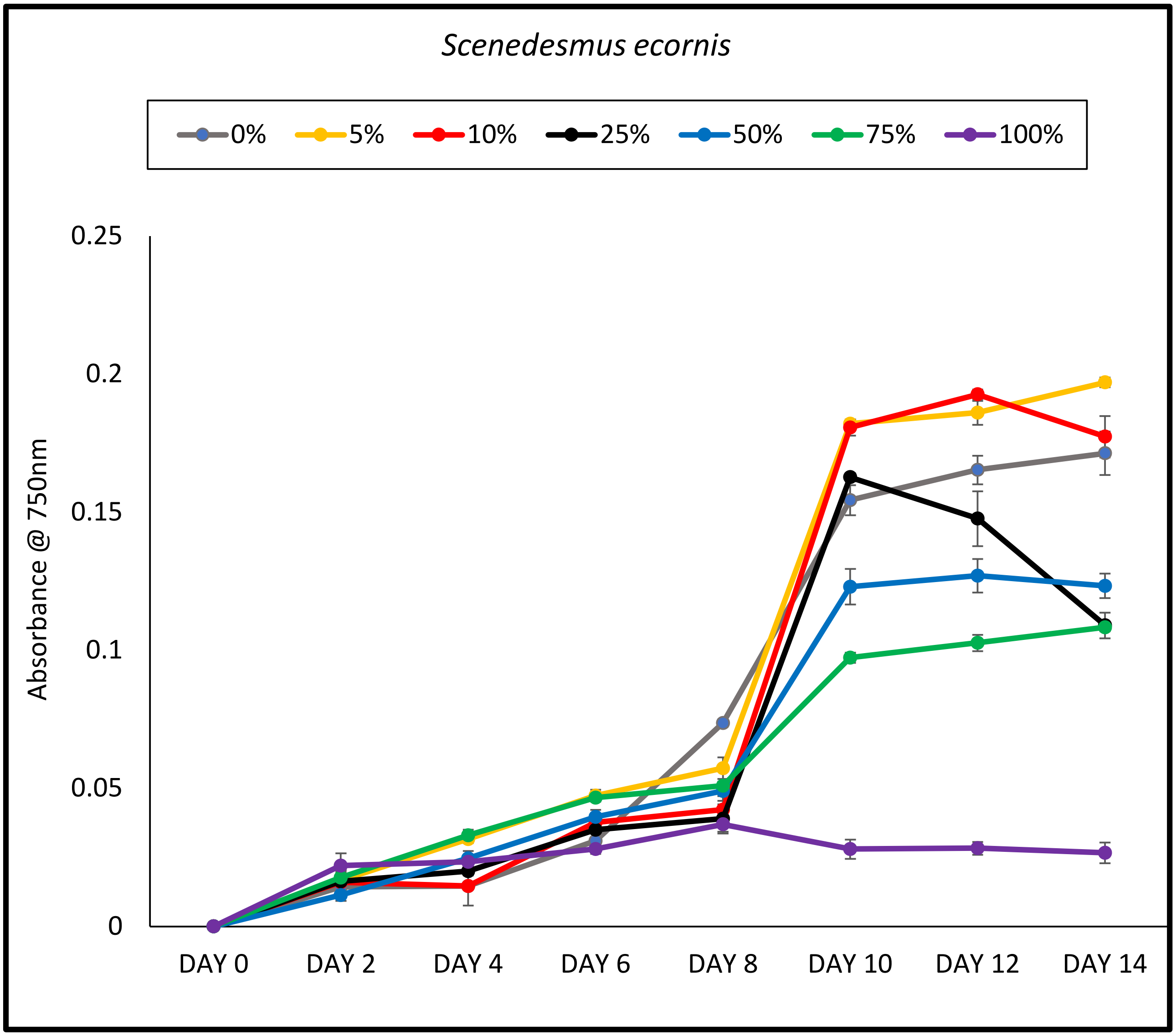

Figure 4.2a: Growth response of *Scenedesmus ecornis* to WSF of unpent engine oil.

4.2b: Growth response of *Chlorella vulgaris* in WSF of unspent engine oil

Figure 4.2b shows the growth response of *Chlorella vulgaris* in the WSF of unspent engine oil. Growth increases gradually up to day 6 for all treatments, representing the adaptation period. The exponential growth phase is marked between day 8 and 12. The low to moderate concentrations 0%, 5%, 10%, and 25% showed the highest growth rate, this was closely followed by 50% and 75% but reached lower peaks, while 100% showed the least growth rate.

Using ANOVA, the difference between the different concentrations were statistically significant in all days ($p < 0.001$).

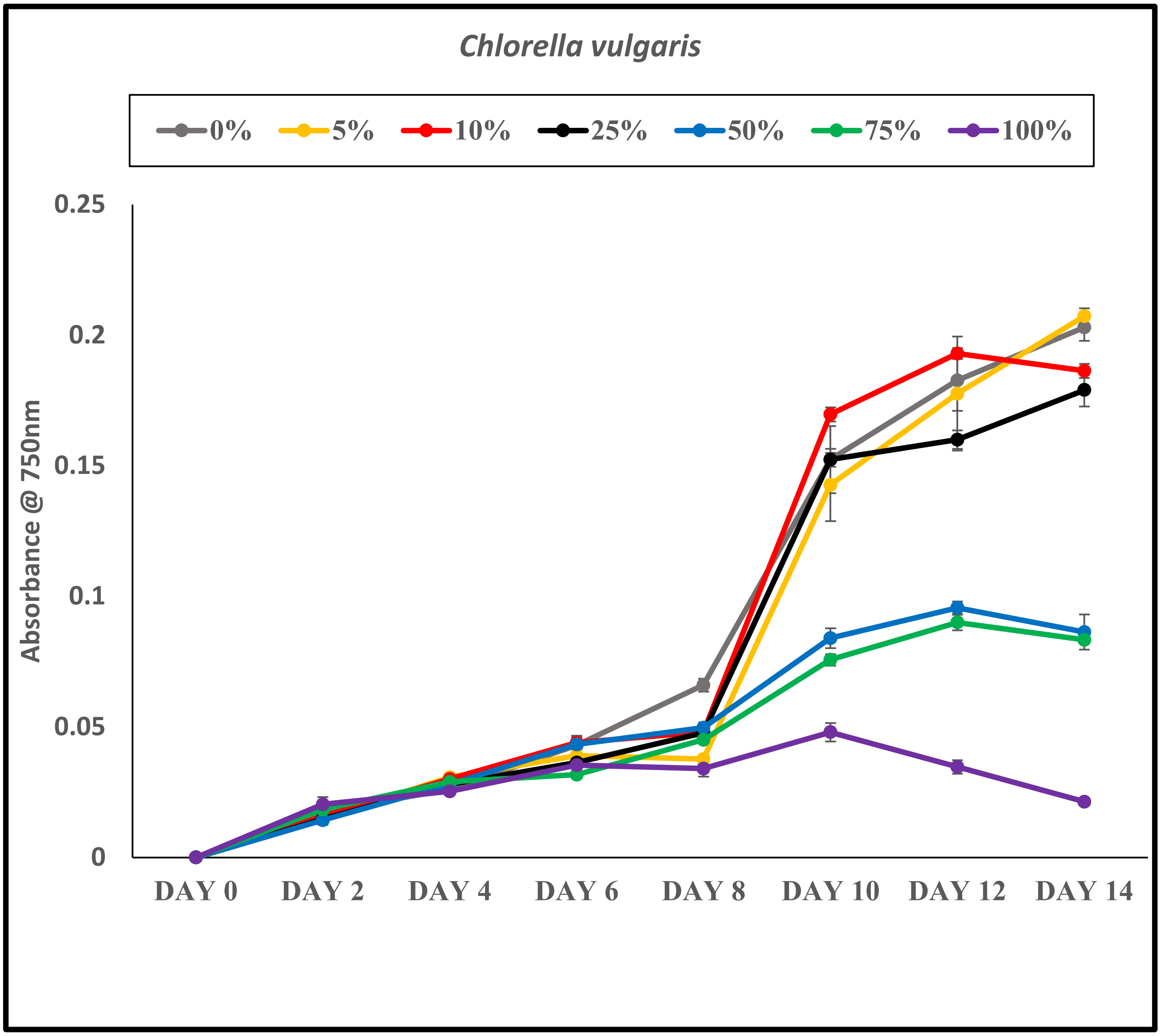

Figure 4.2b: Growth response of *Chlorella vulgaris* to WSF of unpent engine oil.

4.3a Dry weight of *Scenedesmus ecornis* and *Chlorella vulgaris* exposed to spent engine oil

Both *Scenedesmus ecornis* and *Chlorella vulgaris* showed their highest growth in the 0%, 5%, and 10% treatments, with day-14 dry weights reaching 96.29-98.27 mg/L for *S. ecornis, a*nd 92.50-115.08 mg/L for *C. vulgaris*. Growth began to decline at 25%, where both algae showed reduced biomass. Strong suppression occurred at 50-75%, and the lowest growth was recorded at 100%.

Table 4.1: Temporal Variation in Dry weight of *Scenedesmus ecornis* exposed to spent engine oil

TREATMENTS	DRY WEIGHT (mg/L)							
	DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 12	DAY 14
0%	**0**	**17.23**	**19.21**	**25.32**	**50.50**	**85.46**	**97.19**	**96.29**
5%	**0**	**9.69**	**18.85**	**25.32**	**36.28**	**94.84**	**97.19**	**98.27**
10%	**0**	**9.87**	**16.87**	**23.16**	**30.35**	**82.39**	**89.25**	**94.66**
25%	**0**	**8.97**	**18.49**	**24.42**	**25.14**	**74.46**	**79.51**	**82.57**
50%	**0**	**1.79**	**12.56**	**20.47**	**19.57**	**56.26**	**56.80**	**62.38**
75%	**0**	**6.10**	**12.20**	**18.13**	**18.13**	**32.33**	**32.15**	**30.71**
100%	**0**	**6.82**	**15.26**	**17.05**	**20.64**	**8.79**	**7.35**	**4.66**

Table 4.2: Temporal Variation in Dry weight of *Chlorella vulgaris* exposed to spent engine oil

TREATMENTS	DRY WEIGHT (mg/L)							
	DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 12	DAY 14
0%	0	13.28	15.62	30.54	22.45	93.22	104.06	115.08
5%	0	6.82	14.36	16.71	20.47	53.20	102.97	108.75
10%	0	5.923	17.06	22.09	24.42	71.94	95.93	92.50
25%	0	11.86	14.00	17.42	20.47	91.25	90.34	87.99
50%	0	9.87	15.98	20.11	21.73	49.96	73.74	80.41
75%	0	8.26	7.72	12.96	14.36	31.25	36.83	35.57
100%	0	1.97	1.97	3.77	8.08	6.46	15.08	12.03

4.3a Cumulative dry weight of *Chlorella vulgaris* and *Scenedesmus ecornis* in spent engine oil

Figure 4.3a shows that the cumulative dry weight of both *Scenedesmus ecornis* and *Chlorella vulgaris* decreased progressively as the concentration of spent engine oil increased. At 0%, both species exhibited the highest biomass, and a slight reduction in dry weight was observed at 5% and 10%. However, as the concentration reached 25%, a more noticeable decline occurred, while at 50%, the dry weight dropped rapidly. At 75% and 100%, growth was severely suppressed for both species, with *Scenedesmus ecornis* performing marginally better at full concentration.

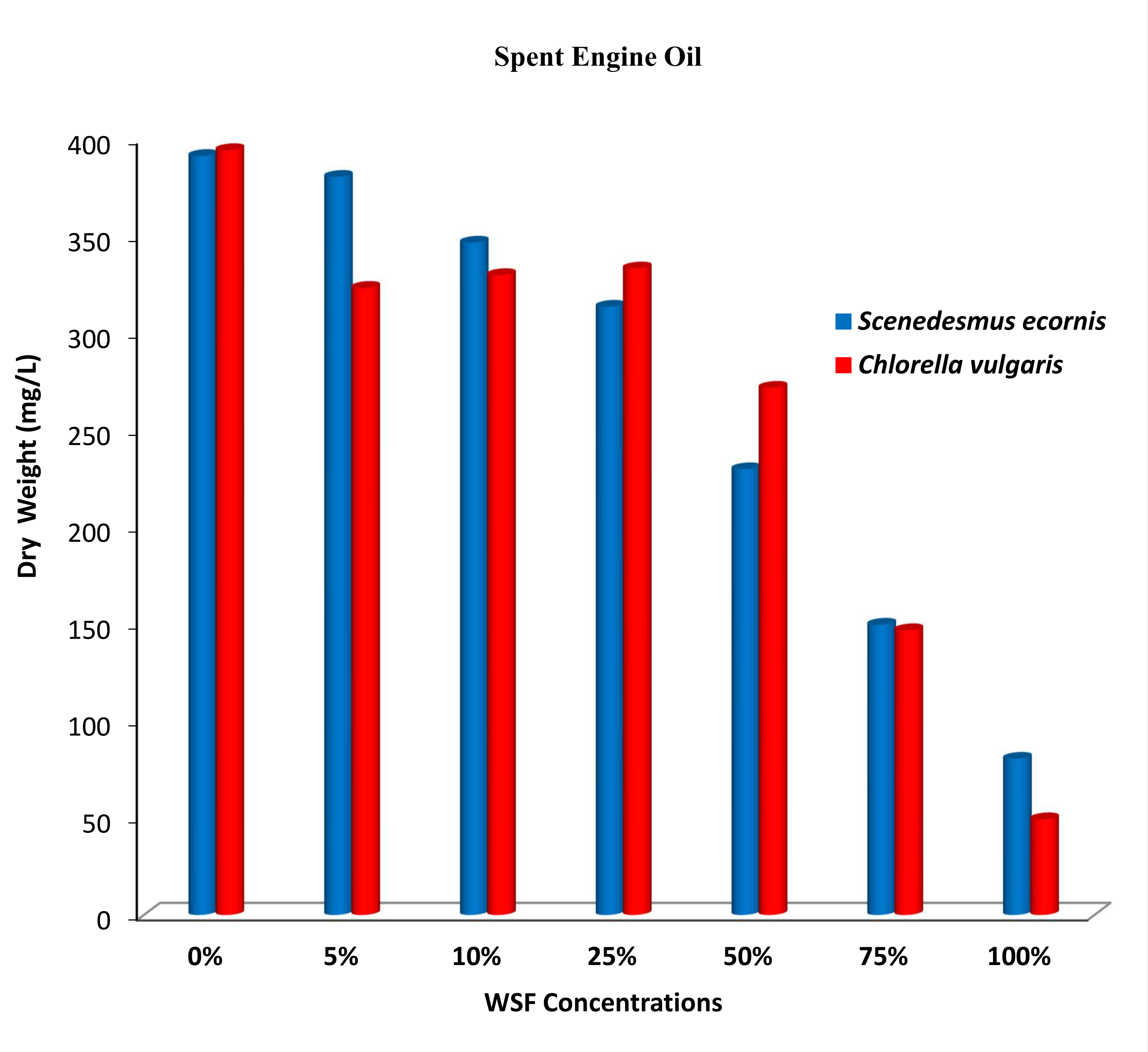

Figure 4.3a: Cumulative dry weight of *Chlorella vulgaris* and *Scenedesmus ecornis* in spent engine oil

4.3b Dry weight of *Scenedesmus ecornis* and *Chlorella vulgaris* exposed to unspent engine oil

Both *Scenedesmus ecornis* and *Chlorella vulgaris* recorded their highest day 14 dry weights at 5% (106.40 mg/L for *Scenedesmus ecornis* and 112.00 mg/L for *Chlorella vulgaris*). Growth declined noticeably at 25%, where *S. ecornis* dropped to 58.79 mg/L and *C. vulgaris* to 96.65 mg/L. Severe suppression occurred at 100%.

