EFFECT OF WATER-SOLUBLE FRACTION OF SPENT ENGINE OIL ON MICROALGAE (*Monoraphidium contortum* and *Dimorphococcus lunatus*)

BY

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ABSTRACT

This study was carried out to assess the effect of spent engine oil on the growth of two microalgae *Monoraphidium contortum* and *Dimorphococcus lunatus*. The test algae were grown in seven concentrations of 0% (control), 5%, 10%, 25%, 50%, 75% and 100% which were set up in triplicates. The growth response was monitored every two days for 14days using a visible spectrophotometer (Model No. HV-721). The percentage inhibition, percentage yield, growth rate, and cumulative growth rate and analysis of variance (ANOVA) were calculated using Microsoft Excel software. Physicochemical parameters were also assessed including, pH, total dissolved solids and electrical conductivity using prescribed methods. The results obtained showed that both microalgae species exhibited a positive growth response to the WSF, with *Monoraphidium contortum* consistently demonstrating higher growth compared to *Dimorphococcus lunatus* across all tested WSF concentrations. Statistical analysis using ANOVA indicated no significant difference ($p > 0.05$) in growth response among the various WSF concentrations for both species. However, temporal differences between measurements were statistically significant ($p < 0.001$). Percentage inhibition analysis revealed growth stimulation at low concentrations (5%, 10%, 25%, and 50%) and inhibition at high concentrations (75% and 100%) for both species, with *Monoraphidium contortum* showing higher inhibitory response relative to *Dimorphococcus lunatus*. Algal yield was enhanced at low to high concentrations (0-75%) for *Monoraphidium contortum* and low to moderate concentrations (0-50%) for *Dimorphococcus lunatus*. Growth rate analysis consistently favored *Monoraphidium contortum* across all WSF concentrations tested. Cumulative growth rate analysis suggested that *Dimorphococcus lunatus* may be less tolerant to WSF components compared to *Monoraphidium contortum*. Furthermore, total dissolved solids, electrical conductivity, and pH increased significantly on termination of the experiment. Based on the findings of this study, both microalgae showed bioremediation potentials and should be further studied.

CHAPTER ONE

1.1 INTRODUCTION

The environment is the intricate web of surroundings and conditions that encompass the Earth and shape the existence of all living organisms. It consists of a myriad of components, both natural and human-made, that interact to create the ecosystems and habitats we rely on for sustenance and survival (Chu and Karr, 2017). These components can be broadly categorized into abiotic, biotic, and anthropogenic elements, each playing a crucial role in shaping the planet's biodiversity, climate, and overall functioning. From the atmosphere that envelops our planet to the microscopic organisms that dwell in soil, every component of the environment contributes to the delicate balance of life on Earth (Manisalidis *et al.*, 2020). Among the components of the environment, the hydrosphere stands out as a vital and dynamic element that sustains life and influences global processes. The hydrosphere encompasses all the water on Earth, from vast oceans to freshwater sources such as rivers, lakes, and groundwater (Doru *et al.*, 2022).

Water is essential to life, serving as a habitat for aquatic organisms, a medium for nutrient transport, and a crucial component of various biochemical reactions (Maestas *et al.,* 2023). The hydrosphere plays a central role in regulating Earth's climate, with oceans acting as heat sinks and redistributing heat around the globe through ocean currents. Furthermore, water is essential for shaping landscapes through erosion and deposition processes, sculpting coastlines, canyons, and other landforms. Without water, human beings cannot live for more than a few days (Turowski and Cook, 2016). It plays a vital role in nearly every function of the body, protecting the immune system – the body’s natural defenses – and helping remove waste matter. To do this effectively, water must be accessible and safe (Imarhiagbe and Eghomwanre, 2023). Reliable access to clean and affordable water is considered as one of the goals for sustainable development and is one of the most vital natural resource necessary for the sustenance of life on the earth (Mushtaq *et al.*, 2020). Of the world's estimated population of about 8.1 billion people, at least 2.2 billion people lack access to safely managed drinking water services (Gordon *et al.*, 2023).

Water is not only a vital commodity, but is also a fundamental human right that is essential for life, health, and well-being. Yet, this right is often violated, ignored, or neglected when water is polluted by human activities like unsustainable agriculture, deforestation, and industrial practices (Palmer *et al.,* 2018). Water is considered polluted when certain substances or conditions are present in concentrations that affect the water quality and can no longer be fit for specific purpose (Bashir *et al.,* 2020). Pollution is a manmade phenomenon wherein naturally occurring substance concentration are enhanced or when harmful non synthetic compounds (xenobiotic) are released into the environment (Štefanac *et al.,* 2021). Water pollution is the release of excessive concentrations of harmful/hazardous substance (pollutants) in water, such that it is no longer acceptable for drinking, bathing, cooking or other purposes (Akhtar *et al.,* 2021).

Water pollution is caused by pollutants which are physical, chemical or biological elements that have an aesthetic or negative impact on aquatic life and those who consume the water. The majority of pollution emanates from industries and different levels of pollution can be released from point and non-point sources into the environment (Eman, 2020). Unplanned establishment of industries and industrialization (Walker *et al.*, 2019), urbanization, higher living standards and population explosion deteriorate the quality as well as quantity of water.

Among the pollutants that can affect water quality, petroleum hydrocarbons are among those with far reaching ecological implications (Gao *et al*., 2022). Environmental pollution by petroleum hydrocarbons has become a serious concern in the developed as well as developing countries, with the exploration and use of fossil fuels and other crude oil fractions (Solomon *et al*., 2020). Crude oil and its derivatives have been considered as important pollutants of aquatic ecosystems. The oil spills in the aquatic environments causes adverse effects and are serious threats to the ecosystem, environment, human health and living organisms (Mehdi, 2020). Living organisms especially aquatic biota that can be affected by oil pollution include, fish, prawns, aquatic macrophytes, zooplankton and algae. Of these, algae are one of the most affected because of the role they play in the aquatic ecosystem. They act as the building blocks in most aquatic food ecosystem, providing a valuable food source for organisms in other trophic levels (Keesing and Edgar, 2018).

Algae are non-vascular plants that range in size from single-celled or colonial microalgae or phytoplankton (such as diatoms and dinoflagellates), to large multicellular macroalgae such as seaweed or kelp. They contain chlorophyll *a* as the major photosynthetic pigments in addition to other pigments (Ankit *et al.,* 2022). Algae have the potential to sequester diverse contaminants (such as heavy metals, pesticides, inorganic and organic toxins). In dilute effluents, they are quite effective in the removal of pollutants, due to their high surface area-to-volume ratio. Also, they are highly efficient at sequestering heavy metals and are suitable even in wastewater containing higher metal concentrations. Additionally, algae are appropriate for aerobic and anaerobic effluent treatment units (Ankit *et al*., 2022).

Algae play a crucial role in aquatic ecosystems by constituting the energy base of the food web for all the aquatic fauna. As autotrophic organisms, algae transform H_2O and CO_2 to sugar by the process of photosynthesis. Photosynthesis also produces oxygen as a by-product, helping in the survival of fish and other aquatic fauna (Ankit *et al.,* 2022). Algae are significant components of various biogeochemical cycles and are important members of aquatic environment food webs. Algae are also used as indicators of water quality owing to their rapid growth, short life cycle and fast response to changes in environmental conditions. Algae has been employed as indicators of quality (Parmar *et al.*, 2016) and the alteration in their species composition, serves as a useful bio indicator of pollution.

Microalgae are microscopic, aquatic organisms that thrive in both marine and freshwater environments, and have a mechanism of photosynthesis that is similar to that of land plants (Abubakar *et al.,* 2020). They produce approximately half of the atmospheric oxygen we breathe. Additionally, they utilize carbon dioxide during photosynthesis. Together with bacteria, microalgae form the foundation of the food web, by providing energy for all trophic levels above them (Giovanni *et al.*, 2023). Their biomass is often measured using chlorophyll *a* concentration. Biomass-wise, they constitute the biggest group of primary producers, accountable for almost 50% of global photosynthesis (Dolganyuk *et al.*, 2020). Microalgae serve as food sources and are important components of the food web for many larval species of mollusks, crustaceans, and fish. The biodiversity of microalgae is vast, with an estimated 200,000 to 800,000 species across different genera (Tounsi *et al.*, 2022). Around 50,000 species have however been described (Tounsi *et al.*, 2022).

They exist independently or in groups and chains, and their size usually ranges between 30 to 400 micrometres (Harun *et al.,* 2014). These tiny organisms yield valuable compounds such as carotenoids, antioxidants, fatty acids, enzymes, peptides, and sterols (Cichoński and

Chrzanowski, 2022). Microalgae are not only essential for the environment but also have practical applications, they play a role in cleaning up pollutants.

Monoraphidium contortum, is a species of green microalgae belonging to the class Chlorophyceae. It is characterized by its filamentous morphology, consisting of long, slender cells arranged in unbranched chains. The cells are typically curved or contorted, giving the organism its distinctive appearance (Ramos *et al.,* 2012). *M. contortum* is commonly found in freshwater environments, including ponds, lakes, and streams, where it thrives under a wide range of environmental conditions. As a photosynthetic microorganism, *Monoraphidium contortum* plays a significant role in aquatic ecosystems by serving as a primary producer and contributing to nutrient cycling. *M. contortum* can tolerate varying levels of light intensity, temperature, and nutrient availability, allowing it to adapt to dynamic environmental conditions (Kirchner *et al.*, 2022). Additionally, it serves as a food source for various aquatic organisms, including zooplankton and small invertebrates, contributing to the transfer of energy through the food web (Taipale *et al.*, 2022).

Dimorphococcus lunatus is a unicellular green alga belonging to the phylum Chlorophyta. It is characterized by its spherical to ovoid cell shape and distinctive morphology, with cells arranged in colonies or aggregates (Mehdizadeh and Peerhossaini, 2022). *Dimorphococcus lunatus* exhibits a unique dimorphic life cycle, alternating between motile, flagellated cells (swarmer cells) and non-motile, vegetative cells (palmelloid cells). This morphological adaptation allows *D. lunatus* to colonize diverse aquatic habitats, including freshwater lakes, ponds, and wetlands. It plays a crucial role in primary production and nutrient cycling in aquatic ecosystems, contributing to the oxygenation of water bodies and providing a food source for herbivorous organisms (Cornwallis *et al.*, 2023). *Dimorphococcus lunatus* can form blooms under favorable environmental conditions, which may have implications for water quality, ecosystem dynamics, and human health.