Table 4.3: Temporal Variation in Dry weight of *Scenedesmus ecornis* exposed to unspent engine oil

Treatments	DRY WEIGHT							
	DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 12	DAY 14
0%	0	7.72	7.90	16.70	39.71	83. 30	89.25	92.50
5%	0	8.98	17.06	25.50	30.90	98.28	100.44	106.40
10%	0	8.62	7.90	20.29	22.81	97.56	104.06	95.75
25%	0	8.80	10.776	18.85	21.01	87.81	79.69	58.79
50%	0	6.10	13.28	21.37	26.40	66.35	68.52	66.53
75%	0	9.51	17.77	25.14	27.48	52.48	55.37	58.43
100%	0	11.85	12.57	15.08	19.93	15.08	15.26	14.36

Table 4.4 Temporal Variation in Dry weight of *Chlorella vulgaris* exposed to unspent engine oil

Treatments	Dry Weight							
	DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 12	DAY 14
0%	0	9.51	14.00	23.17	35.57	82.22	98.64	109.66
5%	0	8.08	16.52	21.01	20.29	76.99	95.93	112.00
10%	0	8.44	16.16	23.70	25.86	91.60	104.24	100.63
25%	0	7.90	15.08	19.57	25.68	82.23	86.37	96.65
50%	0	7.72	15.08	23.35	26.76	45.28	51.58	46.54
75%	0	9.87	15.62	17.06	24.24	40.79	48.52	44.93
100%	0	10.95	13.64	19.03	18.31	25.86	18.67	11.49

4.3b Cumulative dry weight of *Chlorella vulgaris* and *Scenedesmus ecornis* in unspent engine oil

Figure 4.3b shows that the cumulative dry weight of both *Scenedesmus ecornis* and *Chlorella vulgaris* decreased gradually as the concentration of unspent engine oil increased. In the control (0%), both algae exhibited the highest biomass, and at 5% and 10% concentrations the dry weight remained relatively high. At 25%, a reduction in biomass became more noticeable, while at 50% and 75%, the decline in dry weight became more pronounced. At 100%, both species showed the lowest dry weight values.

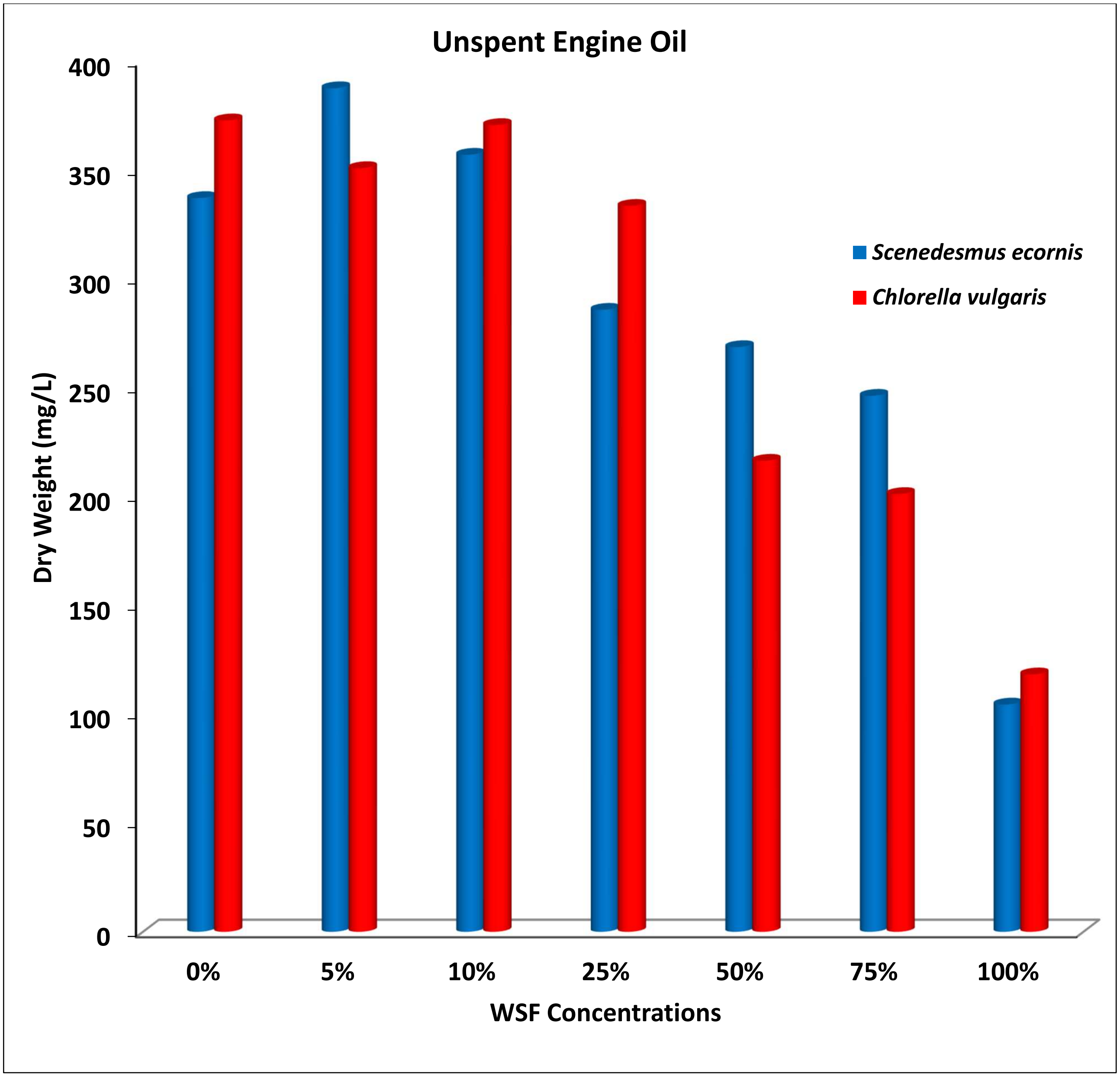

Figure 4.3b: Cumulative dry weight of *Chlorella vulgaris* and *Scenedesmus ecornis* in unspent engine oil

4.4a Percentage inhibition of *Scenedesmus ecornis* and *Chlorella vulgaris* in spent engine oil

Figure 4.4a shows the percentage inhibition of *Scenedesmus ecornis* and *Chlorella vulgaris* to varying concentration of WSF of spent engine oil. Generally, the growth of the microalgal species experienced more inhibition as the concentration of spent engine oil increases. Growth inhibition was relatively low for 0%, 5%, 10% and 25% concentrations, 50% showed stronger inhibitory response for both species, especially for *Scenedesmus ecornis* which reached about 40%. The highest inhibition was recorded at 75% and 100% concentrations for both species.

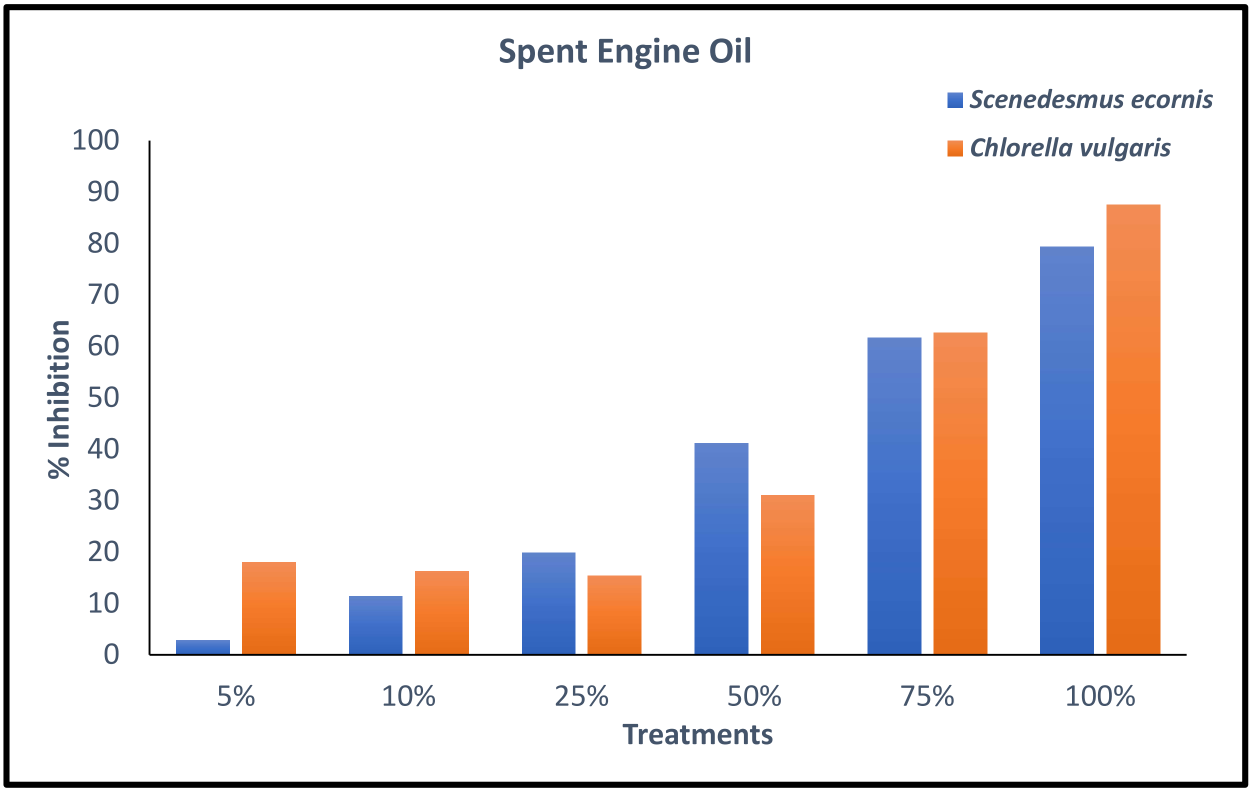

Figure 4.4a: Percentage inhibition of *Scenedesmus ecornis* and *Chlorella vulgaris* in the WSF of spent engine oil

4.4b Percentage inhibition of *Scenedesmus ecornis* and *Chlorella vulgaris* in unspent engine oil

Figure 4.4b shows the percentage inhibition and stimulatory response of *Scenedesmus ecornis* and *Chlorella vulgaris*. Specifically, growth stimulation was recorded at 5% and 10% for *Scenedesmus ecornis,* while *Chlorella vulgaris* showed only slight inhibitory response at those concentrations. From 25% upward, both species began to experience inhibition that increased with higher concentrations (50% and 75%) where *Chlorella vulgaris* surpassed that of *Scenedesmus ecornis*. The highest inhibitory response was observed at 100% concentration.

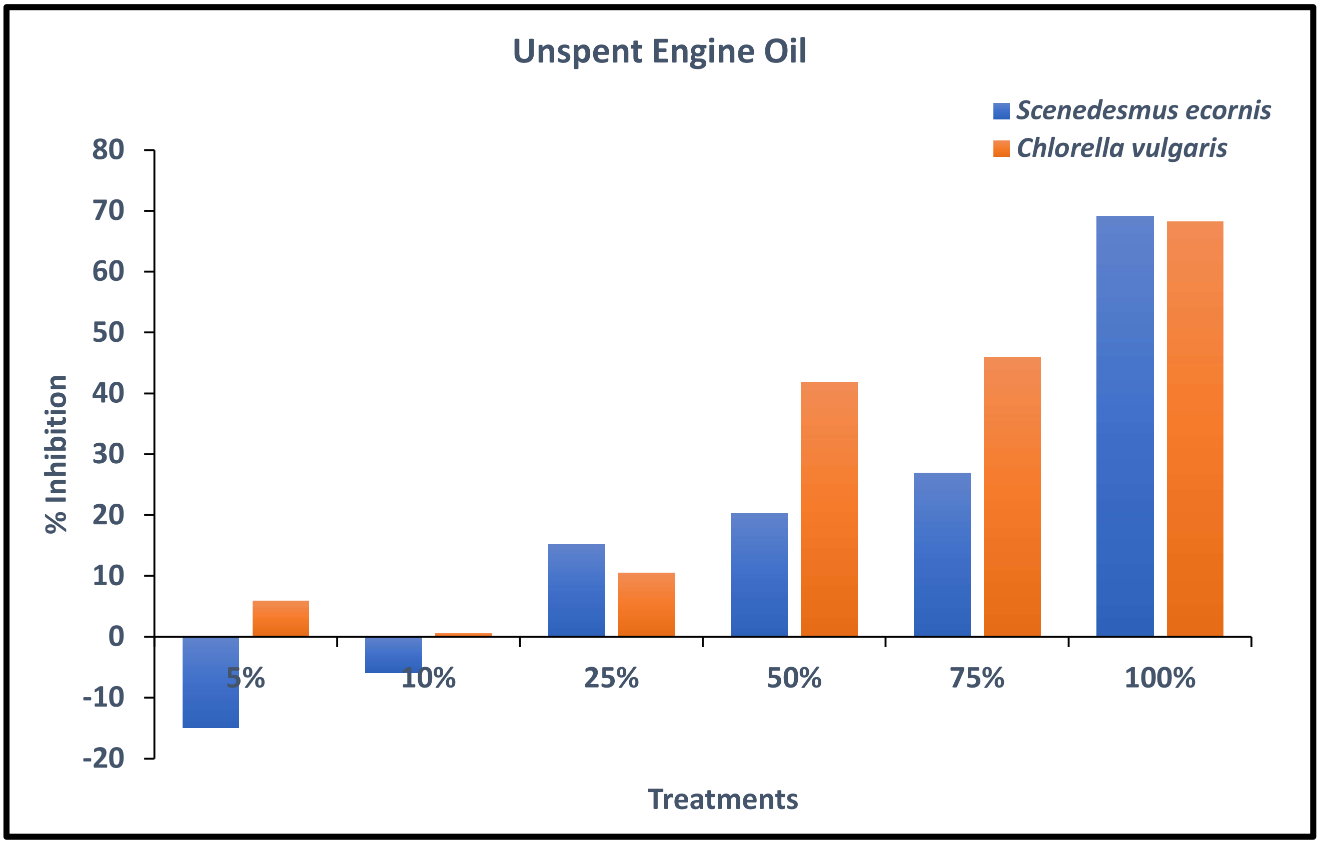

Figure 4.4b: Percentage inhibition of *Scenedesmus ecornis* and *Chlorella vulgaris* in the WSF of unspent engine oil

4.5a Comparative toxicity of spent and unspent engine oil on the growth of *Chlorella vulgaris*

Figure 4.5a shows that as the concentration of both spent and unspent engine oil increased, the percentage inhibition of *Chlorella vulgaris* also increased. At every treatment level, spent engine oil caused greater inhibition than unspent engine oil. At the highest concentration (100%), spent engine oil resulted in nearly complete inhibition, around 90–95%, while unspent engine oil caused about 70% inhibition. At lower concentrations (5% and 10%), inhibition was much lower but still consistently higher for spent engine oil.