Oil spills include any spill of crude oil or oil distilled products such as gasoline, diesel fuels, jet fuels, kerosene, Stoddard solvents, hydraulic oils, and lubricating/engine oils, that can pollute the land, air and water environments (Merv, 2021).

Spills arising from disposal of lubricating/engine oil are becoming a visible problem. Pollution from engine oil is one of the environmental problems in Nigeria and is more widespread than crude oil pollution (Adeleye *et al*., 2018). Engine oil is a petroleum product which is used to reduce the friction between engine surfaces. It is produced by vacuum distillation of crude oil (Kalichesvky and Peters, 1960). Engine oil usually contains chemical additives, these may include; amines, phenols, benzene, calcium, zinc, barium, magnesium, phosphorus, Sulphur, and lead (Ezekwe and Lale, 2014). The main function of engine oil is to lubricate moving parts. However, engine oil also cleans, inhibits corrosion, improves sealing and cools the engine by removing heat away from moving parts of the engine (Hasan-Zadeh and Poshtiban, 2021).

Spent engine oil sometimes referred to as waste engine oil or used motor oil or spent lubricant is produced from automobile mechanic shops and mechanical or electrical engine repairers' shops after servicing the vehicles engines, generating set and other types of engines (Nwachukwu, 2012). It has dark brown to black colour and is viscous. Spent engine oil is a mixture of several different chemicals, including low and high molecular weight (C_{15}-C_{20}) aliphatic hydrocarbons, aromatic hydrocarbons, polychlorinated biphenyls, lubricative additives, chlorodibenzofurans, decomposition products, heavy metal contaminants such as aluminum, chromium, tin, lead, manganese, nickel, and silicon that come from engine parts as they wear down (Onwusiri, 2015).

It gets to the environment due to discharge by motor and generator mechanics and from the exhaust system during engine use and due to engine leaks. The disposal of spent engine oil into open vacant plots and farms, gutters and water drains, is an environmental risk since it is liquid, it easily leaches into the environment and eventually pollutes the soil or water body (Njoku, 2012).

The greatest cause of spent engine oil pollution in water bodies and terrestrial environment comes from anthropogenic sources such as drains and urban run-off caused by improper disposal of spent engine oil (Obi *et al.,* 2022). Improperly disposed spent oil ends up in landfills, sewers, backyards, or storm drains where soil, groundwater and drinking water may become contaminated. Disposal of the spent lubricant into gutters, water drains, open vacant plots and farms is a common practice especially by motor mechanics who change oil from motor vehicles, power generating and other machines (Nwachukwu, 2012).

The discharge of spent engine oil into aquatic environments can have significant negative effects on algal growth and the overall health of aquatic ecosystems. Engine oil contains various harmful substances such as heavy metals, polycyclic aromatic hydrocarbons (PAHs), and other toxic compounds that can contaminate water bodies, disrupting the delicate balance of aquatic ecosystems (Wanda *et al*., 2022). One of the primary impacts of spent engine oil on algal growth is the introduction of pollutants that inhibit photosynthesis; a vital process for algae and other aquatic plants. Poly aromatic hydrocarbons and heavy metals present in engine oil can coat the surface of water bodies, forming a thin film that reduces light penetration (Adeleye *et al.,* 2018). Since algae rely on sunlight for photosynthesis, decreased light availability hampers their ability to produce energy, limiting growth and productivity. This can lead to a decline in algal populations, disrupting the aquatic food web and ecosystem dynamics. Furthermore, the toxic compounds in spent engine oil can directly harm algal cells and inhibit their growth and reproduction. Exposure to these toxicants can impair algal metabolism, damage cell membranes, and disrupt cellular functions, ultimately leading to reduced growth rates, impaired reproductive success, and even cell death (Patel *et al*., 2020).

Over 200 million litres of spent engine oils are generated in Nigeria annually, and are improperly disposed into the environment and yet, adequate attention has not been given to its proper disposal (Echiegu *et al*., 2021). Disposal of the spent lubricant into open vacant plots, farms, gutters, water drains, is a common practice especially by motor mechanics who change oil from motor vehicles, power generating and other machines leading to harm to aquatic ecosystem (Obi *et al.*, 2022). Researchers have widely focused on the effects of effluents, crude oil and petroleum products on *Chlorella vulgaris* and *Scenedesmus obliquus.* However, the effects of WSF of spent/waste engine oil on microalgae algae have not been given proper attention. Consequently, the available literature is very scanty. Previous works include Bott and Rogenmuser (1978) on the effects of water-soluble extracts of No. 2 fuel oil; Akpan and Frank (2003) investigated the effects of waste engine oil on the phytoplankton of Calabar River Estuary; Etim and Okon (2011) studied the effects of diesel oil on phytoplankton species and observed growth inducement in *Coscinodiscus excentricus,* while Kadiri and Eboigbodin (2012) examined the effects of water-soluble fractions (WSF) of refined petroleum products on microalgae (*Isochrysis galbana, Thalassiosira pseudonana and Skeletonema tropicum*). Pollution caused by waste engine oil is an endemic problem in Nigeria, and it is more prevalent than crude oil pollution. This study is therefore necessary to establish the relationship between pollution of water bodies with spent engine oil and the effects on the aquatic biota with particular reference to microalgae.

1.2 Aim and Objectives:

The study is aimed at determining the effect of water soluble fractions of spent engine oil on the growth of the microalgae (*Monoraphidium contortum* and *Dimorphococcus lunatus*).

The objectives were to:

1. evaluate the growth response of the selected fresh water algae (*Monraphidium contortum and Dimorphococcus lunatus*) to various concentrations of WSF of spent engine oil.

2. determine the yield of the test microalgae in various concentrations of spent engine oil WSF

3. examine growth stimulation or inhibition of *Monoraphidium contortum and Dimorphococcus lunatus* on exposure to WSF of spent engine oil.

4. assess the bioremediation potential, if any, of the algae to the WSF of spent engine oil.

CHAPTER TWO

2.1 LITERATURE REVIEW

Due to the recognized toxicity of petroleum products on aquatic ecosystems, including microalgae, this review examines the effects of spent engine oil and other petroleum hydrocarbons, on the growth of microalgae. Owing to the paucity of literature, it also delved into related studies on higher plants and animals, specifically focusing on research conducted in Nigeria, Africa and across other parts of the world. The reports are presented chronologically and nested within regions of the study.

2.2 International Literature

Liu and Vipulanandan (2012), carried out research on the effects of oil and salt water spills on the growth of a fresh-water algae in Texas. The research was carried out by investigating the effects of oil spill and sea water inundation on the growth of a fresh-water algae, specifically *Micractinium pusillum* and cultivating the algae in Proline cultural medium and simulating different growth conditions using closed tube and plate methods. Engine oil was used to simulate oil spill environments, while NaCl solution represented sea water inundation. Algae was grown in varying percentages of NaCl with or without engine oil in the tubes. Light was provided by a bulb with a light density of 2400 lux. Algae growth was measured after 10 days using a UV-vis spectrophotometer at a wavelength of 680 nm. Different concentrations of engine oil were utilized in the study, with the tubes being stirred at a rate of 250 rpm. The study's findings revealed significant effects of oil spill and salt water inundation on the growth of fresh-water algae. Algae growth was notably different in the presence of contaminants, with higher concentrations of engine oil leading to increased inhibition of growth due to light blockage by oil film. Additionally, NaCl solution hindered the growth of fresh-water algae, and the combined presence of oil and salt water further reduced algal growth. Notably, oil content of 0.5% and higher was found to inhibit algal growth in both tube and plate testing conditions. In conclusion, the research demonstrated that both engine oil and NaCl solution adversely affected the growth of the algae, with higher concentrations of engine oil and salt water leading to increased inhibition.

The toxicity of biodiesel, diesel and biodiesel/diesel blends: comparative sub-lethal effects of water-soluble fractions to microalgae species was assessed by Solange *et al.* (2012).The samples from B2, B3, B5 and D were obtained in gas stations from the fuel pump. Chemical analyses were carried out at the LCQ (Quality Control Laboratory All the samples were analyzed for C6–C8 mono-aromatics (BTEX), total heavy aromatic hydrocarbons, and methanol, toxicity tests were carried out using four species of microalgae collected from the algae bank located at the Biology and Biomonitoring Laboratory, Institute of Biology, Federal University of Bahia: a freshwater species, *Pseudokirchneriella subcapitata*, maintained in LC-Oligo medium and three marine species, *Tetraselmis chuii, Nannochloropsis oculata* and *Skeletonema costatum*, maintained at Conway medium, under standard conditions Each test was repeated three times and was fully randomized with regard to vials location during incubation and the order of cell counts. All the tests involved a positive (standard reference toxicant) and a negative (blank) control Possible significant differences in toxicity among the various treatments were determined by ANOVA comparing the IC50 results followed by Tukey test, the result showed a positive correlation with increasing diesel concentrations (B100 \B5\B3\B2\D). Biodiesel interacted with the aqueous matrix, generating methanol, which showed lower toxicity than the diesel contaminants in blends. The WSF caused 50% culture growth inhibition (IC50-96 h) at concentrations varying from 2.3 to 85.6%, depending on the tested fuels and species.

The potential impacts of the deepwater horizon oil spill on lower trophic level food sources, specifically focusing on the toxicological effects of oil (tar mat and MC252 crude oil), dispersant (Corexit 9500A), and dispersed oil on the growth inhibition and motility of two microalgae species, *Isochrysis galbana* and *Chaetoceros* sp was studied by Garr *et al.* (2014). Samples of the microalgae were exposed to oil (tar mat and MC252 crude oil), dispersant (Corexit 9500A), and dispersed oil. The impact on growth inhibition (IC50) and motility of the microalgae was determined. The experiments included examining cell division and percent motility at various time points (0, 24, 48, 72, 96 hours) using specific methods for data analysis. The concentrations of the substances tested were varied, and the effects on the microalgae were monitored over time. Additionally, poly aromatic hydrocarbon (PAH) and total petroleum hydrocarbon (TPH) levels of samples were determined. The experimental setup included using monocultures of the microalgae species in replicates with specific concentrations of the substances being tested. The flasks containing the microalgae were placed in a temperature-controlled room with continuous light exposure to maintain consistent conditions. The research findings indicated that there was no impact on cell division (growth) for the microalgae species, *Isochrysis galbana* and *Chaetoceros* sp., when exposed to both tar mat and MC252 crude oil. The mean motility of *Isochrysis galbana* remained above 79% throughout the experiment. However, the addition of dispersant (Corexit 9500A) inhibited cell division and motility within 24 hours, with *Chaetoceros* sp. being more susceptible to sublethal effects compared to *Isochrysis galbana.* The study highlighted the sensitivity of microalgae to the use of dispersants in bioremediation processes, raising concerns about potential long-term impacts on fisheries recruitment.

The toxicity and oxidative stress induced by used and unused motor oil on freshwater microalgae, was investigated by Ramadass *et al.* (2014). They exposed the microalgae to different concentrations of oil and water-accommodated fractions (WAF) for 2 weeks. The motor oils' toxicity was assessed by directly adding them in various concentrations. Chlorophyll a, an indicator of algal biomass, was measured to monitor algal growth. Additionally, the activity of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), was measured after 96 hours and 7 days of exposure to the motor oil. This measurement of antioxidant enzyme activity aimed to determine the oxidative stress induced in the algae by the motor oil exposure. Findings from the study showed that used motor oil was more toxic to the freshwater microalga, *Pseudokirchneriella subcapitata*, compared to fresh oil, with a 44% inhibition of algal growth at a 0.20% concentration. The water-accommodated fraction (WAF) of used oil showed significant toxicity at concentrations above 50%, while WAF from fresh oil was nontoxic even at 100%. Exposure to used oil and its WAF, even at lower concentrations, increased the levels of antioxidant enzymes in the algal cells, indicating a response to toxicity stress. In contrast, exposure to fresh motor oil did not result in alterations in antioxidant enzyme levels. The TPH concentration in unused oil was significantly more than that in used motor oil and, the extent of metal content increased in used oil compared to unused oil. Overall, the study suggested that contamination of aquatic systems with used motor oil could potentially disrupt ecosystem health by affecting primary producers at the base of the food chain.

The ecotoxicological effects of a mixture of petroleum hydrocarbons were tested by wang and zhang (2015) on the densities of two algae, *Platymonas helgolandica var. tsingtaoensis* and I*sochrysis galbana,* and of a rotifer, *Brachionus plicatilis,* by single-species and customized community experiments. Test concentrations ranged from 0 to 100 mg L^{-1}, while five to seven treatments were assessed in triplicate within 1 month. The petroleum hydrocarbon mixture used in this study was the water-accommodated fraction of crude oil obtained from Bohai offshore oil field, China. A significant decrease in densities during single species toxicity tests were found when concentrations of petroleum hydrocarbons were above 1.0 mg L^{-1}. However, equilibrium densities of algae in the customized community showed a different pattern, which increased with concentration and reached a peak at 20.0 mg L^{-1}. The community-based no observed effect concentration (NOEC; 1.0 mg L^{-1}) was different from the NOEC derived by single-species toxic tests (0.25 mg L^{-1}). This demonstrates that ecotoxicological effects on plankton as part of a community is significantly different from single species toxicity tests owing to ecological interactions.

The effects of motor oil on the growth of three aquatic macrophytes, was studied by Özbay (2016), in the study, the effect of used motor oil on the growth rate of three different species of aquatic macrophytes was investigated for a three-week period under laboratory conditions. Three treatments were used in pots: high oil, low oil, and a control, each with three replicate buckets (three pots per bucket). The relative growth rate of the tested plants, *Potamogeton gramineus* L.*,* *Myriophyllum spicatum* L.*, and Ceratophyllum demersum* L., differed significantly between treatments ($P<0.001$). In the control treatment, *C. demersum, M. spicatum* and *P. gramineus* grew well and produced more lateral shoots than in the high and the low motor oil treatments. The longest shoot lengths were also greater for all three plants in the control than in the low and the high motor oil groups.

Ramadass *et al.* (2016) studied sensitivity and antioxidant response of *Chlorella* sp. MM3 to used engine oil and its water accommodated fraction, the microalgal strain, *Chlorella* sp. MM3, was exposed to unused or used engine oil, or their water accommodated fractions (WAFs) to determine growth inhibition and response of antioxidant enzymes. Oil type and oil concentration greatly affected the microalgal growth. Used oil at 0.04 % (0.4 g L^{-1}) resulted in 50% inhibition in algal growth, measured in terms of chlorophyll *a*, while the corresponding concentration of unused oil was nontoxic. Similarly, used oil WAF showed significant toxicity to the algal growth at 10 % level, whereas WAF from unused oil was nontoxic even at 100 % concentration. Peroxidase enzyme in the microalga significantly increased with used oil at concentrations above 0.04 g L^{-1} whereas the induction of superoxide dismutase and catalase was apparent only at 0.06 g L^{-1}. Activities of the antioxidant enzymes increased significantly when the microalga was exposed to 75 and 100 % WAF obtained from used oil.

Salinas-Whittaker *et al*. (2020) assessed the growth response of the microalga *Dunaliella tertiolecta* on the water-soluble fraction of the mixture fuel oil/diesel, the research involved monitoring the microalgae *Dunaliella tertiolecta* exposed to the water-soluble fraction (WSF) of a fuel oil/diesel mixture over a 15-day period. The study included preparing the WSF stock solution, culturing the microalgae under controlled conditions, conducting toxicity tests, analyzing various parameters, studying pigments (chlorophyll *a*, lutein, β-carotene), total phenolic compounds, protein content, total lipids, and fatty acid composition. Additionally, Fourier transform infrared spectroscopy was used to characterize lipid accumulation. The data obtained were analyzed statistically to understand the effects of the WSF on the growth, pigments, and biochemical components of *Dunaliella tertiolect.* Results from the study showed that exposure to WSF triggered physiological and biochemical responses in *D. tertiolecta*, leading to changes in growth rate, pigments, phenols, lipids, and proteins of the microalgae. Notably, there were significant differences in all the biochemical parameters analyzed between the exposed group and the control. While the fatty acid profile remained unaltered, there was an increase in unsaturated acyl chains within the exposed microalgae, indicating a possible uptake of hydrocarbons from WSF. The variation in pigments and phenol contents was interpreted as an integrated antioxidant response to the stress imposed by WSF.

2.3 African Literature

In study conducted by Chijioke-Osuji (2016) on the impact of waste engine oil on the microflora and physio-chemical quality of soils in two West African countries. The study analyzed the presence of microorganisms in both control soils and soils contaminated with waste engine oil from Kumasi and Owerri. Additionally, the study assessed the levels of polycyclic aromatic hydrocarbons (PAHs) and heavy metals in the soil samples using specific analytical methods. The analysis of microorganisms in the soil samples was conducted through isolation and identification techniques. The PAH content was determined using the cold extraction GC-FID method, while heavy metals content was analyzed using the AAS: APHA methods 3010-3110. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) to compare the means of the different results obtained. The result from the study identified various microorganisms present in both control soils and soils contaminated with waste engine oil from Kumasi and Owerri. For example, *Bacillus subtilis*, *Rhizopus oryzae*, and *Trichoderma spirale* were found in the contaminated soil from Kumasi, while *Bacillus cereus, Aspergillus carbonarius, Cunninghamella*, and *Rhizopus sp*. were present in the control soil. In Owerri, *Bacillus subtilis, Rhizopus oryzae*, and *Rhizopus sp*. were identified in the contaminated soil. The study also found the presence of hydrocarbon-utilizing microorganisms in the contaminated soils, suggesting the adaptation of certain microorganisms to utilize hydrocarbons present in waste engine oil as a carbon source. The analysis of the total hydrocarbon content revealed an increase in the total hydrocarbon content in the soils contaminated with waste engine oil compared to the control soils. This indicates the accumulation of hydrocarbons in the soil due to contamination.

The levels of PAHs in the contaminated soils were measured, showing higher concentrations of certain PAH components, especially high molecular weight PAHs. This suggests that waste engine oil contamination led to an increase in PAH levels in the soil. The study found elevated levels of heavy metals, including iron, manganese, zinc, lead, nickel, and chromium, in the soils contaminated with waste engine oil. This indicates that the presence of waste engine oil contributed to the accumulation of heavy metals in the soil, posing potential risks to human health and the environment. Various physio-chemical parameters such as pH, total organic carbon, phosphate, nitrate, and sulfate content were analyzed in the contaminated soils. Changes in these parameters were observed, reflecting alterations in the soil's chemical composition due to waste engine oil exposure.

Metagenomics' insights into effects of spent engine oil perturbation on the microbial community composition and function in a tropical agricultural soil by Salam *et al.* (2017) provides a comprehensive analysis of the impact of spent engine oil (SEO) contamination on microbial communities in tropical agricultural soil using metagenomics approaches. The study focused on understanding the structural and metabolic shifts in the microbial community due to SEO perturbation. The research methodology involved setting up soil microcosms designated as 1S (agricultural soil) and AB1 (agricultural soil polluted with SEO). Metagenomic DNA extracted from these microcosms was sequenced using Miseq Illumina sequencing and analyzed for taxonomic and functional properties. Based on the results obtained the taxonomic profiling revealed a decrease in microbial diversity in the SEO-polluted soil (AB1) compared to the uncontaminated soil (1S). Dominant phyla identified included Actinobacteria and Proteobacteria, with specific genera such as *Streptomyces*, *Gemmatimonas*, *Geodermatophilus, Burkholderia*, and *Pseudomonas* showing prevalence in either 1S or AB1. The functional analysis of the microbial communities highlighted diverse metabolic potentials, including adaptation to environmental stressors such as hydrocarbon hydrophobicity, heavy metal toxicity, oxidative stress, nutrient starvation, and C/N/P imbalance. The study also revealed the enrichment of hydrocarbonoclastic organisms in the SEO-polluted soil, indicating a shift in community structure and functions due to contamination.