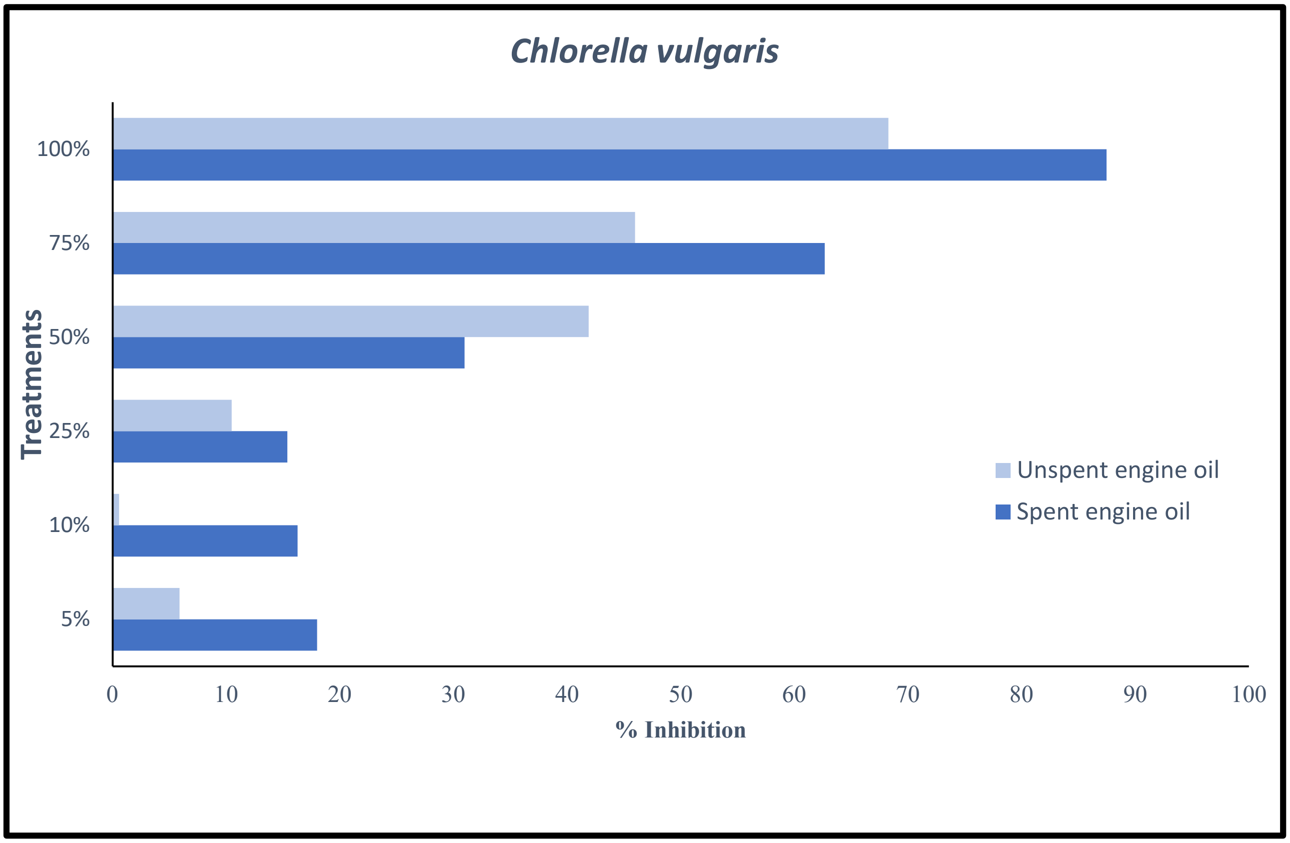

Figure 4.5a: Comparative toxicity of spent and unspent engine oil on the growth of *Chlorella vulgaris*

4.5b Comparative toxicity of spent and unspent engine oil on the growth of *Scenedesmus ecornis*

Figure 4.5b shows the comparative toxicity of spent and unspent engine oil on the growth of *Scenedesmus ecornis*. The growth inhibition of *Scenedesmus ecornis* increased as the concentration of both spent and unspent engine oil increased. At lower concentrations (5% and 10%), the inhibition was very low, with unspent engine oil showing slight stimulation at 5%. As the concentration increases from 25% to 100%, the percentage inhibition becomes much higher. Across all treatments, spent engine oil consistently caused greater inhibition than unspent oil.

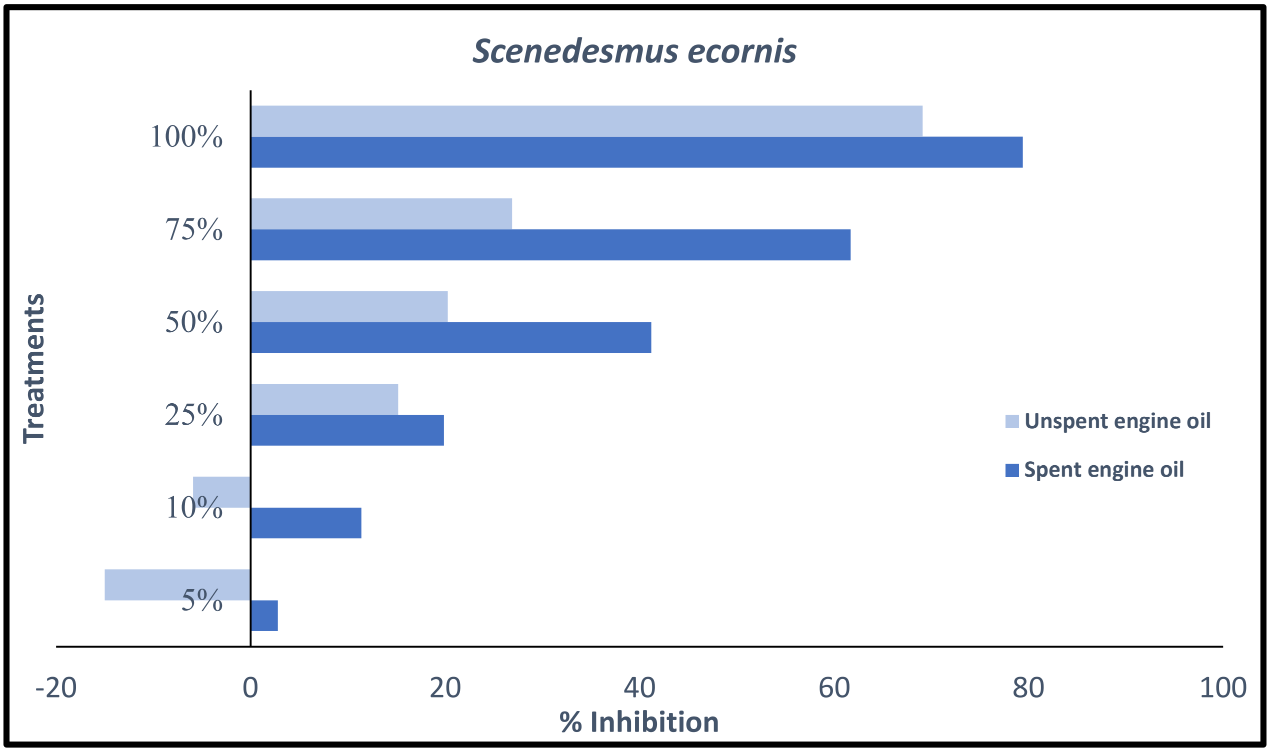

Figure 4.5b: Comparative toxicity of spent and unspent engine oil on the growth of *Scenedesmus ecornis*

4.6a Changes in pH of *Scenedesmus ecornis* exposed to spent engine oil over time

Figure 4.6a shows that at the beginning of the experiment (day 0), all treatments including the control (0%) started with pH values around 7.0. As the days progress, the pH gradually increased across all treatments. By day 8 to 14, the pH values increased to around 8-10, with slight differences among concentrations. Higher concentrations of spent engine oil (100%, 75% and 50%) showed more variation in pH, particularly around day 8, where the 100% treatment peaks at nearly pH 10. The lower concentrations (0%, 5%, 10% and 25%) exhibited a steadier and slightly lower increase in pH compared to higher concentrations.

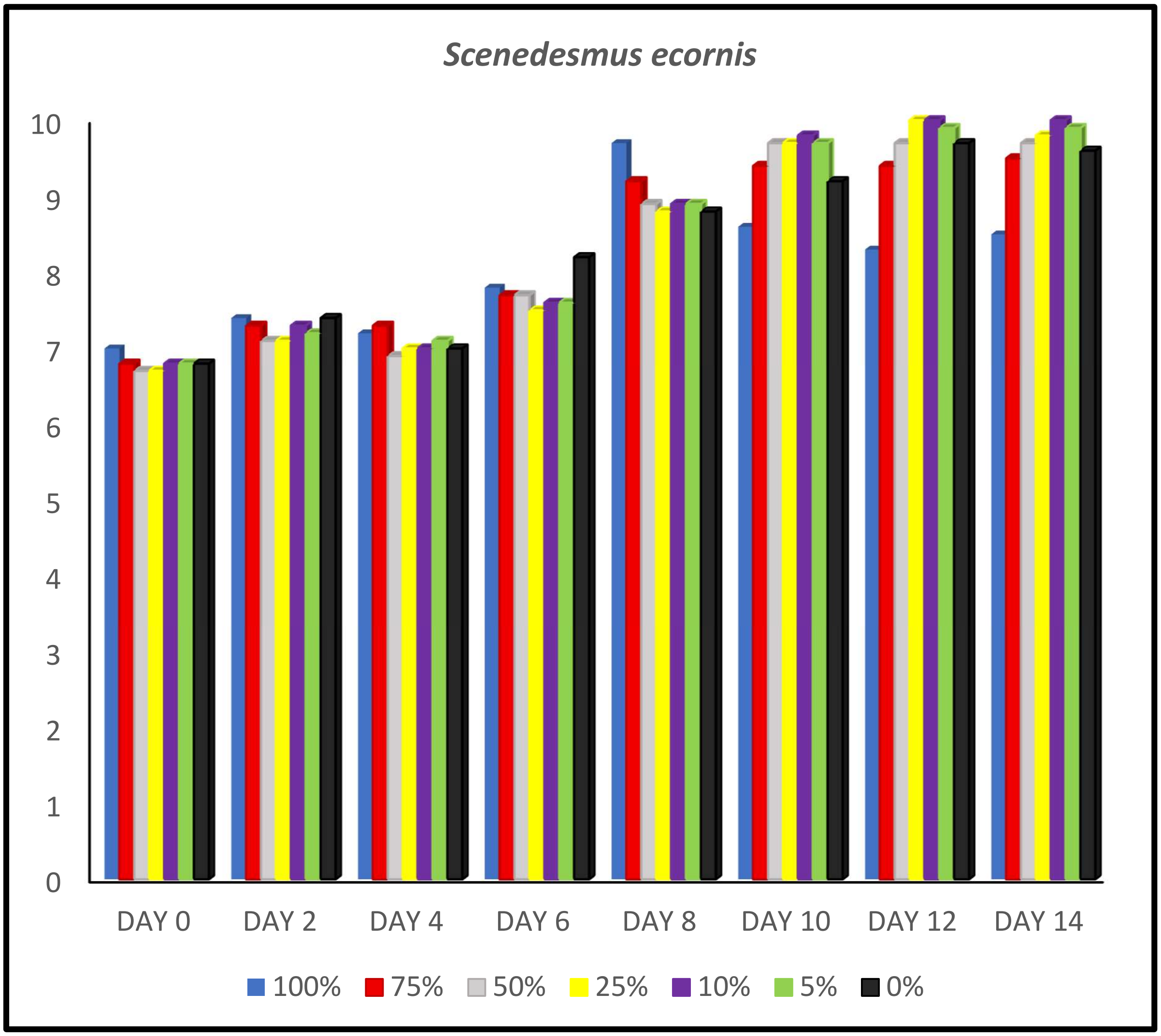

Figure 4.6a: Changes in pH of *Scenedesmus ecornis* exposed to spent engine oil

4.6b Variation in Total Dissolved Solids (TDS) of *Scenedesmus ecornis* exposed to spent engine oil over time

The variation in TDS of *Scenedesmus ecornis* exposed to spent engine oil is represented in Figure 4.6b. The values were relatively low across all treatments at the start of the experiment and as time progressed, the TDS increased steadily. Higher concentrations of spent engine oil (50%, 75% and 100%) consistently showed the highest TDS values throughout the 14-day period, while lower concentrations (0%, 5%, 10% and 25%) had lower but still elevated values.

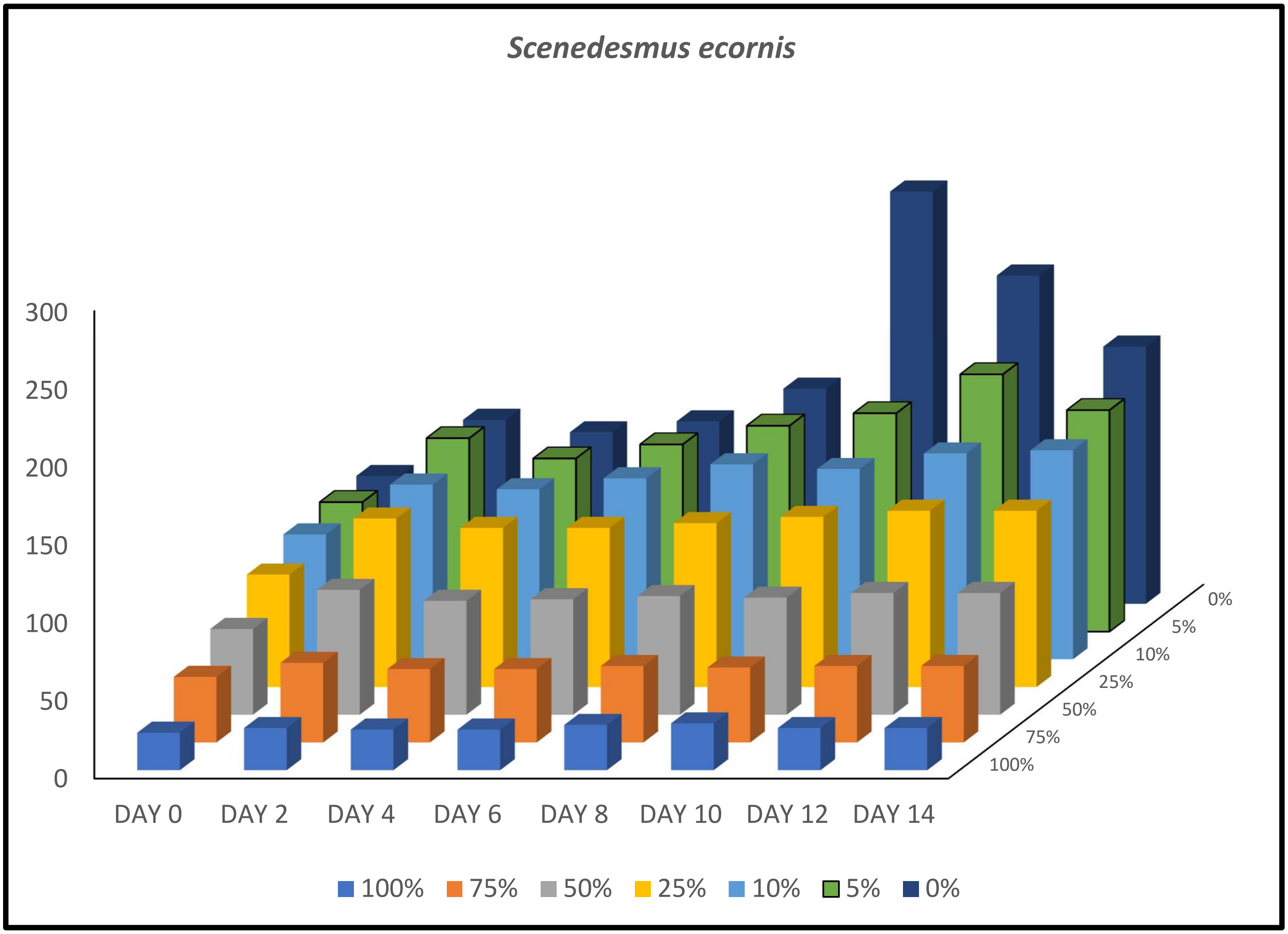

Figure 4.6b: Variation in Total Dissolved Solids (TDS) of *Scenedesmus ecornis* exposed to spent engine oil over time

4.6c Changes in Electrical Conductivity (EC) of *Scenedesmus ecornis* exposed to spent engine oil over time

The changes in electrical conductivity of *Scenedesmus ecornis* exposed to spent engine oil is represented in Figure 4.6c. At the start of the experiment (day 0), the EC values were relatively low across all treatments, and as the experiment progressed it increased gradually for all concentrations. The higher concentrations of spent engine oil (50%, 75% and 100%) showed the greatest increase in EC throughout the 14-day period, while lower concentrations (0%, 5%, 10% and 25%) exhibited smaller but consistent increase in EC over time.

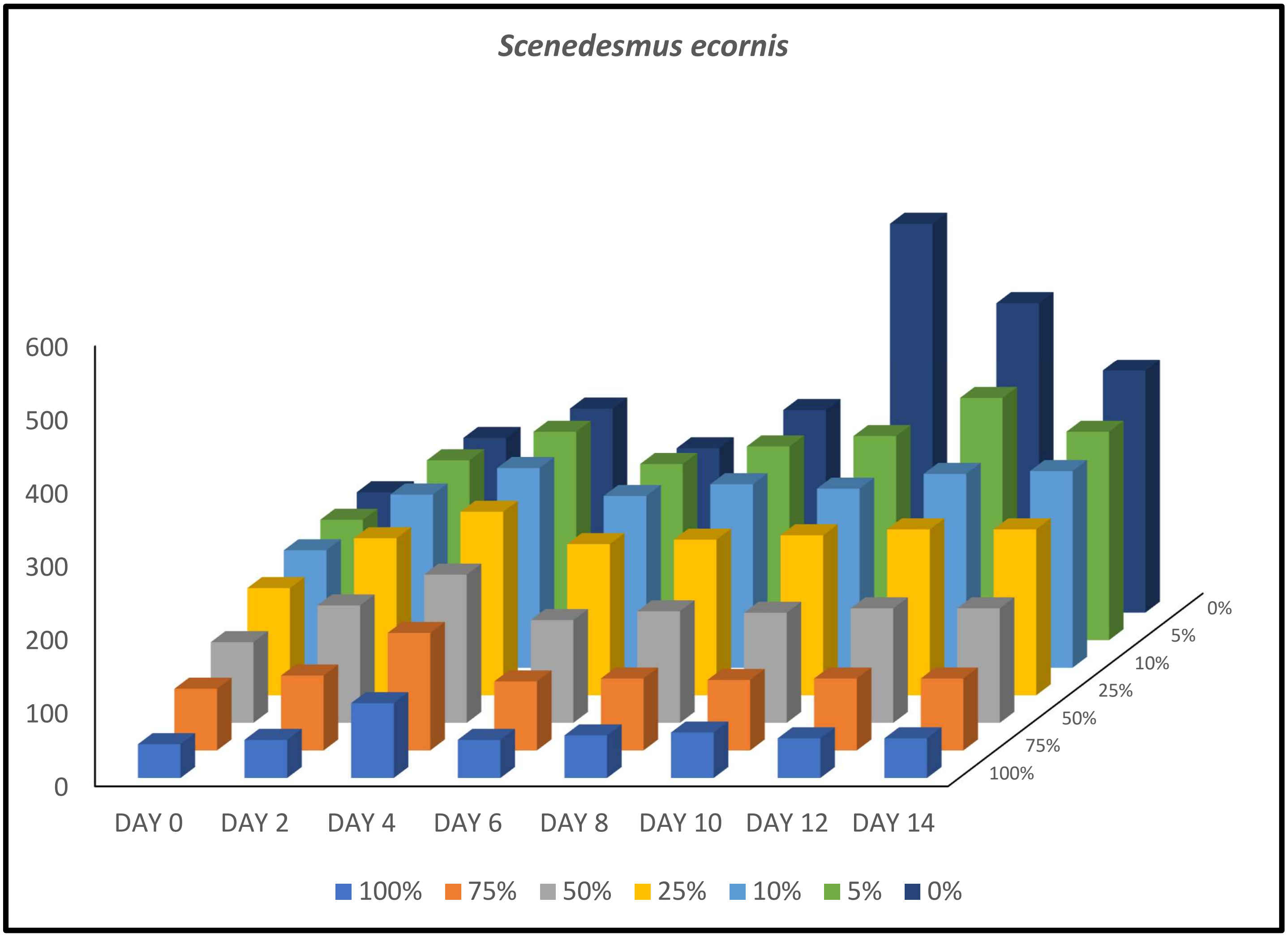

Figure 4.6c: Changes in Electrical Conductivity (EC) of *Scenedesmus ecornis* exposed to spent engine oil over time

4.7a Changes in pH of *Chlorella vulgaris* exposed to spent engine oil over time

Figure 4.7a shows that at the beginning of the experiment, the pH values range between 6.5 and 7.5 for all treatments. Over time, there was a gradual and consistent increase in pH across all concentrations. By day 8, the pH rises noticeably, reaching around 8.5-9.0, and by day 12 and 14, the pH values peak between 9.5 and 10 for most treatments. All treatments showed upward trends, the higher concentrations of spent engine oil (75% and 100%) showed slightly different patterns compared to lower concentrations and the control (0%).

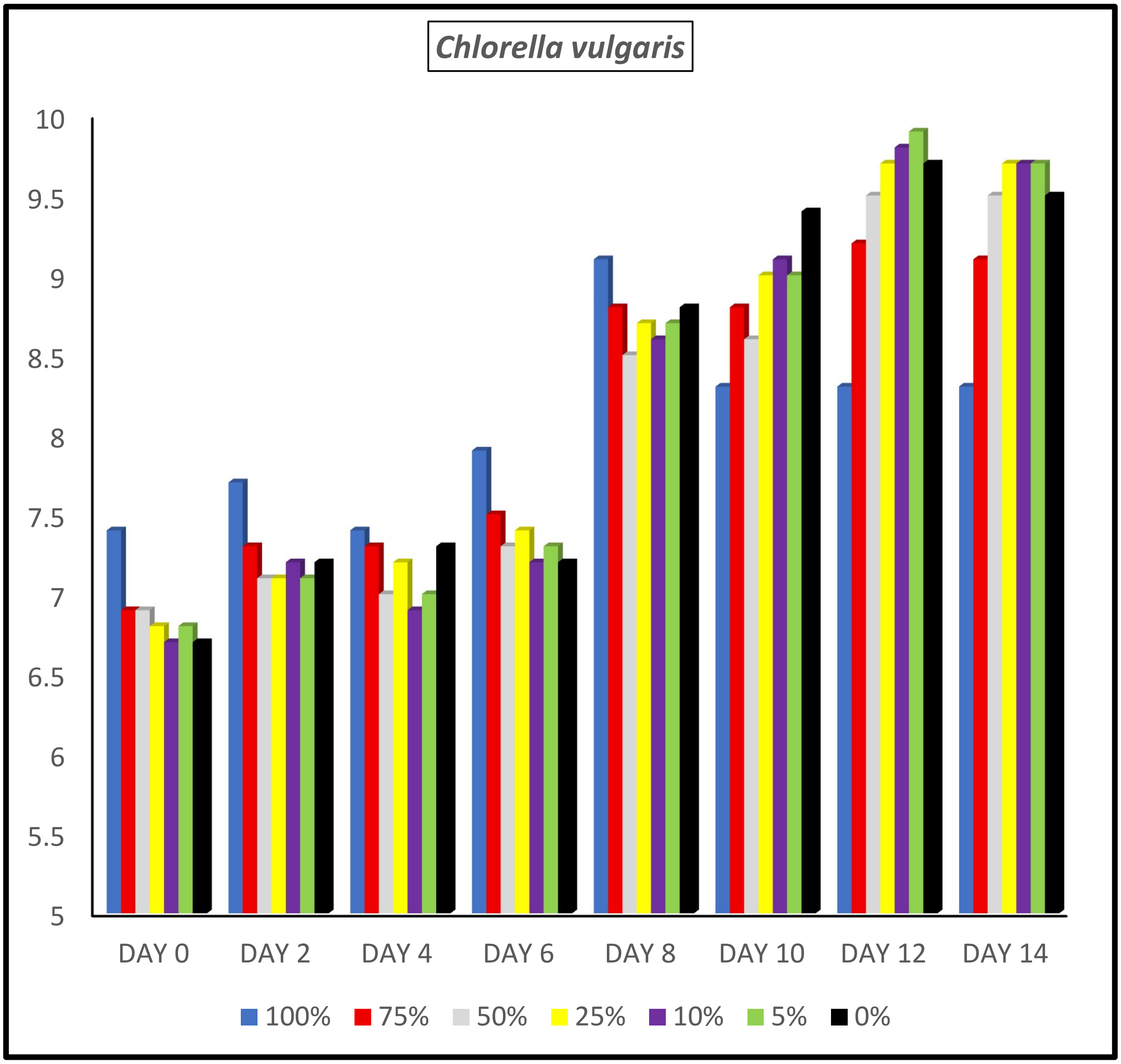

Figure 4.7a: Changes in pH of *Chlorella vulgaris* exposed to spent engine oil over time

4.7b Variation in Total Dissolved Solids (TDS) of *Chlorella vulgaris* exposed to spent engine oil over time

Figure 4.7b shows the variation in TDS of *Chlorella vulgaris* exposed to spent engine oil over time. At the start of the experiment (day 0), TDS values were relatively low across all treatments, and gradually increased as the days progress. Higher concentrations of spent engine oil (50%, 75% and 100%) consistently recorded higher TDS levels compared to the lower concentrations (0%, 5%, 10%, and 25%). By day 14, the 100% concentration exhibited the highest TDS value, while the control remained significantly lower.

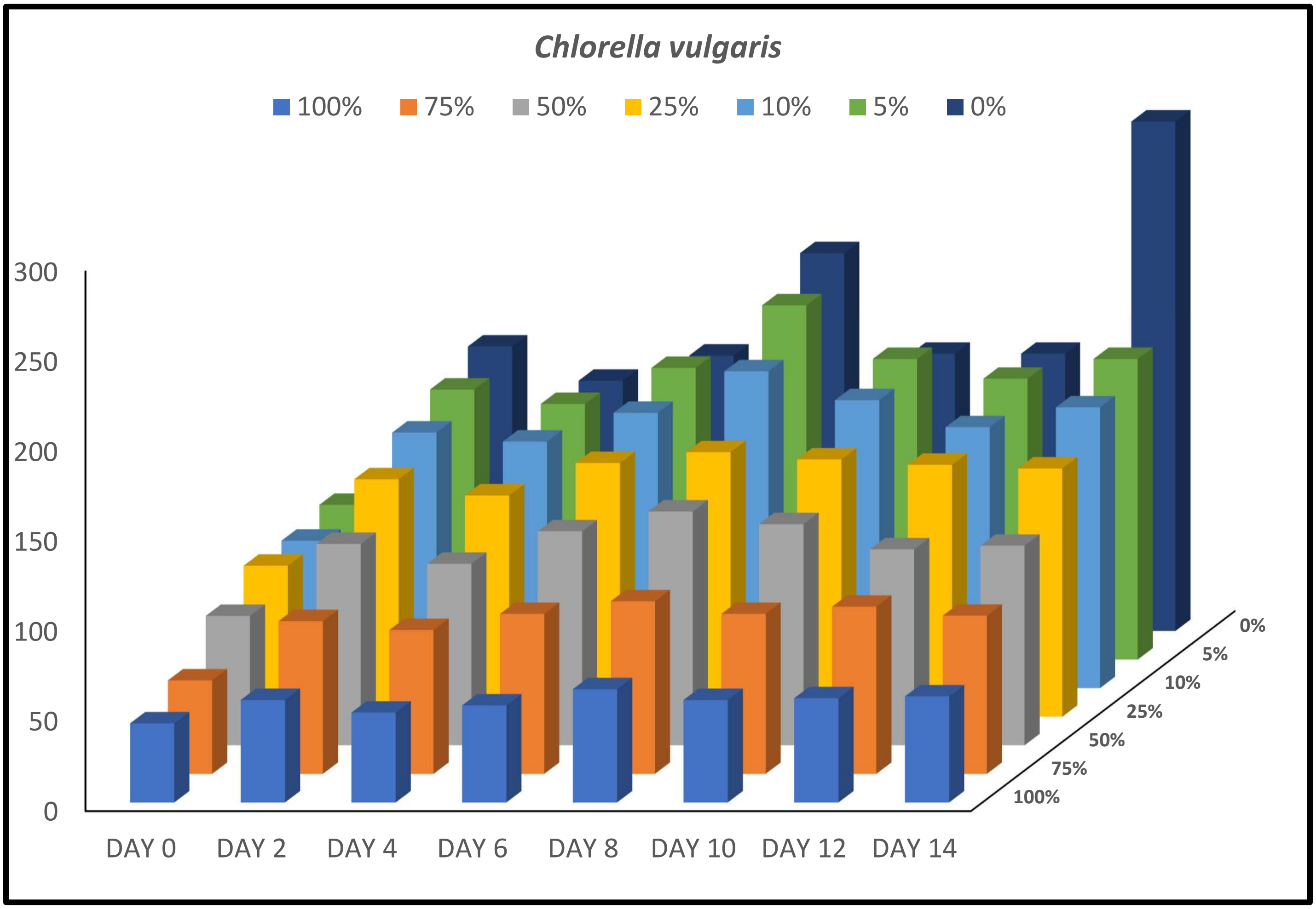

Figure 4.7b: Variation in Total Dissolved Solids (TDS) of *Chlorella vulgaris* exposed to spent engine oil over time

4.7c Changes in Electrical Conductivity (EC) of *Chlorella vulgaris* exposed to spent engine oil over time

The changes in electrical conductivity of *Scenedesmus ecornis* exposed to spent engine oil is represented in Figure 4.7c. The experiment began with all treatments showing relatively low EC values, and increased steadily as time progresses. Higher concentrations of spent engine oil (50%, 75% and 100%) displayed the most significant increase in EC throughout the experimental period. Lower concentrations (5%, 10%, and 25%) and the control (0%) showed smaller but consistent increase in EC, remaining comparatively lower than the higher concentrations.

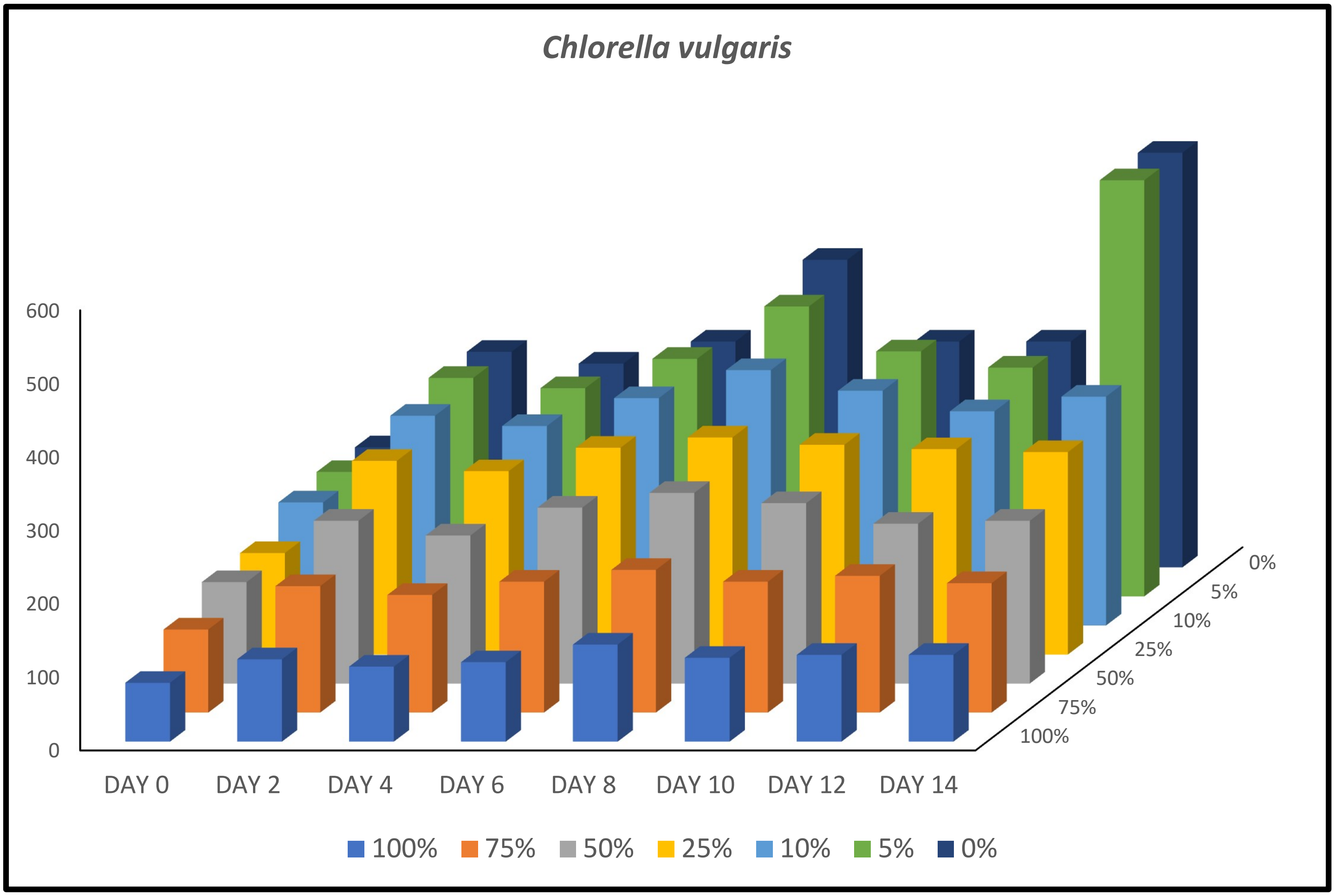

Figure 4.7c: Changes in Electrical Conductivity (EC) of *Chlorella vulgaris* exposed to spent engine oil over time

4.8a Changes in pH of *Scenedesmus ecornis* exposed to unspent engine oil over time

The changes in pH of *Scenedesmus ecornis* exposed to unspent engine oil is represented in Figure 4.8a. All treatments showed pH values between 7.0 and 8.0 at the beginning of the experiment. As the experiment progressed, the pH values gradually increased across all concentrations. By day 6, the pH began to increase, particularly in higher concentrations of unspent engine oil (50%, 75% and 100%). From day 8 onward, pH values remained high across all treatments, with the control (0%) and lower concentrations (5%-25%) showing slightly lower pH compared to the higher oil concentrations.