Wanda *et al.* (2021) studied the Potential Impacts of Oil and Grease on Algae, Invertebrates and Fish in the Bujagali Hydropower Project Area in Uganda. This study determined the concentration of oil and grease and inferred its impacts on algae, invertebrates and fish. Water samples were collected in April and September from 2012 to 2018 at the upstream and downstream transects and in the reservoir, and analyzed for oil and grease following standard procedures. Environmental compliance was compared to NEMA's discharge standard of 10mg/l, and it's PAH effluent discharge standard of ≤ 0.1 mg/L. At all sites, average concentrations of oil and grease were below 10 mg/L throughout the sampling period. Result showed that out of the 14 data sets for each transect, only 3 along the upstream transect, and 2 at each of the downstream transect and the reservoir were compliant with the effluent discharge standard. Although impacts of oil and grease on aquatic biota were not assessed, their relatively high concentration compared to total Polycyclic Aromatic Hydrocarbon (> 0.1 µg/L) is considered hazardous to most aquatic organisms. The diverse activities around the project area implied that sources of oil and grease were proportionately diverse. Hence, the observed trends may not solely be attributed to the hydropower project. Accordingly, assessment of the various sources of oil and grease and their impact on aquatic biota in the area is recommended

2.4 Nigerian Literature

Kadiri and Eboigbodin (2012) conducted a phytotoxicity assessment and study of water soluble fractions of refined petroleum products (diesel, kerosene, and petrol) on two test microalgae, *Desmodesmus quadricauda* and *Eudorina elegans*. The experiment, carried out over a 14-day period in a laboratory setting, involved exposing the microalgae to various concentrations (0%, 10%, 25%, 50%, 75%, and 100%) of diesel, kerosene, and petrol WSF. Growth responses were measured spectrophotometrically. The result showed that high concentrations of water-soluble fractions (WSF) of diesel, kerosene, and petrol negatively impacted the growth of the microalgae *Desmodesmus quadricauda* and *Eudorina elegans*. However, lower concentrations of the fuel oils stimulated the growth of the microalgae. Specifically, maximum growth for *Eudorina elegans* was observed at 10% in diesel, petrol, and kerosene, except for kerosene where maximum growth was at 25%. For *Desmodesmus quadricauda*, maximum growth occurred at 10% in diesel, 25% in kerosene, and 50% in petrol. Both microalgae exhibited minimum growth and were highly inhibited at 100% concentration of the fuel oils' WSF.

A 96-hour bioassay was conducted using the water-soluble fraction of a Nigerian light crude oil sample on *Clarias gariepinus* fingerlings by Olaifa (2012). 0, 2.5, 5.0, 7.5 and 10 ml of water-soluble fractions (WSF) of the oil were added to 1000litres of de-chlorinated tap water to form 0, 25, 50, 75 and 100 parts per million representing treatments 1 to 5 respectively. Each treatment had two replicates with fifteen fish per replicate. At the end of the 96-hour period of exposure, the fish were transferred into separate bowls containing fresh water without oil for recovery for ten more days. Heavy metal and total hydrocarbon contents of the water and fish were analyzed at 96 hour and 14 days which marked the end of the recovery period. No mortalities were recorded on all treatments during the 96-hour period. Mortalities were observed between 120 and

144 hours after the onset of the experiment with the maximum number of dead fish ($p<0.05$) from treatment 5 (100 ppm WSF) during the recovery period indicating a delayed response to the WSF by the fish. No mortalities were recorded after 144 hours till the termination of the experiment at 14 days.

Mishra and Mukherji (2012) conducted a study on the biosorption of diesel and lubricating oil on algal biomass. The research focused on investigating the potential of algae as a biosorbent for diesel and lubricating oil, expanding beyond the traditional use of algae for heavy metal sorption. The studied was carried out through batch experiments. The algal biomass was characterized using BET and Langmuir surface area analysis, pH drift method for pH at the point of zero charge, and FTIR spectroscopy to analyze functional groups on the cell wall of algae samples]. The kinetic study involved using fixed concentrations of oil, specific doses of biomass, and controlled experimental conditions. The study revealed that algae exhibited significant sorption of oil, comparable to other oil spill clean-up sorbents. The sorption rate of lubricating oil was lower than that of diesel due to additives present in lubricating oil. Two-parameter isotherms, such as Langmuir and Freundlich isotherms, were found to be suitable for diesel but not for lubricating oil. The research highlighted the potential of algae as a low-cost sorbent for oil removal, emphasizing its role in influencing the fate and transport of spilled oil.

Obazuaye and Obueh (2014) investigated the impact of waste engine oil on the growth of three local pepper species: *Capsicum frutescens, Capsicum chinense*, and *Capsicum annum*. The study examined the physicochemical properties of the soil under various concentrations of waste engine oil. It also assessed the bacterial population in the soil to understand the impact of pollution on microbial communities. Additionally, the study analyzed the growth parameters of the pepper plants, such as germination rate, plant height, and nutrient uptake, in response to the contaminated soil. The study was carried out by treating the soil with different percentages of waste engine oil before planting the pepper species. The growth of the plants was monitored over time and the changes in soil properties and plant characteristics analyzed. Various analytical techniques were employed to measure nutrient levels, pH, organic matter content, and bacterial populations in the soil. The results of the study showed that the germination, growth, and height of the pepper species were inhibited by treatments of 3%, 6%, and 9% concentrations of waste engine oil. Only the control (0%) had successful germination. Capsicum annum was the most affected pepper species, with germination and growth inhibition observed in all three species in concentrations of 3%, 6%, and 9% waste engine oil. The order of susceptibility to the oil contamination was *C. annum* < *C. chinense* < *C. frutescens*. And there was reduced growth in the pepper species under the 1% treatment of waste engine oil-contaminated soil. While the pepper varieties grew under this concentration, they eventually died before fruiting, indicating a negative impact on overall plant health and development. The nutrient uptake of the pepper plants was hindered in concentrations of 3%, 6%, and 9% waste engine oil-contaminated soil. The levels of nitrogen, calcium, magnesium, iron, lead, and zinc increased in the soil with a 1% treatment of waste engine oil after planting with the pepper species. This increase in heavy metals in the contaminated soils could have toxic effects on the plants. The study also observed a decline in the bacterial population in the soil with increasing concentrations of waste engine oil. This decline in microbial communities could have implications for soil health and nutrient cycling processes.

Nwite and Alu (2015) investigated the effects of different levels of spent engine oil on soil properties, maize yield, and heavy metal uptake in Abakaliki, Southeastern Nigeria. The research was conducted using a Completely Randomized Design (CRD) in a Plant and Screen house at the Faculty of Agriculture and Natural Resources Management, Ebonyi State University, Abakaliki. Perforated poly bags filled with 20 kg of soil were arranged with specific spacing between them and replications. Nine treatment levels of spent engine oil ranging from 0.0 to 1.0 L per poly bag of soil were applied. Maize seeds were planted in the treated soil, and agronomic practices such as fertilization, weeding, and thinning were carried out. The research findings revealed that the application of spent engine oil at 1.0 L per poly bag led to a significant increase in bulk density and organic carbon, while reducing gravimetric moisture content, total porosity, and hydraulic conductivity. Additionally, there were notable decreases in available phosphorus, exchangeable potassium, magnesium, sodium, and calcium in the soil treated with 1.0 L of spent engine oil compared to the control. The study also demonstrated that maize grain yield was significantly lower in plots treated with spent engine oil compared to the control group. There was a substantial reduction in grain yield and delayed germination of maize seeds in soil treated with higher levels of spent engine oil, indicating a negative impact on crop productivity. The research also showed that there was a higher uptake of lead and cadmium by maize seeds in plots receiving spent engine oil treatment. This increased uptake of heavy metals poses a potential risk to human health, as crops grown in oil-contaminated soil may contain elevated levels of these toxic metals. In conclusion, the study underscored the urgent need for proper waste management practices to prevent soil contamination with spent engine oil.

In the study conducted by Onwusiri *et al*. (2017) on the effect of spent engine oil on germination and growth parameters of fluted pumpkin in Makurdi, Benue State, Nigeria. Provides valuable insights into the impact of soil contamination on an important crop species. The experiment was designed as a completely randomized design (CRD) with six replications for each treatment. Soil samples were contaminated with different levels of spent engine oil by thoroughly mixing the soil with 20ml, 40ml, 60ml, 80ml, and 100ml of spent oil to obtain 1%, 2%, 3%, 4%, and 5% spent oil contaminations, respectively. Each treatment, including the control (0%), was replicated six times, and four viable fluted pumpkin seeds were planted in each bucket. The seeds were laid out in the field and watered. The growth parameters assessed included plant height, number of leaves, leaf area, plant fresh weight, and dry weight. The data collected on growth parameters were subjected to analysis of variance (ANOVA) to analyze the effects of different treatments. The findings of the study revealed that contamination of soil with spent engine oil inhibited the germination of fluted pumpkin seeds, with higher concentrations of oil leading to death and non-germination of the seeds.

In the study conducted by Iqbal *et al*. (2018) on the toxic effects of motor oil pollution on the early seedling growth performance of *Adenanthera pavonina*. The research was carried out by treating *Adenanthera pavonina* seeds with different concentrations of motor oil suspension and monitoring their growth over a specified period. Also the seed germination rates, root/shoot ratios, and total plant dry weight to quantify the effects of motor oil pollution on the seedling growth performance of *Adenanthera pavonina*. The results from the study showed that increasing concentrations of motor oil suspension had a detrimental effect on the seed germination of *Adenanthera pavonina*. Additionally, the root/shoot ratio was significantly altered in oil-polluted soil compared to the control treatment, indicating a disruption in normal plant growth patterns. Furthermore, the total plant dry weight of *Adenanthera pavonina* decreased with higher levels of oil pollution in the soil, highlighting the negative impact of motor oil pollution on plant biomass production. Different concentrations of motor oil suspension had a significant impact on the seed germination of *Adenanthera pavonina* compared to the control treatment. The study found that as the concentration of motor oil suspension increased, the seed germination of

A. pavonina was adversely affected. Specifically, the treatment with 5.0% motor oil suspension resulted in a substantial decrease in seed germination compared to the control treatment. This indicates that higher concentrations of motor oil pollution have a detrimental effect on the seed germination of *Adenanthera pavonina*.

Yerima *et al*. (2020) studied bio-remedial effect of specific micro-organisms (bacteria) on used engine oil, some mechanic workshops in Maiduguri metropolitan council (MMC) as case studies. The research involved isolating bacteria from soil contaminated with used engine oil. Various species of bacteria, including species of Pseudomonas, Streptococcus and Bacillus, were identified and characterized based on their morphological and biochemical characteristics. The study also examined the growth response of maize in both control and treated soil across different locations to assess the impact of bioremediation on plant growth. The study was carried out by isolating bacteria obtained from the contaminated soil were identified through biochemical and morphological characteristics. Techniques such as dilution methods and oil spread tests were employed to isolate and confirm the degradative capabilities of the bacteria. The growth response of maize in contaminated soil was monitored weekly, and the effectiveness of bioremediation was evaluated based on the plant growth in treated soil compared to control soil. The research findings indicated that bioremediation was more effective in locations with lower exposure rates of used engine oil, such as Lagos Street and Polo and the isolated bacteria species, including *Pseudomonas aeruginosa, Micrococus sp*., *Bacillus sp.,* and *Serratia sp.,* were found to be effective in bioremediation of soil contaminated with engine oil. The study concluded that the isolated bacteria species, particularly Pseudomonas, Streptococcus, and Bacillus, could be utilized for bioremediation processes to clean up soil contaminated with used engine oil.