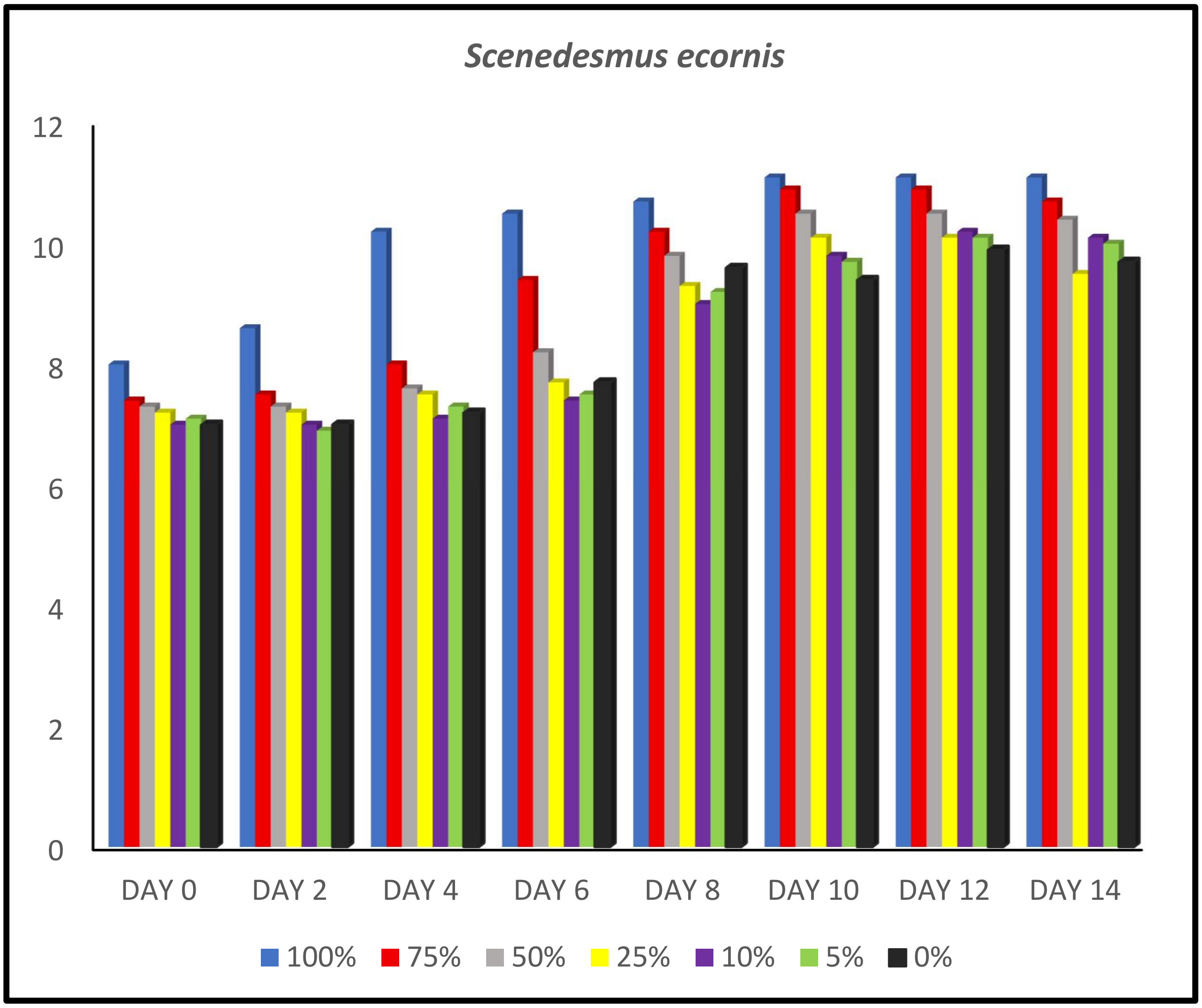

Figure 4.8a Changes in pH of *Scenedesmus ecornis* exposed to unspent engine oil over time

4.8b Variation in Total Dissolved Solids (TDS) of *Scenedesmus ecornis* exposed to unspent engine oil over time

Figure 4.8b shows that across all concentrations, the TDS levels were moderately low at the beginning of the experiments, with the control (0%) showing the highest initial reading. As time progressed, TDS values increased steadily across all treatments. By day 8 onward, the TDS levels in all treatments were relatively stable, though the values remained higher in the control and lower concentration groups (5%–25%) compared to higher concentrations (75% and 100%). The TDS values generally increased over time in all treatments.

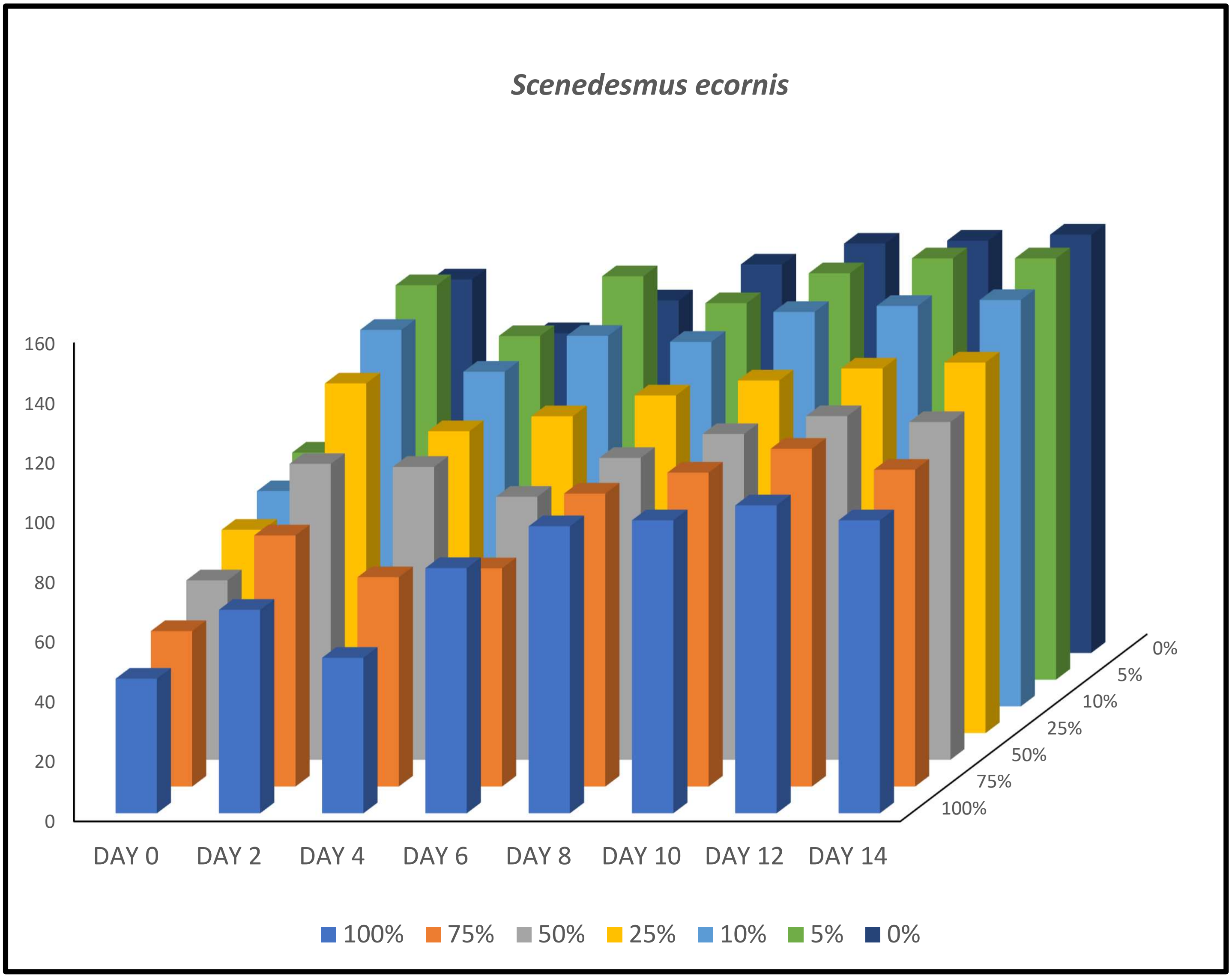

Figure 4.8b: Variation in Total Dissolved Solids (TDS) of *Scenedesmus ecornis* exposed to unspent engine oil over time

4.8c Changes in Electrical Conductivity (EC) of *Scenedesmus ecornis* exposed to unspent engine oil over time

The changes in electrical conductivity of *Scenedesmus ecornis* exposed to unspent engine oil is shown in Figure 4.8c. At day 0, EC values were fairly low across all treatments and as the days progressed, EC levels increased steadily. The control (0%) and lower concentrations (5%-25%) showed the highest EC values by the end of the experiment. In contrast, higher concentrations (75%-100%) exhibited a slower rate of EC increase, and by day 14, all treatments reached relatively stabled EC values.

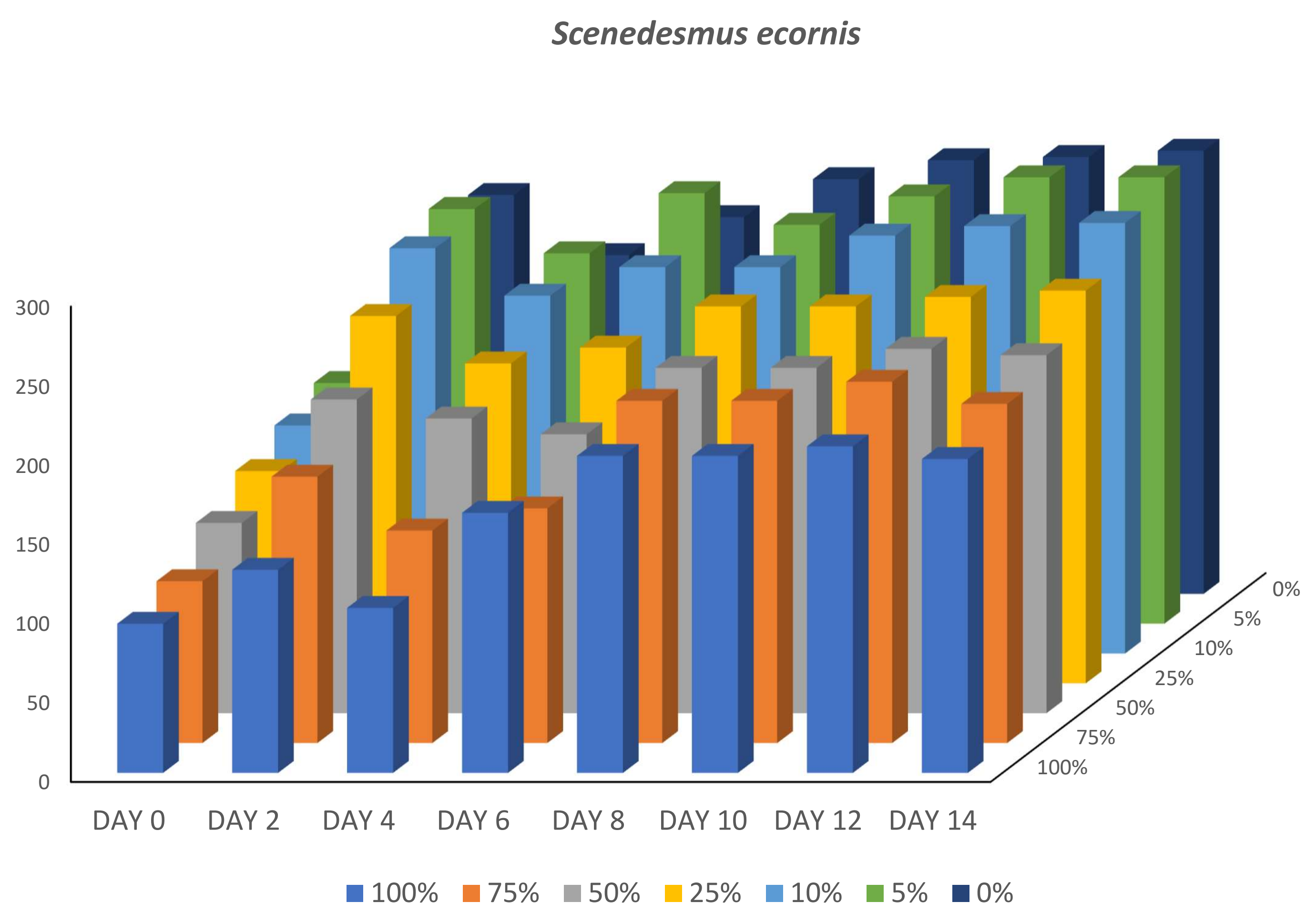

Figure 4.8c: Changes in Electrical Conductivity (EC) of *Scenedesmus ecornis* exposed to unspent engine oil over time

4.9a Changes in pH of *Chlorella vulgaris* exposed to unspent engine oil over time

Figure 4.9a shows that at day 0, the pH values ranged from approximately 7.0 to 8.0 across all treatments and increased over time. By day 6, the pH began to increase more noticeably, especially in the lower concentrations (5%-50%) and the control (0%). At higher concentrations (75% and 100%), the pH also increased but at a slower rate initially. By day 10 onward, all treatments showed pH values between 9 and 11 with the control (0%) and lower concentrations (5%-25%) maintaining slightly higher pH values.

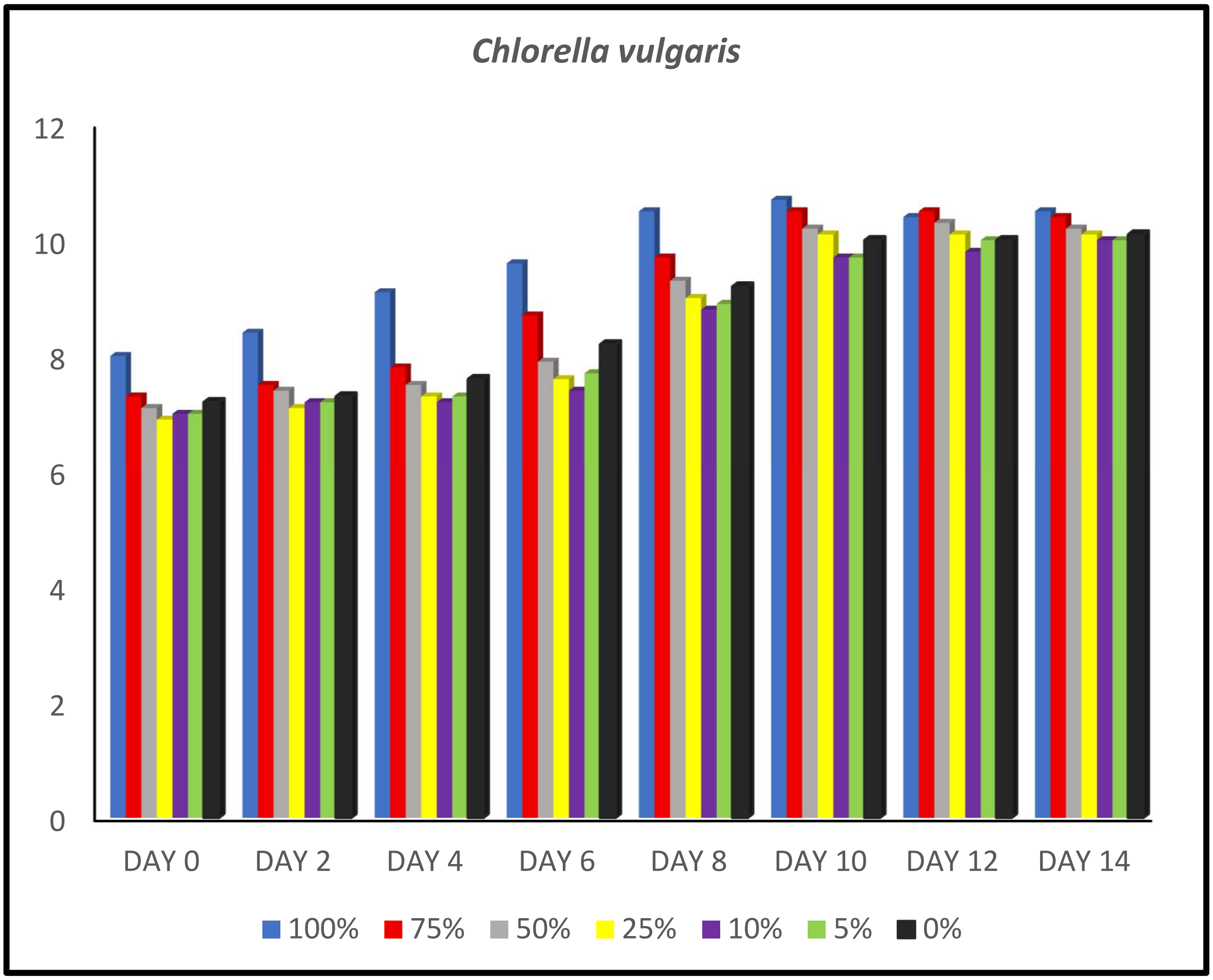

Figure 4.9a: Changes in pH of *Chlorella vulgaris* exposed to unspent engine oil over time

4.9b Variation in Total Dissolved Solids (TDS) of *Chlorella vulgaris* exposed to unspent engine oil over time

Figure 4.9b shows the variation in TDS of *Chlorella vulgaris* exposed to unspent engine oil over time. At day 0, the TDS values showed an initial variation across all the concentrations. As the days progressed, TDS values gradually increased in all treatments. By day 8-14, the TDS stabilized especially at higher concentrations (75% and 100%), while in lower concentrations (5%-25%), the TDS increase was steady and consistent.

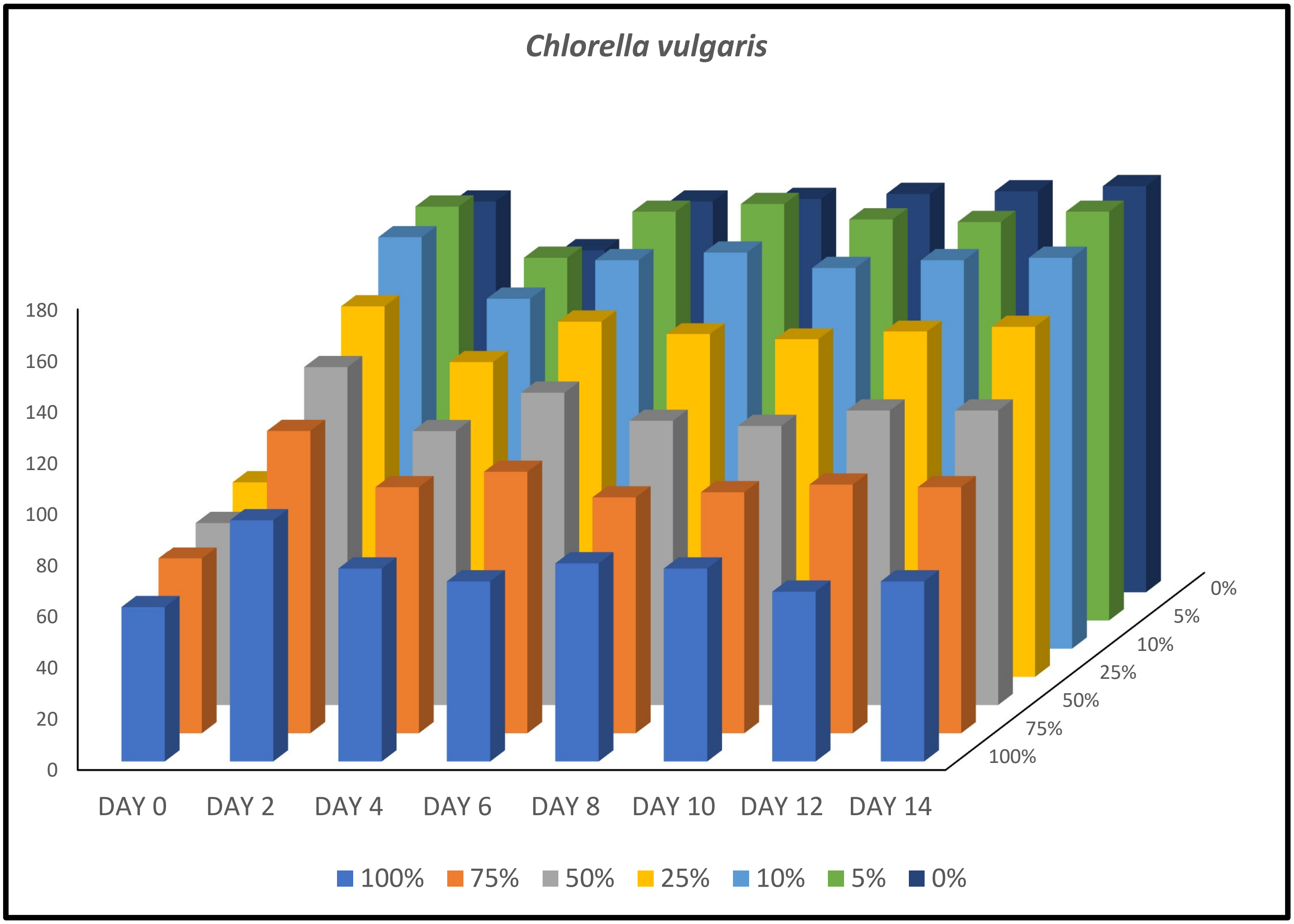

Figure 4.9b: Variation in Total Dissolved Solids (TDS) of *Chlorella vulgaris* exposed to unspent engine oil over time

4.9c Changes in Electrical Conductivity (EC) of *Chlorella vulgaris* exposed to unspent engine oil over time

The changes in electrical conductivity of *Chlorella vulgaris* exposed to unspent engine oil over time is represented in Figure 4.9c. The EC values showed a general increase over the 14-day period across all concentrations. At lower concentrations of unspent engine oil, the increase in conductivity was more pronounced and in contrast, higher concentrations showed a slower increase in conductivity. Toward the end of the experiment, the conductivity values tended to level off.

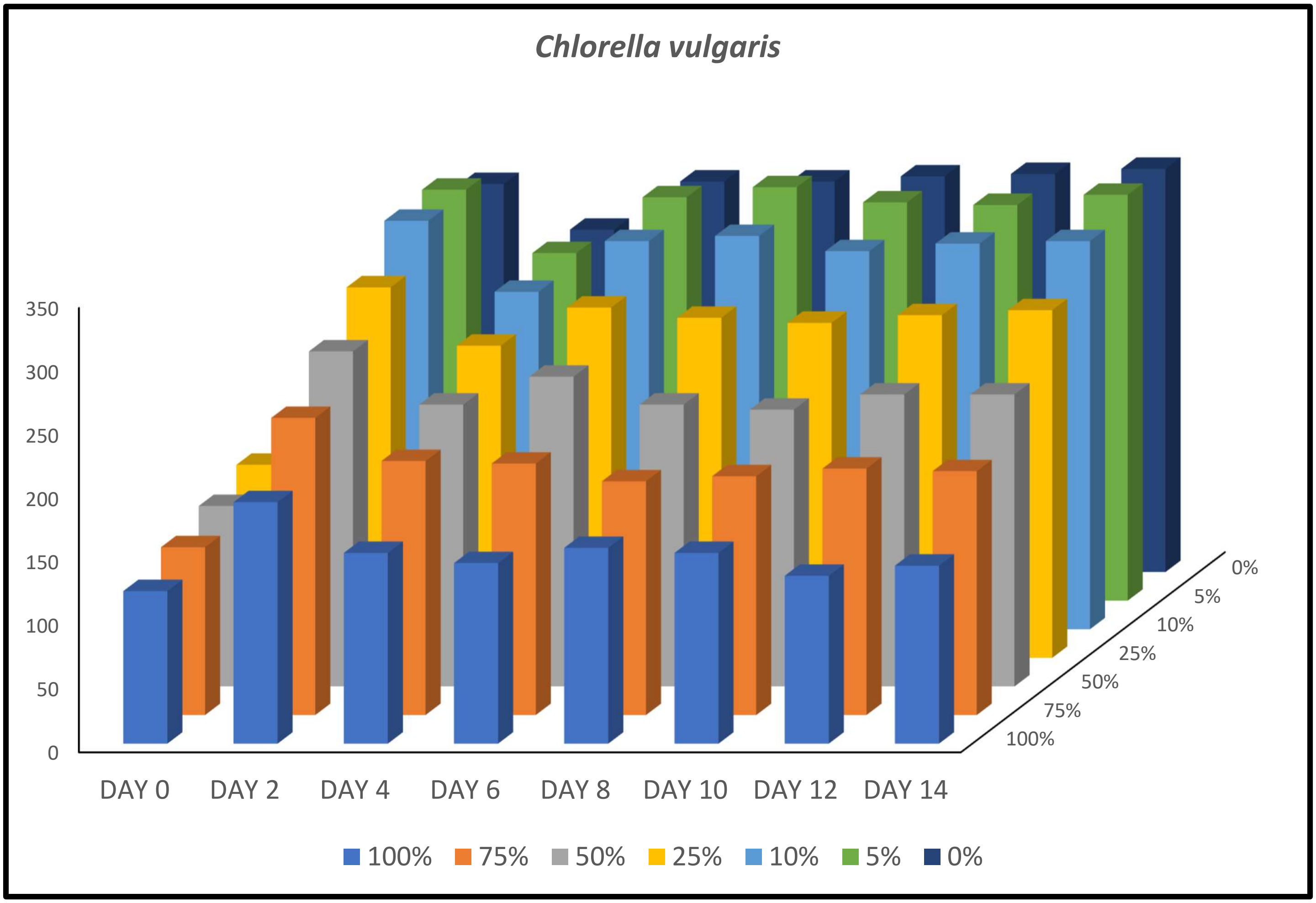

Figure 4.9c: Changes in Electrical Conductivity (EC) of *Chlorella vulgaris* exposed to unspent engine oil over time

CHAPTER FIVE

DISCUSSION

The improper disposal of spent and unspent engine oils is widespread in Nigeria, with mechanics and machine operators commonly releasing oils onto soil, into gutters, and nearby water bodies after servicing engines (Daniel *et al*., 2020). This widespread practice introduces harmful pollutants into the environment, often more frequently than crude oil spills. Once released into aquatic systems, these oils interact with water to form a water-soluble fraction. The water-soluble fraction forms when petroleum products (crude oil, diesel, gasoline, lubricants) enter water, dissolving lighter and more soluble hydrocarbons polycyclic aromatic hydrocarbons (PAHs), phenols, and metallic residues (De Santana *et al*., 2021). Both spent and unspent engine oils pose threats to aquatic life, though spent oil tends to be more toxic due to the accumulation of degraded additives, combustion by-products, and heavy metals. Additionally, it also interferes with photosynthesis, respiration, and nutrient uptake in algae.

The effect of spent and unspent engine oil on the growth of two freshwater microalgae (*Scenedesmus ecornis* and *Chlorella vulgaris*) was studied for a period of two weeks. In this study, the growth response of the test microalga; *Scenedesmus ecornis* and *Chlorella vulgaris* to different WSF concentrations of spent and unspent engine oil were examined.

Growth response of *Scenedesmus ecornis* and *Chlorella vulgaris* in spent engine oil

In the growth response of *Scenedesmus ecornis* in spent engine oil, there was a lag phase up to day 6 which indicated an adaptation period before growth, followed by an exponential growth between day 8 and day 10. Growth was the highest in the control (0%) and 5% treatments, which then progressively declined as concentration increased, showing a concentration dependent gradient. Overall, the lowest growth occurred in the higher (75% and 100%) concentrations. This study aligns with previous work of Kadiri and Eboigbodun (2012), which indicated that both microalgae used were impaired by the high concentrations of WSF of these fuel oils, while lower concentrations stimulated growth. In their studies, maximum growth was observed at 10% and 25%, indicating that at lower concentrations, certain compounds within the WSF provided carbon sources that temporarily supported growth. However, as concentrations increased, toxic compounds such as heavy metals, polycyclic aromatic hydrocarbons (PAHs) and oxidized residues accumulated, reducing photosynthetic efficiency and inhibiting cell division. From this present study it was shown that petroleum derived WSFs initially supported algal growth but became toxic at higher concentrations due to hydrocarbon buildup. This finding is corroborated by Ramadas *et al*. (2016). The ANOVA result revealed that it was statistically significant ($p< 0.001$) across the different WSF concentrations tested. This indicates that *Scenedesmus ecornis* was significantly affected by increasing levels of spent engine oil.

The growth response of *Chlorella vulgaris* in the WSF of spent engine oil increased gradually from day 0 to day 6, peaked between day 8 to day 10 at lower concentrations, and then declined rapidly at higher concentrations (75% and 100%). The lowest concentrations (0%, 5%, 10% and 25%) recorded the highest growth, suggesting that these levels provide nutrient-like conditions that supported the growth of *Chlorella vulgaris*. The stronger growth suppression at high concentrations indicates that *Chlorella vulgaris* is more sensitive to hydrocarbon and heavy metal stress than *Scenedesmus ecornis*. The study of Ramadas *et al*. (2016) found that spent oil WAF showed significant toxicity to the algal growth at 10% level, whereas in this study, the spent engine oil showed significant toxicity at higher concentrations (75% and 100%), which attributed this to oxidative stress that damages cell membrane and chlorophyll pigments. This observation is also supported by Olaleye and Kadiri (2021), who reported that marine microalgae exposed to petroleum

WSF experienced suppressed growth beyond 10%. Additionally, the ANOVA result indicated a statistically significant difference ($p< 0.0001$) across all days suggesting that changes observed were not random. Therefore, the reduced growth at higher concentrations reflects real biological inhibition caused by oil-derived toxicants.

Growth response of *Scenedesmus ecornis* and *Chlorella vulgaris* in unspent engine oil

The growth pattern of *Scenedesmus ecornis* in the WSF of unspent engine oil was similar to that observed in spent engine oil but with milder effects. Growth increased up to day 10 in the lower concentration treatments but declined at higher concentrations. The 5% and 10% recorded the highest growth level showing that these concentrations support optimal growth, followed closely by 0% and 25%, while higher concentrations suppressed growth. This suggests that unspent engine oil, though still containing hydrocarbons has lower toxicity, because it lacks the degraded compounds, metal particles, and oxidized products present in spent oil. This corresponds with Obayori *et al*. (2014), who reported that unspent oil retains its base additives and is less harmful at low concentrations but still capable of causing stress to aquatic microorganisms when concentrations are high. Olaleye and Kadiri (2021) also observed that the WSF of unspent engine oil affected algal photosynthesis mainly at higher concentrations due to light blockage and reduced gas exchange at the medium surface. The statistically significant p-values from ANOVA confirms that growth varied meaningfully with concentration and time. The trend of decreasing growth with increasing concentration demonstrates that despite being less toxic, unspent engine oil still negatively influences algal physiology.

In the growth response of *Chlorella vulgaris* in WSF of unspent engine oil, there was a steady increase from day 0 up to day 8, which was followed by stabilization and eventual decline at higher concentrations. Maximum growth occurred in the control and 5% treatments, while 100%

concentration exhibited minimal activity. This indicates that even unspent engine oil contains compounds that inhibit algal metabolism when sufficiently concentrated. Ramadas *et al*. (2016) similarly found that the WSF of unspent engine oil at low concentrations had negligible toxicity to *Chlorella* sp., but at higher concentrations caused significant inhibition due to the formation of oil film on the surface, reducing light and gas exchange. The study by Kadiri and Eboigbodin (2012) also observed similar trends in other green algae, reinforcing the consistency of this response. The ANOVA results ($p < 0.0001$) confirmed that these variations were statistically significant, showing that *Chlorella vulgaris* was consistently more affected than *Scenedesmus ecornis* across all treatments.