Azorji *et al*. (2021) investigated the effects of spent oil pollution on seed germination and early seedling establishment of *Vigna unguiculata* TGm-50*, Glycine max* and *Zea mays* TZm-30181. The seeds of each accession were placed in petri dish lined with moist tissue paper at 0, 4, 8, 12 and 16% (v/v). The experiment was laid out in complete randomized design (CRD) with three replicates. The effects of spent engine oil on the assayed parameters such as germination percentage, rate of germination, seedling root and shoot elongation, seedling dry and fresh weights of the seeds was determined. The results showed the effects of spent engine oil on the assayed parameters examined which were concentration and accession dependent. Seeds and seedlings of *Vigna unguiculata* TGm-50*, Glycine max* and *Zea mays* TZm-30181 grown at various treatment levels differed significantly ($P<0.05$) from the control. Tolerance levels among the accessions were in the order: *Vigna unguiculata* > *Glycine max* > *Zea mays*. The used engine oil inhibited the germination and early seedlings growth of these crops in a dose dependent manner and those inhibitory effects of spent oil do not imply inhibition of subsequent growth.

Phytotoxicity and the effects of water saturated fractions of hydrocarbons on growth and photosynthetic pigments of microalgae were assessed by Denise *et al.* (2022). Using three species of fresh water microalgae namely *Chlorella vulgaris* (Chlorophyte), *Nitzschiapalea* (Diatom) and *Anabaena flosaquae* (Cyanobacterium). The microalgae were grown separately in ratios 1:9 of 0%, 10%, 25%, 50%, 75% and 100% of water saturated fractions of hydrocarbons (Hexane, Benzene, Toluene and Xylene). Absorbance at 745nm was used to estimate algal growth while 630nm, 645nm, and 665nm were used to evaluate photosynthetic pigments. Phytotoxicity was measured using percentage inhibition. *C. vulgaris* showed growth stimulation at 10% - 50% concentrations of WSFs of hydrocarbon while 75% and 100% concentrations were inhibitory. *A. flosaquae* showed growth stimulation in 10% - 50% concentration of WSF of

(Xylene), 10% - 75% of WSF of Toluene and Hexane and 10% - 100% in WSF of Benzene. *N. palea* showed growth stimulation at all concentrations (10% - 100%) of the WSFs of the various hydrocarbons. Photosynthetic pigment (Chlorophyll a, b and c*) in A. flosaquae* were negatively affected in lower to mid concentration of WSF of Xylene, higher concentrations in WSF of Toluene and Hexane and no effect on all concentrations of WSF of Benzene. *C. vulgaris* showed adverse effects on these growth parameters only at higher concentrations while *N. pale* show no negative effects in these parameters in all concentrations. The order of phytotoxicity of WSFs of hydrocarbons to the microalgae follows this pattern: Toluene > Xylene > Benzene > Hexane while the order of growth stimulation or response is *N. palea > A. flosaquae > C. vulgaris*. Phytotoxicity was higher in WSF of aromatic hydrocarbons (Benzene, Xylene and Toluene) than the WSF of the aliphatic hydrocarbon (Hexane). The results obtained showed mineralization pattern of the four hydrocarbons by the different species of microalgae and thus could be an addition to existing list of indicators microalgae of crude oil hydrocarbon polluted environment and microorganism with hydrocarbon bioremediation potentials.

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 effect of water-soluble fractions (WSF) of the three petroleum fuels was investigated at 0%, 25%, 50% and 100%. The growth response of both species was monitored optically every two days for 14 days using a 721 visible spectrophotometer. Chlorophyll *a,* morphology and antioxidant enzyme activity of the algae were examined using prescribed methods. In both algae, minimum growth was obtained with 100% WSF of the petroleum fuels. In *N. oculata*, there was growth stimulation and the maximum growth was obtained at different concentrations (25% and 50%) depending on the test fuels. The maximum growth of *P. cruentum* was obtained at 10% WSF in all the fuels. ANOVA ($P < 0.05$) showed significant differences in algal growth with changes in concentration of the test fuels. Unpaired t-tests showed that in all the fuels, there was a significant difference ($p < 0.05$) between the growth of *N. oculata* and *P. cruentum*. *N. oculata* showed more tolerance to petroleum fuel pollution than *P. cruentum*. Morphological studies showed that petroleum fuel pollution altered the size of *N. oculata* and caused severe cell clumping in *P. cruentum*. Antioxidant concentration assessment showed that whereas *N. oculata* produced high levels of superoxide dismutase, catalase and peroxidase, *P. cruentum* produced high levels of superoxide dismutase but was less efficient in catalase and peroxidase production.

The productivity and bioremediation potential of *Nannochloropsis oculata* and *Porphyridium cruentum* in Water Soluble Fraction (WSF) of petroleum fuels was examined by Ezenweani and Kadiri (2023), The study was carried out by investigating the growth of *Nannochloropsis oculata* and *Porphyridium cruentum* at 0%, 10% 20% 30% 40% 50% 75% 100% of WSF of kerosene, diesel, and gasoline. Growth was monitored optically every two days for fourteen days using 721 Visible Spectrophotometer. Productivity was measured using prescribed procedure. Bioremediation potential of test algae was examined using standard method for the GC analysis of diesel range organics in 100% WSFs. The minimum growth for both species was recorded at 100% in all the fuels. The maximum growth of *Porphyridium cruentum* was obtained at 10% in all fuels, while the maximum growth of *Nannochloropsis oculata* was obtained at 30% in both kerosene and gasoline and at 50% in diesel. Result revealed that *Porphyridium cruentum* was greatly inhibited by all fuels, *Nannochloropsis oculata* was stimulated at lower concentration of the fuels. *Nannochloropsis oculata* proved more efficient for bioremediation of the petroleum fuels with 84.58%, 65.51% and 70.77% removal efficiency for kerosene, diesel and gasoline respectively, while *Porphyridium cruentum* was 58.94%, 46.64% and 56.67% respectively. *Nannochloropsis oculata* is a very strong and reliable candidate for bioremediation of petroleum hydrocarbons and should be subjected to further examination for sustainable and eco-friendly remediation of petroleum pollution.

A study carried out by Bello *et al.* (2024) assessed the potential of water-soluble fractions (WSFs) of crude oil, diesel, spent engine oil, and their composite mixture, to inhibit the growth of marine microalgae, *Skeletonema costatum*. 72 hours after exposure, the sensitivity of test organisms to the WSF of various petroleum hydrocarbons were assessed using potential inhibition of cellular growth. The inhibition concentrations (IC50) were determined as 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). After exposure for 72 hours, the cell density of *Skeletonema costatum* in control vessels increased from $2x10^4$ cells/ml to a maximum of $203.52x10^4$ cells/ml, with an average of $33.92x10^4$ cells/ml. In the test samples, cell density diminished significantly with increasing concentrations and duration of exposure for vessels containing WSFs of crude oil, diesel, spent engine oil, and their composite mixture. The pH ranged from 7.6 and 8.3 across test samples at 0 - 72hrs. The calculated growth rate (µ) decreased with an increase in test concentrations, while the percentage inhibition increased. Toxicity of WSF of diesel peaked at 72 hours with a corresponding IC50 of 1.08% (10.81 g/L), followed by spent engine oil at 2.27% (22.70g/L) and crude oil at 4.57% (45.72 g/L). However, the WSF of composite mixture was observed to exert the least toxicity and had a corresponding IC50 of 5.54% (55.36 g/l) in the exposed population of *Skeletonema costatum*..

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 tests were conducted in the Limnology and Phycology Laboratory of the department of Plant Biology and Biotechnology.

3.2 Test microalgae

The test microalgae were *Monoraphidium contortum* and *Dimorphococcus lunatus*

3.3 Collection of test microalgae

Water samples from algal blooms of various microalgae used for this studies were collected from ditches around medical street, Uselu Benin City (Lat long 6.367234706878662°, 5.626601696014404°) and a Fish pond at airport road Benin City (Lat, Long 6.290207469178526°, 5.586585166076196°)

3.4 Isolation of pure culture of microalgae

Cultures of test microalgae were obtained by isolating desired alga and inoculating into a growth medium. Unialgal cultures and the resulting cultures were obtained after a series of subcultures were subjected to microscopic examination to confirm the algal species in the samples prior to use.

3.5 Botany of Test Microalgae

3.5.1 *Monoraphidium contortum*

Description and Taxonomic Classification

Monoraphidium contortum consists of single cells, which are 2-182 by 1-8 micrometers. The cell is straight to lunate to sigmoid or helically shaped. Cells contain a single nucleus, a single parietal chloroplast and a single pyrenoid lacking a starch sheath (or no pyrenoid at all). Reproduction occurs asexually by autospores. *Monoraphidium contortum,* is a species of green microalgae belonging to the class Chlorophyceae. It is characterized by its filamentous morphology, consisting of long, slender cells arranged in unbranched chains. The cells are typically curved or contorted, giving the organism its distinctive appearance (Ramos *et al.,* 2012). *M. contortum* is commonly found in freshwater environments, including ponds, lakes, and streams, where it thrives under a wide range of environmental conditions. As a photosynthetic microorganism, *Monoraphidium contortum* plays a significant role in aquatic ecosystems by serving as a primary producer and contributing to nutrient cycling. *M. contortum* can tolerate varying levels of light intensity, temperature, and nutrient availability, allowing it to adapt to dynamic environmental conditions (Kirchner *et al.*, 2022). Additionally, it serves as a food source for various aquatic organisms, including zooplankton and small invertebrates, contributing to the transfer of energy through the food web (Taipale *et al.*, 2022).