Dry weight of *Scenedesmus ecornis* and *Chlorella vulgaris* in spent engine oil

Dry weight is a fundamental metric in biology, ecology, agriculture, and environmental science for quantifying biomass, productivity, and nutrient content. It plays a vital role in energy production, environmental sustainability, and ecological balance (Bashan *et al*., 2016).

In this study, the cumulative dry weight of both *Scenedesmus ecornis* and *Chlorella vulgaris* decreased gradually as the concentration of spent engine oil increased. At 0%, where no oil was present, both species exhibited the highest biomass, indicating healthy growth under normal conditions. A slight reduction in dry weight was observed at 5% and 10%, showing that low levels of spent oil only mildly affected growth, but as the concentration reached 25%, a more noticeable decline occurred, suggesting the beginning of stress due to oil toxicity. At 50%, the dry weight dropped rapidly, while at 75% and 100%, growth was severely suppressed for both species, with *Scenedesmus ecornis* performing marginally better at full concentration. The overall trend shows that increasing levels of spent engine oil have a strong suppressive effect on algal biomass, most likely due to the toxic hydrocarbons and heavy metals in the oil that disrupt photosynthesis and nutrient uptake.

Dry weight of *Scenedesmus ecornis* and *Chlorella vulgaris* in unspent engine oil

The dry weight of both *Scenedesmus ecornis* and *Chlorella vulgaris* decreased gradually as the concentration of unspent engine oil increased. In the control (0%), both algae exhibited the highest biomass, reflecting normal growth in the absence of contaminants. At 5% and 10% concentrations, the dry weight remained relatively high, indicating that the presence of small amounts of unspent oil caused only slight reduction in biomass and that both species tolerated low concentrations well. Beyond this point, at 25%, a reduction in biomass became more noticeable, suggesting the onset of stress as the oil concentration increased. At 50% and 75%, the decline in dry weight became more pronounced, revealing the negative influence of the oil film, which likely restricted light penetration and gas exchange necessary for photosynthesis. At 100%, both species showed the lowest dry weight values, signifying severe suppression of growth under maximum oil exposure. Overall, *Scenedesmus ecornis* tended to perform slightly better than *Chlorella vulgaris* at higher concentrations, implying greater tolerance to unspent engine oil.

Percentage inhibition of *Scenedesmus ecornis* and *Chlorella vulgaris* in spent engine oil

The percentage inhibition result for both *Scenedesmus ecornis* and *Chlorella vulgaris* exposed to the WSF of spent engine oil showed a clear concentration-dependent relationship. At lower concentrations, both microalgae recorded low inhibition, indicating that the cells could still tolerate minimal levels of hydrocarbons. As concentration increased, growth declined progressively, leading to increased inhibition. This trend demonstrates that the toxic effect intensified with concentration, which is typical of petroleum-based pollutants. These findings correspond with those of Kadiri and

Eboigbodin (2012), who observed the growth inhibition of freshwater microalgae with increasing petroleum WSF concentration. In this study, between the two algae, *Chlorella vulgaris* exhibited higher inhibition than *Scenedesmus ecornis*, which means that *Chlorella vulgaris* was more sensitive to hydrocarbon stress. The difference in response can be related to the thinner cell wall and smaller size of *Chlorella*, which allow faster diffusion of toxic compounds, whereas *Scenedesmus* possesses a thicker, more resistant wall structure that offers partial protection.

Percentage inhibition of *Scenedesmus ecornis* and *Chlorella vulgaris* in unspent engine oil

In the unspent engine oil WSF, both *Scenedesmus ecornis* and *Chlorella vulgaris* showed increased percentage inhibition with increasing concentration, but the magnitude was notably lower than that observed in the spent engine oil treatments. Growth stimulations were observed at 5% and 10% concentrations in *Scenedesmus ecornis*, while *Chlorella vulgaris* had very low growth inhibition at same concentrations. The 100% concentration recorded the highest growth inhibition for both microalgae. These results are consistent with the observations of Olaleye and Kadiri (2014), and Obayori *et al*. (2014), who reported that unspent engine oil WSFs suppressed algal growth only at higher concentrations because of the surface film formation and oxygen limitation. Fresh lubricating oil retains stable hydrocarbons and additive compounds that have not undergone thermal degradation, and therefore contains fewer soluble metals and reactive by-products. Between the two microalgae, *Scenedesmus ecornis* again recorded lower inhibition percentages than *Chlorella vulgaris*, confirming higher tolerance to hydrocarbon stress. Similarly, Ramadas *et al*. (2016) found that *Chlorella* sp. exposed to unspent engine oil exhibited moderate inhibition compared with the same species in spent oil, demonstrating that degradation and metal contamination during oil use greatly enhance toxicity.

Comparative toxicity of spent and unspent engine oil on the growth of *Chlorella vulgaris*

The growth response and percentage inhibition of *Chlorella vulgaris* showed that the spent engine oil WSF was more toxic than the unspent engine oil WSF. In the spent oil treatments, growth of *Chlorella vulgaris* declined sharply with increasing concentration, and inhibition was evident even at the lowest concentrations. At 75% and 100% concentrations, growth was almost completely suppressed. In contrast, the unspent engine oil WSF produced only moderate inhibition at low to mid concentrations and stronger suppression only at 75% and 100%. These findings are consistent with Olaleye and Kadiri (2021), who reported that spent engine oil WSFs produced more severe growth suppression in *Chlorella* sp. than unspent oil, due to accumulated heavy metals and degraded hydrocarbons. This pattern indicates that while both oils negatively affected algal growth, the spent oil exhibited a much greater inhibitory effect, confirming that it contains more soluble toxic compounds. During engine oil use, lubricating oil undergoes oxidation, combustion, and contamination leading to the formation of degraded hydrocarbons, metallic additives, and oxidized residues. Similarly, Amaeze *et al*. (2014) observed that used oils have greater solubility of toxic compounds, making them more lethal to aquatic microorganisms.

Comparative toxicity of spent and unspent engine oil on the growth of *Scenedesmus ecornis*

For *Scenedesmus ecornis*, a similar pattern was observed, although this species demonstrated greater resilience to both oil types compared to *Chlorella vulgaris*. Growth in spent oil WSF declined with increasing concentration, showing high inhibition at 75% and 100%. However, the level of inhibition was slightly lower than that seen in *Chlorella vulgaris* under the same conditions. When *Scenedesmus ecornis* was exposed to the WSF of unspent engine oil, it showed lower inhibition values. Growth reduction occurred only at higher concentrations, and the cells appeared to tolerate the lower levels (5%-25%) better suggesting that *Scenedesmus ecornis* has a stronger physiological tolerance to petroleum-induced stress. This trend agrees with Ogbebor and Ekemhankhomhen (2024), who observed that *Scenedesmus* species generally possess thicker cell walls, better adaptive mechanisms, and more efficient detoxification systems than *Chlorella* species, allowing them to tolerate hydrocarbon contamination more effectively. The difference between the spent and unspent engine oil is again linked to the degree of chemical degradation.

Physicochemical Parameters of *Scenedesmus ecornis* in Water-Soluble Fraction of Spent Engine Oil

Physicochemical parameters are essential tools for evaluating the quality and condition of water, soil, and other environmental samples. They provide a scientific basis for environmental monitoring, pollution control, and sustainable management of natural resources (Qureshi *et al*., 2021). The physicochemical parameters used in this study were pH, TDS and EC.

In this study, the pH values for *Scenedesmus ecornis* exposed to the WSF of spent engine oil showed a gradual increase with time and concentration. The pH across all treatments of *Scenedesmus ecornis* ranged around 7.0, indicating a neutral environment. As exposure continued, pH increased progressively in all concentrations. By days 8-14, the pH values increased between 8.0 and 10.0, with the 100% concentration peaking near pH 10.0. The lower concentrations (0%-25%) showed smaller increases (to about pH 8.0-8.5), while higher concentrations (50%-100%) exhibited stronger alkalinization. This consistent increase suggests that chemical and biological processes such as hydrocarbon oxidation and the release of basic ions (Na^+, Ca^{2+}, Mg^{2+}) were altering the culture medium. The breakdown of petroleum hydrocarbons releases alkaline by-products that shift the medium toward basic conditions (Okwuego *et al*., 2025).

In *Scenedesmus ecornis*, the TDS values decreased steadily over time for all treatments. At the beginning of the experiment, TDS values were higher reflecting initial stabilization of oil components, but by day 14 they had declined remarkably. The decrease likely indicates that the dissolved compounds were being taken up and settled as insoluble residues during algal metabolism and hydrocarbon degradation. In the control and low concentrations (0%-25%), the reduction in TDS reflects active algal assimilation of nutrients and organic materials, while in higher concentrations (50%-100%), the sharp decline could result from the adsorption of oil particles, which caused dissolved compounds to settle out. Chinweuba *et al*. (2024) in his study noted that over time, petroleum WSFs can form aggregates that precipitate, leading to apparent TDS reduction even in contaminated water.

The EC followed a similar pattern to TDS, decreasing gradually throughout the experiment. The initial EC readings were higher at day 0, then dropped as exposure continued. This pattern reflects a decline in ionic mobility, likely due to the precipitation or adsorption of charged particles onto oil films or cell surfaces. In polluted aquatic systems, hydrocarbons can trap ions, which reduces conductivity. The decrease in EC also coincides with reduced algal metabolic activity at higher concentrations, resulting in less ionic exchange between the cells and medium. This observation agrees with Amaeze *et al*. (2014), who found that long-term petroleum exposure reduced EC as ions became less available for conduction.

Physicochemical Parameters of *Chlorella vulgaris* in Water-Soluble Fraction of Spent Engine Oil

The pH of *Chlorella vulgaris* cultures started between 6.5 and 7.5 but increased progressively to 9.5-10.0 by days 12-14. The control (0%) and lower concentrations showed steady increases, while higher concentrations (75% and 100%) had slightly irregular patterns but ultimately reached similar alkaline levels. This alkalinity rise indicates accumulation of basic degradation products and a reduction in biological CO_2 production as algal metabolism slowed under toxicity. Kadiri and Eboigbodin (2012) observed comparable increases, explaining that hydrocarbon degradation and ionic buildup shift pH upward in polluted media.

TDS values were highest at day 0 across all concentrations and decreased gradually with time. The reduction was consistent across the entire 14-day period. At lower concentrations (5%-25%), the decline was gradual, while in higher concentrations (75% and 100%), TDS levels dropped sharply between day 6 and day 14. By the final observation day, TDS values were considerably lower than their initial readings in every treatment. This is due to adsorption of dissolved hydrocarbons to cell walls, sedimentation of oil droplets, and biological utilization of soluble organic matter by the algae during the early growth phase. The decreasing trend indicates that *Chlorella* assimilated part of the dissolved substances. Ramadass *et al*. (2015) also observed declining TDS over time in oil-polluted microalgal systems, attributing it to oil-water phase separation during prolonged incubation.

The EC pattern for *Chlorella vulgaris* mirrored the TDS trend. Initial EC values were relatively high, but declined progressively as exposure time increased. The rate of decline was more pronounced in the higher concentrations, with the 100% treatment showing the lowest EC across all days. The control and lower concentrations had smaller differences over time but still showed downward movement. Lower EC values at later stages indicate reduced ionic mobility and possible ion binding to organic molecules. Olaleye and Kadiri (2021) reported similar patterns where EC dropped as ions became less available due to hydrocarbon aggregation. Both TDS and EC decreased throughout, with *Chlorella vulgaris* showing slightly higher declines than *Scenedesmus ecornis* under similar conditions.

Physicochemical Parameters of *Scenedesmus ecornis* in Water-Soluble Fraction of Unspent Engine Oil

The pH values at day 0 ranged between 7.0 and 8.0 across all treatments showing a slightly alkaline but stable environment. With time, pH gradually increased, especially in higher concentrations (50%-100%), stabilizing between 8.0 and 9.0 by day 14. Lower concentrations (0%:25%) maintained slightly lower pH. These modest increase in alkalinity suggests limited chemical dissolution of oil components. Unspent engine oil contains fewer oxidized compounds, so fewer ions were released to alter the acidity of the medium. According to Okwuego *et al*. (2025), small pH shifts in hydrocarbon-exposed water indicates partial oxidation of hydrocarbons and weak ion exchange, consistent with the mild conditions observed here. The slight alkalinization also suggests that *Scenedesmus ecornis* tolerated the unspent oil stress better, maintaining relatively balanced metabolic activity throughout exposure.

In the WSF of unspent engine oil, *Scenedesmus ecornis* showed lower initial TDS values at day 0 compared to the spent oil treatments. These TDS values then declined progressively throughout the 14-day period. The reduction was evident across all concentrations, though it occurred more slowly in lower concentrations (5%-25%) and more rapidly in higher ones (75% and100%). The control remained relatively stable, showing only minor decreases over time.

The EC showed a similar declining trend, as the values were moderate at day 0 and decreased steadily with exposure duration. The drop in EC was consistent across all concentrations, with the highest reduction observed in the 75% and 100% treatments. By day 14, EC values had dropped to their lowest points in all treatments. Overall, both TDS and EC in *Scenedesmus ecornis* cultures exposed to unspent oil showed gradual decreases over time, with higher oil concentrations recording greater reductions.

Physicochemical Parameters of *Chlorella vulgaris* in Water-Soluble Fraction of Unspent Engine Oil

At day 0, the pH values of *Chlorella vulgaris* ranged between 7.0 and 8.0, similar to those of *Scenedesmus ecornis*. With continued exposure, the pH increased between 9.0-11.0 by day 10-14, particularly in higher concentrations (50%-100%). The stronger alkalinization suggest that chemical interactions between the algal metabolites and unspent engine oil compounds were more pronounced in *Chlorella* cultures. This pattern shows that even unspent oil can alter pH, though the trend reflects milder chemical reactions and biological adjustments. A study of Kadiri and Eboigbodin (2012) supports the findings, which observed that algal stress and slowed photosynthesis leads to reduced acidification and thus higher medium pH in polluted cultures.

TDS decreased steadily with time and concentration. The highest initial readings dropped considerably by day 14, showing loss of soluble materials from the water phase. This decline may be attributed to adsorption of hydrocarbons to the algal surface, oil film accumulation, and biochemical transformation of soluble organics. Since unspent oil has limited solubility, much of the dissolved fraction likely degraded under light exposure.

The EC values also declined consistently through the experiment. Early in the exposure, EC was slightly higher, but it dropped in all concentrations as time progressed. This shows that ionic species became less available as hydrocarbons aggregated and as algal activity decreased. The decrease in EC parallels the TDS reduction and confirms a general decline in ionic strength of the medium over time.

CONCLUSION

This study demonstrated that the growth of *Scenedesmus ecornis* and *Chlorella vulgaris* is adversely affected by the water-soluble fractions of spent and unspent engine oils. As concentration increased, the growth reduction became more pronounced with spent engine oil having more toxic effects than unspent engine oil. *Scenedesmus ecornis* exhibited higher tolerance than *Chlorella vulgaris*, suggesting that it might have a higher capacity for adaptation when used in the bioremediation of petroleum-contaminated water. The findings demonstrate that primary producers in aquatic systems can be impacted by even low levels of engine oil contamination, thereby threatening ecosystem balance.

RECOMMENDATION

1. Proper disposal and recycling of spent engine oil should be enforced to prevent entry into aquatic environments.
2. There should be routine monitoring of water bodies in urban and industrial areas to identify hydrocarbon pollution early.
3. Further studies should assess biochemical and antioxidant enzyme responses of *Scenedesmus ecornis* and *Chlorella vulgaris* to hydrocarbon exposure.
4. In aquatic systems contaminated by oil, *Scenedesmus ecornis* should be investigated as a potential candidate species for bioremediation.
5. To reduce the discharge of petroleum products into natural ecosystems, environmental regulations and public awareness should be reinforced.

REFERENCES

Ahmad, A., Tseng, W., Ambartsumov, T., Kumar, A., Siddiqui, S., Michalek, J., Wu, Y., Srikanth, S. and Kalita, T. (2024). Different types of algae beneficial for bone health in animals and in humans – A review. *Algal Research*, **82**: 103593.