Taxonomic Classification

Kingdom: Protista

Division: Chlorophyta

Class: Chlorophyceae

Order: Chlorellales

Family: Scenedesmaceae

Genus: *Monoraphidium*

Species: *Monoraphidium contortum*

Plate 3.1: A photomicrograph of *Monoraphidium contortum*

3.5.2 *Dimorphococcus lunatus*

Description and Taxonomic Classification

Dimorphococcus lunatus is usually found in small colonies of multiples of four cells, surrounded by a gelatinous mass. Groups of four cells are further attached to each other via mucilaginous strands, which are the remnants of the mother cell wall. Cells are kidney-shaped to heart-shaped, 10–25 µm long and 3–8(–15) µm wide. Each cell is uninucleate (containing one nucleus) and has one parietal chloroplast each with one or more pyrenoids. *Dimorphococcus lunatus* reproduces asexually via autospores, with four spores produced per mother cell. *Dimorphococcus lunatus* exhibits a unique dimorphic life cycle, alternating between motile, flagellated cells (swarmer cells) and non-motile, vegetative cells (palmelloid cells). This morphological adaptation allows *D. lunatus* to colonize diverse aquatic habitats, including freshwater lakes, ponds, and wetlands. It plays a crucial role in primary production and nutrient cycling in aquatic ecosystems, contributing to the oxygenation of water bodies and providing a food source for herbivorous organisms (Cornwallis *et al.,* 2023). *Dimorphococcus lunatus* can form blooms under favorable environmental conditions, which may have implications for water quality, ecosystem dynamics, and human health.

Division: Chlorophyta

Class: Chlorophyceae

Order: Sphaeropleales

Family: Scenedesmaceae

Genus: *Dimorphococcus*

Species: *Dimorphococcus lunatus*

Plate 3.2: A photomicrograph of *Dimorphococcus lunatus*

3.6 Preparation of culture media

The micro algae species were grown in an artificial medium, Chu's modified No 10 media which was used in combination with the WSF of spent engine oil. The composition of the modified medium is shown in the table below

3.7 Composition of modified Chu 10 culture medium

3.7.1 Macronutrient Stock Solution

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

3.7.2 Iron Stock Solution

Iron stock was prepared by adding 3.35g of citric acid ($C_6H_8O_7$. H_2O) and 50ml of distilled water, followed by the addition of 3.35g of ferric citrate ($FeC_6H_5O_3$. $5H_2O$). The volume was then brought up to 100ml final volume and autoclaved

3.7.3 Trace Element Stock Solution

A trace element solution was made by dissolving the salts below in the amounts (mg) indicated in 1litre of distilled water. The mixture was autoclaved and kept sterile.

3.7.4 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. Sterilization through filtration or autoclaving might be necessary.

Table 3.1 Preparation of Chu No. 10 medium

Macronutrients	**g/100ml**
$CaCl_{2.}$ $2H_20$	3.67
$MgSO_{4.}$ $7H_20$	3.69
$NaHCO_3$	1.26
K_2HPO_4	0.84
$NaNO_3$	8.50
Na_2SiO_3	2.84
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
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
Vitamin Stock Solution	**g/100ml**
Cyanocobalamin(B_{12})	0.004
Thiamine	0.004
Biotin	0.004

3.8 Culture Vessels

The study utilized brand new plastic culture bottles with 250ml capacity. They were acid washed using dilute hydrochloric acid (HCl-water ratio 1:1) and rinsed several times with distilled water. Thereafter these bottles were allowed to air dry before being used. Following this, the bottles were subsequently labelled according to test algae and effluent concentration.

3.9 Spent Engine Oil Source

The spent engine oil was collected from an auto-mechanic workshop at wire road Benin City, Edo state (Lat, Long 6.347131407442849°, 5.619806093636806°).

3.10 Preparation of water soluble fraction (WSF) of spent engine oil

The procedure of Ezenweani and Kadiri (2023) was followed to obtain the 100% stock solution of the WSF of each of the spent engine oil. The stock solution was prepared by mixing 1 part of spent engine oil to 9 parts of distilled water. The mixture was stirred using a magnetic stirrer hotplate for 24 h. The solution was allowed to stand for 24 h in a separating funnel after which the aqueous phase was separated and regarded as 100% stock solution of WSF.

Plate 3.3: Preparation of WSF of spent engine oil.

3.11 Preparation of different concentration of treatments

Different concentration of treatment samples was prepared by adding the volume of stock solution and growth medium as shown in the table below.

Table 3.2 Preparation of different concentration of treatments.

Concentration (%)	Volume of Stock Added (ml)	Volume of Growth Medium Added (ml)
0	0	720
5	36	684
10	72	648
25	180	540
50	360	360
75	540	180
100	720	0

3.12 Experimental Set up

A total of seven treatments were used in the experiments. These were 0% - control, 5%, 10%, 25%, 50%, 75%, and 100%.

WSF fractions of spent engine oil with each in separate triplicate bottles which were properly labelled. The treatments were obtained through serial dilution of the effluents with growth medium using the measurement as shown in the table 3.5.

3.13 Inoculation

Treatments were inoculated using a brand new syringe. Five (5) milliliters of the unialgal culture were used to inoculate the vessels holding the different treatment and repeated for each replicate. To allow for efficient oxygen exchange and to avoid contamination, the cultures were partly capped after inoculation. Thereafter the culture vessels were positioned next to a north facing window and the replicates were rotated every two days to allow for equal exposure to light.

3.14 Growth response of microalgae

Growth response test of algae was determined optically spectrophotometric ally using a visible spectrophotometer (Model No. HV-721). The equipment was turned on 15-20 minutes before measurements were done, and the wave length was adjusted to 750nm. This was double checked throughout readings. Each reading was preceded by the addition of distilled water and pressing of the rest button on the cuvette in order to zero the equipment. After zeroing the equipment, a 5ml aliquot of the culture was transferred to a matching cuvette and placed in the cell holder of the spectrophotometer. The lid of the equipment was then covered and the displayed readings were recorded. This process was repeated for all replicates of the different concentrations.

Plate 3.4: *Monoraphidium contortum* at Day 0

Plate 3.5: *Monoraphidium contortum* at Day 14

Plate 3.6: *Dimorphococcus lunatus* at Day 0.

Plate 3.7: *Dimorphococcus lunatus* at Day 14.

3.15.1 Percentage Inhibition

The percentage inhibition was calculated for the test cultures at day 14 of the experiment using the formula

$$Percentage\ inhibition = \left(\frac{Measured\ biomass}{Theoretical\ biomass} \times \frac{100}{1}\right)$$

3.15.2 Percentage Yield

The algal yield of the cultures in the test concentrations were determined at the day 14 of the experiment using the formula

$$Percentage\ yield = \left(\frac{Actual\ yield}{Theoretical\ yield} \times \frac{100}{1}\right)$$

3.15.3 Cumulative Growth

Cumulative growth rate of cultures in each test concentration was determined on day 14 of the experiment, by calculating the total value of each concentrations and plotting a bar chart with it.

$$Cummulative\ growith\ rate\ = \left(\frac{Ending\ value}{Beginning\ value}\right)^{\frac{1}{n}} - 1$$

Where n is the number of periods in which the growth rate is growth rate is measured (which can be in days, months, week's e.t.c)

3.15.4 Statistical Analysis

Computations of the above measurement were graphically represented using bar charts, line graphs some singly and comparatively. The variations were analyzed using the Analysis of Variance (ANOVA)

3.16 Physicochemical parameters

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

This was determined using a HACH CO150 TDS/Conductivity/Salinity meter. The samples were shaken vigorously after which the probe was dipped into the cultures in the one 250ml culture vessels. The readings were then recorded when the values steadied.

13.16.2 Electrical Conductivity (µS/cm)

Conductivity values were obtained using a HACH CO150 TDS/Conductivity/Salinity meter. Again, the culture in the 250ml plastic containers were mixed thoroughly for and the probe was then dipped immediately in the cultures and the appropriate values displayed were recorded.

13.16.3 Hydrogen Ion Concentration (pH)

This was determined using a HANNAH field pH meter was dipped into the cultures and the appropriate values were taken.

CHAPTER FOUR

RESULTS

4.1a Growth response of *Monoraphidium contortum* in WSF of spent engine oil

Figure 4.1a shows the growth response of *Monoraphidium contortum* in WSF of spent engine oil.

All the concentrations of the WSF of the spent engine oil were seen to exhibit a lag phase from day 0 to day 2. However, an exponential growth was noticeable in the concentrations of 0 %(control), 5%, 10%, 25%, 50%, 75% in same pattern from day 4 to day 14, with the exception of the 0%(control) which had a drop in growth at day 12. Much depressed growth was seen in 100%. Maximum growth response was recorded in the lowest concentration (5%), this was closely followed by 25%, 50%, 10% and 75% in that order.

Statistical analyses reveals that there was no significant difference (p>0.05) in growth response among the different concentrations. However, the temporal differences were significant (P= 1.43959E-45) or (p< 0.001).

Figure 4.1a: Growth response of *Monoraphidium contortum* in WSF of spent engine oil.

4.1b Growth response of *Dimorphococcus lunatus* in WSF of spent engine oil

The growth response of *Dimorphococcus lunatus* in varying concentrations of WSF of spent engine oil is represented in Figure 4.1b. The growth pattern in different concentration of the WSF of spent engine oil revealed that the alga lagged up to day 2. Between day 2 and day 6 a decreasing trend for all concentration was observed. From day 6 onwards the growth response showed an increasing trend till day 10, for all with the exception of 25% which experienced a drop in growth. The maximum growth was recorded in 5%, followed by a distant second by 10%. Growth at 0%, 50% and 75% were remarkably comparable while growth in 100% showed least growth.

Statistical results of the ANOVA showed that there was no significant difference (*P* = 0.999997881) in growth response between the different concentration. Between columns the differences were found to be (*P* = 9.65895E-46).

Figure 4.1b: Growth response of *Dimorphococcus lunatus* in WSF of spent engine oil

4.2 Percentage inhibition of *Monoraphidium contortum* and *Dimorphococcus lunatus*

Figure 4.2 shows the percentage inhibitory and stimulatory response of *Monoraphidium contortum* and *Dimorpococcus lunatus* to varying concentration of WSF of spent engine oil.

Generally, the growth of the microalgal species was stimulated in the lower concentrations and inhibited in the higher concentrations. Specifically, growth stimulation was recorded at 5%, 10%, and 25% for *D. lunatus* while inhibitory response was observed at 50%, 75%, and 100%. For *M. contortum* the cultures were observed to show stimulatory growth response at 5%, 10%, 25%, and 50% while the reverse was true for 75% and 100%. Overall, the highest stimulatory response was recorded at 50% and the lowest was at 10%. Conversely, the highest inhibitory response was observed at 100% concentration.

Figure 4.2: Percentage inhibition of *Monoraphidium contortum* and *Dimorphococcus lunatus*

4.3a Percentage yield of *Monoraphidium contortum* to varying concentration of WSF of spent engine oil.

Figure 4.3a shows the percentage yield of test alga (*Monoraphidium contortum*) in different concentration of WSF.

Initially at day 0 the alga showed a lag yield, as increase in yield was observed from day 2. Maximum yield was observed for 5%, from day 6 to day 14. Meanwhile lowest yield was seen at 100%, while 5% and 10%, 25% and 50% had similar yield patterns.