Amaeze, N. H., Onadeko, A. and Nwosu, C. C. (2014). Comparative acute toxicity and oxidative stress responses in tadpoles of *Amietophrynus regularis* exposed to refined petroleum products, unused and spent engine oils. *African Journal of Biotechnology*, **13**(45): 4251-4258.

Anwar, A., Samad, O., Mohammed, S., El-Haroun, E., Ragab, S. and Van Doan, H. (2025). Common Carp (*Cyprinus carpio*) exposure to water-soluble fraction (wsf) of crude oil: evaluation of protective effects of dietary *Rosmarinus officinalis* leaf on water quality, growth performance, oxidative stress, stress hormones and heat shock proteins. *Annals of Animal Science*, **25**(3): 1035-1044.

Baggio, G., Smakhtin, V. and Qadir, M. (2021). Freshwater availability status across countries for human and ecosystem needs. *Science of the Total Environment*, **792**: 148230.

Bamidele, J. F. and Eshagberi, G. O. (2015). Effects of water-soluble fractions of crude oil, diesel fuel and gasoline on *Salvinia nymphellula* (Desv.). *Journal of Natural Science Research*, **5**(14): 31-37.

Bashan, Y., Huang, P., Kloepper, J. and De-Bashan, L. (2016). A proposal for avoiding fresh-weight measurements when reporting the effect of plant growth-promoting (rhizo) bacteria on growth promotion of plants. *Biology and Fertility of Soils*, **53**: 1-2.

Bello, B. O., Agbo, S. O., Olumayede, G. E. and Lawal, O. S. (2024). Acute toxicity and inhibitory response of marine microalgae (*Skeletonema costatum* Cleve 1873) exposed to water-soluble fractions of crude oil, diesel, spent engine oil and their composite mixture. *Renewable*, **4**(1): 1-9.

Cavalletti, E., Romano, G., Esposito, F., Barra, L., Chiaiese, P., Balzano, S. and Sardo, A. (2022). Copper effect on microalgae: toxicity and bioremediation strategies. *Toxics*, **10**(9): 527.

Chen, J., Qian, H., Lu, T., Zhang, Z., Chen, J., Zhang, Q. and Hu, B. (2021). Pollutant toxicology with respect to microalgae and cyanobacteria. *Journal of Environmental Sciences*, **99**: 175-186.

Chinweuba, A. J., Okwuego, P. O. and Omoh, T. O. (2024). The comparative toxicities of two petroleum products, automotive gas oil, ago and premium motor spirit, pms on fresh and brackish water habitat. *International Journal of Chemistry and Chemical Processes*, **10**(5): 23-40.

Cui, J., Rajasekar, A., Ganapathy, N., Parthipan, P., Kumar, A., Dhandapani, P., Choi, M., Sarma, H., Min, A., Gao, X., Elumalai, P. and Theerthagiri, J. (2024). Impact of petroleum hydrocarbon and heavy metal pollution on coral reefs and mangroves: a review. *Environmental Chemistry Letters*, **22**(3): 1413-1435.

Daniel, U., Utuh, I. and Nwaichi, E. (2020). Profile of polycyclic aromatic hydrocarbons in soil environment around selected auto-technicians' workshop in Obio-Akpor, Rivers State Nigeria. *Journal of Environmental Protection*, **10**(5): 399-407.

Davies, E., Liu, Y., Niyogi, D., Teuling, A., He, Y., Hejazi, M., Kyle, P., Miralles, D. and Kim, S. (2016). Global and regional evaluation of energy for water. *Environmental Science and Technology*, **50**(17): 9736-9745.

De Santana, D., Perina, F., Lourenço, R., Da Silva, J., Moreira, L. and De Souza Abessa, D. (2021). Levels of hydrocarbons and toxicity of water-soluble fractions of maritime fuels on neotropical invertebrates. *Ecotoxicology*, **30**: 2109-2118.

Ďurišová, S., Papučová, I., Moricová, K. and Janík, R. (2020). Determination of engine oil characteristics. *IOP Conference Series: Materials Science and Engineering*, **776**(1): 012099.

Eshagberi, G. O. (2017). Toxic effects of water-soluble fractions of crude oil, diesel and gasoline on *Ceratophyllum demersum*. *International Journal of Health and Medicine*, **2**(2): 6-9.

Ezenweani, R. S. and Kadiri, M. O. (2023). Resilience and bio-response of two marine algae to petroleum fuel pollution. *Applied Phycology*, **4**(1): 54-77.

Ezenweani, R. S. and Kadiri, M. O. (2024). Evaluating the productivity and bioremediation potential of two tropical marine algae in petroleum hydrocarbon polluted tropical marine water. *International Journal of Phytoremediation*, **26**(7): 1099-1116.

González, R., Häder, D., Villafañe, V., Villafañe, V., Banaszak, A., Narvarte, M., Helbling, E. and Helbling, E. (2020). Anthropogenic pollution of aquatic ecosystems: Emerging problems with global implications. *Science of the Total Environment*, **713**: 136586.

Hassan, A., Aboudi-Mana, S., Hamid, F. and Ossai, I. (2024). Ecotoxicological effects, human and animal health risks of pollution and exposure to waste engine oils: a review. *Environmental Geochemistry and Health*, **46**(10): 416.

Hazaimeh, M. and Ahmed, E. (2021). Bioremediation perspectives and progress in petroleum pollution in the marine environment: a review. *Environmental Science and Pollution Research*, **28**: 54238-54259.

Horvatic, J., Palijan, G. and Lukavsky, J. (2003). Algal responses to nutrient additions in water of Nature Park Kopacki Rit (Croatia) by miniaturized algal growth bioassay. *Algological Studies*, **110**: 117-126.

Isa, K., Hairunnaja, M., Rahman, N., Ali, U., Arifin, M., and Aziz, M. (2023). Innovative formulation and characterisation of grease made from waste engine oil. *Pertanika Journal of Science and Technology*, **3**(5): 17.

Kadiri, M. and Eboigbodin, A. (2012). Phytotoxicity assessment of water-soluble fractions of refined petroleum products using microalgae. *Acta Botanica Hungarica*, **54**(3-4): 301-311.

Khoo, K., Peter, A., Ling, T., Chang, J., Chew, K., Show, P. and Ho, S. (2021). Microalgae for biofuels, wastewater treatment and environmental monitoring. *Environmental Chemistry Letters*, **19**: 2891-2904.

Komolafe, R. J., Akinola, O. M. and Agbolade, O. J. (2015). Effect of petrol and spent oil on the growth of Guinea Corn (*Sorghum bicolor* L.). *International Journal of Plant Biology*, **6**(1): 5883.

Lemuel, A., Hassan, H., Makpo, J., Abdulmalik, A. and Andrew, N. (2020). Sublethal effects of the water-soluble fractions of virgin diesel oil on some physiological parameters of *Clarias gariepinus* juveniles. *Nigerian Annals of Pure and Applied Sciences*, **3**(1): 14-32.

Li, P. and Wu, J. (2023). Water resources and sustainable development. *Water*, **16**(1): 134.

Liu, F., Huang, Q., Du, Y., Li, S., Cai, M., Huang, X., Zheng, F. and Lin, L. (2023). The interference of marine accidental and persistent petroleum hydrocarbons pollution on primary biomass and trace elements sink. *Science of the Total Environment*, **883**: 163812.

Menció, A. and Mas-Pla, J. (2018). Groundwater nitrate pollution and climate change: learnings from a water balance-based analysis of several aquifers in a western Mediterranean region (Catalonia). *Environmental Science and Pollution Research International*, **26**: 2184-2202.

Mendes, M. C., Navalho, S., Ferreira, A., Paulino, C., Figueiredo, D., Silva, D., Gao, F., Gama, F., Bombo, G., Jacinto, R., Aveiro, S. S., Schulze, P. S. C., Gonçalves, A. T., Pereira, H., Gouveia, L., Patarra, R. F., Abreu, M. H., Silva, J. L., Navalho, J. and Speranza, L. G. (2022). Algae as food in europe: an overview of species diversity and their application. *Foods*, **11**(13): 1871.

Mishra, I. and Kundu, P. (2018). Treatment and reclamation of hydrocarbon-bearing oily wastewater as a hazardous pollutant by different processes and technologies: a state-of-the-art review. *Reviews in Chemical Engineering*, **35**: 73-108.

Monteiro, L., Moens, T., Lynen, F. and Traunspurger, W. (2019). Effects of the water-soluble fraction of a crude oil on freshwater meiofauna and nematode assemblages. *Ecotoxicology and Environmental Safety*, **176**: 186-195.

Moreira, L., Lourenço, R., De Souza Abessa, D., De Santana, D., Da Silva, J. and Perina, F. (2021). Levels of hydrocarbons and toxicity of water-soluble fractions of maritime fuels on neotropical invertebrates. *Ecotoxicology*, **30**: 2109-2118.

Mousaviyon, Z., Pourkhabbaz, H. R., Banaee, M., Khodadoust, S., Pourkhabbaz, A. R., Trivedi, A., Faggio, C. and Multisanti, C. R. (2025). Toxicity of crude oil wastewater treated with Nano-ZnO as a photocatalyst on *Labeo rohita*: a biochemical and physiological investigation. *Journal of Xenobiotics*, **15**(1): 25.

Müller, J. B., Melegari, S., Perreault, F. and Matias, W. G. (2019). Comparative assessment of acute and chronic ecotoxicity of water-soluble fractions of diesel and biodiesel on *Daphnia magna* and *Aliivibrio fisheri*. *Chemosphere*, **221**: 640-646.

Musie, W. and Gonfa, G. (2023). Fresh water resource, scarcity, water salinity challenges and possible remedies: A review. *Heliyon*, **9**(8): e18685.

Nour, A., Nour, A., Elamin, E. and Alara, O. (2021). Dataset on the recycling of used engine oil through solvent extraction. *Chemical Data Collections*, **31**: 100598.

Obayori, O. S., Salam, L. B. and Ogunwumi O. S. (2014) Biodegradation of fresh and used engine oils by *Pseudomonas aeruginosa* LP5. *Journal of Bioremediation and Biodegradation*, **5**: 213.

Ogbebor, J. U. and Ekemhankhomhen, O. D. (2024). Toxicity of water-soluble fractions of petrol on some freshwater microalgae. *African Scientist Journal*, **25**(1): 103-109.

Okwuego, P. O., Oshokha, O. T. and Oragwu, I. P. (2025). Analysis of physicochemical properties of water-soluble fractions of automative gas oil (AGO), and premium motor spirit (PMS) in fresh and brackish water habitat. *International Journal of Geography and Environmental Management*, **11**(6): 107.

Olaleye, Y. O. and Kadiri, M. O. (2021). Toxicity of water accommodated fractions of waste engine oil on growth of selected marine algae. *West African Journal of Fisheries and Aquatic Sciences*, **2**(1): 1-8.

Onwusiri, K. C., Aguoru, C. U. and Akomolafe, G. F. (2017). Effect of spent engine oil on germination and growth parameters of fluted pumpkin (*Telfairia occidentalis* Hook F.) in Makurdi, Benue State, Nigeria. *Journal of Research in Forestry, Wildlife and Environment*, **9**(4): 1-8.

Qureshi, S., Channa, A., Memon, S., Khan, Q., Jamali, G., Panhwar, A. and Saleh, T. (2021). Assessment of physicochemical characteristics in groundwater quality parameters. *Environmental Technology and Innovation*, **24**: 101877.

Ramadass, K., Megharaj, M., Venkateswarlu, K. and Naidu, R. (2016). Sensitivity and antioxidant response of *Chlorella* sp. MM3 to used engine oil and its water accommodated fraction. *Bulletin of Environmental Contamination and Toxicology*, **97**(1): 71-77.

Ramadass, K., Megharaj, M., Venkateswarlu, K. and Naidu, R. (2015). Toxicity and oxidative stress induced by used and unused motor oil on freshwater microalga, *Pseudokirchneriella subcapitata*. *Environmental Science and Pollution Research,* **22**: 8890–8901.

Rivilli. P., Alarcon. R., Isasmendi. G. L. and Perez, J. D. (2011). Stepwise isothermal fast pyrolysis (SIFP). Part II. SIFP of peanut shells – Antifungal properties of phenolic fractions. *Bioresources*, 7(1): 1000-1014.

Salinas-Whittaker, S., Gómez-Gutiérrez, C. M., Cordero-Esquivel, B., Luque, P. A. and Guerra-Rivas, G. (2020). Effects of the water-soluble fraction of the mixture fuel oil/diesel on the microalgae *Dunaliella tertiolecta* through growth. *Environmental Science and Pollution Research International*, **27**(28): 35148-35160.

Santana, N., Mothé, M., Silva, G. and Mothé, C. (2022). Evaluation of lubricating oil in marine diesel engine using thermal analysis, FTIR, and rheology. *Journal of Thermal Analysis and Calorimetry*, **147**: 13261-13274.

Sarmento, H., Melão, M., De Araujo Paina, K., Gebara, R. and De Oliveira Gonçalves Alho, L. (2019). Responses of *Raphidocelis subcapitata* exposed to Cd and Pb: mechanisms of toxicity assessed by multiple endpoints. *Ecotoxicology and Environmental Safety*, **169**: 950-959.

Shah, G., Soni, V., Singhal, K. and Sharma, K. (2024). Comprehensive insights into the impact of oil pollution on the environment. *Regional Studies in Marine Science*, **74**(6): 103516.

Soares, A. R., Simas, D. L. R., Gestinari, L. M., Kaiser, C. R. and Duarte, H. M. (2017). Influence of the water-soluble fraction of petroleum on photosynthesis and chemical defenses in two sympatric seaweeds. *Journal of Applied Phycology*, **29**: 799-810.

Stark, J. S. (2022). Effects of lubricant oil and diesel on macrofaunal communities in marine sediments: a five-year field experiment in Antarctica. *Environmental Pollution*, **311**: 119885.

Subedi, S. (2024). Algal diversity in nepal and its applications: current insights and future prospects. *Science Heritage Journal*, **8**(2): 69-78.

Thirumalaisamy, S., Duraisamy, K., Periyasamy, G. and Babuji, P. (2023). Human health risks due to exposure to water pollution: a review. *Water*, **15**(14): 2532.

Wang, C., Onyeaka, H., Miri, T. and Soltani, F. (2024). Chlorella vulgaris as a food substitute: Applications and benefits in the food industry. *Journal of Food Science*, **89**: 823 -824.

Wang, C., Zhang, Y., Li, H., Xing, W. and Yu, H. (2015). The effects of petroleum hydrocarbons on algae can be reversed in the presence of a primary consumer. *Bulletin of Environmental Contamination and Toxicology*, **95**(3): 344-349.

Xia, J., Yang, D. and Yang, Y. (2021). Hydrological cycle and water resources in a changing world: a review. *Geography and Sustainbility*, **2**: 115-122.

Yakub, A. S. and Ajijo, M. R. (2016). Assessment of acute toxicity of water-soluble fraction of diesel on two species of marine microalgae. *Journal of Aquatic Sciences*, **31**(2B): 383-390.

Yasmin, F., Roy, A., Gogoi, N. and Farooq, M. (2022). The use of algae for environmental sustainability: trends and future prospects. *Environmental Science and Pollution Research*, **29**: 40373-40383.

Yassine, M. H., Wu, S., Suidan, M. T. and Venosa, A. D. (2012). Microtox aquatic toxicity of petrodiesel and biodiesel blends: The role of biodiesel's autoxidation products. *Environmental Toxicology and Chemistry*, **31**(12): 2757-2762.

Yim, U., Barron, M., Vivian, D. and Heintz, R. (2020). Long-term ecological impacts from oil spills: comparison of Exxon Valdez, Hebei Spirit and Deepwater Horizon. *Environmental Science and Technology*, **54**(11): 6456-6467.

Zhao, Z., Rasool, M., Chen, C., Wang, L. and Huang, G. (2020). Identification and screening of multiple tropical microalgal strains for antioxidant activity in vitro. *Food Bioscience*, **36**: 100649.

Zulian, G., Pistocchi, A., Roo, A., Liquete, C., Bouraoui, F., Grizzetti, B., Cardoso, A. and Vigiak, O. (2019). Relationship between ecological condition and ecosystem services in European rivers, lakes and coastal waters. *Science of the Total Environment*, **671**: 452-465.