Figure 4.3a: The percentage yield of test algae *(Monoraphidium contortum)* in WSF of spent engine oil.

4.3b Percentage yield of *Dimorphococcus lunatus* to varying concentrations of WSF of spent engine oil.

Figure 4.5 shows the percentage algal yield of *Dimorphococcus lunatus Spent* engine oil WSF concentrations. The highest yield was observed in 10% from day 2 to day 14, except at day 12. However, yield at 5% and 25% concentrations were almost similarly paired as they showed similar yield pattern at same concentrations. There was no consistent pattern in algal yield for 5%, 75%, and 100% concentration, though the lowest yield was seen in 100%.

Figure 4.3b: The percentage algal yield of *Dimorphococcus lunatus* in WSF of spent engine oil.

4.4 Cumulative Growth Rate of *Dimorphococcus lunatus* and *Monoraphidium lunatus* in WSF of spent engine oil.

Figure 4.5 shows the cumulative growth rate of *Dimorphococcus lunatus* and *Monoraphidium lunatus on WSF* of spent engine oil. *Monoraphidium contortum* showed a steady increase in growth from 0% to 50%, reaching a peak value just below 0.35. Beyond 50%, the growth rate appears to plateau, remaining relatively stable around the peak value. *Dimorphococcus lunatus* exhibited a different growth trend. The growth rate rises steadily to a peak value just over 0.15 at 25%. Subsequently, there is a decline in growth rate, with values dropping to approximately 0.1 at 50% and further decreasing to just above 0.05 at 75%. Finally, the growth rate approached 0 at 100%. Overall, the contrasting growth patterns between *Monoraphidium contortum* and *Dimorphococcus lunatus* are evident, with *Monoraphidium contortum* showing a more stable growth trend after reaching its peak, while *Dimorphococcus lunatus* experienced a decline in growth rate after its peak at 25%.

Figure 4.5 The cumulative growth rate of *Dimorphococcus lunatus* and *Monoraphidium lunatus* in WSF of spent engine oil.

4.5. Physicochemical Parameters

From Table 4.1, it can be observed that the stock solution (100% WSF) analyzed at day zero (0) of the experiment recorded relatively low concentrations of total dissolved solids (TDS) and electrical conductivity (EC), with values of 13 mg/L and 26 µS/cm respectively. Additionally, the pH of the solution was 7.76, indicating a slightly alkaline nature.

For *Monoraphidium contortum* cultures it was evident that as the concentration of the WSF of spent engine oil increased, there was a trend of decreasing pH, conductivity, and total dissolved solids. Specifically, at the control (0%), the pH was 9.5, while conductivity and TDS were 276 µS/cm, and TDS was 138 mg/L respoectively. As the concentration increased to 5%, 10%, and 25%, the pH remained relatively stable around 9.5, while conductivity decreased to 272 µS/cm, 256 µS/cm, and 228 µS/cm respectively, and TDS decreased to 136 mg/L, 128 mg/L, and 114 mg/L respectively.

The physicochemical parameters of *Dimorphococcus lunatus* cultures on day 14 of the experiment across various concentrations (mg/L) compared to a control (0%), showed that as the concentration of WSF increased, there were discernible changes in pH, conductivity (µS/cm), and total dissolved solids (TDS, mg/L). At the control (0%), the pH was recorded as 10.0, conductivity as 279 µS/cm, and TDS as 139 mg/L. As the concentration of WSF increased from 5% to 100%, there's was trend of decreasing pH, conductivity, and TDS. Specifically, at 100% concentration, the pH dropped to 7.9, conductivity decreased to 60 µS/cm, and TDS declined to 30 mg/L.

Table 4.1: Physicochemical Parameters of Stock Solution, *Monoraphidium contortum* (MC) and *Dimorphococcus lunatus* (DL) Cultures in different WSF concentrations of Spent Engine Oil

Concentrations	pH	Electrical Conductivity (uS/cm)	Total Dissolved Solids (mg/L)
Stock solution	7.76	26	13
0% (control) MC	9.5	276	138
5% MC	9.6	272	136
10% MC	9.5	256	128
25% MC	9.5	228	114
50% MC	9.4	163	81
75% MC	8.7	98	49
100% MC	6.9	58	29
0 %(control) DL	10.0	279	139
5% DL	10.0	270	135
10% DL	10.1	257	128
25% DL	10.0	224	112
50% DL	10.0	164	82
75% DL	9.6	98	49
100% DL	7.9	60	30

CHAPTER FIVE

5.1 Discussion

The indiscriminate disposal of used engine oil, a widespread environmental issue in Nigeria, surpasses even crude oil spills in its prevalence (Ajayi *et al*., 2008; Michael, 2015). Disposal of the spent lubricant into gutters, water drains, open vacant plots and farms is a common practice especially by motor mechanics that change oil from motor vehicles, power generating machines and other machines. The discharge of spent engine oil into aquatic environments disrupts algal growth and jeopardizes the health of aquatic ecosystems due to the presence of toxic hydrocarbons and bioavailable metallic contaminants within the water soluble fraction (WSF) (Olaleye and Kadiri, 2021).

The effect of spent engine oil on the growth of two freshwater microalgae (*Monoraphidium contortum* and *Dimorphococcus lunatus*) was studied for a period of two weeks. In this study the growth response of the test microalga; *Monoraphidium contortum* and *Dimorphococcus lunatus* to various WSF concentrations of spent engine oil were examined. There was a lag phase for both test algae between day 0 and day 2, which is commonly observed in microalgal cultures. This initial lag likely reflects the time required for the cells to adjust to the new growth environment and initiate cell division (Lam *et al.,* 2017). A study by Chunzhuk *et al.* (2023) investigating the effect of light intensity on *Chlorella minutissima* growth supports this concept. Their research observed a lag phase in all light conditions before the exponential growth phase began. An exponential growth phase was observed in all treatments after the initial lag phase. This rapid rise in cell number likely occurred due to the sufficient availability of nutrients and the successful adaptation of the microalgae to the culture conditions (Nathalie *et al.,* 2010).

Studies by Nisa *et al.* (2021) on optimizing *Nannochloropsis oculata* culturing conditions also reported the same observation. Their study found that cultures achieved exponential growth when essential nutrients were provided. The study found that *Monoraphidium contortum* exhibited significantly depressed growth at 100% concentration of spent engine oil WSF, while demonstrating the most robust growth at 5% concentration. Conversely, *Dimorphococcus lunatus* achieved its peak growth at both 5% and 100% WSF, with the lowest growth observed at 0% concentration. Microalgae's utilization of the water-soluble fraction (WSF) of engine oil as a carbon source for photosynthesis likely explains the observed growth stimulation at low WSF concentrations (Radice *et al.*, 2023; Dell' Anno *et al.,* 2021). Lauritano *et al.* (2020) proposed several explanations for the observed growth patterns. Lower WSF concentrations might have stimulated growth due to two factors including volatilization of some toxic hydrocarbons, reducing their inhibitory effect, and adaptation of the microalgae population adapting to utilize the WSF components as a nutrient source. Conversely, higher WSF concentrations likely resulted in growth depression and inhibition due to the presence of lethal hydrocarbons exceeding the tolerance threshold of the organisms. This aligns with the concept of hormesis, where low doses can be stimulating and high doses become toxic (wang *et al.,* 2016). Supporting this finding, a study by Gomaa *et al*. (2023) which investigated microalgae responses to environmental stressors found that low-dose exposure to pollutants can sometimes enhance growth, while high doses have detrimental effects (wang *et al.,* 2016). The implication of high concentration of fuel oil was also reported by Kato *et al*. (2021) who stated that the carbohydrate content of algal cells decreased significantly as a result of high concentrations of fuel oils.

The observed consistent decrease in algal growth with increasing WSF concentrations aligns with previous work of Kadiri and Eboigbodun (2012) who reported similar growth retardation in algae exposed to higher concentrations of the Water Soluble Fraction (WSF) of kerosene, petrol, and diesel. This suggests that the toxic components within the WSF inhibit algal growth at elevated levels. Additionally, Olaleye and Kadiri (2021) observed a consistent decrease in algal growth with increasing concentrations of Waste Accommodated Fraction (WAF) of waste engine oil. While their study used WAF instead of WSF, both represent water-soluble fractions of petroleum products with similar growth-inhibiting effects on algae. Finally, Özbay (2016) reported a seemingly contradictory finding – higher chlorophyll *a* concentration at low motor oil concentrations. However, chlorophyll *a* concentration can be a stress response in some algae, indicating an attempt to compensate for reduced photosynthetic efficiency at lower oil concentrations.

The statistical analysis using ANOVA revealed interesting findings. For *Monoraphidium contortum*, there was no significant difference ($p > 0.05$) in growth response across the different WSF concentrations tested. This suggests that the growth of *M. contortum* was relatively consistent regardless of the WSF concentration within the experiment's range. However, the temporal differences between measurements were statistically significant ($P < 0.001$). This indicates that the growth rate or response of *M. contortum* changed significantly over the course of the experiment, likely due to factors like nutrient depletion or entry into a different growth phase.

The statistical analysis using ANOVA revealed interesting findings for *Dimorphococcus lunatus*. There was no significant difference ($P = 0.999997881$) in growth response between the different WSF concentrations tested. This suggests that the growth of *D. lunatus* was relatively consistent regardless of the WSF concentration within the experiment's range. This aligns with observations in other studies, such as the one by Olaleye and Kadiri (2021) who reported that certain microalgae species exhibit tolerance to a wide range of water-soluble fractions of used lubricating oil. Additionally, the analysis indicated a highly statistically significant difference (*p* = 9.65895E-46) between the growth responses over time suggesting that the growth rate or response of *D. lunatus* changed significantly throughout the experiment. This observation could be due to factors such as the algae entering a different growth phase, nutrient depletion in the culture medium, or other environmental changes that occurred during the experiment.

The results of percentage inhibition determination of the two microalgal species show that there was growth stimulation at concentrations that are considered low to medium (5%, 10%, 25%, and 50%) for both test algae and growth inhibition for both algae at high concentrations (75%, 100%) with *M. contortum* having the highest inhibitory response and *D. lunatus* having the highest stimulatory responses. The observed growth stimulation at 5%,10%, 25%, and 50% respectively is in consonance with the studies of Özhan *et al.* (2014) where the degree to which crude oil influenced phytoplankton growth varied with the concentration of oil and the algae species. Increasing crude oil concentrations increased the percent growth inhibition rates for all and lowest total petroleum hydrocarbon concentration showed stimulation in growth. Growth stimulation at low concentrations can be explained on the basis of the fact that microalgae are able to use the water soluble fraction as a carbon source for photosynthesis (Filali *et al.*, 2021). Both laboratory researches and field investigations showed that low concentrations of WSF in water bodies had less adverse influence on microalgae, even could promote it growth (Ramadass *et al*., 2014). On the contrary, spent engine oil may be toxic to *Monoraphidium contortum* and *Dimorphococcus lunatus* in higher concentrations (75% and 100%) due to several effects such as decreased growth rate, cell number, biomass, chlorophyll *a*, photosynthesis, respiration, yield, nucleic acids (DNA, RNA), proteins, carbohydrates, dry matter, chlorosis and sometimes death of algae (Kadiri and Eboigbodin, 2012; Lewis and Pryor, 2013). Several authors also observed growth inhibition at high concentrations (Kadiri and Egboigbodin, 2012; Ezeawani and Kadiri, 2023). Toxicity of hydrocarbons on algae occurs via disruption of cellular metabolism (Tomar *et al.,* 2022), as they enter into the lipophilic layer of the cell membrane from properly controlling the transport of ions in and out of the cell. Meanwhile the higher stimulatory response of *D. lunatus* compared to *M. contortum* could be due to the fact that the concentrations of WSF of spent engine oil was not harmful and the microalga was able to acclimatize and metabolize which suggests it has a better bioremediative potential (Perhar and Arhonditsis, 2014).

Alga yield in the both microalgae studied was enhanced at low to high concentrations (0-75%) for *M. contortum* and low to moderate concentrations (0-50%) for *D. lunatus.* This corroborates with reports of chan and Munir *et al.* (2022) that low concentration of British petroleum (BP) light diesel and the oil dispersant BP1100X either alone or in a mixture stimulated the growth rate, bomass yield, chlorophyll a level and photosynthesis of the estuarine alga *Chlorella salina*.

In this study, *Monoraphidium contortum* was seen to have outperformed *Dimorphococus lunatum* in growth. This suggests that *M. contortum* has a generally faster growth rate compared to *D. lunatus* under the conditions of this experiment. The varying degrees of difference between the two species at different WSF concentrations could be due to several factors. *M. contortum* might have a higher tolerance to the WSF components, allowing it to maintain a faster growth rate even at higher concentrations. Additionally, it's possible that *M. contortum* has a more efficient metabolic pathway to utilize the WSF or its components as a nutrient source, leading to a growth advantage over *D. lunatus*. This is in consonance with studies of (Olaleye and Kadiri, 2021; Abreu *et al.,* 2012; Kadiri and Eboigbodin)

The analysis of cumulative growth rate revealed distinct patterns for the two microalgae species. *Monoraphidium contortum* exhibited a steady increase in growth from 0% to 50% WSF concentration, reaching a peak value slightly below 0.35. Beyond 50%, the growth rate plateaued, remaining relatively stable. This initial stimulation followed by a plateau at higher concentrations aligns with the study of Olaleye and Kadiri (2021). In contrast, *Dimorphococcus lunatus* displayed a different growth trend. Its growth rate rose steadily to a peak value just above 0.15 at 25% WSF, followed by a decline in growth rate with increasing concentrations. This suggests that *D. lunatus* might be less tolerant to the WSF components compared to *M. contortum*. The growth rate dropped to approximately 0.1 at 50% and further decreased to just above 0.05 at 75%, finally approaching zero at 100%.

The values obtained for all physiochemical properties assessed in the study including total dissolved solids, electrical conductivity and pH were within the regulatory limits for water quality recommended by the USEPA, (2017) and WHO, (2017). pH, holds immense significance in water quality, defined as the negative logarithm of the concentration of hydrogen ions (H^+) in a solution (Boyd *et al.,* 2011), it acts as a vital indicator of a solution's acidity or alkalinity. Expressed as a dimensionless number ranging from 0 to 14, pH provides a crucial lens through which we understand the health of water resources and the potential impacts of environmental factors (Al-Amshawee *et al.,* 2020). The initial stock solution in the study exhibited a pH of 7.76, falling within the generally favorable neutral to slightly alkaline range (pH 6.5-8.5) preferred by most microalgae. Interestingly, the research also observed that microalga growth itself can influence the culture medium's pH. During photosynthesis, microalgae consume carbon dioxide (CO_2), which can lead to an increase in pH as the concentration of H^+ ions decreases. However, some microalgae species may secrete organic acids or other metabolites that can decrease the culture medium's pH (Nwite and Alu, 2015).

The pH values for *Monoraphidium contortum* ranged from 6.9 (100% concentration) to 9.5 (control), and 7.9 (100% concentration) to 10.0 (control) for *Dimorphococcus lunatus.* Both species showed a decrease in pH with increasing concentrations of the tested substance. However, *Monoraphidium contortum* generally exhibited a broader range of pH values compared to *Dimorphococcus lunatus.* The decrease in pH values observed in both *Monoraphidium contortum* and *Dimorphococcus lunatus* with increasing concentrations of the tested substance can be attributed to several factors, including the release of acidic components from the substance itself, intensified metabolic activity of the microalgae species in response to environmental stress, uptake of nutrients such as carbon dioxide leading to the release of protons, and interactions between dissolved substances in the medium. However, *Monoraphidium contortum* exhibited a broader range of pH values compared to *Dimorphococcus lunatus,* potentially due to differences in their physiological characteristics such as metabolic rates, nutrient requirements, and pH tolerance mechanisms (Bhuyan *et al*., 2019; Schneider *et al.,* 2012).

Total dissolved solids (TDS), refers to all soluble solids, primarily mineral salts, present in water (Sullivan *et al*., 2017). In *Monoraphidium contortum,* total dissolved solids decrease from 138 mg/L (control) to 29 mg/L (100% concentration) and from 139 mg/L (control) to 30 mg/L (100% concentration) for Dimorphococcus *lunatus*. Both species showed a reduction in total dissolved solids as the concentration increased, with similar trends observed in their respective ranges. The decrease in total dissolved solids (TDS) observed in both *Monoraphidium contortum* and Dimorphococcus *lunatus* with increasing concentrations of the tested substance can be attributed to several factors according to the reports of Samuel (2022). As reported by Samuel (2022), the dilution effect resulting from the addition of the substance to the medium led to a proportional increase in water content, thereby lowering the concentration of dissolved solids. Some dissolved solids may adsorb onto the surface of microalgae cells or bind to organic matter, reducing their concentration in the medium. Furthermore, the metabolic uptake of nutrients and ions by the microalgae for growth and cellular processes contributes to the decrease in TDS concentrations as populations increase and the chemical transformations within the experimental system may result in the formation of insoluble precipitates or complex compounds, further reducing TDS levels (Peng *et al.,* 2019; Mohsenpour *et al.,* 2021; Nicula *et al.,* 2023). Conductivity, on the other hand, measures a water sample's ability to conduct electricity. For *Monoraphidium contortum,* conductivity decreased from 276 µs/cm (control) to 58 µs/cm (100% concentration) and from 279 µs/cm (control) to 60 µs/cm (100% concentration) for *Dimorphococcus lunatus*. Both species recorded a decline in conductivity with increasing concentrations, with *Monoraphidium contortum* showing slightly lower conductivity values. The decline in conductivity observed in both *Monoraphidium contortum* and *Dimorphococcus lunatus* with increasing concentrations of the tested substance can be attributed to the addition of the substance to the medium leading to a dilution effect, resulting in a decrease in the concentration of ions and other conductive substances (de Sousa *et al*., 2014; Fayad *et al*., 2023). Additionally, interactions between the substance and the ions present in the medium may result in the formation of complexes or insoluble compounds that are less conductive. Furthermore, metabolic processes and cellular activities of the microalgae, such as nutrient uptake and growth, can contribute to the reduction (Juneja *et al.,* 2013; Wang and Hong, 2022).

Interestingly, the study observed comparable growth rates across all tested TDS concentrations, including the stock solution. The observed connection between TDS and growth aligns with the established role of these dissolved solids as essential nutrients for microalgae. Algal cells actively absorb TDS, utilizing them as minerals and nutrients to fuel their metabolic processes (Kuldip *et al.*, 2018). This uptake of dissolved solids effectively reduces the overall TDS concentration in the culture medium. The study is in consonance with that of Masood and Saleem (2021), who reported a positive correlation between initial TDS concentration and microalgal biomass production, thus indicating that higher levels of dissolved solids (within an optimal range) provided essential nutrients for algal growth.

5.2 Conclusion

This study assessed the growth response of *Monoraphidium contortum* and *Dimorphococcus lunatus* when exposed to water soluble fraction of spent engine oil from a mechanic workshop at wire road, Benin City. Overall there was positive growth response for both species with *Monoraphidium contortum* consistently outperforming *Dimorphococcus lunatus* in growth across all WSF concentrations tested. The statistical analysis using ANOVA revealed interesting findings. For both test algae, there was no significant difference ($p > 0.05$) in growth response across the different WSF concentrations tested. However, the temporal differences between measurements were statistically significant ($p < 0.001$). The results from percentage inhibition of both test algae showed that there was growth stimulation at concentrations that are considered low (5%,10%, 25%, and 50%) for both test algae and growth inhibition for both algae at high concentrations (75%, 100%) with *M. contortum* having the highest inhibitory response and *D. lunatus* having the highest stimulatory responses. Algal yield in the both microalgae studied was enhanced at low to high concentrations (0-75%) for *M. contortum* and low to moderate concentrations (0-50%) for *D. lunatus.* The analysis of growth rates revealed that *Monoraphidium contortum* consistently outperformed *Dimorphococcus lunatus* across all WSF concentrations tested. The analysis of cumulative growth rate revealed *D. lunatus* might be less tolerant to the WSF components compared to *M. contortum.* The values obtained for all physical properties assessed in the study including total dissolved solids, electrical conductivity and pH were within the regulatory limits.

5.3 Recommendations

Based on the findings of the study, the following recommendation are made:

1. Waste engine oil from automobile and other sources should be properly disposed such that they would not constitute environmental problems vis-à-vis land pollution and its attendant effects on soil, plants and public health.

2. Research into recycling of these spent engine oils should be encouraged and funded as this would go a long way in reducing the large volumes and check-mating the unhealthy disposal of these petroleum wastes.

3. Strict disposal regulations and legislations against irregular and reckless disposal of this environmental hazard.